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SIMPLE EXTRACTION OF INDOLE DERIVATIVES FROM AQUEOUS SOLUTION BY ADSORPTION ON NEUTRAL POLYSTYRENE RESIN*

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SUMMARY

Indoles can be desalted and fractionated by adsorption on the neutral polystyrene resin Porapak Q® and eluted with distilled water and aqueous alcohols. A procedure for the rapid extraction of 5-hydroxyindoleacetic acid from urine is described.

The adsorption coefficients of indole, indole-3-acetic acid, 5-hydroxyindole-3-acetic acid, tryptamine, serotonine, tryptophan and 5-hydroxytryptophan have been determined in 0.1 *N* HCl (I), 5 m*M* glycine buffer of pH 9 (II), 1 *M* NaCl–5 m*M* glycine buffer of pH 9 (III), and 0.1 *N* sodium hydroxide (IV). Adsorption was found to depend on pH and salt concentration. Acids are most strongly adsorbed in I, bases in IV, and hydroxyindolic acids run through in IV, allowing their isolation from other indoles.

INTRODUCTION

Molecules bearing a hydrophobic group can be adsorbed from aqueous solution onto neutral polystyrene resin due to nonelectrostatic attraction from hydrophobic or van der Waals–London dispersion forces. Desorption occurs with solvents such as alcohol–water, acetone–water, or pure acetone. This phenomenon can be exploited for the rapid extraction and desalting of steroids and steroid glucuronides[1,2] and dinitrophenyl derivatives of amino acids, peptides and hexosamines[3,4], and as shown in another paper[5] for desalting and fractionation of free non-polar amino acids and oligopeptides containing non-polar amino acid residues. It will be shown here that it is also possible to adsorb indole derivatives on the neutral polystyrene resin Porapak Q®. In this way, time-consuming liquid–liquid extractions may be avoided and hydroxyindoles can be separated easily from non-hydroxylated indoles.

MATERIALS AND PROCEDURES

Porapak Q®, 150–200 mesh (Waters Associates, Framingham, Mass., U.S.A.), was swelled in acetone and washed thoroughly with water and buffer immediately before use. It must be emphasized that a resin which has not been wetted with an

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organic solvent beforehand does not adsorb at all. The organic solvent must then be removed completely by excessive water washings ($\geqslant$40 ml/g) and the resin must be kept wet; otherwise the adsorption capacity will decrease drastically.

All solvents and materials were of the highest grade of purity available. Adsorption coefficients were determined by equilibrium studies as described in another paper[5]. Column effluents were monitored by a refractive index detector (Varian Aerograph, Walnut Creek, Calif., U.S.A.) and colorimetrically at 280 nm using a Uvicord II detector (LKB-Producter AB, Stockholm, Sweden).

EXPERIMENT AND DISCUSSION

The adsorption on neutral polystyrene resin is dependent on pH and salt concentration, as was found in other studies[3,5]. In a preliminary survey on the behavior of indole derivatives, adsorption coefficients of seven neutral, acidic, amphoteric, and basic indole derivatives were determined in acidic and alkaline solvents of low and high salt concentration (Table I).

TABLE I

ADSORPTION COEFFICIENTS (ml/g) OF INDOLE DERIVATIVES[a] ON PORAPAK Q AT ROOM TEMPERATURE

Indole derivative	*Solvent*			
	0.1 N HCl	*5 mM glycine of pH 9*	*1 M NaCl in 5 mM glycine of pH 9*	*0.1 N NaOH*
Indole	>3000	2100	3000	>3000
Indole-3-acetic acid	>3000	27	100	57
5-Hydroxyindole-3-acetic acid	370	7	19	1.5
Tryptamine	270	1400	1300	2000
5-Hydroxytryptamine (serotonine)	29	120	130	22
Tryptophan	240	65	130	120
5-Hydroxytryptophan	35	14	22	1.6

[a] 1 μmole in 10 ml of solvent equilibrated with 0.3 g of Porapak Q. Single and double determinations. High values mean strong adsorption.

At pH values up to 9, adsorption of all the derivatives investigated was strong enough to permit desalting. Acidic indoles are adsorbed more strongly at a low pH, and the basic tryptamine is bound more strongly at high pH. This means that an electric charge on the molecule diminishes the adsorption. A hydroxyl group in the 5-position reduces adsorption further, particularly in alkaline media, where ionization occurs. Hydroxyindolic acids, such as 5-hydroxyindole-3-acetic acid and 5-hydroxytryptophan, possess two negative charges in strong alkaline solvents and, therefore, are only weakly bound. This finding can be exploited for the separation of hydroxyindolic acids from all other indoles at a pH $>$ 11. However, a hydroxyindole is easily separable from its non-hydroxylated parent compound (see below) at low pHs as well.

Addition of 1 *M* sodium chloride increases the adsorption coefficients at pH 9 (Table I) by factors between 1 (tryptamine and serotonine) and 3.7 (indoleacetic acid). Application of a higher salt concentration will increase adsorption still further.

It may be noteworthy that the salting-out effect at this pH is smallest for the basic indoles and greatest for the acids indoleacetic acid and 5-hydroxyindoleacetic acid. This further supports the postulation that the salting-out effect is minimal at the isoelectric point[5]. The advantage of this salting-out effect where extraction from solutions of high ionic strength is required is obvious. Tryptophan, for instance, can be adsorbed on Porapak Q from alkaline hydrolysates in 4 *N* sodium hydroxide[6]; its adsorption coefficient exceeds a value of 1000 under these conditions.

Desorption of indole derivatives from Porapak Q occurs with aqueous solutions of methanol, ethanol or acetone, the latter being the most effective. For cleaning the resin the column is washed afterwards with water–acetone (20:80) and pure acetone. The resin may remain partially contaminated, *e.g.*, by urinary dyes, but can be re-used many times.

The possibility of chromatographic separation of indole derivatives on a column of Porapak Q is suggested by the large differences in adsorption coefficients and the easy desorption of the derivatives by aqueous alcohols or acetone. However, some tailing of peaks is observed, a fact which is typical for adsorption chromatography. Excessive peak broadening is noticed particularly at higher flow rates, presumably due to slow desorption kinetics. Nevertheless, under appropriate conditions complete separations can be achieved. This is exemplified in Fig. 1, showing the separation of sodium chloride, 5-hydroxytryptophan and tryptophan by step-by-step procedures. Tailing of peaks could be best counteracted by continuous gradient elution.

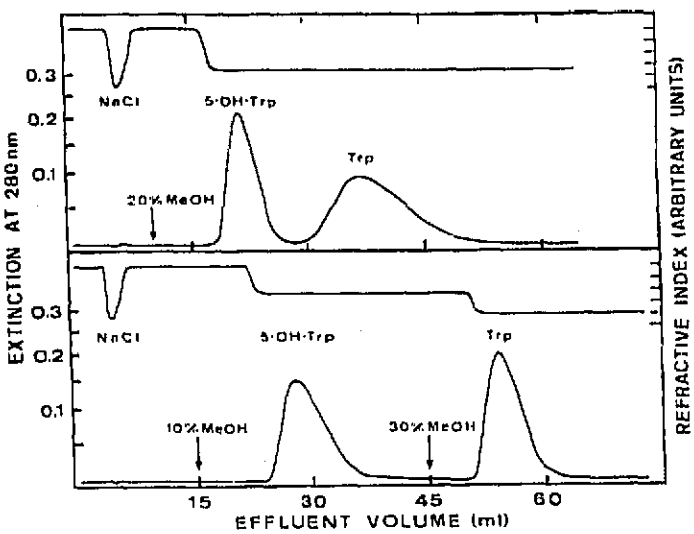

Fig. 1. Desalting and separation of 5-hydroxytryptophan (5-OH-Trp) and tryptophan (Trp) on a column (8 × 150 mm) of 3 g of Porapak Q in 0.1 *N* hydrochloric acid (top) and distilled water (bottom). Elution in both experiments was with aqueous methanol. Load: 3 μmole each of 5-OH-Trp and Trp in 1.1 ml of 1.1 *M* sodium chloride. Flow rate: 0.9 ml/min. Sodium chloride and methanol in the effluent were registered by a refractive index detector, and indole derivatives in the UV at 280 nm.

Desalting and fractionation of indoles

In order to exploit fully the favorable salting-out effect, the sample is concentrated as much as is practical. The column is prepared with a suspension of Porapak Q in acetone and washed prior to use with at least 40 ml of degassed water per gram of

resin (see MATERIALS AND PROCEDURES). If air has penetrated into the adsorbent bed, wetting with acetone and washing with excess water should be repeated to ensure proper adsorption. About 0.2 mmole of substance per gram of Porapak Q can be adsorbed from aqueous solution.

For the extraction of total indoles from aqueous solution the sample (which should always be stabilized by ascorbic acid) is acidified to pH 2–1 just prior to use (see Table I). After washing with distilled water, the indoles are eluted with ethanol–water (1:1).

For desalting and separation of tryptophan and 5-hydroxytryptophan, 1 ml of sample is applied to a column of 1 g of Porapak Q (scaling up or down proportionally when necessary) with a flow rate not exceeding 1 $ml \cdot min^{-1} \cdot cm^{-2}$. The column is washed with 5 ml of distilled water (flow rate up to 5 $ml \cdot min^{-1} \cdot cm^{-2}$) and the 5-hydroxytryptophan is eluted with 10 ml of 10% aqueous methanol and tryptophan with 10 ml of 30% aqueous methanol (Fig. 1).

Extraction of 5-hydroxyindoleacetic acid (5-HIAA) from urine

A column is filled with 2 g of Porapak Q in acetone and the resin is washed thoroughly with distilled water (see above). 1 ml of urine, adjusted to pH 8.0 and stabilized by ascorbic acid, is applied to the column which is then rinsed with 1 ml of distilled water and 6 ml of 0.01 *M* sodium phosphate buffer of pH 8.0 containing 10 mg/l of ascorbic acid. 5-HIAA is eluted with 15 ml of water containing 10 mg/l of ascorbic acid, and this fraction is evaporated to dryness in a 25-ml round-bottomed flask, using a spider multi-evaporator adapter* for a rotary vacuum evaporator, which allows rapid evaporation of at least six samples simultaneously. The residue is ready for quantitative determination. The column is cleaned by rinsing with acetone–water (80:20) and pure acetone.

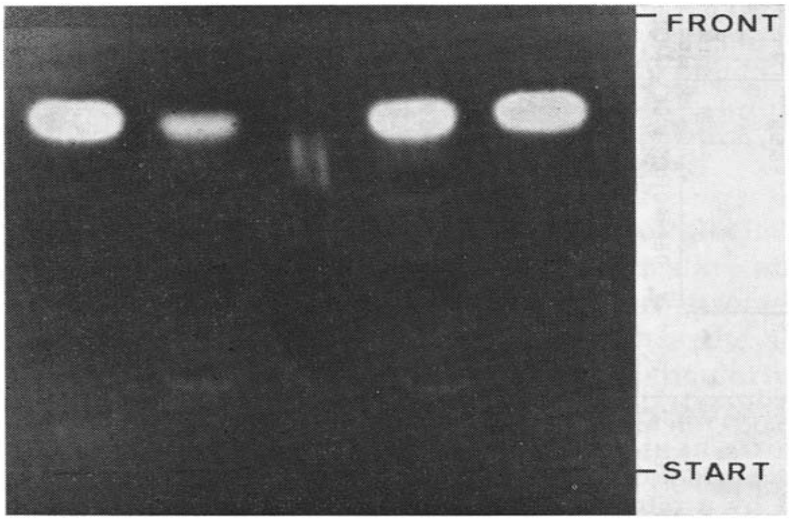

Fig. 2. TLC of 5-hydroxyindoleacetic acid (5-HIAA) extracted from urine by Porapak Q. Photograph taken in the UV at 366 nm. Extraction, solvent, and detection reagent are described in the text. From left to right: 0.6 μg of 5-HIAA in 10 μl of methanol–water (1:1); 10-μl extract of normal urine (equivalent to 20 μl of native urine); 20 μl of native urine; 10-μl extract of normal urine with 6 mg of 5-HIAA added per 100 ml of urine prior to extraction; 0.6 μg of 5-HIAA standard.

* Available, *e.g.*, from W. Büchi, Glasapparatefabrik, CH-9230 Flawil, Switzerland.

For thin-layer chromatography (TLC), the residue is dissolved in 0.5 ml of methanol–water (1:1) and 10 μl are applied onto a cellulose layer without a fluorescent additive (Merck, ready-to-use plate), side-by-side with appropriate standards. One-dimensional TLC is performed in *n*-propanol–water–acetic acid (75:25:2), and the dried plate is then sprayed with a fresh solution of 100 mg of cysteine and 50 mg of *o*-phthalyldialdehyde in 50 ml of methanol–conc. hydrochloric acid (a modification of the technique described in ref. 7), and heated for 5 min at 110°. 5-HIAA fluoresces in UV-light (366 nm).

Fig. 2 demonstrates the usefulness of the above adsorptive extraction. Two-dimensional TLC on cellulose in *n*-propanol–water–conc. ammonia (75:25:2) in the first dimension, and *n*-propanol–water–acetic acid (75:25:2) in the second, revealed only one single fluorescent spot in the area of 5-HIAA.

REFERENCES

1 H. L. Bradlow, *Steroids*, 11 (1968) 265.
2 C. H. L. Shackleton, J. Sjövall and O. Wisén, *Clin. Chim. Acta*, 27 (1970) 354.
3 A. Niederwieser, *J. Chromatogr.*, 54 (1971) 215.
4 A. Niederwieser and H.-Ch. Curtius, *J. Chromatogr.*, 51 (1970) 491.
5 A. Niederwieser, *J. Chromatogr.*, 61 (1971) 81.
6 In preparation.
7 J. Korf and T. Valkenburgh-Sikkema, *Clin. Chim. Acta*, 26 (1969) 301.