Supercritical fluids: puissant media for the modification of polymers and biopolymers

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The use of supercritical fluids as media for several types of polymer modification is demonstrated. Treatment of mixtures of chitosan with glucose or malto-oligosaccharides in supercritical carbon dioxide afforded the corresponding water soluble imine-linked, branched chitosan derivatives with high degrees of conversion. These transformations were substantially more facile and complete than previously reported equivalent reactions in conventional media. Phosphorylations of amylose and poly(vinyl alcohol) were conducted in supercritical carbon dioxide, yielding phosphate ester derivatives with phosphorus contents of 3.70% and 0.88%, respectively. Treatment of poly(vinyl alcohol), starch, maltodextrins, cellulose acetate and paper with mixtures of supercritical carbon dioxide and oxygen (19:1 v/v) led to the corresponding oxidized materials.

(Keywords: supercritical fluids; polymer modification; biopolymers)

Introduction

The use of supercritical fluid (SCF) technology in processes involving the extraction of a broad spectrum of low molecular weight substrates is well established[1]. SCF applications have also been extended to the synthesis and fractionation of polymers[2–5], the preparation of films and fibres[6,7], the extraction of biopolymers from their native matrices[8], and the hydrolytic treatment of glycans[9]. The scope of the SCF technique has clearly transcended the domain of routine isolations. Thus, the merits of supercritical media for chemical reactions have already been recognized[10], although they remain as yet largely unexplored[11–13]. Compared to conventional solvents, supercritical media feature low surface tensions, and high diffusivity[14] and solute capacity[1–5], all factors that may contribute to overall enhanced reactivities and selectivities. This communication describes the use of SCFs as media for various polymer derivatizations.

The inert or intractable nature of polymers frequently restricts or precludes their modification. Prominent examples among biopolymers are several 1-4-β-glycans, such as cellulose and chitosan, which are not readily susceptible to homogeneous reactions in inexpensive solvents[15]. The preparation of commercial products thus requires heterogeneous reaction conditions that afford substitution patterns and functionalities significantly distinct from those of uniformly derivatized materials.

The transformation of chitosan (**1**) into a branched polymer can be accomplished via reductive alkylation of the amine functions, using various carbohydrates[16]. Introduction of these branches disrupts the hydrogen bonding and converts the native material into a water-soluble polymer. Depending on the nature of the substituent, the branching reaction may require 2–72 h under ambient conditions for quantitative conversions. For a given degree of substitution (DS), the alkylation of the chitosan amine functions advances at considerably faster rates for monosaccharides than for oligosaccharides. Moreover, if imine- rather than amine-linked branched products are desired, the modifications proceed only to low DS values (<0.2), and require more rigorous reaction conditions[16–18]. The susceptibility differences of chitosan to branching therefore provide a convenient measure for the utility of SCFs as reaction media.

Experimental

Methods and materials. Chitosan powder (Protan Laboratories) was finely ground (to pass mesh 30) prior to use. Cellulose acetate (90% acetyl content, ASTM viscosity 25) was from Eastman, amylose and starch (corn) from Sigma, poly(vinyl alcohol) (PVA) and sodium tripolyphosphate (TPP) from Aldrich, malto-oligosaccharides (MOS, degree of polymerization (DP) 3–10) from Pfanstiehl, softwood-derived high-alpha Kraft paper from Domtar, and dialysis membranes (M_w cut-off 6000–8000) were from Spectropore. ^{13}C n.m.r. spectra were observed at 100 MHz using a Bruker WH 400.

SCF modifications. All SCF reactions were performed under similar pressure and temperature (27.4–31.4 MPa and 60°C) conditions. The experiments were conducted in a 1 × 45 cm stainless steel tube, fitted at both ends with high pressure swagelock fittings, or in an 8.8 l stainless steel vessel. The SCFs were delivered to the reaction vessels by an air driven gas compressor with a capacity of 78 l min^{-1}. The reactions were carried out by charging the vessels (10–20 g for tube and ~200–400 g for autoclave) and then pressurizing to the desired pressure. Reaction temperatures were maintained within 3°C and monitored inside the reactors by a chromel–alumel thermocouple. For reactions in the tube, Raschig rings were admixed with the solids to prevent pressure build-up. Continuous SCF flow was achieved by cracking needle valves at the reactor outlets, with fluid flow monitoring by a rotameter. The gas flow rates were

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10 and 20 l min^{-1} for the tube and autoclave, respectively. The products were collected in a 1 or 8.8 l trap by lowering the pressure below critical values, and passing SCFs through a glass wool filter before venting.

Preparation of chitosan-glucimine derivative **2**. An aqueous mixture of **1** and glucose (2:1 w/w, 9.8 g total solids, 34.0 ml) was subjected to supercritical carbon dioxide (SCD) treatment for 1.5 h. A clear, light yellow and viscous solution (pH 6.0) was recovered in the trap, which yielded, after dialysis and lyophilization, derivative **2a** as an off-white material (5.0 g). Elemental analysis gave the following results. For **2a** $[(C_8H_{13}NO_5)_{0.02}(C_6H_{11}NO_4)_{0.08}(C_{12}H_{21}NO_9)_{0.90}] \times 1H_2O$ (degree of substitution (DS) 0.9), calculated: C 42.15; H 6.82; N 4.30 (C/N 9.8); found: C 41.58; H 6.63; N 4.33 (C/N 9.6). The identity of **2** was confirmed by comparison of its ^{13}C n.m.r. spectrum with those of previously reported materials[16–18] prepared in conventional media. The reaction was also repeated on a larger scale (375 g total solids, 75 ml water), affording, after work-up, derivative **2b** (140.2 g) with DS 0.9, found: C 41.44; H 6.51; N 4.23 (C/N 9.8). For comparison, an aqueous mixture of **1** (10.18 g) and glucose (6.30 g, 60 ml) was also held at ambient conditions for 20 days, and subsequently dialysed, affording a yellow solid material (7.03 g), which was unmodified **1** based on microanalysis. Elemental analysis gave the following result, found: C 43.55, H 7.04, N 7.81.

Preparation of chitosan-malto-oligosaccharide imine derivative **3**. An aqueous mixture of **1** (6.3 g) and MOS (10.2 g, 40 ml) was treated with SCD for 2 h. The receiving vessel contained a yellow, viscous solution (pH 5.6). A dispersion of unmodified **1** in a similarly viscous, yellow solution was recovered from the reaction vessel. The latter was filtered to remove the residual chitosan (0.6 g), and both the extracted solution, containing derivative (**3a**) and the filtrate (**3b**), were separately dialysed and lyophilized. The branched product **3** (**3a**, 6.2 g; **3b**, 4.7 g) was isolated as off-white material. Elemental analysis gave the following results. For **3a** $[(C_8H_{13}NO_5)_{0.02}(C_6H_{11}NO_4)_{0.18}(C_{24}H_{41}NO_{18})_{0.80}] \times 3.5\ H_2O$ (DS 0.8), calculated: C 40.82, H 7.05, N 2.33; found: C 40.06, H 6.67, N 2.11, and for **3b** $[(C_8H_{13}NO_5)_{0.02}(C_6H_{11}NO_4)_{0.38}(C_{24}H_{41}NO_{18})_{0.60}] \times 2.7\ H_2O$ (DS 0.6) calculated: C 41.03, H 7.04, N 2.84; found: C 40.19, H 6.23, N 2.59. For comparison, an aqueous mixture of **1** (10.35 g) and MOS (6.32 g, 80 ml) was also held at ambient conditions (20 days), and subsequently dialysed, affording a yellow material (6.88 g), that was unmodified **1**, according to microanalysis. Elemental analysis gave the following result, found: C 43.18, H 7.02, N 7.50 (C/N 5.8).

Preparation of amylose phosphate **4**. An aqueous mixture of amylose (15.34 g) and TPP (6.42 g, 38.5 ml) was treated with SCD for 1.5 h. Microanalysis gave the following result for **4**, found (DS ~0.25): C 40.13, H 7.42, P 3.70.

Preparation of PVA-phosphate **5**. An aqueous mixture of PVA (27.33 g) and TPP (10.84 g, 68.8 ml) was treated with SCD for 1.5 h. Microanalysis gave the following result for **5a**, found: C 54.01, H 9.24, P 0.88. A conventional phosphorylation was conducted by treating PVA (3.78 g) and TPP (2.90 g) in water (75 ml, pH 9.4) for 2.5 h at 25°C. The reaction mixture was then dialysed and lyophilized to afford **5b** as a white material. Microanalysis gave the following result for **5b**, found: C 38.95, H 6.46, P 5.34.

Oxidations. Oxidations were performed by blending ~5% (v/v) oxygen with CO_2, and treating the dry polymers with the SCF blend for 2 h. The oxidized materials were fully recovered from the reaction vessel and characterized by their i.r. carbonyl absorptions (KBr) as follows (sample size, product colour, λ_{max}): starch (18.3 g, light yellow, 1648 cm^{-1}); maltodextrins (22.6 g, light yellow, 1632 cm^{-1}); PVA (11.1 g, white, 1743 cm^{-1}); cellulose acetate (11.2 g, white, 1653 cm^{-1}); Kraft paper (11.85 g, white, 1643 cm^{-1}).

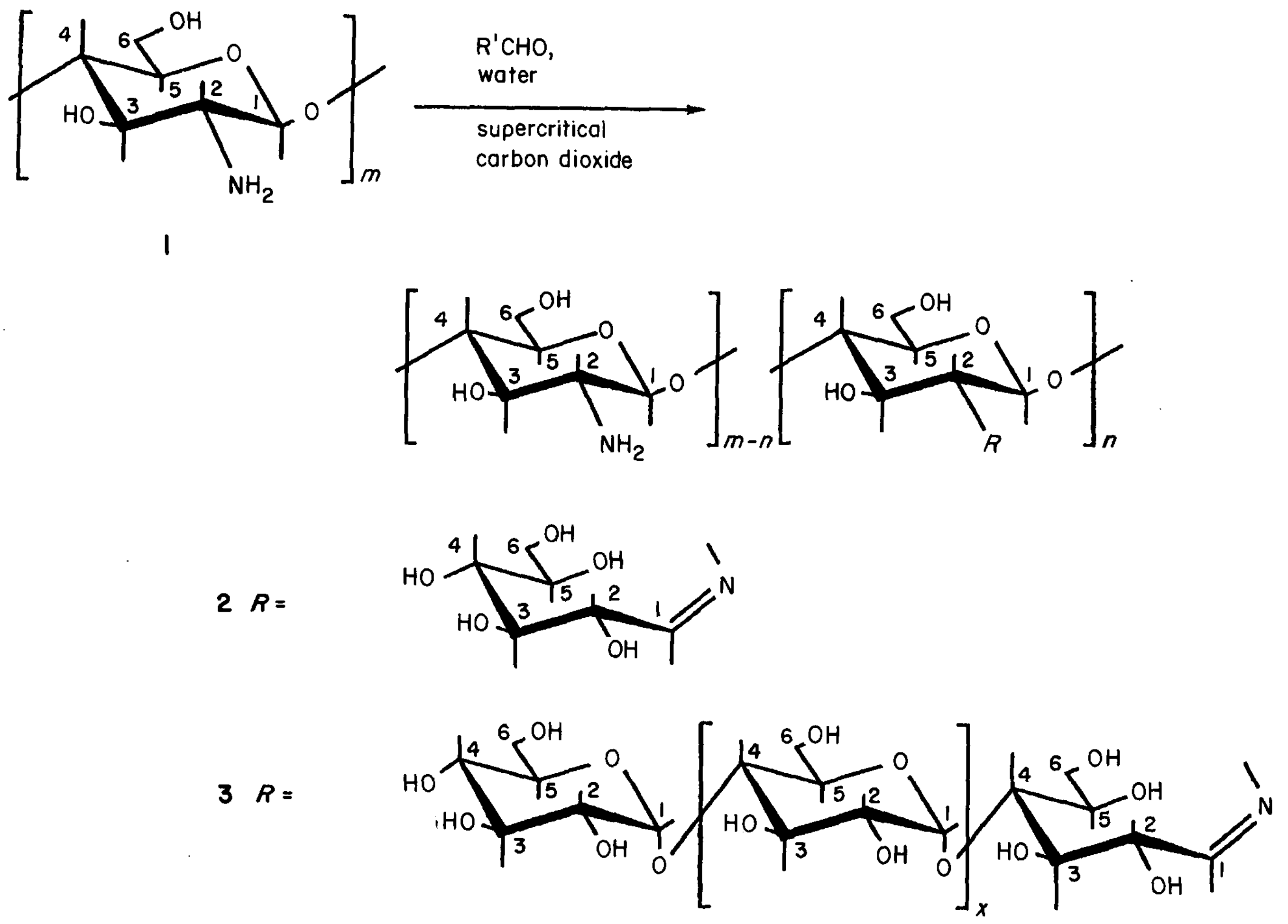
Results and discussion

In an initial probe, an aqueous dispersion of chitosan powder and glucose in a tubular reaction vessel was treated with SCD at 60°C for 1.5 h. Upon completion of the process, the solid biopolymer substrate was unexpectedly found to be completely transformed. The reaction product was recovered as a viscous, light yellow solution in the receiving vessel. After lyophilization, an off-white, water-soluble material was isolated, which was identified as the chitosan derivative **2** (*Scheme 1*). Based on elemental analysis and ^{13}C n.m.r. of **2**, a remarkably high degree of conversion was attained for chitosan (DS 0.9). Identical results were obtained, when the experiment was scaled up about 40-fold.

Spurred by the efficiency of this conversion, the Schiff's-base formation was extended to a less reactive carbohydrate. A mixture of a commercial preparation of malto-oligosaccharides (DP 3–10) and chitosan (1.6:1) was subjected to SCD treatment for 2 h. Again, a viscous, yellow solution was recovered in the trap, and a smaller fraction of an equally viscous, low solids dispersion from the reactor. The residual solids in this dispersion (unmodified **1**, ~10%) were removed, and the branched product **3** was isolated after lyophilization of the respective fractions. Elemental analysis of **3** revealed high conversion levels (**3a**, DS ~0.8), with the materials recovered from the reactor displaying a slightly lower degree of branching (**3b**, DS ~0.6).

The above findings demonstrate the ease with which chitosan can be converted into mono- and even oligosaccharide-branched imine derivatives in these relatively brief SCF reactions. Such transformations, as already noted, are considerably more arduous under conventional reaction conditions. This was further underscored by the observation that similar mixtures of chitosan and either glucose or malto-oligosaccharides in water produced no branched materials at ambient conditions after prolonged reaction periods (20 days).

Another type of modification was exemplified by the phosphorylation of PVA with TPP in SCD. In this reaction, a paste was prepared with water, PVA and TPP, which was subsequently exposed to SCD. The resulting product exhibited a relatively low level of phosphorus incorporation (P 0.88%). This may, in part, be attributable to inter- and intramolecular crosslinking between PVA chains, as the formation of phosphate diesters would result in lower apparent DS values than for the corresponding monoesters. A similar experiment with amylose, on the other hand, afforded a phosphorylated material with a P content (3.70%) that compares



Scheme 1

favourably with levels attainable under regular phosphorylation conditions[19]. While the results of these SCF transformations are not vastly superior to those from equivalent conventional processes, they offer the benefit of obviating the requirement for alkaline pretreatments, which may be critical for applications where the native polymer morphology and ultrastructure need to be maintained. Such changes are generally induced in solid glycans by most activation treatments[20].

In a separate series of experiments, polymer oxidations were investigated. Solid samples of starch and PVA were each treated for 2 h with a supercritical mixture of carbon dioxide and oxygen. The emergence of carbonyl absorption bands in the *FT*i.r. spectra of the recovered solids provided clear evidence for the successful oxidation of the polymers. Analogous results were obtained for oxidized samples of maltodextrins, cellulose acetate and softwood-derived Kraft paper. The facile oxidations performed here are indicative of a variety of other modifications that may be contemplated by combining reactive supercritical components with carbon dioxide or other inert SCFs.

The success of the SCF modifications poses the obvious query for a rationale. A number of factors may be implicated in the three exemplified reaction types, each of which represent different phase systems. Thus, the phosphorylations and, initially, the chitosan reactions constitute solid–solid–liquid–vapour (SSLV) systems, while the oxidations involve solid–vapour–vapour (SVV) systems. Previous observations that supercritical fluids may induce considerable swelling[21], plasticization[22,23], or morphological alterations[24–26] of polymers offer plausible, prima facie explanations that could be relevant to all of the SCF reactions. These factors are likely responsible for enhancing the rapid rates at which the solutes are transported into the polymer matrices[27,28] and, for the chitosan reactions, the extensive hydrogen bonding network of the semicrystalline aminoglycan is apparently disrupted here, as branching proceeds.

The reduced pH environment that is temporarily created in the course of the SCD treatment also merits consideration. Homogeneous reactions under ambient conditions require aqueous acetic acid or equivalent media[17,18], to affect chitosan solubilization. In aqueous solutions[29] SCD can conveniently generate a pH of about 3. While the actual pH under SCF conditions could not be probed here, even a relatively minor pH shift to about 5.5 would suffice to promote polymer solubilization and derivatization. However, while a low pH environment would help to establish homogeneous conditions, it would, in the absence of reducing agents, also disfavour formation of the acid-labile imine products, particularly those with high DS values as attained here. Thus, SCD-induced pH reductions would be expected to reduce, rather than enhance product formation. This factor seems to be therefore of only secondary, if any, relevance for the chitosan reactions.

Other studies have concluded from transition-state analysis that enhanced reaction rates may arise from the unique volumetric nature of heavy solutes in SCF media, and a favourable pressure dependence of the reaction rate constant[30,31]. The latter relation is a function of the difference in the partial molar volumes of the activated transition complex and the reactants. However, since the field of SCF reactions is only at its inception, there is little precedence from which to draw analogies about the thermodynamic equilibria of the complex multiphase systems described here. The present synthetic results therefore need to be complemented by phase studies, in order to resolve the intricacies of the individual solutes and reaction systems. A case in point is the transition of the chitosan SSLV system into a liquid–vapour system in the course of the SCF treatments, where the phase behaviour of the initial reactants will clearly be affected by product formation[32].

Conclusions

The above results provide only a first, intriguing glance at the potential range of SCF-mediated polymer modifications, especially when one considers the choice of strictly innocuous reagents that guided the present selection of experiments. A wide spectrum of other covalent derivatizations is readily conceivable for various types of polymeric fibres, granules, surfaces in either solution, gel or other solvated states. Selective chemical transformations can be contemplated, in view of the relatively short processing periods witnessed here, and the possibility of influencing the course of reactions by controlling pressure and temperature in the supercritical regime. Other applications may arise for uniform, low-level cross-linking of polymer matrices, such as chromatographic and immobilization supports. Potential benefits can also be envisaged for derivatizations of sensitive biopolymers, e.g. proteins[32], considering the efficacy and mild reaction conditions of the method.

It is evident that SCFs provide a versatile and extremely powerful approach to polymer alterations. Supercritical media will likely offer important advantages for commercial applications, where environmental, regulatory or other concerns demand consideration of product homogeneity, the absence of trace amounts of hazardous chemicals or solvents, or simply the effective transformation of materials that would otherwise elude such attempts.

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