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CHROMATOGRAPHIC BEHAVIOUR OF ALKALOIDS ON THIN LAYERS OF ANION AND CATION EXCHANGERS

I. AG 1-X4 AND CELLEX D

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SUMMARY

The chromatographic behaviour of 48 alkaloids has been studied on Bio-Rad AG 1-X4, Cellex D and microcrystalline cellulose, eluting with solutions of different pH but constant ionic strength (0.5). Many interesting separations were effected on both AG 1-X4 and Cellex D layers.

The influence of pH on the chromatographic behaviour of alkaloids has been quantitatively studied and an equation was used that expresses the behaviour of the alkaloids on both AG 1-X4 (AcO^-) and microcrystalline cellulose layers. The non-applicability of this equation to Cellex D layers is discussed.

INTRODUCTION

The chromatographic characteristics of alkaloids on ion exchangers have been studied by column chromatography[1–7] and, to a lesser extent, with modified papers or papers impregnated with ion exchangers[8–12]. Most work, however, has concerned a small number of compounds and an accurate study of the parameters that determine the retention process (pH and ionic strength of the eluent, acid–base characteristics of the compounds) has not been made.

Owing to the lack of studies on thin layers of ion exchangers, we deemed it useful to study methodically the chromatographic behaviour of many alkaloids in order to find the best conditions for their separation. This first paper concerns anion exchangers with polystyrene (Bio-Rad AG 1-X4) and cellulose (Cellex D) matrices. The results achieved with these two exchangers are compared with those obtained on microcrystalline cellulose.

EXPERIMENTAL

The 48 alkaloids studied were dissolved in ethanol, with the exception of theobromine, which was dissolved in 2 *M* hydrochloric acid. The amounts of each alkaloid on the layer (relative to 1 μl of solution) are reported in Table I. A freshly prepared solution of ergotamine must be used in order to prevent the appearance on

the layer of spots (with a yellow fluorescence) other than that (with a blue fluorescence) peculiar to the pure compound.

Of the 48 alkaloids, some (morphine, spermine, spermidine, cevadine, veratrine, protoveratrine A and ephedrine) cannot be detected on AG 1-X4 thin layers and they are therefore not reported in Table I.

Preparation of the layers

Layers of AG 1-X4 in the acetate form (Bio-Rad Labs., Richmond, Calif., U.S.A.) were prepared by mixing 3 g of the exchanger with 9 g of microcrystalline cellulose in 50 ml of water. The AG 1-X4 (AcO^-) was obtained by treating the commercial product (200–400 mesh) in the chloride form with 0.5 *M* sodium acetate solution until the complete disappearance of the chloride ion, then rinsing with water and methanol and drying at room temperature.

DEAE-cellulose (Cellex D) layers were prepared by dissolving 6 g of the exchanger in 50 ml of water. The DEAE-cellulose (Bio-Rad Labs.) was treated, before use, with 0.5 *M* acetate buffer, rinsed with water and methanol and dried at room temperature. Microcrystalline cellulose (E. Merck, Darmstadt, G.F.R.) layers were prepared by dissolving 12 g of the exchanger in 50 ml of water.

Detection

Many alkaloids, when observed under UV light (254 nm), give rise to black spots on a bright background or to fluorescence. Some compounds are fluorescent only in the protonated form; for this reason the layers, after elution and drying, were sprayed with 6 *M* acetic acid solution.

With Dragendorff reagent, modified according to Vágújfalvi[13], many alkaloids can be detected on AG 1-X4 and microcrystalline cellulose layers that have previously been sprayed with the acetic acid solution. This reagent, however, does not give good results on Cellex D layers. Ajmaline was detected by spraying the layer with a saturated solution of ceric sulphate in 60% sulphuric acid. The migration distance was 11 cm unless otherwise stated. The measurements were carried out at 25 ± 0.5°.

RESULTS AND DISCUSSION

AG 1-X4 (AcO^-)

In Table I are reported the R_F values of 41 alkaloids on AG 1-X4 (AcO^-) layers on elution with solutions of different pH values but constant ionic strength ($\mu = 0.5$). Table I also indicates the amounts of each alkaloid deposited on the layer. It is interesting to note that these amounts are generally smaller than those on papers impregnated with inorganic ion exchangers[9]. Alkaloids were classified in order of increasing R_F values on elution with 0.5 *M* ammonium acetate solution.

The affinities of most alkaloids towards the exchanger increase as the pH is increased. Such an effect, which has already been discussed for primary aromatic amines[14], is correlated with the stronger retention of the layer towards the alkaloids in the free base form.

The behaviour of caffeine and colchicine supports such an assumption; in fact, the R_F values of these compounds, which are in the free base form over the whole pH range studied, do not change with changes in pH.

TABLE I

R_F VALUES OF ALKALOIDS ON AG 1-X4 (AcO^-) THIN LAYERS

Alkaloid	*Eluent*				*Amount (μg)*
	1 M NH_3 + 0.5 M CH_3COONa	*0.5 M NH_3 + 0.5 M CH_3COONH_4*	*0.5 M CH_3COONH_4*	*0.5 M acetate buffer*	
Narceine	0.00	0.00	0.00	0.00	0.25
Ergocristine	0.00	0.00	0.00	0.05	0.4
Ergotamine	0.00	0.00	0.00	0.06	0.4
Papaverine	0.01	0.01	0.02	0.46	0.4
Ibogaine	0.02	0.02	0.03	0.36	0.4
Berberine hydrochloride	0.03	0.04	0.04	0.03	0.01
Reserpine	0.00	0.00	0.04	0.19	0.5
Boldine	0.02	0.02	0.07	0.25	0.4
Ergonovine	0.02	0.02	0.08	0.21	0.5
Narcotine	0.10	0.10	0.22	0.72	2.0
Hydrastine	0.21	0.21	0.36	0.73	0.8
Aminophylline	0.03	0.03	0.38	0.53	8.0
Theophylline	0.03	0.03	0.38	0.54	8.0
Colchicine	0.40	0.40	0.42	0.45	4.0
Yohimbine hydrochloride	0.07	0.07	0.44	0.58	4.0
Quinine	0.08	0.10	0.50	0.65	0.3
Quinidine sulphate	0.13	0.15	0.54	0.67	0.3
Brucine	0.23	0.26	0.66	0.70	6.0
Theobromine	0.41	0.52	0.68	0.68	8.0
Caffeine	0.69	0.69	0.68	0.68	8.0
Cinchonine hydrochloride	0.00	0.00	0.71	0.74	1.0
Cinchonidine	0.16	0.20	0.71	0.74	1.0
Strychnine	e.s.*	e.s.	0.77	0.79	6.0
Ajmaline	0.35	0.36	0.78	0.79	2.0
Lobeline hydrochloride	0.00	0.12	0.82	0.87	5.0
Tubocurarine	0.74	0.79	0.85	0.8.	12.0
Cocaine	0.25	0.42	0.90	0.91	5.0
Atropine	0.61	0.88	0.91	0.92	10.0
Hyoscyamine	0.62	0.89	0.91	0.92	10.0
Eucatropine hydrochloride	0.62	0.89	0.91	0.92	10.0
Emetine hydrochloride	0.12	0.21	0.91	0.93	5.0
Ethylmorphine	0.41	0.47	0.91	0.94	8.0
Eserine sulphate (1)	0.28	0.50	0.93	0.94	3.0
(2)	0.54				
Homatropine	0.61	0.90	0.93	0.95	10.0
Scopolamine hydrochloride	0.68	0.72	0.93	0.95	7.0
Arecoline hydrochloride	0.79	0.88	0.94	0.95	10.0
Hyoscyne	0.93	0.96	0.96	0.95	10.0
Scopoline	0.90	0.96	0.98	0.96	7.0
Sparteine sulphate	0.94	0.97	0.98	0.97	10.0
Tropine	0.96	0.97	0.98	0.97	10.0
Prostigmine	0.96	0.97	0.98	0.97	10.0

* e.s. = elongated spot.

Theobromine and theophylline apparently behave anomalously as their R_F values increase as the pH decreases, notwithstanding the fact that their basic characteristics are similar to those of caffeine ($pK_a < 1$ for all three compounds). In order to explain such a result, it must be pointed out that, contrary to caffeine, the other

two compounds may give rise to anionic species that are retained by the exchanger through an anion-exchange process.

The different chromatographic behaviour of theobromine and theophylline may be explained on the basis of their different acidic characteristics: theophylline, in fact, ($pK_{a2} = 8.6$)[15] is retained more strongly than theobromine ($pK_{a2} = 10.0$)[15].

The influence of ionic strength on the chromatographic behaviour of the alkaloids is more marked at low pH values of the eluent, that is, when the alkaloids are prevalently in the protonated form. As the ionic strength is decreased, an increase in the R_F values is observed. Similar behaviour has been found with phenols on polystyrene-based cation exchangers[16]; for these compounds, the greatest influence of the ionic strength is observed when they are in the deprotonated form.

As regards the relationship between the chromatographic behaviour of the alkaloids and their structure, it is difficult to compare compounds with different structures. When such a comparison is possible, it is found that the introduction into the molecule of one or more methoxyl groups results in a stronger retention by the exchanger. Some typical examples are the greater retentions of narcotine, brucine, quinine and quinidine in comparison with those of hydrastine, strychnine, cinchonine and cinchonidine, respectively.

Some alkaloids listed in Table I are also noticeably retained when eluting with 0.5 *M* acetate buffer. For these compounds, a remarkable increase in the R_F values can be achieved by eluting with 1 *M* acetic acid or adding ethanol to the aqueous solutions given in Table I. The increase observed with 1 *M* acetic acid may be attributed mainly to the decrease in the ionic strength of the eluent. With the addition of ethanol, more compact spots are observed, but there is a levelling of the R_F values overall at ethanol concentrations above 50% (v/v). An exception is narceine, which always remains at the starting point; such behaviour may be correlated with the presence in the molecule of a carboxylic group, which gives rise to an anion-exchange process with the exchanger.

The behaviour of eserine with 0.5 *M* sodium acetate and 1 *M* ammonia solution is very interesting from an analytical point of view. In fact, as indicated by the data in Table I, with this eluent eserine gives two spots, the one with higher R_F being red-violet and the other giving a yellow fluorescence under UV light after spraying with acetic acid solution. The presence of the red-violet spot can be used for the qualitative detection of this alkaloid, as such a test is selective and sensitive (3 μg).

Analytical application. Among the separations foreseeable on the basis of the R_F values reported in Table I, the following have been effected: cinchonine and cinchonidine (migration distance = 14 cm; 0.5 *M* sodium acetate + 1 *M* ammonia); tropine and atropine (0.5 *M* sodium acetate + 1 *M* ammonia); caffeine, theophylline and theobromine (0.5 *M* sodium acetate + 1 *M* ammonia); cocaine, scopoline and scopolamine (0.5 *M* sodium acetate + 1 *M* ammonia or 0.5 *M* ammonium acetate + 0.5 *M* ammonia); eserine and prostigmine (0.5 *M* ammonium acetate + 0.5 *M* ammonia); quinine (or quinidine) and cinchonine (or cinchonidine) (0.5 *M* ammonium acetate).

The separation reported in Fig. 1, with 1 *M* acetic acid as eluent, includes many alkaloids and cannot be effected with the eluents listed in Table I.

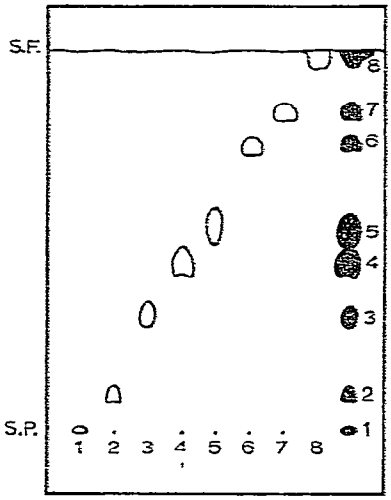

Fig. 1. Thin-layer chromatogram of alkaloids on AG 1-X4 (AcO^-) Eluent, 1 *M* acetic acid. Migration distance, 12.5 cm. Spots: 1, narceine (red); 2, berberine hydrochloride, 3, ergonovine; 4, reserpine; 5, ergocristine; 6, jbogaine; 7, quinine; 8, cocaine. Black spots: mixtures. Yellow fluorescence: berberine, reserpine and jbogaine. Blue fluorescence: ergonovine, ergocristine and quinine. Cocaine gives an orange colour with Dragendorff reagent.

Cellex D and microcrystalline cellulose

Table II gives the R_F values of 28 alkaloids on Cellex D layers with the same eluents as those used on AG 1-X4 (AcO^-). On this exchanger, fewer alkaloids are reported than on AG 1-X4, because Dragendorff reagent cannot be used as the detection agent.

The alkaloids are listed in order of increasing R_F values on elution with 0.5 *M* ammonium acetate + 0.5 *M* ammonia.

Table II also reports the chromatographic characteristics of the alkaloids on microcrystalline cellulose. On this exchanger also the alkaloids that react with Dragendorff reagent can be detected. Such compounds have R_F values $\geqslant 0.7$ with all of the eluents listed in Table II. The amounts of each alkaloid on Cellex D and microcrystalline cellulose layers are generally lower than those on AG 1-X4 (AcO^-).

From the comparison between the chromatographic behaviour of the alkaloids on Cellex D and microcrystalline cellulose, some interesting conclusions can be drawn:

(1) Among the alkaloids that are not protonated in the pH range studied, colchicine, theophylline and aminophylline show a chromatographic behaviour on Cellex D at changing pH values that is different from that on microcrystalline cellulose, while caffeine and theobromine exhibit high R_F values, which are independent of the pH, on both exchangers. The behaviour of colchicine on Cellex D may be ascribed to the progressive protonation of the functional group of the exchanger as the pH of the eluent is decreased, while that of theophylline and theobromine can also be correlated with the anion-exchange process between them and the exchanger with both 0.5 *M* sodium acetate + 1 *M* ammonia and 0.5 *M* ammonium acetate + 0.5 *M* ammonia as eluent.

(2) The alkaloids that are protonated in the pH range studied generally exhibit

TABLE II

R_F VALUES OF ALKALOIDS ON CELLEX D AND MICROCRYSTALLINE CELLULOSE (mC) THIN LAYERS

Alkaloid	*Eluent*							
	1 M NH_3 – 0.5 M CH_3COONa		*0.5 M NH_3 + 0.5 M CH_3COONH_4*		*0.5 M CH_3COONH_4*		*0.5 M acetate buffer*	
	Cellex D	*mC*	*Cellex D*	*mC*	*Cellex D*	*mC*	*Cellex D*	*mC*
Narceine	0.00	0.06	0.00	0.02	0.00	0 04	0.00	0.08
Reserpine	0.00	0.00	0.00	0.00	0.09	0.05	0 41	e.s.*
Ergocristine	e.s.	e s.	0.03	0.01	0.06	0.04	0.41	0.23
Ergotamine	e s.	e.s.	0.12	e.s.	0.17	0.06	0 51	0.31
Boldine	0 33	0.65	0 16	0.30	0.32	0.31	0.49	0.35
Ibogaine	0.14	0.08	0.18	0.08	0 24	0.12	0.84	0.49
Papaverine	0.12	0.32	0.20	0 32	0 37	0.40	0.89	0.82
Berberine hydrochloride	0.16	0 00	0 29	0.00	0.36	0.03	0.49	0.05
Ergonovine	0 30	0.30	0.35	0.31	0.49	0.24	0.65	0 24
Yohimbine hydrochloride	0 35	0 39	0.42	0.45	0.77	0.57	0.86	0.65
Narcotine	0.39	0.50	0.45	0.51	0.72	0.56	0 94	0.83
Aminophylline	0.47	0 63	0.48	0.63	0.81	0.64	0.85	0.68
Theophylline	0.48	0.67	0.49	0.65	0 83	0.66	0.88	0.70
Emetine hydrochloride	0 26	e.s	0.49	e s	0 95	e.s.	0 95	0.93
Brucine	0.42	0.50	0.55	0 52	0 86	0.67	0.90	0.69
Quinidine sulphate	0.37	0 47	0.55	0.49	0.80	0.64	0.89	0.73
Quinine	0.38	0 50	0.56	0 54	0.81	0.67	0.89	0.74
Lobeline hydrochloride (1)	0.00	0.00	0.56	0.61	0.95	0 86	0.95	0.85
(2)	e.s.	e.s.						
Hydrastine	0.53	0.64	0.64	0 66	0 82	0.70	0.94	0.83
Strychnine	0 52	0.63	0.65	0 64	0.87	0.75	0.91	0.79
Cinchonine hydrochloride (1)	0.00	0.00	0 70	0.66	0.92	0.79	0.94	0.83
(2)	e.s.	e s						
Cinchonidine	0.51	0.65	0 70	0.66	0.92	0.79	0.94	0.83
Ajmaline	0.63	0.71	0.73	0.74	0.91	0 79	0.95	0 88
Colchicine	0 58	0 87	0.75	0.86	0.84	0.86	0.88	0 88
Ethylmorphine	n.d.**	0.82	0.80	0.84	0.95	0.95	0.95	0.95
Theobromine	0 83	0.73	0.83	0.73	0 88	0.80	0 89	0.80
Caffeine	0.84	0 81	0 88	0.80	0.89	0 81	0.89	0.83
Eserine sulphate	0 81	0.89	0.90	0.89	0.95	0.95	0 95	0.95

* e s. = elongated spot.
** n.d. = not determined.

an increase in the R_F values on both layers as the pH of the eluent is decreased. Such an increase in R_F is more marked on Cellex D than on microcrystalline cellulose, and the results confirm the influence on the R_F value both of the protonation of the alkaloid and of the progressive protonation of the functional group of Cellex D.

It should be noted that narceine and berberine are strongly retained by microcrystalline cellulose, independently of the pH of the eluent. On both exchangers, the

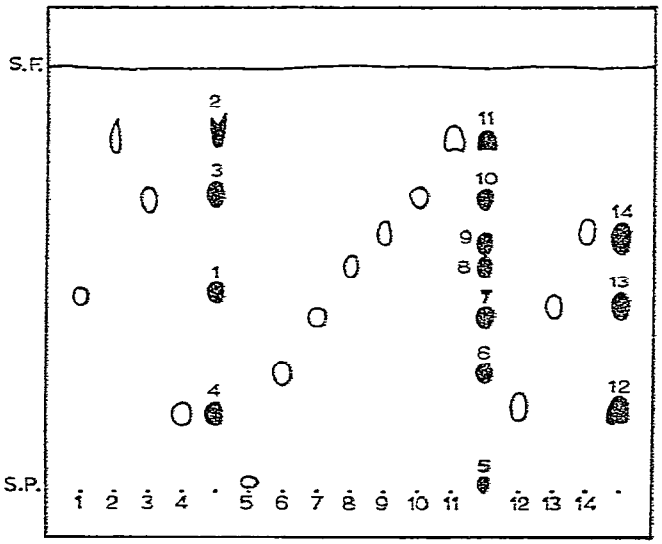

Fig. 2. Thin-layer chromatogram of alkaloids on Cellex D. Eluent, 0.5 *M* ammonia + 0.5 *M* ammonium acetate. Migration distance, 14 cm. Spots: 1, theophylline; 2, caffeine, 3, ajmaline; 4, jbogaine; 5. narceine (red); 6, berberine hydrochloride: 7, yohimbine hydrochloride; 8, brucine; 9, strychnine; 10, colchicine; 11, eserine sulphate; 12, papaverine; 13, narcotine; 14, hydrastine. Black spots: mixtures. Yellow fluorescence: berberine, jbogaine and papaverine. Yellow-green fluorescence: narcotine and hydrastine. Blue fluorescence: yohimbine and eserine. Caffeine, theophylline, brucine. strychnine and colchicine are visible in UV light as dark spots.

chromatographic behaviour of boldine is peculiar because, as the pH changes, its R_F value first decreases and then increases. On eluting with 0.5 *M* sodium acetate + 1 *M* ammonia, eserine, contrary to its behaviour on AG 1-X4 (AcO^-), does not give double spots on either Cellex D or microcrystalline cellulose layers.

Analytical application. Many separations can be effected on Cellex D layers. Most of these separations can also be obtained on microcrystalline cellulose, as it is predictable on the basis of the R_F values. Fig. 2 shows three separations on Cellex D with 0.5 *M* ammonium acetate + 0.5 *M* ammonia as eluent.

The separation of narceine, berberine, yohimbine, brucine, strychnine, colchicine and eserine cannot be effected on microcrystalline cellulose.

R_F versus pH graphs

In order to study quantitatively the influence of pH on the chromatographic characteristics of the alkaloids, we used the following relationship:

$$\left(\frac{1}{R_F} - 1\right) = \left(\frac{1}{R_{Falk}} - 1\right) \frac{K_a}{K_a + [H^+]} + \left(\frac{1}{R_{Fac}} - 1\right) \frac{[H^+]}{K_a + [H^+]} \qquad (1)$$

which has been employed for phenols on Dowex 50-X4 (Na^+)[16], where K_a is the dissociation constant of the protonated amine group and R_{Fac} and R_{Falk} are the R_F values of the protonated and the free base form of the alkaloids, respectively.

AG 1-X4 (AcO^-). Fig. 3 shows the R_F *versus* pH curves obtained by applying

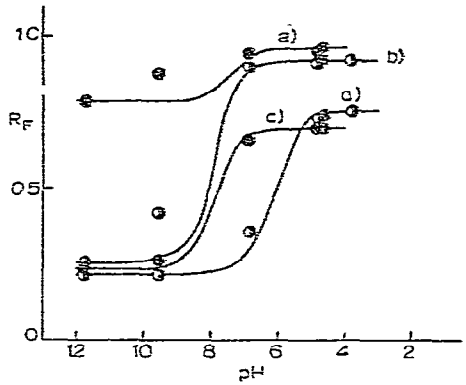

Fig. 3. R_F *versus* pH plots for alkaloids on AG 1-X4 (AcO^-) thin layers. (a) Arecoline; (b) cocaine; (c) brucine; (d) hydrastine.

eqn. 1, where the R_{Fac} and R_{Falk} values are those obtained on eluting with a solution at pH = 4.60 or 3.75 (R_{Fac}) and pH = 11.70 (R_{Falk}), indicated in Table III.

The good agreement between the experimental R_F values and those which can be drawn from the theoretical curves at the same pH values (see Fig. 3) supports the validity of eqn. 1 also in the case of anion exchangers. The R_F values obtained with ammonium acetate + ammonia (pH = 9.50) are an exception; such values (overall in the case of curves a and b in Fig. 3) are noticeably higher than those calculated on the basis of the pH of the eluent. Such differences between the experimental and theoretical values are correlated with the different pH values on the layer compared that of the eluent. In fact, with the method described previously[17], we have measured the pH on the layer and found values between 8.8 and 7.0 from the origin up to the solvent front. It should be noted that the two R_F values at pH = 9.50 for curves a

TABLE III

R_F VALUES OF ALKALOIDS ON AG 1-X4 (AcO^-) THIN LAYERS OBTAINED WITH ELUENTS AT DIFFERENT pH VALUES

1 *M* ammonia – 0.5 *M* sodium acetate (pH = 11.70); 0.5 *M* ammonia + 0.5 *M* ammonium acetate (pH = 9.50); 0.5 *M* ammonium acetate (pH = 6.80); 0.5 *M* acetic acid + 0.5 *M* sodium acetate (pH = 4.75); 0.25 *M* monochloroacetic acid + 0.5 *M* sodium acetate (pH = 4.60); 0.5 *M* monochloroacetic acid + 0.5 *M* sodium acetate (pH = 3.75).

*Arecoline**		*Cocaine***		*Brucine****		*Hydrastine*§	
pH	R_F	*pH*	R_F	*pH*	R_F	*pH*	R_F
11.70	0.79	11.70	0.25	11.70	0.23	11.70	0.21
9.50	0.88	9.50	0.42	9.50	0.26	9.50	0.21
6.80	0.94	6.80	0.90	6.80	0.66	6.80	0.36
4.75	0.95	4.75	0.91	4.75	0.70	4.75	0.73
4.60	0.96	4.60	0.92	4.60	0.70	4.60	0.74
		3.75	0.92			3.75	0.76

* pK_a = 7.41 at 17.5° (ref. 15).
** pK_a = 8.39 at 24° (ref. 15).
*** pK_a = 8.28 at 25° (ref. 15).
§ pK_a = 6.63 at 15° (ref. 15).

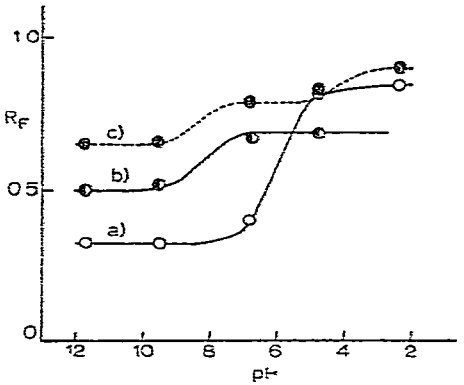

Fig. 4. R_F *versus* pH plots for alkaloids on microcrystalline cellulose thin layers. (a) Papaverine; (b) brucine; (c) cinchonidine.

and b in Fig. 3 correspond to pH values on the layer of 7.3 (curve a) and 8.9 (curve b), in good agreement with the pH values determined experimentally on the layer at distances corresponding to the R_F values of these two compounds.

As regards the possible application of eqn. 1 when using anion exchangers and particularly AG 1-X4 (AcO^-) layers, it must be pointed out that many alkaloids in the free base form are strongly retained by this exchanger ($R_F \leqslant 0.02$), and for this reason the $1/R_{Falk}$ value is not reliable. Other alkaloids, on the other hand, give rise to elongated spots (*e.g.*, strychnine) and therefore eqn. 1 cannot be applied.

Cellex D. On this exchanger, eqn. 1 can be applied only in very few instances, which cannot be predicted, as the retention of the alkaloids does not depend only on the proportions of the protonated and free base forms of the alkaloid, but also on the progressive protonation of the functional group of the exchanger as the pH is decreased.

TABLE IV

R_F VALUES OF ALKALOIDS ON MICROCRYSTALLINE CELLULOSE THIN LAYERS OBTAINED WITH ELUENTS AT DIFFERENT pH VALUES

1 *M* ammonia + 0.5 *M* sodium acetate (pH = 11.70); 0.5 *M* ammonia + 0.5 *M* ammonium acetate (pH = 9.50). 0.5 *M* ammonium acetate (pH = 6.80); 0.5 *M* acetic acid — 0.5 *M* sodium acetate (pH = 4.75), 1 *M* acetic acid + 0.5 *M* sodium chloride (pH = 2.30)

*Papaverine**		*Brucine***		*Cinchoridine****	
pH	R_F	*pH*	R_F	*pH*	R_F
11.70	0.32 (R_{Falk})	11.70	0.50 (R_{Falk})	11.70	0.65 (R_{Falk})
9.50	0.32	9.50	0.52	9.50	0.66
6.80	0.40	6.80	0.66	6.80	0.79 (R_{Fac_1})§
4.75	0.82	4.75	0.69 (R_{Fac})	4.75	0.83
2.30	0.84 (R_{Fac})			2.30	0.90 (R_{Fac_2})§§

* $pK_a = 6.40$ at 25° (ref. 15).
** $pK_a = 8.28$ at 25° (ref. 15).
*** $pK_{-1} = 8.40$ and $pK_{-2} = 4.17$ at 15° (ref. 15).
§ R_F value of monoprotonated form of cinchonidine.
§§ R_F value of diprotonated form of cinchonidine.

Microcrystalline cellulose. Fig. 4 shows the R_F *versus* pH curves for papaverine, brucine and cinchonidine, obtained by applying eqn. 1 on the basis of the R_{Fac} and R_{Falk} values reported in Table IV.

With respect to the other curves, that of cinchonidine (curve c) exhibits two inflection points, which correspond to the protonation of its two basic groups ($pK_{a_1} = 8.40$ and $pK_{a_2} = 4.17$).

With respect to the curves relating to AG 1-X4 layers (Fig. 3), the better agreement between the experimental and theoretical points owing to the disappearance of the pH gradient on the layer should be noted. Such an occurrence is a further confirmation of the assumption that we made in order to explain the differences between theoretical and experimental points on AG 1-X4 (AcO^-) layers and of the applicability of eqn. 1 not only on strong cation[16] and anion exchangers but, in general, on every type of resin whose retention is not affected by the pH of the eluent, as with Cellex D.

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