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## Gel chromatography column packings as observed with the scanning electron microscope

During recent years, some work has been carried out in our laboratory on the separation of eye lens proteins using gel chromatography and polyacrylamide gel electrophoresis[1]. A new apparatus for the analytical applications of the latter technique has been developed[2].

In the course of this work we wished to examine the surface structure of the Sephadex particles used for the protein separations. Transmission electron microscope investigations were not very successful, as the difference between the polymeric part of the Sephadex and the embedding material is difficult to distinguish in this apparatus using thin sections.

The scanning electron microscope (SEM) is a powerful instrument for the observation of the surface structure of various materials. Some applications of this relatively new technique to the study of gas chromatographic supports and column packings have been published[3–6], and we compared the surface structure of Porapak Q and Sephadex beads[6].

This paper describes the results of examinations of the surface structure of Bio-Gel P-2 and dry and swollen Sephadex particles using this technique.

*Experimental*

A few particles of Sephadex or Bio-Gel were stuck on a small plate, which can be placed in the specimen chamber of the SEM (Stereoscan, Cambridge). A thin layer (200–300 Å) of gold is evaporated *in vacuo* on to the surface of the material, while the sample is rotated to ensure a good overall coating. This metal coating is necessary to dissipate heat and electric charge induced by the primary electron beam. The samples were observed using 3–20 kV high tension values.

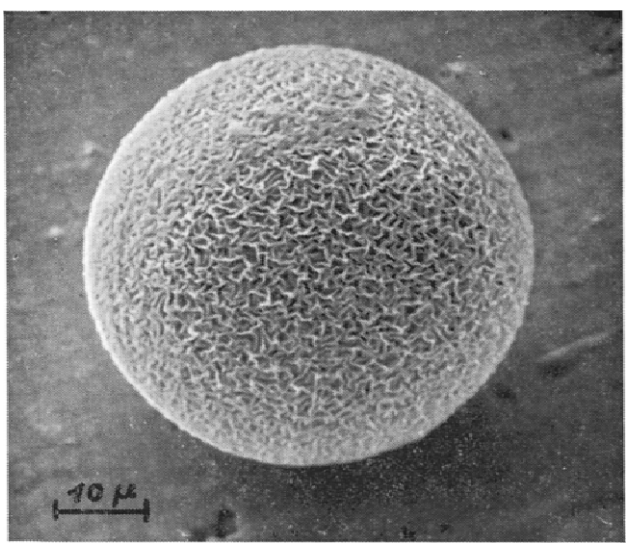


Fig. 1. SEM micrograph of a dry Sephadex G-100 particle.

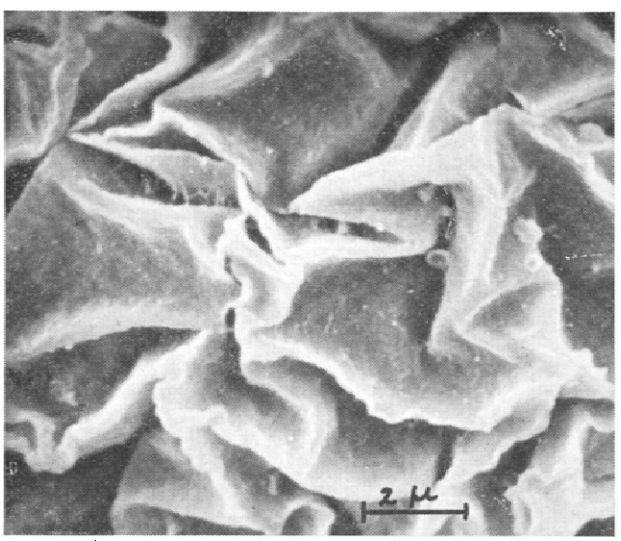


Fig. 2. Detail from Fig. 1.

Swollen Sephadex beads were put on a filter paper to absorb the excess of water, and an impression was obtained using Xantopren (Bayer), a material which is used in dental techniques. Positive replicas were made from the moulding with Technovit (Kulzer & Co), and coated with gold as described for final examination.

*Results and discussion*

Figs. 1 and 2 illustrate the surface structure of a dry particle of Sephadex G-100. This peculiar shrunken shape has been found for the different G-types of Sephadex. Unfortunately, even the best resolution conditions of the SEM did not reveal the small capillary holes in this material.

The surface structure of dry Bio-Gel P-2 polyacrylamide beads is totally different (Figs. 3 and 4). The surfaces are much smoother, and it has been observed[6,7] that these beads tend to form conglomerates of several units, while the grains seem to be fused at the contact points. The latter fact results in the occurrence of Bio-Gel units of different size and geometry, at least in the dry state.

The bead dimensions of these two materials vary considerably (Figs. 3 and 5). Micrographs of Sephadex G-100 were made on a sample of 40–120 $\mu$ diameter beads (as stated on the lot label). It can be seen from Fig. 5 that a large number of particles are smaller than the lowest size indicated by the manufacturer. On the other hand, there were very few particles of 120 $\mu$ diameter present. Sieving commercially available Sephadex is recommended in order to obtain a fraction with a narrower range of particle sizes. The quality of a gel column packed with a narrower range of particle sizes of Sephadex is markedly better as compared with a column made with the same non-screened material.

It was expected that the surface structure and bead dimensions of swollen Sephadex particles would be different from that of a dry sample. However, a wet specimen can not be observed in an electron microscope. Attempts were made to make replicas of the surface of swollen Sephadex G-100 particles. Fig. 6 illustrates the results we obtained in this way. It can be seen that the shrunken topography has disappeared. The surface structure of swollen beads is not so smooth as would be

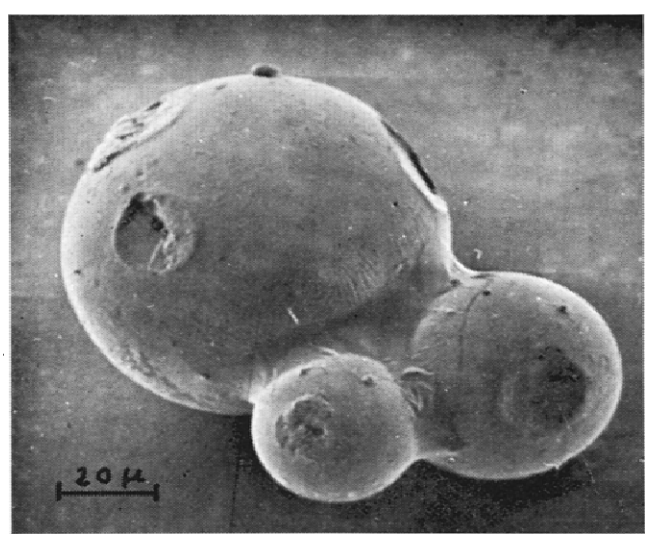


Fig. 3. Dry Bio-Gel P-2 unit.

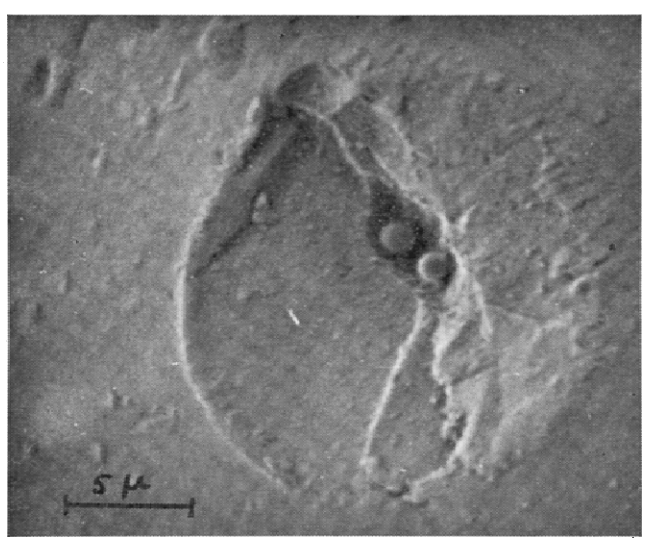


Fig. 4. Detail from Fig. 3.

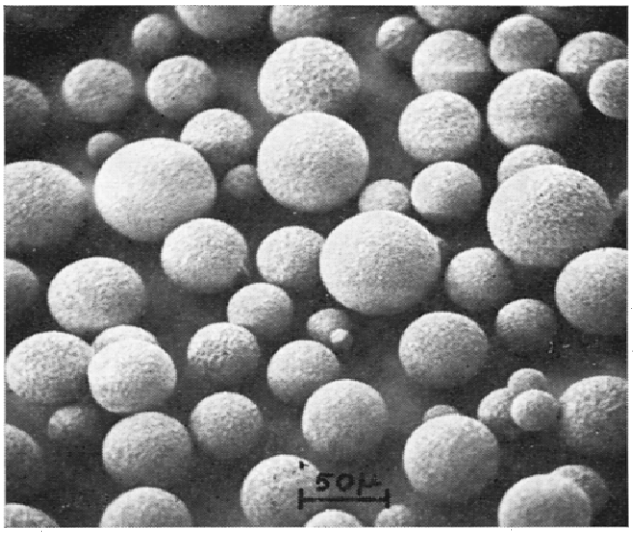


Fig. 5. Size distribution of a commercially available Sephadex G-100 sample.

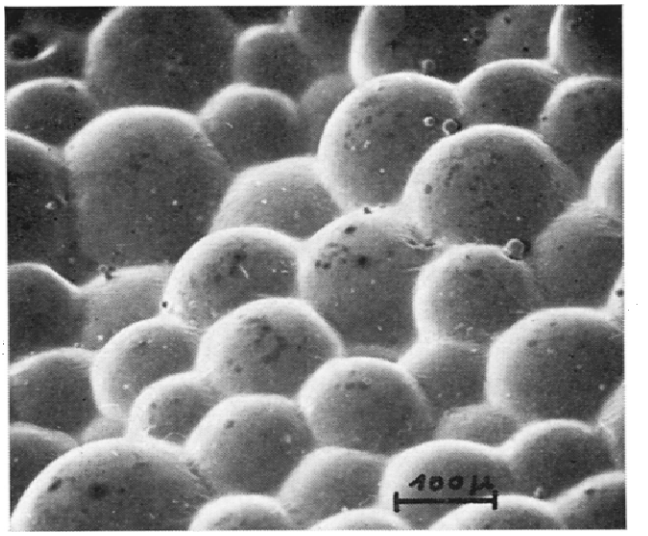


Fig. 6. SEM micrograph of swollen Sephadex G-100 replica.

expected, but this might partly be due to the particular circumstances of making replicas of the wet sample. These pictures also illustrate that the mean diameter of swollen Sephadex G-100 particles is about two times the diameter of dry ones, so that an eightfold increase in bed volume results from the water regain. This fact agrees with the accepted value of volume increase for this type of Sephadex.

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