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Synthesis and characterization of pH-responsive hydrogels based on chemically modified Arabic gum polysaccharide

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Abstract

This work describes the preparation of a pH-responsive hydrogel from Arabic gum (AG) chemically modified with glycidyl methacrylate (GMA). This report first describes the chemical modification of AG and next the synthesis and characterization of the hydrogel obtained. An appropriate mixture of water and DMSO was used to dissolve AG and GMA. The presence of GMA groups in the modified structure of Arabic gum (AG-MA) was detected by ^{13}C NMR, ^{1}H NMR, and FT-IR techniques. The cross-linking reaction of AG-MA leads to formation of an AG-MA hydrogel, which was characterized by solid-state ^{13}C-CP/MAS NMR and FT-IR spectroscopy. Morphology was visualized by scanning electron microscopy. It was observed in water uptake tests that AG-MA hydrogels showed significant pH dependence, which affected the water absorption transport mechanism. In the studied pH range, it was found that the transport mechanism of water into AG-MA hydrogel was controlled by Fickian diffusion and polymer relaxation (anomalous transport). At high pH values, the water transport profile became more dependent on polymer relaxation. This effect was attributed to the increase in the ionized groups of glucuronic acid segments, which contributed to electrostatic repulsion among the groups and led the gel polymer network to expand. AG-MA hydrogels exhibited pH-responsive, demonstrating them to be appropriate materials for further tests as drug carriers.

Keywords: Polysaccharide hydrogel; pH-Responsive hydrogels; Polymeric drug carrier

1. Introduction

Several natural polymers, such as those found in diet, are preferred over synthetic materials for colonic drug delivery because they are more available and more susceptible to microbial biodegradation. In view of this, in the last few years, particular attention has been paid to drug delivery devices based on polysaccharides [1–4]. However, the high solubility of polysaccharides in aqueous media is often responsible for the premature release of solutes. Natural polymers such as dextran [5], pectin [6], guar gum [7], and inulin [8] have been chemically modified in an attempt to tailor desired applications. After modification, these polysaccharides are chemically or physically cross-linked to reduce solubility in aqueous solutions and, thereby, prevent premature drug release. An important fact is that hydrogels prepared by cross-linking a modified polysaccharide have high potential application to colon-specific drug delivery, because they are susceptible to enzymatic biodegradation by the bacteria present in the colon environment [9–11].

The modification of polysaccharides by the insertion of glycidyl methacrylate (GMA) by using DMSO as a solvent has become a common procedure, as previously reported in the literature [12–16]. This method consists in coupling GMA double bonds to a polysaccharide structure, which allows cross-linking (or gelation) [17]. However, that procedure cannot be used to modify polysaccharides insoluble in DMSO like the Arabic gum (AG), which has interesting properties to be used as a biomaterial [18,19].

The main objective of this work is to develop an alternative procedure for the chemical modification of AG and prepare a hydrogel based on the modified polysaccharide for acting as a polymeric drug carrier. Thus, it is essential to investigate factors affecting the molecule transport properties of such a system. In this sense, water uptake experiments, as well as water absorption transport studies at different pH and ionic

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strength were carefully conducted. Attention was primarily focused on the description of the chemical modification of AG and thereafter, the synthesis and characterization of the hydrogel obtained. This work opens up interesting perspectives for the synthesis of a potential polymeric drug carrier and the chemical modification of polysaccharides insoluble in organic solvents as for example, pectin, and chondroitin sulfate.

2. Materials and methods

2.1. Materials

Start materials were obtained as follows: Arabic gum (Company-Sudan), glycidyl methacrylate (GMA, Acros Organics), sodium persulphate (Sigma), dimethylsulfoxide (DMSO, Labsynt—Brazil), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED, Sigma). Dialysis tubes were purchased from Sigma (D-0530, lot 103H0525).

2.2. Chemical modification of Arabic gum (AG)

AG was purified by precipitation in an aqueous solution by the addition of ethanol. The aqueous-DMSO solution was prepared by mixing 50.7 mL distilled–deionized water and 129 mL DMSO. After homogenization, 20 g of purified AG were added to the DMSO/H_2O solution. Afterwards, 0.127 mmol TEMED and 75.2 mmol GMA were added. The resulting mixture was stirred for 72 h at 50 °C. The modified polysaccharide, labeled AG-MA, was purified by precipitation in ethanol and re-mixed in water. This cycle was repeated at least three times. AG-MA samples were dissolved in Milli-Q® water and dialyzed for 5 days at 4 °C. After that, the purified AG-MA was lyophilized and FT-IR, ^{1}H NMR and ^{13}C NMR analyses were carried out.

2.3. Hydrogel synthesis

The 15% AG-MA aqueous solution was added with 0.1 mmol sodium persulfate, stirred for 15 min, and transferred to a test tube. Next, it was heated to 70 °C for 30 min. A consistent gel was formed. The sample was taken out of the test tube and immersed in distilled/deionized water. The water was renewed every 8 h for 72 h, after which the hydrogels were dried at room temperature.

2.4. FT-IR spectroscopy

FT-IR spectra of AG, GMA, AG-MA, and AG-MA hydrogels were taken on a Bomem FT-IR model MB100 spectrometer. Powdered samples were prepared into pellets with KBr. To achieve a 2-cm^{-1} resolution, 128 scans were run for each spectrum.

2.5. ^{1}H and ^{13}C NMR spectroscopy

^{1}H and ^{13}C NMR spectra were obtained on a Varian spectrometer model Oxford 300 at 300 MHz. ^{1}H and ^{13}C NMR of AG, GMA, and AG-MA were obtained by using 100 mg mL^{-1} D_2O solutions. The relaxation time length and the angle pulse used in ^{1}H NMR and ^{13}C NMR spectra were 30 s and 90°, and 1 s and 30°, respectively. ^{1}H NMR and ^{13}C NMR spectra of GMA were obtained in CD_3Cl as a solvent. The intern reference used was 3-(trimethylsilyl) propionic acid-d_4 sodium salt. The chemical shift is given in ppm.

2.6. Solid-state ^{13}C-CP/MAS NMR analysis of AG-MA and AG-MA hydrogel characterization

Solid-state ^{13}C-CP/MAS NMR spectra were obtained on a Varian spectrometer model Oxford 300 at 74.47 MHz. The samples were placed in a 4-mm rotor; other important parameters were adjusted as follows: pulse angle $\theta = 37°$, spinning rate of 12 kHz, contact time of 3 ms, and relaxation time of 3 s.

2.7. Scanning electron microscopy for hydrogel morphology

AG-MA hydrogel morphology assay was conducted on a Shimadzu, model SS 550 scanning electron microscope (SEM) operating at 12 keV. Hydrogel swollen to equilibrium was first frozen in liquid nitrogen and then lyophilized by freeze-drying (Martin Christ, Freeze Dryer, Alpha 1–2/LD) for 24 h. For comparison, a SEM image of an AG-MA film prepared by freezing the dried AG-MA solution with an equivalent amount of polymer used in hydrogel formation was obtained. The AG-MA film and the lyophilized hydrogel were gold sputter-coated before SEM analysis. It was assumed that the morphologies of the swollen samples were preserved in freeze-dried samples.

2.8. Water uptake ratio (W_u) measurement in dependence of ionic strength and pH

Water uptake ratios at equilibrium, W_u, of AG-MA hydrogels were determined as a function of ionic strength and pH. Sodium chloride solutions in concentrations ranging from 0.01 to 0.25 mol kg^{-1} were prepared to investigate the dependence of W_u on ionic strength. The dependence of W_u on pH was determined by using buffer solutions with pH ranging from 2 to 10 at 37 °C. The ionic strength of each buffer solution was adjusted to 0.09 mol kg^{-1} with the addition of potassium chloride. W_u values were estimated from the following relation

$$W_u = \frac{W_s}{W_d} \quad (1)$$

where W_s is the weight of the hydrogel swollen to equilibrium at different pH and ionic strengths, and W_d is the weight of the dried hydrogel (pieces with ca. 150 mg average weight). Swelling experiments were repeated at least three times to check reproducibility.

2.9. Determination of water absorption mechanism

Dried hydrogel cylinders were weighed and immersed in buffer solutions with pH values of 1.2, 5.6, and 10.0 at 37 °C.

The ionic strength of each buffer solution was adjusted to 0.09 mol kg^{-1}. Thereafter, the hydrogel cylinders were removed from the buffer solutions and weighed at specified periods. The water absorption mechanism of AG-MA hydrogel was determined by means of fractional water uptake (W_t/W_∞) as a function of time. W_t/W_∞ data were determined through Eq. (2), which stands for the first 60% of fractional water uptake ($W_t/W_\infty \leq 0.60$) [20–23]:

$$\frac{W_t}{W_\infty} = kt^n \tag{2}$$

where W_t is the mass of the water absorbed by the hydrogel at a specified time, W_∞ is the mass of the water absorbed by the hydrogel at equilibrium, k is a characteristic constant of the hydrogel, and n is the diffusional exponent, which is often used to determine the transport mechanism mode. The experiments were repeated at least three times to check reproducibility.

3. Results and discussion

It has been assumed that chemical modifications of polysaccharides with GMA occur through two different pathway reactions [13,14,24,25]. A schematic drawing of these reaction routes is shown in Fig. 1. When treated with a protic solvent, the polysaccharide reacts with GMA by opening the epoxy ring. It is considered that the whole GMA molecules are coupled to the polysaccharide structure. The other modification route of the polysaccharide with GMA is transesterification reaction, which occurs in an aprotic solvent. In this case, glycidol is formed as a byproduct, and only then, the methacrylate molecule is coupled to the polysaccharide structure. However, the epoxy ring opening reaction route with GMA as a chemical modifier of polysaccharides has been little discussed in the literature. Furthermore, those investigations have hardly addressed branched acidic hetero-polysaccharides of complex structure like AG. The complex structure of AG generates large signals in ^{1}H NMR spectra, and therefore it is not possible to determine the degree of modification from these signals. Consequently, the extent of the vinyl groups incorporated into AG cannot be evaluated by NMR analysis. According to the literature [13,14], this parameter has already been determined by ^{1}H NMR analyses. However, the studied polysaccharides, e.g. dextran and inulin, have macromolecular structures less complex than that of AG. Therefore, the purpose of this work was to incorporate GMA vinyl groups into the AG structure to form polysaccharide hydrogels and to perform their characterization and not to verify whether transesterification and/or epoxy ring opening occur.

3.1. FT-IR analysis

Fig. 2 presents the FT-IR spectra of AG, GMA, and AG-MA. The discrete band at 1711 cm^{-1} in the AG-MA spectrum was attributed to the C=O stretching frequency of the conjugated ester groups. This band is an evidence of the modification of AG.

3.2. ^{1}H and ^{13}C NMR analyses

Fig. 3 shows the ^{1}H NMR spectra of AG, GMA, and AG-MA. The signals observed in the AG-MA spectrum at δ 6.20 and 5.79 ppm were attributed to the vinyl hydrogen from GMA. The signal at δ 1.96 ppm was correlated to the methyl

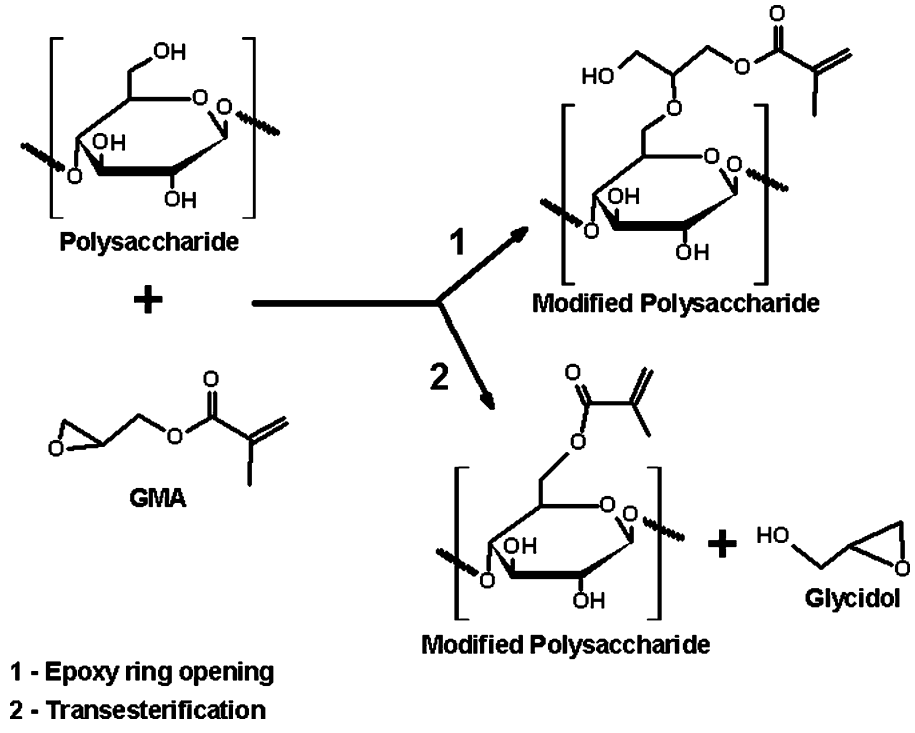

Fig. 1. Schematic representation of feasible modification route of polysaccharides with GMA: (1) epoxy ring opening mechanism, (2) transesterification.

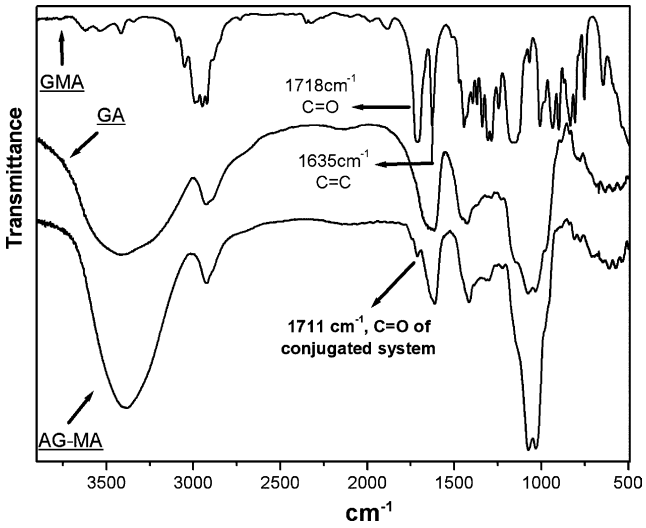

Fig. 2. FT-IR transmittance spectra of GMA, AG, and AG-MA.

hydrogen. The modification of AG with GMA was also confirmed by ^{13}C NMR analysis. As presented in Fig. 4, the signals at δ 136.62 and 128.39 ppm were attributed to the vinyl carbons; and the signals at δ 170.19 and 20 ppm were assigned to the carbonyl groups and methyl carbons, respectively.

3.3. Solid-state ^{13}C-CP/MAS NMR and FT-IR analysis for AG-MA hydrogels characterization

Fig. 5(a) shows the solid-state ^{13}C-CP/MAS NMR spectra of AG-MA and AG-MA hydrogels. The signals at δ 176.7 and 169 ppm in the AG-MA spectrum were attributed to the C=O groups from glucuronic acid originally present in AG and to conjugated ester groups, respectively. The signal at δ 140–120 ppm was assigned to C=C groups. The signal observed at δ 179.6 ppm in the hydrogel spectrum was attributed to C=O groups of non-conjugated ester groups, which is indicative of cross-linking. The absence of the spectrum signal at

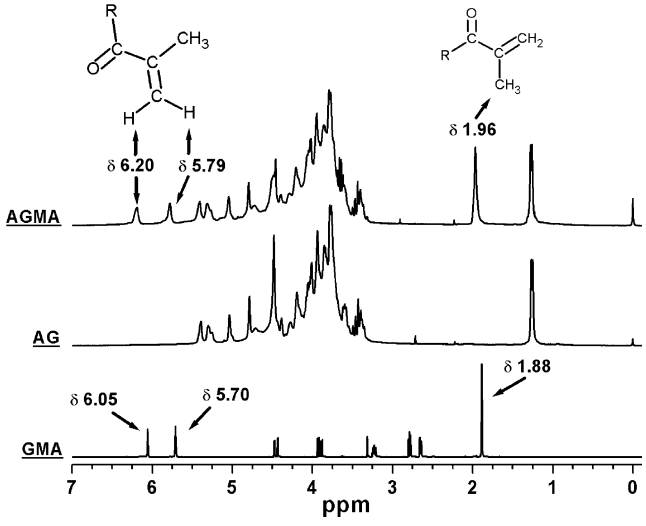

Fig. 3. ^{1}H NMR spectrum of GMA recorded in CD_3Cl. Spectra of AG and AG-MA recorded in D_2O (methyl protons: δ 1.97 ppm; vinyl protons: δ 6.21, and 5.78 ppm).

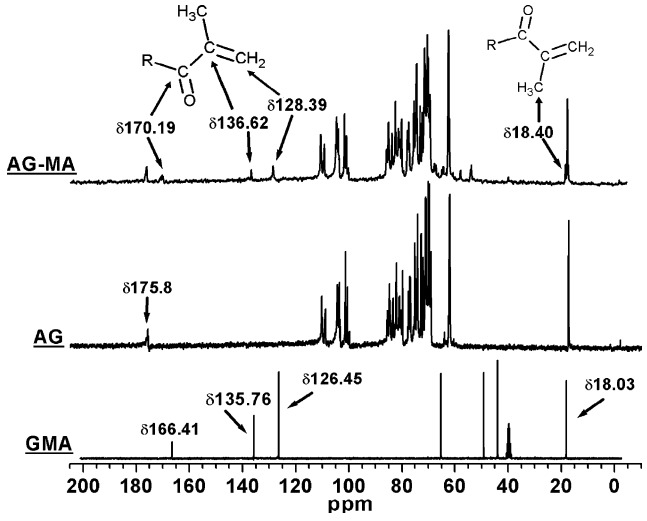

Fig. 4. ^{13}C NMR spectra of AG and AG-MA recorded in D_2O (methyl carbon: δ 18.40 ppm; vinyl carbon: δ 136.62. and 128.39 ppm; carbon carbonyl: δ 170.19 ppm). ^{13}C NMR spectrum of GMA recorded in CD_3Cl.

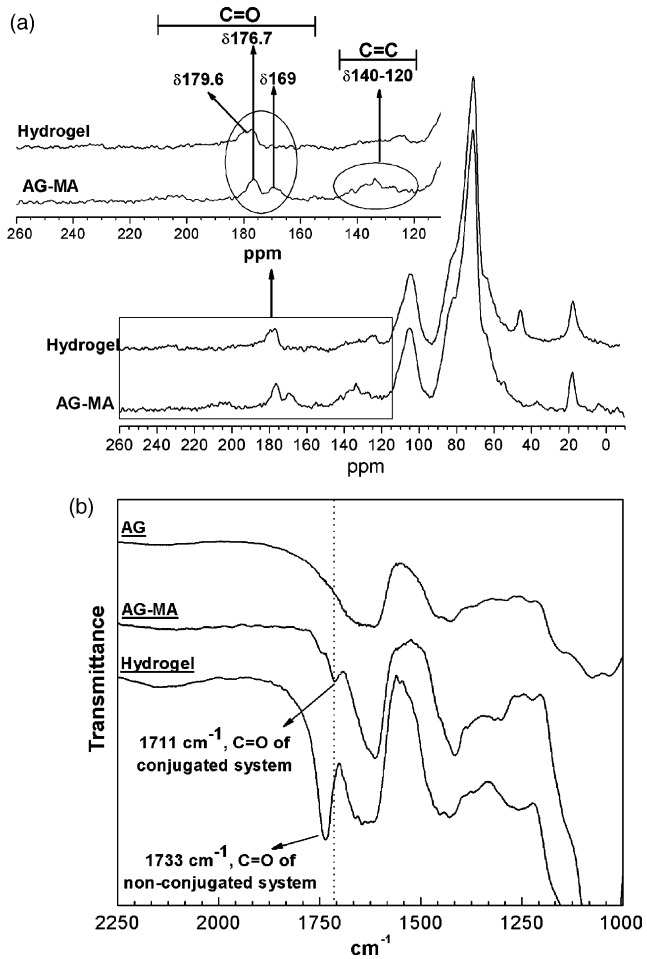

Fig. 5. (a) Shows the solid-state ^{13}C-CP/MAS NMR spectra of AG-MA and AG-MA hydrogel. (b) Shows the FT-IR transmittance spectra of AG, AG-MA, and AG-MA hydrogel. The band at 1711 cm^{-1} in the AG-MA spectrum corresponds to C=O groups of conjugated system, while the band at 1733 cm^{-1} corresponds to C=O groups of non-conjugated system.

140–120 ppm attributed to AG-MA hydrogel C=C groups indicates the consumption of these groups during cross-linking reaction. The characterization of AG-MA hydrogel was followed by FT-IR analyses. The spectra are shown in Fig. 5(b). The cross-linking reaction was characterized by the shift in the C=O stretching band from 1711 to 1733 cm^{-1}, which indicates the loss of conjugation of the ester groups. This finding is an additional evidence of the occurrence of the cross-linking reaction.

3.4. Scanning electron microscopy analysis for hydrogel morphology

The surface morphologies of (a) AG-MA film and (b) AG-MA hydrogel were analyzed by SEM images (Fig. 6). It should be highlighted that the AG-MA film showed a tight structure while the AG-MA hydrogel had a porous structure due to the formation of voids. The pores that became visible on the surface of the hydrogel were attributed to solvent evaporation during dry freezing.

3.5. Ionic strength and pH dependence on water uptake ratio (W_u)

Fig. 7(a) presents the W_u profile of the AG-MA hydrogel determined by sequential ionic strength variation. It is observed that the W_u of hydrogel is significantly affected as the ionic strength of the surrounding liquid changes. This effect is attributed to the AG-MA slightly polyelectrolytic character of the AG-MA hydrogel due to the charged groups of the glucuronic acid segments [9]. A large W_u converging to that in pure water was noted for the hydrogel in solutions with low salt concentration. In higher salt concentration solutions, the larger counterion concentration neutralizes the fixed charged groups, leading the hydrogel deswelling [26].

The W_u of the AG-MA hydrogel evaluated by sequential pH variation and the sigmoid-curve used to estimate the hydrogel p*K*a are shown in Fig. 7(b). The p*K*a data was in the range from ca. 4 to 5. The W_u of the AG-MA hydrogel showed a significant pH-dependence. It is evident that the hydrogel W_u increased with the increase in the surrounding pH. At pH values higher than the hydrogel p*K*a, the COOH groups

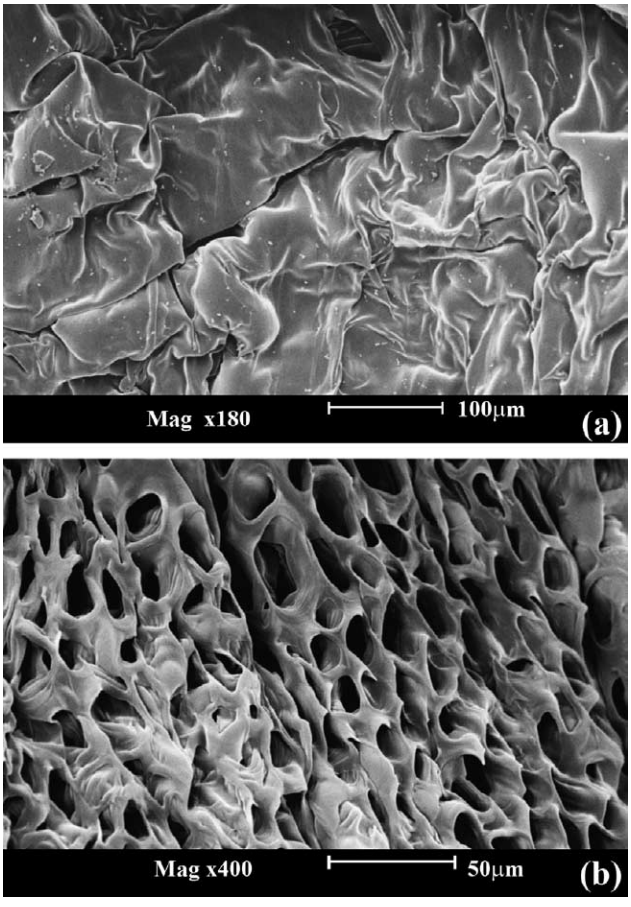

Fig. 6. SEM images of (a) freeze-dried AG-MA film and (b) freeze-dried AG-MA hydrogel.

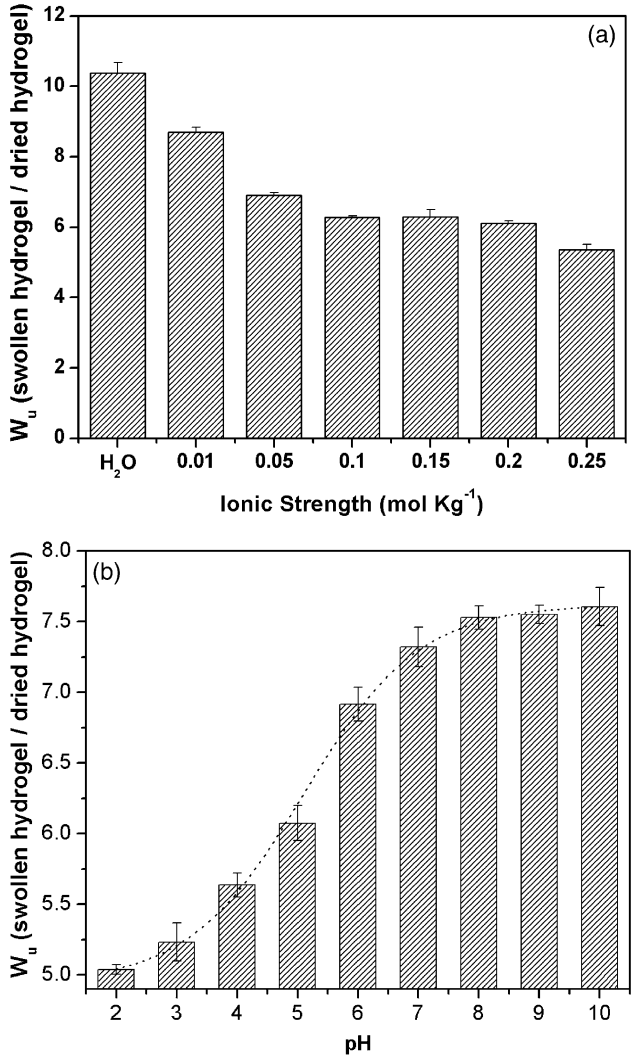

Fig. 7. Water uptake response of AG-MA hydrogels to changes in (a) ionic strength and (b) pH. Sodium chloride solutions in concentrations ranging from 0.01 to 0.25 mol kg^{-1} and buffer solutions at pH ranging from 2 to 10 with ionic strength adjusted to 0.09 mol kg^{-1} were used as swelling media at 37 °C. Data of water uptake were calculated as the average over the three water uptake weighted. Error bars represent standard deviations for the three experiments.

dissociate to COO^-, increasing the number of fixed ionized groups within the hydrogel structure. This generates electrostatic repulsion forces between the adjacent ionized groups in the polymer network, which increases the AG-MA hydrogel W_u significantly.

3.6. Studies of water absorption mechanism

According to the literature [23], n values are dependent on the hydrogel geometry due to changes in the boundary conditions, which in turn affect the water absorption mechanism. In the present work, hydrogels in cylinder form were used. In this case, it is assumed that the n values are given as follows: $n=0.45$ for Fickian diffusion (case I), $0.45<n<0.89$ for non-Fickian transport (or anomalous), $n=0.89$ for zero order (case II), and $n>0.89$ for super case II type of penetrating transport mechanism. The zero order (case II) transport is governed by the controlled-relaxation of the polymer chains, while the anomalous transport is driven by the summation of Fickian diffusion (case I) and controlled-relaxation (case II).

Fig. 8 shows the fractional water uptake in dependence of time for the AG-MA hydrogel in pH values of 1.2, 5.6, and 10.0. Parameters n and k were determined from the slopes and intercepts of the $\ln(W_t/W_\infty)$ versus $\ln(t)$ curve [27]. The data are summarized in Table 1. It was observed that the n values were on the order of $0.45<n<0.89$, which indicates that the water absorption mechanism of the AG-MA hydrogel is governed by anomalous transport in the pH range studied. Increase in the pH of the surrounding liquid from 1.2 to 10 leads the n values to an enhancement. It could be pointed out that at high pH values, the water uptake profile becomes more dependent on the polymer relaxation. This effect was attributed to the increased ionization of COOH groups of glucuronic acid segments of the hydrogel at high pH. An increase in the number of fixed ionized groups within the hydrogel structure gives rise to a major polymer relaxation.

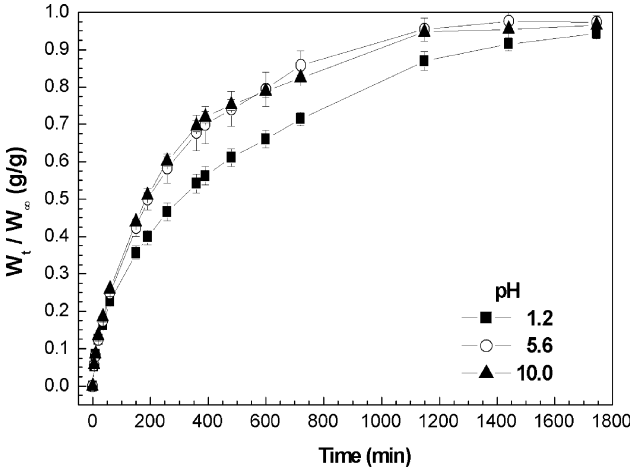

Fig. 8. Fractional water uptake in dependence of time of AG-MA hydrogels swollen in buffer solutions at pH 1.2, 5.6, and 10 with ionic strength adjusted to 0.09 mol kg^{-1} at 37 °C. Data of fractional water uptake were calculated as the average over the three water uptake measured. Error bars represent standard deviations for the three experiments.

Table 1
Parameters n and k determined by Eq. (2) for AG-MA hydrogel swollen in buffer solutions at pH 1.2, 5.6, and 10 with ionic strength adjusted to 0.09 mol kg^{-1} at 37 °C

pH	n	$k\times10^{-2}$ (min^{-n})	R^2
1.2	0.51 ± 0.008	2.68 ± 0.11	0.997
5.6	0.59 ± 0.006	2.29 ± 0.05	0.999
10	0.61 ± 0.007	2.00 ± 0.04	0.998

Errors represent standard deviations for three experiments. R is correlation coefficient.

4. Conclusions

Arabic gum (AG) was chemically modified with glycidyl methacrylate (GMA) with an appropriated mixture of water and DMSO. The presence of GMA groups in the AG-MA structure was detected by ^{13}C NMR, ^{1}H NMR, and FT-IR spectroscopies. The cross-linking reaction of AG-MA gave rise to the formation of an AG-MA hydrogel, which was characterized by solid-state ^{13}C-CP/MAS NMR, FT-IR, and SEM. The AG-MA hydrogel showed significant pH dependence, which had a considerable effect on the water absorption transport mechanism. In the pH range studied, it was found that the water uptake mechanism of the AG-MA hydrogel was controlled by Fickian diffusion and polymer relaxation (anomalous transport). At high pH values, the water uptake profile became more dependent on polymer relaxation. This effect was attributed to the increase in the ionized groups of glucuronic acid segments, which contributed to electrostatic repulsion among the groups and led to the expansion of the gel polymer network. Hydration under alkaline pH favors enzyme access to the hydrogel and its later enzymatic decomposition with the ensuing delivery of the drug entrapped in the hydrogel. The AG-MA hydrogels exhibited pH-responsive, which makes them a potential polymeric drug carrier. However, further in vitro research is needed to prove that AG-MA hydrogels are susceptible to be applied as such a system.

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