CONTENTS

Section A

Introduction

LIFS 2720
INTRODUCTORY BIOCHEMICAL
LABORATORY

LIFS 2720 - Introductory Biochemical Laboratory

General Introduction

Aims and Objectives:

This is a hand-on course in basic biochemical techniques. The primary objective is to expose students to a number of procedures commonly used in biochemical laboratories, and to provide a basis for understanding how biochemical data is obtained, analyzed and presented.

On completion of the course, students would be able to:

- become familiar with relevant applications of standard biochemical equipments
- utilize commonly-used equipments in a standard biochemical laboratory
- acquire specific knowledge of selected biochemical laboratory procedures
- present laboratory data in a concise manner
- undertake interpretation of basic biochemical data

Linkage between LIFS 2720 and LIFS 2820:

While LIFS 2720 has been designed to provide practical experience in fundamental biochemical techniques, LIFS 2820 (Biochemical Laboratory Techniques) is intended to introduce the underlying principles complementary to topics covered in LIFS 2720. Given that the teaching materials of LIFS 2720 and 2820 are closely correlated, you are advised to take cross-reference between the two courses.

Course Assessments:

The methods of assessments are used for the calculation of the final grade.

Method of Assessment	Contribution of Final Grade (%)
Laboratory Logbook and Practical Performance	9
Laboratory Worksheets (Total: 9 per student)	18
Laboratory Report (Total: 1 per student)	20
Final Examination	53

Laboratory logbook:

A lab logbook is very important for a researcher. **You are required to read the relevant part of the lab manual prior to the start of your experiment. Preparation of protocol flowcharts and data tables for recording experimental data, when appropriate, should be conducted in advance of the laboratory session.**

At the end of each laboratory session, you are required to present your logbook for evaluation. Failure to hand in your laboratory logbook for inspection will give you no mark for the particular laboratory session.

Laboratory Performance:

You MUST wear a labcoat in the laboratory at all times. If you do not have a lab coat, you are not allowed to enter the laboratory for the experimental session. As a result, you will not be given any marks for the laboratory performance component. Moreover, your marks for result parts (worksheet and report) of that absent practical might be deducted due to you did not participate in the experiment.

If you have any medical or special reason for your absence, you are required to provide evidence (e.g. medical document issued by doctor). Moreover, please inform instructor in advance if it is possible. In such case, marks for result parts (worksheet and report) of that absent practical might not be deducted.

Laboratory skills and logbook preparation for each practical, organization, disciplines (e.g. following instructions from instructor, teaching assistants and technicians, tidying up the assigned working bench area after each practical, *etc*.) and quality of experimental data are all counted for in each practical.

Laboratory Worksheets:

For each practical conducted, you are required to complete a laboratory worksheet consisting of questions related to the experiment. These worksheets emphasize on data presentation, analysis and interpretation. You are required to complete your own **individual** data sheets and hand in at the beginning of the next practical.

Laboratory Report:

Report writing is an essential skill required in all laboratory work. You are therefore advised to pay particular attention to this aspect of the assessment. Please refer to the "Guide to Laboratory Report Writing" included in this manual.

Each student is required to submit **ONE** pre-assigned full laboratory report, which must be hand in at the beginning of the laboratory session two weeks after a practical. You are required to summit a hardcopy for marking as well as a softcopy to Turnitin for plagiarism checking.

Online CANVAS

In addition to the information contained in this laboratory manual, you are also required to log on to CANVAS website for downloading notes and presentation, data worksheets, announcement-checking as well as Q&A.

Teaching assistants (TA) evaluation

During specific practical sessions, you will be requested to provide us with important feedback on the performance of TAs involved in the teaching of the present course. The evaluation is done in the form of a simple questionnaire. However, you may also include written comments about the course.

LIFS 2720 - Introductory Biochemical Laboratory

A Guide to Laboratory Logbook Writing

A laboratory logbook is often regarded as an experimenter's diary containing all the information necessary for later reporting on an experiment in full. As such, it is considered to be the primary repository of experimental details and data. In order to inculcate a good laboratory practice, you are required to maintain a laboratory logbook for **ALL** practical sessions scheduled for the course of LIFS 2720. The laboratory logbook to be written for each experiment should include the following:

"Pre-Lab" Preparation

Prior to the attendance of each practical, you are required to read the laboratory manual to familiarize yourself with both the objectives and instructions of the experiments. This would allow you to plan and organize your experiment in advance so that the practical could be completed within the allocated period. Moreover, pre-lab preparation often minimizes the chances of making experimental errors. This is particularly important, as **there would be little room for experimental repetition!**

The "pre-lab" preparation is to be accompanied by a hand written account of the general procedural outline, which could be in the form of diagrams, calculations, tables for data entry, graph plotting as well as additional notes relevant to the experiment.

Experimental Procedures

Although the experimental procedures described in the laboratory manual distributed at the beginning of the course have been standardized, it is imperative at times to modify the experimental details on the day of the experiment. Any modifications or alterations of protocol made must be recorded in the laboratory logbook for later reference.

(DO NOT transcribe information directly from the laboratory manual into the logbook).

Experimental Observation and Data Entry

All information to be obtained during a practical session should be recorded directly into the laboratory logbook. This should include all experimental observations as well as data points generated during the course of the experiment. As subsequent writing of laboratory report is to be based on the information entered into the logbook, it is to be written in full accuracy and completeness with all data relevant to the experiment included.

Concluding Statement

You are very much encouraged to briefly inspect your data entries at the end of the experiment and to establish a concluding statement for the practical.

Evaluation

At the end of each practical session, the completed logbook must be presented to the allocated bench supervisor(s) for verification**. Failure to obtain an endorsement from the bench supervisors would result in a complete deduction of 1 mark for the particular practical**.

(As all sections of the logbook listed above (Pre-lab, experimental procedures, experimental observation and data entry and concluding statement) would be examined by the bench supervisors, you are encouraged to present the information in a concise and legible fashion.)

LIFS 2720 - Introductory Biochemical Laboratory

A Guide to Laboratory Report Writing

Each student is required to submit **ONE** pre-assigned full laboratory report as part of the assessment. The following guidelines should be considered during the preparation of a laboratory report.

Structure of a Laboratory Report

A laboratory report should be sub-divided into different sections using sub-headings so that information can be logically organized and presented. A basic laboratory report should include:

Abstract

An abstract is a concise single paragraph summary of your completed work. In brief, a reader can gain the rationale(s) behind the study, general approach to the problem, pertinent results, and important conclusions or new questions.

Writing an abstract

Summarize the study, including the following elements in any abstract. Try to keep the first two items to no more than one sentence each.

- Purpose of the study - hypothesis, overall question, objective
- Model organism or system and brief description of the experiment
- Results, including specific data - if the results are quantitative in nature, report quantitative data; results of any statistical analysis should be reported
- Important conclusions or questions that follow from the experiment(s)

Style:

- Single paragraph, and concise
- As a summary of work done, it is always written in past tense
- An abstract should stand on its own, and not refer to any other part of the paper such as a figure or table
- Focus on summarizing results - limit background information to a sentence or two, if absolutely necessary
- What you report in an abstract must be consistent with what you reported in the paper
- Correct spelling, clarity of sentences and phrases, and proper reporting of quantities (Proper units, significant figures) are just as important in an abstract as they are anywhere else

Introduction

The purpose of an introduction is to acquaint the reader with the rationale behind the work, with the intention of defending it. It places your work in a theoretical context, and enables the reader to understand and appreciate your objectives.

Writing an introduction

- The abstract is the only text in a research paper to be written without using paragraphs in order to separate major points. Approaches vary widely, however for our studies the following approach can produce an effective introduction.
- Describe the importance (significance) of the study - why was this worth doing in the first place? Provide a broad context.
- Defend the model - why did you use this particular organism or system? What are its advantages? You might comment on its suitability from a theoretical point of view as well as indicate practical reasons for using it.
- Provide a rationale. State your specific hypothesis(es) or objective(s), and describe the reasoning that led you to select them.
- Very briefly describe the experimental design and how it accomplished the stated objectives.

Style:

- Use past tense except when referring to established facts. After all, the paper will be submitted after all of the work is completed.
- Organize your ideas, making one major point with each paragraph. If you make the four points listed above, you will need a minimum of four paragraphs.
- Present background information only as needed in order support a position. The reader does not want to read everything you know about a subject.
- State the hypothesis/objective precisely - do not oversimplify.
- Pay attention to spelling, clarity and appropriateness of sentences and phrases.

Materials and Methods

This should be the easiest section to write, but many students misunderstand the purpose. The objective is to document all specialized materials and general procedures, so that another individual may use some or all of the methods in another study or judge the scientific merit of your work. It is not to be a step by step description of everything you did, nor is a section of a set of instructions. In particular, it is not supposed to tell a story. By the way, your notebook should contain all of the information that you need for this section.

Writing a materials and methods section

Materials:

- Describe materials separately only if the study is so complicated in order to save space.
- Include specialized chemicals, biological materials, and any equipment or supplies that are not commonly found in laboratories.
- Do not include commonly found supplies such as test tubes, pipet tips, beakers, etc., or standard lab equipment such as centrifuges, spectrophotometers, pipettors, etc.

- If use of a specific type of equipment, a specific enzyme, or a culture from a particular supplier is critical to the success of the experiment, then it and the source should be pointed out, otherwise no.
- Materials may be reported in a separate paragraph or else they may be identified along with your procedures.
- In biosciences we frequently work with solutions - refer to them by name and describe completely, including concentrations of all reagents, and pH of aqueous solutions, solvent if non-aqueous.

Methods:
- See the examples in the lab manual.
- Report the methodology in a concise paragraph using the given bullet points.
- To be concise, present methods under headings devoted to specific procedures or groups of procedures.
- Be generalize - report how procedures were done, not how they were specifically performed on a particular day. For example, report "samples were diluted to a final concentration of 2 mg/mL protein;" instead of reporting that "135 microliters of sample one was diluted with 330 microliters of buffer to make the protein concentration 2 mg/mL." Always think about what would be relevant to an investigator at another institution, working on his/ her own project.
- If well-documented procedures were used, report the procedure by name, perhaps with reference. For example, the Bradford assay is well known. You need not report the procedure in full - just that you used a Bradford assay to estimate protein concentration, and identify what you used as a standard.

Style:
- It is awkward or impossible to use active voice when documenting methods without using first person, which would focus the reader's attention on the investigator rather than the work. Therefore when writing up methods, most authors use third person passive voice.
- Use normal prose in this and in every other section of the paper - avoids informal lists, and use complete sentences.

Results

The purpose of a result section is to present and illustrate your findings. Make this section a completely objective report of the results, and save all interpretations for the discussion.

Writing a result section

Content
- Summarize your findings in text and illustrate them, if appropriate, with figures and tables.
- In text, describe each of your results, pointing the reader to observations that are most relevant.
- Provide a context, such as by describing the question that was addressed by making a particular observation.
- Describe results of control experiments and include observations that are not presentedin a formal figure or table, if appropriate.
- Analyze your data, then prepare the analyzed (converted) data in the form of a figure such as a graph, table, or in text form.

Style

- As always, use past tense when you refer to your results, and put everything in a logical order.
- In text, refer to each figure and/or table as "Figure/Table 1," "Figure/Table 2," *etc*.
- Place figures and tables, properly numbered, generates an important reference point in the later discussion.
- If you prefer, you may place your figures and tables appropriately within the text of your results section.

Figures and tables

- Either place figures and tables within the text of the result, or include them in the back of the report
- If you place figures and tables at the end of the report, make sure they are clearly distinguished from any attached appendix materials, such as raw data
- Regardless of placement, each figure must be numbered consecutively and complete with caption (caption goes under the figure)
- Regardless of placement, each table must be titled, numbered consecutively and complete with heading (title with description goes above the table)
- Each figure and table must be sufficiently completed in a way that it could stand on its own separate from text

Discussion

The objective here is to provide an interpretation of your results and support all of your conclusions, using evidence from your experiment and generally accepted knowledge, if appropriate. The significance of findings should be clearly described.

Writing a discussion

Interpret your data in the discussion in appropriate depth. This means that when you explain a phenomenon you must describe mechanisms that may account for the observation. If your results differ from your expectations, explain why that may have happened. If your results agree, then describe the theory that the evidence supported. It is never appropriate to simply state that the data agreed with expectations, and let it drop at that.

- Decide if each hypothesis is supported, rejected, or if you cannot make a decision with confidence,do not simply dismiss a study or part of a study as "inconclusive".
- Reports are not accepted if the work is lack of a conclusion.
- You may suggest future directions, such as how the experiment might be modified to accomplish another objective.
- Explain all of your observations as much as possible, focusing on mechanisms.
- Decide if the experimental design adequately addressed the hypothesis, and whether or not it was properly controlled.
- Try to offer alternative explanations if reasonable alternatives exist.
- One experiment will not answer an overall question, so keeping the big picture in mind, where do you go next?
- Recommendations for specific papers will provide additional suggestions.

Style:

- When you refer to information, distinguish data generated by your own studies from published information or from information obtained from other students.
- Refer to work done by specific individuals (including yourself) in past tense.
- Refer to generally accepted facts and principles in present tense. For example, "Doofus, in a 1989 survey, found that anemia in basset hounds was correlated with advanced age. Anemia is a condition in which there is insufficient hemoglobin in the blood".

The biggest mistake that students make in discussions is to present a superficial interpretation that more or less re-states the results. It is necessary to suggest why results came out as they did, focusing on the mechanisms behind the observations.

Literature Cited

Please note that in the introductory laboratory course, you will not be required to properly document sources of all of your information. One reason is that your major source of information is likely to be obtained from various websites, and they are inappropriate as primary sources. Second, it is problematic to provide a hundred students with equal access to potential reference materials. You may nevertheless find outside sources, and you should cite any articles that the instructor provides or that you find for yourself.

List all literature cited in your paper, in alphabetical order, by first author. In a proper research paper, only primary literature is used (original research articles authored by the original investigators). Be cautious about using websites as references - anyone can put just about anything on a website, and you have no certain way of knowing if it is truth or fiction. If you are citing an on line journal, use the journal citation (name, volume, year, page numbers). Some of your papers may not require references, and if that is the case simply state that "no references were consulted".

Academic Misconduct:

Plagiarism is a serious form of academic misconduct. It is defined as the presentation of work which actually originates from other sources as one’s own. Students who take the use of other lab reports as his / her own will be seriously dealt with.

Warning: Plagiarism is strictly prohibited!

LIFS 2720 - Introductory Biochemical Laboratory

Laboratory Disciplines

You are required to read and follow the following laboratory safety rules. It must be stressed that the following rules are mandatorily enforced in the laboratory. Failure to observe such rules will be penalized by subtracting a fraction of points from the total score for each violation.

Attitude and Practice

1. Punctuality: You are required to arrive at the laboratory within the first 5 minutes of the scheduled practical session.

2. Attendance: Full attendance is expected throughout the entire duration of the practical session. No early departure is permitted without the consent of the principle instructor. If you were absent three or more practical among Practical 1 to 9, you will fail this practical course LIFS2720.

3. Food and drinks are strictly prohibited in the laboratory.

4. You are not expected to use cellular phone during the practical session. If necessary, you may use cellular phone at all the way outside the laboratory.

5. Absolute prohibition of mouth pipetting. Pipette bulbs and measuring cylinders will be provided and you are expected to make full use of them.

6. You are required to follow the experimental procedures as described in the laboratory manual to avoid misuse of laboratory equipments.

Personal Protection

1. Laboratory coat with your name card must be worn at all times in the laboratory.

2. You are advised to cover the lower part of your body to as far as possible to minimize danger against chemical spillage. i.e., **no shorts, mini-skirts, sandals**, **slippers** *etc*.

3. Wear protective gloves when handling hazardous materials.

4. Long loose hair must be tied up to minimize contact with corrosive chemicals, *etc*.

Waste Disposal and Cleaning-up procedures

1. Never pour inflammable liquid or water-insoluble organic solvent down the sink.

2. Dispose laboratory wastes separately in proper containers such as those labeled as biohazardous materials, halogenated organic wastes, non-halogenated organic wastes, *etc*.

3. Do not place broken glass in waste paper basket but in specified broken glass container.

4. You are required to report on any cases of broken glassware or chemical spillage to a member of the teaching team.

5. Working bench must be cleaned at the end of each experiment.

6. All equipments and apparatus used during the course of the experiment must be cleaned according to instructions. You are responsible for your own laboratory glassware and are expected to clean and keep them in your designated locker.

Laboratory technicians and teaching assistants will undertake the responsibility of supervising the performance of students. You are expected to ask for advice if necessary during the course of the experiment. Towards the end of the laboratory session, you are required to present your laboratory logbook for evaluation and signature.

Penalty for Absentees

Students enrolled for LIFS 2720 are required to attend all sessions of the laboratory practical scheduled in the laboratory manual. As the method of assessment for the course is based on continuous evaluation, absence from any one of the practicals would certainly have a negative effect on your overall grade.

For absences due to medical reasons, the student is required to submit a copy of the medical certificate issued by a registered doctor. For absences due to other non-medically related causes, students would be required to contact the course instructor and provide a relevant documentation to request for an advanced approval of exemption for the particular laboratory session.

Absences with prior approval or supported by a medical certificate will not be scored for lab performance. However, a written laboratory worksheet and/or report for the unattended practical should be submitted for assessment.

LIFS 2720 - Introductory Biochemical Laboratory

Practical Outlines and List of Teaching Assistants (Fall 2017-2018)

Instructor:	Dr. Helen CHEUNG	cheungh@ust.hk	Ext 7904	Room 5502B
Technicians	Mr. Kelvin SOU	bckasou@ust.hk		
	Mr. Marcus YAN	marcus@ust.hk		
	Ms. Joanne CHAN	lnchan@ust.hk		
	Miss Hoi Yan LEUNG	hoiyan@ust.hk		

Day & Time: Thursday (13:00-16:50)

Venue: Room 4160

Week/ Date	Lectures	Technician in-charge
Practical 00 7 Sep 2017	INTRODUCTION TO LIFS 2720 Briefing, check-out lab wares	-
Practical 01 14 Sep 2017	BUFFERS, TITRATIONS AND pH MEASUREMENT **(Please bring laptop [one per group] for data analysis)**	JC
Practical 02 21 Sep 2017	CHROMATOGRAPHY I AMINO ACID SEPARATION AND IDENTIFICATION	KS
Practical 03 28 Sep 2017	CHROMATOGRAPHY II GEL FILTRATION COLUMN CHROMATOGRAPHY **(Please bring laptop [one per group] for data analysis)**	ALL
Practical 04 12 Oct 2017	CHROMATOGRAPHY III SERUM ELECTROPHORESIS USING CELLULOSE ACETATE	MY
Practical 05 19 Oct 2017	SPECTROPHOTOMETRY ENZYME KINETICS: LACTATE DEHYDROGENASE **(Please bring laptop [one per group] for data analysis)**	KS
Practical 06 26 Oct 2017	ORGANELLE ISOLATION BY CENTRIFUGATION AND MARKER ENZYME ASSAY	JC
Practical 07 2 Nov 2017	DNA MELTING CURVE **(Please bring laptop [one per group] for data analysis)**	MY
Practical 08 9 Nov 2017	METABOLIC SYNDROME (PART 1) GLUCOSE	HYL
Practical 09 16 Nov 2017	METABOLIC SYNDROME (PART 2) LIPID	HYL

Time Schedule for hand-in worksheet and report:

Practical	Worksheet	Report
1	21 Sep 2017 (Thu)	-
2	28 Sep 2017 (Thu)	-
3	**6 Oct 2017 (Fri)**	12 Oct 2017 (Thu)
4	12 Oct 2017(Thu)	19 Oct 2017 (Thu)
5	26 Oct 2017 (Thu)	-
6	2 Nov 2017 (Thu)	9 Nov 2017 (Thu)
7	9 Nov 2017 (Thu)	-
8	16 Nov 2017 (Thu)	**23 Nov 2017 (Thu)**
9	**23 Nov 2017 (Thu)**	**30 Nov 2017 (Thu)**

Note:

1. The deadline for handing in the worksheet/ report is at 13:30 on the above indicated date of each practical at teaching lab (Rm4160).

2. Any worksheet/ report handed in later than 13:30, but on or before 16:50 on the indicated date of each practical **will be marked down by 30%**.

3. Please pay attention to the **BOLD** date in the above table, on those **BOLD** dates, please hand in your worksheet/ report to LIFS General Office during office hours (09:00-12:00 and 14:00-17:00). There will be two collection times (14:30 and 17:00) and **the above point no. 2 still be applied to this situation.**

4. Any worksheet/ report handed in later than the deadline will still be marked by TA, however your mark of that worksheet/ report will be **ZERO**. The marked worksheet/ report will still be useful for you to prepare your examination!

5. We will return the marked report/ worksheet to you one week after you handed in (the above indicated deadline), and if there is any question about the marking, you have one week to contact the in-charged TA of that practical. And all mark will be finalized after that period of time (a week after distribution), and TA has right to reject any mark correction after that period of time.

6. If you have any special reason for early/ late hand-in, please contact LIFS 2720 instructor in advance.

Section B

Experimental Protocol

LIFS 2720
INTRODUCTORY BIOCHEMICAL LABORATORY

Experiment 1
BUFFERS, TITRATIONS AND pH MEASUREMENT

Introduction

Many biologically important molecules contain acidic or basic groups that donate or accept ions resulting in a change of pH. Acidic groups, such as carboxylic, phenolic, and phosphoric acid groups, are converted to anionic groups at pH levels above their pK_a values. The charge on a molecule profoundly affects its chemical and biological properties; hence controlling solution pH is essential if one expects predictable and reproducible behavior from a biomolecule.

A buffer solution is an aqueous solution consisting of a mixture of a weak acid and its conjugate base or a weak base and its conjugate acid. It has the property of maintaining pH of solutions within narrow ranges upon addition of a small amount of strong acid or base. Preparation of buffers belongs to the list of basic techniques that a biochemist must master. A biochemist must know the acid and base properties of common buffer components in order to select buffer formulations that will govern pH over the desired range as well as to maintain their compatibilities with the chemical properties of all other solution components.

Background

Brønsted & Lowry

A conjugate acid is defined as a proton donor: a molecule contains a hydrogen atom (H) which can be released as a hydrogen ion (H^+).

A conjugate base is defined as a proton acceptor: a molecule can form a covalent bond with a hydrogen ion.

Example: the molecule acetic acid, CH_3COOH, is the conjugate acid, while acetate ion, $CHCOO^-$ is the conjugate base.

For 'Pure' H_2O,

$$2\ H_2O \longleftrightarrow OH^- + H_3O^+$$

$$KW = 10^{-14}$$

……..*Equation 1*

(K_W - Ionization constant for H_2O)

H_2O is such a strong base that in aqueous systems, free protons do not exist at all in significant concentrations and hydrogen ion concentration really means hydronium ion (H_3O^+) concentration.

$$pH = -\log[H_3O^+];\ pOH = -\log[OH^-];\ \text{and}\ pH + pOH = 14$$

……..…..*Equation 2*

For a carboxylic acid,

$$RCOOH + H_2O \longleftrightarrow RCOO^- + H_3O^+$$

Conjugate Acid **Conjugate Base**

$$Ka = \frac{[RCOO^-][H_3O^+]}{[RCOOH]}$$

……..*Equation 3*

(K_a - Dissociation constant of acid)

For a primary amine,

$$RNH_{3+} + H_2O \longleftrightarrow RNH_2 + H_3O^+$$

Conjugate Acid **Conjugate Base**

$$Ka = \frac{[RNH_2][H_3O^+]}{[RNH_{3+}]}$$

…….……..*Equation 4*

In either case, $pK_a = -\log [K_a]$

Henderson-Hasselbalch equation

$$HA \longleftrightarrow A^- + H^+$$

$$Ka = \frac{[A^-][H^+]}{[HA]}$$

$$pH = pK_a + \log \frac{[A]}{[HA]}$$

……...……..*Equation 5*

where A is the conjugate base and HA is the conjugate acid.

This equation can be used to calculate the pH of a buffer solution if given the concentration and pK_a value of the appropriate buffer components.

Titration curves

When a strong acid such as HCl is added to water, it is completely dissociated to give H_3O^+ ions. When HCl is added to a solution of weak base (e.g. sodium acetate or imidazole), the pH does not fall as much as when HCl is added to water because H_3O^+ reacts with the conjugate base A to form undissociated HA. If the pH values are plotted against the total added volume of HCl solution (with the pH along the ordinate), a titration curve can be generated.

The equivalent point or stoichiometric point of a titration (V_E) is the point in a titration where the amounts of titrant and solution being titrated are equivalent chemically. The pK_a value corresponds to the pH at which the acid and base forms of the titrated species are in equal concentration. This corresponds to the pH observed at one-half equivalent volume $V_E/2$. The pK_a of the titrated species can be determined from the titration curve as illustrated in Figure 1.1.

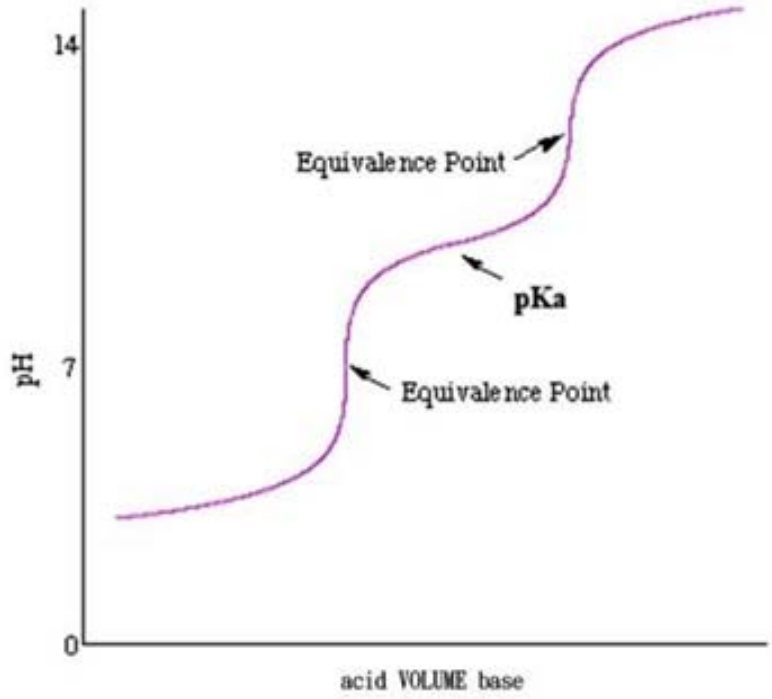

Figure 1.1 Titration Curve

A useful reference link for titration curves of amino acids provided below.

http://cti.itc.virginia.edu/~cmg/Demo/compareAA/compareAAApplet.html