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CHARACTERISTICS OF LITHIUM IODIDE-CONTAINING POLY(ETHYLENE GLYCOL) AS A GAS CHROMATOGRAPHIC STATIONARY PHASE, AND ITS APPLICATION TO ANALYSIS OF AMIDIC DRUGS

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SUMMARY

The characteristics of lithium iodide-containing poly(ethylene glycol) as a gas chromatographic stationary phase have been evaluated in terms of partial free energy of transfer ($\Delta\overline{G}_t^\circ$) from poly(ethylene glycol) to the lithium iodide–poly(ethylene glycol) system for a variaty of amides (*n*-fatty acid amides, lactams, benzamides, anilides, nicotinamides, isonicotinamides, barbiturates, pyrazolones) and several amines. The changes in relative retention and resolution of two solute peaks caused by the addition of lithium iodide to poly(ethylene glycol) are correlated with the difference in their $\Delta\overline{G}_t^\circ$ values. The application to the specific separation of some amidic drugs is demonstrated.

INTRODUCTION

Following the use of silver nitrate-containing poly(ethylene glycol) (PEG) as a stationary phase[1], various inorganic salts have been mixed with a normal gas chromatographic stationary liquid in an effort to achieve specific separation of interesting species[2], for example the use of the specific interaction between acid amides and alkali-metal halides for the selective retardation of the elution of acid amides[3]. Our previous paper[3] showed that the partition coefficients of *n*-fatty acid amides on a stationary column containing alkali-metal halide differ from those on an analogous, but salt-free, column, and that the difference is dependent on the nature and concentration of the alkali-metal halide, the chemical structure of amide, and the polarity of the stationary liquid. Among the alkali-metal halides examined, lithium iodide showed the strongest interaction with acid amides.

Many drugs have an amide group in the molecule. This prompted us to expand the investigation to include the various types of amides and to attempt the specific separation of amidic drugs.

EXPERIMENTAL

Gas chromatography

The apparatus used was a Shimadzu GC-3BF gas chromatograph equipped with a flame ionization detector. The stationary phases, packed in 80 cm × 3 mm I.D. spiral glass tubes, were 2% PEG 20M and 2% LiI–PEG 20 M, both coated on Chromosorb W AW DMCS (60–80 mesh). The LiI–PEG 20M stationary phase contained 1 molal LiI in PEG 20M. The oven temperature was maintained at 180°, 200°, and 220°, the injection port temperature at 250°. Helium was used as carrier gas at a flow-rate of 50 ml/min.

Reagents and materials

All the sample materials were obtained from commercial sources. Most were supplied pure, and a few were obtained by extraction from drug preparations. Lithium iodide of analytical grade and PEG 20M were purchased from Nakarai Chemicals (Kyoto, Japan) and used after thorough drying over phosphorus pentoxide.

The partition coefficient, K, and the partial free energy of transfer from PEG to LiI–PEG, $\Delta\overline{G_t^\circ}$, were calculated from the following equations:

$$K = V_r'/V_L \tag{1}$$

$$\Delta\overline{G_t^\circ} = RT_c \ln (K/K'f) \tag{2}$$

where V_r' is the adjusted retention volume, V_L is the volume of the stationary phase, R is the gas constant, T_c is the column temperature, K' is the partition coefficient of a solute on the LiI–PEG column, and f is a quantity peculiar to the stationary phase. In the present experiment, where the molality of LiI is unity and the column temperature is between 180° and 220°, f is *ca.* 1.005. These equations have been described in detail in the previous paper[3].

RESULTS AND DISCUSSION

It is found from eqn. 2 that the retention of solute B relative to solute A on the salt-free PEG column ($\alpha_{B/A}$) and on the salt-containing PEG column ($\alpha'_{B/A}$) are related to the difference in their values of transfer free energy[3] by the equation

$$\alpha'_{B/A} = \alpha_{B/A} \exp\{\Delta(\Delta\overline{G_t^\circ})_{B/A}/RT_c\} \tag{3}$$

where $\Delta(\Delta\overline{G_t^\circ})_{B/A} = \Delta\overline{G_{t,A}^\circ} - \Delta\overline{G_{t,B}^\circ}$. If $\Delta(\Delta\overline{G_t^\circ})_{B/A} = 0.09$ kcal/mole at 200°, the relative retention value increases by 10%. If $\Delta(\Delta\overline{G_t^\circ})_{B/A} > 0$ and $\Delta(\Delta\overline{G_t^\circ})_{B/A} < -2RT_c \times \ln(\alpha_{B/A})$, the salt-containing column gives a better separation of the solutes than the salt-free column. For $\Delta(\Delta\overline{G_t^\circ})_{B/A} < -2RT_c \cdot \ln(\alpha_{B/A})$, the solutes would be eluted in the reverse order. In the special case where $\alpha_{B/A} = 1$, the elution order of A and B on the salt-containing column is determined by the sign (positive or negative) of $\Delta(\Delta\overline{G_t^\circ})_{B/A}$.

The resolution, R_S, of peaks A and B[4] is also expressed in terms of energy difference and the number of theoretical plates, N', on salt-containing column.

$$R_{s,B/A} = \frac{\sqrt{N'_B}}{2} \frac{(\alpha_{B/A} \cdot \gamma - 1)}{(\alpha_{B/A} \cdot \gamma - \sqrt{N'_B/N'_A})} \tag{4}$$

where $\gamma = \exp\{\Delta(\Delta\overline{G_t^\circ})_{B/A}/RT_c\}$. Eqn. 4 shows that when the solutes A and B are separated on a PEG column with $\alpha_{B/A} = 1.1$ and $N_A = N_B = 800$, the resolution is 0.67. If the same solutes are applied to a salt-containing column with $\Delta(\Delta\overline{G_t^\circ})_{B/A} > 0.05$ kcal/mole or < -0.23 kcal/mole at 200° with the same plate numbers as above for both solutes, the resolution becomes larger than unity, *i.e.* complete separation occurs.

The values for the transfer energy and partition coefficients on PEG and LiI–PEG columns are given in Table I. The $\Delta\overline{G_t^\circ}$ values for *n*-fatty acid amides (C_4–C_{18}) at 220° range from −0.81 to −0.21 kcal/mole, in the order of increasing alkyl carbon number, whereas those for aromatic amides lie between −0.84 and −0.72 kcal/mole,

TABLE I

PARTITION COEFFICIENT (*K*) AND FREE ENERGY OF TRANSFER ($\Delta\overline{G_t^\circ}$)

Compound	*ln K*			*ln K'*			$\Delta\overline{G_t^\circ}$ *(kcal/mole)*		
	220°	*200°*	*180°*	*220°*	*200°*	*180°*	*220°*	*200°*	*180°*
Butyramide	4.23	4.72	5.20	5.05	5.66	6.28	−0.81	−0.88	−0.97
Valeramide	4.57	5.10	5.66	5.37	6.00	6.66	−0.78	−0.85	−0.90
Hexanamide	4.97	5.47	6.03	5.65	6.32	6.99	−0.67	−0.79	−0.85
Heptanamide	5.30	5.86	6.43	5.95	6.63	7.33	−0.63	−0.72	−0.81
Octanamide	5.64	6.26	6.87	6.28	6.97	7.70	−0.62	−0.66	−0.74
Nonanamide	5.97	6.59	7.21	6.52	7.26	8.02	−0.54	−0.62	−0.73
Decanamide	6.28	6.95	7.60	6.82	7.56	8.34	−0.52	−0.57	−0.67
Undecanamide	6.62	7.30	7.98	7.10	7.87	8.68	−0.47	−0.54	−0.63
Dodecanamide	6.94	7.64	8.35	7.38	8.18	9.02	−0.43	−0.50	−0.60
Tridecanamide	7.26	8.00	8.75	7.66	8.49	9.37	−0.39	−0.45	−0.55
Tetradecanamide	7.56	8.32	9.10	7.92	8.77	9.68	−0.35	−0.42	−0.52
Pentadecanamide	7.89	8.69	9.49	8.21	9.09	10.03	−0.31	−0.38	−0.48
Hexadecanamide	8.17	8.99	9.82	8.46	9.37	10.34	−0.28	−0.35	−0.46
Heptadecanamide	8.48	9.33	10.19	8.74	9.69	10.68	−0.25	−0.33	−0.44
Octadecanamide	8.78	9.66	10.55	8.95	9.95	11.00	−0.21	−0.27	−0.40
Succinamide	8.83	—	—	9.94	—	—	−1.11	—	—
α-Phenylacetamide	6.76	7.38	8.03	7.61	8.37	9.19	−0.84	−0.93	−1.04
Benzamide	6.68	7.31	7.96	7.50	8.21	9.05	−0.80	−0.84	−0.97
Salicylamide	7.55	8.29	9.11	8.41	9.24	10.27	−0.84	−0.89	−1.13
o-Ethoxybenzamide	6.98	7.73	8.41	7.72	8.43	9.09	−0.72	−0.64	−0.61
Nicotinamide	7.35	8.11	8.86	8.37	9.08	10.00	−0.99	−0.91	−1.03
Isonicotinamide	7.32	8.12	8.86	8.35	9.19	10.11	−1.01	−0.99	−1.12
N-Ethylnicotinamide	6.56	7.30	7.97	7.56	8.31	9.21	−0.97	−0.94	−1.11
N,N-Diethylnicotinamide	5.87	6.52	7.07	6.32	6.92	7.61	−0.44	−0.37	−0.48
Pyrazinamide	6.17	6.74	7.33	6.72	7.33	8.08	−0.54	−0.55	−0.67
Nicotinic acid hydrazide	7.60	—	—	9.12	—	—	−1.49	—	—
Acetanilide	6.39	7.01	7.68	7.25	8.07	8.91	−0.83	−0.99	−1.10
Phenacetin	7.52	8.32	9.13	8.46	9.29	10.29	−0.92	−0.91	−1.05
Salicylanilide	9.26	—	—	10.01	—	—	−0.73	—	—
Lidocaine	6.80	7.37	8.13	7.08	7.73	8.51	−0.27	−0.34	−0.34
Oxanilide	9.23	—	—	9.29	—	—	−0.05	—	—
α-Acetonaphthalide	8.31	9.07	9.86	9.07	10.01	10.99	−0.74	−0.88	−1.02
β-Acetonaphthalide	8.93	9.73	—	9.89	10.91	—	−0.93	−1.11	—
2-Pyrrolidone	5.01	5.53	5.95	5.91	6.42	7.02	−0.88	−0.83	−0.95
δ-Valerolactam	5.25	5.71	6.22	6.23	6.76	7.38	−0.95	−0.97	−1.04

(Continued on p. 84)

TABLE I *(continued)*

Compound	*ln K*			*ln K'*			$\Delta\overline{G_t^\circ}$ *(kcal/mole)*		
	220°	*200°*	*180°*	*220°*	*200°*	*180°*	*220°*	*200°*	*180°*
ε-Caprolactam	5.42	5.88	6.38	6.28	6.80	7.45	−0.84	−0.86	−0.95
N-Methylpyrrolidone	4.21	4.38	4.96	4.67	5.03	5.63	−0.68	−0.65	−0.60
2,5-Piperazinedione	9.20	—	—	10.81	—	—	−1.57	—	—
Hydantoin	8.31	9.14	9.95	9.35	10.18	11.10	−1.01	−0.98	−1.03
Succinimide	6.11	6.69	7.33	6.66	7.38	8.00	−0.54	−0.64	−0.60
Phthalimide	7.32	7.95	8.62	7.55	8.24	9.09	−0.22	−0.27	−0.42
Barbital	7.88	8.67	9.50	8.08	8.89	9.83	−0.20	−0.20	−0.29
Amobarbital	8.31	9.20	10.10	8.43	9.32	10.36	−0.11	−0.11	−0.23
Mephobarbital	8.42	9.26	10.11	8.57	9.44	10.37	−0.14	−0.17	−0.23
Thiopental	8.46	9.34	—	8.68	9.55	—	−0.20	−0.20	—
Secobarbital	8.66	9.56	10.48	8.73	9.67	10.73	−0.06	−0.10	−0.22
Thiamylal	8.68	9.58	—	8.76	9.60	—	−0.07	−0.01	—
Phenobarbital	10.15	—	—	10.47	—	—	−0.31	—	—
Aminopyrine	7.53	8.22	8.92	7.95	8.62	9.45	−0.41	−0.37	−0.47
Isopropylantipyrine	7.67	8.32	9.01	8.02	8.77	9.56	−0.33	−0.42	−0.49
Antipyrine	8.10	8.81	9.53	9.00	9.68	10.52	−0.88	−0.82	−0.88
Caffeine	7.85	8.57	9.30	8.16	8.88	9.72	−0.31	−0.29	−0.38
Theobromine	9.19	9.88	10.55	9.53	10.26	11.00	−0.33	−0.39	−0.40
Proxyphylline	9.44	10.35	—	10.04	11.00	—	−0.58	−0.61	—
N,N-Diethylaniline	3.49	3.80	4.10	3.49	3.91	4.41	0.00	−0.10	−0.28
2-Ethylaminobenzoate	6.97	7.69	8.40	7.34	8.14	9.04	−0.35	−0.42	−0.58
Chlorpheniramine	7.09	7.79	8.48	7.20	7.86	8.62	−0.10	−0.06	−0.12
Amitriptyline	7.52	8.26	8.98	7.47	8.14	9.02	0.06	0.11	−0.03
Nicotinic acid ethylester	4.32	4.77	5.16	4.45	4.89	5.43	−0.13	−0.11	−0.24
Isonicotinic acid ethyl ester	4.21	4.69	4.97	4.21	4.66	5.22	0.00	0.03	0.16

indicating that the interaction of LiI with aromatic amides is generally stronger than with *n*-fatty acid amides. The five- to seven-membered lactams (2-pyrrolidone, δ-valerolactam, and ε-caprolactam) gave $\Delta\overline{G_t^\circ}$ values between −0.84 and −0.95 kcal/mole, showing a slightly stronger interaction than the corresponding fatty acid amides. Barbiturates, their $\Delta\overline{G_t}$ values ranging from −0.06 to −0.31 kcal/mole, showed weaker interaction than hydantoin. Piperazine-2,5-dione, which has two imide groups in a six-membered ring, gave the strongest interaction with LiI among the amides examined. Antipyrine showed a $\Delta\overline{G_t}$ value about double that of the other pyrazolones. Nicotinamides, isonicotinamides, and anilides are found to have moderate or strong interactions with LiI, whereas esters and amines showed very weak or no interaction.

From the energy data in Table I, the separation profiles of some amide mixtures on LiI–PEG column can be discussed in terms of relative retention and resolution.

A mixture of barbital (A), antipyrine (B), and hexadecanamide (C) (Fig. 1) was eluted from a PEG column at 220° in the order A, B, C with poor resolution (complete overlap of the B and C peaks), whereas complete separation was achieved on a LiI–PEG column at the same temperature. The energy difference, relative retention and resolution data for both columns are listed in Table II. The data for $\Delta(\Delta\overline{G_t^\circ})$ and r_s for B/A, C/B, and C/A indicate that the relative retention values for B/A, C/A and B/C on the LiI–PEG column are larger than those on the PEG column, resulting in

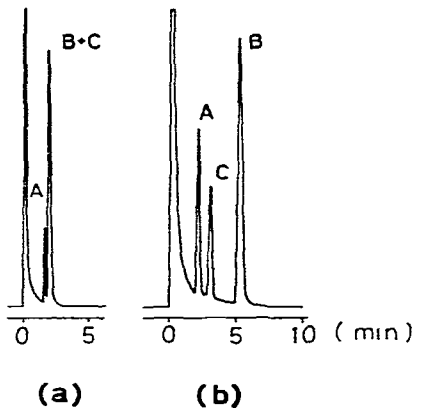

Fig. 1. Separation of a mixture of barbital (A), antipyrine (B) and hexadecanamide (C) on (a) a salt-free PEG column and (b) a LiI–PEG column, at 220°.

the complete separation of the peaks with the elution order A, C, B (Fig. 1b). Table II also lists the results for the number of theoretical plates. It was found that in spite of a slight decrease in the plate number caused by the addition of LiI, the increase in the relative retention value enhanced the separation efficiencies.

TABLE II

SEPARATION CHARACTERISTICS OF A MIXTURE OF BARBITAL (A), ANTIPYRINE (B) AND HEXADECANAMIDE (C) ON A SALT-FREE PEG COLUMN AND A LiI–PEG COLUMN AT 220°

α and α' denote relative retention on PEG and LiI–PEG columns, respectively; R_s and $|R_s|$ denote resolution on PEG and LiI–PEG columns, respectively; N and N' denote plate numbers on PEG and LiI–PEG columns, respectively.

	α	R_s	$RT_c \ln \alpha$ (kcal/mole)	$\Delta(\Delta\overline{G_t^\circ})$ (kcal/mol)	α'	$\lvert R_s \rvert$
B/A	1.25	1.67	0.22	0.68	2.51	6.0
C/B	1.0	0.0	0.0	−0.60	0.58	4.0
C/A	1.25	1.67	0.22	0.08	1.46	2.5

	N	N'
A	900	800
B	900	800
C	1000	800

For a mixture of amitriptyline (A), aminopyrine (B), and salicylamide (C), it must be noted that the $\Delta\overline{G_t^\circ}$ values at 220° (see Table I) indicate that B and C undergo a salting-in effect, whereas the reverse is true for A. The chromatograms for this mixture on PEG and LiI–PEG columns at 220° are shown in Fig. 2. A single peak was observed using a PEG column, whereas A, B, and C were eluted separately from a LiI–PEG column in this order. The energy difference and separation data are

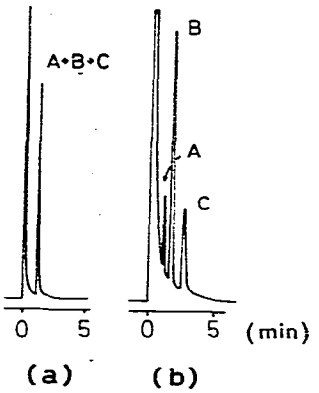

Fig. 2. Separation of a mixture of amitriptyline (A), aminopyrine (B) and salicylamide (C) on (a) a salt-free PEG column and (b) a LiI–PEG column, at 220°.

given in Table III. All the $\Delta(\Delta\overline{G_t^\circ})$ values for B/A, C/B, and C/A are positive, and good resolution is obtained for all the solutes with no change in the elution order. Table I also shows that the partition coefficient of C is almost doubled by changing the stationary phase from PEG to LiI–PEG at 220° as well as by lowering the temperature of the PEG column from 220° to 200°. The subsequent results for the relative retention value ($\alpha_{C/A} = 1.03$ and $\alpha_{C/B} = 1.07$ on PEG column at 200°), however, indicate that a LiI–PEG column is more efficient for the separation of this mixture.

TABLE III

SEPARATION CHARACTERISTICS OF A MIXTURE OF AMITRIPTYLINE (A), AMINOPYRINE (B) AND SALICYLAMIDE (C) ON A SALT-FREE PEG COLUMN AND A LiI–PEG COLUMN AT 220°

For symbols, see Table II.

	α	R_s	$RT_c \ln \alpha$ (kcal/mole)	$\Delta(\Delta\overline{G_t^\circ})$ (kcal/mole)	α'	$\lvert R_s \rvert$
B/A	1.0	0.0	0.0	0.47	1.39	2.2
C/B	1.0	0.0	0.0	0.43	1.61	3.5
C/A	1.0	0.0	0.0	0.90	2.24	6.0

	N	N'
A	800	600
B	1100	800
C	800	700

A mixture of aminopyrine (A), phenacetin (B), and caffeine is widely used as an antifebrile anodyne. The elution profile of this mixture exhibited very poor resolution on the PEG column (Fig. 3a), and it was difficult to attain complete separation of the peaks by changing the column temperature. The use of a LiI–PEG column, however, produced a clear separation (Fig. 3b) accompanied by a reversal of the elution order

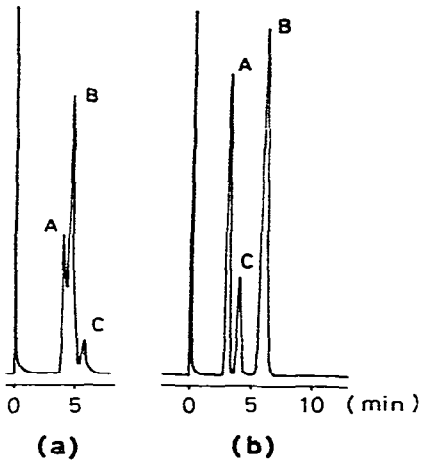

Fig. 3. Separation of a mixture of aminopyrine (A), phenacetin (B) and caffeine (C) on (a) a salt-free PEG column and (b) a LiI–PEG column, at 200°.

of peaks B and C. Table IV summarizes the data for relative retention, resolution, energy difference, and plate number for these solutes. The positive value of $\Delta(\Delta\overline{G_t^\circ})$ for B/A indicates that the LiI–PEG column gives a larger relative retention value for B/A than the PEG column, with the elution order unchanged. $\Delta(\Delta\overline{G_t^\circ})_{C/B}$ (−0.62 kcal/mole) is smaller than $-2RT_c \ln(\alpha_{B/A}) = -0.46$ kcal/mole, and the peak C, therefore, is released from the LiI–PEG column before peak B, and resolution is better than on the PEG column. In the case of A and C, the energy difference −(0.08 kcal/mole) indicates that the elution order remains unchanged, whereas the relative retention value on the LiI–PEG column becomes smaller than on the PEG column.

TABLE IV

SEPARATION CHARACTERISTICS OF A MIXTURE OF AMINOPYRINE (A), PHENACETIN (B) AND CAFFEINE (C) ON A SALT-FREE PEG COLUMN AND A LiI–PEG COLUMN AT 200°

For symbols, see Table II.

	α	R_s	$RT_c \ln \alpha$ (kcal/mole)	$\Delta(\Delta\overline{G_t^\circ})$ (kcal/mole)	α'	$\lvert R_s \rvert$
B/A	1.10	0.69	0.09	0.54	1.95	4.2
C/B	1.28	1.79	0.23	−0.62	0.66	2.6
C/A	1.41	2.24	0.32	−0.08	1.30	1.8

	N	N'
A	900	800
B	800	700
C	900	1000

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