A Laboratory Course in BIOMATERIALS

A Laboratory Course in BIOMATERIALS

Wujing Xian

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

© 2010 by Taylor & Francis Group, LLC
CRC Press is an imprint of Taylor & Francis Group, an Informa business

No claim to original U.S. Government works
Version Date: 20110720

International Standard Book Number-13: 978-1-4200-7615-8 (eBook - PDF)

This book contains information obtained from authentic and highly regarded sources. Reasonable efforts have been made to publish reliable data and information, but the author and publisher cannot assume responsibility for the validity of all materials or the consequences of their use. The authors and publishers have attempted to trace the copyright holders of all material reproduced in this publication and apologize to copyright holders if permission to publish in this form has not been obtained. If any copyright material has not been acknowledged please write and let us know so we may rectify in any future reprint.

Except as permitted under U.S. Copyright Law, no part of this book may be reprinted, reproduced, transmitted, or utilized in any form by any electronic, mechanical, or other means, now known or hereafter invented, including photocopying, microfilming, and recording, or in any information storage or retrieval system, without written permission from the publishers.

For permission to photocopy or use material electronically from this work, please access www.copyright.com (http://www.copyright.com/) or contact the Copyright Clearance Center, Inc. (CCC), 222 Rosewood Drive, Danvers, MA 01923, 978-750-8400. CCC is a not-for-profit organization that provides licenses and registration for a variety of users. For organizations that have been granted a photocopy license by the CCC, a separate system of payment has been arranged.

Trademark Notice: Product or corporate names may be trademarks or registered trademarks, and are used only for identification and explanation without intent to infringe.

Visit the Taylor & Francis Web site at
http://www.taylorandfrancis.com

and the CRC Press Web site at
http://www.crcpress.com

Contents

Preface ... xi
Acknowledgments ... xiii
About the Author ... xv

1 Basic Laboratory Skills I ... 1
I Commonly Used Lab Equipment and Supplies ... 1
Exercises ... 2
II Waste Disposal ... 3
Exercise ... 4
III Liquid Transfer ... 4
III.1 Micropipettes ... 4
III.1.1 Anatomy of a Micropipette ... 4
III.1.2 Pipetting Techniques ... 4
III.1.3 Before You Put Away the Micropipette … ... 5
III.2 Pipet-Aids ... 6
III.3 Pasteur Pipettes ... 6
III.4 Plastic Transfer Pipettes ... 6
III.5 Graduated Cylinders and Volumetric Flasks ... 6
IV Weighing ... 7
IV.1 Electronic Balance ... 7
IV.2 Trip Balance ... 7
Exercises ... 7
V Making Solutions ... 8
V.1 Phosphate Buffered Solution ... 8
Procedures ... 8
V.2 Acetic Acid Solution (0.50 *M*) ... 9
Procedures ... 10
VI Error Analysis ... 10
VI.1 Significant Figures ... 10
Exercises ... 11
VI.2 Accuracy and Precision ... 11
Exercises ... 12
Discussion ... 13
Reference ... 14

2 Module I. Drug Delivery: Controlled Release of Encapsulated Protein from PLGA Microspheres ... 15
Session 1. Encapsulation of BSA in PLGA Microspheres ... 18
Safety Note ... 18
Procedures ... 18
Part 1 (Day 1). The Double Emulsions ... 18
Part 2 (Day 2). Harvesting the Microspheres ... 21
Session 2. Evaluation of the Encapsulation Efficiency and the Drug Load ... 23
Safety Note ... 24

Procedures....25
Data Processing....29
Session 3. Controlled Release of BSA from PLGA Microspheres....29
Procedures....30
Session 4. Evaluation of Controlled Release Profile....32
Procedures....34
Data Processing....35
Questions....37
Appendix. Recipes and Sources for Equipment, Reagents, and Supplies....39
Session 1. Encapsulation of BSA in PLGA microspheres....39
Session 2. Evaluation of the Encapsulation Efficiency and the Drug Load....40
Session 3. Controlled Release of BSA from PLGA Microspheres....40
Session 4. Evaluation of Controlled Release Profile....40
References....41

3 Module II. Natural Biomaterials: Collagen and Chitosan....43
Session 1. Extracting Acid-Soluble Type I Collagen from Bovine Calf Skin....46
Procedures....47
Session 2. Purification of Extracted Collagen....50
Procedures....51
Part 1 (Day 1). Salt Precipitation of Collagen....51
Part 2 (Day 2). Freeze-Drying Collagen for Storage....55
Session 3. Analysis of Collagen Extraction and Purification by Electrophoresis....55
Notes....57
Procedures....57
Part 1 (Day 1). Running the SDS-PAGE....57
Part 2 (Day 2). Drying the SDS-PAGE Gel....63
Part 3 (Day 3). Imaging the Dried Gel....64
Session 4. Fabricating Collagen/Chitosan Sponges....65
Procedures....65
Part 1 (Day 1). Preparing the Calf-Skin Dermis (Corium)....65
Part 2 (Day 2). Making Collagen/Chitosan Sponges....68
(Optional) Part 3 (Day 3). Heat Treatment of the Collagen Sponge....69
Questions....69
Appendix. Recipes and Sources for Equipment, Reagents, and Supplies....70
Session 1. Extracting Acid-Soluble Type I Collagen from Bovine Calf Skin....70
Session 2. Purification of Extracted Collagen....70
Session 3. Analysis of Collagen Extraction and Purification by Electrophoresis....70
Session 4. Fabricating Collagen/Chitosan Sponges....71
References....71

4 Basic Laboratory Skills II. Cell Culture....73
I Cell Culture....73
I.1 Equipment for Cell Culture....74
I.2 Consumable Items....77
I.3 Waste Disposal....78
II Exercises....79
II.1 Know Your Cell Culture Lab Equipment....79

II.2 Waste Disposals in Your Cell Culture Lab ... 80
II.3 Liquid Handling ... 80
Procedures ... 80
II.4 Basic Microscope Operation ... 81
Procedures ... 82
II.5 Preparing the BSC ... 83
Procedures ... 83
II.6 Preparing Cell Culture Medium ... 83
Procedures ... 84
II.7 Starting a Cell Culture from Cryo-Frozen Stock ... 85
Procedures ... 85
II.8 Cell Counting ... 87
Procedures ... 87
II.9 Changing Medium ... 90
Procedures ... 91
II.10 Passaging and Freezing Cells ... 92
Procedures for Cell Passaging ... 92
Procedures for Freezing Cells ... 93
Appendix. Recipes and Sources for Equipment, Reagents, and Supplies ... 95
I.1 Equipment for Cell Culture ... 95
I.2 Consumable (Disposable) Items ... 96
I.3 Waste Disposal ... 97
II.3 Liquid Handling ... 97
II.6 Preparing Cell Culture Medium ... 97
II.7 Starting Cell Cultures from Frozen Stock ... 97
II.8 Cell Counting ... 97
II.10 Passaging and Freezing Cells ... 98

5 Module III. Biocompatibility Testing: Cytotoxicity and Adhesion ... 99
Session 1. Cytotoxicity Evaluation Using Direct Contact Tests ... 103
Safety Notes ... 104
Procedures ... 104
Part 1 (Day 1). Preparing Cell Cultures ... 104
Part 2 (Day 1). Preparing Test Materials ... 106
Part 3 (Day 2). Placing Test Materials in Direct Contact with Cell Cultures ... 107
Part 4 (Day 3). Evaluation of Cytotoxicity ... 108
Data Processing ... 109
Session 2. Cytotoxicity Evaluation Using Liquid Extracts of Materials ... 110
Procedures ... 112
Part 1 (Day 1). Preparing Cell Cultures ... 112
Part 2 (Day 1). Sample Preparation for the Extraction Tests ... 112
Part 3 (Day 2). Extracting the Test Materials ... 113
Part 4 (Day 3). Incubating Cell Cultures with Extracts ... 113
Part 5 (Day 4). Evaluation of Cytotoxicity ... 114
Data Processing ... 115
Session 3. Biofunctionality Evaluation through Cell Adhesion/Proliferation Tests ... 116
Procedures ... 117
Part 1 (Day 1). Preparing Test Materials ... 117
Part 2 (Day 2). Seeding MC3T3-E1 Cells on the Surfaces of Test Materials ... 118

Part 3 (Day 3). Preparing MC3T3-E1 Cultures for MTT Calibration 121
Part 4 (Day 3). MTT Assay 122
Data Processing 125
Questions 126
Appendix. Recipes and Sources for Equipment, Reagents, and Supplies 127
Session 1. Cytotoxicity Evaluation Using Direct Contact Tests 127
Session 2. Cytotoxicity Evaluation Using Liquid Extracts of Materials 127
Session 3. Biofunctionality Evaluation through Cell Adhesion/Proliferation Studies 127
References 128

6 Module IV. Tissue Engineering: Organotypic Culture of Skin Equivalent 129
Session 1. The Dermis Equivalent 133
Procedures 135
Part 1 (Day 1). Encapsulation of NIH 3T3 Cells in Collagen Gel 135
Part 2. Adding Ascorbic Acid and Maintaining the Cultures 138
Session 2. The Epidermis Equivalent 138
Procedures 140
Part 1 (Day 1). Seeding HaCaT Cells onto the Dermis Equivalent 140
Part 2 (Day 2). Adding TGF-α and Maintaining the Cultures 142
Part 3 (Day 3). Air Exposure of the Epidermis Equivalent 142
Part 4 (Day 4). Fixing the Skin-Equivalent Samples for Histology Slide Preparation 144
Session 3. Histological Studies of Natural Skin and Organotypic Skin Equivalent 147
Procedures 147
Part 1 (Day 1). Histology of Human Skin (Thick Skin) 147
Part 2 (Day 1). Histology of Organotypic Skin Equivalents 155
Questions 156
Appendix A. Sterilizing Collagen and Preparing Collagen Solution 157
Procedures 157
Appendix B. Custom-Made Teflon Support for Air Exposure of the Epidermis Equivalent 159
Appendix C. Recipes and Sources for Equipment, Reagents, and Supplies 161
Session 1. The Dermis Equivalent 161
Session 2. The Epidermis Equivalent 163
Session 3. Histological Studies of Natural Skin and Organotypic Skin Equivalent 163
References 163

7 Module V. Bioceramics: Hydroxyapatite 165
Session 1. Synthesis of HA and FA 167
Procedures 168
Part 1 (Day 1). The Wet Precipitation Reaction 168
Part 2 (Day 2). Harvesting and Drying HA/FA 169
Session 2. X-Ray Powder Diffraction of HA and FA 172
Procedures 174
Part 1 (Day 1). Thermal Treatment of HA/FA 174
Part 2 (Day 2). X-Ray Powder Diffraction Measurement 175
Data Processing 176

Session 3. Fabrication of Porous HA/FA-PLGA Composite Using a Salt-Leaching Method 177
Procedures 178
Session 4. Characterization of the Porous HA/FA-PLGA Composites—Acid Degradation and Compression Testing 179
Procedures 182
Part 1 (Day 1). *In Vitro* Acid Degradation of the Composites 182
Part 2 (Day 2). Compression Testing 183
Data Processing 184
Questions 185
Appendix. Recipes and Sources for Equipment, Reagents, and Supplies 186
Session 1. Synthesis of HA and FA 186
Session 2. X-Ray Powder Diffraction of HA and FA 189
Session 3. Fabrication of Porous HA/FA-PLGA Composite Using a Salt-Leaching Method 189
References 189

Appendix: Answers 191

Index 203

Preface

The area of biomedical engineering has vastly expanded in the past couple of decades. The need to educate the multidisciplinary students who will make up the work force in research and industry in biomedical engineering has also correspondingly increased. A brief survey of bioengineering and biomaterials programs in a number of academic institutions reveals a general consensus on topics that are covered by lecture courses. Topics and other teaching aids for laboratory courses, however, are much less readily available. In fact, few biomaterials lab courses exist at present, despite the clear need. It is the intention of this book to provide a laboratory curriculum that is comprehensive in scope as well as current in its perspective. This book is suitable for an undergraduate laboratory course in biomaterials, and bioengineering at the senior or junior level. It is also designed to help lower the barriers for entry into biomaterials for the more "traditional" engineering departments (such as materials science, chemical engineering, and mechanical engineering) since the cost in time and resources required for developing such laboratory courses can be quite high. This course is inherently multidisciplinary, integrates a variety of principles from materials science, mechanical engineering, chemistry, biochemistry, molecular and cell biology, and tissue engineering, and will train students in laboratory skills, data analysis, problem solving, and scientific writing. Experiments in this course are described in the form of flexible modules that can be chosen and adapted for the needs of different departments. Within each module, a range of multidisciplinary knowledge and laboratory practices are organized around a central theme, so that students can see the labs not as a compilation of procedures but rather as a coherent whole consisting of interconnections from various disciplines. Much has happened recently in this dynamic field; many experiments in this lab course are adapted from research papers and reflect recent progress in bioengineering and biomaterials.

As a laboratory manual, this book provides step-by-step descriptions of lab procedures, reagents, equipment, and even data processing guidelines so that a laboratory course can be started from scratch. These descriptions are guidelines rather than rigid prescriptions, and the experiments can be adapted according to the instructional laboratory settings and the students' learning needs. The questions following each module incorporate some of the frequently encountered problems and mistakes made by the students from my own teaching experience. Finally, it is my hope that this laboratory course is a fun and rewarding experience for students as well as teachers. Let's get started!

Acknowledgments

This book is based on the biomaterials laboratory course that I have taught for several years at the Materials Science and Engineering Department (MatSE) at the University of Illinois at Urbana-Champaign. I am very grateful to the people who have helped me with the course and with writing the book. Thanks are in order to Professor J. J. Cheng for the many helpful discussions; to Dr. Joanne Manaster, Sheeny Lan, Dr. Aylin Sendemir-Urkmez, and Dr. Sharon Wong for their help with cell cultures; to Dr. Raju Perecherla and Professor David Cahill for access to and help with instruments; to Spencer Shultz for his expert machine-shop work; and to Zach Culumber, Lanfang Li, Scott Slimmer, and Lihua Yang for their assistance. I am also thankful for the commitment to and support for biomaterials by my department. Especially I'd like to thank Jay Menacher, assistant to the head of MatSE, who has made things easy. And lastly, I am deeply grateful to Gerard Wong, who suggested that I write this book and helped with his expertise and moral support along the way: This book would not have been possible without him.

About the Author

Wujing Xian received a BS degree in chemistry from Sun Yat-Sen (Zhongshan) University, and a PhD degree in chemistry from the University of Nebraska–Lincoln. After postdoctoral work on protein structure and engineering at the Brigham & Women's Hospital of Harvard Medical School and the Institute for Medicine and Engineering at the University of Pennsylvania, Dr. Xian became a lecturer at the University of Illinois at Urbana-Champaign, where she created a new biomaterials laboratory course for the Department of Materials Science and Engineering. Her research interests include protein engineering, tissue engineering, and antimicrobials.

1

Basic Laboratory Skills I

Experiments in this course aim to provide lab skill training in many disciplines. Before we begin, however, it is helpful to familiarize ourselves with some of the basic lab practices so that we can conduct the experiments more efficiently and safely. To get started, we will survey some of the most commonly used equipment and supplies found in our lab. We will also learn about proper waste disposal, an often ignored but extremely important practice that ensures protection of both the environment and ourselves. Next we will learn basic practices in liquid transfer and weighing; then, to integrate these practices, we will make two solutions that will be later used in our experiments. Last, we will review some of the basic practices in data processing.

I Commonly Used Lab Equipment and Supplies

- **Balances.** Top-loading electronic balances are commonly used nowadays. An electronic balance can be categorized as either *regular* or *analytical* depending on its weighing resolution, or "readability." A regular balance typically has a range of hundreds to thousands of grams and readability from milligrams to grams, while an analytical balance's range is typically of tens to hundreds of grams and its readability is ≤0.1 mg. When weighing samples, choose the right balance based on the weight of the sample and the accuracy requirement. A special type of mechanical balance, the trip balance, is also used in lab. (See Section IV for more details.)
- **Centrifuges.** There are a variety of centrifuges for different centrifugation needs, and one of the determining factors for centrifuge selection is the centrifugal force that is required for sedimentation of components in the sample. Typically, mini-centrifuges and microcentrifuges are used for quick processing of small volumes of samples (typically <2 ml each), whereas larger centrifuges, with either bench-top or floor models, accommodate larger volumes and offer more centrifugal power.
- **Glassware.** Glass beakers, Erlenmeyer flasks, test tubes, and bottles of different volumes are examples of typical glassware used in lab. To clean glassware, first wash with detergent until clean—water will flow as a sheet rather than streaks on clean glass surface—then rinse with water, and finally rinse with de-ionized water.
- **Gloves.** Disposable latex and nitrile gloves are the most commonly used in labs. Latex gloves are relatively inexpensive but have poor chemical resistance. Nitrile gloves have much better chemical resistance and should be worn when handling organic solvents or caustic reagents.
- **Liquid transfer.** Liquid transfer is handled in many different ways depending on the liquid volume and the accuracy requirement. Micropipettes, electronic

pipette-aids, transfer pipettes, and graduated cylinders are some of the most commonly used liquid transfer tools. (See Section III for more details.)

- **pH meter.** This is used for measuring the pH of solutions. Operate your pH meter according to the user's manual. It should be calibrated using pH standard buffers from time to time. *Important:* Do not allow the electrode to dry.
- **Mixing.** Vortex mixers, magnetic stirring plates, and shakers are examples of commonly used mixing equipment in the lab. In our experiments, we will also use specialized mixing equipment such as the sonicator and the homogenizer.
  - **Vortex mixer.** The high-speed vibration of a vortex mixer generates vortex in liquid, which results in quick mixing of liquid-liquid or liquid-solid. It is generally used for quick mixing in microcentrifuge tubes, test tubes, etc., in which vortexes can be generated.
  - **Magnetic stirring plate with stirring bar.** This is generally used for mixing in beakers, flasks, bottles, or other similar containers. For efficient stirring, the length of a magnetic stirring bar should be about 2/3 the diameter of the container's bottom.
  - **Shaker.** This is suitable for consistent mixing for prolonged periods of time. Some shakers are equipped with environment control such as water bath that can maintain constant temperature for incubating and shaking.
- **Refrigeration.** Some reagents need to be stored at low temperatures. For a given reagent, refer to the on-bottle label, the user's manual, or the material safety data sheet (MSDS) for the appropriate storage temperature.
- **Water purification.** Requirement of water purity varies depending on the application. Water purity is categorized as type I, II, and III, which typically requires the resistivity to be >18.2 mΩ·cm, >1 mΩ·cm, and >50 kΩ·cm, respectively; other parameters are restricted as well. For reagent preparation, type I water is generally preferred. On the other hand, for less demanding usage of purified water such as glassware rinsing, type III water is usually sufficient. (Note that de-ionized water can be corrosive to metal, thus it may not be suitable for certain applications.) De-ionization can be achieved by filtering water through mixed-bed ion-exchange resins that remove both anions and cations.
- **Disposable items.** Commonly used disposable items include 0.5-ml and 1.5-ml microcentrifuge tubes, pipette tips, serological pipettes (polystyrene), weighing boats, flint glass test tubes, etc. Observe waste disposal guidelines when discarding these items.

Exercises

1. Survey the centrifuges in your lab using Table 1.1 as an example.
2. Locate a vortex mixer, a magnetic stirring plate, and a shaker in your lab.
3. What type(s) of water purification equipment is(are) available in your lab? In your lab notebook, record the make and model of the equipment, the type of water (I, II, or III), and the resistivity threshold of the water purifier.

TABLE 1.1

A Survey of the Centrifuges in My Lab

Make	Model no.	Type[a]	Rotor	Max. RPM[b]	Max. RCF[c]	Vol. Capacity
Fisher Sci.[d]	—	Mini	Fixed-angle	6600	2200 ×g	6 × 2.0 ml
			Strip	6600	2200 ×g	8 × 0.2 ml or 16 × 0.2 ml
…	…	…	…	…	…	…

[a] Centrifuge type can be minicentrifuge, microcentrifuge, general-purpose centrifuge (bench-top or floor model), clinical centrifuge, etc.
[b] RPM: rotation per minute.
[c] RCF: Relative centrifugal force, expressed as a number of times of gravity, or "×g."
[d] Example (Fisher cat. no. 05-090-100). This model includes two rotors.

II Waste Disposal

It is important to observe applicable institutional guidelines when disposing of used supplies and reagents. Always ask your instructor if you are not sure how to dispose of a certain item. The following are some of commonly available waste disposals:

- **Broken glass disposal.** Clean, nonhazardous broken glassware, Pasteur pipettes, disposable glass tubes, etc. should be collected in a designated plastic-lined box clearly marked as "broken glass disposal." Residual chemicals or reagents should be removed from the glassware before disposal. *Example:* A Pasteur pipette was used for transferring chloroform. Before discarding it into broken glass disposal, leave the pipette in a fume hood until the residual chloroform has completely evaporated.
- **Regular trash.** Some disposable items such as plastic serological pipettes, plastic transfer pipettes, paper tissues, etc. that have not been used with toxic, caustic, or carcinogenic reagents may be discarded into regular trash. *Example:* A plastic transfer pipette was used for adjusting pH with HCl solution. Before disposing it into regular trash, rinse the pipette with water to remove any residual HCl solution.
- **Sharps disposal.** Sharps such as scalpels, blades, syringes, and needles need to be disposed of in special heavy-duty plastic sharps containers, which should be clearly marked. (Syringes, though made of plastic, are often required to be disposed of in sharps containers because of the association between syringes and needles.) *Example:* A scalpel was used for cutting up calf skin. When finished, wipe it on a piece of tissue paper to remove any stuck skin tissues and discard it into a sharps container.
- **Sink disposal.** Institutional and local municipal guidelines must be strictly followed for sink disposal. In general, only small amounts of nonhazardous inorganic salts, acids, and bases can be flushed down the sink with a large amount of water. *Example:* In our experiments, phosphate-buffered saline, electrophoresis running buffer, acetic acid solution, dilute HCl solution, NaOH solution, and excess salts such as NaCl taken from the bottles when weighing samples, etc. can generally be flushed down a sink with a generous amount of water.

- **Disposal of solvents, reagents, and chemicals.** These should be collected in specially designated containers and disposed of following proper guidelines. *Example:* 70% ethanol is used for disinfecting calf skin before collagen extraction. When finished, the 70% ethanol should be collected in a container clearly labeled with "70% ethanol waste with trace calf skin" or similar wording.

Exercise

Locate the broken glass disposal and sharps disposal containers in your lab.

III Liquid Transfer

III.1 Micropipettes

Micropipettes, also called micropipetters or pipettes, are piston-driven air-displacement devices that are typically used to transfer liquid volumes in the range of 1 µl to 1000 µl with high precision and accuracy, which can be as low as <0.5% or as high as 5% depending on the micropipette and the pipetted volume. Specialized micropipettes are available for larger or smaller volumes.

III.1.1 Anatomy of a Micropipette

Different micropipettes may have different designs, but they all have certain common features and are operated in similar ways:

- The handle. Grab the handle firmly with your hand.
- A push button, or plunger, on the top of the micropipette. Use the thumb to push the plunger for delivery of the liquid. There should be two stops when pushing down on the plunger. Usually, for delivery of the bulk volume, press the plunger to the first stop and to deliver the residual liquid, press the plunger to the second stop.
- A volume adjustment dial and a numeric display. Turn the dial to set the volume of the liquid to be delivered. For accuracy, turn the dial past the desired setting, and then turn it back down gradually to the correct setting. The unit for the set volumes is µl; make sure that the decimal place is located correctly on the dial.
- A shaft that leads to the tip cone. To attach a tip to the tip cone, firmly push down the pipette (but avoid using excessive force).
- A tip ejector. To eject a tip, point the tip to the waste receptacle and press the ejector with your thumb.

III.1.2 Pipetting Techniques

Observe the following guidelines when using a micropipette:

- Pick the right micropipette. Do not use a micropipette outside its designated range; otherwise it could be damaged mechanically. When a volume to be pipetted is within the ranges of different micropipettes, use the one with the smallest range.

For example, to pipette a volume of 18.2 μl, use a 20-μl micropipette instead of a 100-μl micropipette.

- *Never* allow liquid to get inside the micropipette barrel. This will lead to cross-contamination between samples, and potential damage to the micropipette as well. To avoid this situation, pipette liquid in smoothly, and do not tilt the micropipette too far from vertical when it is holding liquid.
- Press and release the plunger smoothly. Do not allow it to snap back.
- When loading the pipette tip onto the tip cone, make sure that the tip is firmly attached but avoid using excessive force—a light tap or two on the tip rack will usually do.
- Pipetting in: Submerge the pipette tip slightly (several mm) beneath the surface of the liquid. Do not plunge the tip into the liquid.
- Pipetting out: Hold the tip against the inner wall of the receptacle for a steady and smooth delivery of the liquid.

Select the following pipetting techniques based on the volume of the liquid to be transferred, and whether it is nonviscous, viscous, or foamy:

- **The forward technique.** The forward technique is typically used for nonviscous liquid. To pipette using the forward technique, press the plunger to the first stop and release to draw liquid in through the tip. Wipe the tip against the liquid container to remove excess liquid on the outside of the tip. In the new receptacle, press the plunger to the first stop to deliver the liquid. Wait for a second or two, and then press the plunger to the second stop to empty any residual liquid. Release the plunger and eject the tip.
- **The reverse technique.** The reverse technique is suitable for viscous or foamy liquid, or very small volume. Examples of viscous or foamy liquids include glycerol solutions, protein solutions, and detergent solutions. To pipette using the reverse technique, first press the plunger all the way to the second stop, then release slowly to draw liquid in. Again, wipe the tip against the liquid container to remove excess liquid on the outside of the tip. To deliver, press the plunger to the first stop and hold for one or two seconds. The remaining liquid should be released back into the original liquid container or discarded.
- **The pumping technique.** Some liquids, such as whole blood, are viscous and tend to cling to pipette tips. Repeated pumping is necessary to deliver the full volume. To pipette using the pumping technique, first press the plunger to the first stop, then release to draw up liquid. Press the plunger to the first stop to deliver the liquid, and then release it smoothly. Repeat the pumping motion until all the liquid inside the tip is delivered. To finish, press the plunger to the second stop to deliver any residual liquid.

III.1.3 Before You Put Away the Micropipette …

After work is finished, do the following before you put the micropipette away for the day:

- Clean the micropipette. Wipe away any moisture or soiled spots. If the micropipette has been used to handle hazardous materials, make sure that no residue is left behind.

- Turn the volume adjustment to the highest setting. This is to prevent the spring inside the micropipette from being compressed for a long period of time, which helps to maintain the accuracy of micropipette.
- For small-volume micropipettes (<10 μl), it is recommended that they be stored with pipette tips attached, so that in case the micropipettes are dropped, their small tip cones are protected.

III.2 Pipet-Aids

A pipet-aid is a device that is used with a serological pipette to transfer liquid volumes from <1 ml to ~100 ml. Commonly used pipet-aids are electrical and are controlled by two buttons: Press the top button to draw up liquid and the down button to deliver the liquid. A serological pipette is attached to the mouthpiece of a pipet-aid.

Pay attention to the following when using a pipet-aid for our experiments:

- ***Caution:*** Do *not* draw liquid into the mouthpiece of the pipet-aid.
- Pipetting organic solvent: To pipette organic solvents, glass serological pipettes *must* be used. Polystyrene pipettes will dissolve in many organic solvents. To tell a glass pipette from a polystyrene pipette: 1) read the label on the pipette; 2) feel the pipette: glass pipettes are heavier and clink when bounced on a hard surface. Pay attention to whether the glass pipette is reusable or disposable.
- Pipetting in: Liquid should be drawn up smoothly; do not allow it to "spring" (like spring water gushing out of the ground) through the opening.
- Pipetting out: Rest the tip of the serological pipette against the inner wall of the receptacle if possible, and deliver the liquid smoothly.

III.3 Pasteur Pipettes

Pasteur pipettes are glass pipettes with long, fine tips that need to be attached to rubber bulbs for pipetting. These pipettes are inexpensive and have excellent chemical resistance; they are especially suitable for transferring organic solvents and reagents when volume accuracy is not required. ***Caution:*** Pasteur pipettes should be disposed of in broken glass disposal.

III.4 Plastic Transfer Pipettes

These are disposable plastic pipettes with "built-in" suction bulbs. They can be used to transfer small volumes of liquid (<10 ml each transfer) when volume accuracy is not required. (They are also very useful for other tasks; for example, a transfer pipette can be used for resuspending a pellet after centrifugation since it can be used as a stirring rod as well as a pipette.)

III.5 Graduated Cylinders and Volumetric Flasks

Graduated cylinders and volumetric flasks are liquid-measuring devices that are mainly used for making solutions. Volumetric flasks are used when the accuracy requirement for

concentration is very stringent. (For the experiments in this course, graduated cylinders are sufficient for making solutions. See Section V.)

IV Weighing

IV.1 Electronic Balance

Weighing with top-loading electronic balance is straightforward: Place a weighing boat or weighing paper on the platen, tare (zero) the balance, add the sample to the weighing boat or paper, and read the displayed weight. Observe the following guidelines when weighing solid samples:

- **Range:** Each balance has its range and accuracy. Do not use a balance outside its range, and pick a balance with the right accuracy.
- **For analytical balance:** It is important for an analytical balance to be leveled. Check the window on the balance in which an air bubble is sealed in water: if the bubble is not centered, adjust the screws on the feet of the balance until it is centered.
- **Weighing powder sample:** When weighing powder, use spatula to transfer the powder to the weighing vessel; when it is getting close to the target weight, lightly tap on the wrist of the hand that is holding the spatula and allow a small amount of powder to fall into the weighing vessel, and repeat until the target weight is reached.
- **Cleaning up:** Use a tissue or brush to clean up any spilled sample. Tare the balance.

IV.2 Trip Balance

Trip balance is a type of mechanical balance that is often used for measuring the *relative* weight of one sample against another. It is particularly useful when weighing samples for centrifugation, where two centrifuge tubes across from each other in the rotor must be equal in weight. To balance two centrifuge tubes, place two tube holders (such as 100-ml beakers) on the two platens, and then zero the needle in the middle by sliding the weight on the balance beam. Next, place one centrifuge tube in each holder; add or subtract samples until the needle is zeroed again. If the two tubes are holding the same sample, then use a transfer pipette to transfer the sample back and forth until the two tubes are balanced. Remember to include the caps of the centrifuge tubes for the balancing. *Note:* Balancing with a trip balance is a must for high-speed centrifuges, where a small difference in the weight is augmented by the high centrifugal force. For minicentrifuge or microcentrifuge that uses microcentrifuge tubes, balancing is achieved by weighing the microcentrifuge tubes with electronic balance and adjusting the liquid volumes accordingly. For short centrifugation time at low speed (<1000 xg), sometimes "eyeball balancing" is good enough.

Exercises

1. Survey the balances in your lab using Table 1.2 as an example.
2. Add water into two 50-ml centrifuge tubes to roughly 2/3 full. Balance the two tubes on a trip balance.

TABLE 1.2

A Survey of the Balances in My Lab

Make	Model No.	Type[a]	Range (g)	Readability (g/mg)
Ohaus Scout Pro[b]	SP601	Top loading, general	0–600	0.1 g
…	…	…	…	…

[a] Types of balances include general, analytical, top loading, trip balance, etc.
[b] Fisher cat. no. 01-921-13.

V Making Solutions

V.1 Phosphate Buffered Saline

In the following exercise, you will make 500 ml of phosphate-buffered saline (PBS) with the following composition. This solution will be used in Module I (see Chapter 2).

- 155 *mM* NaCl
- 1.0 *mM* KH_2PO_4
- 3.0 *mM* Na_2HPO_4

Procedures

1. *Check-in*
   a. Reagents
      – NaCl, solid
      – KH_2PO_4, solid
      – Na_2HPO_4, solid
      – De-ionized water
      – 1.0 *M* HCl solution
      – 1.0 *M* NaOH solution
   b. Equipment and supplies
      – Balance, with weighing boats and spatula
      – Kimwipe tissue paper
      – Graduated cylinders, 500 ml and 100 ml
      – Beaker, 500 ml or 600 ml
      – 500-ml bottle, glass or plastic
      – Magnetic stirring plate and stir bar
      – pH meter
      – Wash bottle with de-ionized water
      – Labeling tapes

TABLE 1.3

Components of PBS

Component	Concentration (*mM*)	Mol. Formula	Formula Weight	Weight Needed for 500 ml PBS
NaCl	155			
KH_2PO_4	1.0			
Na_2HPO_4	3.0			

2. *Calculations:* Note that some salts are available as either anhydrites or hydrates. Check the labels on the bottles for NaCl, KH_2PO_4, and Na_2HPO_4 for the molecular formula of the salts and their formula weights. Calculate the weight of each salt needed for the PBS. Organize your data in Table 1.3.
3. *Weighing samples:* Use an appropriate balance and proper techniques to weigh NaCl, KH_2PO_4, and Na_2HPO_4 according to Table 1.3. Wipe the spatula clean with a Kimwipe tissue before using it for the next chemical, but use a different weighing boat for each chemical. Add the samples to a 500-ml or 600-ml beaker.
4. *Dissolving the salts:* Add a stir bar of an appropriate size and ~400 ml of de-ionized water to the beaker. When adding water, position the spout of the graduated cylinder so that the water flows down along the inner wall of the beaker. Do not let the water splash. Place the beaker on a magnetic stirring plate and stir the solution with moderate speed until the salts are completely dissolved.
5. *pH adjusting:* Turn on the pH meter, which has already been calibrated by your instructor. Take the electrode out of the storage buffer, open the air inlet (usually located at the upper end of the electrode), and rinse the lower end of the electrode with de-ionized water from a wash bottle. Position the tip of the electrode about 2–3 cm below the surface of the solution and read the pH meter. If it is >7.4, use a plastic transfer pipette to add 1.0 *M* HCl solution drop by drop to adjust it to 7.4; if the pH is <7.4, use 1.0 *M* NaOH solution in the same manner. When finished, rinse the electrode with de-ionized water again, blot the water off with a Kimwipe tissue, and place it back into the storage buffer.
6. *Adjusting the volume:* Pour the solution from the beaker to a 500-ml graduated cylinder. Rinse the beaker with de-ionized water several times and add the rinse to the graduated cylinder as well. Finally, add de-ionized water to the graduated cylinder so that the volume is exactly 500 ml. (Change in pH due to change of the solution volume is assumed to be small. Measure and adjust the pH again if necessary.) Pour the PBS into a 500-ml bottle.
7. *Labeling:* Label the bottle with the name of the solution (PBS), the pH, your group's name, and the date. You may also write the composition of the solution on the label for your own convenience. *Important:* Always label your samples and reagents with at least the names of the chemicals or materials, your (group's) name, and the date; other pertinent information such as the pH of a buffer, the toxicity if any, etc. should be added to the label as well.
8. *Finishing up:* Store the PBS. Wash and rinse the beaker, the graduated cylinders, the stir bar, and the spatula. The used weighing boats and transfer pipettes can be discarded into regular trash after rinsing with tap water.

V.2 Acetic Acid Solution (0.50 *M*)

In the following exercise, you will make 200 ml of 0.50 *M* acetic acid solution, which you will use later in Module II (see Chapter 3).

Procedures

1. *Check-in*
   a. Reagents
      – Glacial acetic acid (pure acetic acid)
      – De-ionized water
   b. Equipment and supplies
      – 200-ml graduated cylinder
      – Glass bottle, ≥200 ml
      – Pipet-aid
      – Serological pipette, polystyrene and glass
      – Fume hood
2. *Calculations:* Glacial acetic acid is in liquid form at room temperature, and its density is 1.049 g/ml. The molecular weight of acetic acid is 60.05 g/mol. Calculate the molarity of glacial acetic acid at room temperature, and then calculate the volume of glacial acetic acid, V_{gaa} in ml, that you need to make 200 ml of 0.50 *M* acetic acid solution.
3. *Making the solution:*
   a. Measure 200 ml de-ionized water with a graduated cylinder and add it to a glass bottle.
   b. With a pipet-aid and a plastic serological pipette, remove V_{gaa} ml of water from the bottle.
   c. In a fume hood, pipette V_{gaa} ml of glacial acetic acid with a dry glass pipette and add it to the de-ionized water. (Remember: When diluting acid, always add acid to water instead of adding water to acid.) Pipette up and down several times for a complete delivery of the acetic acid into the water. Cap the bottles. Swirl the acetic acid solution to mix evenly.
   d. Label the acetic acid solution properly. (See V.1, Step 7.)
4. *Finishing up:* Store the 0.50 *M* acetic acid solution. Rinse the glass pipette with water and de-ionized water. Let the pipettes and the graduated cylinder dry. (The polystyrene pipette may be reused since it was only used for water.)

VI Error Analysis

It is assumed that you have learned error analysis in your previous studies. A paper by Cumming et al. (2007) offers an excellent review with examples. The following exercises are designed as a review and refreshment for the error analysis methods that will be used in this course.

TABLE 1.4
What is the Number of Significant Figures for the Following Measurements?

Parameter	Value (unit)	No. of Sig. Figs.
Density	1.049 g/ml	
Absorbance	0.330	
Readability	0.0001 g	
Volume	1000.0 µl	

VI.1 Significant Figures

Significant figures (or significant digits) indicate the degree of error for a given measurement. For example, a concentration given as 0.50 *M* indicates possible error of 0.01 *M*, whereas a concentration given as 0.5 *M* indicates possible error of 0.1 *M*. In multiplication and division, the number of significant figures in the result should be the same as the smallest number of significant figures among the multiplied or divided quantities; and in addition and subtraction, the result should have the same number of decimal places as the added or subtracted quantity with the fewest number of decimal places.

Exercises

1. Write down the number of significant figures for the measurements in Table 1.4.
2. There is a bottle of commercial NaOH solution with a concentration of 1.000 *N* in your lab. If you accidentally mix it with a bottle of homemade 1.0 *N* NaOH solution, how should you label the concentration of the mixed solution?
3. Check your calculation in Section V.2, Step 2, for the volume of glacial acetic acid needed to make 200 ml of 0.50 *M* acetic acid solution. What should the number of significant figures be in your result?
4. Check your calculation results in Table 1.3 and make sure that the correct numbers of significant figures are used for each results.

VI.2 Accuracy and Precision

Accuracy is defined by how closely a measurement agrees with the true or accepted value, and it reflects the system errors in the measurement. Precision is defined by the reproducibility of the measurement, and it reflects the random errors in the measurement. To evaluate the accuracy and precision of a set of independent measurements, two parameters are frequently used: the mean (M) and the standard deviation (SD). The mean is defined as

$$\mathrm{M} = \frac{1}{N}\sum_{i=1}^{N} X_i \qquad \text{(BLS I.1)}$$

where N is the number of measurements, and X_i is the result of measurement number i.