CHROM. 4811

The effect of temperature on the interaction of phenols with Sephadex gels

In gel chromatography, mixtures of substances are separated according to their molecular sizes. The mixture is eluted through a column of the gel and, since the structure of the gel is a three-dimensional network of meshes, the molecules of largest size are eluted first, as they are least able to diffuse into the pores of the gel. Separations of substances on this principle would not be expected to be dependent on temperature, and, indeed, it has been convincingly shown that elution volumes are unaffected by changes of temperature[1].

However, certain substances are strongly adsorbed by gels, and, in such cases, it is probable that elution volumes will vary with temperature. Many aromatic and heterocyclic compounds are adsorbed by cross-linked dextran[2] and other gels. Mono-substituted phenols interact particularly strongly with Sephadex G-10, a dextran gel which has a high degree of cross-linking[3]. The phenol is adsorbed onto the glyceryl cross-links[4], and it was suggested that the hydroxyl group of the phenol interacts with the cross-links through hydrogen bonds[3]. The interaction can be represented by the equilibrium shown in eqn. (1),

$$\mathrm{P} + \mathrm{D} \overset{K_D}{\rightleftharpoons} \mathrm{P\text{–}D} \qquad (1)$$

where P is the substituted phenol, D is the dextran gel, and P–D the phenol–dextran gel hydrogen-bonded complex. The equilibrium constant is obtained from eqn. (2),

$$K_D = \frac{V_e - V_0}{V_i} \qquad (2)$$

where V_e is the elution volume of the phenol, V_0 the void volume of the column, and V_i the internal aqueous volume of the gel. For normal gel filtration, K_D lies between 0 and 1. When a substance is adsorbed by the gel, K_D is greater than 1.

Equilibrium constants, and hence elution volumes, for reactions of the type shown in eqn. (1) usually depend on temperature. Consequently, the elution volumes of a series of monosubstituted phenols from columns of Sephadex G-10 were measured at various temperatures. In addition, an investigation of the temperature dependence of K_D should lead to values for the free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) by using eqns. (3), (4) and (5).

$$\Delta G = -RT \log_e K_D \qquad (3)$$

$$\mathrm{Log_e}\, K_D = -\frac{\Delta H}{RT} + I \qquad (4)$$

where I is a constant, and

$$\Delta G = H - T\Delta S \qquad (5)$$

Materials and methods

The column, of diameter 1.5 cm, was packed with Sephadex G-10 gel to a height of 11.5 cm as described by DETERMANN[1]. The column was surrounded by a

water-jacket through which water at constant temperature was passed. The temperature of the column was constant to ± 0.1°C.

Samples, 0.5 ml of eluent containing 1 to 5 mg of the substituted phenol and 0.1 mg of Blue Dextran 2000 as internal standard, were introduced onto the column with a hypodermic syringe. The eluent was acetate buffer of pH 4.0.

The effluent from the column was continuously monitored with an L.K.B. Uvicord I flow analyser which measured absorbance at 254 nm. The flow analyser was connected to a Leeds and Northrup "Speedomax H" strip-chart recorder. Elution volumes (V_e) were measured from the recorder chart.

TABLE I

THE VARIATION OF K_D WITH TEMPERATURE OF SUBSTITUTED PHENOLS

Phenol	*K_D value*			
	5° C	*15° C*	*25° C*	*35° C*
Phenol	10.1	9.6	9.1	8.4
m-Fluorophenol	16.5	15.7	14.7	13.5
p-Chlorophenol	36.2	33.4	30.3	26.7
p-Bromophenol	55.0	49.9	45.3	38.7
p-Iodophenol	101.8	89.2	75.6	64.0
p-tert.-Butylphenol	23.9	24.0	24.5	25.1

Results and discussion

The variation of K_D with temperature of a series of substituted phenols is shown in Table I. It was assumed in the calculation of K_D that the internal volume of the gel (V_i) was independent of temperature.

As expected, there was a large variation of K_D with temperature over the range investigated, particularly in the case of the halogen-substituted phenols. Hence, temperature programming should prove to be useful for the separation of mixtures of phenols.

However, *p-tert.*-butylphenol is a notable exception. Although it is strongly adsorbed by the gel, K_D is virtually independent of temperature. This implies that the mechanism of adsorption of *p-tert.*-butylphenol is different from the other substituted phenols. The adsorption effect cannot therefore be due to hydrogen-bonding of the phenolic hydroxyl group to the cross-linking of the gel. It is possible that the interaction of *p-tert.*-butylphenol with the gel is caused by steric effects associated with the *p-tert.*-butyl group. The side chain may be a particularly good fit in the pores of the gel. Alternatively, alkyl groups may have some specific interaction with the gel, since MARSDEN[5] has shown that for a series of alcohols, K_D increases with the length of the alkyl chain. A similar effect may be operating in the case of *p-tert.*-butylphenol.

A plot of log K_D for the substituted phenols against the reciprocal of absolute temperature is shown in Fig. 1. The curves are approximately linear and the slopes of the lines, calculated by a least-squares treatment, gave the enthalpies (ΔH) for the reaction between the substituted phenols and the dextran gel (eqn. 1). The thermodynamic parameters at 25°C are shown in Table II.

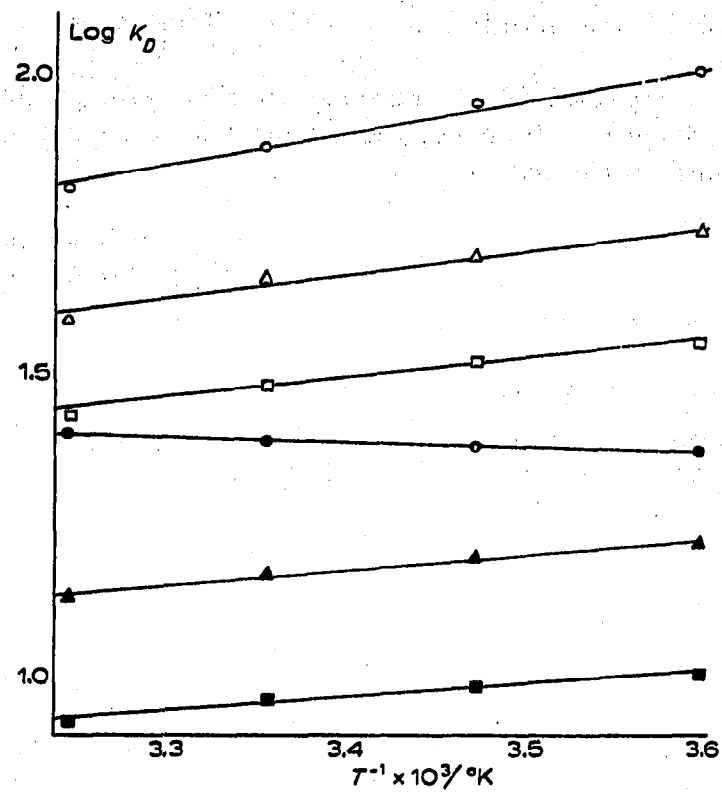

Fig. 1. A plot of log K_D against the reciprocal of absolute temperature for a series of substituted phenols. ■, phenol; ▲, *m*-fluorophenol; ●, *p-tert.*-butylphenol; □, *p*-chlorophenol; △, *p*-bromophenol; ○, *p*-iodophenol.

The value of ΔH should give some idea of the strength of the bond between the phenol and the dextran gel. However, this will not be an exact measure of bond dissociation energy of the complex as solvation effects cannot be taken into account since ΔH is the sum of all bonds formed and all bonds broken. In addition to the formation of the bond of the phenol–dextran gel complex, bonds will be broken between the phenol and its solvation sheath, and between the dextran gel and solvent hydrogen-bonded to the cross-linking.

For phenol, values of ΔH are in reasonable agreement with the suggestion that the phenol is hydrogen-bonded to the dextran gel. However, for the formation of such a complex, ΔS would be expected to be large and negative. In fact, except

TABLE II

THERMODYNAMIC PARAMETERS FOR THE ADSORPTION OF A SERIES OF SUBSTITUTED PHENOLS ON SEPHADEX G-10 AT 25° C

Phenol	$-\Delta G$ (kJmol^{-1})	$-\Delta H$ (kJmol^{-1})	ΔS (Jmol^{-1}deg^{-1})
Phenol	5.4	4.3	3.7
m-Fluorophenol	6.6	4.9	5.8
p-Chlorophenol	8.4	7.1	4.4
p-Bromophenol	9.4	8.2	3.9
p-Iodophenol	10.7	11.1	−1.3
p-tert.-Butylphenol	7.9	−1.2	30.9

for *p*-iodophenol and *p-tert.*-butylphenol, ΔS is small and positive. This must be due to desolvation of the phenol and gel.

Again, *p-tert.*-butylphenol is an exception. The decrease in free energy is entirely associated with an increase in entropy, which suggests that the adsorption of *p-tert.*-butylphenol is controlled by some steric process.

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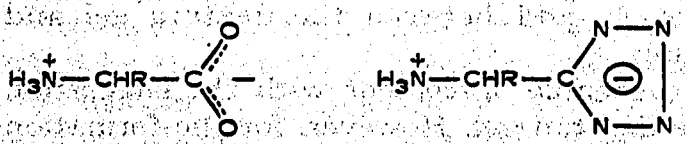
Tetrazole analogues of amino acids and peptides

II. Paper and thin-layer chromatography of tetrazole analogues of amino acids

Like amino acids (I), tetrazole analogues of amino acids (II)[1–3]—the compounds in which the carboxyl group is replaced by a 5-tetrazolyl group—exist in a zwitterion form.



I II

A number of tetrazole analogues of amino acids were prepared and characterised in the first paper[3] of this series. As a further part of the study the chromatographic behaviour of these compounds was investigated, and the sensitivity of the reaction with ninhydrin was determined.

The physical and chemical properties of tetrazole analogues of amino acids are very similar to the properties of amino acids. They are soluble in water, alkalis, aqueous acids, and sparingly soluble or insoluble in organic solvents. All tetrazole analogues of amino acids have high melting points, and all decompose at the melting temperatures. The dissociation constants of the tetrazole analogues of the amino acids are comparable to the respective constants for the amino acids.

We propose that the tetrazole analogues of amino acids, as a group, should be