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The effect of the conditions of preparation of cation-exchange resins on their resolution efficiency in ion-exchange chromatography of amino acids

Attempts to improve the liquid chromatography of amino acids on sulphonated cation-exchange resin[1] have up to now been limited predominantly to the optimization of the apparatus used, the amino acid analyzer[2–4], or the conditions of separation[5–8]. Much less definite information has been published about the role of the resin itself in the resolution, though its importance has often been emphasized[9–11]. The resin was considered suitable providing it reached a certain level of guaranteed properties with respect to the content of the sulphonic groups, the degree of cross-linking and the size distribution or the shape of the particles.

It has been shown, however, that these criteria do not define the quality of the chromatographic material completely. Identical values for various resins do not guarantee the same resolution even when all the other conditions are constant.

So far, the effect of the conditions of preparation of the resin on its resolution efficiency has not been determined. This effect is quite demonstrable, though in a certain range, a change in the conditions of synthesis does not result in a change of the basic resin characteristics which can be detected by analytical methods at present available.

The purpose of this paper is to demonstrate the relation between the reaction conditions of sulphonation of a styrene–divinylbenzene copolymer and the resolution efficiency of the resulting cation-exchange resin in the separation of neutral amino acids.

Materials

Cation exchange resins I–IV were prepared by sulphonation of the same spherical copolymer of styrene with 8 % (w/w) divinylbenzene. The following parameters were varied in the sulphonation process: the swelling agent for the copolymer; the concentration of the sulphonating agent; the duration of sulphonation; and the temperature of sulphonation. After sulphonation, the resins were washed in the same way with 2 *N* HCl and 2 *N* NaOH, alternatively, and classified by a modified HAMILTON method[12]. All the resins showed the following basic properties: total exchange capacity[13], 5.32 ± 0.09 mequiv./g; strong acid exchange capacity, 5.19 ± 0.09 mequiv. per g; weak acid exchange capacity, 0.13 ± 0.03 mequiv./g; true density (H^+ form), 1.201 ± 0.003 $g \cdot cm^{-3}$; mean particle diameter, 18.0 ± 1.3 μm.

Separation conditions

Apparatus: AAA type 6020/A, a product of the Development Works of the Czechoslovak Academy of Sciences, Prague; column, 0.8 × 55.0 cm; resin bed depth, 52.0 cm; elution buffers, sodium citrate pH 3.25, 4.25, and 5.28; flow rates, buffers 60 ml/h, ninhydrin 30 ml/h; temperature, 53°.

Amounts: 100 nmole of each amino acid.

Definitions and units[5]

Peak volume (ml) $\bar{v} = AZ\,(K_d + F_1)$ (1)

where AZ is the total column volume (ml)
K_d is distribution coefficient at equilibrium (dimensionless)
F_1 is the void fraction (dimensionless).

Resolution of components a and b (dimensionless):

$$R_{ab} = \frac{\bar{v}_a - \bar{v}_b}{\sigma_a + \sigma_b} \quad (2)$$

where $\bar{v}_a$, $\bar{v}_b$ are the peak elution volumes of components a and b respectively (ml)
σ_a, σ_b are the half widths of the eluted peaks of the components a and b, respectively, measured at a distance of $c_{max.} \cdot e^{-1/2}$ from the base line (ml).

Results and discussion

Under the operating conditions described above, the resolution of a standard series of acidic and neutral amino acids is possible within 140–150 min, but the main problem comprises the resolution of the pair threonine–serine and, to a lesser extent, of the pairs glycine–alanine and tyrosine–phenylalanine. The degree of resolution of these pairs was used as a criterion in the investigation of the effect of the reaction conditions during the sulphonation of the styrene–divinylbenzene copolymer on the resolution efficiency of the cationic resin.

Changes in the sulphonation conditions led to resins which differ considerably in the peak elution volume differences (*i.e.* distances between the peaks) of the amino acid pairs in question (see Table I). As the measurements were carried out using the

TABLE I

PEAK ELUTION VOLUME DIFFERENCES OF THREONINE–SERINE, GLYCINE–ALANINE, AND TYROSINE–PHENYLALANINE (ml)

The resins in this table are arranged in such a way that product No. I was prepared using a swelling agent and at a lower level of variables, while product No. IV was synthesized without a swelling agent and at a higher level of variables.

Resin No.	$\bar{v}_{Ser} - \bar{v}_{Thr}$	$\bar{v}_{Ala} - \bar{v}_{Gly}$	$\bar{v}_{Phe} - \bar{v}_{Tyr}$
I	2.03 ± 0.13	6.59 ± 0.08	5.58 ± 0.06
II	2.55 ± 0.07	6.34 ± 0.07	4.53 ± 0.18
III	2.60 ± 0.06	5.97 ± 0.22	3.86 ± 0.07
IV	2.84 ± 0.03	3.60 ± 0.20	2.29 ± 0.01

TABLE II

RESOLUTION OF THREONINE–SERINE, GLYCINE–ALANINE, AND TYROSINE–PHENYLALANINE

Resin No.	$R_{Thr/Ser}$	$R_{Gly/Ala}$	$R_{Tyr/Phe}$
I	1.72 ± 0.11	3.66 ± 0.05	3.10 ± 0.05
II	2.20 ± 0.08	3.64 ± 0.03	2.46 ± 0.12
III	2.28 ± 0.05	3.39 ± 0.23	2.07 ± 0.05
IV	2.49 ± 0.02	2.04 ± 0.18	1.23 ± 0.02

same column and all the resins had the same particle size distribution, and therefore an identical F_1 value, the reason for the differences found must, according to eqn. 1, be the different affinities of the cation-exchange resins to the amino acids tested under the conditions employed. It follows from the definition of the resolution (2), that the differences in the elution volumes of adjacent amino acids are of primary importance for the degree of their resolution. Due to the fact that the peak widths of the amino acids examined differ only slightly with all four cationic resins, it is innate, that the R values should differ considerably one from another (see Table II).

HAMILTON[5] considers a resolution sufficient for quantitative evaluation, if R exceeds 2. This requirement is met for all three amino acid pairs with the resins II and III. As the data in Tables I and II were selected from a larger factorial experiment, it was possible to compare quantitatively the contributions of the individual variables to the change in the resolution, R, of the amino acids used.

A higher temperature, longer sulphonation time, and higher concentration of sulphonating agent permit the preparation of resins which show a better resolution of threonine–serine, but a worse one of glycine–alanine and particularly tyrosine–phenylalanine. The intensity of the effect of the individual reaction conditions decreases in the order stated above.

The use of a swelling agent for the copolymer prior to sulphonation has the reverse effect. The resolution of threonine–serine becomes worse while that of glycine–alanine and tyrosine–phenylalanine improves. The magnitude of this effect is comparable with that of the sulphonation temperature.

The optimum conditions between the extreme effects of the reaction conditions are represented by resin No. II. To illustrate its resolution ability more distinctly, a tracing of a separation of a standard series of acidic and neutral amino acids is shown in Fig. 1.

Conclusions

By varying the conditions of sulphonation of a styrene–divinylbenzene copolymer, the affinity of the resulting cation-exchange resin towards some neutral

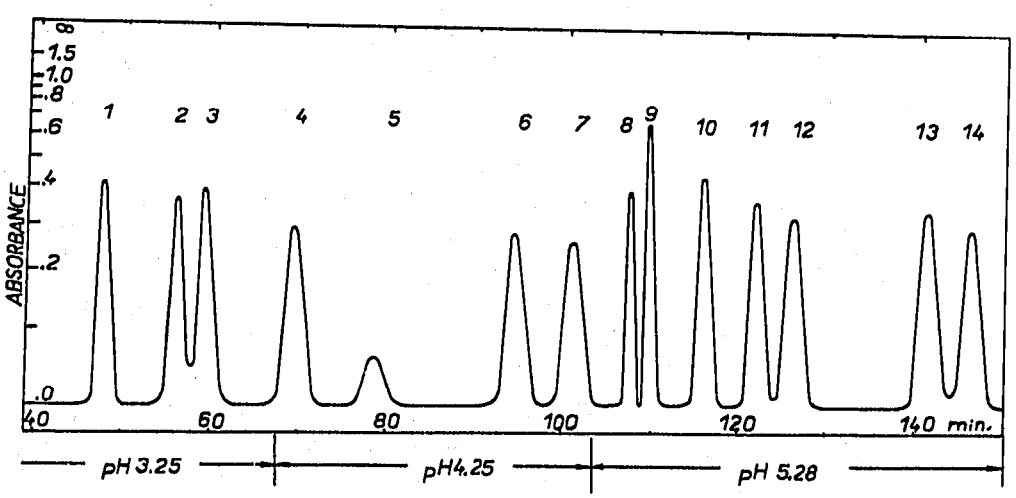

Fig. 1. Chromatogram of a standard series of acidic and neutral amino acids using resin No. II. 1 = Asp; 2 = Thr; 3 = Ser; 4 = Glu; 5 = Pro; 6 = Gly; 7 = Ala; 8 = Cys; 9 = Val; 10 = Met; 11 = Ile; 12 = Leu; 13 = Tyr; 14 = Phe.

amino acids alters. It represents a further method of effecting a resolution of amino acid pairs which are difficult to separate in ion-exchange chromatography according to MOORE AND STEIN[1].

The results presented here by no means exhaust the possibilities of this new approach.

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