Z Katedry Chemii Nieorganicznej Wydziału Farmaceutycznego AM w Lublinie
Kierownik: prof. dr Andrzej Waksmundzki

Edward SOCZEWIŃSKI
i Andrzej WAKSMUNDZKI

Some Differences and Analogies in Linear, Radial and Reversed Phase Chromatography

Różnice i analogie w chromatografii liniowej, krążkowej i odwróconych faz

Различия и аналогии в хроматографии линеарной, радиальной и обращенными фазами

Making the assumptions of the plate theory (modified correspondingly for radial chromatography: the cross-sectional areas of the two liquid phases are proportional to their distances from the center of the circle), and assuming that in all three techniques the same organic and water phases are used, at constant ratio of their cross-sectional areas (r), we may write the following equations concerning the relative distances travelled by a given substance in linear (R_{FL}), radial (R_{FC}) and reversed phase chromatography (R_{FR}):

$$R_{FC} = \sqrt{R_{FL}}$$

$$R_{FR} = 1 - R_{FL} \qquad (1)$$

Therefore, for buffered paper chromatography [1] we have:

$$CC \qquad R_{FC} = \sqrt{\frac{kr}{kr + 1 + 10^{\pm(pH - pK_A)}}}$$

$$LC \qquad R_{FL} = \frac{kr}{kr + 1 + 10^{\pm(pH - pK_A)}} \qquad (2)$$

$$RC \qquad R_{FR} = 1 - \frac{kr}{kr + 1 + 10^{\pm(pH - pK_A)}}$$

(upper sign in the power refers to acids, lower — to bases, for which $pK_A = pK_W — pK_B$).

Thus, in all three techniques the relative rate of migration (R_F) is a function of four parameters:

1. The partition coefficient k (ratio of concentrations of unionized substance in organic and water phase, at equilibrium, $k = c_{org}/c_w$).

2. The volume coefficient r (ratio) of cross-sectional areas of organic and water phase, or their volumes in a sufficiently short section of the system) $(kr$ — partition number of unionized electrolyte).

3. The ionization constant.

4. The pH of water phase.

By mathematical analysis of the function (2) it is possible to anticipate the theoretical effect of the four parameters and their variations upon the position and sequence of $R_F = f(pH)$ curves $(R_F = R_{FL}, R_{FC}$ or $R_{FR})$, the range of changes of R_F, the pH of optimal separation etc. The knowledge of these relations may simplify and make easier the choice of optimal conditions of separation and the estimation of its efficiency; moreover, the results of the remaining techniques may be predicted on the basis of the results of either of these techniques; and the value of any of the four parameters of distribution may be calculated when the remaining parameters are known.

EFFECT OF PARTITION NUMBER OF UNIONIZED ELECTROLYTE kr

The effect of the product kr upon $R_F = f(pH)$ curves in the three different chromatographic techniques is illustrated in Fig. 1 $(K_A = \text{const.})$. The partition number influences both the shape and position of $R_F = f(pH)$ curves.

The height of a curve increases with kr and is equal to $\dfrac{kr}{kr+1}$ (comp. form. 2) in case of linear and reversed phase chromatography $(R_F$ vs. pH curves in reversed phase chromatography may be obtained by turning R_{FL} vs. pH curves by $180°$ around a horizontal axis passing through $R_F = 0.5$). In radial chromatography the height of a curve is equal to $\sqrt{\dfrac{kr}{kr+1}}$; thus R_{FC} vs. pH curves are always higher than R_{FL} vs. pH curves and the difference is more pronounced at lower values of kr. At kr values higher than 100 the height of R_F vs. pH curves practically does not increase any more with kr $(R_F = 1)$ and the shape is already invariable: with the increase of kr the curves are only shifted along the pH axis, an n-fold increase in kr causing a shift by log n pH units:
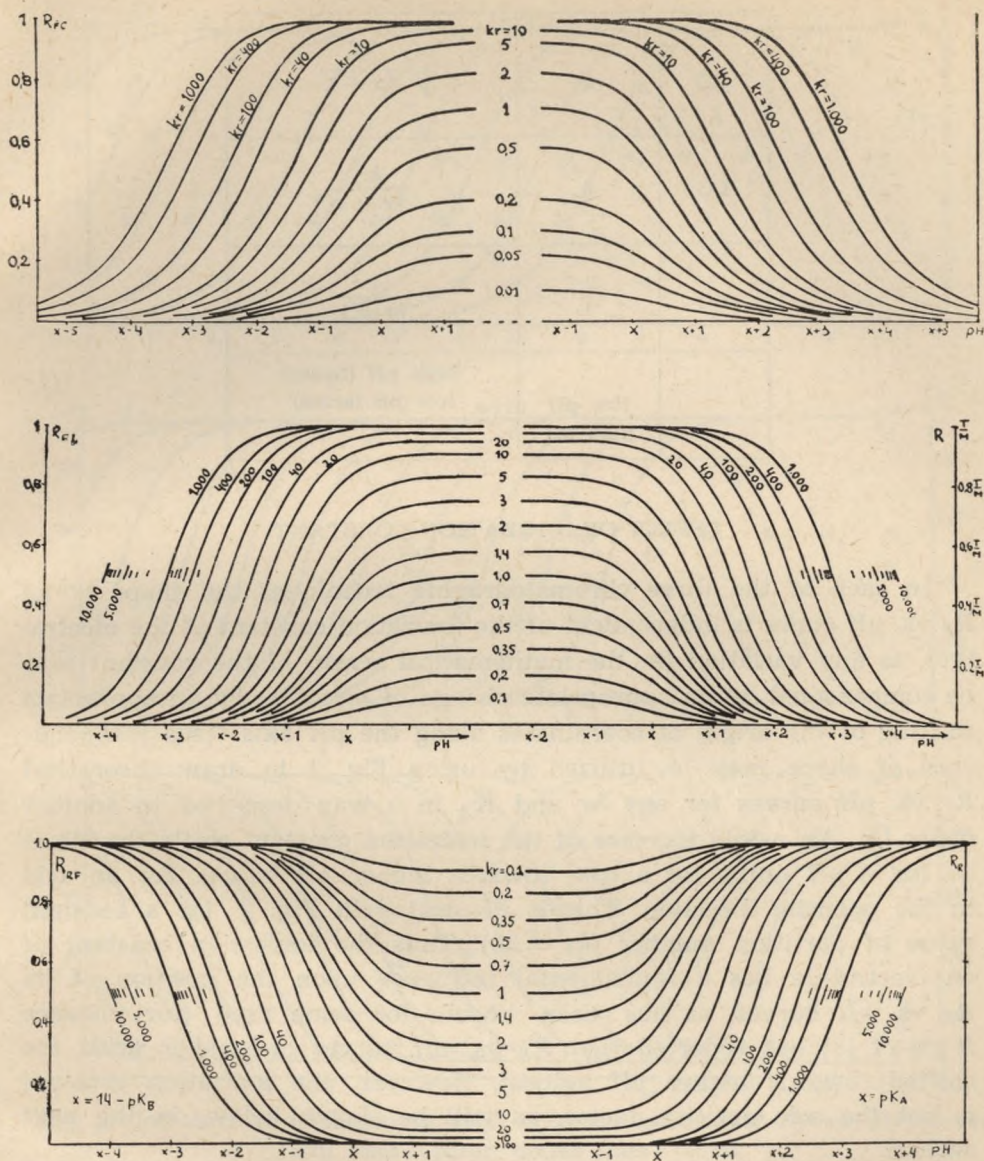
Fig. 1. Influence of partition number upon the shape and position of R_F vs. pH curves; R_{FC} — radial, R_{FL} — linear (conventional), R_{RF} — reversed phase chromatography

bases — towards lower pH values, acids — in the opposite direction (comp. form. 3).

By appropriate variations of pH one can change the R_F values within the following ranges:

CC	0	$\sqrt{\dfrac{kr}{kr+1}}$
LC	0	$\dfrac{kr}{kr+1}$
RC	1	$\dfrac{1}{kr+1}$
	low pH high pH	high pH (bases) low pH (acids)

EFFECT OF IONIZATION CONSTANT

In each of the three chromatographic techniques the shape of an R_F vs. pH curve is independent of the ionization constant of the electrolyte, as any variation (in the mathematical sense) of the constant may be compensated by an appropriate change of pH, i. e., by an appropiate shifting of the origin of coordinates along the pH axis. This independence of shape may be utilized by using Fig. 1 to draw theoretical R_F vs. pH curves for any kr and K_A in a way described in another paper [2]. An n-fold increase of the ionization constant shifts the curve by log n pH units; for a base towards higher pH values, for an acid in the opposite direction. This is illustrated in Fig. 2 for a constant value of partition number $(kr = 20)$. Thus the ionization constant of an electrolyte has a fundamental influence upon the position of its R_F vs. pH curves, as has been known for some time (for instance R y b a ř [3] has reported that R_F vs. pH curves of weaker acids are shifted towards higher pH values). However, the ionization constant is not the sole decisive factor, as will be shown below, in the next section.

THE JOINT EFFECT OF IONIZATION CONSTANT AND PARTITION NUMBER UPON THE POSITION AND SEQUENCE OF R_F VS. pH CURVES

In case of linear chromatography (conventional or reversed phase) the position of an R_F vs. pH curve is most conveniently defined by its inflexion point [4], which lies at its half-height, and, as can be easily demonstrated, is the center of its axial symmetry. From the condition
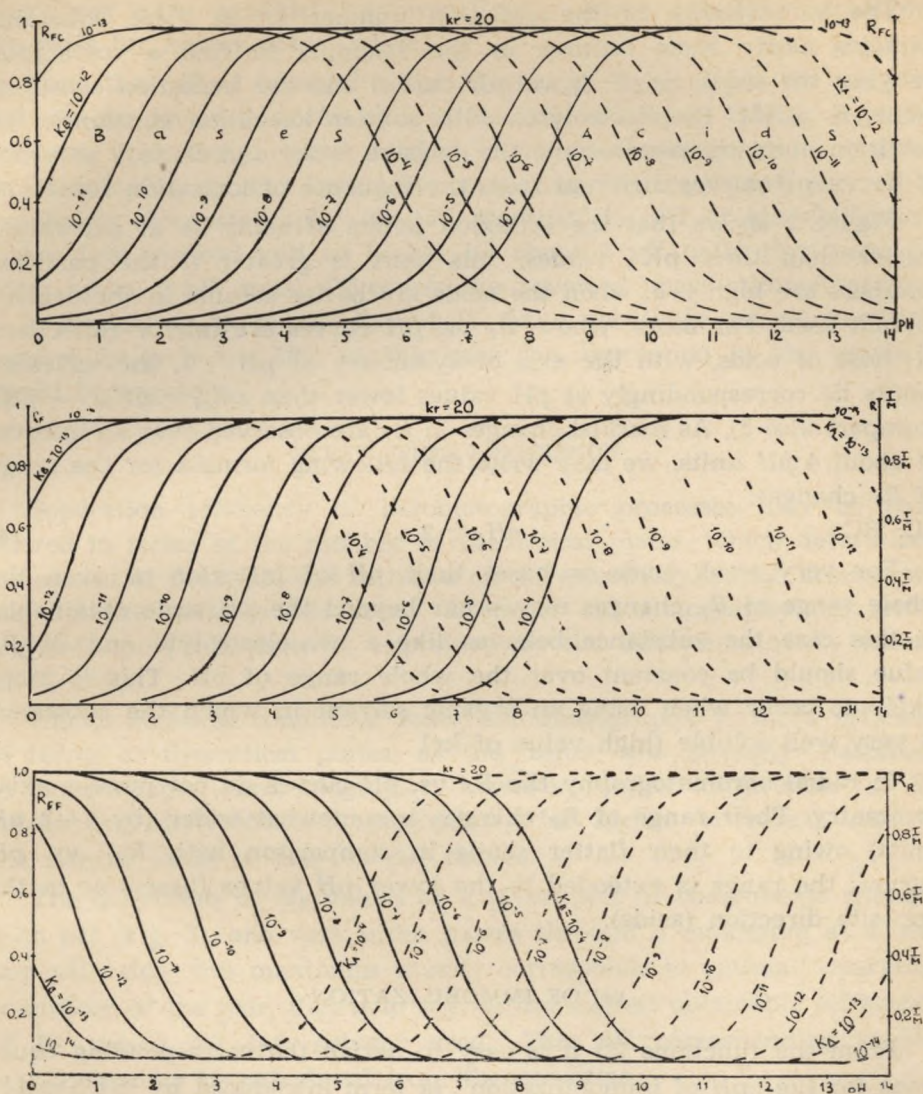
Fig. 2. Influence of ionization constant upon the position of R_F vs. pH curves. Full lines — bases; dotted lines — acids.

$R_F = \dfrac{1}{2}\dfrac{kr}{kr+1}$ we have for the pH value (pH_i) corresponding to the inflexion point:

LC, RC
$$pH_i = pK_A \pm \log(kr + 1). \tag{3}$$

For high partition numbers $(kr > 100)$ the unity may be neglected and then

$$pH_i = pK_A \pm \log kr. \tag{3a}$$

The participation of the partition number kr in these equations explains easily some failures in the attempts to find a correlation between the sequence of R_F vs. pH curves and the ionization constants alone, e. g. [5]: for electrolytes with similar ionization constants, the partition numbers may become the decisive factor and cause a sequence of R_F vs. pH curves different from the sequence of ionization constants.

Figure 2 shows that the inflexion points of acids lie at pH values higher than their pK_A values; this shift is greater if the partition numbers are high i. e. when the acids are better soluble in the organic solvent used. For bases, whose R_F vs. pH curves are mirror reflections of those of acids, with the axis of symmetry at $pH = 7$, the inflexion points lie correspondingly at pH values lower than $pK_A = pK_W - pK_B$ (compare also 3). As marked changes in R_F are observed over an interval of about 4 pH units, we may write the following formula for the range of R_F changes:

LC, RC $\qquad\qquad\qquad pH_i \pm 2.$ $\qquad\qquad\qquad$ (4)

For very weak acids or bases their pH of inflexion or even the whole range of R_F changes may occur beyond the pH scale obtainable. In this case the substance behaves like a non-electrolyte and its R_F value should be constant over the whole range of pH. This is more likely to occur when using an organic solvent in which the substance is very well soluble (high value of kr).

In radial chromatography the R_F vs. pH curves do not possess axial symmetry. Their range of R_F changes is somewhat wider (by 1—2 pH units) owing to their flatter shape in comparison with R_{FL} vs. pH curves; the range is extended to the lower pH values (bases) or in the opposite direction (acids).

pH OF IMMOBILIZATION

From the functions (2) it is easy to derive the corresponding equations for the „pH of immobilization" (a term introduced by S c h m a l l and co-workers [6, 7] in their papers on multi-buffered paper chromatography), that denotes the pH at which the migration of the electrolyte is stopped. Assuming that at pH of immobilization the R_F value of an electrolyte is equal to 0.01 we may derive the following approximate equations:

CC $\qquad pH_O = pK_A \pm \log (10.000 \ kr - 1) = pK_A \pm 4 \pm \log kr$

LC $\qquad pH_O = pK_A \pm \log (100 \ kr - 1) \doteq pK_A \pm 2 \pm \log kr$ $\qquad$ (5)

RC $\qquad pH_O = pK_A \pm \log \left(\dfrac{kr}{100} - 1\right)$

(the upper signs — acids; the lower signs — bases; simplified equations are valid for higher values of kr).

Thus the pH of immobilization is always higher than the pK_A of the acid (or lower than the pK_A in the case of bases). It also depends, however, upon the partition number of unionized electrolyte, i. e. upon the developing solvent used. By comparing the equations for radial and linear chromatography we can see that the pH of immobilization in the first method will be higher by about 2 pH units (for bases — lower by ca. 2 pH units). Of course, for reversed phase chromatography it is impossible to obtain $R_F = 0.01$ for a substance whose partition number kr is below 100; in this case the expression in the brackets would be negative.

SEPARATION EFFICIENCY

Separation efficiency of chromatographic processes may be considered in terms of the number of theoretical plates, which determines the spread of the zone, and in terms of the selectivity, which determines the distance between two zones. In paper chromatography the latter factor is most conveniently expressed by the difference of R_F values ($\triangle R_F \geqslant 0$).

Under identical conditions radial chromatography is more efficient in terms of theoretical plates, as the zones are strongly flattened. In linear paper chromatography, however, higher differences in R_F values are often obtained, particularly for higher kr values, due to the steeper shape of R_{FL} vs. pH curves.

The difference of R_F values of a given pair of components changes with pH (Fig. 3), and very often passes through a maximum at a certain pH value; this maximum usually corresponds to optimal separation conditions of the pair, that is to say, to the highest obtainable difference of R_F values for the solvent used. This pH corresponding to the maximum may be calculated from the condition:

$$\frac{d(R_F' - R_F'')}{dpH} = 0. \tag{6}$$

Substituting (1) into (6) the following formulae are obtained:

$$CC \qquad pH_{max} = \log \frac{\dfrac{k'r + 1}{K'_A}\sqrt[3]{\dfrac{k''r}{K''_A}} - \dfrac{k''r + 1}{K''_A}\sqrt[3]{\dfrac{k'r}{K'_A}}}{\sqrt[3]{\dfrac{k'r}{K'_A}} - \sqrt[3]{\dfrac{k''r}{K''_A}}}$$
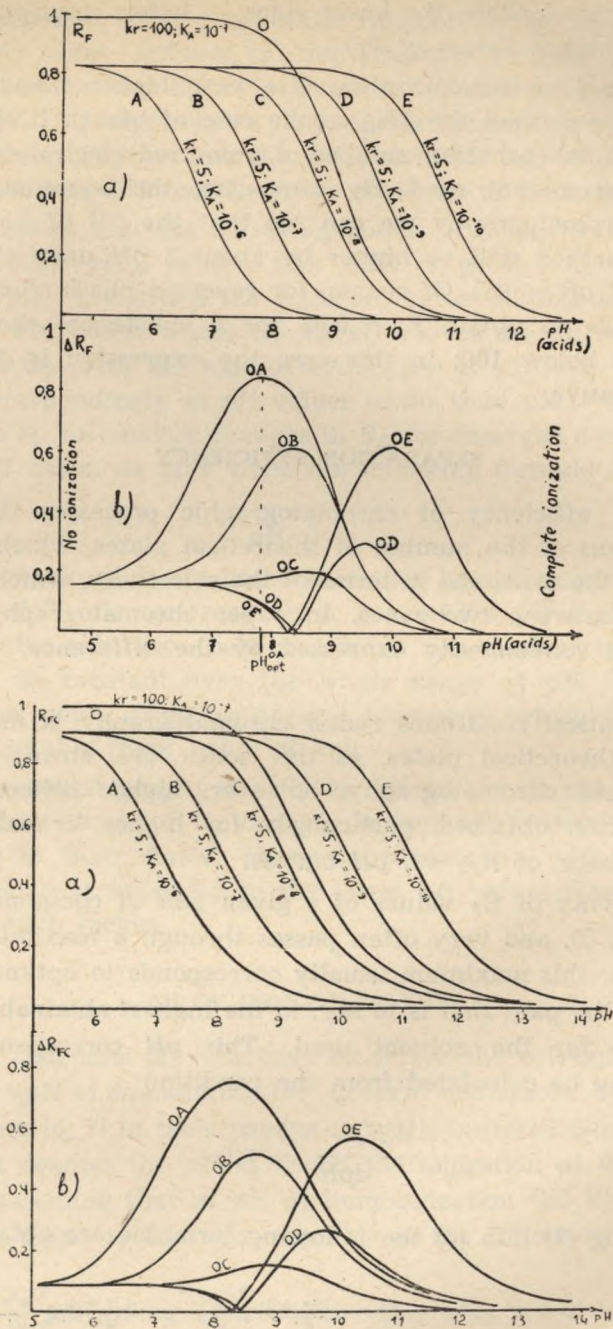
Fig. 3. R_F vs. pH curves (top) and $\triangle R_F$ vs. pH curves (bottom) for several pairs of components in linear (upper diagram) and radial chromatography (lower diagram. The maxima correspond to optimal separation conditions: $pH_{max} = pH_{opt}$.

$$\text{LC, RC} \qquad pH_{\max} = \log \frac{\frac{k'r+1}{K'}_A \sqrt{\frac{k''r}{K''}_A} - \frac{k''r+1}{K''}_A \sqrt{\frac{k'r}{K'}_A}}{\sqrt{\frac{k'r}{K'}_A} - \sqrt{\frac{k''r}{K''}_A}}. \qquad (7)$$

Of course, for the same parameters of distribution the differences of R_F values obtained in both conventional and reversed phase linear chromatography will be identical, only the sequence of zones will be reversed. Thus the equation for $pH_{\max}$ will be the same for both techniques. If, however, one of the parameters of distribution is changed (e. g. the coefficient r), then other distribution conditions are obtained and the maximum will be shifted to another pH value; the extent and direction of the shift may be anticipated on the basis of Fig. 1. The maximal obtainable difference of R_F values may also change.

On the other hand, in radial chromatography the pH of optimal separation will be different from that in linear chromatography, even with the same parameters of distribution. It can be seen from Fig. 3 that for higher partition numbers kr the maxima are lower and longer. The position of $pH_{\max}$ is somewhat shifted to the lower pH (bases) or the higher pH (acids).

However, in certain cases, particularly when the partition numbers of both substances are low (below $kr = 0.5$), it may be possible to obtain higher differences of R_F values by the radial method. Such a case is illustrated in Fig. 4.

Thus, with low partition numbers (when the substance is better soluble in water than in the developing solvent), radial chromatography
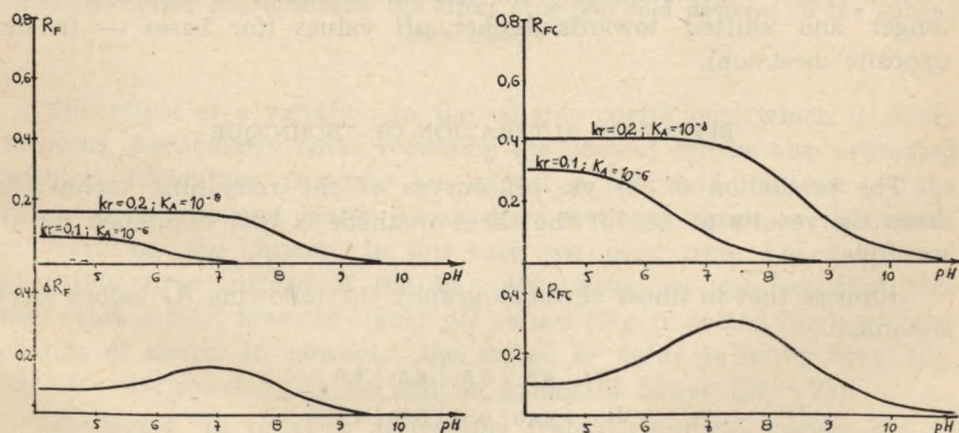

Fig. 4. Case of greater R_F differences in circular chromatography:
$$\max \triangle R_{FC} > \max \triangle R_{FL}$$

may prove more efficient both in terms of theoretical plates and in terms of differences of R_F values.

The calculation of pH_{max} from form. 7 would be tedious; moreover, in certain cases pH_{max} does not correspond to optimal separation conditions, and higher differences in R_F values may be obtained when the substances are in their unionized forms (an analogous case is also possible in paper electrophoresis, cf. [8]). Furthermore, for multicomponent mixtures different pH_{opt} are obtained for each pair of components. Therefore, it would be simpler to plot theoretical R_F vs. pH curves from Fig. 1, using the simplified method [2] (on the basis of the distribution parameters kr and K_A or on the basis of experimental R_F values determined at a few values of pH), and then to choose the pH value at which all the substances are best separated.

The case when partition numbers are high ($kr \geqslant 100$) is less complicated, particularly for linear chromatography (conventional or reversed phase); pH_{max} is then identical with pH_{opt} and the equations are much simpler; for linear chromatography:

$$\text{LC, RC} \qquad pH_{max} = pH_{opt} = \frac{1}{2} \log \frac{k'r \cdot k''r}{K'_A \cdot K''_A}.$$

Furthermore, in each technique the horizontal distance between two curves is then equal to the logarithm of the coefficient of separation of the pair of components:

$$\beta = \frac{k' K''_A}{k'' K'_A} \geqslant 1$$

and in radial chromatography the maxima of $\triangle R_F$ are always lower, longer and shifted towards higher pH values (for bases — in the opposite direction).

EFFECT OF ALTERATION OF TECHNIQUE

The prediction of R_F vs. pH curves of the remaining techniques from the results of any of the three methods is best explained by an example.

Suppose that in linear chromatography the following R_F values were obtained:

pH	4,5	5,5	6,5	8,0
R_{FL}	0,10	0,52	0,90	1,00

Plotting these values on a sheet of tracing paper, the coordinate scales identical with those in Fig. 1, and making the points fit to

Fig. 1 LC, as described in, [2], we can see that any of the curves with $kr > 100$ may be the theoretical R_{FL} vs. pH curve for these points. For example, we copy the $kr = 400$ curve, marking also the X point on the pH axis. The sheet of tracing paper is now transferred over Fig. 1 CC, and with pH axes and X points overlapping, the $kr = 400$ curve is copied, giving R_{FC} vs. pH relationship for the same substance, under the same distribution conditions, in radial chromatography. The sheet of tracing paper is then transferred over Fig. 1 RC, and with pH axes and X points overlapping, the $kr = 400$ curve is copied, giving this time R_{FR} vs. pH relationship for reversed phase chromatography under identical distribution conditions (the same parameters k, r and K_A). Thus we have obtained R_F vs. pH relationship for all three techniques (Fig. 5, full lines).

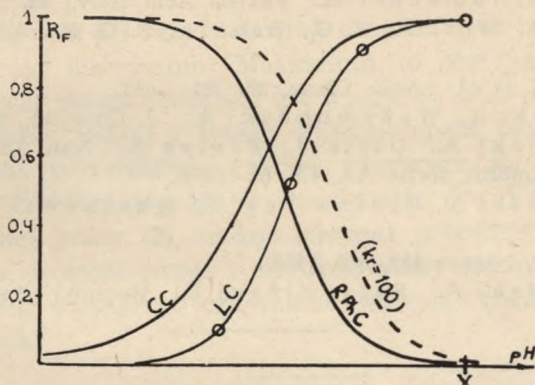Fig. 5. Prediction of R_{FC} and R_{FR} vs. pH relationships from linear chromatography data. The dotted line illustrates the effect of a four-fold decrease in the volume coefficient r

The effect of a variation in the volume coefficient (which is likely to occur, particularly when reversing the phases) can be also estimated without difficulties. Suppose we expect a four-fold decrease of the ratio of organic and water phase cross-sectional areas as the result of reversing the phases. In this case we copy from Fig. 1 RC the $kr = 100$ curve instead of the $kr = 400$ curve; we can see that this will cause a shift towards higher pH values (Fig. 5, dotted line), without change of shape. If, however, the actual kr value is lower than 400, say 100, the resulting curve will be somewhat lower ($kr = 25$).

Of course, in practice deflections from theoretical curves are to be expected due to departures from idealizing assumptions of the plate theory: adsorption, formation of r and pH gradients, demixion etc.

It seems that the „moist paper method" developed in this laboratory [2, 9—11] may give conditions similar to those of countercurrent extraction processes assumed in the plate theory. Good agreement of experimental and theoretical curves was obtained in the works cited (linear conventional paper chromatography), and the values of the volume coefficient r were defined and reproducible [12].

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STRESZCZENIE

Zakładając słuszność wzorów (1), wiążących wartości R_F otrzymane w trzech technikach chromatograficznych: liniowej (konwencjonalnej), krążkowej i odwróconymi fazami, omówiono wpływ stosowanej techniki na wartości R_F elektrolitów organicznych (buforowana faza polarna) oraz na zdolność rozdzielczą procesu.

1. Wpływ liczby podziału *(kr)* niezdysocjowanej substancji na przebieg krzywych R_F vs. *pH* zilustrowano na ryc. 1. Krzywe dla chromatografii odwróconych faz otrzymać można przez obrót wykresu dla chromatografii konwencjonalnej (polarna faza nieruchoma) o 180° wokół osi poziomej, leżącej na wysokości $R_F = 0,5$. Krzywe dla chromatografii krążkowej są wyższe od krzywych dla chromatografii liniowej (szczególnie przy niskich liczbach podziału *kr)* i bardziej wydłużone.

2. Stała dysocjacji nie wpływa na kształt krzywych we wszystkich trzech technikach, a tylko na ich położenie wzdłuż osi *pH*. Dla chromatografii liniowej (LC) i odwróconych faz (RC) położenie krzywych

określone ich punktami przegięcia wyraża się wzorem (3), zaś zakres zmian R_F wzorem (4). Dla chromatografii krążkowej zakres zmian R_F jest rozszerzony w kierunku niższych pH (zasady) lub wyższych pH (kwasy).

3. Wzory (5) wyrażają wpływ parametrów podziału na pH potrzebne do zatrzymania elektrolitu organicznego na linii startu.

4. Zdolność rozdzielcza, wyrażona w postaci różnicy wartości R_F, ze względu na bardziej płaski przebieg krzywych F_{FC} vs. pH jest na ogół niższa w chromatografii krążkowej niż liniowej. Jednak przy niskich liczbach podziału, np. poniżej 0,5, możliwe jest uzyskanie wyższych różnic R_F w chromatografii krążkowej (por. ryc. 4). Z punktu widzenia liczby półek teoretycznych, jak wiadomo, chromatografia krążkowa jest na ogół bardziej skuteczna ze względu na zwężenie pasm w czasie rozwijania.

Wzór (7) wyraża pH fazy polarnej, przy których różnica wartości R_F przechodzi przez maksimum. Maksimum to jest zawsze równoważne optymalnym warunkom rozdziału dla wysokich liczb podziału $(kr > 100)$.

5. Z wyników jednej z trzech wymienionych technik chromatograficznych można przewidzieć przebieg krzywych R_F vs. pH dla pozostałych technik chromatograficznych, stosując ryc. 1 i metodę graficzną opisaną w innej pracy (2), można również przewidzieć wpływ zmiany współczynnika objętościowego r spowodowanej zmianą stosowanej techniki, a ponadto wyznaczyć optymalne warunki rozdziału dla poszczególnych technik.

Р Е З Ю М Е

Предполагая применимость уравнений (1) связывающих величины R_f получаемые при трёх хроматографических методах: линеарном (конвенциальном) R_{FL}, радиальном R_{FC} и с обращенными фазами R_{FR}, рассмотрено влияние применяемой техники на величины R_F органических электролитов (забуференная полярная фаза) и на разделительную способность процесса.

1. Влияние числа распределения (kr) недиссоциированного вещества на ход кривых R_F vs pH показано на рис. 1. Кривые для хроматографии обращенными фазами можно получить поворотом графика для конвенциональной хроматографии (полярная неподвижная фаза) на $180°$ вокруг горизонтальной оси, проходящей через $R_F = 0,5$. Кривые для радиальной хроматографии выше чем кривые для линеарной хроматографии (особенно при низких числах распределения) и более растянутые.

2. Константа ионизации не влияет на вид кривых при всех трёх способах, а только на их положение вдоль оси pH. Для линеарной хроматографии (LC) и хроматографии обращенными фазами (RC) положение кривых определенное их точками перегиба выражает формула 3, интервал изменений R_F формула 4. Для радиальной хроматографии интервал изменений R_F расширен в направлении более низких pH (основания) или более высоких pH (кислоты).

3. Формулы 5 устанавливают связь между параметрами распределения и pH нужном для задержания электролита на линии старта.

4. Разделительная способность выраженная в виде разности величин R_F, обычно ниже в радиальной хроматографии из-за более плоского хода кривых R_{FC} vs pH. Однако для низких чисел распределения, например ниже 0,5, возможны более высокие разности коэффициентов R_F в радиальной хроматографии (сравни рис. 4). С точки зрения теоретических тарелок, как известно, радиальная хроматография является обычно более эффективной из-за суживания полос во время проявления.

Формула (7) определяет pH полярной фазы при которой разность коэффициентов R_F достигает максимума. В случае высоких чисел распределения $(kr > 100)$ этот максимум всегда соответствует оптимальным условиям разделения.

5. Из результатов одной из трёх рассматриваемым техник нетрудно теоретически установить ход кривых R_F vs. pH для остальных техник, применяя рис. 1 и графический метод описанный в другой работе [2]; возможно также предвидеть влияние изменения коэффициента объемности r вызванного изменением применяющейся техники, а также определить оптимальные условия разделения для остальных двух методик.