Journal of Chromatography, 238 (1982) 377–383
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,623

MICROSCOPIC ANALYSIS OF FOUR COMMERCIAL COLUMN PACKINGS UNCOATED AND COATED WITH A THIN ALGINATE ESTER FILM

J. A. BARNES*, S. A. BARKER and J. F. KENNEDY
Department of Chemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT (Great Britain)
(First received June 12th, 1981; revised manuscript received November 30th, 1981)

SUMMARY

The structures of four commercial column supports, uncoated and coated with an alginate ester film, have been examined with plain and polarized light microscopy. Some potential advantages of this simple technique for the preparation and use of this water permeable but water insoluble film are assessed.

INTRODUCTION

The efficiency of many solid supports employed in adsorption chromatography is limited by various structural defects in the supports. Some column parameters (*e.g.* flow-rate, exclusion limit and non-specific retention) are influenced by the shape and porosity of polymeric matrices. In addition, many anomalous chromatographic results can be attributed to the structure of the column matrix[1,2]. Nevertheless, microscopic data on many commercial column packings are scant.

The procedure for preparing agarose beads was first reported by Hjertén[3] who provided photomicrographs illustrating the spherical shape of the beads. Neame *et al.*[4] and Gribnau *et al.*[5] reported the presence of vacuoles of various sizes in Sepharose beads. Neame *et al.*[4] described the presence of a large number of highly motile, refractive particles in some of the vacuoles. However, these vacuolar inclusions appeared to be non-motile in the freeze-dried cyanogen bromide-activated Sepharose 4B[4,5]. Some of the vacuoles were shown to be filled with bacteria[4–6]. The difficulty in reconstituting the spherical shape of re-swollen cyanogen bromide-activated Sepharose was attributed to microbial digestion of the internal structure of the beads resulting in a collapsed disc-like structure[4]. Neame *et al.*[4] also published photomicrographs illustrating severe fracture and shrinkage of the CNBr-activated beads. This resulted in major distortion in pore sizes.

In an attempt to prepare improved adsorbents for use in immunoadsorption, we found it necessary to examine the microscopic features of some commonly used

* Present Address: Department of Biochemistry, Memorial University of Newfoundland, St. John's, NF, Canada A1B 3X9

0021-9673/82/0000–0000/$02.75

column packings (*i.e.* Sepharose 6B, Spheron P100,000, Porasil C and silica gel) with the aid of plain light with a green filter and polarized light. Numerous structural aberrations were observed in all the supports examined. This inspired us to coat the solid supports with a propylene glycol alginate ester to obtain a water-insoluble but water-permeable film created by base-catalysed transesterification at 20°C[7]. With the Sepharose and Spheron coated beads, the alginate ester film cross-linked with ethylenediamine proved to be very stable at pH 11.5. However, the durability of the alginate film on the Porasil beads was rather poor. Greater mechanical, chemical and thermal stability was achieved with the cross-linked alginate film. In addition, the non-specific adsorptive properties of Sepharose and Spheron was considerably reduced[7,8].

MATERIALS AND METHODS

Propylene glycol alginate ester (Manucol E/RE, batch 41652) was obtained from Alginate Industries (London, Great Britain). Sepharose® 6B was purchased from Pharmacia (Upsala, Sweden). Spheron® P100,000 was bought from Koch Light (Colnbrook, Great Britain). Silica gel, 66–100 mesh, ethylenediamine, *n*-butyl acetate and cyanogen bromide were obtained from BDH (Poole, Great Britain) and Porasil C was purchased from Waters Assoc. (Milford, MA, U.S.A.). Photomicrographs were taken with a Leitz Wetzlar Orthomat microscope and camera on Ilford PANF ASA 50 professional film using polarized light and plain light with a green filter at 20× magnification.

Encapsulation of solid supports with propylene glycol alginate ester cross-linked with ethylenediamine

Aqueous alginate-solution (5%, w/v) was used for the optimal coating of the following supports:

Silica gel 60–120 mesh. To silica gel (4.0 g) washed with distilled water and suspended in distilled water (20 ml), aqueous ethyleneiamine solution (10 ml, 2% v/v, pH 7.0) and alginate ester solution (20 ml) were added.

Porasil C. To Porasil C beads (2.5 g) washed with distilled water and suspended in distilled water (20 ml), aqueous ethylenediamine solution (10 ml, 2% v/v) and alginate ester solution (20 ml) were added.

Spheron P100,000. To Spheron beads (3.0 ng) were added distilled water (20 ml) aqueous ethylenediamine solution (15.0 ml, 2% v/v) and alginate ester solution (20 ml).

Sepharose 6B. To an aqueous suspension of Sepharose 6B beads (20 ml) were added distilled water (10 ml), aqueous ethylenediamine solution (20 ml, 2% v/v) and alginate ester solution (40 ml).

Coating procedure

To a 500-ml quick fit round bottom flask containing *n*-butyl acetate (150 ml), the alginate ester mixture for a given support was added and the solution stirred vigorously with a vibro-mixer for 10 min before the addition of sodium carbonate solution (100 ml, 10%, v/v) to effect polymerization of the alginate film by transesterification. After settling, the butyl acetate was decanted and the remaining aqueous mixture neutralized with HCl (1 *M*). The encapsulated beads were then

washed free of butyl acetate with phosphate buffered saline (PBS 0.2 *M*, pH 7.2) by successive centrifugation at 2000 *g*. The coated Sepharose, Porasil and silica beads which sedimented freely as discrete particles were stored in PBS. The much lighter coated Spheron beads did not settle easily and had to be filtered on a No. 3 sintered Büchner funnel and dried with acetone before storing in a dry state.

RESULTS

In general, it was observed that the coated beads tended to settle as discrete particles and the smaller uncoated beads such as Spheron tended to clump together. There was also a noticeable increase in the volume of the coated beads compared with the uncoated beads. In view of the observed heterogenicity in the Sepharose bead size, an overall increase in volume is not apparent from the photomicrographs. Nevertheless, an increase in volume was noticeable in several batches of coated beads.

The results of the microscopic observations of the coated and uncoated column packings viewed with plain light and a green filter and with polarized light are illustrated in Fig. 1 and Fig. 2, respectively. With the exception of the uncoated Porasil and silica supports which were examined in the dry state all the other beads were dispersed in PBS during microscopic examination.

DISCUSSION

A comparison of the photomicrographs of the coated and uncoated beads viewed with plain light and a green filter (Fig. 1) reveal a variable bead size in the commercial Sepharose 6B specimen (Fig. 1A and B). The difference between the coated and uncoated Sepharose beads was also less apparent. Nevertheless, some beads were observed with one or more small vacuoles which were optically empty. Other beads possessed larger vacuoles with strongly refractive particles. These observations confirm the findings reported by Gribnau *et al.*[5]. The coated Spheron beads appeared mostly as discrete particles (Fig. 1C) whereas the uncoated particles were clumped (Fig. 1D). The acetone-dried coated Spheron beads were also satisfactorily reswollen.

Structural inhomogeneities similar to those observed on the coated and uncoated Sepharose beads were also noticeable on the coated and uncoated Porasil beads. Some of the Porasil beads were broken and others contained numerous small pits. Other beads had larger cavities containing strongly refracting matter. The coated Porasil beads, unlike the uncoated beads, possessed a well-defined film of alginate ester (Fig. 1E and F). The edges and faces of the coated crystalline silica gel beads were more darkly shaded than those of the uncoated beads. In both the coated and uncoated silica beads less structural inhomogeneities were observed.

In contrast, more convincing evidence of positive coating was observed with the coated beads photomicrographed with polarized light (Fig. 2). The high water content of Sepharose and other polymeric matrices used in affinity chromatography contributes to their low optical diffraction. Consequently, examination of these materials with bright field illumination is unsatisfactory. The use of polarized light afforded a significant improvement in the photomicrographs of Sepharose. A quasi

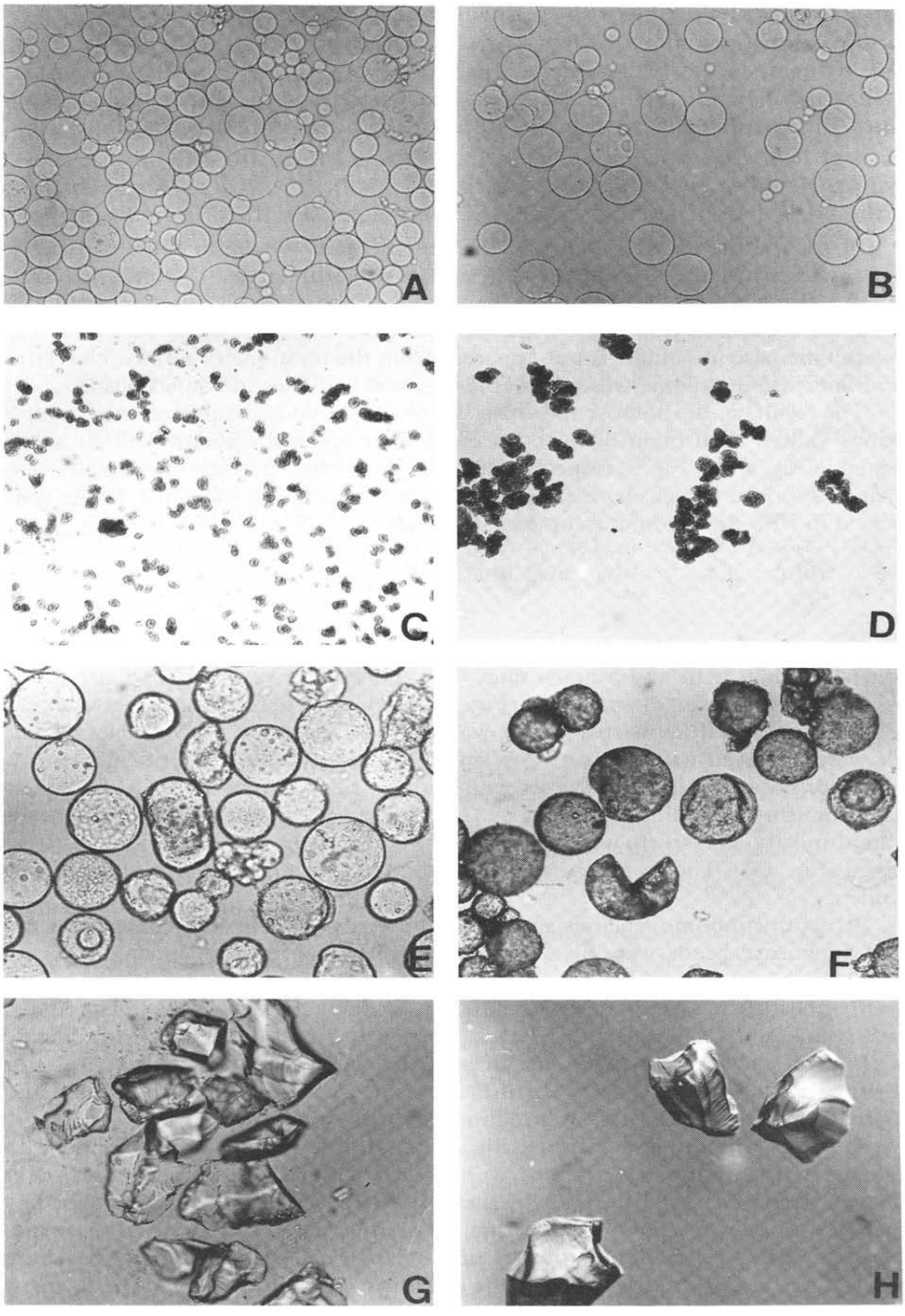

Fig. 1. Photomicrographs of coated and uncoated column packings viewed with plain light and a green filter: Sepharose 6B (A, B); Spheron P100,000 (C, D); Porasil C (E, F); and silica gel (G, H).

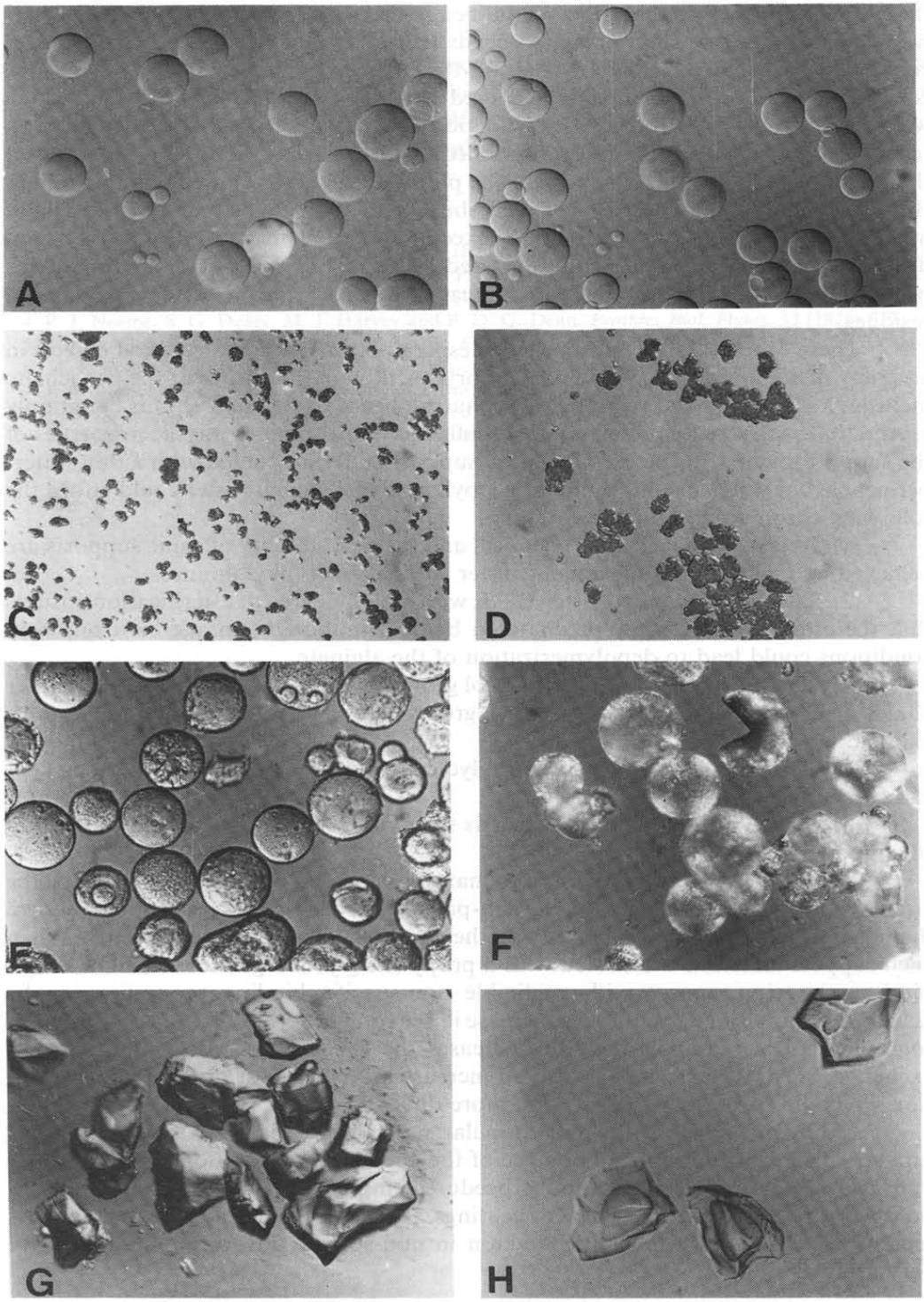

Fig. 2. Photomicrographs of coated and uncoated column packings viewed with polarized light: Sepharose 6B (A, B); Spheron P100,000 (C, D); Porasil C (E, F); and silica gel (G, H).

three-dimensional representation of the beads was obtained and the coated beads were somewhat larger than uncoated beads (Figs. 2A and B). The polarizing effect could be varied reproducibly by rotating the polarizer attached to the microscope condenser. This was dramatically observed with the uncoated Porasil beads which were found to be anisotropic. The distinction between the coated and uncoated isotropic silica gel beads was more pronounced with polarized light (Fig. 2G and H) than with plain light (Fig. 1G and H). The photomicrographs viewed with plain light (Fig. 1) highlight the contrast between the use of plain light and polarized light microscopy (Fig. 2). Moreover, with the exception of Sepharose, there is a significant difference between the coated and uncoated beads in Fig. 1. Positive coating of the beads with an alginate ester film was confirmed by the carbazole test for uronic acid residues.

These observations confirm the widespread occurrence of structural defects in a variety of commonly used column supports. Although the nature of the particulate inclusions in some of the vacuoles of Sepharose beads is disputed[4,5], it is commonly agreed that such structural defects could influence the chromatographic properties of the support materials. The use of column support materials coated with a thin water-permeable but water-insoluble film of propylene glycol alginate ester could afford the following chromatographic advantages:

(1) Improved mechanical, chemical and thermal stability of some supports are achieved by cross-linking the alginate ester with suitable alkyl amines.

(2) The use of Manucol ester E/RE with a high degree of esterification ensures that the esters are not easily precipitated by acids although storage in strong acid conditions could lead to depolymerization of the alginate.

(3) The substituent propylene glycol groups hinder the aggregation of polymer chains and ensure that fewer carboxyl groups are available for interaction with cations[9].

(4) The stability of the propylene glycol alginate film at high pH could make it less susceptible to ligand leakage.

(5) Conservation of a beaded matrix structure which enhances the preparation of supports of high binding capacity.

The structural defects common to many commercial column packings could be circumvented by the use of suitable non-porous beaded solid supports of uniform diameter which could be employed for the synthesis of efficient adsorbents. When such support materials are coated with a propylene glycol alginate ester film, a high binding capacity support with negligible non-specific binding properties can be achieved (unpublished results). An increase in the total bead surface area of a chromatographic column support material increases the capacity for adsorption and ion exchange. Nevertheless, there is also an increase in the sites for non-specific adsorption which, in turn, makes desorption more difficult.

Although agarose is the most popular support material, it exhibits significant non-specific adsorption, partly because of the chemical composition of the polymer and also because of derivatization procedures employed. With the available technology for producing stable, efficient coatings, the use of a suitable hydrophilic polymer which affords a significant reduction in non-specific adsorption should prove very useful.

ACKNOWLEDGEMENTS

The authors thank Dr. H. A. Kordan of the Department of Plant Biology at the University of Birmingham for his kind interest in the work and his generous assistance with the microscopic studies. We also thank the Endowment Fund Medical Research Committee, University of Birmingham for a research grant to J.A.B.

REFERENCES

1 C. R. Lowe and P. D. G. Dean, *FEBS Lett.*, 18 (1971) 31–34.
2 D. B. Craven, M. J. Harvey, C. R. Lowe and P. D. G. Dean, *Eur. J. Biochem.*, 41 (1974) 329–333.
3 S. Hjertén, *Biochim. Biophys. Acta*, 79 (1964) 393–398.
4 P. J. Neame, S. G. Doley, M. J. Harvey and P. D. G. Dean, *Protides Biol. Fluids*, 33 (1976) 513.
5 T. C. J. Gribnau, C. Stumm and G. I. Tesser, *FEBS Lett.*, 57 (1975) 301–303.
6 M. Malmqvist and B. V. Hofsten, *J. Gen. Microbiol.*, 87 (1975) 167–169.
7 J. A. Barnes, *Ph. D. Thesis*, University of Birmingham, Birmingham, 1980.
8 J. A. Barnes, J. F. Kennedy and S. A. Barker, *J. Solid-Phase Biochem.*, in press.
9 R. H. McDowell, *Properties of Alginates*, Alginate Industries Ltd., Lithofax, 4th ed., 1955, p. 39.