Journal of Chromatography, 469 (1989) 396–398
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 368

Note

Simple method for the preparation of spherical agarose and composite gel particles

ELENA PRESECAN
Institute of Hygiene and Public Health, R-3400 Cluj-Napoca (Romania)
HOREA PORUMB
Department of Biophysics, Medical and Pharmaceutical Institute, R-3400 Cluj-Napoca (Romania)
and
IOAN LASCU*
Department of Biochemistry, Medical and Pharmaceutical Institute, 6 Pasteur Street, R-3400 Cluj-Napoca (Romania)
(First received May 26th, 1988; revised manuscript received January 31st, 1989)

Agarose gel in spherical form is used extensively in chromatography and as a carrier for biocatalysts. The methods for obtaining agarose beads are based either on phase separation[1] or on gelation of an agarose solution after dispersion in an "oil" phase[2] or by spraying it in diethyl ether[3]. The last two methods are particularly attractive for the preparation of "tailor-made" agarose beads and composite beads on a laboratory scale, not only for determining the performance of new derivatives, but also for the preparation of moderate amounts (a few litres) of commercially available but expensive chromatographic materials. We describe here a modification of the latter technique; instead of applying pressure to force the agarose solution through a needle, we used an electrical paint blower for the generation of the agarose droplets.

EXPERIMENTAL

A 5% agarose solution was obtained by swelling the agarose overnight and then autoclaving it for 10 min at 120°C. This procedure is particularly useful for gel concentrations above 6%, when simple addition of agarose to boiling water does not suffice. For the preparation of composite gels, the hot agarose solution (200 ml) was mixed with either 30 g of wet hydroxyapatite (equivalent to about 10 g of dry material, prepared as described by Hirano *et al.*[4]), 15 g of activated carbon or 15 g of magnetite.

The set-up for producing the particles is shown in Fig. 1. The upper diethyl ether layer cools the agarose droplets before they reach and become accumulated into the aqueous layer at the bottom. To avoid gelation of the agarose solution in the blower, it was pre-heated by running hot distilled water through the blower before starting the experiment. The nozzle of the blower was fitted to the neck of the glass vessel by using a flexible rubber collar. During operation, the blower was contin-

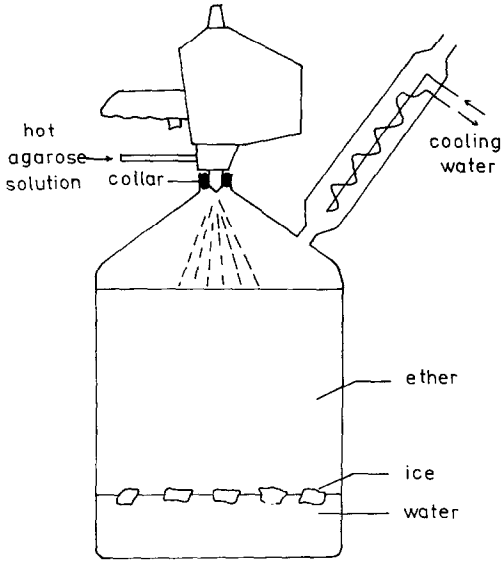

Fig. 1. Set-up for producing agarose beads using a paint blower.

uously moved from one side to another in order to avoid clumping of particles. In spite of this, the agarose beads tended to form a layer at the diethyl ether–water interface, which was dispersed by vigorously rotating the whole vessel after spraying about 50 ml of solution. Other mixing methods (magnetic stirring, bubbling of air) were found to be less efficient. The water phase was replaced after spraying about 300 ml of agarose solution. Finally, the beads were sorted according to size by passing them under a water jet through successive sieves of decreasing mesh size.

RESULTS AND DISCUSSION

With respect to the method described by Bengtsson and Philipson[3] we made two improvements. The first was to use a closed container where the cooling with diethyl ether occurs. This ensures the safety of the procedure. Diethyl ether is highly flammable but it cannot be replaced with other organic solvent as it is convenient to have both a boiling point below or about the same as the gelation temperature of the agarose solution and a density lower than that of water. The second improvement was the use of a paint blower for generating the agarose droplets. The blower develops a considerable pressure, and therefore viscous solutions and thick suspensions can be conveniently handled. Professional paint blowers are inexpensive and offer the facility for varying the pressure, which makes it possible to obtain particles of predetermined size. In the experiments reported, the diameters of the particles obtained from a 5% agarose solution ranged between 50 and 400 μm. The addition of hydroxyapatite, activated carbon or magnetite did not have any significant effect on the size of the particles. The bead size distribution did not seem to be related to the agarose concentration or to be affected by the balancing of the blower, although a thorough investigation was not carried out. Beads cross-linked as described previous-

ly[5] and liganded to Cibacron red 3B-P were successfully used in the purification of nucleoside diphosphate kinase form human erythrocytes (to be reported elsewhere).

The composite gels have not been tested yet. It is believed that the same set-up, with appropiate gelling solutions, could be useful for the preparation of spherical particles made of agarose–alginate, cellulose and other polymers, and of other composite gels.

REFERENCES

1 H. Pertoft and A. Hallén, *J. Chromatogr.*, 128 (1976) 125–131.
2 S. Hjertén, *Biochim. Biophys. Acta*, 79 (1964) 393–398.
3 S. Bengtsson and L. Philipson, *Biochim. Biophys. Acta*, 79 (1964) 399–404.
4 H. Hirano, T. Nishimura and T. Iwamura, *Anal. Biochem.*, 150 (1985) 228–232.
5 T. Porumb, H. Porumb, I. Lascu and I. Proinov, *J. Chromatogr.*, 319 (1985) 218–221.