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USE OF NEUTRAL POLYSTYRENE RESIN FOR RAPID DESALTING AND FRACTIONATION OF NON-POLAR AMINO ACIDS AND NON-POLAR OLIGOPEPTIDES*

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SUMMARY

Rapid desalting and chromatographic fractionation of nonpolar amino acids and nonpolar oligopeptides is possible by adsorption on the neutral polystyrene resin Porapak Q. Elution occurs with distilled water and mixtures of water with methanol, ethanol or acetone.

The adsorption coefficients, K, of the amino acids in 0.1 N HCl decrease in the following order:

$K \geqslant 10$: Trp > Phe > Nle, Tyr, Leu, Ile;
$10 > K > 1$: Met > Nval, Val;
$K < 0$: Gly, $(Cys)_2$, His, Lys, Orn.

Adsorption of an aliphatic CH_2-group is stronger than that of an aromatic CH-group. Within the homologous series of aliphatic amino acids each CH_2-group increases the K value by a constant factor of 5.1.

Adsorption of neutral or acidic oligopeptides in 0.1 N HCl is stronger than, or at least equal to, that of the corresponding most nonpolar amino acid. The repeating unit $-NH-CH_2-CO-$ in the series of oligoglycines increases K by a constant factor of 1.3. The isomers Gly–Pro and Pro–Gly are adsorbed differently.

The effect of pH was investigated for Phe, Trp and Tyr. At low salt concentration, a minimum of adsorption was found near pH 7 and loss of adsorption of tyrosine was observed above pH 10 (ionization of the phenolic hydroxyl). Addition of salt increases adsorption. The effect of salting-out onto the adsorbent depends on the pH; it is minimal near pH 6 for Phe, Trp and Tyr, and is postulated to be minimal at the isoelectric point. The salting-out effect can be described for Phe at pH 10.9 by $\log K/K_0 = a\,C_s$, where K and K_0 are the adsorption coefficients in salt-containing and salt-free solution, respectively, a is a constant and C_s is the salt concentration.

INTRODUCTION

Adsorption on neutral polystyrene resin, caused by non-electrostatic attraction due to hydrophobic or van der Waals–London forces, offers a rapid means for the extraction of organic substances from aqueous solution. This technique was first employed for the extraction of steroids, and has also been applied recently to the

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extraction of 2,4-dinitrophenyl derivatives[2,3] of amino acids, peptides and hexosamines, and for 2,4-dinitrophenylhydrazones as well. Whereas most of these rather non-polar substances can also be extracted by organic solvents (though somewhat tediously), non-electrostatic adsorption is of still greater interest for hydrophilic compounds. With the idea of developing simple techniques for desalting and fractionation of low molecular weight hydrophilic substances we have investigated the adsorption of 5-hydroxyindoles[4], amino acids and several peptides (this paper) on the neutral resin Porapak Q®. Porapak Q is a porous copolymer of styrene and ethylvinylbenzene crosslinked with divinylbenzene. In addition to its practical importance, such an investigation is of interest for the theoretical explanation of ion-exchange chromatography of amino acids and peptides on resins based on polystyrene.

MATERIALS AND PROCEDURES

Porapak Q®, 150–200 mesh (Waters Associates, Framingham, Mass., U.S.A.), was suspended in acetone and washed thoroughly with water and buffer before use. It should be emphasized that a resin which has not been wetted with an organic solvent beforehand does not adsorb at all. The organic solvent must then be removed completely by excessive washing with water (at least 40 ml/g of resin); otherwise the adsorption capacity is decreased drastically. Adsorbed substances are best eluted with acetone–water(1:1 or 2:1), or pure acetone, but aqueous ethanol and aqueous methanol can also be employed.

All solvents and materials were of the highest grade of purity available. Buffers used were 0.05 *M* (based on the acid). They were: pH 3–5, citric acid – NaOH; pH 7, KH_2PO_4 – Na_2HPO_4; pH 9–11, glycine – NaOH.

Adsorption coefficients were determined by equilibrium studies and by column chromatography. For equilibrium studies 300 mg of Porapak Q were packed into a glass column, 10 mm I.D., provided with a porous glass disk, were washed with acetone, excess of distilled water (see above), and with the corresponding aqueous solution. Then, 10 ml of sample solution were repeatedly (10 times) run through the column for equilibration. The concentration of the sample in the mobile phase was measured photometrically in the UV (phenylalanine, tryptophan, tyrosine and derivatives). The result was corrected for the outer column volume (correction factor $F = 1.05$) and, if necessary, for the low self-absorption of the solvent in the UV. The adsorption coefficient K was calculated from: $K = (c/c_m - 1)$ 10 ml/0.3 g; where c and c_m are the mean concentrations in the solution before and after equilibration, respectively.

For the column chromatographic experiments, 3 g of Porapak Q were used in a 10-mm I.D. column except when otherwise stated. The column was connected with a refractive index detector (Varian Aerograph, Walnut Creek, Calif., U.S.A.) and a UV detector (Uvicord II, LKB-Producter AB, Stockholm-Bromma, Sweden) and showed a void volume $V_0 = 5.7$ ml, as judged by the negative "water" peak. This peak was registered by the refractive index detector if free amino acids or peptides were dissolved in and eluted with 0.1 *N* hydrochloric acid. Hydrochloride formation in the sample causes the concentration of free hydrochloric acid in the sample solution to be lowered and the break-through of this solvent could be registered when the ad-

sorption coefficient of the sample was $K > 1$. Adsorption coefficients were calculated by $K = (V_e - V_0) / 3$ [ml/g].

The units of the adsorption coefficient are ml per g and K is the ratio of the equilibrium concentrations of adsorbed (in mole per g) and unadsorbed substance (in mole per ml), or, in column chromatography, the ratio of corrected elution volume ($V_e - V_0$, in ml) and weight of Porapak Q in g.

K is concentration dependent. For example, in the case of leucine an increase of the load by a factor 10, from 1.33 to 13.3 μmole/g of Porapak Q, lowered the adsorption coefficient by about 30%. In order to be able to compare data, the same molar concentration of samples was used whenever possible. For practical reasons, however, the concentrations of tryptophan, tyrosine, phenylalanine and their derivatives had to differ in the ratio 1:2:20, when measured by UV photometry.

RESULTS AND DISCUSSION

Effect of pH

The influence of the pH on the adsorption of amino acids on Porapak Q was investigated for phenylalanine, tryptophan and tyrosine (Fig. 1). At low salt concentration (0.05 M buffers) the influence of the pH on the adsorption of phenylalanine is remarkably low: between pH 1 and 13 the adsorption coefficient changes only by a factor of about 2. The effect is more pronounced for tryptophan and tyrosine, and is much greater at high salt concentrations (salting-out effect, see below). At low salt concentrations a minimum of adsorption is observed near pH 7. It is interesting to note that ionization of the phenolic hydroxyl of tyrosine ($pK_a = 10.05$) causes loss of adsorption.

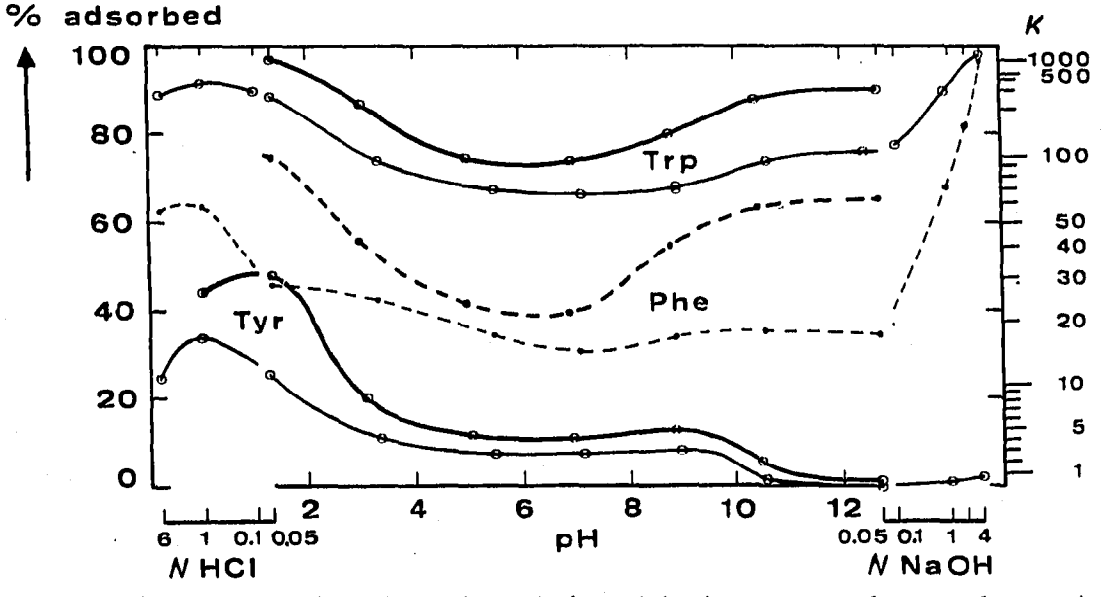


Fig. 1. Effect pH on the adsorption of phenylalanine, tryptophan and tyrosine on Porapak Q at low salt concentrations (lower lines, 0.05 M buffers, see MATERIALS AND PROCEDURES) and high salt concentrations (higher lines, 1 M sodium chloride + 0.05 M buffers). The pH-scale is interrupted to indicate the increase of the "buffer" concentration from 0.05 M at low and high pH values; in fact, the very strong increase in adsorption of phenylalanine is mainly caused by the increase of ion strength and not by the pH. Adsorption coefficients K are determined by equilibration of 1 μmole of tryptophan, 2 μmole of tyrosine, and 20 μmole of phenylalanine, respectively, in 10 ml of aqueous solution with 0.3 g of Porapak Q at 23°.

Obviously, the absolute amount, Z, of the electric charges on the adsorbate (but not the net charge) is an important parameter for adsorption on Porapak Q. For bifunctional amino acids, Z and, hence, also the degree of hydration and the tendency to remain in the aqueous mobile phase are maximal at the isoelectric point. The strong hydrated electrical change on the tyrosine phenolic hydroxyl prevents the adsorption of this residue. As a consequence, acids are more strongly adsorbed at low pH and bases at high pH values[4].

Effect of salt concentration

A very pronounced effect of 'salting-out onto the adsorbent' was noted earlier[3] in the case of 2,4-dinitrophenyl derivatives. A similar effect is also demonstrable for non-polar amino acids, and is of practical importance for desalting. The phenomenon of salting-out (*e.g.* proteins from aqueous solution by addition of ammonium sulfate) can be described by the Setschenow equation:

$$\log \frac{S_o}{S} = k_s C_s$$

where S_o and S refer to the solubility of the substance in water and salt solution, respectively, k_s is the salting-out constant, and C_s is the salt concentration. The behavior of phenylalanine and tyrosine in the presence of the adsorbent Porapak Q can be approximated by the analogous expression (1):

$$\log \frac{C_{m,o}}{C_m} = k_{s,A} C_s \qquad (1)$$

where $C_{m,o}$ and C_m are the concentration of the substance in the salt-free mobile phase and in the salt-containing mobile phase, respectively, and $k_{s,A}$ is the constant for salting-out onto the adsorbent. This approximation is applicable up to a salt concentration of about 3 M sodium chloride, $k_{s,A}$ being 0.18 for phenylalanine at pH 10.9 and 0.14 for tyrosine at pH 1.4. It is a general observation that k_s decreases with increasing polarity of the solute.

If the logarithm of the adsorption coefficient K is plotted against the salt concentration (in moles per l), a straight line is obtained which seems to be valid for salt concentrations still higher than 3 M. However, in the case of tyrosine (at pH 1.4) a deflection from the straight line was observed at salt concentrations lower than 0.5 M. These straight lines are described by eqn. 2:

$$\log \frac{K}{K_0} = aC_s + b \qquad (2)$$

where K and K_0 are the adsorption coefficients in salt solution and in water, respectively, and a and b are constants, with $b = 0$ for phenylalanine. In the case of dinitrophenylcysteic acid at pH 9 neither eqn. 1 nor 2 was applicable.

As can be seen from Fig. 1, the salting-out effect is also a function of pH. This is demonstrated more clearly in Fig. 2, where $\log K_1/K_0$ is plotted against the pH; K_1 is the adsorption coefficient in 1 M sodium chloride + 0.05 M buffer and K_0 is the coefficient in 0.05 M buffer alone. The salting-out effect is minimal for phenylalanine, tryptophan and tyrosine between pH 5 and 7, and it is postulated that this minimum coincides with the isoelectric point. However, the data presented are not precise

enough for such a decision, and values for acidic and basic amino acids are still missing. On the other hand, this postulation is given further support by the behavior of indole amines at higher pH values[4].

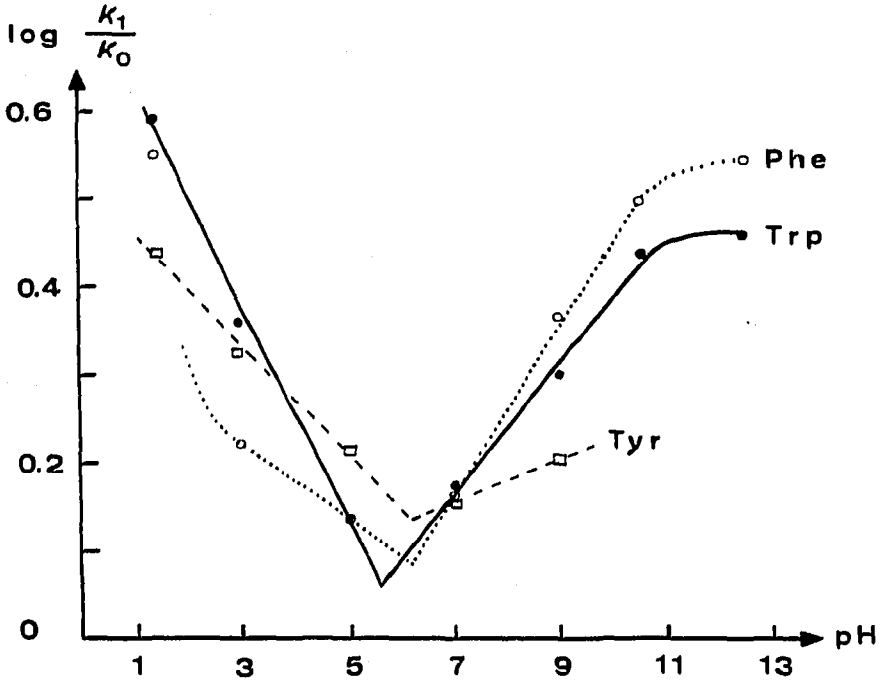


Fig. 2. Effect of salting-out onto adsorbent as a function of pH. K_1, adsorption coefficient in 1 M sodium chloride + 0.05 M buffer; K_0, adsorption coefficient in 0.05 M buffer. The same data as in Fig. 1. Note the minimum near pH 6, in the region of the isoelectric points.

In either case, the observed salting-out effect is very useful as a means for the desalting of non-polar amino acids and peptides. In contrast to the conventional desalting of amino acids and oligopeptides by ion exchange[5], the higher the salt concentration, the better the method described here works. It enables one, for instance, to extract tryptophan from alkaline hydrolysates in 4 N sodium hydroxide[6].

Adsorption of amino acids from 0.1 N hydrochloric acid

Preliminary experiments (see also Fig. 1) have shown that at low salt concentrations adsorption is strongest in acid medium for most amino acids. Hence 0.1 N hydrochloric acid was chosen as the solvent for the following comparative study.

Adsorption coefficients of amino acids in 0.1 N hydrochloric acid are collected in Table I. In decreasing order of adsorption coefficients, the following amino acids exhibit values of $K \geqslant 10$: tryptophan, phenylalanine, norleucine, tyrosine, leucine and isoleucine; followed by values of $10 > K > 1$ for methionine, norvaline and valine. The first group of amino acids can be extracted very easily from aqueous solutions by adsorption on Porapak Q (see below, desalting).

In order to analyse the adsorptive behavior of amino acids on Porapak Q, the logarithm of the adsorption coefficient in 0.1 N hydrochloric acid was plotted against the number of carbon atoms in the amino acid side chain (Fig. 3). A straight line was obtained for the homologous series from alanine to norleucine and a parallel one from the isomers valine to leucine. Each CH_2-group of the aliphatic side chain con-

TABLE I

ADSORPTION COEFFICIENTS OF AMINO ACIDS ON PORAPAK Q IN 0.1 *N* HYDROCHLORIC ACID AT 23°

Amino acid	*Abbrev.*	*By column chromatography*[a]	*By equilibration*[b]
α-Aminoadipic acid	Aad	0.936 ± 0.013	
α-Amino-*n*-butyric acid	Abu	0.518 ± 0.004	
α-Aminoisobutyric acid	Aib	0.519 ± 0.021	
Alanine	Ala	0.102 ± 0.008	
Arginine·HCl	Arg	0.02	
Asparagine	Asn	0	
Aspartic acid	Asp	0.097 ± 0.007	
Cystine	$(Cys)_2$	−0.04	
Glutamic acid	Glu	0.249 ± 0.008	
Glutamine	Gln	0.06	
Glycine	Gly	−0.099 ± 0.004	
Histidine·HCl	His	−0.04	
Hydroxyproline	Hyp	0.13	
Isoleucine	Ile	9.6	
Leucine	Leu	11.25 ± 0.15	
Lysine·HCl	Lys	−0.04	
Methionine	Met	6.0	
Methionine sulfoxide	MetO	0.36	
Methionine sulfone	$MetO_2$	0.52	
Norleucine	Nle	14.7	
Norvaline	Nval	2.586 ± 0.023	
Ornithine·HCl	Orn	−0.09	
Phenylalanine	Phe	52[c]	37
Proline	Pro	0.80	
Serine	Ser	0	
Threonine	Thr	0.12	
Tryptophan	Trp		240
Tyrosine	Tyr	12	10.6
Valine	Val	2.126 ± 0.031	

[a] See MATERIALS AND PROCEDURES. Load 1.33 μmole/g of Porapak Q. Where the standard deviation is given, the number of determinations was $n \geqslant 6$.

[b] See MATERIALS AND PROCEDURES. Load of Trp, Tyr and Phe was 3.33, 6.67, and 66.7 μmole/g of Porapak Q, respectively.

[c] On a small column of 0.3 g of resin. Load: 1.33 μmole/g of Porapak Q.

tributes the same amount, 0.71, to the logarithm of the absorption coefficient or, in other words, increases the adsorption coefficient by a factor of 5.1. By extrapolation to zero carbon atoms, a theoretical adsorption coefficient $K_{theor.} = 0.02$ is obtained for glycine. This value is in contrast to the observed coefficient $K_{obs.} = -0.1$.

The negative value is quite surprising and means that glycine moves faster than the aqueous solvent through a column of Porapak Q. Although of no practical importance, this finding deserves some theoretical interest. Negative values have also been found in the case of cystine, histidine, lysine and ornithine (Table I), amino acids all bearing two positive electrical charges in 0.1 *N* hydrochloric acid. A positive adsorption coefficient near zero was found for arginine, but within the experimental error a negative value would still be possible. At present these findings cannot be explained. One could assume that water is adsorbed on the Porapak Q and would then not be a good reference for the determination of adsorption coefficients (see MATERIALS AND PROCEDURES). However, semilogarithmic plots of the adsorption coefficients of

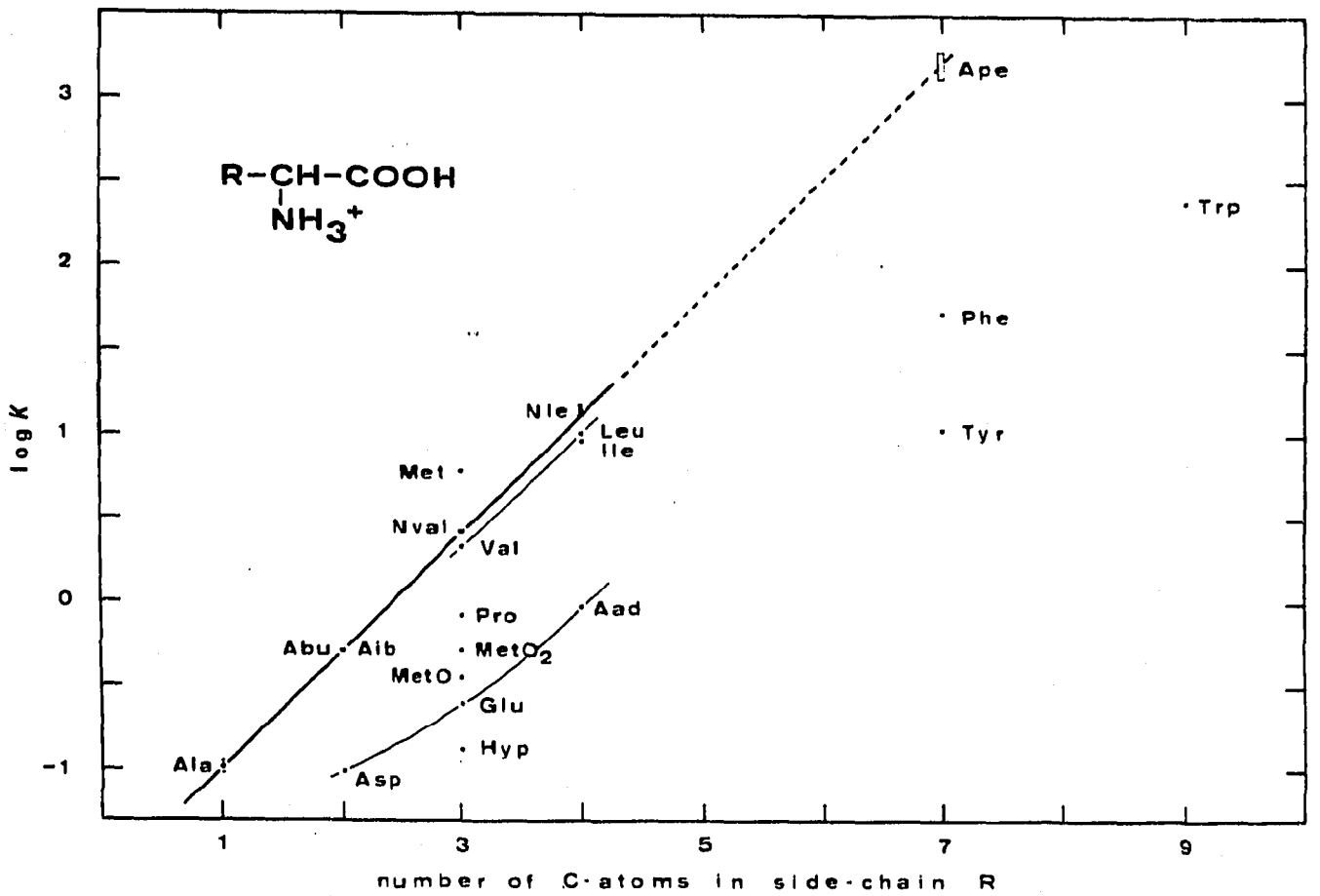


Fig. 3. Adsorption coefficients K of amino acids on Porapak Q as a function of the number n of carbon atoms in the amino acid side chain, in 0.1 N hydrochloric acid at 23°. The equation to the straight line of the homologous series of aliphatic amino acids is given by $\log K = -1.71 + (0.709 \pm 0.009)\, n$. The position of α-aminopelargonic acid (Ape) is found by extrapolation $\pm$ 3 s. Note the weak adsorption of the aromatic amino acids when compared with the aliphatic straight-chain amino acids.

aliphatic amino acids (Fig. 3) and of glycine peptides (Fig. 4, see below) against the number of repeating units give straight lines if the adsorption coefficients are referred to water, but no straight lines when they are referred to glycine. Inaccessibility of the adsorbent pores due to steric hindrance by the hydrated positively charged groups could be possible in the case of basic amino acids, but can be excluded for glycine. However, in the case of the basic amino acids and cystine, repulsion by a partial positively charged adsorbent matrix at pH 1 could also be considered. Weak adsorption of protons is indicated by the observation that hydrochloric acid moves somewhat more slowly than sodium chloride through a column of Porapak Q in distilled water ($K_{HCl} = 0.06$, when related to sodium chloride).

The lower adsorption coefficients of valine, leucine, and isoleucine compared with their straight-chain isomers can be explained by the decrease in molecular area concomitant with chain-branching. Also for the low value of proline, a decrease in molecular area due to ring closure may be responsible but the steric hindrance of

adsorption of the aliphatic ring by the hydrated ammonium ion seems to be more important.

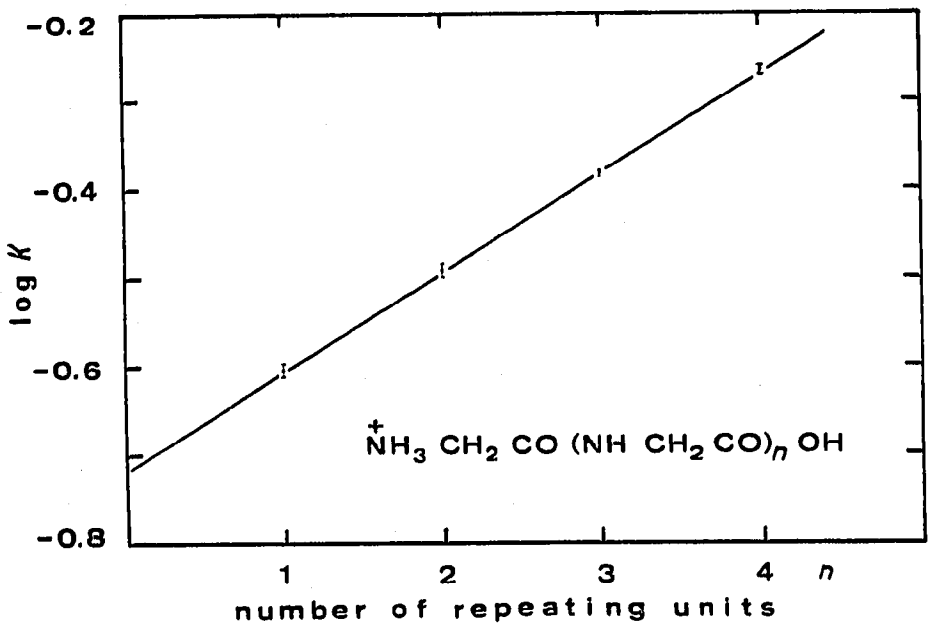


Fig. 4. Adsorption coefficients K of oligoglycines on Porapak Q as a function of the number n of repeating units –NH–CH_2–CO–. In 0.1 N hydrochloric acid at 25°. The straight line is given by $\log K = -0.719 + (0.113 \pm 0.003)\, n$.

The aromatic amino acids tryptophan and phenylalanine are adsorbed more strongly than the leucines, while tyrosine behaves similarly to the leucines. However, these aromatic compounds should be compared with the corresponding straight-chain amino acids. At pH 1, phenylalanine ($K_{Phe} = 5$) is adsorbed considerably more weakly than the corresponding α-aminopelargonic acid (Ape). Although at present the adsorption coefficient of Ape is accessible only by extrapolation of the data in Fig. 3, $K_{Ape,\,theor.} = 1750$, there is no doubt regarding the order of magnitude of this value. Tryptophan, bearing the largest apolar residue, is adsorbed only about 5 times as strongly as phenylalanine. The phenolic tyrosine is able to form hydrogen bonds with the mobile phase and is adsorbed about 5 times less than phenylalanine. The unexpectedly low adsorption of the aromatic amino acids is surprising since aromatic adsorption is known to occur on many other chromatographic materials, *e.g.* on hydrophobic charcoal[7] as well as on hydrophilic dextran gels[8]. However, comparable data for Porapak Q at higher pH values are still lacking.

Methionine is bound about twice as strongly as norvaline. This means that although the contribution of the thioether sulfur to log K is positive, it is less than that of a methylene group. Oxidation produces a polar, strongly hydrated sulfoxide or sulfone group within the aliphatic side chain and therefore decreases the adsorption markedly (see MetO and $MetO_2$ in Fig. 3).

The contribution of a carboxyl group to log K is negative and varies from zero in aspartic acid to about −0.44 in α-aminoadipic acid. The observed inconstancy of this contribution and the decline of the theoretical straight line (Fig. 3) presumably is due to intramolecular interaction in aspartic acid and glutamic acid. Amide for-

mation (asparagine, glutamine) further decreases adsorption. Introduction of a hydroxyl group decreases (hydroxyproline, tyrosine) or increases (serine, threonine) adsorption on Porapak Q. The unexpected increase could be caused by intramolecular interaction.

Comparison with ion-exchange chromatography of amino acids

It can be expected that the adsorption phenomena observed on the neutral polystyrene resin Porapak Q are also effective in the separation of amino acids on the sulfonated polystyrene resins used for the amino acid analysis according to MOORE AND STEIN. Most neutral amino acids are separated easily by ion-exchange chromatography, although the pK_1 values as well as the isoelectric points of these amino acids are close together. In point of fact the chromatographic behavior of many neutral amino acids cannot be explained by their pK_1 or pI values. For example; if the pK_1 values were responsible for the chromatography, Val, Met and Tyr should run faster than Gly, and Gly should be inseparable from Ala. According to the pI values, Phe, Tyr and Trp should run faster than Gly, and Gly, Val as well as Ile, Leu should be inseparable; furthermore, Pro should elute last. On the other hand, the similarity of the elution sequence on the ion exchanger and on the neutral Porapak Q is noteworthy: Gly, Ala, Abu, Val, Nval, Met, Ile, Leu, Tyr, Phe and Trp (last) and also of MetO, $MetO_2$, Met. This indicates strongly that the adsorption on the ion-exchange matrix is considerable and presumably dominates for the amino acids of this series with molecular weights higher than Abu.

From the observation that adsorption on the neutral polystyrene resin is negligibly small for the polar amino acids $(Cys)_2$, Gly, Ser, Asn, Gln, His, Lys, Orn, Arg it can be assumed that their behavior on the sulfonated polystyrene resin is determined only by ion exchange. This could explain, *e.g.*, the particularly high sensitivity of the position of cystine (and citrulline) to the pH. Although excluded from the neutral resin, cystine runs near valine on the sulfonated resin, presumably because it carries two positive charges.

The ionic strength shows an opposite effect: whereas it increases the adsorption on the neutral resin, it speeds up the elution from the ion exchanger. Only tryptophan, adsorbed the most strongly on polystyrene, is eluted faster from the ion exchanger with decreasing ionic strength.

Adsorption of oligopeptides from 0.1 N hydrochloric acid

In connection with an investigation on urinary peptides[2] the above adsorption study was extended to include oligopeptides. In order to compare the adsorption behavior of peptides with that of the amino acids, 0.1 *N* hydrochloric acid was again used as solvent. Despite of the strongly acidic nature of this solvent, it should also be useful for desalting of rather labile peptides because adsorption and desorption can be finished within a few minutes.

The only homologous series of peptides at hand was that of oligoglycines. Their adsorption coefficients, from diglycine to pentaglycine, were measured and log K was plotted *versus* the number of repeating units, as shown in Fig. 4. A straight line was again obtained (compare Fig. 3). This means that the repeating unit $-NH-CH_2-CO-$ contributes a constant amount, *i.e.* + 0.113, to the logarithm of the adsorption co-

TABLE II

ADSORPTION COEFFICIENTS OF PEPTIDES ON PORAPAK Q IN 0.1 *N* HYDROCHLORIC ACID AT 25°

Peptide	*By column chromatography*[a]	*By equilibration*[b]
Ala–Asp	0.40	
Ala–Gly	0.22	
Ala–Pro	5.3	
Ala–Tyr		24[c]
Glu–Gly–Phe		150[d]
Gly–Gly	0.257 ± 0.005	
Gly–Gly–Gly	0.331 ± 0.006	
Gly–Gly–Gly–Gly	0.422 ± 0.004	
Gly–Gly–Gly–Gly–Gly	0.551 ± 0.007	
Gly–Leu–Tyr		460[e]
Gly–Phe–Phe		800[d]
Gly–Pro	3.8	
Gly–Trp		440[e]
Gly–Val	6.4	
Leu–Tyr		420[e]
Met–Gly	8.1	
Phe–Phe		810[d]
Pro–Gly	1.2	
Pro–Phe		140[d]
Tyr–Gly–Gly		11[e]

[a] See MATERIALS AND PROCEDURES. Load: 1.33 μmole/g of Porapak Q. Where standard deviation is given, the number of determinations was $n \geqslant 9$.
[b] See MATERIALS AND PROCEDURES.
[c] Load: 6.67 μmole/g of PorapakQ.
[d] Load: 33.3 μmole/g of Porapak Q.
[e] Load: 3.33 μmole/g of Porapak Q.

efficient. This is a further indication that it should be possible at a later stage to calculate the adsorption coefficients of organic compounds on the neutral resin.

The adsorption coefficients of a few peptides are given in Table II. These data should be considered as guide values only. However, it is clear from Tables I and II that the adsorption of peptides is stronger than that of the corresponding amino acids. Non-polar peptides can easily be separated from polar ones. Yet, dipeptides can be desalted when they contain at least one valine, leucine, isoleucine, methionine, tyrosine, phenylalanine or tryptophan residue. A valuable fractionation of peptide mixtures according to their polarity should be possible, and very useful because the separation proceeds in a salt-free aqueous or aqueous–alcoholic solution.

It should be emphasized that the isomers Gly–Pro ($K = 3.8$) and Pro–Gly ($K = 1.2$) are adsorbed differently; their behavior may be compared with proline ($K = 0.80$). Naturally, the observed increase in the adsorption coefficient of Gly–Pro must be due to steric effects. In proline and prolylglycine the hydrated ammonium group is able to disturb the adsorption of the pyrrolidine ring. This hindrance is reduced or lacking in Gly–Pro. Moreover, in Gly–Pro there are many more atoms in positions which can effect steric hindrance of the hydration of the N-terminal ammonium group (Table III). Adsorption of Gly–Pro should also be enhanced by the asymmetric position of the apolar residue.

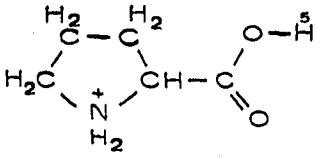
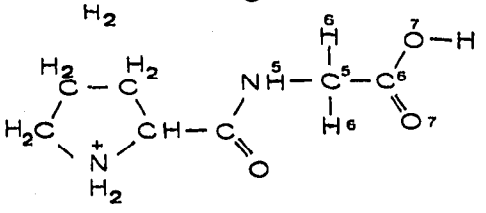
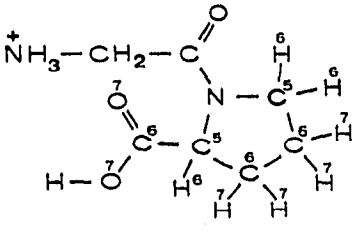
Desalting and fractionation according to nonpolarity

In practice, when dealing with desalting with Porapak Q it is important that the adsorption increases with the salt concentration. Hence, a sample to be desalted should be concentrated as much as is practical. The resulting small sample volume is also of advantage in the chromatographic step. A dead volume as small as possible in the exit of the column is decisive in producing good results, particularly when desalting a compound with $K < 10$.

TABLE III

STRUCTURE OF PROLINE, PROLYL-GLYCINE, AND GLYCYL-PROLINE AND THEIR ADSORPTION COEFFICIENTS K ON PORAPAK Q IN 0.1 N HYDROCHLORIC ACID

Adsorption of the pyrrolidine ring of proline and prolyl-glycine is hindered by the hydrated electric charge. Adsorption of glycyl-proline is enhanced by steric hindrance of the hydration of the N-terminal ammonium group.

Substance	*Structure*	K	*Steric hindrance of N-terminal ammonium group: number of atoms*	
			Total	*Without H-atoms*
			In position *	*In position* *
			5 6 7	5 6 7
Proline		0.80	1 0 0	0 0 0
Prolyl-glycine		1.2	2 3 2	1 1 2
Glycyl-proline		3.8	2 6 6	2 3 2

* Numbering from the ammonium ion. The greater the number of atoms in the 6-position the greater will be the steric effect[9].

It must be emphasized once again that wetting of the absorbent with acetone or ethanol followed by thorough washing with distilled water (40 ml/g of adsorbent) prior to use is essential for adsorption (see MATERIALS AND PROCEDURES). The total capacity for extraction and desalting is at least about 0.2 mmole of adsorbed substances per g of Porapak Q, as indicated by the data in Fig. 6. Elution is fast with deaerated water–ethanol (50:50) or water–acetone (50:50) and the resin should be cleaned with water–acetone (20:80) and pure acetone.

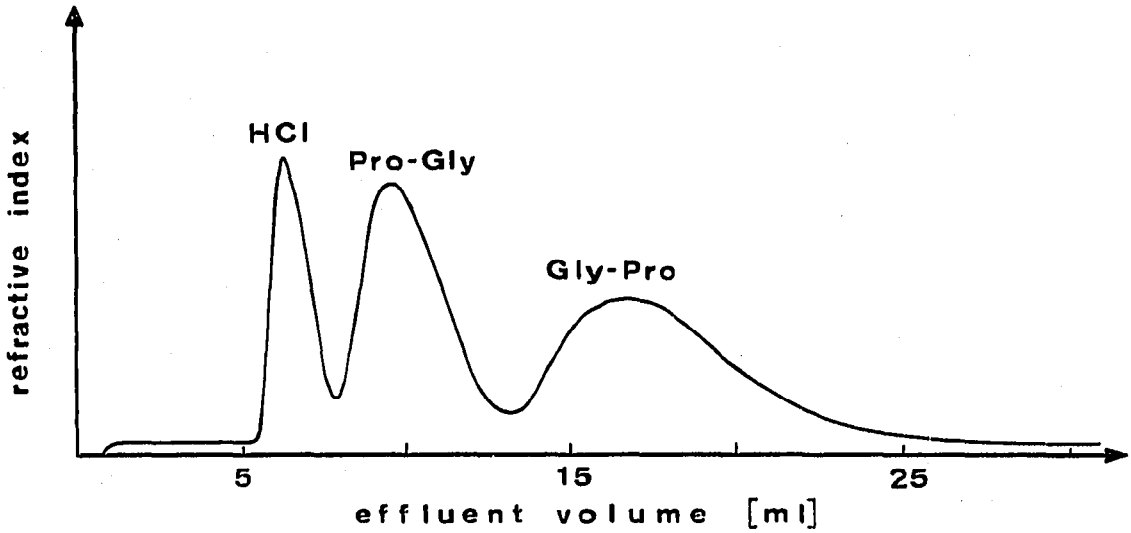


Fig. 5. Separation of Pro-Gly and Gly–Pro on a column of 3 g of Porapak Q in 0.1 *N* hydrochloric acid. Load: 1 mg of each in 0.2 ml of 0.2 *N* HCl. Elution with 0.1 *N* hydrochloric acid.

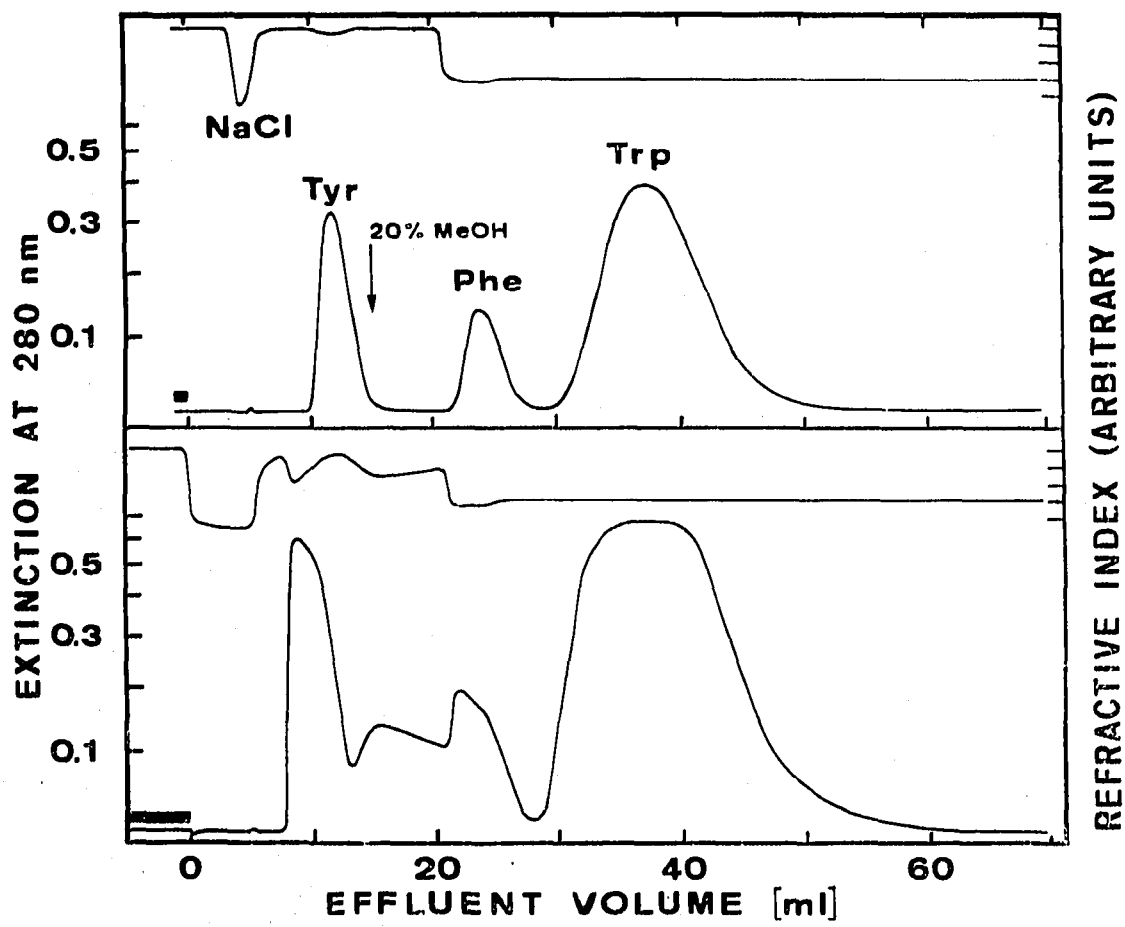


Fig. 6. Desalting and separation of phenylalanine, tryptophan and tyrosine on a column of 2.5 g of Porapak Q. Top: Optimal load: 14 mg of Phe, 2 mg of Trp, and 3.6 mg of Tyr in 1 ml of 2 *M* NaCl + 0.1 *N* HCl. Bottom: Overloaded with respect to phenylalanine: 70 mg of Phe, 10 mg of Trp, and 18 mg of Tyr in 5 ml of 2 *M* NaCl + 0.1 *N* HCl. After application of the sample (indicated by the bar) the column was rinsed in each case with 15 ml of water followed by 20% aq. methanol. The breakthrough of methanol gives rise to the appearance of a double peak of phenylalanine when overloaded (bottom). Note: at 280 nm phenylalanine absorbs much less than tryptophan and tyrosine.

Examples of fractionations on Porapak Q according to non-polarity are shown for compounds with adsorption coefficients (in 0.1 N HCl) of $K < 4$ in Fig. 5 and $K > 10$ in Fig. 6. The separation of the dipeptide isomers Pro–Gly and Gly–Pro in Fig. 5 demonstrates the operation of steric effects in the adsorption mechanism. The tailing, which is typical of adsorption, should also be noted. The tailing can be made good by gradient elution with water → aq. alcohol or aq. acetone. Degassed solvents must be used for the gradient elution. The effect of overloading (with respect to phenylalanine) can also be seen in Fig. 6.

CONCLUSIONS

The principles of adsorption on the neutral polystyrene resin Porapak Q can be recognized qualitatively by the experimental data presented here. The straight lines obtained in the semilogarithmic plots of adsorption coefficient *versus* the number of repeating units in a homologous series of aliphatic amino acids and in the series of oligoglycines strongly indicate that log K (or, in other words, the sample adsorption energy) is made up of the sum of constant contributions due to the individual functional groups of the molecule plus a ground constant (Martin relationship). In 0.1 N hydrochloric acid, positive contributions to log K are effected by the following groups:

aliphatic $-CH_2-$ > aromatic $=CH-$ > $-S-$

and negative by:

$-OH, -COOH < -NH_3^+$

All electric charges, *i.e.*, including $-COO^-$, strongly reduce adsorption. Acids are more strongly adsorbed at low and bases at high pH values. The zwitterionic amino acids should be adsorbed weakest at the isoelectric point.

The determination of the group constants according to the Martin relationship allows one to calculate the order of magnitude of the adsorption coefficient of a substance [10] (exceptions being cystine and the basic amino acids). Intermolecular interaction of polar groups, *e.g.* of –COOH in aspartic acid and glutamic acid, and –OH in serine and threonine, increases log K to an extent depending on the nature and position of the corresponding group. These and other steric effects (*e.g.* in Gly–Pro, Pro–Gly, see above) render a more precise calculation of the adsorption coefficient difficult.

The observed, remarkably high, group contributions of the aliphatic CH_2- and aromatic CH-groups enables in the adsorption of non-polar amino acids and non-polar oligopeptides from aqueous solution and permits the fractionation of these compounds according to their polarity. Since the adsorption is favored by high salt concentration and elution is possible with salt-free solvents, this simple technique should be of value for many extractions and clean-up procedures in biochemistry.

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