Journal of Chromatography, 177 (1979) 263–270

CHROM. 12,029

SIMPLE PREPARATION OF A BONDED CATION-EXCHANGE PACKING MATERIAL AND ITS APPLICATION TO THE SEPARATION OF PHENOTHIAZINES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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(Received May 14th, 1979)

SUMMARY

A method for introducing sulphonic acid groups on to a microparticulate silica is described. This involves formation of a mercaptopropyl bonded phase followed by oxidation to the corresponding sulphonic acid. The packing material so prepared displays cation-exchange properties and has been used to separate phenothiazines.

INTRODUCTION

The strong cation-exchange materials used in high-performance liquid chromatography (HPLC) are mainly based on styrene–divinylbenzene copolymer beads that have been sulphonated, or alternatively utilise microparticulate silicas which have ion-exchanging groups (usually aromatic sulphonic acids) chemically bonded to their surface[1–3]. A reaction that does not appear to have been used to introduce sulphonic acid groups on to silica is the oxidation of a thiol group. This type of reaction proceeds very rapidly at room temperature and, as this paper will show, can provide the basis for preparing a bonded strong cation-exchanger for HPLC.

To study the properties of the cation-exchanger it was used to separate phenothiazines. These compounds, used as psychosedative drugs, contain a phenothiazine moiety with various side-chains attached to the nitrogen atom and in some cases substituents in the 2-position. Some of these compounds are very difficult to analyse by gas chromatography, and HPLC separations based on adsorption ion-pair partition, ion-exchange, and reversed-phase partition processes, have been described. The methods appearing before 1977 have been reviewed[4,5] and since that time there have been few papers dealing with phenothiazine separation[6,7].

EXPERIMENTAL

An amount of 50 g of microparticulate silica of 5 μm nominal particle size (Partisil 5, Whatman, Maidstone, Great Britain) was dried for 24 h at 150°. The dried material was added to 250 ml of hexane in a conical flask and 2 ml of water added.

The flask was stoppered and shaken for about 3 h to equilibrate the contents; 25 ml of 3-mercaptopropyltrimethoxysilane was added and the mixture was heated under reflux for 1 h. The product was filtered through a glass sinter, washed several times with hexane and finally with acetone and was then dried in a vacuum oven.

20 g of the dried product was transferred to a beaker containing 100 ml of 1 *M* sulphuric acid and stirred magnetically. A filtered, saturated, solution of potassium permanganate in 1 *M* sulphuric acid was then slowly added until in slight excess. The excess permanganate was removed by adding a saturated solution of oxalic acid in 1 *M* sulphuric acid until the solution became colourless. The resulting cation-exchange material was filtered, washed several times with 0.1 *M* nitric acid and then with water until the washings were neutral. After a final wash with acetone the product was vacuum dried.

The ion-exchange capacity of the product was determined by titrating with 0.1 *M* sodium hydroxide, the product having been suspended in a 5% solution of sodium chloride. The level of organic material was determined by ashing at 600°.

The chromatographic properties of the packing material were determined by packing it into 25 cm × 5 mm I.D. stainless-steel columns terminated with ZDV (Zero Dead Volume) endfittings (1/4 to 1/16 in.). The separation of solutions of phenothiazines was studied using methanol–aqueous ammonium nitrate mixtures of different ionic strength, pH, and ratios of organic to aqueous phase. The eluent was monitored at 254 nm using a variable wavelength UV detector (Cecil 212, Cecil Instruments, Cambridge, Great Britain). Injections were made using a stop-flow injection technique.

RESULTS AND DISCUSSION

The reaction described above was found to be reproducible, giving rise to a mercapto bonded-phase material with about 10.5% organic loading. The resulting cation-exchanger was found to contain about 9.1% of organic loading and had an ion-exchange capacity of about 0.5–0.6 mequiv./g. The idealised reaction (assuming a non-polymerised mercapto layer) can be considered to be:

This reaction proceeds rapidly at room temperature.

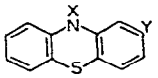
The ion-exchange capacity of the material was very similar to that obtained by the sulphonation of a 2-phenylethyl bonded-phase silica[2]. However, by using additional water in the primary reaction with the mercaptopropyl silylating reagent, it is possible to build up a thicker layer of organic material on the silica and it is to be expected that this would, in turn, give rise to ion exchangers having a greater capacity. Another unusual feature of the preparation is that it produces an aliphatic sulphonic acid, whereas virtually all the other methods of making strong cation-exchangers produce a sulphonated aromatic species. Although no direct comparison has been made, it is possible that the packing material described here will give slightly different separations from those achieved on packings containing aromatic sulphonic

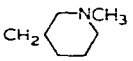
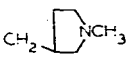
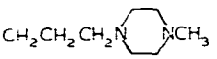
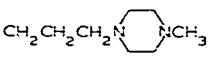
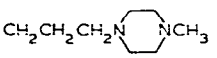
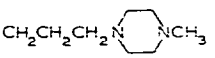
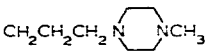
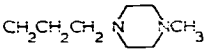
acids; for few separations rely solely on one mechanism and, if hydrophobic interactions play a part in addition to simple ion-exchange, it is to be expected that an *n*-propyl chain and an aromatic ring system will display different lipophilic properties.

The chromatographic properties of the cation exchanger were good. Column efficiencies for phenothiazines measured at 1 ml/min ranged from $N = 7000$ to 11,000 depending on factors such as the phenothiazine used for measurement, and the solvent composition. Thus the plate heights, *i.e.* from 0.036 tp 0.022 mm, compare very favourably with those achieved on other cation exchangers. The retention time data for various phenothiazines (see Table I for the chemical structures), as a function of pH, ionic strength and the methanol content of the eluent, are shown in

TABLE I

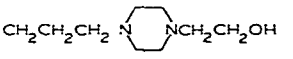
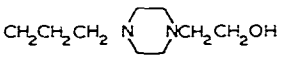
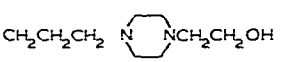
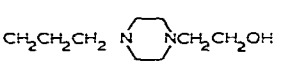
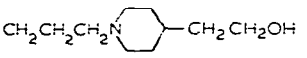
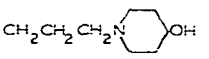
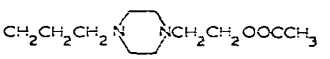
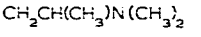
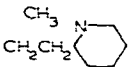
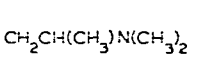
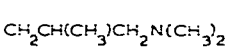
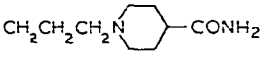
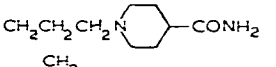
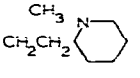
STRUCTURES OF THE PHENOTHIAZINES STUDIED, ARRANGED IN THEIR ELUTION SEQUENCE



Phenothiazines	*X*	*Y*
Parent compounds		
Diethazine	$CH_2CH_2N(C_2H_5)_2$	H
Proquamazine	$CH_2CH[N(CH_3)_2]CH_2N(CH_3)_2$	H
Trimeprazine	$CH_2CH(CH_3)CH_2N(CH_3)_2$	H
Pecazine		H
Dimethoxanate	$COOCH_2CH_2OCH_2CH_2N(CH_3)_2$	H
Promazine	$CH_2CH_2CH_2N(CH_3)_2$	H
Methdilazine		H
Perazine		H
Derivatives of perazine		
Trifluperazine		CF_3
Thiethylperazine		SCH_2CH_3
Butaperazine		$COCH_2CH_2CH_3$
Prochlorperazine		Cl
Thioperazine		$SO_2N(CH_3)_2$

(Continued on p. 266)

TABLE I *(continued)*

Phenothiazines	*X*	*Y*
Derivatives of promazine		
Trifluprομazine	$CH_2CH_2CH_2N(CH_3)_2$	CF_3
Chlorpromazine	$CH_2CH_2CH_2N(CH_3)_2$	Cl
Methoxypromazine	$CH_2CH_2CH_2N(CH_3)_2$	OCH_3
Acetylpromazine	$CH_2CH_2CH_2N(CH_3)_2$	$COCH_3$
Derivatives of phenazine		
Fluphenazine		CF_3
Perphenazine		Cl
Carphenazine		$COCH_2CH_3$
Acetophenazine		$COCH_3$
Miscellaneous phenothiazines		
Piperacetazine		$COCH_3$
Pericyazine		CN
Thiopropazate		Cl
Dimethothiazine		$SO_2N(CH_3)_2$
Thioridazine		SCH_3
Propiomazine		$COCH_2CH_3$
Methotrimeprazine		OCH_3
Pipamazine		Cl
Metopimazine		SO_2CH_3
Mesoridazine		SCH_3 ↓ O

Tables II–IV. These variables, together with temperature, which was not studied, are the major factors influencing ion-exchange separations. Ammonium nitrate was used to vary the ionic strength although this is not really an ideal material, for the nitrate ion displays appreciable UV absorption and is a poor buffering agent. Nevertheless, it has a high solubility in methanol which is not the case with phosphate buffers.

TABLE II

THE INFLUENCE OF ELUENT pH ON THE RETENTION OF PHENOTHIAZINES ON AN ALKYL SULPHONIC ACID STRONG CATION EXCHANGER

Eluent: Methanol–1 *M* ammonium nitrate (9:1). The ammonium nitrate solution was adjusted to the pH shown before dilution with methanol. The solvent was pumped at 1 ml/min.

Phenothiazines	*Elution volume (ml)*			
	pH 10	*pH 8*	*pH 6*	*pH 4*
Parent compounds				
Diethazine	—	8.7	9.8	9.5
Proquamazine	5.0	9.3	9.9	10.1
Trimeprazine	—	8.7	10.1	9.9
Pecazine	4.2	9.9	11.2	11.2
Dimethoxanate	4.8	10.8	11.7	11.9
Promazine	4.4	10.9	12.0	12.3
Methdilazine	5.2	12.0	12.6	13.2
Perazine	4.2	9.6	15.0	16.3
Derivatives of perazine				
Trifluperazine	4.0	7.3	9.2	11.9
Thiethylperazine	—	8.7	11.1	11.9
Butaperazine	—	8.0	11.1	11.9
Prochlorperazine	—	8.7	11.9	13.2
Thioperazine	3.8	9.1	12.2	13.6
Derivatives of promazine				
Triflupromazine	—	8.2	9.0	9.2
Chlorpromazine	—	9.6	10.6	11.0
Methoxypromazine	4.2	10.3	11.2	11.2
Acetylpromazine	4.4	10.9	11.8	12.0
Derivatives of phenazine				
Fluphenazine	3.8	5.7	6.7	9.0
Perphenazine	—	6.4	8.8	10.3
Carphenazine	—	6.4	8.9	9.6
Acetophenazine	—	6.7	9.6	10.9
Miscellaneous phenothiazines				
Piperacetazine	—	7.2	7.8	8.1
Pericyazine	—	7.6	9.2	9.8
Thiopropazate	—	7.5	9.6	9.6
Dimethothiazine	—	7.4	9.6	10.6
Thioridazine	4.7	9.5	9.7	10.2
Propiomazine	—	8.4	9.7	9.7
Methotrimeprazine	—	8.4	9.7	9.6
Pipamazine	—	7.9	10.4	10.9
Metopimazine	4.1	9.6	11.1	13.7
Mesoridazine	4.9	12.6	13.6	13.9

The data in Table II show that the retention of all the phenothiazines is strongly pH-dependent over the range 10–4 and is at a maximum in the region of pH 6–4. This type of behaviour is what would be expected of a strongly basic group of compounds. In addition to causing greater retention, increasing the acidity of the eluent also gives rise to minor changes in elution order.

The tabulation sequence for each group in Table I is identical with the

elution sequence of the drugs at pH 6.0 and it enables some correlation to be made between the nature of the chemical substituents and the degree of retention. For the parent compounds, the diversity of substituents in the 10-position (*i.e.* on the tertiary nitrogen) prevents any simple conclusions, although the long retention time associated with the piperazinyl side-chain is notable. Substitution of the phenothiazine ring in the 2-position gives rise to compounds which elute more rapidly than the parent drugs, and from the limited range of compounds available, the influence of each substituent group on the retention decreases in the following order: $-H > -COCH_3 > -SO_2N(CH_3)_2 > -OCH_3 > -COCH_2CH_3 > -Cl > -SCH_2CH_3 > -COCH_2CH_2CH_3 > -CF_3$. In the absence of suitable compounds it was not possible to assign methylthio, methylsulphonyl and methylsulphinyl groups into the above sequence although the miscellaneous compounds yield the following information: $-SCH_3 > -\overset{\downarrow}{\underset{O}{S}}CH_3$ and $SO_2CH_3 > -Cl$.

As would be expected of an ion-exchange process, a decreasing ionic strength leads to longer retention of all compounds and this pattern of elution can be observed in Table III.

TABLE III

THE INFLUENCE OF ELUENT IONIC STRENGTH ON THE RETENTION OF PHENOTHIAZINES ON AN ALKYL SULPHONIC ACID STRONG CATION EXCHANGER

The eluent used consisted of methanol–aqueous ammonium nitrate pH 6.0 (80:20) and was pumped at 1 ml/min.

Compound	*Elution volume (ml) at various ammonium nitrate concentrations*			
	1.0 M	*0.5 M*	*0.25 M*	*0.125 M*
Trifluperazine	6.7	10.0	15.8	26.0
Perazine	8.3	13.2	21.6	37.0

The effect of varying the methanol content of the eluent is shown in Table IV. It gives rise to some complex phenomena. Not only does the proportion of methanol influence the retention of the phenothiazines, it also acts on the peak shape

TABLE IV

THE INFLUENCE OF THE METHANOL CONTENT OF THE ELUENT ON THE RETENTION OF PHENOTHIAZINES ON AN ALKYL SULPHONIC ACID STRONG CATION EXCHANGER

The aqueous portion of the eluent was 0.5 *M* ammonium nitrate adjusted to pH 6.0. The eluent was pumped at 1 ml/min.

Compound	*Elution volume (ml) at various methanol concentrations*				
	90%	*80%*	*70%*	*60%*	*40%*
Triflupromazine	12.0	8.6	7.0	9.2	9.8
Promazine	15.6	10.7	8.4	10.4	11.0
Perazine	24.2	13.2	9.8	13.8	14.8
Pressure drop (p.s.i.)	1350	1650	1900	2350	2300
Number of theoretical plates, *N* (for promazine)	10,400	7500	5781	600	670

and the background UV absorption. Most of the drugs can be efficiently eluted at methanol concentrations in the range 90–70% but at lower concentrations appreciable peak-broadening occurs, presumably because of the low water-solubility of the free base form of the drugs. In the case of perazine and its derivatives there was some tendency for double peaking to occur at the higher methanol concentration, but a good peak shape could be attained with eluents containing 80–70% of methanol.

With so many variables it is almost impossible to assign optimum separation conditions for the phenothiazines as a whole but some typical results are shown in Figs. 1–3. In Fig. 1 it can be seen that several of the drugs having different substituents in the 10-position are separable. Figs. 2 and 3 illustrate the separation of a series of promazine and perazine derivatives. Columns packed with the alkyl sulphonic acid cation exchanger were found to be stable in performance and provide a useful means of separating phenothiazines.

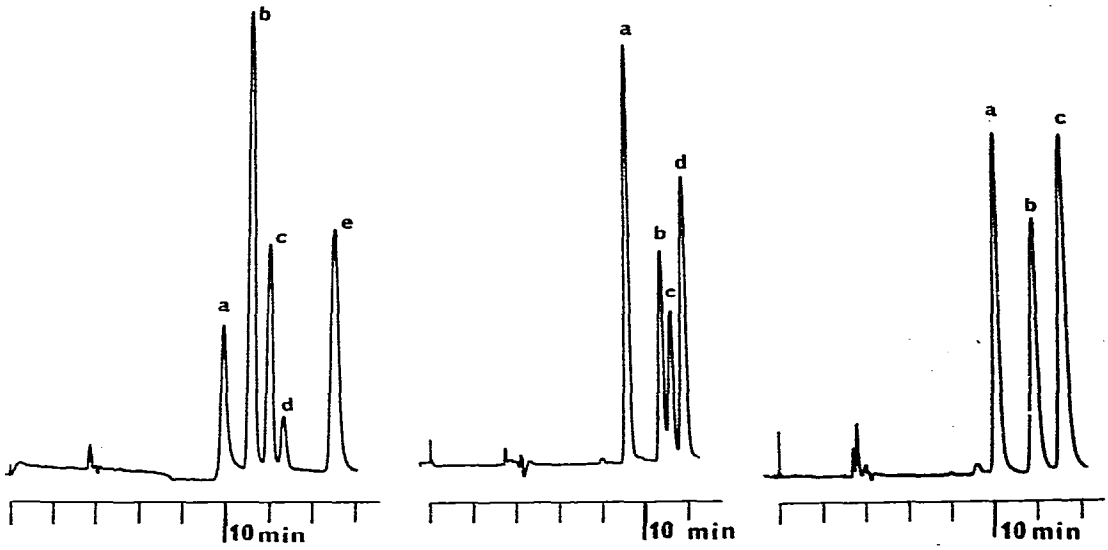


Fig. 1. A separation of phenothiazines on an alkyl sulphonic acid strong cation exchanger. Column: 25 cm × 0.5 cm I.D.; eluent: methanol–1 *M* ammonium nitrate (pH 6.0) (9:1); flow-rate: 1 ml/min; pressure: 1350 p.s.i. Detection: UV at 254 nm. Compounds: a = proquamazine; b = pecazine; c = promazine; d = methdilazine; e = perazine.

Fig. 2. A separation of promazine and related compounds on an alkyl sulphonic acid strong cation exchanger. Conditions as in Fig. 1. Compounds: a = triflupromazine; b = chlorpromazine; c = methoxypromazine; d = promazine.

Fig. 3. A separation of perazine and related compounds on an alkyl sulphonic acid strong cation exchanger. Column: 25 cm × 0.5 cm I.D.; eluent: methanol–0.5 *M* ammonium nitrate (pH 6.0) (4:1); flow-rate: 1 ml/min; pressure: 1750 p.s.i. Detection: UV at 254 nm. Compounds: a = trifluperazine; b = butaperazine; c = perazine.

ACKNOWLEDGEMENT

Some of the practical work in this project was carried out by my assistant Paul Worts and his help is gratefully acknowledged.

REFERENCES

1 G. B. Cox, *J. Chromatogr. Sci.*, 15 (1977) 385.
2 G. B. Cox, C. R. Loscombe, M. J. Slucutt, K. Sugden and J. A. Upfield, *J. Chromatogr.*, 117 (1976) 269.
3 M. Caude and R. Rosset, *J. Chromatogr. Sci.*, 15 (1977) 405.
4 B. B. Wheals and I. Jane, *Analyst (London)*, 102 (1977) 1218.
5 A. Pryde and M. T. Gilbert, *Applications of high-performance liquid chromatography*, Chapman and Hall, London, 1979.
6 W. F. Heyes and J. R. Salmon, *J. Chromatogr.*, 156 (1978) 309.
7 A. G. Butterfield and R. W. Sears, *J. Pharm. Sci.*, 66 (1977) 1117.