Cellulose Phosphates as Biomaterials. I. Synthesis and Characterization of Highly Phosphorylated Cellulose Gels

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ABSTRACT: The present study concerns the investigation of a material expected to be biocompatible and able to promote bone regeneration. For this purpose, cellulose was chemically modified by phosphorylation. Once implanted, phosphorylated cellulose could promote the formation of calcium phosphates, thus having closer resemblance to bone functionality. In a previous investigation, the obtention and the preliminary characterization of cellulose phosphate gels were reported. In the present study, the synthesis by the $H_3PO_4/P_2O_5/Et_3PO_4$/hexanol method was optimized in terms of reaction parameters. The structure of materials was investigated by FTIR, Raman, and solid-state ^{31}P– and ^{13}C–NMR spectroscopic studies, and X-ray diffraction. Water swelling and stability to sterilization by gamma-radiation were also assessed. It was demonstrated that the present method allows highly phosphorylated cellulose derivatives to be obtained. Cellulose triphosphates gels are described here for the first time. Products obtained were poorly crystallized monoesters, apparently not significantly affected by gamma sterilization and showed a high water swelling capability. Chemical bonding was confirmed by FTIR, Raman, and both ^{31}P– and ^{13}C–NMR spectroscopies. It was also shown that the $H_3PO_4/P_2O_5/Et_3PO_4$/hexanol method provides a versatile and interesting alternative route to some of the more widely used techniques for the phosphorylation of cellulose. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 82: 3341–3353, 2001

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INTRODUCTION

Cellulose is the world's most abundant natural, renewable, and biodegradable polymer. Polysaccharides, like cellulose, are the polymer group with the longest and widest medical applications experience because of their unique properties[1,2]: nontoxicity (monomer residues are not hazardous to health), water solubility or high swelling ability by simple chemical modification, stability to temperature and pH variations, and a broad variety of chemical structures. In medicine, membranes for blood purification (hemodialysis) made of cellulosics are among the most widely used polymeric devices in therapy.[3,4] Cellulose and its

derivatives have found several other biomedical applications, either unmodified or as esters, ethers, and as either crosslinked or graft copolymers.[1–8] Microcrystalline cellulose Avicel®, the type of cellulose used in the present study, is widely used in the pharmaceutical and food industries for several applications and enjoys the *generally recognized as safe* (GRAS) status. This material is hydrophilic, not biodegradable, and insoluble in water, dilute acids and alkali solutions, and common organic solvents.[5,6,9–13] In orthopedic surgery, oxidized cellulose is used as a wound dressing.[14–16] Cellulose viscose sponges have also been proposed as implantable tissue matrices for connective tissue regeneration.[17] Cellulose regenerated by the viscose process (CRV®) has also been investigated as an implantable material in orthopedic surgery, as a sealing material for the femoral component in hip prostheses, in place of acrylic cement.[18–22] It was envisaged to take advantage not only of its good mechanical matching with the properties of bone but also of its hydroexpansivity, thus allowing a satisfactory fixation to hard tissue. The osteoconduction of this material has also been demonstrated.[19–21] Nevertheless, a full bioactive character cannot be attributed to normally occurring cellulose because of its lack of osteoinduction.

Phosphorylation was therefore envisaged as the means to enhance cellulose bioactivity. The use of cellulose phosphates for biomedical applications is not new. Cellulose phosphates have been used for decades in the treatment of calcium metabolism–related diseases, taking advantage of their high ability to bind calcium ions.[23–25] Several other biomedical applications have been proposed, always profiting from their high ion exchange capacity.[26–34]

The present study concerns the investigation of a material for orthopedic applications. This material is expected to be biocompatible and able to promote bone regeneration. For this purpose, the selected material was cellulose, a biocompatible naturally occurring polymer, chemically modified by phosphorylation. To stimulate bone induction by cellulose-based materials, its chemical modification via phosphorylation was envisaged. Once implanted, phosphorylated cellulose could promote the formation of calcium phosphates, thus having closer resemblance to bone functionality and ensuring a satisfactory bonding at the interface between hard tissue and the biomaterial.

In orthopedic applications, mineralization of biomaterials is usually desirable, that is, the ability to induce the formation of a calcium phosphate layer, which will then contact the living tissue. Synthetic calcium phosphates generally induce the formation of an apatite layer in simulated plasma solutions. Some other synthetic biomaterials have also shown the capability of inducing the formation of an apatitic mineral, namely polymers containing phosphate functionalities,[35–38] including phosphorylated cellulose derivatives.[39–41] However, only low degrees of phosphorylation were used because of the lack of techniques that would allow highly phosphorylated products to be obtained.

Methods for cellulose phosphorylation have been consecutively reviewed since the early decades of the 20th century, when they were first proposed as flame retardants.[42–45] In a previous investigation, the preparation and the preliminary characterization of cellulose phosphate gels was reported.[46,47] The synthesis was carried out by the $H_3PO_4/P_2O_5/Et_3PO_4$/hexanol method, first proposed by Touey and Kingsport[48] in 1956, in which the hemisynthesis of a water-soluble and nondegraded flame-resistant textile material was described. The choice of this method was based on the possibility of obtaining products free of biologically hazardous chemical compounds present in products obtained by other available methods.[49–57] Our preliminary results indicated good comparative reaction yieldings.[46,47] Reaction parameters were optimized using microcrystalline cellulose, and cellulose phosphates of relatively high degrees of substitution were obtained.[46,47] These materials were characterized for their structure and cytocompatibility.

In the present study, the synthesis of these materials was optimized in terms of reaction parameters. Cellulose triphosphates are reported here for the first time. The transparent and high swelling gels thus obtained were further characterized in terms of their water swelling, and their structure was studied by FTIR, Raman, and solid-state ^{31}P– and ^{13}C–NMR spectroscopies, and X-ray diffraction. Their stability to gamma-radiation was also assessed.

EXPERIMENTAL

Microcrystalline cellulose Avicel® PH-101 type was purchased from Fluka Chemie (Buchs, Switzerland). All chemicals were of research-grade purity and used without further purification.

Synthesis of Cellulose Phosphate

The cellulose powder was dried in vacuum in the presence of phosphorous pentaoxide. Samples were swollen for 24 h in hexanol, *N,N*-dimethyl-formamide (DMF), or 85% orthophosphoric acid, to assess the influence of swelling prior to chemical modification. Hexanol is the solvent of the reaction and H_3PO_4 is the phosphorylating agent, besides being a well-known cellulose solvent. DMF is widely used in cellulose chemistry and was already reported in several other systems, in conjunction with H_3PO_4, for preparing cellulose phosphates. The phosphorylation reaction was carried out in a four-neck round-bottom flask, equipped with a nitrogen inlet, a condenser, a $CaCl_2$ drying tube, a thermometer, and a mechanical stirrer. The highest degree of phosphorylation was obtained using the following procedure: to a stirring suspension of cellulose (4 g) in hexanol (29 mL) was added portionwise a solution of phosphorus pentaoxide (50 g) in triethyl phosphate (37 mL) and orthophosphoric acid (42 mL). The mixture was stirred at 30°C for 72 h and then filtered. The filter cake was washed successively with hexanol and ethanol, and then rinsed repeatedly with water, to wash out the excess H_3PO_4. The filtrate was then centrifuged prior to purification. Purification was carried out by Soxhlet extraction with deionized water and ethanol, for at least 24 h, until tests for inorganic phosphate were negative. The gel obtained was finally freeze-dried.

The reaction parameters were investigated and products characterized. In previous studies,[46,47] the correct sequence of addition of reagents was established. The maximum degree of substitution [DS ($0 \leq DS \leq 3$)] previously obtained (~ 1.0) was comparable to the DS obtained by the most widely used methods. An experimental design was then conceived to optimize the maximum DS. Parameters investigated were: temperature (30, 50, and 70°C), reaction time (24 and 72 h), phosphoric acid to cellulose ratio (18 and 28 mL/g), phosphorus pentaoxide to phosphoric acid ratio (0.4 and 0.7 g/mL), phosphoric acid to hexanol ratio (1 and 3), triethyl phosphate to hexanol ratio (0.5 and 1.5), and, finally, the swelling agent (hexanol, DMF, and phosphoric acid were tested). These limits were established according to our experience obtained from preliminary tests, by varying the values used in previously reported works.[46,47]

Preparation of Amorphous Cellulose

Amorphous cellulose was prepared by anhydrous deacetylation of cellulose triacetate (CTA), according to a previously published method.[58] Briefly, 3.5 g of CTA (Kodak, Japan) was dispersed in ethanol for 10 min, under strong stirring, and filtered. The filtrate was then dissolved in 9 mL of methylene chloride and diluted in 37.5 mL of pyridine. Saponification was carried out under strong stirring, using 0.1*M* NaOH (30 mL), acetone (45 mL), and 90 mL of a 1.0*M* ethanolic NaOH solution (50%, v/v), and then left overnight. The precipitate was filtered, washed with anhydrous ethanol, and dried in air.

All dried materials were stored at 23°C and 50% relative humidity, until constant weight was attained, before further examination.

Characterization

Phosphorus Content

Elemental analyses for phosphorus were run separately by potentiometric titration[59] with 0.1*M* NaOH and spectrophotometrically by the Kjeldhal method[60] and values obtained by both methods were found to be in good agreement. In the latter case, organic phosphates were dissolved in sulfuric acid. Phosphorus was then converted to phosphomolybdate, and the reaction with ascorbic acid resulted in a blue complex, the optical density of which was measured by spectrophotometry at 880 nm. The DS was calculated as previously reported.[46]

Morphology

Scanning electron microscopy (SEM) observations were carried out at an accelerating voltage of 15 kV in an S-2500 scanning electron microscope (Hitachi, Japan). Observations were made on Au-sputtered specimens.

Water Sorption

These materials swell to such an extent that they become too fragile to handle. Thus, 0.1 mg of the dried powdered material was allowed to hydrate in excess distilled water, at room temperature, in centrifuge polyethylene tubes. The weight of the hydrating samples was measured at several times, following removal of excess water after centrifugation at 4000 rpm for 10 min. Subsequently, water sorption was determined via the difference of weight before and after swelling.

Sterilization Resistance

After packing, samples were gamma-irradiated for 8.5 h at room temperature, either in the presence or in the absence of oxygen (in a nitrogen atmosphere), using a CIS Bio-Industries equipment, model IBL 337 (Gif-sur-Yvette, France), loaded with 13,000 Ci of ^{137}Cs, to absorb a total dose equal to 25 kGy. The effect of gamma sterilization on materials structure was evaluated by FTIR spectroscopy.

Crystallinity Index

X-ray diffraction (XRD) was carried out to crystallographically characterize cellulosic powders, by means of an X'Pert X-ray diffractometer (Phillips, The Netherlands), using a CuK_α radiation (source conditions: 40 kV and 50 mA). The crystalline-to-amorphous ratio of materials was determined using the empirical procedure first proposed by Segal et al.[61] and modified by Nelson and O'Connor.[62] This method consists of estimating the crystallinity index (Cr.I.), according to the following equation:

$$\text{Cr.I.}(\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \qquad (1)$$

where I_{002} is the maximum intensity (in arbitrary units) of the diffraction from the (002) plane at $2\theta = 22.6°$, and I_{am} is the intensity of the background scatter measured at $2\theta = 19°$.

Spectroscopic Analyses

Fourier transform infrared (FTIR) and Raman spectra were recorded on Perkin–Elmer models 137B Infracord and 2000 Nir FT-Raman spectrometers (Perkin Elmer Cetus Instruments, Norwalk, CT), respectively. Samples for FTIR analysis were prepared as dispersions in spectroscopic-grade potassium bromide (KBr).

Solid-state ^{31}P magic angle spinning (MAS) and ^{13}C CP/MAS NMR spectra were recorded at 9.4 T with a Bruker DPX-400 NMR spectrometer (Bruker Instruments, Billerica, MA) operating at 161.97 MHz (^{31}P–NMR) and 160.612 MHz (^{13}C–NMR). Studies were carried out at room temperature with zirconia rotors, using MAS rates of 8 and 4 kHz. Chemical shifts were determined using 85% H_3PO_4 ($\delta_{31P} = 0$ ppm) for phosphorus and TMS (tetramethylsilane, Me_4Si; $\delta_{13C} = 0$ ppm) for carbon, as external reference substances. ^{31}P MAS NMR spectra of solid samples were recorded

Table I   Influence of Reaction Time on the DS Obtained

Reaction Time (days)	DS
1	1.42 ± 0.59
2	1.35 ± 0.21
3	2.50 ± 0.64
5	1.39 ± 0.31
8	1.14 ± 0.27
10	1.34 ± 0.27

using Bruker's microprogram hpdec.drx (proton decoupled) with the following parameters: 120 scans, a 6.5-μs pulse with a delay time of 5.0 s, 31 ms of acquisition time, and a sweep width of 400 ppm. For ^{13}C–NMR spectra, the cross-polarization (CP) MAS method was used (Bruker's microprogram cp31ev.drx) with the following parameters: 5000 scans, a contact time of 1.5 ms, a 4.5-μs pulse with a delay time of 4.0 s, 68 ms of acquisition time, and a sweep width of 368 ppm. Concerning gel samples, ^{13}C–NMR spectra were recorded by the liquid NMR technique, using the Bruker's microprogram hpdec.drx, with the following parameters: 5000 scans, a 5.0-μs pulse with a delay time of 4.0 s, 68 ms of acquisition time, and a sweep width of 300 ppm.

RESULTS AND DISCUSSION

In previous works, the synthesis of cellulose phosphates by the $H_3PO_4/P_2O_5/Et_3PO_4$/hexanol method was successfully carried out.[46,47] The correct sequence of addition of reagents was established.[46,47] The method proposed then showed to be adequate for obtaining products with DS values up to 1.0, which is comparable to the maximum DS obtained by the most widely used methods.[49–57] Using the present methodology, an average DS of approximately 2.5 was obtained for the first time (Table I). As previously observed for lower DS values, the temperature and reaction time influenced the maximum DS.[46,47] Only one combination of the tested parameters allowed the achievement of this high DS, as indicated in the previous section. Experiments carried out at 50 and 70°C promoted insoluble and visibly degraded products. All other combinations at 30°C promoted only poorly phosphorylated products, with DS values ranging from 0.1 to 0.2. Longer

reaction durations (Table I) and higher temperatures enhanced the DS, despite the fact that this progression seems to reach a maximum point at which a maximum DS was obtained. Above this maximum point the DS decreased as a result of degraded products being obtained. This indicates that there is probably a competition between phosphorylation and hydrolysis of preexisting phosphoester bonds. Similar findings were also described previously for products having low DS values.[46,47]

The swelling pretreatment, as well as the swelling agent itself, also influenced the DS considerably (results not shown here). Using the same conditions, preswelling in DMF or phosphoric acid yielded degraded end products, whereas the use of hexanol, the solvent used in the reaction, allowed highly phosphorylated products. Cellulose triphosphates were not obtained if this treatment was not carried out as described. Possibly, other combinations of swelling agents[63,64] would allow even higher swelling degrees and maybe shorter reaction durations for the same DS. However, given that the purpose of this investigation was to develop a material for biomedical applications, where high purity is desirable, the sequence of water, ethanol, and hexanol was thought to be the best choice. Water is one of the best cellulose swelling agents, although traces of water are deleterious to the present reaction.[46,47] Hence, water was exchanged by ethanol, where it is soluble. Ethanol, on the other hand, is soluble in hexanol and was washed by the latter given that hexanol is the solvent of the reaction itself. Hexanol is not a good swelling agent for cellulose but the material kept its swelling in water after the exchange in the sequence of ethanol followed by hexanol.

The macroscopic aspect of dried and hydrated samples of unmodified microcrystalline Avicel cellulose, cellulose triphosphate gel, and lyophilized cellulose triphosphate is shown in Figure 1. Avicel cellulose has swollen very little in water. The decrease in density of lyophilized cellulose triphosphate gels is observable since the same weight of Avicel and lyophilized cellulose triphosphate is shown in Figure 1. Furthermore, it can be observed that cellulose triphosphates swell considerably in water, forming a consistent translucent gel. SEM micrographs (Fig. 2) show the spongy-like aspect of the lyophilized products, at high DS (2.9).

Potentiometric titration curves (Fig. 3) were used to determine phosphorus content and
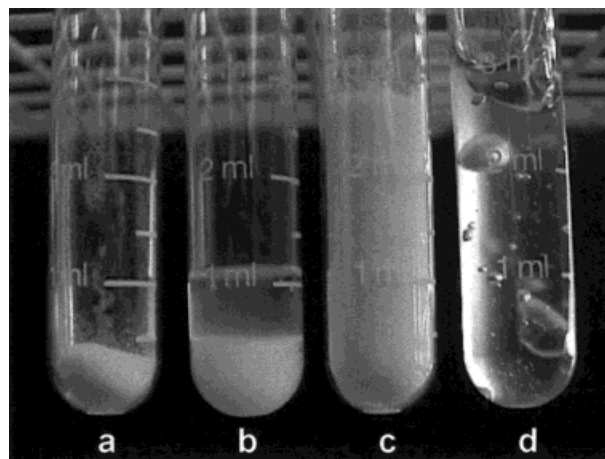
Figure 1 Macroscopic aspect of (a) unmodified microcrystalline cellulose Avicel®, (b) hydrated Avicel, (c) lyophilized cellulose triphosphate, and (d) cellulose triphosphate gel (hydrated lyophilized cellulose triphosphate). The same weights of Avicel and cellulose phosphate were introduced in the test tubes.

showed that the second equivalence point, determined by the calculation of the first derivative, has approximately doubled the first, independently of the phosphate content. The second equivalence point, which is double the first [Fig. 3(b)], indicates that titrable phosphorus is bound by a unique link to the cellulose chain, leaving two hydrogens available for titration. The DS did not affect the relative distance between the two equivalence points on the potentiometric titration curves, demonstrating that monophosphates are obtained in every case.

Water swelling of phosphorylated materials was significantly higher compared to that of unmodified cellulose (Fig. 4). After phosphorylation, a decrease in crystallinity was expected, and changes in water swelling could result from this phenomenon. To assess the influence of crystallinity on water swelling, amorphous cellulose was prepared and its water swelling was also determined. It has been shown that highly phosphorylated cellulose absorbed water in greater amounts than did amorphous cellulose, confirming that its high water sorption is not solely the result of its low crystallinity. Hence, the high water swelling observed is closely related to the functionality of phosphate groups themselves. A more comprehensive study of the influence of the DS on water sorption is being carried out in an ongoing investigation and will be reported in a future study.
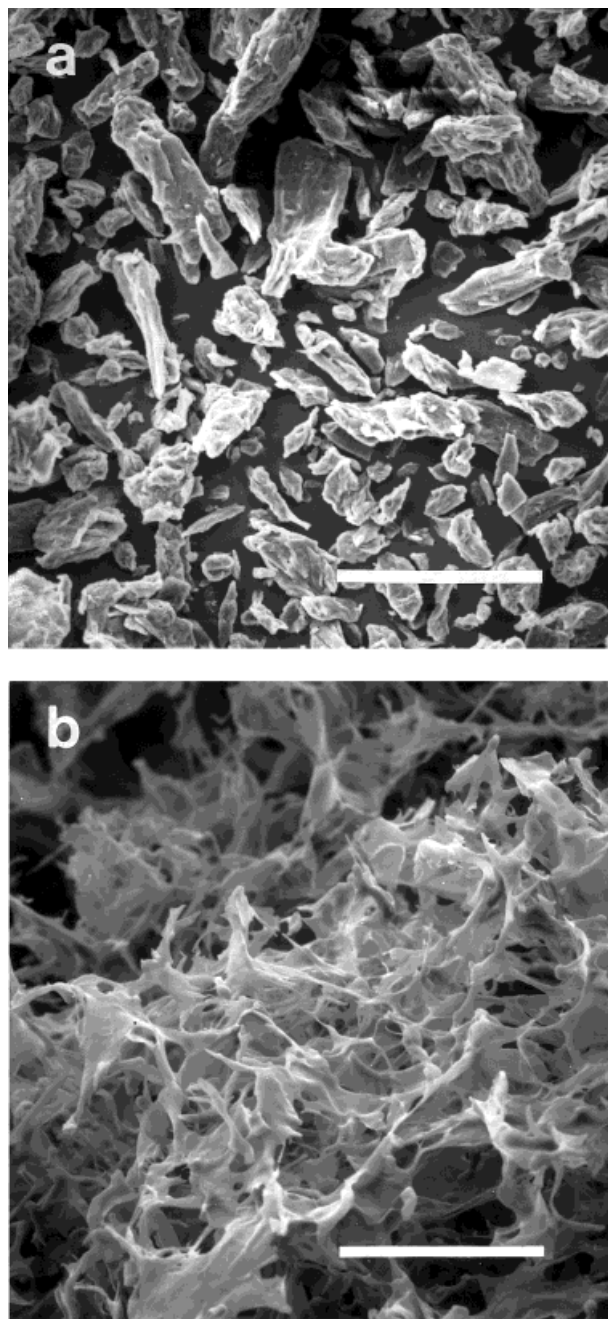
Figure 2 SEM micrographs of (a) unmodified micro-crystalline cellulose Avicel and (b) lyophilized cellulose triphosphates (DS = 2.9). White bars in the pictures correspond to 100 μm.

The FTIR spectra of phosphorylated samples (Fig. 5) showed main absorption bands (in cm^{-1}) at 3402 (O—H stretching), 2891 (C—H stretching), 1625 (absorbed H_2O), 1418, 1382, 1152, and 1029 (C—O stretching).[65–69] The sharp peak at 1383 cm^{-1} can be attributed to P=O stretching, indicating the presence of a phosphate ester.[66,67]

A C—O—P stretching signal, usually found at 1055–950 cm^{-1}, was not observed, probably resulting from a broadening of the typical cellulose bands. C—O—P stretching may have caused the decrease in the O—H bending and C—O stretching bands, attributed to substitution of C—O—H bonds by C—O—P bonds. FTIR does not seem to be an adequate technique to identify this type of modification in cellulose structure, given that typical phosphate bands are usually more pronounced in the area of the spectrum where cellulose already has several bands, that is, between 900 and 1200 cm^{-1}.[66–68] The sharp peak found at 1384 cm^{-1} and attributed to phosphoryl functionalities is not very often attributed to this group. Probably, the modification introduced promoted a shift of the band usually found at 1250 cm^{-1} to a higher wavenumber.

The Raman spectra of phosphorylated samples (Fig. 6) showed main bands (in cm^{-1}) at 3353 (O—H stretching), 2901 (C—H stretching), 1457, 1374, 1272 (CH_2—OH deformation), 1113, 1089 (C—OH deformation), 960, 896 (Cl—H, CH_2, and C—OH deformation), 840, 433, and 348 (C—O stretching). The band at 840 cm^{-1} on phosphorylated materials can be attributed to P—O functionalities from phosphate groups.[68] Both Raman and FTIR spectra showed that cellulose phosphates were typically less crystalline, given the broad aspect of the spectra obtained. Broadening of the peaks increased with increasing phosphate contents.

Sterilization is a critical step in the evaluation of a medical device because it renders the product free of microbial contamination.[70] Gamma-radiation is generally designated as the most adequate method for cellulose sterilization, although chain scission may occur.[71–73] Hence, FTIR studies were performed to investigate the effect of gamma-radiation on phosphorylated cellulose (Fig. 7). It was shown that even an oxidizing atmosphere did not induce noticeable modifications in the structure, detectable by this technique.
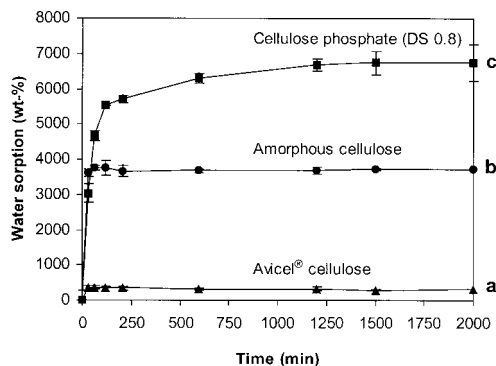
XRD spectra obtained (Fig. 8) allowed the determination of the crystallinity index (Table II). Results obtained indicated that crystallinity decreased with increasing DS, although not significantly in that, at low DS, materials are already considerably amorphous, compared to the original crystalline Avicel cellulose (Fig. 8). However, some remarks must be made about the use of this method. If crystallinity was determined using the maximum and minimum intensity peaks, values for amorphous and phosphorylated cellulose
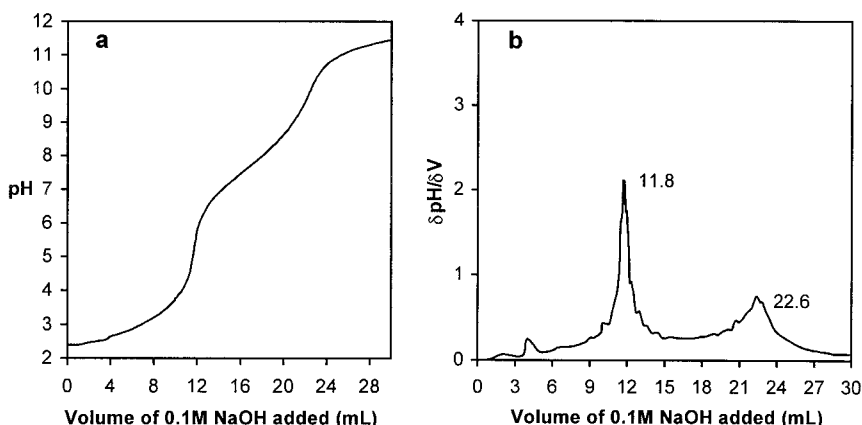
Figure 3 Potentiometric titration data of the neutralization of cellulose phosphate (DS = 1.4) by 0.1M NaOH. (a) Potentiometric titration curve and (b) first derivative, indicating the two equivalence points.
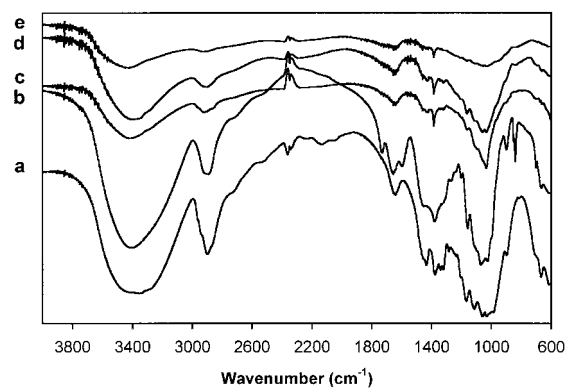
would be significantly higher than those reported, given that the 2Θ angles used for the calculation do not correspond to the peaks of maximum and minimum intensity, as can be seen in Figure 8. For comparative purposes, results from Table II were kept, although it is the authors' conviction that quantitative alternative methods should be used, like thermal methods, and NMR or FTIR spectroscopies.[58,74-78]

The ^{31}P NMR MAS spectrum (Fig. 9) exhibited a signal at −0.4 ppm. This latter chemical shift value is generally expected for ^{31}P in phosphate functionalities.[65,79-81] The ^{31}P MAS NMR studies confirmed that phosphate groups are chemically bonded to the material. Moreover, MAS rate variation showed that symmetrical side bands found at ±50 and ±24 ppm are rotational side bands resulting from powder anisotropy (Fig. 9).[46]

^{13}C CP/MAS NMR studies were carried out to elucidate regioselectivity of this reaction. The spectra of solid powdered phosphorylated cellulose can be seen in Figure 10. It was observed that for high DS values, chemical shifts of carbons bearing OH groups available for substitution (i.e., C-2, C-3, and C-6) were all shifted, thus confirming that cellulose triphosphates were obtained (Table III). At low DS values [Fig. 10(b)], however, different resonance values were preferentially observed in the C-6 carbon, indicating preferential substitution in that position, as could be expected after the previous results from Nehls et al.[82,83] Similar findings were reported using model phosphorylated compounds (D-glucose-1,6-diphosphate and D-glucose-6-phosphate).[83] At high DS values [Fig. 10(c)], considerable changes
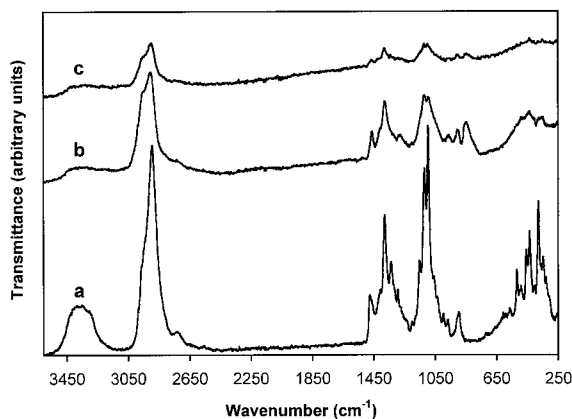

Figure 4 Water swelling of (a) unmodified microcrystalline cellulose Avicel, (b) amorphous cellulose, and (c) cellulose phosphate (DS = 0.8).
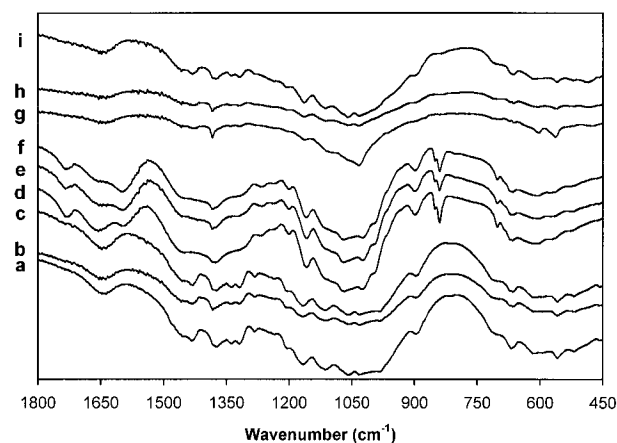

Figure 5 FTIR spectra of (a) unmodified microcrystalline cellulose Avicel, (b) amorphous cellulose, and cellulose phosphates with varying DS values: (c) 0.4, (d) 0.8, and (e) 1.4.

Figure 6   Raman spectra of (a) unmodified microcrystalline cellulose Avicel and cellulose phosphates with varying DS values: (b) 0.8 and (c) 1.4.

in the spectrum could be observed, in positions C-1, C-4, and C-6. In the case of positions C-1 and C-4, which are the carbons responsible for the glycosidic linkage and hence not available to substitution, this is usually attributed to the gamma effect,[84–86] which is a steric effect that indicates a modification in the C-2 or C-3 positions (in the vicinity of C-1 and C-4, respectively), resulting in the shift of the C-1 and C-4 carbons to a higher resonance. It is known that the esterification of a hydroxyl group of glucopyranosic compounds

Figure 7   FTIR spectra of samples sterilized by gamma radiation in air and in a nitrogen atmosphere. (a) unmodified microcrystalline cellulose Avicel; (b) Avicel sterilized in air; (c) Avicel sterilized in nitrogen; (d) amorphous cellulose; (e) amorphous cellulose sterilized in air; (f) amorphous cellulose sterilized in nitrogen; (g) cellulose phosphate (DS = 0.4); (h) cellulose phosphate (DS = 0.4) sterilized in air; and (i) cellulose phosphate (DS = 0.4) sterilized in nitrogen.
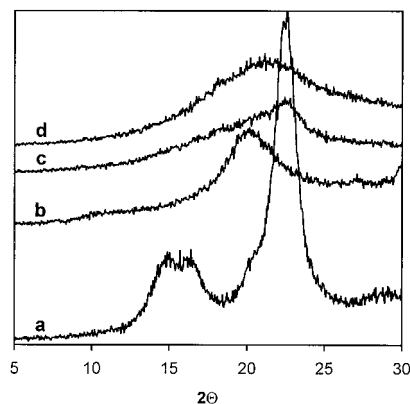
Figure 8   XRD patterns of (a) unmodified microcrystalline cellulose Avicel, (b) amorphous cellulose, and cellulose phosphate at different DS values: (c) 0.8 and (d) 1.4.

causes an upfield shift of the resonance of the adjacent carbons.[83,87–93] Concerning position C-4, the original peak seemed to join the C-2, C-3, and C-5 central peak when the spectra were observed in the solid state but, at higher resolution using hydrated samples (Fig. 11), a shift of about 10 ppm from its original peak could be distinguished. In the case of C-1, a shift of about 4 ppm was observed. In the case of the C-6 position, a downfield shift of the C-6 carbon signal was observed (of ~ 5 ppm), which also was already observed in other cellulose esterification reactions.[83,87–93] This was described as the beta effect, which is a substitutional effect, and it is consistent with the fact that esterification of hydroxyl groups in cellulose causes a strong deshielding. The C-2, C-3, and C-6 carbon peaks moved to a lower resonance region because the electron density of these carbons decreased by the phosphate group, which is an electron-attractive group.

The splitting of the C-6 peak in two (Fig. 11) is the result of grafting in this position and the presence of the original peak means that some

Table II   Crystallinity Index, Determined by XRD, as a Function of the DS Obtained

| Sample	Crystallinity Index (%)
Avicel®	82.9
Amorphous cellulose	0
Cellulose phosphate (DS = 0.8)	32.4
Cellulose phosphate (DS = 1.4)	14.5
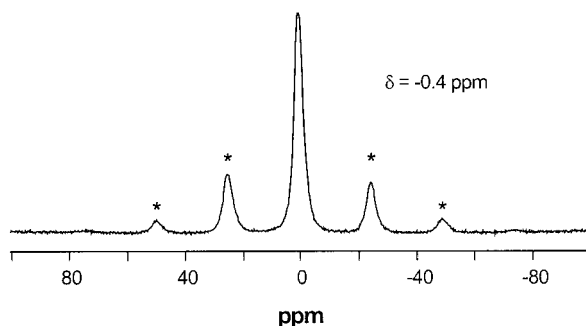
Figure 9 Solid-state ^{31}P MAS NMR spectra of cellulose phosphate (DS = 2.9) at 4 kHz. The asterisk shows spinning side bands. 85% H_3PO_4 was used as the external reference.

C-6 positions are still available. Given that the DS obtained was approximately 3.0, all the C-6 positions (and both C-2 and C-3 as well) should be substituted by phosphate groups. This could mean that some of the free H_3PO_4 was not effectively washed and was kept entrapped in the gel structure. Hence, the DS obtained was probably

not so close to 3.0 but lower, which can also explain the high standard deviation obtained for the DS after 3 days, from the experiments listed in Table I.

In Table III the peaks designated C-1′ and C-4′ may be assigned to C-1 and C-4 carbons adjacent to C-2 and C-3 carbons bearing a substituted hydroxyl group, respectively. These assignments may be supported by the fact that the relative intensities of C-1, C-4, and C-6 peaks decreased as the value of the total DS increased, whereas those of C-1′, C-4′, and C-6s peaks increased. Another aspect to be taken into account in observing the spectra obtained is the splitting observed in some peaks after the chemical modification. Although the reaction was carried out under heterogeneous conditions, the highly homogeneous products obtained suggest that cellulose was probably dissolved in the reaction medium. Phosphoric acid is a well-known cellulose solvent, although degradation may also occur.[63] Hence, cellulose may have been dissolved and then regenerated, which justifies its lower crystallinity after
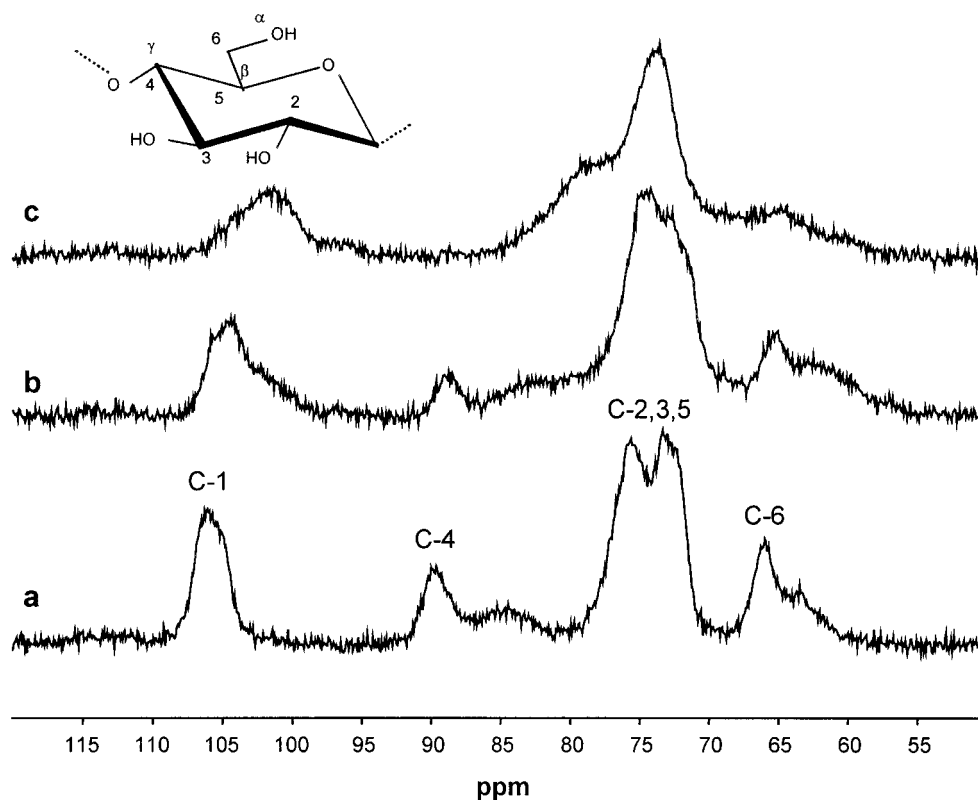
Figure 10 High-resolution solid-state ^{13}C CP/MAS NMR spectra of (a) microcrystalline cellulose Avicel and cellulose phosphate at various DS values: (b) 1.8 and (c) 2.9. TMS was used as the external reference.

Table III Chemical Shifts Obtained from ^{13}C–CP/MAS NMR Data, Indicating the Carbon Positions on Avicel® Cellulose and Cellulose Phosphates

	Chemical Shift (ppm)				
Sample	C-1/ C-1′	C-2, 3, 5		C-4/ C-4′	C-6/ C-6s[a]
Avicel®	106.0	75.6,	73.4,70.4	89.6	66.0
Avicel®, from Ref. 87	104.8	74.4,	71.6,71.4	88.5	64.9
Cellulose phosphate gel (DS = 0.2)	105.0	75.1,	72.3	89.1	65.1
				83.7	61.3
Cellulose phosphate gel (DS = 1.8)	103.0	79.5,	77.5,76.1	81.0	63.8
					61.4
Cellulose phosphate gel (DS = 2.9)	102.4	75.3,	73.7	—	64.6
	101.0			78.3	60.5
Cellulose phosphate (DS = 0.3), from Ref. 82	103.2	73.8,	74.9,75.5	79.6	63.0
					60.9
Cellulose phosphate (DS = 0.3), from Ref. 42	104.1	75.4,	76.7,76.5	78.7	63.9
	102.7				61.7

[a] s = substituted.

phosphorylation by the present method. According to several studies reported in the literature, these splittings were observed as a function of lattice type and state of order of cellulose used.[74,94–96] It was described that changes in conformation and chain packing exert a rather strong influence on the electronic environment of the different carbon atoms. Using solid samples, at low DS values there were no significant differences, compared to unmodified cellulose, except the broadening of the peaks, usually attributed to lower crystallinity.[74,94–96] XRD results (Fig. 9) confirmed that phosphorylation promoted a lower crystallinity compared to that of unmodified cellulose.

It was also confirmed by the NMR experiments that a much higher resolution can be obtained if the NMR experiments are carried out in hydrated products, as previously described in several other works.[97–99] Figure 11 shows that, for gel-forming materials, liquid NMR afforded spectra of high quality, thus enabling the use of a simpler and more easily available technique (solution-state) for obtaining high-resolution spectra of insoluble materials.

The relatively high levels of grafted phosphate groups attainable with this method may be of interest in several applications, in which the materials performance is directly correlated to the degree of substitution. The functionality for biomedical applications is under separate investigation. The use of a gel in orthopedic applications can be an interesting alternative as an injectable material, in minimally invasive surgery. Furthermore, the synthesis of anionic, phosphorus-containing carbohydrate polymers is of interest for biomedical applications for a variety of reasons, including the preparation of peptide- or calcium-binding materials, hemostatic agents, blood stabilizers, or antimicrobial surfaces.[23–34,100–102]

CONCLUSIONS

It was demonstrated that the $H_3PO_4/P_2O_5/$ Et_3PO_4/hexanol method allows highly phosphorylated cellulose derivatives to be obtained. Cellulose triphosphate gels are described here for the first time. Products obtained were poorly crystal-
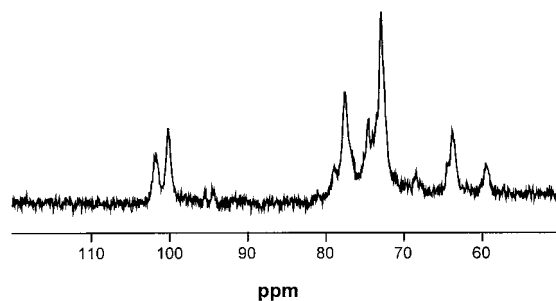
Figure 11 ^{13}C–NMR solution-state spectrum of cellulose phosphate (DS = 2.9) gel. TMS was used as the external reference.

lized monoesters, apparently not significantly affected by gamma sterilization and showed a high water swelling capability. Chemical bonding was confirmed by FTIR, Raman, ^{31}P–, and ^{13}C–NMR spectroscopies.

It was shown here that the $H_3PO_4/P_2O_5/Et_3PO_4$/hexanol method provides a versatile and interesting alternative route to some of the more widely used techniques for the phosphorylation of cellulose. The relatively high levels of phosphorus incorporation attainable with this method may be of interest in applications in which the materials performance is directly correlated to the degree of substitution.

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