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ION-PAIR ADSORPTION CHROMATOGRAPHY OF BASIC DRUGS USING STRAIGHT-PHASE SYSTEMS ON SILICA GEL THIN LAYERS

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SUMMARY

A general approach to the ion-pair adsorption chromatography of basic drugs on silica gel thin layers is described. Under acidic or neutral conditions, basic drugs will migrate as uncharged ion pairs if a suitable inorganic counter ion such as Br^- or Cl^- is present in sufficient amounts. The latter can be achieved by dissolving halide salts in the developing solvent, or by impregnating the sorbent with halide salts prior to development. The ion-pair chromatographic systems tested are compared with systems in which the drugs migrate in their basic form and the results suggest that the use of ion-pair systems has great potential as a general screening technique for basic drugs when carried out in combination with a general basic development system.

INTRODUCTION

Basic drugs play a major role in medicine and pharmacy. Their extensive use as therapeutic agents, their toxicity and their potential addictive properties make their detection and identification highly important. Thin-layer chromatography (TLC) has been shown to be a useful technique for this purpose, but the large number of basic drugs in use, together with the fact that in some sub-classes structural differences between the individual components are minimal, has resulted in the publication of numerous TLC systems[1–6]. In most of these systems the drugs are assumed to migrate in their unionized, basic form*; silica gel and alumina are the sorbents of choice and the differences between the various systems are found in the composition of the developing solvent. However, few systematic attempts seem to have been made to investigate whether the TLC of basic drugs can also be performed under conditions in which the drug molecules are not present in their basic form.

We recently described the ion-pair adsorption chromatography of quaternary ammonium compounds on silica gel thin layers[7], in which quaternary cations migrated as uncharged ion pairs, based on the reaction

$$Q^+ + X^- \rightleftarrows QX$$

* Such systems will be referred to as basic systems.

As this approach seemed to be applicable to other organic components if they can be converted into a stable, ionized form, we have investigated if basic drugs (primary, secondary and tertiary amines) can be chromatographed according to the same principle, namely

$$BH^+ + X^- \rightleftarrows BHX$$

As this equation indicates, the basic drugs should be present in their protonated form (BH^+) to give ion pairs with a suitable counter ion (X^-). The latter may be the case in chromatography under acidic conditions.

The work presented here describes our first experiences with a selection of basic drugs and their ion-pair chromatographic behaviour is compared with that in basic systems.

EXPERIMENTAL

Chemicals

Basic drugs were obtained as salts from commercial suppliers or were gifts, and were used as received. The free bases were obtained by extraction from aqueous alkaline media (pH 12 for amphetamines, pH 9.5 for the remaining drugs) with chloroform, yielding free base concentrations of approximately 1 mg/ml in chloroform.

All other chemicals and solvents were of analytical grade and were obtained from E. Merck (Darmstadt, G.F.R.).

Thin-layer chromatography

Plates were 20 × 20 cm pre-coated silica gel 60-F_{254} plates (Merck), or were hand-made using the same sorbent with a layer thickness of 0.25 mm.

Chromatography under acidic conditions was achieved by means of a 0.1 *M* phosphate buffer (pH 2). Pre-coated plates were immersed in about 200 ml of buffer solution for 15 sec, blotted and dried. For hand-made plates, the buffer was used in preparing the spreading slurry.

The inorganic counter ions tested were acetate, sulphate, nitrate, chloride, bromide, iodide and perchlorate. They were applied as their potassium, sodium or lithium salts and added to the chromatographic system in one of the following modes:

(a) by using an aqueous solution of the desired counter ion in the spreading procedure of hand-made plates; this aqueous solution may also be the phosphate buffer as described above;

(b) by dipping pre-coated plates in a methanolic solution of the desired counter ion;

(c) by dissolving the counter ion in the developing solvent, provided that the latter is able to dissolve the required amount (this is usually the case with solvents containing large percentages of methanol).

The concentrations of the counter ions tested ranged from 0.025 to 0.5 *M*.

After all of the spreading or dipping procedures, the plates were dried for 30 min at 105° in an oven with a fan, except for those containing iodide and perchlorate, which were dried overnight at ambient temperature in order to avoid oxidation to

iodine and to reduce explosion risks, respectively. The dried plates were stored in a desiccator until required for use.

Spotting was effected with the aid of Microcaps (Drummond, Broomall, Pa., U.S.A.). Volumes of 1 or 5 μl were spotted, 2 cm from the bottom edge of the plate and at least 0.5 cm apart.

Development was carried out in 24 × 22 × 5 cm tanks to a height of 10 cm over the starting points in unsaturated chambers[8]. The solvent systems used were chloroform–methanol (90:10), chloroform–methanol (90:10) on 0.1 *M* sodium hydroxide-impregnated plates[9], methanol, methanol on 0.1 *M* sodium hydroxide-impregnated plates[10] and methanol–25% ammonia (100:1.5).

Detection was carried out under UV light of 254 nm and/or by spraying with an appropriate reagent, such as acidified iodoplatinate, Dragendorff or ninhydrin reagent[5].

RESULTS AND DISCUSSION

Excellent ion-pair chromatography could be obtained with chloride, bromide, iodide or perchlorate as counter ions, provided that the counter ion concentration applied to the system was at least 0.1 *M*. The ion pairs thus formed had good stability, did not decompose on silica gel and had adequate mobility in the solvent systems used. Compact spots were obtained with counter ion concentrations of 0.1 *M* and above. At lower concentrations, tailing of the spots occurred. With higher counter ion concentrations (0.2–0.5 *M*), the R_F values of the individual components had a tendency to increase slightly.

Other inorganic counter ions, such as sulphate and nitrate, did not give adequate ion pairs as their use resulted in a markedly reduced migration, if any, of the basic drugs, usually accompanied by pronounced tailing. Organic acids, such as acetic acid, toluenesulphonic acid, bromothymol blue and bromocresol purple, were also tested as counter ions, dissolved in the developing solvents, but were also unsatisfactory. This may be due to lack of ionization of the organic acids under acidic conditions, so that ion pairs with the basic drugs cannot be formed, or to interactions of the organic acids with the sorbent.

As the use of iodide and perchlorate had practical disadvantages because of iodine formation and explosion risks, bromide and chloride systems were selected for general use and were compared with a series of basic development systems. The systems studied are summarized in Table I. The use of phosphate as acidic buffer resulted in no migration of the components investigated with the chloroform–methanol system; with methanol as solvent most of the components again remained at the starting point, but some components tended to migrate slightly as streaks. The absence of migration can be explained by the fact that the protonated bases formed under the acidic conditions are strongly bound to the sorbent. When the buffer was omitted, as was done in systems E and M, all substances migrated, as indicated in Table II. If the methanolic system E is compared with system D, it can be seen that the addition of OH^- had hardly any influence on the R_F values, nor on the separation sequence. If system M is compared with system L, it can be seen that the addition of OH^- to the chloroform–methanol system resulted in an increase in most R_F values, possibly owing to an increase in polarity, but again the separation sequence remained essential-

TABLE I

ION-PAIR AND BASIC TLC SYSTEMS STUDIED

System	*Silica gel prepared or dipped in*	*Solvent*
A	Aqueous phosphate buffer (pH 2)	Methanol
B	No special treatment	Br^- in methanol
C	No special treatment	Cl^- in methanol
D	0.1 *M* NaOH in methanol	Methanol
E	No special treatment	Methanol
F	No special treatment	Methanol–ammonia (100:1.5)
G	Aqueous phosphate buffer (pH 2)	Chloroform–methanol (90:10)
H	Aqueous phosphate buffer (pH 2), followed by Br^- in methanol	Chloroform–methanol (90:10)
I	Br^- in methanol	Chloroform–methanol (90:10)
K	Cl^- in methanol	Chloroform–methanol (90:10)
L	0.1 *M* NaOH in methanol	Chloroform–methanol (90:10)
M	No special treatment	Chloroform–methanol (90:10)

ly the same. Therefore, it can be assumed that under the "neutral" conditions of systems E and M the drugs migrate in their basic form, despite the absence of a strongly basic component in the sorbent or in the solvent.

This observation is of special importance for the chloroform–methanol (90:10) system. This system, applied to sodium hydroxide-impregnated silica gel, has

TABLE II

$R_F \times 100$ VALUES OF BASIC DRUGS IN ION-PAIR AND BASIC TLC SYSTEMS

Concentrations of the counter ions or hydroxides in the spreading slurry or in the solvent were 0.1 *M*.

Drug	*System**									
	B	*C*	*D*	*E*	*F*	*H*	*I*	*K*	*L*	*M*
Oxycodone	44	39	35	32	76	25	34	34	68	51
Ethylmorphine	37	30	28	27	59	29	40	30	35	32
Codeine	30	30	24	27	57	21	34	25	32	32
Morphine	25	24	22	24	55	06	16	09	32	31
Quinine	59	55	34	36	74	22	55	41	23	16
Cocaine	55	47	41	44	89	23	24	30	74	52
Aminophenazone	75	74	75	74	89	37	59	60	65	61
Phenazone	74	73	73	73	86	51	50	52	59	55
Amphetamine	67	65	28	24	61	36	27	29	34	20
Methylamphetamine	64	59	19	17	51	42	31	31	27	14
Ephedrine	67	61	19	18	50	35	23	24	08	07
Amitriptyline	48	41	28	34	77	56	39	38	58	40
Nortriptyline	68	65	11	15	49	56	39	36	37	20
Imipramine	44	36	24	29	72	61	45	39	55	40
Desipramine	62	56	11	12	41	61	46	39	30	18
Yohimbine	79	77	73	73	93	30	29	31	65	52
Dextromoramide	82	82	82	80	93	69	69	70	94	80

* See Table I for descriptions. Systems B and C are ion-pair systems using methanol as solvent; systems D, E and F are "basic" systems using methanol as solvent. Systems H, I and K are ion-pair systems using chloroform–methanol (90:10) as solvent; systems L and M are "basic" systems using chloroform–methanol (90:10) as solvent.

been recommended by Moffat and Clare[11] as one of the best thin-layer systems for basic drugs, based on its discriminating power, but the need to use impregnated plates has been regarded a time-consuming disadvantage. With the above evidence, indicating that no impregnation is needed, it can be expected that chloroform–methanol (90:10) will gain further prominence as a general system for basic drugs[12]. As already indicated[11], this chloroform–methanol system has a much better discriminating power than methanol–ammonia (100:1.5), which was confirmed in our work by comparing the spread of the components in either system L or M with that in system F. On the other hand, it should be noted that the addition of 1.5% of ammonia to methanol (F) results in a marked increase in all R_F values compared with methanol alone (E) or methanol on sodium hydroxide-impregnated plates (D). This is also represented graphically in Figs. 1 and 2. As this increase can hardly be explained by an increase in polarity alone, the reason for this phenomenon remains unknown.

When a suitable counter ion, such as Br^-, was added to the pH 2-buffered chloroform–methanol system, all substances migrated as compact spots and yielded the R_F values given in Table II, system H. The protonated bases can now form relatively stable ion pairs which migrate as such. When Br^- was added to the pH 2-buffered methanol system, again migration of all substances was observed, but as the solvent had a tendency to wash out the buffer the spot shapes became irregular and showed tailing.

When trying to find the optimal pH of the buffer system and to avoid the wash-out effect, we then observed that the use of the buffer is not essential for ion-pair chromatography: The mere presence of a suitable amount of Br^- in the system, either in the sorbent layer or in the solvent, is sufficient. This is reflected in systems I (Br^- in the sorbent) and B (Br^- in the solvent). If the R_F values in system I (chloroform–methanol plus Br^-) are compared with those in system M (chloroform–methanol), it can be seen that ion-pair chromatography gives distinctly different patterns from those obtained in basic systems. The same applies to the methanol systems B (ion pair) and E (basic).

Excellent ion-pair chromatography was also obtained with Cl^- as counter ion, as can be seen from the R_F values in systems C and K. Although the R_F values in the Cl^- systems tended to be similar to those in the Br^- systems, there were some marked differences, such as a decrease in the R_F values of ethylmorphine, codeine, morphine and quinine in system K compared with system I. A decrease in the R_F values of cocaine, ephedrine, amitriptyline and imipramine is also noticeable.

Some of the separation patterns are depicted graphically in Figs. 1 and 2 for the methanol systems and in Figs. 3 and 4 for the chloroform–methanol systems. Fig. 1 shows the R_F patterns of a variety of basic drugs. It can be seen that some drugs show little or no difference in R_F values between ion-pair and basic development systems (dextromoramide, yohimbine and morphine), whereas others, such as ephedrine and quinine, show substantial changes in R_F values when going from ion-pair systems (B and C) to basic systems (D and E). The latter effect is also observed with other ephedrine-like substances, such as amphetamine and methylamphetamine, but these have been omitted from the figures for simplicity.

Another class of components that shows considerable changes in R_F values when changing from ion-pair to basic systems is that of the tricyclic antidepressants, as illustrated in Fig. 2. All four components show relatively high R_F values in the ion-

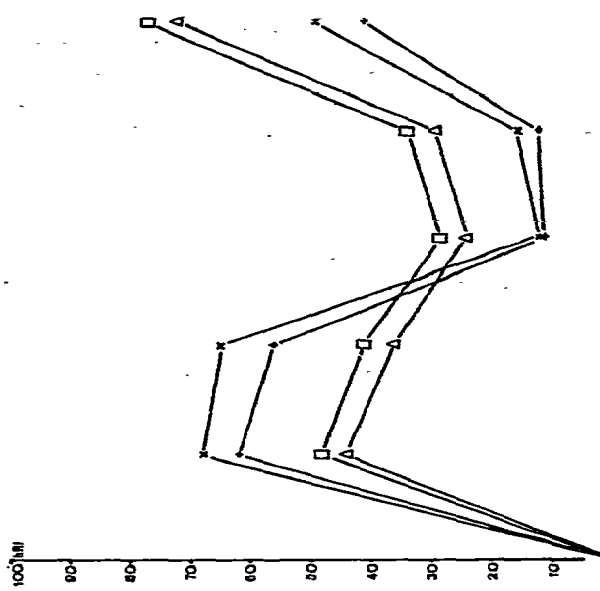

Fig. 1. R_F values of some basic drugs in ion-pair TLC systems and in basic TLC systems using methanol as solvent. See Table I for description of systems A–F. ×, Dextromoramide; ○, yohimbine; ■, cocaine; □, quinine; □, morphine; △, ephedrine.

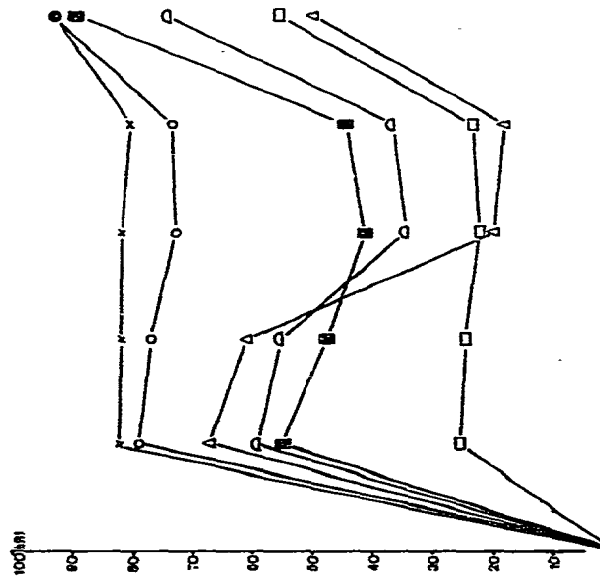

Fig. 2. R_F patterns of some tricyclic antidepressants in ion-pair TLC systems and in basic TLC systems using methanol as solvent. See Table I for description of systems A–F. □, Amitriptyline; △, imipramine; ×, nortriptyline; +, desipramine.

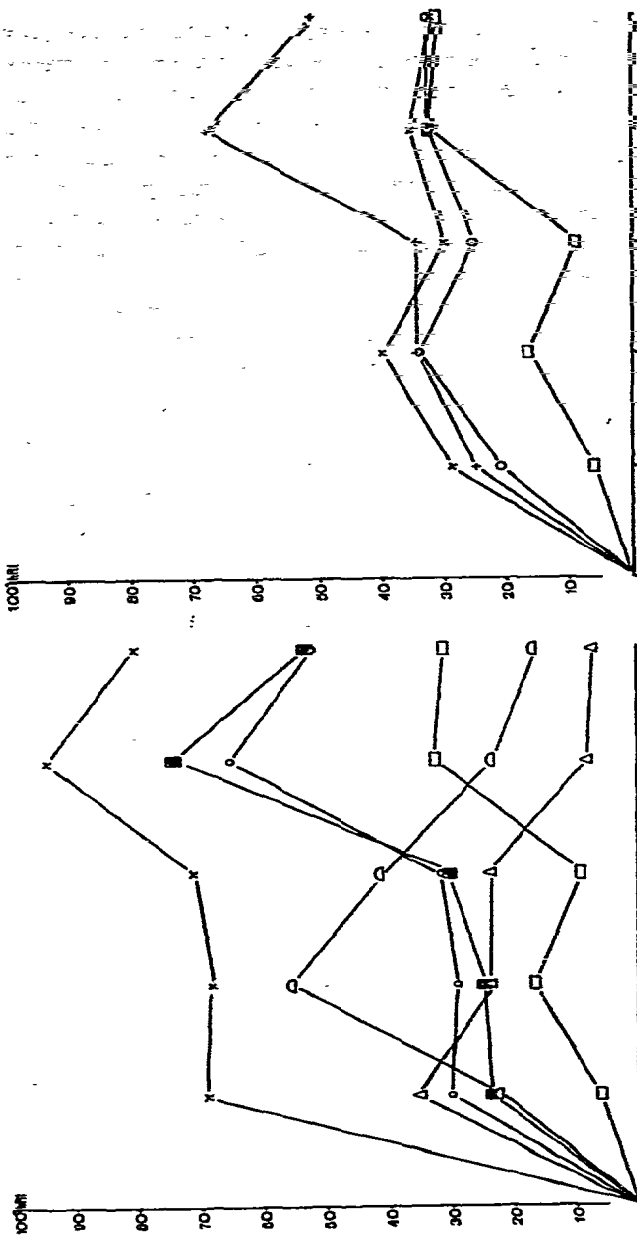

Fig. 3. R_F patterns of some basic drugs in ion-pair TLC systems and in basic TLC systems using chloroform–methanol (9:1(v)) as solvent. See Table I for description of systems G–M. ×, Dextromoramide; ■, cocaine; ○, yohimbine; □, morphine; ◇, quinine; △, ephedrine.

Fig. 4. R_F patterns of some opiates in ion-pair TLC systems and in basic TLC systems using chloroform–methanol (9:1(v)) as solvent. See Table I for description of systems G–M. +, Oxycodone; ×, ethylmorphine; ○, codeine; □, morphine.

pair systems B and C, with the secondary amines nortriptyline and desipramine running higher than their corresponding tertiary derivatives. In the basic systems D and E, all components have lower R_F values, which is much more pronounced for the secondary than for the tertiary amines. However, this results in a reversal of the separation sequence, with the tertiary amines now running ahead of the secondary amines.

Fig. 3 shows the R_F values of the same substances as in Fig. 1, but in the chloroform–methanol system. Again, marked differences can be observed when changing from ion-pair systems (H, I and K) to basic systems (L and M). However, it should be noted that there are substantial differences between the ion-pair systems as such, indicating that the choice of counter ion can play a role (compare I and K), as well as the pH (compare H and I). Apparently, these factors are more pronounced in the chloroform–methanol systems than with methanol alone. Structurally related substances may show identical patterns, as can be seen with the opiates and the amphetamines, but aminophenazone and phenazone display a different behaviour. In all methanol systems and in the basic chloroform–methanol systems L and M, these two drugs run closely together, with aminophenazone having slightly higher R_F values. The separation becomes slightly better in the ion-pair systems I and K, and is reasonable in system H, albeit that in the latter system the separation sequence is reversed, with phenazone now running faster. Within the group of tricyclic antidepressants another interesting phenomenon can be noted. In the ion-pair systems H, I and K, amitriptyline and nortriptyline run together, as do imipramine and desipramine, so that the migration rate is determined by changes in the tricyclic ring structure. However, in the basic systems L and M, the two tertiary amines amitriptyline and imipramine run together despite the differences in their ring structures, whereas the secondary amines nortriptyline and desipramine have much lower R_F values, indicating that the secondary amine function has a predominant effect on the migration in basic systems.

Fig. 4 depicts the separation behaviour of the four opiates studied. The ion-pair systems give good separations between morphine and codeine. The separation of ethylmorphine and codeine, which differ by only one methylene group, is best in system H, provided that oxycodone is absent. The latter compound can be tested for easily in the basic system L or M.

Development times for the ion-pair systems were of the same order as those for the basic systems, namely 30–45 min. The spot shapes and sizes as well as the reproducibility were also comparable to those in the basic systems. Detection can be carried out under UV light (not recommended for phosphate-containing plates) or with the usual detection reagents.

CONCLUSION

The straight-phase ion-pair adsorption systems presented here are rapid, simple and rather insensitive to small changes in temperature and relative humitidy, and therefore seem to be more advantageous than reversed-phase ion-pair partition systems, which require carefully standardized conditions[13–16]. Moreover, the ion-pair adsorption systems are able to utilize the high separation selectivity of the silica gel sorbent.

The major advantage of ion-pair adsorption chromatography is that the separation is based on different physico-chemical principles compared with separa-

tions in basic systems, thus providing a new and independent parameter for the identification of a particular drug. Therefore, the use of ion-pair adsorption systems can be recommended as additional general screening techniques, especially in combination with the already existing basic systems[11]. Ion-pair systems may also offer increased possibilities in specific separation problems in which basic systems do not provide adequate resolution.

Further investigations to evaluate the further potential of this technique are being carried out.

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REFERENCES

1 K. Macek, I. M. Hais, J. Kopecký and J. Gasparič (Editors), *Bibliography of Paper and Thin-Layer Chromatography, 1961–1965*, Elsevier, Amsterdam, 1968.
2 K. Macek, I. M. Hais, J. Kopecký, J. Gasparič, V. Rábek and J. Churáček (Editors), *Bibliography of Paper and Thin-Layer Chromatography, 1966–1969*, Elsevier, Amsterdam, 1972.
3 K. Macek, I. M. Hais, J. Kopecký, V. Schwarz, J. Gasparič and J. Churáček (Editors), *Bibliography of Paper and Thin-Layer Chromatography, 1970–1973*, Elsevier, Amsterdam, 1976.
4 I. Sunshine (Editor), *Handbook of Analytical Toxicology*, CRC Press, Cleveland, Ohio, 1969.
5 E. G. C. Clarke (Editor), *Isolation and Identification of Drugs*, Pharmaceutical Press, London, 1969.
6 G. Zweig and J. Sherma (Editors), *Handbook of Chromatography*, Vol. 1, CRC Press, Cleveland, Ohio, 1972.
7 R. A. de Zeeuw, P. E. W. van der Laan, J. E. Greving and F. J. W. van Mansvelt, *Anal. Lett.*, 9 (1976) 831.
8 R. A. de Zeeuw, *Anal. Chem.*, 40 (1968) 915.
9 G. F. Phillips and J. Gardiner, *J. Pharm. Pharmacol.*, 21 (1969) 793.
10 W. W. Fike, *Anal. Chem.*, 38 (1966) 1967.
11 A. C. Moffat and B. Clare, *J. Pharm. Pharmacol.*, 26 (1974) 665.
12 M. L. Bastos, G. E. Kananen, R. M. Young, J. R. Montforte and I. Sunshine, *Clin. Chem.*, 16 (1970) 931.
13 K. Gröningsson and G. Schill, *Acta Pharm. Suecica*, 7 (1969) 447.
14 K. Gröningsson, *Acta Pharm. Suecica*, 8 (1970) 635.
15 K. Gröningsson and M. Weimers, *Acta Pharm. Suecica*, 12 (1975) 65.
16 K. Gröningsson, H. Westerlind and R. Modin, *Acta Pharm. Suecica*, 12 (1975) 97.