AN IMMUNOSORBENT BASED ON POROUS CELLULOSE BEADS

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UDC 615.373:616-097.5-078.73:661.183

KEY WORDS: immunosorbent; cellulose.

Various types of immunosorbents have now been described. Immunosorbents based on specific particles of agarose (Sepharose) and polyacrylamide gel (Biogel P), marketed by Western firms, are the most widely used. These bases are often not easily available, whereas cheap granulated cellulose [5] has not been used for this purpose.

The writers previously suggested a method of obtaining immunosorbents on the basis of a cellulose suspension obtained by reprecipitation from cuprammonium solution [2, 3, 4]. An essential shortcoming of these sorbents is that they cannot be used in columns, because it is almost impossible for aqueous solutions to flow through layers of such immunosorbents.

This paper describes a method of obtaining a cellulose immunosorbent which preserves the high capacity of the suspension immunosorbent, but which can permit fluid to flow through it, so that it is suitable for experiments with columns. Porous cellulose beads (PCB) were used as the basis for the immunosorbent.

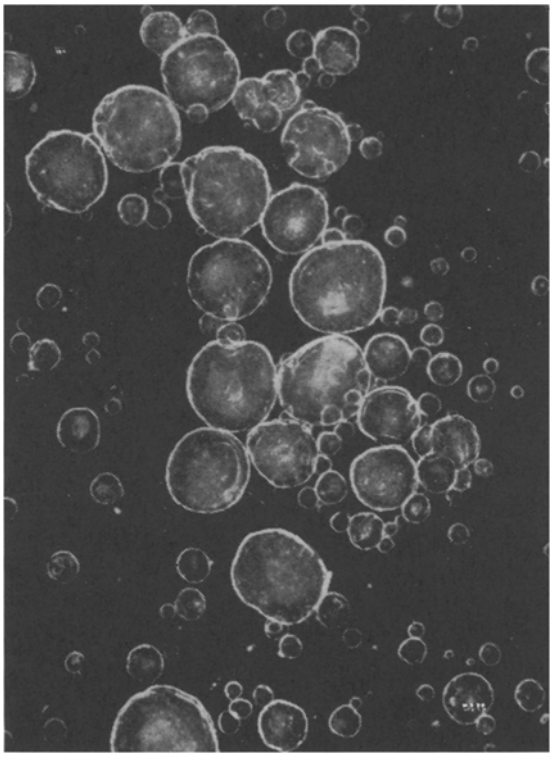

Fig. 1. Photomicrograph of PCB obtained from 3% solution of cellulose (DP 200), magnification 48.

Laboratory of Chemistry and Biosynthesis of Antibodies, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 92, No. 12, pp. 752-754, December, 1981. Original article submitted June 16, 1981.

 0007-4888/81/9212-1742$07.50

TABLE 1. Characteristics of Immunosorbent Based on PCB

Preparation No.	Cellulose concentration, %		DP of cellulose	Quantity of fixed protein		Column				
	in solution	in particles		mg/ml freely settling preparation	mg/g dry weight	weight of immunosorbent, mg	volume of immunosorbent, ml	rate of flow of liquid with pressure of 32 cm water, ml/h/cm²	quantity of antibodies extracted from column, mg	capacity, mg antibodies/g dry preparation
1	3,0	1,7	200	0,54	32	50	3,0	64	15,2	260
2	1,5	0,7	200	0,31	45	20	3,0	51	23,0	1150
3	1,0	0,7	5 000—10 000	1,26	126	50	6,0	115	21,2	422

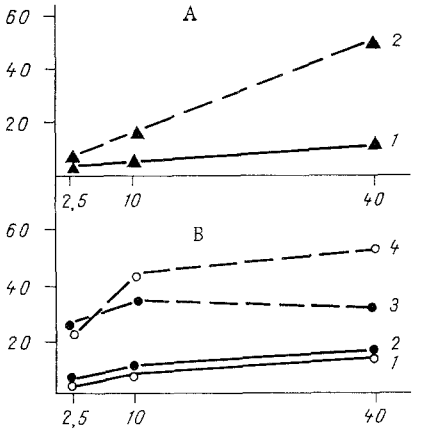

Fig. 2. Dependence of properties of immunosorbent on quantity of protein added to PCB. Abscissa, quantity of protein added (in mg); ordinate, protein content in mg/100 mg sorbent. A) PCB from medicinal cotton, cellulose content 0.7%; B) PCB from cellulose powder (DP 200), cellulose content 2% (2, 3) and 1.1% (1, 4). Continuous line indicates quantity of fixed antigen, broken line — quantity of bound antibodies.

EXPERIMENTAL METHOD

Preparations of cellulose with different degrees of polymerization (DP) served as the original material: cellulose powder (DP 200) and floccular hygroscopic medicinal cotton (DP 5000-10,000).

Solutions of cellulose were prepared as follows: 1 g of cotton was soaked in 20 ml 15% NH_4OH solution for 2 h. Next 13.5 g of wet precipitate of freshly prepared $Cu(OH)_2$ [2] and 10 ml of 15% NH_4OH solution were added. After 1 h a further 10 ml of 25% NH_4OH solution was added to the swollen gel and the mixture was allowed to stand overnight. Next day 10 ml of 25% NH_4OH solution was added and the total volume of the resulting solution was made up to 100 ml with 15% NH_4OH solution. Solutions with concentrations of 6.0, 3.0, or 1.5% were prepared in the same way from cellulose powder with DP 200, by dissolving 6.0, 3.0, or 1.5 g of material respectively.

One volume of solution from medicinal cotton was introduced into a flask containing 2 volumes of a chloroform—benzene mixture (1:5), to which 0.15% of a surface-active substance (polyhydroxyethylene sorbitan monoleate, or Tween 80) was added. A mixer was lowered into the vessel and the mixture was emulsified and let out through the lower side tube of this flask into a vessel with regenerating solution (6 volumes of 6% sulfuric acid solution in acetone). The liquid was poured off 10 min later and the beads thus formed were washed with 10 volumes of water. The product was fractionated by free sedimentation

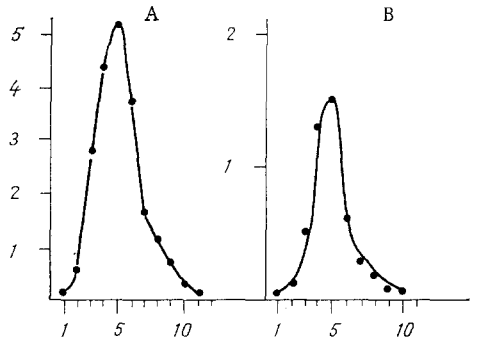

Fig. 3. Isolation of antibodies and antigen on columns with immunosorbent. Abscissa, Nos. of fractions; ordinate, protein content (in mg). PCB obtained from 1% solution of medicinal cotton. Volume of fractions 2.5 ml. A) Isolation of antibodies against IgG_{rab} from donkey antiserum; B) isolation of IgG_{rab} from rabbit serum on PCB with fixed antibodies against IgG_{rab}.

in a cylinder and fractions of particles measuring 100-400 μ were separated. The cellulose content in the final preparation of PCB was usually half that in the original cellulose solution used for their preparation.

The PCB (1 g) were activated with 0.2 M $NaIO_4$ solution (100 ml) for 30 min at room temperature and washed on a Büchner funnel with water (200 ml) [4, 6]. The product can be kept at 4°C for many months in water without loss of activity.

Before combination with protein the product was washed with 0.1 M carbonate–bicarbonate buffer, pH 9.0, and a solution of protein was added to it (from 2.5 to 40 mg/100 mg dry weight of carrier). The unreacted protein was removed 16-18 h later on a Büchner funnel and the preparation was reduced with 0.2 M $NaBH_4$ solution in 0.15 M NaCl for 1 h at room temperature. The synthesized immunosorbent was washed off with 0.15 M NaCl.

The quantity of protein bound to the carrier was determined with the aid of bromphenol blue [1]. Rabbit immunoglobulin G (IgG_{rab}) was used as the antigen. Protein specifically bound to the immunosorbent was eluted with 0.01 N HCl in 0.15 M NaCl and determined by Lowry's method [7]. Antibodies were extracted from donkey serum against IgG_{rab}. The immunosorbent was used to pack a column measuring 15 × 200 mm (from Whatman, England).

EXPERIMENTAL RESULTS

PCB containing from 0.7 to 3% of cellulose were obtained by the method described above from cellulose solutions with different degrees of polymerization and concentration (from 1 to 6%) (Fig. 1).

The relationship of the capacity of the immunosorbent obtained as described above to cellulose concentration in the porous beads and to the quantity of protein bound to them was studied (Fig. 2). Experiments showed that if the quantity of added protein was increased, initially (up to 10 mg) it was practically entirely bound, but later binding was only partial. The capacity of immunosorbents based on PCB containing 0.7-1% cellulose increased with an increase in the quantity of antigen fixed on them and it exceeded 300-500 mg/g sorbent. A different picture was observed when PCB with a high cellulose content (2%) were used. In this case an increase in the quantity of antigen fixed to the carrier above a certain level was not accompanied by any further increase in the quantity of bound antibodies. The reason was evidently that fixation of a large number of antigen molecules in the pores of the denser carrier prevented the penetration of antibody molecules into them.

PCB can be used not only to extract antibodies, but also to isolate individual antigens from complex protein mixtures. For example, if pure donkey antibodies against IgG_{rab} are

fixed to PCB, the corresponding immunoglobulins can be extracted by means of this immunosorbent from rabbit serum in a yield of 50-70 mg/g sorbent.

The preparations as described above readily allow fluid to flow through them in columns: The volume of liquid flowing through the column per hour is 20 times greater than the volume of sorbent with which it is packed. Saturation of the immunosorbent with antibodies and antigen takes place quickly when the appropriate solutions are passed through, and eluted material comes away from the column in a sharp peak (Fig. 3).

Data for samples of immunosorbents based on PCB tested in column experiments are given in Table 1.

The immunosorbent possesses adequate mechanical strength and is suitable for re-use, although the quantity of antibodies extractable decreases (by 33-50%) toward the 10th cycle.

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EVALUATION OF THE ATRAUMATIC PROPERTIES OF DRESSING MATERIALS

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UDC 615.468.015.4

KEY WORDS: dressings; method of studying atraumatic qualities.

Production of modern dressing materials necessitates the evaluation of their atraumatic qualities. The method of determining the degree of adhesion of materials in the lower layer of dressings on models of the wound surface [1] (Authors' Certificate No. 685292 of 1979), developed in the "Polymers in Medicine" Laboratory, gives results which requires more precise experimental verification.

The object of this investigation was to obtain the fullest and most objective assessment of the atraumatic properties of some specimen dressing materials by cytological analysis of squash preparations from the wound surface and by histological and electron-microscopic investigation of the dressings.

EXPERIMENTAL METHOD

The degree of adhesion of the materials under laboratory conditions was determined as follows: The test samples were glued to the conventional wound surface, dried, and removed with the aid of a mobile dynamometer. The force required to remove the samples characterized the degree of their adhesion. The results were compared with the results of determination of the adhesive properties of regulation medical gauze. The degree of adhesion of the gauze was taken to be 100%. Samples of dressings with better atraumatic qualities than medical gauze were selected for detailed experimental study.

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 12, pp. 754-757, December, 1981. Original article submitted August 21, 1981.

0007-4888/81/9212-1745$07.50