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Influence of lignin modifications on physically crosslinked lignin hydrogels for drug delivery applications

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ABSTRACT

So far, the possibility of synthesizing hydrogels based on multiple biopolymers has been investigated, and among them lignin has proven to be one of the potentials for this purpose due to the multiple advantages it offers. However, because of its high molecular weight, steric hindrance and few reactive sites on its structure, it is sometimes necessary to improve its reactivity though chemical modifications. On the basis of previous results, two chemical modifications were selected in order to enhance almond, walnut and commercial alkaline and organosolv lignins' reactivity: a peroxidation reaction for alkaline ones and a hydroxymethylation for organosolv ones. Both reactions were confirmed by multiple techniques (i.e. FTIR, GPC and TGA). Hydrogels were synthesized from these lignins according to previous works. The high lignin waste of the synthesized hydrogels suggested that despite the modification of the lignins, just the highest molecular weight fractions reacted with the matrix polymer. Moreover, the swelling capacity of modified alkaline lignin-based hydrogels was negatively affected, whereas the one for organosolv lignin-based samples improved. The SEM micrographs explained the aforementioned, and the results from the DSC and compression tests were in accordance with them. Self-extracted quercetin loading and release studies suggested that these samples could be used for controlled drug delivery.

1. Introduction

The insatiable demand for energy and fossil resources has driven the current society to many global environmental and social concerns. In this context, lignocellulosic biomass has opened an alternative door to the production of chemicals, materials and fuels [1]. This biomass is constituted by lignin, hemicelluloses and cellulose. Although biorefineries, the sustainable combination of processes able to transform biomass into a great variety of commercial products [2], have mostly been focused on cellulose and hemicelluloses for the production of paper and bioethanol [3], for instance, the conversion of lignin into value-added compounds is vital for the cost-competitiveness of biorefineries [4]. In fact, lignin can constitute up to 40% of woody biomass and 15% of herbal one [5] and it has demonstrated to possess interesting properties not just in energetic terms but also for the synthesis of new bio-based materials [6].

Lignins' structure is an intricate and random combination of phenylpropanoid units (i.e. coniferyl, coumaril and sinapyl alcohols), which varies according to the source and kind of plant, its culture conditions and the used lignin isolation method [7]. In addition, the lignin structure is highly branched and has multiple functional groups including carbonyl (C=O), hydroxyl (-OH), carboxyl (-COOH) and methoxy ($-CH_3O$) groups [5], which have a direct effect on its reactivity [8]. Moreover, the reactivity of this biopolymer is usually not high enough owing to its high molecular weight, steric hindrance and few reactive sites [2,9]. Therefore, in order to overcome this drawback it is sometimes necessary to perform a chemical modification of its structure [10]. For this aim, there are four main ways: the first one involves its depolymerisation or fragmentation, the second one focuses on the creation of chemically active sites, the third one is related to the modification of the hydroxyl groups in its structure; and the last one would be through the production of graft copolymers [11].

According to various studies, lignin can confer interesting properties to lignin-based composite materials such as antioxidant or antibacterial capacity [12]. This fact makes lignin attractive for the formulation of materials to be used in the biomedical field. A clear example of this is the rising trend of lignin addition into hydrogels [13], which are very useful materials for drug delivery [14], wound dressing [15] and tissue

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engineering and regenerative medicine [16], for instance.

Recently, modified lignins have been used for the formulation of hydrogels in order to overcome some drawbacks such as agglomeration or water insolubility [12], although many of them have been applied for pollutant adsorption [10,17]. Moreover, lignin modifications can be crucial to avoid the use of chemical crosslinking agents, promoting the synthesis of physically-crosslinked hydrogels, which are usually more environmentally friendly and economical [18].

Previously, the influence of the source and characteristics of alkaline and organosolv nut-shell (almond and walnut) lignins was studied [19] as well as the impact of commercial ones [20] on the characteristics of the hydrogels. Nevertheless, a significant lignin waste was generally observed in all the systems as well as a lower swelling degree in organosolv lignin-containing hydrogels. Thus, the objective of this work was to overcome the aforementioned weaknesses by synthesizing physical hydrogels with lower lignin wastes and higher swelling capacity for organosolv lignin-based ones modifying previously employed lignins [19,20]. For this aim, alkaline lignins were fragmented via an oxidation reaction and the organosolv ones were hydroxymethylated so as to introduce new reactive sites into them. The modified lignins were characterised and the modifications were confirmed via various techniques (FTIR, GPC and ^{31}P NMR). Then, hydrogels were synthesized from modified lignins and their lignin wastes, swelling capacities, morphology, glass transition temperatures and mechanical properties were studied. In addition, the possibility of using these hydrogels as drug deliverers was studied by analysing their release kinetics of self-extracted quercetin.

2. Materials and methods

2.1. Materials

Organosolv lignin (powder) was purchased from Chemical Point. Poly (vinyl alcohol) (beads) (M_w = 83,000–124,000 g/mol, 99+ % hydrolyzed), alkaline lignin (powder) and phosphate buffer saline (PBS) tablets were supplied by Sigma Aldrich. Sodium hydroxide (NaOH, analysis grade, ≥ 98%, pellets), hydrogen peroxide (30% *w/v*, for analysis), formaldehyde (37–38% *w/w*, stabilized with methanol, for analysis) and hydrochloric acid (37%, for analysis) were purchased from PanReac Química SLU. All reagents were employed as supplied.

Almond (AS) and walnut shell (WNS) alkaline and organosolv lignins (powder) extracted in a previous work via subsequent autohydrolysis and delignification processes were used in this work [19].

2.2. Lignin modification

Alkaline lignins from AS and WNS as well as the commercial one were subjected to a microwave assisted peroxidation reaction with hydrogen peroxide as described by Infante et al. (2007) [21]. Briefly, lignin and hydrogen peroxide were introduced into a high-pressure vessel keeping a LSR of 10:1 (mL:g). After sealing the vessel, it was subjected to three irradiation cycles of 10 s at 1100 W with a 30 s suspension period between them. Afterwards, the vessel was cleaned with distilled water and the collected mixture was left to dry over an oven.

Organosolv lignins (from AS, WNS and the commercial one) were exposed to a hydroxymethylation reaction with formaldehyde following the procedure reported by Chen et al. (2020) [9] with slight modifications. Concisely, 0.6 g of lignin were dissolved in an aqueous NaOH solution (140 mL). Then, 0.495 mL of formaldehyde were added and the solution was heated up to 80 °C under magnetic stirring and refrigeration. The reaction was left for 3.5 h. Afterwards, the modified lignin was precipitated with 2% hydrochloric acid, filtered, neutralized and dried.

2.3. Lignin characterisation

All the lignins were characterised employing the methods described in previous works. Their purity and composition [22], average molecular weights and total phenolic contents [23], thermal degradation, crystallinity and chemical structure [20] were determined. In addition, in order to confirm the chemical modification of organosolv lignins, ^{31}P NMR was employed following the protocol described by Meng et al. (2019) [8].

2.4. Hydrogel synthesis

The synthesis of the hydrogels was performed based on a previously detailed method [19,20]. In brief, 60 mL of a 2% (*w/w*) NaOH aqueous solution containing 9.87 w. % PVA was prepared and heated up to 90 °C until complete dissolution of PVA. Then, 9.12 w. % of lignin was added. After the lignin was dissolved, the blends were poured into silicon moulds, eliminating the remaining internal bubbles via ultrasound and the superficial air bubbles manually.

Five freeze-thawing cycles were then performed: firstly, the blends were completely frozen (2.5 h) at −20 °C and, then, they were thawed at 28 °C (1.5 h). During the second and last cycles, the samples were left at the freezer overnight. Finally, the hydrogels were washed into distilled water and dried at room temperature.

2.5. Hydrogel characterisation

The characterisation of the hydrogels was also done based on previous works [19,20,24]. Their lignin waste, swelling capacity, morphology, thermal behaviours and compression modules were studied.

2.6. Drug extraction and loading-release tests

Quercetin was extracted as a drug combining the methods reported by George et al. (2019) and Jin et al. (2011) [25,26]. Firstly, onion peels were cleaned and dried at 50 °C before being powdered. Quercetin, together with other compounds, was then extracted by microwave assisted extraction (MAE), which was based on previous experiments (data not shown) modifying the microwave power reported by Jin et al. (2011) [26]. The extraction was done with a 70% ethanol/water (*v/v*) solution, keeping a LSR of 40:1 (*v/w*). Since the used equipment was not a commercial microwave oven, the employed power for intermittent 10 s irradiations was fixed at 375 W. The total reaction time was 2 min, leaving a 20 s interval between the irradiations. After the reaction, the solid was filtered and the liquid phase was rotary evaporated for the complete elimination of ethanol. The remaining aqueous solution was considered as quercetin extract (QE).

The concentration of the quercetin extract was determined by UV spectrophotometry. For this purpose, a calibration curve was constructed using some solutions of certain concentrations of commercial quercetin (CQE) and measuring their absorbances at 375 nm [26]. The total phenolic and flavonoid contents (TPC and TFC, respectively) of QE were estimated as described by Sillero et al. (2019) [27], although in the case of TFC the standard was done with CQE. QE was freeze-dried and analyzed by FTIR and compared with the spectrum of CQE.

The loading tests were performed by introducing dry hydrogel samples into diluted QE (1 mL QE into 250 mL distilled water) solutions for 24 h. The absorbed QE amount was calculated by the difference on the concentrations of the initial and final solutions [25]. After the loaded hydrogels were dried, they were weighted and again immersed into PBS at 37 °C, simulating in vitro conditions, for 24 h. The release kinetics was performed by measuring the concentration of QE in PBS at certain times. All release tests were done in triplicates. The obtained results were introduced into various kinetic models including zero order, first order, Korsmeyer–Peppas and Higuchi (see Eqs. 1–4) so as to understand the release mechanism for QE [25,28,29].

$$F = k_0 t \quad (1)$$

$$ln(1-F) = -k_1 t \quad (2)$$

$$\frac{M_t}{M_\infty} = k_{kp} t^n \quad (3)$$

$$F = k_h t^{1/2} \quad (4)$$

Being F the percentage of quercetin released at time t, n the diffusion exponent and k_0, k_1, k_{kp} and k_h the rate constants of zero order, first order, Korsmeyer-Peppas and Higuchi kinetic models, subsequently.

3. Results and discussion

3.1. Lignin characterisation

As shown in Table 1 the purity of the lignins was altered after the modification reaction, especially in self-extracted lignins. This might be due to the employed reagents. As for their molecular weight, their weight average molecular weights augmented in all cases, especially in alkaline lignins. Their number average molecular weights got decreased for modified self-extracted alkaline lignins and for commercial organosolv lignin, leading to a more meaningful rise in their polidispersity index. Surprisingly, commercial organosolv lignin (COL) and its modified version (MCOL) were the most heterogeneous lignins, whereas the self-extracted native and modified organosolv lignins (AAOL, AWOL, MAAOL and MAWOL) were the most homogeneous ones.

Although the peroxidation reaction was supposed to fractionate lignin, a high percentage of the chains seemed to undergo recondensation reactions, which made the total weight average molecular weights increase. However, no certain evidence of this has been found in literature. The change on the total phenolic content suggested the degradation of aromatic rings in lignin [21,30]. However, CAL presented the opposite trend, which could be related to the differences on the pH of the solutions. According to the results reported by Xinping et al. [30], the reaction might have yielded more degradation compounds under alkaline conditions [11], but as Infante et al. (2007) had demonstrated that this could also be achieved with the non presence of a catalyser, the present work was done according to the latter [21]. In addition, the differences on the range 1600–1730 cm^{-1} on their FTIR spectra confirmed the reaction (see Fig. 1a). In fact, the band around 1710 cm^{-1} moved to higher wavenumbers in all cases, which was attributed to the –OH oxidation of side chains, together with the weakening of the peak at 1599 cm^{-1} corresponding to aromatic C═C stretching vibration. In addition, as reported by Infante et al., the appearance of the band around 1640 cm^{-1} was also representative of the degradation of aromatic rings [21].

Table 1
Summary of the purity, GPC, TPC and TGA results for the modified lignins.

Lignin Sample	Purity (%)	M_w[a] (g/mol)	M_n[b] (g/mol)	M_w/M_n[c]	TPC (% GAE[d])	T_{max}[e] (°C)
AAAL	88.2 ± 0.6	12,793	1528	8.4	33.1 ± 1.1	355
AAOL	95.2 ± 2.2	9020	1520	5.9	26.2 ± 0.1	357
AWAL	95.7 ± 1.9	16,670	1604	10.4	33.8 ± 0.4	354
AWOL	95.2 ± 0.8	7644	1359	5.6	27.2 ± 0.1	365
CAL	91.5 ± 1.2	9333	1365	6.8	20.3 ± 0.1	379
COL	92.5 ± 2.9	32,933	1123	29.3	19.3 ± 0.3	343
MAAAL	85.2 ± 1.1	17,675	1348	13.1	25.3 ± 0.1	384
MAAOL	92.3 ± 0.6	9557	1636	5.8	20.4 ± 0.1	383
MAWAL	84.0 ± 1.9	19,939	1369	14.6	27.6 ± 0.4	384
MAWOL	83.6 ± 2.9	8187	1420	5.8	23.6 ± 0.5	383
MCAL	91.9 ± 1.7	12,141	1718	7.1	25.8 ± 0.9	396
MCOL	96.5 ± 0.5	32,997	968	34.07	21.5 ± 0.4	390

[a] M_w: weight average molecular weight.
[b] M_n: number average molecular weight;.
[c] M_w/M_n: polydispersity index.
[d] % GAE: percentage of gallic acid equivalents.
[e] T_{max}: maximum degradation temperature from TG/DTGA curves.

As for the hydroxymethylated lignins, it is known that formaldehyde may react with lignin in alkaline medium in two ways: the first one, by substituting the free ortho positions in the aromatic rings, and the second one, by reacting with the side chains containing carbonyl groups [9,31,32]. Nevertheless, if reactivity of the lignin is wanted to increase, the latter reaction should be avoided [32]. Moreover, hydroxymethyl groups can also react at free positions of other lignin units forming methylene bonds and leading to the condensation of the structure [31,32]. The variation on the distributions and average molecular weights suggested that the modification occurred [32]. Despite de fact that the change on the polydispersity of the lignins was not representative of having obtained more homogeneous modified lignins, their molecular weight distributions (Supplementary data) evoked a trend of homogenization of the highest molecular weight fractions towards the ones with lower molecular weights. This behaviour was also observed by other authors [33]. Moreover, the increase on the number and weight average molecular weights was observed for MAAOL and MAWOL samples, which was also reported by Capraru et al. (2012) for grass lignins.

The FTIR spectra of native and modified organosolv lignins also indicated the success of the reaction (Fig. 1b) [33]. In fact, the intensification of the –OH band (at 3400 cm^{-1}), the one corresponding to C—H (around 2930 cm^{-1}), the one attributed to methoxyl and hydroxymethyl groups (around 2850 cm^{-1}) and the one related to the C—O stretching vibration of aliphatic C–OH and hydroxymethyl C–OH (around 1030 cm^{-1}) were a clear evidence of the introduction of hydroxymethyl groups via the modification reaction [9,32]. The appearance of a shoulder at 3660 cm^{-1} suggested the presence of free –OH groups within the modified lignin structures [34]. These results were in agreement with those obtained from ^{31}P NMR analyses, in which an increase on the aliphatic hydroxyl signal between 150 and 145.4 ppm was observed for all the samples after the hydroxymethylation reaction [8] (Supplementary data).

The thermal stability of all the samples was altered through the modification reactions. In fact, the maximum degradation step was shifted to higher temperatures in all cases. However, in the case of alkaline lignins, another degradation step appeared between the stage corresponding to moisture evaporation (< 100 °C) and the maximum degradation stage. This peak was detected around 300 °C, and was attributed to the lower molecular weight fractions of lignin generated during the modification step [22,35]. It was also observed that organosolv lignins were more thermally stable and started to lose weight at higher temperatures than alkaline lignins, although their maximum degradation temperatures were slightly lower. This fact was attributed to their molecular weight distributions and polydispersity indexes, since alkaline samples were more heterogeneous and, as aforementioned, the lowest molecular weight fractions could have started to degrade firstly. Compared to native lignins, modified organosolv lignins presented higher thermal stability and final residue, which was also observed by Chen et al. (2020) [9]. This was related to the increase on their molecular weights. In addition, despite all the left residues being of around the 40% of the initial sample weight, modified organosolv lignins left higher residues than alkaline ones, conversely to what happened for native lignins, which may be attributed to the modification and lignin precipitation stages.

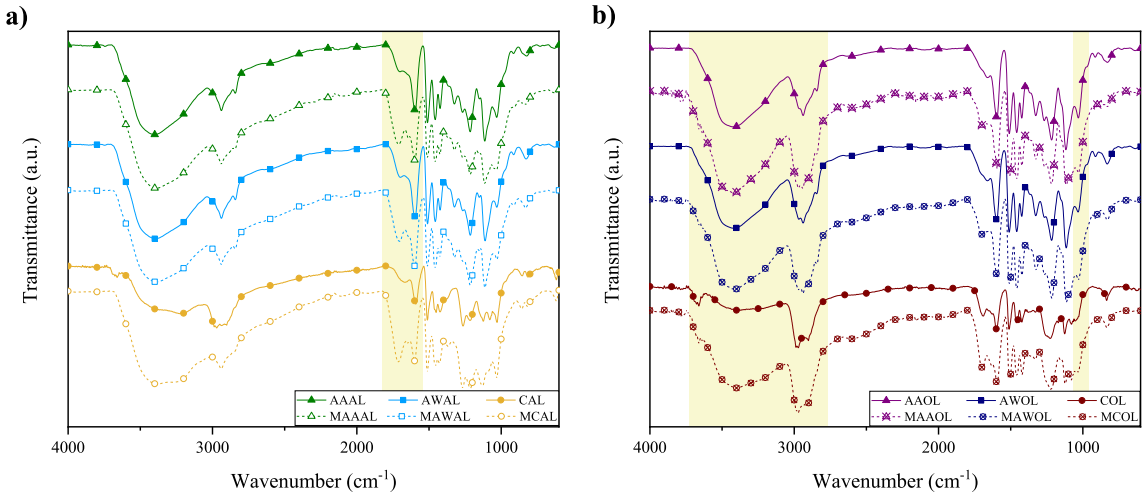

Fig. 1. FTIR spectra of modified and native alkaline (a) and organosolv (b) lignins.

The crystallinity of the samples was almost unaltered by the modification reactions (Supplementary data). All the samples presented a wide peak around 22°, which is related to the amorphous structure of lignin [36,37].

3.2. Hydrogel characterisation

3.2.1. Lignin waste

As in previous works, the lignin waste of the synthesized hydrogels was determined through UV spectroscopy (Table 2) [19,20,24]. It was expected to have lower lignin wastes than in the previous works due to the higher reactivity of the modified lignins. Nevertheless, the results proved that the hypothesis was incorrect, since the lignin waste determined for all the samples resulted to be higher than those reported previously. This change was significantly greater for the samples containing MCAL and MCOL, which presented a loss of almost 89 and 97% of their initial amount of lignin, respectively. The samples containing MAWA did also show a huge increase. The rest of the samples exhibited lower lignin waste raises, ranging from 12 to 14%.

As the observed lignin wastes were so unexpected and so as to study the reusability of lignins in the washing solutions, it was decided to precipitate these lignins and study their molecular weights. These results are shown in Table 3. It was observed that in all cases the lost lignins had a lower weight average molecular weight than the native ones, and they were also more homogeneous, since their polydispersity indexes were lower. These results suggested that the polymeric matrix could have reacted with the highest molecular weight fractions, leading to a big elimination of the lowest molecular weight fractions.

3.2.2. Swelling capacity

In order to determine the effect that the lignin modifications had on the properties of the synthesized hydrogels, their swelling capacity was studied. The results are depicted in Fig. 2.

Comparing to previous results [19], it was observed that in the case of the alkaline lignin-containing samples, the swelling capacity got significantly reduced when modified lignins were employed for their synthesis. These results suggest that the peroxidation of lignin performed in the present work was not an appropriate modification to obtain hydrogels with a high swelling capacity. On the other hand, when modified organosolv lignins were used, their swelling capacity was enhanced, especially in the case of the samples with MCOL (450%), which had also presented the highest lignin waste. Moreover, MAAO

Table 2
Lignin waste (%) of native and modified lignin-containing hydrogels.

Sample	Native (%)	Modified (%)
AAA	59.6 ± 2.8	73.5 ± 0.5
AAO	71.1 ± 3.0	83.4 ± 4.6
AWA	44.2 ± 1.6	71.6 ± 0.5
AWO	59.9 ± 4.0	74.0 ± 4.8
CA	67.8 ± 2.0	88.7 ± 3.4
CO	77.4 ± 1.7	96.5 ± 0.3

Table 3
Average molecular weights and polydispersity indexes of the lignins recovered from the washing solutions.

Lignin Sample	M_w[a] (g/mol)	M_n[b] (g/mol)	M_w/M_n[c]
MAAAL	10,134	1317	7.7
MAAOL	7250	1592	4.5
MAWAL	10,350	1643	6.3
MAWOL	6804	1569	4.5
MCAL	9710	1985	4.9
MCOL	3685	636	5.8

[a] M_w: weight average molecular weight.
[b] M_n: number average molecular weight.
[c] M_w/M_n: polydispersity index.

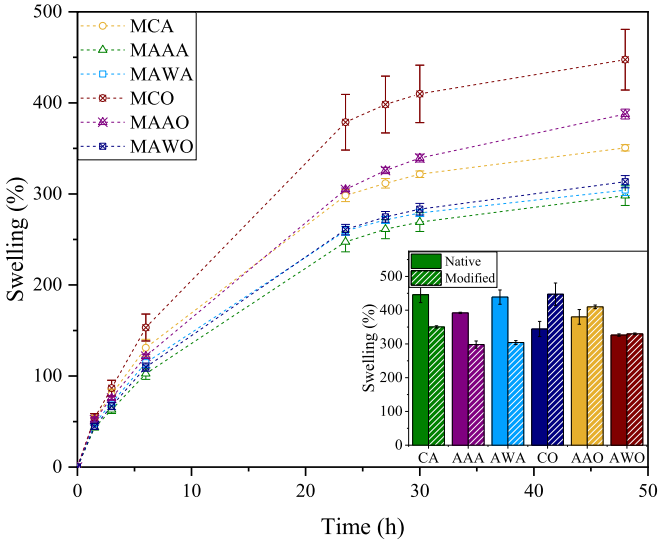

Fig. 2. Swelling performance of modified lignin-based hydrogels during the first 48 h.

samples were the second ones with improved swelling capacity (410%), which also coincided with the second highest lignin waste. Similarly, MAWO samples exhibited the lightest enhancement on their swelling degree and had displayed the lowest lignin waste among the samples containing modified organosolv lignins. Thus, it could be concluded that hydroxymethylation could be a good method to enhance the swelling ability of organosolv lignin-based hydrogels. As reported previously, the molecular weight and the phenolic/aliphatic hydroxyl group content in lignin are important factors when synthesizing hydrogels [19,38]. Looking at the results in Tables 1 and 3, it was concluded that the highest molecular weight fractions were attached to the matrix. In the case of alkaline samples, as the molecular weights and TPC contents were higher, these fractions might have promoted the interactions with PVA. Therefore, a compact structure with esteric hindrance could have been obtained, leading to a lower swelling capacity and a lower lignin waste. On the contrary, for organosolv lignins the molecular weights were much lower and more homogeneous, leading to a decrease on their interactions with the matrix and enabling higher swelling capacities [39,40].

Looking at the present results and comparing them with previous ones, it should be mentioned that although hydroxymethylation demonstrated to be effective for improving the swelling capacity of lignin-hydrogels, other variations during the synthesis process (lengthening the last thawing step, for instance) leaded to higher improvements on this property, without needing to modify the native lignins.

3.2.3. Morphology

Scanning Electron Microscopy (SEM) permitted studying the morphology of the samples. The obtained micrographs at 500× and 1500× magnifications are shown in Fig. 3.

The images, in general, did not reveal highly porous structures; indeed they showed quite dense and continuous morphologies with scarcely detectable voids. This fact was more evident in the samples containing alkaline lignins, which may be attributed to a greater crosslinking density, which would also clarify the decline on their swelling ability. For the samples containing organosolv lignins, especially for MAAO samples, the created voids were more obvious, which were probably responsible for the augment on their water absorption capacity. However, as the swelling study was performed at the same conditions as previously, the obtained microstructures might have just hindered the water diffusion through the matrix, and after leaving them longer times immersed, they may present higher swelling capacities.

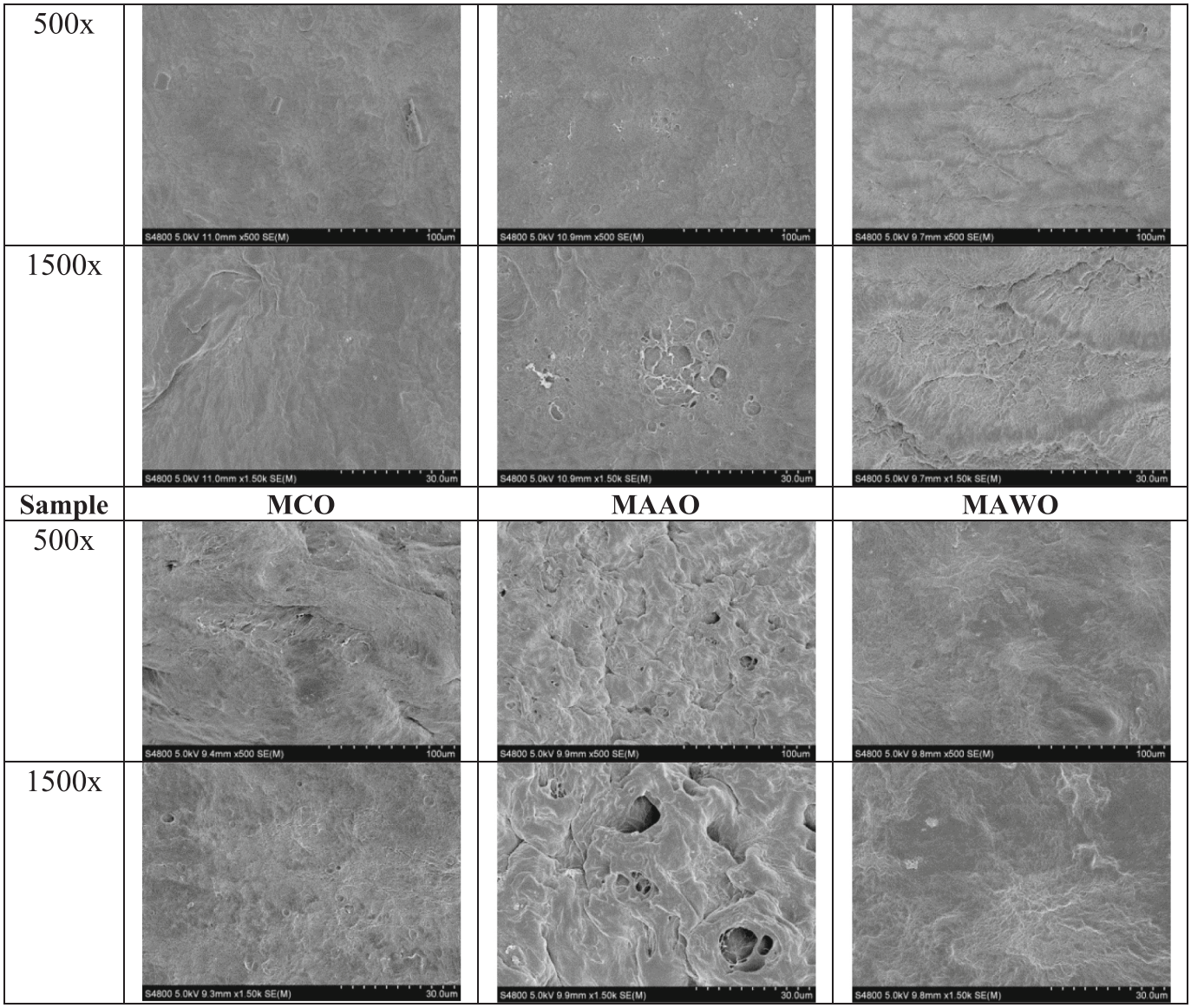

Fig. 3. SEM micrographs for modified lignin-based hydrogels at 500× and 1500× magnifications.

Hence, it is clear that the lignin modifications had direct impact on the microstructures of the synthesized hydrogels, which also clearly affected their swelling capacity.

3.2.4. Thermal behaviour

For some applications, the glass transition (T_g) and melting temperatures (T_m) of polymeric materials are determining features. Hence, these parameters together with the melting enthalpy (ΔH_m) and the crystallinity indexes (χ_c) for each sample were defined by Differential Scanning Calorimetry (DSC) and the results are displayed in Table 4. The T_g was found on the inflection point of the specific heat increment during the second heating scan, after removing the thermal memory of the samples, but the rest of the parameters were identified from the first heating scan.

All the determined T_g values were in the range of 77–103 °C. These values were higher than those reported previously [19], suggesting that lignin modifications leaded to more compact structures in which the movement of the amorphous polymeric chains was hindered, which was also related to the obtained SEM micrographs. In addition, it was observed that the hydrogels containing modified organosolv lignins presented lower T_g values than the ones containing alkaline ones. Moreover, these results were aligned with the ones reported for the swelling capacity of the samples, being the aforementioned higher for the samples with lower T_g. Despite all the melting temperatures being similar (≈ 235 °C), they were quite close to pure the T_m of PVA hydrogels [20,24], and their melting enthalpies were also high, suggesting the existence of many crystalline regions [41]. The crystallization indexes were calculated based on a well-known equation [20,42], and the results suggested that a great part of the hydrogels was crystalline. In addition, the samples with higher filler contents seemed to have higher crystallinity degrees, which would be in accordance with previous results [20] and could be due to an enhancement of interfacial interactions via hydrogen bonding between the multiple hydroxyl groups on the matrix polymer and lignin [42]. It was also observed that the samples containing alkaline lignin presented higher crystallinity indexes, which would explain their lower swelling abilities and higher T_g values.

3.2.5. Compression tests

The compression tests of the samples were performed in order to determine the impact that lignin modification had on their compression modulus at 80% of strain. Once again, all the tested hydrogels were able to keep total integrity and good recoverability thanks to their elastic behaviour.

From the results in Fig. 4 it was concluded that hydrogels containing alkaline lignins had greater compression modulus than those containing organosolv lignins. The latter is consistent with the results obtained for their crystallinity, since the more crystalline and compact the sample is the higher its compression modulus should be [43]. Nevertheless, this improvement of the compression modulus could also be attributed to the higher solid content (m_{filler}) on alkaline hydrogels, as explained by Queiroz et al. (2021) [44]. Moreover, all the modules values for the samples with alkaline lignin were in the range of 10–12 MPa, whereas the ones for hydrogels with organosolv lignin were between 4.5 and 6 MPa. Comparing to the previous work [19], an enhancement of its compression modulus was especially observed for MAWA samples, although MAAO also got slightly higher. All these values were aligned with the results reported for lignin-hydrogels by some authors [45] but they were also higher than those obtained by others [44,46,47].

Table 4
Summarized results for the analyzed parameters by DSC and calculations.

Sample	1st heating scan				2nd heating scan
	T_m (°C)	ΔH_m (J/g)	m_{filler} (%)	χ_c (%)	T_g (°C)
MAAA	235	64	25	53	103
MAAO	235	59	15	43	92
MAWA	236	62	27	52	81
MAWO	234	61	25	50	77
MCA	235	60	11	42	91
MCO	234	59	4	38	88

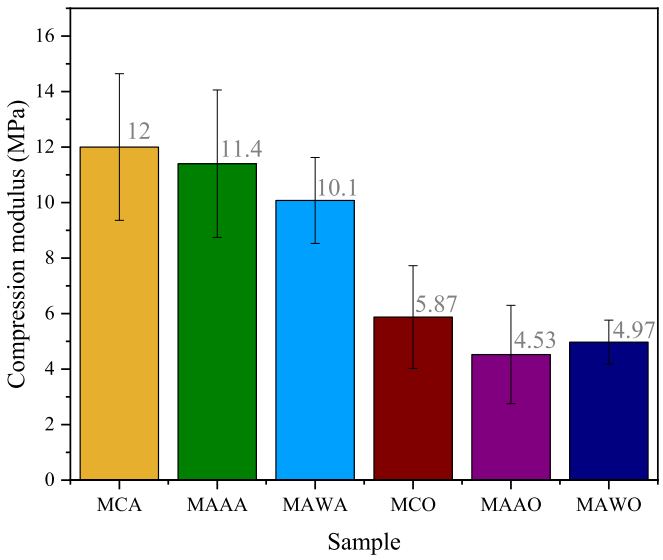

Fig. 4. Compression behaviours of modified lignin-based hydrogels.

3.2.6. Drug loading and delivery tests

Quercetin (QE) is a bioflavonoid present in fruits and vegetables with interesting anti-inflammatory, antioxidant, anti-carcinogenic and anti-obesity properties [25,26,48]. Due to the aforementioned characteristics and the current trend of preferring natural compounds rather than synthesized drugs, quercetin has recently gained great attention. This compound can be found in onion peels, which are an abundant waste all over the world. Thus, the obtaining of a flavonoid-rich extract from this waste would give an added-value to it, contributing to circular economy.

Among the extraction strategies that have been studied for QE, microwave assisted extraction (MAE) has proven to be a promising sustainable and green process [26]. Therefore, QE was extracted though MAE.

3.2.6.1. Characterisation of QE extract. The solid content on the extract was determined through gravimetric analyses, drying 1 ml of the extract at 105 °C for 24 h. This measurement revealed a solid content of 18.3 ± 0.2 mg of solid/g of liquid extract. TPC and TFC analyses showed high phenolic and flavonoid contents for the extract (576.4 ± 75.4 mg GAE/g dry extract and 470.1 ± 22.5 mg CQE/g dry extract). These values for TPC were higher than those reported by other authors and the ones for TFC were similar [25]. In addition, the characteristic peaks of CQE reported by George et al. (2019) were also present on the FTIR spectra of QE, confirming the existence of this compound in the extract (see Fig. 5a) [25].

3.2.6.2. Drug loading tests. The drug loading tests were performed by immersing dry hydrogels into diluted QE solutions (68.4 mg quercetin/L). The objective of these tests was to analyse the capacity of these samples of absorbing and releasing this drug, not to optimize the loading-release kinetic.

According to the absorbance difference between the initial and final solutions, all the samples were capable of trapping between 26 and 34% of the drug in the initial solution (see Table 5), being this percentage higher for the hydrogels containing organosolv lignins, which had also presented the highest swelling capacities.

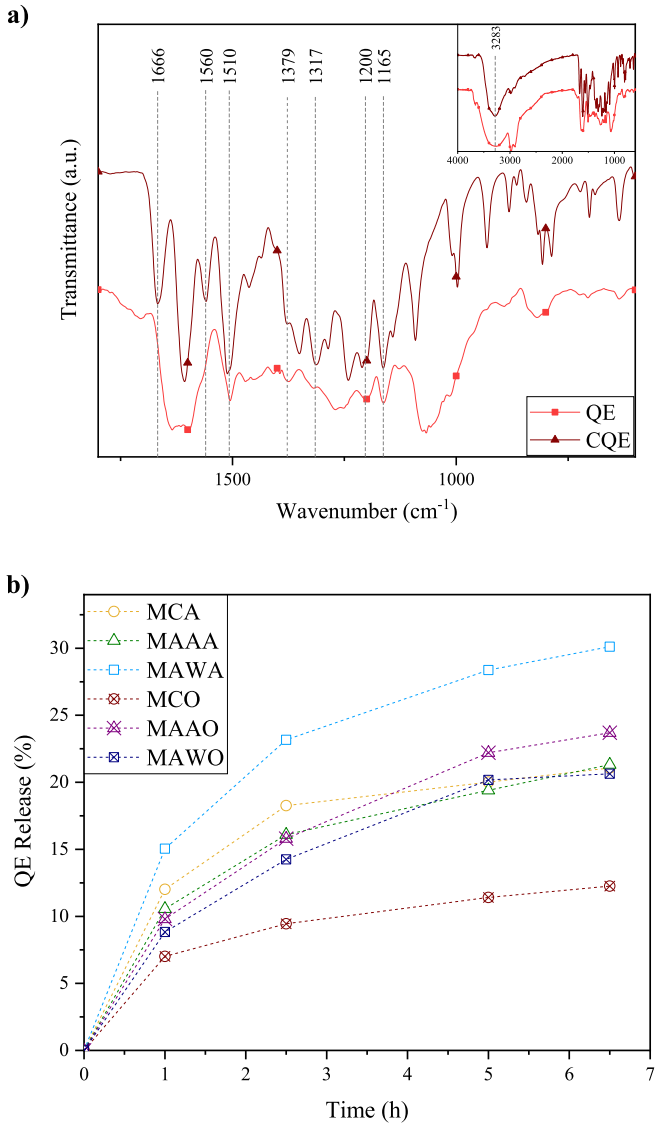

Fig. 5. (a) FTIR spectra of self-extracted (QE) and commercial (CQE) quercetin extract and (b) QE release profiles of modified lignin-based hydrogels.

3.2.6.3. Drug release tests. After the loaded hydrogels had dried, they were immersed in PBS at 37 °C for drug release, simulating in vitro conditions. The absorbance of the release medium was performed at several times during the first 6.5 h. As the hydrogels contained lignin, and this lignin presented an absorbance peak at 330 nm, at high concentrations this peak sagged the results of the peak corresponding to QE at 375 nm. Thus, the release kinetics was performed during the first 6.5 h, while the released lignin was negligible.

From the release profiles shown in Fig. 5b it was concluded that although all the samples were able to be loaded with similar drug amounts, the release capacity was completely different for each sample. In fact, with respect to the loaded amounts of drug, the released drug percentages ranged from 12 to 30%. The samples displaying the highest release drug percentage were MAWA, followed by MAAO and MAAA. The lowest release was observed for MCO samples, suggesting that despite having higher drug loading abilities, the interactions with the drug made its release difficult. Nevertheless, it should be noted that these profiles were just for the first 6.5 h, and cannot be extrapolated to longer times.

So as to determine the release kinetics, several models were applied (i.e. zero order, first order, Korsmeyer–Peppas and Higuchi) [25,28,29]. The estimated kinetic parameters for each of these models are displayed in Table 5 and the graphic representations of the four kinetic models in the Supplementary data. From the original release profile, it was inferred that the release kinetics would not fit correctly to a zero order model, which was confirmed by the determination coefficients (R^2). Among the rest of the models, Korsmeyer–Peppas model was the one fitting the best, except for MCA sample, whose fitting did not improve either with Higuchi model.

As indicated by Saidi et al. (2020) [29], the release exponents (n) and the logarithms of the rate constants (ln k_{kp}) were determined from the slopes and intercepts of the plots (ln (QE %) versus ln t) of the experimental data. As shown in Table 5, all the estimated values for n were below 0.5. Although Fickian diffusion is usually considered when $n = 0.5$ [25,28], in this case it could also be said that the QE release followed a Fickian diffusion [25]. Thus, it could be said that the synthesized hydrogels could be used as controlled drug delivery systems.

4. Conclusions

On the basis of the results obtained for the properties of previously synthesized alkaline and organosolv lignin-based hydrogels, two chemical modifications were performed to these lignins in order to enhance their reactivity. The peroxidation of alkaline lignin was confirmed by FTIR, but the molecular weight studies suggested that condensation reactions had also happened during the reaction, leading to fractions with higher average molecular weights. The hydroxymethylation of organosolv lignin was also confirmed by FTIR, ^{31}P NMR and GPC. Moreover, this reaction leaded to more thermally stable lignins, which supported the success of the reaction. From the modified organosolv lignins, hydrogels with improved swelling capacities (up to 450%) were synthesized, although their lignin wastes were higher than the ones reported previously. On the contrary, modified alkaline lignins leaded to a decrease on the swelling capacity of the hydrogels under the employed assay conditions. These results were explained by the continuous and compact structures seen on SEM micrographs, being more obvious for alkaline lignin containing samples. These samples also presented higher compression moduli than the organosolv ones, which would be desirable depending on the application field, but they still presented promising results in drug loading and release studies. Although all the tested samples allowed a Fickian diffusion of QE, MAWA sample was the one with the most adequate release profile.

Table 5
Kinetic parameters estimated from models for QE release from hydrogels.

	Loading	Zero order	First order	Korsmeyer–Peppas			Higuchi
Sample	(%)	R^2	R^2	R^2	n[a]	ln k_{kp}[a]	R^2
MAAA	29.0	0.8194	0.8438	0.9852	0.37 ± 0.13	2.38 ± 0.18	0.9764
MAAO	31.8	0.8946	0.9165	0.9947	0.48 ± 0.11	2.30 ± 0.14	0.9962
MAWA	30.4	0.8134	0.849	0.9809	0.37 ± 0.16	2.74 ± 0.21	0.9741
MAWO	32.0	0.8781	0.8968	0.9868	0.47 ± 0.16	2.20 ± 0.22	0.9904
MCA	26.0	0.7248	0.7473	0.9284	0.29 ± 0.25	2.53 ± 0.33	0.9294
MCO	34.5	0.7725	0.7872	0.9978	0.30 ± 0.04	1.96 ± 0.06	0.9562

[a] Estimated intervals at 95% of confidence level.

However, they could all be employed as potential controlled drug delivery systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.susmat.2022.e00474.

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