

Hydrogels from Polysaccharides. II. Beads with Cellulose Derivatives

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SYNOPSIS

The generation of hydrogels from solutions of cellulose ester derivatives of various types using an atomization device, in which the solution travels through air before phase inversion occurs in a nonsolvent, was unable to produce beads in spherical shape. Instead, irregularly shaped particles were formed. The cellulose derivatives included a series of cellulose esters with a high and low degree of substitution (DS) and with acyl substituents ranging in size from C3 to C12 (laurate). Starch was tested as a noncellulosic polysaccharide, and the list of cellulose derivatives included a fluorine containing ester. The formation of spherical hydrogel beads by this method became possible only when cellulose derivatives or starch were blended in solution with unmodified cellulose. The resulting beads had dynamic flow characteristics, for instance, linear velocity vs. pressure drop relationships, that closely followed the rule of mixing. An exception was cellulose laurate, which retained most of the strength of unblended cellulose even at 60% derivative content. The results suggest (a) that cellulose is needed for the formation of droplets with sufficiently high surface tension capable of withstanding the collision with the nonsolvent surface; (b) that cellulose derivatives require the presence of unmodified cellulose to attain sufficient gel strength; and (c) that waxy ester substituents provide the basis for hydrophobic interactions which contribute to gel strength retention. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

Hydrogels in bead form are important separation devices used in biotechnology. Spherical hydrogel beads have been produced from various polysaccharides including cellulose.¹⁻⁴ Cellulose has been demonstrated to be particularly useful for beads of large size (greater than 300 μm diameter), beads with low solids content and with high mechanical strength, without a need for crosslinking.⁵ Following functionalization by chemical modification, cellulosic hydrogel beads have proven to be useful for ion exchange and affinity chromatography. The previous manuscript in this series established the utility of cellulosic hydrogels in bead form for chromatographic purposes.⁵ Beads from cellulose were found to have excellent flow characteristics at low solids

content; they had resolution capacity that was independent of bead diameter above 300 μm ; and they exhibited flow behavior indicative of convective through-pore transport. Without activation, cellulose beads were found to have no significant binding characteristics, and this is an important feature of high-performance chromatographic support materials.

Unmodified cellulose, however, is neither useful for selective binding nor for dissolution in usual solvents.⁶ This is why cellulose derivatives, chemically modified celluloses with (active) functional groups installed prior to bead formation, would offer advantages for the formation of preactivated separations materials, with uniform distribution of active sites and from simpler solvent systems.

The commercial regeneration of cellulose in fiber or film form employs the transitory modification as xanthate, and cellulose beads have previously been produced from cellulose derivative solutions, xanthate and acetate, followed by hydrolysis or deace-

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tylation. Thus, cellulose modification prior to regeneration is common, as it prevents the use of complicated solvent systems such as *N,N*-dimethyl acetamide and lithium chloride (DMAc/LiCl) or *N*-methyl morpholine oxide (N,MMO).

It was, therefore, the objective of the current study to explore the feasibility of producing spherical hydrogel beads from cellulose derivatives, and from blends of cellulose with cellulose derivatives, using the previously adopted method of atomization in turbulent air followed by phase inversion in a non-solvent.

EXPERIMENTAL

Materials

Cellulose (CF-11, DP_n 200) was purchased from Whatman Paper Ltd.; cellulose triacetate (CTA, DS 2.8), cellulose propionate (CP, DS 2.8), and cellulose acetate-butyrate (CAB, DS 2.8) were purchased from Aldrich Chemical Company; cellulose hexanoate (CH, DS 0.9–1.1), cellulose acetate hexanoate (CAH), cellulose trifluoroethoxyacetate (CFA), cellulose acetate trifluoroethoxyacetate (CAFA), and cellulose laurate (CL, DS 1.3) were synthesized in our laboratory;⁷ starch was purchased from a local supermarket; *N,N*-dimethylacetamide (DMAc) reagent grade was obtained from Fisher Scientific; and isopropyl alcohol was purchased from Baxter Scientific Products. All materials were used without further purification.

Methods

The dissolution of cellulose was based on the procedure of McCormick and Lichatowich.⁸ In short, the cellulose sample was solvent exchanged in DMAc for at least 1 week. Excess solvent was removed by filtration and the dry weight was determined. (Cellulose esters did not require a solvent-exchange step prior to dissolution.) The cellulose dissolution process consisted of adding a weighed amount of the polysaccharide into a solution of 8–10% LiCl in DMAc at 80°C. After 1 h under continuous stirring, the heat source was removed and the mixture stirred for 10–15 h at room temperature till the dissolution was completed. With starch, an extra addition of 15 wt % water at the end of the 10–15 h stirring period was necessary for complete solubilization. Cellulose derivatives were dissolved in LiCl-free DMAc.

Blends of the polysaccharides were prepared by mixing known amounts of solutions prior to atom-

ization. The blended beads were identified according to their composition. For instance, 80% CF-11/20% CP represents the blend containing 80 wt % CF-11 and 20 wt % of CP as determined by the concentrations of the respective solutions and the volumes mixed prior to regeneration.

The beading process and pressure drop measurements were as described elsewhere.⁵

RESULTS AND DISCUSSION

Bead Generation Procedure

The optional methods for producing spherical hydrogel beads from cellulose have been summarized by Stamberg et al.⁹ These include the steps of (a) solvation of cellulose (or cellulose derivative), (b) formation of a sphere from the solution, (c) stabilization of the spherical droplet, (d) solidification of the polysaccharide solids in the droplet by phase inversion, and (e) postgeneration treatment including washing. Two generic process types are distinguished, one employing the principles of the dry jet wet (fiber) spinning process in which droplets are ejected into air prior to phase inversion in a non-solvent (ejection process) and one dispersing the cellulose solution in a solvent medium that does not mix with the cellulose-containing liquid phase (suspension process).^{10–22} The suspension process constitutes a current state of art for the commercial production of hydrogel beads on the basis of polysaccharides; however, many reports in the literature have also dealt with bead production by the ejection method.^{10–13} Whereas the suspension process favors the formation of gel particles of small size (less than 300 μm diameter) and of irregular shape, the ejection process appears to be limited by viscosity and surface tension considerations. The commonly high viscosity of all polysaccharide solutions favors the formation of fibers and complicates the formation of spherical droplets in air. However, with the emergence of new solvent systems for cellulose, and with a variety of pretreatments to reduce the cellulose molecular weight while increasing its solubility, the adoption of a so-called “two fluid annular atomizer” as described by Su et al., has become feasible.²³ Using this technique, which was described and advocated for aqueous solutions of sodium alginate, polysaccharide solutions are ejected from a capillary with the aid of a flowing (turbulent) gas which assists in the formation of spherical droplets. The atomized polysaccharide solution then travels through air before undergoing phase inversion in a nonsolvent.

While the method of Su et al. failed to produce the expected spherical droplets from cellulose solutions, apparently because of solvent evaporation and the resulting clogging and fouling of capillary tips, a modified atomization device (Fig. 1) resulted in successful bead generation. This method, which adopts the principles of the dry jet wet spinning fiber technology, directs a high velocity gas stream at the tip of the capillary such that a shearing force is applied to a protruding viscous liquid without causing the build up of solids in the spinnerette, which frequently results in clogging. The gas capillary jets impart a Venturi effect across the liquid being ejected from the capillary tip, thus drawing the cellulose solution into a continuous stream as it leaves the liquid capillary. The shear force of the gas capillary jets is responsible for uniform flow at high rate and for steady disintegration of the stream into droplets of narrow size distribution. The introduction of capillary jets, and the manner in which they are directed at the tip of the liquid capillary, form the major distinction of the apparatus shown in Figure 1 with the device of Su et al.²³ Parameters contributing to the successful formation of spherical droplets by this modified dry jet wet ejection procedure include viscosity (acceptable range between 50 and 700 cS, preferably around 300 cS) and surface tension.

When this method was used for the ejection of solutions of cellulose ester derivatives of all types, nonspherical particles (resembling flattened "pancakes" and other disintegrated droplets) were formed. This suggests that cellulose derivative droplets have insufficient mechanical resistance (surface tension) to withstand the impact of the collision with the nonsolvent surface. In contrast to cellulose, which forms high surface tension droplets, cellulose derivative droplets deform upon impact with the nonsolvent surface. However, when cellulose derivatives were blended in solution with varying amounts of cellulose in the same solvent prior to regeneration, the spherical nature of the droplet was preserved through the process of phase inversion.

Cellulose-Supported Cellulose Derivative Beads

The cellulose derivatives used in the present investigation involved commercially available cellulose esters (acetate, propionate, butyrate) as well as several novel derivatives prepared by homogeneous phase reaction in DMAc/LiCl solution. The latter included cellulose hexanoate (CH) with a degree of substitution (DS) of 1.1 (i.e., a derivative having, on

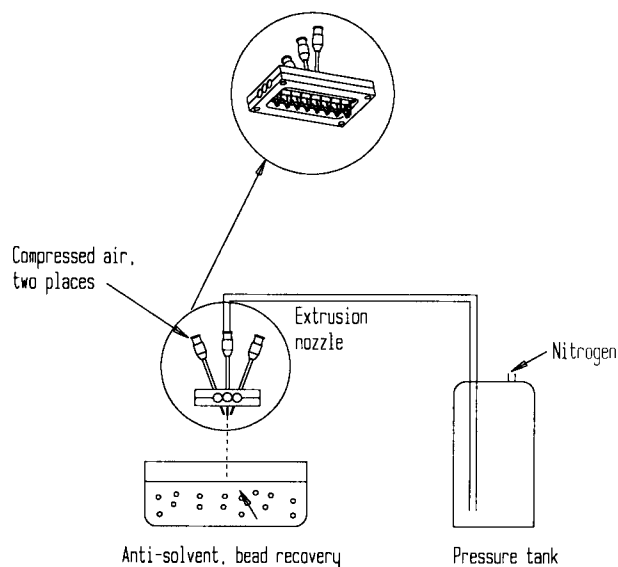


Figure 1 Schematic representation of the beading manifold device showing the capillary gas jets pointing directly at the capillary tip from which carbohydrate solution protrudes.

average, 1.9 OH groups per anhydroglucose repeat unit), cellulose acetate hexanoate (CAH), cellulose trifluoroethoxyacetate (CFA), cellulose acetate trifluoroethoxy acetate (CAFA), cellulose laurate (CL) (DS 1.3), and starch (Fig. 2). These derivatives were selected for the purpose of examining the potential for preactivating cellulose in homogeneous phase (i.e., DMAc/LiCl solution) using established esterification methods,⁷ followed by the regeneration in bead form.

All solution mixtures of cellulose and cellulose derivatives and starch produced spherical beads when regenerated from aqueous isopropanol. Bead strength was found to vary with composition. Typical results regarding the relationship between linear flow velocity and pressure drop (Fig. 3) revealed a gradual decline of maximum linear velocity with cellulose derivative content. The results suggest that CP (and other cellulose derivatives) is incapable of contributing to the strength characteristics of cellulose-containing beads. Although the blended beads have retained their spherical form during collision with the nonsolvent surface, the beads lose strength in relation to rising cellulose derivative content.

The capacity of cellulose/cellulose derivative blend beads to withstand linear (aqueous) flow prior to pressure exponentiation was found to be related to cellulose content (Fig. 3). On the assumption that cellulose alone is responsible for gel strength, and defining the point of pressure exponentiation as the

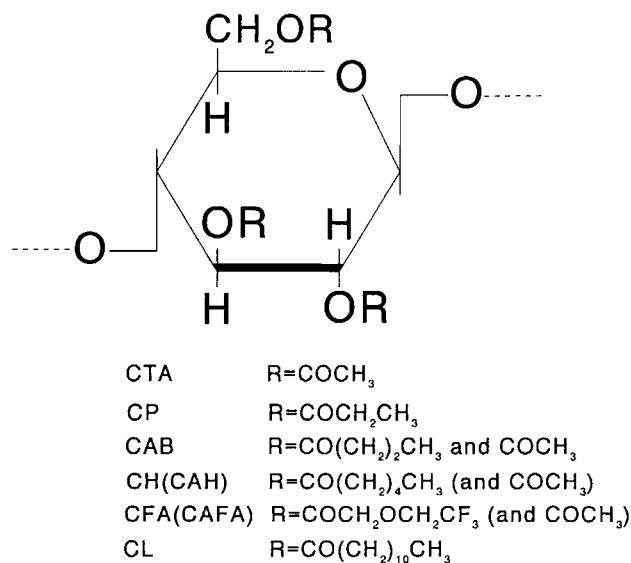


Figure 2 Repeating unit structures of cellulose derivatives used in this study: CTA = cellulose triacetate; CP = cellulose propionate; CAB = cellulose acetate butyrate; CH (CAH) = cellulose hexanoate and cellulose acetate hexanoate; CFA (CAFA) = cellulose trifluoroethoxyacetate and cellulose acetate trifluoroethoxyacetate; and CL = cellulose laurate.

linear velocity at which beads permanently deform ("crushing velocity"), the theoretical relationship between crushing velocity and cellulose derivative content can be expected to follow the rule of mixing. Actual strength data for blend beads (Fig. 4) reveal a slightly to significantly greater loss of strength

with cellulose derivative content rising for all derivatives and starch except CL. Whereas CAB follows most closely the rule of mixing, the highly hydrophobic CAFA indicates the most dramatic reduction in strength as cellulose content diminishes (Fig. 4).

The surprising retention of strength in support of dynamic flow of the CF-11/CL blend beads, which suggests undiminished crushing velocity at cellulose contents as low as 40% (Fig. 4), suggests a significant strengthening effect of large hydrophobic ester substituents. Laurate groups, with their bulky hydrocarbon residue (C₁₁H₂₃), appear to organize in hydrophobic aggregates capable of providing mechanical support similar to chemical crosslinks (Fig. 5). This is consistent with the observations of Sau and Landoll dealing with the presumed pseudonetwork structures of hydrophobically modified hydroxyethyl celluloses,²⁴ and it is also in agreement with the work by Berni et al. who found that lightly alkanoylated textile fibers have improved crease resistance on account of their hydrophobic interactions²⁵.

The atomization of polymer containing fluids, and the formation of spherical gel particles, is understood in relation to such factors as fluid elasticity (vs. inelasticity) and macromolecular rigidity. The breakup performance of an alginate-containing liquid was illustrated by Hulst et al. photographically.²⁶ Important features of fluid breakup involve (a) the rapid formation of droplets, a process that is usually assisted by high air currents creating shear forces on a protruding fluid stream; and (b) the distance a droplet travels before it collides with the surface of a nonsolvent. Early droplet formation and adequate

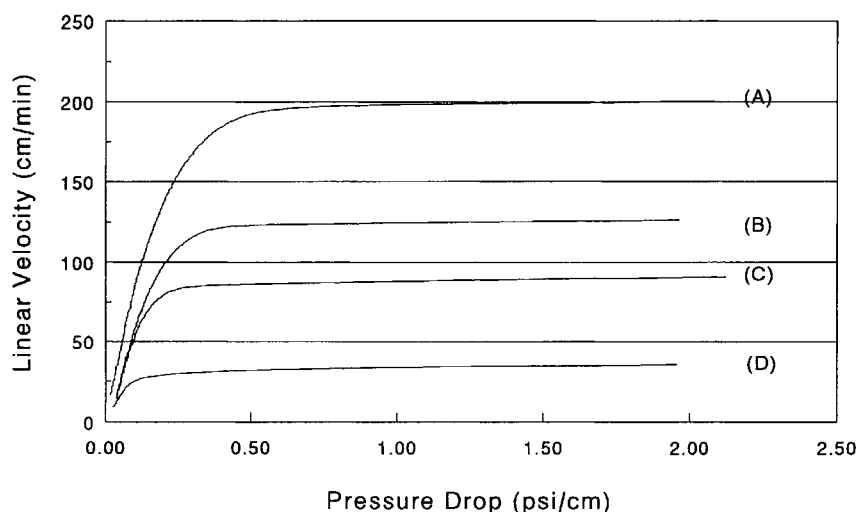


Figure 3 Pressure-flow curves for cellulose/cellulose propionate blend beads: (A) 100% CF-11; (B) 80% CF-11/20% CP; (C) 60% CF-11/40% CP; (D) 40% CF-11/60% CP. All the beads tested had an average of 7% solids content and average bead diameter of 500 μ m.

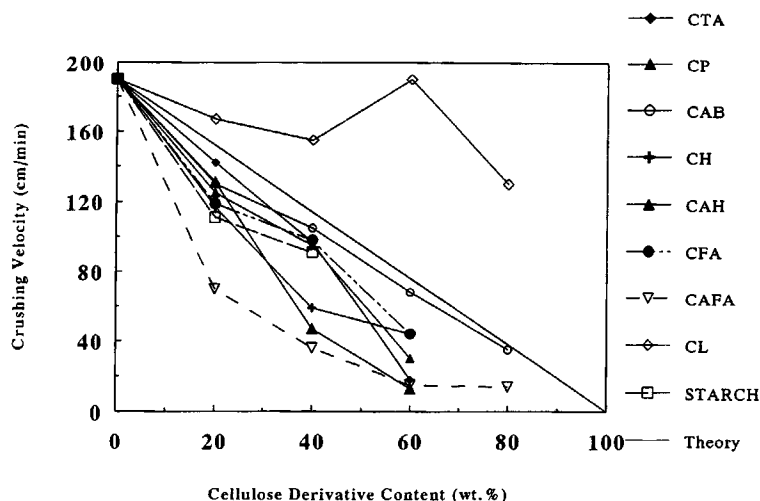


Figure 4 The effect of the chemistry of the cellulose derivative (or starch) on the crushing velocity of the cellulose/cellulose derivative blend beads. The theoretical model represents the predicted crushing velocities according to the rule of mixing.

droplet separation are necessary for preventing droplet coagulation and fusion in the nonsolvent. Various breakup characteristics were illustrated by Goldin et al.²⁷ The fluid breakup behavior is said to be dependent on the elasticity of fluids. Goldin et al. demonstrated that fluid elasticity with shear-independent viscosity is required for the formation of stable Newtonian liquids that possess adequate breakup characteristics. Because it is known that polysaccharides form inelastic (viscoelastic) fluids at concentrations that depend on chemical and molecular structure parameters, the inability of cellulose derivatives and noncellulosic polysaccharides to form beads may be attributed to the formation of unstable Newtonian liquids.

The ideal matrix for bioaffinity chromatographic support should provide ease of derivatization for the

incorporation of specific functionality. The generation of beaded hydrogels containing mixtures of cellulose and cellulose derivatives creates new opportunities for the production of beads that are preactivated, or that may be activated on the basis of derivative functionality present within the gel structure. There is also a need for bioaffinity support materials that (a) have, in addition to the foregoing desired properties, high mechanical strength to resist crushing at high column flow rates; (b) do not suffer from the nonspecific adsorption of complex solution components; (c) are made from an inexpensive and readily accessible material; and (d) are produced by a simple and inexpensive process that is readily adaptable for large scale production.

CONCLUSIONS

Cellulose ester derivatives fail to produce spherical hydrogel particles (beads) when subjected to a bead forming procedure involving (a) shear forces created by turbulent air flow, (b) travel through air prior to collision with nonsolvent, and (c) phase inversion.

The addition of dissolved cellulose to cellulose derivative solutions serves to build gel strength, and this effect is significantly or slightly more pronounced than the prediction based on the rule of mixing.

Starch behaves like cellulose derivatives by failing to exhibit (significant) inherent strength.

Cellulose laurate with DS 1.3 fails to obey the rule of mixing by exhibiting virtually undiminished strength for beads having cellulose contents as low

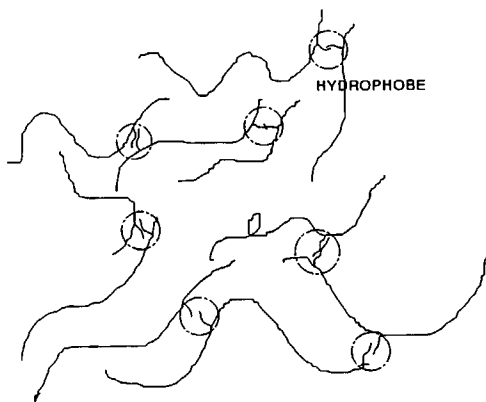


Figure 5 Schematic representation of a hydrophobically modified cellulose network according to Sau and Landoll.²⁴

as 40%. This is explained with hydrophobic interactions serving as pseudocrosslinks, which stabilize hydrogel-forming molecules against permanent deformation.

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