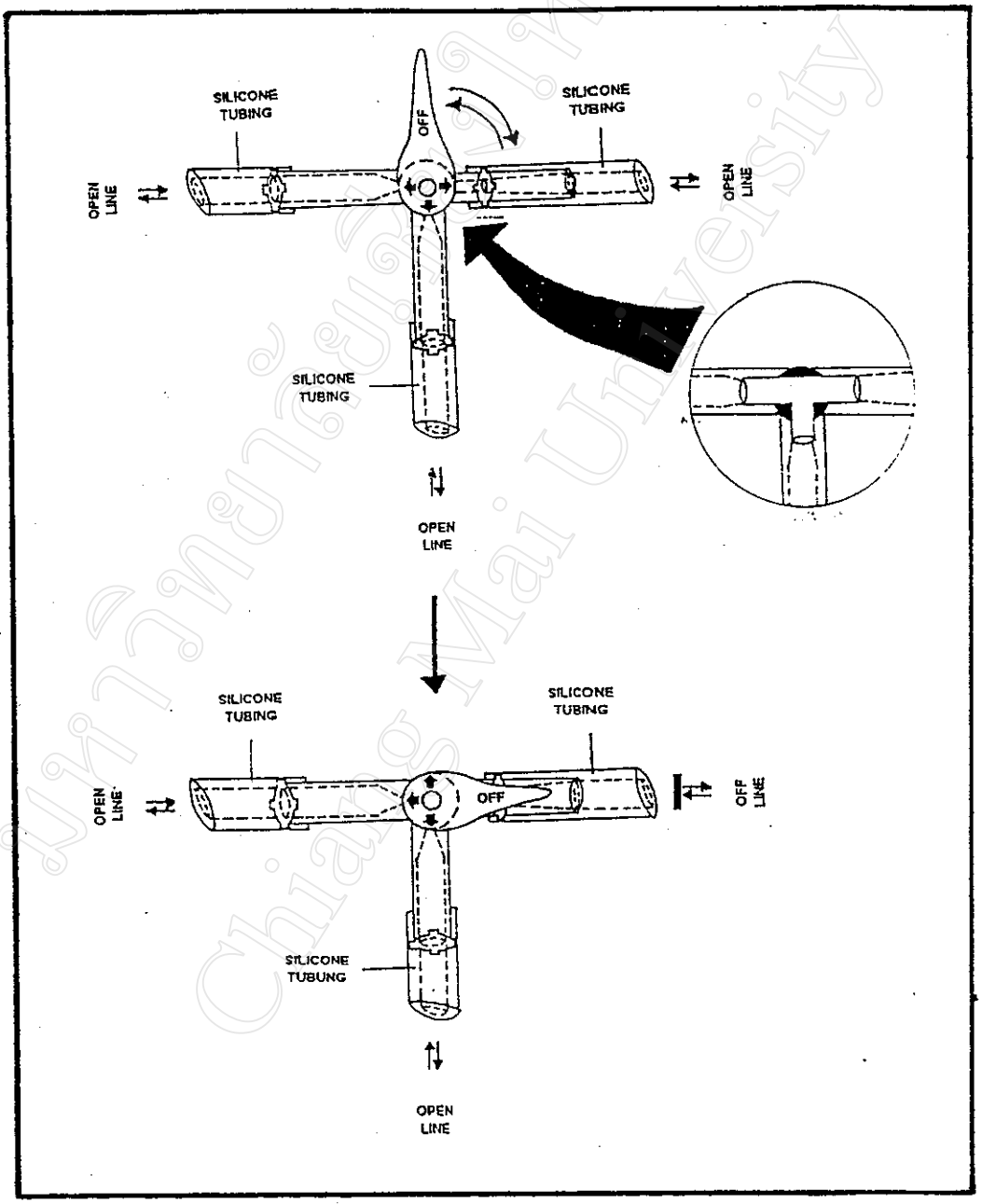
APPENDICES

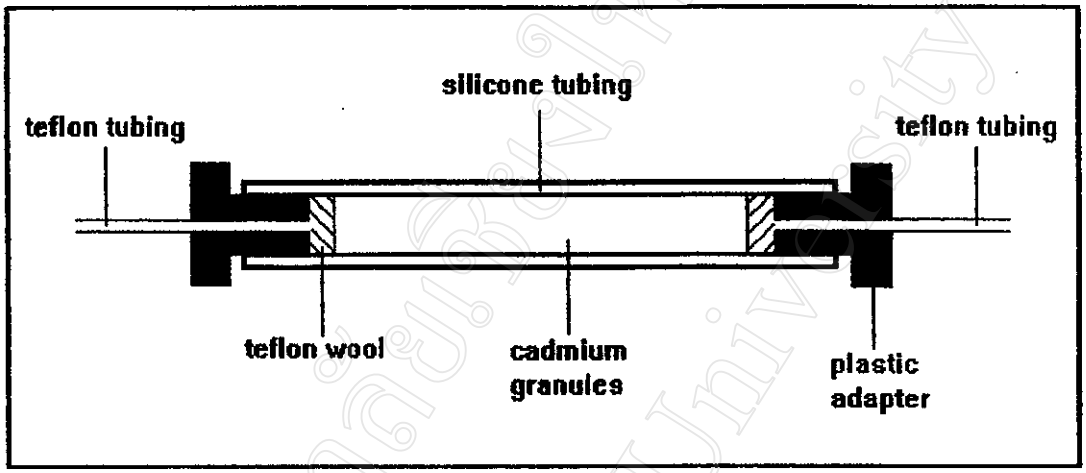
APPENDIX A

Three way valve (used in clinical purposes, Connecta®, Sweden)



APPENDIX B

Cadmium reduction column



The cadmium reduction column is prepared as following :

(1). Cadmium Cleanning

Cadmium was prepared by placing 10-20 g of cadmium granules (0.83-1.65 mm diameter) in a 250 ml beaker, washed with 50 ml of acetone, then water, following by two 50 ml portions of 1 M HCl. It was rinsed several times with water.

(2). Copperization

The cadmium copperization was made by adding a 100 ml portion of 2 % (w/v) copper sulfate solution to the cadmium previously prepared as described above; swirling for about 5 minutes, then the solution was decanted and repeating with a fresh 100 ml portion of the 2 % (w/v) copper sulfate solution. The procedure was continued until the blue aqueous copper persisted. Decanting and washing with at least five portions of NH_4Cl buffer (pH 8.5) to remove colloidal copper. The cadmium should be black or dark gray. The copperized cadmium granules may be stored under NH_4Cl buffer.

(3). Packing the Column

The cadmium column was packed into silicone tubing (3.5 mm i.d., 15 cm long) of which the two ends were closed with teflon wool pulgs and covered with plastic adapters.

APPENDIX C

(1). Solid Phase Extraction (SPE) with LiChrolut®. [66]

The preliminary goal of solid phase extraction with LiChrolut® is the selective extraction of the component of interest from the complex sample or much larger sample volume prior to actual analysis (e.g, HPLC, GC, TLC). As solid extraction works on the principle of liquid chromatography, this is acheived by using strong but reversible interactions between analyte and surface of the stationary phase. Typical interaction are e.g. hydrophobic (Van-der waals forces), polar (hydrogen bonding, dipole-dipole forces) or ion exchange interaction between stationary phase and matrix should not occure. It is thus meaningful to carry out appropriate sample pretreatment as this emphasises the differences in chemical properties between the substance to be analysed and matrix components so that these are then adsorbed to different extents. This can be achieved by altering the pH or the ionic strength of the sample solution. under these conditions, the analyte is enriched as a narrow zone on the stationary phase. Subsequent to a washing step, which serves to remove possibly adsorbed sample components, the actual selective elution of the analytes takes place.

A total of 4 steps are necessary for solid phase extraction :

1. Conditioning of the absorbent

conditioning means the treatment of the support with an organic solvent, e.g. methanol or acetonitrile. This causes solvation of the hydrocarbon chains of the modified support and the surface accessibility to the analyte, which is essential for reproducible adsorption of the analyte. Excess organic solvent is removed using water or a buffer solution.

2. Application of the sample

The sample solution is forced through the conditioned extraction cartridge. In this process the substance to be analysed concentrates itself as a narrow zone on the sorbent.

3. Washing step

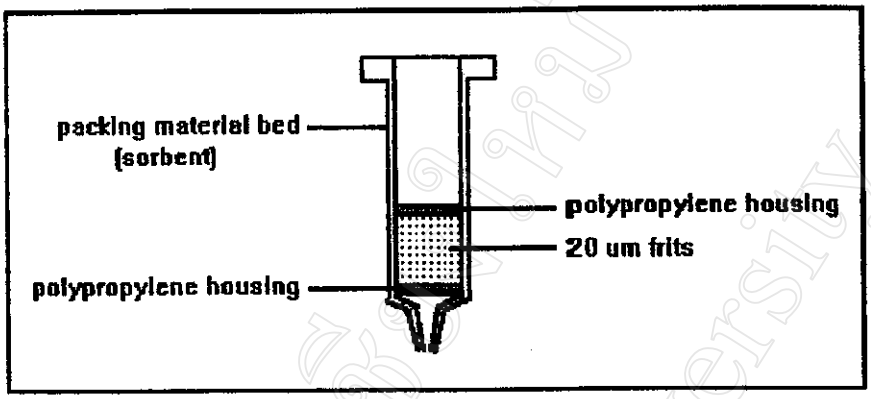
Other interfering matrix components are removed from the surface of the stationary phase with a small volume of water or buffer. A water buffer mixture containing a small quantity of methanol can also be used.

4. Elution of adsorbed analytes

In this final step of solid phase extraction, the substance to be analysed is desorbed with a suitable solvent and eluted as a narrow zone.

The range of LiChrolut® sorbents comprised non-modified, various non-polar, medium-polar and ion exchanging sorbents base on silica and a polymer. A synthetic silica with a particle size distribution of 40-63 μm or 25 μm for RP-select B and high porosity is the basic material used in the manufacture of modified stationary phases and is especially suitable for sample preparation.

Schematic of a LiChrolut® extraction column is as following :



LiChrolut® selective guide is as following :

	LiChrolut® extraction column	typical sample matrix	typical sample substance	typical elution solvent
Non-polar extraction	RP-select B RP-18 RP-18e (end-capped) CN	Aqueous; buffer solution	Aromatic ring systems, compounds with alkyl chains	Acetonitrile, methanol, ethyl acetate
Polar extraction	Si 60 CN NH_2	Hexane, oils, chlorinated hydrocarbons	Hydroxyl groups, amines, compounds with hetero atoms (S,N,O)	Methanol, 2-Propanol
Cation exchange extraction	SCX (strong)	Methanolic/ aqueous buffer with low(0.1 mol/l) ionic strength ; 2 pH units under pK value of the sample substance	Cations : amines, pyrimidines	Aqueous buffer of high ionic strength (>0.1 mol/l); 2 pH units over pK value of the sample substance
Mixed mode extraction	TSC	Body fluids	Cationic and neutral analytes	Chloroform-acetone, NH_3-ethyl acetate or NH_3-methanol
Anion exchange extraction	SAX (strong) NH_2 (weak)	Methanolic/ aqueous buffer with low(0.1 mol/l) ionic strength ; 2 pH units over pK value of the sample substance	Anions: carboxylic acids, sulphonic acids, Phosphates	Aqueous buffer of high ionic strength (>0.1 mol/l); 2 pH units under pK value of the sample substance
Non-polar extraction on a polymer phase	EN	Drinking, ground and surface water	Polar contaminants: pesticides, phenol, exprosives, anilines	Ethyl acetate, methanol, acetonitrile-methanol (1:1)

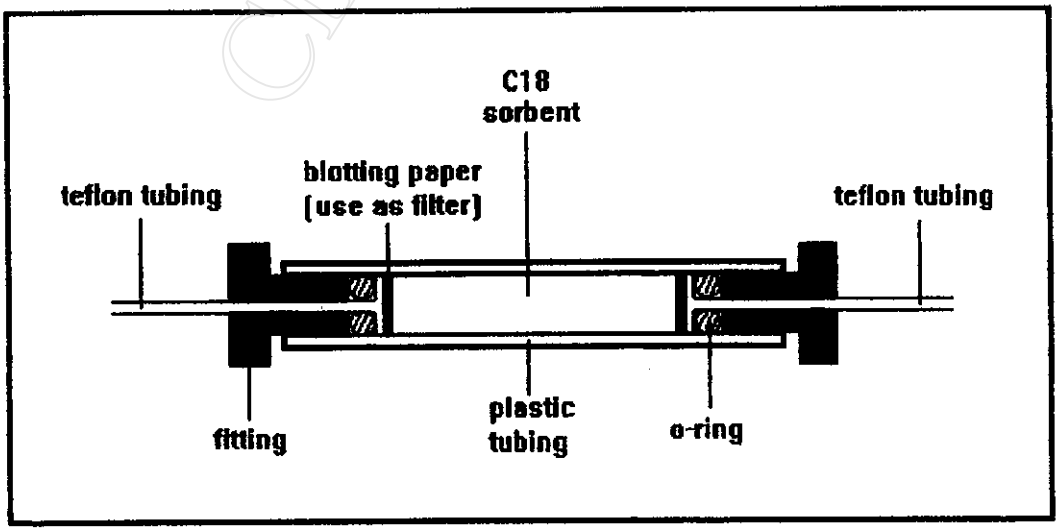
The details on the unique properties of each sorbent are as following :

Non-polar	C18	Octadecyl	$-Si-C_{18}H_{37}$
	C8	Octyl	$-Si-C_8H_{17}$
	C2	Ethyl	$-Si-C_2H_5$
	CH	Cyclohexyl	
	PH	Phenyl	
Polar	CN	Cyanopropyl	$-Si-CH_2CH_2CH_2CN$
	2OH	Diol	$-Si-CH_2CH_2CH_2OCH_2\underset{OH}{CH}-\underset{OH}{CH_2}$
	Si	Silica	$-Si-OH$
	NH_2	Aminopropyl	$-Si-CH_2CH_2CH_2NH_2$
	PSA	N-propylethylenediamine	$-Si-CH_2CH_2CH_2N\underset{H}{}CH_2CH_2NH_2$
Ion exchange	SCX	Benzenesulfonylpropyl	
	PRS	Sulfonylpropyl	$-Si-CH_2CH_2CH_2-SO_3^-$
	CBA	Carboxymethyl	$-Si-CH_2COO$
	DEA	Diethylaminopropyl	$-Si-CH_2CH_2CH_2\overset{+}{N}\underset{H}{}(CH_2CH_3)_2$
	SAX	Trimethylaminopropyl	$-Si-CH_2CH_2CH_2\overset{+}{N}(CH_3)_3$

Non-polar interactions are those that occur between the carbon-hydrogen bonds of the sorbent functional groups and the carbon-hydrogen bonds of the isolate. These forces are commonly know as "Van der Waals forces". Since most organic molecules have some non-polar structure , non-polar interactions are often used to retain isolates on sorbents offering non-polar functional groups on the surface. The most widely used sorbent for non-polar interactions is octadecyl silane bonded to the silica substrate , called C18.

(2). C18 SPE Column

The proposed C18 SPE column (LiChrolut® RP-18) used in this work was packed as depicted :

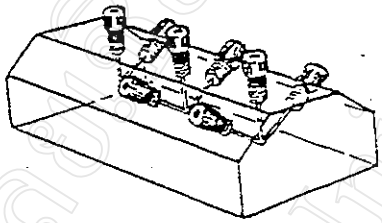


APPENDIX D

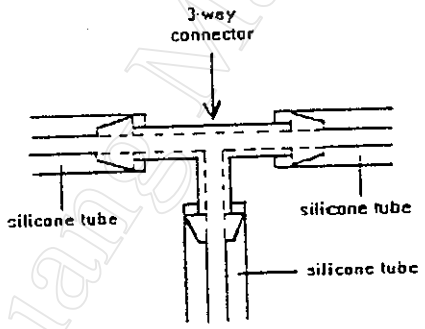
A three way connector is used to connect the different streams of the flow diagram of an FIA system. Three kinds of three way connectors are as following :

(1) Y or W-Configuration

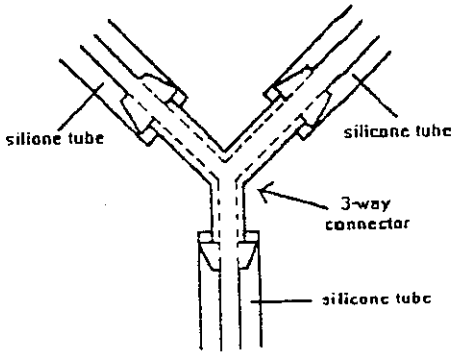
A commercialized one by Tecator (Hoganas, Sweden) is termed a " chemifold " [58].



(2). T-Configuration



(3). Y-Configuration



APPENDIX E

Determination of dead volume (or internal volume ; Sd) of the injection valve

A single line FIA manifold (Figure A.1) was used for this experiment. All of the conditions are as described in 2.3.3.8 (on Table 2.8).

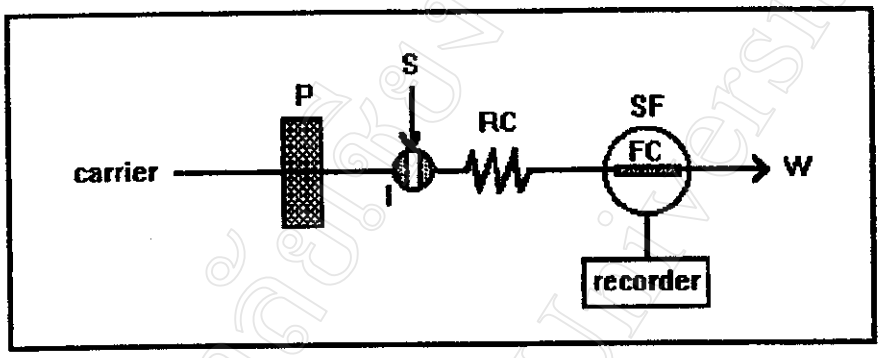


Figure E.1 Single line FIA system for determination of injection valve dead volume.

Water was used as carrier while RB (0.0003 % w/v) solution being injected. Firstly, the RB solution was allowed to flow undispersed through the carrier stream of the FIA manifold and the fluorescence intensity measured at λ_{ex} = 560 nm and λ_{em} = 580 nm. The fluorescence intensity which was observed corresponded to C^0 being the concentration of undispersed RB solutions of different volumes were then made into the carrier stream, the volumes being (Sd+100) µl, (Sd+200)µl, (Sd+300) µl, and (Sd+400) µl, where Sd = dead space in the injection valve. C^{max} (obtained from the height) was concentration of the injected RB solution during dispersion. The dispersion coefficient (D) of an FIA system is defined as following :

$$D = C^0/C^{max} + \text{const. } H^0/H^{max}$$

It has been shown by Ruzicka and Hansen [4] that : $1/D = 1-e^{-kSv}$. If the dead volume of the valve Sd, is incorporated, then :

$$1/D = 1-e^{-k(Sv+Sd)}$$

$$e^{-k(Sv+Sd)} = 1-1/D$$

$$-k(Sv+Sd) = \ln(1-1/D)$$

Hence, $$-\ln(1-1/D) = kSv+kSd$$

A plot of -ln(1-1/D) versus Sv for a range of injection volumes should yield a linear plot with a gradient of k and intercept of kSd.

Table E.1 The determination of injection valve dead volume, (H^0 = 6.20 mV).

Sv *	peak height (mV)	mean=H^{max}	D=const. H^o/H^{max}	-ln(1-1/D)
100	3.90 4.60 4.70	4.40	1.41	1.24
200	5.10 5.40 5.80	5.20	1.12	1.83
300	5.45 5.50 5.80	5.58	1.11	2.31
400	5.55 5.80 6.00	5.78	1.06	2.87

* Sv = sample volume (external injection loop)

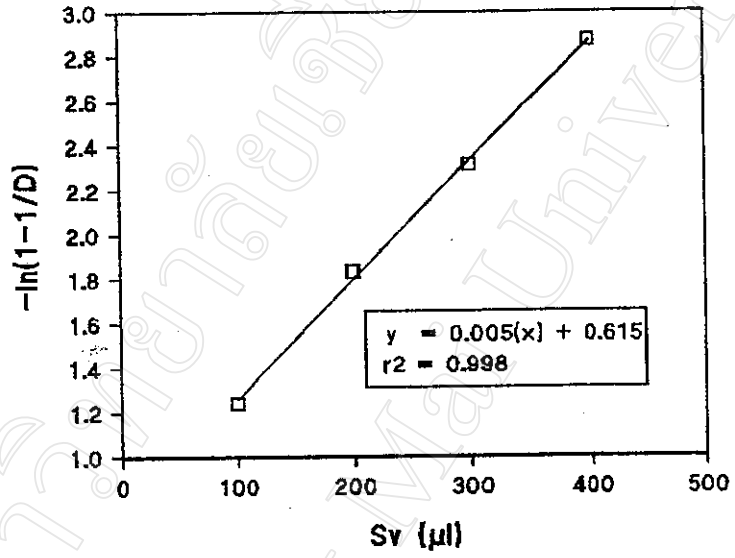


Figure E.2 The plot for determination of injection valve dead volume.

The results are :

Slope = k = 0.005

intercept = kSd = 0.615

$0.005 \times Sd = 0.615$

$Sd = 0.615 + 0.005$,

Hence, $Sd = 123$

APPENDIX F

The cadmium column efficiency can be determined by [60] :

(1). Concentration and Peak Height Ratio

$$(C_{NO3^-} / C_{NO2^-}) \times 1.35 \times 100 = (H_{NO3^-}/H_{NO2^-}) \times 1.35 \times 100 = E$$

when ; C_{NO3^-} , H_{NO3^-} = concentration and peak height of NO_3^- standard.
C_{NO2^-} , H_{NO2^-} = concentration and peak height of NO_2^- standard.
E = % efficiency

(2). Slope Ratio

$$(S_{NO3^-}/S_{NO2^-}) \times 1.35 \times 100 = E$$

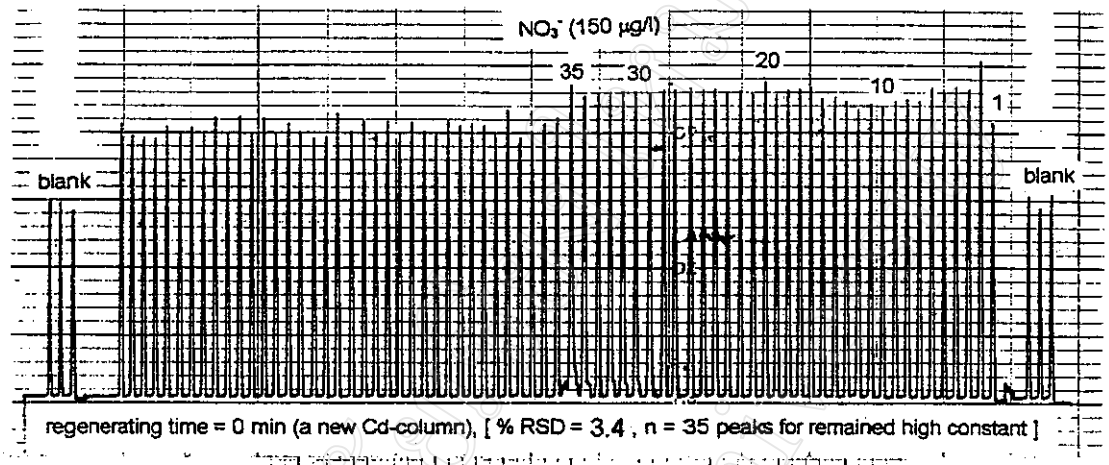
where ; S_{NO3^-} = slope of NO_3^- calibration graph
S_{NO2^-} = slope of NO_2^- calibration graph
E = % efficiency
1.35 = factor (M.W. of NO_3^- / M.W. of NO_2^- = 62/46)

For example in 2.4.4.1 (Table 2.26) ;

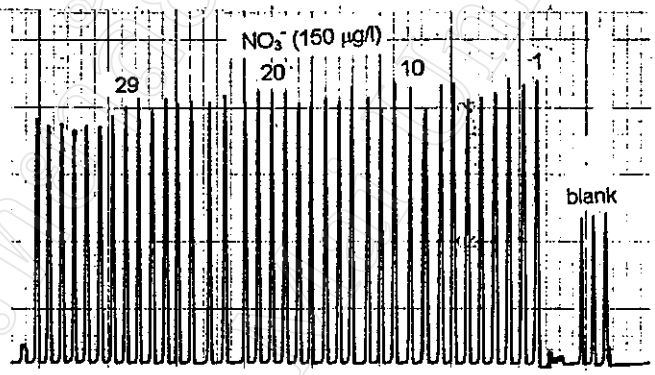
$$E = (H_{NO3^-}/H_{NO2^-}) \times 1.35 \times 100$$
$$= (0.7/1.1) \times 1.35 \times 100$$
$$= 79\ \%$$

APPENDIX G

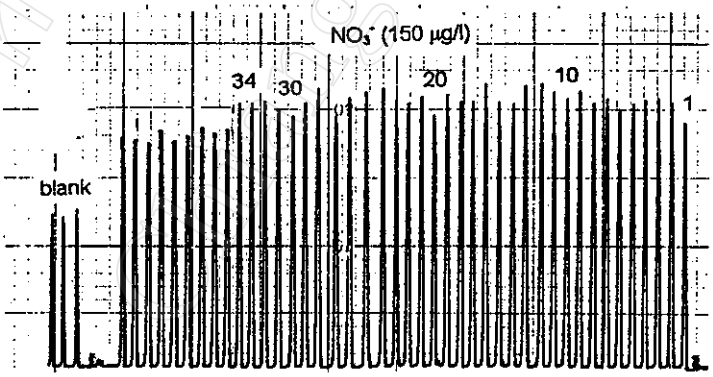
(1). FIA signals for the effect of regenerating time (in 2.4.4.4 of Table 2.29).



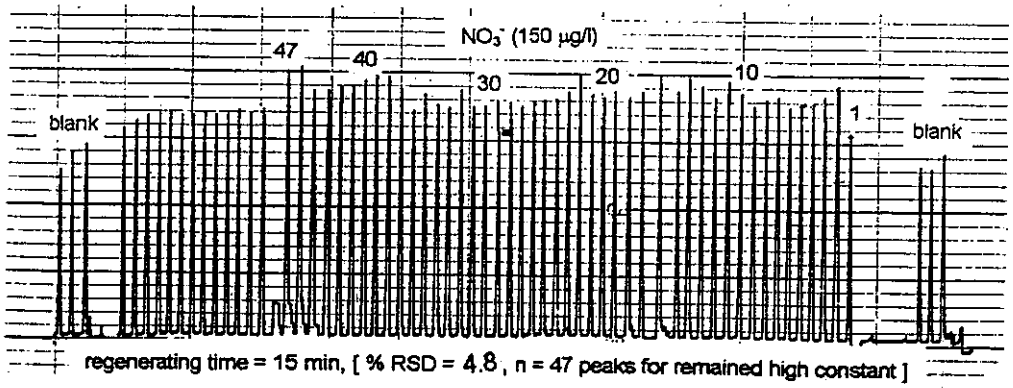
regenerating time = 0 min (a new Cd-column), [% RSD = 3.4 , n = 35 peaks for remained high constant]



regenerating time = 5 min, [% RSD = 3.6 , n = 29 peaks for remained high constant]

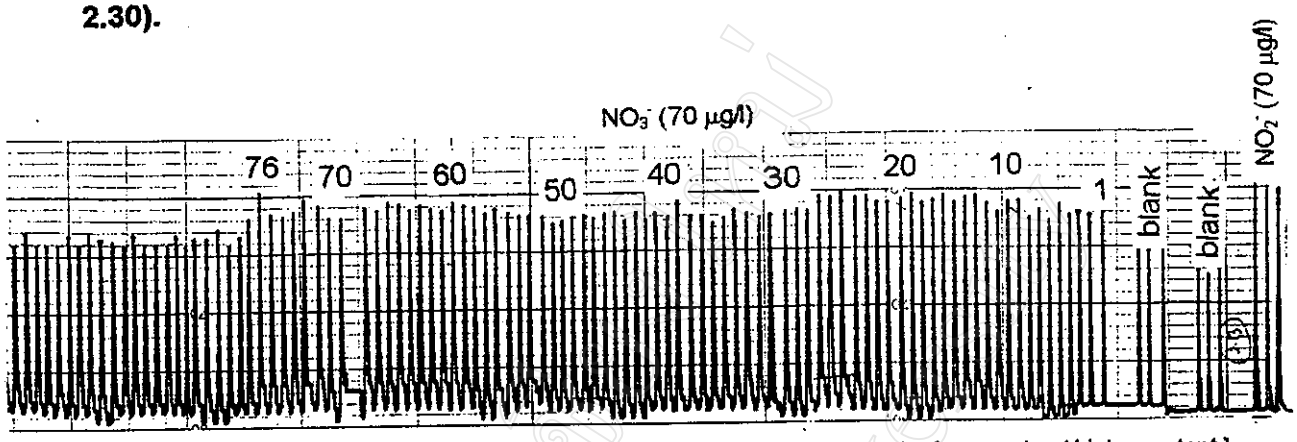


regenerating time = 10 min, [% RSD = 3.5 , n = 34 peaks for remained high constant]

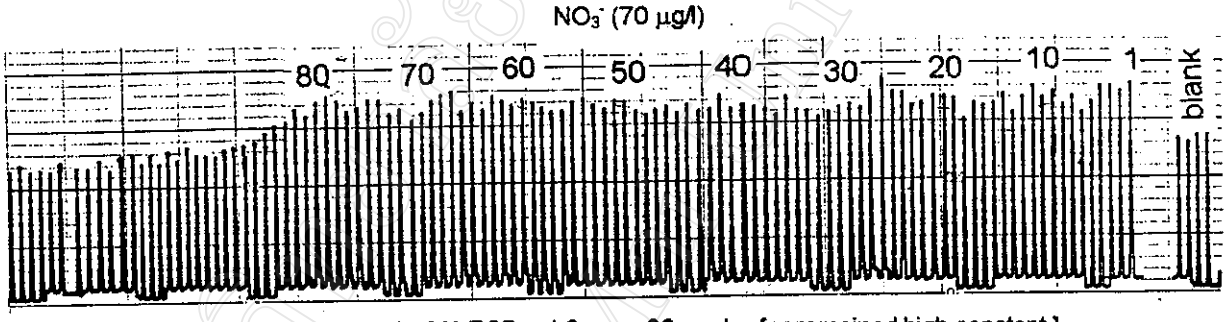


regenerating time = 15 min, [% RSD = 4.8 , n = 47 peaks for remained high constant]

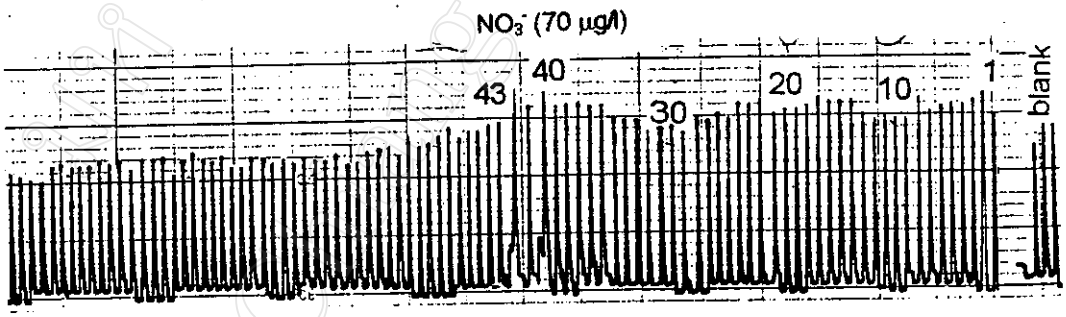
(2). FIA signals for the effect of regenerating efficiency (in 2.4.4.4 of Table 2.30).



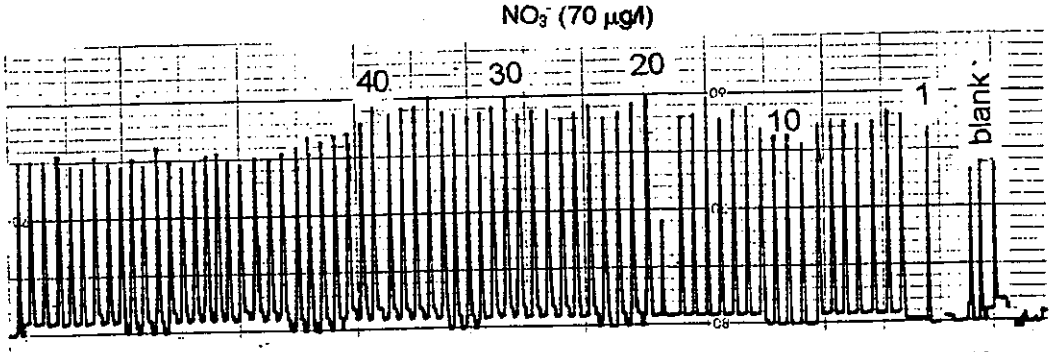
regenerating round = 0 min (a new Cd-column), [% RSD = 4.5 , n = 76 peaks for remained high constant]



regenerating round = 1 min, [% RSD = 4.8 , n = 80 peaks for remained high constant]



regenerating round = 2 min, [% RSD = 7.7 , n = 43 peaks for remained high constant]



regenerating round = 3 min, [% RSD = 6.8 , n = 40 peaks for remained high constant]

APPENDIX H

The calculation of molar absorptivity (ε) is as following :

The fundamental equation of the Beer-Lambert law can be stated as :

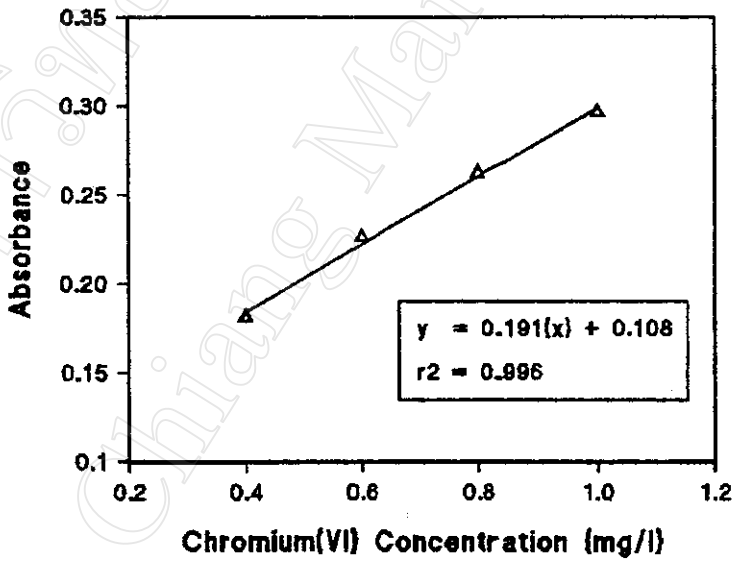
$$A = \varepsilon bc$$

where, A = absorbance
ε = molar absorptivity or molar absorption coefficient (cm^{-1} $mol^{-1}l$ or cm^{-1} M^{-1})
b = cell path length = 1 cm
c = concentration (mg/l)

or $\varepsilon = A/bc$ (cm^{-1} $mg^{-1}l$)
$= slope/b$ (cm^{-1} $mg^{-1}l$)

Hence, $\varepsilon = slope \times (M.W.\ of\ the\ species) \times 10^3$ (cm^{-1} M^{-1})

For example in Figure 2.26 :



$\varepsilon = 0.191 \times (M.W.\ of\ Cr^{6+}\ as\ K_2Cr_2O_7\ used) \times 10^3$
$= 0.191 \times 104 \times 10^3$
$= 2.0 \times 10^4\ cm^{-1}\ M^{-1}$

APPENDIX I

Evaluation for chromium concentration (in 2.5.4.11) :

(1). Chromium(VI)

Cr(VI) concentration can be directly determined from Cr(VI) versus H_2O stream as equation :

$$y_1 = a_1x_1 + b_1 \quad \text{(i)}$$

Hence, Cr(VI) concentration (x1) = $(y_1-b_1)/a_1$

(2). Chromium(III)

From the equation of Cr(VI) versus R2 reagent stream is ;

$$y_2 = a_2x_2 + b_2 \quad \text{(ii)}$$

From the equation of Cr(III) versus Ce(IV) reagent stream (R2) is ;

$$y_3 = a_3x_3 + b_3 \quad \text{(iii)}$$

From the reaction of (I), (ii) and (iii), Cr(III) can be determined as following :

$$H_T = (H_{Cr6+.H2O} \times F_1) + [(a_1x_1 + b_1) \times F_2]$$

$$x = \{[(H_T - (H_{Cr6+.H2O} \times F_1)) / F_2] - b_1\} / a_1 \quad \text{(iv)}$$

When, H_T = the total Cr(VI) peak height when versus Ce(IV) reagent stream (R2)
$H_{Cr6+.H2O}$ = the Cr(VI) peak height when versus H_2O stream
F_1 = slope a_2/slope a_1
F_2 = slope a_3/slope a_1

Hence, Cr(III) concentration = $X \times F_1$ (v)

For example of sample No.6 in Table 2.6.1 ;

$y_1 = a_1x_1 + b_1 = 459.12(x) + 2.63$
$y_2 = a_2x_2 + b_2 = 378.32(x) + 37.37$
$y_3 = a_3x_3 + b_3 = 314.17(x) + 46.46$
$F_1 = 378.32 / 459.12 = 0.82$
$F_2 = 314.17 / 459.12 = 0.68$

Hence, $x_1 = (144.8 - 2.63) / 459.12 = 0.3$ mg Cr^{6+} /l

and $x = \{ [(289.7 - (144.8 \times 0.82)) / 0.68] - 2.63 \} / 459.12 = 0.5$
Hence, $X = 0.5 \times 0.82 = 0.4$ mg Cr^{3+}/l

APPENDIX J

The detection limit was described by Miller and Miller [59] as following :

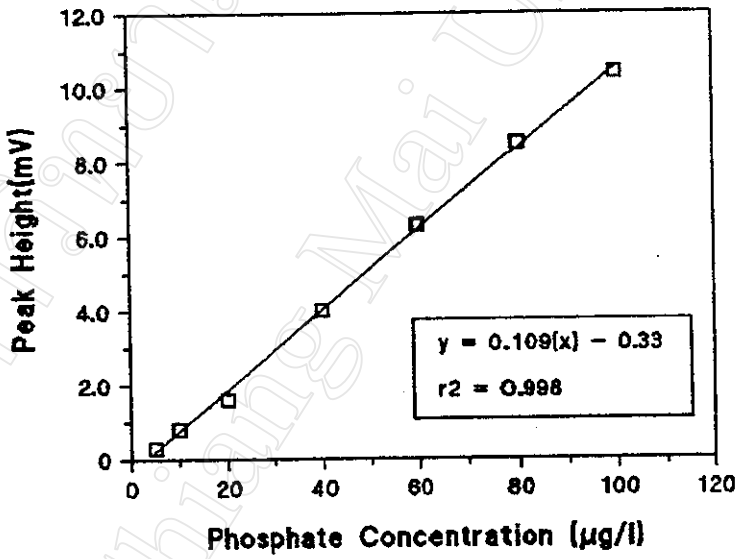
(1). Definition

A commonly used definition in the literature of analytical chemistry is that the detection limit is "the analyte concentration giving a signal equal to the blank signal, y_B, plus three standard deviations of the blank, S_B".

$$y = y_B + 3S_B$$

(2). Calculation

For example, the detection limit calculation of phosphate determination in the range of 5-100 μg PO_4^{3-} /l (from the data in 2.3.3.9).



$$y = y_B + 3S_B = -0.33 + 3S_B \quad \text{(i)}$$

From a regression line equation ;

$$y = a(x) + b = 0.109(x) - 0.33 \quad \text{(ii)}$$

when, a,b = slope and intercept of regression line
r = correlation coefficient

The datas are presented in a table as following ;

n	x_i	y_i*	y_i^ **	y_i-y_i^	$(y_i-y_i^{\wedge})^2$
1	0	0	-0.33	0.33	0.109
2	5	0.3	0.2	0.1	0.010
3	10	0.8	0.7	0.1	0.010
4	20	1.6	1.8	-0.2	0.040
5	40	4.0	4.0	0.0	0.000
6	60	6.3	6.2	0.1	0.010
7	80	8.5	8.4	0.1	0.010
8	100	10.4	10.5	-0.1	0.010
					0.199

* y_i = the observed vaule correspond to the x_i

** y_i^ = the point on the calculated regression line corresponding to the individual x_i values or $y_i^{\wedge} = 0.109(x_i) - 0.33$.

The statistic S_B calculates by :

$$S_B = S_{y/x} = [(\Sigma_i(y_i-y_i^{\wedge})^2)/(n-2)]^{1/2}$$
$$= (0.199/6)^{1/2} = 0.182$$

The $S_B = 0.182$ is inserted into the equation (i) :

$$y = -0.33 + 3(0.182) = 0.216$$

From the equation (ii) :

$$0.216 = 0.109(x) - 0.33$$
$$x = 5.0$$

Hence, the detection limit of phosphate determination is 5 μg PO_4^{3-}/l.

VITA

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Paper Presented	- Orawan Tue-Ngeun, Kate Grudpan and Pimol Rienvatana, "Flow Injection Analysis of Nitrite and Nitrate* ", 21st Congress on Science and Technogy of Thailand, 1995, G-09. * A part of research supported by NSTDA. - Orawan Tue-Ngeun, Kate Grudpan and Ponlayuth Sooksamiti, "Flow Injection Determination of Hardness of Water Samples* ",22nd Congress on Science and Technology of Thailand, 1996, A-75. * A part of research supported by NSTDA.