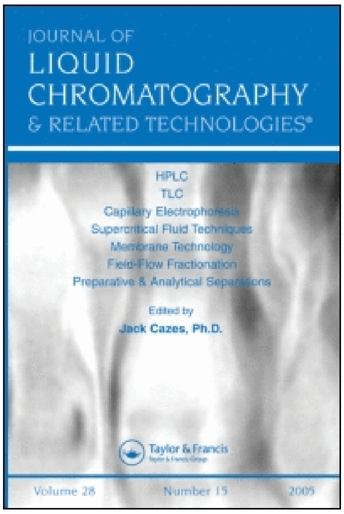

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Srivastava, S. P. and Reena(1985) 'TLC Separation of Barbiturates on Impregnated Plates', Journal of Liquid Chromatography & Related Technologies, 8: 7, 1265 — 1278
To link to this Article: DOI: 10.1080/01483918508067142
URL: http://dx.doi.org/10.1080/01483918508067142

JOURNAL OF LIQUID CHROMATOGRAPHY, 8(7), 1265-1278 (1985)

TLC SEPARATION OF BARBITURATES ON IMPREGNATED PLATES

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ABSTRACT

Barbiturates are of immense physiological importance and because of their adverse reactions leading even to death by poisoning, separation and identification of barbiturates in trace amounts is of great importance. In this work impregnation technique has been employed for working out better separation scheme for barbiturates on TLC plates. The different barbiturates studied were - phenobarbital, barbituric acid, 1:3 dimethyl barbituric acid, disodium barbitone, diethyl barbituric acid, thio barbituric acid and pentobarbital. All these seven barbiturates are satisfactory separated on 2 % copper sulphate as well as 2 % ethylenediamine impregnated plates by using the solvent system benzene-methanol (40:3) and benzene-acetone (40:12) respectively. The correlation between chromatographic behaviour and the weak interaction between the ethylenediamine and different barbiturates due to hydrogen bonding has been attempted.

INTRODUCTION

Upto 1971 Kirchner (1) has reviewed the work on TLC separation of barbiturates. Chmel in 1978 (2) separated eleven barbiturates by TLC on silufol sheets using

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chloroform-dimethyl ketone - aq. ammonia (50:50:2) as mobile phase and used $HgSO_4$ - diphenyl carbazone as developer, while Abu-Zittah (3) tried alkaline silica layers and a Hg(II) spray reagent. Bress and coworkers (4) separated seven barbiturates by reverse phase TLC on silica gel plates coated with mineral oil and using (80:20:2) H_2O - MeOH - NH_4OH as the solvent system. Recently Ekiert et al (5) separated 20 barbituric acid derivatives with four types of substitution by thin layer non polar adsorbents.

It is thus evident that most of the work on the TLC separation of barbiturates has been carried out on plain silica G, treated or untreated with alkali. No work seems to have been done on the TLC behaviour of barbiturates on silica gel plates impregnated with metal salts. Hence the present study on the use of metal salts as well as aliphatic amines as impregnants for the TLC separation of barbiturates was undertaken.

EXPERIMENTAL

The TLC plates (thickness 0.5 mm) were prepared by means of a stahl type applicator by spreading a slurry of 50 g silica gel G (SISCO made) and varying amounts of impregnants in 100 ml of distilled water. The plates were dried for 24 hours at a constant temp. of 60 $\pm$ 1^{o}c.

Various barbiturates were obtained through the courtesy of I.D.P.L. Hyderabad, May and Baker Pvt. Ltd. (India), and Department of Physiology, Indian medical Institute, B.H.U. and were used after recrystallization. A 0.2 % solution of the compounds in ethanol was applied to the plates by means of a micro pipette manufactured by Clay Admas (U.S.A.).

Detection

The plates were visualized by spraying with 1 % aqueous solution of mercurous nitrate. Blackish grey spots on white background, appeared.

Copper salts impregnation

The various impregnants tried were-copper sulphate, copper acetate and copper chloride. The results obtained on plates impregnated with different copper salts are given in Table 1.

Amine impregnation

In case of amine impregnation methyl amine, ethylenediamine, diethylenetriamine, triethyl amine used as impregnants. The results obtained on plates impregnated with different these aliphatic amines are given in Table 2.

In case of copper salt impregnation the best solvent system was found to be benzene: methanol (40:8). In case of amine impregnation the best solvent system was found to be benzene:acetone (40:12).

RESULT AND DISCUSSION

Comparison of the data for different impregnates in this Table (Table 1) shows that by using copper salt as impregnant the hR_f value for almost all barbiturates are decreased than that obtained on plain silica gel plate. Further it is seen that best results are obtained on 0.2 % copper sulphate impregnated plate on which the spots are not only well separated but also the size of the spots is minimal. There is no tailing on 0.2 % copper sulphate impregnated plate. Besides this the hR_f value of all barbiturates do not change when they are present in a mixture also.

In order to find out whether any improvement in the separation of barbiturates could be made by varying the concentration of copper sulphate as impregnants, three different concentrations of copper sulphate were employed. The results of these runs are giving in (Table 3) that on 0.1 % and 0.25 % copper sulphate impregnation slight or medium tailing was observed and the hR_f values are also close to each other. However, on 0.2 / copper sulphate impregnation the spots showed no tailing and all the seven barbiturates were separated.

A perusal of the hR_f values in Table 3 for different barbiturates showed that tailing was observed to a lesser or greater for different barbiturates on plain silica gel layer and on amine impregnated layers except in case of 2.0 % ethylene diamine impregnated plate. All the barbiturates are well separated on 2.0 % ethylene diamine impregnated plate. These results showed that ethylenediamine with the present solvent system, was proably the most suitable impregnant among those tried.

TABLE 1

Solvent system - Benzene-methanol (40:8)

hR_f

Barbiturate	Plain silica gel	Copper sulphate 0.2 %	Copper chloride 0.2 %	Copper acetate 0.2 %	Detection limit (µg) on 2 % copper sulphate
Phenobarbital	81	57	50	48	.97
Barbituric acid	17mT	16	15	18	.44
1:3 dimethyl barbituric acid	20	23	20mT	19	.19
Disodium barbitone	40	36	35	32	1.0
Diethyl barbituric acid	40	40	36	33	.77
Thiobarbituric acid	18	12	7sT	14sT	.19
Pentobarbital	93sT	61	65mT	55mT	.78

mT - medium tailing

sT - slight tailing

TABLE 2

hR_f on silica gel layer impregnated with various aliphatic amines

Solvent system - benzene-acetone (40:12)

hR_f

Barbiturate	Silica gel plain	Ethylene diamine 2.0 %	Methyl amine 2.0 %	Diethylene triamine 2.0 %	Trimethyl amine 2.0 %
Phenobarbital	59	31	52	30	52
Barbituric acid	9	6	16	5	12
1:3 dimethyl barbituric acid	22LT	3	20	6	17mT
Disod. barbitone	52mT	40	30mT	35sT	30sT
Diethyl barbituric acid	45	35	32	32	31
Thiobarbituric acid	6sT	0	5	3sT	2
Pentobarbital	61sT	48	50sT	42	52

LT - large tailing

mT - medium tailing

sT - small tailing

TABLE 3

Solvent system - Benzene-methanol (40:8)

hR_f

	Barbiturate	Plain silica gel	Concentration of copper sulphate used as impregnant		
			0.1 %	0.2 %	0.25 %
1.	Phenobarbital	81	74	57	76mT
2.	Barbituric acid	17mT	20	16	15
3.	1:3 dimethyl barbituric acid	20	28	23	22
4.	Disodium barbitone	40	43	36	36
5.	Diethyl barbituric acid	40	45	40	42
6.	Thiobarbituric acid	18	13sT	12	6mT
7.	Pentobarbital	93sT	81	61	77

sT - slight tailing

mT - medium tailing

Rate of development 10 cm in 30 min.

In order to decide optimum concentration of ethylenediamine as impregnant, it was considered necessary to carry out the chromatographic runs of these barbiturates on silica gel layers impregnated with different concentration of ethylenediamine as shown in Table 4.

An examination of the hR_f values in Table 4 showed that on 2 % ethylene diamine impregnated layers no tailing was observed. It can be inferred that maximum number of barbiturates got separated when ethylendiamine concentration employed was 2.0 %.

TABLE 4

Effect of different concentration of ethylenediamine on hR_f

Solvent system - benzene-acetone (40:12)

hR_f

	Barbiturate	Silica gel plain	% Ethylenediamine			+ Detection limit (µg)
			1	1.5	2.0	
1.	Phenobarbital	59	45	40	31	1.30
2.	Barbituric acid	9	3	2	6	.5
3.	1:3 dimethyl barbituric acid	22LT	16LT	10mT	3	1.48
4.	Disodium barbitone	53mT	44	42	40	1.9
5.	Diethyl barbituric acid	45	39	38	35	.9
6.	Thiobarbituric acid	6sT	2	1	1	.25
7.	Pentobarbital	61sT	52sT	50sT	48	1.7

LT - large tailing

mT - medium tailing

sT - slight tailing

+on 2.0 % ethylenediamine impregnated plate

On 1% and 1.5 % ethlenediamine impregnation tailing was observed and most of the hR_f values are close to each other.

Thus for the separation of barbiturates the most suitable solvent system was benzene: acetone (40:12) on 2.0 % ethylenediamine impregnated layer.

The separation of barbiturates on ethylenediamine impregnated plate may be due to hydrogen bond formation between the amine and barbiturates.

To verify this possibility and to establish any correlations between the chromatographic behaviour of barbiturates on ethylenediamine impregnated plates and the hydrogen bonding there in equilibrium studies were carried out on hydrogen bond formation between barbiturates and aliphatic amines, using the spectroscopic method of Baba and Suzuki (6).

The increase in absorbance or the shifted, band with increase in donor concentration was employed for the calculation of equilibrium constants for hydrogen bond formation. The equilibrium constnats were calculated from the following equation given by Baba and Suzuki (6):

$$\frac{1}{\varepsilon - \varepsilon_f} = \frac{1}{k(\varepsilon_b - \varepsilon_f)} \cdot \frac{1}{C} + \frac{1}{(\varepsilon_b - \varepsilon_f)}$$

where

ε_f molar extinction coefficient of the non hydrogen bonded or free molecule.

ε_b molar extinction coefficient of the hydrogen bonded molecule.

ε molar extinction coefficient as observed in solution. in which the concentration of electron donor is c.

By keeping the concentration of electron acceptor and the cell length constant, throughout a set of spectra, the ε terms in Baba and Suzuki's equation can be replaced with the corresponding absorbences (A) and the equilibrium constant can be evaluated from a plot of $1/(A - A_f)$ versus 1/C.

All of the studies were confined to the benzenoid absorption region in the range 210-250 mm and a constant temperature of 30 $\pm$ 0.5 ^{o}C. The solutions of ethylenediamine and barbiturates taken were prepared in methanol (Spectroscopic grade).

A typical spectrum of the system ethylenediamine-phenobarbital is shown in (Fig. 1). This shows the presence of isobestic points which indicate the existence of a hydrogen bonded complex between the hydrogen of the -NH group of phenobarbital and the lone pair of electrons of N atoms of the amine. Similar behaviour was observed with four other barbiturates.

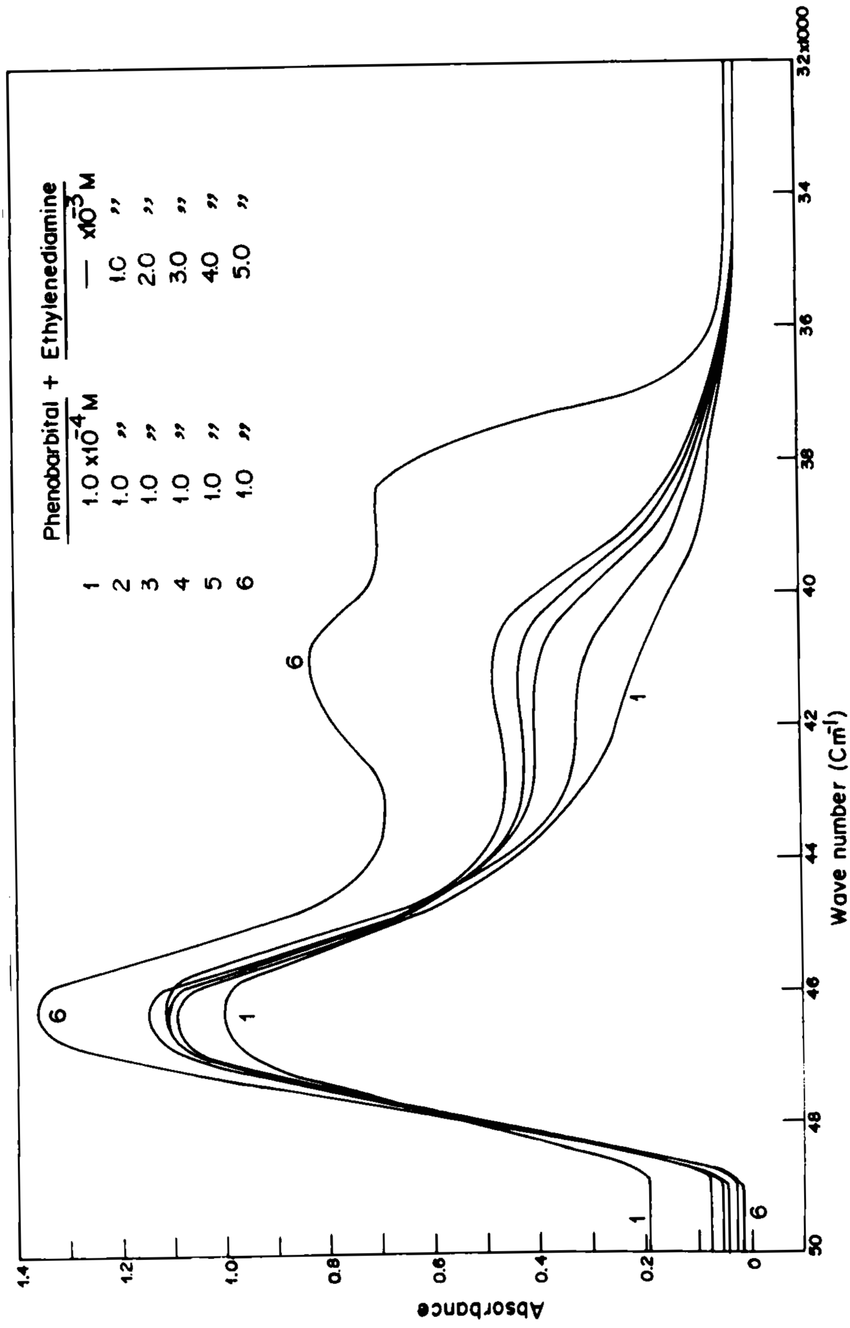

FIGURE 1. Absorption spectra of phenobarbital-ethylenediamine system.

TABLE 5

Calculation of $(\frac{1}{A-A_f})$ and $\frac{1}{C}$ for different barbiturates with ethylenediamine

Sl. No.	A	A_f	$(A-A_f)$	C	$1/(A-A_f)$	1/C
Phenobarbital = $1x10^{-4}$M						
1.	.48	.45	.03	10^{-3}	33.3	1000
2.	.49	.45	.04	$2x10^{-3}$	25	500
3.	.49	.45	.04	$3x10^{-3}$	25	333.3
4.	.52	.45	.07	$4x10^{-3}$	14.3	250
5.	.72	.45	.27	$5x10^{-3}$	3.8	200
1:3 dimethyl barbituric acid = 2.10^{-4}M						
1.	18.7	15.5	3.2	$1x10^{-3}$	3.12	1000
2.	20.0	15.5	4.5	$2x10^{-3}$	2.12	500
3.	21.3	15.5	5.8	$3x10^{-3}$	1.72	333.1
4.	40.0	15.5	24.5	$4x10^{-3}$	.41	250
Diethyl barbituric acid = $2x10^{-4}$M						
1.	.38	.23	.15	$2x10^{-3}$	6.67	500
2.	.42	.23	.19	$3x10^{-3}$	5.26	333.3
3.	.45	.23	.22	$4x10^{-3}$	4.54	250
4.	.53	.23	.30	$5x10^{-3}$	3.33	200
Thio barbituric acid = $1x10^{-4}$M						
1.	14.0	13.0	1.0	$1x10^{-3}$	10.0	1000
2.	14.8	13.0	1.8	$2x10^{-3}$	5.5	500
3.	15.8	13.0	2.8	$3x10^{-3}$	3.33	333.3
4.	27.8	13.0	14.8	$4x10^{-3}$	.7	250
Pentobarbital = $2x10^{-4}$M						
1.	.355	.25	.105	$2x10^{-3}$	10	500
2.	.38	.25	.13	$3x10^{-3}$	7.7	333.3
3.	.41	.25	.16	$4x10^{-3}$	6.25	250
4.	.41	.25	.16	$5x10^{-3}$	6.25	200
5.	.44	.25	.19	$6x10^{-3}$	5.3	167

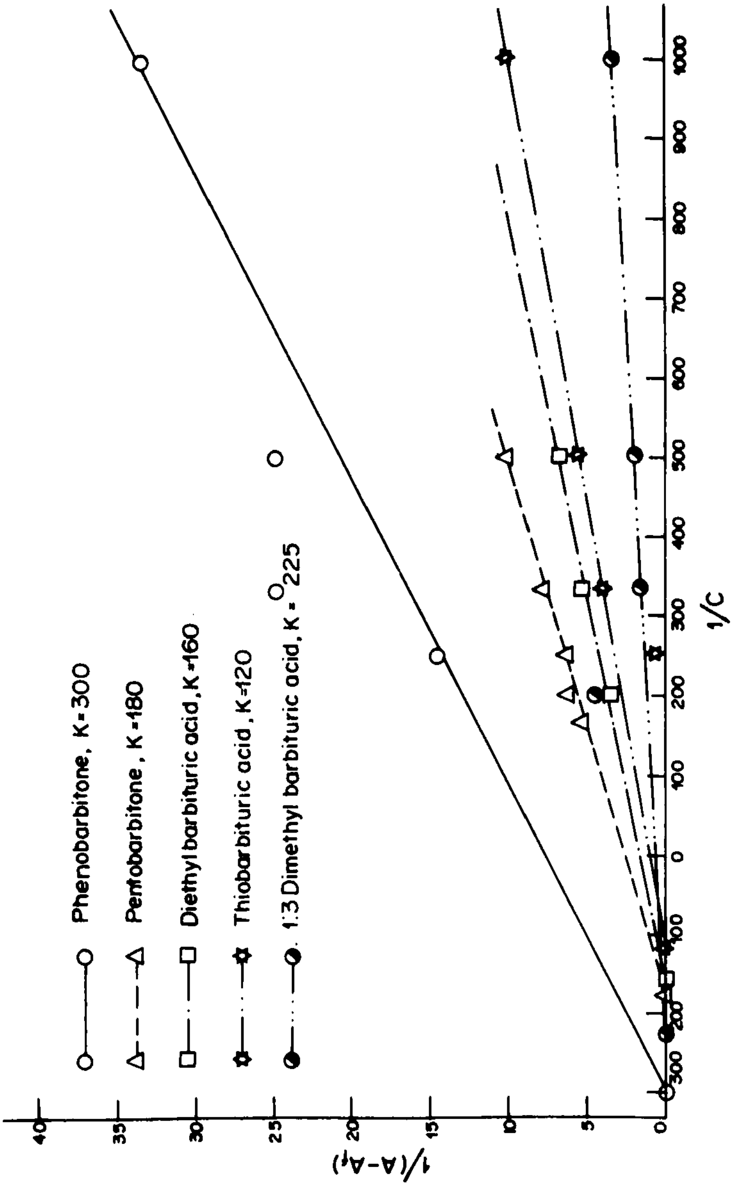

FIGURE 2. Plot between $1/(A-A_f)$ and 1/C.

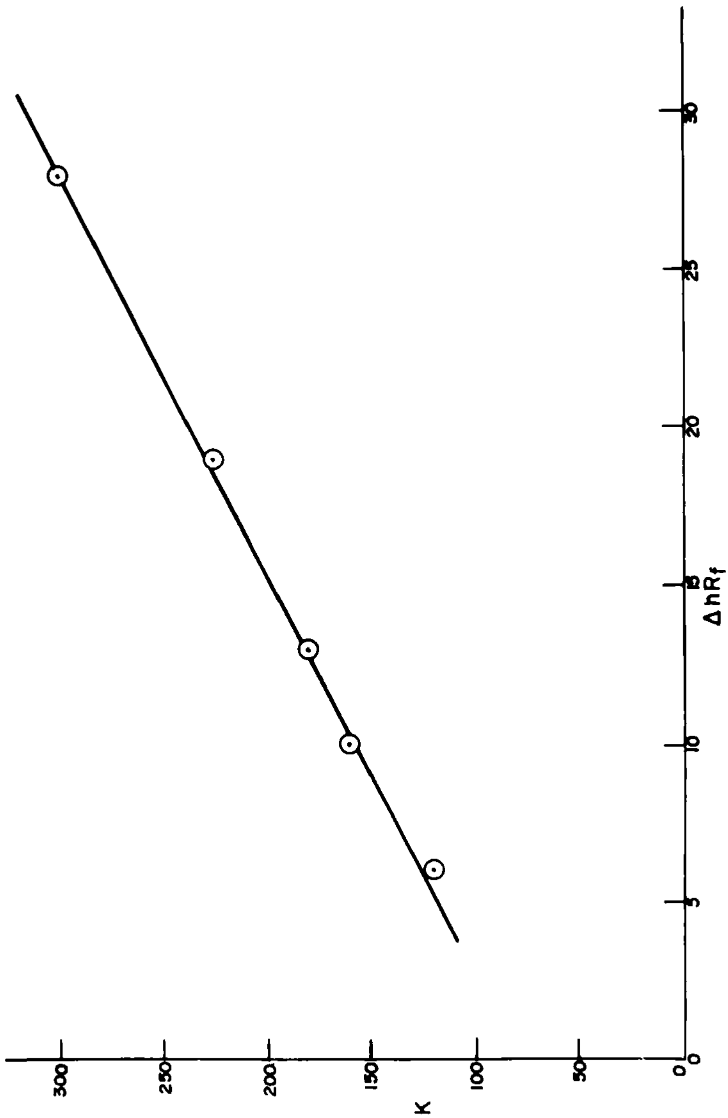

FIGURE 3. Relationship between K and hR_f.

TABLE 6

Barbiturates	Equilibrium constant of adducts K	Adsorbent		
		Silica gel hR_f	Silica gel + ethylene diamine hR_f	ΔhR_f
Phenobarbital	300	59	31	28
1:3 dimethyl barbituric acid	225	22LT	3	19
Diethyl barbituric acid	160	45	35	10
Thiobarbituric acid	120	6sT	0	6
Pentobarbital	180	61sT	48	13

LT - large tailing

sT - slight tailing

The value of A and A/f, read at 44 x 1000 cm^{-1} in each case and these values for each barbiturate are tabulated in Table 5.

For calculating the equilibrium constant for hydrogen bond formation in each instance, $1/(A - A_f)$ was plotted against $1/C$ (Fig 2). By extrapolation of the value of the equilibrium constant (k) of hydrogen bond formation for each barbiturate was evaluated (Table 5 and 6).

To find out the relationship between k values of hydrogen bond formation and ΔhR_f where, ΔhR_f denote the difference, in the hR_f between (those on plain silica gel plate and on the impregnated plate) the values of ΔhR_f are calculated and are given in Table 6. The plot of kvs. ΔhR_f is depicted in (Fig.3). It is seen that hR_f for any particular barbiturate is lower on the impregnated plate than on the plain silica gel plate and further, hR_f increases linerarly with k. It, therefore, suggests that among the various forces responsible for TLC separation of barbiturates, on ethylenediamine impregnated layers, hydrogen bond formation plays a prominant role.

REFERENCES

1. Kirchner, J.G., Thin layer Chromatography, Wiley Inter-Science publication, John Wiley and Sons, New York, 1978.

2. Chemel, K., Cesk. Farm. 22(10), 456-459, 1973.

3. Abu - Eittah, R., Osman, A., E l - Behare, A., Analyst. 103(1231), 1083-1087, 1978.

4. Bress, W., Ziminski, K., Long, W., manning, T., Lukarty, L., Clin. Toxicol. 16(2), 219-221, 1980.

5. Ekiert, L., Grodzinska - Zachwieja Z., Bojarski, J., Chromatographia. 13(8), 472-478, 1980.

6. Baba, H. and Suzuki, S., J. Chem. Physics, 35(3), 1118-1127, 1961.