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Effect of the pH of Buffered Paper on the R_f of Alkaloids*

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The influence of pH upon the R_f and the nature of the buffer used for the pretreatment of the paper have been investigated for belladonna, strychnos, and opium alkaloids. R_f values at the same pH of the same buffer are correlated to their chemical structure. Variation of R_f with the pH of the buffer, variation of R_f for different buffers of the same pH, the influence of certain anions and cations, molarity of buffer, and the kind of paper used for chromatography are discussed.

ALKALOIDS and other basic substances with a dissociation constant of about 10^{-3} to 10^{-10} , chromatographed on untreated paper with neutral developing solvents, give diffuse elongated spots. This is due to their existence on the paper as ions and as undissociated molecules. These two forms have different R_f values and therefore give elongated spots (1-4).

Strong or very weak bases on the other hand, when chromatographed on untreated paper with neutral solvents, give sharp round spots because they exist in only one form either as ions or as undissociated molecules.

Alkaloids with a dissociation constant between 10^{-3} and 10^{-10} can be chromatographed on paper without "tailing" by addition of an acid to the developing solvent, or by pretreatment of the filter paper with neutral salts or buffer solutions of appropriate pH.

The addition of an acid to the developing solvent generally produces good chromatograms. The dissociation constant of the base determines the amount of the acid to be used. The addition of strong mineral acids or polycarboxylic acids to the developing solvent gives rise to the effect of "demixion" (Entmischung) (1). The ions of the slower moving strong acid remain behind the

solvent front forming a second front, the front of "demixion." If the alkaloids appear above or below the front of "demixion," this effect does not interfere in most cases with the identification and separation of the substances. However, if an alkaloid appears in the vicinity of the front of "demixion," double spots are formed which make the measurement of the R_f and separation of a mixture difficult. By increasing or decreasing the concentration of the acid, and thus moving the front of "demixion" above or below the spot of the alkaloid, the formation of double spots can be prevented.

The use of filter paper pretreated with neutral salts or buffer solutions also prevents "tailing." In the first case, the acid of the anion of the salt is added to the developing solvent; whereas in the second case, neutral developing solvents are used.

The R_f of an alkaloid chromatographed on paper pretreated with buffer solutions with *n*-butyl alcohol or isobutyl alcohol saturated with the same buffer solution as the nature of solvent, depends on the pH and the nature of the buffer used (5). The lower the pH, the lower the R_f value of the alkaloid will be since the ion has lower R_f value than the undissociated base. Thus the R_f increases with increasing pH and becomes constant at a higher pH.

The purpose of this investigation was to determine the variation of the R_f for various alkaloids with different buffers. The alkaloids investigated were apatropine, atropine, hyoscyamine, scopolamine, brucine, strychnine, codeine, morphine, papaverine, and 1-benzyl-3-ethyl-6,7-dimethoxy-isoquinoline (isaverine, Merck).

EXPERIMENTAL

Apparatus and Reagents.—Beckman model G pH meter, *n*-Butyl alcohol reagent grade, chloroform

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reagent grade, McIlvaine's buffer (6), citrate buffer (7), phosphate buffer 0.2 *M* U. S. P. XV, tartrate buffer 0.2 *M* (7), acetate buffer 0.2 *M* (7), lactate buffer 0.2 *M* (7), phthalate buffer U. S. P. XV, and Dragendorff's reagent as modified by Munier and Macheboeuf (1).

Preparation of Papers.—Strips of Whatman No. 2 paper, 4 x 30 cm., were immersed in different buffer solutions, air dried, sewn lengthwise with a white cotton thread in order of ascending pH, formed to fit the glass cylinder, and then placed therein.

Chromatography.—Glass cylinders (13 cm. diam., 45 cm. height) were used. The substances were applied with a micropipet as free bases in a chloroform solution (20 mcg. each time). The ascending method was used with *n*-butyl alcohol saturated with water as the developing solvent. A small beaker containing the water phase was placed at the bottom of the chromatographic jar, the hollow paper form was placed therein, and left six to fifteen hours for equilibration. The developing solvent was then added and the chromatograms were developed in the dark for six to eight hours (height: 20–25 cm.). The chromatograms were air dried and sprayed with Dragendorff's reagent and the R_f values were calculated, measuring the distance from the point of application to the leading edge of the spot. All chromatograms were run in duplicate and the R_f values, although varying with temperature, did not differ by more than 7%.

The pH of the buffer solutions was measured before and after immersion of the filter paper and after shaking the buffer solution with *n*-butyl alcohol. As Table I shows for citrate buffer, the pH remains unchanged after immersion of the paper, but increases slightly after shaking with *n*-butyl alcohol.

TABLE I.—pH OF BUFFER AFTER IMMERSION OF PAPER AND AFTER SHAKING WITH *n*-BUTYL ALCOHOL

Initial pH	pH After Immersion	pH After Shaking With <i>n</i> -Butyl Alcohol
1.22	1.22	1.32
2.20	2.15	2.30
3.20	3.20	3.30
4.30	4.30	4.40
5.40	5.40	5.50
6.00	5.95	6.10

Use of Whatman Nos. 1 and 4 and Schleicher and Schüll No. 2043b paper gave slightly different R_f values, but showed the same variation of R_f with the pH of the buffer solution used for the pretreatment of the paper. R_f values were always lower on Whatman No. 1 and Schleicher and Schüll No. 2043b than those obtained on Whatman No. 2 paper. On Whatman No. 4 paper they were highest. Whatman No. 1 paper sometimes gave "tailing," whereas Whatman No. 4 paper gave more diffuse spots. Changes in the molarity of the buffer did not bring about any appreciable changes in the R_f values, but the lower the molarity, the more elongated were the spots. The use of citrate buffer, prepared from secondary sodium citrate with the addition of HCl and NaOH, respectively (7), gave dif-

ferent R_f vs. pH graphs from those obtained when using citrate buffer prepared from citric acid by adding tertiary sodium citrate. Figure 1 shows that the R_f values up to about a pH of 5.0 are higher for the first buffer than those for the second. This is due to the influence of chlorine ions of HCl used for the preparation of the first buffer.

With pH's above 5.0 there is no influence of chlorine ions because NaOH instead of HCl was added to the buffer. The use of KOH instead of NaOH in the buffer gave higher R_f values which indicated some influence of the cations on the R_f values of the alkaloids. Figures 2, 3, 4, and 5 illustrate the dependency of the R_f values of the alkaloids on the pH for: citrate, McIlvaine's, phosphate, and tartrate buffers. The R_f values of the alkaloids at dif-

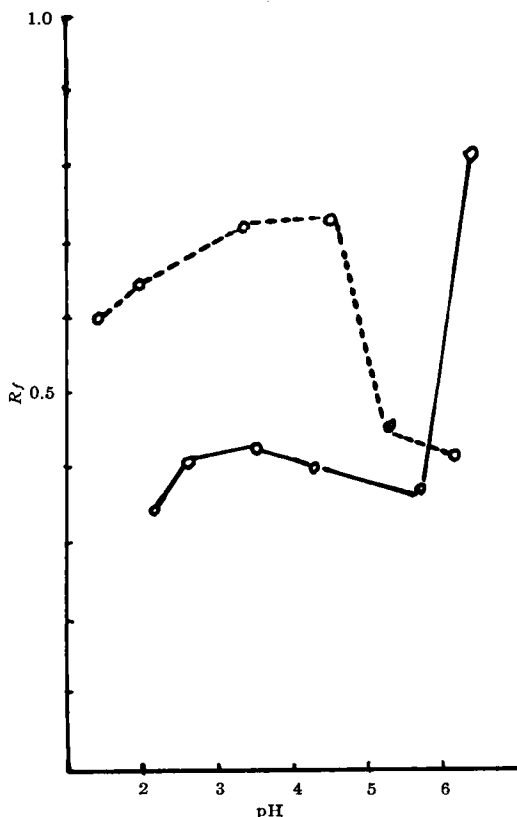


Fig. 1.— R_f vs. pH of atropine, O---O, buffer prepared from secondary sodium citrate with the addition of HCl and NaOH; O—O, buffer prepared from citric acid and tertiary sodium citrate.

ferent pH's for acetate and lactate buffers are shown in Tables II and III. The R_f values of all alkaloids increased steadily with increasing pH for those two buffers. The R_f values for phthalate buffer are not shown because they caused only slight variation.

DISCUSSION

The R_f values of the compounds studied depended primarily on the partition between the stationary

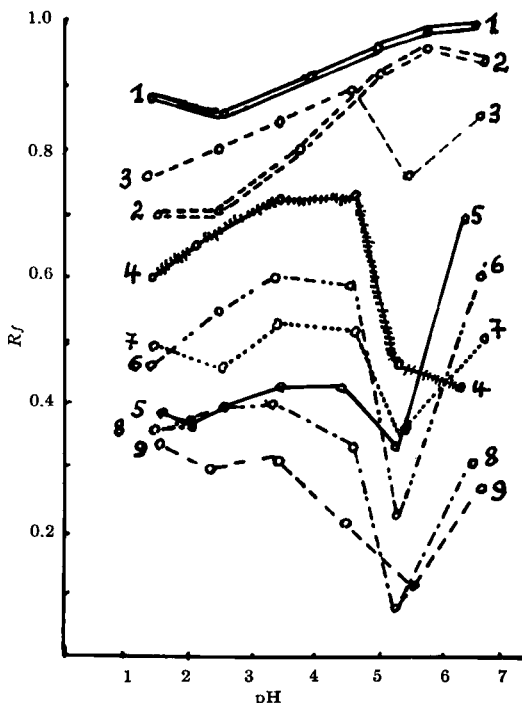


Fig. 2.— R_f vs. pH of alkaloids, citrate buffer.

See Fig. 5 for legend of curves

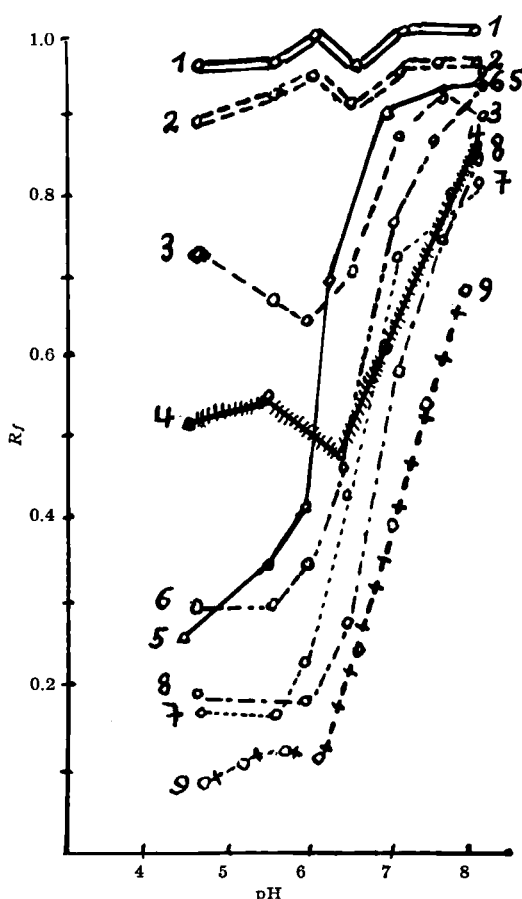


Fig. 4.— R_f vs. pH of alkaloids, phosphate buffer.

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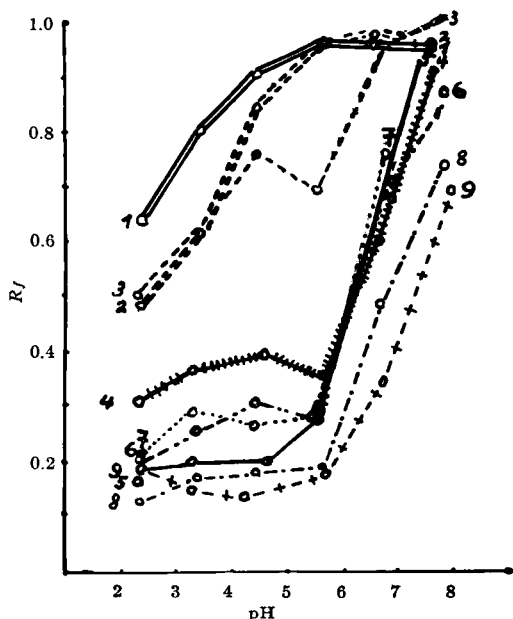


Fig. 3.— R_f vs. pH of alkaloids, McIlvaine's buffer.

See Fig. 5 for legend of curves

phase—adsorbed water—and the moving phase—organic solvent. The highly lipophilic compounds travelled as fast as the organic solvent having high R_f values. The hydrophilic ones having low R_f values remained close to the starting line. Thus the larger the alkyl group, the higher the R_f . Isoalkyl

compounds had lower R_f values than their *n*-alkyl analogs. Hydroxy compounds also had lower R_f values than their alkyl analogs. Cations and anions gave lower R_f values than the undissociated molecules, bases, and acids (8).

In the group of belladonna alkaloids, apoatropine has atropic instead of tropic acid in the molecule, the $\text{CH}-\text{CH}_2\text{OH}$ group being substituted by a $\text{C}=\text{CH}_2$ group. Apoatropine has therefore higher R_f values than atropine at all pH's of all buffers used. Scopolamine has, instead of the tropanol nucleus, an oscine nucleus with an oxygen bridge. Scopolamine has, at pH's above 5.50 and in acetate buffer, higher R_f values than atropine, but in all other buffers below pH 5.50, lower R_f values than atropine; probably because of the formation of oxonium ions at the lower pH levels.

In the group of strychnos alkaloids, brucine, a dimethoxystrychnine, has lower R_f values than strychnine.

The opium alkaloid codeine, is more lipophilic than morphine and therefore has higher R_f values. In the group of opium alkaloids with an isoquinoline nucleus, papaverine, because of the two additional $-\text{OCH}_3$ groups and the lack of the $-\text{C}_2\text{H}_5$ group, has lower R_f values than isaverine (1-benzyl-3-ethyl-6,7-dimethoxy-isoquinoline).

TABLE II.— R_f VALUES OF ALKALOIDS AT DIFFERENT pH'S FOR ACETATE BUFFER

pH	C	M	P	I	ST	B	AT	SC	AP ^a
3.3	0.84	0.64	0.86	1.00	0.74	0.60	0.60	0.84	0.76
3.7	0.89	0.70	0.88	1.00	0.80	0.64	0.74	0.90	0.87
4.3	0.94	0.72	0.91	1.00	0.88	0.76	0.82	0.94	0.94
4.9	0.97	0.82	0.92	1.00	0.95	0.85	0.94	0.99	0.97
5.6	0.97	0.80	1.00	1.00	0.95	0.90	0.96	1.00	1.00
6.1	0.95	..	1.00	1.00	0.95	0.86	0.98	1.00	1.00

TABLE III.— R_f VALUES OF ALKALOIDS AT DIFFERENT pH'S FOR LACTATE BUFFER

pH	C	M	P	I	ST	B	AT	SC	AP ^a
2.5	0.42	0.32	0.63	0.72	0.42	0.40	0.48	0.32	0.62
3.0	0.46	0.39	0.66	0.76	0.48	0.42	0.54	0.38	0.67
3.4	0.54	0.39	0.72	0.82	0.60	0.50	0.60	0.46	0.76
4.2	0.62	0.45	0.82	0.85	0.72	0.62	0.74	0.56	0.86
4.8	0.64	0.40	0.84	0.86	0.76	0.68	0.82	0.66	0.90
5.2	0.64	0.44	0.84	0.78	0.78	0.70	0.82	0.67	0.90

^a C, Codeine; M, Morphine; P, Papaverine; I, Isaverine; ST, Strychnine; B, Brucine; AT, Atropine; SC, Scopolamine; AP, Apoptropine.

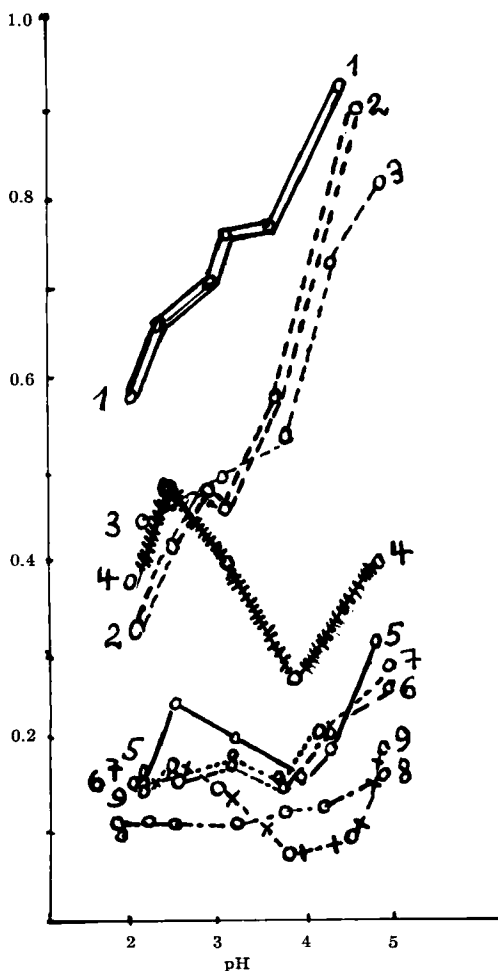


Fig. 5.— R_f vs. pH of alkaloids, tartrate buffer. Figures 2, 3, 4, and 5: 1---1 isaverine, 2---2 papaverine, 3---3 apoatropine, 4---4 atropine, 5---5 scopolamine, 6---6 strychnine, 7---7 codeine, 8---8 brucine, 9---9 morphine.

l-Hyoscyamine, in spite of its difference in the R_f values in certain buffers, especially tartrate, cannot be separated from atropine, its *dl*-isomer.

All alkaloids investigated, with the exception of papaverine and isaverine, belong to the group of alkaloids with dissociation constants from 10^{-3} to 10^{-7} and show a variation of R_f with pH, the R_f generally increasing with increasing pH. Papaverine and isaverine, which belong to the group of alkaloids with dissociation constants from 10^{-7} to 10^{-10} , being weaker bases, show this variation at lower pH levels. The R_f values of each alkaloid show differences at the same pH levels depending on the buffer used. These differences can not be explained by the difference in ionic strength because they exist even in buffers of approximately the same ionic strength, but they may also be due to differences in the water solubility of the solvent (9) or a chemical interaction between the anion of the buffer and the alkaloid. In the case of buffers of di- and poly-carboxylic acids, the R_f does not always increase by increasing the pH and the graphs R_f vs. pH show minima at certain pH levels (Figs. 2, 3, and 5). This may be due to the fact that di- and, in general, poly-carboxylic acids exist in different ionic forms depending on the pH.

The graphs R_f vs. pH are characteristic of a compound or a group of compounds and may be used for identification. They may also be used to find the optimum pH for the separation of compounds in a mixture.

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