

THE EFFECT OF SOLVENT CONCENTRATION ON THE  
SEPARATION OF RADIOIODINE LABELLED TRIIODOTHYRONINE  
AND THYROXINE BY ADSORPTION CHROMATOGRAPHY

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Separation of radioiodine ( $^{131}\text{I}$  and  $^{125}\text{I}$ ) labelled 3,3',5-L-triiodothyronine and L-thyroxine was performed by adsorption chromatography using Sephadex LH-20 as adsorbent and aqueous solution of organic solvents (ethanol, methanol, acetone and tetrahydrofuran) as eluent. The relationship found between the volumetric distribution coefficient ( $k$ ) and the concentration of organic solvent  $/S/$ :  $\log k = \log k^0 - n \cdot \log /S/$  was derived on theoretical bases and was used to optimize the separation conditions.

INTRODUCTION

Dextran gels like Sephadex LH-20 are widely used for the separation of triiodothyronine (T3) and/or thyroxine (T4) either from a mixture of T3, T4, monoiodothyrosine (MIT), diiodothyrosine (DIT) and iodide formed when chloramine-T labelling method is used, or from patient serum in the in vitro tests aiming at the laboratory assesment of thyroid function<sup>1-4</sup>.

Albeit other methods, like ion-pair partition chromatography<sup>5</sup> or the use of dextran ion exchangers<sup>6,7</sup> are also often applied for the separation of iodoaminoacids, they proved to be excellent methods rather for analytical than for preparative purposes. Especially the ion-pair partition chromatography suffers from the drawback of contaminating the separated

products with reagents influencing their further application in a disadvantageous manner.

The application of organic solvents as eluent usually proves to result in good resolution in case of dextran gel column chromatography, however, no attempts were made to establish a relationship between the distribution coefficient and/or the elution volume on one hand, and the solvent concentration on the other one.

The aim of this paper is to show that the relationship between the distribution coefficient and solvent concentration can be derived theoretically as well and to make prediction of optimum separation conditions possible.

## MATERIALS AND METHODS

### Reagents

3, 3', 5-L-triiodothyronine (Koch-Light), L-thyroxine (Sigma) were used to produce  $^{125}\text{I}$  and/or  $^{131}\text{I}$  labelled T3 and T4 according to the chloramine-T method<sup>8</sup>. Methanol, ethanol, acetone and tetrahydrofurane were reagent grade products. The pH of the eluents were adjusted to pH = 4 with citrate buffer.

### Column

Sephadex LH-20 was used as bed material. The dry powder was allowed to swell for 12-24 hours, and the slurry of the gel was poured into a 10 x 150 mm glass tube equipped with a porous disc at the bottom. The height of the filling was 100 mm. The effluent was collected by an automatic fraction collector in 1 ml fractions the T3 and T4 content of which was determined by radiometric assay using a scintillation counting system and NaI(Tl) detector.

### Evaluation of experimental results

The volumetric distribution coefficient was calculated by the use of Eq. (1):

$$k = \frac{V_e - V_o}{V_t - V_o} \quad (1)$$

where  $V_e$  stands for the elution volume,  $V_o$  for the dead volume and  $V_t$  is the total volume.

Since the separation of T3 and/or T4 is governed in our case by adsorption and not by molecular sieving, the total volume of the liquid phase present in the column is to be considered as a mobile phase.

## RESULTS AND DISCUSSION

Fig. 1 shows the distribution coefficient vs. eluent concentration plots for T3 and T4.

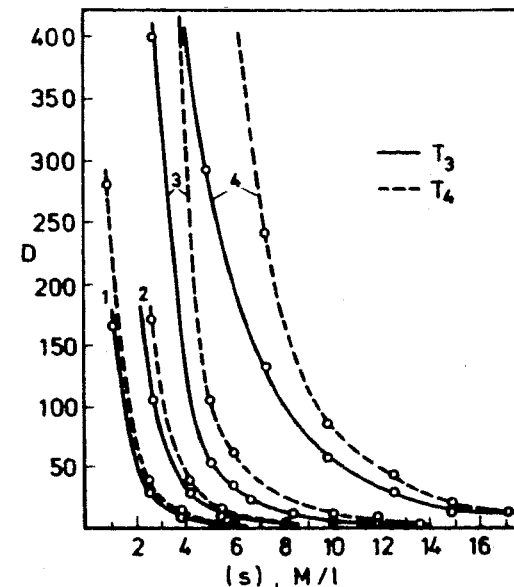


Fig. 1. The dependence of the distribution coefficient on the solvent concentration. 1-Tetrahydrofuran, 2 - acetone, 3 - ethanol, 4 - methanol

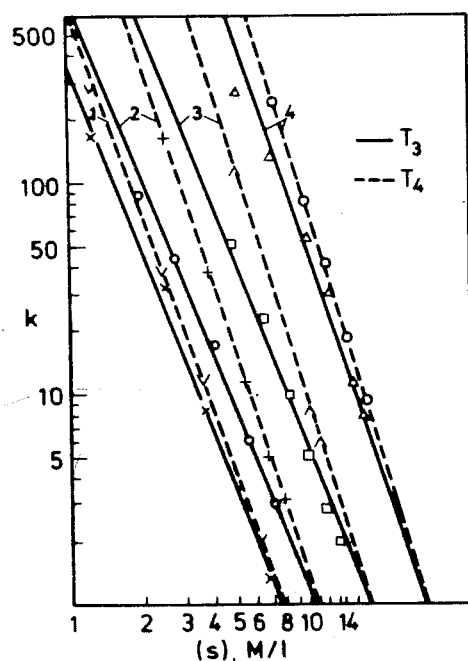
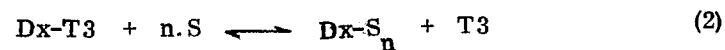


Fig. 2. The  $\log k$  vs.  $\log /S/$  plots for T3 and T4. 1 - Tetrahydrofuran, 2 - acetone, 3 - ethanol, 4 - methanol

As it can be seen from Fig. 2, the curves of Fig. 1 can be linearized in log - log plots.

The linear  $\log k$  vs.  $\log /S/$  relationship can be derived theoretically as well taken linear adsorption isotherms and reversible adsorption for granted. An equation describing the dependence of  $k$  on  $/S/$  can be obtained by formulating the elution as a reversible reaction



expressing the equilibrium constant

$$K = \frac{[Dx-S_n] / [T3]}{[Dx-T3] / [S]^n} = \frac{1}{k} \cdot \frac{[Dx-S_n]}{[S]^n} \quad (3)$$

and neglecting the change of the term  $[Dx-S_n]$ . In Eqs. (2), (3)  $Dx-T3$  stands for T3 adsorbed on the dextran gel,  $Dx-S_n$  for the solvent adsorbed on the gel and  $k$  for the distribution coefficient of T3 and/or T4 defined as follows:

$$k = \frac{[Dx-T3]}{[T3]}$$

In order to express Eq. (3) in a useful form the term

$$\log k^0 = -\log K + \log [Dx-S_n]$$

may be introduced, both sides are logarithmized and  $\log k$  expressed:

$$\log k = \log k^0 - n \cdot \log /S/ \quad (4)$$

The term  $k^0$  is - from practical point of view - the distribution coefficient at 1 mole/dm<sup>3</sup> eluent concentration and  $n$  - the slope of the  $\log k$  vs.  $\log /S/$  plots - is the stoichiometric factor in Eq. (2).

Inspection of the curves shown in Fig. 1 and those referring to the pH dependence of  $k^0$  leads to the conclusion that change of the solvent concentration results in much more considerable variation of  $k$  than the shift of the pH. On the other hand, in the latter case the distribution coefficient varies with the pH in the vicinity of the isoelectric point only, while varia-

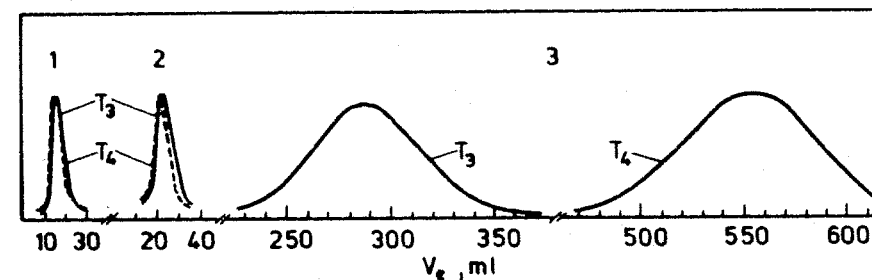


Fig. 3. The elution curves of T3 and T4. Eluent: 1 - 70% methanol, 2 - 60% methanol, 3 - 20% methanol

tion of the eluent concentration gives rise a continuous and - by the use of Eq. (4) - predictable change of  $k$  practically over the whole possible concentration range.

As an example for the dependence of the elution volume on solvent concentration the elution curves of T3 and T4 are shown in Fig. 3 for three different methanol concentrations.

The resolution, the best measure of the performance of a separation system can be expressed as follows<sup>10</sup>:

$$R_s = \frac{1}{4} \left[ \frac{k_2}{k_1} - 1 \right] \sqrt{N} \left[ \frac{k'}{1+k'} \right] \quad (5)$$

where  $k_2/k_1$  is the selectivity of separation,  $N$  is the number of theoretical plates in the column and  $k'$  the capacity factor. Since the effect of the term  $k'/(1+k')$  in Eq. (5) is in our case negligible, it is only  $N$  and  $k_2/k_1$  which is to be taken into account when the optimum resolution is predicted. Out of these two parameters  $k_2/k_1$  can be predicted using Eq. (4):

$$\frac{k_2}{k_1} = \frac{k_2^0}{k_1^0} \cdot /S/^{n_1 - n_2} \quad (6)$$

for any solvent concentration provided  $k^0$  and  $n$  are known.

The dependence of the resolution on the solvent concentration is out of the scope of this paper, it will be dealt with elsewhere.

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