Homogeneity of Ion Distribution in, and Macromolecular Exclusion from Sephadex® G-25 Beads

The more tightly cross-linked dextran (Sephadex®) gels apparently exclude large molecules and exhibit selectivity to both penetrant small neutral molecules[1–3] and ions[4–10].

The distribution of solutes within the hydrated, or solvated gel beads is of considerable theoretical and practical interest. Theoretical analysis of solute behaviour is usually made with the assumption of homogeneity of distribution within the gel phase[2,11] and would be much more complicated otherwise. The intraparticulate distribution is also important in the kinetics of transfer processes to and from the gel, and hence in chromatographic operation[12].

Specific ion concentrations can be determined in very small volumes by spectrometry of the X-ray fluorescence excited by the electron beam of the scanning electron microscope (SEM)[13]. In this way, the concentration profile of an ion across an object may be determined; and this communication reports preliminary results of radial distribution analyses on freeze-dried sections of Sephadex® G-25 beads. The behaviour of a fluorescent macromolecule (dextran) is also discussed.

Methods. Beads of Sephadex® G-25 (the dry bead diameter range was about 80–120 μm) were immersed in 0.2 *M* solutions of LiCl, NaCl, KCl, CsCl, NaBr and NaI for not less than 24 h. Most of the interstitital solution was then removed by centrifugation at about 600 *g* for 5 min in a tube closed at the bottom with a 40 μm mesh nylon net[14]. The adherent wet gel beads were then frozen at −20 °C in a cryostat where they were cut into 10μm thick sections. The sections have a loose texture and must be supported during subsequent drying. The frozen bead mass was therefore first cut to obtain a flat surface, to which was stuck a short strip of tape with adhesive on both sides (Type 665, 3M, Minnesota, USA). The block was then cut under the tape, so producing a frozen section attached to the tape[15]. This was then dried at −20 °C over silica gel for about 24 h before mounting on a brass specimen holder for the SEM. To obtain better thermal and electrical contact, the edges of the tape and adjacent parts of the specimen holder were painted with a colloidal silver preparation (Dag® 1415, Acheson Colloiden NV, Scheemda, Holland). An Aluminium foil was also tested as a support, using the colloidal silver preparation as adhesive. Gold-palladium was then sublimed in vacuo on to the freeze dried sections before examination in a Scanning electron microscope (Jeolco JSM-U3). The X-ray emission at the required energy levels was measured with an energy dispersive X-ray analyser (XS-165, Nuclear Diodes, Prairie View, Illinois, USA) and a multichannel analyser (Edax 707, Nuclear Diodes).

Gel beads were also allowed to swell in a 2% aqueous solution of fluoresceinyl thiocarbamoyl dextran ($\overline{M}_w$ = 208,000). The beads were sectioned as above except that the frozen section was cut without the tape support, transferred immediately to a quartz microscope slide and then dried at −20 °C. The fluorescence of the dried section was then photographed under UV-excitation.

Results and discussion. The surfaces of the sections appeared to be corrugated (Figure 1, a), having a similar fold pattern to the surface of a dry bead[16]. This fold pattern is most probably caused by shrinkage during drying.

The thickness of the sections of the frozen water-swollen beads was 10 μm. There is about a five-fold decrease in volume on drying. The dry section thickness thus will be about 5 μm.

It was often not possible to distinguish an abrupt edge at the circumference of the SEM image of a gel particle; this probably depended on the angle between the section

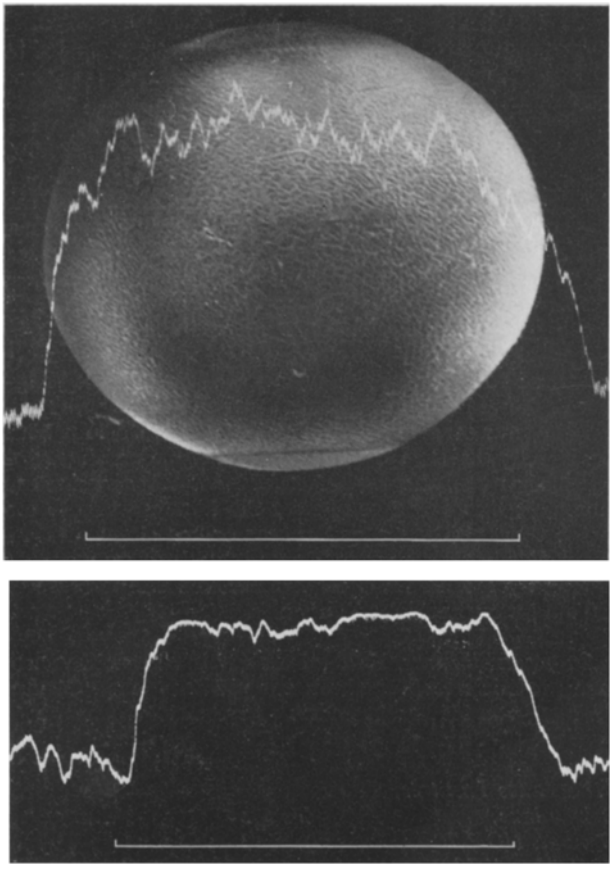

Fig. 1. a) Scanning electron microscope view of a dried frozen section originally 10 μm thick from a bead of Sephadex® G-25 showing the surface fold pattern. Superimposed on the SEM image of the section is a line scan of the ClK α-peak. The gel bead had been immersed previously in 0.2 *M* KCl for about 24 h. The white scale line corresponds to 100 μm. b) Line scan of the Cl K α-peak from another section of a G-25 bead pretreated as in (a). The white scale line corresponds to 50 μm.

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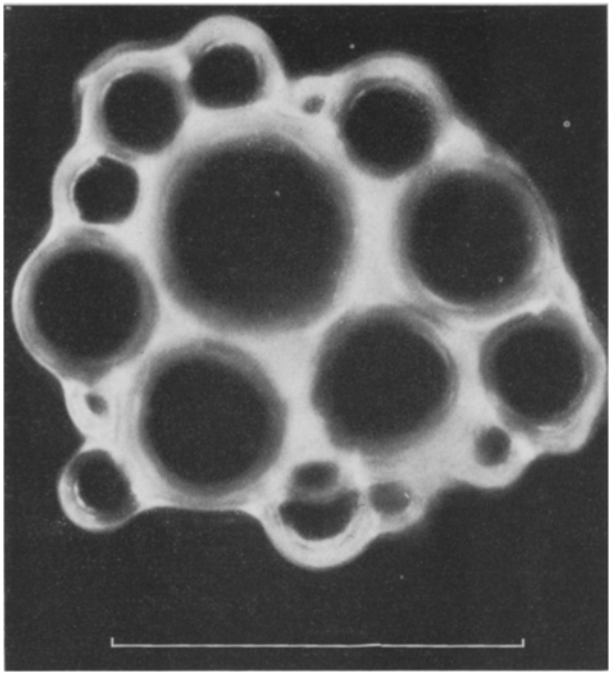

Fig. 2. Dried frozen section originally 10 μm thick of a cluster of Sephadex® G-25 beads which had been immersed previously in an aqueous fluorescent dextran solution. The fluorescent material is excluded from the gel particles. Note fluorescence around outer border of cluster. The white scale line correponds to 200 μm.

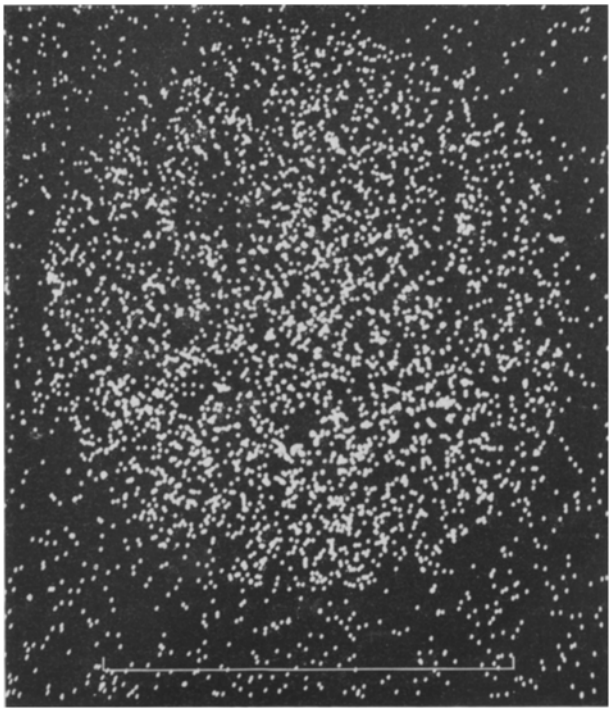

Fig. 3. X-ray picture of a dried frozen section originally 10 μm thick of Sephadex® G-25; the beads were pretreated in 0.2 *M* NaI. The points represent counts in the IL α-peak. The white scale line corresponds to 100 μm.

plane and the surface of the bead. Most, if not all of the surfaces where edges could not be identified were, however, almost certainly sectioned. The folding pattern on the surface of a bead of Sephadex® G-25 exhibits obvious changes as the observing angle varies, due to the curvature of the bead. Such a phenomenon was not observed on the sectioned material.

Indirect evidence that the bead surfaces were sectioned was provided by the distribution of the fluorescent dextran. When wet beads are centrifuged some interstitial water remains in clusters of beads. A section of such a cluster is shown in Figure 2, where it is evident that a thin layer of fluorescent material surrounds the whole cluster. This suggests that each bead is also surrounded by fluorescent material, in which case fluorescence should occur over both the gel and interstitial areas of the preparation if it consisted of whole, unsectioned gel particles. Fluorescence is, however, very largely if not entirely confined, to the interstitial region, as would be expected from sections and also from the exclusion theory[1].

Excellent linearity between X-ray emission and ionic concentrations has been reported by BÄCKMAN and LEHTINEN[17]. Line scans across the diameters of sections indicated that for all salts studied the ion distribution was probably homogeneous within the limits of resolution at the magnification used. A typical result of a line scan is shown in Figure 1, where the Cl Kα-line of KCl is super-imposed on an ordinary SEM image of the section. A similar Cl scan is shown in Figure 1b. These profiles are almost certainly more rectangular than is apparent, the slopes at the edges being due mainly to an unavoidable disparity between a too fast scanning speed and the long time constant of the rate meter used to minimize fluctuations in the counting rate along the scan; local overheating of the specimen precluded an optimally low scanning speed.

Figure 3 is an X-ray picture of a section of a bead exposed to NaI. Each point represents an I Lα photon and the picture was obtained by line scans covering the whole section. The SEM image is not shown but was well delineated by the spot density contour.

While the resolution of the SEM using the secondary electrons is about 200 Å, that of the X-ray fluorescence is poorer due to its larger generation volume. The latter is difficult to assess but is likely to correspond to about 1 μm in the plane of the section. X-rays were presumably generated from the whole thickness of the section, since a Si Kα-peak from the mounting tape was present in the line scan over the section.

The high efficiency of the energy dispersive X-ray analyser makes it possible to detect X-ray fluorescence excited by the relatively weak electron beam of the SEM[18]. The thin beryllium entrance window absorbs the weaker X-rays, however, setting a lower atomic number detection limit above Li, which thus could not be determined. Considerable improvement in the solid detecting angle would be obtained with an anylser placed nearer than 90 mm, which was the fixed distance used here.

With thick preparations[18], such as here, where electron absorption occurs predominantly near the surface of a material with poor electrical and thermal conductivity, the surface must be covered with a conducting layer. Carbon was used in the first tests but heat dissipation was inadequate; at scanning rates sufficiently slow to give adequate stability of the counting rate, local heating

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was too great, fissures appearing along the scanning line. There was considerable improvement with gold-palladium, although at the slowest scanning speeds there was still evidence of local heating with higher accelerating voltages. Mounting the section on aluminium foil with the silver adhesive gave further improvement in this respect. An Ag(Lα) peak at 3,0 keV was, however, evident and this overlapped to some extent the neighbouring KKα-peak at 3,3 keV; the ClKα-peak at 2,6 keV was, however, unaffected.

Considerable improvement in the peak to background bremsstrahlung ratio should be obtained if a lighter element such as aluminium or beryllium were used for covering.

The accelerating voltage was usually 15 kV with a beam current of 1 nA. The electron absorption was not apparently affected significantly by surface irregularities (Figure 1a) since the absorbed electron current was essentially constant during a line scan.

With a resolution limit of about 1 μm, the method can only give information about grosser heterogeneities in the gel structure. It is essential for the validity of the method that ions do not migrate to any significant extent during preparation of the samples since this would presumably tend to even out any heterogeneities. Freezing effects will be studied by using much lower temperature. It seems unlikely that cutting will disturb solutes significantly, since this procedure appears satisfactory for producing biological samples suitable for autoradiography where small displacements would be easily evident. Although the results so far obtained can be improved considerably, they do not suggest the presence of any major radial ionic heterogeneities.

Little seems to be known about the microscopic properties of beads of gels or ion exchange resins, although heterogeneities have been reported[19]. The gel Sephadex® G-25, although fairly highly cross-linked, is not the most ion selective of this series and studies on the more selective gels G-15 and G-10 are planned. The use of freeze-dried gel sections is also being extended to autoradiographic distributional studies of radioisotope labelled nonelectrolytes[20].

Zusammenfassung. In gefriergetrockneten Schnitten von Sephadex G-25 wurde die Verteilung einiger Alkalihalogenverbindungen mit Hilfe ihrer Röntgenstrahlenfluoreszenz im Raster-Elektronenmikroskop untersucht. Es ergab sich eine ionisch homogene radiale Verteilung und fluoreszierende Makromoleküle (Dextran) wurden von den Gelschichten ausgeschieden.

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Registrierung von Saharastaubfällen mit Hilfe einer Burkard Pollen- und Sporenfalle

Das Niederfallen von Saharastaub in Europa ist, wie bekannt, oft beschrieben worden[1]. Über weite Gebiete von Süd- und Mitteleuropa erstreckten sich intensive Staubfälle in den Jahren 1901[2,3] und 1902[3]. Weniger ausgeprägte Niedergänge von Saharastaub wurden beispielsweise 1936 und 1937 in Arosa[4], ferner 1926 und 1930 in Barcelona[5] beobachtet. 1962 gab es Saharastaubfälle in Freiburg i/Breisgau, resp. in Höhenkurorten des Schwarzwaldes[6a], an den gleichen Tagen auch in Oberitalien, in der Schweiz[6b], in Österreich und ebenso in weiten Gebieten von Frankreich bis zur Bretagne[6c]. Auch in England wurde 1968 Saharastaub festgestellt[7]. Meist wurden derartige Staubfälle nach Niederschlägen – Regen oder Schnee – registriert. Es gab dann einen Schlammregen, oder einen bräunlich, bezw. rötlich gefärbten Schnee, resp. Schmutzflecken nach dem Auftrocknen von Regentropfen auf «blanken Oberflächen»[6a].

Ein Transport von Sahara-Staub nach Süd- und Mitteleuropa, oder noch weiter nach Norden, setzt natürlich bestimmte meteorologische Konstellationen voraus: In der Sahara müssen Staubteilchen aufgewirbelt werden. Mit Südströmungen – Scirocco und einer Föhnlage – kann es dann zu einer Beförderung der Staubteilchen bis über die Alpen, und auch sonst nach Gebieten von Europa kommen. Nach Ansicht von GÖTZ (1936)[4], ferner GLAWION (1937, 1939)[4] ist der Transport von Saharastaub bis über die Alpen, resp. bis nach Mitteleuropa beim Vorliegen der erwähnten Windverhältnisse – d.h. beim Einströmen subtropischer Warmluftmassen – vielleicht häufiger als das meist angenommen wird. Beim Fehlen von Niederschlägen – vor allen in tieferen Lagen – und beim Ausbleiben absteigender Luftströmungen wäre Staub dieser Art in geringeren Konzentrationen nicht nachweisbar. Die Sedimentation derartiger Staubteilchen benötigt viel Zeit: Bei mittleren Dichten zwischen 2,75[6c] und 2,589[5] (oder 2,43[8]) und Grössen zwischen 5 und 1 μm würden bei Schwebehöhen von einigen hundert Metern bei ruhiger Luft Tage, bezw. Wochen, oder noch sehr viel längere Zeiträume bis zum Erreichen der Erdoberfläche vergehen. Ganz ähnlich wie manche Pollenkörner oder Pilzsporen würden sich die Staubpartikel sehr lange schwebend erhalten, oder leicht wieder aufgewirbelt oder

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