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SEPARATION OF DRUGS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH POROUS POLYMER RESINS

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SUMMARY

The separation of cold drugs and neuroleptics by high-performance liquid chromatography with the porous polymer resin DVB-MCL-O (or 11-30-0), which is a styrene–divinylbenzene–methyl methacrylate copolymer substituted with hydroxymethyl groups, was studied. This copolymer was compared with the commercial porous polymers Hitachi gel 3011, 3011-0 and 3030.

A very small theoretical plate height was obtained by using DVB-MCL-O and methanol–ammonia solution (99:1) as the stationary and mobile phases, respectively. This combination was found to be the most suitable for the rapid separation of condensed aromatic ring compounds.

INTRODUCTION

Recent developments in high-performance liquid chromatography (HPLC) can be attributed to advances in detectors of high sensitivity and packing materials of high performance. Of the available packing materials, porous polymer resins are widely used. They are classified into several types, *e.g.*, poly(vinyl acetate), poly(methyl methacrylate) and styrene–divinylbenzene copolymers. The first type has a high polarity, the second has medium polarity and the last has the lowest polarity. By introducing hydroxymethyl groups in styrene–divinylbenzene copolymer, more hydrophylic gels are obtained[1,2].

We have prepared a new packing material of high performance for the rapid separation of condensed aromatic ring compounds consisting of a styrene–divinylbenzene–methyl methacrylate copolymer substituted with hydroxymethyl groups, and separations of alkaloids and phenothiazine-type drugs have been carried out on it.

In this paper, the character of the new porous polymer resin, the effects of the composition and pH of the mobile phase and a comparison with conventional porous polymer resins are described. It has been found that the new porous polymer has not
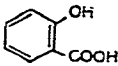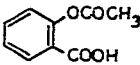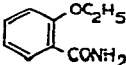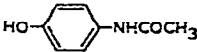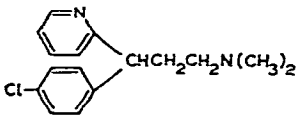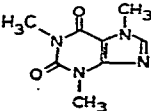
only the character of the parent polymers but also a small theoretical plate height, and that it is suitable for the rapid separation of condensed aromatic ring compounds[3-8].

EXPERIMENTAL

Materials

Analgesics and neuroleptics (shown in Tables I and II) were purchased from commercial sources.

TABLE I

STRUCTURES OF SOME OF THE SAMPLES

Name	Formula
Salicylic acid	OH, COOH
Aspirin	OCOCH$_3$, COOH
Ethoxybenzamide	OC$_2$H$_5$, CONH$_2$
Acetaminophen	HO—⟨ ⟩—NHCOCH$_3$
Chlorpheniramine	CHCH$_2$CH$_2$N(CH$_3$)$_2$, Cl—
Caffeine	H$_3$C, CH$_3$, CH$_3$

Apparatus

A Nihon Seimitsu Kagaku NSLC-102 high-performance liquid chromatograph with a variable-wavelength UV detector was used.
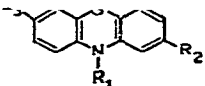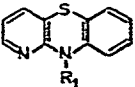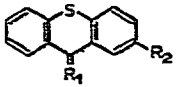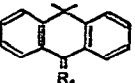
Column preparation

Glass columns (50 cm × 5 mm or 3 mm I.D.) with a septum injection port were packed with a methanol slurry of packing material through a 20-ml stainless-steel packer at the rate of 2 ml/min for 2 h.

Stationary and mobile phases
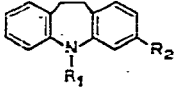
DVB-MCL-O (or 11-30-0) (styrene–divinylbenzene–methyl methacrylate copolymer substituted with hydroxymethyl groups, 10 μm), Hitachi gel 3011 (styrene–divinylbenzene copolymer, 10 μm), Hitachi gel 3011-0 (styrene–divinylbenzene copolymer substituted with hydroxymethyl groups, 10 μm), Hitachi gel 3030 [poly-(methyl methacrylate), 20 μm] and LiChrosorb RP-18 were used as stationary phases.

TABLE II

STRUCTURES OF SOME OF THE SAMPLES

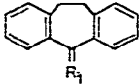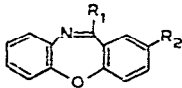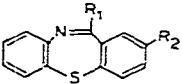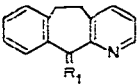| Formula	Name	R
Phenothiazine 	Phenothiazine	$R_1, R_2, R_3 = -H$
	~~Fenethazine~~	~~$R_1 = -CH_2CH_2N(CH_3)_2$~~
	Promethazine	$R_1 = -CH_2CHN(CH_3)_2$ $\quad\quad\quad\vert$ $\quad\quad\quad CH_3$
	Alimemazine	$R_1 = -CH_2CHCH_2N(CH_3)_2$ $\quad\quad\quad\quad\vert$ $\quad\quad\quad\quad CH_3$
	Chlorpromazine	$R_1 = -CH_2CH_2CH_2N(CH_3)_2$ $R_2 = -Cl$
	Fluphenazine	$R_1 = -CH_2CH_2CH_2N\diagup\diagdown NCH_2CH_2OH$ $R_2 = -CF_3$
	Perphenazine	$R_1 = -CH_2CH_2CH_2N\diagup\diagdown NCH_2CH_2OH$ $R_2 = -Cl$
	Propericiazine	$R_1 = -CH_2CH_2CH_2N\diagup\diagdown OH$ $R_2 = -Cl$
	Thioridazine	$R_1 = -CH_2CH_2-\langle N$ ring $\rangle$ $\quad\quad\quad\quad\quad\quad\vert$ $\quad\quad\quad\quad\quad\quad CH_3$ $R_2 = -SCH_3$
	Protizinic acid	$R_1 = -CH_3$ $R_2 = -CHCOOH$ $\quad\quad\quad\vert$ $\quad\quad\quad CH_3$ $R_3 = -OCH_3$
Azaphenothiazine 	Isothipendyl	$R_1 = -CH_2CHN(CH_3)_2$ $\quad\quad\quad\vert$ $\quad\quad\quad CH_3$
Thioxanthene 	Flupentixol	$R_1 = =CHCH_2CH_2N\diagup\diagdown NCH_2CH_2OH$ $R_2 = -CF_3$
Anthracene 	Melitracene	$R_1 = =CHCH_2CH_2N(CH_3)_2$

TABLE II *(continued)*

Formula	Name	R
Dihydrodibenzazepine	Imipramine	$R_1 = -CH_2CH_2CH_2N(CH_3)_2$
	Rophepramine	$R_1 = -CH_2CH_2CH_2\underset{CH_3}{N}CH_2\underset{O}{C}\!-\!\!\!\bigcirc\!\!-Cl$
	Clocapramine	$R_1 = -CH_2CH_2CHN$ ... CONH$_2$
		$R_2 = -Cl$
Dihydrodibenzocycloheptane	Piroheptine	$R_1 = $ ... $\underset{CH_3}{N}-C_2H_5$
Dibenzoxazepine	Amoxapine	$R_1 = -N$ ... NH
		$R_2 = -Cl$
Dibenzothiazepine	Clothiapine	$R_1 = -N$ ... NCH$_3$
		$R_2 = -Cl$
Dihydropyridobenzocycloheptane	Azatazine	$R_1 = $ ... NCH$_3$

The mobile phase was prepared by addition of ammonia solution or buffer solution to methanol or acetonitrile. The buffer solutions consisted of mixture of equal portions of 0.04 M orthophosphoric acid, 0.04 M glacial acetic acid and 0.04 M boric acid, 0.2 M hydrochloric acid and 0.2 M sodium hydroxide solution. The composition and pH of buffer solutions are listed in Table VI. The composition and apparent pH of the mobile phase are also listed in Table VI. If a precipitate separates out, the solution is filtered before use.

RESULTS AND DISCUSSION

Comparison of porous polymers

Table III shows the k' and H values of the cold drugs and phenothiazine-type compounds with various stationary phases. The highest k' value is observed with

TABLE III

CAPACITY FACTORS (k') AND THEORETICAL PLATE HEIGHTS (H) OF SOME DRUGS ON A POROUS POLYMER COLUMN WITH BASIC METHANOL AND BASIC ACETO-NITRILE MOBILE PHASES

Column size: 50 cm × 5 mm I.D. Linear flow-rate: 5.0 cm/min.

| Drug | Parameter | Stationary phase | | | | 3011-0: | 3030: |
| | | 11-30-0 | | 3011 | | I* | I* |
		I*	II**	I*	II**		
Acetaminophen	k'	1.16	2.23	0.60	0.67	0.72	0.46
	H (mm)	0.087	0.233	0.357	0.368	0.313	0.454
Ethoxybenzamide	k'	1.51	1.37	1.37	0.93	1.13	0.78
	H (mm)	0.065	0.129	0.367	0.303	0.319	0.437
Caffeine	k'	2.22	1.27	2.56	0.94	1.83	1.67
	H (mm)	0.076	0.152	0.385	0.300	0.380	0.463
Chlorpheniramine	k'	1.78	2.29	3.36	2.40	1.47	0.88
	H (mm)	0.109	0.126	0.484	0.392	0.463	0.656
Alimemazine	k'	4.19	3.05	10.18	4.75	3.77	2.25
	H (mm)	0.179	0.148	0.768	0.508	0.964	0.906
Promethazine	k'	3.94	3.19	8.52	4.07	3.49	2.11
	H (mm)	0.167	0.144	0.703	0.481	0.852	0.893
Fenethazine	k'	4.47	3.19	10.48	4.35	4.59	2.32
	H (mm)	0.136	0.132	0.741	0.485	1.000	0.757
Isothipendyl	k'	3.56	3.43	8.92	4.58	3.28	1.76
	H (mm)	0.145	0.141	0.639	0.498	0.808	0.819
Phenothiazine	k'	7.35	3.68	5.83	2.87	6.66	3.60
	H (mm)	0.101	0.132	0.696	0.393	0.706	0.542
Thioridazine	k'	8.15	5.57	34.50	10.71	9.06	4.00
	H (mm)	0.276	0.302	1.245	0.754	1.742	1.294

* I: mobile phase = methanol–ammonia solution (99:1).
** II: mobile phase = acetonitrile–ammonia solution (99:1).

Hitachi gel 3011, which has the lowest polarity. The order of k' value is 3011 > 11-30-0 > 3011-0 > 3030. On the other hand, the H values increase in the order 11-30-0 < 3011 < 3030 < 3011-0. Especially for tricyclic compounds, 11-30-0 resin has a much smaller H value than 3011 and 3011-0 gels, and has a small k' value, which means that rapid chromatographic analysis is possible.

In addition, the k' values are reduced with acetonitrile–ammonia solution as the mobile phase and the H value is also reduced with Hitachi gel 3011, but with 11-30-0 resin the variation of H is relatively small and for some compounds it increases.

Structure and separation

The k' values of phenothiazine and similar tricyclic compounds were measured using 11-30-0 resin and Hitachi gel 3030 and 3011-0 and are listed in Table IV.

The k' values depend on the tricyclic skeleton, and decrease in the order
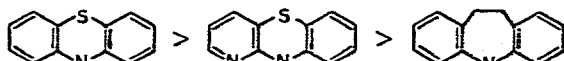
TABLE IV

CAPACITY FACTORS (k') OF SOME DRUGS ON A POROUS POLYMER COLUMN WITH A BASIC METHANOL MOBILE PHASE

Mobile phase: methanol–ammonia solution (99:1). Column size: 50 cm × 5 mm I.D. Linear flow-rate: 2.5 cm/min. Detection: 254 nm.

Drug	Stationary phase		
	3030	3011-0	11-30-0
Protizinic acid	0.0	0.0	0.28
Promethazine	2.09	3.53	4.00
Alimemazine	2.22	3.78	4.20
Fenethazine	2.25	4.57	4.41
Chlorpromazine	2.57	5.52	4.45
Phenothiazine	3.52	6.48	7.29
Fluphenazine	1.41	2.15	2.08
Propericiazine	2.22	3.04	2.97
Perphenazine	2.24	4.10	3.55
Thioridazine	3.95	9.30	8.09
Isothipendyl	1.75	3.31	3.60
Imipramine	1.67	3.06	2.61
Rophepramine	4.92	9.56	8.06
Flupenthixol	1.51	2.23	2.14
Melitracene	1.58	2.68	2.64
Piroheptine	1.66	2.77	2.61
Clocapramine	2.57	3.75	3.58
Azatazine	0.83	1.46	1.42
Amoxapine	2.50	3.39	3.13
Clothiapine	2.72	4.49	3.91
Height equivalent to a theoretical plate (H) (mm)	0.318–0.778	0.378–1.479	0.077–0.361

There is also a considerable influence of the side-chain on the nitrogen atom in the centre ring on the k' values. Phenothiazine, which lacks such a side-chain, has high affinity for the stationary phase, and therefore has a high k' value. Terminal piperidine, piperazine and benzene rings in the side-chain result in high k' values. Groups in position 2, *e.g.*, SCH_3 and CF_3, also influence the k' value. Polar substituents, *e.g.*, OH or COOH, reduce the k' value.

Influence of linear velocity of the mobile phase

The H values of cold drugs and phenothiazine-type compounds were measured with Hitachi gel 3011-0 and 3030 and 11-30-0 resin at various flow-rates. The results are shown in Fig. 1. Methanol–ammonia solution (99:1) was used as the eluent at flow-rates of 2.5, 5.0, 7.5, 8.5 and 10.0 cm/min. On each stationary phase the H values increased with an increase in flow-rate. The H value at a flow-rate of 2.5 cm/min approximately doubled when the flow-rate was increased to 10.0 cm/min.

Influence of the composition of the mobile phase

The variation in the k' values of cold drugs and phenothiazine-type compounds with 11-30-0 resin was studied using various mobile phases:

    (1) methanol–ammonia solution (99:1);

    (2) acetonitrile–ammonia solution (99:1);

(3) methanol–acetonitrile–ammonia solution (25:75:1);
(4) methanol–acetonitrile–ammonia solution (50:50:1);
(5) methanol–acetonitrile–ammonia solution (75:25:1);
(6) methanol–water–ammonia solution (95:4:1);
(7) methanol–water–ammonia solution (90:9:1).

The addition of water or acetonitrile (Fig. 2) has a strong effect on the k' values of phenothiazine-type drugs, which have large k' values with (1), but has only a slight effect with compounds that have small k' values. Isothipendyl (IT), thioridazine (TZ), etc., belong to the former group and acetaminophen (PA), ethoxybenzamide (EB), chlorpheniramine maleate (CP) and caffeine (CF) to the latter. In general, the addition of acetonitrile reduces and the addition of water increases k' values. Compounds that have no side-chain or with a cyclic amine group in the side-chain are strongly influenced by the composition of the mobile phase. Phenothiazine and thioridazine are examples of such compounds.

The k' values of PA and CP with acetonitrile (mobile phase 2) are larger than with methanol (mobile phase 1).
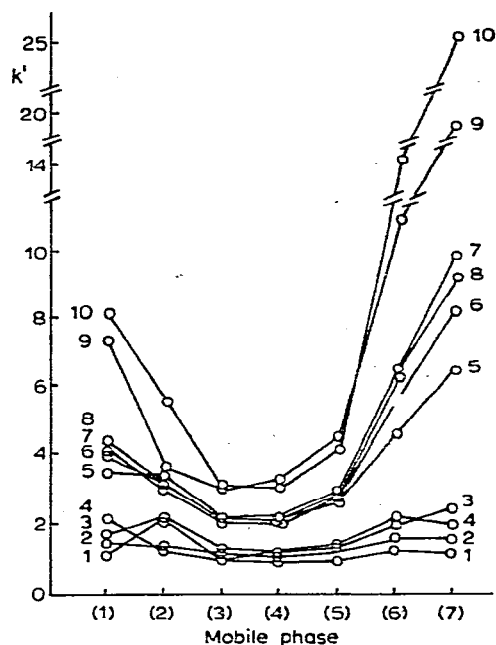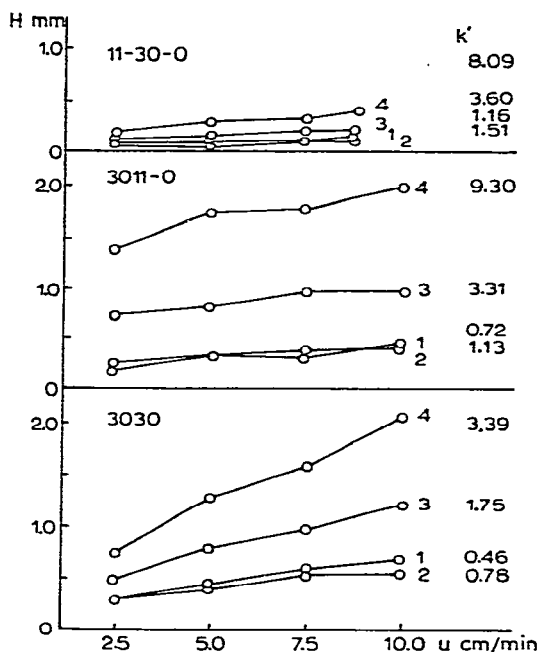

Fig. 1. Relationship between linear flow-rate (u) and theoretical plate height (H). Stationary phase: 11-30-0, 3011-0 and 3030. Column size: 50 cm × 5 mm I.D. Mobile phase: methanol–ammonia solution (99:1). 1 = Acetaminophen; 2 = ethoxybenzamide; 3 = isothipendyl; 4 = thioridazine.

Fig. 2. Relationship between capacity factor (k') and the composition of the mobile phase. Mobile phase: (1) methanol–ammonia solution (99:1); (2) acetonitrile–ammonia solution; (3) methanol–acetonitrile–ammonia solution (25:75:1); (4) methanol–acetonitrile–ammonia solution (50:50:1); (5) methanol–acetonitrile–ammonia solution (75:25:1); (6) methanol–water–ammonia solution (95:4:1); (7) methanol–water–ammonia solution (90:9:1). 1 = Acetaminophen; 2 = ethoxybenzamide; 3 = chlorpheniramine; 4 = caffeine; 5 = isothipendyl; 6 = promethazine; 7 = alimemazine; 8 = fenethazine; 9 = phenothiazine; 10 = thioridazine.

TABLE V

CAPACITY FACTORS (k') OF DRUGS WITH BASIC AND ACIDIC METHANOL MOBILE PHASES

Column size: 50 cm × 5 mm I.D. Linear flow-rate: 5.0 cm/min.

Drug	Methanol–aq. ammonia		Methanol–acetic acid	
	3011	11-30-0	3011	11-30-0
Salicylic acid	0.05	0.88	0.86	—
Aspirin	0.05	0.88	0.60	2.98
Acetaminophen	0.60	1.16	0.33	1.20
Ethoxybenzamide	1.37	1.51	0.85	1.38
Caffeine	2.56	2.22	1.71	1.87
Chlorpheniramine	3.36	1.78	0.05	0.03
Alimemazine	10.18	4.19	0.03	0.01
Promethazine	8.52	3.95	0.00	0.01
Fenethazine	10.48	4.47	0.04	0.00
Isothipendyl	8.92	3.56	0.08	0.02
Phenothiazine	5.83	7.35	3.87	5.88
Thioridazine	36.54	8.15	0.38	0.01

Influence of pH

Table V shows the k' values of drugs with acidic and basic methanol mobile phases. Acidic compounds such as aspirin and salicylic acid have very small k' values in a basic mobile phase and larger k' values in an acidic mobile phase. In particular, with 11-30-0 resin salicylic acid is not eluted. The k' values of chlorpheniramine and phenothiazine-type drugs are higher with a basic than with an acidic methanol mobile phase.

Hence it is to be expected that the k' values of basic compound will be higher with basic and lower with acidic mobile phases on a porous polymer resin stationary phase. The k' values were measured at various pH values, using mobile phases adjusted to pH 1–12 by mixing buffer solution and methanol in a volume ratio of 1:9 (Table VI). The results are shown in Fig. 3.

The k' value of PA hardly varies between pH 1 and 10 but is lower at pH 12. This decrease in k' results from the dissociation of OH group at high pH and from the consequent reduction in the affinity of PA for the stationary phase. The k' values of EB and CF do not vary with variation in pH. The k' value of phenothiazine, which

TABLE VI

MOBILE PHASE SYSTEMS AND THEIR APPARENT pH VALUES

Component	Proportions (ml)						
0.2 M HCl	10	0	0	0	0	0	0
0.04 M mixed acids*	0	100	85	75	70	60	0
0.2 M NaOH	0	0	15	25	30	40	10
Methanol	900	900	900	900	900	900	900
pH	1.20**	3.20	5.50	7.10	8.60	10.50	12.80**

    * Mixed acids: 0.04 M H$_3$PO$_4$–0.04 M CH$_3$COOH–0.04 M H$_3$BO$_3$ (1:1:1).
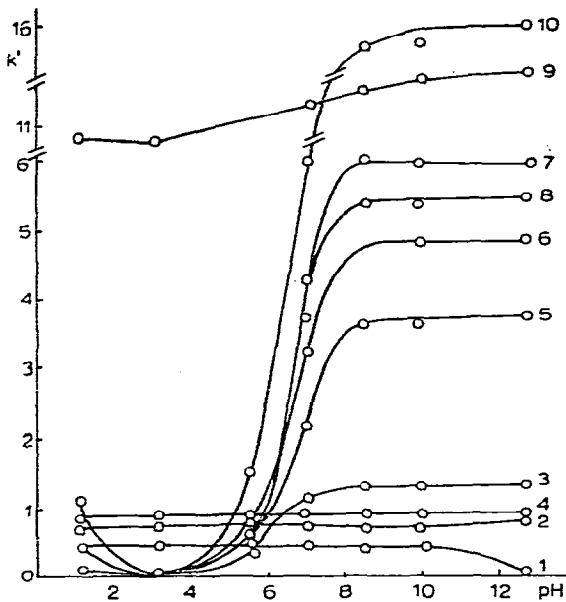   ** 90 ml of water added.

Fig. 3. Relationship between capacity factor (k') and pH of the mobile phase. Stationary phase: 11-30-0. Column size: 50 cm × 5 mm I.D. Mobile phase: methanol–buffer solution (9:1). Linear flow-rate: 5.0 cm/min. 1 = Acetaminophen; 2 = ethoxybenzamide; 3 = chlorpheniramine; 4 = caffeine; 5 = isothipendyl; 6 = promethazine; 7 = alimemazine; 8 = fenethazine; 9 = phenothiazine; 10 = thioridazine.
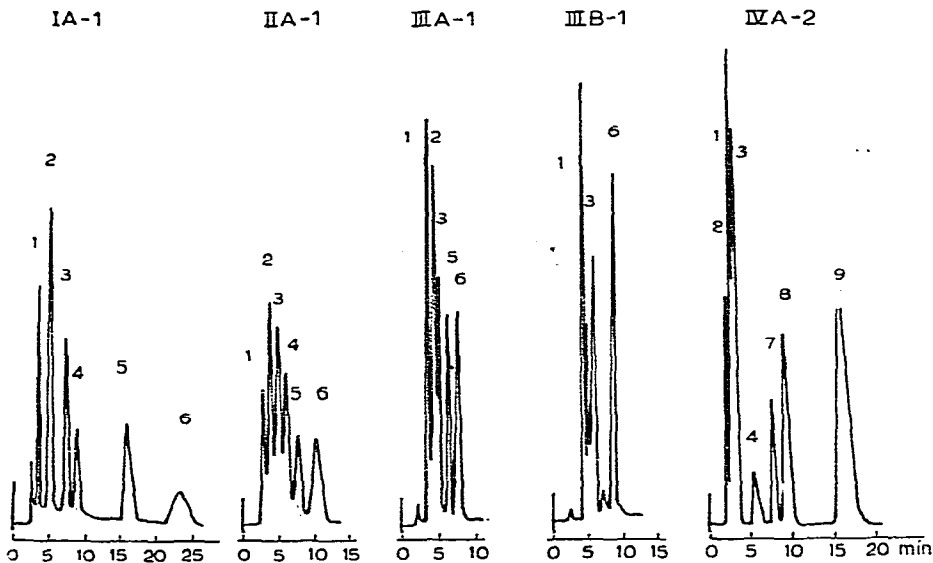

Fig. 4. Separation of some drugs. Stationary phase: I, Hitachi gel 3011; II, Hitachi gel 3011-0; III, 11-30-0 resin; IV, LiChrosorb RP-18. Mobile phase: 1, methanol–ammonia solution (99:1); 2, methanol–water–ammonia solution (90:9:1). Detection: A, 230 nm; B, 254 nm. 1 = Acetaminophen; 2 = ethoxybenzamide; 3 = caffeine; 4 = chlorpheniramine; 5 = dextromethorphan; 6 = promethazine; 7 = isothipendyl; 8 = alimemazine; 9 = thioridazine.

lacks a side-chain, is also not influenced by pH. Other phenothiazine-type drugs that have amine groups in the side-chain exhibit very low k' values between pH 1 and 6, then k' increases with increase in pH and remains constant from pH 8 to 12. CP shows a similar tendency. This pattern of the variation of the k' values of basic compounds is attributed to the dissociation of amino groups, that is, the ammonium ion form at low pH, which has a low affinity for the stationary phase, dissociates into molecular form at high pH, and hence acquires a high affinity for the stationary phase.

Examples of separation

Fig. 4 shows chromatograms of a mixture of PA, EB, CF, CP, dextromethorphan (DX) and promethazine (PR), which are usually compounded in cold drug preparations. Hitachi gel 3011 and 3011-0, 11-30-0 resin and LiChrosorb RP-18 were used. DX and PR have condensed ring structures, and hence possess a high affinity for Hitachi gel 3011 owing to its low polarity. Accordingly, the peaks of DX and PR with Hitachi gel 3011 are broad and their retention times are long. Using Hitachi gel 3011-0, with a relatively high polarity, the retention times of DX and PR are lower, but the peaks are broad because of the large theoretical plate height. 11-30-0 resin possesses both a high polarity and small H value, which result in lower retention times of DX and PR and sharp peaks. The chromatogram obtained with LiChrosorb RP-18 is similar to that with 11-30-0 resin. As DX and PR are generally present in only small amounts in the usual medicinal preparations, the sharpness of the peaks, that
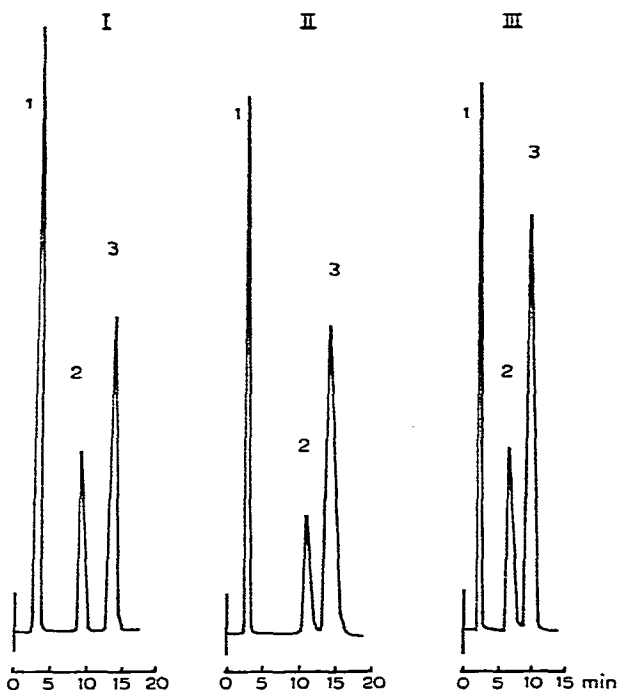

Fig. 5. Separation of some drugs. Stationary phase: I, 11-30-0 resin; II, Hitachi gel 3011-0; III, Hitachi gel 3030. Mobile phase: methanol–ammonia solution (99:1). Flow-rate: 1.0 ml/min. Detection: UV (254 nm). 1 = Protizinic acid; 2 = promethazine; 3 = phenothiazine.
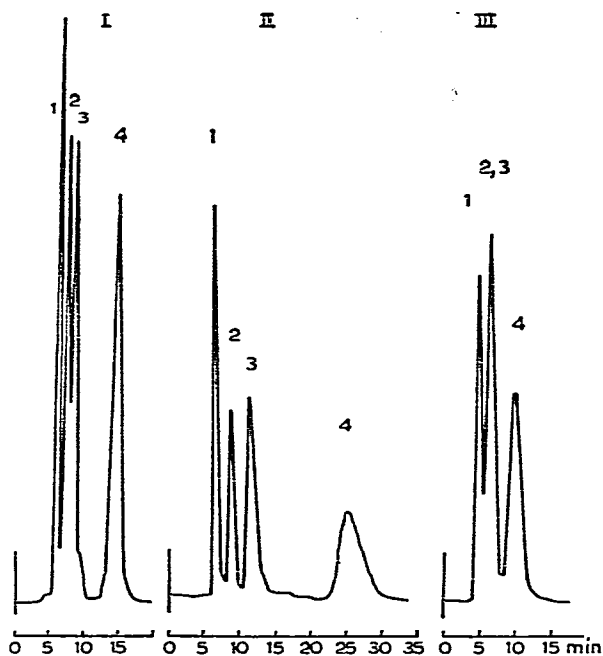
Fig. 6. Separation of some drugs. Stationary phase: I, 11-30-0 resin; II, Hitachi gel 3011-0; III, Hitachi gel 3030. Mobile phase: methanol–ammonia solution (99:1). Flow-rate: 1.0 ml/min. Detection: UV (254 nm). 1 = Fluphenazine; 2 = propericiazine; 4 = perphenazine; 4 = thioridazine.

means high peaks and a high sensitivity, are necessary for this determination. For this purpose, the selection of the wavelength for detection is an important factor. In Fig. 4, chromatogram IIIB represents detection at 254 nm, which is the wavelength of maxi-
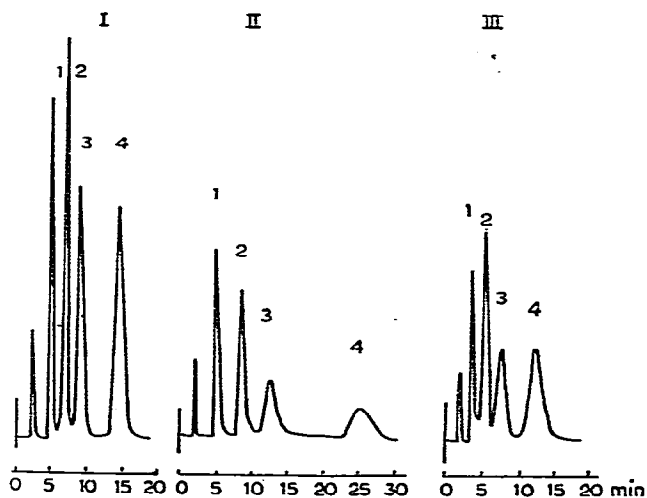

Fig. 7. Separation of some drugs. Stationary phase: I, 11-30-0 resin; II, Hitachi gel 3011-0; III, Hitachi gel 3030. Mobile phase: methanol–ammonia solution (99:1). Flow-rate: 1.0 ml/min. Detection: UV (254 nm). 1 = Azatazine; 2 = piroheptine; 3 = amoxapine; 4 = rophepramine.

mum absorption of PR. A higher peak of PR is obtained than in IIIA, which represents detection at 230 nm.

Typical chromatograms of phenothiazine-type drugs are shown in Figs. 5–7, obtained using Hitachi gel 3030 and 3011-0 and 11-30-0 resin packed in a 50 cm × 3 mm I.D. column and eluted with methanol–ammonia solution at the rate of 1.0 ml/min. The detection wavelength was 254 nm. Just as with the separations shown in Fig. 4, 11-30-0 resin gives the best results in terms of resolution, peak height and retention time.

Fig. 8 shows the chromatograms of noscapine in a commercial cold drug and thioridazine (internal standard) obtained with 11-30-0 resin. Two mobile phases were used: methanol–ammonia solution (99:1) and methanol–water –ammonia solution (95:5:1). The addition of water to the mobile phase results in an increased resolution between noscapine and the other components.
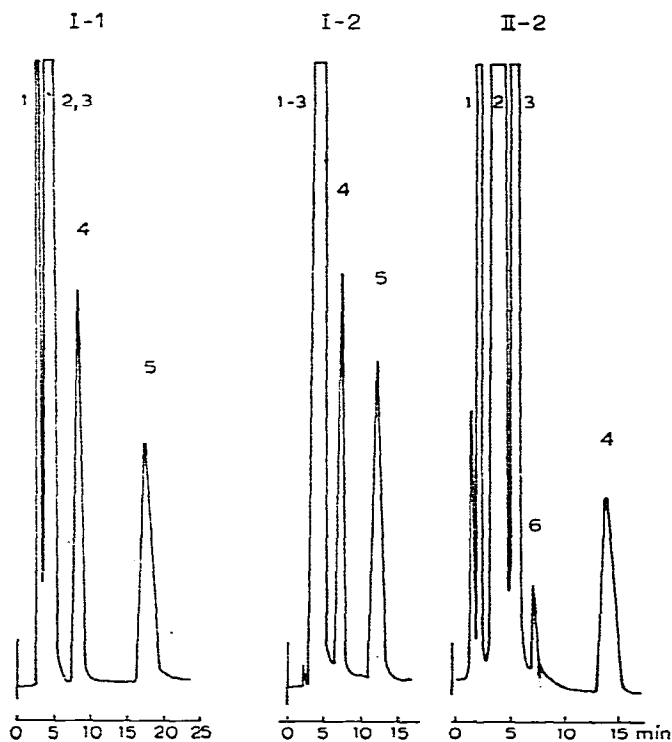

Fig. 8. Separation of some drugs. Stationary phase: I, 11-30-0 resin (25 cm × 3 mm I.D. column);II, Hitachi gel 3011 (50 cm × 3 mm I.D. column). Mobile phase: 1, methanol–water–ammonia solution (95:5:1), 2, methanol–ammonia solution (99:1). Flow-rate: (a) 0.6 ml/min; (b) 1.5 ml/min. Detection: 230 nm. 1 = Barbital; 2 = aminopyrine; 3 = caffeine; 4 = noscapine; 5 = thioridazine; 6 = chlorpheniramine maleate.

From the examples of separations presented above, it can be concluded that the porous polymer resin 11-30-0 is more suitable than conventional resins for the separation of condensed aromatic ring compounds.

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