

A SIMPLE METHOD OF PLOTTING THEORETICAL CURVES OF R_F AS A FUNCTION OF pH IN BUFFERED PAPER CHROMATOGRAPHY

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In buffered paper chromatography of organic electrolytes it is often possible to obtain optimal separation conditions by changing the partition ratios of the substances between both phases (and thus the rate of migration) by the use of buffer solutions as the stationary phase¹. The R_F -pH relationship is then expressed by the formula²:

$$R_F = \frac{k\nu}{k\nu + 1 + \frac{K_A}{[H^+]}} \text{ (acid)} \quad \text{or} \quad R_F = \frac{k\nu}{k\nu + 1 + \frac{K_B}{[OH^-]}} \text{ (base)}$$

where k is the partition coefficient of unionized substance between the mobile and immobile phase, ν is the ratio of cross-sectional areas of mobile and immobile phases ($k\nu$ is thus the partition number), and K_A and K_B are ionization constants.

When R_F measurements are interpreted, these functions should be plotted. Determinations of parameters involved (k , ν , K_A) and calculation and plotting of the curves takes a lot of time. Particularly when there are many determinations to be interpreted much time and work can be saved by taking advantage of a property of the function $R_F = f(\text{pH})$. If the ionization constant K_A is increased x times, it is sufficient to increase the hydrogen ion concentration x times (or to decrease the pH by $\log x$ units) for the R_F value to remain unchanged. This is valid for the whole curve; thus the ionization constant does not influence the shape of the curve but only its position: an x -fold increase of K_A shifts the curve, without changing its shape, by $\log x$ pH units towards lower pH values.

$R_F = f(\text{pH})$ curves of organic bases are mirror reflections of curves of acids and an x -fold increase of K_B shifts these curves, unchanged, by $\log x$ pH units towards higher pH values.

This property permits the use of a simple method of plotting $R_F = f(\text{pH})$ curves (see Fig. 1).

Fig. 1, representing plots of $R_F = f(\text{pH})$ curves for acids, at various $k\nu$ values (partition number of the unionized substance), is copied onto a transparent sheet. On a second sheet identical coordinates are drawn. The second sheet is laid upon the first sheet and then shifted horizontally, with pH axes overlapping, until the point marked X on the first sheet coincides with the pH value equal to the $\text{p}K_A$ of the

acid under consideration. Then the curve with respective k_r value is copied. If the substance considered is a base, the first sheet (with Fig. 1) is turned over, curves for bases being mirror reflections of curves for acids. Point X must then coincide with a pH value on the second sheet equal to $14 - \text{p}K_B$.

Working backwards, when theoretical curves from Fig. 1 are made to fit points found experimentally, for an acid of a known partition number k_r , the point X will

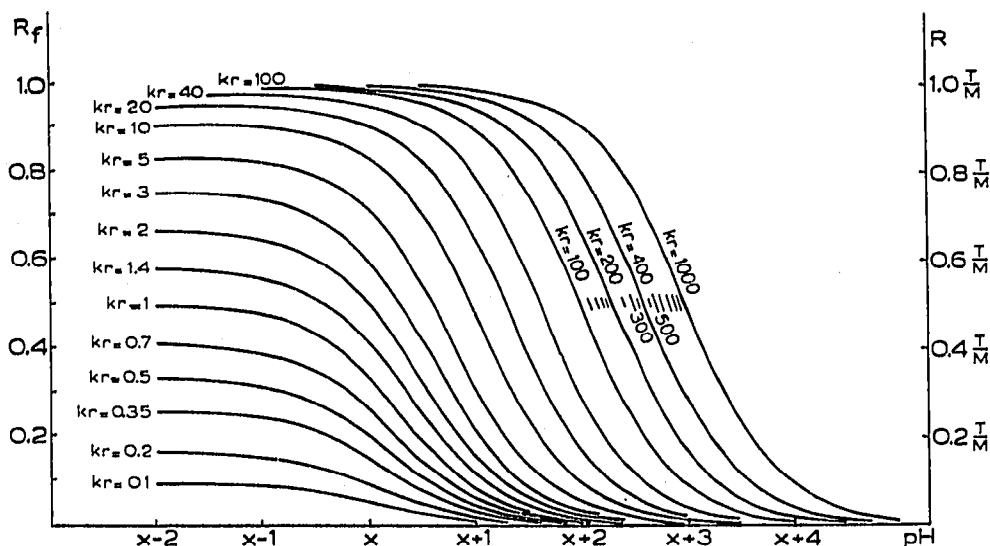


Fig. 1. The influence of the partition number k_r on the shape and position of $R_F = f(\text{pH})$ curves.

indicate a pH value on the pH coordinate of the experimental plot equal to the $\text{p}K_A$ value of the acid (or $14 - \text{p}K_B$ value if the substance is a base).

Comparison of the theoretical and experimental curves may give information concerning the mechanism of chromatography and make it possible to find ionization constants of acids and bases and isoelectric points of ampholytes. Moreover, if the experimental $R_F = f(\text{pH})$ curve is in accordance with theory (which holds if paper chromatography is a continuous extraction process in the strict sense of the word) only very few R_F values found experimentally at various pH values are needed to predict the whole curve. In fact, for an acid or base only two such points are sufficient to allow one to plot the whole curve: one at the pH in which the substance shows its maximum R_F value (for a given solvent and humidity of the paper), the second at the pH at which the substance has an intermediate R_F value (0.2–0.8 R_F max.). For an ampholyte three R_F values are needed: (1) at the pH at which the substance shows its maximum R_F value, (2) at the pH at which the substance has an intermediate R_F value owing to acidic ionization, and (3) at the pH at which the substance has an intermediate R_F owing to basic ionization.

Examples

(a) For an organic base it was found that at pH 6 the R_F is 0.83. This is its highest R_F since at pH 7 also the R_F is 0.83. At pH 3 the R_F was found to be 0.42. After turning over Fig. 1 (since the substance is a base) we find that only one curve can be

TABLE I

k_r pH	0.1	0.2	0.35	0.5	0.7	1	1.4	2	3	5	10	20	40	100	200	400	1000
X-2	0.09	0.17	0.26	0.33	0.41	0.50	0.58	0.67	0.75	0.83	0.91	0.95	0.98	0.99	1.0	1.0	1.0
X-1.5	0.09	0.16	0.25	0.33	0.41	0.49	0.58	0.66	0.75	0.83	0.91	0.95	0.98	0.99	1.0	1.0	1.0
X-1	0.08	0.15	0.24	0.31	0.39	0.48	0.56	0.65	0.73	0.82	0.90	0.95	0.97	0.99	1.0	1.0	1.0
X-0.5	0.07	0.13	0.21	0.28	0.35	0.43	0.52	0.60	0.70	0.79	0.89	0.94	0.97	0.99	1.0	1.0	1.0
X	0.05	0.09	0.15	0.20	0.26	0.33	0.41	0.50	0.60	0.71	0.83	0.91	0.95	0.98	0.99	1.0	1.0
X+0.5	0.02	0.05	0.08	0.11	0.14	0.19	0.25	0.32	0.42	0.55	0.71	0.83	0.91	0.97	0.98	0.99	1.0
X+1	0.01	0.02	0.03	0.04	0.06	0.08	0.11	0.15	0.21	0.31	0.48	0.65	0.79	0.90	0.95	0.98	0.99
X+1.5	0	0.01	0.01	0.02	0.02	0.03	0.04	0.06	0.08	0.13	0.23	0.38	0.55	0.76	0.86	0.93	0.96
X+2	0	0	0	0	0.01	0.01	0.01	0.02	0.03	0.05	0.09	0.17	0.28	0.50	0.67	0.80	0.91
X+2.5	0	0	0	0	0	0	0	0.01	0.01	0.02	0.03	0.06	0.11	0.24	0.39	0.56	0.76
X+3	0	0	0	0	0	0	0	0	0	0	0.01	0.02	0.04	0.09	0.17	0.29	0.50
X+3.5	0	0	0	0	0	0	0	0	0	0	0	0.01	0.01	0.03	0.06	0.12	0.24
X+4	0	0	0	0	0	0	0	0	0	0	0	0	0	0.01	0.02	0.04	0.09
X+4.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.01	0.01	0.03

made to fit these experimental points: the $k' = 5$ curve (Fig. 2). Point X of Fig. 1 is situated at pH 3.7 on the experimental plot; so $\text{p}K_B = 14 - 3.7 = 10.3$; $K_B = 5 \cdot 10^{-11}$.

(b) For an ampholyte it was found that $R_F = 1$ at pH 7, $R_F = 0.5$ at pH 11 (acidic ionization) and $R_F = 0.4$ at pH 1 (basic ionization). The partition number of the substance is here above 50 and it is impossible to estimate it from $R_F \text{ max.}$ because k' values above 50 no longer influence the shape of the curve, only its position. So we may copy onto the experimental plot any of the curves of Fig. 1 whose $k' > 50$, for instance the $k' = 100$ curve. At higher pH we copy the curve in its normal position (the ampholyte behaves like an acid), at lower pH in reversed position (the ampholyte behaves like a base). So we get the $R_F = f(\text{pH})$ relationship in the whole pH range (for a given solvent and humidity of the paper) in the form of a bell-shaped curve

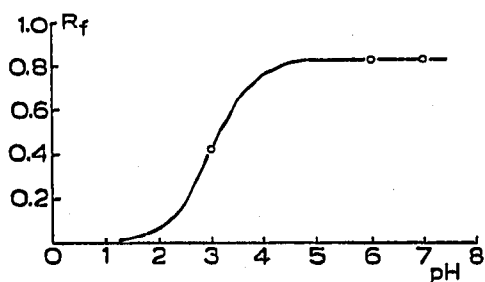


Fig. 2.

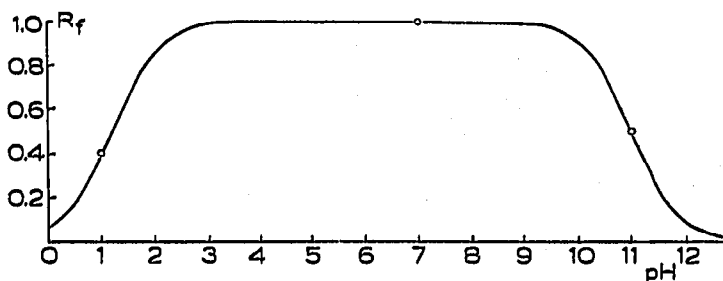


Fig. 3.

(Fig. 3). The isoelectric point of the ampholyte lies on the axis of symmetry of the curve. It is impossible, however, to estimate ionization constants of the ampholyte since we do not know its partition number k' ; to find this other experiments will be necessary. For instance, if we find that $k' = 100$, point X of Fig. 1 indicates $\text{p}K_A = 9.0$; $\text{p}K_B = 14 - 3.2 = 10.8$; when $k' = 500$ we find that $\text{p}K_A = 8.3$; $\text{p}K_B = 14 - 3.9 = 10.1$. For plotting theoretical curves in any scale, Table I may also be used. The method described is also applicable to partition chromatography on buffered columns. Ordinate values must then be multiplied by T/M (T is the total cross-sectional area of column, M is the cross-sectional area of the mobile phase), and the R value used in column chromatography is obtained instead of R_F (see coordinates on the right-hand side of Fig. 1). The method described has been confirmed experimentally for quinoline derivatives.

SUMMARY

A simple method of drawing theoretical $R_F = f(\text{pH})$ curves of organic electrolytes is described.

REFERENCES

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