MICROSCOPIC OBSERVATIONS ON COMMERCIAL SEPHAROSE: DEVIATIONS FROM NORMAL BEAD-STRUCTURE

T.C.J. GRIBNAU, C. STUMM*, G.I. TESSER

*Department of Organic Chemistry and *Department of Microbiology*
Catholic University, Toernooiveld, Nijmegen, The Netherlands

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1. Introduction

The limitations of the cyanogen bromide activation as a method for the covalent attachment of amine ligands to polysaccharides (e.g. agarose-beads, Sepharose®) gave rise to the development of alternative procedures [1–3]. In the various stages of the coupling reactions conservation of the bead form of the matrix was examined microscopically. The observed phenomena were reasons for further microscopic investigations into different lots of blank Sepharose. A detailed description of matrix structure deviations is given here.

2. Materials and methods

Sepharose-2B(lot no.: 7641), Sepharose-4B(lot no 2684, 8820, 2184, 8061, 3373, 3861, 5448), Sepharose-6B(lot no.: 7739); CNBr-activated Sepharose-4B-(batch no.: 3581) and Con A-Sepharose-4B(lot no.: 2572) were purchased from Pharmacia Fine Chemicals.

Tests for bacterial contamination were performed by means of routine plating and staining techniques at the Medical Microbiology Department.

Bright field, phase contrast and Nomarski interference contrast photomicrographs were taken with the aid of a Leitz Ortholux microscope on 35 mm Agfapan 25 Professional film.

3. Results

The first sample of Sepharose-4B(lot no.: 2684) examined microscopically, showed at bright field and

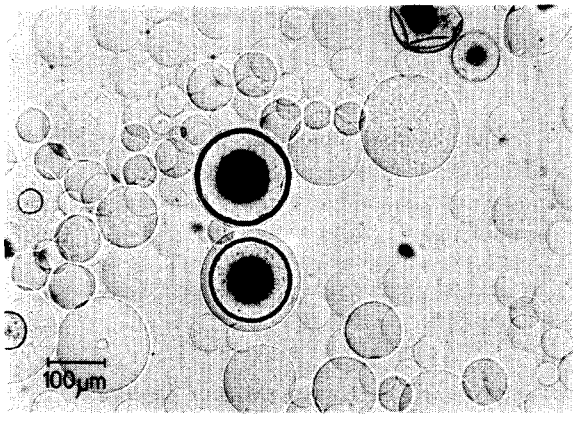

Fig.1. Blank Sepharose-4B, lot no.: 2684. Bright field.

with moderate magnification the following structures (fig.1):

- normal, clear beads
- beads with one or more small vacuoles; the latter are optically empty
- beads with one or more bigger vacuoles, containing strongly refractive, mobile, non-mobile particles of varying shape and magnitude
- collapsed beads

Other lots of varying dates, including samples of Sepharose-2B and -6B, were examined in order to get an impression of the frequency of these phenomena. All of them showed to a more or less extent, the same results, with the exception of lot numbers 5448 and 7641 which contained only few beads with vacuoles.

Two commercial Sepharose-4B derivatives were also investigated microscopically:

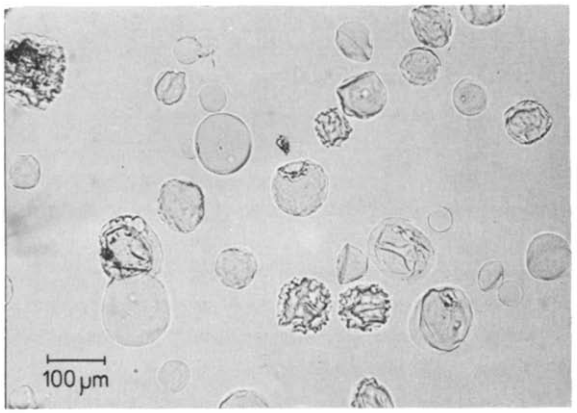

Fig.2. CNBr-activated Sepharose-4B, batch no.: 3581, after reswelling. Bright field.

– CNBr-activated Sepharose-4B(batch no.: 3581; freeze-dried) was reswollen and photomicrographs were taken. It was evident that the spherical structure of the beads had not been reconstituted(fig.2). The same strongly refractive particles were present but they were not mobile (fig.3). Obviously they are fixed to the agarose matrix. In order to test the effect of the CNBr-activation alone, a sample of freshly activated Sepharose-4B was also examined microscopically. In comparison with blank Sepharose-4B of the same lot, no change of mobility of the particles could be detected.

– Con A-Sepharose-4B(lot no.: 2572) showed again

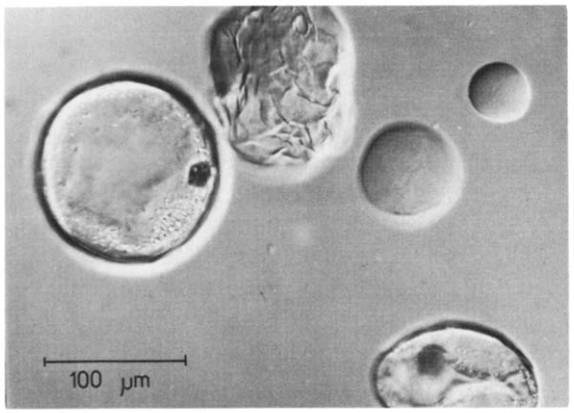

Fig.3. CNBr-activated Sepharose-4B, batch no.: 3581, after reswelling. Nomarski interference contrast.

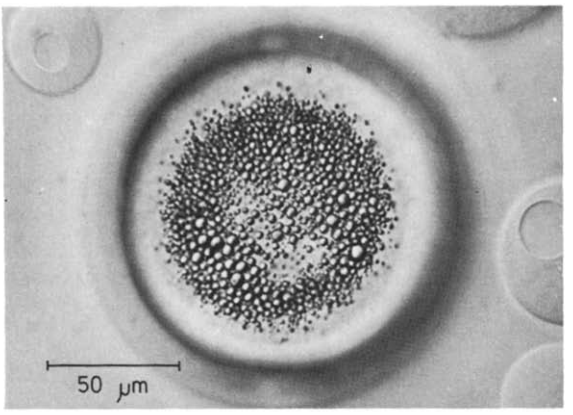

Fig.4. Agarose globule (Sepharose-4B, lot no.: 2684) filled with fluid and numerous particles; at the left and right hand side other beads with vacuoles only. Nomarski interference contrast.

the same vacuolated beads and collapsed beads. The refractive particles were less mobile.

Routine plating and staining methods, executed to check upon bacterial contamination, showed in some cases the presence of Gram negative bacteria and indifferent *Streptococci*.

For a more detailed observation of the irregular structures, more advanced microscopic techniques (phase contrast and Nomarski interference contrast) were applied to samples of the representative lot no.: 2684.

The refractive particles show a higher specific gravity than the surrounding fluid since in turning beads they tend to remain at the 'bottom' of the vacuole. When the vacuolated beads were flattened by pressure of the coverglass, the particles frequently are arranged at the periphery of the vacuole. This effect is caused by the collapsing wall. More pressure may finally result in rupture of the beads. The diameter of the particles varies between 0.5 and 5 µm (fig.4). They are non-motile but show Brownian movement.

In the same lot of Sepharose-4B bacteria could be detected microscopically as typical shorter or longer rods adhering to the outside of the beads or floating in the fluid of a vacuole (fig.5). In the latter case the rods are spread throughout the whole lumen of the vacuole. The number of bacteria-filled vacuolated beads was far less than that of vacuolated beads filled with particles.

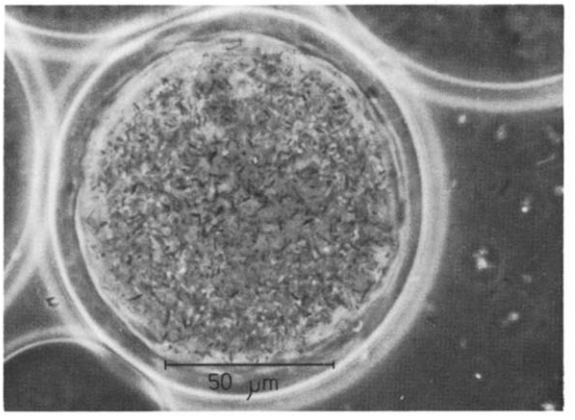

Fig.5. Agarose globule (Sepharose-4B, lot no.: 2684) filled with fluid and bacteria. Phase contrast.

4. Discussion

Until recently microscopic data on beaded agarose (Sepharose®) were rather scarce in the literature. Hjertén [4] illustrated his standard procedure for the preparation of agarose spheres with a photomicrograph to show the spherical shape of the grains. A similar photograph has been published by Pharmacia Fine Chemicals [5]. Neither of them show many details. Capel [6] published fluorescence patterns and electron microscopical pictures of IgG_1-Sepharose beads after successive incubations with rabbit anti-human IgG serum and fluorescein isothiocyanate labelled horse anti-rabbit Ig serum, or peroxidase labelled horse anti-rabbit Ig serum. David et al. [7] published radioautographs of Sepharose-4B–protein conjugates. Excellent scanning electron photomicrographs, showing Sepharose-4B beads before and after incubation with an agarose degrading bacterium, have been published by Malmqvist and Hofsten [8]. None of these authors mentioned the here described deviations from the normal bead structure. Recently we indicated some strange phenomena in commercial Sepharose-4B [9]. Neame et al. [10,11] reported observations, some of which are consistent with our microscopical findings, published in full detail here. There are, however, differences in details and interpretation. In the lots described here the diameter of the refractive particles obviously varies continuously, whereas Neame et al. [10,11] described two classes of much smaller size (0.2 and 0.4 µm). We were not able to observe active motility of the particles. Their mobility is not influenced by CNBr-activation. In contrast to bacteria and yeast cells the particles show a relative high specific gravity.

The results of the described investigations make it rather unlikely that the particulate inclusions are bacteria or yeasts. The origin of the vacuoles and the particles deserves further investigation, especially in connection with the importance of Sepharose for affinity chromatography. The structural deviations may influence the chromatographic properties of this support material. Diffusion of ligand-solution from the vacuolated beads could also be a reason for leakage and for the necessity of prolonged washing procedures.

Acknowledgements

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