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Retention of basic drugs on porous polymers in high-performance liquid chromatography

Barbara Gawdzik

Faculty of Chemistry, Maria Curie Skłodowska University, Pl. M. Curie-Skłodowskiej 3, 20031 Lublin (Poland)

ABSTRACT

The behaviour of basic drugs on two polymeric high-performance liquid chromatographic columns in the presence of alkaline mobile phases was studied. The packings were PRP-1 and di(methacryloyloxymethyl)naphthalene–divinylbenzene copolymer. Using the alkyl aryl ketone scale, the retention indices for ephedrine, clonidine, lidocaine, benzocaine, procaine, propranolol, diazepam, strychnine, cyproheptadine, amitriptyline and promethazine were calculated. To determine the influence of the pH of the mobile phase on the retention of these compounds, peak asymmetry factors and reduced plate heights were measured.

INTRODUCTION

In recent years there has been growing interest in polymeric materials for use in reversed-phase high-performance liquid chromatography (HPLC), the most popular being macroporous styrene–divinylbenzene copolymers [1–9]. Unfortunately, polymeric sorbents are characterized by some important drawbacks such as swelling in strong organic solvents owing to the presence of micropores in the polymeric matrix and low column efficiencies [8,9]. On the other hand, porous polymers are stable with eluents over the whole pH range [10]. This important feature permits the direct separation of basic compounds at high pH.

Commonly used alkyl-bonded silica cannot be employed for the chromatography of amines in an uncharged from because the pH limit of silica is about 8 and many of these supports possess strongly adsorptive properties. To diminish these effects, deactivating agents such as aliphatic amines were added to the eluent [11] or eluents containing a high proportion of an organic component [12] and different buffers were applied [13–16]. In spite of this, limited selectivity and poor peak shapes were often achieved.

According to Jane [17], the retention of basic compounds is predominantly controlled by the analyte pK_a. To determine them in a non-ionized form, a mobile phase with $pH - pK_a > 2$ should be used [18]. For this reason, PRP-1 and Amberlite XAD-2 porous copolymers have been applied for the determination of basic drugs in alkaline eluents [18,19].

In this work, a comparison of two polymeric columns in the reversed-phase HPLC separation of basic drugs was made. The retention of basic drugs was studied on a column packed with a copolymer of two cross-linking agents, di(methacryloyloxymethyl)naphthalene and divinylbenzene (DMN–DVB), whose properties were studied previously [20–22], and on a PRP-1 styrene–divinylbenzene column (Hamilton, Reno, NV, USA). Methanol–aqueous sodium hydroxide (90:10) was employed according to De Biasi *et al.* [18] as the optimum mobile phase for reasonably short retention times of the studied basic drugs on the PRP-1 column.

Using mobile phases of different pH (8.9, 10.3, 11.5 and 12.5), the effect of pH on retention data and peak symmetry was determined.

In order to determine the selectivities of the columns, retention indices for the studied basic drugs based on the alkyl aryl ketone scale were calculated.

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EXPERIMENTAL

Chemicals and mobile phases

Methanol of HPLC grade was obtained from Merck (Darmstadt, Germany) and sodium hydroxide of laboratory-reagent grade from POCh (Gliwice, Poland). Mobile phases were made up by volumetric mixing of methanol with 0.001, 0.005, 0.05 and 0.25 *M* sodium hydroxide solution, prepared from sodium hydroxide pellets. The pH of the mobile phases was measured with a PHM-64 pH meter (Radiometer, Copenhagen, Denmark). All mobile phases were filtered through suitable filters and degassed by agitation in an ultrasonic bath and kept under a weak stream of helium. Alkyl aryl ketones were of laboratory-reagent grade from a range of sources.

Basic drugs were kindly provided by the Medical Academy in Lublin, Poland. Except for benzocaine, diazepam and strychnine, they were in a form of hydrochlorides. Sample solutions were prepared at a concentration of *ca.* 0.1 mg/ml by dissolving the bases in methanol–water (90:10, v/v) [18]. Sodium nitrate (30 mg/ml) in the same mobile phase was used as a void volume marker [23].

HPLC equipment

Chromatographic measurements were carried out using an HPLC system consisting of a Techma-Robot (Warsaw, Poland) Model 302 syringe pump fitted with a Rheodyne (Cotati, CA, USA) Model 7125 injection valve equipped with a 10-μl sample loop and a Laboratórni Přístroje (Prague, Czechoslovakia) LCD 2563 UV–visible detector set at 254 nm, [16,23] and 0.04 a.u.f.s.

The columns used were a 150 × 4.1 mm I.D. column packed with PRP-1 (5 μm) and a 100 × 4 mm I.D. column packed with DMN–DVB porous copolymer (3–10 μm). The columns were maintained at 30°C in a circulating water-bath. The measuring cell of the detector was also water thermostated at 30°C.

TABLE I

PEAK ASYMMETRY FACTORS (A_s) AND REDUCED PLATE HEIGHTS (h) FOR BASIC DRUGS MEASURED ON DMN–DVB AND PRP-1 COLUMNS

Column	Drug	pH 8.9		pH 10.3		pH 11.5		pH 12.5	
		A_s	h (μm)	A_s	h (μm)	A_s	h (μm)	A_s	h (μm)
DMN–DVB	Ephedrine	1.2	51	1.0	51	1.0	30	1.0	29
	Clonidine	1.0	32	1.0	35	1.0	31	1.0	23
	Lidocaine	1.2	57	1.0	37	1.0	35	1.0	31
	Benzocaine	1.0	38	1.0	39	1.0	35	1.0	29
	Procaine	1.7	71	1.5	71	1.2	35	1.2	34
	Propranolol	–	–	–	–	1.4	54	1.3	36
	Diazepam	1.8	95	1.8	95	1.6	92	1.3	84
	Strychnine	2.4	178	2.2	178	1.8	109	1.4	69
	Cyproheptadine	2.0	178	2.0	142	1.8	109	1.8	109
	Amitriptyline	–	–	1.7	142	1.0	71	1.0	51
	Promethazine	1.8	95	1.8	102	1.6	95	1.2	84
PRP-1	Ephedrine	2.0	186	2.0	171	1.2	37	1.0	30
	Clonidine	1.5	58	1.3	29	1.2	34	1.2	31
	Lidocaine	1.5	42	1.3	40	1.3	36	1.3	36
	Benzocaine	1.4	42	1.0	27	1.0	30	1.0	29
	Procaine	2.0	44	1.2	31	1.2	31	1.2	30
	Propranolol	–	–	2.5	64	1.6	61	1.5	52
	Diazepam	3.4	166	3.2	166	2.8	150	2.8	153
	Strychnine	3.4	230	3.0	162	2.7	142	2.5	136
	Cyproheptadine	3.4	214	2.8	187	1.8	166	1.7	150
	Amitriptyline	2.0	214	1.5	156	1.5	115	1.2	117
	Promethazine	3.0	187	2.0	142	1.8	100	1.4	109

Peaks were recorded on a Laboratórni Přístroje Model 4001 recorder. The recorded retention distances were the means of three determinations. The flow-rate of the mobile phase was 0.5 ml/min. At the end of each working day the column was flushed with methanol–water (90:10, v/v).

Titration

A 1-g amount of the DMN–DVB copolymer in 20 ml of 1 *M* NaCl and 10 ml of methanol was titrated potentiometrically using an automatic titrator set and PHM-64 digital pH meter (Radiometer); 0.01 *M* sodium hydroxide solution and 0.01 *M* hydrochloric acid were used for titration. For each point on the potentiometric titration curve, the equilibrium state with respect to the display of the pH meter was achieved.

Chromatographic measurements

Capacity factors (k') were calculated as $k' = (l_R - l_0)/l_0$, where l_R and l_0 are the retention distances on the chromatogram of the sample and a non-retained sample (sodium nitrate), respectively. Retention indices (I) derived from the alkyl aryl ketones from butyrophenone to heptanophenone were calculated as described previously [20,22]. Peak asymmetry factors (A_s) were measured at 10% of the peak height [24]. Reduced plate heights, $h = H/d_p$, where H is the theoretical plate height and d_p (μm) is the particle diameter, were calculated to compare column packings of different particle diameters [24].

RESULTS AND DISCUSSION

Accordding to De Biasi *et al.* [18], basic drugs can be successfully separated on a PRP-1 polymeric column using mobile phases of high pH and with a high percentage of the organic component. Following their work, the chromatographic behaviour of a range of basic drugs with methanol–aqueous sodium hydroxide solutions (90:10, v/v) as mobile phases was studied. In order to investigate te influence of pH on retention four mobile phase of different pH were used.

In Table I, peak asymmetry factors (A_s) and reduced plate heights (h) for the bases determined on the DMN–DVB and PRP-1 columns are summarized. At pH 8.9 on both columns significant asymmetry of the peaks is visible. The peak shapes improve with increasing pH of the mobile phase. As the pK_a values of the studied amines do not exceed 10 (Table II), in a mobile phase of pH 12.5 they should be in a non-ionized form and peak asym-

TABLE II

IONIZATION CONSTANTS OF BASIS DRUGS [23,25]

Drug	Ionization constant	Drug	Ionization constant
Ephedrine	9.6	Diazepan	3.3
Clonidine	8.2	Strychnine	2.3; 8.0
Lidocaine	7.9	Cyproheptadine	–[a]
Benzocaine	2.5	Amitryptyline	9.4
Procaine	9.0	Promethazine	9.1
Propranolol	9.5		

[a] Not available.

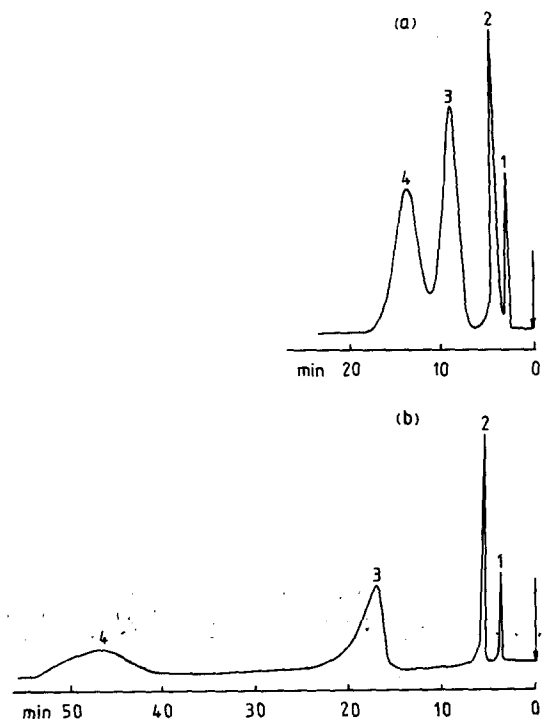

Fig. 1. Separation of basic drugs on polymeric columns: (a) DMN–DVB; (b) PRP-1. Peaks: 1 = ephedrine; 2 = benzocaine; 3 = strychnine; 4 = promethazine. Mobile phase, methanol–0.25 *M* NaOH (90:10, v/v); flow-rate, 0.5 ml/min; column inlet pressure, 17.4 MPa for DMN–DVB and 10.2 MPa for PRP-1; detection, 254 nm.

metry should disappear. Unfortunately, even at this pH significant asymmetry is observed, for cyproheptadine, strychnine and diazepam on the PRP-1 column and for cyproheptadine on the DMN–DVB column.

From a comparison of the chromatograms (Fig. 1), one can conclude that the efficiency of the PRP-1 column is much better but the values of the reduced plate heights are very similar. On both columns the reduced plate heights depend on the pH of the mobile phase, with the lowest values occurring at pH 12.5. Simultaneously, the efficiency of these columns decreases with increasing retention.

Tables III and IV give the capacity factors for the basic drugs and the alkyl aryl ketone standards used for calculation of retention indices. On both copolymers, changes in the pH of the mobile phase cause marked changes in the capacity factors of the basic drugs. Only diazepam and benzocaine behave exceptionally, their capacity factors remaining nearly constant independently of the mobile phase used. This lack of sensitivity to pH changes is due to their low ionization constants (Table II). For the other bases a decrease in the capacity factors is characteristic. On the PRP-1 column it occurs below pH 10.3 and on the DMN–DVB column below pH 11.5.

Changes in the pH of the mobile phase have a smaller influence on the k' values for alkyl aryl ketones. On the DMN–DVB column, an increase in pH causes a regular decrease in the k' values of alkyl aryl ketones but on the PRP-1 column their retentions are fairly constant. Such a behaviour of these copolymers results from their different chemical character. PRP-1 copolymer does not possess any functional groups whereas DMN–DVB copolymer has ester groups in a polymeric network and these groups are responsible for the sensitivity

TABLE III

CAPACITY FACTORS (k') AND RETENTION INDICES (I) FOR BASIC DRUGS OBTAINED ON THE DMN–DVB POLYMERIC COLUMN

Compound	pH 8.9		pH 10.3		pH 11.5		pH 12.5	
	k'	I	k'	I	k'	I	k'	I
Alkyl aryl ketones								
Acetophenone	1.18	841	1.16	856	1.14	854	1.09	854
Propiophenone	1.71	950	1.61	966	1.56	965	1.52	965
Butyrophenone	2.04	1002	1.80	1001	1.74	1001	1.69	1001
Valerophenone	2.82	1098	2.41	1099	2.30	1098	2.25	1098
Hexanophenone	3.98	1198	3.25	1200	3.10	1201	3.09	1203
Heptanophenone	5.67	1302	4.41	1301	4.13	1300	4.10	1299
Basic drugs								
Ephedrine	2.00	997	1.16	856	0.47	500	0.30	410
Clonidine	1.98	993	1.21	869	0.61	638	0.55	621
Lidocaine	0.98	788	0.80	733	0.65	673	0.63	653
Benzocaine	0.94	784	0.94	773	0.86	759	0.79	739
Procaine	6.56	1345	3.57	1230	0.94	792	0.90	784
Propranolol	–	–	–	–	1.45	940	1.25	901
Diazepam	2.43	1062	2.33	1088	2.18	1081	2.18	1086
Strychnine	16.33	1613	9.36	1552	2.82	1169	2.70	1158
Cyproheptadine	8.58	1426	4.86	1333	3.20	1213	3.06	1200
Amitriptyline	–	–	6.57	1434	3.50	1244	3.45	1241
Promethazine	8.24	1412	5.85	1395	3.73	1266	3.69	1263
Void volume for sodium nitrate (ml)	0.93		0.93		0.92		0.92	
Correlation coefficients								
Acetophenone–heptanophenone	0.9963		0.9934		0.9936		0.9937	
Butyrophenone–heptanophenone	0.9998		0.9999		0.9999		0.9998	

TABLE IV

CAPACITY FACTORS (k') AND RETENTION INDICES (I) FOR BASIC DRUGS OBTAINED ON THE PRP-1 POLYMERIC COLUMN

Compound	pH 8.9		pH 10.3		pH 11.5		pH 12.5	
	k'	I	k'	I	k'	I	k'	I
Alkyl aryl ketones								
Acetophenone	2.97	796	2.90	797	2.90	795	2.92	796
Propiophenone	5.00	922	4.85	921	4.83	921	4.90	921
Butyrophenone	7.00	1003	6.77	1002	6.71	1002	6.85	1002
Valerophenone	10.29	1096	10.00	1096	9.87	1097	10.18	1098
Hexanophenone	15.79	1199	15.49	1201	15.07	1201	15.54	1200
Heptanophenone	24.14	1301	23.39	1300	22.57	1300	23.53	1301
Basic drugs								
Ephedrine	2.80	782	1.09	562	0.69	449	0.65	435
Clonidine	2.58	766	1.19	583	1.10	557	1.07	556
Lidocaine	2.21	725	2.06	716	2.14	721	2.10	718
Benzocaine	1.53	636	1.55	619	1.37	615	1.39	619
Procaine	2.17	720	1.68	666	1.66	658	1.66	661
Propranolol	–	–	4.36	896	3.74	858	3.49	841
Diazepam	7.29	1013	7.27	1019	7.50	1023	7.25	1017
Strychnine	9.93	1087	6.29	985	6.36	989	6.11	975
Cyproheptadine	23.70	1297	18.07	1242	18.03	1241	18.20	1237
Amitriptyline	29.43	1356	21.27	1283	21.11	1278	21.37	1276
Promethazine	23.57	1296	18.42	1243	18.19	1242	18.15	1238
Void volume for sodium nitrate (ml)	1.18		1.18		1.18		1.18	
Correlation coefficients								
Acetophenone–heptanophenone	0.9987		0.9989		0.9988		0.9987	
Butyrophenone–heptanophenone	0.9997		0.9998		0.9998		0.9998	

of the copolymer to pH changes. The weakly acidic character of the DMN–DVB copolymer, confirmed by the titration curve (Fig. 2), increases the dissociation of basic drugs. Hence a mobile phase of higher pH than for the PRP-1 copolymer is required for the separation of bases in a non-ionized form. Nevertheless, the decrease in k' for basic drugs observed on the PRP-1 column also suggests the existence of weakly acidic groups in this copolymer.

On comparing the retention indices of the basic drugs (Figs. 3 and 4), differences in the retention mechanism on these two copolymers can be seen. On both copolymers, an increase in the mobile phase pH generally causes a decrease in retention indices. The retention indices of diazepam and benzocaine do not change with increase in pH. The other amines are more or less ionized, so their retention changes in comparison with alkyl aryl ketones. The influence of the mobile phase pH on the retention indices of basic drugs is especially noticeable with the DMN–DVB copolymer (S-shapes curves). In a mobile phase of pH 12.5, in which interactions of ion-exchange character disappear, the differences in copolymer selectivities are more visible. At this pH the retention indices of the basic drugs are greater on the DMN-DVB than on the PRP-1 column. This means that some specific interactions between the copolymer with ester functional groups and the solutes take place.

The results presented here indicate that the retention of basic drugs depends on the pH of the mobile phase but the mechanism is complex and involves the degree of ionization of the analytes and the chemical character of the polymeric phase. In mobile phases of high pH interactions of ion-exchange character disappear but the separation efficiency of basic drugs on both of the copolymers studied is still poor.

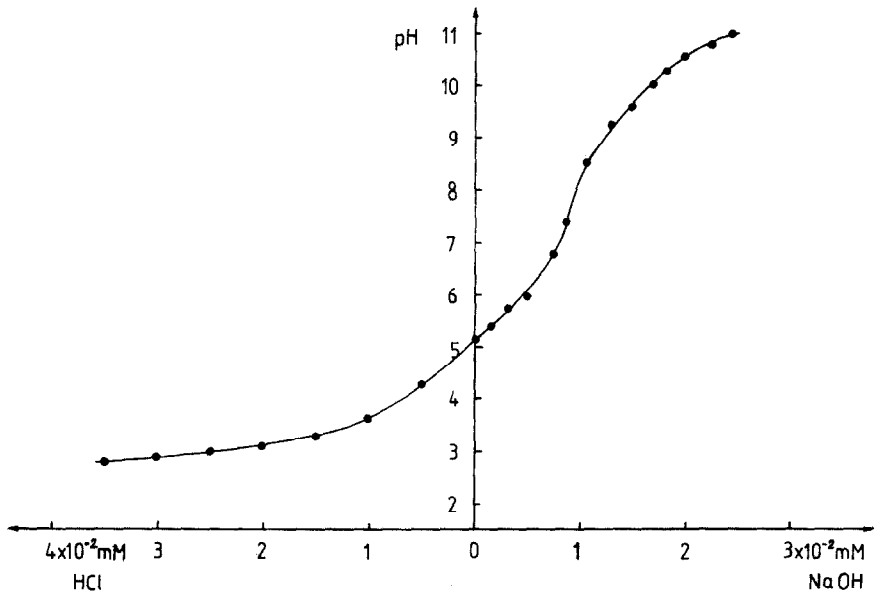

Fig. 2. Potentiometric titration of DMN–DVB porous copolymer.

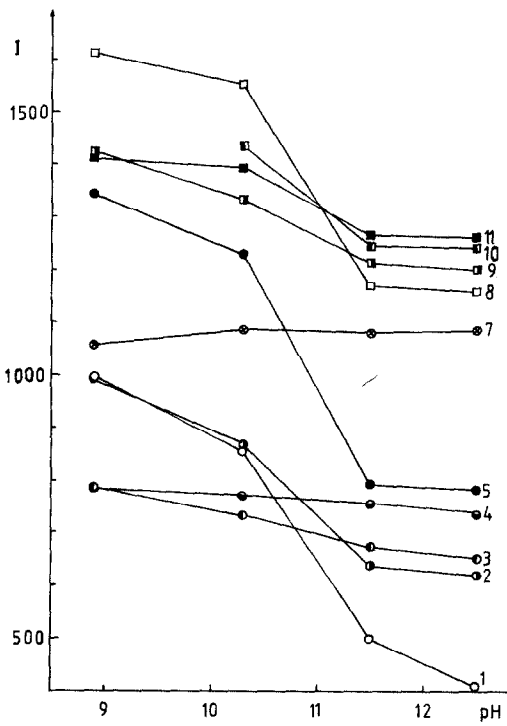

Fig. 3. Effect of mobile phase pH on retention indices of basic drugs obtained on the DMN–DVB column. Drugs: 1 = ephedrine; 2 = clonidine; 3 = lidocaine; 4 = benzocaine; 5 = procaine; 7 = diazepam; 8 = strychnine; 9 = cyproheptadine; 10 = amitriptyline; 11 = promethazine.

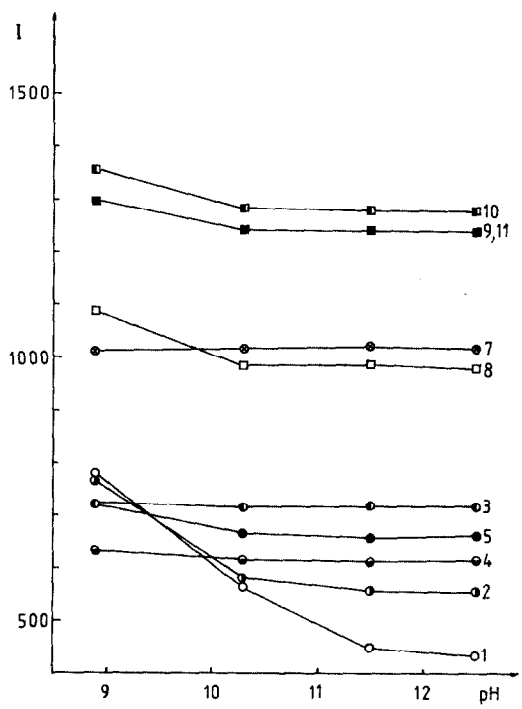

Fig. 4. Effect of mobile phase pH on retention indices of basic drugs obtained on the PRP-1 column. Drugs as in Fig. 3.

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