Topics in Pharmaceutical Quality and Clinical Laboratory Medicine Laboratory Manual

PHM 241H1 2018

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University of Toronto

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Contributions to the PHM 241H1 Laboratory Manual were also made by James Rogers, University of Alberta.

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Preface

There are a lot of rules and guidelines that accompany working in a laboratory, since there are chemicals and equipment that can be dangerous if handled improperly. Rising above the details, there are three basic tenets that will permeate through each laboratory:

1) Be aware of the specific hazards and protect yourself accordingly;
2) Think about the exercises as you are doing them, and learn the techniques and principles behind them;
3) **Have fun!** A lab is a refreshing change from the classroom, where you have an opportunity to observe concepts in action, rather than just being told how they work.

Pharmaceutics is one of the foundations upon which the profession of pharmacy has been built. This laboratory has been designed to allow you to examine some of the basic physiochemical properties of common dosage forms. The laboratory environment offers what the classroom can never achieve: a working knowledge. The experiences gained in this course will find utility in community and hospital practice, and sometimes everyday life. The techniques and principles are also extremely relevant to so many other areas of study: cosmetics, chemical engineering, and therapeutics to name a few. My role as Laboratory Coordinator is not only to make sure everything runs smoothly, but also to try to instill in you some of the enthusiasm that I have for this area of pharmacy practice.

This manual will help guide you to learn fundamental pharmaceutics principles. Although a brief introduction is provided for each lab exercise, this manual does not contain all the necessary information for you to completely understand the theory behind each lab. To make learning easier, you should read the manual, and learn how to perform the necessary calculations prior to attending the lab. References are provided for more in-depth knowledge and utility in pharmacy practice.

The Introduction section of this manual includes an outline of the laboratory formats, safety considerations, methods of evaluation, and an outline of the duties of the Teaching Assistants. Following the Introduction section are the procedures for the six laboratories in this course. These labs, each with their own themes, are to be performed in small groups. You are encouraged to work together with your lab partner(s) through each section. The labs have been carefully designed to fit into their allotted time frames. Avoid assigning a section of the lab to a specific person – this will exclude you from the full educational benefit of the exercises. In order to work efficiently, subtasks in a section may be delegated.

The appendix to this manual contains useful reference data, and is provided as an extra resource to complement learning during the lab.

Subtle methods in the labs may be changed by your instructor, TA, or even by you, depending on the equipment and supplies available to you on your lab day. There is room for creativity.

If you find a specific section, step, or explanation in this manual vague or difficult to follow, ask your TA or instructor for help. Please let us know, so we can improve the manual for future editions.
The following icons are used in the margins throughout this manual:

<table>
<thead>
<tr>
<th>Icon</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Tip Icon]</td>
<td>Useful tip on an experimental method. Read carefully.</td>
</tr>
<tr>
<td>![Discussion Icon]</td>
<td>Important discussion point that is particularly useful in understanding the exercise.</td>
</tr>
<tr>
<td>![Safety Icon]</td>
<td>Important safety tip.</td>
</tr>
<tr>
<td>![Time Icon]</td>
<td>Time-critical experimental step.</td>
</tr>
</tbody>
</table>
General Information

The laboratory section of PHM 241H1 consists of six laboratory sessions, scheduled throughout the term. The class is divided into 4 groups of 60 students, and further divided into sub-groups of approximately 30 students. This enables us to most efficiently make use of laboratory resources.

To prepare for a lab, read the part of the lab manual pertaining to that lab exercise, understand the rationale of the exercise, watch any associated videos on the laboratory website, perform any calculations that are needed, be aware of any potential hazards, review the questions at the end of the lab, and go to bed early the night before. The laboratories start on time. There will be a closed-book quiz at the beginning each lab.

The group and locker assignments are posted on Quercus. Please visit Quercus in order to see your Group assignment. Because of the size of the class, we cannot make any changes to the group assignment list. Switching lab groups with other students is not permitted.

Recommended Textbooks

There are no required textbooks for the PHM 241 laboratory. This manual will serve as the primary reference. The following textbooks are recommended to clarify concepts or to serve as useful general references:


**Teaching Staff**

The following people will be teaching, helping, and evaluating your work in the lab:

<table>
<thead>
<tr>
<th>PHM 241H1 Laboratory Coordinator</th>
<th>E-mail Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>David Dubins</td>
<td><a href="mailto:d.dubins@utoronto.ca">d.dubins@utoronto.ca</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PHM 241H1 Teaching Assistants</th>
<th>E-mail Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taksim Ahmed</td>
<td><a href="mailto:taksim.ahmed@mail.utoronto.ca">taksim.ahmed@mail.utoronto.ca</a></td>
</tr>
<tr>
<td>Nellie Han</td>
<td><a href="mailto:nellie.han@mail.utoronto.ca">nellie.han@mail.utoronto.ca</a></td>
</tr>
<tr>
<td>Julian Gilmore</td>
<td><a href="mailto:julian.gilmore@mail.utoronto.ca">julian.gilmore@mail.utoronto.ca</a></td>
</tr>
<tr>
<td>Anthony Ku</td>
<td><a href="mailto:anthonysy.ku@mail.utoronto.ca">anthonysy.ku@mail.utoronto.ca</a></td>
</tr>
<tr>
<td>Jacinda Kwok</td>
<td><a href="mailto:jacinda.kwok@mail.utoronto.ca">jacinda.kwok@mail.utoronto.ca</a></td>
</tr>
<tr>
<td>Charles Yen</td>
<td><a href="mailto:tinyo.yen@mail.utoronto.ca">tinyo.yen@mail.utoronto.ca</a></td>
</tr>
</tbody>
</table>

**Role of Teaching Assistants**

Three Teaching Assistants (lab TAs) will be assigned to each laboratory period. Lab TAs have many demanding responsibilities before, during, and after the labs. The following outlines some of the roles and duties of your lab TAs:

**Before the lab:**
- Ensure the availability of chemicals and supplies, and inform the Instructor if orders are required in advance
- Work with the Instructor to ensure equipment in their assigned section is set up, functional and serviced
- Prepare buffers, reagents, and indicators in advance
- Arrive before the lab in order to warm up any relevant equipment and appropriately set up the lab
- Set up and (re)stock individual student workstations

**During the lab:**
- Take attendance, checking student TCards
- Ensure laboratory safety
- Notify the Instructor of any injuries or hazards in the lab
- Handling of the disposal of hazardous chemicals
- Provide pre-laboratory lectures in an interactive format
- Administering, invigilating, and collecting lab quizzes
- Supervising students regarding procedure, process, technique and safety elements of laboratory session
- Coordinate equitable access to equipment
- Supervise the progress of student work - by asking appropriate questions, not only by providing answers
- Provide directions and clarify instructions and protocols
- Ensure the cleanliness of the lab, and coordinate laboratory clean-up
• Ensure equipment is clean and shut down at the end of the lab (especially spectrophotometers)
• Collect student products/hand-ins for evaluation

After the lab:
• Assist with lab check-in and check-out
• Evaluate submitted laboratory reports, quizzes, and products
• Recording and entering marks on the Quercus system
• Marking midterm and final exams for PHM241

Attendance
Attendance will be recorded as you enter the lab. You are required to present your TCard, be properly dressed (lab coat, safety glasses) and sign the attendance sheet in order to enter the lab.

Outer coats, laptops, and backpacks are not permitted in the laboratory. These should be kept in your locker, on the B levels of the building.

Attendance at each lab is mandatory. You are expected to be available, and attend your entire lab period on your scheduled dates. In some instances, you will be permitted to leave early provided your work is submitted, your lab area is properly cleaned, and the post-lab talk has been completed.

If you are not able to attend a lab, you need to submit a petition with the appropriate supporting documentation (e.g. the U of T Student Medical Certificate) to the faculty registrar. If you are too sick to attend the lab, please do not come to the lab. If you have questions about the petition process, please contact the registrar.

Lab Safety Lecture
Safety training is an important safety and legal requirement in order to participate in laboratory activities. A mandatory introductory safety lecture FOR ALL GROUPS will be held on:
Tuesday September 6th 2018, from 4-5 pm in Room O1 G162 (OISE, 252 Bloor St. W.
Attendance will be taken. There will be a safety quiz. Absences from the safety lecture will require a formal petition, and a separate safety quiz.
Laboratory Schedule

*All laboratory announcements and experiment schedules will be posted on Quercus.*

Groups A and C have their laboratory sessions in PB 860 on **Fridays from 8:30 a.m. to 12:30 p.m.**

Groups B and D have their laboratory sessions in PB 860 on **Fridays from 1:00 p.m. to 5:00 p.m.**

---

### Fall Term 2018

**Room PB860 - Patheon Pharmaceutics Teaching Laboratory**

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Group – Lab #</th>
<th>Group – Lab #</th>
</tr>
</thead>
<tbody>
<tr>
<td>06-Sep-18</td>
<td>4:00p – 5:00p</td>
<td>Safety Lecture, TA Intro</td>
<td>Room OI G162 (OISE, 252 Bloor St. W.</td>
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<tr>
<td></td>
<td></td>
<td>All Groups</td>
<td></td>
</tr>
<tr>
<td>07-Sep-18</td>
<td>9:30a – 11:00a</td>
<td>Locker Check-in: Groups A &amp; B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3:00p – 4:30p</td>
<td>Locker Check-in: Groups C &amp; D</td>
<td></td>
</tr>
<tr>
<td>14-Sep-18</td>
<td>8:30a – 12:30p</td>
<td>A1 – Lab 1</td>
<td>A2 – Lab 2</td>
</tr>
<tr>
<td></td>
<td>1:00p – 5:00p</td>
<td>B1 – Lab 1</td>
<td>B2 – Lab 2</td>
</tr>
<tr>
<td>21-Sep-18</td>
<td>8:30a – 12:30p</td>
<td>C1 – Lab 1</td>
<td>C2 – Lab 2</td>
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<tr>
<td></td>
<td>1:00p – 5:00p</td>
<td>D1 – Lab 1</td>
<td>D2 – Lab 2</td>
</tr>
<tr>
<td>28-Sep-18</td>
<td>8:30a – 12:30p</td>
<td>C1 – Lab 2</td>
<td>C2 – Lab 1</td>
</tr>
<tr>
<td></td>
<td>1:00p – 5:00p</td>
<td>D1 – Lab 2</td>
<td>D2 – Lab 1</td>
</tr>
<tr>
<td>05-Oct-18</td>
<td>8:30a – 12:30p</td>
<td>A1 – Lab 2</td>
<td>A2 – Lab 1</td>
</tr>
<tr>
<td></td>
<td>1:00p – 5:00p</td>
<td>B1 – Lab 2</td>
<td>B2 – Lab 1</td>
</tr>
<tr>
<td>12-Oct-18</td>
<td>8:30a – 12:30p</td>
<td>C1 – Lab 3</td>
<td>C2 – Lab 4</td>
</tr>
<tr>
<td></td>
<td>1:00p – 5:00p</td>
<td>D1 – Lab 3</td>
<td>D2 – Lab 4</td>
</tr>
<tr>
<td>19-Oct-18</td>
<td>8:30a – 12:30p</td>
<td>A1 – Lab 3</td>
<td>A2 – Lab 4</td>
</tr>
<tr>
<td></td>
<td>1:00p – 5:00p</td>
<td>B1 – Lab 3</td>
<td>B2 – Lab 4</td>
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<tr>
<td>26-Oct-18</td>
<td>8:30a – 12:30p</td>
<td>A1 – Lab 4</td>
<td>A2 – Lab 3</td>
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<tr>
<td></td>
<td>1:00p – 5:00p</td>
<td>B1 – Lab 4</td>
<td>B2 – Lab 3</td>
</tr>
<tr>
<td>02-Nov-18</td>
<td>8:30a – 12:30p</td>
<td>C1 – Lab 4</td>
<td>C2 – Lab 3</td>
</tr>
<tr>
<td></td>
<td>1:00p – 5:00p</td>
<td>D1 – Lab 4</td>
<td>D2 – Lab 3</td>
</tr>
<tr>
<td>09-Nov-18</td>
<td>8:30a – 12:30p</td>
<td>A1 – Lab 5</td>
<td>A2 – Lab 6 &amp; Check-Out</td>
</tr>
<tr>
<td></td>
<td>1:00p – 5:00p</td>
<td>B1 – Lab 5</td>
<td>B2 – Lab 6 &amp; Check-Out</td>
</tr>
<tr>
<td>16-Nov-18</td>
<td>8:30a – 12:30p</td>
<td>C1 – Lab 5</td>
<td>C2 – Lab 6 &amp; Check-Out</td>
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<tr>
<td></td>
<td>1:00p – 5:00p</td>
<td>D1 – Lab 5</td>
<td>D2 – Lab 6 &amp; Check-Out</td>
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<td>23-Nov-18</td>
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<td>C1 – Lab 6 &amp; Check-Out</td>
<td>C2 – Lab 5</td>
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<td></td>
<td>1:00p – 5:00p</td>
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<td>D2 – Lab 5</td>
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<tr>
<td>30-Nov-18</td>
<td>8:30a – 12:30p</td>
<td>A1 – Lab 6 &amp; Check-Out</td>
<td>A2 – Lab 5</td>
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<tr>
<td></td>
<td>1:00p – 5:00p</td>
<td>B1 – Lab 6 &amp; Check-Out</td>
<td>B2 – Lab 5</td>
</tr>
</tbody>
</table>

Labs will begin with a ten-minute pre-lab quiz, so make sure you arrive on time and ready for the lab.
**Introduction**

**Locker Check-In / Check-Out**

**Check-In: Friday 07-Sep-18**

Your Locker #: ____________

You have been assigned a group number and a locker number. It is your privilege to use the locker, and your responsibility to maintain its contents. On your check-in day, make sure you have all the glassware according to the “Content of your Locker” list. You may also want to clean the glassware. Replacement of damaged equipment can be obtained from the back shelves, or the lab TAs.

**Equipment orders placed during the summer can be picked up at announced times during the first laboratory sessions.**

Take time to review the laboratory safety section of this manual and locate the following safety equipment in the laboratory. Indicate the location in the space provided below:

<table>
<thead>
<tr>
<th>Safety Equipment</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fire Extinguishers</td>
<td></td>
</tr>
<tr>
<td>Fire Alarm</td>
<td></td>
</tr>
<tr>
<td>Eye Wash Fountains</td>
<td></td>
</tr>
<tr>
<td>Safety Shower</td>
<td></td>
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<tr>
<td>First Aid Box</td>
<td></td>
</tr>
</tbody>
</table>

When your group has completed the locker check-in, notify your teaching assistant and he/she will ask you a few safety questions before you go.

**Locker key issued _________________________ (Student Signature)**

**Locker Check-Out (During Lab 6)**

A fee of $10.00 will be charged for locker key replacement. You are encouraged to attach the key to a secure key ring or case.

**Lab Check-Out Procedure**

- Clean your lab bench and any dirty glassware;
- Throw out any remaining formulations or garbage;
- Empty your locker water bottle;
- Verify that your locker contents are complete;
- Return any extra glassware to the laboratory back shelves;
- Get a TA or Instructor to verify the above, and sign your check-out list (next page);
- Be assigned a special area in the lab to clean;
- Lock your locker, and return your lab key when the above is completed.

Keep this and the following page in your laboratory manual.
# Locker Contents 2018

<table>
<thead>
<tr>
<th>Student Name</th>
<th>Locker #</th>
<th>Date</th>
</tr>
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<tbody>
<tr>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Name of Apparatus/Item</th>
<th>Qty</th>
<th>In</th>
<th>Out</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mL Volumetric Flask</td>
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<td></td>
<td></td>
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<tr>
<td>100 mL Volumetric Flask</td>
<td>2</td>
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<tr>
<td>200 mL or 250 mL Volumetric Flask</td>
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<tr>
<td>10 mL Graduated Cylinder</td>
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<tr>
<td>25 mL Graduated Cylinder</td>
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<tr>
<td>100 mL Graduated Cylinder</td>
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<tr>
<td>50 mL Erlenmeyer Flask</td>
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<tr>
<td>125 mL Erlenmeyer Flask</td>
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<tr>
<td>250 mL Erlenmeyer Flask</td>
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<tr>
<td>500 mL Erlenmeyer Flask</td>
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</tr>
<tr>
<td>5 cm Glass Funnel</td>
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<tr>
<td>7.5 cm Glass Funnel</td>
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</tr>
<tr>
<td>10 cm Glass Funnel</td>
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</tr>
<tr>
<td>Test Tube Rack</td>
<td>1</td>
<td></td>
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<tr>
<td>1 mL Bulb Pipette</td>
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<tr>
<td>5 mL Bulb Pipette</td>
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<tr>
<td>10 mL Bulb Pipette</td>
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<tr>
<td>20 mL or 25 mL Bulb Pipette</td>
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<tr>
<td>1 mL Graduated Pipette</td>
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<tr>
<td>10 mL Graduated Pipette</td>
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<tr>
<td>Thermometer (°C)</td>
<td>1</td>
<td></td>
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<tr>
<td>Watch Glass small</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watch Glass large</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8” Glass Stirring Rod</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>50 mL Beaker</td>
<td>2</td>
<td></td>
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<tr>
<td>150 mL Beaker</td>
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<tr>
<td>250 mL Beaker</td>
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<tr>
<td>400 mL Beaker</td>
<td>2</td>
<td></td>
<td></td>
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<tr>
<td>600 mL Beaker</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass Slab</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3” Ceramic Evaporating Dish</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>6” Ceramic Evaporating Dish</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plastic Wash Bottle</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceramic Mortar &amp; Pestle Set (Glass set additional in some cases)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Funnel Clamp and Holders</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sharpie Lab Marker</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TA Signature - Lab Check-In</th>
<th>TA Signature - Lab Check-Out</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>
Assignment of Grades

The laboratory portion of PHM 241H1 has been assigned a 40% weight of the course grade (PHM 241H1). Performance in the laboratory will be evaluated from a combination of activities. In order to receive a grade for the laboratory portion of the course, students submit work for each of the graded areas noted.

<table>
<thead>
<tr>
<th>% of total</th>
<th>( \text{PHM 241H1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory Quizzes ((2.5% \times 6))</td>
<td>15%</td>
</tr>
<tr>
<td>Lab Products ((2.5% \times 6))</td>
<td>15%</td>
</tr>
<tr>
<td>Formal Group Laboratory Report (\text{(Lab 3)})</td>
<td>10%</td>
</tr>
<tr>
<td>Total Lab Component:</td>
<td>40%</td>
</tr>
</tbody>
</table>

**PHM241 Lecture Component**

<table>
<thead>
<tr>
<th>% of total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid-term Examination</td>
<td>30%</td>
</tr>
<tr>
<td>Final Examination (\text{(MCQs)})</td>
<td>30%</td>
</tr>
<tr>
<td>Total Lecture Component:</td>
<td>60%</td>
</tr>
</tbody>
</table>

Recording Data, Analysis, and Presenting Results

**Individual Laboratory Worksheets \(\text{(Labs 1 – 5)}\)**

At the beginning of Labs 1 to 5, your TA will give you a lab worksheet in which you will record your data and observations for that laboratory. Laboratory worksheets will be handed out at the beginning of Labs 1 to 5, to provide you with space to collect data, perform calculations, and to help guide you with probing questions regarding the lab. These worksheets will not be evaluated. They are yours to take home. The worksheet for Lab 3 will help you document data for the formal lab report. Once you have taken a worksheet home, do not bring it back into the lab.

During the laboratory, you will be working in small groups. Any data that is collected during the lab period will be recorded in each member of the group’s lab worksheet. The analysis of the data, presentation and calculations are to be done individually, and recorded in the appropriate section in the lab worksheet. In many cases, you will also be using the laboratory computers to download, complete, and print spreadsheets from the laboratory website. In some cases, these spreadsheets will be handed in with your product(s).

Try to work cooperatively with others in your group. If there are unresolved conflicts, approach your TA or the lab coordinator to seek a solution. Remember, we are attempting to reach a goal together: to prepare you for pharmacy practice two years after you complete this laboratory.

Plagiarism and Falsification

At some point in your laboratory, you might look at your results and think,

“OH NO! This can’t be right!”

You will be nervous. You will wonder what happened. What went wrong? Worse off, you might be tempted to hide or change the results for that ONE point that should have fallen on the line.

However, you are reminded to **always report what you observed**, rather than what you would
have liked to observe. Provided you made the correct calculations and performed your exercises meticulously and carefully, you will not lose marks for less than perfect looking observations. Real data rarely look perfect. Things don’t always work. If they did, there would be no need for formulation scientists.

If you encounter suspicious looking data, identify your concerns in your analysis, and explain where you think things may have gone wrong (sources of error). If your entire data set is concerning you, seek the assistance of your TA. There could be a malfunction in the equipment, a problem with your method, or a systematic error in your calculations. If you have time, you can repeat the outlying measurements to refute or confirm their validity.

**DO NOT PLAGIARISE OR FALSIFY YOUR DATA.** Doing so is an offence under the University of Toronto Governing Council’s Code of Behaviour on Academic Matters.

**Unauthorized Aids – Important Note**

During the lab, you are welcome to bring in resources to help complete laboratory activities. The following materials are examples of unauthorized aids in the laboratory:

- Other people’s past labs
- Other people’s data/answers
- Other people’s notes
- Answers to questions from previous year lab reports
- Electronic notes on personal handheld devices
- Programmable calculators

You may write answers to lab questions directly in this manual.

**If you are suspected of copying data or answers from another source, you will be removed immediately from the laboratory, and may face an academic penalty.**

**Product Evaluation**

Where applicable, your compounded product and supportive documentation will be submitted for evaluation and constitute a proportion of your lab mark. Your final preparations are to be handed in, and will be graded for their pharmaceutical elegance and label (Labs 1-5). Products will not be accepted after the lab period is over.

**Product Label**

The **product label** should be neat, clearly legible, written in pen, and should include:

- Name and strength of the preparation, including any active ingredients
- Course Code, Lab Number, & Lab Group Number (e.g. “PHM241 Lab 1, Group A2”)
- Name(s) of Group Member(s)
- Date Compounded
- Beyond Use Date (BUD)

For determination of the BUD, see the appendix of this manual.
Often, in addition to handing in the product, you will be required to submit supportive documentation (e.g. a printed calibration curve, QC sheet, and/or graph).

**Strength vs. Dose**

The difference between “strength” and “dose” can be confusing at first in a formulations lab. The strength of a formulation is the amount of API one unit of the formulation contains. API stands for “Active Pharmaceutical Ingredient”, and generally means drug in the context we are using it (also sometimes called the “active”).

The dose is how much drug in total that is delivered to a patient at the time of administration.

For instance, a patient can take 2 x 200 mg ibuprofen tablets for a headache. The API is ibuprofen. The strength of this formulation is 200 mg (one tablet contains 200 mg ibuprofen), and the dose is 400 mg (because the patient is taking two tablets).

The same patient can alternately take one extra-strength 400 mg ibuprofen tablet. The strength of this formulation is 400 mg (one tablet contains 400 mg ibuprofen), and the dose is also 400 mg (the total amount of API the patient is taking – in this case, only one tablet).

\[
\text{Dose} = \text{Strength} \times \#\text{Units Administered}
\]

**Product Marking Scheme**

The following marking scheme will be used to evaluate each product and document submitted:

<table>
<thead>
<tr>
<th>Product Mark</th>
<th>Document Mark</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0 – No product handed in</strong></td>
<td><strong>0 – No document handed in</strong></td>
</tr>
<tr>
<td><strong>1 – “At Least You Tried”</strong></td>
<td><strong>1 – “At Least You Tried”</strong></td>
</tr>
<tr>
<td>• Product is ugly / incorrect / no label</td>
<td>• Document incomplete / missing fields</td>
</tr>
<tr>
<td>• Inappropriate container / incorrect amount</td>
<td>• Wrong precision of data / wrong units / absence of units</td>
</tr>
<tr>
<td><strong>2 – “The Right Idea”</strong></td>
<td>• Major errors</td>
</tr>
<tr>
<td>• Satisfactory product / labeled</td>
<td>• Proper procedure followed</td>
</tr>
<tr>
<td>• Appropriate container / correct amount</td>
<td>• Minor errors / data entry errors</td>
</tr>
<tr>
<td>• Inaccuracy found on label / label messy or illegible</td>
<td><strong>3 – “Good Documentation Practices”</strong></td>
</tr>
<tr>
<td><strong>3 – Suitable for Dispensing in a Compounding Pharmacy</strong></td>
<td>• No errors detected</td>
</tr>
<tr>
<td>• Pharmaceutically elegant</td>
<td></td>
</tr>
<tr>
<td>• No labelling errors / label legible and accurate</td>
<td></td>
</tr>
</tbody>
</table>

Supportive documents (e.g. QC sheets) will be weighted equivalently to products (3 marks). The total raw score of submitted products and supportive documentation will be reported for each lab on Quercus. They will be weighted equally, and together will constitute 15% of your final grade in PHM 241 (see “Assignment of Grades” for more details).
Clean-up Check-List

Your experiment is done. Are you all ready to go? Here are some helpful tips on leaving the lab clean for the next group of students:

☐ I closed all open bottles of chemicals and cleaned up my lab station for the next group.
☐ I cleaned my station balances, so other students can use them.
☐ I rinsed out my pipettes and burettes with water, so crystallization won’t gum up the tips.
☐ I washed all of my glassware, and returned it my locker so it’s clean for my next lab.
☐ I wiped my work area, lab bench, and bench top (including the balances I used).
☐ I properly labeled and handed in my preparation (if there is one to hand in).
☐ I properly disposed of all chemicals:
  • solid and semi-solid inert waste in the garbage,
  • liquid inert waste down the sinks
  • hazardous chemicals in appropriately labeled waste bottles in the fume hoods
☐ I double-checked the fume hood. It’s clean, and I didn’t leave anything in it.

Guidelines for Writing a Formal Group Laboratory Report (Lab 3)

For Lab 3, a formal group laboratory report consisting of individual identified sections will be submitted at the due date (to be announced on Quercus).

Distribute the sections accordingly in your group. Clearly identify who completed each section in the final report.

Rationale of Laboratory Reports

The purpose of writing a scientific report is to communicate your findings with the outside world. Enough detail should be conveyed so that someone who did not do the experiment could repeat it, and be able to fairly compare their results with yours. Writing laboratory reports (and technical writing in general) is an extremely useful and valuable skill to develop. Avoid providing one word answers and bullet points. Use sentence form, and summarize where appropriate. The ability to condense the purpose, observations, and results into an abstract will help the reader connect with the material, and will put your results in perspective for the reader. This process will help prepare you for writing scientific publications.

Be consistent with grammar. For events that happened in the lab, use the past tense for reports, and the passive voice.

e.g.: “1 mg of the free acid of sulfathiazole was incubated at 25 °C in 10 mL of phosphate buffer for 1 hour, with agitation every 15 minutes.”

For scientific principles, use the present tense.

e.g.: “Ethanol is a co-solvent, and disrupts the hydrogen bonding between water molecules and the surface of the drug molecule.”
Typical Components of a Laboratory Report

1. Title Page (1%)
   - Please include lab number and title, a list of student names, date submitted, course code.

2. Abstract (10%)
   - No more than 200 words, an abstract is a mini version of the entire lab report. It provides a brief introduction, purpose, a summary of results (not the raw data itself but parameters estimated), conclusions, and the relevance of the conclusions to the field of study. It is usually the last section that you will write, although it comes first in the report.

3. Introduction (5%)
   - This section should be 1-2 paragraphs long, and include the purpose of the experiment and a brief overview.
   - What is the main purpose of the lab? Which scientific principles are being investigated? What is the value of the results to the field of study? A good introduction will spark the interest of the reader and explain the purpose of the work.

4. Methods (10%)
   - **Do not copy and paste the methods section from the lab manual** – this is a protocol. The purpose of the methods section is to summarize what you did with sufficient detail for someone to repeat the experiment, without getting into step-by-step instructions.
   - Provide details of the chemicals you used. Key equipment (e.g. a UV spectrophotometer) should be mentioned; however, glassware (e.g. 100 mL graduated cylinder) should not unless it was integral to the method.
   - e.g.: “A standard curve of salicylic acid was prepared by diluting a standard solution of 0.2 M sodium salicylate at ratios of 1:50, 1:100, 1:200, 1:250, and 1:500. The assay procedure involved adding 1 mL of sample with 5 mL of de-ionized water and 2 drops of ferric chloride TS. Absorbance was measured at 525 nm in a UV spectrophotometer.”
   - Provide **sample calculations** for key elements of the lab. Provide the following tables in the Methods section:
     - Table for preparation of the selected ointment bases
     - Table for preparation of the salicylic acid standards for the calibration curve
   - Document what you actually did, not what you were supposed to do. If there was a change or deviation from the lab manual, describe it. Explain what you did in chronological order (the order that you did things in the lab).
5. Results (35%)

- The length of your results section will depend on the experiment.
- *All of your data and observations* go into this section, in table form. Attach any graphs printed out in the lab as source documents. This should be the easiest section to write.
- Make sure you:
  - Properly label all graph axes, with units;
  - **Always** report the units with each measurement;
  - Report your parameters with the appropriate number of significant digits (e.g. if the pH meter reads 2 decimals, don’t report a pK\textsubscript{a} of 6.39281).
  - Provide **printouts** of your source documents:
    - [http://phm.utoronto.ca/~ddubins/DL/calibration.xls](http://phm.utoronto.ca/~ddubins/DL/calibration.xls)
    - [http://phm.utoronto.ca/~ddubins/DL/ointmentdiff.xls](http://phm.utoronto.ca/~ddubins/DL/ointmentdiff.xls)
  - in an appendix of the report. Even though you are providing these documents in the appendix, present and summarize graphs and tables in the results section. Provide summary statements for each graph.
  - State final estimated parameters in sentence form briefly.
    - e.g.: “The equation of the best-fit line of the calibration curve was OD(@123nm)=0.123*Concentration(in mg/L) + 0.04.”

6. Discussion (35%)

The discussion section will likely be the longest section, and should be **no less than 2 pages long**. This section is your chance to demonstrate your understanding of the lab. For the majority of labs, the scientific principles are discussed in the *background* section of each lab in this manual. They will lay the foundation of your discussion, but it is up to you to make the link between the scientific principles, and the data you collected in the lab.

a) Summarize the key scientific idea(s) behind the lab. If there was a key equation (e.g. Beer’s Law), report it here and describe its significance.

b) Did the results confirm or refute the scientific principles involved? Discuss the precision of your data (e.g. how good the \( r^2 \) was of a fitted linear regression). Were the results obtained what you expected? Sometimes in the lab you may observe a trend opposite to what you were expecting. It is up to you to either re-evaluate your understanding of the phenomena, or try to identify the **sources of error**. Some reasons may include:
  - Limitations on the sensitivity of the instruments (noise)
  - Improperly performed calculations before or during the lab
  - Deviations from the lab protocol
  - Errors in the lab protocol
  - Limitations of the method used to evaluate the phenomena of study
  - Equipment malfunction or improper use of the equipment

If the error was a result of experimental design, suggest how the design could be improved.
c) If relevant, put your results in the context of literature values. Were they in agreement?

  e.g.: The pKₐ of sulfathiazole was estimated to be 5.98. This is not in good agreement with a published value of 7.14 (reference 1).

d) Answer the discussion questions provided at the end of the lab worksheet. These answers will be evaluated separately in the discussion section.

e) You may also discuss other related theories.

7. Conclusions (4%)

Conclusions are relatively short compared to the discussion. They are typically one to two paragraphs, and serve as the bottom line of the lab. In sentence form, report any final estimated values of parameters, and summarize the results/discussions with a closing thought. Recommendations for future work or how the lab could change may also be included here.

8. References

Include literature references you referred to in this section. If you did not refer to the references in the laboratory manual, you do not need to include them here.

  e.g.:
  

9. Appendices

You may include extra calculations, additional information, and supplementary analyses attached as appendices.

The report is to be handed in by paper copy only. A drop box will be provided. Make sure you staple your lab report together, and that you present your work neatly. At your option, you may submit the report in a folder.

**Laboratory Safety**

**Chemical Inventory**

- A complete chemical inventory for PB 860 is located through the lab website: [http://pb860.pbworks.com/w/page/41084070/PB860-Chemical-Inventory](http://pb860.pbworks.com/w/page/41084070/PB860-Chemical-Inventory)
- In consideration for others, be frugal with chemicals and buffers – take only what you need.
- Replace the caps of chemicals when you are finished weighing them.
- Use the fume hood when handling flammable or volatile solvents.
- Get into the habit of labeling a weighing boat with a sharpie marker to identify the chemical it will hold. Many chemicals in the lab are white to off-white powders. This will help you keep track of your work.
- Don’t leave unlabelled weighing boats filled with white powder by the scales. Not only is this wasteful, but it is dangerous as well.

**Labeling of Preparations**

“What was in that beaker again? It looks like water…”

Nothing is more frustrating than spending an hour to make a product, and then forgetting which
beaker you poured it in. It will save you aggravation to get in the habit early of clearly labeling your preparations as you go along. Use a sharpie marker on bare glass to label your solutions. Do not use paper labels, as they are more difficult to remove.

**Chemical Disposal**

- There are large green buckets available for broken glassware in the lab. Please use them instead of the garbage, to respect the safety of the cleaning staff.
- There will be designated waste jars for hazardous waste and organic solvents in the fume hoods for each lab. When appropriate, there will also be a designated container for sharps (e.g. needles).
- Solid and semi-solid chemically inert waste (e.g. petrolatum) will gum up the drains, and are properly disposed of in the garbage.
- If you are unsure how to properly dispose something, ask your TA or instructor.

**ACIDS CORRODE PIPES, AND SHOULD BE DISPOSED OF IN WASTE BOTTLES ONLY.**

**Dress Code**

For your protection, you are **required** to wear the following protective gear, **at all times during the lab**:

- A lab coat
- Safety Glasses or Safety Goggles (even if you wear glasses)
- Closed-Toed Shoes (no sandals or open-toed shoes are permitted)
- Clothing that covers your legs

A **3-mark penalty** will be applied to the quiz mark of the corresponding lab for the first time a student is found not wearing appropriate eye protection while inside the pharmaceutics labs (PB860 or PB819). The second time a student is found not wearing appropriate eye protection while inside the pharmaceutics labs, the student will receive a **zero on the entire lab**, and will be asked to leave.

The following special protective equipment is available for specific tasks, or on your request:

- Latex (and non-allergenic neoprene) gloves
- N95 Masks
- Face shields
- Oven mitts

**Dress Code Rationale**

If you have ever taken a laboratory course, you have likely already heard the following safety advice at some point. Common sense plays a large part in lab safety. However, it is useful to outline a few principles that pertain to the labs in this manual, so they are fresh in your mind.

- **Laboratory coats** offer first line protection to your clothes and body against chemical burns. They work best when they are done up – an open lab coat will not properly protect you from a spill.
- **Closed-toed shoes** protect your feet from chemical spills.
- **Safety glasses** will help to shield your eyes from any chemical splashes, including boiling solutions.
• **Nitrile gloves** are available for use in the laboratory. In particular, hydrochloric acid (HCl), potassium hydroxide (KOH), and sodium hydroxide (NaOH) are extremely corrosive. Gloves should be worn if you are going to be handling these solutions. Gloves also offer protection if you have a known specific allergy or sensitivity to a certain chemical.

• **Wear clothing that covers your legs.**

**Working with Hazardous Chemicals**

• When in doubt, treat all chemicals as hazardous, until you are familiarized with their properties. Consult the **Material Safety Data Sheets (MSDS)** or your TA for relevant information.

• Whenever possible, or necessary, handling chemicals in a fume hood will protect you as well as those around you from toxic and flammable fumes.

• Handle all volatile and flammable solvents in a fume hood.

• Do not put a sealed container over any heat source, as it may explode.

• If you are not sure how to use something, ask your TA.

• Notify your TA if there is any broken glassware, so they can safely clean and dispose of any chemical or sharps hazards.

• Notify your TA immediately if there is a mercury spill. They will have access to a mercury spill kit.

• Be cautious when testing for odours. Never inhale a chemical directly. Fan the vapours towards your nose. Many vapours can cause irreparable damage.

• No food or drinks are allowed in the lab. This includes water bottles.

• Never ingest any excipients or products in the teaching laboratory.

• Other safety references:
  - Merck Index
  - Material Safety Data Sheets (MSDS), a part of the WHMIS (Workplace Hazardous Material Information System) right-to-know system
  - Fisher Scientific Catalog
  - Sigma-Aldrich MSDS

**Follow these guidelines to decrease the risks of working with chemicals:**

• Work with a minimum amount of chemicals necessary.

• Read the warning labels and/or consult the MSDS before using a chemical. A database of common MSDSs used in this course is available on the lab website:
  - [http://phm.utoronto.ca/~ddubins/PB860_MSDS.htm](http://phm.utoronto.ca/~ddubins/PB860_MSDS.htm)

• When storing, using or disposing of chemicals, avoid accidental mixing of incompatible chemicals such as acids and bases, flammables and toxics, flammables and oxidizers, oxidizers and reducers.

• Highly toxic and flammable chemicals must be stored in ventilated areas in unbreakable, chemically resistant containers.
**Emergency Response**
The University Emergency phone number is 416-978-2222.

**In Case of Personal Injury**
- Inform the Teaching Assistant, or the Laboratory Coordinator of any injury acquired during a lab, no matter how slight it may appear.
- An open or even partially healed cut is dangerous, since it allows easier penetration of chemicals. Cover any exposed areas with a bandage when working in the laboratory. **Protective nitrile gloves are available from your TA.**
- In case of chemical eye injury, hold the eye open in the eye-wash, even if painful, and wash the eye for 15-20 minutes.
- In case of chemical body burns, use cold water to wash chemicals from the skin immediately, and thoroughly. Hot water may increase the absorbency of the chemical.

**In Case of Spills**
- Chemicals spilled in the laboratory must be cleaned up immediately to reduce and eliminate hazards. The Chemical Spill Cart is located in the laboratory outside the entrance of Room 865.

In the event of a localized, minor spill, use the following procedure:

<table>
<thead>
<tr>
<th>Responding to a Minor Spill</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Report all spills to the TA or instructor. Notify other students who are working in the area.</td>
</tr>
<tr>
<td>• Confine the spill to a small area. Do not allow the spill to spread.</td>
</tr>
<tr>
<td>• If the material involved is flammable, turn off any ignition sources/electrical equipment present.</td>
</tr>
<tr>
<td>• Ventilation should be established to dispel vapour, if necessary, and if safe to do so.</td>
</tr>
<tr>
<td>• Absorb and neutralize the spilled liquid chemical. For example, strong acids should first be neutralized with sodium bicarbonate, then washed with water. It is always advisable to add acid into water when mixing, since water has a much larger heat capacity and will therefore be able to absorb any resulting heat much better. You can always remember the catch phrase: “Do as you aughta, add acid to watah”.</td>
</tr>
<tr>
<td>• The TA or Instructor should handle a mercury spill. Spilled mercury is collected with a mercury collector. Sprinkle the affected area with sulfur powder. The sulfur-mercury powder is then swept up and discarded in the appropriate labeled container.</td>
</tr>
<tr>
<td>• When cleaning up a spill, wear the proper protective equipment, such as gloves and goggles.</td>
</tr>
<tr>
<td>• After the spilled chemicals have been removed, wash the area with warm, soapy water to remove any residue left behind.</td>
</tr>
</tbody>
</table>

In the event of a major spill that exceeds the clean-up capabilities of the laboratory, the following procedure is to be followed:

<table>
<thead>
<tr>
<th>Responding to a Major Spill</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Notify everyone to evacuate the area immediately.</td>
</tr>
<tr>
<td>• Contact the University of Toronto Emergency Number 416-978-2222 and state the location of spill, extent of the spill, and the chemical involved.</td>
</tr>
<tr>
<td>• Or, call 911.</td>
</tr>
<tr>
<td>• Wait in a safe area until the response team arrives.</td>
</tr>
</tbody>
</table>
**In Case of Fire**

- If the fire is contained in beakers or flasks, smother the fire simply by covering the vessels so that no oxygen can enter.
- If electrical equipment is on fire, unplug it quickly or cut the power if possible.
- If your clothing is on fire, do not run. Stop, drop, and roll. If the clothing of someone next to you is on fire, help him to the floor and use your lab coat or fire blanket, or whatever is available to smother the fire. Once the fire is extinguished, help the person away from the general fire area.
- If the fire is small and contained, a qualified person should attempt to use a fire extinguisher to eliminate the fire. There are 4 classes of fire extinguishers:

<table>
<thead>
<tr>
<th>Fire Extinguisher Class</th>
<th>Appropriate for:</th>
<th>Examples:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class A</td>
<td>Ordinary combustibles</td>
<td>(paper, wood, cardboard)</td>
</tr>
<tr>
<td>Class B</td>
<td>Flammable/Combustible Liquids and Gasses</td>
<td>(gasoline, organic solvents)</td>
</tr>
<tr>
<td>Class C</td>
<td>Electrical Equipment</td>
<td>Computers, monitors, melting point apparatus</td>
</tr>
<tr>
<td>Class D</td>
<td>Combustible metals</td>
<td>Magnesium, titanium, potassium, sodium</td>
</tr>
<tr>
<td>Class K</td>
<td>Grease fires</td>
<td>Cooking Oils and Fats</td>
</tr>
</tbody>
</table>

- Many fire extinguishers can handle multiple classes. The fire extinguishers in Room 860 are rated for Classes A, B, and C. They are located by each exit, and outline the following procedure: **(PASS)**
  - Pull the pin out
  - Aim at the base of the fire
  - Squeeze the handle
  - Sweep the nozzle back and forth
- If the fire is too large to be contained with a fire extinguisher, pull the fire alarm, and evacuate the building. Once out of harm’s way, call the University of Toronto Emergency 978-2222 or call 911. Specify the site and extent of the fire.
- Wait outside the building, away from the main entrance so that you do not block the entrance when the fire personnel arrive.

**If the Fire Alarm Sounds**

- Evacuate the building quickly, using the stairwells. The elevators will automatically go out of service. Do not try to use them.
- Wait in the designated emergency area (the area between the Medical Sciences Building and the Leslie L. Dan Pharmacy Building),
- Keep clear of the building.
- Do not re-enter the building until authorized by a Fire Officer.
Lab 1: The Use of Suspending Agents and Surfactants in Suspensions

### Preparing for the Lab
Read the introduction and lab protocol completely.
Propose and calculate resultant HLB values for 7 blends of matched span/tween surfactants, varying from pure span to pure tween.
Complete the “Purpose” column for the table in Part B.

### Group Allocation
You will be working in groups of 2 students

### What You’ll Be Doing
- **Part A:** Prepare your predetermined series of 7 surfactant blends (1 set per group of 2 students). Prepare test emulsions for each blend, shake, and observe the stability of the emulsion over time.
  - Demo: Proper use of a pipette bulb
- **Part B:** Prepare 7 test suspensions, to observe the effect of suspension excipients on the sedimentation and redispersion of calamine powder.
- **Part C:** Prepare an otic suspension for treatment of Otitis Externa (Swimmer’s Ear).

### Spreadsheets You Will Need
http://phm.utoronto.ca/~ddubins/DL/sedimentation.xls

### What You’re Handing In
- There will be a closed-book quiz at the beginning of the lab
- Final Unknown Oral Oil/Surfactant Blend, dispensed in a properly labeled scintillation vial (1 per group of 2 students)
- Calamine 2% Topical Suspension, dispensed in a properly labeled 120 mL amber oval bottle (1 per group of 2 students)
- Completed sedimentation.xls spreadsheet (1 per group of 2 students)
- Ciprofloxacin 0.2%, Hydrocortisone 1% and 0.14% Benzocaine Otic Suspension, dispensed in a properly labeled Droptainer® (1 per student)

### Introduction
Suspensions are heterogeneous systems consisting of at least two phases. The continuous or external phase is generally a liquid or a semi-solid, and the dispersed or internal phase is made up of particulate matter which is essentially insoluble in, but dispersed throughout, the continuous phase. Pharmaceutical suspensions can be taken orally, applied topically, or injected (only intramuscular, subcutaneous, or as reservoirs in trans-dermal patches). The particle sizes are generally greater than 0.2 μm in diameter. With time, all suspension systems separate on standing. The formulation pharmacist’s main concern therefore is not to try to eliminate separation, but rather to decrease the rate of settling and to permit easy re-suspension of any settled particulate matter. Although solutions have a higher absorption rates, suspensions can provide improved drug stability, improved taste and palatability, ease of administration, and in some cases enhanced bioavailability.

Oral suspensions should meet a set of basic requirements indicative of a quality formulation including:
- The dispersed particles should be small and uniform - they should not settle fast.
- If the particles settle, they should be easily re-dispersed.
- There should be no excess viscosity to interfere with pouring and re-dispersal.
- The re-dispersal should produce a uniform dose for administration.
- The suspension should be chemically and physically stable for the shelf-life of the
In this exercise, the effect of different excipients on the sedimentation rates of suspensions is explored.

Surface-active agents (surfactants) form micelles in aqueous solution above a critical concentration called the critical micelle concentration (CMC). In aqueous solution, the micelle has a hydrophobic core and a dielectric gradient towards the surface of the micelle, making the micelle surface hydrophilic. Thus, the micelle can act as a soluble phase for non-polar solutes (core), semi-polar solutes (palisade layers) and polar solutes (surface). As a result, the efficiency of a particular surfactant as a solubilizing agent varies from substance to substance. The process of increasing the water solubility of a solute (drug) using a surfactant is called micellar solubilization. This laboratory will demonstrate some of the characteristics of surface-active agents and the use of the HLB System.

References


Background

In addition to the active drug, a typical suspension may contain several excipients including: wetting agents, suspending agents, protective colloids, flocculating agents, sweeteners, preservatives, a buffer system, flavourants, and colourants.

<table>
<thead>
<tr>
<th>Excipients of a Suspension</th>
<th>Typical examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wetting agents: To wet the solid-liquid interface in order to reduce surface tension. Surfactants tend to make good wetting agents as they are designed to lower the surface tension of liquid-liquid and solid-liquid interfaces.</td>
<td>Glycerin, surfactants</td>
</tr>
<tr>
<td>Flocculating agents: To coat the surface of solid particle and, in the presence of counter ions, produces flocculates.</td>
<td>Sodium lauryl sulfate, sodium dioctyl sulfosuccinate, Tweens®, Spans®, polyethylene glycols, Pluronics®</td>
</tr>
<tr>
<td>Protective colloids: By increasing the viscosity of the vehicle, forms a mechanical barrier or sheath around the particles; used in higher concentration than surfactants.</td>
<td>Silica gel (up to 10%, in topical formulation only), aluminum hydroxide gel</td>
</tr>
<tr>
<td>Suspending agents: To retard settling and caking of particles by minimizing interparticle attraction and ultimate flocculation; a combination of various types of agents may be needed to achieve the desired rheological</td>
<td>protective colloids, viscosity inducing agents (e.g. sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, polyacrylic acid), clays (e.g. aluminum and/or magnesium silicates)</td>
</tr>
</tbody>
</table>
Buffers: To maintain suspension in the desired pH range, as the change in pH can alter drug stability as well as suspension stability.

<table>
<thead>
<tr>
<th>Buffers</th>
<th>Phosphate buffer, citrate buffer</th>
</tr>
</thead>
</table>

Salts: To achieve isotonicity or to create flocculation.

<table>
<thead>
<tr>
<th>Salts</th>
<th>Sodium chloride, potassium chloride, aluminum chloride</th>
</tr>
</thead>
</table>

Colour and flavouring agents: To impart pleasant organoleptic properties to the formulation

<table>
<thead>
<tr>
<th>Colour and flavouring agents</th>
<th>Raspberry flavour, chocolate flavour, peppermint oil, orange oil (working range of water soluble flavours is 1-3%, ~0.1% for flavoured oils)</th>
</tr>
</thead>
</table>

Antioxidants

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Propyl, octyl and dodecyl esters of gallic acid, butylated hydroxyanisole (BHA), vitamin E (or tocopherols)</th>
</tr>
</thead>
</table>

Sequestering Agents (for trace metals)

<table>
<thead>
<tr>
<th>Sequestering Agents (for trace metals)</th>
<th>Citric acid, EDTA</th>
</tr>
</thead>
</table>

Preservatives

<table>
<thead>
<tr>
<th>Preservatives</th>
<th>Ethanol (&gt;10%), propylene glycol (15-30%), chlorobutanol, phenylethyl alcohol, benzoic acid (pH &lt;4.5), parabens (e.g. methyl, ethyl, propyl and butyl parabens in acidic environment), benzalkonium chloride (pH 4-10, external use only)</th>
</tr>
</thead>
</table>

A flocculated suspension is one in which the particles appear as floccules or like tufts of wool with a loose fibrous structure. When such a system settles, two distinct layers form: a clear, particle-free supernatant and a sediment. In deflocculated systems (where flocculation does not occur), particles settle as distinct entities and form a dense sediment. This sediment becomes considerably more compact than the corresponding sediment of a flocculated system after a given particular time interval. Flocculated suspensions are preferred, compare to deflocculated systems, because they are less likely to cake on standing and are therefore more readily dispersible. In addition to maintaining a suspended system, the formulation pharmacist needs to be aware of incompatibilities between excipients, e.g. the decrease of preservative efficiency in the presence of certain surfactants.

**Surfactants**

A surface-active molecule possesses approximately an equal ratio between the polar and non-polar portions of the molecule:

![Surfactant Diagram](image)

When such a molecule is placed in an oil-water system, the polar groups are attracted to or oriented toward the water phase, and the nonpolar groups are oriented toward the oil phase. This physicochemical characteristic consequently lowers the interfacial tension between the oil and water phase:

<table>
<thead>
<tr>
<th>SDS</th>
<th>CH₂OOC₁₂H₃₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃</td>
<td></td>
</tr>
<tr>
<td>(CH₂)₁₀</td>
<td></td>
</tr>
<tr>
<td>CH₂</td>
<td></td>
</tr>
<tr>
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<tr>
<td>CH₂</td>
<td></td>
</tr>
<tr>
<td>CH₃</td>
<td></td>
</tr>
<tr>
<td>(CH₂)₁₆</td>
<td></td>
</tr>
</tbody>
</table>
If a molecule, such as glycerin, possesses a dominance of polar groups, it will not be surface-active as it will dissolve in the aqueous phase and will not be oriented at the oil-water interface. If a molecule, such as glyceryl tristearate, possesses a dominance of non-polar groups, it will also not be surface-active as it will dissolve in the oil phase. A molecule, such as glyceryl monostearate, which possesses approximately an equal balance between the polar and non-polar groups will be oriented at the interface and will be surface active. There are two general types of surfactants: nonionic and ionic surfactants. Glycerol monostearate is a nonionic surfactant, whereas sodium lauryl sulfate is an ionic surfactant.

Surface-active agents (surfactants) form micelles in aqueous solution above a critical concentration called the critical micelle concentration (CMC). Since the surfactant molecules would much rather live at an interface than be in either solution alone, when the interface becomes saturated with surfactant, any additional surfactant molecules create more hydrophobic/hydrophilic interface by folding into micelles. In aqueous solution, the micelle has a hydrophobic core and a dielectric gradient towards the surface of the micelle making the micellar surface hydrophilic. Thus, the micelle can act as a soluble phase for non-polar solutes (core), semi-polar solutes (palisade layers) and polar solutes (surface). As a result, the efficiency of a particular surfactant as a solubilizing agent varies from substance to substance. The process of increasing the water solubility of a solute (drug) using a surfactant is called micellar solubilization. This process is illustrated in the following figure.

*Example of an O/W (oil in water) emulsion being formed as the concentration of surfactant increases above the CMC*

Since a surface-active agent is adsorbed or oriented at an interface, it is logical that the concentration of a surface-active substance at the surface of a solution would be greater than the concentration in the bulk solution. Mathematically, such a relationship has been described by Gibbs adsorption equation:

\[
\Gamma = - \frac{c}{RT} \frac{d\gamma}{dc}
\]  

where \(\Gamma\) is the difference in concentration (i.e., excess surface concentration) of the solute in the surface layer and the bulk solution expressed in moles/cm\(^2\), \(d\gamma/dc\) is the rate of change of surface tension with surfactant concentration, \(c\), \(T\) is the absolute temperature, and \(R\) is the gas constant.
A surfactant can be viewed as any material that lowers the surface tension at the interface. Therefore, if \( \frac{dy}{dc} \) is negative (i.e., decreasing surface tension, \( \gamma \), with increasing surfactant concentration, \( c \)), then, \( \Gamma \) is positive. The result is that the concentration of surfactant at the surface is greater than that in the bulk phase.

In dilute solutions, the surfactant molecules move to the interface of a two phase-system and reduce the surface free energy. The reduction of surface tension at:

(a) liquid/air interface facilitates wetting and foaming;
(b) liquid/liquid interface facilitates emulsification;
(c) solid/liquid interface facilitates dispersion.

Above the critical micelle concentration, surface-active agents form clusters, e.g., micelles, which can cause solubilization. These amphiphilic compounds are soluble in water and non-polar solvents. Generally, the solubility in one solvent predominates. The hydrocarbon chain or ring is the hydrophobic portion. The ionic, alcoholic ester or ether portion is hydrophilic.

An important feature of the CMC is that at surfactant concentrations below it, the osmotic properties of the liquid change drastically with surfactant concentration. However, once the air-liquid interface is saturated, changes in osmolarity, solution conductance, and surface tension are much less pronounced. Consequently, the CMC may be found by measuring these properties as a function of surfactant concentration.

The figure to the right shows the several physical changes that occur near the CMC.

**Hydrophilic-Lipophilic Balance (HLB) System**

The orientation and positioning of a surfactant molecule at the oil-water interface would depend on the interactions of the hydrophilic and lipophilic segments with the environment. The molecules’ hydrophilic portion can be expected to dissolve in, or associate with the aqueous phase of the system. On the other hand, the lipophilic portion would dissolve in the oil phase. The balance between the hydrophilic and lipophilic properties of a surfactant has been codified by the hydrophilic-lipophilic balance (HLB) system. In the 1950s, Griffin established an empirical scale of HLB values for a variety of nonionic surfactants. The original concept defined HLB as the percentage (by weight) of the hydrophile, divided by 5 to yield more manageable values:

\[
(2) \quad \text{HLB} = \frac{\text{wt.\% hydrophile}}{5}
\]

The HLB system provides a rational means for identifying combinations of emulsifiers and facilitates the formulation of stable emulsion. Surfactants with a low HLB dissolve or disperse in
oil, while those with a high HLB dissolve or disperse in water.

- Surfactants with an HLB from 1-10 are considered lipophilic.
- Surfactants with an HLB from 10-20 are considered hydrophilic.

A list of the average HLB values of some common surfactants is provided in the appendix of this manual. The HLB of a surfactant will help determine what application it will be most useful for:

<table>
<thead>
<tr>
<th>HLB Range</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–3</td>
<td>Antifoaming agents</td>
</tr>
<tr>
<td>4–6</td>
<td>W/O emulsifying agents</td>
</tr>
<tr>
<td>7–9</td>
<td>Wetting agents</td>
</tr>
<tr>
<td>8–18</td>
<td>O/W emulsifying agents</td>
</tr>
<tr>
<td>13–15</td>
<td>Detergents</td>
</tr>
<tr>
<td>10–18</td>
<td>Solubilizing agents</td>
</tr>
</tbody>
</table>


The HLB numbers are additive so that the HLB value of a blend of emulsifying agents can be calculated, e.g., the HLB of 40% Tween 20 and 60% Span 20 is:

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>HLB</th>
<th>Weight Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween 20</td>
<td>16.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Span 20</td>
<td>8.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Total: 11.8

The estimated HLB of the Tween 20-Span 20 mixture is 11.8.

The HLB requirement for a mixture of oils to be emulsified can be calculated in a similar manner:

<table>
<thead>
<tr>
<th>Oil</th>
<th>HLB Requirement</th>
<th>Weight Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral Oil</td>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td>Cetyl Alcohol</td>
<td>15</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Total: 12.5

Now a suitable blend of surfactants should be selected so an HLB of about 12.5 is achieved. Let $y$ be the fraction of Tween 20 and $(1 - y)$ is the fraction of Span 20. Therefore:

$$16.7y + 8.6(1-y) = 12.5$$

Thus the emulsifying mixture will be:

- Tween 20 = 0.48
- Span 20 = 0.52

The emulsifying mixture consists of 48% of Tween 20 and 52% of Span 20.

Please note that components in the water phase of an emulsion are not included in the HLB requirement calculation.

Spans and tweens pack together to form a monolayer copolymer at the oil/water interface. Spans have HLBs between 1–9, and are more soluble in oil. Tweens have HLBs between 11–17.
They have bulkier hydrophilic heads, and are thus more water soluble. Spans and tweens with higher numbers have longer carbon chain tails. In general, spans and tweens of the same number are paired together for optimal packing of their aliphatic tails.

See the Appendix of this lab manual for the molecular structures of common spans and tweens, and HLB values of common surfactants. Spans and Tweens are typically blended in the same chemical class (e.g. Span 20 with Tween 20) in order to maximize packing density and geometry of the surfactant molecules at the oil/water interface.

- Span or Tween "20s" are laurate (C12) esters.
- Span or Tween "40s" are palmitate (C14) esters.
- Span or Tween "60s" are stearate (C16) esters.
- Span or Tween "80s" are oleate (C18) esters.

**Limitations of HLB**

Although useful, the HLB system does not consider components in the aqueous phase which may impact stability of the emulsion. It also does not take into account the amount of surfactant required. A quantitative method is described below. In the absence of other information, 2–4% by weight is a good starting point for creating an emulsion.

**Selection of the Proper Chemical Type of Nonionic Surfactant**

Once the proper HLB requirement for the formulation is determined, in this case for an o/w emulsion, the proper chemical type of nonionic emulsifiers should be tried, *i.e.* those with different hydrophilic and lipophilic groups to give the properties desired such as ease of emulsification, and stability of the emulsion.

In summary, the steps in formulating an o/w emulsion are:

1. Determine the purpose - oral or topical
2. Assess the globule size
3. Calculate the theoretical amount of surfactant (will probably need more than this)
4. Select the type of ionic surfactant class
5. Estimate the HLB value
6. Try different chemical groups

Surfactants can be chemically classified into four groups by the nature of the hydrophilic head group: nonionic, anionic (-), cationic (+), and zwitterionic (both + and – charges on the same molecule). The table on the following page summarizes the four surfactant types.

Solubilizing agents employed in preparations intended for internal use are confined to Tween 20® and Tween 80® (Polysorbate, USP) at present because they have been shown to have low toxicity yet have HLBs of 16.7 and 15, respectively. In external preparations, Cetomacrogol, Spans®, Tweens®, and sodium lauryl sulfate are commonly used.
<table>
<thead>
<tr>
<th>Surfactant Type</th>
<th>Examples</th>
<th>Applications</th>
</tr>
</thead>
</table>
| **Nonionic:**  | - Least irritating  
                 - Compatible with other three surfactant classes  
                 - Compatible over a broad range of pH values  
                 - Calcium tolerant  
                 - Inert, lower toxicity, some suitable for oral administration, some have better palatability  
  **Esters:** (oil-soluble)  
  - Ethylene Glycol Esters and Propylene Glycol Esters  
  - Glyceryl Esters (e.g. Glyceryl monostearate)  
  - Polyglyceryl esters, Sorbitan esters, Sucrose esters, and Ethoxylated esters  | - Emulsifiers  
  - Most widely used in topical preparations (O/W, W/O emulsions)  
  - Oil soluble, not water soluble  |
| **Ethers:** (generally derived from PEG and PPG)  
  - Ethylene oxide (e.g. OCT, Tween 80)  
  - Span 80, Tween 80  
  - Ester linkage subject to hydrolysis  
  - May hydrogen bond to preservatives and undesirably reduce antimicrobial activity  
  - Viscous liquids, bitter taste  | - Solubilizing agents for oil, detergents, wetting agents, emulsifying agents  |
| **Ether-Esters:** (water soluble)  
  - Span 80 and Tween 80  
  - Ester linkage subject to hydrolysis  
  - May hydrogen bond to preservatives and undesirably reduce antimicrobial activity  
  - Viscous liquids, bitter taste  | - Useful for solubilization and emulsification  
  - Viscous liquids, bitter taste  |
| **Fatty alcohols:** (C₆-C₁₈) | - Only marginal surfactant effect, useful as co-surfactant  |
| **Anionic:**  | - Personal care products, industrial purposes  
                 - Cleansing and detergency  
                 - Not for oral use  
  **Alkali Soaps:** (R COO· M⁺ (M=Na, K, NH₄⁺))  
  - 12-18 carbon atoms  
  - Sensitive to/precipitate in hard water (Ca₂⁺ intolerant)  
  - Gives an alkaline pH (~10), pH sensitive  
  - Incompatible with acidic drugs  
  - Micelles break up at elevated temperatures  | - External use only (laxative effect, poor taste, hemolytic properties)  
  - Irritating to the eye  
  - Useful in enemas/liniments as a counter-irritant  
  - Good O/W emulsifiers at 5-10%  
  - Good solubilizers at 20%  |
| **Alkaline Soaps:** (R COO⁺ M⁻ (M = Ca, Mg, Zn))  
  - 12-18 carbon atoms  
  - Oil soluble  
  - Similar disadvantages to Alkali soaps  | - Form W/O emulsions (e.g. calamine lotion BP)  |
| **Amine Soaps:** (e.g. triethanolamine stearate)  
  - Lack calcium intolerance  
  - Less alkaline, pH ~8  
  - Less hydrophilic, not as temperature dependent  | - Hair creams, lotions, cosmetic creams  
  - Better O/W emulsions due to HLB  |
| **Sulfuric Acid Esters:**  
  - e.g. Sulfated fatty alcohols R OSO₃ M⁺  
  - e.g. Sodium Lauryl Sulfate (SLS, SDS)  
  - Salt of strong acid and base  
  - Highly water soluble, ionized at low pH  
  - Does not precipitate with Ca²⁺  
  - May precipitate with large positive drugs  | - Preparations of creams, emulsifying wax, emulsifying ointment  
  - Can be used with neutral or negatively charged drugs  
  - Generally requires an auxiliary substance (e.g. cetyl alcohol)  |
| **Sulfonic Acid Derivatives:**  
  - e.g. Dioctyl sodium sulfosuccinate (aerosol OT)  
  - Similar to sulfated alcohols  
  - Less prone to hydrolysis, Ca²⁺ tolerant  
  - Hydrophilic  | - Emulsifying agent in creams and ointments  
  - Wetting agent, solubilizer  |
| **Cationic:**  | - External preparations  
  **Simple Amine Salts** (R NH₂ HCl)  
  - e.g. Octadecylamine HCl  
  - pH sensitive  | - Wetting, foaming, detergent properties  |
| **Quaternary Ammonium Salts** (NR₄⁺ R₂R₃R₄⁻ X⁻)  
  - e.g. Cetyltrimethyl ammonium bromide  
  - Soluble over wide pH range  
  - Requires secondary emulsifier/stabilizer  
  - Incompatible with large anionic compounds  | - Emulsifiers  
  - Not for oral or parenteral administration  
  - In creams or emulsions containing nonionic or cationic drugs for external application  
  - Bactericidal activity  |
| **Zwitterionic/Amphoteric:**  | - e.g. Alkyl Betaine  
  - Positive charge almost always ammonium, negative charge carboxylate, sulphate, sulphonate  
  - pH sensitive – charge can change depending on pH  
  - Compatible with other surfactant classes  
  - Soluble and effective in the presence of high concentrations of electrolytes, acids and alkalis  | - Personal care and household cleaning products  
  - Excellent dermatological properties  
  - Frequently used in shampoos and cosmetics products, and also in hand dishwashing liquids because of their high foaming properties  |
Estimate the Amount of Surfactant Required to Surround Oil Globules in an Emulsion

Since surfactants are adsorbed at the oil-water interfaces, the minimum amount of surfactant required to form a complete monolayer around the oil droplets can be estimated. Sodium lauryl sulphate has a cross sectional area of $22 \times 10^{-16}$ cm$^2$ and has a molecular weight of 288 g/mole. Say for example you would like to emulsify 100 mL of oil in water, with an average oil droplet diameter of 1 µm (or $1 \times 10^{-4}$ cm). We can calculate the average volume of an oil droplet:

$$V_i = \frac{4}{3} \pi r^3 = \frac{4}{3} \pi (0.5 \times 10^{-4})^3 = 5.24 \times 10^{-13} \text{ cm}^3$$

The number of droplets in 100 mL is:

$$\frac{\text{Total Volume}}{\text{Volume of a Globule}} = \frac{100 \text{ cm}^3}{5.24 \times 10^{-13} \text{cm}^3/\text{droplet}} = 1.91 \times 10^{14} \text{ droplets}$$

The surface area of a drop is:

$$S_i = 4 \pi r^2 = 4 \times 3.14 \times (0.5 \times 10^{-4})^2 = 3.14 \times 10^{-8} \text{ cm}^2$$

Total surface area of all the emulsified droplets of oil is the total number of drops of dispersed oil x surface area per drop:

$$\text{Total Surface Area} = 1.91 \times 10^{14} \times 3.14 \times 10^{-8} = 6 \times 10^6 \text{ cm}^2$$

The number of surfactant molecules required to cover the surface is equal to:

$$\frac{\text{Total Surface Area}}{\text{Surface Area per Molecule}} = \frac{6 \times 10^6}{22 \times 10^{-16}} = 2.72 \times 10^{21} \text{ molecules}$$

The number of moles of surfactant required is equal to the total number of molecules adsorbed at the interface divided by Avogadro's number:

$$\frac{\text{Total Number of Molecules}}{\text{Number of Molecules per Mole}} = \frac{2.72 \times 10^{21}}{6.02 \times 10^{23}} = 4.5 \times 10^{-3} \text{ moles}$$

The weight of surfactant required:

$$4.5 \times 10^{-3} \times 288 = 1.3 \text{ g}$$

The actual amount used would be more than 1.3 g because some surfactant would adhere to equipment and thus not be available to solubilize the oil.

**Phase Inversion**

Provided a system has enough surfactant to prevent phase separation, an interesting phenomenon occurs when an emulsion is diluted with the dispersed phase. Eventually, micelles of the disperse phase can coalesce, resulting in a phase inversion. The dispersed phase becomes the continuous phase, and vice versa. This can be problematic in compounding, as a formulation scientist may believe they have created a W/O emulsion when in fact the opposite is present:
The vast majority of drugs are hydrophobic. However, often aqueous solutions, suspensions, or emulsions are required (e.g. intravenous and topical formulations). Surfactants, when organized into micelles, solubilize drugs by entrapping them in their hydrophobic core. Drugs which would never exist in aqueous solution can be wetted and effectively dissolved using an appropriate type and concentration of surfactant. Micelle formation and phase inversion is dependent upon the surfactant’s concentration. Too low a concentration will leave surfactant only at the interface of the formulation.

Particularly with emulsions, compounding must involve knowledge of the concentration of surfactant required in order to achieve the desired emulsion (e.g. O/W, W/O, O/W/O, W/O/W) as well as the target micellar size. Some negative consequences of diluting a carefully balanced emulsion could involve phase separation, phase inversion, or drug precipitation. For an intravenous formulation, drug precipitates can cause local irritation or perhaps stroke and death. This can occur simply because the formulation is diluted below the CMC. In the case of intravenous injections, drug solubility must be considered not only in the formulation itself but also at the site of delivery. For instance, a drug dissolved in an organic solvent can precipitate upon injection. Moreover, compounding intravenous admixtures is often practiced by hospital pharmacists. Instability and physiochemical stability can happen in these admixtures owing to the changes of surfactant concentration, pH, solvent properties, and ingredient compositions, etc.

Surfactants can also impart stability to the drug itself. If a drug is sensitive to hydrolysis or oxidation in aqueous medium, the addition of a non-ionic surfactant can protect the drug from degradation. In addition to improving the stability of formulation and the drug, determination of the phase inversion point and CMC is used to help characterize the HLB of surfactants. In particular, the HLB of non-ionic surfactants has been shown to influence the phase inversion temperature. Other constituents in the formulation can affect these values, such as the concentration of ionic species present (e.g. NaCl). Surfactant type, heat, time, evaporation, and surfactant/excipient or surfactant/drug interactions can affect the CMC and result in phase inversion and/or precipitation. This makes the selection of surfactant type and concentration a crucial component for a successful liquid or semi-solid formulation. Phase inversion can be minimized by using the proper emulsifying agent and keeping the volume ratio of the dispersed phase well below the phase inversion point.
Stoke’s Law: Sedimentation Rate

Stoke’s Law describes a relationship between the settling rate of particles in a liquid to particle size, their respective densities, and the viscosity of the liquid. Inherently, larger/heavier particles will fall out of suspension faster. Settling rate will also depend on the relative density of the particles and the fluid they are suspended in. For instance, if the particles are less dense than the fluid, they will rise instead of fall. Stoke’s law is expressed using the following mathematical relationship:

\[ V = \frac{2r^2(\rho_p - \rho_L)g}{9\eta} \]

Note: Watch your units!

- \( V \) is the particles’ settling velocity (m/s)
- \( r \) is the radius of the particle
- \( g \) is the gravitational constant (9.81 m/s²)
- \( \rho_p \) is the density of the particles (kg/m³)
- \( \rho_L \) is the density of the liquid (kg/m³)
- \( \eta \) is the dynamic viscosity (Pa·s, or kg/(m·s))

Although most drugs in suspensions are not perfect spheres and the suspensions are not dilute enough to follow Stokes’ law, the equation is still useful qualitatively. Three methods can be used to control sedimentation: 1) particle size reduction; 2) density matching; 3) viscosity building.

Example Topical Suspension: Calamine

The model drug in Part B of this lab is calamine (Calamine USP Powder). Calamine is a mixture of zinc oxide with iron (III) oxide, giving it a characteristic pink colour. Calamine is the principle ingredient in Calamine lotion, and is used as an antipruritic to treat insect bites, chicken pox lesions, acne, sunburn, rashes, and other exogenous skin irritations.


Calamine is being used in Part B as a model of a hydrophobic drug compound, to illustrate caking, settling time, and flocculation. Its pink colour will facilitate measurement and qualitative evaluation of different suspension excipients. The suspensions you will be compounding in Part B of the laboratory are teaching formulations not designed for therapeutic use. The planned concentration in the lab (2 %w/v) is much lower than the USP formulation (8 %w/v). The following is the USP formulation for calamine lotion:

- Calamine Powder USP 80.0 g
- Zinc Oxide 80.0 g
- Glycerin 20 mL
- Bentonite Magma 250 mL
- Calcium Hydroxide Topical Solution qs to 1000 mL

Source: [http://www.pharmacopeia.cn/v29240/usp29nf24s0_m11260.html](http://www.pharmacopeia.cn/v29240/usp29nf24s0_m11260.html)

Example Otic Suspension: Ciprofloxacin/Hydrocortisone

Swimmer’s Ear is caused by water entering the ear during swimming, and is characterized by pain and discharge from the ear. Treatment involves careful cleaning, and the use of medicated ear drops. Ear drop formulations often contain an antibiotic and a steroid (e.g. Ciprodex® -
Ciprofloxacin and Dexamethasone, and Ciproxin® - Ciprofoxacin and Hydrocortisone). Exterior ear infections offer unique formulation considerations to drug delivery. Another common treatment for Otitis Externa is a solution of 2% ammonium acetate solution and boric acid 2.75% in isopropyl alcohol, or alternately a solution of 2-2.5% acetic acid in 70% ethanol.

The vehicles for otic preparations are typically liquids, but should be viscous enough to remain in the ear canal when applied. Polyethylene glycol 300, propylene glycol, glycerin, and mineral oil are typically used as vehicles, although propylene glycol should not be used if there is an open lesion in the ear (e.g. a ruptured eardrum) as there is toxicity associated with systemic absorption. The vehicles used (e.g. propylene glycol, glycerin) tend to be hygroscopic (water absorbing), which helps reduce swelling associated with inflammation, and also thus play a factor in pain relief.

In this lab, you will be compounding ear drops for treatment of Otitis Externa, containing ciprofloxacin, hydrocortisone, and a topical anesthetic to reduce pain. Pramoxine is preferred in patients with sensitivities to benzocaine. Glycerin and propylene glycol are used as suspending agents.

**Experiment Protocol**

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Supplies</th>
<th>Special Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calamine Powder USP</td>
<td>2 x 15 mL Droptainers (e.g. 15 mL STERI-DROPPER®, EPS® Inc.)</td>
<td>1 Metal Ruler</td>
</tr>
<tr>
<td>Potassium Phosphate Monobasic (MW 136.09)</td>
<td>3-4 Plastic Droppers</td>
<td></td>
</tr>
<tr>
<td>Food colouring</td>
<td>1 x 100 mL Amber Prescription Bottle</td>
<td></td>
</tr>
<tr>
<td>1 set of:</td>
<td>7 x 20 mL Scintillation Vials</td>
<td></td>
</tr>
<tr>
<td>• Span 20, Tween 20</td>
<td>7 x 100 mL DigiTUBEs</td>
<td></td>
</tr>
<tr>
<td>• Span 80, Tween 80</td>
<td>2 x 1 mL Plastic Syringe</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin HCl</td>
<td>2 x 5 mL Plastic Syringe</td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone USP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Topical Anesthetic (Benzocaine USP, or Pramoxine USP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzyl Alcohol BP</td>
<td></td>
<td></td>
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<tr>
<td>Propylene Glycol USP</td>
<td></td>
<td></td>
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<tr>
<td>Glycerin USP</td>
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</tr>
</tbody>
</table>

The following solutions are prepared or provided by the TA:

- 0.2% Dioctyl Sodium Sulfosuccinate solution (MW 444.6 g/mol)
- 1% Aluminum Chloride Hexahydrate solution (AlCl₃ · 6H₂O; MW 241.4 g/mol)
- 0.2% Sodium Carboxymethylcellulose solution
- Unknown oil

**Part A. Determination of the HLB required for an Unknown Oil for an Oral Emulsion**

In this part of the experiment, you will prepare a series of trial emulsions, using emulsifier combinations of known HLB values. You can then estimate the HLB of an unknown substance by observing the interaction of the unknown with your series of trial emulsions.

**Making Trial Emulsions**

- To determine the suitable HLB, select a matched pair of Span and Tween emulsifiers,
Lab 1: The Use of Suspending Agents and Surfactants in Suspensions

( either Span 20+Tween 20 or Span 80+Tween 80).

1. Make up small batches (1.00 g each) of seven emulsifier combinations, ranging in HLB from pure span to a pure tween. Calculate your series of seven blends before you come to the lab, by filling out the following table. Invariably, you and your partner will have two different sets of calculations for the 7 blends. Choose one of them (consider emulsifier availability) and compound one series of 7 solutions between you and your partner.

NOTE: Use the HLB table in the appendix of this manual to find out HLB values for your selected Span and Tween.

<table>
<thead>
<tr>
<th>Blend</th>
<th>Span (%w/w)</th>
<th>Tween (%w/w)</th>
<th>Span (mg)</th>
<th>Tween (mg)</th>
<th>Calculated HLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2</td>
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<td>6</td>
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<tr>
<td>7</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

2. To make each emulsifier blend, tare a small weighing boat on a closed-air analytical balance. Using a clean microspatula, measure out both the span and the tween directly onto the weighing boat, taring the scale between ingredients. Keep the location of the span and the tween separate on the weighing boat. Mix with a microspatula until uniform. Using a wax pencil, label the weighing boat with the appropriate blend number.

3. For each surfactant blend, prepare a test emulsion by taring a clean scintillation vial on the closed-window analytical balance, and directly weighing in:
   - 0.25 g of surfactant blend + 1.5 g of unknown oil + 15 g of de-ionized water.

   Tare the scale after adding each ingredient.

4. Add 1 drop of food colouring to each of the 7 emulsifier blends. The food colouring is water soluble, and will dissolve in the aqueous phase only. To obtain a better contrast between phases, avoid using the colour yellow.

5. Shake each mixture thoroughly for 1 minute.

6. Note the time (t=0), and allow the mixture to stand.

7. Record brief qualitative observations at t=0, 10, 20, and 30 minutes regarding the stability or separation of the mixture. Also observe the thickness of each layer, and boundary characteristics. Note the presence of foam on the top of a suspension.

8. Compare your emulsions for stability i.e., for separation of ingredients during the
remains of the laboratory.

- Evaluation of your resulting emulsions may include other factors such as clarity/cloudiness, width of oil/water interface, viscosity, and ease of preparation.

9. Consider all observations made for each emulsion, and select the emulsion with the best HLB value to hand in.

10. Properly label and hand in your best emulsifier blend. Be sure to include the HLB blend (e.g. 25% Span 80/75% Tween 80) and Calculated HLB of the surfactant blend on the label.

11. Wash the remaining scintillation vials, and dispose of them in the laboratory glass recycling container.

**Part B. Excipients of Pharmaceutical Suspensions (Calamine Topical Suspension)**

1. Obtain 7 x plastic 100 mL DigiTUBEs. Label them A through G with a sharpie marker.

2. Prepare the following test suspensions in the labeled DigiTUBEs, according to the following chart. Fill in the purpose of each excipient before the lab.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Suspension</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calamine Powder USP (g)</td>
<td>A B C D E F G</td>
<td></td>
</tr>
<tr>
<td>0.2% Dioctyl sodium sulfosuccinate (DSS) solution (mL)*</td>
<td>- 98.0 - 60.0 60.0 60.0 -</td>
<td></td>
</tr>
<tr>
<td>0.2% Sodium carboxymethylcellulose (NaCMC) solution (mL)*</td>
<td>- - 30.0 - 30.0 30.0 75.0</td>
<td></td>
</tr>
<tr>
<td>1% Aluminum chloride hexahydrate (AlCl₃·6H₂O) solution (mL)*</td>
<td>- - - 5.0 5.0 5.0 25.0</td>
<td>Add with vigorous stirring.</td>
</tr>
<tr>
<td>Potassium phosphate monobasic (KH₂PO₄) (g) Add with vigorous stirring.</td>
<td>- - - - - 0.1 -</td>
<td></td>
</tr>
<tr>
<td>De-ionized water q.s. to 100 mL with Deionized Water</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Solutions prepared by TA prior to lab.

**Note:** For suspensions A-F, make sure to add the calamine powder first, and the remaining ingredients in order, from top to bottom.

3. Cap the tops of the DigiTUBEs. Invert the tubes repeatedly until the calamine is completely dispersed, and not caked at the bottom of the tube. Set the tube down on the lab bench, and start the lab timer at this point.

4. For suspensions A to F, measure the sedimentation height of the suspensions at t=0, 2, 5, 10, 15, and 30 minutes, using a metal ruler.

**Note:** There is no drug in Suspension G. Observations are only taken at time=0.

Sedimentation volume ratio = $H_u/H_o$

$H_u$: ultimate height of the sediment, i.e., the height of the sediment at a particular time
Lab 1: The Use of Suspending Agents and Surfactants in Suspensions

H₀: initial height of the total suspension

5. Download sedimentation.xls from the downloads section on the laboratory website, and plot Hᵢ/H₀ vs. time for each of the suspensions.

6. Examine the ease of re-dispersibility of each formulation after the last measurement. To do this:
   - Make sure the plastic cap is snug on the mouth of the DigiTUBE.
   - Count the number of inversions it takes to completely re-disperse the drug.
   - Record your observations on the sedimentation.xls worksheet.

7. What phenomenon is each test suspension a demonstration of?

8. Which suspension displayed the most desirable properties? Select the winning formulation and transfer to an appropriate container for dispensing. Properly label the container for handing in at the end of the laboratory.

Part C. Otic Drops

The following formulation is a custom otic suspension of ciprofloxacin (antibiotic), hydrocortisone (corticosteroid), and benzocaine (anesthetic) for treatment of Otitis Externa (Swimmer’s Ear). It is an improvement on the commercial product Ciproxin HC®, as it has the added benefit of an anesthetic for acute pain relief.

Ciprofloxacin 0.2%, Hydrocortisone 1% and 0.14% Benzocaine Ear Drops for Otitis Externa

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin HCl</td>
<td>23.3 mg</td>
</tr>
<tr>
<td>Hydrocortisone USP (Micronized)</td>
<td>100 mg</td>
</tr>
<tr>
<td>Benzocaine USP</td>
<td>14 mg</td>
</tr>
<tr>
<td>Benzyl Alcohol (Preservative)</td>
<td>90 mg</td>
</tr>
<tr>
<td>Propylene Glycol USP</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Glycerine USP</td>
<td>10.0 g</td>
</tr>
</tbody>
</table>

1. Accurately weigh the ciprofloxacin, hydrocortisone, and benzocaine.

2. Calibrate a Droptainer by measuring and pouring exactly 10 mL of deionized water into the Droptainer, and marking the liquid level with a wax pencil or lab marker.

3. Empty and shake out the Droptainer so that there is no fluid remaining. Tare the Droptainer on a sensitive lab scale. Using a 1 mL syringe, weigh 90 mg of benzyl alcohol directly into the Droptainer.

4. Tare a clean, dry 50 mL beaker on a lab scale. Using a plastic dropper, weigh 10.0 g of propylene glycol directly into the beaker. Re-tare the beaker. Using a clean plastic dropper, weigh 10.0 g of glycerine directly into the beaker. Mix until the vehicle is clear and uniform.

5. Transfer the weighed drug components (Step 1) into a small glass mortar and pestle.
Using a 5 mL syringe (with no needle), slowly add ~2 mL of the vehicle (Step 4) to the mortar, and mix until the drugs are evenly dispersed into the vehicle. There should be no larger particle sizes visible in the suspension.

6. Using a clean plastic dropper, transfer as much as possible of the suspension from the mortar into the Droptainer.

7. In increments (sequential steps), continue adding ~2 mL aliquots of the vehicle (Step 4) to the mortar, mixing with the pestle until uniform, and transferring to the Droptainer, in order to recover the residual drug adhering to the sides of the mortar and pestle. Continue until the Droptainer is QS’d to the calibrated 10 mL line (Step 2).

8. Cap the Droptainer by snapping on the nozzle tip, and screwing on the cap. Invert the Droptainer until the suspension is uniform. Do not shake, to avoid bubble formation.

9. Wipe off your calibrated line and properly label the bottle for handing in at the end of the laboratory.

Questions

1. Which of the following properties could be used to measure the critical micelle concentration?
   a) Osmolarity
   b) Interfacial tension
   c) Conductivity
   d) Surface tension
   e) All of the above

2. The HLB requirement of an oil is too high for the surfactant blend prepared. Which of the following would lower the HLB?
   a) Increasing the proportion of span
   b) Decreasing the amount of oil
   c) Increasing the proportion of tween
   d) Increasing the amount of surfactant

3. What is the HLB scale out of?
   a) 10
   b) 20
   c) 50
   d) 100
4. How does a salt affect the dispersibility of a suspension? What if sodium carboxymethylcellulose is added to the suspension?

5. What is the difference between an emulsion and a suspension?

6. Why are Spans and Tweens used in mixtures rather than as single emulsifying agents?

7. What is interfacial tension and why is it important?

8. Calculate the mass of sodium lauryl sulfate required to emulsify 1 mL of oil, with an average oil droplet diameter of 10 \( \mu \text{m} \).

   **Properties of Sodium Lauryl Sulphate:**
   - Cross-sectional area: \( 22 \times 10^{-16} \text{ cm}^2 \)
   - Molecular Weight: 288 g/mol

6. By means of a diagram, show three forms in which the excess emulsifying agent can exist in an o/w emulsion.

7. Calculate the minimal amount of sodium lauryl sulfate required to emulsify 7 mL of oil to an average oil droplet diameter of 1.5 \( \mu \text{m} \). How does the amount of emulsifying agent affect the globular size of an emulsion?

8. You are designing a topical emulsion and have chosen to use Tween and Span 60 as the surfactant combination. Your drug dissolves in a 90:10 mixture of mineral oil and cetyl alcohol.

   Determine the ratio of the two surfactants and describe how you will prepare 100 mL of the preparation. Use point form wherever possible.

   **HLB**
   - **Tween 60:** 14.9, **Span 60:** 4.7, **mineral oil:** 10, and **cetyl alcohol:** 15

9. Stoke’s law measures the sedimentation rate of a sphere in a liquid. What is the effect of the particle size of the sphere on the rate of sedimentation? Would a sphere with a low density 0.5 g/cm\(^3\) and a larger particle diameter of 8 \( \mu \text{m} \) settle faster than one with a density six times as great but with a diameter of 2 \( \mu \text{m} \)?

   \[
   d_L = 1 \text{ gm/cm}^3 \quad V = \frac{2r^2(d_r - d_L)g}{9\eta}
   \]
Lab 2: Formulating Using Molds

Preparing for the Lab
Read the introduction and lab protocol completely.

Group Allocation
You will be working in groups of 2 students

What You’ll Be Doing

Part A: Formulating 325 mg acetaminophen suppositories in PEG base using the *Calibrated Batch Volume method*

Part B: Formulating 20 mg hydrocortisone troches using the *Displacement Factor Method*, and QC’ing your work.

Spreadsheets You Will Need
http://phm.utoronto.ca/~ddubins/DL/moldcalcs.xls

What You’re Handing In

- There will be a closed-book quiz at the beginning of the lab
- 1 x QC Sheet to accompany each formulation handed in, folded up into a square and attached to the proper formulation vial with adhesive tape.
- 6 x 325 mg acetaminophen suppositories, individually wrapped in foil, dispensed in a properly labeled 16 Dram prescription vial, or dispensed in the mold, packed in a properly labeled suppository shell box (1 per group of 2 students)
- 30 x 20 mg hydrocortisone troches, dispensed in a properly labeled troche mold and mold cover (1 per group of 2 students)

NOTE: Do not taste, use, or ingest any preparations made in the lab.

Do not write on, cut, or dispose of any of the molds in this lab. They are re-useable.

Introduction

There are many interesting opportunities to design and compound custom medications using molds, both in a compounding pharmacy and within the pharmaceutical industry. In addition to the realm of suppositories, formulations involving molds have found their way into pediatrics (lollipops, gummy bears, and hard candies), adult medicines (troches, rectal rockets, lozenges, and lip balms), veterinary applications, and even custom rapid-dissolve tablets. The calculations for formulating each design share a common theme, and once understood, are applicable to new mold types. A solid understanding of the types of excipients and calculations involved can unlock a myriad of possibilities for the compounding pharmacist and prescriber to improve efficacy and compliance.

References

5. http://www.pharmacopeia.cn/v29240/usp29nf24s0_m54856.html

Background

If the densities of substances did not change during solidification, and if volumes of components were truly additive when combined, then mold calculations would be straightforward. However, we know that not to be the case. Effects such as hydration, electrostriction, contraction upon cooling, and changes in density of substances upon mixing complicate what we think might work when pouring a liquid into a mold to solidify. It is therefore important to somehow take volume...
into account when compounding the formulation. The consequence of not doing so would be an inaccurate dose. Under-potent formulations could result in a lack of efficacy, and over-potency runs the danger of unwanted adverse events. Some molds come “pre-calibrated” to specific base types. For instance, PCCA suppositistrips® are available in blue (1.4 g) and pink (1.9g). The calibrated weights are based on PEG suppository base (PEG 1450). However, if different bases are used, the calibration provided would not be applicable.

The first step in using a mold is therefore calibrating it to the specific vehicle that is selected by the formulator. Three methods will be presented here to deal with the calculations: the **displacement factor method**, the **calibrated batch volume method**, and the **double casting method**. Simplifications may also be made when the mass of drug or a particular excipient is negligible compared with the weight of the overall formulation. As with any mold formulation, always compound extra units to account for losses due to adherence to the glassware, broken/malformed units, and units that fall out of ±10% of their target mass. You are responsible for understanding how each of these methods work for the prelab quiz.

**Displacement Factor Method**

The displacement factor method (also called the Density Factor method) is a popular approach to formulating most mold-type preparations. This method first requires calibration of the mold with vehicle alone. The placebo units are formed, and then weighed. Knowledge (or determination) of a displacement factor is required to calculate how much vehicle is required for the medicated batch. To the right is a published table of the density (displacement) factors for various drugs in cocoa butter vehicle.

The following procedure is typically used:

1. Determine the average weight of 1 unit with vehicle alone (or average placebo weight) by calibrating the mold with an excess of empty melted vehicle.

2. Obtain the displacement factors for the drug (and each excipient) in the selected base (tabular, or provided).

**NOTE:** If the amount of drug or a particular excipient in the unit dose is small (e.g. 1-5%) and the displacement factor is unknown, then the formulator may assume that adding the drug will not significantly affect the total volume or density of the formulation, and a displacement factor of 1 may be used to arbitrarily account for the volume displacement of the drug. This is a convenient simplification, but may not be as accurate at higher doses. Note that this is different than disregarding the mass of the drug completely, as the mass of the drug is still accounted for.

3. The weight of vehicle required is the total theoretical amount - the mass of each excipient divided by the respective displacement factor in the vehicle.
4. The final formulation is calculated as:

\[ m_{\text{vehicle}} = m_{\text{placebo}} - (m_{\text{drug}}/DF_{\text{drug in vehicle}}) \]

Where:
- \( m_{\text{vehicle}} \) is the total batch weight of vehicle required for the medicated batch;
- \( m_{\text{placebo}} \) is the total weight of vehicle required for a placebo batch;
- \( m_{\text{drug}} \) is the amount of drug required to produce the right concentration of drug in vehicle for the correct dose in the medicated batch; and
- \( DF_{\text{drug in vehicle}} \) is the displacement factor for the drug in the vehicle.

Note that if excess is compounded to account for losses, \( m_{\text{drug}} \) is not simply the dose \( \times \) # finished/intended units compounded. This would result in a sub-potent formulation.

**Calibrated Batch Volume Method**

The calibrated batch volume method for determining the quantities of excipients and drug required is simple, as it does not require advance knowledge or determination of displacement factors. Calibration with empty vehicle is first performed in order to determine the volume of melted vehicle required. Briefly, a first (placebo) batch is made using the vehicle alone. The units are formed, cooled, removed, weighed, and subsequently re-melted in a volume-calibrated vessel (e.g. a small beaker). A second batch is then made in a new vessel, starting with adding the drug itself. A portion of the melted vehicle is added, mixed, and then brought up to the same volume as the melted placebos, with sufficient mixing. The mixture is then poured into molds and cast. The units are cooled, removed, and weighted to check for accuracy.

This method requires that the liquid/solid transition of the vehicle is reversible. Although this can be the case for some suppository and troche vehicles, it is not the case for lollipops or gummy bears. When the dosage form is relatively small and the script is small, it may also be difficult to measure and calibrate the total batch volume reliably. This makes the Calibrated Batch Volume impractical for small batches.
Double Casting Method

One method that circumvents the need for mold calibration is the double casting (or double pour) method. This method involves mixing the required amount of drug with a quantity of melted base that is known to be insufficient for the entire suppository batch. The drug/base mixture is poured into the desired number of mold cavities, incompletely filling them. The empty space at the top of each mold is then overfilled with plain melted base. The suppositories are then trimmed with a hot spatula and removed, and then subsequently re-melted, mixed, and re-cast to ensure uniformity. The same mold must be used in both casting steps. Although calibration is circumvented, the work involved is essentially the same as the other methods, and this method has the added disadvantage of exposing the drug to heat much longer than the other methods. Like the calibrated batch volume method, this method also requires that the liquid/solid transition of the vehicle be reversible. It requires a reasonable estimate of the approximate mold volume so the formulator can estimate the amount of base to incorporate with the drug for the first step.

Determining Your Own Displacement Factor

Provided you know the final weights of the placebo and medicated formulations using any of the methods above, a displacement factor may be calculated for future batches. This will save the pharmacist time for script refills, even if the strength changes. The displacement factor is calculated using the following formula:

\[
DF = \frac{Dose}{m_{\text{placebo}} - m_{\text{medicated}} + Dose}
\]

Where \(m_{\text{placebo}}\) and \(m_{\text{medicated}}\) are the weights of 1 placebo unit and 1 medicated unit, respectively, and Dose is the mass of drug in one medicated unit. You can see in this formula that if the medicated units weigh the same as the blank units, the displacement factor simplifies to \((\text{Dose}/\text{Dose}) = 1\), which will be true as the percentage of drug decreases compared to the overall weight of the formulation. Thus, the determined displacement factor for a drug in vehicle will not be reliable (noisy, and highly variable) if the dose is relatively small. In this case, you can disregard the mass contribution of the drug to the overall formulation.
Disregarding the Mass of a Drug or Excipient

As mentioned above, the displacement factor approaches 1 as the dose decreases. Similarly, the mass of vehicle required for the entire medicated batch:

\[ m_{\text{vehicle}} = m_{\text{placebo}} - \left( \frac{m_{\text{drug}}}{DF_{\text{drug in vehicle}}} \right) \]

will approach \( m_{\text{placebo}} \) (the mass of vehicle required for a placebo batch) as the dose decreases. At a certain point, subtracting the mass of drug from the vehicle and recalculating the amount of vehicle required will result in a negligible adjustment. USP 795 specifies that “compounded preparations are to be prepared to ensure that each preparation shall contain not less than 90.0% and not more than 110.0% of the theoretically calculated weight or volume per unit of the preparation.” When the mass of drug is negligible, it allows the pharmacist to ignore subtracting the displaced amount of vehicle while still falling well within USP 795 limits.

Consequently, after calibrating a mold with empty vehicle, if the mass of drug or excipient is negligible (e.g. <1%), it can simply be added to the vehicle formula without subtracting off the mass of vehicle that would be displaced by it. The compounding steps are then simply to calibrate the mold with empty vehicle, weigh the placebos to obtain the average weight, and then compound the medicated version by adding the drug without adjustment.

Formulating Suppositories

A suppository is defined as a small plug of medication, designed to melt or dissolve at body temperature within a body cavity other than the mouth, typically the rectum or vagina. The word suppository comes from the latin word *suppositus*, the past participle of *supponere*, “to put something under or next to something else”. Suppositories offer an alternative pathway to deliver a drug locally (e.g. antifungals, laxatives, antibiotics) and also systemically (e.g. NSAIDs, antiemetics, opioid analgesics). The ideal suppository vehicle does not interact with or degrade the active ingredients, is a solid at room temperature and melts or dissolves at body temperature, is stable under ambient storage conditions, and promotes release and absorption (if applicable) of the active. The first suppositories were compounded in cocoa butter. A formulator now has the flexibility to choose the vehicle type:

<table>
<thead>
<tr>
<th>Suppository Vehicle Type</th>
<th>Examples</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleaginous</td>
<td>• Cocoa butter</td>
<td>• Easy to prepare</td>
<td>• Anal leakage (rectal)</td>
</tr>
<tr>
<td></td>
<td>• Hydrogenated vegetable oils</td>
<td>• Self-preserving</td>
<td>• Poor systemic absorption of hydrophobic drugs</td>
</tr>
<tr>
<td></td>
<td>e.g. Theobroma oil</td>
<td>• Better for inflammation/irritation (emollient)</td>
<td>• Difficult polymorphs of cocoa butter (overheating + cooling too quickly results in wrong polymorph being formed, and base won’t solidify)</td>
</tr>
<tr>
<td></td>
<td>• Synthetic triglycerides (Witepsol H-15)</td>
<td>• most common</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• “Base F” (PCCA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrophilic</td>
<td>• Glycerinated gelatin</td>
<td>• Dissolves slowly at 37°C (doesn’t melt)</td>
<td>• Not self-preserving</td>
</tr>
<tr>
<td></td>
<td>• Hydrogels (PVA, hydroxyethyl methacrylate, polyacrylic acid, polyoxethylene)</td>
<td>• Doesn’t melt as quickly as oleaginous bases</td>
<td>• Hygroscopic (pain)</td>
</tr>
<tr>
<td></td>
<td>• PEG (Macrogols)</td>
<td>• Well suited to vaginal prolonged release</td>
<td>→ Wet before using</td>
</tr>
<tr>
<td></td>
<td>• High MW or mixtures</td>
<td>• Chemically stable, non-irritating</td>
<td>• Poor/sensitive mechanical properties</td>
</tr>
<tr>
<td></td>
<td>• “Base A” (PCCA)</td>
<td></td>
<td>• Glycinerated gelatin requires mold lubrication</td>
</tr>
</tbody>
</table>

PHM 241H1 Lab Manual 2018
In general, the guiding principle of formulating a suppository is that if systemic delivery is the goal of the formulation, to select a base that is opposite in hydrophilicity than the drug. In other words, to optimize systemic absorption of a hydrophobic drug, a hydrophilic base is selected, and vice versa. Systemic absorption via the rectal mucosa can also bypass the first-pass effect (FPE); however, the effect is not always evident, and the bioavailability of rectally-administered drugs can vary widely depending on the physiochemical properties of the drug, and the suppository vehicle.

For PEG suppositories, a low molecular weight PEG is typically mixed with a higher molecular weight PEG to produce a suppository with a tailorable hardness, melting point, and dissolution time. Micronized silica may be used (1-2% of formulation by weight, typically 25 to 35 mg in a 2.1 g suppository), to stiffen a formulation that is too soft.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Typical Suppository Bases</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG 8000</td>
<td>70% 70% 70% 70% 70% 70% 70% 70%</td>
</tr>
<tr>
<td>PEG 6000</td>
<td>47% 47% 47% 47% 47% 47% 47% 47%</td>
</tr>
<tr>
<td>PEG 4000</td>
<td>33% 33% 33% 33% 33% 33% 33% 33%</td>
</tr>
<tr>
<td>PEG 1540</td>
<td>--- --- --- --- --- --- --- ---</td>
</tr>
<tr>
<td>PEG 1000</td>
<td>--- --- --- 75% 95% 95% 95% 95%</td>
</tr>
<tr>
<td>PEG 400</td>
<td>--- --- --- --- --- 10% 48% 48%</td>
</tr>
<tr>
<td>Water</td>
<td>--- --- --- --- --- --- --- ---</td>
</tr>
</tbody>
</table>

Acetaminophen is a very important antipyretic and analgesic. In this lab, you will be preparing 8 x 325 mg acetaminophen suppositories using the calibrated batch volume method, in PEG vehicle. These would be appropriate for an adult patient in need of pain or fever control, who is unable to swallow.
Examples of Rectal Suppositories

<table>
<thead>
<tr>
<th>SUPPOSITORY</th>
<th>COMMERCIAL PRODUCT</th>
<th>ACTIVE CONSTITUENT</th>
<th>TYPE OF EFFECT</th>
<th>CATEGORY AND COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisacodyl</td>
<td>Dulcolax (Boehringer-Ingelheim)</td>
<td>10 mg</td>
<td>Local</td>
<td>Cathartic. Base: hydrogenated vegetable oil</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Thorazine (GlaxoSmithKline)</td>
<td>100 mg</td>
<td>Systemic</td>
<td>Antiemetic; tranquilizer. Base: glyciner, glyceryl monopalmitate, glycerol monostearate, hydrogenated fatty acids of coconut and palm kernel oils</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>Anusol-HC (Salix)</td>
<td>25 mg</td>
<td>Local</td>
<td>Pruritis ani, inflamed hemorrhoids, other inflammatory conditions of the anorectum. Base: hydrogenated glycerides</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td>Dilaudid (Abbott)</td>
<td>3 mg</td>
<td>Systemic</td>
<td>Analgesic. Base: cocoa butter with silicon dioxide</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Indocin</td>
<td>50 mg</td>
<td>Systemic</td>
<td>Anti-inflammatory: Base: polyethylene glycols</td>
</tr>
<tr>
<td>Mesalamine</td>
<td>Canasa (AxcanScandipharm)</td>
<td>500 mg</td>
<td>Local</td>
<td>Anti-inflammatory. Base: hard fat</td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>Numorphan (Endo)</td>
<td>5 mg</td>
<td>Systemic</td>
<td>Analgesic. Base: polyethylene glycols 1000 and 3350</td>
</tr>
<tr>
<td>Prochlorperazine</td>
<td>Compazine (SmithKline Beecham)</td>
<td>2.5, 5, 25 mg</td>
<td>Systemic</td>
<td>Antiemetic. Base: glyciner, glyceryl monopalmitate, glycerol monostearate, hydrogenated fatty acids of coconut and palm kernel oils</td>
</tr>
<tr>
<td>Promethazine HCl</td>
<td>Phenergan (Wyeth)</td>
<td>12.5, 25 mg</td>
<td>Systemic</td>
<td>Antihistamine, antiemetic, sedative: used to manage allergic conditions; preoperative or postoperative sedation or nausea and vomiting; motion sickness. Base: cocoa butter, white wax</td>
</tr>
</tbody>
</table>


Formulating Lollipops

Lollipops are a preferred formulation when compliance is an issue for pediatric use, especially when delivery is desired locally to the oral mucosa, teeth, gums, or throat. One drawback of compounding a lollipop is that the drug must be capable of surviving the high temperatures of the melted base – sometimes upwards of 135 °C. For candy lollipops, temperature control is critical – a thermometer is required to know when to stop heating. Too high or too low a temperature will result in an unsatisfactory product.

Lollipops can be formulated for analgesics, antipyretics, antibiotics, antifungals, and many other drug classes. Some examples of drugs that have been compounded as lollipops include:
### Lab 2: Formulating Using Molds

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine, Tetracaine, Benzocaine</td>
<td>Dental/Buccal Pain (local)</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>Systemic pain</td>
</tr>
<tr>
<td>Tranexamic acid</td>
<td>Excessive Bleeding (local)</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>Sedation / relaxant / insomnia</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>Oral Thrush</td>
</tr>
<tr>
<td>Dextromethorphan</td>
<td>Cough suppressant</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>Fever / pain</td>
</tr>
<tr>
<td>Ketamine</td>
<td>Conscious sedation (pediatrics)</td>
</tr>
</tbody>
</table>

Several different fun mold shapes can be purchased for compounding. As with any mold, the mold must be calibrated prior to use in order to compound the correct dose. A training formula for PEG/sorbitol lollipops is included in the appendix of this manual.

**Formulating Troches**

Troches (pronounced troh-keys) are another term for oral lozenges. The troche shape will vary depending on the mold. The two major vehicle types for troches are gelatin-based, and higher molecular weight polyethylene glycol-based formulations. Each will have different properties and potential drug-excipient interactions.

Oral hydrocortisone (e.g. 10 and 20 mg Cortef® Hydrocortisone Tablets, Pfizer Canada Inc.) is indicated for a very wide variety of conditions, including endocrine and rheumatic disorders, collagen and dermatologic diseases, allergic states, hematologic disorders, ophthalmic, respiratory, neoplastic, and gastrointestinal diseases, and edematous states.

Compounding the correct dose for a troche requires knowledge of the displacement factor for hydrocortisone in troche vehicle, and proper calibration of the mold. In this lab, you will calibrate a troche mold with empty vehicle to determine the blank troche weight, and then use the displacement factor method to determine the amount of vehicle required for the medicated batch. You will then compound 20 mg hydrocortisone troches. The troches you will be compounding in this laboratory are a training formulation, not designed for therapeutic use.

**Formulating Lip Balms**

Lip balms or lip salves are excellent for local delivery of drug to the topical surface of the lips, and can also be useful for other parts of the body (e.g. sunscreen sticks). Although many cosmetic lip balm products are available, there are also medicated formulations listed in the Therapeutic Products Directorate of Health Canada as over-the-counter products, that have a Drug Identification Number (DIN). These include preparations with octinoxate, oxybenzone, homosalate, and avobenzone (all used for absorption of ultraviolet light in sunblock). A training formula for beeswax lip balm is included in the appendix of this manual.

**Experiment Protocol**

**Materials and Special Equipment**

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Supplies</th>
<th>Special Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen USP (MW 151.17)</td>
<td>Non-Stick Cooking Spray 2 × Suppository Molds 1 x Troche Mold</td>
<td>Hot Plate Thermometer Retort Stand, Vinyl Retort Clamp Test tube rack Stability Chamber (set to 5°C)</td>
</tr>
<tr>
<td>Hydrocortisone USP (MW 362.46)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The following solutions are prepared or provided by the TA:

- Not Applicable (TA to turn on stability chamber to 5 °C for cooling formulations)

**Part A. Calibrated Batch Volume Method: 325 mg Acetaminophen Suppositories**

**PEG Suppository Base**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Weight Percent (%w/w)</th>
<th>Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyethylene Glycol 3350, NF</td>
<td>58.8 %w/w</td>
<td></td>
</tr>
<tr>
<td>Polyethylene Glycol 400 (Liquid)</td>
<td>39.2 %w/w</td>
<td></td>
</tr>
<tr>
<td>Silica Gel (Micronized)</td>
<td>2 %w/w</td>
<td></td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>100 %w/w</td>
<td>~35 g</td>
</tr>
</tbody>
</table>

**Mold Calibration**

1. Fill a 600 mL beaker approximately a third of the way with tap water on a hot plate, set to high. Set up a large ceramic dish on the beaker. This should result in a dish temperature between 70-80 °C when the water boils.

2. Weigh the required amounts of PEG 3350 and PEG 400. Transfer the PEG 3350 to the ceramic dish. An excess amount of vehicle is required for the anticipated amount; in this case 35.0 g will be more than enough base to compound 12 suppositories. Mix with a glass rod until the PEG 3350 melts completely. Add the PEG 400 and silica gel, and mix with a glass rod until homogenous.

**NOTE:** With any fusion preparation, constant stirring will help prevent excipient separation.

3. Turn a test-tube rack upside down, and use it to lightly support the suppository mold (do not press the mold into the rack with too much pressure, or the mold cavities will deform). In a slow, continuous stream, pour the homogenous mixture (Step 2) into the dry mold and **overfill** each cavity so that there is a thick bead of continuous melted suppository vehicle across the top of the mold. This is to account for contraction of the base during cooling.
4. When the suppositories begin to solidify, place the filled suppository mold in the lab stability chamber (at 5 °C) for at least 15 minutes. Set the hot plate to low and allow the hot plate to cool down.

5. Remove mold from the stability chamber. Using a metal microspatula, remove the excess of base by scraping firmly across the top of the mold.

6. Smooth the surface, then carefully remove the suppositories from the mold by gently pushing upwards from the bottom.

7. Weigh the well-formed placebo suppositories individually to determine the average placebo (blank) weight.

Medicated Suppositories

8. Weigh out 3.90 g of acetaminophen in a small weighing boat.

**NOTE:** 12 suppositories x 325 mg acetaminophen/suppository = 3.90 g acetaminophen. A total of 12 units are planned to compensate for losses in compounding, in order to prepare 6 well-formed suppositories. If you were unable to recover and re-melt all 12 suppositories, scale down the mass of drug in Step 8 to the correct dose (325 mg * # recovered suppositories).

9. Re-melt your well-formed placebo suppositories in a 50 mL beaker (Beaker A), directly on the hot plate on medium heat. Remove the beaker from the hot plate, and mark the liquid level with a wax pencil.

10. **Now discard** the melted placebo suppository mix from Beaker A. Clean Beaker A, being careful not to erase your calibration mark. Weigh out a fresh batch of vehicle for your medicated batch of suppositories (using the same proportions above). Repeat step 2, preparing a fresh batch of the same amount of vehicle in a large ceramic dish, on the water bath.

11. Transfer the 3.90 g of acetaminophen into Beaker A. This step is performed without heating, to limit heat exposure to the API. Add the fresh melted vehicle, stirring while pouring, to Beaker A, filling it approximately ¾ of the way to the calibrated mark (including the acetaminophen volume). Stir with a glass rod until an opaque, homogenous mixture is produced.

12. Continue to fill Beaker A to the calibrated mark with vehicle, and stir with a glass rod until homogenous and starting to thicken.
13. While stirring **Beaker A**, pour the homogenous mixture from **Beaker A** into a clean, dry suppository mold, and over-fill the mold cavities (to avoid formation of holes that could take place due to contraction of the base on cooling). Do not use the same mold as your placebo batch, as any remaining vehicle may interfere with suppository ejection.

**NOTE:** You might not have enough melted suppository mixture to fill every mold cavity. Fill each one sequentially in the same manner as in mold calibration, generously overfilling each cavity, and when you run out of the mixture, leave the remaining cavities empty.

14. When the suppositories begin to solidify, place the filled suppository mold in the lab stability chamber (at 5 °C) for at least 15 minutes.

15. Remove mold from the stability chamber. Using a metal microspatula, remove the excess of base by scraping firmly across the top of the mold.

16. Smooth the surface, then carefully remove the suppositories from the mold by gently pushing upwards from the bottom.

17. Complete a QC spreadsheet for your suppository batch (use the “Mold QC” worksheet in moldcalcs.xls) and hand it in with your final formulation. Use the average placebo and medicated suppository weights to calculate a displacement factor for acetaminophen in this vehicle. You may also use the “Displacement Factor Method” worksheet in moldcalcs.xls to verify your calculations.

**How could you use the calculated Displacement Factor to compound more quickly, if you were to prepare this formulation again?**

18. Wrap the suppositories individually in aluminum foil, and properly label. To wrap, cut a small aluminum square (approx. 5 cm x 5 cm) and place the suppository in the centre of the square, diagonally. Fold the corners in towards the suppository ends, and then roll the suppository applying a slight pressure during rolling. Alternately, you may replace the medicated suppositories inside the suppository mold, and pack the mold in a suppository shell box.

19. Rinse the molds in **hot water** in the lab sinks to clean them and remove any material. Return the molds to your TA or instructor. **Do not dispose of the molds.** They are reusable.

---

**Part B. Displacement Factor Method: 20 mg Hydrocortisone Troches**

**Troche Gelatin Base**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Weight Percent (%w/w)</th>
<th>Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica Gel - Micronized (stiffener)</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Stevia Powder (sweetener)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Acacia NF (thickener)</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Gelatin (gelling agent)</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>Glycerin (plasticizer, sweetener)</td>
<td>46.0</td>
<td></td>
</tr>
</tbody>
</table>
### 70% Sorbitol USP (plasticizer, sweetener)

**Note:** This is sorbitol solution, not powder.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>De-ionized Water</td>
<td>23.4</td>
</tr>
<tr>
<td>Flavour of Choice</td>
<td>(1-2 drops per batch)</td>
</tr>
<tr>
<td>Colour of Choice</td>
<td>(1-2 drops per batch)</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>100 %w/w</td>
</tr>
</tbody>
</table>

**About Stevia:**

Stevia powder (or stevioside) is a sweetener that is rapidly gaining popularity in North America. It is 250-300 times sweeter than sugar, and does not affect blood sugar metabolism. It is water and alcohol soluble, heat stable, and has a working range of 0.1-0.8% (above which it becomes bitter).

---

### Mold Calibration

1. Calculate an excess amount of ingredients for at least 45 placebo troches (~50 g total batch weight). Even though you are only making 30 troches, 45 is planned to account for losses in compounding.

2. Set a hot plate on high. Tare a 150 mL beaker on a lab scale, and weigh in the measured amounts of de-ionized water, glycerin and 70% sorbitol solution. Stir with a glass rod until clear. Place the beaker on the hot plate. Set up a thermometer on a retort stand at the middle of the solution (not touching the sides or bottom) to monitor the temperature while heating. Heat mixture to 80°C.

3. Remove the 150 mL beaker from the hot plate. Add the gelatin, and mix with a glass rod. Stir with periodic heating to prevent the gelatin from gelling or burning on the bottom of the beaker.

4. When the gelatin appears smooth and homogenous, remove it from the hot plate. The mixture will not appear completely transparent when the gelatin has melted.

5. Using a glass mortar and pestle, triturate the silica gel, stevia powder, and acacia to a fine powder.

6. Add the powders from Step 5 into the melted gelatin. Stir with a glass rod until evenly dispersed and uniform.

7. Add the colourant and flavourant of choice. Mix well.

8. As soon as the ingredients are properly mixed, pour mixture (Step 7) into mold, ensuring the cavities are full.

**NOTE:** Do not allow the mixture to cool before filling the mold, or it will thicken. Remove excess vehicle with the back of a warmed metal spatula. Molds should be filled flush to the top with the partitions visible and not covered with vehicle, to allow for proper separation of troches. Avoid overfilling the troche mold. Do not close the lid before the troches have solidified.
9. Allow the troches to cool. Carefully remove and weigh the placebo troches individually to determine the average placebo troche weight.

**Medicated Troches**

10. Based on the average weight of vehicle per troche, calculate how much hydrocortisone and vehicle is required to compound 45 x 20 mg hydrocortisone troches for the final medicated batch. Use a displacement factor of 1.50 for hydrocortisone in troche gelatin base.

11. In a small glass mortar and pestle, triturate the required amount of hydrocortisone USP (powder), silica gel, stevia powder, and acacia powder together, and reduce the particle size to a smooth powder.

12. With a clean 140 mL beaker, repeat the above steps for mold calibration (Steps 1-8), adding the medicated powdered mixture at the appropriate time (Step 6). The drug is added after the mixture has been removed from the heat, to limit heat exposure of the API and help prevent thermal degradation.

13. Complete a QC spreadsheet for your troche batch (in the “Mold QC” tab in moldcalc.s.xl) and hand it in with your final formulation. Did the batch pass weight/dosage specifications?

**Questions**

1. Which of the following is not a step in the calibrated batch volume method?
   a) Casting placebo units
   b) Incompletely filling the molds with the correct dose
   c) Marking a volume line on the melted placebos
   d) Weigh formed medicated units to check for accuracy

2. Which of the following are disadvantages of oleaginous bases for suppositories?
   a) Hygroscopic
   b) Irritating to the anal mucosa
   c) Anal leakage
   d) Not self-preserving

3. The USP 795-defined weight or volume limits on compounded preparations are:
   a) 95.0 - 105.5%
   b) 90.0 - 110.0%
   c) 85.0 - 115.0%
   d) 80.0 - 125.0%

4. A patient returns to the pharmacy with a troche formulation that has grown mold. How might you change the formulation for future batches?

5. You fill the suppository script above and the patient returns to the pharmacy complaining that the medication doesn’t seem to be working. What might be the problem(s) and what follow up steps would you take to improve drug efficacy?

6. What does the displacement factor you calculated for the suppository formulation assume that may be incorrect for future batches?
7. A coffee company is developing a caffeine suppository formulated in cocoa butter, for busy executives who have no time for a coffee break. The displacement factor for caffeine in cocoa butter is 1.2. The target dose is 250 mg caffeine – equivalent to ~2 cups of coffee. The suppositories would be sold in strips of 12. Calculate how much base is required to make 14 caffeine suppositories. The calibrated placebo weight is 2.1 g per suppository.
# Lab 3: Topical Formulations

| Preparing for the Lab | Read the introduction and lab protocol completely. Watch the following related lab videos on the laboratory website:  
- UV/Vis Spectrophotometry - Determining Absorbance  
- UNC Eshelman School of Pharmacy Video - How to Pack an Ointment Jar  
Calculate the volume of stock required for each standard solution in the salicylate calibration curve. |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Group Allocation</td>
<td><strong>You will be working in groups of 4 students</strong></td>
</tr>
</tbody>
</table>
| What You’ll Be Doing | **Part A:** Prepare a calibration curve for your salicylic acid release study  
**Part B:** Prepare 4 of the 6 ointment bases  
**Demonstration:** levigation, geometric dilution  
**Part C:** Formulating 3% salicylic acid preparations of 4 ointment bases. Ointment Release Studies on 3 ointment bases. Ointment base evaluation (texture, ease of removal, etc.)  
**Part D:** Preparation of a 40% Urea Ointment and Demonstration of an Ointment Mill |
[http://phm.utoronto.ca/~ddubins/DL/ointmentdiff.xls](http://phm.utoronto.ca/~ddubins/DL/ointmentdiff.xls) |
| What You’re Handing In | **There will be a closed-book quiz at the beginning of the lab**  
**Four ointment bases, each dispensed in a properly labeled white plastic 50 cc ointment jar:**  
1) 40% Urea in [Hydrocarbon (Base #1) or Absorption (Base #2)]  
2) 1 of: W/O Emulsion (Base #3) or O/W Emulsion (Base #4) – Non-medicated  
3) Water Soluble (Base #5) – Non-medicated  
4) 3% Salicylic Acid in Poloxamer Cream (Base #6)  
**The [calibration.xls](http://phm.utoronto.ca/~ddubins/DL/calibration.xls) and [ointmentdiff.xls](http://phm.utoronto.ca/~ddubins/DL/ointmentdiff.xls) spreadsheets, printed out and attached to your first formulation.**  
**A formal group lab report, due date to be announced on Quercus.** |

## Introduction

Semisolid dosage forms are those preparations intended for spreading on the skin for the purpose of: (1) providing lubrication (emollients); (2) bringing into contact with the skin drugs required for healing skin disorders; (3) acting as protective coverings to prevent contact of the skin surface with chemicals, solutions and organic solvents. The preparations include primarily ointments/creams (salves), cerates, jellies, pastes, plasters and poultices. Ointments are of such a consistency that they may be readily applied to the skin by inundation. They should be of such composition that they soften but not necessarily melt when applied to the body. Creams and jellies generally have a lower viscosity than ointments whereas cerates, pastes, plasters and poultices generally have a higher viscosity.

When semisolid preparations are applied to local areas of the skin, a special beneficial effect is the intended result. The effect produced may be due to the therapeutic action of the medicament (e.g., keratolytic agent, antipyretic agent, antiseptic) which must be released from the base, or due to the base itself. The base usually has a more general action on the skin, providing occlusion to water loss from the skin, emollient and lubricating action, or a drying action.
The purpose of this laboratory is to prepare examples of the pharmaceutical classes of ointments and investigate some of their physicochemical properties.

References


Background

There are several factors which influence the selection of the types of preparations to be used topically. Among these are:

- the diagnosis;
- the effect desired;
- the condition of the skin area to be treated;
- its ability to release the medicament to the skin surface;
- the chemical and physical stability of active ingredients contained therein.

In addition, cosmetic appearance and hypo-allergenic properties of the base can be important. The selection of bases for the extemporaneous preparation of a semisolid dosage form is the privilege of the physician, but in this area particularly, the knowledge and wisdom of the pharmacist is frequently called upon to assist the physician in making a suitable selection. It is the responsibility of the pharmacist to prepare a quality product that is pharmaceutically correct. In exercising this responsibility, the pharmacist may be required to make minor changes in composition in order to produce a superior product. This may involve the use of small amounts of inert materials as levigating and/or solubilizing agents. Levigation is the process of reducing the size of solid particles, made into a paste by the addition (with the aid of a spatula and ointment slab) of a small amount of a liquid ointment base. This liquid or ointment base is known as a levigating agent.

The preparation of semisolid dosage forms often involves two procedures:

- Fusion
- Mechanical Incorporation

The medicament and the physical properties of the constituents of the base usually determine the extent to which each procedure is used.

Preparation by Fusion

Ointment bases consisting of hard, waxy ingredients, such as beeswax, spermaceti, paraffin, fatty alcohols, or high molecular weight polyethylene glycols, and soft or liquid ingredients such
as petrolatum, mineral oil, glycols or hydrocarbons which are gently heated together over a water-bath until a melt is produced. Drugs and adjuvants which are soluble in the melt may be added at this point and mixed in. The melt is removed from the heat and stirred continuously as it cools until congealing has occurred. Heat-sensitive or volatile ingredients should be added just prior to the congealing point which is about 35 – 45 °C.

The method for preparing creams by the fusion process is slightly more complicated. In this case, both the aqueous phase and the oil phase are heated separately, to somewhere between 60 – 80 °C. As a general rule, the oil phase should be heated to at least 5°C above the melting point of the highest melting waxy ingredient. The water phase is heated to 5°C above the temperature of the oil phase to prevent premature solidification prior to mixing and emulsification. Water-soluble adjuvant is dissolved in the heated aqueous phase with stirring while nonvolatile oil-soluble ingredients are dissolved in the heated oil phase. Generally, the internal phase is gradually added to the external phase and vigorously mixed. Mechanical dispersion techniques may be used to increase the state of dispersion. Many excellent creams have also been produced by the reverse order of combination, but it varies from one formula to another.

The method for the preparation of the poloxamer/lecithin isopropyl palmitate bases is unique. The poloxamers are white waxy free-flowing granules. They exhibit reverse thermal gelling. In other words they are free flowing when cold at refrigerator temperatures (4 – 8 °C). They are soluble in water and are prepared by placing the flakes in a closed bottle and adding cold water. The mixture is mixed gently and placed in a refrigerator overnight. More water is added up to the designated volume and the gel is ready for use. The soya lecithin is a yellow sticky granule. It is weighed and placed in a sealed bottle and isopropyl palmitate is added as the solvent. This dissolves overnight at room temperature. Usually either potassium sorbate or sorbic acid is added to each of the above as a preservative.

In order to prepare the cream/gel, the drug is dissolved or dispersed in one of either of the above and the second liquid component is added. At room temperature a smooth cream or gel is formed as an oil/water emulsion. Usually, the lipophilic portion of the final cream constitutes approximately 25% of the final weight of the preparation. Two methods are used to decrease the particle dimensions of the lipophilic phase: vigorous trituration in a mortar or extrusion through a small bore syringe opening.

**Levigation: Preparing the Drug for Mechanical Incorporation**

Mechanical incorporation may be performed with a mortar and pestle, or on a glass slab with a spatula. The drugs being incorporated into the base are frequently insoluble in the base, and it is necessary to reduce them to a fine paste by levigation. This is best accomplished using the *slab and spatula technique* by using a levigating agent. A levigating agent can be a wetting agent that is compatible with the base or simply a small portion of the base itself (or the melted base). Levigation will be demonstrated to you in the lab. If very small amounts of drug are to be incorporated, a small amount of mineral oil or glycerin can be used as a levigating agent. Using too much of a levigating agent can result in over-diluting or over-softening the finished product.

**Geometric Dilution: Mechanically Incorporating the Drug into the Base**

After the powder is levigated and worked to form a very smooth nucleus, the drug can then be incorporated (by geometric serial dilution) with the remainder of the base. Briefly, the first step of geometric dilution starts with mixing the drug with a roughly equal volume of base. Mixing is
performed using a figure 8 pattern with the spatula, and periodically the mix is scraped to the
centre of the glass slab to maintain a small working area. Once the drug is evenly distributed in
the base with no evidence of lumps, more base is added, with a roughly equal volume to the
current mix of drug+base. In this way, the amount of material doubles for each round of mixing
(hence the term “geometric” dilution). Mixing is performed until uniform, and this continues
until all of the base is incorporated into the drug+base mixture.

The mortar and pestle are probably not as efficient as the ointment slab and spatula for
incorporating insoluble powders in an ointment base because of the small surface area levigated
at any contact point of the mortar and pestle. However, when a liquid is to be incorporated into
a base, the mortar is often preferred since the percentage of ointment exposed to the air is
much less by this method, and the possibility of liquid loss by evaporation, due either to friction
or thinness of film, is reduced.

Alternatively, the solid can be dissolved in a little solvent, usually water, and incorporated as a
solution (i.e., its water number must be high enough). The base, of course, must have the
capacity to take up the solution. Volatile aromatic materials, such as essential and perfume oils,
camphor and menthol, will volatilize if dissolved in the hot oil phase. These ingredients are
usually incorporated in creams as alcoholic solutions added with mixing at the point when the
emulsion begins to solidify upon cooling. In the case of ointments, lanolin or some other w/o
emulsifier may have to be substituted for a fraction of the base to allow aqueous solutions to be
incorporated.

In the case of the poloxamers, the drug is incorporated into one of the two phases during
gel/cream formation. It is usually levigated with a solvent such as alcohol or propylene glycol.

A summary chart of the properties of ointment bases is provided on the following page.
### SUMMARY CHART: PROPERTIES OF OINTMENT BASES

<table>
<thead>
<tr>
<th>Property of Base</th>
<th>Oleaginous Ointment Bases (Hydrocarbon)</th>
<th>Absorption Ointment Bases</th>
<th>Water/Oil Emulsion Ointment Bases</th>
<th>Oil/Water Emulsion Ointment Bases</th>
<th>Water-miscible Ointment Bases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition</td>
<td>oleaginous compounds</td>
<td>oleaginous base + w/o surfactant</td>
<td>oleaginous base + water (&lt; 45% w/w) + w/o surfactant (HLB &lt;8)</td>
<td>oleaginous base + water (&gt; 45% w/w) + o/w surfactant (HLB ≥9)</td>
<td>Polyethylene Glycols (PEGs)</td>
</tr>
<tr>
<td>Water Content</td>
<td>anhydrous</td>
<td>hydrous</td>
<td>hydrous</td>
<td>hydrous</td>
<td>anhydrous, hydrous</td>
</tr>
<tr>
<td>Affinity for Water</td>
<td>hydrophobic</td>
<td>hydrophilic</td>
<td>hydrophilic</td>
<td>hydrophilic</td>
<td>hydrophilic</td>
</tr>
<tr>
<td>Ability to Take up Water</td>
<td>very little</td>
<td>some, forms a w/o emulsion</td>
<td>will take up more water, eventually the emulsion may break (phase separation) or form an o/w emulsion (phase inversion)</td>
<td>will take up a great deal of water becoming fluid</td>
<td>will only take up a small amount of water before becoming fluid</td>
</tr>
<tr>
<td>Spreadability</td>
<td>difficult</td>
<td>difficult</td>
<td>moderate to easy</td>
<td>easy</td>
<td>moderate to easy</td>
</tr>
<tr>
<td>Washability</td>
<td>non-washable</td>
<td>non-washable</td>
<td>non- or poorly washable</td>
<td>washable</td>
<td>washable</td>
</tr>
<tr>
<td>Stability</td>
<td>oils poor; hydrocarbons better</td>
<td>oils poor; hydrocarbons better</td>
<td>unstable, especially alkali soaps and natural colloids</td>
<td>unstable, especially alkali soaps and natural colloids; nonionics better</td>
<td>stable</td>
</tr>
<tr>
<td>Drug Incorporation Potential</td>
<td>solids or oils (oil solubles only)</td>
<td>solids, oils, and aqueous solutions (small amounts)</td>
<td>solids, oils, and aqueous solutions (small amounts)</td>
<td>solid and aqueous solutions (small amounts)</td>
<td>solid and aqueous solutions</td>
</tr>
<tr>
<td>Occlusiveness</td>
<td>yes</td>
<td>yes</td>
<td>sometimes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Uses</td>
<td>protectants, emollients, vehicles for hydrolyzable drugs</td>
<td>protectants, emollients, vehicles for aqueous solutions, solids, and non-hydrolyzable drugs</td>
<td>emollients, cleansing creams, vehicles for solid, liquid, or non-hydrolyzable drugs</td>
<td>emollients, vehicles for solid, liquid, or non-hydrolyzable drugs</td>
<td>drug vehicles</td>
</tr>
<tr>
<td>Examples</td>
<td>White Petrolatum, White Ointment</td>
<td>Hydrophilic Petrolatum, Anhydrous Lanolin, Aquabase™, Aquaphor®, Polysorb®</td>
<td>Cold Cream type, Hydrous Lanolin, Rose Water Ointment, Hydrocream™, Eucerin®, Nivea®</td>
<td>Hydrophilic Ointment, Dermabase™, Velvachol®, Unibase®</td>
<td>PEG Ointment, Polybase™</td>
</tr>
</tbody>
</table>

Source: [http://pharmlabs.unc.edu/labs/ointments/bases.htm](http://pharmlabs.unc.edu/labs/ointments/bases.htm)
**Experiment Protocol**

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Supplies</th>
<th>Special Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylic Acid (MW 138.12)</td>
<td>Elastic bands</td>
<td>Dermamill 100 Ointment Mill</td>
</tr>
<tr>
<td>Excipients as indicated in each section</td>
<td>Cellophane membrane (dialysis tubing), 45 mm flat width</td>
<td>Hot plate</td>
</tr>
<tr>
<td>Urea (MW 60.06)</td>
<td>MWCO 12-14,000</td>
<td>Evaporating Dish</td>
</tr>
<tr>
<td>Glycerin (MW 92.09)</td>
<td>4 × 25cc ointment jars</td>
<td>Stopwatch</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>4 × 50cc ointment jars</td>
<td>Spectrophotometer</td>
</tr>
<tr>
<td>Ferric Chloride TS USP</td>
<td>Plastic Cuvettes</td>
<td>Hard Rubber Spatula</td>
</tr>
<tr>
<td></td>
<td>Plastic Transfer Pipets</td>
<td>Burette</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Retort stand with Burette Clamp</td>
</tr>
</tbody>
</table>

The following solutions are prepared or provided by the TA:

- Salicylic Acid (1.00 mg/mL)
- Ferric Chloride TS USP (9% w/w FeCl₃ in water)

**Part A. UV Absorbance Standard Curve of Salicylic Acid**

**Preparing the Standards**

1. Prepare 50 mL of each of the following salicylic acid concentrations in de-ionized water, *in triplicate*:

   5, 25, 50, 75, and 100 µg/mL

To clarify, this means that you prepare each solution three times (including 100 µg/mL), rather than measure the absorbance of the same solution three times, to get an estimate of the error associated with creating the standard solutions. Measuring the standards *in triplicate* will allow you to report the average, standard deviation, and %RSD at each standard concentration. An efficient way to accomplish this is to have three different people prepare dilutions in parallel. The same spectrophotometer must be used.

The TA will prepare a 1.00 mg/mL salicylic acid stock solution. You will be preparing 200 mL of the highest standard concentration (100 µg/mL), and then using that solution to prepare 50 mL each of the less concentrated standards. This is a more accurate (and easy) approach than preparing each standard from the 1.00 mg/mL stock. **You will need to calculate** the volumes of stock or 100 µg/mL solution required for each of the standard curve concentrations. The following figure illustrates the dilution scheme:
Note: Prepare each solution using volumetric flasks. Beakers and Erlenmeyer flasks are only accurate to 5%, and should not be used to prepare standard concentrations. Use a burette or bulb pipette to dispense your calculated volumes of solution required, and dilute to the mark with a plastic dropper.

2. Add 2 drops of indicator solution (ferric chloride) to 50 mL of each concentration of your standard curve. **Ensure that the concentration of indicator is the same in every standard, 2 drops to 50 mL.**

**NOTE:** Allow 10-15 minutes for the colour to completely develop before measuring.

3. Show details of your preparation and calculation.

**Preparing the Blank**

4. Measure 25.0 mL de-ionized water into a beaker using a pipette and add 1 drop of the indicator ferric chloride solution.

5. As the colour of the blank may intensify with time, prepare a new blank as you start the drug release experiment in the second period.

**Colourimetric Absorbance Measurement**

Your TA will demonstrate how to use the spectrophotometer. A video is also available on the laboratory website.

1. Set the wavelength of the spectrophotometer to 525 nm (visible light).

2. Zero the absorbance of the spectrophotometer using the blank solution.

**NOTE:** Some time should be allowed for colour to develop in each of the samples: typically 10-15 minutes should suffice.

3. Measure the absorbance of each concentration of the standard curve and record the absorbance.
4. Plot a calibration curve of absorbance vs. known concentration of salicylic acid, using the laboratory computers. You may use the file calibration.xlsx in the “Downloads” section of the laboratory website.

**Part B. Ointment Base Preparation**

You will be melting components for your ointments on a steam bath. Set up the steam bath as follows:

1. Pour ~100 mL de-ionized water in a 400 mL beaker.
2. Set the beaker on a hot plate, and set it to high heat.
3. Place a large evaporating dish on the mouth of the 400 mL beaker.

When the water starts boiling, set the hot plate to medium. The temperature of the evaporating dish will be approximately 60 °C. This will be sufficient for melting solid components in the bases.

4. **Stir with a glass rod, not a thermometer.** Do not leave the material on the hot plate without mixing.
5. Materials should be weighed on weighing boats, waxed weighing paper, or tared glass beakers. Never put chemicals directly on any scale or balance pan.

**NOTE:** Dispose of all ointments in the garbage, **not** in the sink.

You will be compounding and testing 4 bases:

- 1 of: Hydrocarbon base (Base #1) or Absorption Base (Base #2)
- 1 of: W/O emulsion base (Base #3) or O/W Emulsion Base (Base #4)
- Water Soluble (Base #5)
- Poloxamer base (Base #6)
1. **Hydrocarbon Base**

* e.g. White Petrolatum USP

White petrolatum USP is a purified mixture of semi-solid hydrocarbons from petrolatum and is decolourized. It may contain a stabilizer.

Synonyms: white soft paraffin, white petroleum jelly, Vaseline®, white ointment USP

Yellow soft paraffin is often used in eye ointments, as it is not bleached and probably does not contain a stabilizer.

1. Prepare White Ointment USP XXII

   - White wax 5 g
   - White petrolatum 95 g
   - to make: 100 g

2. Melt the white wax in the *Ointment Melting Apparatus.*

   **NOTE:** Boiling wax is extremely flammable. Do not bring the wax to a boil.

3. Add the white petrolatum, warming until liquefied.

4. Remove the evaporating dish from the steam bath.

5. Allow to cool, and stir until the mixture begins to congeal.

**NOTES:**

- The fusion method is usually used in this class of ointments.
- *The material with the highest melting point is melted first* and the other ingredients are incorporated in decreasing order of melting point (or range). Using this method the cooling process is quicker and all the ingredients are not subjected to the highest temperature.
- If a lower temperature should be used, then the lowest melting ingredient is heated first and then the materials of highest melting point are added.
- Hydrocarbon ointments are the most occlusive, meaning that they form a physical barrier on the skin, thus hydrating it because body water is unable to evaporate.
2. Absorption Base

e.g. Hydrophilic Petrolatum USP

1. Prepare hydrophilic petrolatum as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>3 g</td>
</tr>
<tr>
<td>Stearyl alcohol</td>
<td>3 g</td>
</tr>
<tr>
<td>White wax</td>
<td>8 g</td>
</tr>
<tr>
<td>White petrolatum</td>
<td>86 g</td>
</tr>
<tr>
<td><strong>to make:</strong></td>
<td><strong>100 g</strong></td>
</tr>
</tbody>
</table>

2. Melt the stearyl alcohol and white wax together in the Ointment Melting Apparatus.

**NOTE:** Boiling wax is extremely flammable. Do not bring the wax to a boil.

3. Add the cholesterol, and stir until completely dissolved.
4. Add the white petrolatum, and mix.
5. Allow to cool, and stir until the mixture begins to congeal.

**NOTES:**

- This base contains no water.
- Absorption means that the base can absorb water i.e., it has nothing to do with drug absorption.
- Because cholesterol is a surfactant with a low HLB, a certain amount of water can be added (see tests) to form a w/o emulsion.
- The addition of stearyl alcohol, a surfactant with very low HLB, acts as a co-emulsifier and, along with white wax, gives firmness and heat stability to the product.
- The anhydrous base is suitable for water unstable drugs.
- A commercial absorption base is Aquaphor®.
3. Emulsion Bases W/O Type

e.g. Petrolatum Rose Water Ointment USP XVI (Cold Cream)

1. Prepare cold cream as follows:
   - Cetyl esters wax (Spermaceti) 12.5 g
   - White wax 12 g
   - Mineral oil (heavy) 56 g
   - Sodium tetraborate 0.5 g
   - Purified water 19 g
   to make: 100 g

2. Reduce the cetyl esters wax and the white wax to small pieces.
3. Melt the cetyl esters wax and white wax together in the Ointment Melting Apparatus.

NOTE: Boiling wax is extremely flammable. Do not bring the wax to a boil.

4. Once the waxes are melted, add the mineral oil.
5. Continue heating until the temperature of the mixture reaches 70°C; maintain at 70°C for 5 minutes.
6. In a separate 100 mL beaker, dissolve the sodium tetraborate in the purified water, warmed to 70°C.
7. Gradually add this warm solution to the melted oil mixture.
8. Remove from heat. While the mixture is cooling, use a hand blender to emulsify the phases. Run the hand blender on the lower speed until the mixture appears milky.
9. Stir rapidly and continuously until it has congealed (thickened up). Otherwise the phases will separate.

NOTES:

- The oil phase is prepared by fusion.
- The aqueous solution is at about the same hot temperature as the oil phase, so when the aqueous phase is added, a suitable homogeneous w/o emulsion will form without congealing.
- In this method of preparation, the internal phase is added to the external phase and a suitable product is formed. Usually the aqueous phase is added to the oil phase because it is more convenient to pour the aqueous phase and thus a minimal loss of ingredients.
- Nivea Cream and Pond’s Cold Cream are commercial examples of w/o emulsions.
- The emulsifier is formed in situ and is the sodium salts of the acids in white wax, any acids in the cetyl esters wax, and acids formed during heating.
- Because of the phase volume ratio, an o/w emulsion is formed in spite of the high HLB of the emulsifier.
4a. Emulsion Bases O/W Type

e.g. Hydrophilic Ointment USP

1. Prepare hydrophilic ointment as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>O/W Type</th>
<th>Alternate Formulas for a Softer Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylparaben (methyl p-hydroxy benzoate)</td>
<td>0.025 g</td>
<td>0.025 g</td>
</tr>
<tr>
<td>Propylparaben (n-propyl p-hydroxy benzoate)</td>
<td>0.015 g</td>
<td>0.015 g</td>
</tr>
<tr>
<td>Sodium lauryl sulfate</td>
<td>1 g</td>
<td>1 g</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>25 g</td>
<td>24 g</td>
</tr>
<tr>
<td>Stearyl alcohol</td>
<td>25 g</td>
<td>19 g</td>
</tr>
<tr>
<td>White petrolatum</td>
<td>25 g</td>
<td>19 g</td>
</tr>
<tr>
<td>purified water</td>
<td>37 g</td>
<td>37 g</td>
</tr>
<tr>
<td>to make:</td>
<td>100 g</td>
<td>100 g</td>
</tr>
</tbody>
</table>

2. Melt the stearyl alcohol and white petrolatum together in the Ointment Melting Apparatus.

3. Dissolve the other ingredients in water, warmed to 75 °C.

4. Discontinue heating. Add the water to the melted oil phase with agitation.

5. Allow to cool, and stir until congealed.

NOTES:

- The parabens are used as preservatives. Ointments containing water should/must have a preservative.
- Because the oil phase contains some solids, fusion is used.
- This is an o/w emulsion because of the phase volume ratio and the high HLB of the surfactant.
- Stearyl alcohol functions as the adjuvant emulsifier and provides smoothness (with the petrolatum) on the skin. (reference 3, p. 1575)
- Propylene glycol, a humectant, tends to increase the viscosity of the aqueous phase and binds the water, thus lessening the evaporation.
4b. Emulsion Bases O/W Type

e.g. Vanishing Cream

1. Prepare the vanishing cream as follow:
   - Stearic acid: 18.0 g
   - Potassium hydroxide: 0.80 g
   - Glycerin: 5.0 g
   - Methylparaben: 0.020 g
   - Propylparaben: 0.010 g
   - Purified water: 76.0 g
   - to make: 100 g

2. Melt the stearic acid in the Ointment Melting Apparatus.
3. In a separate beaker, dissolve the remaining ingredients in the water at 75 °C.
4. Discontinue heating. Add the aqueous solution to the oil phase with agitation.
5. Allow to cool, and stir until congealed.

NOTES:
- The stearic acid is reacted with alkali, e.g. potassium hydroxide, to form a soap. The unreacted stearic aid forms the oily dispersed phase, which is left as a film on the skin after the water evaporates.
- The glycerin serves as a humectant.

5. Hydrophillic/Water Soluble Bases

e.g. Polyethylene Glycol Ointment

1. Prepare polyethylene glycol ointment as follows:
   - Carbowax® PEG 3350 (formerly polyethylene glycol 4000): 40 g
   - PEG 400 (also known as PEG-8): 60 g
   - to make: 100 g

2. Prepare 100 g of the above ointment.
3. Heat the PEG3350 in the Ointment Melting Apparatus (at 65°C) in the ceramic dish until it completely melts (the mixture will go clear).
4. Add the PEG 400, and mix until uniform.
5. Allow to cool, and stir until congealed. You may use a room temperature water bath to accelerate cooling.

NOTES:
- A firmer preparation can be prepared by replacing up to 10 g of the PEG 400 with PEG 3350.
- If more than 5% water is to be added, i.e., from 6 to 25%, then 5 g of PEG 3350 is replaced with an equal amount of stearyl alcohol.
- The PEGs are characterized by their number referring to their approximate molecular weight. Smaller numbers indicate the number of ethylene glycol repeat units.
6. Poloxamer Gel/Cream

The two precursors to poloxamer base cream are:

- Pleuronic gel 20% (F127) in water
- LIPS (lecithin and isopropyl palmitate), or Lipmax®

These precursors will be provided to you in the lab, but also can be made from scratch in your pharmacy. Why, might you ask, would you bother preparing them from scratch, when you can simply buy them pre-made? The answer lies in cost savings – translating a higher profit to your pharmacy. The method for preparing PLO 20% and LIPS is provided here for your future reference:

**Poloxamer (PLO 20%)**
- Weigh 20 g of poloxamer 407 (pluronic F127) and place in an amber 220 mL graduated prescription bottle.
  
  **NOTE:** pluronic F127 powder is a respiratory irritant. Wear an N95 mask when dealing with poloxamer 407 powder.
- Weigh 0.3 g of potassium sorbate and place in the same bottle.
- Cap the amber bottle and gently mix the pluronic F127 and potassium sorbate together so that it is adequately blended.
- Open the cap, and while gently agitating, slowly add cold de-ionized water (5-10 °C) to approximately 100 mL (use the graduated markings on the amber bottle).
- Gently agitate the mixture, label, and place in a refrigerator.
- The PLO will disperse and dissolve in the fridge in 1-2 days.

**Lecithin/Isopropyl Palmitate (LIPS, or Lipmax®)**
- Weigh 22.7 g of soya lecithin and place in a 100 mL prescription bottle.
- Add 0.45 g of sorbic acid to the bottle
- Add 22.7 g (26.6 mL) of isopropyl palmitate to the bottle, label, and store.
- The lecithin beads will dissolve at room temperature in 1-2 days.

Preparation of the Poloxamer Gel/Cream

Poloxamer gels have wide-ranging applications from teeth-bleaching to artificial skin. Pluronic F-127 is a nonionic surfactant, MW 12,500, which forms gels of tailororable strength and gelling temperature, depending on the concentration and excipients used.

Poloxamer creams are very versatile. If the drug is hydrophobic, it is levigated with a suitable hydrophobic levigating agent and incorporated into the oily (LIPS) phase. If it is hydrophilic, it is levigated with a suitable hydrophilic levigating agent and incorporated into the watery (PLO) phase. The two phases are then combined. Use the following protocol to prepare the salicylic acid poloxamer cream:

1. In a glass mortar, combine 1.2 g salicylic acid in an equal quantity (1.2 g) of 95% ethanol, and mix with a pestle until smooth.
2. Shake the Lipmax® solution prior to using.
3. Add 8.8 mL of the Lipmax® solution, and continue mixing until smooth.
4. Add 28.8 g of PLO 20% (about 29 mL), and triturate until a smooth gel/cream is made.

Gel formation happens as the mixture heats to room temperature. The lecithin gives the mixture
an opaque light beige colour. You may have to place your hands around the mortar to bring the ingredients up to room temperature. The pestle should be able to stand upright when the gel is finished.

Part C. Salicylic Acid Base Compounding and Drug Release

Your TA will demonstrate levigation, geometric dilution, and preparing the diffusion cell during the laboratory.

NOTE: Start the release experiment as soon as you finished making your base. Depending on your base type, it may take up an hour to collect the data.

In the next part of the experiment, the prepared ointment base will be used as a vehicle into which a drug, salicylic acid, will be incorporated. The release characteristics of this dosage form will be evaluated using a simple dialysis cell method.

1. For all ointments compounded, prepare 3.0% (w/w) Salicylic Acid Ointments:
   - Salicylic Acid (powder)  1.253 g
   - Levigating Agent (with or without)  0.500 g
   - Ointment base  40.00 g

2. Neatly pack the remaining amount (60 g) of unused ointment base into a clean ointment jar. Label properly and retain. You will be handing your unused ointments in for evaluation. Do not discard the unused ointment.

3. Using a plastic dropper and a hard rubber spatula, levigate the salicylic acid powder with enough drops to turn it into a smooth mass, with a toothpaste-like consistency (~10-15 drops for this amount). The levigating agent, usually liquid, should be compatible with the ointment base. If the base is soft, it alone may be used as the levigating agent. For this lab, you can use the following levigating agents depending on the base you selected:

<table>
<thead>
<tr>
<th>Ointment Base</th>
<th>Levigating Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hydrocarbon Base</td>
<td>Mineral Oil (heavy)</td>
</tr>
<tr>
<td>2. Absorption Base</td>
<td>Mineral Oil (heavy)</td>
</tr>
<tr>
<td>3. W/O Emulsion Base</td>
<td>Glycerin</td>
</tr>
<tr>
<td>4. O/W Emulsion Base</td>
<td>Glycerin</td>
</tr>
<tr>
<td>5. Hydrophilic Base</td>
<td>PEG 400</td>
</tr>
</tbody>
</table>

4. The mass is then incorporated into the ointment base by the method of geometric dilution. The drug must be uniformly distributed.

Preparation of the Diffusion Cell

5. The diffusion cell consists of a a suitable ointment jar, PC jar or snapsafe vial filled with the ointment under study. A membrane is used to allow for the diffusion of the drug (salicylic acid) but not the base itself.

6. The ointment jar cell is packed to avoid air-pockets and is rounded slightly with a spatula.

7. The dialysis cellophane membrane is moistened with de-ionized water, opened by vigorous rubbing, cut, and the excess water is removed by blotting between 2 sheets of
8. The moist membrane is then spread smoothly over the ointment, removing all wrinkles and air-pockets at the ointment-membrane interface. Care should be taken to avoid damaging the diffusion surface of the membrane (handle membrane by the edges).

9. Secure the membrane in place with an elastic band.

Release and Analysis of Salicylic Acid

You will be conducting release studies on three of your bases. Retain the unused portion of your bases to hand in at the end of the lab.

<table>
<thead>
<tr>
<th>Release Study 1</th>
<th>3.0% Salicylic Acid in Hydrocarbon (Base #1) or Absorption (Base #2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Release Study 2</td>
<td>3.0% Salicylic Acid in W/O emulsion (Base #3) or O/W emulsion (Base #4)</td>
</tr>
<tr>
<td>Release Study 3</td>
<td>3.0% Salicylic Acid in Water Soluble (Base #5)</td>
</tr>
</tbody>
</table>

10. Using a retort stand and clamp, the completed diffusion cell is immersed in a large ceramic dish or 400 mL beaker containing exactly 100 mL of de-ionized water to which has been added 4 drops of ferric chloride TS.

11. Fill a cuvette with the above solution to blank the spectrophotometer with. Blank the spectrophotometer, and retain the cuvette with the blank solution in order to zero the spectrophotometer prior to each sample measurement. This is your baseline sample (time=0). You will need to use a new blank for each release study.

12. The membrane is maintained approximately 0.5 cm below the surface of the solution. This permits the removal of 5 mL of the solution for analysis without exposing the membranes to the atmosphere and prevents bubbles at the solution-membrane interface.

13. Timing is started when the cell is in contact with the release solution. A purple colour is formed by the reaction of the salicylic ion with the FeCl₃.

14. Gently agitate the release solution to make the colour uniform prior to taking your sample for assay.
15. Using ointmentdiff.xls, plot the concentration of salicylic acid released vs. time for the three bases on the same graph.

Ointment Base Evaluation

16. Test a small amount of the prepared ointment base (on the tip of your finger) and tabulate the results in chart form for the following:
   - Texture
   - Ease of application
   - Ease of removal
   - Type of film, if any, remaining on the skin
   - Is the base washable?
   - How is the ointment removed from the skin?

17. Place 5 g of the ointment on a glass slab. Determine approximately how much water can be incorporated before a 2-phase preparation is observed or when the ointment becomes fluid.

Part D. Using an Ointment Mill

Ointment mills are used in compounding pharmacies to help reduce the particle size of insoluble APIs or excipients, and ultimately produce a smoother, more pharmaceutically elegant cream or ointment. Ointment mills are not effective at mixing ointments – the ointment must still be properly mixed prior to milling. In Part D, you will be preparing a 40%w/w urea ointment. With the assistance of your instructor, you will be running the cream through the ointment mill in order to reduce the grittiness of the preparation.

1. In a small glass mortar, triturate 16.0 g urea.
2. Using geometric dilution, incorporate 24 g of your remaining Base (1) or Base (2).
3. Mix with a pestle until uniform. Try to make the ointment as smooth as possible.
4. Note the grittiness of the formulation.
5. Bring your Part D base to the ointment mill. With the help of your instructor, mill your ointment, and note the final consistency.
SAFETY TIP: Make sure loose hair is tied back, and do not touch the ointment mill while it is operating. Do not operate the ointment mill without assistance.

6. Pack, label and hand in your final 40% urea ointment.

Due at the End of the Lab:

You will be handing in your ointment bases for evaluation at the end of the laboratory. The following bases are to be handed in:

Four ointment bases, each dispensed in a suitable and properly labeled container:

1) 40% Urea in [Hydrocarbon (Base #1) or Absorption (Base #2)]
2) 1 of: W/O emulsion (Base #3) or O/W emulsion (Base #4) – Non-medicated
3) Water Soluble (Base #5) – Non-medicated
4) 3% Salicylic Acid in Poloxamer Cream (Base #6)
5) The calibration.xls and ointmentdiff.xls spreadsheets, printed out and attached to your first formulation.

It is your responsibility to hand in your bases.

Due on Thursday November 16th by 6pm: Formal Group Lab Report

• See “Guidelines for Writing a Formal Group Laboratory Report” in this manual for tips on writing the final group laboratory report.

Questions

1. The standard curve will be performed in triplicate. This means...
   a) Three different spectrophotometers will be used
   b) The dilution factor for each standard is 1:3
   c) The same solution will be measured 3 times
   d) There will be three solutions per standard

2. Which of the following formulations is mandatory to compound in today's lab?
   a) Base #1 (Hydrocarbon base)
   b) Base #5 (Water soluble base)
   c) Base #4 (O/W Emulsion)
   d) Base #2 (Absorption base)

3. What is the strongest indicator that the poloxamer gel has formed properly?
   a) The gel becomes less viscous when properly mixed
   b) The PLO 20%/LIPS mixture will appear homogenous
   c) The pestle will stand upright unsupported in the mortar
   d) The resultant mixture clarifies to a transparent finish
Lab 4: Formulation and Pharmaceutical Quality of Powders and Capsules

Preparing for the Lab
Read the introduction and lab protocol completely. Watch the following related lab videos on the laboratory website:
- UV/Vis Spectrophotometry - Determining Absorbance
- Capsule Making (CAP-M-QUIK Method)
- Calculate the volume of stock required for each standard solution of the calibration curve.
- Calculate the batch drug and excipient masses required for the 3 powder blends in Part A, and understand how this calculation is performed.

Group Allocation
You will be working in groups of 3 students

What You’ll Be Doing
Part A: Compounding 3 powder blends, and preparing 50 x 100 mg acetaminophen capsules with Blend B or C
Part B: Preparing an acetaminophen standard curve. Performing dissolution test on 5 x 100 mg capsules vs. 1 x 500 mg commercial acetaminophen tablet
Demo: Proper use of Parafilm®
Part C: Determining the bulk density, tapped density, and speed of consolidation, and flowability of 3 blends. Mix blend A in a laboratory blender for 1 minute, and repeat flowability measurements. You will be sharing flowability data only with one other lab group.

Spreadsheets You Will Need
http://phm.utoronto.ca/~ddubins/ DL/Capsule_Filling.xls
http://phm.utoronto.ca/~ddubins/ DL/calibration.xls
http://phm.utoronto.ca/~ddubins/ DL/tapdensity.xls
http://phm.utoronto.ca/~ddubins/ DL/dissolution.xls

What You’re Handing In
- There will be a closed-book quiz at the beginning of the lab
- 10 units of your capsule formulation, dispensed in a properly labeled 7 Dram prescription vial
- 1 x Capsule Filling Worksheet for your capsules (“Capsule Filling - %vol” worksheet, in Capsule_Filling.xls)
- 1 x Quality Control Worksheet for your capsules (“Capsule QC” worksheet, in Capsule_Filling.xls)
- 1 x Calibration Curve Worksheet (calibration.xls)
- 1 x Tap Density Worksheet (tapdensity.xls)
- 1 x Dissolution Worksheet (dissolution.xls)

Introduction
Scale-up of a formulation from development to production requires a fundamental understanding of the interrelationships between ingredient behaviour and processing equipment parameters. Selecting the correct combination of ingredients to satisfy these interrelationships involves careful examination at the pre-formulation stage. Powder blends can be used for capsule formulations, insufflations, douche powders and dusting powders. Additives, such as diluents, binders, lubricants, glidants, disintegrants, and colourants, are usually included to facilitate handling, enhance physical appearance, improve stability, and aid in absorption. This exercise demonstrates the measure of a few experimental parameters to characterize powder flow properties.

PHM 241H1 Lab Manual 2018
Prior to the lab, watch the video “Capsule Making (CAP-M-QUIK Method)” on the laboratory website.

References

Background
In order to make a capsule on the industrial scale, it is necessary to (a) make the powder flow from a hopper into a feed frame, (b) make the powder flow from the feed frame into the die holding the capsule shell, (c) lock the capsule shell with the capsule cap without dislodging the filled powder, and (d) eject the filled capsule from the die. Flow rates of powders are a function of particle size, particle shape, and surface roughness. In addition to the inherent powder characteristics, there are numerous processing variables that must also be considered such as the time and speed of mixing, type of mixing dynamics, and temperature and humidity effects. Scale-up of a formulation from development to production remains an inexact discipline. Proportionality does not necessarily apply. In early development, supplies of bulk active are limited and capsule formulations must be developed on a small scale. Difficulty can be encountered if the formulations are not designed with consideration of the stresses of high speed manufacturing equipment.

The degree of mixing affects the lubricity and wettability of magnesium stearate-containing capsule blends, and stressing a powder blend in a mixer can mimic the changes in blend properties that may occur on scale-up. Measuring tapped bulk density, wettability and disintegration of stressed blends identifies robust formulations which are unaffected by long “lubrication” times and scale. In this fashion, the industrial formulating pharmacist can quickly assess the impact of various parameters on the suitability of a formulation.

<table>
<thead>
<tr>
<th>Types of Additive in a Powder Formulation</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diluents</strong></td>
<td>dicalcium phosphate, calcium sulfate, lactose, cellulose, kaolin, mannitol, inositol, sodium chloride, dry starch, powdered sugar, hydroxypropylmethylcellulose</td>
</tr>
<tr>
<td>− increase the bulk to a practical size for handling</td>
<td></td>
</tr>
<tr>
<td><strong>Binders or Granulators</strong></td>
<td>starch, gelatin, sucrose, glucose, dextrose, molasses, lactose, acacia, sodium alginate, carboxymethylcellulose, methylcellulose, polyvinylpyrrolidone (occasionally, polyethylene glycol, waxes, water,</td>
</tr>
</tbody>
</table>
“Avicel” excipients in the Appendix of this manual are excellent binders. An extremely desirable characteristic of Avicel product is compressibility under pressure. Under compaction, Avicel PH undergoes a plastic deformation process that facilitates hydrogen bonding between the adjacent cellulose particles.

**Lubricants**
- prevent the adhesion of material to the surface of equipment, reduce interparticle friction, may improve the flow of granulation
- talc, magnesium stearate, calcium stearate, stearic acid, hydrogenated vegetable oils and polyethylene glycol

**Glidants**
- improve the flow characteristics of a powder mixture
- usually added in the dry state just prior to compression
- colloidal silicon dioxide, talc

**Disintegrants**
- facilitate the disintegration of a tablet
- starches, clays, cellulosics, aligns, gums, and cross-linked polymers

**Colourants**
- make the product identifiable
- FD & C red, FD & C yellow, FD & C green, FD & C blue, iron oxide, titanium dioxide

### Bulk Density

Bulk density is the mass of powder per unit of bulk volume which consists of the void volume and the true volume occupied by the particles. Although there is no direct linear relationship between the potential flowability of a powder and its bulk density, other properties of the substance can affect the bulk density and flowability. By comparing both the initial and final bulk volumes of powder subjected to tapped compression, Carr defined the compressibility index (CI):

\[
\text{Compressibility Index (or, Consolidation Index)} = \left(1 - \frac{V_{\text{tap}}}{V_{\text{bulk}}} \right) \times 100
\]

Where \( V_{\text{tap}} \) is the volume of the tapped powder
\( V_{\text{bulk}} \) is the volume of the bulk powder when placed in a container (includes the true volume, volume of the internal pores, volume of spaces between the particles)

Compressibility is strictly a misnomer since compression is not involved and “consolidation” might be a more suitable description. It is a simple index and the interpretation is shown in the following table:

<table>
<thead>
<tr>
<th>Consolidation Index after 50 Taps (%)</th>
<th>Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-15</td>
<td>Excellent</td>
</tr>
<tr>
<td>12-16</td>
<td>Good</td>
</tr>
<tr>
<td>*18-21</td>
<td>Fair to passable</td>
</tr>
<tr>
<td>*21-35</td>
<td>Poor</td>
</tr>
<tr>
<td>33-38</td>
<td>Very Poor</td>
</tr>
<tr>
<td>&gt;40</td>
<td>Extremely Poor</td>
</tr>
</tbody>
</table>

* adding a glidant should improve flow
Blends having CI’s less than 15% usually exhibit good flow tendencies while those with a CI value greater than 26% most likely have poor flow characteristics. The index of Carr is a one-point determination and does not reflect the ease or speed of consolidation. Some materials may have a high index suggesting poor flow but may consolidate rapidly which is essential in tableting. An empirical relationship can be drawn between the percentage change in bulk density: \( (V_0-V_n)/(V_0-V_{50}) \times 100 \) and the log of the number of taps, \( (\log n) \), where \( V_0 \) is the initial bulk volume and \( V_{50} \) is the bulk volume after 50 taps. Non-linearity occurs up to two taps and after 30 taps, when the bed consolidates more slowly. The slope is a measure of the speed of consolidation and is useful for assessing powders or blends with similar indices, the beneficial effect of glidants, and the design of capsule formulations. Although counter-intuitive, when comparing two blends, a steeper slope indicates a slower speed of consolidation.

\[
\log(n) \propto \frac{V_0 - V_n}{V_0 - V_{50}} \times 100
\]

**Angle of Repose**

A static heap of powder, when only gravity acts upon it, will tend to form a conical mound. One limitation exists; the angle to the horizontal cannot exceed a certain value, and this is known as the angle of repose (\( \theta \)). The angle depends on the mutual friction between the particles. With an increase in the friction, there is an increase in the angle of repose. As the irregularity of the particles become greater, the friction and the resistance to flow is increased. Accordingly there is an implied relationship between \( \theta \) and flow and particle shape. By measuring the diameter of the base, \( D \), and the height of the heap (h) the angle of repose can be calculated using the trigonometric relationship:

\[
\text{Angle of Repose} = \tan^{-1}\left(\frac{h}{0.5 \times D}\right)
\]

The exact value for \( \theta \) depends on the method of measurement but in general the values in the table below may be used as a guide:

<table>
<thead>
<tr>
<th>Angle of Repose</th>
<th>Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25°</td>
<td>Excellent</td>
</tr>
<tr>
<td>25-30°</td>
<td>Good</td>
</tr>
<tr>
<td>*30-40°</td>
<td>Passable</td>
</tr>
<tr>
<td>&gt;40°</td>
<td>Very Poor</td>
</tr>
</tbody>
</table>

* adding a glidant should improve flow

**Powder Flowability through a Hole in a Plate**

Many powders are marketed with “viscosity” of the powder specified. This does not refer to the viscosity of the material when dissolved into a liquid, but rather the flowability of the powder itself. The viscosity of a powder may be estimated by observing its ability to fall freely through a
hole of known dimensions in a plate. The powder is tested with different hole sizes, ranging from small (e.g. 5 mm) to large (eg 22 mm). The diameter of the smallest hole through which the powder passes three times out of three is taken as the flowability index. The diameter may be used as an empirical measure, or converted into viscosity, which is a comprehensive industry standard.

Mathematically, a “core” cylinder of powder will flow through a hole if the weight of the powder above the hole is greater than the friction of the side surface of the powder:

\[
\pi r^2 h g \geq 2 \pi rh K
\]

Where:
- \( h \) = height of core cylinder of powder
- \( r \) = radius of hole
- \( \pi r^2 h \) = volume of core cylinder
- \( g \) = acceleration due to gravity (981 cm/s\(^2\))
- \( d \) = non-tapped bulk density of powder
- \( 2 \pi rh \) = surface area of core cylinder of powder
- \( K \) = coefficient of friction/cm\(^2\) (powder viscosity)

The above equation can be simplified to:

\[
r \geq \frac{K (\frac{g}{\text{cm.s}^2})}{490.5 \left(\frac{\text{cm}}{\text{s}^2}\right) \times d \left(\frac{g}{\text{cm}^3}\right)}
\]

Solving for \( K \) (powder viscosity):

\[
K (\text{Poise}) \leq 490.5 \left(\frac{\text{cm}}{\text{s}^2}\right) \times r (\text{cm}) \times d \left(\frac{g}{\text{cm}^3}\right)
\]

The answer in Poise (P) can be multiplied by 100 to obtain the measurement in centipoise (cP), a more typical viscosity unit. Thus the viscosity of the powder can be estimated by finding the minimum hole diameter the powder will freely flow through.

**Wettability**

The time for a fluid to penetrate a bed of the consolidated powder can be an effective measure of the degree of dispersion of the hydrophobic lubricant (magnesium stearate). The extent of lubrication of the blend is likely to increase with duration of mixing and is limited only by complete surface coverage of the excipients by magnesium stearate. Thus the hydrophobicity of the powder bed will increase with a subsequent decrease in wettability. Occasionally, over-mixing can result in the loss of the lubricating effect if the excipients are able to trap the magnesium stearate in inner pores rather than the particle surfaces.

**Dissolution**

Although disintegration time of a tablet may influence the rate of drug release to the body, the dissolution rate of the drug from the primary particle is fundamentally important because dissolution of the drug is essential for subsequent absorption to occur. Dissolution testing is a critical test for measuring the in vitro performance of a drug product. It is a quality control tool and an effective aid to formulation development. Dissolution testing can detect changes on stability, and is used to establish an in-vitro and in-vivo correlation for modified release products. When release at a single pH is desired (e.g. for a quick-release formulation),
commonly used dissolution methods include the basket method (USP/NF Apparatus 1) and the paddle method (USP/NF Apparatus 2). For controlled-release drugs that may release slowly over time through the entire GI tract, more complicated models are required, where the pH during drug dissolution can be varied (USP/NF Apparatus 4). Only the paddle method will be employed in this laboratory exercise.

The sample is withdrawn midway through the dissolution vial. Varying the sample withdrawal location can very strongly influence the dissolution profile, so care must be taken when manually withdrawing samples to do so in the same location. Measurements are taken spectrophotometrically, and a standard curve is used to convert absorbance at a given wavelength into concentration. Concentration is then converted to mass by multiplying the known volume remaining of the dissolution vessel. This mass is then converted to % released by dividing by label claim of the dosage form. Typically this test is conducted 6 times for a given formulation. In this laboratory due to resource limitations, you will only need to conduct one dissolution test per formulation.

<table>
<thead>
<tr>
<th>Uses of Dissolution Testing in Industry</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single-point specifications:</strong></td>
</tr>
<tr>
<td>• As a routine quality control test. (For highly soluble and rapidly dissolving drug products.)</td>
</tr>
<tr>
<td>• “Qt”=NLT 75%/30 minutes, means that a “pass” is that not less than 75% of the dose will be released into the dissolution medium by 30 minutes.</td>
</tr>
<tr>
<td>• Different Q values for different drugs and formulations (USP defined).</td>
</tr>
<tr>
<td><strong>Two-point specifications:</strong></td>
</tr>
<tr>
<td>1. For characterizing the quality of the drug product.</td>
</tr>
<tr>
<td>2. As a routine quality control test for certain types of drug products (e.g., slow dissolving or poorly water soluble drug product like carbamazepine).</td>
</tr>
<tr>
<td><strong>Dissolution profile comparison: (Different methods used: Similarity factors, model independent, model dependent)</strong></td>
</tr>
<tr>
<td>1. For accepting product sameness under SUPAC-related changes.</td>
</tr>
<tr>
<td>2. To waive bioequivalence requirements for lower strengths of a dosage form.</td>
</tr>
<tr>
<td>3. To support waivers for other bioequivalence requirements.</td>
</tr>
<tr>
<td><strong>IV/IVC: In Vitro/In Vivo Correlations</strong></td>
</tr>
<tr>
<td>-Only really works if PK is absorption-limited</td>
</tr>
</tbody>
</table>

**Typical Dissolution Test Parameters**

- **Volume:** 500, 900, or 1000 mL
- **Release Medium:**
  - To Simulate Intestinal Fluid (SIF): pH 6.8
  - Pancreatin may be added
  - To Simulate Gastric Fluid (SGF): pH 1.2
  - Pepsin may be added
  - SDS may be added (must be justified)
- **Temperature:** For all IR dosage forms, 37±0.5°C
• Agitation Speed: Basket: 50-100 rpm, Paddle: 50—75 rpm
• Sampling Interval: typically 15 minute intervals, 5 minute intervals for quick dissolving.
• Last time point: typically 60 min (30 min is used in this laboratory).

Experiment Protocol

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Supplies</th>
<th>Special Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen USP (MW 151.17)</td>
<td>Hard Gelatin Capsules Size# 1</td>
<td>Powder Flow Apparatus</td>
</tr>
<tr>
<td>Corn Starch</td>
<td>Parafilm®</td>
<td>Metal funnel</td>
</tr>
<tr>
<td>Lactose NF (MW 360.3)</td>
<td>Cardboard</td>
<td>Ceramic bowl</td>
</tr>
<tr>
<td>Avicel: various grades (will be</td>
<td>Plastic UV Cuvettes</td>
<td>13 metal powder flow disks: 5, 7,</td>
</tr>
<tr>
<td>announced in the lab)</td>
<td>Plastic Droppers</td>
<td>9, 11, 12, 16, 19, 22, 24, 26, 30,</td>
</tr>
<tr>
<td>Dibasic Calcium Phosphate</td>
<td></td>
<td>35, 40 mm diameter</td>
</tr>
<tr>
<td>(CaHPO₄)</td>
<td></td>
<td>Dissolution Bath</td>
</tr>
</tbody>
</table>

The following solutions are prepared or provided by the TA:

• Hydrochloric Acid (0.1 N)
• 5 L of 100 mg/L acetaminophen stock solution

Note: To prepare for Part B, pre-warm your dissolution medium by adding 500 mL of 0.1 N HCl in each of 2 dissolution vessels in one of the dissolution apparatus machines. Label your vessels with your group name.

Part A. Preparing Powder Blends and Capsules

Three separate pre-mixes for 100 mg acetaminophen capsules (Size 01) are to be prepared and evaluated according to the following tables. Note: Size 01 capsules have a fill volume of 0.5 mL. To determine the mass of each ingredient in one capsule, the following formula is used:

\[
\text{Fill Weight} \times \%\text{Fill Volume} = \text{Mass in Capsule}
\]

Fill weights are dependent on the capsule volume, and can either be looked up in tables, or determined in the lab by completely filling 5 capsules with the excipient of interest and weighing the resulting powder weight determined (minus the weight of the empty capsules). Fill volumes are selected by the formulator. It is important to understand that %Fill Volume is not equal to %w/w, because different powders have different densities.

Once the mass of each excipient in the capsule is determined, the %w/w may be calculated by dividing by the total powder weight in one capsule.

Once the %w/w of each component is calculated, they can be multiplied by the total batch weight to compound your powder blend.
Lab 4: Formulation and Pharmaceutical Quality of Powders and Capsules

<table>
<thead>
<tr>
<th>Formulation A</th>
<th>Fill Weight (Size 01)</th>
<th>Planned %Fill Volume</th>
<th>Mass in Capsule (mg) (=Fill Weight × %Fill Volume)</th>
<th>%w/w (Mass of component in capsule ÷ total capsule weight)</th>
<th>Batch Mass (g) (=%w/w × total batch mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen USP (powder)</td>
<td>295 mg</td>
<td>33.9% (=100 mg/295 mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose NF</td>
<td>450 mg</td>
<td>5.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>154 mg</td>
<td>1.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn Starch (Diluent)</td>
<td>375 mg</td>
<td>60.1% (=100-33.9-5-1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td></td>
<td>100%</td>
<td></td>
<td></td>
<td><strong>120.0 g</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Formulation B</th>
<th>Fill Weight (Size 01)</th>
<th>Planned %Fill Volume</th>
<th>Mass in Capsule (mg)</th>
<th>%w/w</th>
<th>Batch Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen USP (powder)</td>
<td>295 mg</td>
<td>33.9% (=100 mg/295 mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose NF</td>
<td>450 mg</td>
<td>5.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn Starch</td>
<td>375 mg</td>
<td>10.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>154 mg</td>
<td>1.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose Component*</td>
<td>230 mg</td>
<td>50.1% (=100-33.9-5-10-1)</td>
<td></td>
<td></td>
<td><strong>120.0 g</strong></td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td></td>
<td>100%</td>
<td></td>
<td></td>
<td><strong>120.0 g</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Formulation C</th>
<th>Fill Weight (Size 01)</th>
<th>Planned %Fill Volume</th>
<th>Mass in Capsule (mg)</th>
<th>%w/w</th>
<th>Batch Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen USP (powder)</td>
<td>295 mg</td>
<td>33.9% (=100 mg/295 mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn Starch</td>
<td>375 mg</td>
<td>15.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dibasic Calcium Phosphate</td>
<td>350 mg</td>
<td>15.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>154 mg</td>
<td>1.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose Component*</td>
<td>230 mg</td>
<td>35.1% (=100-33.9-15-15-1)</td>
<td></td>
<td></td>
<td><strong>120.0 g</strong></td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td></td>
<td>100%</td>
<td></td>
<td></td>
<td><strong>120.0 g</strong></td>
</tr>
</tbody>
</table>

*Groups will be assigned a grade of Avicel for use in this experiment. See the background section of this lab, and the Appendix for more information about these excipients.

1. Prepare **120 g of each blend above (3 in total)**. Make sure to properly label each blend.

**For each blend:**
2. Carefully pour the blend into a plastic laboratory bag.
3. Twist or seal the bag shut with at least as much air trapped in the bag as product.
4. Shake the sealed bag for **5 minutes** by repeatedly inverting the bag.

**Capsule Making**
5. Select either formulation **B** or **C** for capsuling. Calculate and weigh out enough powder to fill **60 x Size 01 hard gelatin capsules** of your selected formulation. Use the capsule machines to fill 50 capsules. The extra powder is compounded to account for losses due to leakage and processing error in filling.

**Note:** Your TA or instructor will demonstrate how to use capsule machines. **Wear gloves when making and handling capsules.** Also watch the video **Capsule Making (CAP-M-QUiK Method)**, available on the laboratory website.
Capsule Quality Control (QC)

6. Using the “QC” spreadsheet in Capsule_Filling.xls, follow the protocol to QC your batch. You will be individually weighing 5 empty gelatin capsules, then ten randomly selected capsules from your formulation as part of the QC process.

Note: Use the sensitive scales for QCing – the open-air scales (without glass enclosures) will not provide enough precision for QC.

Part B. USP Paddle Dissolution Test

Note: To save time, start Parts B and C simultaneously.

UV Absorbance Standard Curve of Acetaminophen

Preparing the Standards

Note: The TA will prepare 100 mg/L stock solution of acetaminophen. You will be preparing a dilution in series, meaning that you will prepare the highest concentration first (20 mg/L) and then use that solution to prepare the more dilute solutions. This is a more accurate (and easy) approach than preparing each concentration from the 100 mg/L stock. **You will need to calculate** the volumes of stock or 20 mg/L solution required for each of the standard curve concentrations.

1. Using the stock solution, prepare each of the following acetaminophen concentrations in de-ionized water, **in triplicate**:

   1, 5, 10, 15, and 20 mg/L

To clarify, this means that you create each solution three times, rather than measure the absorbance of the same solution three times, in order to get an estimate of the error associated with creating the standard solutions. Measuring the standards **in triplicate** will allow you to report the average, standard deviation, and %RSD at each standard concentration. As you are working in groups of 3, each student will prepare one series of standards above, and measure the absorbance. The same spectrophotometer must be used.

Prepare 100 mL of the most concentrated standard solution first (20 mg/L), and then dilute that solution to prepare 50 mL each of the less concentrated standard solutions:
Fill in the following table to prepare your dilution calculations:

<table>
<thead>
<tr>
<th>Concentration ($C_2$)</th>
<th>$C_1$ (mg/L)</th>
<th>$V_2$ (mL)</th>
<th>$V_1$ (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/L</td>
<td>20 mg/L</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>5 mg/L</td>
<td>20 mg/L</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>10 mg/L</td>
<td>20 mg/L</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>15 mg/L</td>
<td>20 mg/L</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>20 mg/L</td>
<td>100 mg/L</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Note: Prepare each solution in a volumetric flask. Along with the flasks in your lockers, your station has been provided with enough 50 mL volumetric flasks for all three curves. Beakers and Erlenmeyer flasks are only accurate to 5%, and should not be used to prepare standard concentrations. Use bulb pipettes to dispense your calculated volumes of solution required.

Preparing the Blank

2. Prepare the blank solution. The blank solution consists of 5 mL of de-ionized water in a clean plastic UV cuvette.

UV Absorbance Measurement

Obtain a pair of small volume plastic UV cuvettes from your TA or instructor. Your TA will demonstrate how to use the spectrophotometer. A video is also available on the lab website.

Set the wavelength of the spectrophotometer to 243 nm (UV range).

3. Zero the absorbance of the spectrophotometer using the blank solution.

4. Measure the absorbance of each concentration of the standard curve and record the absorbance.

*SPECTROSCOPY NOTES*

- Fill the cuvette to the etched line (approx ¾ full)
- Make sure the cuvette is facing the correct way (the light path should go through the clear windows, not the ridged sides)
- To avoid fingerprints, only handle the cuvettes by the ridged sides, not the clear windows.
- Fill the cuvette slowly, and gently tap to release bubbles clinging to the sides of the cuvette
- Gently wipe the clear windows with a Kimwipe prior to measuring
- Make sure the sample door is closed before measuring absorbance
- Make sure you use the same UV spectrophotometer for calibration and sample measurements.

*NOTE: Plastic UV cuvettes are tapered towards the bottom, to accommodate a smaller sample volume. The fill line is just above the clear part of the cuvette window. The “V” shaped arrow on the Plastic UV cuvette indicates the side of the cuvette that the UV beam will travel through the
entire 1 cm path length (not widthwise, which is only 0.5 cm):

5. Plot a calibration curve of absorbance vs. known concentration of acetaminophen, using the laboratory computers. You may use the file calibration.xls in the “Downloads” section of the laboratory website.

Dissolution Runs

For the dissolution runs, you will be assigned two dissolution vessels per group. You will be comparing the dissolution profiles of 5 x 100 mg acetaminophen capsules (your own selected formulation) vs. 1 x 500 mg commercial tablet:
The Teaching Assistants will help you prepare and load the dissolution vessels, as they are very fragile. Do not load or run these tests without the assistance of the TAs.

1. Place 500 mL of the dissolution medium (0.1 N HCl) into each of the 1000 mL dissolution vessels.
2. Insert 5 capsules into one of the dissolution vials. Commence rotation of the paddles at 100 rpm. Start timing the dissolution test.
3. At 2, 5, 10, 20, and 30 minutes after starting the dissolution test:
   - Withdraw a 1 mL sample of the dissolution medium using a 3 mL syringe.
   - Use a syringe filter to filter only 1 mL of the sample into a 50 mL volumetric flask (see diagram below on the proper use of a syringe filter). For the first sample, run de-ionized water through the syringe filter to prime it.

**Note:** Syringe filters are re-useable. One will be allocated to your selected formulation, and the other will be allocated to the commercial product. You will be using one syringe and syringe filter for each formulation, for the entire dissolution test.

5. Dilute the 50 mL volumetric flask to the mark with deionized water.
6. Agitate, then using a plastic dropper, transfer ~3 mL of the diluted sample to a UV cuvette.
7. Blank the spectrophotometer, then measure and record the absorbance of the sample at 243 nm.
8. In your report, record the time when the capsules break or appear to disintegrate.
9. Obtain one extra-strength (500 mg) commercial acetaminophen tablet from your TA or instructor. Repeat the dissolution test for 1 x 500 mg acetaminophen commercial tablet.

**Note:** You may run the capsule and tablet test at the same time. You may find it easier to start the tablet test 5 minutes later to stagger the sampling times.

10. Using **dissolution.xls** on the laboratory website, plot the absorbance vs. time for the two formulations, and % released vs. time. According to the f-values calculated, would the two curves be deemed similar by the FDA?

### Part C. Determining Powder Characteristics

**NOTE:** We are using 15 taps instead of 50 taps, as described in the Background section.

1. Loosely place an aliquot of the blend into a dried, tared 25 mL graduated cylinder, filling the cylinder to the 25 mL graduation mark.
2. Weigh the cylinder again to determine the weight of powder. Cover the mouth of the cylinder with Parafilm®. Calculate and record \( d \), the untapped powder density.

**NOTE:** The TA will demonstrate how to properly use Parafilm® during the lab.

3. Obtain a retort stand and rubberized ring clamp. Place a piece of corrugated cardboard on top of the retort stand base.
4. Clamp the rubberized ring on the retort stand 10 cm above the cardboard.
5. Hold the 25 mL graduated cylinder within the ring clamp so that the base is at the level of the clamp. Allow the cylinder to drop onto the cardboard. (The ring clamp should act as a guide to prevent the cylinder from tipping over.)
6. Tap the 25 mL cylinder in this manner ONCE. Record the volume of the blend in the 25 mL cylinder ($V_1$).
7. Continue tapping using the fixed-height drop technique for a total of 1, 2, 5, 10, and 15 times. Record the volume of the blend at different times.
8. Calculate the tapped density of the blend powder: $(\text{powder weight}/V_2)$.

NOTE: Use the rubberized ring clamp (shown above) to determine tapped density.

9. Use tapdensity.xls on the laboratory website to plot $\log n$ vs. $(V_0-V_n)/(V_0-V_{15})$, and to calculate the speed of consolidation for each formulation. Print off the graphs and staple them to your worksheets. Report the slope of the curve.

Powder Flowability

NOTE: Partner up with one other group. Measure the powder flowability of two formulations, and share data with the second group to obtain flowability of the other two formulations, so that you have flowability data for Blend A, Blend B, Blend C, and Blend A (subjected to 1 minute of high-shear mixing, in a laboratory blender).

Setting up the Powder Flow Apparatus: (TA performs this set-up)

1. The powder flow apparatus will be set up in the fume hoods using the following procedure:
2. Obtain a series of disks and a metal ruler from your instructor or TA. As these disks are custom made, take special care not to lose them.
3. Place the powder collection bowl at the base of a retort stand. 
4. Attach the powder flow apparatus on a retort stand using a vinyl retort clamp. 
   - The apparatus should be high enough so that the trap door swings open freely. With the trap door shut, the body of the powder flow apparatus cylinder should be ~10 cm from the bottom of the ceramic bowl.
   - The trap door should be facing downwards.
   - The trap door release hinge should be pointing forward (towards you).
5. Attach the metal funnel above the powder flow apparatus using a ring clamp, so that the funnel is 4-5 cm above the top of the top of the powder flow apparatus. The final assembly should look like this:
6. The disks are labelled with their hole diameter in millimeters stamped on the disk.
7. Insert the 16 mm disk into the powder flow apparatus. Make sure the disk is flush with the bottom of the powder flow apparatus cylinder.
8. Close the trap door by sliding the middle hole of the trap door release hinge into the pin attached to the bottom of the trap door:

Test Procedure:

1. Fill a clean, dry 400 mL beaker approximately half way with the powder test mix.
2. Pour the powder test mix into the metal funnel, until the powder flow apparatus is filled ~1 cm from the top. If the powder becomes trapped in the funnel, tap the funnel gently with a spatula until all of the powder falls loosely into the centre of the cylinder of the powder flow apparatus. Pouring the powder in the funnel disrupts powder aggregates due to long term storage or sitting.

NOTE: Be careful not to touch the sides of the powder flow apparatus or tap it once it is loaded.
3. After the cylinder is filled, allow 30 seconds for possible formation of individual flocculi or mass flocculation of the whole powder mass.
4. Flip the trap door release hinge up to open the trap door.
5. A “positive” result is deemed if the powder flows through the hole, and the hole is visible from the top of the cylinder. A “negative” result is deemed if the hole is not visible from the top of the cylinder:
6. For positive results, repeat the test with smaller and smaller disks until a negative result is obtained. For negative results, repeat the test with larger and larger disks until a positive result is obtained.

**Determining Flowability:**

7. Three positive results in a row are required to determine flowability. Repeat the test two more times on the smallest disk that produces a positive result. If a negative result is obtained, advance to the disk having the next largest diameter, and proceed testing.
   - Calculate and record the viscosity of the three formulations.
   - Comment on the flowability of the samples.

**The Effect of High Shear Mixing on Formulation A**

8. *After* you have performed all flowability tests on Formulation A, transfer all of the formulation to a laboratory blender. Subject Formulation A to 1 minute of high shear mixing. Use a lab timer to accurately measure the mixing time.

9. Repeat the procedures outlined in Part C (speed of consolidation, bulk density, tapped density, and powder flow) for the blended Formulation A.

10. Comment on the flowability of the samples. What was the effect of mixing on powder density and flowability?

**To Hand In: (by the end of the lab)**

- 10 units of your capsule formulation, **properly labelled** in a 7 Dram prescription vial for evaluation.
- 1 x Capsule Filling Worksheet for your capsules (“Capsule Filling - %vol” worksheet, in Capsule_Filling.xls)
- 1 x Quality Control spreadsheet for your capsules (“Capsule QC” worksheet in Capsule_Filling.xls).
- 1 x completed Calibration Curve Worksheet (calibration.xls)
- 1 x completed Tap Density Worksheet (tapdensity.xls)
- 1 x completed Dissolution Worksheet (dissolution.xls)

Clean your work area, the spectrophotometer area (in PB 819), and any scales you used.
Questions

1. A Q-value of "NLT 80%/45 minutes" means...
   a) At least 80% of the dose will be released by 45 minutes
   b) Each 45 minutes, 80% of the remaining dose will be released
   c) After 45 minutes, exactly 80% of the dose will be released
   d) No more than 80% of the dose will be released by 30 minutes

2. A higher angle of repose is indicative of....
   a) Poorer powder compressibility
   b) Better powder compressibility
   c) Poorer powder flowability
   d) Better powder flowability

3. How many capsules are planned to be compounded in Part A of this lab, to account for losses in compounding?
   a) 40
   b) 50
   c) 60
   d) 70

4. Why do we use dibasic calcium phosphate in the experiment?

5. Why do we prepare plots of \((V_0 - V_n) / (V_0 - V_{15})\) vs log n, what does the slope indicate? Define bulk density.

6. What is the purpose of having a rubber ring clamp instead of a regular metal ring clamp in the tapped density procedure?

7. How do we calculate powder viscosity? Why do we measure it in this experiment?

8. What does a Q-value of \(Q_1 = \text{NLT 50%}/30\) minutes means in a USP dissolution test? Did your results satisfy this criteria?

9. According to the f-values calculated in dissolution.xlsx, would the two curves be deemed similar by the FDA?

10. Thinking about the powder properties, and given that the potencies are the same, which formulation(s) would you pick for:

<table>
<thead>
<tr>
<th>Method</th>
<th>Formulation</th>
<th>Reason / Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand-Filling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand Capsule Machine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Industrial Capsule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Machine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

11. Using the “Capsule Filling - %vol” spreadsheet on Capsule Filling.xlsx, calculate how much of each excipient would be required to compound 50 x Size 01 capsules of the following formulation:
   - Acetaminophen 160 mg
   - Corn Starch 30 %fill volume
   - Lactose 10 %fill volume
   - Avicel PH-105 (diluent)
Lab 5: The Effect of Particle Size on Tableting Properties

Preparation for the Lab

Read the introduction and lab protocol completely.

Group Allocation

You will be working in groups of 6 students.

What You’ll Be Doing

Part A: Watching a demo of low and high shear granulation, and performing particle size analysis on one of the dried granulates.

Part B: Adding post-granulation excipients to your assigned granulate, and tableting the mixture. Determining weight uniformity, hardness, friability, and disintegration time.

Part C: Determining the melting point of acetaminophen.

Resources You Will Need

http://phm.utoronto.ca/~ddubins/DL/probit.xls
http://phm.utoronto.ca/~ddubins/DL/probit.pdf
http://phm.utoronto.ca/~ddubins/DL/tablets.xls

What You’re Handing In

• There will be a closed-book quiz at the beginning of the lab.
• 10 tablets of your final formulation, dispensed in a properly labeled 7 Dram prescription vial (1 per group of 6 students)
• 1 x QC Sheet for your final formulation (in the “Tablet QC” worksheet of tablets.xls), folded up into a square and attached to the formulation vial with adhesive tape
• Both of the spreadsheets in probit.xls, printed out and attached to the final formulation with adhesive tape (not collected until after the post-lab talk).

Introduction

In the preparation and characterization of many pharmaceuticals, one is vitally concerned with the size distribution of particles. In the preparation of a solution the time required for a given weight of material to dissolve depends on the degree of subdivision of the solute. The texture, taste, and rheology of an oral suspension depend on the size-frequency distribution of the dispersed phase. The automatic machine-filling of bulk powders into bottles and vials is affected by the shape and the size of the particles of the powder. The uniformity of weight of compressed tablets and hard gelatin capsules depends on the proper flow of the granulation from the hopper into the die cavity. Particle size affects the rapidity of extraction from crude drugs. Particle size is one of the major factors which influence the physiological availability of drugs from oral and parenteral pharmaceuticals.

Most starting materials used in the manufacture of solid dosage forms are fine powders with wide size distributions. Pharmaceutical wet granulation can agglomerate powders in order to enhance some of the material handling characteristics. Agglomeration (defined as the assemblage of particles in a powder) primarily serves to prepare powders for tableting by rendering them free-flowing, non-segregating and easily compressible. It may also serve to modify solubility and dissolution rate and to reduce the formation of dust.

In this laboratory, the effect of particle size on tableting properties is investigated by comparing some of the physicochemical properties of un-granulated versus granulated material. The results should indicate the importance of selecting the appropriate particle size of drug for the development of tablet dosage forms.

References

Background

Particle Sizing

The major techniques for particle size determination are microscopy, sieving, and sedimentation. Sieving is the simplest and most widely used method for determining particle size since the analysis can be completed in a short time. The appendix of this manual has a chart of sieve number vs. particle size, and the inventory available in PB 860.

The sieve number refers to the number of meshes to a linear inch of the sieve through which the powder will pass. The number of openings per linear inch, however, is not always an indication of the size of the openings in the sieve due to the variation in the diameter of the wire used in various sieve cloths. For this reason the Bureau of Standards has established specifications for standard sieves, as given in the above table.

The integrity of a tablet is dependent on the strength and resistance of the compacted powder in withstanding external disruptive forces until the tablet is administered. The purpose of compaction is to bring particle surfaces into close proximity and to enhance intermolecular forces, thereby enabling inter-particulate bonding. Compactibility of powder is dependent on both the intra- and inter-particulate bond strength and on the area of inter-particle bonding resulting from powder compaction and decompression. Compactibility may be affected by both physicochemical characteristics of material under consolidation as well as tableting conditions. Important functional characteristics include the ability of particles to bond following deformation, particle roughness and shape, particle size and size distribution, moisture and amount of elastic recovery occurring during decompression. In general, excipients that deform quickly and permanently facilitate inter-particle bonding, and produce tablets with improved mechanical strength. Several techniques used to enhance compactibility of excipient have been cited in the literature. Changes in process equipment or conditions can influence the compaction behaviour of the materials.

Particle size evaluation involves placing a sample of the test material on the upper stack of standard sieves and shaking the sieves for a given time. A Tyler or Cenco-Meinzer sieve shaker holds seven standard sieves and will classify a powder in five or ten minutes. The weight of powder retained on each sieve is determined. The size assigned to the powder retained is arbitrary, but by convention the size of the particles retained on a sieve is taken as the arithmetic or geometric mean size of the two sieves. Thus, for a powder passing a 30-mesh sieve and retained on a 45-mesh sieve, we look up their sieve openings on the table above:

11. Sieve # 30 has a sieve opening of 600 µm
12. Sieve # 45 has a sieve opening of 355 µm

The particles weighed on the #45 sieve will have an arithmetic mean diameter of:
The particles weighed on the #45 sieve will have a geometric mean diameter of:

$$\sqrt[10]{\frac{\log(600) + \log(355)}{2}} = \sqrt{600 \times 355} = 461.5 \mu m$$

Simple statistical analysis can be performed on sieve data. You should be familiar with the following statistical calculations; arithmetic mean, geometric mean, median, mode, percentile, standard deviation. Investigate these definitions on your own, and be prepared to use these concepts in the lab.

A number of graphs may also be drawn with this data in order to facilitate evaluation. The simplest of these is the frequency distribution curve which plots the percentage of particles retained on a sieve versus the mean particle size for the particles. In order to make a visual estimation as to whether or not the particles approximate a normal distribution curve, it is necessary that the range of sizes of the sieves be uniform. To do this, an adjusted frequency distribution curve is prepared by dividing the fraction retained by the size range of the two sieves between which the particles fell. This value is then plotted versus the mean particle size. A visual comparison of size distribution can now be made between different materials or the same material subjected to different agglomeration processes. From this data, a series of cumulative plots may now be drawn. Cumulative distributions are used to determine percentiles, i.e., the proportion of a test material which is above or below a specified size value.

**Granulation**

Powder can be directly compressed or granulated prior to compression into tablets. The purpose of granulation is to agglomerate particles, improve powder flow, prevent segregation, and with the use of appropriate excipients, enhance compressibility. The binding mechanism of the granulation fluid and the resultant granule growth conditions will affect the properties of the granules. With wet granulation, the presence of inter-granular moisture causes adhesion of the particles, where the voids among the powders are only partially filled with liquid. Liquid bridges exist to hold the particles together. When the void space is completely filled with liquid, which extends to the edge of the pores, bonding is affected by interfacial forces at the surface of the granule and a negative capillary pressure throughout the liquid space. During drying, although some residual moisture may remain, solid bridges may be formed in the granule by fusion of the point contacts or contact areas similar to sintering, or by curing of any adhesive added in the form of a binding agent, or by crystallization of dissolved materials.
Hardness

Hardness may be defined as the resistance of a solid to attrition or breakage. It has been used in characterizing tablet as it provides a simple measure of the effectiveness of the compression process. Both the Strong Cobb and the Stokes hardness testers are used in the pharmaceutical industry to measure the force required to break the tablet. Hardness values obtained from these two instruments for a given set of tablets are not equivalent but they can be correlated. There appears to be a linear relationship between the tablet hardness and the logarithm of the compression force. One would anticipate that as a tablet becomes more dense, its hardness would increase. Other relationships among hardness, compression force and pack density are empirical and can be obtained experimentally.

The desirable tablet hardness depends on the formulation intended, and tablet weight. Small compressed tablets should have lower hardness (~5 kP for 100 mg tablet) and larger compressed tablets should have a higher hardness (15-20 kP for ~1000 mg tablet). Tablet hardness should be high enough to keep tablet integrity in the further processing, such as coating and packaging, and in product transportation. The following table lists approximate values for different formulations, and serves only as an approximate guideline:

<table>
<thead>
<tr>
<th>Formulation</th>
<th>N (Newton)</th>
<th>kg (kilogram) or kp (kilopond)</th>
<th>Sc (Strong-Cobb)</th>
<th>lb or lbf (pounds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast-Disintegrating (e.g. sublingual) Tablet</td>
<td>20-70</td>
<td>2.7</td>
<td>2.9-10.0</td>
<td>4.5-15.7</td>
</tr>
<tr>
<td>Chewable Tablet</td>
<td>30</td>
<td>3</td>
<td>4.3</td>
<td>6.7</td>
</tr>
<tr>
<td>Small compressed tablet (100 mg), uncoated</td>
<td>50</td>
<td>5</td>
<td>7.1</td>
<td>11.2</td>
</tr>
<tr>
<td>Large compressed tablet (1000 mg), uncoated</td>
<td>150-200</td>
<td>15-20</td>
<td>21.4-28.6</td>
<td>33.7-45.0</td>
</tr>
<tr>
<td>Small compressed tablet, coated</td>
<td>105</td>
<td>10.7</td>
<td>15.0</td>
<td>23.6</td>
</tr>
<tr>
<td>Unit conversion factor</td>
<td>1</td>
<td>0.101971</td>
<td>0.142812</td>
<td>0.224737</td>
</tr>
</tbody>
</table>

Friability

Tablet friability is related to hardness. A friability tester measures the ability of the tablet to resist abrasion which is important to packaging, shipping and handling. Friability tests the strength of tablets against wear.

The number of tablets used in a friability test depends on the tablet weight. For tablets weighing <= 650 mg each, the minimum number of tablets to reach 6.5 grams are used for the test. For tablets > 650 mg each, 10 tablets are used in the test. The total initial weight of the tablets is determined (W₀).

The tablets are loaded into a friability tester, where they are...
subjected to controlled falls. The test involves rotating the drum exactly 100 times, over 4 minutes (25 rpm). The intact tablets are then removed and weighed again (W).

The measure of abrasion resistance or % Friability is expressed as a percentage loss in tablet weight:

\[
\% \text{Friability} = 100 \times \left[ 1 - \frac{W}{W_0} \right]
\]

A tablet weight loss of 1% is the USP-defined upper limit for friability; however, a target of less than 0.3% is desirable for tablet processing and resilience.

Disintegration Testing

When a powdered drug is granulated and compressed into a tablet, the effective surface area of the medicinal compound is decreased. An immediate-release tablet must break up or disintegrate in the gastrointestinal fluids into granules, which then must disintegrate into primary particles. The drug needs to dissolve from the primary particles before the molecules or ions of the medicinal compound can be absorbed by the gastrointestinal mucosa. Improperly formulated and improperly processed compressed tablets may retard drug release with a decrease in bioavailability. If a tablet does not disintegrate, the surface available for dissolution is restricted only to the surface area of the tablet.

Disintegration time specification is a useful tool for quality control, but disintegration of a tablet does not imply that the drug has dissolved. A tablet may pass a disintegration test and yet the drug may be biologically unavailable. The disintegration time is a rapid indicator of the effect caused by changes in formulation parameters or stability of the final dosage form.

In the lab, we use Nessler Tubes to determine the disintegration time. It is an empirical measure as the end-point is the visual confirmation of the largest piece of tablet breaking into smaller pieces. In industry, a more robust measure is used. A basket travels up and down in a 37 °C water bath. The basket holds six vertical glass tubes. At the bottom of each glass tube is a 10-mesh screen. A tablet is placed in each tube to start the test. The disintegration time is expressed as the time it takes for the last piece of tablet to fall through the 10-mesh screen.
**Weight Uniformity**

Variation in processing and powder flow can lead to a variation in tablet weight. Assessing weight uniformity provides a measure of the variability in the tableting process. The test is performed as follows:

1. Weigh 10 tablets together:
   \[ \bar{x} = \frac{\text{weight}}{10} \]
2. Weigh each tablet separately:
   \[ x_1, x_2, x_3, \ldots, x_{10} \]
3. Calculate the Standard Deviation:
   \[ SD = \sqrt{\frac{(x_i - \bar{x})^2}{n}} \]
4. Calculate the Relative Standard Deviation:
   \[ \%RSD = \frac{SD}{\bar{x}} \]

A large %RSD is indicative of processing problems (e.g. >5%). This can be because of the formulation itself (e.g. poor powder flowability, poor batch mixing, hold-up in the upper), or because of improper equipment performance or settings (e.g. machine lubrication and wear, ventilation, environment).

**Stability Testing**

A formulation can demonstrate favourable properties on the day of manufacture; however, stability tests need to be performed to demonstrate safety and efficacy over time. Stability testing methods will depend on the formulation (e.g. tablet, capsule, suspension, etc.), anticipated storage conditions, and type of drug. Simply speaking, a group of drug products are stored in different controlled environments for a certain period of time. Typical storage conditions are:

<table>
<thead>
<tr>
<th>Study</th>
<th>Storage Condition</th>
<th>Minimum Time Period Covered by Data at Submission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long-term</td>
<td>25 ± 2°C / 60 ± 5 %RH or 30 ± 2°C / 65 ± 5 %RH</td>
<td>12 months</td>
</tr>
<tr>
<td>Intermediate</td>
<td>30 ± 2°C / 65 ± 5 %RH</td>
<td>6 months</td>
</tr>
<tr>
<td>Accelerated</td>
<td>40 ± 2°C / 75 ± 5 %RH</td>
<td>6 months</td>
</tr>
</tbody>
</table>


At planned time points, samples are retrieved for testing. Timepoints such as 1M(month), 2M, 3M, 6M, 9M, 12M, 18M and 24M are typically used.

The tests of the products at each time point must include physical appearance, assay (drug content), relative substances (impurities) and other product specific properties such as hardness, disintegration time for tablets, and pH/flow properties for liquids. The stability test will determine the resilience and shelf life of the formulation, and identify the degradation products.

Accelerated stability tests involve incubating the formulation at a higher temperature to increase degradative processes that lead to drug decomposition, such as chemical incompatibilities with excipients, or drug hydrolysis. Briefly, k, the first-order rate constant for decomposition, can be estimated at different temperatures (in this lab, 40°C and 60 °C). The k
values for decomposition over time at a given temperature can be calculated by plotting ln(C) vs. time, using the drug content determined from the assay at room temperature for time=0. The slope of the graph at that temperature will be equal to –k.

As temperature rises, decomposition of the drug occurs more rapidly. The temperature dependence of k can be described using the Arrhenius equation:

\[
    k = A e^{\left(\frac{-E_a}{RT}\right)}
\]

Where A is the pre-exponential frequency factor, \(E_a\) is the activation energy, and \(R\) is the universal gas constant. An Arrhenius plot can be constructed for ln(k) vs. 1/T. The slope of this graph is \(-E_a/R\), and the intercept is ln(A). Once the Arrhenius constants are determined from the Arrhenius plot, the decomposition rate may be estimated at room temperature using the above relationship. Then, the shelf life may be calculated as the time to 10% degradation of the drug (or 90% of the drug remaining, \(t_{90\%}\)) using this k value:

\[
    t_{90\%} = \frac{-\ln(0.9)}{k_{T=21^\circ C}}
\]

We are not testing formulation stability in this laboratory. This section is provided for informational purposes.

Content Uniformity

A content uniformity test evaluates if the strength of the drug is within acceptable limits of the label claim. It is determined either by weight variation or by assay of individual units. Acceptance limits vary depending on formulation, and the label claim on the product monograph of the drug. The following table provides acceptance criteria for different dosage forms.

We are not testing content uniformity in this laboratory. This section is provided for informational purposes.

<table>
<thead>
<tr>
<th>Table 1. Acceptance Criteria</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Stage</th>
<th>EP and USP common dosage forms(^1)</th>
<th>USP suppositories</th>
<th>USP transdermal systems and inhalations packaged in premetered dosage units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1((n=10))</td>
<td>Acceptance value ≤ maximum allowed acceptance value L1 (L1 is 15.0 by default.)</td>
<td>No unit outside 85.0–115.0% of the label claim(^2) and RSD ≤ 6.0%</td>
<td>No unit outside 75.0–125.0% of the label claim;(^3) not more than one unit outside 85.0–115.0% of the label claim(^2) and RSD ≤ 6.0%</td>
</tr>
<tr>
<td>2((n=30))</td>
<td>Final acceptance value ≤ L1 and no individual content of any dosage is less than ((1-0.01\times L2)) M nor more than ((1+0.01\times L2)) M (L2 is the maximum allowed range for deviation of each dosage unit from the reference value and is 25.0 by default.)</td>
<td>No unit outside 75.0–125.0% of the label claim;(^3) not more than 1 of 30 units outside 85.0–115.0% of the label claim(^2) and RSD of the 30 units ≤ 7.8%</td>
<td>No unit outside 75.0–125.0% of the label claim;(^3) not more than 3 of 30 units outside 85.0–115.0% of the label claim(^2) and RSD of the 30 units ≤ 7.8%</td>
</tr>
</tbody>
</table>

\(^1\)Common dosage forms include: uncoated, coated, or molded tablets; capsules; oral solutions in unit-dose containers; suspensions, emulsions, or gels in single-unit containers (that are intended for systemic administration only); and solids (including sterile solids) in single-unit containers.

\(^2\)Dependent on the average of the limits specified in the potency definition in the individual monograph and mean value of the results, "label claim" is replaced by "label claim multiplied by the average of the limits specified in the potency definition in the individual monograph divided by 100" or by "label claim multiplied by the average value of the units tested (expressed as a percent of label claim) divided by 100".
Melting Point

An important part of characterizing the stability of a drug is determining its melting point. Knowledge of the thermostability of a drug is a critical factor in compounding, as the process may involve high temperatures and pressures (e.g. high shear granulation, tableting). The melting point of a substance is the temperature at which it changes from a solid to a liquid state. The capillary method is commonly used, as it is reproducible, relatively inexpensive, fast, and simple. Briefly, a sample is loaded into a capillary tube, which is inserted into a capillary tube melting point apparatus. The temperature is increased at a controlled rate while an observer (or video recording device) monitors the sample.

Relatively small amounts of impurities can change the melting temperature of a substance, or broaden its range. As a general guideline, 1% of a foreign substance will result in a 0.5 °C depression in the melting point. We take advantage of this fact to identify an unknown chemical. Most melting point units have three chambers so that an unknown substance can be identified: one chamber for the unknown test substance (to be identified), one for the reference substance (100% pure), and one for an equal combination of both. If the melting ranges and melting point in all three chambers agree, then the conclusion of the test is that the test and reference substances are the same.

However, the effect of mixing two substances together does not always broaden the melting point range. In general, mixing two substances together will result in a depression of the melting temperature. For the mixing of two substances, the melting temperature hits a minimum at a specific combination of both substances. This is known as the “eutectic point”, and is not necessarily at a 50/50 mixture.

It is important to mention, because the mixture of two substances will not necessarily exhibit a broad melting point range. A eutectic mixture has the characteristic of a sharp melting temperature. The dash line in the above phase diagram indicates where melting begins, and the solid line indicates the clear point.

Important observations are made throughout the melting process, which should be recorded. The following distinct stages are usually present:
<table>
<thead>
<tr>
<th>Stages of Melting</th>
<th>Image</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting Point</td>
<td><img src="image1" alt="Image" /></td>
<td>No liquid or condensation present. Sample is completely solid/crystalline.</td>
</tr>
<tr>
<td>First Signs of Change</td>
<td><img src="image2" alt="Image" /></td>
<td>Early changes may be due to solvent loss, dehydration, change in crystallization state, decomposition (darkening of colour), condensation of solvent on cool parts of the capillary.</td>
</tr>
<tr>
<td>Sintering Point</td>
<td><img src="image3" alt="Image" /></td>
<td>A few surface crystals melt.</td>
</tr>
<tr>
<td>Onset Point / Collapse Point</td>
<td><img src="image4" alt="Image" /></td>
<td>The “official” start of the melt: liquid clearly appears in equilibrium with crystals. This is the lower temperature recorded in the melting point range.</td>
</tr>
<tr>
<td>Meniscus Point</td>
<td><img src="image5" alt="Image" /></td>
<td>Enough crystals melt to form a clear meniscus in the capillary tube. There is a solid phase at the bottom and a liquid phase at the top. Sometimes bubbles will prevent a clear meniscus from forming. In Europe, it is also recorded as the melting point.</td>
</tr>
<tr>
<td>Clear Point / Liquefaction Point</td>
<td><img src="image6" alt="Image" /></td>
<td>The substance becomes completely liquid. There are no longer any solid crystals. This is the higher temperature recorded in the melting point range. In North America, it is also recorded as the melting point.</td>
</tr>
<tr>
<td>Chemical Degradation</td>
<td><img src="image7" alt="Image" /></td>
<td>It is important to note that some substances will degrade before they melt, and thus a melting temperature for these compounds cannot be recorded. Darkening or blackening during the melting process is a clear indication of thermal degradation. Degradation may also occur after the Clear Point.</td>
</tr>
</tbody>
</table>

The melting point and melting range are dependent upon the heating rate used in the melting point apparatus (also called the ramp rate). The ramp rate must be recorded along with the melting range in order to ensure reproducibility and in order to identify the test. In general, a slower ramp rate will provide better resolution and a narrower melting range.

Other parameters that need to be considered during pre-formulation include the type of sterilization used and the choice of packaging materials.
Lab 5: The Effect of Particle Size on Tableting Properties

Experiment Protocol

There will be a demonstration of high and low shear granulation. The samples from the previous week’s demonstration will be used for the particle size analysis and tableting.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Supplies</th>
<th>Special Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen USP (MW 151.17)</td>
<td>Glass Capillary Tubes</td>
<td>Standard Sieves (set 1: #12, 20, 40, 60, 80;</td>
</tr>
<tr>
<td>Polyvinyl Pyrrolidone (PVP K30)</td>
<td>Nessler Tube</td>
<td>set 2: #18, 40, 60, 80, 100)</td>
</tr>
<tr>
<td>Magnesium Stearate (MW 591.24)</td>
<td></td>
<td>Quadro Comil</td>
</tr>
<tr>
<td>Lactose NF (MW 360.3)</td>
<td></td>
<td>Rotary Tablet Press</td>
</tr>
<tr>
<td>Corn Starch</td>
<td></td>
<td>Planetary Mixer</td>
</tr>
<tr>
<td>1 %w/v Magnesium Stearate/Ethanol (optional)</td>
<td></td>
<td>Friability Tester</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hardness Tester</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 mL Nessler Tube</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Melting Point Apparatus</td>
</tr>
</tbody>
</table>

The following solutions are prepared or provided by the TA:

13. High and Low Shear Granulations

Part A. Particle Size Analysis: Low and High Shear Granulation

Granulating Demonstrations

The TA will prepare low and high shear granulations of the following formulation as a demonstration:

Granule Compositions

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>weight (g)</th>
<th>% w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen USP (powder)</td>
<td>90.68</td>
<td>30.2%</td>
</tr>
<tr>
<td>Lactose NF</td>
<td>75.68</td>
<td>25.2%</td>
</tr>
<tr>
<td>Avicel PH101</td>
<td>117.95</td>
<td>39.3%</td>
</tr>
<tr>
<td>Polyvinylpyrrolidone</td>
<td>15.68</td>
<td>5.2%</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td><strong>300 g</strong></td>
<td><strong>100.0%</strong></td>
</tr>
</tbody>
</table>

The following processing parameters will be used:

<table>
<thead>
<tr>
<th>High Shear</th>
<th>Low Shear</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-Mixing Stage:</strong></td>
<td>Impellor Speed: 240 rpm</td>
</tr>
<tr>
<td>Impellor Speed: 500 rpm</td>
<td>Carrier Fluid: Distilled Water</td>
</tr>
<tr>
<td>Chopper Speed: 0 rpm</td>
<td>Dosing Rate: Add slowly in small quantities using a 100 mL grad cylinder (not controlled, total amount should be &gt;= 100 mL)</td>
</tr>
<tr>
<td>Dosing Rate: 0 mL/min</td>
<td></td>
</tr>
<tr>
<td><strong>Granulating Stage:</strong></td>
<td></td>
</tr>
<tr>
<td>Impellor Speed: 900 rpm</td>
<td></td>
</tr>
<tr>
<td>Chopper Speed: 1800 rpm</td>
<td></td>
</tr>
<tr>
<td>Carrier Fluid: Distilled Water</td>
<td></td>
</tr>
<tr>
<td>Dosing Rate: 10 mL/min</td>
<td></td>
</tr>
</tbody>
</table>

PHM 241H1 Lab Manual 2018
Lab 5: The Effect of Particle Size on Tableting Properties

**Protocol Overview:**

- **MILL** the powder, 18 mesh
- **SEIVE** your powder to determine PSD
- **WEIGH** out 47.25g of granulate and MIX with post-granulation excipients
- **PRESS** tablets with your formulation
- Determine the melting point of acetyaminophen
- **Measure** tablet properties: mass, hardness, friability, disintegration time

**Powder Milling**

1. With the assistance of your TA, **mill the particles** using the Quadro Comil. Select a mesh equivalent to a US mesh # of 18.

**Particle Size Analysis**

2. Select 5 sieves to conduct your particle size analysis. Use the appendix of this manual to select sieves appropriate for the anticipated particle sizes (choose mesh numbers between 12 and 120). For the top sieve, select a size **12 or 16 mesh**. Place the bottom tray + sieves on the mechanical shaker.

3. **Pre-weigh each sieve** to determine the empty sieve weight, and record the weights on your worksheet. You will need to use the kilogram scales in the lab (there are two of them – ask your TA or instructor where they are located).

4. Accurately weigh your assigned granulate, and pour it into the top sieve.

5. Affix the lid on the sieving column, and vibrate the column for 3 minutes.

6. Accurately weigh each sieve and record the final sieve weights. Calculate and record the amount of powder retained on each sieve.

7. Complete the following table in the Laboratory worksheet:

<table>
<thead>
<tr>
<th>Sieve # (Passed / Retained)</th>
<th>Mean Size, (d^*) (µm)</th>
<th>Weight Retained (g)</th>
<th>Fraction Retained, (n)</th>
<th>Cumulative Fraction Retained (cfr) (frequency distribution)</th>
<th>Arithmetic Weighted Size, (n \times d)</th>
<th>Geometric Weighted Size, (n \times \log d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\vdots)</td>
<td>(\vdots)</td>
<td>(\vdots)</td>
<td>(\vdots)</td>
<td>(\vdots)</td>
<td>(\vdots)</td>
<td>(\vdots)</td>
</tr>
<tr>
<td>(\Sigma n)</td>
<td></td>
<td></td>
<td></td>
<td>(\Sigma (nd))</td>
<td>(\Sigma (n \times \log d)) = (\vdots)</td>
<td></td>
</tr>
</tbody>
</table>

* use arithmetic mean size

8. Reconstitute your assigned granulate by pouring each sieve fraction into the same formulation plastic bag, and shake by inverting for 1 minute.

9. Use the “probit” spreadsheet in **probit.xls** from the laboratory website to prepare a graph to determine the Mean Mass Diameter, using probit analysis.

**Note:** Remember to read about probit analysis on the laboratory website (**probit.pdf**).

- Determine the arithmetic mean diameter:
  
  \[
  d_{av} = \frac{\Sigma nd}{\Sigma n}
  \]
where \( n \) is the fraction retained and \( d \) is the mean pore opening.

What is the arithmetic average diameter of your sample?

- Also, determine the standard deviation:

\[
\sigma = \sqrt{\frac{\sum (n(d - d_{av})^2)}{\sum n}}
\]

- Determine the geometric mean diameter, \( d_{geo} \):

\[
\log(d_{geo}) = \frac{\sum (n \log d)}{\sum n}
\]

\[
d_{geo} = 10\left(\frac{\sum (n \log d)}{\sum n}\right)
\]

- Use the “fracplot” spreadsheet in probit.xls from the laboratory website to prepare a graph of the fraction retained vs. mean particle size. Indicate the mode, MMD, arithmetic mean particle size and geometric mean particle size.

- Compare your results with a group who analyzed the other granulate (low vs. high shear granulation). Were the mean particle sizes and particle size distributions comparable? If both granulates were milled using the same hole size, why might their resultant particle size distributions be different?

**Part B. Preparation and Testing of Tablets**

1. Weigh out your assigned granulate, and post-granulation excipients according to the following formula into a plastic formulation bag:

   **Tablet Acetaminophen Formulation (will make ~140 x 100 mg acetaminophen tablets, with an overall tablet weight of 350 mg):**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Batch Mass (g)</th>
<th>% w/w</th>
<th>Mass in 1 Tablet (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulation (high or low shear):</td>
<td>47.250</td>
<td>94.50%</td>
<td></td>
</tr>
<tr>
<td>• Acetaminophen (in granulate)</td>
<td>14.29</td>
<td>28.57%</td>
<td></td>
</tr>
<tr>
<td>• Lactose (in granulate)</td>
<td>11.92</td>
<td>23.84%</td>
<td></td>
</tr>
<tr>
<td>• Avicel PH101 (in granulate)</td>
<td>18.58</td>
<td>37.15%</td>
<td></td>
</tr>
<tr>
<td>• Polyvinylpyrrolidone (in granulate)</td>
<td>2.47</td>
<td>4.94%</td>
<td></td>
</tr>
<tr>
<td>Corn starch</td>
<td>2.50</td>
<td>5.00%</td>
<td></td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.25</td>
<td>0.50%</td>
<td></td>
</tr>
<tr>
<td>Total (g):</td>
<td>50.0</td>
<td>100%</td>
<td>350 mg</td>
</tr>
</tbody>
</table>

**Important Notes:**

- **DO NOT** add acetaminophen, lactose, PH101 or PVP at this step – those are already in the granulate (pre-granulation excipients).

- **DO NOT use your entire granulate for your tablet batch.** This formulation requires exactly 47.25 g.
2. Shake the powder mixture in the bag for 5 minutes. Alternately, you may shake the formulation in a sealed plastic bag with headspace.

3. Take the compression mix to your TA at the Rotary Tablet Press, fill the die cavity with the “running powder” and compress the powder. (TA NOTE: you may lubricate the punch die with 1% w/w magnesium stearate in ethanol)

4. Determine the weight uniformity of 10 tablets by weighing each tablet separately. Report the weight of each tablet in tabular form, and report the mean ± %RSD.

5. Take 10 tablets and determine the tablet hardness (mean ± %RSD) using the Hardness Tester and record in tabular form. The tablet should be placed FLAT in the hardness tester (so you can see the diameter of the circle from above). The piston squeezes the tablet on the edges.

6. Determine the number of tablets required for the friability test. If the tablet weight is less than 650 mg, then the total number of tablets will be the lowest number sufficient to attain 6.5 g total. Weigh the tablets collectively, to obtain the total mass of tablets before the friability test. Bring your tablets to a TA or instructor to load and run the friabilator. After running the friabilator for 4 minutes (4 min x 25 rpm = 100 revolutions), weigh the remaining intact tablets to obtain the total mass of tablets after the friability test. Calculate and report the %friability.

7. Determine the disintegration time by placing one tablet in a Nessler tube. Fill the tube with about 50 mL of 0.1 N HCl and invert the tube repeatedly until the tablet disintegrates. This is defined as the point at which the largest visible tablet piece reduces to a particle size not visibly discernible from the other particles present (in other words, you can no longer see the remaining tablet). Record the time it takes for the tablet to disintegrate.

Note: The particles do not need to dissolve.

8. Comment on the physical characteristics of your tablets, such as the hardness and disintegration time. How does the particle size distribution impact the characteristics of the tablets? How would the method of granulation impact the tablet properties? You will need to consult the results of your classmates to draw conclusions.

Part C. Determining the Melting Point of Acetaminophen

1. Place a small amount (~0.1 g) of acetaminophen on a clean, dry watch glass.

2. Crush the solid to a fine powder by gently rubbing it with the flat end of a spatula or pestle.

3. Open a glass capillary tube by scoring it around the middle, and gently bending.

4. Tap the capillary, open end downwards, repeatedly onto the dry powder.

Notes:
- only use a small amount (overfilling will result in uneven heating, broader ranges)
- Pack the sample well (loose samples will heat unevenly)

5. Turn the capillary over (closed end down) and gently tap so that enough powder (1-2 mm) falls to the bottom.

6. Load the capillary, closed-end downwards, into the melting point apparatus.
7. Set the melting point apparatus to heat. Your TA or instructor will demonstrate proper use of the melting point apparatus.

8. A “coarse” or preliminary run will be conducted to obtain the approximate range.
   - For the coarse run, set the starting temperature to 150 °C, the ramp rate to 20 °C/min, and the end temperature to 260 °C.
   - Observe and record the melting temperature range of the solid powder for the coarse run. (HINT: The melting point of acetaminophen is above 160 °C.)
   - Continue heating the sample to 260 °C. Is there a temperature at which chemical degradation (darkening) is present?
   - Once a sample has been melted, discard it (decomposition, oxidation, or polymorphism conversion are likely)

9. On a new sample, a “fine” run is then conducted, with a slowed heating rate once the temperature is within 10 °C of the coarse melting point.
   - For the fine run, set the starting temperature to 10 °C below your observed onset point in the coarse run, the ramp rate from 1-2 °C/min, and the end temperature to 10 °C above your observed melting temperature in the coarse run.
   - Do not insert the sample until the temperature is about 10 °C below the coarse melting point. This will minimize product degradation.
   - Observe and record the melting temperature range of the solid powder for the fine run.
   - Discard the sample.

Questions

1. What is the purpose of adding magnesium stearate to a powder mixture?
   a) To promote powder flow
   b) To act as a tablet binder
   c) To act as a formulation diluent
   d) To form interparticulate bridges
2. Which technique will be used in today's lab to characterize granulate particle size distribution?
   a) Sedimentation
   b) Microscopy
   c) Light Scattering
   d) Sieving

3. What is defined as the melting point in North America?
   a) The meniscus point
   b) The eutectic point
   c) The sintering point
   d) The clear point

4. If the final tablet weight is 350 mg, what is the tablet strength of your formulation?

5. Given the average tablet weight you determined, what is the theoretical percent potency of your formulation, assuming zero degradation and perfect mixing?

6. How did the method of granulation (high vs. low) affect tablet hardness?

7. How did the particle size distribution affect tablet hardness?

8. How does granulation affect the disintegration of a tablet?

9. What is the purpose of adding magnesium stearate to a powder mixture? How would the magnesium stearate help?

10. What is the difference between a lubricant and a glidant?

11. What was the peak temperature during high shear granulation? Was it close to the melting temperature of acetaminophen? Is this a particular concern for degradation?
Lab 6: Analysis of Fab Fragments of Trastuzumab (Herceptin®)

Preparing for the Lab | Read the introduction and lab protocol completely.
---|---
Group Allocation | You will be working in groups of 3 students

What You’ll Be Doing

- TA Demo: Preparing, Loading, and Running a Gel
- **Part A**: SDS PAGE - Loading and Running your Gel
- **Part B**: Zinc Staining your Gel

**Locker Check-Out**: The last hour of the lab is allocated for ensuring your locker is complete, checking out your locker, and returning your lab key.

Spreadsheets You Will Need | Not Applicable
---|---

What You’re Handing In

- There will be a closed-book quiz at the beginning of the lab
- Your completed, stained gel will be presented to the teaching assistant at the end of the lab

Introduction

Proteins are large biological macromolecules consisting of chains of amino acids. Most biological systems are mediated by proteins, which participate in metabolic cycles, DNA replication, cell signalling, cell adhesion, immune response, and other important functions. Proteins are composed of linear sequences of amino acids, but ultimately the way the protein is folded (i.e. the secondary and tertiary three-dimensional structure) will determine and influence its activity. The following four model proteins will be used in this lab:

<table>
<thead>
<tr>
<th>Albumin (MW 67 kDa)</th>
<th>Human albumin is produced in the liver, and is the most abundant protein in human plasma. The primary function of albumin is to regulate osmotic pressure in blood serum. Albumin also acts as a transporter in the bloodstream for a variety of drugs (e.g. warfarin and phenytoin) and endogenous substances (e.g. hormones, bilirubin, bile salts, fatty acids, calcium ions). Albumin levels are used as a clinical measure to detect hepatic and renal abnormalities.</th>
</tr>
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<tbody>
<tr>
<td>Apo-transferrin (MW 80 kDa)</td>
<td>Transferrin is also produced in the liver. It is an iron-binding blood plasma glycoprotein that controls the level of free iron in biological fluids. Transferrin not bound to iron is called apo-transferrin.</td>
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</table>
Immunoglobulin G (150 kDa)

Immunoglobulin G (or simply IgG) is an antibody isotype secreted by plasma B cells, and controls bacterial, viral, fungal, and other pathogenic infection of body tissues. Monoclonal antibodies have emerged as new therapies to treat in a variety of clinical areas, including cancer. Herceptin® (trastuzumab IgG) is a humanized monoclonal antibody used for the treatment of HER2+ (human epidermal growth receptor 2-positive) breast cancer.

Trastuzumab Fab Fragment (50 kDa)

The Fc (fragment crystallisable) domain of an antibody binds to immune system cells (e.g. macrophages). The Fab (fragment antigen-binding) domain binds to the antigen. A Herceptin Fab fragment is the Fab portion of trastuzumab that binds to the “invader”, in this case HER2+ breast cancer cells. It is equivalent to one of the upper stems of the Y-shaped IgG molecule. We are using SDS-PAGE in this lab to assess the purity of Trastuzumab, to demonstrate the utility of gel electrophoresis as a quality control measure.

**Protein Structure**

Saying that proteins are composed of linear sequences of amino acids does not do the complexity of a working protein justice. The 3-dimensional structure of a protein is instrumental in its functionality, and protein folding has long been an area of fascination, interest, and importance in the biophysical sciences. The following is a summary of the protein structure hierarchy:

**Primary Structure**

The sequence of amino acids in a polypeptide chain. Peptides have an N-terminal (basic) and a C-terminal (acidic). The peptide bond is non-ionic. A completely unfolded peptide is said to be in a “random coil” formation, and does not have the higher-order structural features described below.
**Secondary Structure**

The secondary structure of a protein is dependent on the primary sequence. Chains of amino acids may assemble to form \( \beta \)-sheets (pleated), and \( \alpha \)-helices. These secondary structures form because of intra-residue hydrogen bonding.

**Tertiary Structure**

Tertiary structure involves further 3-dimensional constraining of a protein’s shape. Some types of links between protein chains include disulphide bonds, salt bridges, and hydrogen bonds. Hydrophobic burial also plays a role in stabilizing a protein’s tertiary structure. The tertiary structure of a protein is extremely dependent on temperature and solvent conditions. The folded state is often referred to as the “globular” state. A ligand binding to a protein also influences the tertiary structure.

**Quaternary Structure**

Most biological enzymes do not exist in the body as single monomers. Proteins can exist grouped in pairs (dimers), threes (trimers), fours (tetramers), and higher order groupings. For example, hemoglobin is a tetramer.

Images: [https://biochemanics.wordpress.com/2013/04/02/proteins-have-levels-of-structure/](https://biochemanics.wordpress.com/2013/04/02/proteins-have-levels-of-structure/)  
[http://academic.brooklyn.cuny.edu/biology/bio4fv/page/prot_gb.htm](http://academic.brooklyn.cuny.edu/biology/bio4fv/page/prot_gb.htm)  
[http://kvhs.nbcd.nb.ca/gallant/biology/tertiary_structure.jpg](http://kvhs.nbcd.nb.ca/gallant/biology/tertiary_structure.jpg)  
SDS-PAGE

Electrophoresis is a process in which proteins are separated in an electric field. It is a simple, rapid and sensitive analytical tool which can be used to separate, to determine the relative abundance, and to estimate the molecular mass of proteins in a sample.

A common method to separate proteins by electrophoresis is with the use of a polyacrylamide gel and sodium dodecyl sulfate (SDS), known as SDS-PAGE (Polyacrylamide Gel Electrophoresis). Since protein shape will influence migration across a gel, the 3D structure of the protein must be disrupted in order for the peptide’s mobility across a gel to be truly proportional to its molecular weight. The protein must also be charged in order for it to migrate across an electric field. SDS is an anionic detergent, which primarily serves to bind to the proteins, providing them with an overall negative charge, and also participates in protein denaturation. The samples may also be subjected to β-mercaptoethanol and heat to disrupt α-helices, β-sheets, and disulphide bonds that determine a protein’s secondary and tertiary (3 dimensional) structures. This ensures complete denaturation (unfolding to a random coil state). Proteins are loaded using a pipette at the top of the gel. Once the electric field is turned on, the negatively charged proteins are attracted to the anode (the positive side), at the bottom of the gel. Migration across the gel therefore occurs downwards. Polyacrylamide gels inhibit larger proteins from migrating as fast as smaller molecules. The separation of proteins is dependent on the difference in the relative molecular mass (unit: Dalton) of the polypeptides since their charge-to-mass ratio is almost the same. The migration distance of a protein is negatively proportional to the log of its mass.

A useful summary of the experimental setup is here:

Source: http://biolympiads.blogspot.ca/2015/09/sds-page-tutorial.html

The speed of migration in an electrical field depends on the dimension, form and charge of the molecules.

For deaggregation and denaturation of the proteins, SDS, β-mercaptoethanol or DTT (reducing agents), and heat is used.

SDS (strongly anionic detergent) provides negative charge to the proteins.
The smaller proteins migrate faster and further down the gel than larger proteins. Therefore, the masses of proteins can be estimated if they are run simultaneously with standards of known molecular masses. Usually these standards are supplied commercially, in complex mixtures of dye-labelled protein “ladders”:


A protein ladder can therefore help quickly visually identify the molecular weight of an unknown protein.

Running a protein on an SDS-PAGE gel can therefore be used to identify a protein by characterizing its molecular weight, and it can also be used to identify impurities or degradation in a protein sample. SDS-PAGE is therefore a valuable tool to determine and assess the quality of a biologic therapy such as an antibody.

Anode vs. Cathode: Devices with Polarity

Identifying a device’s anode and cathode can be quite confusing. A cathode is thought of as a charge emitter, in the sense of conventional current. It is the part of the device that emits “positive charge”. We can picture positive charge leaving our voltage source in the diagram to the right, from its positive terminal. This terminal is known as the voltage supply’s cathode. The direction of conventional current is depicted as “I”. The other side of the voltage supply is where the “positive charge” flows into – this is known as the anode. From the perspective of the gel, the roles are reversed. Have a look at the gel in the circuit to the right. The charge emitter then is still the cathode. The gel is not producing energy, it’s using it up. The anode and cathode terminals therefore change signs. The gel’s anode is positive, taking on the role as the charge acceptor, and the gel’s cathode is negative, taking on the role of the charge emitter. The roles

---

1 We know that the actual direction of current is opposite – the positive side of a battery (cathode) receives electrons, and the negative side (anode) emits them. However, we are using conventional current definitions for this discussion.
are the same, but the polarity is reversed.

Now consider the flow of a negatively-charged protein in this system. If loaded onto the cathode of the gel (the positive side), the protein will migrate across the gel towards the gel anode, because that is the actual direction of current.

References

Experiment Protocol

**Chemicals** | **Supplies** | **Special Equipment**
--- | --- | ---
Tris-HCl | Weighing paper | Spatula
Tris-base | 1.5 mL centrifuge tube | Analytical balance
Sodium dodecyl sulfate (SDS) | gel loading tips | 500 mL beaker
Glycerol | Pre-cast gel (10%) | 1L beaker
Glycine | | 5 uL pipette
Bromophenol blue | | 10 uL pipette
(β-mercaptoethanol – optional) | | Mini-Gel System (including electrode assembly, clamping frame, buffer dam, mini-tank and lid)
Protein standards | | Power supply
Protease inhibitor cocktail | | Container for gel staining
Apo-transferrin (Sigma) | | 
Herceptin IgG and Fab (obtained from Prof. Reilly’s lab) | | 
Albumin (Sigma) | | 
Zinc stain kit [contains Imidazol (Solution A) and Zinc sulfate (Solution B)] | | 

Teaching Assistant Preparation

The following procedure will be followed by the TAs to prepare reagents. You will not be performing these steps.

1. **2X Sample loading buffer (Laemmli loading dye):** Dissolve 1.25 mL of 0.5 M Tris-HCl pH 6.8, 2 mL of 10% SDS, 2.5 mL of Glycerol, 0.2 mL of 0.5 % of Bromophenol blue and 3.55 mL of distilled and deionized water. *(To prepare samples in the reduced form, another 5% (v/v) of β-mercaptoethanol is added right before mixing with the protein samples – this is optional).*

2. **10X Running buffer (Laemmli buffer):**
   - This is purchased as a 10X Buffer from Biorad (Tris/ Glycine/ SDS 10X Buffer, 5 LT). Dilute 1:10 with deionized water, in a large 30L container (with spigot).
   - To prepare this buffer manually: (not required) In a one-liter beaker, weigh 30.3 g of Tris-base, 144 g of Glycine, 10 g of SDS and add water to 1 L. The 1X running buffer is made before the electrophoresis by diluting the 10X stock solution with distilled and deionized water.
3. Weigh 100 mg of each of the apo-transferrin or albumin powder and dissolve in 50 mL of 50 mM Tris-HCl, pH 6.8 with protease inhibitor cocktail (1:200, v/v). This stock solution can be saved in -80 °C. Before the electrophoresis, each of the samples is mixed with the same volume of the 2X sample loading buffer to make the final concentration as 1 mg/mL. Similarly, obtain a sample of Herceptin IgG and Fab from Prof. Reilly’s laboratory (Conrad or Deborah) and dilute with sample loading buffer to 1 mg/mL. Transfer 12 uL of each sample into a 1.5 mL centrifuge tube. Put the tubes on a 95 °C heating block for 10 minutes. After the heating, the samples are immediately transferred on ice. Briefly spin down the evaporated liquid by centrifuge, and keep the samples on ice until loading.

4. Dilute 50 mL of zinc stain solution A with 450 mL of deionized water. Transfer 50 mL of the solution into a labeled container.

5. Prepare zinc stain solution B by dissolving 16.15 g of zinc sulfate in distilled water, and diluting to the mark in a 500 mL volumetric flask. Transfer 50 mL of the solution into a labeled container.

**Part A - SDS Polyacrylamide Gel Electrophoresis**

**Apparatus Setup:**

1. Obtain 4 tubes of sample (Albumin, Apo-transferrin, Herceptin, and Herceptin Fab) and 1 tube of protein standards from one of the teaching assistants. The samples and protein standards have already been denatured by heating and need to be kept on ice before loading.

2. Remove ready pre-cast Gel from the storage pouch. Use a razor blade to cut the tape at the bottom of the gel along the black “cut here” line. Cut all the way to the edge of the cassette where the pull tab begins. Pull the tape tab along the cut line, up from the cassette and at an angle towards the comb end of the gel.

3. Place gel cassette into the slot at the bottom of electrode assembly with the short plate facing inward. Push the gel cassette up into place against the green gasket (U-shape) to form a tight, leak-proof seal.

4. When preparing only one gel, in the other side of electrode assembly place the buffer dam. After the gel cassette and the buffer dam are in place, slide the assembled electrode assembly into the clamping frame.

5. Using both index fingers, push down gently on the electrode assembly to seat in place. At the same time, use your thumbs to close the clamping frame’s cam levers and lock the gels in place. Lower the whole inner chamber assembly into the mini tank.

6. Pour gel running buffer into the inner chamber (the space between gel and buffer dam) and let it reach the halfway between the tops of taller and shorter plate of gel cassette.

7. Add gel running buffer to the lower buffer chamber until covering the two cam levers. After pouring the buffer, carefully observe the buffer level of the middle space for sign of leakage.

8. At this point, remove the comb by pulling evenly upwards, being careful not to distort or damage the gel well sides in the process. Take a syringe with an angle needle to rinse each well with gel running buffer.
Sample Loading:
You will be loading the following samples:

Protein Ladder / Albumin / Apo-transferrin / Herceptin IgG / Herceptin Fab

1. Use the 5 uL pipette with gel loading tip, and load the protein ladder in the first well.
2. Use the 10 uL pipette with gel loading tip, and load sample 1 in the second well beside the protein standards. Continue the loading until all 4 samples are loaded in 4 continuous wells (total 5 wells, use a fresh loading tip for each loading).

Note: The pre-cast gel should have **10 wells** in total. It is recommended to load the standards and samples continuously in the middle wells for better comparison.

Gel running:

1. Place the green lid on the tank, aligning the color-coded banana plugs and jacks. Insert the electrical leads into the power supply.
2. Apply power to the Mini-PROTEAN 3 cell and begin electrophoresis at 100 volts, 3 amps for 10 minutes to stack the gel, and then 200 volts, 3 amps for about 40 minutes, or until the dye front reaches the bottom of the gel.
3. Record the current reading at the beginning and the end of gel running. Occasionally watch the dyes of sample and molecular weight standard during the electrophoresis to make sure the gel is running properly.

Note: if the electrophoresis needs to be stopped in the middle of running due to buffer leaking or other reasons, **Call your Teaching Assistant or Instructor for assistance. Turn off the power supply and disconnect the electrical leads first before fixing the problem.**

Removing the gel:

Note: **Do not remove the gel without the assistance of a TA. Gels are very fragile and rip easily.**

1. After electrophoresis is complete, turn off the power supply and disconnect the electrical leads.
2. Remove the tank lid and lift out the inner chamber assembly. Pour off and discard the running buffer.
3. Open the cams of the clamping frame. Pull the electrode assembly out of the clamping frame and remove the gel cassettes.
4. Remove the gels from the gel cassette by inserting a metal spatula and lift up one of the plate and gently separating the two plates of the gel cassette. (Note: To remove the gel from a Ready Gel cassette, first slice the tape along the sides of the Ready Gel cassette where the inner glass plate meets the outer plastic plate.)
5. Remove the gel by floating it off the gel plate; invert the gel and place in a container filled with distilled and deionized water, agitating gently until gel separates from the plate.

**Part B – Gel staining (Zinc Stain)**

Note: All the solutions for staining, except water, are biohazardous and should be recycled in specific containers after use.
Lab 6: Analysis of Fab Fragments of Trastuzumab (Herceptin®)

1. Discard water in the container, and re-fill with 50 mL of the diluted 1X Imidazole (solution A). Place the container on a rocker with low speed and incubate at room temperature for 10 minutes.

2. Carefully transfer the gel into another container filled with 50 mL of the diluted 1X Zinc sulfate (solution B). Make sure the gel is completely immersed in the solution. Place the gel on rocker with low speed and allow 30 seconds for the gel to develop.

3. Transfer the gel to another container filled with distilled and deionized water and rinse for 3 minutes. Replace with fresh distilled and deionized water again.

4. Drain the water (not completely dry), and place the gel against a black background for visualizing the bands.

5. The gel image can be taken by a scanner and analysed with software.

Questions

1. In which direction do the negatively charged samples flow in SDS-PAGE?
   a) Towards the sample wells
   b) Towards the protein ladder
   c) Towards the anode at the bottom
   d) Towards the cathode at the top

2. The goal of sample pre-treatment in SDS-PAGE is to...
   a) Denature proteins and equalize their charge-mass ratio
   b) Cleave proteins into smaller fragments
   c) Stabilize proteins and link disulphide bridges
   d) Neutralize protein charge

3. Which of the following best describes the tertiary structure of a protein?
   a) Intra-residue bonding forming sheets/helices
   b) The folded globular state of the protein
   c) The assembly of multiple protein units
   d) The specific sequence of amino acids

4. Based on your results, plot a standard curve for the protein standards [migration distance (Rf) vs. log molecular mass (log M_r)] with the equation and r square value. Determine the molecular mass of these 4 samples by reference to this standard curve.

5. How does the molecular mass obtained from the SDS-PAGE gel compared with the theoretical molecular weight of each sample? If there is a difference, explain why.

6. Comment on the purity of each sample. Which sample appeared to be the least pure? Why? Hypothesize what the impurity or impurities could be (using the molecular masses).

7. Which factors of equipment and sample can affect the accuracy of the outcome?

8. What will the result be on the gel if sample X was contaminated with another unknown protein (i.e. could be an un-related protein or an antibody against sample X)?

9. Select and describe a biotechnology-based pharmaceutical (biopharmaceutical) that is currently used to treat cancer. Indicate its chemical properties, how it is produced, the formulation in which it is included [manufacturer, strength, excipients (if known), reconstitution procedure]. Briefly, indicate what type of cancer it is used to treat and the
dose/dosage regimen. How effective is it for treating this type of cancer? Are there any adverse reactions/side effects? Propose two independent methods which could be used to analyse the purity of this biopharmaceutical product.
# APPENDIX

## U.S. Standard Sieve Sizes and Lab Sieve Inventory

<table>
<thead>
<tr>
<th>Nominal Designation No. (mesh #)</th>
<th>Sieve Opening (µm)</th>
<th>Nominal Designation No. (mesh #)</th>
<th>Sieve Opening (µm)</th>
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<th>Sieve</th>
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## Quadro Comil Meshes

<table>
<thead>
<tr>
<th>Serial Number</th>
<th>Hole</th>
<th>Inches/Microns</th>
<th>Particle Size (µm)</th>
<th>US Mesh#</th>
<th>US Mesh (µm)</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>7B250Q03750*</td>
<td>Square</td>
<td>0.25/6350</td>
<td>3175</td>
<td>6</td>
<td>3350</td>
<td>Square holes are used for wet granulation, dispersion, moist food reclaim, cheeses, de-wrapping candy</td>
</tr>
<tr>
<td>(6350) 2006-11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7B187Q03472*</td>
<td>Square</td>
<td>0.187/4750</td>
<td>2375</td>
<td>8</td>
<td>2360</td>
<td></td>
</tr>
<tr>
<td>(4750)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7B156Q03746*</td>
<td>Square</td>
<td>0.156/3962</td>
<td>1981</td>
<td>10</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>(3962)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7B125G03123*</td>
<td>Cheese Grater</td>
<td>0.125/3175</td>
<td>1587.5</td>
<td>12</td>
<td>1700</td>
<td>Grater screen is used for harder products or more ductile, powderizing cream filled cookies, grating cheese, chopping nuts, sizing compressed slugs, cereal flakes</td>
</tr>
<tr>
<td>(3175)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7B083R03472*</td>
<td>Round</td>
<td>0.083/2107.7</td>
<td>1053.8</td>
<td>18</td>
<td>1000</td>
<td>Round holes are used for dry granulation, powders, fat dispersion, bulk density tuning, de-agglomeration</td>
</tr>
<tr>
<td>0601</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7B045R03137*</td>
<td>Round</td>
<td>0.045/1143.8</td>
<td>571.9</td>
<td>30</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>0601</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Methocel and Avicel Grades

METHOCEL™ Premium methylcellulose and hypromellose products are a broad range of water-soluble cellulose ethers. They enable pharmaceutical developers to create formulas for tablet coatings, granulation, controlled release, extrusion, molding, and for controlled viscosity in liquid formulations.

METHOCEL™ Premium Products for Pharmaceutical Applications - Hypromellose Grades

<table>
<thead>
<tr>
<th>METHOCEL™ Product</th>
<th>Chemical Type</th>
<th>Methoxyl Content, %</th>
<th>Hydroxypropoxyl Content, %</th>
<th>Viscosity of 2% solution in water, mPa·s (USP/EP/JP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>METHOCEL™ E3 Premium LV</td>
<td>Hypromellose 2910</td>
<td>28.0 - 30.0</td>
<td>7.0 - 12.0</td>
<td>2.4 - 3.6</td>
</tr>
<tr>
<td>METHOCEL™ E5 Premium LV</td>
<td>Hypromellose 2910</td>
<td>28.0 - 30.0</td>
<td>7.0 - 12.0</td>
<td>4.0 - 6.0</td>
</tr>
<tr>
<td>METHOCEL™ E6 Premium LV</td>
<td>Hypromellose 2910</td>
<td>28.0 - 30.0</td>
<td>7.0 - 12.0</td>
<td>4.8 - 7.2</td>
</tr>
<tr>
<td>METHOCEL™ E15 Premium LV</td>
<td>Hypromellose 2910</td>
<td>28.0 - 30.0</td>
<td>7.0 - 12.0</td>
<td>12 - 18</td>
</tr>
<tr>
<td>METHOCEL™ E50 Premium LV</td>
<td>Hypromellose 2910</td>
<td>28.0 - 30.0</td>
<td>7.0 - 12.0</td>
<td>40 - 60</td>
</tr>
<tr>
<td>METHOCEL™ E5M Premium</td>
<td>Hypromellose 2910</td>
<td>28.0 - 30.0</td>
<td>7.0 - 12.0</td>
<td>2663 - 4970</td>
</tr>
<tr>
<td>METHOCEL™ E10M Premium</td>
<td>Hypromellose 2910</td>
<td>28.0 - 30.0</td>
<td>7.0 - 12.0</td>
<td>9525 - 17780</td>
</tr>
<tr>
<td>METHOCEL™ E15 Premium</td>
<td>Hypromellose 2906</td>
<td>27.0 - 30.0</td>
<td>4.0 - 7.5</td>
<td>40 - 60</td>
</tr>
<tr>
<td>METHOCEL™ F50 Premium</td>
<td>Hypromellose 2906</td>
<td>27.0 - 30.0</td>
<td>4.0 - 7.5</td>
<td>2663 - 4970</td>
</tr>
<tr>
<td>METHOCEL™ F4M Premium</td>
<td>Hypromellose 2208</td>
<td>19.0 - 24.0</td>
<td>7.0 - 12.0</td>
<td>2.4 - 3.6</td>
</tr>
<tr>
<td>METHOCEL™ K100 Premium</td>
<td>Hypromellose 2208</td>
<td>19.0 - 24.0</td>
<td>7.0 - 12.0</td>
<td>80 - 120</td>
</tr>
<tr>
<td>METHOCEL™ K15M Premium</td>
<td>Hypromellose 2208</td>
<td>19.0 - 24.0</td>
<td>7.0 - 12.0</td>
<td>13275 - 24780</td>
</tr>
</tbody>
</table>

1USP XXII
2Also available in faster hydrating CR (controlled release) grade


METHOCEL™ Cellulose Ethers are the first choice for the formulation of hydrophilic matrix systems, providing a robust mechanism for the slow release of drugs from oral solid dosage forms. With a choice of viscosity grades, METHOCEL™ provides a simple solution to meet a range of drug solubility needs. Tablets are easily manufactured with existing, conventional equipment and processing methods.


METHOCEL™ Premium Products for Pharmaceutical Applications - Methylcellulose Grades

<table>
<thead>
<tr>
<th>METHOCEL™ Product</th>
<th>Chemical Type</th>
<th>Methoxyl Content, %</th>
<th>Hydroxypropoxyl Content, %</th>
<th>Viscosity of 2% solution in water, cPs (USP)</th>
<th>Viscosity of 2% solution in water, mPa·s (EP/JP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>METHOCEL™ A15 Premium LV</td>
<td>Methylcellulose, USP</td>
<td>27.5 - 31.5</td>
<td>0</td>
<td>12 - 18</td>
<td>12 - 18</td>
</tr>
<tr>
<td>METHOCEL™ A4C Premium</td>
<td>Methylcellulose, USP</td>
<td>27.5 - 31.5</td>
<td>0</td>
<td>300 - 560</td>
<td>320 - 480</td>
</tr>
<tr>
<td>METHOCEL™ A15C Premium</td>
<td>Methylcellulose, USP</td>
<td>27.5 - 31.5</td>
<td>0</td>
<td>1125 - 2100</td>
<td>1298 - 2422</td>
</tr>
<tr>
<td>METHOCEL™ A4M Premium</td>
<td>Methylcellulose, USP</td>
<td>27.5 - 31.5</td>
<td>0</td>
<td>3000 - 5600</td>
<td>2663 - 4970</td>
</tr>
</tbody>
</table>

1USP XXII

## AVICEL™ Products

<table>
<thead>
<tr>
<th>Process</th>
<th>Product Grades</th>
<th>Nominal Particle Size, µm</th>
<th>Moisture, %</th>
<th>Loose Bulk Density, g/cc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Roller Compaction</strong></td>
<td>Avicel DG</td>
<td>45</td>
<td>NMT 5.0</td>
<td>0.25 - 0.40</td>
</tr>
<tr>
<td><strong>Wet Granulation</strong></td>
<td>Avicel PH-101</td>
<td>50</td>
<td>3.0 to 5.0</td>
<td>0.26 - 0.31</td>
</tr>
<tr>
<td><strong>Direct Compression</strong></td>
<td>Avicel PH-102</td>
<td>100</td>
<td>3.0 to 5.0</td>
<td>0.28 - 0.33</td>
</tr>
<tr>
<td></td>
<td>Avicel HFE*-102</td>
<td>100</td>
<td>NMT*** 5.0</td>
<td>0.28 - 0.33</td>
</tr>
<tr>
<td><strong>Superior Compactibility</strong></td>
<td>Avicel PH-105</td>
<td>20</td>
<td>NMT 5.0</td>
<td>0.20 - 0.30</td>
</tr>
<tr>
<td><strong>Superior Flow</strong></td>
<td>Avicel PH-102 SCG**</td>
<td>150</td>
<td>3.0 to 5.0</td>
<td>0.28 - 0.34</td>
</tr>
<tr>
<td></td>
<td>Avicel PH-200</td>
<td>180</td>
<td>2.0 to 5.0</td>
<td>0.29 - 0.36</td>
</tr>
<tr>
<td><strong>High Density</strong></td>
<td>Avicel PH-301</td>
<td>50</td>
<td>3.0 to 5.0</td>
<td>0.34 - 0.45</td>
</tr>
<tr>
<td></td>
<td>Avicel PH-302</td>
<td>100</td>
<td>3.0 to 5.0</td>
<td>0.35 - 0.46</td>
</tr>
<tr>
<td><strong>Low Moisture</strong></td>
<td>Avicel PH-103</td>
<td>50</td>
<td>NMT 3</td>
<td>0.26 - 0.31</td>
</tr>
<tr>
<td></td>
<td>Avicel PH-113</td>
<td>50</td>
<td>NMT 2</td>
<td>0.27 - 0.34</td>
</tr>
<tr>
<td></td>
<td>Avicel PH-112</td>
<td>100</td>
<td>NMT 1.5</td>
<td>0.28 - 0.34</td>
</tr>
<tr>
<td></td>
<td>Avicel PH-200 LM</td>
<td>180</td>
<td>NMT 1.5</td>
<td>0.30 - 0.38</td>
</tr>
<tr>
<td><strong>Mouthfeel Improvement</strong></td>
<td>Avicel CE-15</td>
<td>75</td>
<td>NMT 8</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*High Functionality Excipient
**Special Coarse Grade
***Not More Than

Source: [http://www.fmcbiopolymer.com/Pharmaceutical/Products/Avicelorsoliddoseforms.aspx](http://www.fmcbiopolymer.com/Pharmaceutical/Products/Avicelorsoliddoseforms.aspx)
### Avicel Grade Usage Chart

<table>
<thead>
<tr>
<th>Method</th>
<th>Desirable Properties</th>
<th>Recommended Product</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Roller Compaction</strong></td>
<td>- Increase tablet hardness</td>
<td>Avicel DG in intra-granular phase No extra-granular binders required.</td>
</tr>
<tr>
<td></td>
<td>- Provide good flow and a strong ribbon during compaction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Reduce number of excipients</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Improved yields</td>
<td></td>
</tr>
<tr>
<td><strong>Direct Compression</strong></td>
<td>- Improve flow</td>
<td>Avicel PH-102 SCG</td>
</tr>
<tr>
<td></td>
<td>- Better compressibility</td>
<td>Avicel HFE-102</td>
</tr>
<tr>
<td></td>
<td>- Accomodation of moisture-sensitive actives</td>
<td>Avicel PH-200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Avicel PH-302</td>
</tr>
<tr>
<td><strong>Wet Granulation</strong></td>
<td>- Rapid, even wetting as a result of the wicking action of microcrystalline cellulose</td>
<td>Avicel PH-101</td>
</tr>
<tr>
<td></td>
<td>- Reduced sensitivity of the wet masss to over-wetting</td>
<td>Avicel PH-301</td>
</tr>
<tr>
<td></td>
<td>- Faster drying</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Fewer screen blockages or case hardenings</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Reduce dye migration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Faster disintegration</td>
<td></td>
</tr>
<tr>
<td><strong>Disintegration</strong></td>
<td>- Enhance drug dissolution by speeding tablet disintegration</td>
<td>Ac-Di-Sol</td>
</tr>
<tr>
<td></td>
<td>- Provide the highest level of disintegration force at low use levels</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Utilize dual disintegration mechanisms of wicking and swelling for more rapid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>disintegration</td>
<td></td>
</tr>
</tbody>
</table>

### Liquids and Suspensions

<table>
<thead>
<tr>
<th>Suspending Agent</th>
<th>Desirable Properties</th>
<th>Recommended Product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- Maintain suspension uniformity by preventing settling</td>
<td>Avicel RC-591</td>
</tr>
<tr>
<td></td>
<td>- Impart thixotropic viscosity profile</td>
<td>Avicel CL-611</td>
</tr>
</tbody>
</table>

### Capsule Properties

#### Empty Hard Gelatin Capsule Dimensions

<table>
<thead>
<tr>
<th>Size</th>
<th>Outer Diameter (mm)</th>
<th>Height or Locked Length (mm)</th>
<th>Actual Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Su07</td>
<td>23.4</td>
<td>88.5</td>
<td>28</td>
</tr>
<tr>
<td>7</td>
<td>23.4</td>
<td>78.0</td>
<td>24</td>
</tr>
<tr>
<td>10</td>
<td>23.4</td>
<td>64.0</td>
<td>18</td>
</tr>
<tr>
<td>11</td>
<td>20.9</td>
<td>47.5</td>
<td>10</td>
</tr>
<tr>
<td>12el</td>
<td>15.5</td>
<td>57.0</td>
<td>7.5</td>
</tr>
<tr>
<td>12</td>
<td>15.3</td>
<td>40.5</td>
<td>5</td>
</tr>
<tr>
<td>13</td>
<td>15.3</td>
<td>30.0</td>
<td>3.2</td>
</tr>
<tr>
<td>000</td>
<td>10.0</td>
<td>26.1</td>
<td>1.37</td>
</tr>
<tr>
<td>00</td>
<td>8.5</td>
<td>23.3</td>
<td>0.95</td>
</tr>
<tr>
<td>0</td>
<td>7.7</td>
<td>21.7</td>
<td>0.68</td>
</tr>
<tr>
<td>1</td>
<td>6.9</td>
<td>19.4</td>
<td>0.50</td>
</tr>
<tr>
<td>2</td>
<td>6.4</td>
<td>18.0</td>
<td>0.37</td>
</tr>
<tr>
<td>3</td>
<td>5.8</td>
<td>15.9</td>
<td>0.30</td>
</tr>
<tr>
<td>4</td>
<td>5.3</td>
<td>14.3</td>
<td>0.21</td>
</tr>
<tr>
<td>5</td>
<td>4.9</td>
<td>11.1</td>
<td>0.13</td>
</tr>
</tbody>
</table>

**CAPSULE FILL WEIGHS (MG) BASED ON SIZE AND DENSITY**

<table>
<thead>
<tr>
<th>POWDER DENSITY (g/mL)</th>
<th>CAPSULE VOLUME (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>00</td>
<td>225</td>
</tr>
<tr>
<td>0.01</td>
<td>240</td>
</tr>
<tr>
<td>0.1</td>
<td>260</td>
</tr>
<tr>
<td>0.2</td>
<td>280</td>
</tr>
<tr>
<td>0.3</td>
<td>300</td>
</tr>
<tr>
<td>0.4</td>
<td>320</td>
</tr>
<tr>
<td>0.5</td>
<td>340</td>
</tr>
<tr>
<td>0.6</td>
<td>360</td>
</tr>
<tr>
<td>0.7</td>
<td>380</td>
</tr>
<tr>
<td>0.8</td>
<td>400</td>
</tr>
<tr>
<td>0.9</td>
<td>420</td>
</tr>
<tr>
<td>1.0</td>
<td>440</td>
</tr>
<tr>
<td>1.1</td>
<td>460</td>
</tr>
<tr>
<td>1.2</td>
<td>480</td>
</tr>
<tr>
<td>1.3</td>
<td>500</td>
</tr>
<tr>
<td>1.4</td>
<td>520</td>
</tr>
<tr>
<td>1.5</td>
<td>540</td>
</tr>
</tbody>
</table>
**Working Ranges of Typical Granulating Fluids**

<table>
<thead>
<tr>
<th>Granulating Fluid</th>
<th>Typical Concentration Used (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>acacia</td>
<td>10-20</td>
</tr>
<tr>
<td>alcohol</td>
<td>(up to 100%)</td>
</tr>
<tr>
<td>cellulose derivatives</td>
<td>5-10</td>
</tr>
<tr>
<td>gelatin</td>
<td>10-20</td>
</tr>
<tr>
<td>glucose</td>
<td>25-50</td>
</tr>
<tr>
<td>polyvinylpyrrolidone</td>
<td>3-15</td>
</tr>
<tr>
<td>starch</td>
<td>5-10</td>
</tr>
<tr>
<td>sugar</td>
<td>70-85</td>
</tr>
<tr>
<td>water</td>
<td>(up to 100%)</td>
</tr>
</tbody>
</table>

**Viscosities of Typical Fluids**

<table>
<thead>
<tr>
<th>Newtonian Viscosities: Temperature (°C)</th>
<th>Viscosity (poise)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water 20</td>
<td>0.0100</td>
</tr>
<tr>
<td>Water 50</td>
<td>0.0055</td>
</tr>
<tr>
<td>Water 99</td>
<td>0.0028</td>
</tr>
<tr>
<td>Ethanol, absolute 20</td>
<td>0.0120</td>
</tr>
<tr>
<td>Ethanol, absolute 50</td>
<td>0.0070</td>
</tr>
<tr>
<td>Ethanol, 40% w/w 20</td>
<td>0.0291</td>
</tr>
<tr>
<td>Ethanol, 40% w/w 50</td>
<td>0.0113</td>
</tr>
<tr>
<td>Ethyl ether 20</td>
<td>0.0024</td>
</tr>
<tr>
<td>Glycerin, anhydrous 20</td>
<td>15.00</td>
</tr>
<tr>
<td>Glycerin, 95% w/w 20</td>
<td>5.45</td>
</tr>
<tr>
<td>Castor oil 20</td>
<td>10.3</td>
</tr>
</tbody>
</table>

**Powder Flowability Indices**

<table>
<thead>
<tr>
<th>Consolidation Index (%)</th>
<th>Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-15</td>
<td>Excellent</td>
</tr>
<tr>
<td>12-16</td>
<td>Good</td>
</tr>
<tr>
<td>*18-21</td>
<td>Fair to passable</td>
</tr>
<tr>
<td>*21-35</td>
<td>Poor</td>
</tr>
<tr>
<td>33-38</td>
<td>Very Poor</td>
</tr>
<tr>
<td>&gt;40</td>
<td>Extremely Poor</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Angle of Repose</th>
<th>Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25</td>
<td>Excellent</td>
</tr>
<tr>
<td>25-30</td>
<td>Good</td>
</tr>
<tr>
<td>*30-40</td>
<td>Passable</td>
</tr>
<tr>
<td>&gt;40</td>
<td>Very Poor</td>
</tr>
</tbody>
</table>
# Average HLB Values of Some Surface Active Agents

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Compound</th>
<th>HLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia</td>
<td>Sorbitan monooleate</td>
<td>12.0</td>
</tr>
<tr>
<td>Arlacel 80</td>
<td>Sorbitan monostearate</td>
<td>4.3</td>
</tr>
<tr>
<td>Arlacel 60</td>
<td>Sorbitan monopalmitate</td>
<td>4.7</td>
</tr>
<tr>
<td>Arlacel 40</td>
<td>Sorbitan monolaurate</td>
<td>6.7</td>
</tr>
<tr>
<td>Arlacel 20</td>
<td>Sorbitan monolaurate</td>
<td>8.6</td>
</tr>
<tr>
<td>Brij 30</td>
<td>Polyoxyllylene lauryl ether</td>
<td>9.5</td>
</tr>
<tr>
<td>Brij 35</td>
<td>Polyoxyllylene lauryl ether</td>
<td>16.9</td>
</tr>
<tr>
<td>Methocel 15 cps</td>
<td>Methylcellulose</td>
<td>10.5</td>
</tr>
<tr>
<td>Myrj 45</td>
<td>Polyoxyllylene monostearate</td>
<td>11.1</td>
</tr>
<tr>
<td>Myrj 49</td>
<td>Polyoxyllylene monostearate</td>
<td>15.0</td>
</tr>
<tr>
<td>Myrj 51</td>
<td>Polyoxyllylene monostearate</td>
<td>16.0</td>
</tr>
<tr>
<td>Myrj 52</td>
<td>Polyoxyllylene monostearate</td>
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</tr>
<tr>
<td>Myrj 53</td>
<td>Polyoxyllylene monostearate</td>
<td>17.9</td>
</tr>
<tr>
<td>PEG 400 monooleate</td>
<td>Polyoxyllylene monooleate</td>
<td>11.4</td>
</tr>
<tr>
<td>PEG 400 monostearate</td>
<td>Polyoxyllylene monostearate</td>
<td>11.6</td>
</tr>
<tr>
<td>PEG 400 monolaurate</td>
<td>Polyoxyllylene monolaurate</td>
<td>13.1</td>
</tr>
<tr>
<td>Pharmagel B</td>
<td>Gelatin</td>
<td>9.8</td>
</tr>
<tr>
<td>SDS (or SLS)</td>
<td>Sodium dodecyl sulfate (or Sodium lauryl sulfate)</td>
<td>40</td>
</tr>
<tr>
<td>Span 85</td>
<td>Sorbitan trioleate</td>
<td>1.8</td>
</tr>
<tr>
<td>Span 65</td>
<td>Sorbitan tristearate</td>
<td>2.1</td>
</tr>
<tr>
<td>Span 80</td>
<td>Sorbitan monooleate</td>
<td>4.3</td>
</tr>
<tr>
<td>Span 60</td>
<td>Sorbitan monostearate</td>
<td>4.7</td>
</tr>
<tr>
<td>Span 40</td>
<td>Sorbitan monopalmitate</td>
<td>6.7</td>
</tr>
<tr>
<td>Span 20</td>
<td>Sorbitan monolaurate</td>
<td>8.6</td>
</tr>
<tr>
<td>Tween 61</td>
<td>Polyoxyllylene sorbitan monostearate</td>
<td>9.6</td>
</tr>
<tr>
<td>Tween 81</td>
<td>Polyoxyllylene sorbitan monooleate</td>
<td>10.0</td>
</tr>
<tr>
<td>Tween 65</td>
<td>Polyoxyllylene sorbitan tristearate</td>
<td>10.5</td>
</tr>
<tr>
<td>Tween 85</td>
<td>Polyoxyllylene sorbitan trioleate</td>
<td>11.0</td>
</tr>
<tr>
<td>Tween 21</td>
<td>Polyoxyllylene sorbitan monolaurate</td>
<td>13.3</td>
</tr>
<tr>
<td>Tween 60</td>
<td>Polyoxyllylene sorbitan monostearate</td>
<td>14.9</td>
</tr>
<tr>
<td>Tween 80</td>
<td>Polyoxyllylene sorbitan monooleate</td>
<td>15.0</td>
</tr>
<tr>
<td>Tween 40</td>
<td>Polyoxyllylene sorbitan monopalmitate</td>
<td>15.6</td>
</tr>
<tr>
<td>Tween 20</td>
<td>Polyoxyllylene sorbitan monolaurate</td>
<td>16.7</td>
</tr>
</tbody>
</table>

---

**Table 22-6. Relationship between HLB Range and Surfactant Application**

<table>
<thead>
<tr>
<th>HLB RANGE</th>
<th>USE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–3</td>
<td>Antifoaming agents</td>
</tr>
<tr>
<td>4–6</td>
<td>W/O emulsifying agents</td>
</tr>
<tr>
<td>7–9</td>
<td>Wetting agents</td>
</tr>
<tr>
<td>8–18</td>
<td>O/W emulsifying agents</td>
</tr>
<tr>
<td>13–15</td>
<td>Detergents</td>
</tr>
<tr>
<td>10–18</td>
<td>Solubilizing agents</td>
</tr>
</tbody>
</table>

General Physical Properties of Spans and Tweens

Table 1.3 The general physical properties of sorbitan esters and sorbitan ester ethoxylates

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Chemical composition</th>
<th>Mineral oil solubility</th>
<th>HLB</th>
<th>Water solubility</th>
<th>Sur. tension in water (Conc = 1%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Span-20</td>
<td>Sorbitan monolaurate</td>
<td>Soluble</td>
<td>8.6</td>
<td>Insoluble</td>
<td>28</td>
</tr>
<tr>
<td>Span-40</td>
<td>Sorbitan monopalmitate</td>
<td>Insoluble</td>
<td>6.7</td>
<td>Insoluble</td>
<td>36</td>
</tr>
<tr>
<td>Span-60</td>
<td>Sorbitan monostearate</td>
<td>Insoluble</td>
<td>4.7</td>
<td>Insoluble</td>
<td>46</td>
</tr>
<tr>
<td>Span-65</td>
<td>Sorbitan tristeartate</td>
<td>Insoluble</td>
<td>2.1</td>
<td>Insoluble</td>
<td>48</td>
</tr>
<tr>
<td>Span-80</td>
<td>Sorbitan monooleate</td>
<td>Soluble</td>
<td>4.3</td>
<td>Insoluble</td>
<td>30</td>
</tr>
<tr>
<td>Span-85</td>
<td>Sorbitan trioleate</td>
<td>Soluble</td>
<td>1.8</td>
<td>Insoluble</td>
<td>32</td>
</tr>
<tr>
<td>Tween-20</td>
<td>POE-(20)-sorbitan monolaurate</td>
<td>Insoluble</td>
<td>16.7</td>
<td>Soluble</td>
<td>36</td>
</tr>
<tr>
<td>Tween-21</td>
<td>POE-(4)-sorbitan monolaurate</td>
<td>Insoluble</td>
<td>13.3</td>
<td>Dispersible</td>
<td>32</td>
</tr>
<tr>
<td>Tween-40</td>
<td>POE-(20)-sorbitan monopalmitate</td>
<td>Insoluble</td>
<td>15.6</td>
<td>Soluble</td>
<td>40</td>
</tr>
<tr>
<td>Tween-60</td>
<td>POE-(20)-sorbitan monooleate</td>
<td>Insoluble</td>
<td>14.9</td>
<td>Soluble</td>
<td>43</td>
</tr>
<tr>
<td>Tween-61</td>
<td>POE-(4)-sorbitan monooleate</td>
<td>Insoluble</td>
<td>9.6</td>
<td>Dispersible</td>
<td>38</td>
</tr>
<tr>
<td>Tween-65</td>
<td>POE-(20)-sorbitan tristeartate</td>
<td>Insoluble</td>
<td>10.5</td>
<td>Dispersible</td>
<td>31</td>
</tr>
<tr>
<td>Tween-80</td>
<td>POE-(20)-sorbitan monooleate</td>
<td>Insoluble</td>
<td>15</td>
<td>Soluble</td>
<td>41</td>
</tr>
<tr>
<td>Tween-81</td>
<td>POE-(5)-sorbitan monooleate</td>
<td>Soluble</td>
<td>10</td>
<td>Dispersible</td>
<td>38</td>
</tr>
<tr>
<td>Tween-85</td>
<td>POE-(20)-sorbitan trioleate</td>
<td>Soluble</td>
<td>11</td>
<td>Dispersible</td>
<td>42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Molecular Formula</th>
<th>Molecular Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Span 20</td>
<td>C_{18}H_{34}O_{6}</td>
<td><img src="image" alt="Span 20 Structure" /></td>
</tr>
<tr>
<td>Span 40</td>
<td>C_{22}H_{42}O_{6}</td>
<td><img src="image" alt="Span 40 Structure" /></td>
</tr>
<tr>
<td>Span 60</td>
<td>C_{24}H_{46}O_{6}</td>
<td><img src="image" alt="Span 60 Structure" /></td>
</tr>
<tr>
<td>Span 80</td>
<td>C_{24}H_{46}O_{6}</td>
<td><img src="image" alt="Span 80 Structure" /></td>
</tr>
<tr>
<td>Tween 20</td>
<td>C_{18}H_{34}O_{6}(C_{2}H_{4}O)_{20}</td>
<td><img src="image" alt="Tween 20 Structure" /></td>
</tr>
<tr>
<td>Tween 40</td>
<td>C_{22}H_{42}O_{6}(C_{2}H_{4}O)_{20}</td>
<td><img src="image" alt="Tween 40 Structure" /></td>
</tr>
<tr>
<td>Tween 60</td>
<td>C_{24}H_{46}O_{6}(C_{2}H_{4}O)_{20}</td>
<td><img src="image" alt="Tween 60 Structure" /></td>
</tr>
<tr>
<td>Tween 80</td>
<td>C_{24}H_{46}O_{6}(C_{2}H_{4}O)_{20}</td>
<td><img src="image" alt="Tween 80 Structure" /></td>
</tr>
</tbody>
</table>
## HLB Requirement for Common Oil Components

<table>
<thead>
<tr>
<th>Oil Component</th>
<th>Required HLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almond Oil</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Apricot Kernal Oil</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Avocado (Persea Gratissima) Oil</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Beeswax</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>C12-15 Alkyl Benzoate</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Canola Oil</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Caprylic/Capric Triglyceride</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Carinauba (Copernicia Cerifera) Wax</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Carrot (Daucus Carota Sativa) Root Extract</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Carrot (Daucus Carota Sativa) Seed Oil</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Castor (Ricinus Communis) Oil</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>Ceresin</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Cetearyl Alcohol</td>
<td>15.5 ± 1</td>
</tr>
<tr>
<td>Cetyl Alcohol</td>
<td>15.5 ± 1</td>
</tr>
<tr>
<td>Cetyl Esters</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Cetyl Palmitate</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Cocoa (Theobroma Cacao) Butter</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Coconut (Cocos Nucifera) Oil</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Cyclomethicone</td>
<td>7.5 ± 1</td>
</tr>
<tr>
<td>Disopropyl Adipate</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>Dimethicone</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Isopropyl Myristate</td>
<td>11.5 ± 1</td>
</tr>
<tr>
<td>Isopropyl Palmitate</td>
<td>11.5 ± 1</td>
</tr>
<tr>
<td>Jojoba (Buxus Chinensis) Oil</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Kukui Nut (Aleurites Moluccana Seed) Oil</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Lanolin</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Macadamia (Macadamia Ternifolia) Nut Oil</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Mango (Mangifera Indica) Seed Butter</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Mango (Mangifera Indica) Seed Oil</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Mineral Oil</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Myristyl Myristate</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Olive (Olea Europaea) Oil</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Petrolatum</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Retinyl Palmitate</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Safflower (Carthamus Tinctorius) Oil</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Sesame (Sesamum Indicum) Oil</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Shea Butter (Butyrospermum Parkii)</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Soybean (Glycine Soja) Oil</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>Stearyl Alcohol</td>
<td>15.5 ± 1</td>
</tr>
<tr>
<td>Sunflower (Helianthus Annuus) Seed Oil</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Sweet Almond (Prunus Amygdalus Dulcis) Oil</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Tocopherol</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Wheat Germ (Trictum Vulgare) Oil</td>
<td>7 ± 1</td>
</tr>
</tbody>
</table>

**Buffer Solution Preparation: Polyprotic Acids and Bases**

These buffer problems should be looked upon exactly as those for monoprotic acids and bases. For simplicity, assume that each ionization step goes to completion before the next ionization commences. In these cases, choose the salt/acid pair with $pK_a$ close to the desired pH of the buffer. Working buffer concentration are typically on the order of 0.1 M.

For example, citric acid has the following $pK_a$ values:

- $pK_{a,1} = 3.13$
- $pK_{a,2} = 4.76$
- $pK_{a,3} = 6.40$

Looking only at the second $pK_a$:

$$\text{HO}_3\text{C}-\text{C(=O)}-\text{OH} \quad \text{p}K_{a,2} \quad \text{HO}_3\text{C}-\text{C(=O)}\text{O}^- + \text{H}^+$$

The buffer capacity is greatest for ± 1.0 pH around the $pK_a$ value. Thus, the salt/acid ratio from the Henderson-Hasselbach equation can be calculated:

$$\text{pH} = pK_a + \log\left(\frac{\text{[conjugate base]}}{\text{[acid]}}\right)$$

In order to have a buffer solution of pH 4.78, one would need an equimolar concentration of the above salt/acid couple. To generate the components of this couple, the addition of HCl to the sodium citrate, or NaOH to the citric acid would be required.

**Example:**

Say we want a 0.1 M, pH 5 citric buffer and decided to add NaOH to citric acid, the calculation is as follows:

1. Citric acid is fully protonated (i.e., 3 H$^+$), and we wish to prepare a buffer concentration of 0.1 M, then 0.1 M of NaOH is required for the first ionization.

2. From the above equation, the salt/acid ratio required is:
   
   \[ 5.0 = 4.78 + \log \left(\frac{\text{[salt]}}{\text{[acid]}}\right) \]
   
   $\frac{\text{[salt]}}{\text{[acid]}} = 1.66$

   i.e., $[\text{salt}] = 1.66 [\text{acid}]$

3. Since we want $[\text{salt}] + [\text{acid}] = 0.1$ M, there are 2 equations and 2 unknowns.
   
   therefore, $1.66 [\text{acid}] + [\text{acid}] = 0.1$ M
   
   $[\text{acid}] = 0.038$ M
   
   $[\text{salt}] = 1.66 \times 0.038 = 0.063$ M

4. Therefore, the total amount of NaOH required is $0.1 + 0.063 = 0.163$ M.
### Dissociation Constants of Acids in Aqueous Solutions at 25°C

<table>
<thead>
<tr>
<th>Substance</th>
<th>$K_a$</th>
<th>$pK_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>$1.75 \times 10^{-5}$</td>
<td>4.76</td>
</tr>
<tr>
<td>Acetylsalicylic</td>
<td>$3.27 \times 10^{-4}$</td>
<td>3.49</td>
</tr>
<tr>
<td>Aluminum Hydroxide</td>
<td>$6.3 \times 10^{-13}$</td>
<td>12.20</td>
</tr>
<tr>
<td>Benzoic</td>
<td>$6.3 \times 10^{-5}$</td>
<td>4.2</td>
</tr>
<tr>
<td>o-Chlorobenzoic Acid</td>
<td>$1.20 \times 10^{-3}$</td>
<td>2.92</td>
</tr>
<tr>
<td>Cinnamic (Trans)</td>
<td>$1.32 \times 10^{-4}$</td>
<td>3.88</td>
</tr>
<tr>
<td>Formic</td>
<td>$1.76 \times 10^{-4}$</td>
<td>3.75</td>
</tr>
<tr>
<td>Fumaric</td>
<td>$9.3 \times 10^{-4}$</td>
<td>3.03</td>
</tr>
<tr>
<td>Glycine</td>
<td>$1.67 \times 10^{-10}$</td>
<td>9.78</td>
</tr>
<tr>
<td>Lactic</td>
<td>$1.39 \times 10^{-4}$</td>
<td>3.86</td>
</tr>
<tr>
<td>Maleic</td>
<td>$1.0 \times 10^{-2}$, $5.5 \times 10^{-7}$</td>
<td>2.00, 6.26</td>
</tr>
<tr>
<td>Malic</td>
<td>$4 \times 10^{-4}$, $9 \times 10^{-6}$</td>
<td>3.4, 5.05</td>
</tr>
<tr>
<td>Phenol</td>
<td>$1.3 \times 10^{-10}$</td>
<td>9.89</td>
</tr>
<tr>
<td>Phosphoric</td>
<td>$7.5 \times 10^{-3}$, $6.2 \times 10^{-8}$</td>
<td>2.12, 7.21</td>
</tr>
<tr>
<td></td>
<td>$4.8 \times 10^{-13}$</td>
<td>12.32</td>
</tr>
<tr>
<td>Salicylic</td>
<td>$1.06 \times 10^{-3}$</td>
<td>2.97</td>
</tr>
<tr>
<td></td>
<td>$3.6 \times 10^{-14}$</td>
<td>13.44</td>
</tr>
<tr>
<td>Sulfanilic</td>
<td>$6.5 \times 10^{-4}$</td>
<td>3.19</td>
</tr>
<tr>
<td>Tartaric</td>
<td>$9.6 \times 10^{-4}$</td>
<td>3.02</td>
</tr>
<tr>
<td></td>
<td>$2.9 \times 10^{-5}$</td>
<td>4.54</td>
</tr>
<tr>
<td>Valeric</td>
<td>$1.56 \times 10^{-5}$</td>
<td>4.81</td>
</tr>
</tbody>
</table>

### Dissociation Constants of Bases in Aqueous Solutions at 25°C

<table>
<thead>
<tr>
<th>Substance</th>
<th>$K_b$</th>
<th>$pK_b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium Hydroxide</td>
<td>$1.75 \times 10^{-5}$</td>
<td>4.74</td>
</tr>
<tr>
<td>Atropine</td>
<td>$4.47 \times 10^{-5}$</td>
<td>4.35</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>$6 \times 10^{-12}$</td>
<td>11.22</td>
</tr>
<tr>
<td>Caffeine</td>
<td>$4.1 \times 10^{-14}$</td>
<td>13.39</td>
</tr>
<tr>
<td>Codeine</td>
<td>$9 \times 10^{-7}$</td>
<td>6.05</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>$2.77 \times 10^{-5}$</td>
<td>4.56</td>
</tr>
<tr>
<td>Hydroquinine</td>
<td>$4.7 \times 10^{-6}$</td>
<td>5.33</td>
</tr>
<tr>
<td>Nicotine</td>
<td>$7 \times 10^{-7}$</td>
<td>6.15</td>
</tr>
<tr>
<td>Procaaine</td>
<td>$7 \times 10^{-6}$</td>
<td>5.15</td>
</tr>
<tr>
<td>Quinine (first)</td>
<td>$1 \times 10^{-6}$</td>
<td>6.0</td>
</tr>
<tr>
<td>Quinine (second)</td>
<td>$1.3 \times 10^{-10}$</td>
<td>9.89</td>
</tr>
<tr>
<td>Urea</td>
<td>$1.5 \times 10^{-14}$</td>
<td>13.82</td>
</tr>
</tbody>
</table>

(p$K_a + pK_b = 14$)
**Sorensen Phosphate Buffers**

Prepare M/15 solutions and mix appropriate quantities to make 100 mL of buffer at the desired pH.

<table>
<thead>
<tr>
<th>pH</th>
<th>mL M/15 Na₂HPO₄</th>
<th>mL M/15 KH₂PO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.8</td>
<td>7.8</td>
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**Fundamental Lab Calculations**

**Preparing a Known Molar Concentration**

To prepare 500 mL of a 0.2 M solution of dibasic sodium phosphate, we first need to know the number of moles to add:

\[ n = C \times V \]

\[ n = 0.2 \text{ mol/L} \times 500 \text{ mL} \times \left( \frac{1 \text{ L}}{1000 \text{ mL}} \right) = 0.1 \text{ mol} \]

Unfortunately the lab scales measure grams, not moles of substance. The molecular weight of dibasic sodium phosphate is 268.07 g/mol. In order to convert moles into grams, a compound’s molecular weight is used in the following equation:

\[ m = n \times MW \]

\[ m = 0.1 \text{ mol} \times 268.07 \text{ g/mol} \]

\[ m = 26.81 \text{ g} \]

In order to prepare 500 mL of a 0.2 M dibasic sodium phosphate solution, dissolve 26.81 g into a 500 mL volumetric flask, then dilute to the mark.

**Weight-Volume Percent (%w/v)**

Usually when a preparation is liquid and a percentage is specified, by default it is assumed that it
is percent weight-volume (%w/v). Weight volume percent is one of the easiest concentration units to work with. It is equivalent to saying “g/100 mL total solution”. Note, this is different than saying g / 100 mL solution added. The total solution includes the volume of the solute added (thus effects like electrostriction are taken into account). The measure %w/v is great to work with, because you don’t have to look up the molecular weight of the solute to perform the dilution. All you have to do is multiply the final desired volume (in mL) by the %w/v, and your answer will be in grams.

For example, to prepare a 2% w/v solution of any solute in a volume of 200 mL:

\[ m = CV = \frac{2 \text{ g}}{100 \text{ mL}} \times 200 \text{ mL} = 2 \% \times 200 \text{ mL} \]

\[ m = 4 \text{ g} \]

Weigh out 4 g into a 200 mL volumetric flask, and dilute to the mark.

**Weight-Weight Percent (%w/w)**

When a preparation is solid and a percentage is specified, by default it is assumed that it is percent weight-weight (%w/w). Similar to %w/v, it means “g/100g”. The mathematics are similar. To prepare a 2% w/w mixture of any compound for a total mass of 200 g:

\[ m = CV = 2\% \times 200 \text{ g} \]

\[ m = 4 \text{ g} \]

Weigh out 4 g of the compound to be mixed with the remaining 196 g of remaining components. Note that as long as you keep the units consistent, they need not be in grams.

**Dilution Equation**

Although fundamental, the dilution equation is easy to forget, especially if you haven’t been in a lab in a long time. The equation is really just a mass balance. It states that although the concentration will change when you dilute, the mass of the solute added will remain constant:

\[
C_1 = 1 \text{ M} \\
V_1 = ? \\

C_2 = 2 \text{ mM} \\
V_2 = 100 \text{ mL}
\]

The most common problem in a wet lab is usually, how much of Solution 1 do I take to make Solution 2?


The equation used is:

\[
\begin{align*}
(1) & \quad m_1 = m_2 \\
(2) & \quad C_1 V_1 = C_2 V_2 \\
(3) & \quad V_1 = V_2 \times \left( \frac{C_2}{C_1} \right)
\end{align*}
\]

For this example, a 1 M stock solution is to be diluted to a 2 mM solution. How much of that solution you make is up to you, however, it will depend on:

14. How much of Solution 2 will you need? This will depend on the lab.
15. How much of Solution 2 can you make? This will depend on the volume of Solution 1.
16. Is the resulting dilution feasible/realistic/doable given the equipment you have?

Let’s say we need 100 mL of Solution 2, as the example states. We use Equation (3), and calculate the volume of \( V_1 \) required:

\[
V_1 = V_2 \times \left( \frac{C_2}{C_1} \right)
\]

Let’s try making a 100 mL at \( C = 0.1 \text{ M} \), first:

\[
\begin{align*}
V_1 &= 100 \text{ mL} \times \left( \frac{0.1 \text{ M}}{1 \text{ M}} \right) \\
V_1 &= 10 \text{ mL}
\end{align*}
\]

10 mL is no problem with the equipment you have. Take 10 mL of Solution 1 and dilute it to 100 mL to get a 0.1 M solution. Now for the second step:

\[
\begin{align*}
V_1 &= 100 \text{ mL} \times \left( \frac{1 \text{ M}}{1000 \text{ mM}} \right) \\
V_1 &= 0.2 \text{ mL}
\end{align*}
\]

Now take 0.2 mL of Solution 1 and pour into a volumetric flask, then dilute to 100 mL.

The problem is that you look around the lab, and realize the smallest bulb pipette you can find is 1 mL. Here is where dilution in series comes in handy. You can make an intermediate concentration, so that you can use the equipment you have and still get reasonably accurate results. Let’s try making a 100 mL at \( C = 0.1 \text{ M} \), first:

\[
\begin{align*}
V_1 &= 100 \text{ mL} \times \left( \frac{0.1 \text{ M}}{1 \text{ M}} \right) \\
V_1 &= 10 \text{ mL}
\end{align*}
\]

10 mL is no problem with the equipment you have. Take 10 mL of Solution 1 and dilute it to 100 mL to get a 0.1 M solution. Now for the second step:

\[
\begin{align*}
V_1 &= 100 \text{ mL} \times \left( \frac{1 \text{ M}}{1000 \text{ mM}} \right) \\
V_1 &= 2 \text{ mL}
\end{align*}
\]

Now take 2 mL of the 0.1 M solution, add it to a 100 mL volumetric flask, then dilute to the mark.
**pKa and Intrinsic Solubility**

It is important to know the aqueous solubility of a new drug substance. The solubility of a drug substance must be improved if it is less than the required concentration necessary for the recommended dose. This can be done by altering the pH of the delivery vehicle, using co-solvents (e.g. alcohols, propylene glycol, glycerin, sorbitol, and polyethylene glycols), using surfactants to help solubilize the drug, or re-crystallizing the drug into a different salt form (e.g. acetate, citrate, hydrochloride, or sulfate for anions; sodium or calcium salts for cations).

Since many chemical substances of pharmaceutical interest are weak acids and bases, it is important for the pharmacists to be aware of various fundamental physicochemical properties of such substances to fully appreciate their behaviours under the diverse conditions of storage, compounding, administration, and absorption. Knowledge of the pK\(_a\) and intrinsic solubility (solubility of the non-ionized form of the drug) for a weakly acidic or basic drug will allow a better understanding and prediction of the performance and stability of a pertinent pharmaceutical preparation.

Knowing the pK\(_a\) and intrinsic solubility of the drug in solution will help us to understand how solubility changes with pH. Typically, the pK\(_a\) may be determined in aqueous solution using pH titration. However, how can the pK\(_a\) of a drug be determined if the drug is sparingly soluble in water? One option is to determine the pK\(_a\) of the drug at different concentrations of co-solvents. We can then find out what the pK\(_a\) would be in water alone, by calculating the Y-intercept of a graph of pK\(_a\) vs. % co-solvent. This is a valid approach provided the graph is linear. This method is an adaptation of the Yasuda-Sheldovskiy Extrapolation. Computer spreadsheet programs such as alcohol.xls found on the laboratory website may be used to judge the linearity of the data, and calculate the Y intercept (pK\(_a\) in water alone).

The following relationship describes the relationship between solubility, pH, and pK\(_a\):

\[
\log \left( \frac{S_T - S_0}{S_0} \right) = \text{pH} - \text{pK}_a \quad (\text{for weak acids})
\]

The following is a discussion of the derivation of this equation, and will help in answering the discussion questions.

An acid will dissociate in water to a conjugate base and proton. Consequently, acids are typically thought of as proton donors:

\[
\begin{align*}
\text{HA} & \leftrightarrow \text{H}^+ + \text{A}^- \\
\text{Acid} & \quad \text{Proton} \quad \text{Conjugate Base}
\end{align*}
\]

K\(_a\) is the equilibrium constant that determines the extent that the acid will dissociate in water:

\[
(2) \quad K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}
\]

Recall that the pH of a solution in water is the negative log of the concentration of hydrogen ions, and is a more convenient way to express tiny concentrations. Similarly, the pK\(_a\) is also the negative log of the equilibrium constant K\(_a\):

\[
(3) \quad \text{pH} = -\log[\text{H}^+], \quad \text{or alternatively,} \quad 10^{-\text{pH}} = [\text{H}^+]
\]

\[
(4) \quad \text{pK}_a = -\log(K_a), \quad \text{or alternatively,} \quad 10^{-\text{pK}_a} = K_a
\]
By substituting Equations (3) and (4) into Equation (2), we can derive the Henderson-Hasselbalch equation:

\[
10^{-pK_a} = \frac{10^{-pH}[A^-]}{[HA]}
\]

\[
\frac{[HA]}{[A^-]} = 10^{pK_a-pH}
\]

According to the Henderson-Hasselbalch buffer relationship, pH, pK_a, and the buffer component concentrations for a weak acid are related as follows:

\[
\frac{[\text{acid}]}{[\text{base}]} = 10^{pK_a-pH}
\]

Here, the ‘acid’ is the proton donor (HA), and the ‘base’ is the conjugate base (A^-) in Equation (1). This is a very convenient form of the equation, because it allows us to see the following:

If the pK_a is greater than the pH, there will be more of the acid (or conjugate acid) form in the solution.
If the pK_a is equal to the pH, there will be an equal amount of acid and base in the solution.
If the pK_a is less than the pH, there will be more of the base (or conjugate base) form in the solution.

A strong acid is defined as one that will dissociate completely. Consequently, the lower the pK_a of the acid, the stronger the acid.

The same scheme can be re-written to describe the reaction of a base with water, to form its conjugate acid:

\[
K_b \quad \text{[B] + H}_2\text{O } \leftrightarrow \text{[B-H]^+} + \text{[OH^-]}
\]

Bases are thought of as proton acceptors. A similar derivation can be made for the Henderson-Hasselbalch equation of a weak base; however, the equilibrium constants for bases are now more commonly reported using K_a, which allows Equation (8) to be used for bases as well. We can start with the Equilibrium expression for Equation (9), and then substitute the following identities in order to obtain Equation (8).

\[
pOH = -\log[OH^-]; \quad pOH = 14 - pH;
pK_b = -\log[K_b]; \quad pK_a = 14 - pK_b
\]

Try it out for yourself. This saves us having to remember two sets of Henderson-Hasselbalch equations. If the pK_a of a base is greater than the pH, there will be more conjugate acid. It need only be remembered that [B-H^+] is the concentration of conjugate acid and [B] is the concentration of base. The higher the pK_a of a base, the stronger the base.

By using the appropriate experimental conditions, the pK_a of a drug may be determined with a pH meter. A titration with either strong acid or strong base should yield a flat region as the pH is adjusted. The centre of this region is where the drug is acting like a buffer – because there are
both acid and basic forms of the drug present. Baselines can be fit to determine the midpoint of the flat region, or alternately, a derivative of this curve (dV/dpH) will provide a maximum at the pKₐ.

**Calculation of pHₚ**

To prepare a solution of weak electrolyte, the formulator would usually use the salt form of the drug, such as the sodium or hydrochloride salt. However, the addition of other ingredients may alter the final pH of the solution, causing the active ingredient to precipitate from the solution. To prevent unwanted precipitation, the pH of the solution should be buffered so that the drug remains dissolved. A calculation of pHₚ, the pH below which a weak acid will precipitate from the solution or above which a weak base will precipitate from the solution, must be made and the pH of the solution adjusted accordingly. How is this pH calculated?

We start with an expression of total solubility. The total solubility ($S_T$) of a weak acid is the summation of the un-ionized form, [HA], and the ionized form, [A⁻]:

$$S_T = [HA] + [A^-] \quad (11)$$

We would like to derive a relationship between total solubility, pH, and pKₐ. We can re-arrange Equation (2) to solve for [A⁻]:

$$[A^-] = \frac{K_a[HA]}{[H^+]} \quad (12)$$

Now substitute Equation (12) into Equation (11):

$$S_T = [HA] + \frac{K_a[HA]}{[H^+]} \quad (13)$$

$$S_T = [HA]\left(1 + \frac{K_a}{[H^+]}\right) \quad (14)$$

Using the identities in (3) and (4),
(15) \[ S_T = [HA] \left( 1 + \frac{10^{-pK_a}}{10^{-pH}} \right) \]

(16) \[ S_T = [HA] \left( 1 + 10^{pH-pK_a} \right) \]

We define \( S_0 \) as the **intrinsic solubility** of the acid. This is, quite literally, the solubility limit of the drug if it were completely in the un-ionized \((HA)\) form. Therefore, Equation (16) becomes:

(17) \[ S_T = S_0 \left( 1 + 10^{pH-pK_a} \right) \]

Finally, we can re-arrange this equation into a form similar to the Henderson-Hassellbalch equation:

(18) \[ \log \left( \frac{S_T - S_0}{S_0} \right) = pH - pK_a \] (for weak acids)

What does this mean for a weak acid? Let’s take a step backwards. Water is polar, and would form hydrogen bonds with a molecule that is ionized. So, it makes sense that the charged form of a drug \((A^-)\) will be more soluble than the un-ionized form \((HA)\). If the pH of the solution keeps the drug un-ionized, the total solubility will be equal to the intrinsic solubility. As the pH of a solution of weak acid increases, more and more of the drug will be in the ionized form. From Equation (17), we can see that when \( pH = pK_a \), the total solubility of the drug should be double the intrinsic solubility, because:

(19) \[ S_T = S_0 \left( 1 + 10^0 \right) = 2 \times S_0 \]

When the pH rises above the \( pK_a \), Equation (17) implies that the solubility keeps increasing without limit. In reality this does not occur, because eventually there are not enough counterions in solution to support more ions dissolving.

The whole concept can sound rather confusing, but becomes simple when you think of it in terms of **percent of drug in ionized form**. Here is an example of the solubility of a weak acid \((pK_a = 4, \text{ intrinsic solubility } = 0.1 \text{ mg/mL})\) at different pH values, and a corresponding graph of the percent of drug ionized:

**Weak Acid:**
- **Total Solubility vs. pH**
- **% Ionized vs. pH**

The left graph was calculated using Equation (17). Upon visual inspection, it becomes evident that when the pH dips below the \( pK_a \), the acid is mostly un-ionized (right panel), and the solubility reduces asymptotically to the intrinsic solubility (left panel). So if you have a saturated solution of this drug at \( pH 5 \) and the pH drops, you can expect precipitation.
A similar derivation can be performed for a weak base, yielding the equation:

\[
\log \left( \frac{S_T - S_0}{S_0} \right) = pK_a - pH \quad \text{(for weak bases)}
\]

A weak base behaves the opposite way. The base will be in the ionic form when the pH is less than the pK\(_a\). Therefore, the total solubility of a weak base increases as pH decreases. Here is an example of the solubility of a weak base (pK\(_a\) = 8, intrinsic solubility = 0.1 mg/mL) at different pH values, and a corresponding graph of the percent of drug ionized:

In summary, a weak acid will be more soluble at a high pH, and a weak base will be more soluble at a low pH. You can see that a small shift in pH can have a drastic effect, particularly when the pH is close to the pK\(_a\). Equations (18) and (20) may be used to calculate pH\(_p\), the pH below which a solution of a weak acid is likely to precipitate, and above which a saturated solution of a weak base is likely to precipitate. Simply substitute the drug concentration of your solution as “S\(_T\)” into the equation, and calculate the pH at which this concentration is the total solubility limit.

- A saturated solution of a weak acid will precipitate if the pH falls below the pH\(_p\).
- A saturated solution of a weak base will precipitate if the pH rises above the pH\(_p\).

**pK\(_a\) and pH\(_p\) Sample Questions**

1. Phenobarbital is a weak acid. It has a pK\(_a\) of 7.41 and an intrinsic solubility of 1 g in 987 mL of water at 25 °C. You have to formulate a 5 mg/mL solution. Calculate what pH will be required to solubilize the drug.

2. Calculate the pH\(_p\) of a 10 mg/mL phenobarbital sodium solution. What would happen if while in storage, the pH of the solution rises to 9? How about if the pH falls to 7?

3. What is the pH\(_p\) of a 7 mg/mL dobutamine hydrochloride solution? Dobutamine increases cardiac output during short-term use. Its pK\(_a\) and its intrinsic solubility are 9.4 and 1 mg/mL, respectively. What happens if the pH of the solution is adjusted to 10.2?

4. Derive the formula for the fraction of drug ionized in solution if the drug is a weak acid, given the pK\(_a\) and of the drug and the pH of the solution.

**Hint 1:** Fraction of drug ionized = \( \frac{[A^-]}{[HA]+[A^-]} \)
Hint 2: For a weak acid, \[ \frac{[HA]}{[A^-]} = 10^{pK_a - pH} \]

Hint 3: There should not be any concentrations in the final formula.

5. If a weak base \((pK_a = 8.5)\) is in a solution buffered at pH 8.5, what proportion of the drug will be in the ionized form?

6. Does a weak base precipitate above or below the pH of the solution?

Excerpt from USP 795:

**Compounded Preparations**

The term compounded preparations includes the terms compounded dosage forms, compounded drugs, and compounded formulations, and means finished forms that are prepared by or under the direct supervision of a licensed compounding pharmacist.

When controlled substances are used, check with state and federal authorities concerning their policies. Unless otherwise indicated or appropriate, compounding preparations are to be prepared to ensure that each preparation shall contain not less than 90.0% and not more than 110.0% of the theoretically calculated and labeled quantity of active ingredient per unit weight or volume and not less than 90.0% and not more than 110.0% of the theoretically calculated weight or volume per unit of the preparation. Compounded preparations include, but are not restricted to, the following pharmaceutical dosage forms described under Pharmaceutical Dosage Forms (1151).

Capsules, Powders, Lozenges, and Tablets

Source: [http://www.pharmacopeia.cn/v29240/usp29nf24s0_c795.html](http://www.pharmacopeia.cn/v29240/usp29nf24s0_c795.html)

**Recommended Maximum Beyond-Use Dates (BUDs) — Non-Sterile Compounded Preparations**

In the absence of stability information that is applicable to a specific drug and preparation, the following table presents maximum BUDs recommended for nonsterile compounded drug preparations that are packaged in tight, light-resistant containers and stored at controlled room temperature, unless otherwise indicated: (USP 795)

<table>
<thead>
<tr>
<th>Non-Sterile Preparation</th>
<th>Beyond-Use Date (BUD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-aqueous Formulations (such as ointments, suppositories, troches, and others where no water is contained)</td>
<td>Not later than the time remaining until the earliest expiration date of any ingredient or 6 months, whichever is earlier</td>
</tr>
<tr>
<td>Water-containing Oral Formulations</td>
<td>Not later than 14 days when stored at controlled cold temperatures</td>
</tr>
<tr>
<td>Water-containing Topical/Dermal and Mucosal Liquid and Semisolid Formulations (such as preparations for topical application, like creams, gels, ointments, etc.)</td>
<td>Not later than 30 days</td>
</tr>
</tbody>
</table>


5.0 Policy Statement
This policy document is intended to embody the following guiding principles (key concepts are shown in bold):

**General Guiding Principles**
- Compounding must be a legitimate part of the practice of regulated healthcare professionals and must not be used as a means to bypass the federal drug review and approval system.
- All drug compounding and manufacturing activities performed are to be regulated and fall under either the federal or the provincial/territorial jurisdiction.
- The distinguishing between compounding and manufacturing activities is made on a case-by-case basis.

5.1 Compounding
Factors to be considered when assessing whether an activity is compounding:

a) Healthcare professionals who provide compounding related services and products to patients/clients must be able to demonstrate that a *patient-healthcare professional relationship* exists.

b) Activity is *regulated* and facility may be inspected by provincial/territorial regulatory authorities.

c) It is expected that healthcare professionals who compound products will have appropriate *risk management* processes in place to manage risks associated with the compounded product and the workplace (facilities, safety etc.), in line with the standards set by their provincial/territorial regulatory bodies (for example but not limited to the toxicology, pharmacology, therapeutic value, stability, adverse reactions, labelling requirements etc. of the compounded product).

d) A pharmacy may prepare drugs in very *limited quantities*, in anticipation of a prescription. For the purpose of this Policy, preparation involves compounding or repackaging of multiple units, not for immediate use, in a single process, by the same operator in accordance with a standardized batch preparation procedure.

e) Compounding should only be done if there is a *therapeutic need* or *lack of product* availability and should not be done solely for economic reasons for the healthcare professionals.

f) The compounded product must provide a *customized therapeutic solution* to improve patient care without duplicating an approved drug product.

g) When there is a *shortage or no supply of a commercially* available product and the healthcare professional has determined a medical need for this product, the product may be compounded during the period of shortage or no supply only.

h) Drugs should not be compounded in order to be sold to *third parties* who will in turn sell/deliver to patients outside of their defined patient-healthcare professional relationship (see definition of “sell”). Pharmacists that do not provide specific compounding services may contract this activity to another pharmacist who provides this type of specific compounding service.

i) Compounding of *clinical trial drugs* is only permitted if this activity is authorized in the clinical trial application or experimental or investigational authorization.

j) Product should be produced from an *authorized drug* or Active Pharmaceutical Ingredient (API) used in an authorized drug for use in Canada or listed in a *recognized Pharmacopoeia* (USP, PhEur, Phi, BP, CF, NF, Codex - Schedule B Food and Drugs Act.)

k) Those engaged in sterile compounding should be knowledgeable and obtain specialized technical training in this area (The Canadian Society of Hospital Pharmacists as well as United States Pharmacopoeia (USP) have developed guidelines for the preparation of sterile preparations. Compounding of *sterile products* is only permitted in hospitals or other practice settings where carefully established standards for the operation of clean rooms and the preparation of sterile products are in place and documented, in accordance with a recognized source. The products are dispensed directly to patients or to those who administer to patients, and are operating within a demonstrated patient-healthcare professional relationship. Pharmacists may delegate some of the compounding responsibilities to pharmacy technicians if they are adequately trained in compounding sterile products or if the provincial/territorial laws authorize it.

l) Pharmacists in hospitals providing compounding *services to other hospitals* should be within the same province, and operate under the same hospital management board (ie. inter-hospital transfer, where the hospital may be composed of several facilities at different locations).

m) The compounded product must comply with all relevant sections of the *Food and Drugs Act* including sections 3 - Prohibited advertising; 8 - Prohibited sales of drugs; 9 - Deception regarding drugs; and 11 - Unsanitary manufacture of drug.

n) The expiration date of the compounded product is based on known stability data. If stability data is not available, the expiration date should be short, usually limited to the duration of the prescription or use.
Compounding Recipes

20 mg Benzocaine Lollipops

Children find the concept of lozenges difficult, as their cue for candy is to chew and swallow. Lollipops offer a favourable alternative that can be individually flavoured to their liking. This improves both compliance and effectiveness of the topical delivery of anesthetics to the buccal cavity. Drugs may also be delivered systemically in the same manner, provided the drug in question can withstand exposure to heat in the compounding process.

In this procedure the lollipop mold is first calibrated with the base. Then, 10 x 20 mg benzocaine lollipops are formulated in order to compound 6 lollipops (the extra is compounded to account for losses).

**Sorbitol – PEG Lollipop Base**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Weight Percent (%w/w)</th>
<th>Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorbitol (Powder), NF</td>
<td>64.7 %w/w</td>
<td></td>
</tr>
<tr>
<td>Polyethylene Glycol 3350, NF</td>
<td>32.3 %w/w</td>
<td></td>
</tr>
<tr>
<td>Silica Gel (Micronized)</td>
<td>2.0 %w/w</td>
<td></td>
</tr>
<tr>
<td>Total amount of flavourant(s) (target ~1-3% for water based, ~0.1% for oil based)</td>
<td>1.0 %w/w</td>
<td></td>
</tr>
<tr>
<td>Colourant of Choice</td>
<td>1-2 drops per batch</td>
<td>(trace)</td>
</tr>
<tr>
<td>Total:</td>
<td>100%w/w</td>
<td>90.0 g</td>
</tr>
</tbody>
</table>

In this procedure, the weight of the drug is small compared to the overall weight of the lollipop (<0.2%). You will be calibrating the mold with an excess amount of empty vehicle (90 g), calculating the batch mass of benzocaine required, and then scaling your final batch to compound 10 x 20 mg benzocaine lollipops. A total of 10 is planned in order to account for breakage and losses, with a target of 6 perfect lollipops.

**Mold Calibration**

1. Set a 600 mL beaker directly on a hot plate, set to medium. Set up a thermometer inside the beaker, on a retort stand using a vinyl retort clamp.

2. Add the polyethylene glycol 3350 to the 600 mL beaker and stir with a glass rod until completely melted. Add the sorbitol powder, and continuously mix while maintaining a temperature of 100-110°C to form a homogeneous liquid-like dispersion. The mixture will not become clear, but should be smooth.

**NOTE:** Don’t overheat the mixture, or it will separate into 2 phases. If this happens, remove the mixture from the hot plate and mix vigorously until one phase is obtained.

3. Add the silica gel, colouring, and flavouring to Step 2. Continuously mix until a homogeneous liquid-like dispersion is obtained. Remove the beaker from the hot plate and continue monitoring the temperature.
4. Holding the mold at arm’s length, prepare the lollipop molds by lubricating with a **light** coating of non-stick cooking spray. There should not be enough oil to pool in the mold cavity. A thin layer is all that is required. Insert the lollipop sticks into the empty molds.

5. Stir the mixture until the vehicle begins to thicken (~90°C) and appears uniform. Fill the 6 mold cavities with Step 3 so that the molds are filled flush to the top, and the lollipop sticks are positioned with the tip at the circle centre and submerged. If the mixture starts to solidify while filling, reheat to ~100°C and continue.

6. Place the filled lollipop mold in the lab stability chamber (at 5 °C) or freezer in PB 819 for at least 15 minutes.

7. Carefully remove the placebo lollipops from the mold. Individually weigh the lollipops to determine the average placebo lollipop weight, taring the scale with a lollipop stick to subtract off its contribution to the formulation.

**Medicated Lollipops**

8. Based on the average weight of vehicle per lollipop, calculate how much benzocaine and vehicle is required to compound 10 lollipops for the final medicated batch.

9. If the benzocaine appears lumpy, triturate the benzocaine to reduce the particle size to a smooth powder in a small glass mortar and pestle.

10. With a clean 600 mL beaker, repeat the above steps for mold calibration (Steps 1-7), adding the benzocaine at the appropriate time (Step 3). Use a fresh mold – you do not need to wait for the calibration batch to cool. The drug is added after the vehicle has been melted (usually with the flavourant), to limit exposure of the API to heat.

11. At the end of the lab period, remove from mold, and weigh each recovered lollipop, first taring the scale with an empty lollipop stick.

12. Dispense the lollipops in an appropriate container, and label.

13. Complete a QC spreadsheet for your lollipop batch (in the lab worksheet, or the “Mold QC” tab in `moldcalcs.xls`) and hand it in with your final formulation. Did the batch pass weight/dosage specifications?

14. Rinse the molds in **hot water** in the lab sinks to clean them and remove any material. Return the molds to your TA or instructor. **Do not dispose of the molds**, they are reusable.
70 mg Hydrocortisone/150 mg Lidocaine Lip Balm (Double Casting Method)

Lip Balm Base

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Weight Percent (%w/w)</th>
<th>Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petrolatum (occlusive/moisturizing)</td>
<td>48.9</td>
<td></td>
</tr>
<tr>
<td>Beeswax (stiffener)</td>
<td>20.2</td>
<td></td>
</tr>
<tr>
<td>Polyethylene Glycol 400 (hydrophilic)</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td><strong>Note:</strong> PEG 400 is liquid at room temperature,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>and is used to levigate the drugs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lemon Oil or Orange Oil (fragrance)</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td><strong>Note:</strong> Do not weigh out in weighing dish (the</td>
<td></td>
<td></td>
</tr>
<tr>
<td>oil will dissolve the dish)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>100 %w/w</td>
<td>16 g</td>
</tr>
</tbody>
</table>

Lip Balm Preparation

**Note:** The Double Casting Method does not require calibrating the mold.

1. Set up a hot water bath by filling two x 250 mL beakers with ~100 mL tap water, and placing them on the same hot plate (diagonally). Set the hot plate on high, and set a small ceramic dish on each.

2. Remove the caps from the lip balm casings. Check that each lip balm casing has a fill elevator inside it. Ensure each lip balm casing is ready by twisting the bottom ring (called the “knurl”) counter-clockwise all the way, until it becomes difficult to turn. At this point, the fill elevator will be at the bottom of the casing. If the fill elevator is at the top and does not lower, gently apply downward pressure while turning the knurl. **Weigh and record the mass of the casing without the cap.**

3. Calculate an excess amount of ingredients for two lip balm sticks (16 g total batch weight). This should be enough material to make two units in excess.

4. Place and tare the first ceramic dish (dish 1) on an open-air (2 decimal) lab scale. Directly weigh in the calculated amounts of beeswax, petrolatum, and lemon oil (or orange oil), in that order. Tare the scale between measurements.

**Note:** For the beeswax, break off tiny pieces (break or shave larger pieces with a metal weighing spatula if required). For the petrolatum, transfer in small amounts on a clean
spot of dish 1, with a small metal weighing spatula. For the lemon (or orange) oil, use a plastic transfer pipette.

5. Place dish 1 back on the hot water bath. Stir the mixture with a metal spatula until clear, and homogenous. Do not overheat the mixture.

6. Using the sensitive scales, weigh out enough hydrocortisone USP and lidocaine USP for two lip balm sticks (140 mg hydrocortisone USP and 300 mg lidocaine USP) in separate, small weighing boats. Transfer both powders into the second small ceramic dish (dish 2).

7. Weigh out the required amount of PEG 400 in a small beaker. Levigate the drug in dish 2 with the PEG 400, slowly adding it dropwise, and mixing with a spatula until the drug particles are completely wet. Add the remaining amount of PEG 400 from the small beaker to dish 1.

8. Using an oven mitt, pour off approximately one quarter of the vehicle from dish 1 into dish 2, on the second water bath. This should be less than the amount required to compound two lip balm sticks. Mix dish 2 until uniform. If two phases form, remove dish 2 from the hot plate, and stir continuously while cooling, until uniform (but not solidified).

Note: Before pouring the melted vehicle into the lip balm casing, dab the bottom of the ceramic dish onto a lab diaper or tissue, to remove any condensate from the bottom of the dish.

9. Remove dish 2 from the hot plate. Carefully fill the two lip balm casings with the medicated, melted mixture, dividing it approximately equally between them. Ensure all of the liquid is transferred.

10. Top up the lip balm casings with the remaining empty vehicle (from dish 1). Fill to the very top of the casing, without overfilling. The base will contract slightly as it cools. After the base forms a skin on the top, you may transfer it to the lab stability chamber set at 5 (set at 5 °C) for faster cooling (~10 minutes).

11. After the lip balm sticks have cooled and solidified, remove them from the casings by twisting the knurls clockwise, until completely ejected. You will see a small plastic piston (called the “fill elevator”) at the bottom of each casing – remove these as well by continuing to twist the knurls clockwise.

12. Place the solidified formulation back into dish 2, recovering any vehicle stuck on the fill elevator with a small metal weighing spatula. Re-melt the solidified lip balm sticks on dish 2. Stir until homogenous.

13. Reload the fill elevators into the lip balm casings. To accomplish this, push the fill elevator back into the casing, as you twist the knurl counter-clockwise, until the fill elevator starts to descend. Continue twisting the knurl counter-clockwise until the fill
elevator reaches the very bottom. At this point, you will no longer be able to twist the knurl counter-clockwise.

14. Carefully refill the lip balm casings with all of the medicated mixture, being careful to transfer as much of the vehicle as possible.

15. Allow the formulations to cool. Weigh the final casings (not including caps) to determine the medicated lip balm weight. Replace the cap, and properly label the formulation.

16. Complete a QC spreadsheet for your lip balm units (in the “Lip Balm QC” tab in moldcalcs.xls) and hand it in with your final formulations. Did the units pass weight/dosage specifications?
YOUR NOTES

Use these pages as a notepad if you need extra space.