



CHAPTER II

MATERIALS AND METHOD

MATERIALS

1. Aluminum hydroxide gels from
 - Gel 1, Reheis Chemical Co, U.S.A. (Control No.5162)
 - Gel 2-4 were prepared under the support of the Government Pharmaceutical Organization, Thailand
 - Gel 5, Hardman, Australia (Lot. No. H711)
 - Gel 6, Giuliani chemie GmbH, Germany. (Batch No. 88-602-30)
 - Gel 7, T.S. Polyproducts Co, Ltd., Thailand (Lot No. 60108)
2. Ammonium acetate, E. Merck, Germany (Lot No. CC536116)
3. Butyl paraben, Ueno. Fine Chemicals industry, Japan (Lot No Unknown)
4. Chlorhexidine acetate, ICI Pharma, U.S.A. (Lot No. Unknown)
5. Chlorhexidine gluconate, ICI Pharma, U.S.A. (Lot No. Unknown)
6. Disodium ethylenediaminetetraacetic acid, E. Merck, Germany (Lot No. K 11622418)
7. Dithizone, E. Merck, Germany (Lot No. TT 438992)

8. Ethanol, E. Merck, Germany, (Lot No. K 13126383)
9. Glacial acetic acid, E. Merck, Germany (Lot No. Unknown)
10. Heptanesulfonic acid, FSA Laboratory Supplies, (Lot No. 33247270)
11. Hydrochloric acid, E. Merck, Germany (Lot No. K12576717)
12. Methanol (HPLC grade), E. Merck, Germany. (Lot No. I 114907)
13. Methyl paraben, Ueno. Fine Chemicals industry, Japan (Lot No. Unknown)
14. Potassium chloride, J.T. Baker Chemical Co, U.S.A. (Lot No. 533960)
15. Potassium dihydrogen phosphate, E. Merck, Germany. (Lot No. A 459773)
16. Potassium hydroxide, May & Baker Ltd., England. (Lot No. 37038)
17. Propyl paraben, Ueno. Fine Chemicals industry, Japan (Lot No. Unknown)
18. Sodium fluoride, Farmitalia Carlo-Erba, Italy. (Lot No. 1989D100)
19. Sodium hydroxide, E. Merck, Germany (Lot No. 734598)
20. Sorbitol, Vidhyasom Co., Ltd., Thailand (Lot No. 001230)
21. Zinc sulfate heptahydrate, BDH Chemical Ltd., England (Lot No. 4921120 H)

APPARATUS

1. Analytical balance, Sartorius (A 200S), Germany
2. Centrifuge, Safeguard centrifuge, Clay-Adams Co.,
Inc., U.S.A.
3. Column C₁₈ (18 cm x 5 mm), Chanon, U.S.A.
4. Detector (SM 4000), Milton Roy^(R), LDC Division,
U.S.A.
5. High Performance Liquid Chromatography (CM 4000),
Milton Roy^(R), LDC Division, U.S.A.
6. Incubator, Laboratory Thermal Equipment Ltd.
7. Infrared spectrophotometer (IR-440), Shimazu, Japan
8. Magnetic stirrer, Sybron corporation, U.S.A.
9. pH meter, Hanna instruments, U.S.A.
10. Precolumn C₁₈ (8 cm x 5 mm), Chanon, U.S.A.
11. Recorder (CI 4100), Milton Roy^(R), LDC Division,
U.S.A.
12. Transmission Electron Microscope (JEM 200CX), JEOL,
Japan
13. X-Ray Diffractometer (JDX-8030), JEOL, Japan

METHOD

Physicochemical Properties Determinations

Physical Properties Determinations

1. X-Ray Diffraction (Nail et al., 1975)

For X-Ray Diffraction (XRD) analysis, a sample of aluminum hydroxide gel was air dried at room temperature and ground to a fine powder with an agate mortar and pestle. Powder mounts were prepared using aluminum McCreery mounts. The diffraction pattern was recorded from 5° to 40° 2θ under the following conditions:

CuK α radiation, 40 kV, 20 mamp,

500 cps full scale, and 2° /min scan speed.

2. Transmission Electron Microscope

(Liu et al., 1987)

The same powder samples from XRD were fixed onto carbon films supported by copper grids. Several particles were examined in each sample and the particles shown were representative. Micrographs were made at magnification of 270,000.

3. Infrared Spectrum (Scholtz et al., 1984a, 1984b.)

The same powder samples from XRD were diluted in potassium bromide (2 mg/300 mg of KBr) and examined by

Infrared spectroscopy.

4. pH of the Aluminum Hydroxide Gel (USP XXII)

The pH of the ~ 4% equivalent aluminum oxide of each aluminum hydroxide gel was measured. The pH meter was calibrated prior to each measurement.

Chemical Properties Determinations

1. Preliminary Antacid Test (USP XXII)

- Place and accurately weighed amount of the aluminum hydroxide gel equivalent to 0.6 g of aluminum oxide into a 100-ml beaker. Add sufficient water to obtain a total volume of about 40 ml and mix on magnetic stirrer at 300 ± 30 rpm for about 1 minute. Analyze the sample according to the test procedure.

- Test Procedure

(1) Add 10.0 ml of 0.5 N HCl to the test solution while stirring on the magnetic stirrer at 300 ± 30 rpm.

(2) Stir for exactly 10 minutes after addition of acid

(3) Read and record pH

(4) If pH is below 3.5, the product shall not be labeled as an antacid. If the pH is 3.5 or greater, determine the acid neutralizing capacity according to the acid neutralizing capacity test.

2. Acid Neutralizing Capacity Test (USP XXII)

2.1 Place an accurately weighed amount of the aluminum hydroxide gel equivalent to 0.6 g of aluminum oxide into a 250-ml beaker.

2.2 Add sufficient water to obtain a total volume of about 70 ml and mixed on the magnetic stirrer at 300 ± 30 rpm for about 1 minute.

2.3 Transfer 30 ml of 1.0 N HCl VS from buret into the sample solution while stirring on the magnetic stirrer at 300 ± 30 rpm.

2.4 Stir for exactly 15 minutes after addition of acid

2.5 Begin titration immediately and in a period not exceed and additional 5 minutes titrate the excess 1.0 N HCl VS with 0.5 N NaOH VS to stable pH of 3.5.

2.6 Check the sample solution 10 to 15 seconds after obtaining a pH of 3.5 to make sure the pH is stable

2.7 Calculate the number of mEq of acid neutralized by the sample as follow:

$$\text{Total mEq.} = (30.0 \text{ ml})(N \text{ HCl}) - (\text{ml of NaOH})(N \text{ NaOH})$$

2.8 Calculate the percent of theoretically acid neutralizing capacity (% TANC) by the sample as follow:

$$\% \text{ TANC} = \frac{\text{Total mEq.} \times 10}{\text{g of sample} \times \% \text{ Al(OH)}_3 \times 0.0385}$$

2.9 The ln's of the remained concentrations versus times was treated by linear regression statistical technique (Nail, White, and Hem, 1975, 1976a, 1976f.). Then the first order rate constant of each sample at ambient temperature and 45°C were calculated.

Note : The preliminary antacid test and Acid Neutralizing Capacity test should be conducted at 37° ± 3° C

3. Reaction Velocity Test

- According to the modified Reheis reaction velocity test (Parab et al., 1985).

3.1 Weight the aluminum hydroxide gel containing the equivalent of 0.5 g of aluminum oxide.

3.2 Add 100 ml of 0.1 N HCl at 37.5°C while stirring on the magnetic stirrer at 300 ± 30 rpm.

3.3 The time to reach pH 3.5 was noted.

4. Assay for Aluminum Oxide and Aluminum Hydroxide

4.1 Transfer about 25 g, accurately weighed, of aluminum hydroxide gel to a beaker.

4.2 Add 15 ml of hydrochloric acid, and heat gently until solution is complete.

4.3 Cool, transfer to a 500 ml volumetric flask, dilute with water to volume and mix.

4.4 Pipet 20 ml of the solution into a 250-ml beaker, and add, in the order named and with continuous stirring, 25 ml of disodium ethylenediaminetetraacetic acid VS and 20 ml of acetic acid-ammonium acetate buffer TS, then heat the solution near the boiling point for 5 minutes.

4.5 Cool, and add 50 ml of alcohol and 2 ml of dithizone TS.

4.6 Titrate the solution with 0.05 M Zinc sulfate until the color changes from green-violet to rose-pink.

4.7 Perform a blank determination, substituting 20 ml of water for the sample, and make any necessary correction.

4.8 Calculate the Aluminum oxide and Aluminum hydroxide content as follow :

$$\% \text{Al}_2\text{O}_3 = \frac{0.002549 \times 25 (V_b - V_s) \times M_o \times 2,500}{0.05 \times m \times V_b}$$

$$\% \text{Al}(\text{OH})_3 = \frac{0.0039 \times 25 (V_b - V_s) \times M_o \times 2,500}{0.05 \times m \times V_b}$$

V_b = Volume of ZnSO_4 used in blank (ml)

V_s = Volume of ZnSO_4 used in sample (ml)

M_o = Concentration of disodium ethylenediaminetetraacetic acid solution (M)

m = Weight of sample (g)

4.9 Calculate Aluminum content as follow :

$$\text{Al (mmole/g)} = \frac{20 \times \% \text{Al}_2\text{O}_3}{m}$$

m = molecular weight of Aluminum oxide

5. Determine the Molar Ratio of Bound Hydroxide to Aluminum in the Aluminum Hydroxide Gel

(Nail et al, 1976b.)

5.1 Place 50 ml of 0.5 N sodium fluoride (NaF) in a 100-ml beaker. Add 2 drops of phenolphthalein indicator and measure the pH of the solution. If necessary, adjust the pH to about 7.4 with 0.1 N HCl.

5.2 Weighing about 0.5-1.0 g of the aluminum hydroxide gel and add it to the sodium fluoride solution. Agitate throughly. Prepare a 50-ml aliquot of sodium fluoride solution as a blank.

5.3 Immerse the pH electrode in the sample and titrate to the original pH of the sodium fluoride solution (about 7.4) with standard 0.1 N HCl VS.

5.4 Cover the beaker containing the sample with aluminum foil and place on a water bath for 1 hour.

5.5 Allow the sample to cool and again titrate to the original pH. Repeat this heat and titrate operation until no more than 1 drop of 0.1 N HCl is

required. The phenolphthalein serves as a visual indicator of the extent of reaction taking place with each heat and titrate step.

5.6 Record the total mEq of acid used and subtract from this value the number of mEq consumed by the blank. The net mEq of acid consumed equal the mEq of hydroxide liberated from the sample.

5.7 Calculate the molar ratio of bound hydroxide to aluminum as follow :

$$\text{OH}^-/\text{Al} = \frac{M. (V_s - V_b)}{w \times m}$$

M = Concentration of HCl (M)

V_s = Volume of HCl used in the sample (ml)

V_b = Volume of HCl used in the blank (ml)

m = The millimoles of aluminum present in the sample

w = Weight of sample (g)

6. Determine Point of Zero Charge

(Feldkamp et al., 1981)

The titration technique used to measure the PZC was based on the principle that charges in the ionic strength produced by the addition of an indifferent electrolyte, such as KCl, will have no effect on proton adsorption or release by the gel when the gel surface

charge is zero. Thus, titration curves (pH versus volume of KOH or HCl added) obtained at several ionic strengths intersect at a common point, calling the PZC.

The specific procedure to determine PZC was as follow :

6.1 A 60-ml aliquot containing \sim 1% equivalent aluminum oxide was transferred to a 100 ml beaker.

6.2 The initial pH was recorded with stirring after stable value was achieved.

6.3 Sufficient 0.1 N KOH was added from a 10-ml buret to cause a pH change of 0.05-0.10 unit. Usually, increments of titrant ranging from 0.2 to 1.0 ml were required. When the pH was stable, the volume of titrant added and the pH were recorded.

6.4 Step 1-3 were repeated but titration was performed with 0.1 N HCl.

6.5 Step 1-4 were repeated except that, in step 1, 6 ml of 1 M KCl was added before making the final volume of the \sim 1% equivalent aluminum oxide aliquot.

6.6 Step 1-4 were repeated except that, in step 1, 12 ml of 1 M KCl was added before making the final volume of the \sim 1% equivalent aluminum oxide aliquot. The data were plot on the same graph. A plot was constructed with pH on the ordinate and the volume of acid or base added on the abscissa.

Effect of Aging at Ambient Temperature

The aluminum hydroxide gel from various sources were stored in airtight container at ambient temperature, and sampling at predetermined time. All aluminum hydroxide gels were determined of their physicochemical properties. The measurements were as follow:

1. pH, preliminary antacid test, acid neutralizing capacity test, reaction velocity test, and OH^-/Al ratio were recorded at the beginning of storage and approximately one month intervals for six months.

2. X-ray diffraction, Infrared spectrum, and Transmission electron microscope were recorded before and after being stored at ambient temperature for six months.

3. Point of Zero Charge was measured at the beginning of storage and two more times at three and six months of aging.

Effect of Aging at 45°C

The feasible aluminum hydroxide gels were incubated and sampling at predetermined times. Then the physicochemical properties were measured as follow:

1. pH, preliminary antacid test, acid neutralizing capacity test, reaction velocity test, and OH^-/Al ratio of each sample were examined at the beginning of storage and approximately one month intervals for four months.

2. Infrared spectrum and Transmission electron microscope were determined before and after aging at 45°C for six months.

Effect of Sorbitol on the Stability of Aluminum Hydroxide Gel

Two samples were selected to determine the effect of sorbitol on the stability of aluminum hydroxide gel. Sorbitol was added to the gel at 2% and 5% w/w, then stored at 45°C in the incubator. The stability of the sample with and without sorbitol were compared.

The Surface Adsorption of Preservatives by Aluminum Hydroxide

1. Adsorption Studies

Four different preservatives were used in this study, they are methyl, propyl, butyl paraben and chlorhexidine gluconate. The methyl paraben solution at various concentrations were added to ~ 4% equivalent to aluminum oxide suspension which adjust pH equal, low and above the PZC by 0.1 M HCl or 0.1 M NaOH. The ~ 4% equivalent to aluminum oxide suspension (without preservative) and phosphate buffer (with various concentrations of preservative) were used as a blank. The tube were shaken vigorously after equilibrium had been attained (~ 72 hrs). The suspension were centrifuged at 2,000 rpm for 30 minutes and the supernatant was filtered

through a 0.45 um millipore filter before assay by HPLC.

A similar technique was used for propyl paraben, butyl paraben and chlorhexidine gluconate except for the suspension containing chlorhexidine gluconate was kept in the dark.

The concentration of preservatives were 10, 20, 30 and 40 mg/100 ml.

2. Assay for Preservatives

HPLC was used for this studies because of its sensitivity. The conditions are shown in Table 1 (Schmidt and Benke, 1988a).

Operating Conditions of HPLC

Apparatus : CM 4000, Milton Roy^(R), U.S.A.
Column : ODS chanon (C₁₈), U.S.A.
Precolumn : ODS Chanon (C₁₈), U.S.A.
Detector : SM 4000, Milton Roy^(R), U.S.A.
Injection volume : 20 microliters

Calibration Curve

The absorbance followed Beer's law in the concentration range of study. The peak area or peak high ratio of preservatives solution as a function of concentrations shown linear relationship. The calibration curve of preservatives after regression analysis are illustrated in Figures 7-10.

Table 1

The conditions for HPLC analysis of various preservatives

PARAMETER	MP	PP	BP	CG
Mobile phase:				
methanol	70	70	70	90
0.25% acetic acid	30	30	30	10*
Heptanesulfonic acid	-	-	-	180 mg.
Flow rate(ml /min)	1.0	1.0	1.0	2.5
Wavelength(nm.)	254	254	254	257
Retention time(mins)	2.93	4.08	5.70	4.06
Concentration(mg /100ml)	0.2-1.0	0.2-1.0	0.2-1.0	1.5-4.0
Internal standard	PP	MP	MP	**

* = 1.0% acetic acid, ** = external standard(chlorhexidine acetate)
 (MP = methyl paraben, PP = propyl paraben, BP = butyl paraben, -
 CG = chlorhexidine gluconate)

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Calibration curve of methyl paraben

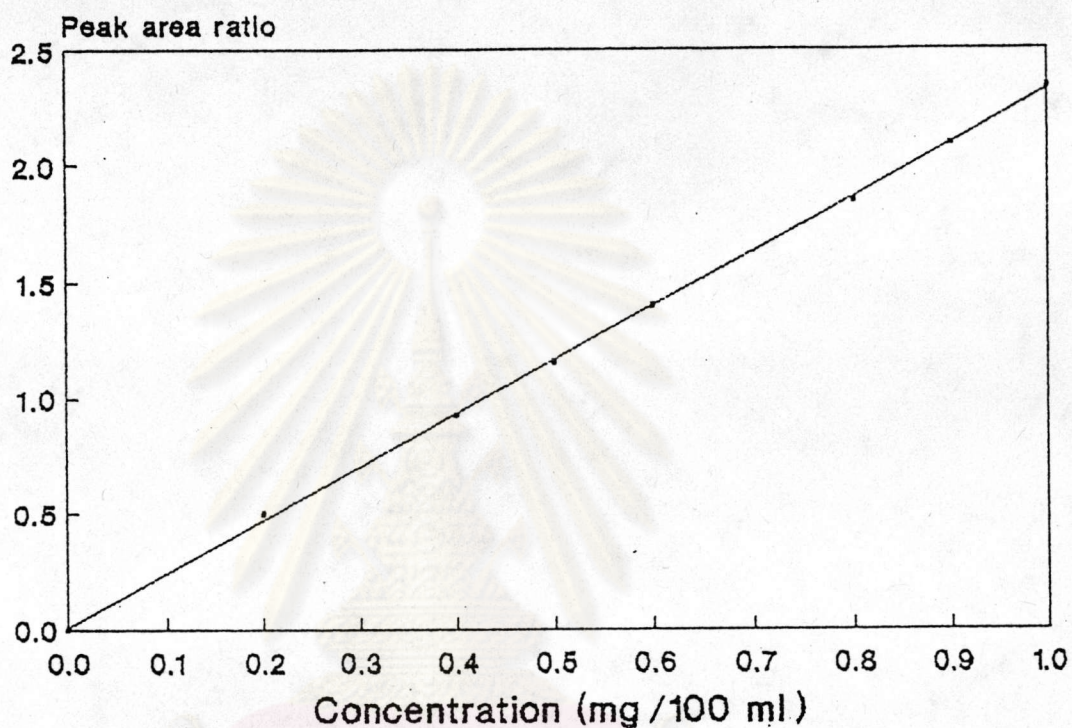


Figure 7 The calibration curve of methyl paraben

$$Y = 2.3134X + 0.0077, (r^2 = 0.9997)$$

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Calibration curve of propyl paraben

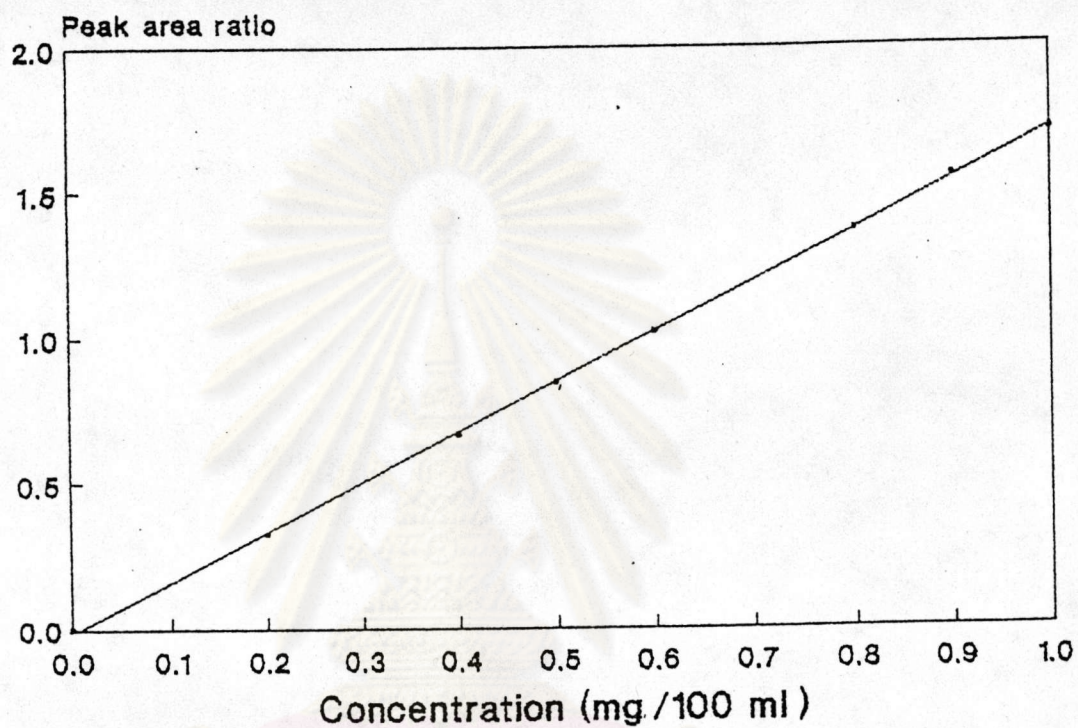


Figure 8 The calibration curve of propyl paraben

$$Y = 1.7204X - 0.0130, (r^2 = 0.9998)$$

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Calibration curve of butyl paraben

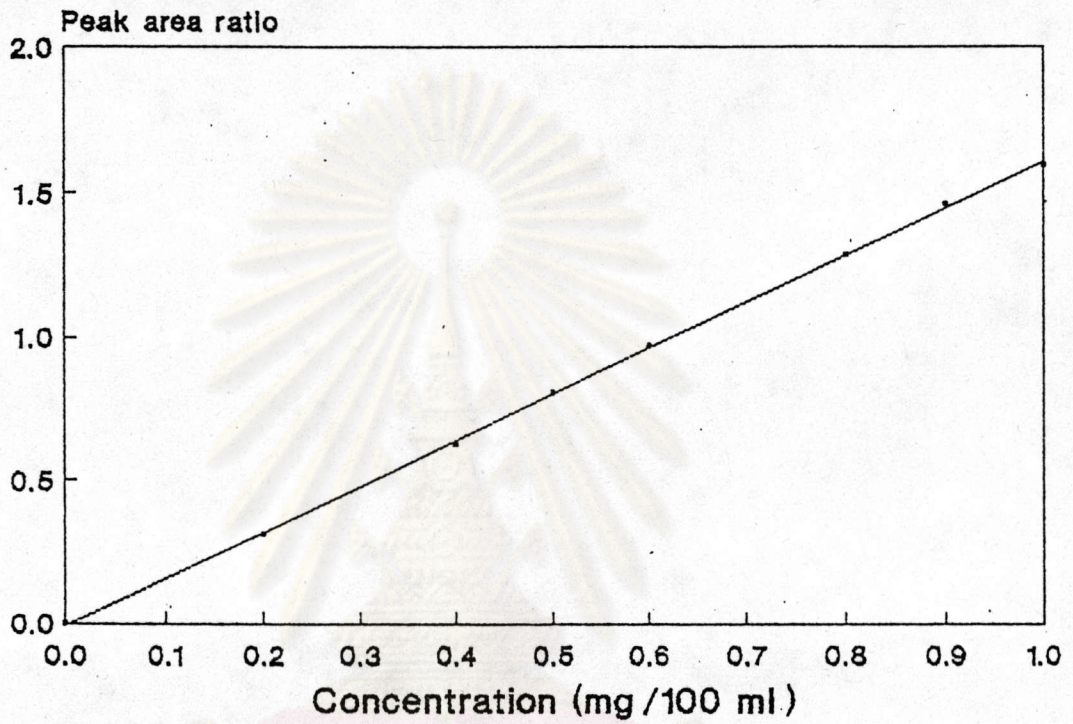


Figure 9 The calibration curve of butyl paraben

$$Y = 1.6141X - 0.0066, (r^2 = 0.9997)$$

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Calibration curve of chlorhexidine

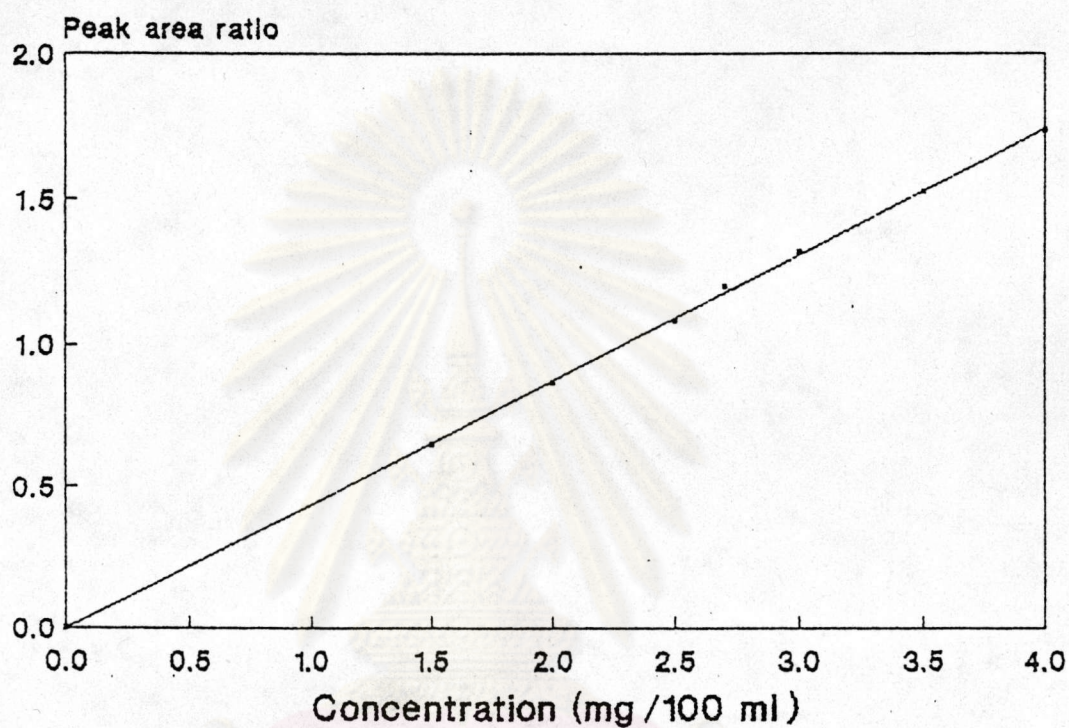


Figure 10 The calibration curve of chlorhexidine gluconate

$$Y = 0.4368X - 0.0013, (r^2 = 0.9996)$$

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3. Adsorption Isotherm Determination

The equilibrium concentrations (C) versus adsorption of preservatives (x) was determined. The adsorption of preservatives from suspension was treated by the Langmuir equation in the following equation :

$$\frac{C}{x/m} = \frac{1}{ab} + \frac{C}{b}$$

where x = weight of adsorbate (mg) adsorbed by m grams of adsorbent; C= the equilibrium concentration (mg/100 ml); and a and b are constant. Therefore a plot of C/(x/m) versus C is a straight line of slope 1/b and intercept 1/ab. (Clarke and Armstrong, 1972).

The calculation of unionized of the esters of parabens can be expressed as :

$$pH = pKa + \log \frac{[\text{ionize}]}{[\text{Unionize}]}$$

Aluminum Hydroxide Gel Preparation

Gel 2

Gel 2 was prepared by the addition of 3,200 liters of alum solution ($AlK(SO_4)_2 \cdot 12H_2O$) (25% w/v) to 1,500 liters of sodium carbonate solution (25% w/v). Alum solution was added with agitation to a final pH of 6.2-

6.5. After precipitation, the gel was washed with deionized water, and reduced the particle size by micronizer.

Gel 3

The alum solution (25% w/v) and sodium carbonate solution (25% w/v) were injected simultaneously into the reactor at a constant rate of 33 liters/min and 15 liters/min, respectively. Experimental laboratory gel was precipitated at 25°C. The solution was added with agitation to a final pH of 6.7. After precipitation, the gel was washed with deionized water, and reduced the particle size by micronizer.

Gel 4

Gel 4 was prepared in an identical manner of gel 3 except that the flow rate of alum solution and sodium carbonate solution was changed to 25 liters/min and 15 liters/min, respectively. In addition, the final pH was 7.2.

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