การหาค่าสัมประสิทธิ์การแบ่งส่วนและค่าคงที่การแตกตัวของกรดอ่อนที่ไม่มีประจุโดยการไทเทรตครั้งเดียว

เรืออากาศเอกหญิง นิสา ภู่ทอง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชเคมี ภาควิชาอาหารและเภสัชเคมี
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2551

DETERMINATION OF PARTITION COEFFICIENTS AND DISSOCIATION CONSTANTS OF NEUTRAL WEAK ACIDS IN SINGLE TITRATION

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A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Pharmacy Program in Pharmaceutical Chemistry

Department of Food and Pharmaceutical Chemistry

Faculty of Pharmaceutical Sciences

Chulalongkorn University

Academic Year 2008

Thesis Title	DETERMINATION OF PARTITION COEFFICIENTS AND DISSOCIATION CONSTANTS OF NEUTRAL WEAK ACIDS IN SINGLE TITRATION
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Field of Study	Pharmaceutical Chemistry
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นิสา ภู่ทอง : การหาค่าสัมประสิทธิ์การแบ่งส่วน และค่าคงที่การแตกตัวของกรดอ่อนที่ไม่มีประจุโดยการไทเทรตครั้งเดียว (DETERMINATION OF PARTITION COEFFICIENTS AND DISSOCIATION CONSTANTS OF NEUTRAL WEAK ACIDS IN SINGLE TITRATION) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ. ดร.มิตร ปทีปวณิช, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : อ. ดร.บดินทร์ ติวสุวรรณ, 121 หน้า.

ค่าพารามิเตอร์สามค่าได้แก่ จุดสมมูลหรือค่าความบริสุทธิ์ ค่าคงที่การแตกตัว และค่าสัมประสิทธิ์การแบ่งส่วนของกรดอ่อนหนึ่งโปรตอนที่ไม่มีประจุในการไทเทรตครั้งเดียว คำนวณได้ด้วยสมการความถดถอยแบบหลายตัวแปรเชิงเส้นกับข้อมูลจำลองการไทเทรต โดยใช้โปรแกรมคอมพิวเตอร์สามโปรแกรม (Excel®2007, Wessa (2009) และ MINITAB®15) และนำผลที่ได้มาเปรียบเทียบกัน ในหลายกรณีการวิเคราะห์ข้อมูลจำลองการทดลองให้ผลไม่ถูกต้อง อาจจะเป็นผลมาจากการกำหนดลำดับขั้นตอนของคำสั่งและ/หรือข้อจำกัดของโปรแกรมที่ต่างกัน สมการถดถอยแบบตัวแปรเดียวเชิงเส้นสองสมการถูกดัดแปลงขึ้นมาเพื่อหาค่าจุดสมมูลของการไทเทรตและค่าสัมประสิทธิ์การแบ่งส่วนจากการไทเทรตที่มีสองวัฏภาค จากสองสมการที่ถูกดัดแปลงร่วมกับสมการของแกรนสามารถคำนวณจุดสมมูลหรือค่าความบริสุทธิ์ ค่าคงที่การแตกตัว และค่าสัมประสิทธิ์การแบ่งส่วนของกรดอ่อนหนึ่งโปรตอนที่ไม่มีประจุได้ในการทดลองครั้งเดียว ในการหาค่าพารามิเตอร์เหล่านี้ของกรดอ่อนห้าชนิด ได้แก่ กรดอะซีติก กรดเบนโซอิก กรด 2-เมทอกซีเบนโซอิก กรดซาลิซิลิก และกรดเมตา-โทลูอิก โดยใช้วิธีอ้างอิงและวิธีโพเทนชิโอเมทรี ผลที่ได้แสดงให้เห็นว่าวิธีนี้สามารถใช้ได้ดีและมีประโยชน์ในการประหยัดเวลา

ภาควิชา.....อาหารและเภสัชเคมี..... ลายมือชื่อนิสิต.....
สาขาวิชา...เภสัชเคมี..... ลายมือชื่ออ. ที่ปรึกษาวิทยานิพนธ์หลัก.....
ปีการศึกษา .....2551…… ลายมือชื่ออ. ที่ปรึกษาวิทยานิพนธ์ร่วม.....

4976574433 : MAJOR PHARMACEUTICAL CHEMISTRY
KEYWORDS : POTENTIOMETRIC TITRATION/ MULTIPLE LINEAR REGRESSION/ EQUIVALENT POINT/ PARTITION COEFFICIENT/ DISSOCIATION CONSTANT/ SIMULATED TITRATION DATA/ MODIFIED GRAN EQUATION

NISA PHUTONG: DETERMINATION OF PARTITION COEFFICIENTS AND DISSOCIATION CONSTANTS OF NEUTRAL WEAK ACIDS IN SINGLE TITRATION. THESIS ADVISOR :
ASST. PROF. MITR PATHIPVANICH, Ph.D., THESIS CO-ADVISOR :
BODIN TUESUWAN, Ph.D., 121 pp.

The three parameters, equivalent point or purity, dissociation constant and partition coefficient, were calculated applying multiple linear regression equation to simulated titration data by three computer programs (Excel® 2007, Wessa (2009) and MINITAB® 15) and all obtained results were compared. In many cases, analysis of simulated data yield inaccurate results, possibly due to programming algorithms. The two modified simple linear regression equations were derived for determination of equivalent point of titration and partition coefficient using titration data from dual phase titration. The two modified simple linear equations, together with Gran equation, can be employed to determine equivalent point or purity, dissociation constant and partition coefficient of weak monoprotic acid from one single titration experiment. The obtained parameters of five weak monoprotic acids, acetic acid, benzoic acid, *2*-methoxybenzoic acid, salicylic acid and *m*-toluic acid, were determined using reference method and potentiometric methods. These results suggest that this method is effective and useful as its saving time.

Department:...Food and Pharmaceutical Chemistry..... Student's Signature: .....
Field of Study:...Pharmaceutical Chemistry..... Advisor's Signature:.....
Academic Year:…..2008…… Co-Advisor's Signature:.....

ACKNOWLEDGEMENTS

With the submission of this manuscript I am hanging up my lab coat and gloves. Although my future work may steer me away from the world of Chemistry, I will look back on my time as a graduate student with great memories regarding experience, people and the pursuit of finding things out. I am very fortunate to be surrounded by people willing their time, to provide encouragement, and support.

I was extraordinarily fortunate in having Assistant Professor Dr. Mitr Pathipvanich, my professor in Chulalongkorn University, who gave me an opportunity to work with him as master student in his research group. I would like to thank him for his understanding, patience, support and very kind help during the difficult time of my studies. Very often I kept him very busy with many issues regarding my work and its progress. He was the main creator of the great ideas, techniques and whole background of this thesis. I could never have embarked and started all of this without his prior teachings in Chemistry and thus opened up unknown areas to me. Thank you very much. I really appreciate.

I would like to thank Dr. Bodin Tuesuwan, my co-advisor, for his support, willing time, valuable advice, kindness and understanding throughout this research.

I would also like to thank Assistant Professor Dr. Chamnan Patarapanich, Assistant Professor Dr. Walaisiri Muangsiri and Assistant Professor Dr. Chaiyavat Chaiyasut for their kind suggestions and discussions to complete this thesis.

My life at the department was extremely facilitated by the work and friendship with students in the research group. Many thanks to you all. I would also like to express my appreciation to the staffs of the Pharmaceutical Chemistry Department for their advice and help.

Outside the doors of the university are also many people who have encouraged and supported this journey of mine. I have to especially thank the Directorate of Medical Services, Royal Thai Air Force for giving me a chance to pursuit my graduate study and financial support. This thesis would never have been completed without the love and encouragement from my family. Their tremendous love and support during the time I pursued my master degree have always been a source of strength and inspiration.

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aq	Aqueous phase
^{o}C	Celsius degree
Eq.	Equation
$[H_3O^+]$	Concentration of hydronium ion (mole/L)
$[HA]_0$	Initial concentration of HA (mole/L)
$[HA]_{aq}$	Concentration of HA in aqueous phase (mole/L)
$[HA]_{oct}$	Concentration of HA in octanol phase (mole/L)
HA	Neutral weak monoprotic acid
g	Gram
K_a	Dissociation constant of acid
K_w	Dissociation constant of water
L	Litr
M	Molarity (mole/L)
ml	Milliliter
meq	Milliequivalent
N	Normality of titrant (meq/ml)
oct	Octanol phase
P	Partition coefficient
R	The ratio of volume octanol to aqueous
R^2	Correlation of determination
Wt	Weight (g)
V	Volume of titrant (ml)
V_a	Initial volume of weak acid solution (ml)
V_e	Volume of titrant at end point (ml)
V_{oct}	Volume of octanol added (ml)

CHAPTER I

INTRODUCTION

Knowledge of the dissociation constant, K_a or pK_a, and lipophilicity in the term of partition coefficient, P or log P, of substances is a primary concern in pharmaceutical sciences. In pharmaceutical researches, pK_a and log P can be used for prediction of their pharmacokinetics properties, i.e., absorbance, distribution, metabolism, elimination and toxicity (Poole, 2003; Wiczling, 2006). Each parameter is usually determined in a completely separate experiment by various methods.

Many researchers have their own preferred method for measuring each parameter but there are no single method is suitable under all circumstances. There was an interesting method that could determine parameter P by potentiometric method, pH-metric method, using known pK_a. The dissociation constants calculated from aqueous and octanol-aqueous titration were different. The difference was used for partition coefficient determination (Kaliszan, 2002; Wiczling, 2006; Seiler, 1974; Kaufman, 1975; Clarke, 1984; Hersey, 1989; Clarke and Cahoon, 1996; The Organization for Economic Co-operation and Development, 2000).

Potentiometric method is a highly precise and accurate technique with simple to achieve and fast (Babic et al., 2007). The purities of substances could be determined from equivalent points of their titrations. It is a challenge to design an experimental procedure which would allow us to simultaneously determined purity, K_a and P in single experiment. We have derived multiple linear regression equation which theoretically would allow us to determined purity, K_a and P of substances in single aqueous-octanol system titration.

Based on only unionized species could partition into the octanol phase and the dissociation constant of water, K_w, was constant along the titration. The concentration of unionized species presence in octanol phases depended on its partition coefficient. Meanwhile in aqueous phase, the concentration of unionized species depended on its partition coefficient and dissociation constant. The equilibrium of neutral weak acid, HA, was shown in Eq. 1.

$$HA_{oct} \underset{\longrightarrow}{\overset{P}{\longleftarrow}} HA_{aq} \underset{\longrightarrow}{\overset{K_a}{\longleftarrow}} H^+ + A^- \qquad \text{(Eq. 1)}$$

Octanol phase Aqueous phase

The dissociation constant in aqueous phase is given by

$$K_a = \frac{[A^-][H^+]}{[HA]_{aq}} \qquad \text{(Eq. 2)}$$

and the partition coefficient is given by

$$P = \frac{[HA]_{oct}}{[HA]_{aq}} \qquad \text{(Eq. 3)}$$

Then $[HA]_{oct}$ = concentration of HA in octanol phase.

$[HA]_{aq}$ = concentration of HA in aqueous phase.

Consider the titration of weak acid, HA by strong base, NaOH,

$$NaOH \longrightarrow Na^+ + OH^-$$

$$HA + OH^- \rightleftharpoons A^- + H_2O$$

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

$$HA + H_2O \rightleftharpoons H_3O^+ + A^-$$

When a neutral weak acid was titrated with strong base, unionized species in aqueous phase were ionized and its concentration decreased. More ionized species were pulled out of the octanol phase to maintain equilibrium between two phases. Based on charge balance, mass balance and equilibrium equation in aqueous-octanol system, the equation were derived as follows below:

Mass balance

$$[HA]_o V_a = V_e N = [HA]_{oct} V_{oct} + [HA]_{aq}(V_a+V) + [A^-](V_a+V) \qquad \text{(Eq. 4)}$$

Volumes are denoted by V, which

$[HA]_o$ = Initial concentration of HA (mole/L)

V_e = volume of titrant at equivalent point

V_a = initial volume of aqueous phase

V_{oct} = octanol volume added

V = cumulated titrant volume added

N = normality of titrant

Substitution Eq. 3 into Eq. 4 and assigning $R = V_{oct}/ V_a$

$$[HA]_o V_a = V_e N = P[HA]_{aq} RV_a + [HA]_{aq}(V_a+V) + [A^-](V_a+V)$$

$$= [HA]_{aq}\{PRV_a + (V_a+V)\} + [A^-](V_a+V)$$

$$[HA]_{aq} = \frac{V_e N - [A^-](V_a+V)}{\{PRV_a + (V_a+V)\}} \quad \text{(Eq. 5)}$$

Charge balance

$$[Na^+] + [H_3O^+] = [A^-] + [OH^-] \quad \text{(Eq. 6)}$$

Substitution $[Na^+] = \frac{VN}{(V + V_a)}$ into Eq. 6

$$\frac{VN}{(V + V_a)} + [H_3O^+] = [A^-] + [OH^-] \quad \text{(Eq. 7)}$$

$$[A^-] = \frac{VN}{(V + V_a)} + [H_3O^+] - [OH^-] \quad \text{(Eq. 8)}$$

Substitution Eq. 5, Eq. 8 into Eq. 2 gave

$$K_a = \frac{\left\{ \frac{VN}{(V + V_a)} + [H_3O^+] - [OH^-] \right\} [H_3O^+]}{\frac{V_e N - [A^-](V_a+V)}{\{PRV_a + (V_a+V)\}}} \quad \text{(Eq. 9)}$$

Substitution Eq. 8 into Eq. 9 gave

$$K_a = \frac{\dfrac{\left\{ VN + \{[H_3O^+] - [OH^-]\}(V_a+V) \right\}[H_3O^+]}{(V+V_a)}}{V_eN - \dfrac{\left\{ VN + \{[H_3O^+] - [OH^-]\}(V_a+V) \right\}(V_a+V)}{(V+V_a)}} \qquad \text{(Eq. 10)}$$

$$\{PRV_a + (V_a+V)\}$$

Let $G = VN + \{[H_3O^+] - [OH^-]\}(V_a+V)$

Then rearrangement gave

$$K_a = \frac{\dfrac{G\,[H_3O^+]}{(V_a+V)}}{\dfrac{V_eN - G}{\{PRV_a + (V_a+V)\}}} = \frac{G\,[H_3O^+][PRV_a + (V_a+V)]}{(V_eN - G)(V_a+V)} \qquad \text{(Eq. 11)}$$

$$K_a(V_eN - G)(V_a+V) = G\,[H_3O^+][PRV_a + (V_a+V)] = G\,[H_3O^+][(PR+1)V_a + V]$$

$$K_aV_eN(V_a+V) - K_aG(V_a+V) = G\,V_a[H_3O^+](PR+1) + GV[H_3O^+]$$

Divided both sides by (V_a+V) gave

$$K_aV_eN - K_aG = \frac{G\,V_a\,(PR+1)\,[H_3O^+]}{(V_a+V)} + \frac{GV[H_3O^+]}{(V_a+V)} \qquad \text{(Eq. 12)}$$

$$\frac{GV[H_3O^+]}{(V_a+V)} = K_aV_eN - K_aG - \frac{(PR+1)\,G\,V_a\,[H_3O^+]}{(V_a+V)} \qquad \text{(Eq. 13)}$$

Eq. 13, it is a multiple linear regression model with two independent variables, $Y = a_0 + a_1X_1 + a_2X_2$. The dependent variable was $GV[H_3O^+]/(V_a+V)$, independent variables, X_1, was G, and X_2 was $GV_a[H_3O^+]/(V_a+V)$. The regression coefficients, a_0 was K_aV_eN, a_1 was $-K_a$ and a_2 was $-(PR+1)$. By regression model fitting, equivalent point, dissociation constant and partition coefficient were calculated from regression coefficients, a_0, a_1 and a_2, respectively.

In theory, Eq. 13 would allow us to simultaneously determine all purity (V_e), dissociation constant (K_a), and partition coefficient (P) from one single experiment. However, we must first test Eq. 13 with simulated titration data to make certain that Eq. 13 can be used for calculation all three parameters under ideal condition. Three software packages (EXCEL® 2007, WESSA (2009) and MINITAB® 15) were chosen for analyzing the simulated data to test their computation ability in yielding the correct theoretical values.

CHAPTER II

LITERATURE REVIEWS

Commonly, the dissociation constant (K_a), equivalent point (V_e) and partition coefficient (P) are often determined from separate experimentation procedures. Many researchers have successfully shown than K_a and V_e can be accurately determined with potentiometric titration while shake flask method is often used in determination of P.

Potentiometric Titration

Potentiometric titration is the method which involves measuring the potential or pH of solution by suitable indicator electrode as a function of titrant volume (Christian, 1986; Skoog, West, and Holler, 1996). This technique is a simple, fast, accurate and reproducible analytical method (Rouessac and Rouessac, 2007; Babic, 2007). From the titration data, determination of the equivalent volume and the dissociation constant can be achieved by various methods.

1. Determination of Equivalent Point

1.1 Methods Based on the Sigmoid Form of the Titration Curve.

The equivalent point will be located on the steeply rising portion of the titration curve. This curve must show a very clearly marked steep portion at the equivalent region and then an approximate value of the equivalent volume will be given. Thus, a titration curve of weak acid which has poorly defined inflection point, will give an unreasonable end point value (Tubbs, 1954; Alfalt and Jagner, 1971; Jeffery et al., 1989; Skoog, Holler, and Crunch, 2007).

1.2 Differential Methods

These methods can be used to determine the equivalent volume by plotting the change in potential or pH per unit volume of titrant ($\Delta E/\Delta V$). A plot of these data as a function of the average volume of titrant added produces a curve with maximum that responds to the end point of inflection (Skoog, 1996). The assumption is then made that this function is a maximum at the equivalent point by the first derivative method. For the second derivative method, the equivalent point can be determined by estimating the point where the second derivative of the voltage with respect to the volume ($\Delta^2 E/\Delta V^2$) becomes zero (Robert, 2001; Skoog, 2007)

1.3 Gran Method

In 1952, Gran proposed a graphical equivalent detection method using linear regression analysis to data before the equivalent point. Gran plot, a better alternative method to detect the equivalent point, does not need the data point near the end point.

The advantages of using Gran's method are simplicity of measurement, simplicity of calculation, versatility and precision. (Gran, 1952; Rossotti and Rossotti, 1965; Ingman and Still, 1966; Arttamangkul, 1986; and Sukbuntherng, 1988)

The equivalent point can be determined by extrapolation of straight line before equivalent point. Gran equation was derived from basis on charge balance, mass balance and equilibrium equation for fitting titration curves of neutral weak acid. When neutral weak acid, HA, was titrated with sodium hydroxide, NaOH, there are dissociated species as the conjugate base, A^-, hydroxide ion, OH^-, and hydronium ion, H_3O^+, in the solution. The chemical reactions are as follows below:

$$NaOH \longrightarrow Na^+ + OH^-$$

$$HA + OH^- \rightleftharpoons A^- + H_2O$$

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

$$HA + H_2O \rightleftharpoons H_3O^+ + A^-$$

Dissociation reaction, K_a, of weak acid was

$$K_a = \frac{[A^-][H_3O^+]}{[HA]} \qquad \text{(Eq.14)}$$

The square brackets mean for concentration (mole/L).

Charge balance

$$[Na^+] + [H_3O^+] = [A^-] + [OH^-] \qquad \text{(Eq.15)}$$

At equivalent point

$$V_eN = (V_a + V)[HA]_0 \qquad \text{(Eq.16)}$$

Where V_e = volume of base equivalent to weak acid, called equivalent volume

N = normality of titrant

V_a = the initial volume of the solution

V = volume of titrant added.

$[HA]_0$ = the initial concentration of weak acid

The concentration of sodium ion at any volumes of titrant was

$$[Na^+] = \frac{VN}{(V + V_a)} \quad \text{(Eq.17)}$$

Mass balance

$$[HA]_0 = [HA] + [A^-] \quad \text{(Eq.18)}$$

Combining Eq.15 and Eq.17 gave

$$[A^-] = \frac{VN}{(V + V_a)} + [H_3O^+] - [OH^-] \quad \text{(Eq.19)}$$

Substitution of Eq.19 into Eq.18 and combine it with Eq.16 gave

$$\frac{V_eN}{(V + V_a)} = [HA] + \frac{VN}{(V + V_a)} + [H_3O^+] - [OH^-]$$

$$[HA] = \frac{V_eN}{(V + V_a)} - \left\{ \frac{VN}{(V + V_a)} + [H_3O^+] - [OH^-] \right\} \quad \text{(Eq.20)}$$

Substitution of Eq.19 and Eq.20 into Eq.14 gave

$$K_a = \frac{[H_3O^+]\left\{\frac{VN}{(V+V_a)} + [H_3O^+] - [OH^-]\right\}}{\frac{V_eN}{(V+V_a)} - \left\{\frac{VN}{(V+V_a)} + [H_3O^+] - [OH]\right\}}$$

$$K_a = \frac{[H_3O^+]\left\{\frac{VN + \{[H_3O^+] - [OH^-]\}(V_a+V)}{(V+V_a)}\right\}}{\frac{V_eN - \left\{VN + \{[H_3O^+] - [OH^-]\}(V_a+V)\right\}}{(V_a+V)}}$$

Let $G = VN + \{[H_3O^+] - [OH^-]\}(V_a+V)$

And rearrangement gave

$$K_a = \frac{G[H_3O^+]}{(V_eN - G)}$$

$$K_a(V_eN - G) = G[H_3O^+]$$

$$G[H_3O^+] = K_aV_eN - K_aG \qquad \text{(Eq.21)}$$

Eq. 21, is in the form of a linear equation, Y = aX + b. Y was $G[H_3O^+]$, X was G, the intercept was K_aV_eN and slope was $-K_a$. When $G[H_3O^+]$ was plotted against G to obtain linear line. The dissociation constant, K_a, could be calculated from the slope and the equivalent point, V_e, could be obtained from the X-intercept. By this equation, the equivalent point and dissociation constant were simultaneously determined.

With all three of the graphical methods the equivalent point was estimated from the graph. The most important data points were the ones surrounding the equivalent point but also were the most difficult to obtained. Gran method has advantages over the others. Since the regression analysis is a straight line rather than a curve, so fewer points are needed to define equivalent point and the points can be taken at regular intervals instead of being bunched in a narrow region around the equivalent point. The treated portions of the curve are before the equivalent point; thus, the linear extrapolation used in this method can provide more precise result than those obtained by the methods based on Sigmoid form and the differential methods, especially for asymmetrical titration curves.

2. Determination of Dissociation Constant

Since most of chemical substances have acidic and or basic functionalities, their ionization state is controlled by both pH of solution and acidic dissociation constants, K_a or pK_a. The extent of ionization is one of several important properties used to estimate the absorption, distribution, metabolism and excretion of compounds in biological system and the environment (Babic, 2007). Knowledge of the aqueous ionization constant, in term of pK_a, of substance is very important in the pharmaceutical industry and environmental field. In the past, potentiometric titration was standard method for pK_a measurement but the alternative methods have been also developed (Avdeef, 2003; Babic, 2007).

There are several methods for measurement dissociation constant. Traditionally, potentiometric titration, Ultraviolet-Visible (UV-VIS) absorption spectrophotometry method have been the most useful techniques due to its accuracy and reproducibility. Some alternative techniques based on separation methods such as liquid chromatography (LC), capillary electrophoresis (CE) have been developed. In

addition, pK_a values can also be predicted by computational methods on the basis of molecular structure (Wiczling, 2006; Avdeef, 2003; Quiang and Adam, 2004).

In potentiometric method, the dissociation constant can be estimated from the titration curve and linear equation (Skoog et al., 1996; Babic et al., 2007) as follows below:

2.1 A Half-Neutralization

The plot of pH against titrant volume added is called a potentiometric titration curve. The shape of such a curve can suggest the amount of substance present and its characteristic acid-base ionization properties. At the equivalent point the sample is almost complete in one state of ionization. At a half of this point or half-neutralization, the molecule present in two states of protonation in equal concentration. The dissociation constant, pK_a, is most easily determined from the pH at this point. However, a titration curve does not always reveal all the pK_a values that the molecule has (Avdeef, 2003).

2.2 Gran's plot

In previous study, by Eq.21, there was no statistically difference between the results obtained from G plot and the official method in USP XX (Arttamangkul, 1986). The dissociation constant and the equivalent point could be simultaneously determined. This is an advantage of Gran equation.

2.3 Second-Derivative (Δ^2pH/ΔV^2)

Plotting Δ^2pH/ΔV^2 against V. Where V = the volume of titrant added and ΔV = the constant volume increment. The dissociation constant corresponds to the point where the value of Δ^2pH/ΔV^2 equals zero. (Qiang and Adam, 2004).

2.4 Least-Squares Non-Linear Regression

This method was developed from acid-base equilibrium. Taking a monoprotic weak acid as example, the volume of base added when the titration is at any pH can be calculated using the following equation; (Qiang, 2004)

$$V = V_a \left\{ \frac{C_A \alpha_1 - [H^+] + [OH^-]}{C_B + [H^+] - [OH^-]} \right\} \qquad \text{Eq. 22}$$

The fraction of conjugate base, α_1, is expressed by

$$\alpha_1 = \frac{K_a}{[H^+] + K_a} \qquad \text{Eq. 23}$$

Where V_a = the total volume of a monoprotic acid

V = the volume of titrant added during titration

K_a = the acid dissociation constant

C_A = the concentration of acid

C_B = the concentration of base

The theoretical volume of base added to reach a certain pH can be calculated by Eq. 23. Meanwhile, the experimental volume of base added (V_{EB}) to reach the same

pH can be directly measured during titration. Thus, the dissociation constant, K_a, can be computed by minimizing the sum of $(V_{EB}–V)^2$.

2.5 Bjerrum Plots

The Bjerrum plots are probably the most important tools in the initial stages of titration data analysis. At each titrant added, the quantities of strong base have been added to the solution and dissociable proton from sample bring to the solution were known and then the total hydrogen ion concentration in solution could be calculated by equilibrium reaction. By measuring pH, the free hydrogen ion concentration was known. The difference between the total and the free concentrations is equal to the concentration of the bound hydrogen ions. The bound concentration divided by that of the sample gives the average number of bound hydrogen atoms per molecule of substance n_H. The Bjerrum curve is a plot of n_H versus pH (Testa, 2001; Avdeef, 2003). The pH values at which the curves intercept the half-integral numbers of bound protons per molecule give approximations of pK_a. These values are then refined by a generalized non-linear least-squares procedure which took other variable factors into account (Chamberlain, 1996).

2.6 The Extrapolation Method

For poorly soluble substances, this method used to estimate their pK_a values in aqueous-organic solvent mixture. To estimate aqueous pK_a values, several titrations of different ratios of aqueous-organic solvent mixtures should be performed. Plotting the

obtained cosolvent ionization constant (p_oK_a) from each ratios versus the ratio of aqueous-organic solvent mixtures, and the aqueous pK_a is obtained by extrapolation to the zero organic solvent (Avdeef, 1999; Babic, 2007; Volgyi, 2007).

3. Determination of Partition Coefficient

Octanol-water partition coefficient, P or log P, is widely used as a measurement of lipophilicity and is one of the most commonly reported physicochemical properties of drugs and industrial chemicals. It is most often used in establishing quantitative structure-activity relationships (QSARs). Although the generally accepted standard method for log P measurement is still the shake flask technique, several other log P measurement method have been developed (Takacs-Novak and Avdeef, 1996; Caron et al., 1999; Avdeef, 2003; Poole, 2003; Hartmann and Svhmitt, 2004).

For the partition coefficient determination, the shake flask method is probably the most widely used techniques as a standard method (Hersey, 1989). In addition, the alternative method such as imbobilized artificial membrane (IAM) chromatography, octanol-coated HPLC colume, (Avdeef, 2003; Testa et al., 2001), slow-stirring method (Ellington and Floyd, 1995), solid-phase microextraction (Dean, Tomlinson and Makovskaya, 1996), electrokinetic method (Jia et al., 2003; Ikonen, Murtomak, and Kontturi, 2007) and a computational method (Benfenati, 2003) were developed.

Brandstrom (1963) performed constant pH titration to determine log P. Seiler (1974) proposed the determination of pK_a and log P from single titration, where octanol is added half-way through the assay, approximately at the half-ionization point by non-linear refinement procedure of a monoprotic substance with partition coefficient and dissociation constant and the sample weight were unknown parameters. However, these methods are not the recommended procedure for establishing partition

coefficient. Kaufman et al. (1975) introduced the use of different plots to the interpretation of log P from titration data. This method has been extensively studied (Comer et al., 1995; Chamberlain, 1996; Takacs-Novak, 1996; Degim, 2001; Barzanti et al., 2007). Moreover, the pH-method was applied for being a guideline for partition coefficient of the chemical testing (OECD, 2000).

pH-metric method, can determine partition coefficient based on pK_a correlation. The pH-Metric method consists of two titrations. The first titration is find out pK_a from the aqueous titration. The difference value of pK_a from the two titration were estimated and related to the value of log P as shown in the equation below

For weak acid;

$$P = \frac{10^{(pKa'-pKa)} - 1}{r} \qquad \text{(Eq.24)}$$

where r is the ratio of volume of octanol to aqueous phase.

pK_a' is the apparent pK_a in the presence of octanol titration.

In the best of our knowledge, there were no studies that suggest the approach determine equivalent point, dissociation constant and partition coefficient in single titration with an equation. Therefore, if there was an equation that could determine equivalent point, dissociation constant and partition coefficient from single titration it will save cost and time with the easy, reliable and accuracy method.

CHAPTER III

EXPERIMENTAL

3.1 Multiple Linear Regression Model Analysis

A. Computer Simulation

To test regression model, we need to simulate titration data. We need to calculate the hydronium concentration, $[H_3O^+]$, for each volume of titrant added. It can be calculated starting with the principle of charge balance, mass balance and equilibrium equation of neutral weak acid to give polynomial equation (see Appendix A) as equation below,

$$\{V_a(1 + PR) + V\}[H_3O^+]^3 + \left\{ \frac{VN\{V_a(1 + PR) + V\}}{V_a + V} + K_a(V_a + V) \right\}[H_3O^+]^2$$

$$+ \left\{ K_aVN - K_a[HA]_0V_a - K_w\{V_a(1 + PR) + V\} \right\} [H_3O^+]$$

$$- K_wK_a(V_a + V) = 0 \qquad \text{(Eq. 33)}$$

The only variable in polynomial equation (Eq. 33) was hydronium ion concentration. The parameters in the coefficients of polynomial equation (N, R, V_a, $[HA]_0$, K_w and V_{oct}) were fixed excluding V. Hence, the hydronium ion concentration can be solved as a function of each volume of titrant added.

Setting regression model, the experiment parameters, N, R, V_a, $[HA]_0$, K_w, $[H_3O^+]$, V and V_{oct} were set and inserted into Eq. 33 to produce $[H_3O^+]$ at each titrant volume addition. In all simulations, the fixed known quantities N, V_a, $[HA]_0$, K_w were

fixed at 0.1000 normality, 50.0 ml, 0.1000 M and 10^{-14}, respectively, but V_{oct} were set as 5.00 and 10.00 ml (R were 0.1 and 0.2, respectively). The values of P, K_a were varied between 0.001 to 10,000 and 1×10^{-2} to 1×10^{-10}, respectively. The constant incremental volume added was 0.100 ml. (See the Table 1.)

B. Multiple Linear Regression Model Testing

The simulated $[H_3O^+]$ at each volume titrant added with all 15 significant figures were inputted together with parameters in Table 1 (excluding V_e, K_a and P) to produce variables of Eq. 13 (X_1, X_2, Y). The variables of all data set were applied to regression model, Eq. 13, for equivalent point, dissociation constant and partition coefficient determination. In rounding off error testing, the simulated $[H_3O^+]$ at each volume titrant added with all 15 significant figures were transformed into its negative logarithm (pH value at each volume titrant added). Each pH value was rounded off to 3 decimal points and then also input together with parameters in Table 1 (excluding V_e, K_a and P) to produce variables of Eq. 13 (X_1, X_2, Y). The variables of all data set were applied to regression model, Eq. 13.

Fitting multiple linear regression models was done using the computer software package. In this study, three programs Excel® 2007, Wessa (2009), and MINITAB® 15 were used for fit the regression coefficients and yielded the calculated V_e, K_a and P which were compared with the theoretical values.

3.2 Modified Gran Equation Analysis

The experiment parameters, N, R, V_a, $[HA]_0$, K_w, $[H_3O^+]$, V and V_{oct} were set and input into Eq. 33 to simulate $[H_3O^+]$ at each titrant volume added. In all simulations, the fixed known quantities N, V_a, $[HA]_0$, K_w were fixed at 0.1000

normality, 50.0 ml, 0.1000 M and 10^{-14}, respectively, but V_{oct} were set as 5.00 and 10.00 ml (R were 0.1 and 0.2, respectively). The constant incremental volume added was 0.100 ml and 0.200 ml. The values of P, K_a were varied. (See the Table 2).

Simulated $[H_3O^+]$ at each titrant volume added were then analyzed with Eq. 38 together with parameters in Table 2 (excluding V_e, K_a and P) to calculate values of variables :X_1, X_2, Y. (For Eq. 38, see Appendix B)

$$\frac{G\,[H_3O^+]V_a}{(V_a+V)} = K'V_eN - K'G \qquad \text{(Eq. 38)}$$

3.3 Materials

3.3.1 Instruments

3.3.1.1 Automatic titrator (Orion 960) and exchange units model (Orion 940)

3.3.1.2 Spectrophotometer (UV-160 A Shimadzu)

3.3.1.3 Electrode (Beckman, lot. no. S804A 511060)

3.3.1.4 Magnetic stirrer (Heidolph MR 3001)

3.3.2 Chemicals (Table 3)

3.3.2.1 Benzoic acid, AR grade (Merck Germany, lot. No. 237 K17360336)

3.3.2.2 Salicylic acid, AR grade (Merck Germany, lot. No. 006 K13000331)

3.3.2.3 *m*-toluic acid (Fluka, lot. No. 05508P)

3.3.2.4 *2*-methoxybenzoic acid (Fluka, lot. No. 048801/1)

3.3.2.5 Glacial acetic acid (Labscan, lot. No. 05050128)

3.3.3 Reagents

3.3.3.1 Sodium hydroxide AR (Merck Germany lot. No. B462698 410)

3.3.3.2 Potassium chloride AR (Ajax Finechem lot .no. AF 501338)

3.3.3.3. Potassium hydrogen phthalate AR (Fluka, lot. no. 226270) Puriss

3.3.3.4 Potassium chloride solution, 4 M, electrolyte for combined glass electrode (Beckman lot. no. 566467)

3.3.3.5 Standard buffer solution pH 4 (Beckman, lot . no. M801295)

3.3.3.6 Standard buffer solution pH 7 (Beckman, lot .no. M11385)

3.3.3.7 Standard buffer solution pH 10 (Beckman, lot .no. M801628)

3.3.3.8 *n*-Octanol AR (Fluka, lot.no. 1243837)

3.3.3.9 Hydrochloric acid AR (Labscan, lot.no. 06080388)

3.4 Procedures

3.4.1. Preparation and Standardization of Titrant (0.1 N NaOH)

Ten grams of sodium hydroxide were weighed and dissolved in 20 ml distilled de-ionized water. The supernatant was decanted after precipitated sodium carbonate had settled out. Eight milliliters of this liquid was pipetted into 1 L volumetric flask and then diluted with distilled de-ionized water to the volume.

Five hundred milligrams potassium hydrogenphthalate (dried at 120°C, 2 hrs) was weighed and dissolved with distilled de-ionized water in 500 ml volumetric flask and then adjust to volume. Pipette 50 ml of potassium hydrogenphthalate solution for standardization and titrate with the sodium hydroxide standard solution.

3.4.2 Aqueous Titration of Neutral Weak Acidic Compound

3.4.2.1 Blank Titration

Fifty milliliters of pipetted distilled de-ionized water were titrated with 0.1 N sodium hydroxide standardized solution. The beaker was placed into a water bath on a magnetic stirrer then the combined electrode, thermometers, dispenser probe and a stirrer were inserted.

The electrodes were submerged into titrated solution for 5 minutes prior to commencement of titration to assure that electrodes were in equilibrium with titrated solution. The water bath was continuously stirred with a magnetic bar to carry out the titrated solution at 25±0.1°C. The titrated solution was mixed with a stirrer after each addition of titrant for 5 seconds and the pH value was measured after the stirrer off for 30 seconds. The constant increment of each addition of titrant was 0.050 ml. Blank solutions were titrated in quintuplicate.

3.4.2.2 Preparation of Neutral Weak Acid Compound Solution

Each weak acidic compound was accurately weighed in suitable quantities (according to their molecular weights and its solubility) to produce desired stock concentration (0.001 – 0.009 M).

Fifty milliliters of stock solution were pipetted and titrated with 0.1 N sodium hydroxide standardized solution. The beaker was placed into a water bath on a magnetic stirrer then the combined electrode, thermometers, dispenser probe and a stirrer were inserted.

The electrodes were submerged into sample solution for 5 minutes prior to commencement of titration to assure that electrodes were in equilibrium with the solution. The water bath was continuously stirred with a magnetic bar to carry out the titrated solution at 25±0.1°C. The titrated solution was mixed with a stirrer after each

addition of titrant (0.1 ml) for 15 seconds and the pH value was measured after the stirrer off for 70 seconds. The constant increment of each addition of titrant was 0.100 ml. All samples were titrated in quintuplicate.

3.4.2.3 Data Analysis to Determine the Parameters

From the raw data that obtained from the aqueous titration of weak acid solution, the equivalent volume and the dissociation constant of each weak acid could be determined by the extrapolation of linear plot of Gran method (according to Eq. 21). The data range which gave maximum R^2 and not less than 10 points was used for the Gran method. The dissociation constant could be determined from the slope and the equivalent point could be determined from the intercept of this plot.

The percent of purity of each weak acidic compound were determined as follows:

$$\% \text{ Purity} = \frac{V_e \times N \times \text{Eq.Wt.} \times 100}{\text{Wt.}} \qquad \text{(Eq. 42)}$$

Where V_e = equivalent point volume (ml).

N = normality if titrant (meq/ml).

Eq.Wt. = equivalent weight of weak acid (mg/meq).

Wt. = weight of weak acid (mg).

3.4.3 Determination of Reference Value of Partition Coefficients by Shake Flask Method

Benzoic acid

Preparation of stock solution: stock solution of benzoic acid was prepared by accurately weighing 0.37922 grams benzoic acid reference substance into 1 L volumetric flask and then was dissolved by 0.1 N HCl in *n*-octanol-saturated distilled de-ionized water (pH = 1.06)

Calibration curve: Aliquots (1, 1, 1, and 3 ml) of the benzoic acid stock solution were transferred volumetrically into 250, 100, 50, and 100 ml flask with final concentration of 0.0124, 0.0310, 0.0621 and 0.0932 mM. The stock solution which concentration 0.0621 mM were pipetted 20 ml and then were transferred to 25 ml volumetric flask with final concentration 0.0497 mM. Each concentration was adjust to final volume with 0.1 N HCl in *n*-octanol-saturated distilled de-ionized water (pH = 1.06) in triplicate. Each benzoic acid solution were measured by UV spectrophotometer at suitable maximum wavelength, λ_{max}, 230.3 nm. The standard curve was plot between the concentrations of substances (mole/L) and the absorbance values. (Table 4.1, Fig. 1.1)

Sample preparation: Fifty milliliters benzoic stock solution were pipetted and transferred to 250 ml separation funnel and then 10 ml water-saturated *n*-octanol was added and mixed by shaking for 10 minutes. The phases were allowed to separate on standing for 4 hours at $25 \pm 0.1^{\circ}C$. Concentrations of weak acids in the aqueous phase were determined by UV spectrophotometer at suitable maximum wavelength, λ_{max}, 230.3 nm. (Fig. 1.2)

_2_-Methoxybenzoic acid

***Preparation of stock solution**:* stock solution of *2*-methoxybenzoic acid was prepared by accurately weighing 0.11048 grams *2*-methoxybenzoic acid reference substance into 500 ml volumetric flask and then was dissolved by 2 N HCl in *n*-octanol-saturated distilled de-ionized water (pH < 0.40). The concentration of stock solution was 0.00145 M.

Calibration curve : Aliquots (1, 3, 1, 5, 3 and 2 ml) of the *2*-methoxybenzoic acid stock solution were transferred volumetrically into 50, 100, 25, 100, 50 and 25 ml flask with final concentration of 0.0291, 0.0437, 0.0582, 0.0728, 0.0873 and 0.116 mM. Each concentration was adjust to final volume with 0.1 N HCl in *n*-octanol-saturated distilled de-ionized water pH < 0.40) in triplicate. Each *2*-methoxybenzoic solution were measured by UV spectrophotometer at suitable maximum wavelength,λ_{max}, 234 nm. The standard curve was plot between the concentrations of substances (mole/L) and the absorbance values. (Table 4.2, Fig. 1.3)

Sample preparation: Fifty milliliters *2*-methoxybenzoic solution were pipetted and transferred to 250 ml separation funnel and then 10 ml water-saturated *n*-octanol was added and mixed by shaking for 10 minutes. The phases were allowed to separate on standing for 4 hours at 25$\pm$0.1°C. Concentrations of weak acids in the aqueous phase were determined by UV spectrophotometer at suitable maximum wavelength,λ_{max}, 234 nm. (Fig. 1.4)

Salicylic acid

Preparation of stock solution : stock solution of salicylic acid was prepared by accurately weighing 0.15234 grams salicylic acid reference substance into 500 ml volumetric flask and then was dissolved by 0.1 N HCl in *n*-octanol-saturated distilled de-ionized water (pH = 0.80).

Calibration curve: Aliquots (1, 3, 1, and 5 ml) of the salicylic acid stock solution were transferred volumetrically into 50, 100, 25, and 100 ml flask with final concentration of 0.0248, 0.0441, 0.0662, 0.0882 and 0.1103 mM. The stock solution which concentration 0.0882 mmol/mL^{-1} were pipetted 10 ml and then were transferred to 20 ml volumetric flask with final concentration 0.0265 mM. Each concentration was adjust to final volume with 0.1 N HCl in *n*-octanol-saturated distilled de-ionized water (pH = 0.80) in triplicate. Each salicylic acid solution were measured by UV spectrophotometer at suitable maximum wavelength,λ_{max}, 237.4 nm. The standard curve was plot between the concentrations of substances (mole/L) and the absorbance values. (Table 4.3, Fig. 1.5)

Sample preparation: Fifty milliliters salicylic stock solution were pipetted and transferred to 250 ml separation funnel and then 10 ml water-saturated *n*-octanol was added and mixed by shaking for 10 minutes. The phases were allowed to separate on standing for 4 hours at 25$\pm$0.1°C. Concentrations of weak acids in the aqueous phase were determined by UV spectrophotometer at suitable maximum wavelength,λ_{max}, 237.4 nm. (Fig. 1.6)

_m_-Toluic acid:

Preparation of stock solution: stock solution of *m*-Toluic acid was prepared by accurately weighing 0.05502 grams *m*-Toluic acid reference substance into 1 L volumetric flask and then was dissolved by 0.1 N HCl in *n*-octanol-saturated distilled de-ionized water (pH = 0.901). The concentration of stock solution was 0.000404 M.

Calibration curve: Aliquots (2, 3, 2, 3, 10 and 5 ml) of the *m*-Toluic acid stock solution were transferred volumetrically into 50, 50, 25, 25, 25 and 25 ml flask with final concentration of 0.0162, 0.0242, 0.0323, 0.0485, 0.0647 and 0.0808 mM. Each concentration was adjust to final volume with 0.1 N HCl in *n*-octanol-saturated distilled de-ionized water (pH = 0.901) in triplicate. Each *m*-Toluic acid solution were measured by UV spectrophotometer at suitable maximum wavelength, λ_{max}, 234 nm. The standard curve was plot between the concentrations of substances (mole/L) and the absorbance values. (Table 4.4, Fig 1.7)

Sample preparation: Fifty milliliters *m*-Toluic acid solution were pipetted and transferred to 250 ml separation funnel and then 5 ml water-saturated *n*-octanol was added and mixed by shaking for 10 minutes. The phases were allowed to separate on standing for 4 hours at $25 \pm 0.1^{\circ}C$. Concentrations of weak acids in the aqueous phase were determined by UV spectrophotometer at suitable maximum wavelength, λ_{max}, 234 nm. (Fig. 1.8)

By fitting linear equation, the concentration of each weak acid in sample aqueous phase was determined. The concentration of the sample in *n*-octanol phase was divided by the concentration in aqueous phase and then the reference value partition coefficient was obtained.

Acetic acid:

Determination of concentration of stock solution: Pipette 5 ml of acetic acid stock solution (0.4 M) transferred to 100 ml beaker and then 50 ml of octanol-saturated water added. The concentration of the stock solution was determined by titrating with 0.1 N sodium hydroxide standardized solution in quintuplicate to determine equivalent point. From the equivalent point, the concentration of stock solution can be calculated.

Determination partition coefficient by shake flask method: Five milliliters *m*-acetic acid solution were pipetted and transferred to 250 ml separation funnel. Pipetted 5 ml of 0.2 N HCl *n*-octanol saturated distillated-deionized water (pH = 0.804) and 10 ml of water-saturated *n*-octanol was added and mixed by shaking for 10 minutes. The phases were allowed to separate on standing for 4 hours at 25$\pm$0.1°C. Five milliliters octanol phase were collected and then 50 ml *n*-octanol distillated-deionized water were added. The mixture was titrated with 0.1 N sodium hydroxide standardized solution in quintuplicate to determine equivalent point of sample to determine the concentration of acetic acid in octanol phase. From the known concentration of acetic acid in stock solution and octanol phase, the concentration of acetic acid in aqueous phase can be calculated. The reference partition coefficient of acetic acid can be calculated from dividing the concentration of acetic acid in octanol phase by the concentration in aqueous phase.

3.4.4 Dual Phase Aqueous-Octanol Titration of Weak Acidic Compounds with 0.1 N Sodium Hydroxide

3.4.4.1. Blank Titration

Blank titration was performed as described in 3.4.2.1

3.4.4.2 Aqueous-Octanol Titration of Weak Acidic Compounds

The Calculation of Equivalent Point, Dissociation Constant and Partition Coefficient

Each titration was divided into two stages: aqueous stage and aqueous-octanol stage (marked by addition octanol to the system). At initial stage of titration the system was defined by aqueous phase. After octanol was added into sample solution the system was defined by dual phase (aqueous-octanol phase). The solution of each weak acid was titrated in quintuplicate.

The dissociation constant could be determined by the slope of linear plot of Gran's method (Eq. 21) using aqueous titration data in the aqueous stage which gave maximum R^2. This is similar to our single phase aqueous titration. The titration data obtain from the aqueous solvent portion of the titration were analyzed with Eq. 21 to determine of the dissociation constant, K_a of the weak acid. The titration data range from 0 to around 1.200 ml of volume of titrant added were not used in calculation because the curvature is in the initial plot of the titration which may lead to error in determination of K_a. In aqueous titration with no *n*-octanol added, titration data of 30-95% of V_e can be determined for K_a by Eq. 21 to determine K_a. The calculated K_a obtained from single aqueous phase titration was used as a reference K_a value.

The aqueous titration was initially titrated with 0.1 N NaOH then was paused approximately 40% (before midpoint) or 60% (after midpoint) of equivalent point for *n*-octanol addition. The volume of *n*-octanol was pipetted 10 ml for before midpoint addition and 5 or 10 ml for after midpoint addition. The aqueous-octanol sample solution was stirred for 1 minute and then let stand undisturbed for 3

minutes to re-establish equilibrium between the electrode and the solvents before the dual-phase titration was begun with 0.1 N NaOH to complete titration.

In sequential aqueous-octanol titration which octanol added after midpoint, octanol were added after 65% of titration and data points in the range of 30-65% of V_e were used for determination of K_a. In sequential aqueous-octanol titration which octanol were added before midpoint (approximately 45% of $V_e/2$), the data points in the range of 25-45% of V_e were used for K_a calculation. The data range which gave maximum correlation coefficient was analyzed by Eq. 21 for determination of K_a. In this experiment, the number of data points in the range must be at least 10 points. For the determination of equivalent point and partition coefficient will be described in result and discussion.

The calculated equivalent point, %purity, dissociation constant, partition coefficient value of each weak acid were compared with its respective reference values . Reference value of purity was obtained from single phase aqueous titration and the P value was obtained from shake flask method. The results were tested for statistical significant by ANOVA analysis at 95% confidence interval.

CHAPTER IV

RESULTS AND DISSCUSSION

Multiple Linear Regression Model Testing

The concentration of hydronium ions, $[H_3O^+]$, at each titrant volume added can be obtained by employing Newton's Approximation to find root of a polynomial equation, Eq. 33, on EXCEL® 2007 computer program. The parameters in the coefficients of polynomial equation (N, R, V_a, $[HA]_0$, K_w and V_{oct}) are shown in Table 1. The parameters of these variables were fixed with exception of the volume of titrant, V. Simulated $[H_3O^+]$ at each volume of titrant added can be obtained from employing Eq. 33. These simulated titration data were input to the multiple linear regression model, Eq. 13 to calculate the coefficients of the multiple linear equation. $GV[H_3O^+]/(V_a+V)$ were treated as the dependent variable and G, $GV_a[H_3O^+]/(V_a+V)$ as the independent variables, X1, X2, respectively. From the coefficient $a_o = K_aV_eN$, $a_1 = -K_a$ and $a_2 = -(1+PR)$, the values of V_e, K_a, and P can be obtained. All titration data series were simulated on EXCEL® 2007 worksheets.

$$\{V_a(1+PR)+V\}[H_3O^+]^3 + \left\{\frac{VN\{V_a(1+PR)+V\}}{V_a+V} + K_a(V_a+V)\right\}[H_3O^+]^2$$

$$+ \left\{K_aVN - K_a[HA]_0V_a - K_w\{V_a(1+PR)+V\}\right\}[H_3O^+]$$

$$- K_wK_a(V_a+V) = 0 \qquad \text{(Eq. 33)}$$

$$\frac{GV[H_3O^+]}{(V_a+V)} = K_aV_eN - K_aG - \frac{(PR+1)\,G\,V_a[H_3O^+]}{(V_a+V)} \quad \text{(Eq. 13)}$$

A. Determination of V_e, K_a and P with No Rounding off Error

To minimize rounding off error, all data simulated with EXCEL® 2007 to test limitations of multiple linear regression programs were set at maximum fifteen significant figures (maximum available for Excel®2007). In this study, three multiple linear regression programs, Excel®2007, Wessa (2009), and MINITAB®15 were used for calculating regression coefficients and to yield the equivalent volume, dissociation constant and partition coefficient for comparison with the theoretical values. The results of the calculated equivalent point, dissociation constant and partition coefficient obtained from three multiple regression program packages were summarized in Table 5.

Excel ® 2007 multiple linear regression achieved correct values for data sets with $pK_a = 4$ or below, P = 0.001 up to 1. In these cases, we obtained the values of V_e, K_a, and P as theoretical values. In the data set that P = 10, just $pK_a = 2$, and $pK_a = 3$ could yield the theoretical values. For set that P = 100, only $pK_a = 2$ with $V_{oct} = 5$ ml yielded the correct values. For all other data sets which Excel ® 2007 failed to give the correct values (V_e, K_a and P), X_2 are very small values as compare to X1 (typically X_2 less than 0.001% of X_1), and in these data sets, Excel ® 2007 seems to deduce that changes in X_2 do not significantly effect changes of values in Y. In these cases, Excel ® 2007 report the value of $a_2 = 0$ and fit model as simple linear regression ($Y = a_0 + a_1X_1$). The rejection of multiple linear regression model (rejecting X_2 data) by Excel ® 2007 and then proceed to fit only the X_1 data to simple linear regression model led to negative value of calculated K_a, V_e (See Table 5).

There are many free online regression programs. In this study, Wessa (2009) is the one we choose because of its well-known to be accurate and convenience to use. It need just only copying data from Excel® worksheet, paste to the online page window and then click "compute". The dominant feature of this package is that the output data sheets could be reported on Microsoft Excel or Microsoft Word or internet window. Wessa (2009) yielded correct values of V_e, K_a and P with all simulation data sets excluding two data sets with P = 10000, pK_a = 10 , for V_{oct}= 5, 10 ml. In these two cases, Wessa (2009) failed to compute and also no error information was presented on page window. The output showed only blank page. When Wessa (2009) was tested by data set which P=10000, pK_a = 9 V_{oct}= 5 or 10 ml, it also would compute and yielded correct values of V_e, K_a and P.

Moreover, the other limitation of Wessa (2009) is that every row represents only 71 characteristics and every column represents a different variable which must be delimited by a space or Tab. Therefore, when the number of variables increases, the significant figures must decrease to be within 71 characteristics. However, since we have only two predictor variables, Wessa (2009) was found suitable for solving multiple regression model, Eq. 13.

Minitab® 15 regression program has limitation that the display of coefficients were limited only maximum of eight decimal places. This mean that data sets with any P, pK_a more than 8, the regression coefficients should be displayed zero (0.00000000) but the actual values of a_o and a_1 were not. To solve this problem, the values of Y were multiplied by 1.0×10^6. Therefore, to calculate the correct values of V_e, K_a and P, the coefficients a_o, a_1 and a_2 must be divided by 1.0×10^6. By this approach, Minitab® 15 is the only multiple linear regression program that can yield correct values of all data sets. Even the most difficult data set (P = 10000, pK_a = 10, V_{oct} = 5.0 ml) which showed extreme different in values of X_1 to X_2 and Y (X_1 are 10^{13}, 10^{11} larger

than Y and X_2, respectively), the Minitab® 15 still able to yield correct theoretical values of V_e, K_a and P.

B. Effect of Rounding off Error

In previous regression program testing all 15 significant figures of each variables were used for calculation. In actual titration data the pH value at each volume of titrant added could be read only maximum of three decimals places. Thus, we need to test the effect of rounding off error of simulated titration data. From employing Eq. 33, the concentration of hydronium ion at each volume titrant added was obtained at the maximum fifteen significant figures. These concentrations of hydronium ion were transformed into the negative logarithm as pH value. The pH values were then rounded off to 3 decimal places and then were transformed back to hydronium ion which were then submitted as concentrations of hydronium ion in Eq. 13.

It is possible that rounding off error may result in increasing errors in calculating V_e, K_a and P by all three multiple linear regression programs.

From previous study, some of simulated data sets were used for study how strongly influence of the effect of rounding of error could be. The simulated titration data sets with P equal 0.001, 1, 100, 10000 with pK_a equal 2, 3, 4, 5, 7 and V_{oct} equal 5 ml were used to simulate theoretical data. The hydronium ion concentration of each data set were changed to pH value and then rounded off to 3 decimal points. These rounded titration data sets were input to multiple linear regression model, Eq. 13, for estimation of equivalent volume, dissociation constant and partition coefficient by fitting regression model using statistical software packages, Excel ® 2007, Wessa (2009) and Minitab®15. The results and their percent of error were summarized on Table 6 and Table 7, respectively. Rounding errors are not handled equally well by all

packages. The results of Minitab® 15 and Wessa (2009) were exactly like each other and differed from Excel® 2007. The discussion as followed below.

In rounding off error testing, all regression programs failed to yield correct values. With the introduction of rounding off error, the equivalent point, dissociation constant and partition coefficient could not be calculated from Eq. 13 employing any regression programs. Therefore, applying Eq. 13 to a real laboratory condition which pH meters can read only maximum of 3 decimal places would definitely lead to erroneous calculation of V_e, K_a and P. We cannot determine V_e, K_a and P using Eq. 13 and we must modify Eq. 13 to a simpler equation.

Modified of Multiple Linear Regression to Simple Linear Regression Model

Since Eq. 13 can not accurately yield V_e, K_a, and P due to limitation of measuring pH of solution (pH meter can measure only maximum of 3 decimal places), we must find an alternate way of determining all three parameters from one single experiment. To do this, we need to simplify Eq. 13 into a simple linear equation. Rearranging Eq. 13, we can obtain Eq. 41 (See Appendix C).

$$\frac{K_a\{V_eN(V_a+V) - G(V_a+V)\}}{G} - V[H_3O^+] = V_a(PR+1)[H_3O^+] \quad \text{(Eq. 41)}$$

Eq. 41, it is a simple linear regression model with one independent variable. The term $(K_a\{V_eN(V_a+V) - G(V_a+V)\})/G - V[H_3O^+]$ is the dependent variable (Y) and $[H_3O^+]$ is the independent variable (X). By simple linear regression model fitting, partition coefficient can be calculated from regression coefficient, a_1. Knowing a_1, we can readily calculate P. However, we must determine the values of K_a and V_e before Eq. 41 can be useful in calculating P.

A. Determination of Dissociation Constant

Previous workers have shown that Gran equation (Eq. 21) can be used to accurately determine the dissociation constant in aqueous titration. Therefore, if we first titrate our weak acids in aqueous solvent (up to approximately 40% - 70% of equivalent volume before adding octanol to the system) we should be able to determine K_a from the slope of Gran's plot using titration data in the aqueous phase (prior to $V_e/2$).

To test Eq. 41, five neutral weak acid compounds with pK_a between 3 and 5 were chosen as test compounds (Table 3).

In the case of *m*-toluic acid, the dissociation constant cannot be obtained from the 20-45% aqueous titration data range because the poor solubility limit the concentration of the sample solution and consequently, the number of data points in the linear portion of the Gran plot which were to be analyzed by Eq. 21 were far less than our set limit of 10 data points (Fig. 2). When sufficient number of titration was available as in the case which *n*-octanol was added at approximately 65% of V_e, ANOVA test showed that there was no statistical difference between the reference K_a and K_a calculated with Eq. 21 for this data range of *m*-toluic acid (Fig. 3)

In addition, we also determined K_a of all 5 weak acidic compounds by half neutralization method. ANOVA test also showed that with exception of salicylic acid, the K_a were not significantly different from their respective reference K_a obtained from Gran method, Eq. 21. (Table 8.1-8.5)

In the case of salicylic acid, the calculated K_a using a half neutralization method was significantly different from K_a values determined from aqueous titration and sequential aqueous-octanol titration. There is due to inherent error in using pH at half-neutralization as pK_a for acidic compound whose pK_a is 3 or below. To proof this, we simulated titration data of the weak acid (Eq. 33) with $V_{oct} = 0$ and $pK_a = 3.00$ and

found that error in using pH at half-neutralization as its pK_a value is 4.49% and the error decreased as pK_a of the weak acid increased (Table 8.6). Table 8.6 showed that pH at midpoint was not equal to pK_a. The pK_a as determined with half-titration method using simulated data is consistent with our salicylic acid experimental result.

Moreover ANOVA test showed that K_a obtained from aqueous portion (35-70% of V_e) of the sequential aqueous-octanol titration (after midpoint) and K_a obtained from aqueous portion (25-45% of V_e) of the sequential aqueous-octanol titration (before midpoint) by Eq. 21 did not pass variance test (Table 8.4). It is possible that the significantly different in standard deviation may have resulted from most of the data used to calculate K_a (with Eq. 21) was around 3 or below which were significantly below the pH calibration range (pH 4-10).

ANOVA test clearly showed that Eq. 21 was the suitable for K_a calculation using titration data 25-45% and 30-65% of V_e regardless whether the data were obtained from the aqueous or sequential aqueous-octanol titration. Therefore, to determination of V_e, K_a and P in a single experiment, K_a of the weak acid can be determined with the titration data from the aqueous portion of the sequential aqueous-octanol titration.

B. Determination of Equivalent Point of the Sequential Aqueous-Octanol Titration

In order to calculate P in Eq. 41, we need the values of K_a and V_e. As we have shown in our discussion of determination of dissociation constant that if we first titrate our weak acids in aqueous solvent, up to approximately 40% - 70% of equivalent volume before adding octanol to the system, we can determine K_a from the slope of Gran's plot using titration data in the aqueous phase (prior to $V_e/2$ or midpoint).

The second value which we need for Eq. 41 is V_e. Although it is possible that the equivalent volume can be determined from the inflection points (Figure 4.1-4.5), accurate determination of titration inflection point would be difficult for weak acids with pK_a above 8 due to the leveling effect of the titrant normality. Therefore, if we can come up with a linear equation similar to Gran equation for determination of V_e, it would allow us to accurately determine pK_a of weak acids with pK_a above 8. To derive our modified Gran equation for dual phase aqueous-octanol solvent system, see Appendix II.

$$\frac{G\,[H_3O^+](\{\,1+PR\,\}V_a + V)}{\{1+PR\}(V_a+V)} = K'V_eN - K'G \qquad \text{(Eq. 37)}$$

$$\frac{G\,[H_3O^+]V_a}{(V_a+V)} = K'V_eN - K'G \qquad \text{(Eq. 38)}$$

For Eq. 38, partition coefficient was not required for equivalent point determination. This was a main advantage over Eq. 37. Eq. 37 and Eq. 38 were very similar to Gran's equation (Eq. 21) which was derived for aqueous titration

$$G\,[H_3O^+] = K_aV_eN - K_aG \qquad \text{(Eq.21)}$$

When the linear equation was extrapolated to Y = 0 for Eq. 37, Eq. 38 and Eq. 21, the equivalent point of titration can be calculated by

$$V_e = \frac{\text{X-intercept}}{N \times \left|\ \text{slope}\ \right|} \qquad \text{(Eq.39)}$$

With Eq. 39, the equivalent point of aqueous-octanol titration can be determined from intercept and slope of Eq. 38.

To test the significant of error in assuming $\{1 + PR\}V_a >> V$ (for $V \leq V_e$) in Eq. 38, titration data ($[H_3O^+]$ at each titrant addition) was simulated with Eq. 33 using the titration parameters as shown in Table 2. The simulated values of $[H_3O^+]$ at each volume of titrant added were then inserted to Eq. 38 to calculate equivalent point from its intercept. The results are summarized in Table 9.1 and Table 9.2. In Eq. 38 testing, all of equivalent point calculation shown very high correlation coefficient (R >0.999) with both V_{oct} = 5 and 10 ml. Only data range of 2.200 – 9.800 ml of the data set that V_e = 10 ml, P = 0.100 and pK_a more than 5 were used yielded R less than 0.9995. In all other cases, obtained R was above 0.9995.

In all of the data ranges tested, as P increases, the error decreases due to the value of $G[H_3O^+]V_a/(V_a+V)$ approaches $G[H_3O^+](\{1+PR\}V_a + V) /\{(1+PR)(V_a+V)\}$. The simulated titration data were divided into 8 ranges and analyzed with Eq. 38 to determine which data range would yield V_e with the least deviation from theoretical value. The analysis show that the closer the data range is to the equivalent point, the more accurate is the V_e which could be calculated from Eq. 38 (Table 9.1 and Table 9.2).

The plot of simulated value of $G[H_3O^+]V_a/(V_a+V)$ against G for the data sets with P=0.1, V_{oct}= 5 ml, V_e = 10 ml showed the slight curved but R still more than 0.999. Nevertheless, the closer titrant volume to equivalent point were used the line becomes more linear and and the calculated V_e also becomes closer to the theoretical value.

In application of Eq. 38 to simulated titration data in the range of titrant volume more than 70% of V_e (more than 3.5 or 7.5 ml for data set that V_e = 5 ml and 10 ml, respectively) the percent of error in calculated V_e was less than 0.25% even with P was low as 0.1 (Table 9.1, Table 9.2). The closer the data range is to the X-intercept, the

more accurate the calculated V_e can be obtained. The curved characteristic of the plot was often exhibited in data set which P = 0.1 and seemed to disappear when P increase (Figure 4.6). When P increased, the value of $G[H_3O^+]V_a/(V_a+V)$ close to the value of $G[H_3O^+]\{(1+PR)V_a+ V\}/\{(V_a+V)(1+PR)\}$, and thus the calculated V_e nearer to correct V_e.

In actual titration of weak acids in dual phase aqueous-octanol solvent system, the ANOVA analysis showed that equivalent volumes obtained with Eq. 38 (dual phase aqueous-octanol solvent system) were statistically indifferent from the respective equivalent volumes of the stock solutions which were determined with Gran equation (Eq. 21) for the single phase aqueous solvent system. The summaries of V_e were shown in Table 9.3 - Table 9.7. The linear plot of each weak acid was showed in Fig. 5.1- Fig. 5.5. The addition of octanol before or after midpoint of titration does not affect the accuracy of V_e determinations. The volume of octanol used (5 ml, 10 ml) also has no effect.

In the case of benzoic acid, our titration data came from two different batches of stock solutions. Our first batch of stock solution ran out before we could complete the sequential aqueous-octanol titration which 10 ml octanol were added before midpoint. Therefore, Table 9.4 showed only calculated V_e from the aqueous titration and the sequential aqueous-octanol titrations which octanol were added after midpoint with no V_e for the sequential aqueous-octanol titrations which octanol were added before midpoint. The second batch of stock solution was prepared for the sequential aqueous-octanol titration which octanol was added before midpoint. Since the concentrations of the two stock solutions were different, we could not use V_e from the second stock solution to compare with V_e from the first stock solution (Table 9.4). However, the purity of second stock solution can be compared with the purity of the first stock solution (Table 9.9). Therefore, purities instead of V_e's should be used for statistical testing to compare application of Eq. 38 to determine equivalent points

among single phase aqueous titration with addition of octanol before and after midpoint of titrations. ANOVA test of the percentage of purities shows there is no statistically difference between the single aqueous phase titration and the dual phase aqueous-octanol, both addition of octanol before or after midpoint (Table 9.8- 9.12).

Our investigations clearly showed that Eq. 38 can be employed for dual phase aqueous-octanol titration to determine V_e which are statistically equivalent to V_e determined with Gran equation using titration data from single phase aqueous solvent system.

We have shown that K_a and V_e which are needed for Eq. 41 can be determined accurately with Eq. 21 and Eq. 38, respectively using data of the sequential aqueous-octanol titration. The dissociation constant can be accurately determined from the titration data in the aqueous portion and V_e can be accurately determined from the dual phase titration of sequential aqueous-octanol titration.

C. Determination of Partition Coefficient by Eq. 41

K_a and V_e obtained with Eq. 21 and Eq. 38 are used as inputs into Eq. 41 to calculate P. From our dual phase titration data, we can also calculate P using OECD guideline, Eq. 24. All P values calculated with Eq. 41 and Eq. 24 will be statistically against our reference method, shake flask.

The partition coefficients of the 5 weak acids in our study were determined as follow:

1. Shake flask method

This is our reference method for determination of partition coefficient. This is labeled Gr. 1 in Table 10.1-10.5.

2. Eq. 41 method

P was calculated with the titration data obtained after addition of octanol to the aqueous solvent. V_e were obtained with Eq. 38 as discussed in previous section. The aqueous dissociation constants K_a were obtained as follow:

2.1 Sequential aqueous-octanol titration with 5 ml octanol added after midpoint.

The reference K_a value obtained from single phase aqueous titration was used for calculation of the partition coefficient. This is labeled Gr. 3 in Table 10.1-10.5.

2.2 Sequential aqueous-octanol titration with 10 ml octanol added after midpoint.

The reference K_a value obtained from single phase aqueous titration was used for calculation of the partition coefficient. This is labeled Gr. 8 in Table 10.1-10.5.

2.3 Sequential aqueous-octanol titration with 10 ml octanol added before midpoint.

The reference K_a value obtained from single phase aqueous titration was used for calculation of the partition coefficient. This is labeled Gr. 13 in Table 10.1-10.5.

2.4 Sequential aqueous-octanol titration with 5 ml octanol added after midpoint.

The K_a value determine with Eq. 21 using its corresponding aqueous titration data. Both P and K_a are determined from the same titration experiment. This is labeled Gr. 2 in Table 10.1-10.5.

2.5 Sequential aqueous-octanol titration with 10 ml octanol added after midpoint.

The K_a value determine with Eq. 21 using its corresponding aqueous titration data. Both P and K_a are determined from the same titration experiment. This is labeled Gr. 7 in Table 10.1-10.5.

2.6 Sequential aqueous-octanol titration with 10 ml octanol added before midpoint.

The K_a value determine with Eq. 21 using its corresponding aqueous titration data. Both P and K_a are determined from the same titration experiment. This is labeled Gr. 12 in Table 10.1-10.5.

3. OECD guideline method – The partition coefficient was calculated using Eq. 24 From slope of Eq. 38, we can obtained K' which is equal to $K_a/(1+PR)$. (Kaufman, 1975) in their derivation of:

$$P = \frac{10^{|pK' - pKa|} - 1}{r} \qquad \text{(Eq. 24)}$$

Also had shown that $K_a' = K_a/(1+PR)$. Therefore, it is of interest to test if our K' can be used to calculate P with the same accuracy as K_a' which is taken to be pH at half-neutralization of the aqueous-octanol dual phase solvent system. The following K_a, K' and K_a' were employed for the calculation of P using Eq. 24.

3.1 Both K_a and K' were determined using titration data from the same experiment. K_a was determined with Eq. 21 and K' (determined with Eq. 38) was obtained from the dual phase aqueous-octanol sequential titration.

3.1.2 The sequential aqueous-octanol titration with 5 ml octanol added after midpoint. This is labeled Gr. 4 in Table 10.1-10.5.

3.1.3 The sequential aqueous-octanol titration with 10 ml octanol added after midpoint. This is labeled Gr. 9 in Table 10.1-10.5.

3.1.4 The sequential aqueous-octanol titration with 10 ml octanol added before midpoint. This is labeled Gr. 14 in Table 10.1-10.5.

3.2 K_a and K′ were determined from different titration experiments. Reference K_a (obtained with Eq. 21 using titration data of the single phase aqueous titration) was used in combination with K′ (determined with Eq. 38) was obtained from the dual phase aqueous-octanol sequential titration.

3.2.1 The sequential aqueous-octanol titration with 5 ml octanol added after midpoint. This is labeled Gr. 5 in Table 10.1-10.5.

3.2.2 The sequential aqueous-octanol titration with 10 ml octanol added after midpoint. This is labeled Gr. 10 in Table 10.1-10.5.

3.2.3 The sequential aqueous-octanol titration with 10 ml octanol added before midpoint. This is labeled Gr. 15 in Table 10.1-10.5.

3.3 Both K_a and K′ were determined using titration data from the different titration experiments. pH value at half-neutralization of the single phase aqueous titration was used as pK_a and K′ (determined with Eq. 38) was obtained from the dual phase aqueous-octanol sequential titration.

3.3.1 The sequential aqueous-octanol titration with 5 ml octanol added after midpoint. This is labeled Gr. 6 in Table 10.1-10.5.

3.3.2 The sequential aqueous-octanol titration with 10 ml octanol added after midpoint. This is labeled Gr. 11 in Table 10.1-10.5.

3.3.3 The sequential aqueous-octanol titration with 10 ml octanol added before midpoint. This is labeled Gr. 16 in Table 10.1-10.5.

3.4 Both K_a and K_a' were determined using titration data from the same experiment. K_a was determined with Eq. 21 using titration data from the aqueous portion of the dual phase aqueous-octanol sequential titration 10 ml of octanol was added before midpoint and the pH value at half neutralization was use as pK_a'. This is labeled Gr. 17 in Table 10.1-10.5.

3.5 K_a and K_a' were determined using titration data from the different experiment. Reference K_a (determined with Eq. 21 using titration data from the single phase aqueous titration) was used for calculating P. 10 ml of octanol was added before midpoint and the pH value at half neutralization was use as pK_a'. The sequential aqueous-octanol titration with 10 ml octanol added before midpoint. This is labeled Gr. 18 in Table 10.1-10.5.

3.6 K_a and K_a' were determined using titration data from the different experiment. pH at half-neutralization of the single phase aqueous titration was used as pK_a for calculating P. 10 ml of octanol was added before midpoint and the pH value at half neutralization was use as pK_a'. The sequential aqueous-octanol titration with 10 ml octanol added before midpoint. The sequential aqueous-octanol titration with 10 ml octanol added before midpoint. This is labeled Gr. 19 in Table 10.1-10.5.

The linear plot of Eq. 41 of each weak acid was showed in Fig. 6.1 to Fig 6.5.

Salicylic Acid

In the case of salicylic acid, ANOVA test showed that Gr. 12 and Gr. 17 did not pass variance. It is possible that the significantly different in standard deviation may have resulted from most of the data used to calculate K_a (with Eq. 21) was around

3 or below which were significantly below the pH calibration range (pH 4-10). Moreover, ANOVA test showed significant statistically difference between calculated P from the method using reference K_a by half neutralization estimation (Gr. 6, Gr. 11, Gr. 16, and Gr. 19) and other groups (Table 10.4). The calculated P in these groups were obviously significantly lower than others. Since pH at midpoint was not equal to pK_a (As discussed in Determination of K_a, page 37) the P calculated according to half neutralization was incorrect and caused erroneous P.

Acetic Acid

In the case of acetic acid, the ANOVA test showed that P calculated with K' (from Eq. 38) are statistically different from P calculated with other methods (Table 10.1). K' can be calculated from the slope of Eq. 38.

$$\frac{G\,[H_3O^+]V_a}{(V_a+V)} = K'V_eN - K'G \qquad \text{(Eq. 38)}$$

The assumption of Eq. 38 is that $(1 + PR)V_a >>> V$. For acetic acid which its P around 0.4-0.5, the values of $(1 + PR)V_a$ was not much higher than V. The percent of the values of V to $(1 + PR)V_a$ was around 9%. For substances that P value was higher, the percent of the values of V to $(1 + PR)V_a$ was closed to zero. For this case, it seems the limitation of Eq. 38 was shown. This equation was not success in applying to the substance that its P was very low. Therefore, in our further study, the limitation of Eq. 38 should be studied more.

Benzoic Acid, *m*-Toluic Acid and *2*-methoxybenzoic Acid

For the result of benzoic acid, *2*-methoxybenzoic acid and *m*-toluic acid, all calculated P by any method were not statistically significant (Table 10.2, Table 10.3 and Table 10.5). However, in the case of *m*-toluic acid, K_a cannot be obtained from the sequential aqueous-octanol titration in which 10 ml of *n*-octanol was added before midpoint because of too few data points. Due to poor aqueous solubility of *m*-toluic acid, V_e was limited to only approximately 3 ml. Therefore addition of octanol before midpoint of titration means octanol must be added at approximately V = 1.2 ml which would not allow sufficient data point for accurately determination of K_a with Eq. 21.

From these five compounds with P ranges from 0.5 – 320 (as determined with reference shake flask method), Eq. 41 can accurately determine P using titration data from the sequential aqueous-octanol titration which *n*-octanol-added before midpoint and after midpoint.

OECD guideline works well for determination of P for all tested acids excluding salicylic acid. This is due to using pH at half-neutralization as pK_a. As pK_a of the acid becomes lower than 3.0, the error in using pH at half-neutralization as pK_a becomes significant.

It has been shown that the method applying Eq. 21, Eq. 38 and Eq. 41 to the sequential aqueous-octanol titration can be useful to determine K_a, V_e (or purity) and P of monoprotic weak acid compound in single titration. Therefore, the method in this study has an advantage over the OECD guideline in saving time.

CHAPTER V

CONCLUSIONS

Although theoretically, Eq. 13 (a multiple linear equation) can be employed for determination of dissociation constant (K_a), purity and partition coefficient (P) from one single titration, Eq. 13 cannot accurately yield calculated K_a, P and purity under laboratory conditions due to limitation of accuracy in measurements of pH and volume of titrant (both of which are limited to three decimal places).

However, by employing Gran equation (Eq. 21) and modified Gran equations (Eq.38 and Eq. 41), we are able to determine dissociation constant (K_a), purity and partition coefficient (P) from one single titration. Eq. 21 allow us to determine aqueous dissociation constants from the initial aqueous phase titration data of the sequential aqueous-octanol dual phase solvent system which are statistically equivalent to the dissociation constant determined from the titration in single phase aqueous solvent system (also determined with Eq. 21). The purities determined (Eq. 42) from the dual phase aqueous-octanol solvent system are statistically equivalent to purities determined with single phase aqueous titration (Gran equation, Eq. 21). Using K_a (determined with Eq. 21) and V_e (determined with Eq. 38) as inputs into Eq. 41 , we were able to calculate partition coefficient (P) which were statistically equivalent to P values determined with the shake flask method.

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APPENDICES

APPENDIX A

POLYNOMIAL EQUATION DERIVATION

The initial steps of derivation were in the same of pattern as for derivation of Eq. 13 which based on mass balance, charge balance and equilibrium equation as follows below;

From octanol-aqueous equilibrium equation

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

Oil phase Aqueous phase

$$Ka = \frac{[A^-][H^+]}{[HA]_{aq}} \quad \text{(Eq. 25)}$$

$$P = \frac{[HA]_{oct}}{[HA]_{aq}} \quad \text{(Eq. 26)}$$

Mass balance

$$[HA]_o V_a = V_e N = [HA]_{oct} V_{oct} + [HA]_{aq}(V_a+V) + [A^-](V_a+V) \quad \text{(Eq. 27)}$$

Substitution $[HA]_{oct}$ from Eq. 26 into Eq. 27

$$[HA]_o V_a = V_e N = P[HA]_{aq} R V_a + [HA]_{aq}(V_a+V) + [A^-](V_a+V)$$

$$= [HA]_{aq}\{PRV_a + (V_a+V)\} + [A^-](V_a+V) \quad \text{(Eq. 28)}$$

Substitution $[HA]_{aq}$ from Eq. 25 into Eq. 28 give

$$[HA]_o V_a = \frac{[A^-][H_3O^+]\{PRVa + (V_a+V)\}}{K_a} + [A^-](V_a+V)$$

$$= [A^-]\left\{\frac{[H_3O^+]\{PRV_a + (V_a+V)\}}{K_a} + (V_a+V)\right\}$$

$$[HA]_o V_a = [A^-]\left\{\frac{PRV_a[H_3O^+] + (V_a+V)[H_3O^+] + K_a(V_a+V)}{K_a}\right\}$$

$$[A^-] = \frac{[HA]_o V_a K_a}{PRV_a[H_3O^+] + (V_a+V)[H_3O^+] + (V_a+V)K_a} \quad \text{(Eq. 29)}$$

Charge balance

$$[Na^+] + [H_3O^+] = [A^-] + [OH^-] \quad \text{(Eq. 30)}$$

Replace $[Na^+] = \frac{VN}{(V+V_a)}$ and $[OH^-] = \frac{K_w}{[H_3O^+]}$ to Eq. 30

$$\frac{VN}{(V+V_a)} + [H_3O^+] = [A^-] + \frac{K_w}{[H_3O^+]}$$

$$[A^-] = [H_3O^+] + \frac{VN}{(V+V_a)} - \frac{K_w}{[H_3O^+]} \quad \text{(Eq. 31)}$$

since Eq. 29 = Eq. 31 and then

$$\frac{[HA]_o V_a K_a}{PRV_a[H_3O^+] + (V_a+V)[H_3O^+] + (V_a+V)K_a} = [H_3O^+] + \frac{VN}{(V+V_a)} - \frac{K_w}{[H_3O^+]}$$

Solved this equation

$$\frac{[HA]_o V_a K_a}{PRV_a[H_3O^+] + (V_a+V)[H_3O^+] + (V_a+V)K_a} = [H_3O^+] + \frac{VN}{(V+V_a)} - \frac{K_w}{[H_3O^+]}$$

$$\frac{[HA]_o V_a K_a}{\{PRV_a + (V_a+V)\}[H_3O^+] + K_a(V_a+V)} = \frac{(V+V_a)[H_3O^+]^2 + VN[H_3O^+] - K_w(V+V_a)}{(V+V_a)[H_3O^+]}$$

$$[HA]_o V_a K_a(V_a+V)[H_3O^+] = [(V+V_a)[H_3O^+]^2 + VN[H_3O^+] - K_w(V+V_a)]$$

$$\times [\{PRV_a + (V_a+V)\}[H_3O^+] + K_a(V_a+V)]$$

$$[HA]_o V_a K_a(V_a+V)[H_3O^+] = [(V+V_a)[H_3O^+]^2\{PRV_a + (V_a+V)\}[H_3O^+] +$$

$$(V+V_a)[H_3O^+]^2 K_a(V_a+V)] + [VN[H_3O^+]\{PRV_a + (V_a+V)\}[H_3O^+] +$$

$$VN[H_3O^+]K_a(V_a+V)] - K_w(V+V_a)\{PRV_a + (V_a+V)\}[H_3O^+] +$$

$$K_w K_a(V+V_a)^2]$$

$$[HA]_o V_a K_a(V_a+V)[H_3O^+] = (V+V_a)\{PRV_a + (V_a+V)\}[H_3O^+]^3 + K_a(V+V_a)^2[H_3O^+]^2$$

$$+VN\{PRV_a + (V_a+V)\}[H_3O^+]^2 + VN\,K_a(V_a+V)[H_3O^+]$$

$$-K_w(V+V_a)\{PRV_a + (V_a+V)\}[H_3O^+] + K_w K_a(V+V_a)^2 \qquad \text{(Eq. 32)}$$

Rearrange to give

$$[PRV_a + (V_a+V)]\,\mathbf{[H_3O^+]^3} + \{K_a(V+V_a) + \frac{VN\ \{PRV_a + (V+V_a)\}}{(V+V_a)}\}\,\mathbf{[H_3O^+]^2}$$

$$+[VN\,K_a - K_w\{PRV_a + (V_a+V)\} - [HA]_o V_a K_a]\,\mathbf{[H_3O^+]} - K_w K_a(V+V_a) = 0 \quad \text{(Eq. 33)}$$

APPENDIX B

MODIFIED GRAN EQUATION

To derive our modified Gran equation for dual phase aqueous-octanol solvent system, we start with Eq. 11 below.

$$K_a = \frac{G\,[H_3O^+](\{1+PR\}V_a + V)}{(V_eN - G)\,(V_a+V)} \qquad \text{(Eq. 11)}$$

If we define a parameter K' such that

$$K' = \frac{[H_3O^+][A^-]V_a}{[HA]_oV_o + [HA]_aV_a} \qquad \text{(Eq. 34)}$$

Substituting Eq. 26 and $R = V_o/V_a$ into Eq. 34, follow by rearranging, gave

$$K' = \frac{[H_3O^+][A^-]}{[HA]_a\,\{1+PR\}} = \frac{K_a}{\{1+PR\}} \qquad \text{(Eq. 35)}$$

or

$$\{1+PR\}K' = K_a \qquad \text{(Eq. 36)}$$

Since K_a and $\{1 + PR\}$ are constants, K' also has constant value. Let's Eq. 11 to Eq. 34, follow by rearranging into the form of simple linear equation,

$$\frac{G\,[H_3O^+](\{1+PR\}V_a + V)}{\{1+PR\}(V_a+V)} = K'V_eN - K'G \qquad \text{(Eq. 37)}$$

If we assume that $\{1 + PR\}V_a >> V$ (for $V \leq V_e$) and Eq. 37 became

$$\frac{G\,[H_3O^+]V_a}{(V_a+V)} = K'V_eN - K'G \qquad \text{(Eq. 38)}$$

The equivalent point of titration can be calculated by

$$V_e = \frac{\text{X-intercept}}{N \times |\text{ slope }|} \qquad \text{(Eq.39)}$$

APPENDIX C

SIMPLE LINEAR EQUATION DERIVATION

According to the result of multiple linear regression, Eq. 13. Based on charge balance, mass balance and equilibrium equation to obtain Eq.10, the equation for determination of partition coefficient must be derived as followed.

$$K_a = \frac{\dfrac{\left\{VN + \{[H_3O^+] - [OH^-]\}(V_a+V)\right\}}{(V+V_a)}[H_3O^+]}{V_eN - \dfrac{\left\{VN + \{[H_3O^+] - [OH^-]\}(V_a+V)\right\}}{(V+V_a)}}{\{PRV_a + (V_a+V)\}} \qquad \text{(Eq.10)}$$

Let $G = VN + ([H_3O^+] - [OH^-])(V_a+V)$ and rearrange to

$$K_a = \frac{\dfrac{G[H_3O^+]}{(V_a+V)}}{\dfrac{V_eN - G}{\{PRV_a + (V_a+V)\}}} = \frac{G[H_3O^+][PRV_a + (V_a+V)]}{(V_eN - G)(V_a+V)} \qquad \text{(Eq. 40)}$$

$$K_a(V_eN - G)(V_a+V) = G[H_3O^+][PRV_a + (V_a+V)] = G[H_3O^+][(PR+1)V_a + V]$$

$$K_aV_eN(V_a+V) - K_aG(V_a+V) = GV_a[H_3O^+](PR+1) + GV[H_3O^+]$$

$$K_a V_e N(V_a+V) - K_a G(V_a+V) - GV[H_3O^+] = G\,V_a[H_3O^+](PR+1)$$

Both sides was divided by G results

$$\frac{K_a\{V_e N(V_a+V) - G(V_a+V)\}}{G} - V[H_3O^+] = V_a(PR+1)\,[H_3O^+] \qquad \text{(Eq. 41)}$$

APPENDIX D

INDEX OF EQUATION

Table 1 – The fixed quantities parameters of simulated titration data for multiple linear regression model (Eq. 13)

pK_w	14	
Initial aqueous volume, V_a (ml)	50.000	
Normality of titrant, N	0.10000	
Constant incremental volume (ml)	0.100	
$[HA]_0$ (M)	0.010000	
V_e (ml)	5.000 ml	
Volume of *n*-octanol added, V_{oct} (ml)	5.000	10.000
pK_a	2, 3, 4, 5, 6, 7, 8, 10	
P	0.001, 0.01, 0.1, 1, 10, 100, 1000, 10000	

Table 2 – The fixed quantities parameters of simulated titration data for modified Gran Equation, Eq. 38

pK_w	14	
Initial aqueous volume, V_a (ml)	50.000	
Normality of titrant, N	0.10000	
Constant incremental volume (ml)	0.100	
$[HA]_0$ (M)	0.010000	0.020000
V_e (ml)	5.000	10.000
Volume of *n*-octanol added, V_o (ml)	5.000	10.000
pK_a	3, 5, 7, 9	
P	0.1, 50, 200, 500	

Table 3 – the value of pK_a and P of neutral weak acids

compound	pK_a [b]	reported P
acetic acid	4.731	0.50[a]
benzoic acid	4.163	74[b]
2-methoxybenzoic acid	4.051	50[c]
salicylic acid	2.936	182[d]
m-toluic acid	4.238	234[b]

[a]Barzanti et al. 2007, [b]Fujta et al. 1964, [c]Hansch et al. 1967, [d]Guanghua et al. 2001.

Table 4.1 The concentrations of benzoic acid (mM) and absorbance for the plot of calibration curve

Concentration (mM)	Absorbance
0.0124	0.145
0.0310	0.353
0.0497	0.575
0.0621	0.711
0.0932	1.063

Table 4.2 The concentrations of *2*-methoxybenzoic acid (mM) and absorbance for the plot of calibration curve

Concentration (mM)	Absorbance
0.0290	0.212
0.0436	0.310
0.0581	0.418
0.0726	0.515
0.0871	0.607
0.116	0.813

Table 4.3 The concentrations of salicylic acid (mM) and absorbance for the plot of calibration curve

Concentration (mM)	Absorbance
0.0265	0.226
0.0441	0.383
0.0662	0.556
0.0883	0.777
0.110	0.932

Table 4.4 The concentrations of *m*-toluic acid (mM) and absorbance for the plot of calibration curve

Concentration (mM)	Absorbance
0.0162	0.163
0.0243	0.242
0.0323	0.326
0.0485	0.483
0.0647	0.650
0.0808	0.820

Table 5 The summary of V_e, K_a and P obtained from three regression program packages using simulated titration data for Eq. 13 testing

	theoretical		Excel® 2007			Wessa (2009)			MINITAB® 15		
V_o	P	pK_a	V_e	K_a	P	V_e	K_a	P	V_e	K_a	P
5	0.001	2	5.000	1.000E-02	0.001	5.000	1.000E-02	0.001	5.000	1.000E-02	0.001
		3	5.000	1.000E-03	0.001	5.000	1.000E-03	0.001	5.000	1.000E-03	0.001
		4	5.000	1.000E-04	0.001	5.000	1.000E-04	0.001	5.000	1.000E-04	0.001
		5	-95.118	-8.109E-09	-10.000	5.000	1.000E-05	0.001	5.000	1.000E-05	0.001
		6	-80.979	-9.509E-10	-10.000	5.000	1.000E-06	0.001	5.000	1.000E-06	0.001
		7	-76.591	-1.004E-10	-10.000	5.000	1.000E-07	0.001	5.000	1.000E-07	0.001
		8	-66.048	-1.161E-11	-10.000	5.000	1.000E-08	0.001	5.000	1.000E-08	0.001
		10	-8.634	-7.851E-13	-10.000	5.000	1.000E-10	0.001	5.000	1.000E-10	0.001
10	0.001	2	5.000	1.000E-02	0.001	5.000	1.000E-02	0.001	5.000	1.000E-02	0.001
		3	5.000	1.000E-03	0.001	5.000	1.000E-03	0.001	5.000	1.000E-03	0.001
		4	5.000	1.000E-04	0.001	5.000	1.000E-04	0.001	5.000	1.000E-04	0.001
		5	-95.107	-8.109E-09	-5.000	5.000	1.000E-05	0.001	5.000	1.000E-05	0.001
		6	-80.971	-9.509E-10	-5.000	5.000	1.000E-06	0.001	5.000	1.000E-06	0.001
		7	-76.584	-1.004E-10	-5.000	5.000	1.000E-07	0.001	5.000	1.000E-07	0.001
		8	-66.042	-1.161E-11	-5.000	5.000	1.000E-08	0.001	5.000	1.000E-08	0.001
		10	-8.633	-7.851E-13	-5.000	5.000	1.000E-10	0.001	5.000	1.000E-10	0.001
5	0.01	2	5.000	1.000E-02	0.010	5.000	1.000E-02	0.010	5.000	1.000E-02	0.010
		3	5.000	1.000E-03	0.010	5.000	1.000E-03	0.010	5.000	1.000E-03	0.010
		4	5.000	1.000E-04	0.010	5.000	1.000E-04	0.010	5.000	1.000E-04	0.010
		5	-95.013	-8.111E-09	-10.000	5.000	1.000E-05	0.010	5.000	1.000E-05	0.010
		6	-80.908	-9.509E-10	-10.000	5.000	1.000E-06	0.010	5.000	1.000E-06	0.010
		7	-76.528	-1.004E-10	-10.000	5.000	1.000E-07	0.010	5.000	1.000E-07	0.010
		8	-65.994	-1.161E-11	-10.000	5.000	1.000E-08	0.010	5.000	1.000E-08	0.010
		10	-8.627	-7.849E-13	-10.000	5.000	1.000E-10	0.010	5.000	1.000E-10	0.010
10	0.01	2	5.000	1.000E-02	0.010	5.000	1.000E-02	0.010	5.000	1.000E-02	0.010
		3	5.000	1.000E-03	0.010	5.000	1.000E-03	0.010	5.000	1.000E-03	0.010
		4	5.000	1.000E-04	0.010	5.000	1.000E-04	0.010	5.000	1.000E-04	0.010
		5	-94.897	-8.113E-09	-5.000	5.000	1.000E-05	0.010	5.000	1.000E-05	0.010
		6	-80.830	-9.509E-10	-5.000	5.000	1.000E-06	0.010	5.000	1.000E-06	0.010
		7	-76.459	-1.004E-10	-5.000	5.000	1.000E-07	0.010	5.000	1.000E-07	0.010
		8	-65.935	-1.160E-11	-5.000	5.000	1.000E-08	0.010	5.000	1.000E-08	0.010
		10	-8.620	-7.847E-13	-5.000	5.000	1.000E-10	0.010	5.000	1.000E-10	0.010
5	0.1	2	5.000	1.000E-02	0.100	5.000	1.000E-02	0.100	5.000	1.000E-02	0.100
		3	5.000	1.000E-03	0.100	5.000	1.000E-03	0.100	5.000	1.000E-03	0.100
		4	5.000	1.000E-04	0.100	5.000	1.000E-04	0.100	5.000	1.000E-04	0.100
		5	-93.982	-8.128E-09	-10.000	5.000	1.000E-05	0.100	5.000	1.000E-05	0.100
		6	-80.213	-9.508E-10	-10.000	5.000	1.000E-06	0.100	5.000	1.000E-06	0.100
		7	-75.911	-1.003E-10	-10.000	5.000	1.000E-07	0.100	5.000	1.000E-07	0.100
		8	-65.467	-1.160E-11	-10.000	5.000	1.000E-08	0.100	5.000	1.000E-08	0.100
		10	-8.565	-7.831E-13	-10.000	5.000	1.000E-10	0.100	5.000	1.000E-10	0.100
10	0.1	2	5.000	1.000E-02	0.100	5.000	1.000E-02	0.100	5.000	1.000E-02	0.100
		3	5.000	1.000E-03	0.100	5.000	1.000E-03	0.100	5.000	1.000E-03	0.100
		4	5.000	1.000E-04	0.100	5.000	1.000E-04	0.100	5.000	1.000E-04	0.100
		5	-92.882	-8.145E-09	-5.000	5.000	1.000E-05	0.100	5.000	1.000E-05	0.100
		6	-79.467	-9.505E-10	-5.000	5.000	1.000E-06	0.100	5.000	1.000E-06	0.100
		7	-75.248	-1.003E-10	-5.000	5.000	1.000E-07	0.100	5.000	1.000E-07	0.100
		8	-64.899	-1.159E-11	-5.000	5.000	1.000E-08	0.100	5.000	1.000E-08	0.100
		10	-8.496	-7.812E-13	-5.000	5.000	1.000E-10	0.100	5.000	1.000E-10	0.100

Table 5 The summary of V_e, K_a and P obtained from three regression program packages using simulated titration data for Eq. 13 testing — continue

	theoretical		Excel® 2007			Wessa (2009)			MINITAB® 15		
V_o	P	pK_a	V_e	K_a	P	V_e	K_a	P	V_e	K_a	P
5	1	2	5.000	1.000E-02	1.000	5.000	1.000E-02	1.000	5.000	1.000E-02	1.000
		3	5.000	1.000E-03	1.000	5.000	1.000E-03	1.000	5.000	1.000E-03	1.000
		4	5.000	1.000E-04	1.000	5.000	1.000E-04	1.000	5.000	1.000E-04	1.000
		5	-85.474	-8.218E-09	-10.000	5.000	1.000E-05	1.000	5.000	1.000E-05	1.000
		6	-74.355	-9.432E-10	-10.000	5.000	1.000E-06	1.000	5.000	1.000E-06	1.000
		7	-70.677	-9.911E-11	-10.000	5.000	1.000E-07	1.000	5.000	1.000E-07	1.000
		8	-60.949	-1.145E-11	-10.000	5.000	1.000E-08	1.000	5.000	1.000E-08	1.000
		10	-7.994	-7.659E-13	-10.000	5.000	1.000E-10	1.000	5.000	1.000E-10	1.000
10	1	2	5.000	1.000E-02	1.000	5.000	1.000E-02	1.000	5.000	1.000E-02	1.000
		3	5.000	1.000E-03	1.000	5.000	1.000E-03	1.000	5.000	1.000E-03	1.000
		4	5.000	1.000E-04	1.000	5.000	1.000E-04	1.000	5.000	1.000E-04	1.000
		5	-78.708	-8.192E-09	-5.000	5.000	1.000E-05	1.000	5.000	1.000E-05	1.000
		6	-69.545	-9.257E-10	-5.000	5.000	1.000E-06	1.000	5.000	1.000E-06	1.000
		7	-66.333	-9.693E-11	-5.000	5.000	1.000E-07	1.000	5.000	1.000E-07	1.000
		8	-57.120	-1.121E-11	-5.000	5.000	1.000E-08	1.000	5.000	1.000E-08	1.000
		10	-7.460	-7.475E-13	-5.000	5.000	1.000E-10	1.000	5.000	1.000E-10	1.000
5	10	2	5.000	1.000E-02	10.000	5.000	1.000E-02	10.000	5.000	1.000E-02	10.000
		3	5.000	1.000E-03	10.000	5.000	1.000E-03	10.000	5.000	1.000E-03	10.000
		4	-69.823	-5.543E-08	-10.000	5.000	1.000E-04	10.000	5.000	1.000E-04	10.000
		5	-57.709	-6.744E-09	-10.000	5.000	1.000E-05	10.000	5.000	1.000E-05	10.000
		6	-53.707	-7.236E-10	-10.000	5.000	1.000E-06	10.000	5.000	1.000E-06	10.000
		7	-51.643	-7.515E-11	-10.000	5.000	1.000E-07	10.000	5.000	1.000E-07	10.000
		8	-43.224	-8.927E-12	-10.000	5.000	1.000E-08	10.000	5.000	1.000E-08	10.000
		10	-5.026	-6.339E-13	-10.000	5.000	1.000E-10	10.000	5.000	1.000E-10	10.000
10	10	2	5.000	1.000E-02	10.000	5.000	1.000E-02	10.000	5.000	1.000E-02	10.000
		3	5.000	1.000E-03	10.000	5.000	1.000E-03	10.000	5.000	1.000E-03	10.000
		4	-58.625	-4.428E-08	-5.000	5.000	1.000E-04	10.000	5.000	1.000E-04	10.000
		5	-50.527	-5.150E-09	-5.000	5.000	1.000E-05	10.000	5.000	1.000E-05	10.000
		6	-47.967	-5.417E-10	-5.000	5.000	1.000E-06	10.000	5.000	1.000E-06	10.000
		7	-46.024	-5.638E-11	-5.000	5.000	1.000E-07	10.000	5.000	1.000E-07	10.000
		8	-36.938	-6.968E-12	-5.000	5.000	1.000E-08	10.000	5.000	1.000E-08	10.000
		10	-3.676	-5.476E-13	-5.000	5.000	1.000E-10	10.000	5.000	1.000E-10	10.000
5	100	2	5.000	1.000E-02	100.000	5.000	1.000E-02	100.000	5.000	1.000E-02	100.000
		3	-51.766	-1.356E-07	-10.000	5.000	1.000E-03	100.000	5.000	1.000E-03	100.000
		4	-45.417	-1.570E-08	-10.000	5.000	1.000E-04	100.000	5.000	1.000E-04	100.000
		5	-42.199	-1.688E-09	-10.000	5.000	1.000E-05	100.000	5.000	1.000E-05	100.000
		6	-41.001	-1.735E-10	-10.000	5.000	1.000E-06	100.000	5.000	1.000E-06	100.000
		7	-37.502	-1.890E-11	-10.000	5.000	1.000E-07	100.000	5.000	1.000E-07	100.000
		8	-23.559	-2.942E-12	-10.000	5.000	1.000E-08	100.000	5.000	1.000E-08	100.000
		10	-1.226	-3.354E-13	-10.000	5.000	1.000E-10	100.000	5.000	1.000E-10	100.000
10	100	2	-41.274	-8.132E-07	-5.000	5.000	1.000E-02	100.000	5.000	1.000E-02	100.000
		3	-48.877	-7.601E-08	-5.000	5.000	1.000E-03	100.000	5.000	1.000E-03	100.000
		4	-42.993	-8.694E-09	-5.000	5.000	1.000E-04	100.000	5.000	1.000E-04	100.000
		5	-40.820	-9.144E-10	-5.000	5.000	1.000E-05	100.000	5.000	1.000E-05	100.000
		6	-39.601	-9.414E-11	-5.000	5.000	1.000E-06	100.000	5.000	1.000E-06	100.000
		7	-34.237	-1.082E-11	-5.000	5.000	1.000E-07	100.000	5.000	1.000E-07	100.000
		8	-17.967	-1.989E-12	-5.000	5.000	1.000E-08	100.000	5.000	1.000E-08	100.000
		10	-0.640	-2.646E-13	-5.000	5.000	1.000E-10	100.000	5.000	1.000E-10	100.000

Table 5 The summary of V_e, K_a and P obtained from three regression program packages using simulated titration data for Eq. 13 testing ─ continue

	theoretical		Excel® 2007			Wessa (2009)			MINITAB® 15		
V_o	P	pK_a	V_e	K_a	P	V_e	K_a	P	V_e	K_a	P
5	1000	2	-48.883	-1.562E-07	-10.000	5.000	1.000E-02	1000.000	5.000	1.000E-02	1000.000
		3	-43.448	-1.790E-08	-10.000	5.000	1.000E-03	1000.000	5.000	1.000E-03	1000.000
		4	-40.402	-1.923E-09	-10.000	5.000	1.000E-04	1000.000	5.000	1.000E-04	1000.000
		5	-39.296	-1.975E-10	-10.000	5.000	1.000E-05	1000.000	5.000	1.000E-05	1000.000
		6	-36.275	-2.132E-11	-10.000	5.000	1.000E-06	1000.000	5.000	1.000E-06	1000.000
		7	-23.628	-3.205E-12	-10.000	5.000	1.000E-07	1000.000	5.000	1.000E-07	1000.000
		8	-7.583	-9.000E-13	-10.000	5.000	1.000E-08	1000.000	5.000	1.000E-08	1000.000
		10	-0.078	-1.606E-13	-10.000	5.000	1.000E-10	1000.000	5.000	1.000E-10	1000.000
10	1000	2	-47.604	-8.156E-08	-5.000	5.000	1.000E-02	1000.000	5.000	1.000E-02	1000.000
		3	-42.021	-9.301E-09	-5.000	5.000	1.000E-03	1000.000	5.000	1.000E-03	1000.000
		4	-39.905	-9.781E-10	-5.000	5.000	1.000E-04	1000.000	5.000	1.000E-04	1000.000
		5	-38.755	-1.006E-10	-5.000	5.000	1.000E-05	1000.000	5.000	1.000E-05	1000.000
		6	-33.766	-1.148E-11	-5.000	5.000	1.000E-06	1000.000	5.000	1.000E-06	1000.000
		7	-18.118	-2.065E-12	-5.000	5.000	1.000E-07	1000.000	5.000	1.000E-07	1000.000
		8	-4.786	-6.671E-13	-5.000	5.000	1.000E-08	1000.000	5.000	1.000E-08	1000.000
		10	-0.022	-1.363E-13	-5.000	5.000	1.000E-10	1000.000	5.000	1.000E-10	1000.000
5	10000	2	-43.240	-1.815E-08	-10.000	5.000	1.000E-02	10000.000	5.000	1.000E-02	10000.000
		3	-40.212	-1.950E-09	-10.000	5.000	1.000E-03	10000.000	5.000	1.000E-03	10000.000
		4	-39.116	-2.002E-10	-10.000	5.000	1.000E-04	10000.000	5.000	1.000E-04	10000.000
		5	-36.141	-2.160E-11	-10.000	5.000	1.000E-05	10000.000	5.000	1.000E-05	10000.000
		6	-23.630	-3.235E-12	-10.000	5.000	1.000E-06	10000.000	5.000	1.000E-06	10000.000
		7	-7.617	-9.048E-13	-10.000	5.000	1.000E-07	10000.000	5.000	1.000E-07	10000.000
		8	-1.330	-3.496E-13	-10.000	5.000	1.000E-08	10000.000	5.000	1.000E-08	10000.000
		9	-0.079	-1.610E-13	-10.000	5.000	1.000E-09	10000.000	5.000	1.000E-09	10000.000
		10	0.000	-1.092E-13	-10.000	Error	Error	Error	5.000	1.000E-10	10000.000
10	10000	2	-41.921	-9.366E-09	-5.000	5.000	1.000E-02	10000.000	5.000	1.000E-02	10000.000
		3	-39.811	-9.849E-10	-5.000	5.000	1.000E-03	10000.000	5.000	1.000E-03	10000.000
		4	-38.668	-1.013E-10	-5.000	5.000	1.000E-04	10000.000	5.000	1.000E-04	10000.000
		5	-33.716	-1.155E-11	-5.000	5.000	1.000E-05	10000.000	5.000	1.000E-05	10000.000
		6	-18.132	-2.073E-12	-5.000	5.000	1.000E-06	10000.000	5.000	1.000E-06	10000.000
		7	-4.799	-6.687E-13	-5.000	5.000	1.000E-07	10000.000	5.000	1.000E-07	10000.000
		8	-0.672	-2.699E-13	-5.000	5.000	1.000E-08	10000.000	5.000	1.000E-08	10000.000
		9	-0.022	-1.364E-13	-5.000	5.000	1.000E-09	10000.000	5.000	1.000E-09	10000.000
		10	0.000	-1.048E-13	-5.000	Error	Error	Error	5.000	1.000E-10	10000.000

Table 6 The summary of multiple linear regression results in the step of data simulation with rounding pH off to 3 decimal points.

Theoretical value			Excel® 2007			Wessa (2009)			MINITAB® 15		
V_{ect}	P	pK_a	V_e	K_a	P	V_e	K_a	P	V_e	K_a	P
5	0.001	2	5.000	9.684E-03	-0.324	5.000	9.684E-03	-0.324	5.000	9.684E-03	-0.324
	0.001	3	5.000	1.001E-03	0.010	5.000	1.001E-03	0.010	5.000	1.001E-03	0.010
	0.001	4	5.000	9.944E-05	-0.058	5.000	9.944E-05	-0.058	5.000	9.944E-05	-0.058
	0.001	5	-94.324	-8.176E-09	-10.000	5.000	1.007E-05	0.077	5.000	1.007E-05	0.077
	0.001	7	-76.601	-1.004E-10	-10.000	5.000	1.005E-07	0.050	5.000	1.005E-07	0.050
	0.001	10	-8.638	-7.848E-13	-10.000	5.001	9.907E-11	-0.093	5.001	9.907E-11	-0.093
5	1	2	5.000	1.003E-02	1.041	5.001	9.724E-03	-0.269	5.000	1.003E-02	1.041
	1	3	5.000	1.004E-03	1.043	5.000	9.932E-04	-0.060	5.000	1.004E-03	1.043
	1	4	5.001	9.897E-05	0.888	5.000	9.918E-05	-0.075	5.001	9.897E-05	0.888
	1	5	-85.464	-7.661E-13	-10.000	5.000	1.003E-02	1.041	5.000	1.005E-05	1.058
	1	7	-70.656	-9.914E-11	-10.000	5.000	1.004E-03	1.043	5.001	9.832E-08	0.813
	1	10	-7.992	-7.661E-13	-10.000	5.001	9.897E-05	0.888	5.002	9.808E-11	0.790
5	100	2	5.005	5.420E-03	49.602	5.005	5.420E-03	49.602	5.005	5.420E-03	49.602
	100	3	-51.622	-1.359E-07	-10.000	5.005	5.927E-04	55.203	5.005	5.927E-04	55.203
	100	4	-45.526	-1.567E-08	-10.000	5.002	7.104E-05	68.111	5.003	7.104E-05	68.111
	100	5	-42.074	-1.693E-09	-10.000	5.002	9.437E-06	93.863	5.002	9.437E-06	93.863
	100	7	-37.481	-1.892E-11	-10.000	5.003	7.386E-08	71.248	5.003	7.386E-08	71.248
	100	10	-1.226	-3.353E-13	-10.000	5.035	3.607E-11	29.883	5.035	3.607E-11	29.883
5	10000	2	-43.362	-1.810E-08	-10.000	5.013	6.400E-05	54.045	5.013	6.400E-05	54.045
	10000	3	-40.177	-1.951E-09	-10.000	5.154	5.729E-07	-4.246	5.148	5.730E-07	-4.246
	10000	4	-39.136	-2.002E-10	-10.000	4.969	-2.808E-07	-38.082	5.000	-2.800E-07	-38.080
	10000	5	-36.215	-2.156E-11	-10.000	5.020	4.254E-08	32.591	5.021	4.254E-08	32.591
	10000	7	-7.617	-9.048E-13	-10.000	5.037	3.049E-10	20.609	5.038	3.049E-10	20.609
	10000	10	-0.001	-1.092E-25	-10.000	0.015	-1.095E-25	-10.000	0.015	-1.095E-13	-10.033

Table 7 The percent different of the results of multiple linear regression, Eq. 13 in the step of data simulation with rounding pH to 3 decimal points.

Theoretical value			Excel® 2007			Wessa (2009)			MINITAB® 15		
V_{ect}	P	pK_a	V_e	K_a	P	V_e	K_a	P	V_e	K_a	P
5	0.001	2	-0.01	3.16	32545.25	-0.01	3.16	32545	-0.01	3.16	32545
	0.001	3	0.00	-0.09	-885.40	0.00	-0.09	-885	0.00	-0.09	-885
	0.001	4	-0.01	0.56	5877.01	-0.01	0.56	5877	0.00	0.56	5877
	0.001	5	1986.48	100.08	1000100	0.00	-0.72	-7586	0.00	-0.72	-7586
	0.001	7	1632.01	100.10	1000100	0.00	-0.48	-4920	0.00	-0.48	-4920
	0.001	10	272.76	100.78	1000100	-0.01	0.93	9440	-0.01	0.93	9440
5	1	2	0.00	-0.30	-4.07	0.00	-0.30	-10307	0.00	-0.30	-10307
	1	3	-0.01	-0.37	-4.34	-0.01	-0.37	-10334	-0.01	-0.37	-10334
	1	4	-0.01	1.03	11.19	-0.01	1.03	-8781	-0.01	1.03	-8781
	1	5	1809.28	100.08	1100.00	0.00	-0.51	-10481	0.00	-0.50	-10481
	1	7	1513.12	100.10	1100.00	-0.02	1.68	-8029	-0.02	1.68	-8029
	1	10	259.83	100.77	1100.00	-0.04	1.92	-7796	-0.04	1.92	-7796
5	100	2	-0.10	45.80	50.40	-0.10	45.80	-495920	-0.10	45.80	-495920
	100	3	1132.44	100.01	110.00	-0.10	40.73	-551931	-0.10	40.73	-551931
	100	4	1010.53	100.02	110.00	-0.05	28.96	-681010	-0.06	28.96	-681010
	100	5	941.48	100.02	110.00	-0.03	5.63	-938533	-0.03	5.64	-938533
	100	7	849.62	100.02	110.00	-0.06	26.14	-712379	-0.06	26.14	-712379
	100	10	124.53	100.34	110.00	-0.69	63.93	-298726	-0.69	63.93	-298726
5	10000	2	967.23	100.00	100.10	-0.25	99.36	-540350	-0.25	99.36	-540350
	10000	3	903.54	100.00	100.10	-3.08	99.94	42556	-2.97	99.94	42556
	10000	4	882.72	100.00	100.10	0.63	100.28	380921	0.64	100.28	380921
	10000	5	824.30	100.00	100.10	-0.40	99.57	-325812	-0.42	99.57	-325812
	10000	7	252.34	100.00	100.10	-0.74	99.70	-205987	-0.75	99.70	-205987
	10000	10	100.01	100.11	100.10	99.70	100.11	100434	99.70	100.11	100434

Table 8.1 The summary of K_a of acetic acid from aqueous portion of various types of titration with 0.1 N sodium hydroxide

No.	$V_e/2$	Gran Equation (Eq. 21)		
		Aqueous Titration	Aqueous-Octanol Titration	
			After midpoint	Before midpoint
1	1.810E-05	1.830E-05		
1	1.830E-05	1.840E-05		
1	1.880E-05	1.880E-05		
1	1.840E-05	1.860E-05		
1	1.850E-05	1.870E-05		
2	1.830E-05		1.890E-05	
2	1.830E-05		1.850E-05	
2	1.830E-05		1.860E-05	
2	1.830E-05		1.840E-05	
2	1.830E-05		1.730E-05	
3	1.890E-05		1.890E-05	1.810E-05
3	1.890E-05		1.850E-05	1.830E-05
3	1.890E-05		1.860E-05	1.880E-05
3	1.890E-05		1.840E-05	1.830E-05
3	1.890E-05		1.730E-05	1.850E-05
Mean	1.854E-05	1.856E-05	1.834E-05	1.840E-05
SD	3.019E-07	2.074E-07	5.758E-07	2.646E-07

No.1 the aqueous titration with no octanol added in quintuplicate

No.2 the sequential of aqueous-octanol titration with 5 ml octanol added in quintuplicate

No.3 the sequential of aqueous-octanol titration with 10 ml octanol added in quintuplicate

Table 8.2 The summary of K_a of benzoic acid from aqueous portion of various types of titration with 0.1 N sodium hydroxide

No.	$V_e/2$	Gran Equation (Eq. 21)		
		Aqueous Titration	Aqueous-Octanol Titration	
			After midpoint	Before midpoint
1	6.470E-05	6.920E-05		
1	6.500E-05	6.970E-05		
1	6.920E-05	6.680E-05		
1	8.580E-05	6.690E-05		
1	6.430E-05	7.140E-05		
2	6.230E-05		6.620E-05	
2	6.550E-05		6.280E-05	
2	6.940E-05		6.530E-05	
2	6.170E-05		7.100E-05	
2	6.520E-05		6.320E-05	
3	6.200E-05		6.580E-05	6.680E-05
3	6.380E-05		6.270E-05	6.750E-05
3	6.570E-05		6.460E-05	6.470E-05
3	6.690E-05		6.670E-05	6.500E-05
3	6.680E-05		6.840E-05	6.920E-05
Mean	6.655E-05	6.880E-05	6.567E-05	6.664E-05
SD	5.796E-06	1.958E-06	2.617E-06	1.856E-06

No.1 the aqueous titration with no octanol added in quintuplicate

No.2 the sequential of aqueous-octanol titration with 5 ml octanol added in quintuplicate

No.3 the sequential of aqueous-octanol titration with 10 ml octanol added in quintuplicate

Table 8.3 The summary of K_a of *2*-methoxybenzoic acid from aqueous portion of various types of titration with 0.1 N sodium hydroxide

No.	$V_e/2$	Gran Equation (Eq. 21)		
		Aqueous Titration	Aqueous-Octanol Titration	
			After midpoint	Before midpoint
1	8.580E-05	8.970E-05		
1	8.722E-05	9.060E-05		
1	9.053E-05	8.790E-05		
1	8.645E-05	8.930E-05		
1	8.091E-05	8.440E-05		
2	9.090E-05		8.980E-05	
2	8.830E-05		8.740E-05	
2	8.980E-05		8.590E-05	
2	8.890E-05		8.940E-05	
2	8.940E-05		8.670E-05	
3	8.610E-05		9.090E-05	8.580E-05
3	8.460E-05		8.830E-05	8.720E-05
3	8.230E-05		8.980E-05	9.050E-05
3	8.590E-05		8.890E-05	8.650E-05
3	9.060E-05		8.940E-05	8.090E-05
Mean	8.718E-05	8.838E-05	8.865E-05	8.618E-05
SD	3.021E-06	2.428E-06	1.562E-06	3.458E-06

No.1 the aqueous titration with no octanol added in quintuplicate

No.2 the sequential of aqueous-octanol titration with 5 ml octanol added in quintuplicate

No.3 the sequential of aqueous-octanol titration with 10 ml octanol added in quintuplicate

Table 8.4 The summary of K_a of salicylic acid from aqueous portion of various types of titration with 0.1 N sodium hydroxide

No.	$V_e/2$[d]	Gran Equation (Eq. 21)		
		Aqueous Titration	Aqueous-Octanol Titration	
			After midpoint	Before midpoint[e]
1	7.688E-04	1.107E-03		
1	8.261E-04	1.186E-03		
1	7.780E-04	1.083E-03		
1	7.917E-04	1.121E-03		
1	8.921E-04	1.126E-03		
2	7.843E-04		1.117E-03	
2	8.067E-04		1.154E-03	
2	7.715E-04		1.102E-03	
2	7.830E-04		1.111E-03	
2	8.436E-04		1.207E-03	
3	7.903E-04		1.110E-03	1.076E-03
3	8.005E-04		1.129E-03	1.298E-03
3	7.962E-04		1.126E-03	1.341E-03
3	8.104E-04		1.157E-03	8.312E-04
3	7.901E-04		1.116E-03	1.079E-03
Mean	8.022E-04	1.125E-03	1.133E-03	1.125E-03
SD	3.188E-05	3.816E-05	3.165E-05	2.046E-04

No.1 the aqueous titration with no octanol added in quintuplicate

No.2 the sequential of aqueous-octanol titration with 5 ml octanol added in quintuplicate

No.3 the sequential of aqueous-octanol titration with 10 ml octanol added in quintuplicate

d Group which statistically significant

e Group which are rejected for variances

Table 8.5 The summary of K_a of *m*-toluic acid from aqueous portion of various types of titration with 0.1 N sodium hydroxide

No.	$V_e/2$	Gran Equation (Eq. 21)		
		Aqueous Titration	Aqueous-Octanol Titration	
			After midpoint	Before midpoint
1	5.195E-05	5.340E-05		
1	5.707E-05	5.950E-05		
1	5.647E-05	5.890E-05		
1	5.698E-05	5.950E-05		
1	5.582E-05	5.850E-05		
2			5.850E-05	
2			5.540E-05	
2			5.860E-05	
2			5.750E-05	
2			5.730E-05	
3			5.820E-05	
3			5.890E-05	
3			5.380E-05	
3			5.540E-05	
3			5.330E-05	
Mean	5.566E-05	5.796E-05	5.669E-05	
SD	2.133E-06	2.584E-06	2.063E-06	

No.1 the aqueous titration with no octanol added in quintuplicate

No.2 the sequential of aqueous-octanol titration with 5 ml octanol added in quintuplicate

No.3 the sequential of aqueous-octanol titration with 10 ml octanol added in quintuplicate

Table 8.6 The summary of pK_a of simulated data by a half neutralization method (Setting V_e = 5.0 ml, P =0 and R=0)

Fixed pKa	Calculated pH at $V_e/2$	Calculated pK_a	% Error
3.0	3.135	3.135	4.49
4.2	4.211	4.211	0.27
4.8	4.803	4.803	0.06
5.0	5.002	5.002	0.00
6.0	6.000	6.000	0.00
8.0	8.000	8.000	0.00

Table 9.1 The summary of calculated equivalent point ($V_{e, cal}$) from Eq. 38 using simulated titration data (fixed V_e = 5.000 ml)

pK_a	V_{oct}	data range (ml)	P= 0.100		P= 50.000		P= 200.000		P= 500.00	
			$V_{e, cal}$	%error	$V_{e, cal}$	%error	$V_{e, cal}$	%error	$V_{e, cal}$	%error
3	5.000	1.1 - 2.1	4.799	4.02	4.961	0.77	4.989	0.22	4.995	0.09
		1.1 - 3.9	4.909	1.82	4.982	0.36	4.995	0.10	4.998	0.04
		1.1 - 4.9	4.957	0.86	4.991	0.17	4.997	0.05	4.999	0.02
		2.1 - 3.9	4.939	1.21	4.988	0.24	4.997	0.07	4.999	0.03
		2.1 - 4.9	4.975	0.50	4.995	0.10	4.999	0.03	4.999	0.01
		3.1 - 3.9	4.964	0.71	4.993	0.14	4.998	0.04	4.999	0.02
		3.1 - 4.9	4.989	0.23	4.998	0.04	4.999	0.01	5.000	0.01
		3.5 - 4.9	4.993	0.15	4.999	0.03	5.000	0.01	5.000	0.00
		4.1 - 4.9	4.997	0.06	4.999	0.01	5.000	0.00	5.000	0.00
	10.000	1.1 - 2.1	4.801	3.99	4.979	0.42	4.994	0.11	4.998	0.05
		1.1 - 3.9	4.910	1.81	4.990	0.20	4.997	0.05	4.999	0.02
		1.1 - 4.9	4.957	0.85	4.995	0.10	4.999	0.03	4.999	0.01
		2.1 - 3.9	4.940	1.20	4.993	0.13	4.998	0.04	4.999	0.01
		2.1 - 4.9	4.975	0.49	4.997	0.06	4.999	0.02	5.000	0.01
		3.1 - 3.9	4.965	0.71	4.996	0.08	4.999	0.02	5.000	0.01
		3.1 - 4.9	4.989	0.22	4.999	0.02	5.000	0.01	5.000	0.00
		3.5 - 4.9	4.993	0.15	4.999	0.02	5.000	0.00	5.000	0.00
		4.1 - 4.9	4.997	0.06	5.000	0.01	5.000	0.00	5.000	0.00
5	5.000	1.1 - 2.1	4.793	4.14	4.962	0.75	4.989	0.22	4.996	0.09
		1.1 - 3.9	4.900	2.01	4.982	0.36	4.995	0.11	4.998	0.04
		1.1 - 4.9	4.950	1.00	4.991	0.18	4.997	0.05	4.999	0.02
		2.1 - 3.9	4.933	1.34	4.988	0.24	4.996	0.07	4.999	0.03
		2.1 - 4.9	4.972	0.56	4.995	0.10	4.999	0.03	4.999	0.01
		3.1 - 3.9	4.961	0.79	4.993	0.14	4.998	0.04	4.999	0.02
		3.1 - 4.9	4.987	0.25	4.998	0.05	4.999	0.01	5.000	0.01
		3.5 - 4.9	4.992	0.16	4.999	0.03	5.000	0.01	5.000	0.00
		4.1 - 4.9	4.997	0.07	4.999	0.01	5.000	0.00	5.000	0.00
	10.000	1.1 - 2.1	4.795	4.11	4.979	0.41	4.994	0.11	4.998	0.05
		1.1 - 3.9	4.901	1.99	4.990	0.20	4.997	0.05	4.999	0.02
		1.1 - 4.9	4.951	0.99	4.995	0.10	4.999	0.03	4.999	0.01
		2.1 - 3.9	4.934	1.32	4.993	0.13	4.998	0.04	4.999	0.01
		2.1 - 4.9	4.972	0.56	4.997	0.06	4.999	0.02	5.000	0.01
		3.1 - 3.9	4.961	0.78	4.996	0.08	4.999	0.02	5.000	0.01
		3.1 - 4.9	4.987	0.25	4.999	0.03	5.000	0.01	5.000	0.00
		3.5 - 4.9	4.992	0.16	4.999	0.02	5.000	0.00	5.000	0.00
		4.1 - 4.9	4.997	0.07	5.000	0.01	5.000	0.00	5.000	0.00

Table 9.1 The summary of calculated equivalent point ($V_{e, cal}$) from Eq. 38 using simulated titration data (fixed V_e = 5.000 ml) ─ continue

pK_a	V_{oct}	data range (ml)	P= 0.100		P= 50.000		P= 200.000		P= 500.00	
			$V_{e, cal}$	%error	$V_{e, cal}$	%error	$V_{e, cal}$	%error	$V_{e, cal}$	%error
7	5.000	1.1 - 2.1	4.793	4.13	4.962	0.75	4.989	0.22	4.996	0.09
		1.1 - 3.9	4.900	2.01	4.982	0.36	4.995	0.11	4.998	0.04
		1.1 - 4.9	4.950	1.00	4.991	0.18	4.997	0.05	4.999	0.02
		2.1 - 3.9	4.933	1.34	4.988	0.24	4.996	0.07	4.999	0.03
		2.1 - 4.9	4.972	0.57	4.995	0.10	4.998	0.03	4.999	0.01
		3.1 - 3.9	4.961	0.79	4.993	0.14	4.998	0.04	4.999	0.02
		3.1 - 4.9	4.987	0.25	4.998	0.05	4.999	0.01	5.000	0.01
		3.5 - 4.9	4.992	0.16	4.998	0.03	5.000	0.01	5.000	0.00
		4.1 - 4.9	4.997	0.07	4.999	0.01	5.000	0.00	5.000	0.00
	10.000	1.1 - 2.1	4.795	4.09	4.979	0.41	4.994	0.11	4.998	0.05
		1.1 - 3.9	4.900	1.99	4.990	0.20	4.997	0.05	4.999	0.02
		1.1 - 4.9	4.950	0.99	4.995	0.10	4.999	0.03	4.999	0.01
		2.1 - 3.9	4.934	1.32	4.993	0.13	4.998	0.04	4.999	0.01
		2.1 - 4.9	4.972	0.56	4.997	0.06	4.999	0.02	5.000	0.01
		3.1 - 3.9	4.961	0.78	4.996	0.08	4.999	0.02	5.000	0.01
		3.1 - 4.9	4.987	0.25	4.999	0.03	5.000	0.01	5.000	0.00
		3.5 - 4.9	4.992	0.16	4.999	0.02	5.000	0.01	5.000	0.00
		4.1 - 4.9	4.997	0.07	5.000	0.01	5.000	0.00	5.000	0.00
9	5.000	1.1 - 2.1	4.786	4.28	4.962	0.77	4.988	0.23	4.995	0.11
		1.1 - 3.9	4.887	2.27	4.980	0.39	4.993	0.13	4.997	0.07
		1.1 - 4.9	4.928	1.44	4.988	0.23	4.996	0.09	4.997	0.05
		2.1 - 3.9	4.921	1.58	4.987	0.27	4.995	0.09	4.997	0.05
		2.1 - 4.9	4.952	0.96	4.993	0.15	4.997	0.06	4.998	0.04
		3.1 - 3.9	4.949	1.02	4.992	0.17	4.997	0.06	4.998	0.04
		3.1 - 4.9	4.971	0.59	4.996	0.08	4.998	0.04	4.999	0.03
		3.5 - 4.9	4.977	0.47	4.997	0.07	4.998	0.04	4.999	0.03
		4.1 - 4.9	4.984	0.33	4.998	0.04	4.999	0.03	4.999	0.02
	10.000	1.1 - 2.1	4.794	4.11	4.979	0.43	4.994	0.13	4.997	0.06
		1.1 - 3.9	4.899	2.02	4.989	0.23	4.996	0.08	4.998	0.04
		1.1 - 4.9	4.947	1.07	4.993	0.14	4.997	0.06	4.998	0.04
		2.1 - 3.9	4.932	1.35	4.992	0.16	4.997	0.06	4.998	0.03
		2.1 - 4.9	4.969	0.63	4.995	0.10	4.998	0.04	4.999	0.03
		3.1 - 3.9	4.960	0.81	4.995	0.10	4.998	0.04	4.999	0.03
		3.1 - 4.9	4.985	0.31	4.997	0.06	4.998	0.03	4.999	0.02
		3.5 - 4.9	4.989	0.21	4.998	0.05	4.999	0.03	4.999	0.02
		4.1 - 4.9	4.995	0.11	4.998	0.03	4.999	0.02	4.999	0.02

Table 9.2 The summary of calculated equivalent point ($V_{e, cal}$) from Eq. 38 using simulated titration data (fixed V_e = 10.000 ml)

pK_a	V_{oct}	data range (ml)	P= 0.100		P= 50.000		P= 200.000		P= 500.00	
			$V_{e, cal}$	%error	$V_{e, cal}$	%error	$V_{e, cal}$	%error	$V_{e, cal}$	%error
3	5.000	2.2 - 4.2	9.240	7.60	9.850	1.50	9.956	0.44	9.991	0.09
		2.2 - 7.8	9.649	3.51	9.929	0.71	9.979	0.21	9.996	0.04
		2.2 - 9.8	9.830	1.70	9.965	0.35	9.990	0.10	9.998	0.02
		4.2 - 7.8	9.767	2.33	9.953	0.47	9.986	0.14	9.997	0.03
		4.2 -9.8	9.903	0.97	9.980	0.20	9.994	0.06	9.999	0.01
		6.2 - 7.8	9.863	1.37	9.972	0.28	9.992	0.08	9.998	0.02
		6.2 - 9.8	9.956	0.44	9.991	0.09	9.997	0.03	9.999	0.01
		7.0 - 9.8	9.972	0.28	9.994	0.06	9.998	0.02	10.000	0.00
		8.2 -9.8	9.989	0.11	9.998	0.02	9.999	0.01	10.000	0.00
	10.000	2.2 - 4.2	9.246	7.54	9.917	0.83	9.978	0.22	9.991	0.09
		2.2 - 7.8	9.652	3.48	9.961	0.39	9.989	0.11	9.996	0.04
		2.2 - 9.8	9.832	1.68	9.981	0.19	9.995	0.05	9.998	0.02
		4.2 - 7.8	9.769	2.31	9.974	0.26	9.993	0.07	9.997	0.03
		4.2 -9.8	9.904	0.96	9.989	0.11	9.997	0.03	9.999	0.01
		6.2 - 7.8	9.864	1.36	9.984	0.16	9.996	0.04	9.998	0.02
		6.2 - 9.8	9.957	0.43	9.995	0.05	9.999	0.01	9.999	0.01
		7.0 - 9.8	9.972	0.28	9.997	0.03	9.999	0.01	10.000	0.00
		8.2 -9.8	9.989	0.11	9.999	0.01	10.000	0.00	10.000	0.00
5	5.000	2.2 - 4.2	9.241	7.59	9.852	1.48	9.957	0.43	9.982	0.18
		2.2 - 7.8	9.630	3.70	9.928	0.72	9.979	0.21	9.991	0.09
		2.2 - 9.8	9.815	1.85	9.965	0.35	9.990	0.10	9.996	0.04
		4.2 - 7.8	9.754	2.46	9.952	0.48	9.986	0.14	9.994	0.06
		4.2 -9.8	9.896	1.04	9.980	0.20	9.994	0.06	9.998	0.02
		6.2 - 7.8	9.856	1.44	9.972	0.28	9.992	0.08	9.997	0.03
		6.2 - 9.8	9.953	0.47	9.991	0.09	9.997	0.03	9.999	0.01
		7.0 - 9.8	9.970	0.30	9.994	0.06	9.998	0.02	9.999	0.01
		8.2 -9.8	9.988	0.12	9.998	0.02	9.999	0.01	10.000	0.00
	10.000	2.2 - 4.2	9.247	7.53	9.918	0.82	9.978	0.22	9.991	0.09
		2.2 - 7.8	9.633	3.67	9.960	0.40	9.989	0.11	9.996	0.04
		2.2 - 9.8	9.816	1.84	9.980	0.20	9.995	0.05	9.998	0.02
		4.2 - 7.8	9.756	2.44	9.974	0.26	9.993	0.07	9.997	0.03
		4.2 -9.8	9.897	1.03	9.989	0.11	9.997	0.03	9.999	0.01
		6.2 - 7.8	9.857	1.43	9.984	0.16	9.996	0.04	9.998	0.02
		6.2 - 9.8	9.954	0.46	9.995	0.05	9.999	0.01	9.999	0.01
		7.0 - 9.8	9.970	0.30	9.997	0.03	9.999	0.01	10.000	0.00
		8.2 -9.8	9.988	0.12	9.999	0.01	10.000	0.00	10.000	0.00

Table 9.2 The summary of calculated equivalent point ($V_{e, cal}$) from Eq. 38 using simulated titration data (fixed V_e = 10.000 ml) ─ continue

pK_a	V_{oct}	data range (ml)	P= 0.100		P= 50.000		P= 200.000		P= 500.00	
			$V_{e, cal}$	%error	$V_{e, cal}$	%error	$V_{e, cal}$	%error	$V_{e, cal}$	%error
7	5.000	2.2 - 4.2	9.242	7.58	9.852	1.48	9.957	0.43	9.982	0.18
		2.2 - 7.8	9.630	3.70	9.928	0.72	9.979	0.21	9.991	0.09
		2.2 - 9.8	9.814	1.86	9.964	0.36	9.990	0.10	9.996	0.04
		4.2 - 7.8	9.754	2.46	9.952	0.48	9.986	0.14	9.994	0.06
		4.2 -9.8	9.895	1.05	9.980	0.20	9.994	0.06	9.997	0.03
		6.2 - 7.8	9.855	1.45	9.972	0.28	9.992	0.08	9.997	0.03
		6.2 - 9.8	9.953	0.47	9.991	0.09	9.997	0.03	9.999	0.01
		7.0 - 9.8	9.970	0.30	9.994	0.06	9.998	0.02	9.999	0.01
		8.2 -9.8	9.988	0.12	9.998	0.02	9.999	0.01	10.000	0.00
	10.000	2.2 - 4.2	9.248	7.52	9.918	0.82	9.978	0.22	9.991	0.09
		2.2 - 7.8	9.633	3.67	9.960	0.40	9.989	0.11	9.996	0.04
		2.2 - 9.8	9.816	1.84	9.980	0.20	9.995	0.05	9.998	0.02
		4.2 - 7.8	9.756	2.44	9.974	0.26	9.993	0.07	9.997	0.03
		4.2 -9.8	9.896	1.04	9.989	0.11	9.997	0.03	9.999	0.01
		6.2 - 7.8	9.857	1.43	9.984	0.16	9.996	0.04	9.998	0.02
		6.2 - 9.8	9.953	0.47	9.995	0.05	9.999	0.01	9.999	0.01
		7.0 - 9.8	9.970	0.30	9.997	0.03	9.999	0.01	10.000	0.00
		8.2 -9.8	9.988	0.12	9.999	0.01	10.000	0.00	10.000	0.00
9	5.000	2.2 - 4.2	9.240	7.60	9.850	1.50	9.955	0.45	9.980	0.20
		2.2 - 7.8	9.627	3.73	9.925	0.75	9.976	0.24	9.989	0.11
		2.2 - 9.8	9.806	1.94	9.958	0.42	9.985	0.15	9.992	0.08
		4.2 - 7.8	9.752	2.48	9.950	0.50	9.983	0.17	9.992	0.08
		4.2 -9.8	9.888	1.12	9.974	0.26	9.990	0.10	9.994	0.06
		6.2 - 7.8	9.853	1.47	9.969	0.31	9.989	0.11	9.994	0.06
		6.2 - 9.8	9.947	0.53	9.986	0.14	9.994	0.06	9.996	0.04
		7.0 - 9.8	9.964	0.36	9.990	0.10	9.995	0.05	9.997	0.03
		8.2 -9.8	9.983	0.17	9.994	0.06	9.997	0.03	9.997	0.03
	10.000	2.2 - 4.2	9.246	7.54	9.916	0.84	9.976	0.24	9.989	0.11
		2.2 - 7.8	9.630	3.70	9.958	0.42	9.987	0.13	9.993	0.07
		2.2 - 9.8	9.808	1.92	9.975	0.25	9.991	0.09	9.995	0.05
		4.2 - 7.8	9.754	2.46	9.971	0.29	9.990	0.10	9.995	0.05
		4.2 -9.8	9.889	1.11	9.984	0.16	9.994	0.06	9.996	0.04
		6.2 - 7.8	9.854	1.46	9.982	0.18	9.993	0.07	9.996	0.04
		6.2 - 9.8	9.947	0.53	9.991	0.09	9.996	0.04	9.997	0.03
		7.0 - 9.8	9.964	0.36	9.993	0.07	9.996	0.04	9.997	0.03
		8.2 -9.8	9.983	0.17	9.996	0.04	9.997	0.03	9.998	0.02

Table 9.3 The summary of V_e of acetic acid from aqueous portion of various types of titration with 0.01 N sodium hydroxide at 95% significant level.

Experiment	Equivalent point (ml)			
	titration method			
	Eq.21	Eq. 38		
	A	B	C	D
1	4.897	4.891	4.892	4.904
2	4.910	4.894	4.904	4.906
3	4.933	4.898	4.894	4.901
4	4.903	4.905	4.898	4.925
5	4.908	4.888	4.902	4.898
Mean	4.910	4.895	4.898	4.907
S.D.	0.014	0.007	0.005	0.010
ANOVA analysis	Not sig.			

A the aqueous titration with no octanol added

B the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint

C the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint

D the sequential of aqueous-octanol titration with 10 ml octanol added before midpoint

Table 9.4 The summary of V_e of benzoic acid from aqueous portion of various types of titration with 0.01 N sodium hydroxide at 95% significant level.

Experiment	Equivalent point (ml)			
	titration method			
	Eq.21	Eq. 38		
	A	B	C	D
1	4.978	4.992	4.953	-
2	4.975	4.950	4.950	-
3	4.963	4.936	4.945	-
4	4.989	4.924	4.955	-
5	4.958	4.924	4.948	-
Mean	4.972	4.945	4.950	-
S.D.	0.012	0.028	0.004	-
ANOVA analysis	Not sig.			

A the aqueous titration with no octanol added

B the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint

C the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint

D the sequential of aqueous-octanol titration with 10 ml octanol added before midpoint

f no data

Table 9.5 The summary of V_e of *2*-methoxybenzoic acid from aqueous portion of of titration with 0.01 N sodium hydroxide at 95% significant level.

Experiment	Equivalent point (ml)			
	titration method			
	Eq.21	Eq. 38		
	A	B	C	D
1	4.959	4.946	4.942	4.979
2	4.964	4.961	4.952	4.973
3	4.873	4.954	4.992	4.974
4	4.969	4.962	4.953	4.954
5	4.980	4.972	4.963	4.959
Mean	4.949	4.959	4.960	4.968
S.D.	0.043	0.010	0.019	0.011
ANOVA analysis	Not sig.			

A the aqueous titration with no octanol added

B the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint

C the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint

D the sequential of aqueous-octanol titration with 10 ml octanol added before midpoint

Table 9.6 The summary of V_e of salicylic acid from aqueous portion of various types of titration with 0.01 N sodium hydroxide at 95% significant level.

Experiment	Equivalent point (ml)			
	titration method			
	Eq.21	Eq. 38		
	A	B	C	D
1	5.005	5.015	4.995	5.015
2	5.026	5.010	5.016	5.002
3	5.019	5.016	5.011	4.984
4	5.014	4.988	4.995	4.990
5	5.008	4.987	5.025	5.004
Mean	5.014	5.003	5.008	4.999
S.D.	0.008	0.014	0.013	0.012
ANOVA analysis	Not sig.			

A the aqueous titration with no octanol added

B the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint

C the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint

D the sequential of aqueous-octanol titration with 10 ml ctanol added before midpoint

Table 9.7 The summary of V_e of *m*-toluic acid from aqueous portion of various types of titration with 0.01 N sodium hydroxide at 95% significant level.

Experiment	Equivalent point (ml)			
	titration method			
	Eq.21	Eq. 38		
	A	B	C	D
1	3.027	3.007	3.015	2.996
2	3.039	3.000	3.023	3.029
3	3.017	3.000	3.008	3.003
4	3.007	3.006	3.010	3.003
5	3.014	3.009	3.003	3.000
Mean	3.021	3.005	3.012	3.006
S.D.	0.012	0.004	0.007	0.013
ANOVA analysis	Not sig.			

A the aqueous titration with no octanol added

B the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint

C the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint

D the sequential of aqueous-octanol titration with 10 ml octanol added before midpoint

Table 9.8 The summary of percent of purity of acetic acid at 95% significant level.

Experiment	Purity (%)			
	titration method			
	Eq. 21	Eq. 38		
	A	B	C	D
1	100.2	100.1	100.1	100.3
2	100.5	100.1	100.3	100.4
3	101.0	100.2	100.2	100.3
4	100.3	100.4	100.2	100.8
5	100.4	100.0	100.3	100.2
Mean	100.5	100.2	100.2	100.4
S.D.	0.287	0.140	0.102	0.214
ANOVA analysis	Not sig.			

A the aqueous titration with no octanol added

B the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint

C the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint

D the sequential of aqueous-octanol titration with 10 ml octanol added before midpoint

Table 9.9 The summary of percent of purity of benzoic acid at 95% significant level.

Experiment	Purity (%)			
	titration method			
	Eq. 21	Eq. 38		
	A	B	C	D
1	99.99	100.3	99.49	99.55
2	99.93	99.44	99.43	99.55
3	99.70	99.16	99.34	100.1
4	100.2	98.91	99.54	99.67
5	99.60	98.92	99.40	99.60
Mean	99.89	99.34	99.44	99.68
S.D.	0.244	0.563	0.078	0.211
ANOVA analysis	Not sig.			

A the aqueous titration with no octanol added

B the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint

C the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint

D the sequential of aqueous-octanol titration with 10 ml octanol added before midpoint

Table 9.10 The summary of percent of purity of *2*-methoxybenzoic acid at 95% significant level.

Experiment	Purity (%)			
	titration method			
	Eq. 21	Eq. 38		
	A	B	C	D
1	99.44	99.17	99.10	99.84
2	99.54	99.47	99.30	99.72
3	97.72	99.34	100.1	99.73
4	99.64	99.49	99.32	99.34
5	99.86	99.70	99.52	99.44
Mean	99.24	99.43	99.47	99.61
S.D.	0.865	0.198	0.382	0.215
ANOVA analysis	Not sig.			

A the aqueous titration with no octanol added

B the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint

C the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint

D the sequential of aqueous-octanol titration with 10 ml octanol added before midpoint

Table 9.11 The summary of percent of purity of salicylic acid at 95% significant level.

Experiment	Purity (%)			
	titration method			
	Eq. 21	Eq. 38		
	A	B	C	D
1	99.48	99.68	99.28	99.69
2	99.89	99.58	99.69	99.42
3	99.75	99.70	99.59	99.06
4	99.67	99.14	99.29	99.18
5	99.54	99.22	99.88	99.46
Mean	99.67	99.47	99.55	99.36
S.D.	0.167	0.286	0.258	0.245
ANOVA analysis	Not sig.			

A the aqueous titration with no octanol added

B the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint

C the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint

D the sequential of aqueous-octanol titration with 10 ml octanol added before midpoint

Table 9.12 The summary of percent of purity of *m*-toluic acid at 95% significant level.

Experiment	Purity (%)			
	titration method			
	Eq. 21	Eq. 38		
	A	B	C	D
1	99.68	99.02	99.27	98.64
2	100.1	98.78	99.53	99.73
3	99.35	98.79	99.06	98.88
4	99.03	98.99	99.10	98.88
5	99.24	99.09	98.89	98.77
Mean	99.47	98.93	99.17	98.98
S.D.	0.403	0.140	0.245	0.433
ANOVA analysis	Not sig.			

A the aqueous titration with no octanol added

B the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint

C the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint

D the sequential of aqueous-octanol titration with 10 ml octanol added before midpoint

Table 10.1 The summary of calculated partition coefficient of acetic acid

Shake flask	Titration method							
	Eq. 41		OECD guildline					
	Ka^a	Ka^b	K' (Eq. 38)			Ka' (half neutralization method)		
			Ka^a	Ka^b	Ka^c	Ka^a	Ka^b	Ka^c
Gr. 1	Gr. 2	Gr. 3	Gr. 4^d	Gr. 5^d	Gr. 6^d			
0.516	0.503	0.558	1.016	1.192	1.182			
0.512	0.530	0.489	1.263	1.239	1.150			
0.512	0.539	0.681	1.204	1.244	1.183			
0.510	0.513	0.821	1.377	1.253	1.193			
0.508	0.516	0.615	2.053	1.222	1.121			
	Gr. 7	Gr. 8	Gr. 9^d	Gr. 10^d	Gr. 11^d			
	0.499	0.327	0.935	0.876	0.858			
	0.522	0.554	0.865	0.898	0.886			
	0.580	0.500	1.036	0.935	0.875			
	0.635	0.636	1.191	1.077	0.839			
	0.507		0.965	0.857	0.832			
	Gr. 12	Gr. 13	Gr. 14^d	Gr. 15^d	Gr. 16^d	Gr. 17	Gr. 18	Gr. 19
	0.608	0.475	0.954	0.819	0.907	0.612	0.484	0.567
	0.484	0.389	0.820	0.724	0.774	0.489	0.398	0.445
	0.450	0.449	0.748	0.747	0.703	0.462	0.461	0.418
	0.493	0.693	0.908	1.114	0.861	0.517	0.711	0.473
	0.507	0.710	0.929	1.139	0.882	0.523	0.719	0.479

a Calculated K_a by Eq. 21 from each sequential aqueous-octanol titration

b Reference K_a by Eq. 21 from aqueous titration

c Reference K_a by half neutralization method from aqueous titration

d Group which statistically significant

Gr. X The category of calculated P in quintuplicate

Gr. 1 Calculated P obtained from shake flask method

Gr. 2-6 Calculated P obtained from the sequential aq/oct titration which 5 ml octanol added after midpoint

Gr. 7-11 Calculated P obtained from the sequential aq/oct titration which 10 ml octanol added after midpoint

Gr. 12-19 Calculated P obtained from the sequential aq/oct titration which 10 ml octanol added before midpoint

Table 10.2 the summary of calculated partition coefficient of Benzoic acid

Shake flask	Titration method							
	Eq. 41		OECD guildline					
	Ka^a	Ka^b	K' (Eq. 38)			Ka' (half neutralization method)		
			Ka^a	Ka^b	Ka^c	Ka^a	Ka^b	Ka^c
Gr. 1	Gr. 2	Gr. 3	Gr. 4	Gr. 5	Gr. 6			
79.6	83.9	87.6	85.8	89.4	83.0			
81.4	81.3	90.0	82.1	90.8	81.3			
79.3	81.8	84.2	81.1	85.9	81.4			
81.4	82.7	79.7	88.4	85.3	86.1			
81.6	83.3	91.5	86.3	94.8	84.1			
	Gr. 7	Gr. 8	Gr. 9	Gr. 10	Gr. 11			
	81.0	84.9	80.8	84.7	80.0			
	81.8	90.2	82.3	90.7	81.3			
	82.1	87.7	82.4	88.0	81.3			
	82.6	85.3	84.2	87.0	82.9			
	86.2	86.7	87.9	88.3	85.8			
	Gr. 12	Gr. 13	Gr. 14	Gr. 15	Gr. 16	Gr. 17	Gr. 18	Gr. 19
	82.6	82.8	82.8	83.7	81.0	79.0	79.8	77.3
	82.9	79.9	79.7	83.8	81.1	76.6	80.6	78.0
	84.0	81.8	83.4	86.4	83.6	78.1	80.9	78.4
	82.3	76.9	77.1	83.6	80.9	73.4	79.6	77.0
	81.8	80.6	80.0	82.8	80.2	76.4	79.1	76.5

a Calculated K_a by Eq. 21 from each sequential aqueous-octanol titration

b Reference K_a by Eq. 21 from aqueous titration

c Reference K_a by half neutralization method from aqueous titration

Gr. X The category of calculated P in quintuplicate

Gr. 1 Calculated P obtained from shake flask method

Gr. 2-6 Calculated P obtained from the sequential aq/oct titration which 5 ml octanol added after midpoint

Gr. 7-11 Calculated P obtained from the sequential aq/oct titration which 10 ml octanol added after midpoint

Gr. 12-19 Calculated P obtained from the sequential aq/oct titration which 10 ml octanol added before midpoint

Table 10.3 The summary of calculated partition coefficient of *2*-Methoxybenzoic acid

Shake flask	Titration method							
	Eq. 41		OECD guildline					
	Ka^a	Ka^b	K' (Eq. 38)			Ka' (half neutralization method)		
			Ka^a	Ka^b	Ka^c	Ka^a	Ka^b	Ka^c
Gr. 1	Gr. 2	Gr. 3	Gr. 4	Gr. 5	Gr. 6			
17.0	17.4	17.0	18.3	17.8	17.1			
17.3	16.7	17.6	17.3	17.6	16.5			
17.1	17.3	16.7	18.0	18.9	16.9			
17.2	17.3	17.6	18.0	17.7	17.0			
17.2	16.8	18.3	17.5	18.0	18.7			
	Gr. 7	Gr. 8	Gr. 9	Gr. 10	Gr. 11			
	17.8	17.0	18.1	17.5	17.3			
	16.9	17.0	17.2	17.3	16.6			
	17.1	18.1	17.5	17.1	16.6			
	17.1	17.0	17.4	17.2	16.7			
	17.4	17.3	17.8	17.5	16.9			
	Gr. 12	Gr. 13	Gr. 14	Gr. 15	Gr. 16	Gr. 17	Gr. 18	Gr. 19
	17.5	17.5	17.4	17.8	17.2	16.6	17.0	16.4
	17.7	17.7	18.0	18.1	17.5	17.1	17.2	16.7
	18.0	18.0	17.1	18.3	17.7	16.2	17.4	16.8
	17.6	17.6	17.8	17.9	17.3	16.9	16.9	16.4
	17.0	17.0	18.7	17.4	16.8	17.6	16.4	15.8

a Calculated K_a by Eq. 21 from each sequential aqueous-octanol titration

b Reference K_a by Eq. 21 from aqueous titration

c Reference K_a by half neutralization method from aqueous titration

Gr. X The category of calculated P in quintuplicate

Gr. 1 Calculated P obtained from shake flask method

Gr. 2-6 Calculated P obtained from the sequential aq/oct titration which 5 ml octanol added after midpoint

Gr. 7-11 Calculated P obtained from the sequential aq/oct titration which 10 ml octanol added after midpoint

Gr. 12-19 Calculated P obtained from the sequential aq/oct titration which 10 ml octanol added before midpoint

Table 10.4 The summary of calculated partition coefficient of Salicylic acid

Shake flask	Titration method							
	Eq. 41		OECD guildline					
	Ka^a	Ka^b	K' (Eq. 38)			Ka' (half neutralization method)		
			Ka^a	Ka^b	Ka^c	Ka^a	Ka^b	Ka^c
Gr. 1	Gr. 2	Gr. 3	Gr. 4	Gr. 5	Gr. 6[d]			
215	208	209	205	206	141			
215	212	207	213	207	146			
216	212	216	211	216	145			
219	205	207	203	206	140			
212	217	207	217	201	149			
	Gr. 7	Gr. 8	Gr. 9	Gr. 10	Gr. 11[d]			
	212	214	210	213	148			
	213	213	211	210	148			
	217	217	211	211	148			
	219	213	214	208	148			
	211	214	212	214	149			
	Gr. 12[e]	Gr. 13	Gr. 14[e]	Gr. 15	Gr. 16[d]	Gr. 17[e]	Gr. 18[d]	Gr. 19[d]
	200	210	201	210	150	195	204	146
	246	213	246	212	152	237	205	146
	256	217	256	214	153	247	206	147
	155	212	155	212	151	152	208	148
	196	209	196	205	146	189	197	141

a Calculated K_a by Eq. 21 from each sequential aqueous-octanol titration

b Reference K_a by Eq. 21 from aqueous titration

c Reference K_3 by half neutralization method from aqueous titration

d Group which statistically significant

e Group which are rejected for variances

Gr. X The category of calculated P in quintuplicate

Gr. 1 Calculated P obtained from shake flask method

Gr. 2-6 Calculated P obtained from the sequential aq/oct titration which 5 ml octanol added after midpoint

Gr. 7-11 Calculated P obtained from the sequential aq/oct titration which 10 ml octanol added after midpoint

Gr. 12-19 Calculated P obtained from the sequential aq/oct titration which 10 ml octanol added before midpoint

Table 10.5 The summary of calculated partition coefficient of *m*-Toluic acid

Shake flask	Titration method							
	Eq. 41		OECD guildline					
	Ka^a	Ka^b	K' (Eq. 38)			Ka' (half neutralization method)		
			Ka^a	Ka^b	Ka^c	Ka^a	Ka^b	Ka^c
Gr. 1	Gr. 2	Gr. 3	Gr. 4	Gr. 5	Gr. 6			
297	296	293	304	300	296			
295	307	303	309	301	299			
297	305	327	325	348	316			
296	303	315	332	345	313			
293	307	333	313	338	297			
	Gr. 7	Gr. 8	Gr. 9	Gr. 10	Gr. 11			
	305	310	316	311	304			
	301	313	324	337	310			
	302	317	322	315	309			
	312	313	333	333	319			
	295	300	307	308	295			
	Gr. 12	Gr. 13	Gr. 14	Gr. 15	Gr. 16	Gr. 17	Gr. 18	Gr. 19
	-	300	-	302	292	-	294	284
	-	296	-	298	288	-	287	278
	-	314	-	315	304	-	310	299
	-	312	-	312	302	-	303	293
	-	306	-	307	296	-	296	286

a Calculated K_a by Eq. 21 from each sequential aqueous-octanol titration

b Reference K_a by Eq. 21 from aqueous titration

c Reference K_a by half neutralization method from aqueous titration

Gr. X The category of calculated P in quintuplicate

Gr. 1 Calculated P obtained from shake flask method

Gr. 2-6 Calculated P obtained from the sequential aq/oct titration which 5 ml octanol added after midpoint

Gr. 7-11 Calculated P obtained from the sequential aq/oct titration which 10 ml octanol added after midpoint

Gr. 12-19 Calculated P obtained from the sequential aq/oct titration which 10 ml octanol added before midpoint

Figure 1.1 Calibration curve for benzoic acid

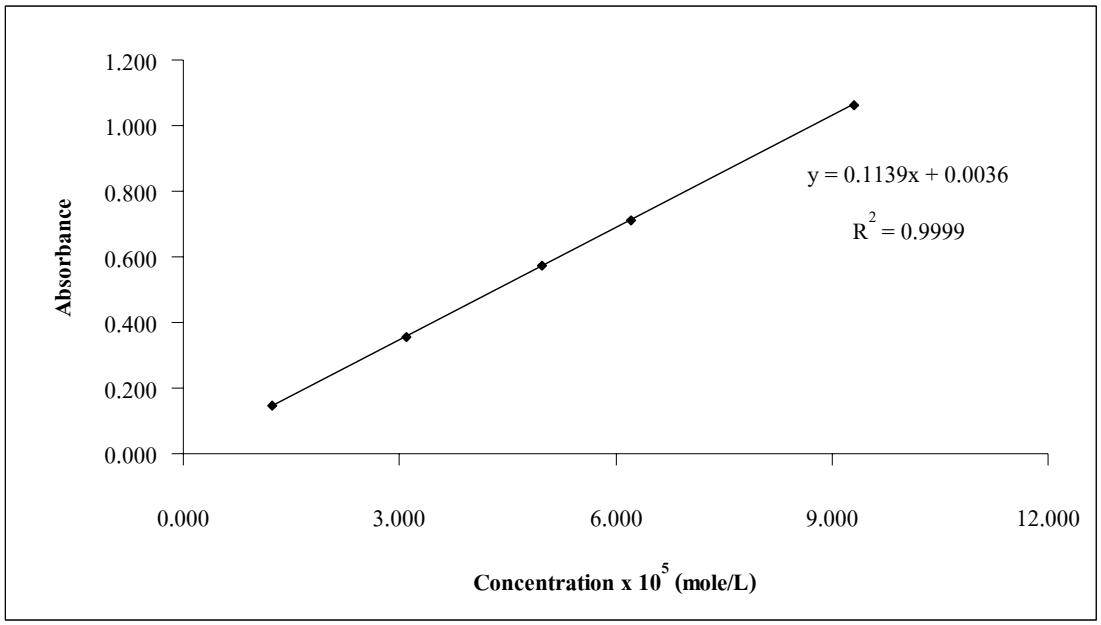


Figure 1.2 UV spectrum of benzoic acid at λ_{max} 230.3 nm

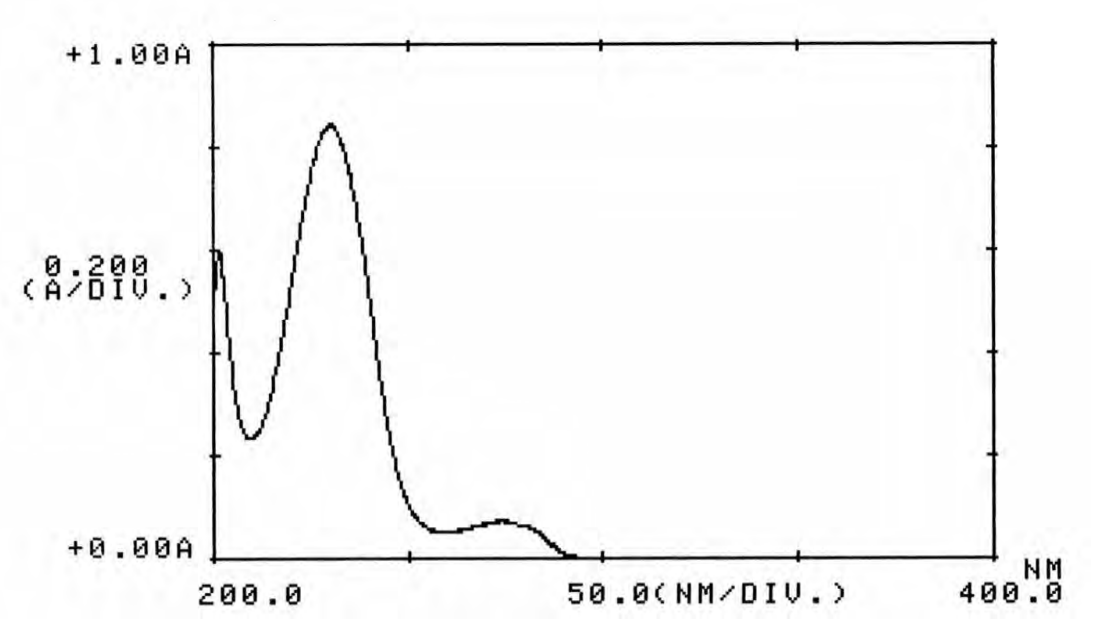


Figure 1.3 Calibration curve for *2*-methoxybenzoic acid

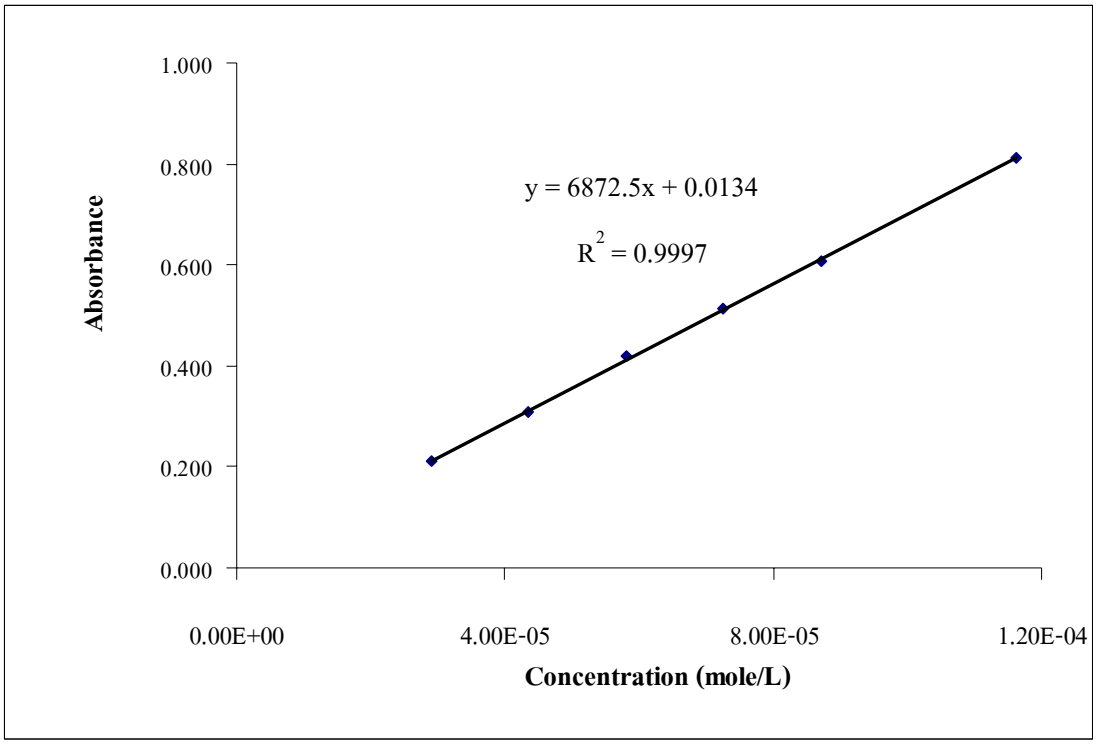


Figure 1.4 UV spectrum of *2*-methoxybenzoic acid at λ_{max} 234 nm

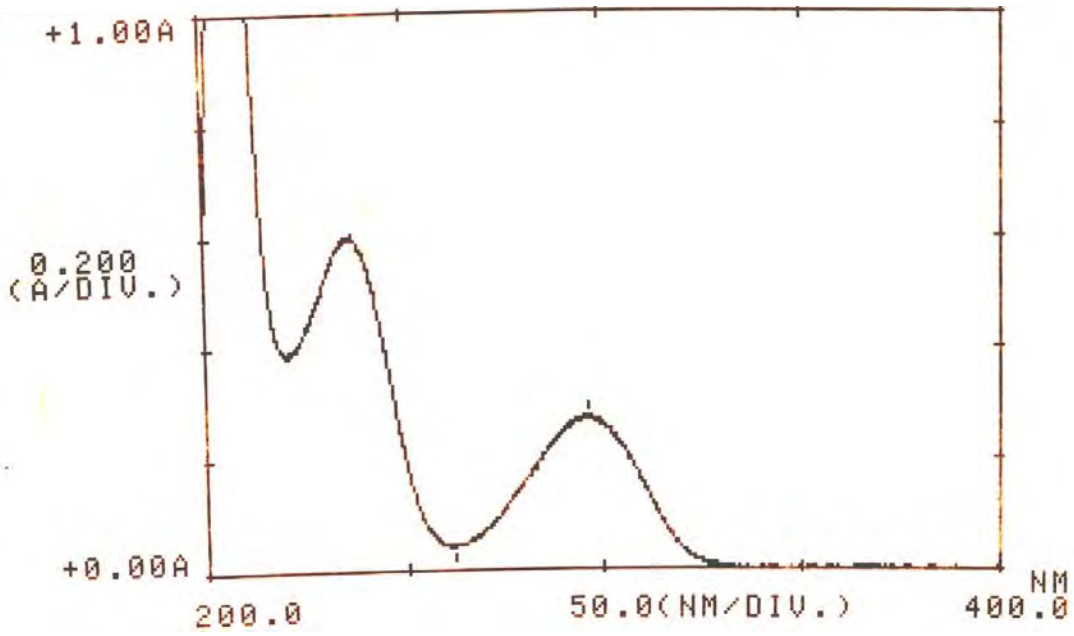


Figure 1.5 Calibration curve for salicylic acid

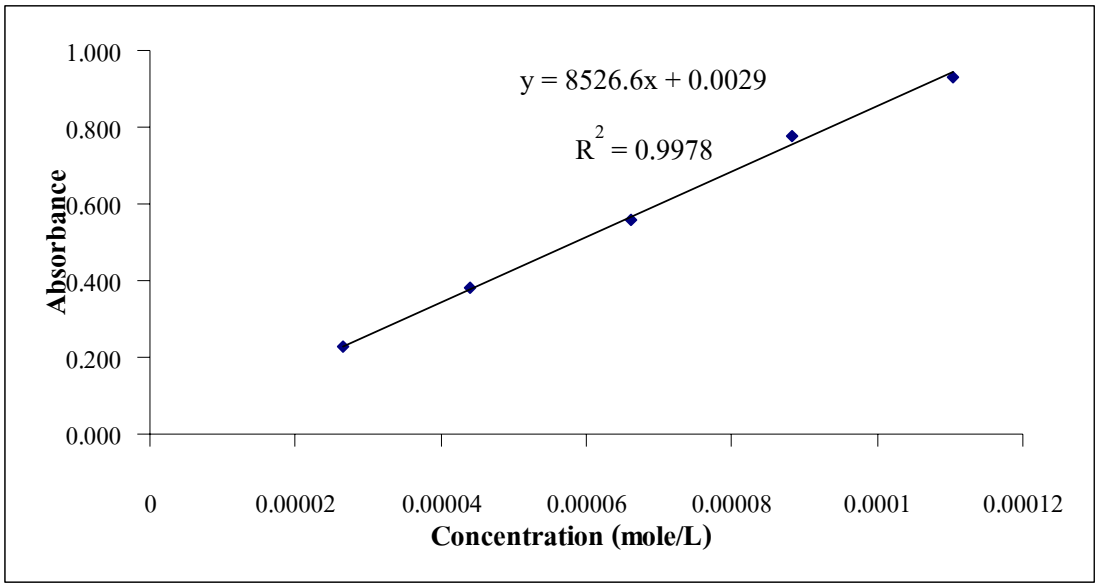


Figure 1.6UV spectrum of salicylic acid at λ_{max} 237.4 nm

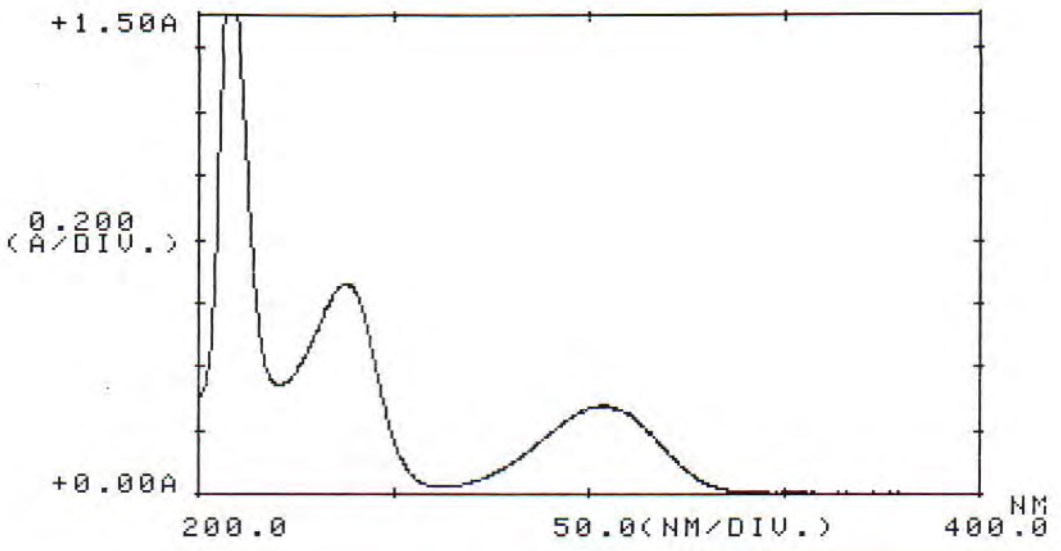


Figure 1.7 Calibration curve for *m*-toluic acid

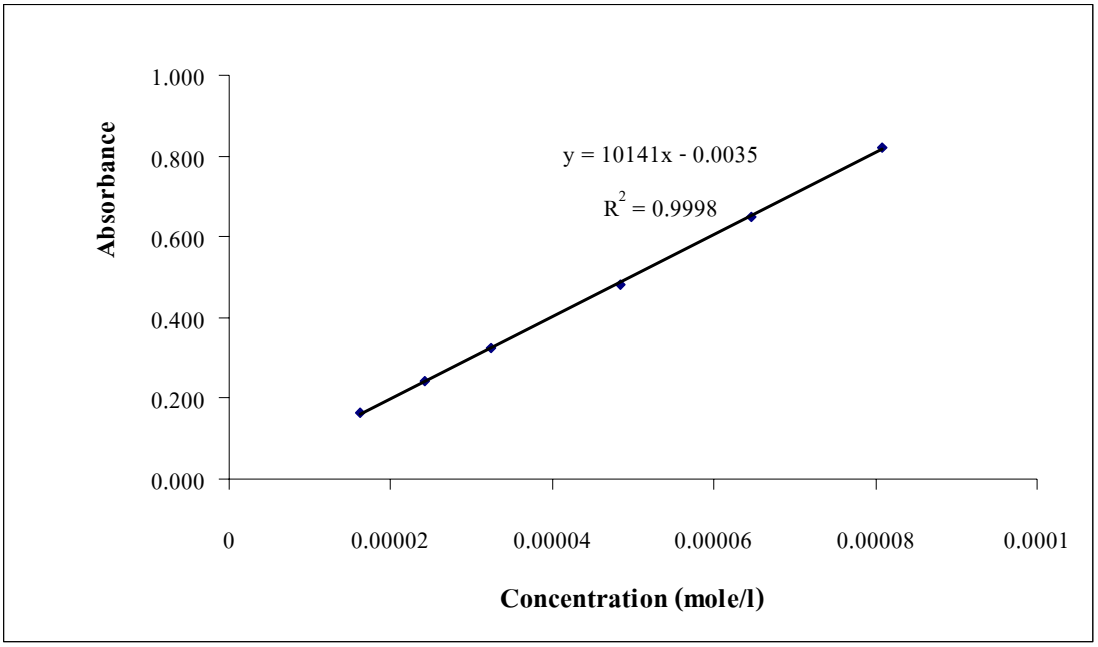


Figure 1.8 UV spectrum of *m*-toluic acid at λ_{max} 234 nm

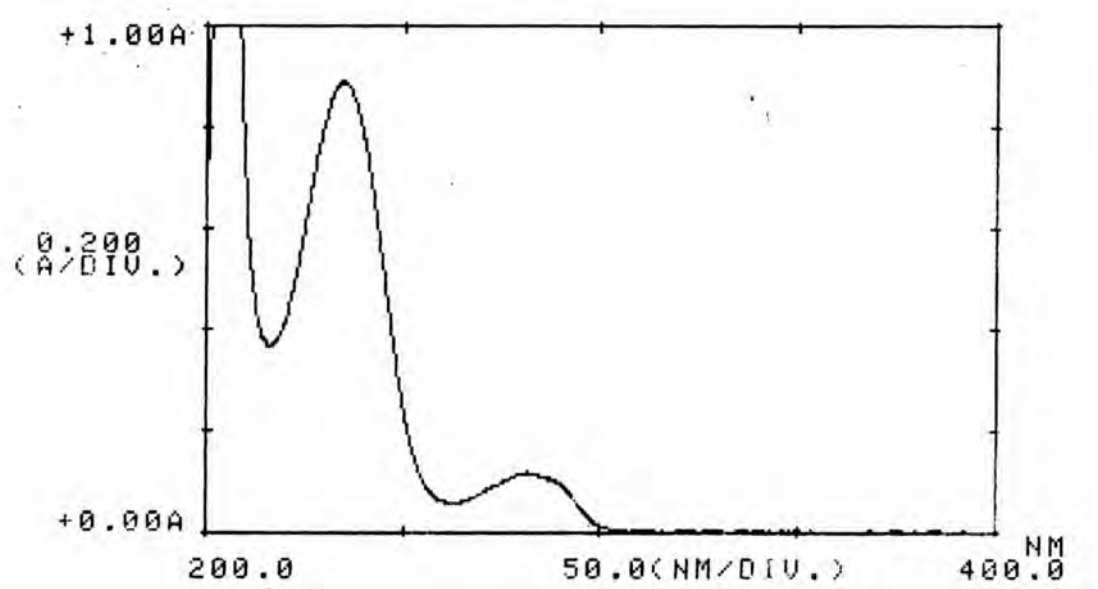


Figure 2 Titration curve of the sequential aqueous-octanol titration of *m*-toluic acid with 10 ml octanol added before midpoint with 0.1 N sodium hydroxide

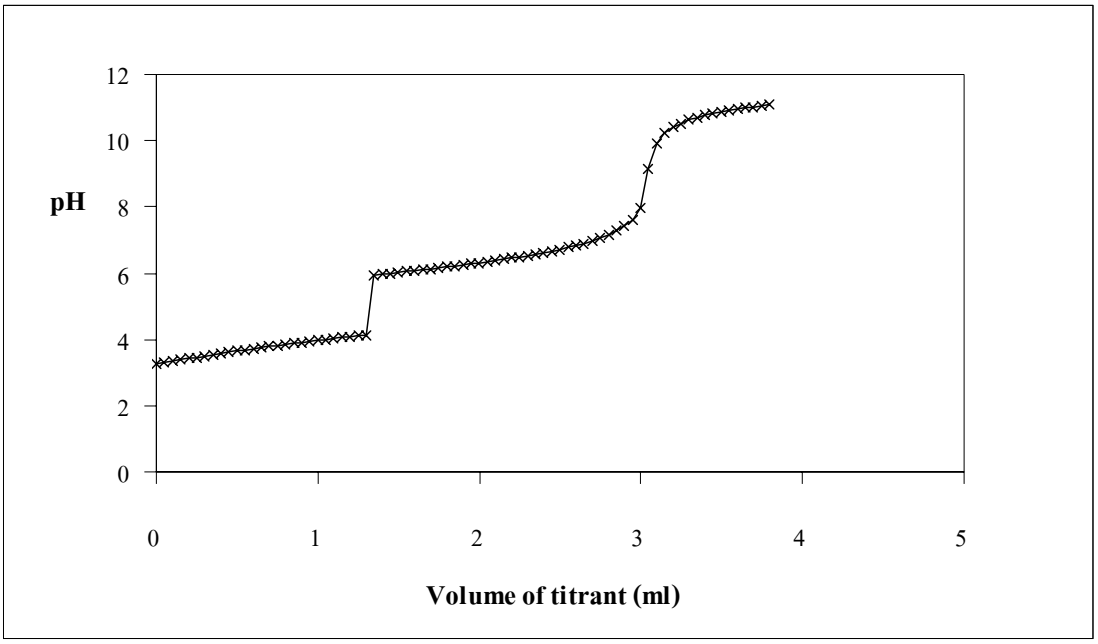
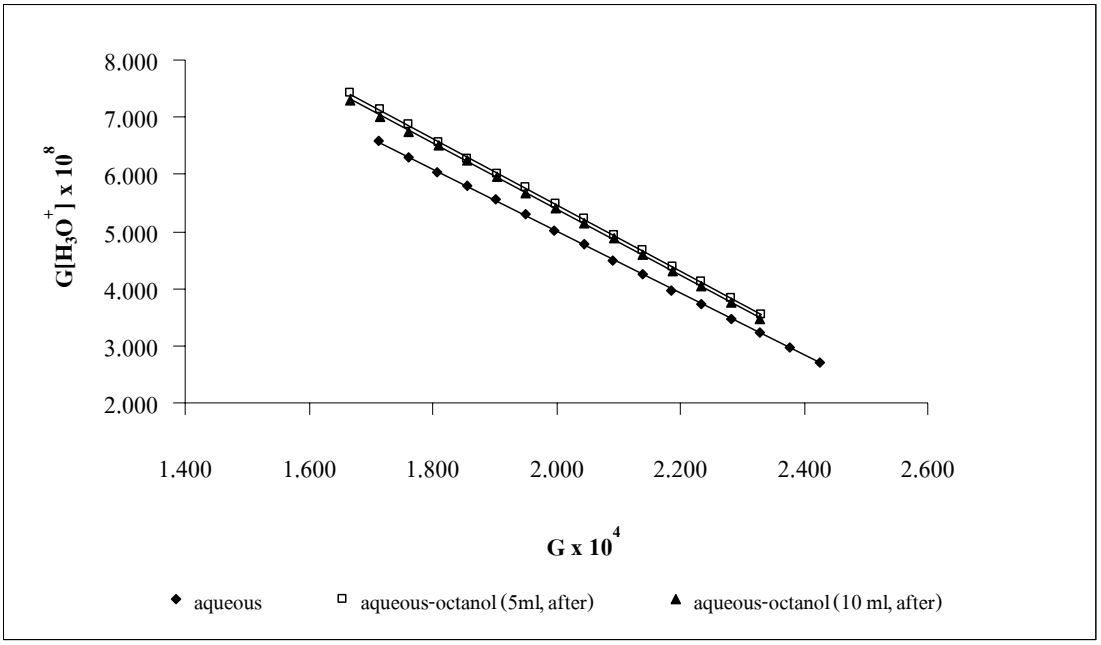
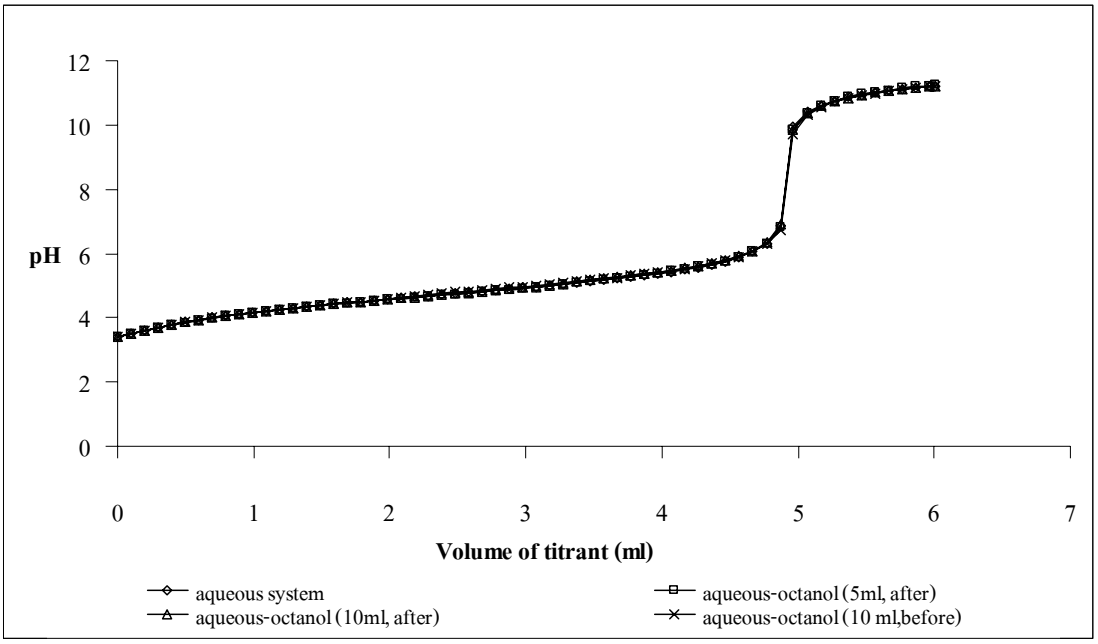


Figure 3 Gran plot for the aqueous portion of the aqueous and the sequential aqueous-octanol titration of *m*-toluic acid with 0.1 N sodium hydroxide



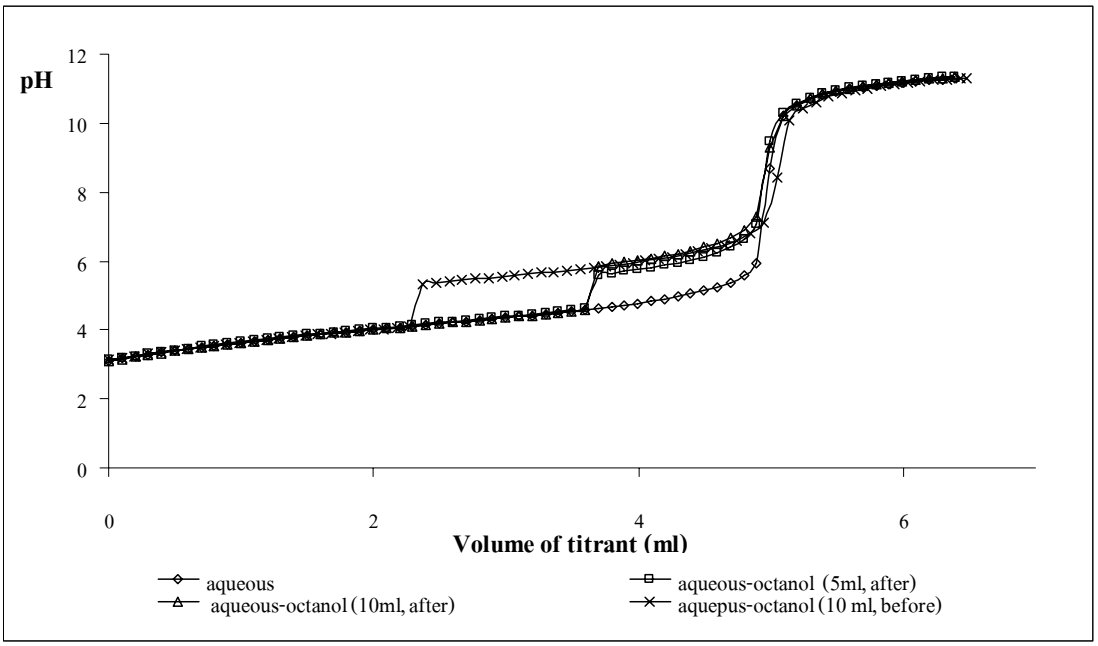
aqueous	the aqueous titration with no octanol added
aqueous-octanol (5 ml, after)	the sequential of aqueous-octanol titration with octanol added after mid point 5 ml
aqueous-octanol (10 ml, after)	the sequential of aqueous-octanol titration with octanol added after mid point 10 ml

Figure 4.1 Titration curve of the aqueous titration and the sequential aqueous-octanol titration of acetic acid with 0.1 N sodium hydroxide



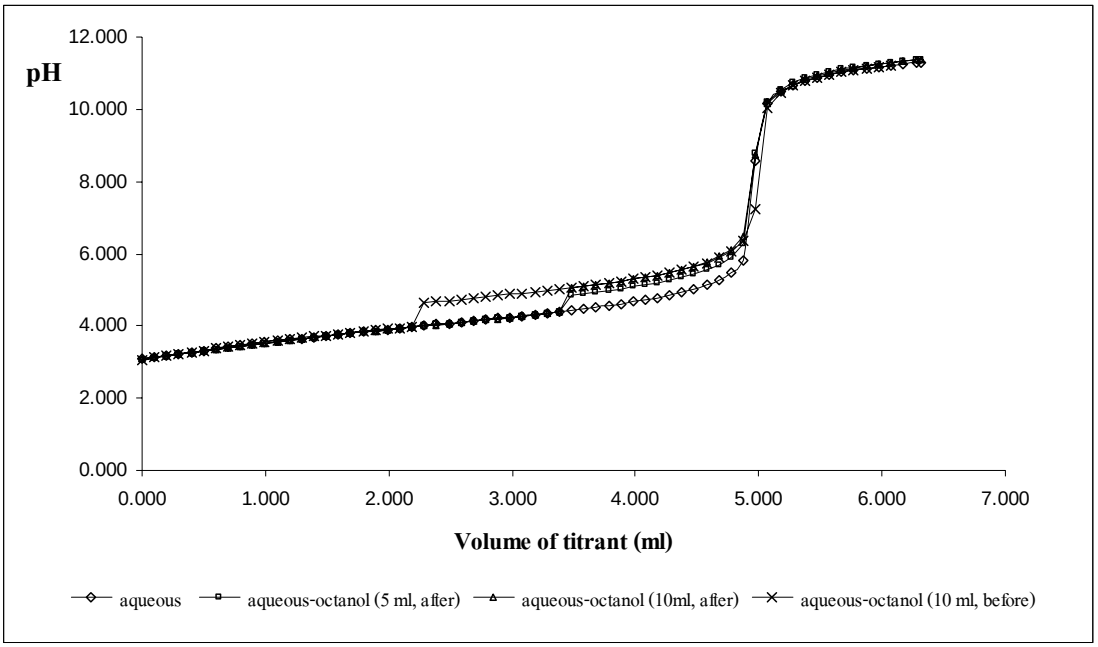
aqueous	the aqueous titration with no octanol added
aqueous-octanol (5 ml, after)	the sequential of aqueous-octanol titration with octanol added after mid point 5 ml
aqueous-octanol (10 ml, after)	the sequential of aqueous-octanol titration with octanol added after mid point 10 ml
aqueous-octanol (10 ml, before)	the sequential of aqueous-octanol titration with octanol added before mid point 10 ml

Figure 4.2 Titration curve of the aqueous titration and the sequential aqueous-octanol titration of benzoic acid with 0.1 N sodium hydroxide



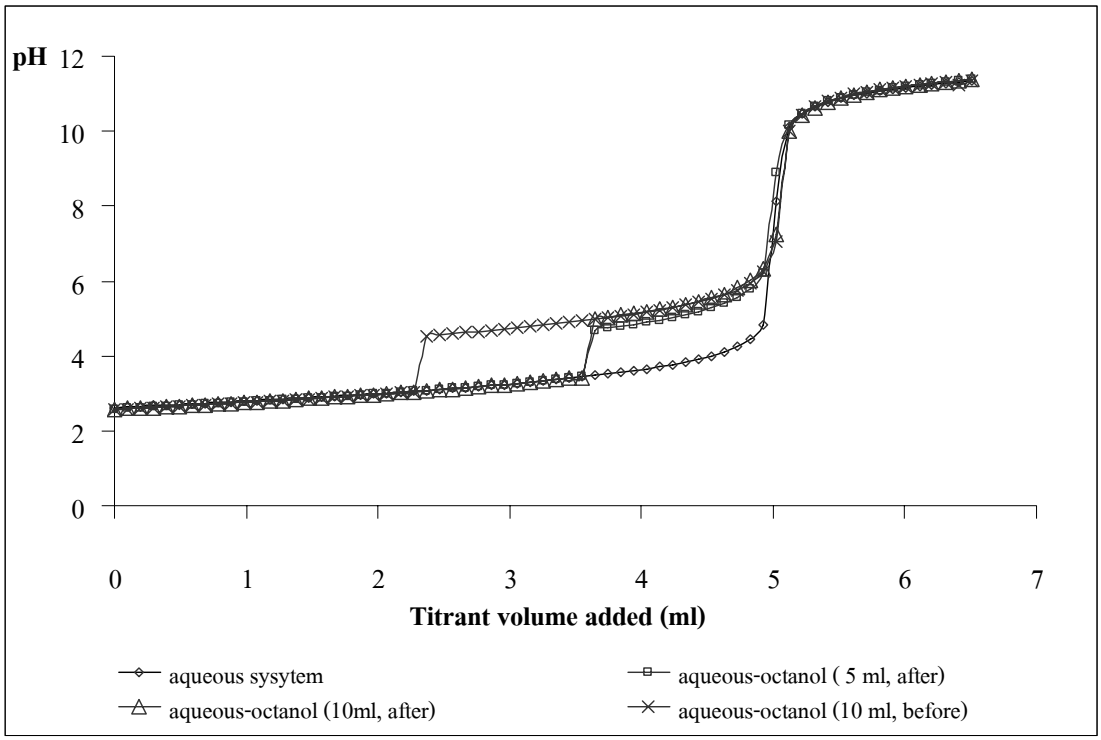
aqueous	the aqueous titration with no octanol added
aqueous-octanol (5 ml, after)	the sequential of aqueous-octanol titration with octanol added after mid point 5 ml
aqueous-octanol (10 ml, after)	the sequential of aqueous-octanol titration with octanol added after mid point 10 ml
aqueous-octanol (10 ml, before)	the sequential of aqueous-octanol titration with octanol added before mid point 10 ml

Figure 4.3 Titration curve of the aqueous titration and the sequential aqueous-octanol titration of *2*-methoxybenzoic acid with 0.1 N sodium hydroxide



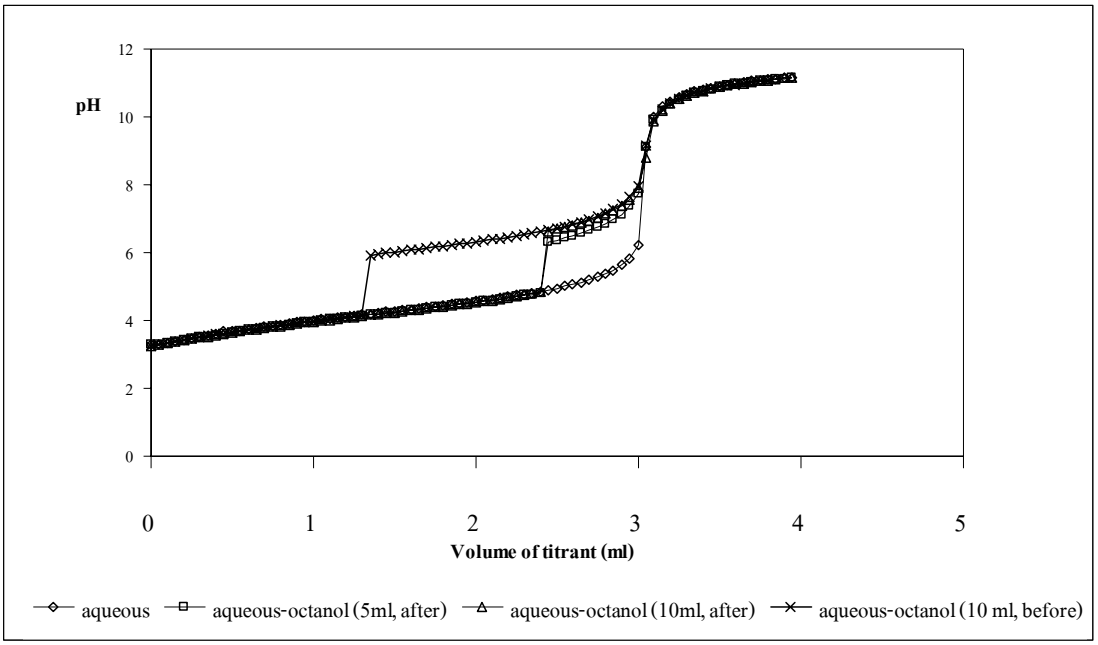
aqueous	the aqueous titration with no octanol added
aqueous-octanol (5 ml, after)	the sequential of aqueous-octanol titration with octanol added after mid point 5 ml
aqueous-octanol (10 ml, after)	the sequential of aqueous-octanol titration with octanol added after mid point 10 ml
aqueous-octanol (10 ml, before)	the sequential of aqueous-octanol titration with octanol added before mid point 10 ml

Figure 4.4 Titration curve of the aqueous titration and the sequential aqueous-octanol titration of salicylic acid with 0.1 N sodium hydroxide



aqueous	the aqueous titration with no octanol added
aqueous-octanol (5 ml, after)	the sequential of aqueous-octanol titration with octanol added after mid point 5 ml
aqueous-octanol (10 ml, after)	the sequential of aqueous-octanol titration with octanol added after mid point 10 ml
aqueous-octanol (10 ml, before)	the sequential of aqueous-octanol titration with octanol added before mid point 10 ml

Figure 4.5 Titration curve of the aqueous titration and the sequential aqueous-octanol titration of *m*-toluic acid with 0.1 N sodium hydroxide



aqueous	the aqueous titration with no octanol added
aqueous-octanol (5 ml, after)	the sequential of aqueous-octanol titration with octanol added after mid point 5 ml
aqueous-octanol (10 ml, after)	the sequential of aqueous-octanol titration with octanol added after mid point 10 ml
aqueous-octanol (10 ml, before)	the sequential of aqueous-octanol titration with octanol added before mid point 10 ml

Figure 4.6 Plot of simulated data (Eq. 38): $pK_a = 9$, P=0.100, $V_{oct} = 5.0$ ml, $V_e = 10$ ml

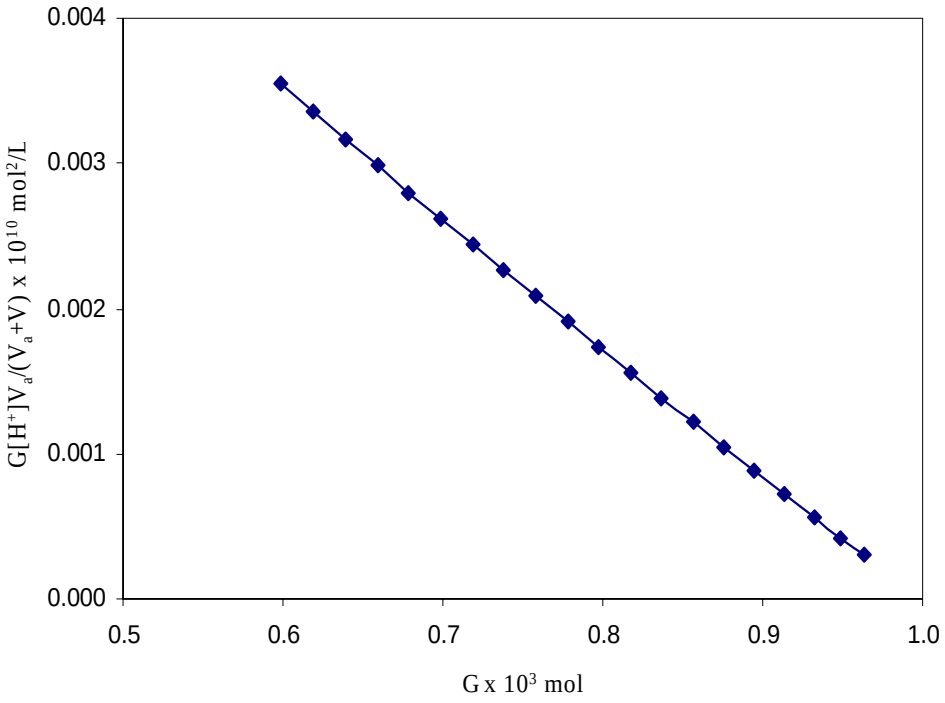
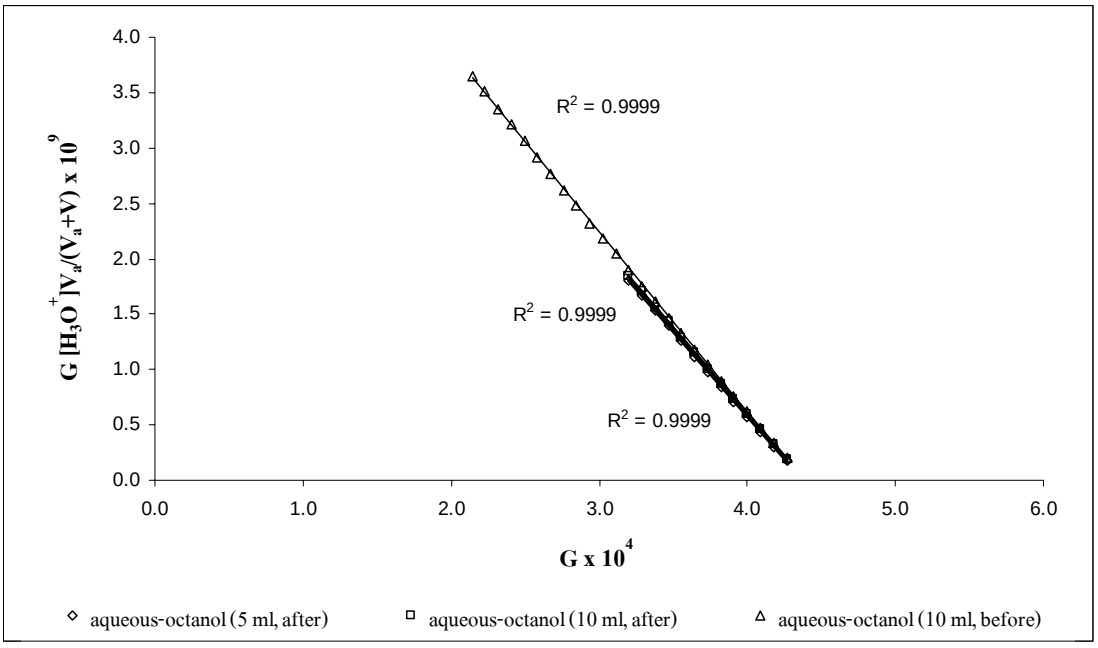


Figure 5.1 The modify Gran plot (Eq. 38) for acetic acid to determine V_e from the aqueous-octanol portion of the sequential aqueous-octanol titration with 0.1 N sodium hydroxide

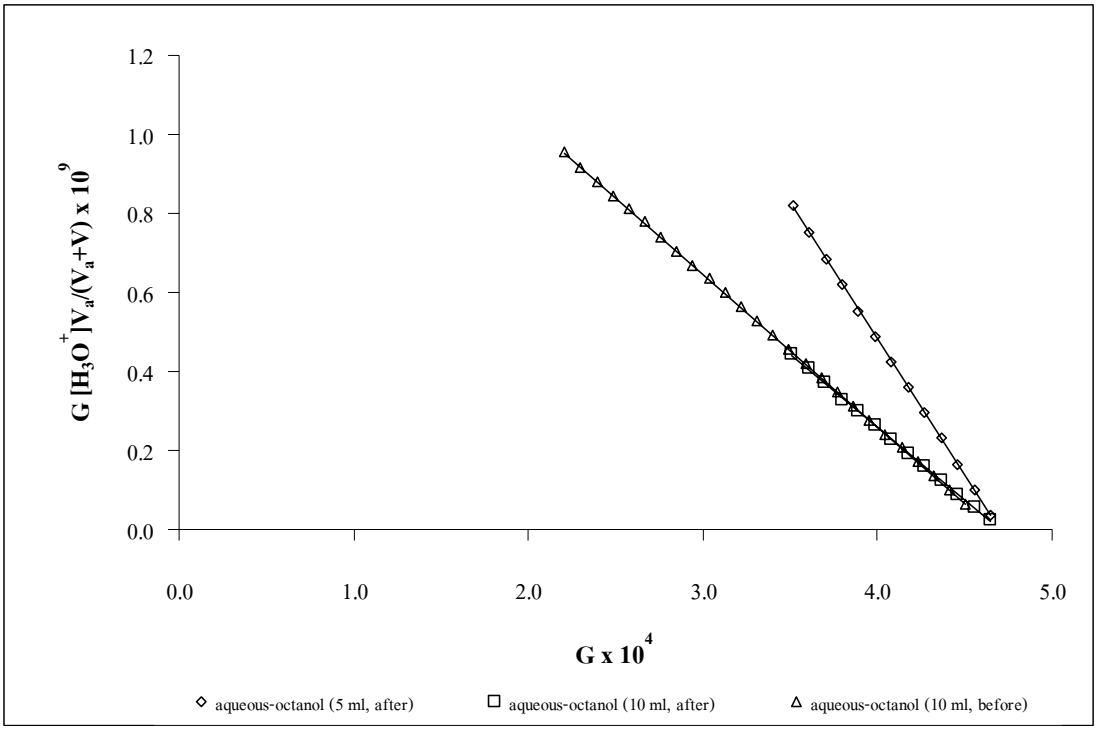


aqueous-octanol (5 ml, after) the sequential of aqueous-octanol titration with 5 ml octanol added after mid point

aqueous-octanol (10 ml, after) the sequential of aqueous-octanol titration with 10 ml octanol added after mid point

aqueous-octanol (10 ml, before) the sequential of aqueous-octanol titration with 10 ml octanol added before mid point

Figure 5.2 The modify Gran plot (Eq. 38) for benzoic acid to determine V_e from the aqueous-octanol portion of the sequential aqueous-octanol titration with 0.1 N sodium hydroxide

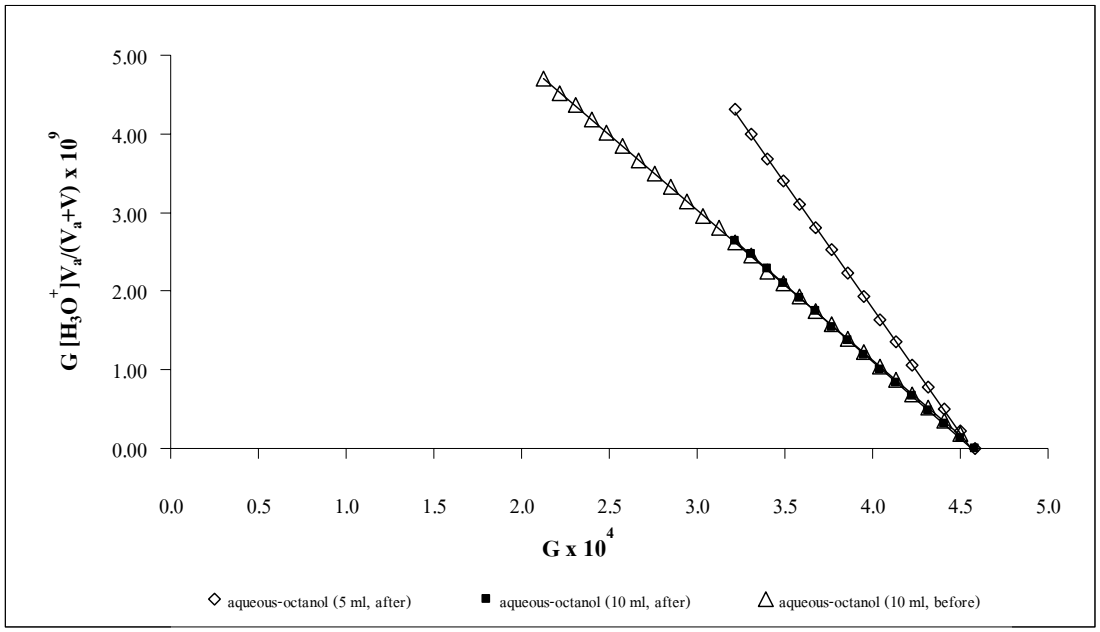


aqueous-octanol (5 ml, after) the sequential of aqueous-octanol titration with 5 ml octanol added after mid point

aqueous-octanol (10 ml, after) the sequential of aqueous-octanol titration with 10 ml octanol added after mid point

aqueous-octanol (10 ml, before) the sequential of aqueous-octanol titration with 10 ml octanol added before mid point

Figure 5.3 The modify Gran plot (Eq. 38) for *2*-methoxybenzoic acid to V_e from the aqueous-octanol portion of the sequential aqueous-octanol titration with 0.1 N sodium hydroxide

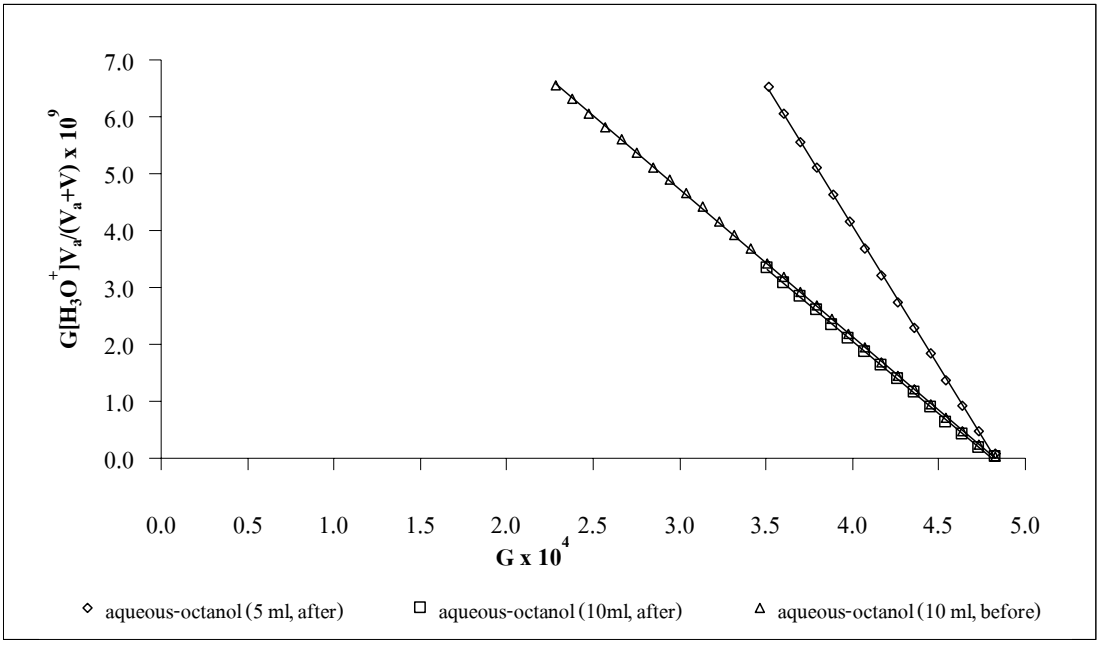


aqueous-octanol (5 ml, after) the sequential of aqueous-octanol titration with 5 ml octanol added after mid point

aqueous-octanol (10 ml, after) the sequential of aqueous-octanol titration with 10 ml octanol added after mid point

aqueous-octanol (10 ml, before) the sequential of aqueous-octanol titration with 10 ml octanol added before mid point

Figure 5.4 The modify Gran plot (Eq. 38) for salicylic acid to V_e from the aqueous-octanol portion of the sequential aqueous-octanol titration with 0.1 N sodium hydroxide

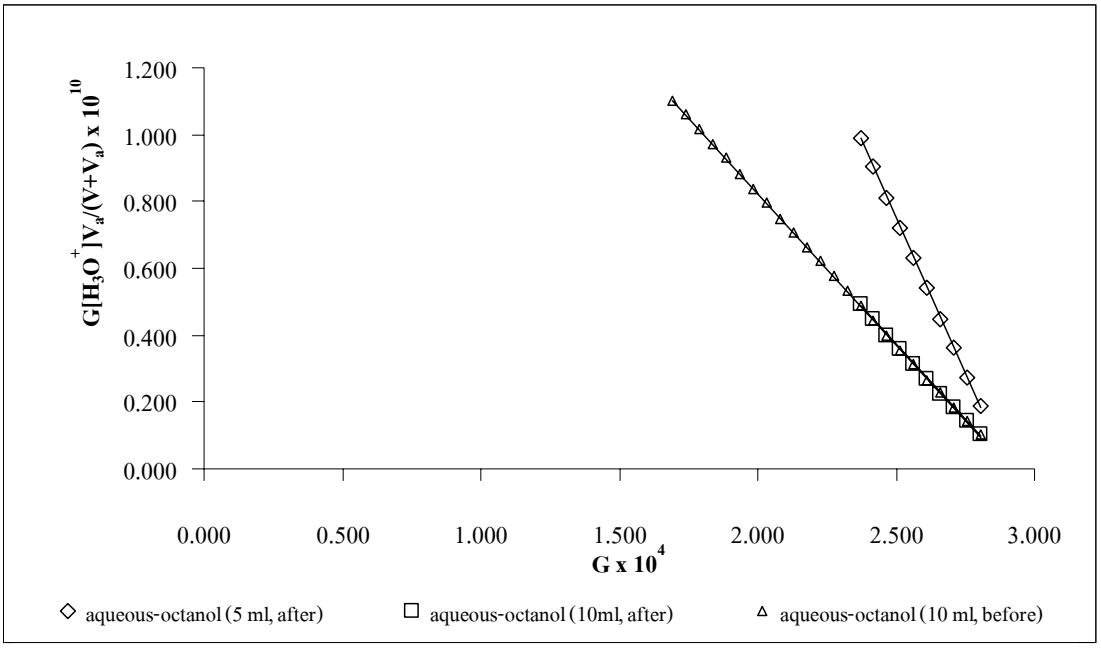


aqueous-octanol (5 ml, after) the sequential of aqueous-octanol titration with 5 ml octanol added after mid point

aqueous-octanol (10 ml, after) the sequential of aqueous-octanol titration with 10 ml octanol added after mid point

aqueous-octanol (10 ml, before) the sequential of aqueous-octanol titration with 10 ml octanol added before mid point

Figure 5.5 The modify Gran plot (Eq. 38) for *m*-toluic acid to determine V_e from the aqueous-octanol portion of the sequential aqueous-octanol titration with 0.1 N sodium hydroxide

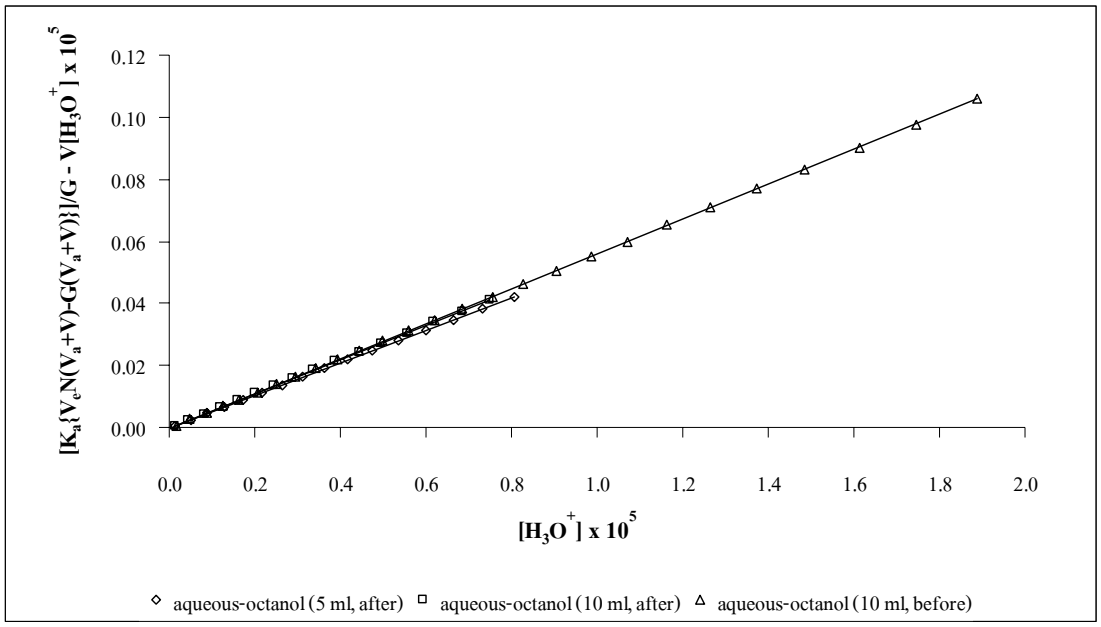


aqueous-octanol (5 ml, after) the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint

aqueous-octanol (10 ml, after) the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint

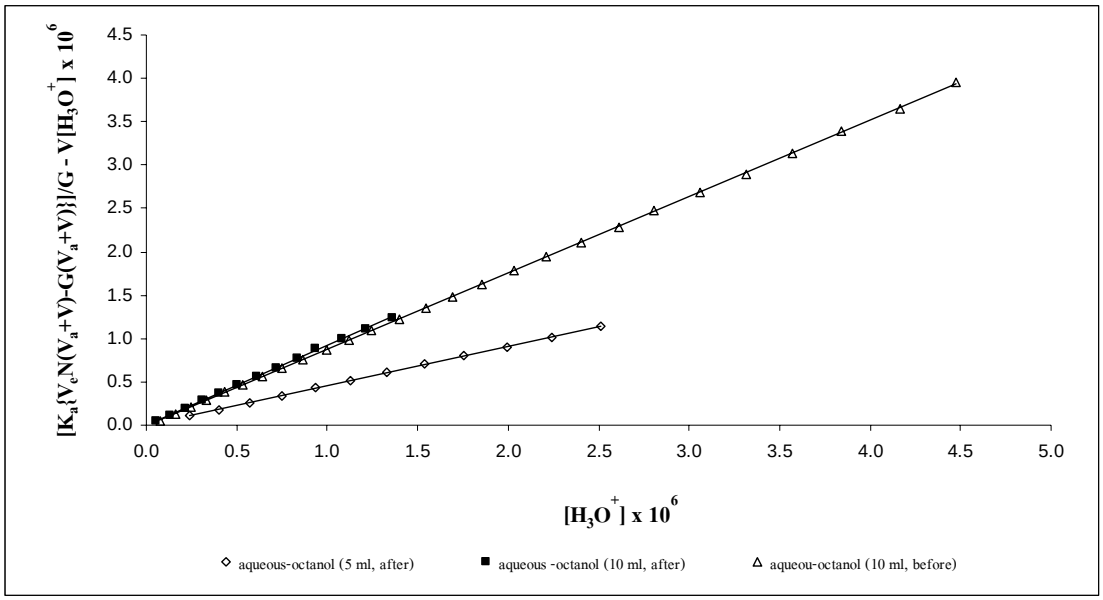
aqueous-octanol (10 ml, before) the sequential of aqueous-octanol titration with 10 ml octanol added before midpoint

Figure 6.1 Linear plot of Eq. 41 for acetic acid to determine P from the aqueous-octanol portion of the sequential aqueous-octanol titration with 0.1 N sodium hydroxide



aqueous-octanol (5 ml, after)	the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint
aqueous-octanol (10 ml, after)	the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint
aqueous-octanol (10 ml, before)	the sequential of aqueous-octanol titration with 10 ml octanol added before midpoint

Figure 6.2 Linear plot of Eq. 41 for benzoic acid to determine P from the aqueous-octanol portion of the sequential aqueous-octanol titration with 0.1 N sodium hydroxide

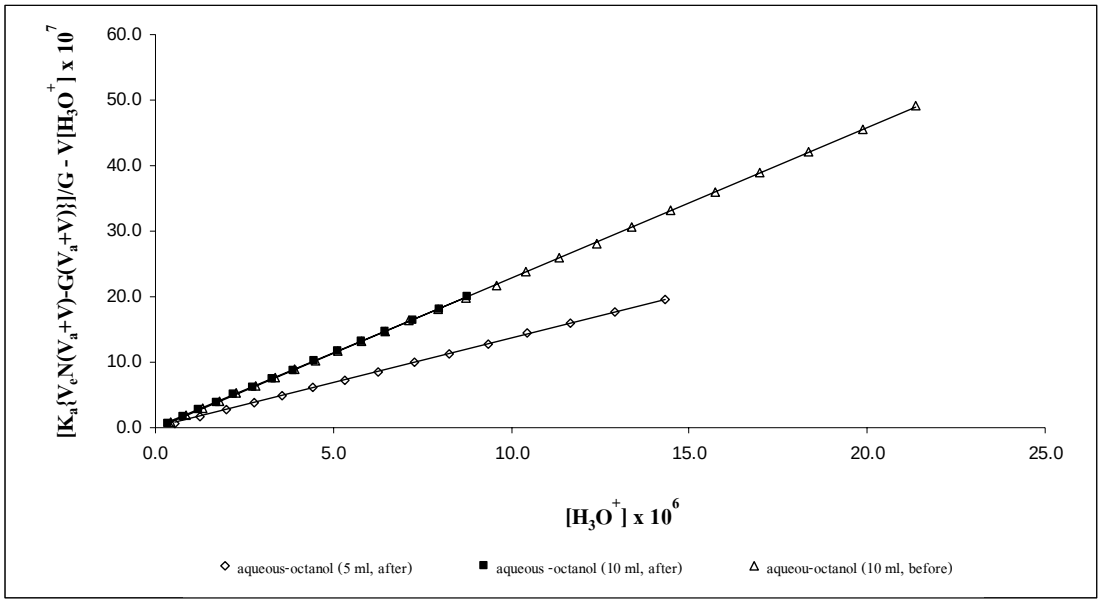


aqueous-octanol (5 ml, after) the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint

aqueous-octanol (10 ml, after) the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint

aqueous-octanol (10 ml, before) the sequential of aqueous-octanol titration with 10 ml octanol added before midpoint

Figure 6.3 Linear plot of Eq. 41 for *2*-methoxybenzoic acid to determine P from the aqueous-octanol portion of the sequential aqueous-octanol titration with 0.1 N sodium hydroxide

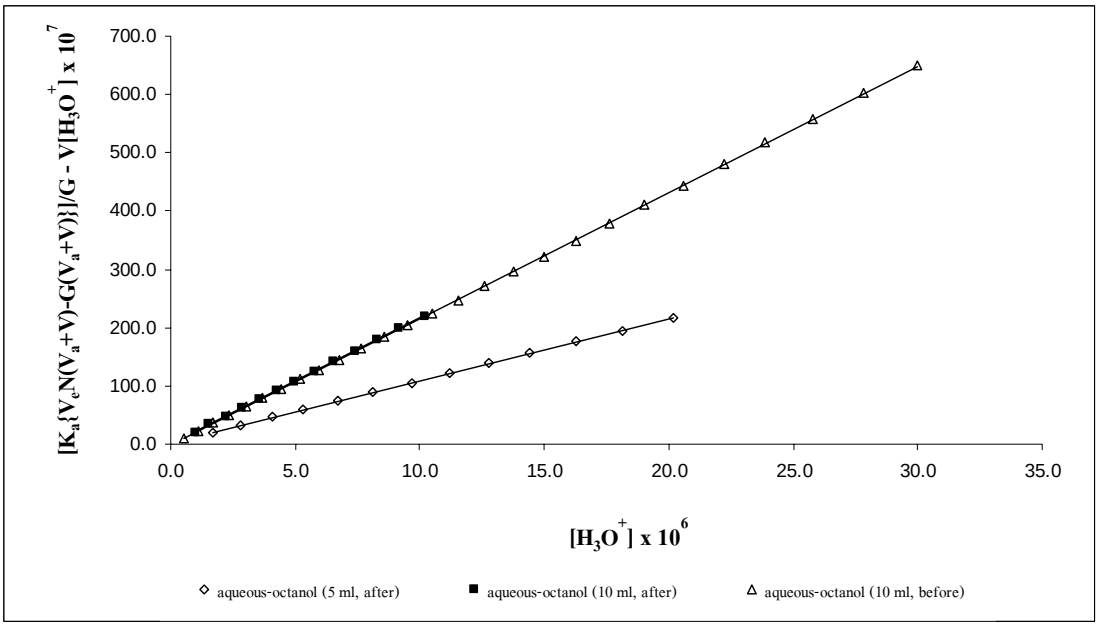


aqueous-octanol (5 ml, after) the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint

aqueous-octanol (10 ml, after) the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint

aqueous-octanol (10 ml, before) the sequential of aqueous-octanol titration with 10 ml octanol added before midpoint

Figure 6.4 Linear plot of Eq. 41 for salicylic acid to determine P from the aqueous-octanol portion of the sequential aqueous-octanol titration with 0.1 N sodium hydroxide

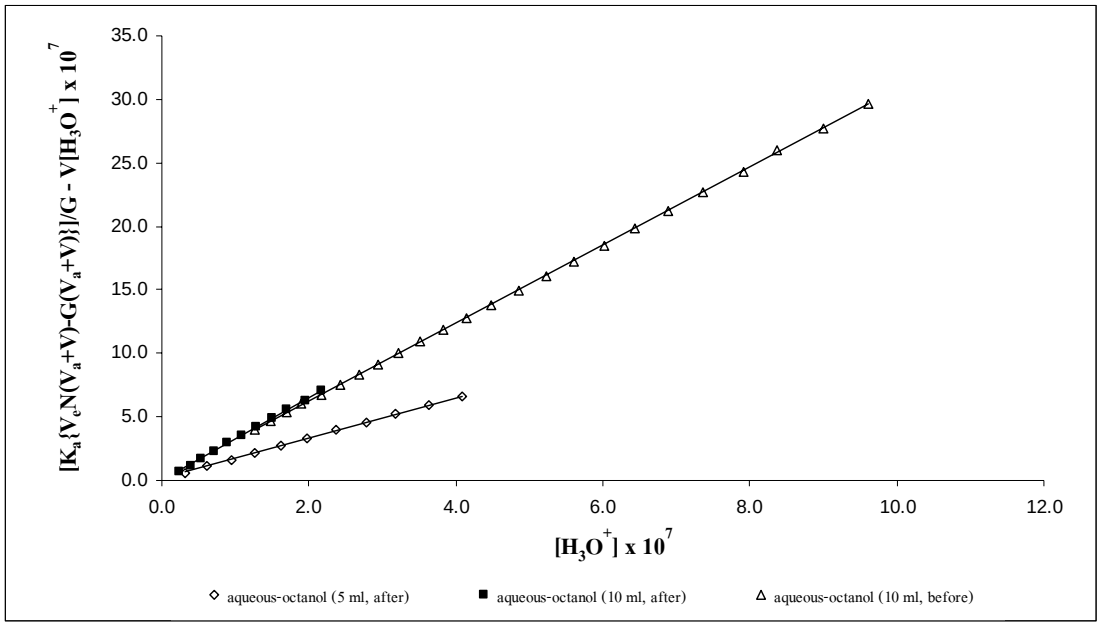


aqueous-octanol (5 ml, after) the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint

aqueous-octanol (10 ml, after) the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint

aqueous-octanol (10 ml, before) the sequential of aqueous-octanol titration with 10 ml octanol added before midpoint

Figure 6.5 Linear plot of Eq. 41 for *m*-toluic acid to determine P from the aqueous-octanol portion of the sequential aqueous-octanol titration with 0.1 N sodium hydroxide



aqueous-octanol (5 ml, after) the sequential of aqueous-octanol titration with 5 ml octanol added after midpoint

aqueous-octanol (10 ml, after) the sequential of aqueous-octanol titration with 10 ml octanol added after midpoint

aqueous-octanol (10 ml, before) the sequential of aqueous-octanol titration with 10 ml octanol added before midpoint

VITAE

Flight Lieutenant Nisa Phutong was born on October 12, 1981 in Nakhonpathom, Thailand. She graduated with Bachelor Degree of Pharmacy from Faculty of Pharmacy, Chiangmai University, Chiangmai in 2004. She had started her work at Wing 4 hospital, Nakhonsawan from 2004 to 2006. In 2006, she was admitted to Pharmaceutical Chemistry Master Degree Program in Chulalongkorn University.