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Note

Starch gel for gel filtration

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For gel filtration, polysaccharide derivatives are commonly used in the form of a chemically treated gel in order to obtain the required porosity and good column permeability[1,2]. The use of polysaccharide gels that have not previously been chemically treated has not yet been described.

In this paper, the employment of starch gel without any chemical modifications for gel filtration is described.

MATERIALS AND METHODS

All chemicals used were of laboratory grade, while the proteins were purified by a commonly used procedure. Elution volumes were estimated from the UV analyzer record (Development Workshops, CSAV, Prague, Czechoslovakia), the flow-rate through the column being constant. For estimation of the void volume, blue dextran (Pharmacia, Uppsala, Sweden) and for the estimation of total volume, potassium chromate (Lachema, Brno, Czechoslovakia) were used.

Starch gel was prepared by the following procedure, which was developed by the Starch Factories Research Centre, Červená Řečice, Czechoslovakia. To 50 ml of water heated in a boiling water-bath, a suspension of 10 g of starch in 50 ml of water was added with stirring (for 10% gel: 5 g of starch is used for 5% gel, etc.). The mixture was heated in order to complete gelling, then the gel was cooled to room temperature and frozen to −20° (12 h). The gel was subsequently thawed at room temperature and then frozen again (24 h). When thawed again, the gel was mixed in a laboratory mixer and freed from the too fine portion by decantation. Gels of concentration 10, 5, 3 and 1% were prepared.

RESULTS AND DISCUSSION

No differences were found among the K_{av} values of different proteins by gel filtration on starch gels of various concentrations (Table I). This can be explained by the fact that the porosity of a gel depends on the molecular structure of starch rather than on its initial concentration.

TABLE I

COMPARISON OF K_{av} VALUES OF VARIOUS PROTEINS ON STARCH GEL PREPARED AT DIFFERENT CONCENTRATIONS

Protein	*Gel concentration (%)*			
	10	*5*	*3*	*1*
Blue dextran	V_0	V_0	V_0	V_0
Potassium chromate	V_t	V_t	V_t	V_t
Chymotrypsin	0.17	0.14	0.16	0.16
RNase	0.21	0.20	0.21	0.19

TABLE II

COMPARISON OF STARCH GEL PROPERTIES WITH PROPERTIES OF VARIOUS TYPES OF SEPHADEX (PHARMACIA, UPPSALA, SWEDEN)

Protein	K_{av}			
	Starch gel (10%)	*Sephadex*		
		G-25	*G-50*	*G-75*
Blue dextran	V_0	V_0	V_0	V_0
Potassium chromate	V_t	V_t	V_t	V_t
Chymotrypsin	0.17	0.05	0.16	0.25
RNase	0.21	0.11	0.22	0.35

Comparing the K_{av} values of various proteins obtained by gel filtration on starch gel with values obtained by gel filtration on Sephadex, it was found that starch gel corresponds, in terms of gel filtration characteristics, to Sephadex G-50 (Table II) irrespective of the initial concentration of the starch from which it had been prepared.

The flow characteristics were determined and evaluated according to the equation

$$U = K \frac{d_p}{L} \tag{1}$$

where U is the linear flow-rate through the column (cm/h), d_p is the hydrostatic pressure in the column (cm H_2O), L is the height of the bed of gel (cm) and K is a constant characteristic for a gel.

Fig. 1 shows the relationship between U and d_p. It is apparent that the flow-rate through the column is directly proportional to the pressure and the flow resistance does not increase when the pressure increased over the whole range of pressures studied. The value of K calculated according to eqn. 1 remains constant at 22 cm/h over the whole range measured. The K value depends on the conditions of mixing, but it is always independent of the pressure used.

Preparation of the gel in the presence of ammonium sulphate showed that starch gel can be prepared in the presence of this salt up to a concentration of 20% of saturation. In the presence of casein, starch gel was formed as well as in its absence (the maximal concentration measured was 1% of casein).

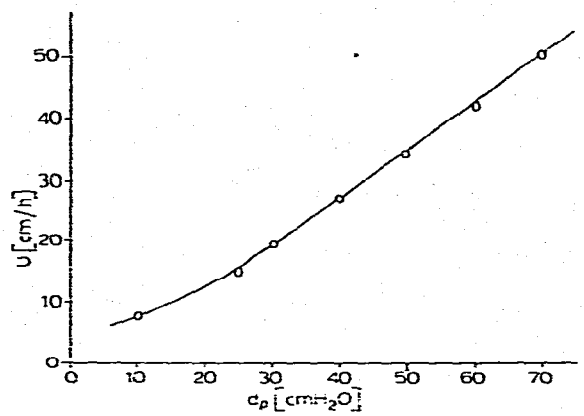

Fig. 1. Relationship between hydrostatic pressure and flow-rate.

The results obtained show that starch gel prepared by the procedure described can be used for the gel filtration of low-molecular-weight proteins and peptides and for the desalting of high-molecular-weight proteins.

This starch gel can also be used for immobilization of the enzymes by entrapping them in the gel by the method described by Bauman *et al.*[3].

A disadvantage is that gels of different densities cannot be prepared by the above method.

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