Carbohydrate Polymers 88 (2012) 1033–1040

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Rheological characterization and release properties of inulin-based hydrogels

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ARTICLE INFO

Article history:
Received 26 October 2011
Received in revised form 12 January 2012
Accepted 19 January 2012
Available online 28 January 2012

Keywords:
Inulin
UV irradiation
Chemical hydrogels
Rheological measurements
Drug release

ABSTRACT

In the present study the rheological properties of hydrogels obtained through UV irradiation of an aqueous solution of methacrylated inulin (INUMA) alone or in the presence of four different crosslinkers, have been recorded as a function of irradiation time, at two different wavelengths (320 and 365 nm) and fixed frequency and amplitude by oscillatory experiments. Each hydrogel has been analyzed by measurements of the frequency-dependency of the elastic modulus, G', in the linear viscoelastic region. The amount of polymeric chains giving an elastic response has been correlated to the chemical nature of the crosslinker used together with INUMA. Finally the release properties of the hydrogel exhibiting the highest elastic modulus have been tested by using 5-Fluorouracil, as a model drug, and two different loading procedures. The rheological characterization allowed us to determine the most effective condition (appropriate crosslinker, wavelength and irradiation time) to obtain a strong hydrogel that was able to give a modified drug release in simulated gastrointestinal fluids.

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1. Introduction

Inulin (INU) is a natural polysaccharide belonging to the fructan group. It mainly, if not exclusively, consists of β(2 → 1) fructyl fructose units (F_m) with normally, but not necessarily, one glucopyranose unit at the reducing end (GF_n) of the chain (De Leenheer, 1996; Hirst, McGilvray, & Percival, 1950; Stevens, Meriggi, & Booten, 2001; Van Loo, Coussement, De Leenheer, Hoebregs, & Smits, 1995). It is present in many plants, including onion, garlic, leek, chicory and artichoke. Its average chain length and molecular weight distribution depend on the plant species, the growth phase and preparation method. Average chain lengths of 30 fructose units are usual (Kunz & Begli, 1996).

The growing interest in INU is due to its properties: it is non-toxic, biocompatible, water soluble, specifically degraded in the colon and very cheap polymer (Biedrzycka & Bielecka, 2004; Gibson & Roberfroid, 1995; Wang & Gibson, 1993).

Moreover, there are many beneficial effects of INU and oligosaccharides in general, for human health. It must be mentioned, particularly:

(1) reduction of blood lipid levels;
(2) selective stimulation of growth of bifidobacteria population in the intestine;
(3) enhancement of the absorption of minerals in the colon.

INU is also used in diagnostics for the determination of the renal clearance and in several foodstuffs as a dietary fiber (Knudsen & Hessov, 1995) and no significant inulin side effects have been noted in the medical literature except the case of very high concentrations.

Series of animal studies demonstrate that inulin-type fructans affect the metabolism of lipids primarily by decreasing triglyceridaemia (Delzenne et al., 1993; Fiordaliso et al., 1995). Recent studies have shown that the effects on serum triglycerides are due to the reduced secretion of the very low density lipoprotein (VLDL) particles from the liver and associated with decreased activity and gene expression of the key regulatory enzyme, fatty acid synthetase (Kok, Roberfroid, & Delzenne, 1996a, 1996b). Although the data obtained from animal studies suggest convincing lipid-lowering properties of INU and oligofructose compounds in general, much less information is available from human studies, in which the

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doi:10.1016/j.carbpol.2012.01.059

doses that can be applied are much lower than those used to elicit effects in animals (Williams, 1999).

Both inulin and oligofructoses have been demonstrated to be effective prebiotics, as demonstrated by *in vitro* and *in vivo* studies in several laboratories (Cummings, Macfarlane, & Englyst, 2001; Gibson & Wang, 1994; Gibson, Beatty, Wang, & Cummings, 1995; Roberfroid, Bornet, Bouley, & Cummings, 1995). They exert prebiotic properties, especially towards the selective stimulation of colonic bifidobacteria having a high role against the gut pathology. Inulin and oligofructose are increasingly used in many foods such as drinks, yogurts, biscuits and table spreads.

The effects of INU and oligofructose compounds or their combination, on the absorption and balance of minerals have also been investigated in animals (Coudray, Tressol, Gueux, & Rayssiguier, 2003; Delzenne, Aertssens, Verplaetse, Roccaro, & Roberfroid, 1995; Ohta et al., 1995; Younes et al., 2001). These studies show that INU and oligofructoses are able to increase the Mg absorption from the colon even in Mg-deficient rats (Ohta et al., 1995). Further, they have also shown that the absorption of calcium is enhanced by the oligofructose compounds (Ohta et al., 1994).

In consideration of these beneficial effects, in recent years, the interest in INU has been concentrated on its chemical derivatization in order to obtain systems suitable for various purposes such as surfactants, complexes with minerals, microspheres and hydrogels (Maris et al., 2001; Mandracchia et al., 2011; Pitarresi, Tripodo, Cavallaro, Palumbo, & Giammona, 2008; Pitarresi et al., 2008; Pitarresi, Tripodo, Triolo, Fiorica, & Giammona, 2009; Poulain et al., 2003; Rogge & Stevens, 2004; Stevens et al., 2001; Vervoort et al., 1997; Wu & Lee, 2000)

The aim of this work has been the preparation and rheological characterization of new hydrogels based on INU derivatives designed as potential drug delivery systems for oral administration. Particularly, to allow the crosslinking *via* UV irradiation, INU molecules have been derivatized with methacrylic anhydride (MA) to introduce, along its chains, pendent double bonds reactive towards radical reactions activated by UV rays that cause the formation of a chemical hydrogel (see in Fig. 1 the chemical structure of INUMA derivative).

Furthermore, aqueous solutions of INUMA thus obtained, have been used as such or with the addition of four different crosslinker molecules in order to obtain hydrogels with a wide range of elasticity depending on the chemical nature of the crosslinker and the irradiation time. Further, the release properties of the elastically strongest obtained hydrogel have been tested *in vitro* by using 5-Fluorouracil, chosen as a model drug.

2. Experimental

2.1. Materials

All reagents were of the best available commercial grades. Methacrylic anhydride (MA), INU from Dahlia Tubers $M_w \approx 5000$ Da, triethylamine (TEA) were from Fluka (Italy). Anhydrous *N*,*N*-dimethylformamide (DMF) 99.9%, 5-Fluorouracil and D_2O (isotopic purity 99.9%) were purchased from Aldrich Chemical Co. (Italy). Diethyl ether and acetone were purchased from Merck (Germany). Pullulan GPC standards were from Polymer Laboratories (Germany). The used crosslinkers (Fig. 2) were:

1. poly(ethylene glycol) diacrylate (PEGDA, $M_w \approx 700$ Da) (Sigma Aldrich);
2. poly(ethylene glycol) dimetacrylate (PEGDMA, $M_w \approx 750$ Da) (Sigma Aldrich);
3. N-N′ methylene-bis-acrylamide (BIS, $M_w = 154.17$ Da) (Sigma Aldrich);

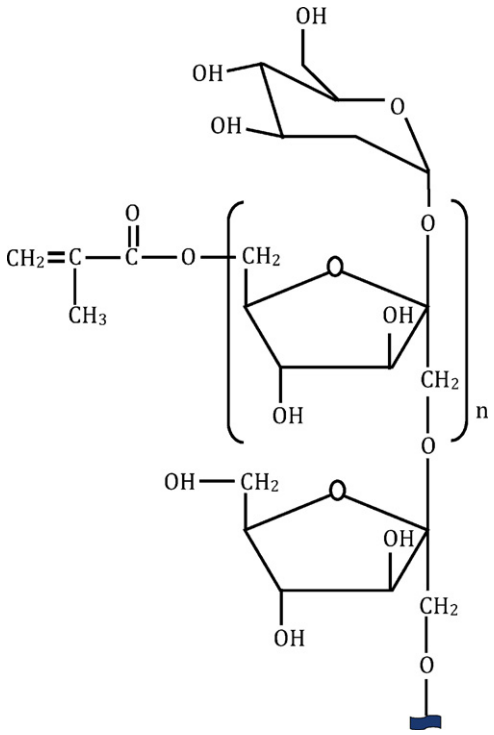

Fig. 1. Chemical structure of INUMA derivative.

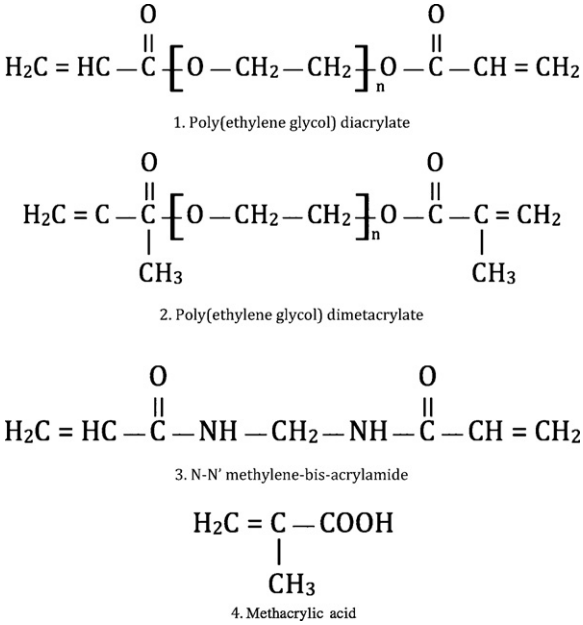

Fig. 2. Chemical structures of crosslinkers added to INUMA aqueous solution before UV irradiation.

4. metacrylic acid (AcMA, $M_w = 86$ Da) (Sigma Aldrich).

2.2. Apparatus

Molecular weight of INUMA was determined by a SEC system equipped with a pump system, a Ultrahydrogel 1000 (size exclusion range 10,000–500,000 Da) and a Ultrahydrogel 250 (size exclusion range 1,000–50,000 Da) as columns and a 410 differential refractometer (DRI) as a concentration detector, all from Waters (USA). The following conditions were employed: phosphate buffer solution 0.05 M pH 7.2 as a mobile phase, 35 °C, flow 0.6 ml/min. The

molecular weight was determined by using Pullulan standards (range 300–150,000 Da).

^{1}H NMR(D_2O) spectra were obtained with a Bruker AC-250 instrument.

FT-IR spectra were obtained, after 100 scans, with a Perkin-Elmer 1720 Fourier Transform Spectrophotometer in the range 4000–400 cm^{-1} with a resolution of 1 cm^{-1}. Samples were in KBr pellets.

Centrifugations were performed with an International Equipment Company Centra MP4R equipped with an 854 rotor and temperature control.

Release studies were performed in a Benchtop 80 °C Incubator Orbital Shaker model 420 at 37 ± 0.1 °C and at 100 rpm.

High-pressure liquid chromatography (HPLC) analyses were carried out by using an Agilent 1100 Liquid Chromatograph equipped with a Rheodyne 7125 injector (fitted with a 20 μl loop) and an Agilent 1100 HPLC detector in line with a computerized workstation. Column: reversed-phase C_{18} (μBondapak; 10 μm of 250 × 4.6 mm internal diameter, obtained from Waters). Mobile phase: H_3PO_4 0.05%v/v/CH_3OH, 98:2, flow 1 ml/min, λ = 266 nm.

The amount of 5-Fluorouracil released from hydrogel was evaluated by using two standard calibration curves: in HCl 0.1 N ($y = 66{,}532x + 22{,}499$; $R^2 = 0.9958$) and in HCl 0.1 N/Na_3PO_4 0.2 M/NaOH 1 N pH 6.8 ($y = 54{,}992x - 31{,}539$; $R^2 = 0.9961$).

The particle size was studied using a Leica Quantimet Q 500 image processing and analysis system equipped with a Leica Wild 3D stereomicroscope. The image processor used for the determination of the particle size also calculates the roundness index, which was always below 1.3. The roundness index (R_i) is a parameter which gives information about particle shape. It was calculated using following ratio:

$$R_i = \frac{p^2}{4 \times \pi \times A \times 1.064} \quad (1)$$

where p and A are the perimeter and the area of the particle, respectively, and 1.064 is a correction factor for the angles produced by the image digitalization. Values of the roundness index close to 1 indicate an almost spherical shape.

2.3. Derivatization of INU with MA (INUMA)

Before use, INU was dried for 24 h at 70 °C. 1 g of INU was then dissolved in 14 ml of anhydrous DMF under argon for at least 3 h. After complete solubilization, suitable amounts of TEA, as a catalyst, and MA were added according to $X = 0.5$ and $Y = 0.5$, being:

$$X = \frac{\text{moles of MA}}{\text{moles of INU repeating unit}} \quad (2)$$

$$Y = \frac{\text{moles of TEA}}{\text{moles of MA}} \quad (3)$$

The reaction mixture was stirred at 25 °C under argon for 24 h. After this time, the reaction mixture was precipitated in 140 ml of a mixture diethyl ether/acetone (2:1) and centrifuged for 15 min at 12,000 rpm at the temperature of 4 °C. The product was recovered, washed several times with the same mixture of solvents and then dried under reduced pressure.

2.4. Characterization of INUMA derivative

FT-IR (KBr): 3300 (br, υ_{as} OH); 1717 (υ_{as} COO), 1304 (scissoring −C═*C*−H) cm^{-1}.

^{1}H NMR (D_2O): $\delta = 1.95$ (3H, s, CH_3—C), 3.5–4.0 (5H, m, −*C*H_2—OH; C*H*—CH_2—OH; −CH_2—*C*H_2—O—), 4.14 (1H, t, C*H*—OH), 4.25 (1H, d, C*H*—OH), 5.79 and 6.22 (2H, 2s, CH_2═C).

The degree of derivatization in MA groups linked to INU (*DD*) was determined by ^{1}H NMR by comparing the integral of the peaks at $\delta = 5.79$ and 6.22 (2H, 2 s, CH_2═C, belonging to the linked MA) with the integral of the peaks between $\delta = 3.5$ and 4.25 (belonging to the INU unit). The value of *DD* was found to be 20 ± 2 mol%.

2.5. Sample preparation

INUMA powder was dissolved in Millipore Super Q H_2O to obtain a concentration of 60 mg/ml. All crosslinkers (Fig. 2) have been added at the concentration corresponding to the 20 mol% with respect to the moles of MA linked to INU. Methacrylic acid (AcMA) has been also added at the concentration corresponding to the 40 mol% with respect to the moles of MA linked to INU.

2.6. UV irradiation and rheological measurements

All samples have been irradiated *in situ* on the quartz plate of the TA Instruments AR-G2 rheometer equipped with the UV lamp OmniCure S 2000 system. The UV beam was carried on the rheometer plate through an optical fiber. The wavelengths were selected at 320 or 365 nm through two optical filters placed immediately below the quartz window. The power of the lamp was set at 180 mW/cm^2 measured with an UVmeter. The geometry was a plate-plate system with a diameter of 20 mm.

Kinetic measurements were obtained during UV irradiation up to 100 min because of the polymer degradation occurring 120 min after the beginning of the irradiation procedure, as evidenced by the sudden decrease in the G' value. The frequency was 1 Hz and the strain 0.08.

Mechanical spectra were performed at the temperature of 25 ± 0.1 °C through a built-in electric system cooled by a Haake thermostat. The frequency sweeps have been performed at different irradiation times chosen as the time at which the 5, 25, 50, 75 (data not shown) and 100% of the maximum excursion of the elastic modulus occurred. The time was obtained by the kinetic measurements.

The frequency range was 0.02–30 Hz, with a strain of 0.08. The measurements started with a delay of 10 min after the switching off of the lamp, to allow the extinguishing of the radical reactions, as confirmed by experimental measurements. All measurements were performed at least in triplicate.

2.7. Drug loading procedures

In the case of drug incorporated hydrogel (sample A), the 5-Fluorouracil was added to the fresh aqueous solution of INUMA + PEGDA to obtain a final drug concentration of 0.65 μM. Then, the solution containing polymer, crosslinker and drug was placed on the rheometry plate and irradiated at 320 nm for 100 min in which the values of the viscoelastic parameters, as a function of time, have been recorded. Then, after a 10 min stop, the G' and G'' values have been recorded as a function of frequency in the range 0.2–30 Hz with a strain of 0.08. After the rheological measurements, the samples were recovered and treated with acetone in order to obtain a powder that has been dried at 10^{-1} mmHg in the presence of P_2O_5 until a constant weight was reached. Preliminary studies have showed that 5-Fluorouracil does not undergo alteration after UV irradiation. In particular, an aqueous solution of 5-Fluorouracil (10 mg/ml) has been irradiated for 100 min at 320 nm. After irradiation, the solution has been analyzed by HPLC, then lyophilized and the solid residue characterized by FT-IR analysis. The absence of alteration of drug was confirmed by comparing the HPLC chromatogram and FT-IR spectrum with those of non irradiated 5-Fluorouracil.

In the case of drug loading by soaking procedure (sample B), the 5-Fluorouracil was added to the dried sample (hydrogel of INUMA/PEGDA irradiated at 320 nm for 100 min) obtained after

its recovery from the rheometry plate. Therefore, a concentrated solution of 5-Fluorouracil in twice distilled water was added to INUMA/PEGDA hydrogel. The mixture was maintained at room temperature under stirring for 3 days. After this time, the solvent was removed by filtration and the sample was rapidly washed with twice distilled water in order to remove exteriorized 5-Fluorouracil, then with acetone as in the case of sample A.

2.8. Determination of drug amount entrapped into INUMA/PEGDA hydrogel

50 mg of drug loaded INUMA/PEGDA hydrogel (sample A or sample B) were extensively extracted at room temperature with twice distilled water. The liquids of extraction were filtered with 0.45 μm acetate cellulose membrane filter. The amount of 5-Fluorouracil loaded into each hydrogel was evaluated by HPLC analysis and it was expressed as drug loading (DL) percentage calculated as:

$$\mathrm{DL}(\%) = \frac{W_d}{W_t} \times 100 \quad (4)$$

where W_d is the weight of loaded drug and W_t is the weight of drug loaded hydrogel.

Each experiment was performed in triplicate. DL resulted to be 9.2% (w/w) for sample A and 8.9% (w/w) for sample B.

2.9. Drug release at pH 1.0 and 6.8 from INUMA/PEGDA hydrogel

Aliquots (10 mg) of the drug loaded INUMA/PEGDA hydrogel (sample A or sample B) were dispersed in flasks containing 40 ml of HCl 0.1 N (pH 1.0, simulated gastric fluid) and maintained at 37 ± 0.1 °C in an incubator for 2 h (100 rpm). Since the drug release was not complete after 2 h of incubation at pH 1.0, a solution of 0.2 M tribasic sodium phosphate/NaOH 1 N was added to raise the pH to 6.8 (simulated intestinal fluid), according to the method reported in USP XXII (drug-release test, method A for enteric-coated particles). Then the experiment was continued until 24 h. Sink conditions were maintained throughout the experiment. Then, at suitable time intervals, samples were filtered through a 0.45 μm cellulose membrane filter and analyzed by HPLC.

Each experiment was carried out in triplicate.

3. Results and discussion

The dynamic storage and loss moduli as a function of frequency before UV irradiation for INUMA aqueous solution (60 mg/ml) are reported in Fig. 3.

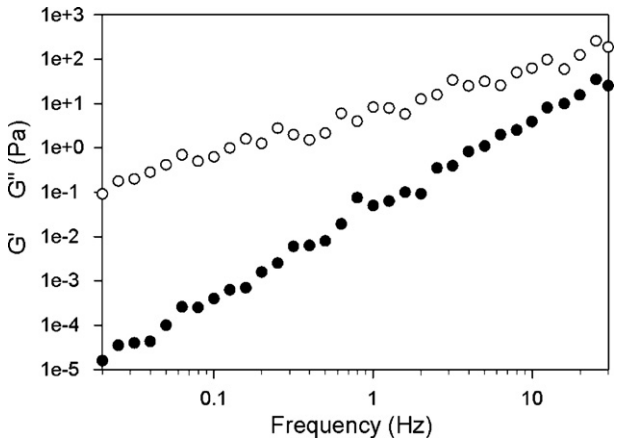

Fig. 3. Mechanical spectrum of a INUMA aqueous solution (60 mg/ml) before UV irradiation (G': solid circles; G'': empty circles).

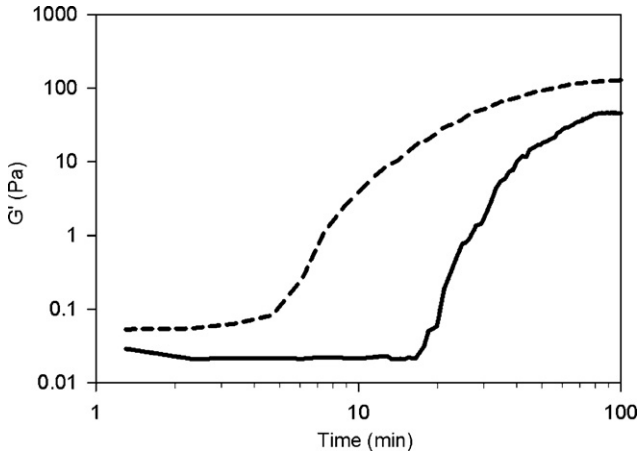

Fig. 4. G' values for INUMA hydrogel recorded as a function of UV irradiation time at 320 (dashed line) and 365 nm (continuous line).

As expected, before UV irradiation, the sample exhibits a typical liquid like behavior with an increase in both moduli at increasing the frequency. The same behavior is observed for all investigated samples, i.e. aqueous solutions containing INUMA mixed with each crosslinker (PEGDA, PEGDMA, BIS or AcMA).

When an aqueous solution of INUMA is photo-irradiated, thanks to the presence of pendent double bonds reactive towards the radical reactions activated by UV rays, a crosslinking occurs with formation of a chemical hydrogel.

Fig. 4 shows G' values obtained as a function of time for the two wavelengths used, 320 and 365 nm.

As it can be observed, the formation of intermolecular crosslinks is evidenced by the increase in the elastic modulus for both the wavelengths tested in our experiments.

The value of G' is a measure of the energy stored and recovered per cycle of oscillatory deformation and it represents the elastic fraction of the material (Ferry, 1961). Beside intermolecular crosslinks, this parameter is also related to physical chain entanglements, intramolecular crosslinks and chains attached by a single bond.

As expected, due to the higher energy content, the irradiation is more effective at the lower wavelength (320 nm), thus leading to a faster formation of a more elastic network.

Fig. 5A and B shows the G' values of INUMA hydrogels in comparison with those of samples obtained starting from INUMA irradiated in the presence of the different crosslinkers.

Also in this case, as expected, the UV irradiation of INUMA in the presence of each crosslinker at 320 nm is more effective than 365 nm.

Referring to the INUMA sample, both at 320 (Fig. 5A) and 365 nm (Fig. 5B), three different behaviors, due to the addition of crosslinkers, can be observed:

(i) INUMA/PEGDA hydrogel, suddenly exhibits a very strong solid-like character few min after the UV irradiation and, in about 5 min, the plateau value is reached;
(ii) the presence of PEGDMA and BIS also accelerates the UV rays effect. As soon as the UV irradiation is applied, the value of G' of INUMA/PEGDMA and INUMA/BIS hydrogels gradually increases and reaches the plateau value in 100 min from the beginning of the kinetic measurement.
(iii) on the contrary, the presence of AcMA, for both the investigated concentrations (20 or 40 mol% with respect to the moles of MA linked to INU), inhibits the network formation. No G' signal increase is detected up to 10 min from the UV irradiation

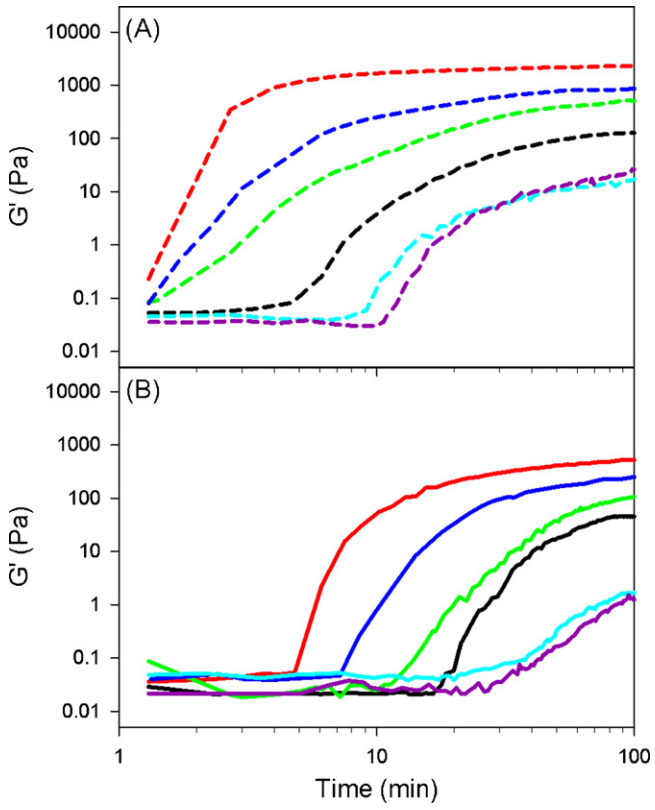

Fig. 5. G' values recorded as a function of UV irradiation time at 320 (A) (dashed line) and 365 nm (B) (continuous line) for INUMA/PEGDA (red), INUMA/BIS (blue), INUMA/PEGDMA (green), INUMA (black), INUMA/AcMA 20 mol% (sky blue) and INUMA/AcMA 40 mol% (violet) hydrogels. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

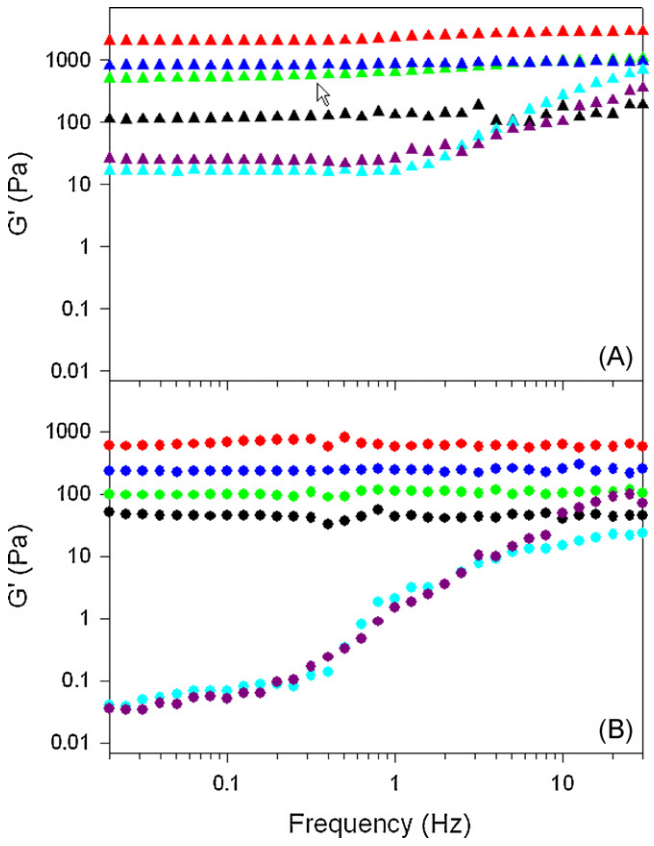

Fig. 6. G' values as a function of oscillation frequency obtained at the end of the irradiation time at 320 (A) (triangles) and 365 nm (B) (circles) for INUMA/PEGDA (red), INUMA/BIS (blue), INUMA/PEGDMA (green), INUMA (black), INUMA/AcMA 20 mol% (sky blue) and INUMA/AcMA 40 mol% (violet) hydrogels (G'' values are not shown for an easier reading of the data). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

and only a weak elastic behavior is reached 100 min after the beginning of the measurements.

G' values recorded as a function of frequency at the end of the kinetic measurements are in agreement with these findings (Fig. 6).

The results (G'' values are not shown for an easier reading of the data) show that the elastic moduli are independent of the frequency for the INUMA, INUMA/PEGDA, INUMA/PEGDMA, and INUMA/BIS samples in the investigated time, thus indicating that a three dimensional network is formed.

On the contrary, the addition of AcMA (20 and 40 mol%) is not able to originate a strong network, in fact, at the high frequencies, a behavior considerably dependent on frequency can be observed.

The obtained results suggest that the addition of crosslinkers having different chemical structure and molecular weight facilitates or inhibits the network formation. In particular:

(i) the high molecular weight of PEGDA (700 Da) added to INUMA aqueous solution makes easy the approaching of the INUMA chains that react with each other and with PEGDA molecules, thus facilitating the crosslinking reaction;
(ii) although the PEGDMA molecules have a molecular weight similar to that of PEGDA (750 vs 700 Da), the presence of methacrylic group in the immediacy of the reactive vinyl group, could cause a slowdown of the radical reaction due to its steric hindrance. The hydrogel formed in this case, is less elastic and the G' value is lower than those observed for INUMA/PEGDA and INUMA/BIS hydrogels;
(iii) when N-N′ methylene-bis-acrylamide (BIS) is added to the INUMA solution, an intermediate behaviour between those found in the presence of PEGDA and PEGDMA is observed. We can suppose that in this case the data are the results of two opposite effects: for one hand, the absence of steric hindrance in the proximity of the reactive groups could favour the linking formation; for the other hand, the low molecular weight of BIS (154.17 Da) makes more difficult the approaching of the INUMA chains that should react with each other and with BIS molecules.

Finally, the addition of methacrylic acid inhibits the network formation. This behavior could be explained considering that, in aqueous solution, the carboxyl group of the crosslinker is ionized. Once linked to the polymer chain, the electrostatic repulsion, due to the negative charges, moves away the polymer molecules thus slowing down the radical reaction and as a consequence the crosslinking process.

The rheological characterization has permitted us to determine the most effective condition to obtain a strong hydrogel to be to employed as drug delivery system. As evidenced by data above reported, under the present experimental conditions, the INUMA/PEGDA hydrogel irradiated at 320 nm for 100 min fulfils this requirement.

First of all, a test has been done to verify that the presence of a drug does not modify the rheological properties of the INUMA/PEGDA hydrogel. At this aim, 5-Fluorouracil has been chosen as a model drug and added to INUMA/PEGDA solution before UV irradiation (sample A); no rheological modification has been recorded in comparison with INUMA/PEGDA hydrogel obtained in the absence of drug, after *in situ* irradiation at 320 nm for 100 min.

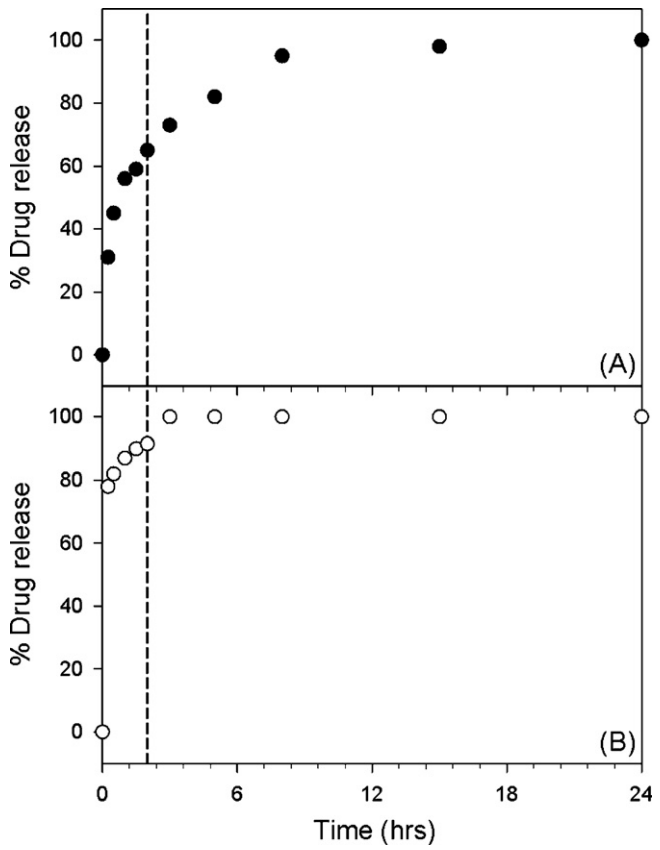

Fig. 7. Release profiles of 5-Fluorouracil from INUMA/PEGDA hydrogel (incorporated sample) (A) and soaked sample (B). The release study has been performed at pH 1.0 for the first 2 h to simulate the gastric fluid, then at pH 6.8 to mimic the intestinal medium. The dashed line indicates the pH change.

Moreover, in order to evaluate the effect of a different method of drug loading on release properties of INUMA/PEGDA hydrogel, besides drug incorporation before UV irradiation (sample A), a soaking procedure has been also employed, i.e. 5-Fluorouracil has been entrapped after UV irradiation into a preformed empty hydrogel (sample B).

Both drug loaded INUMA/PEGDA hydrogels (sample A and sample B) have been investigated by *in vitro* release studies under experimental conditions which simulate gastrointestinal fluids. The experiments have been carried out at 37 °C at pH 1.0 (simulated gastric fluid) and pH 6.8 (simulated intestinal fluid) by using the pH change method. Fig. 7A depicts drug release from drug incorporated sample (sample A), expressed as the percent of drug delivered (related to the entrapped total dose) as a function of time.

It is possible to observe a gradual release of 5-Fluorouracil; in particular, at pH 1.0, after 2 h, 65% of drug is released and, when the pH jumps to 6.8, the complete drug release is achieved within 24 h.

Fig. 7B depicts drug release from drug soaked sample (sample B), expressed as the percent of drug delivered (related to the entrapped total dose) as a function of time.

This sample showed a pronounced burst effect; about 91.5% of entrapped drug was released within 2 h, at pH 1.0, and the release was complete within 3 h.

These data, in comparison with those obtained for the release of 5-Fluorouracil from sample A, in which the drug was loaded before UV irradiation, confirm that the drug release is affected by the method of drug loading.

The kinetics of drug release was analysed by using the power law equation, often employed for identifying the release mechanism (Korsmeyer, Gurny, Doelker, Buri, & Peppas, 1983; Peppas & Korsmeyer, 1987; Ritger & Peppas, 1987; Sinclair & Peppas, 1984).

Table 1
Fitting results of release data with Eqs. (5) and (6).

$M_t/M_\infty = Kt^n$	(5)	K (min^{-n})	n	R^2
		6.83 ± 1.00	0.48 ± 0.04	0.994
$M_t/M_\infty = K_1 t^{1/2} + K_2 t$	(6)	K_1 ($\text{min}^{-1/2}$)	K_2 (min^{-1})	R^2
		7.45 ± 0.16	-0.14 ± 0.01	0.996

In this equation, the drug fraction released is related to time according to the expression:

$$\frac{M_t}{M_\infty} = Kt^n \tag{5}$$

with $M_t/M_\infty \leq 0.6$. Where M_t/M_∞ is the drug fraction released at time t, and K and n are the constant and the kinetic exponent of drug release, respectively. Due to the rapid drug release from sample B, it was not possible to perform the mathematical fitting for this sample. On the contrary, for sample A it was possible to calculate the value of exponent n that gives an indication on release kinetics. Since the sample has been recovered as microparticulate powder with a good roundness (see Section 2.2), the value of exponent n should range from Fickian ($n = 0.43$) to case II transport ($n = 0.85$), being anomalous for intermediate values (Ritger & Peppas, 1987). The obtained n value was 0.48 (Table 1), that being very near to 0.43, suggests an essentially Fickian mechanism. However to perform a more complete analysis of experimental data, we have adopted the equation proposed by Peppas & Sahlin for swellable systems (Peppas & Sahlin, 1989) and used by several authors (Colombo, Catellani, Peppas, Maggi, & Conte, 1992; Giammona, Pitarresi, Craparo, Cavallaro, & Buscemi, 2001; Peppas & Korsmeyer, 1987; Pitarresi et al., 2002; Spadaro, Dispenza, Giammona, Pitarresi, & Cavallaro, 1996; Vandelli, Forni, Iannuccelli, & Cameroni, 1991). Indeed, drug release from swellable matrices is often anomalous and depends on two processes: (i) drug diffusion into the swollen polymer; and (ii) matrix swelling due to the penetrant. Calculation of the approximative contribution of the diffusional and relaxational mechanisms to the anomalous release process is carried out by fitting the data to the heuristic model proposed by Peppas and Sahlin (1989). The equation of this model is:

$$\frac{M_t}{M_\infty} = K_1 t^{1/2} + K_2 t \tag{6}$$

with $M_t/M_\infty \leq 0.95$. In this equation, the first term of the right hand side is the Fickian contribution, and the second term is the case II relaxational contribution, due to the swelling in aqueous medium.

Table 1 reports n, K_1 and K_2 values calculated according to Eqs. (5) and (6).

It is noticeable that the term $K_1 t^{1/2}$ is more greater than the term $K_2 t$, thus indicating, as expected, that the mechanism for 5-Fluorouracil release from sample A is essentially a Fickian diffusion through the swollen microparticles. The percentage of drug release due to the Fickian mechanism, F, can be clearly calculated as (Peppas & Sahlin, 1989):

$$F = \frac{1}{1 + (K_2/K_1 t^{1/2})} \tag{7}$$

and was found to be 98% about.

Finally, to calculate the apparent diffusion coefficient, D_i, the final portion of the release profile ($0.6 \leq M_t/M_{inf} \leq 1$) was analyzed

by means of the approximate diffusion equation for spherical matrices (Baker & Lonsdale, 1974):

$$1 - \frac{M_t}{M_\infty} = \left(\frac{6}{\pi^2}\right) \exp\left[-\left(\frac{\pi^2 D_i t}{r^2}\right)\right] \quad (8)$$

r being the mean radius of the swollen particles.

For $0.6 \leq M_t/M_{inf} \leq 1$ values, the pH of release medium changes from 1.0 to 6.8, and in these conditions r was equal to 3.5 μm, determined by observing the variation of the microparticle diameter during the whole release experiment with a stereomicroscope connected to an image analyzer. The calculated D_i value was found to be 4.14×10^{-13} cm^2/s, in accordance with values found for other swellable polymeric microparticles (Gander, Gurny, Doelker, & Peppas, 1989; Giammona et al., 2001; Pitarresi et al., 2002; Vandelli et al., 1991).

4. Conclusions

In the present work the rheological properties of chemical hydrogels, obtained through UV irradiation of aqueous solutions of a methacrylated inulin (INUMA) with or without four different crosslinkers (PEGDA, PEGDMA, BIS and acMA) have been reported. It has been found that the crosslinkers modify in a different way the rate of crosslinking under UV irradiation at 320 and 365 nm and the elasticity of resulting hydrogels. Therefore it is possible to modulate the mechanical properties of INUMA hydrogel by choosing an appropriate crosslinker.

INUMA/PEGDA hydrogel obtained through irradiation at 320 nm for 100 min has shown the highest mechanical strength arising from the increased amount of crosslinked bonds formed during the UV irradiation. Drug molecules, like 5-Fluorouracil, can be loaded into this hydrogel before or after UV irradiation, without alteration of their structure and rheological properties of sample. *In vitro* experiments in simulated gastrointestinal fluids demonstrate that the kinetics of drug release depends on loading procedure, resulting rapid for soaked sample and slower when the active agent is incorporated before UV irradiation. In the second case, drug release occurs within 24 h by a mechanism controlled essentially by diffusion. All obtained data suggest that INUMA/PEGDA hydrogel is a good candidate for an oral delivery of biologically active substances.

Acknowledgements

The authors thank the Italian Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) for financial support and Fulvio Ferrante and Alessia Provenzano for technical support.

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