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Synthesis and characterization of some new homo- and co-poly(vinylsaccharides). Some preliminary studies as drug delivery

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Abstract

Homopolymers **P1** and **P3** were prepared by free radical polymerization reactions of 1,3,4,6-tetra-*O*-acetyl-2-acrylamido-2-deoxy-α-D-glucopyranose monomer (**M1**) and 1,2:3,4-di-*O*-isopropylidene-6-*O*-acryloyl-α-D-galactopyranose monomer (**M2**), respectively. Both monomers were also copolymerized at two different (**M1**/**M2**) feed ratios (1:1, and 3:1) to yield **P4** and **P5**, respectively. The free radical polymerization reactions were carried out in a mixed solvent system under either nitrogen atmosphere or vacuum at 60°C using AIBN as free radical initiator. **P1** was deacetylated to **P2**, while **P4** was first deacetylated to **P6** and then deacetonated to hydrophilic **P7**. The new poly(vinylsaccharides) were characterized by elemental analysis, DSC, GPC, and FT IR, ^{1}H- and ^{13}C-NMR spectroscopies. Inherent viscosities and specific optical rotations were also recorded. Some preliminary rheological studies have demonstrated that the hydrophobic, non-swelling acrylic polymer **P4** seems to be a good substrate for employment as matrix-forming material for controlled release tablets as drug delivery systems.

Keywords: Poly(vinylsaccharides); Acrylic carbohydrates; Carbohydrate matrixes

1. Introduction

A wide variety of biodegradable polymers has been investigated as potential biomaterials, particularly in the field of controlled drug delivery systems [1–3]. In recent years numerous polymers containing carbohydrate residues have been reported for this purpose, for the coating industry, biomedical engineering, biotechnology, and molecular recognition reactions [4–6]. The polyvalency inherent to carbohydrates is an important feature for the sugar–protein interactions, and most polymeric materials bearing pendant carbohydrates serve, in essence, as cell surface mimics [7].

Sustained release dosage forms have been developed in order to avoid the problems associated with plasma level fluctuations and to increase the intervals between dosage regimens, especially for chronic diseases requiring repeated doses. Such systems have been formulated using various resins, plastics and polymers and applying different techniques. In this way, insoluble plastics such as methyl acrylate–methyl methacrylate or polyvinyl chloride, and hydrophilic polymers such as methyl cellulose and other polysaccharide-based polymers, have been used as matrix-forming materials for tablets. It would be important to find out, whether the typical saccharide properties (such as polarity, multifunctionality, biodegradability and biocompatibility) can be advantageously combined with those of the classic polymers above mentioned. For this reason, we consider of interest the use of poly(vinylsaccharides) in drug delivery.

Most of the reported glycopolymers [poly(vinylsaccharides)] have been prepared by radical polymerization of olefin or acrylamide components attached to the sugar residues via flexible extenders. Carbohydrates have been derivatized through their hydroxy and amino functions including *O*- and *C*-glycosides [8–10], and poly(vinylsaccharides) have been obtained as ethers [11,12], esters [13,14], and amides [15–18]. We now report on the synthesis and characterization of the new acetylated homopolymer **P1** as well as the new copolymers **P4** and **P5**, and also the de-*O*-protected **P6** and **P7**. The poly(vinylsaccharide) **P4** could be the first semi-synthetic polymer of a variety of important carbohydrate-derived matrix-forming materials for controlled drug delivery systems.

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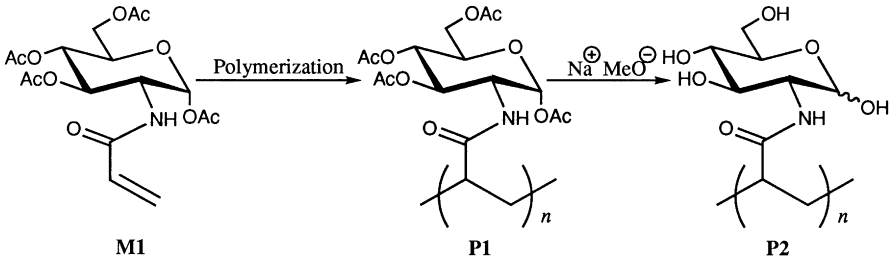
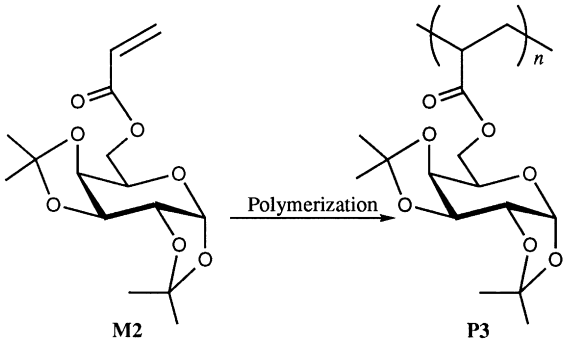

Polymerization
Na MeO
M1
P1
P2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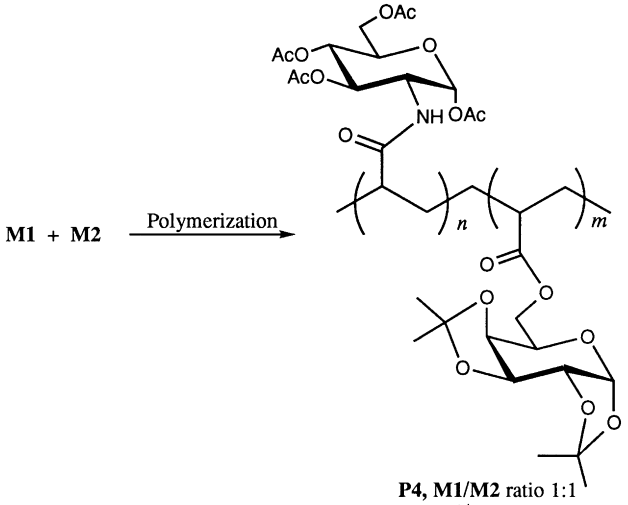
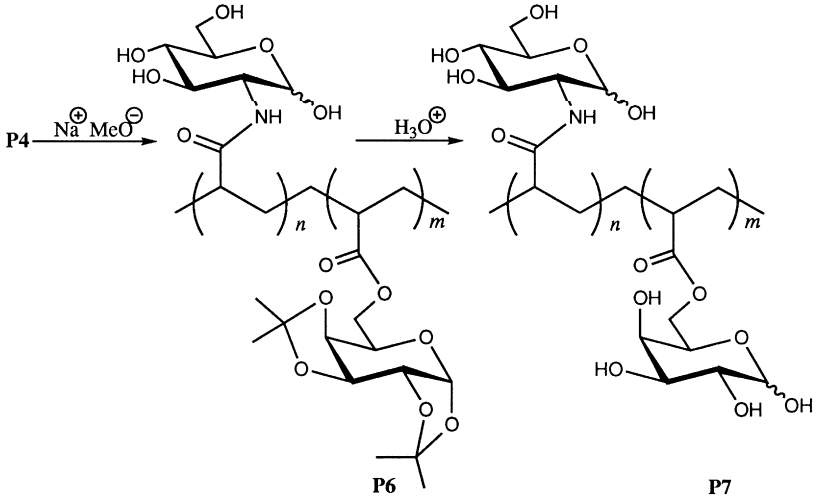
M2
P3


M1 + M2
Polymerization
P4, M1/M2 ratio 1:1
P5, M1/M2 ratio 3:1


P4
Na MeO
H3O
P6
P7

2. Methods

2.1. General

Solvents were purified and dried when necessary, by appropriate standard procedures. 2,2-Azo-Bis-isobutironitrile (AIBN) was recrystallyzed from methanol. FT-IR spectra were recorded with a Michelson 100 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker 200 AC-P spectrometer. Chemical shifts are reported as parts per million down field from tetramethylsilane. Mass spectra were obtained using a Kratos MS80RFA instrument. Optical rotations were mesured at 25 ± 5°C with a Bellingham Stanley Ltd., P20 polarimeter (5 cm cell). Intrinsic viscosity measurements were determined in chloroform or 0.1 M Na_2SO_4 solutions with a Cannon-Ubbelohde semimicroviscometer at a temperature of 25.0 ± 0.1°C. Differential scanning calorimetric (DSC) measurements were carried out in a Perkin–Elmer DSC-7 instrument at a heating rate of 20°C min^{-1} under a nitrogen atmosphere. Gel permeation chromatography (GPC) analyses of **P1**, **P3**, **P4**, and **P5** were carried out in a Waters apparatus fitted with a Waters model 410 RI detector, and a Millenium 2010 computerized data station. Three GPC columns (Waters HR 05, HR 3, and HR 4) were placed in series, and the analysis was performed in chloroform-*O*-chlorophenol (95:5) at a flow rate of 1 ml min^{-1}. Molecular weight studies were determined relative to polystyrene, calibration was done using polystyrene samples of narrow molecular weight distribution, with molecular weights ranging from 687 to 931780 g mol^{-1}. Aqueous GPC was used for **P2**, **P6**, and **P7**. This set-up comprised a Polymer Laboratories HPLC pump, a Pharmacia Biotech. Superdex® 200HR 10/30 FPLC® column and Polymer Laboratories ERC-7515A RI detector. The eluent was a 1 M NaCl/0.014 M $(HOCH_2)_3CNH_2{\cdot}HCl$/0.036 M $(HOCH_2)_3CNH_2$ solution in double distilled water at a flow rate of 0.5 ml min^{-1}. The samples were dissolved in the same solution referred above. Calibration was carried out using a series of poly(ethylene oxide) (PEO) standards (Polymer Laboratories), with molecular weights ranging from 620 to 22 800 g mol^{-1}. Potassium chloride (Acofarma, Tarrasa, E-Barcelona) was used as a model water-soluble drug. The polymer **P4**, hydrophobic and non-swelling, was employed as matrix-forming material. Potassium chloride was sieved (Retsch, type Vibro) and the 200–250 μm granulometric fraction was employed. The 1:1 mixture of KCl and **P4**, was compressed on an eccentric machine (Bonals A-300) without any further excipients. Cylindrical tablets (600 mg) were prepared. The in vitro release assay of the elaborated tablets was performed in the USP XXII apparatus (Turu Grau, model D-6) using the rotating disk method (50 rpm) so that only one surface of the tablet was exposed to the dissolution medium (deaerated water at 37 ± 0.5°C). The amount of KCl released was detected by the increase in conductance of the dissolution medium using a Crison micro CM-2201 digital conductivity-meter [19].

2.2. Monomer syntheses

*1,3,4,6-Tetra-O-acetyl-2-acrylamido-2-deoxy-α-D-glucopyranose (**M1**):* To a suspension of 1,3,4,6-tera-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrochloride (4 g, 10.4 mmol) in dichloromethane (100 ml) were added triethylamine (4 ml, 31.2 mmol) and acryloyl chloride (1.2 ml, 15.6 mmol) and the mixture was stirred at room temperature for 5 min. The resulting solution was diluted with dichloromethane (50 ml), washed with saturated sodium hydrogencarbonate solution (75 ml), and water, then dried (magnesium sulfate). Evaporation gave pure the title compound as a syrup which solidified on standing (4 g, 95%). Several recrystallizations from ethyl acetate afforded the crystalline material (181–182°C); $[\alpha]_D$ +66 (*c* 1, dichloromethane); IR (KBr disk): ν_{max} 1 650 and 1 540 (amide), 1 645 ($CH{=}CH_2$) cm^{-1}; Mass spectrum: m/z 342 $(M-CH_3COO)^+$. 1H-NMR ($CDCl_3$): δ 1.95, 2.05, 2.10, 2.30 (OAc), 3.98 (m, 1H, H^5), 4.03 (dd, 1H, H^{6a}, $J_{5,6a}$ 2.3, $J_{6a,6b}$ 12.6 Hz), 4.23 (dd, 1H, H^{6b}, $J_{5,6b}$ 4.2 Hz), 4.52 (m, 1H, H^2), 5.20 (m, 2H, H^3, H^4), 5.66 (dd, 1H, $=CH_2$(*cis*), J_{gem} 10.2, J_{cis} 1.5 Hz), 5.75 (d, 1H, NH, $J_{NH,2}$ 8.9 Hz), 5.96 (dd, 1H, $=CH_2$(*trans*), J_{trans} 16.9 Hz), 6.18 (d, 1H, H^1, $J_{1,2}$ 3.6 Hz), and 6.23 (dd, 1H, =CH); ^{13}C-NMR ($CDCl_3$): δ 20.5, 20.6, and 20.8 (OAc), 51.1 (C^2), 61.5 (C^6), 67.4 (C^5), 69.7 (C^4), 70.6 (C^3), 90.5 (C^1), 128.0 ($=CH_2$), 129.7 (=CH), 165.2, 168.5, 169.0, 170.7, and 171.8 (C=O). Anal. Calcd. for $C_{17}H_{23}NO_{10}$: C, 50.87; H, 5.77; N, 3.49. Found: C, 50.83; H, 5.58; N, 3.45.

*1,2:3,4-Di-O-isopropylidene-6-O-acryloyl-α-D-galactopyranose (**M2**):* To a solution of the commercially available 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (1.07 g, 4.1 mmol) in dichloromethane (25 ml), triethylamine (1.73 ml, 12.4 mmol) and acryloyl chloride (0.5 ml, 6.2 mmol) were added, and the mixture was stirred at room temperature for 15 min. After work-up as described for **M1**, a syrup was obtained, which was purified by flash column chromatography [1:7 diethyl ether–petroleum ether (50–70°C)] to give **M2** as a solid (0.8 g, 67%); mp 57–58°C [from diethyl ether–petroleum ether (50–70°C)] {Ref. [20] 57–57.5°C (from ethanol–water).}

2.3. Polymer syntheses

General polymerization procedure. The reactions were carried out in a mixture of toluene–dichloromethane (3:1) with AIBN (10^{-3} M) as free radical initiator in a sealed glass tube, which was either degassed under a few freez–thaw cycles or nitrogen gas flashes, at 60°C for 24 h. The polymerization mixtures were added to a large volume of diethyl ether or methanol, filtered, purified by successive reprecipitations in diethyl ether from tetrahydrofuran, and finally dried under vaccum at room temperature for 24 h.

General hydrolysis procedures. Removal of the protecting

Table 1
Yields and some physical properties of polymers **P1**–**P7**

Polymer	Yield (%)	$[\alpha]_D$[a]	$[\eta]$ (dl g^{-1})	T_m (°C)	ΔH (J g^{-1})	$\bar{M}_n$[b]	$\bar{M}_w$[b]	$\bar{M}_w/\bar{M}_n$
P1	100	+20	0.80	183[c]	141	3900	7300	1.9
P2	100	+39	0.35	177[d]	83	4300	9000	2.1
P3	66	−111	0.78	210[e]		4600	22 800	4.9
P4	98	+32	0.82	224[f]	109	17 000	11 4000	6.7
P5	86	+47	0.81	216[f]	63	41 000	153 000	3.7
P6	100	−207	0.81	179	53	8500	18 700	2.2
P7	100	+30	0.80	193	79	7500	11250	1.5

[a] Measured at 20 ± 5°C.
[b] Determined by GPC analysis (see Section 2.1).
[c] After annealing at 150°C for 45 min.
[d] After annealing at 110°C for 15 min.
[e] Decomposition.
[f] After annealing at 130°C for 15 min.

groups was conducted as described in Ref. [21]. For deacetylation, the polymers were dissolved in a mixture of chloroform–methanol containing sodium methoxide as catalyst. The mixtures were stirred for 45 min. The resulting polymers were precipitated and reprecipitated in acetone from methanol, and finally dried under vaccum at 35°C overnight. For deacetonation, the polymer was dissolved in 80% formic acid, and water was gradually added as the reaction proceeded. The solution was stirred for 2 days at 60°C and then the polymer was reprecipitated in ethanol from water, filtered, washed with diethyl ether, and finally dried over P_2O_5 under high vaccum for 2 days at room temperature.

Characterization of the polymers **P1**–**P7** was done on the bases of the data summarised in Table 1, and the following data:

P1: $[\alpha]_D$ +20 (*c* 1.16, chloroform); $[\eta]$ (dl g^{-1}) 0.80 (chloroform); IR (KBr disk): ν_{max} 1747 (C=O, ester), 1665 and 1529 cm^{-1} (C=O, amide). ^{13}C-NMR ($CDCl_3$): δ 20.6 (OAc), 50.5 (C^2 α), 61.6 (C^6 α), 90.3 (C^1 α), 67.6, 68.0, and 69.6 ($C^{3/4/5}$ α), 169.2 (OAc), and 170.6 (C=O, amide). Anal. calcd. for $(C_{17}H_{23}NO_{10})_n$: C, 50.87; H, 5.77; N, 3.48. Found: C, 49.60; H, 5.77; N, 3.25.

P2: Hygroscopic; $[\alpha]_D$ + 39 (*c* 1.35, water); $[\eta]$ (dl g^{-1}) 0.35 (0.1 M Na_2SO_4) {Ref. [17] 52 and 58 (ml g^{-1}) (0.1M Na_2SO_4)}; IR (KBr disk): ν_{max} 3430 (broad, O–H), 1665 and 1529 cm^{-1} (C=O, amide). ^{13}C-NMR (DMSO-d_6): δ 54.1 (C^2 α), 61.0 (C^6 α), 70.7, 71.48, and 76.5 ($C^{3/4/5}$ α), 90.2 (C^1 α), and 174.8 (C=O amide). ^{13}C-NMR (D_2O): δ 54.6 and 57.2 (C^2 α and C^2 β), 61.2 and 63.1 (C^6 α and C^6 β), 91.4 and 95.5 (C^1 α and C^1 β), and 177.7 (C=O, amide). Anal. calcd. for $(C_9H_{15}NO_6)_n$: C, 40.14; H, 7.06; N, 5.20. Found: C, 41.53; H, 6.71; N, 5.12.

P3: $[\alpha]_D$ −111 (*c* 1.13, chloroform) {Ref. [20] −50.1 (*c* 0.5 tetrachloroethane)}; $[\eta]$ (dl g^{-1}) 0.78 (chloroform); IR (KBr disk): ν_{max} 1738 cm^{-1} (C=O, ester). ^{13}C-NMR ($CDCl_3$): δ 24.4, 24.9, and 25.9 (CH_3), 63.4 (C^6 α), 70.4–71.0 (C^2 α, C^3 α, C^4 α and C^5 α), 96.1 (C^1 α), 108.6, and 109.2 (quatern. C, isopropylid.), and 174.2 (C=O, ester). Anal. calcd. for $(C_{15}H_{22}O_7)_n$: C, 57.31; H, 7.05. Found; C, 56.22; H, 6.99.

P4: $[\alpha]_D$ +32 (*c* 1.12, chloroform); $[\eta]$ (dl g^{-1}) 0.82 (chloroform); IR (KBr disk): ν_{max} 1750 (C=O, ester) 1681 and 1529 cm^{-1} (C=O, amide). ^{13}C-NMR ($CDCl_3$): δ 20.6, 24.4, 24.9, and 25.9 (CH_3 of **M1** and **M2**), 50.6 (C^2 α **M1**), 61.6 (C^6 α **M1**), 63.4 (C^6 α **M2**), 90.5 (C^1 α **M1**), 96.2 (C α **M2**), 108.7 and 109.3 (quatern. C, isopropylid.), 169.3 (C=O, acetate), 170.6 (C=O, amide), and 174.5 (C=O, ester). Anal. calcd. for $(C_{17}H_{23}NO_{10})_n$: C, 53.70; H, 6.33; N, 1.95. Found: C, 52.90; H, 6.22; N, 2.20.

P5: $[\alpha]_D$ +47 (*c* 1.12, chloroform); $[\eta]$ (dl g^{-1}) 0.81 (chloroform). IR and ^{13}C-NMR spectra were identical to P4. Anal. calcd. for $(C_{51}H_{69}N_3O_{30})_n$ $(C_{15}H_{22}O_7)_m$: C, 52.20; H, 6.04; N, 2.76. Found: C, 51.45; H, 5.92; N, 2.91.

P6: Hygroscopic; $[\alpha]_D$ −207 (*c* 1.15, dimethylsulfoxide); $[\eta]$ (dl g^{-1}) 0.81 (dimethylsulfoxide); IR (KBr disk): ν_{max} 3340 (broad, O–H), 1728 (C=O, ester), 1653 and 1540 cm^{-1} (C=O, amide). ^{13}C-NMR (DMSO-d_6): δ 20.4–25.9 (CH_3 of **M2**), 50.6 (C^2 α **M1**), 61.6 (C^6 α **M1**), 63.4 (C^6 α **M2**), 90.5 (C^1 α **M1**), 96.2 (C^1 α **M2**), 108.7 and 109.3 (quatern. C, isopropylid.), 170.6 (C=O, amide), and 174.7 (C=O, ester). Anal. calcd. for $(C_9H_{15}NO_6)_n$ $(C_{15}H_{22}O_7)_m \cdot 1.5\ H_20$: C, 50.17; H, 7.02; N, 2.44. Found: C, 50.02; H, 6.75; N, 3.01.

P7: Hygroscopic; $[\alpha]_D$ + 30 (*c* 1.09, water); $[\eta]$ (dl g^{-1}) 0.80 (0.1 M Na_2SO_4); IR (KBr disk): ν_{max} 3340 (broad, O–H), 1721 (C=O, ester) 1654 and 1550 cm^{-1} (C=O, amide). ^{13}C-NMR (DMSO-d_6): δ 50.6 (C^2 α **M1**), 61.6 (C^6 α **M1**), 63.4 (C^6 α **M2**), 90.3 (C^1 α **M1**), 96.2 (C^1 α **M2**), 170.6 (C=O

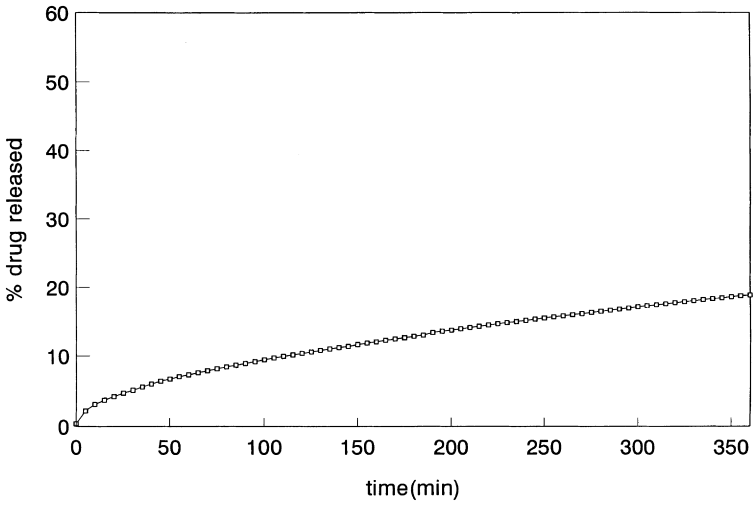


Fig. 1. In vitro release profile of a tablet prepared with polymer P4 and KCl, 1:1.

amide), and 174.7 (C=O, ester). Anal. calcd. for $(C_9H_{15}NO_6)_n$ $(C_9H_{14}O_7)_m$ 1.5 H_20: C, 43.73; H, 6.52; N, 2.83. Found: C, 43.01; H, 5.89; N, 2.70.

2.4. Determination of the solid state properties

Bulk and tapped densities: In order to determine the bulk density of **P4**, 2 g of the polymer were put into a 10 ml graduated cylinder. The bulk density was 0.2304 g ml^{-1} (calculated from the volume occupied by the solid).

To determine the tapped density, the product was subjected to three series of 500 percussions. The tapped density was 0.2723 g ml^{-1} (calculated from the final volume).

Haussner index (HI) and percentage compressibility (%C) determination:

HI = tapped density/bulk density = 1.18

%C = (tapped density − bulk density) × 100/tapped density = 15.39%

3. Results and discussion

The synthesized monomers **M1** and **M2** were homopolymerized to **P1** and **P2**, respectively, and copolymerized with two monomer feed ratios (**M1**/**M2**), i.e. 1:1 and 3:1, to **P4** and **P5**, respectively. The polymerization reactions were all carried out in a mixed solvent consisting of toluene–dichloromethane (3:1) at 60°C with AIBN as initiator. The conversion of **M1** in the homopolymerization was quantitative, while lower degree of conversion was obtained with **M2** (66%).

The homopolymer **P1** was deacetylated in a mixture of chloroform–methanol with sodium methoxide, to a water-soluble polymer **P2**, while the copolymer **P4** was first deacetylated to **P6** followed by deacetonation to a water-soluble polymer **P7**. All polymers were obtained as white materials except **P7** which was obtained as a pinkish powdery product. **P1**, **P3**, **P4** and **P5** were all water insoluble, while **P6** was soluble in both polar and non-polar solvents.

The thermal behavior of the synthesized polymers was investigated by DSC (see Table 1). Homopolymer **P1** did not show endotherms corresponding to melting transitions during the first heating cycle between 50 and 215°C. After this temperature, decomposition was observed. However, when the polymer was subjected to annealing at 150°C for 45 min an endotherm corresponding to melting was observed at 183°C. The deacetylated polymer **P2** did not show melting transitions during the first heating cycle, but after annealing at 110°C for 15 min showed an endotherm at 177°C. The polymer **P3** did not show any endotherm of melting even after annealing at different temperatures. The only observed transition was a broad exotherm at 191°C and decomposition after 210°C. Copolymers **P4** and **P5** also showed a similar behavior. After annealing at 130°C for 15 min both polymers presented melting peaks, preceded by crystallization exotherms, at 224 and 216°C, respectively. The partially deprotected polymer **P6** showed a melting endotherm at 179°C preceded by a crystallization exotherm centered about 170°C. After 190°C decomposition occurred. Similarly, the fully deprotected polymer **P7** showed a melting endotherm at 193°C preceded by a crystallization exotherm at 185°C. After 200°C decomposition occurred.

The intrinsic viscosity results and the specific optical rotation data were found to be in good agreement to what has been reported for other poly(vinylsaccharides) of the type esters and amides [13–18]. The weight-average molecular weights of copolymers **P4** and **P5** were found to be 1.14×10^5 and 1.53×10^5, respectively. The elemental analyses for polymers **P1–P5** are in accordance with the anticipated chemical composition. The de-*O*-protected polymers **P6** and **P7** were very hygroscopic and the elemental

analytical data always showed some residual amount of water. The IR, and ^{13}C-NMR spectra showed that these polymers had lost their respective *O*-protecting groups, as expected. When the ^{13}C spectra of the de-*O*-protected **P2**, **P6** and **P7** were taken in DMSO-d_6, only α-anomeric forms were detected; however, **P2** in D_2O showed a mixture of α- and β-anomers as was also expected (see Section 2).

Some preliminary studies on rheology were carried out with the obtained polymers. The ability to flow of these substances was observed and their compressibility was estimated; the obtained data were used only for comparison purposes at a qualitative level. The hydrophobic and non-swelling polymer **P4** showed the best results, therefore this polymer was selected to carry out additional studies. The bulk and tapped densities, HI and %C, were calculated for this drug following the procedure described in Section 2. Under these conditions, the bulk density of the polymer **P4** was 0.2304 g ml^{-1}, the tapped density being 0.2723 g ml^{-1}, HI = 1.18 and %C = 15.39%. These results showed the adequate flow properties of the polymer, especially by taking into account the bad flow that most hydrophobic compounds exhibit. These adequate flow properties were confirmed by compressing a mixture of **P4** and potassium chloride (employed as hydrophilic and water-soluble drug model) in a 50% (w/w), on an eccentric machine. The obtained tablets exhibited very slow release of the hydrophilic drug (see Fig. 1), showing no disintegration during the release assay. These results demonstrated that the polymer **P4** had adequate rheological and compressional properties, which afforded a slow release inert matrix-tablet without addition of any other excipient. Tablets remained intact after the release assay.

On the basis of these preliminary results, the polymer **P4** is a good candidate to be used as matrix-forming material in the pharmaceutical industry. The synthesis of new polymers, which can be used to prepare controlled release drug delivery systems, is very interesting in the design of pharmaceutical dosage forms, because their mechanical and biopharmaceutical properties may lead to the improvement of the design of a great number of pharmaceutical formulations. Additional studies in this field are currently in progress.

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