Short Communication

CHROM. 5958

Membrane chromatography of cells on sponge-like poly(2-hydroxyethyl) methacrylate

The problem of separating cells by simple and rapid techniques has attracted the attention of many investigators. Chromatography seems to be one of the most promising methods in this field[1-4]. In the present work we investigated whether the micro-technique of membrane chromatography[5] and the principle of chromatographic sieving could be utilized with particles of the magnitude of cells. The difficult search for a suitable model system led us finally to the use of red blood cells of different species, because the erythrocytes are very uniform in their specific size and shape and have a distinct colour. Porous membranes of poly(2-hydroxyethyl)methacrylate (HEMA) were found to be uniquely suitable; they were inert and possessed both the suitable pore size and porosity as well as convenient mechanical properties.

Materials and methods

Sample preparation. 40 volumes of blood were collected in 6 volumes of the standard ACD anticoagulant solution containing 2.2% of trisodium citrate, 0.8% of citric acid and 2.45% of glucose in water. Human, murine and chicken erythrocytes were washed twice with a standard phosphate balanced solution (PBS), pH 7.5, on a low speed centrifuge. PBS, which was used as the eluant in chromatography, contained 0.8% NaCl, 0.02% KCl, 0.11% Na_2HPO_4, 0.02% KH_2PO_4 in water. The final volume of each cell suspension was adjusted to that of the original sample. The erythrocytes were stored at +4° and were used for chromatography within 24 h after blood collection. Blue dextran 2000 (Pharmacia, Uppsala) was dissolved in PBS to a 2% solution. Due to its distinct blue colour and known molecular weight ($2 \cdot 10^6$) it served as a good orientation standard in chromatography.

Membrane preparation. HEMA membranes were prepared by a direct block copolymerization of defined amounts of the monomers and water[6,7] between two glass plates which were planparallel within a deviation of 0.01 mm. A mixture of ethylene glycol methacrylate containing a given amount (*e.g.* 2%) of ethylene dimethacrylate as the cross-linking agent, was used as monomer. Potassium persulphate and diethylaminoethyl acetate served as the initiating system.

The polymerization of a mixture containing 30% of the monomer and 70% of water resulted in the formation of a sponge-like, elastic, hydrophilic polymer with a porous structure[8,9]. The polymerization lasted 24 h at laboratory temperature and the system was then heated to 80° for 2 h. The membrane was taken from the glass mould and the remnants of the low-molecular-weight reagents were removed by several repeated extractions in boiling water for 5 h.

Membrane chromatography. A swollen strip of the HEMA membrane about 1 cm wide, 2 cm long and 1.5 mm thick was washed in the PBS solution. Excess PBS was soaked away with a wick of filter paper. Standard suspensions of fresh erythrocytes and blue dextran and their mixtures were applied (about 0.2–1 μl) by a careful

touch of a thin glass capillary onto one end of the strip. The other end of the strip was then attached to a glass slide so that the strip hung vertically with the start at the lower end. Another strip of HEMA was squeezed between filter papers to remove about 50–70% of its maximum water-content. This strip was laid on the upper end of the chromatogram and the glass slide to achieve a standard upward suction of the eluant from below. Small portions (about 2–4 μl) of PBS were repeatedly applied with a capillary to a plexiglass slide and allowed to soak, with short interruptions (about 2–5 sec) into the lower end of the chromatogram[10]. A sufficient development of the spots required about 5 min. For photographic documentation, the wet strips were stored in a moist chamber. The same strip could be used repeatedly (*e.g.* 20 times) after a thorough washing and squeezing in PBS or water.

Results and discussion

Fig. 1 shows the marked differences between the size and shape of the three types of erythrocytes used as standard particles in our experiments. Most of the fixed

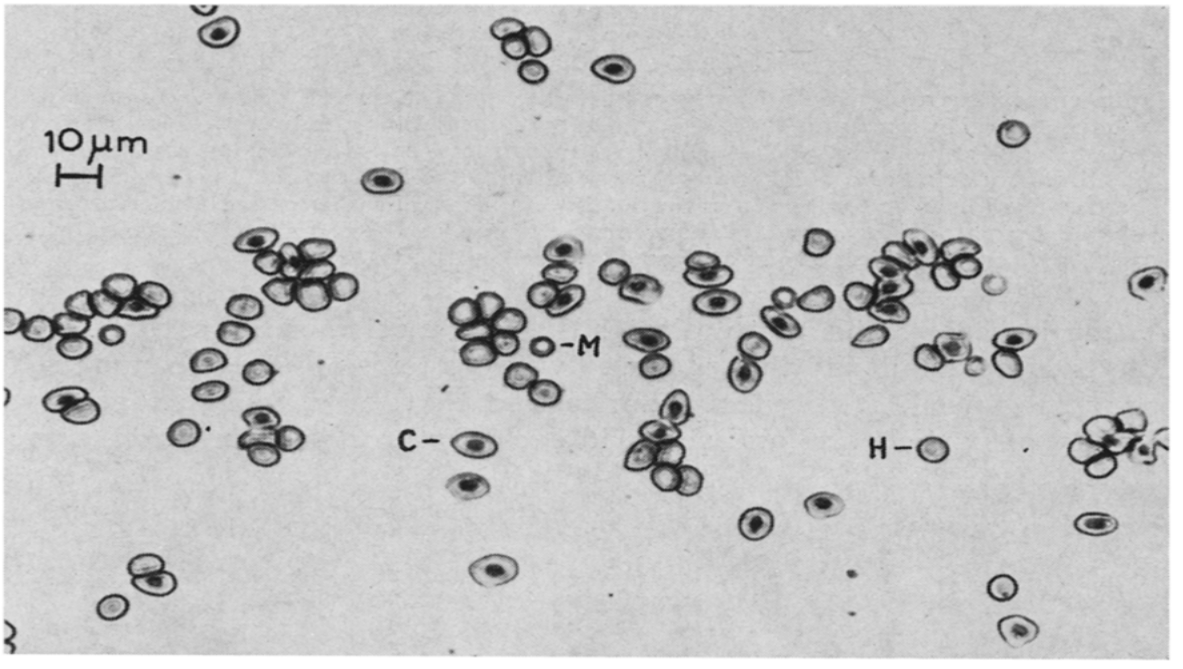

Fig. 1. Microphotograph of a mixture of fixed human, murine and chicken erythrocytes. Note the marked differences in the sizes and shapes of the human (H), murine (M) and chicken (C) erythrocytes as well as the slight differences within each species-specific group. The smear was stained by the standard May–Grünwald and Giemsa–Romanowski techniques.

cells are seen only from their flat side here. However, in reality, the human and murine erythrocytes have the general form of a thin (about 1–2 μl) biconcave disk. The disk of chicken erythrocytes is wider at the center. The formation of loose cell aggregates visible in Fig. 1 was an artifact caused by the fixation technique. In the fresh suspension, the mixed erythrocytes did not interact significantly. The cells had the following mean diameters: chicken, 11 μm; human, 7.2 μm and murine, 5.5 μm with a deviation of about 10%.

Fig. 2 demonstrates that under the given conditions a good separation of cells and blue dextran can be achieved. It is evident that as the particle size decreases the

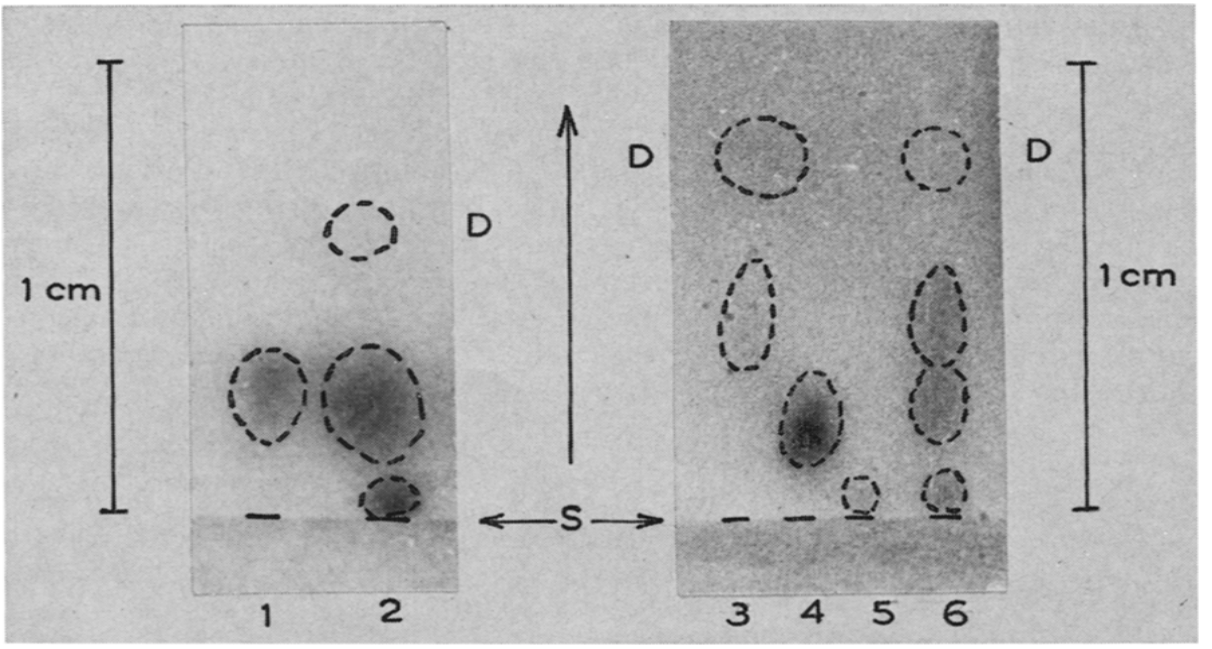

Fig. 2. Membrane chromatography of erythrocytes and blue dextran on HEMA. 1 = human erythrocytes; 2 = mixture of human and chicken erythrocytes and blue dextran; 3 = mixture of murine erythrocytes and blue dextran; 4 = human erythrocytes; 5 = chicken erythrocytes; 6 = mixture of human, murine and chicken erythrocytes and blue dextran (*cf.* Fig. 1.); S = Position of the start at the lower end of the membrane. D = Spots of blue dextran. The chromatogram was developed by parallel upward-development for 5 min in PBS, pH 7.5. The membrane was prepared with 2% cross-linking agent and 70% water (see text).

rate of migration increases. This fact indicates that the mechanism of separation is based on direct sieving, similar to that observed with proteins on wedge-compressed nitrocellulose membranes[11]. It should be emphasized that, even though there is a high homogeneity of particle size and shape in each erythrocyte population, there

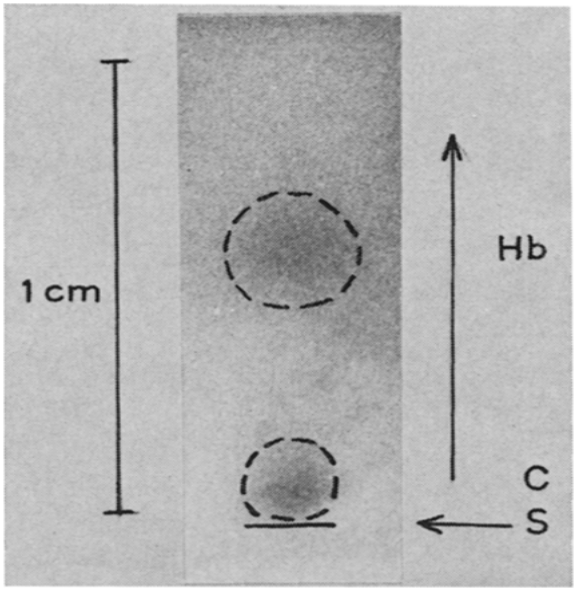

Fig. 3. Chromatography of erythrocytes and free hemoglobin. C = chicken erythrocytes; Hb = human hemoglobin; S = start. The chromatogram was developed for 5 min in PBS, pH 7.5 and the same membrane was used as in Fig. 2.

exists a certain (about 10%) deviation in their size and disk-like shape from the mean. This is probably one of the reasons why the spots of erythrocytes tended to elongate towards the front. Another reason might be a certain dispersion of the pore size in the HEMA carrier. In contrast to that, the spots of freely migrating blue dextran (mol. wt. $2 \cdot 10^6$) and of even lower-molecular-weight substances *e.g.* hemoglobin, showed no tailing (*cf.* Fig. 3). Hemoglobin and blue dextran had the same rate of migration here.

The separation of large particles in HEMA membranes depends evidently on the porosity and pore size of the sponge-like sieve. This was observed in orientation experiments with different batches of "tougher" and "looser" HEMA polymers. With the tougher polymers the cells did not enter the membrane at all (however, low molecular substances, *e.g.* hemoglobin, copper sulphate, sodium nitroprusside etc., passed through in well defined zones), while the looser HEMA caused greater diffusion of the spots. In HEMA prepared with 2% of cross-linking agent and 70% water a fair separation of the four components under study was achieved (Fig. 2), whereas in HEMA prepared with 1% cross-linking agent and 70% water there was practically no separation of blue dextran and murine erythrocytes, while chicken and human erythrocytes were well separated.

The pulsating upward development seems to be favorable for the microchromatography of cells, because it helps to eliminate the unwanted effects of particle sedimentation and the mechanical blocking of the pores. Overcoming this obstacle is generally a serious problem in the separation of larger particles in foam-like carriers[12].

Further experiments dealing with the separation of various cells on different types of HEMA carriers will be reported later.

The authors are grateful to Dr. E. HERMANOVÁ and Mrs. V. ŘEŠÁTKOVÁ for photographic documentation.

*Institute of Hematology and Blood Transfusion**, *Prague (Czechoslovakia)*
T. I. PŘISTOUPIL
M. KRAMLOVÁ

Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague (Czechoslovakia)
M. KUBÍN
P. ŠPAČEK

1 K. SHORTMAN, *Aust. J. Exp. Biol. Med. Sci.*, 44 (1966) 271.
2 P. PLOTZ AND N. TALAL, *J. Immunol.*, 99 (1967) 1236.
3 H. WIGZELL AND O. MÄKELÄ, *J. Exp. Med.*, 132 (1970) 110.
4 T. I. PŘISTOUPIL, V. FRIČOVÁ, A. HRUBÁ AND M. KRAMLOVÁ, *J. Chromatogr.*, 67 (1972) 63.
5 T. I. PŘISTOUPIL, *Chromatogr. Rev.*, 12 (1970) 109.
6 O. WICHTERLE AND D. LÍM, Pat. ČSSR No. 91918.
7 J. KOPEČEK, J. JOKL AND D. LÍM, *J. Polym. Sci. Part C*, 16 (1968) 3877.
8 M. KUBÍN, P. ŠPAČEK AND R. CHROMEČEK, *Collect. Czech. Chem. Commun.*, 32 (1967) 3881.
9 P. ŠPAČEK, *Thesis*, Czechoslovak Academy of Sciences, Prague, 1965.
10 T. I. PŘISTOUPIL AND M. KRAMLOVÁ, *Experientia*, 26 (1970) 1045.
11 T. I. PŘISTOUPIL, *J. Chromatogr.*, 28 (1967) 89.
12 W. H. EVANS, M. G. MAGE AND E. A. PETERSON, *J. Immunol.*, 102 (1969) 899.

Received January 29th, 1972

* Director: Prof. J. HOŘEJŠÍ, MD., Dr. Sc.