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PREPARATION OF OPTICALLY ACTIVE ION-EXCHANGE RESINS AND RESOLUTION OF RACEMATES

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SUMMARY

Optically active anion-exchange resins based on a porous, low-crosslinked styrene–divinylbenzene copolymer matrix were synthesized and were used in the frontal and displacement chromatographic resolution of *dl*-racemates of sodium mandelate and mandelic acid. The synthetic aspects and the results of the resolution experiments are discussed in the light of the nature of the functional group and the matrix of the optically active resin. The nature of the matrix of the resin was found to play a significant role in resolution.

INTRODUCTION

Many mixtures that are difficult to separate by conventional methods can be separated successfully by ion-exchange techniques[1,2] and these techniques have been extended to the separation of optically active isomers from racemates.

An active stationary phase for the resolution of *dl*-racemates should be asymmetric and have a functional group to bind the molecules of the racemates. Dalgliesh[3] used cellulose as the stationary phase in the resolution of some amino acids by paper chromatography and postulated that an amino acid, in order to be resolved, must establish a "three-point contact" with the cellulose. The first attempt by Bunnett and Marks[4] to separate racemates with an optically active ion-exchange resin, prepared by condensing D- and L-forms of β-(*p*-hydroxyphenyl)butyric acid or N-*p*-toluenesulphonyl-L-tyrosine with phenol and formaldehyde failed to bring about any detectable resolution of racemates.

Later, attempts were made to modify the available ion-exchange resins by incorporating optically active alkaloids such as quinine, and the partial resolution of mandelic acid was reported[5–7]. Other investigators treated chloromethylated polystyrene resins with optically active tertiary amines to synthesize optically active strongly basic anion-exchange resins; Suda and Oda[8] used brucine for this purpose, while Lott and Rieman[9] used (—)-N,N-dimethyl-α-phenylethylamine. Most of the optically active resins that have been synthesized are based on polystyrene[5,6,8,9], owing to the

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flexibility offered by the matrix. This paper describes the preparation of optically active anion-exchange resins based on styrene–divinylbenzene porous polymers and resolution of racemates (sodium mandelate and mandelic acid).

EXPERIMENTAL

Preparation of polymers

The polymers used were prepared as described by Renganathan *et al.*[10]. The fractional number refers to the fraction of the monomer in its solution in the diluent; A and E refer to amyl alcohol and Essotherm-500 oil, respectively, used as the diluent, and the percentage of crosslinking with divinylbenzene is represented by X. A styrene–divinylbenzene mixture was polymerized in the presence of a diluent with poly(vinyl alcohol) as a suspension stabilizer in water and with benzoyl peroxide as the initiator.

Preparation of resins

MARP 10X Ep. This weakly basic anion-exchange resin was prepared by condensing chloromethylated styrene–divinylbenzene copolymer[10] (0.6 A MARP 10X, −40 to +60 B.S.S.) with *l*-ephedrine at 40° for 8 h using dioxan as the solvent.

MARP 2X Bru. Chloromethylated styrene—divinylbenzene copolymer[10] (0.5 E MARP 2X, −40 to +60 B.S.S.) was condensed with (—)-brucine in dimethylformamide at 50–60° for 12 h to give a strongly basic anion-exchange resin.

MARP 2X Me Gl. This resin was prepared by aminating a chloromethylated styrene–divinylbenzene copolymer[10] (0.5 E MARP 2X, −40 to +60 B.S.S.) with D(−)-N-methylglucamine using dimethylformamide as the solvent at 90° for 3 h.

Capacity and nitrogen content of the resins

The ion-exchange capacity of the resins was determined after conditioning by following Kunin's procedure[11]. The nitrogen content of the samples was determined by Kjeldahl's method[12] (see Table I).

Resolution of racemates

Frontal chromatographic analysis. A glass column provided with a sintered disc support and a stopcock was filled to a known height with the free base or chloride form of the resin. The column was fixed on a stand provided with a fraction collector. Mandelic acid or sodium mandelate of known concentration was passed through the bed from an aspirator bottle (kept at a suitable height) until the concentration of the effluent was the same as that of influent. Fractions of 3 ml, at a desired flow-rate, were collected and analyzed for the optically active component by using a polarimeter and for the total concentration by using a spectrophotometer.

The exhausted resin was washed thoroughly with distilled water and mandelate was eluted by passing sodium chloride solution of a specified concentration through the column. Fractions of 3 ml, collected at the desired flow-rate, were analyzed. The elution was continued until the absorbance at 258 nm ($\lambda_{max.}$ of mandelate) was zero. The results of the frontal chromatographic analysis are given in Table II.

Displacement chromatographic analysis. The resin (chloride form) was packed in a glass column and a known amount of sodium mandelate (*ca.* 1/10th of the column capacity) was added carefully without disturbing the bed. The sodium mandelate

was displaced by passing 0.1 *N* hydrochloric acid, 1.0 *N* sodium chloride solution or 1.0 *N* potassium nitrate solution through the column, depending upon the resin used. Fractions of 3 ml, collected at a flow-rate of 0.4 ml/cm^2·min, were analyzed, as described for frontal analysis. When potassium nitrate solution was used as the displacing agent, corrections for the absorbance of nitrate ion at 258 nm were made by means of a calibration graph (see Table II).

Methods of analysis

Determination of the concentration of optically active component in partially resolved racemates. A 5-cm polarimeter tube (2-ml capacity) was filled with the solution and placed in a Zeiss polarimeter provided with a circular scale reading to 0.01°. The angle of rotation was measured and the concentration of optically active component was calculated using the standard equation[13].

Determination of total sodium mandelate or mandelic acid concentration using a spectrophotometer. To determine the unknown concentration of sodium mandelate or mandelic acid, the solution was diluted approximately with distilled water and its absorbance was determined at 258 nm using a Hilger Uvispek spectrophotometer. The concentration was then calculated from the extinction coefficient determined with standard solutions[12].

RESULTS AND DISCUSSION

Table I gives the nitrogen contents and capacities of the anion-exchange resins prepared for optical resolution. The values for the theoretical nitrogen content were calculated on the assumption of 100% condensation of the amines used with the chloromethylated copolymer.

TABLE I

PROPERTIES OF ANION-EXCHANGE RESINS PREPARED FOR RESOLUTION OF RACEMATES

Resin	*Nitrogen content (%)*		*Capacity (mequiv./g)*		
	Theoretical	*Observed*	*Theoretical (calculated on 100% condensation)*	*Calculated on observed nitrogen content*	*Experimentally determined*
MARP 10X Ep	4.38	1.25	3.13	0.89	0.91
MARP 2X Bru	5.12	1.99	1.83	0.71	0.73
MARP 2X Me Gl	4.02	3.50	2.89	2.50	2.17

The estimated nitrogen contents in the anion-exchange resins reveal that with N-methylglutamine, *l*-ephedrine and (−)-brucine the condensation proceeded to the extent of 87, 39, and 28.5%, respectively. On this basis, it is also observed that the capacity determined experimentally and that calculated from the actual nitrogen content agrees reasonably well. It is felt that the degree of condensation can possibly be increased by employing more rigorous amination conditions, leading to anion-exchange resins of higher capacity.

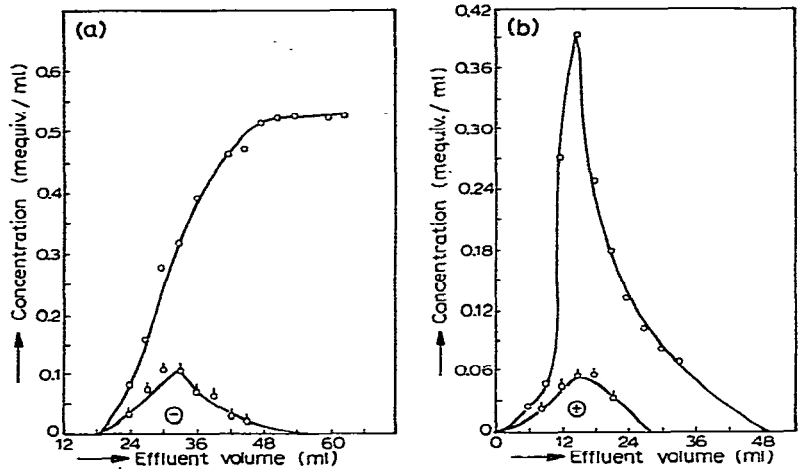

Fig. 1. Frontal chromatographic resolution of sodium mandelate by MARP 10X Ep. (a) Exhaustion cycle; (b) elution cycle. In each instance, the upper curve represents total concentration and the lower curve optically active concentration ($\times 10$).

When a solution of racemic mandelate was passed through the chloride form of the resin, the mandelate ion displaced the chloride ion from the resin and, after breakthrough, the concentration of mandelate ion in the effluent increased sharply and approached the influent concentration. With MARP 10X Ep it was observed

TABLE II

RESULTS OF FRONTAL AND DISPLACEMENT CHROMATOGRAPHIC ANALYSES

Ambient temperature during experiments: 28 ± 1°.

Parameter	*Frontal analysis*				*Displacement analysis*	
	MARP 10X Ep	*MARP 2X Me Gl*			*MARP 10X Ep*	*MARP 2X Bru*
		Expt. No. 1	*Expt. No. 2*	*Expt. No. 3*		
Height of column (cm)	47.8	41.5	41.5	35.0	43.5	56.7
Cross-sectional area (cm^2)	0.56	0.56	0.56	0.56	0.56	0.56
Column capacity (Q_T) (mequiv.)	13.67	14.46	14.46	14.46	11.78	16.21
Concentration of racemate	0.5 *N**	0.1 *N**	0.1 *N**	0.1 *N***	1.18 mequiv.*	1.46 mequiv.*
Flow-rate ($ml/cm^2 \cdot min$)	0.2	0.2	0.4	0.4	0.4	0.4
Form of resin	Cl^-	Cl^-	Cl^-	Free base	Cl^-	Cl^-
Eluting/displacing agent	1.0 *N* NaCl	1.0 *N* NaCl	1.0 *N* NaCl	1.0 *N* NaCl	0.1 *N* HCl	1.0 *N* KNO_3
Optically active isomer in exhaustion cycle (μmole)	(−) 133	(+) 90	(+) 164	(+) 127	—	—
Optically active isomer in elution cycle (μmole)	(+) 59	(+) 69	(+) 136	(−) 133	(−) 66 (+) 64	(−) 126 (+) 99
E based on (+)	1.024	—	—	1.029	1.006	1.014
E based on (−)	1.047	—	—	1.025	1.011	1.019

* Sodium mandelate.

** Mandelic acid.

that in the exhaustion cycle, fractions with definite negative rotation were obtained. Seven fractions containing 150, 274, 320, 390, 425, 465 and 475 μmole of sodium mandelate with 9.32, 7.66, 6.25, 3.58, 1.53, 1.26 and 0.857% optical purity, respectively, were isolated. The elution cycle gave five fractions with positive rotation containing 42, 270, 393, 250 and 180 μmole of sodium mandelate with 10, 3.3, 2.5, 4.4 and 3.31% optical purity, respectively. The exhaustion and elution cycles are presented in Fig. 1.

The selectivity coefficient for a resin in resolution experiments is defined by the equation[9].

$$E = \frac{Q_T + \Delta L}{Q_T - \Delta L}$$

where Q_T = column capacity and ΔL = excess of one isomer over the other in milli-equivalents.

The values for MARP 10X Ep (Table II) are E [based on (−)] = 1.047 and E [based on (+)] = 1.024, which are in reasonable agreement with the results reported for a phenylethylamine condensed resin[9]. It is observed from the data in Table II that there is discrepancy between the total positive and negative isomers found. This is in accordance with the work of Lott and Rieman[9], and is attributed to small errors in the polarimeter readings.

Fig. 2 shows how the cumulative optical purity decreased with increase in the amount of mandelate ion emerging from the column. The term cumulative optical purity at any given point means the optical purity that would be found if all of the mandelate collected in the effluent up to this point were mixed and examined, and is easily calculated from the analysis of individual fractions of the effluent[9]. The abscissa

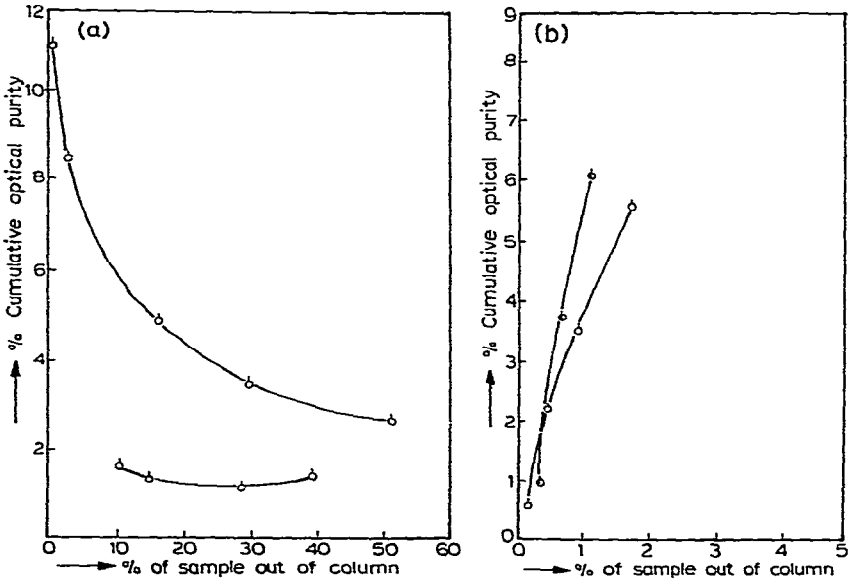

Fig. 2. Cumulative optical purity. (a) Frontal chromatography: upper curve, MARP 10X Ep; lower curve, MARP 2X Me Gl. (b) Displacement chromatography: ○, MARP 10X Ep; ◐, MARP 2X Bru.

in Fig. 2 shows the amount of mandelate ion (+-antipode for MARP 2X Me Gl and —-antipode for MARP 10X Ep during the exhaustion cycle) emerging from the column, expressed as a percentage of the "sample" of racemic mandelate used in the experiment. As the exhaustion cycle was continued until the column became saturated (complete conversion of the resin into the mandelate form), the "sample" is equal to the total column capacity in milliequivalents.

With MARP 10X Ep, the cumulative optical purity decreased sharply up to 15% of "sample" in the effluent. It can be concluded that fractions enriched with a relatively high concentration of one isomer can be obtained shortly after the breakthrough of mandelate.

The results of two displacement chromatographic analyses with MARP 10X Ep and MARP 2X Bru are presented in Figs. 3 and 4, respectively. The pattern obtained with the former resin is in accordance with the findings of Manecke and Lamer[14]. Further, the cumulative optical purity with the ephedrine condensed resin is found to increase with increase in the percentage of mandelate in the effluent (Fig. 2), probably because, unlike in frontal experiments, the rate of exchange of mandelate with the anion (Cl^-) of the resin is greater. This possibility fulfils the prerequisite of better contact of the racemate molecules with the optically active resin.

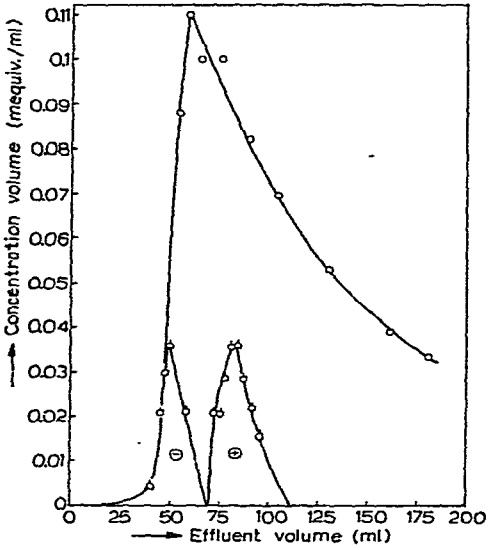

Fig. 3. Displacement chromatographic resolution of sodium mandelate by MARP 10X Ep. Upper curve, total concentration (×10); lower curves, optically active concentration (×10).

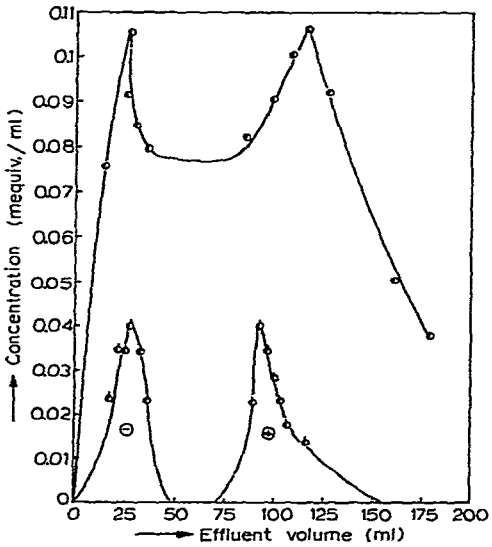

Fig. 4. Displacement chromatographic resolution of sodium mandelate by MARP 2X Bru. Upper curve, total concentration (×10); lower curves, optically active concentration (×5).

With MARP 2X Bru resin (Fig. 4), seven fractions of mandelate with negative rotation of 50–75% optical purity were obtained, followed by seven fractions with positive rotation of 22–77% optical purity. This resin was observed to give well separated bands of positive and negative isomers (Fig. 4) compared with MARP 10X Ep (Fig. 3), and this improved efficiency of resolution can be attributed to the nature

of the functional group, the eluting agent, the matrix in which the alkaloid is incorporated and the column capacity. Contrary to reported evidence of superior properties of a weakly basic anion-exchange resin[15], in this investigation the strongly basic resin MARP 2X Bru proved to be superior, possibly owing to the low-crosslinked porous matrix, which permits facile diffusion and hence better contact of racemic molecules with the asymmetric centre.

An interesting observation is that MARP 10X Ep performed better than a phenylethylamine condensed resin[9,15]. This result is probably due to the presence of an additional –CHOH group in ephedrine compared with phenylethylamine, affording more discriminating interactions with a racemic molecule[16].

With MARP 2X Me Gl resin, mandelic acid was partially resolved (Fig. 5) into its antipodes and the cumulative optical purity initially decreased but remained steady thereafter. This result suggests that this resin can be used with advantage for enriching fractions with the same percentage of cumulative optical purity. However, with sodium mandelate in both frontal and displacement experiments, only the positive isomer could be isolated with this resin. It was felt that as the column was kept suspended for some time after the resolution of mandelic acid, some bacteria might have grown and consumed one of the isomers[17]. This effect was supported by the absence of a material balance.

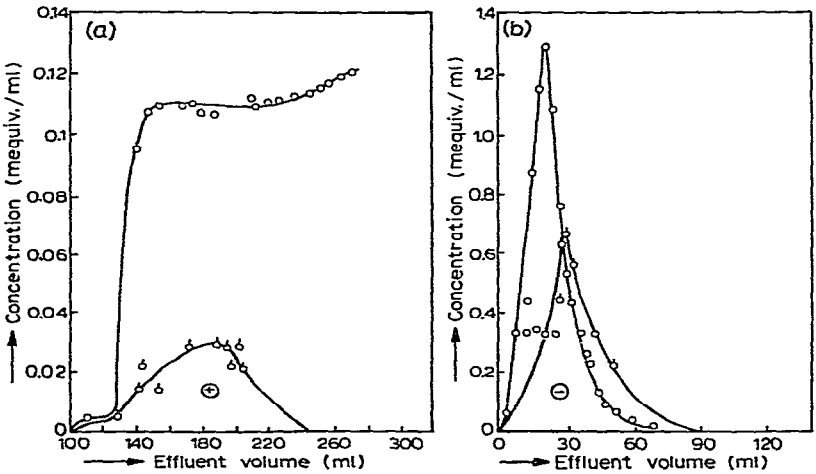

Fig. 5. Frontal chromatographic resolution of mandelic acid by MARP 2X Me Gl. (a) Exhaustion cycle; (b) elution cycle. In each instance, the upper curve represents total concentration and the lower curve optically active concentration (×10 in (a) and ×100 in (b)).

CONCLUSION

The results were confirmed by repeating these experiments five times and are in agreement with the data reported in the literature, thus confirming the possibility of partially resolving racemates through optically active ion-exchange resins. However, an important inference is that the nature of the matrix in which the optically

active component of the resin is incorporated plays a significant role in determining the efficiency of resolution, and this aspect has not been reported in the literature so far.

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