CHROM. 5616

An aqueous two-phase system of dextran/hydroxypropyldextran as a model in adsorption studies of Sephadex® gels

In most cases, aqueous solutions of two water-soluble polymers are not miscible and result in an aqueous two-phase system when brought into contact. ALBERTSON has established a very effective separation method for viruses and cell particles[1] by making use of this effect. Dextran (Dex) and hydroxypropylated dextran (HPD) form such a pair of incompatible polymers. The aqueous Dex/HPD system provided us with a simple model for Sephadex gels, because Sephadex gel consists of Dex as the basic polymer with hydroxyglycerol ether bridges as cross-links; in the case of Sephadex LH-20 the Dex units carry hydroxypropyl ether groups.

DETERMANN AND WALTER[2] have demonstrated in dialysis experiments the affinity of a solution of polyethylene glycol for phenols as compared with a Dex solution. Our new experiment omits the uncertainty of the dialysis membrane and allows a simple measurement of the affinity between benzoic acid, as test substance, and Dex or HPD, respectively. 1.5 g Dextran 500 (mol. wt. 500000) and 2 g HPD (33 % hydroxypropylated dextran, mol. wt. 546000) are dissolved separately, each in 15 ml of buffer solution. 2 ml of an 0.1 % benzoic acid–buffer solution are added to 4 ml of each polymer solution, mixed by shaking and then centrifuged. The two phases have almost the same volume. An aliquot of each phase is taken, diluted, and the extinction of benzoic acid is measured at its UV maximum against a reference polymer solution. Ammonium formate and ammonium acetate buffers of pH 3.04, 3.95, 4.55, and 5.60 are used. The ionic strength of the buffers is kept constant.

In the case where the benzoic acid undergoes no interaction with the polymer solutions, it should behave as if there were no polymers present and the same concentration of acid should be found in each phase. But the experiment shows that more benzoic acid is found in the upper phase, which per volume contains more HPD than Dex. The affinity of the benzoic acid towards HPD depends on pH, as demonstrated by Fig. 1. The pK of 4.4, found experimentally is in good agreement with the literature

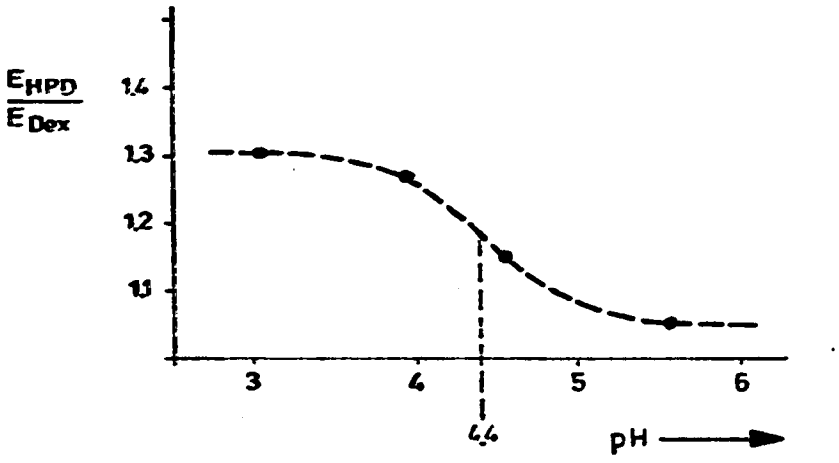

Fig. 1. Quotient of extinction values of benzoic acid in the aqueous two-phase system HPD/Dex plotted against pH at a constant ionic strength of 0.2.

value of pK 4.2 for benzoic acid. The partition of the benzoate ion is almost equal in both phases, the relation between the extinction values of the phases approaches 1.0. A similar pH dependence of ionisable substances of low molecular weight was observed by BROOK AND HOUSLEY in adsorption gel chromatography on Sephadex gels[3]. This behaviour stresses the value of our model.

These experiments add support to the idea that the hydroxypropyl ether groups in the Sephadex gels are the source of adsorption noticed during gel chromatography. A solvent containing similar ether groupings should overcome all the adsorption effects when used as an eluant. With a large excess of eluant it could be expected that the interactions between the solute and the gel matrix would be overruled by similar but much more probable interactions between solute and eluant. Substances retarded in water should not be retarded in such a solvent.

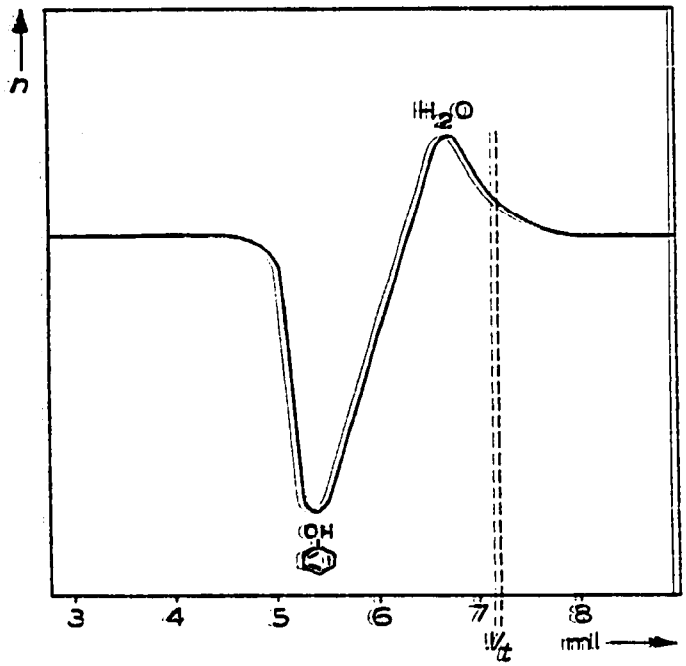

Fig. 2. Elution diagram of phenol and water chromatographed on Sephadex LH-20 (2 g) with monoethyl diglycol ether as eluant. Column, 0.67 × 20.5 cm; V_t, 7.2 ml; sample volume, 0.1 ml; concentration, 0.5 mg/ml; flow rate, 5.0 ml/h.

TABLE I

MEASURED (V_e) AND CALCULATED (K_{av}) ELUTION PARAMETERS FOR SEPHADEX LH-20 WITH MONOETHYL DIGLYCOL ETHER AS ELUANT

1.98 g gel; column, 0.67 × 20.5 cm; V_t, 7.2 ml.

Substance	V_e (*ml*)	K_{av}
Anthracene	4.9	0.64
Cyclohexane	5.2	0.68
Phenol	5.3	0.70
Glucose	5.8	0.74
Water	6.8	0.94

To investigate this we used monoethyl diglycol ether as the eluant and Sephadex LH-20 as the gel since it gives the strongest adsorption effects of all the Sephadex gels[2]. Fig. 2 shows that phenol is eluted before water, both without retardation, which means well before the total volume of the column (V_t). Table I gives data about some more substances run with this eluant on Sephadex LH-20. The basic criterion of the separation in this solvent should be that of size difference only, as in common gel chromatography.

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1 P. Å. ALBERTSON, *Partition of Cell Particles and Macromolecules*, New York, 1960.
2 H. DETERMANN AND I. WALTER, *Nature*, 219 (1968) 604.
3 A. J. W. BROOK AND S. HOUSLEY, *J. Chromatogr.*, 42 (1969) 112.

Received August 6th, 1971