

Chitosan, xanthan and locust bean gum matrices crosslinked with β -cyclodextrin as green sorbents of aromatic compounds

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ABSTRACT

Three different polysaccharides, xanthan gum, chitosan and locust bean gum, were crosslinked with or without β -cyclodextrin, using citric acid in different ratios, to create 'green' hydrogel matrices. The crosslinking of these polysaccharides was produced through an inexpensive and innocuous solvent-free synthesis process. A favorable swelling behavior of the hydrophilic matrices facilitates the sorption of the solutes tested. Interestingly, the amount of β -cyclodextrin groups is not the only factor to yield the best sorption capability for hydrophobic model molecules: polysaccharides themselves also influence the sorption depending on their characteristic functional groups, the conformation of their chains and, as mentioned above, their degrees of swelling. In order to ascertain the effect of the polysaccharides on the sorption capabilities of a model sorbate (1-naphthol), isotherms using a wide range of solute concentrations were analyzed, and the Hill equation yielded the best fitting results and provided some insight into the mechanisms of interaction.

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1. Introduction

Among the natural resources that can be exploited, those that can be grown, farmed and renewed or recycled as quickly as they are collected, need to be the building blocks of the materials used in the near future. A relevant family of those molecules produced by the biomass (animals, plants or bacteria) is that of polysaccharides; made of carbohydrate units, their hydroxyl functional groups allow them to possess a high reactive power. Their constituent units can possess other chemical functions and different types of glycosidic bonds, which provide them with different physicochemical and mechanical properties. These structural or chemical modifications derive from the biological source of the molecule [1].

Chitosan (CS) is a polysaccharide obtained by the deacetylation of chitin, the main component of insect/crustacean shells. This linear polymer is composed of a random mixture of D-glucosamine and N-acetyl-D-glucosamine with a ratio above 50% of the former. The amine groups can be easily protonated to produce a polycation, to yield the unique "natural" cationic polysaccharide [2]. Also, its biocompatibility and antimicrobial effect makes it useful in several fields [3]. The recent literature shows their uses as drug carriers [4], biosorbents [5,6], food packaging materials [7] or in textile fabrics functionalization [8].

Xanthan gum (XG) is a branched exopolysaccharide produced by *Xanthomonas campestris*. Its main chain is composed of D-glucose linked

by β -1,4 glycosidic bonds. Each two units, a trisaccharide branch is attached, composed of two mannoses separated by one glucuronic acid. The pyruvate group, present on the last mannose, and the glucuronic acid confer an anionic character [9]. Its particular non-Newtonian rheological properties make it useful in the food industry as a thickener [10,11] but also in the field of regenerative medicine [12].

Locust bean gum (LBG), extracted from the carob fruit, is generally produced as a mixture of several components. The polysaccharide is about 85% of the total mass and the rest is protein (ca. 5%), and small amounts of fat, crude fibres plus 10% moisture [13]. The galactomannan is composed of a linear chain of D-mannose with a D-galactose branch every four mannose units [14]. Locust bean gum is especially useful in the food industry as a thickener. Because of its synergistic interaction with xanthan gum [15], films [16], nanoparticles [17] or food thickeners are prepared using this mixture.

Cyclodextrins (CD) are produced by an enzymatic reaction between starch and the CGTase protein. These cyclic oligosaccharides, formed by 6, 7 or 8 glucose units (named α -, β -, γ -cyclodextrin, respectively), possess a hydrophilic external structure and a hydrophobic cavity which allows them to form inclusion complexes with different molecules depending on their polarity, size or affinity with the cyclodextrin ring. These inclusion capabilities make them useful in different fields such as food packaging, drug delivery or separation technologies [18]. In addition, these oligosaccharides can be easily crosslinked or chemically modified to produce a wide variety of products, including stimuli responsive materials and molecular imprinted polymers [19], and magnetic nanoparticles [20].

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Even though epichlorohydrin is the most common crosslinker utilized to produce cyclodextrin polymers [21], sustainable and non-toxic reagents, such as citric acid and other polycarboxylic acids have also been tested [22]. As demonstrated in the recent literature, matrices made of cyclodextrin crosslinked with citric acid are able to capture hydrophobic and hydrophilic organic solutes, as well as heavy metals [23]. In addition, the possibility of attaching cyclodextrin moieties to cellulose derivatives has been recently explored [24].

The synthesis of polyesters by the crosslinking reaction of citric acid and a polyfunctional hydroxyl containing reagent such as glycerol has been known for years [25]. The theory of gelation and the formation of infinite networks by the condensation reaction of polyfunctional units, including glycerol, had been well established many years before [26]. Several polysaccharides have been previously crosslinked using citric acid [24,27–29], and so has cyclodextrin itself [22,23,30] but the feasibility of combining both materials in the same matrix by a solvent-free procedure has not been explored yet, to the best of our knowledge.

In this study, the aforementioned polysaccharides (chitosan, xanthan gum and locust bean gum) have been crosslinked with or without β -cyclodextrin, using citric acid in different ratios, to create novel hydrogel matrices. The presence of the polysaccharide networks in the formulation could modify the interactions with different solutes. Thus, synergistic and/or selective sorption capacities towards solutes of different nature can be expected. The synthetic path chosen for this approach follows the 'greenest' and most inexpensive procedure: citric acid crosslinks the polysaccharides plus the cyclodextrin molecules by a thermally induced esterification process in a short amount of time [31], and only water is used to separate and purify the insoluble products. By varying the composition in the reactive mixtures, we intend to analyze their differences in their swelling behaviors and to test their sorption capacities towards phenolic model molecules. Besides the fact that the process chosen to prepare these matrices is simple, inexpensive and solvent-free, all the reagents are already used in the food industry so these materials would be suitable for applications in which health and safety concerns are crucial.

2. Materials and methods

2.1. Materials

Citric acid (CTR) was purchased from Panreac AppliChem, Spain (99.5%); sodium phosphate dibasic (Na_2HPO_4) anhydrous ($\geq 98\%$) was from Sigma-Aldrich, Germany. Xanthan gum (XG) (lot#SLBG3388V), locust bean gum (LBG) (lot#SLBC7065V), were obtained also from Sigma-Aldrich. β -Cyclodextrin (purity $\geq 97\%$, humidity 12.5%) was purchased from Wacker, Germany; chitosan (CS) (deacetylation degree 90%, measured by ^1H NMR; Bruker Advance 400 MHz) was a donated sample of unknown origin. Additional characterization data of the three polysaccharides used in this work (viscosities, pyruvate and acetylate contents for xanthan gum and mannose/galactose ratio for locust bean gum) can be found in the Supporting Information section. Phenolphthalein and 1-naphthol ($\geq 99\%$) were from Merck, Germany.

2.2. Synthesis of matrices

Cyclodextrin/polysaccharide-based insoluble matrices were prepared with β -cyclodextrin (βCD), a polysaccharide (PS) and citric acid (CTR) plus the catalyst (Na_2HPO_4) as shown in Scheme S1 (Supplementary data). The crosslinked resins were prepared using different feed ratios of those three main components and a constant CTR: Na_2HPO_4 ratio (4.6:1). The total mass of the three constituents ranged between 3 and 5 g, and PS/ βCD /CTR ratios varied as shown in the ternary plot. For each polysaccharide, we ended up with three types of matrices: the binary CTR- βCD and CTR-PS samples, and the ternary CTR- βCD -PS samples. After each individual mixture was analyzed according to the

procedures shown below, the results of the ternary diagram were fitted to obtain the corresponding contour plots using the smoothing device of OriginPro (2016) software (total points increase factor = 1000, smoothing parameter = 0.05). On each contour plot, the experimental points will be also shown (their colors corresponding to the actual value of the property tested).

The mixtures of the reagents (approximate particle size: 50–100 μm) and the catalyst, once crushed together, were baked in an oven at 170 $^\circ\text{C}$ for 20 min. The obtained solid product was grinded and the powder was washed twice using 100 mL of deionized water, filtered through paper using a Büchner funnel and dried in an oven at 102 $^\circ\text{C}$ to constant weight. The insoluble dry matrices were finally pulverized using a Retsch MM300 ball mill for 30 s (approximate particle size: 10–50 μm). The filtration process is somewhat problematic in the case of matrices prepared with the highest ratios of xanthan gum due to excessive swelling.

2.3. Swelling studies

10 mg of each insoluble dry sample (xerogel) were placed in flask tubes with 10 mL of deionized water for 24 h. Once the swelling equilibrium is reached, the swollen gel mass is measured. Finally, the swollen and initial mass ratios (q_w) were used to analyze the swelling behavior.

2.4. Infrared analysis

Infrared spectra of reagents (see Supplementary data, Fig. S1) and products were obtained using a Shimadzu IRAffinity-1S spectrometer, coupled with an ATR (Specac) device and the software LabSolution-IR. Spectrums were acquired between 4000 and 600 cm^{-1} with 32 scans and a resolution of 4 cm^{-1} . Curve fitting procedures have been performed using the "Multiple Peak Fit" tool in the Origin software (see Supplementary data, Figs. S2–5). In the case of the xanthan matrices, in order to improve the peak analysis, a chemical modification procedure was performed on the samples. Briefly, 20 mg of each solid matrix were placed for 1 h in a pH 2 buffered solution (10 mL); after centrifugation, the solid residue was dried at 70 $^\circ\text{C}$ and its infrared spectrum was collected. With this procedure, the carboxylate band at 1600 cm^{-1} converts into a carboxylic acid band at ca. 1710 cm^{-1} , which is easier to resolve from the other bands in this region (see Supplementary data, Figs. S4–5).

2.5. Phenolphthalein absorption

0.1 mol/L sodium hydrogen carbonate buffer (adjusted to pH 10.5 using NaOH 6 mol/L) is employed to prepare 3.6×10^{-5} mol/L of phenolphthalein (with an addition of 0.4 mL of ethanol per liter to solubilize it). For each polymer, samples were measured in triplicate using different weights (2, 5 and 10 mg) in 7 mL of the aforementioned solution. After 24 h of mixing, the samples were centrifuged during 5 min at 10,000 rpm and the supernatant was analyzed using UV-vis spectrometry (Cary 8454, Agilent Tech.).

2.6. Sorption of 1-naphthol

10 mg of each matrix were added to 10 mL of the 1-naphthol solutions for 5 h, then filtered using a 0.1 μm PVDF membrane (Durapore®), and analyzed with HPLC (Agilent 1100). With regard to the sorbate concentrations used, two levels of 1-naphthol were selected, 20 and 200 ppm. In addition, ten point isotherms (2; 5; 7.5; 10; 20; 50; 100; 150; 200; 300 ppm of 1-naphthol) were obtained using four matrices with the same CTR amount (46.5%) but with different PS/ βCD ratios (i.e. 0%, 33%, 67%, 100% of PS) (see Scheme S1, star marked mixtures). The sorption data were fitted using the Hill isotherm [32].

3. Results and discussion

3.1. Synthesis procedure

As mentioned in the introduction, the aim of this work was to prepare insoluble networks using polysaccharides and cyclodextrin, in order to combine their main features, by using the most inexpensive and innocuous procedure possible. Although an esterification process carried out in aqueous solutions using common acidic catalysts was a possibility, and that was the common practice in the previous literature [24], we decided to avoid even that step and attempt a high temperature reaction using a solid mixture. Homogenization of such blends can be the main issue in such a procedure but the use of a thoroughly dispersed solid made of extremely fine grain size and reaction temperatures above the melting point of citric acid proved to be successful. The selection of the reaction conditions (temperature, time, catalysts) and their influence on the yields and characteristics of the resulting products have been the subject of a different work [33]. We found that, as expected, the results for three different batches produced using the same reaction conditions were not identical. The variability found is manageable at the laboratory scale but it could be a problem if the scaling-up of the process is intended. The use of kneading techniques (i.e. preparing a paste using a small amount of water) would be advisable in that case.

Once the unreacted chemicals and the crosslinked soluble fraction are washed away in the purification process, three ingredients remain in the crosslinked resin: the polysaccharide (i.e. xanthan gum, locust bean gum or chitosan), the cyclodextrin moieties, and the citrate groups (partly in its ester form and in its unreacted acidic form). Ternary plots seemed to be the best choice to analyze the characteristics of the products, by covering all composition ratios. It has been assumed that, as in a general mixture problem, the measured responses depend only on the proportions of the ingredients present and not on the amount of the mixture [34]. The latter was not kept strictly constant in our design though. In all cases, the feed ratios of the three components are plotted in the ternary diagrams (see Scheme S1) and a computational interpolation has been carried out to fill the whole triangle (the extrapolated regions, beyond the outer mixtures assayed with the smallest amounts of the CTR crosslinker, will be shown in lighter tones).

After the reaction conditions were selected, identical in all cases, and the insoluble matrices were isolated, the reaction yields could be compared. As seen in Fig. S6 (Supplementary data), the two component CTR + β CD matrices (right sides of the three triangles) present a maximum yield around 30% corresponding to a feed ratio of ca. 45/55 (CTR/ β CD, w/w). Above and below this ratio, the amount of insoluble resin obtained decrease (down to less than 10%, in some instances). The fact that citric acid cannot self-react and the deficiency of cyclodextrin will prevent the formation of the networks. On the other hand, for higher β CD ratios, the crosslinking process cannot be optimal either because of the low amounts of citric acid. Indeed, the probability of establishing citrate bridges between two crosslinked cyclodextrin units is lower when the latter is in excess.

When a ternary mixture is prepared by adding a polysaccharide to the formulation, the yields always increase. In fact, for the ternary mixtures with LBG or CS, the maximum yields were obtained in the absence of β CD (i.e. bottom sides of triangles in Fig. S6). In the case of XG, the maximum yield corresponds to a 40/60 CTR/XG blend with a 20% of CD in the feed ratio. This difference can be explained by the excessive swelling capacity of 'pure' xanthan hydrogels. Matrices with high xanthan gum ratios absorb a large amount of water (see below), which prevents a proper polymer filtration and recuperation of the whole insoluble product.

For the three polysaccharides, yield increases steadily with the PS/ β CD ratio when we keep the amount of citric acid constant. The maximum yield for the XG matrices is about 60%, for LBG ones is close to 30%, while for CS mixtures is over 70%. It becomes evident that the long polysaccharide chains provide a higher probability of becoming

crosslinked through citrate bridges in at least two positions when compared to the smaller and sterically hindered cyclodextrin moieties.

3.2. Swelling capabilities

The amount of absorbed water for xanthan ternary matrices is remarkably higher than those for the two other polysaccharides. Moreover, this swelling is considerable for networks that possess only crosslinked xanthan gum and no cyclodextrin. Xanthan gum, with its acid functional groups, is known to swell up to a great extent [27]. For dual CTR/XG matrices a maximum swelling is achieved at about 25/75 (CTR/XG). At this concentration, the crosslinking yield is relatively low. For a higher citric acid content (i.e. a lower ratio of xanthan gum), the probability of intrachain crosslinking and/or the possibility of unsuccessful esterification reactions are higher. On the other hand, when the citric acid percentage is low, interchain crosslinking is more probable and the effective crosslinking density will be low. This corresponds to a higher value of the molecular weight between crosslinks and the corresponding extent of the swelling of these hydrophilic materials.

With respect to the other polysaccharides, 'pure' locust bean gum matrices also present a higher swelling for higher gum ratios, although in a moderate fashion if we compare it to xanthan gum. Finally, for the chitosan resins, the same result is achieved: higher swelling degrees are found for CTR-CS samples with higher chitosan ratios. It was observed that these chitosan matrices coagulate when water is added so they are easier to purify by washing and filtration.

The swelling behavior of the ternary polysaccharide/cyclodextrin/citric acid matrices is shown in Fig. 1, where the contour plots have been obtained using a log scale due to the large differences found. The binary cyclodextrin/citric acid samples show much smaller swelling ratios compared to those of the polysaccharide/citric acid matrices described above. Nevertheless, in the case of the ternary mixtures, although it could be expected that, for a certain citric acid ratio, the degree of swelling was higher for a larger polysaccharide/cyclodextrin ratio, this is not the case, and additional maxima are found within the triangles. Thus, using the smallest amounts of the crosslinker (ca. 10%), the mixtures prepared using 50–60% of cyclodextrin present a swelling ratio of about 20 (lower in the case of chitosan).

3.3. Characterization of citrate contents

Infrared spectroscopy is an appropriate technique, both reliable and affordable, to characterize the composition of polymeric matrices provided that the components are present in a significant amount and possess distinct vibration modes that appear well resolved in the spectrum. In this case, cyclodextrin share its functional groups with other polysaccharides, so it is complicate to ascertain the actual cyclodextrin contents of the matrices. This essential parameter (since the sorption capability of the matrix is its most important property) will be characterized by an indirect measurement using specific sorbates (see below). Thus, locust bean gum infrared spectrum is similar to that of cyclodextrin with intense bands at 1020 cm^{-1} (alcohol C—O stretching) and 1050 cm^{-1} (ether C—O stretching). Xanthan gum has additional carboxylic moieties, which are shown in the 1600 cm^{-1} band. Chitosan possess its own specific vibrational modes corresponding to its amine and amide groups (1585 and 1540 cm^{-1}). The fingerprint region of the reagents can be found in the Supplementary data section (Fig. S1).

In contrast to these 'sugar' compositions, the citrate/citric acid contents, which will provide an interesting indication on the extent of the crosslinking reactions, are more easily studied by means of the carbonyl/carboxyl bands. LBG/CD crosslinked matrices have been selected to analyze the extent of the crosslinking reactions. The 1550 – 1800 cm^{-1} region of the locust bean gum matrices present four characteristic bands: two intense bands located at ca. 1740 and 1715 cm^{-1} , which correspond to citrate and citric acid carboxyl groups,

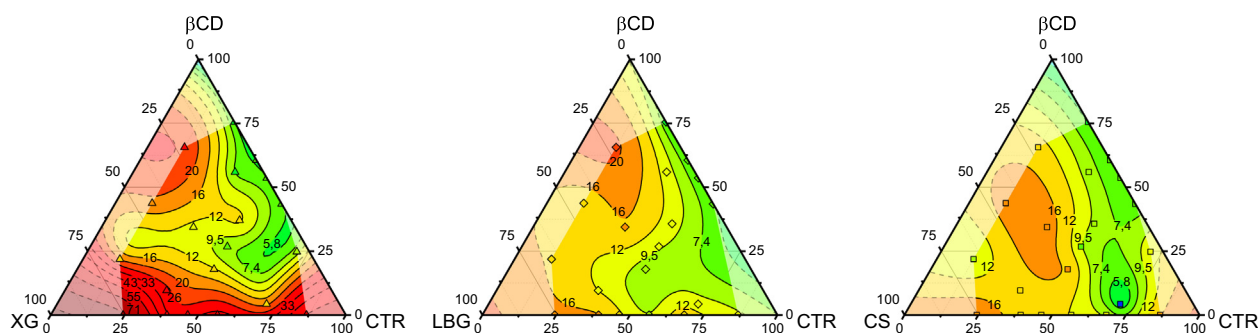


Fig. 1. Swelling ratios (q_w values shown on the corresponding contour lines, in log scale) of matrices of cyclodextrin (β CD), citric acid (CTR) and polysaccharide (XG: xanthan gum, LBG: locust bean gum, CS: chitosan). A common color scale has been used for the three plots.

a medium band located at ca. 1650 cm^{-1} for the stretching of hydroxyl groups of water, and a small band at 1580 cm^{-1} , characteristic of LBG samples, and attributed to the protein residues [35]. Because of the overlapping of the vibration model in this region, a fitting procedure has been carried out to separate these contributions, and the relative areas of several samples have been analyzed (see Figs. S2–S5, Supplementary data section). In order to simplify the comparisons, four families of samples have been selected. Three of them correspond to three different fixed feed ratios of citric acid (10%, 40% and 50%). For each of these three groups of samples, the LBG/CD ratios have been varied. Finally, a fourth group of samples possess the same LBG/CD feed ratio but the CTR contents are different. It can be observed that, as expected, the relative areas of the citrate and citric acid bands increase with the CTR contents in the samples. On the other hand, for a given CTR ratio, it is clearly observed that these bands are not the same when LBG/CD ratios are varied. For the three families of samples, the 1710 cm^{-1} band steadily increases with the amount of cyclodextrin in the mixture.

A much simpler way of comparison is to produce a contour plot (Fig. 2) using the ratio of intensity of the 1710 cm^{-1} band (corresponding to the citrate groups) over the sum of that intensity plus the one of the 1010 cm^{-1} band. The latter gives us an idea of the total sugar contents, since those groups are present both in the cyclodextrin moieties and in the polysaccharide chains.

3.4. Amount of cyclodextrin available

The amount of β -cyclodextrin present in the insoluble matrices is a key parameter to characterize these materials. Moreover, it is their relatively hydrophobic cavities which adds a particular functionality to the hydrogels. Wherever the cavities are not accessible due to steric hindrance, the cyclodextrin moieties will behave as ‘regular’ saccharide functional groups, capable of establishing specific interactions via hydrogen bonding. It has been shown in the literature that

phenolphthalein is a very simple and convenient sorbate to analyze the amount of cyclodextrin sites available [36].

For both the locust bean gum and chitosan ternary plots (Fig. 3), the highest amount of phenolphthalein sorbed corresponds to the same spot, located on the right side of the triangles (i.e. CTR-crosslinked cyclodextrin matrices with no polysaccharide). In contrast, xanthan-based polymers present a maximum of phenolphthalein absorbed for a feed ratio that contains a small amount of the gum, i.e. in a region within the triangle. Interestingly, the available cyclodextrin calculated for the three systems is remarkably lower than the corresponding initial β CD feed ratio. In our previous investigations [31] it has been proved that this solid state esterification reaction is not totally effective and a significant amount of the initial cyclodextrin is not crosslinked to become part of the insoluble crosslinked resin, so it is lost along the purification steps. Moreover, some of the cyclodextrin cavities that can be found in the matrices will not be accessible after the crosslinking process due to steric hindrance, so their inclusion interactions, such as those expected for phenolphthalein, will not be feasible. Nevertheless, for these matrices the swelling ratios seem to be high enough to facilitate the access to the cavities, so the differences found in the available β CD values might be mainly due to the crosslinking reaction efficiency.

Finally, when the three matrices are compared, chitosan and xanthan gum have almost the same concentration of free cyclodextrin for the same initial compositions. Locust bean gum polymers, on the contrary, present a lower percentage of free cyclodextrin than the other two polysaccharides. In particular, for a LBG feed ratio of 30% and above, the maximum cyclodextrin available present is quite small.

3.5. Matrices characterization using 1-naphthol as a model sorbate

If a better characterization of the different sorptive characteristics of these matrices is intended, a model sorbate should be selected so the effect of both the cyclodextrin (‘primary’) cavities and the rest of the saccharide/citrate networks (‘secondary cavities’) can be ascertained. For

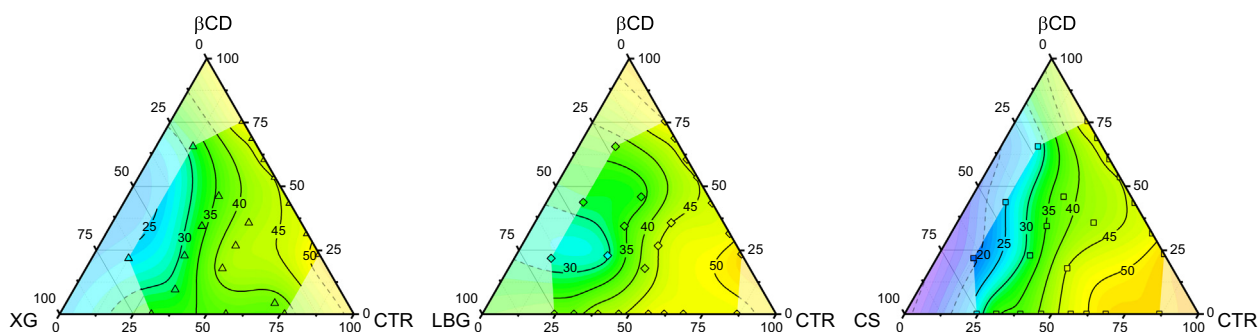


Fig. 2. Ternary diagrams for the percent of carbonyl citric acid (CTR) infrared band crosslinked polysaccharides (CS: chitosan, XG: xanthan gum, LBG: locust bean gum) functionalized with β -cyclodextrin (β CD).

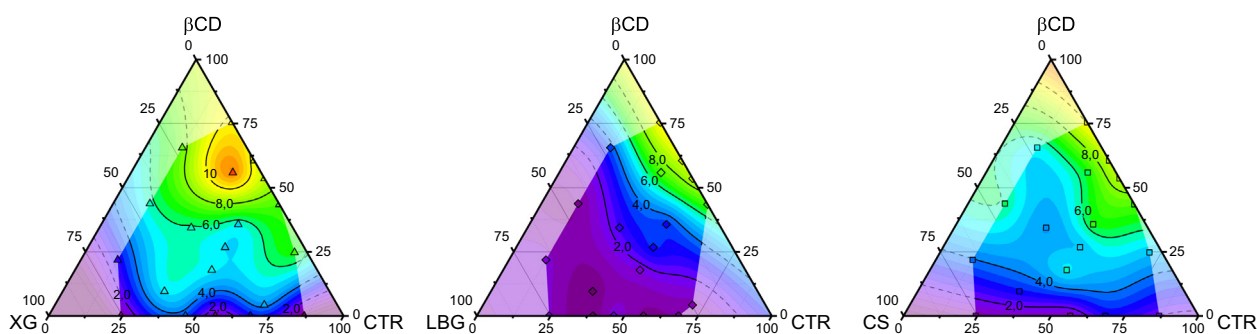


Fig. 3. Ternary diagrams for the sorption of phenolphthalein (mg/g polymer) onto citric acid (CTR) crosslinked polysaccharides (CS: chitosan, XG: xanthan gum, LBG: locust bean gum) functionalized with β -cyclodextrin (β CD).

the latter, the influence of the citrate carbonyl groups and the hydrophilic hydroxyls can be tested if the model sorbate contains a chemical function that can establish specific interactions via hydrogen bonding. In previous works [37], 1-naphthol has been chosen as a model molecule because it fulfils both requisites: a hydroxyl group to bond with polar moieties and a naphthalene ring suitable for a favorable complexation within the cyclodextrin rings.

Two initial concentrations of 1-naphthol have been selected to test the sorption behavior of the matrices (Fig. 4). For a low level such as 20 ppm, the most favorable sites are expected to be occupied. On the other hand, a higher initial concentration of 200 ppm will allow us to obtain an idea on the maximum sorption capacity of the resins, as this concentration is assumed to be well above their saturation capabilities. This characteristic will obviously depend on the sorbate.

Fig. 4 shows that, for both the xanthan gum and the chitosan matrices, 1-naphthol absorption is mainly attributable to β CD but the citric/citrate groups are also important (compare this to Fig. 3, where the maximal sorptions of phenolphthalein were located much closer to the β CD corners). The 1-naphthol absorbed amount is always lower for the ternary mixtures than for the binary CTR- β CD ones. As can be

seen, the absorbed amounts steadily decrease with the polysaccharide ratio. In fact, both types of matrices will absorb similar amounts of 1-naphthol for the same β CD/polysaccharide ratios. Nevertheless, there is also a remarkable difference for the bottom region of the triangles of the samples tested using the high level of 1-naphthol.

In contrast, for the locust bean gum matrices, the maximum of 1-naphthol absorption is reached within the ternary plot, for ternary mixtures with a high LBG ratio. Two reasons can be advantageous for this distinct behavior. From an interactional point of view, a synergistic interaction between 1-naphthol and LBG/ β CD networks can favor the sorption since both hydrogen bonds and inclusional interactions can occur at the same time. The medium level swelling of CTR- β CD-LBG matrices favors the availability of cyclodextrin cavities and the simultaneous interaction with neighboring saccharide networks.

For the high concentration level tested (Fig. 4, bottom), the total 1-naphthol mass in the 10 mL vials is 2 mg, an amount obviously well above the sorption capabilities of the 10 mg samples of the matrices. For instance, in the case of β -cyclodextrin polymers prepared using epichlorohydrin, the most common crosslinker used in the literature, it was found that the sorption capacity was about 30 mg of phenol per gram of

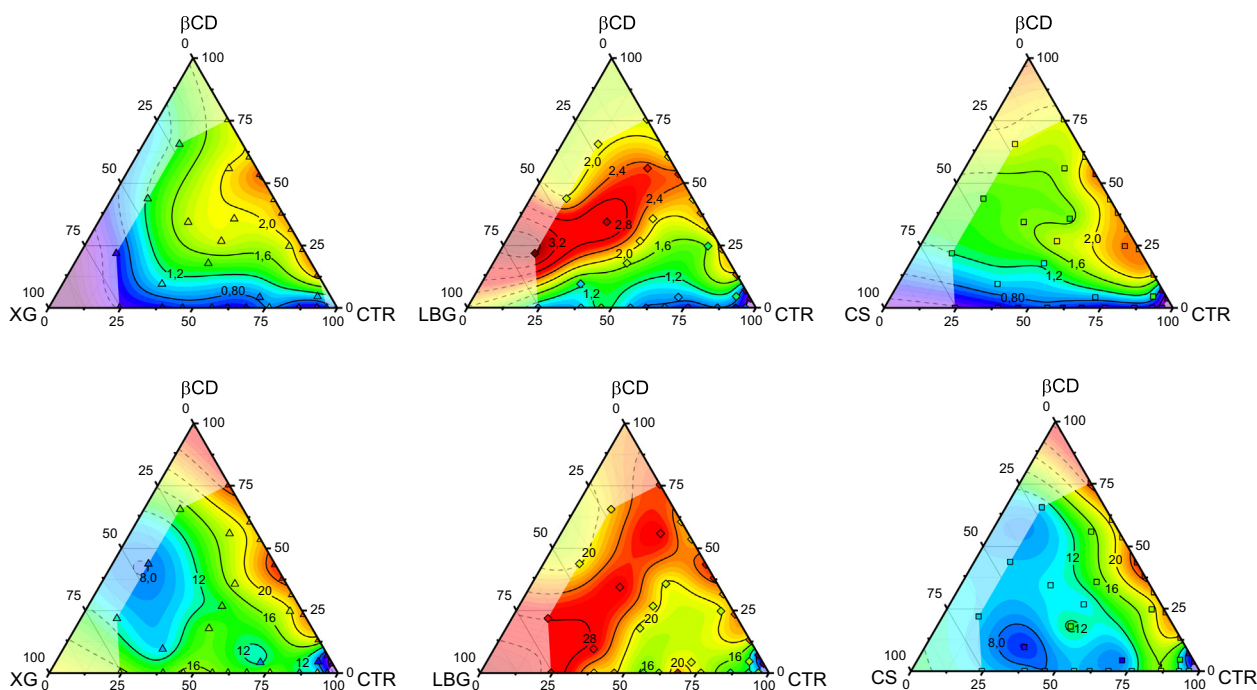


Fig. 4. Ternary diagrams for the sorption of 1-naphthol (mg/g polymer) onto citric acid (CTR) crosslinked polysaccharides (CS: chitosan, XG: xanthan gum, LBG: locust bean gum) functionalized with β -cyclodextrin (β CD). Initial 1-naphthol concentrations: 20 ppm (top) and 200 ppm (bottom).

polymer using effluents with ca. 500 ppm of phenol; higher capacities were achieved in batch experiments using considerably higher sorbate concentrations [38,39]. For 1-naphthol, typical values found for its sorption in cyclodextrin/epichlorohydrin polymers are somewhat higher, depending on the initial sorbate concentration but in the same order of magnitude. As mentioned above, in the case of CTR- β CD-CS and CTR- β CD-XG matrices, the maximum capacity is found for the binary CTR- β CD samples, with 0.24 mg absorbed in 10 mg of polymer (i.e. 24 mg/g), so it becomes evident that adding the polysaccharide as a third component of the matrices somewhat dilutes the favorable interactions with the other two. As a matter of fact, the contour lines are almost parallel to the β CD-CTR side of these triangles, so the sorption steadily decreases as the amount of the polysaccharide is increased (note that this was not the case for the low concentration level of 1-naphthol, where the contour lines were curved). The differences between these two (CS and XG) matrices become evident for a polysaccharide ratio higher than 25%, when xanthan-based matrices absorb more than the chitosan ones. Besides, the amounts absorbed decrease further for the high concentration level of 1-naphthol.

3.6. Sorption isotherms for 1-naphthol

The previous results point to remarkable differences in the sorption behavior of the ternary mixtures with respect to the model compound chosen. With the aim of achieving a better characterization of the matrices themselves, the sorption isotherms at room temperature have been analyzed over a wide concentration range. For each polysaccharide, four resins have been analyzed. All of them with the same CTR ratio (46.5%) but four different CD/PS proportions: 0%, 33%, 67% and 100%. The 'pure' β CD matrices (with a β CD:CTR ratio close to 1:1) possesses the better absorption capacity for all 1-naphthol concentrations tested, up to 300 ppm, with one exception: the CTR-LBG (with no β CD) matrix will absorb more 1-naphthol between 100 and 250 ppm (see Fig. 5).

As can be seen in Fig. 5, chitosan/ β CD matrices absorption mainly depends on their β CD contents, which confirms the results of the previous section as shown in the ternary plots. Thus, for a given concentration of the sorbate, the amount absorbed per unit mass of the polymer increases with the β CD proportion. In that ternary plot, the contour lines are almost parallel to the right side of the triangle, meaning that the sorption here is mainly due to the cyclodextrin moieties, and the polysaccharide acts quite like a diluent of the former.

In comparison, xanthan matrices show a more complex behavior. The mixed xanthan/ β CD matrices, plus the one with no cyclodextrin, absorb less than those with 'pure' β CD but, interestingly, the three isotherms are very close to each other. At the low concentration range, between 0 and 50 ppm, pure xanthan matrices sorb the lowest amounts but, for concentrations above 100 ppm, the values of q are similar. In other words, both components are capable of interacting with 1-naphthol when the sorbent is closer to its saturation capacity. Nevertheless, the most favorable sites will be always filled first, as reflected in the significant differences found in the low concentration range (see Fig. 4, top).

Finally, for the LBG matrices the sorption behavior is similar to that of the XG ones for the low concentration range. Thus, the isotherms show again that the most favorable sites for the guest molecules are the cyclodextrins. In contrast, the isotherms cross each other at high concentrations of this sorbate (ca. 80–100 ppm). The interactions between LBG and 1-naphthol are more important for these matrices. In the ternary plots, it was observed that the regions with the highest amounts sorbed were those with a low CTR ratio and a high LBG amount (see Fig. 4). Interestingly, if all matrices are compared for a concentration range between 100 and 200 ppm, the sorbent prepared with LBG and no cyclodextrin absorbs the highest amount of 1-naphthol because of their favorable specific interactions and its particular degree of swelling. Above this range, the 'pure' cyclodextrin (with no LBG) matrix isotherm is again above the others. Xanthan matrices are also good

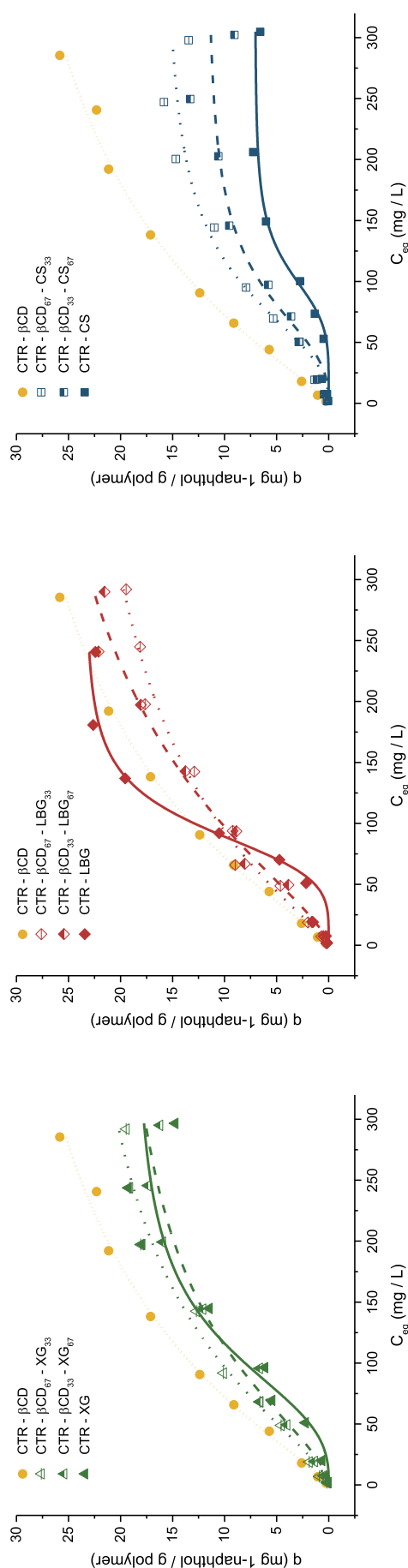


Fig. 5. Sorption isotherms of 1-naphthol (up to 300 ppm) of sorbate onto ternary matrices of cyclodextrin (β CD, yellow line) and polysaccharide (XG: xanthan gum, LBG: locust bean gum, CS: chitosan) in different ratios (33%, dotted lines; 67%, dashed lines; 100% solid lines) crosslinked with the same amount of citric acid. Lines represent fitting results using the Hill isotherm.

sorbents for 1-naphthol by themselves, same as the LBG ones in contrast to chitosan, but their excessive swelling might be detrimental in their sorption capabilities because the network is too 'loose' to hold this particular sorbate.

Several models have been proposed to fit sorption data. The classical Langmuir and Freundlich models represent two typical behaviors: homogeneous sorbents, in the former and heterogeneity for the latter. Those have been successfully applied to cyclodextrin resins in the literature. In some cases, a dual model combining both is more convenient when the primary cavities of the cyclodextrins behave as homogeneous sites and the rest of the network possess also sorption capabilities through 'secondary cavities', which behave as a heterogeneous sorbate, as expected [40].

The peculiar shape of some of the curves points to the fact that, although a saturation plateau seems to be reached in all cases, a two-parameter equation such as Langmuir's will not produce satisfactory fitting results. Both the Langmuir and Freundlich models have been tested for these ternary matrices, but it was found that the best fitting results were obtained using the Hill isotherm (see Supplementary data, Fig. S7). This model, which is convenient to explain the cooperative binding of single molecules to a macromolecule or other adsorbents [32], allows us to extract the maximum quantity that these matrices can adsorb at saturation (q_{sat}) and the cooperativity coefficient of the binding interaction (n), as can be seen in Fig. S8 (Supplementary data), plus the Hill constant value.

As expected, the 'pure' β CD matrix shows the highest q_{sat} value. For the LBG series, the values are below that, and the synergistic behavior of the favorable sorption in this gum is shown in the higher value of q_{sat} for an intermediate 1:2 β CD/LBG matrix. On the other hand, for the two other polymers types, the lower the β CD proportion, the lower the value of q_{sat} . Those sorbents mainly show an additive behavior. Xanthan gum matrices will adsorb better than chitosan ones, as reflected in the higher values found for this parameter. With respect to the other characteristic parameter obtained by fitting this model, n is the number or fraction of adsorbed molecules per site [41]. The 'pure' cyclodextrin sorbent presents an n value close to unity, i.e. the 1-naphthol molecules are adsorbed individually in this matrix. The opposite is found with the 'pure' polysaccharide matrices (with no cyclodextrin). The three of them show a more cooperative binding, with n values close to or above 3. In these matrices, where the sorption is certainly non-inclusional, the hydrophobic interactions between these sorbate molecules must play a significant role in the process, so the binding is more cooperative. Finally, in the case of the 'combined' β CD/polysaccharide matrices the n values are closer to unity again for the LBG and XG matrices, while they lie between the two extreme values of the parent polymers for the chitosan ones (note that in Fig. 5 these isotherms are also equally spaced between those of the 'pure' matrices).

4. Conclusions

The main goal of this investigation was to prepare new sorbents using common and harmless (food grade) raw materials through a green and inexpensive procedure. Cyclodextrins themselves present an interest due to their complexation capabilities with many hydrophobic molecules. Its immobilization within an insoluble matrix permits to use them for many applications, such as sorbents of drugs or flavors, among others. Matrices prepared by crosslinking the cyclodextrin units with citric acid have demonstrated these capabilities but the incorporation of a polysaccharide can make them much more versatile.

Besides the remarkable yields (close or above 50%) achieved by this simple and low-cost method of synthesis, the addition of a hydrophilic polysaccharide permits to obtain materials suitable to achieve different degrees of swelling, which has an influence both on the kinetics of the sorption process and, as shown in this investigation, in their sorption capacities and mechanisms of interaction.

Two model molecules have been chosen to test the properties of these new sorbents. On the one hand, phenolphthalein behaves as a specific sorbate for the cyclodextrin cavities. Matrices with the highest β CD ratios absorb more phenolphthalein, which serves as a good indicator of the amount of cyclodextrin available for inclusional interactions. On the other hand, 1-naphthol shows also a good affinity for the β CD-rich matrices, but especially for those with intermediate to high crosslinker ratios. Interestingly, locust bean gum mixtures present the highest sorption for high gum (i.e. lower cyclodextrin) contents and for high sorbate concentrations, while chitosan matrices show a progressive increase of sorption as the ratio of cyclodextrin increases, and, finally, xanthan gum matrices present similar sorption capacities, irrespective of their composition.

Thus, the three selected polysaccharides have produced significant differences in the crosslinked resins. Chitosan-based matrices are produced with better yields due to a higher probability of citric acid and other types of crosslinking thanks to their amino groups. Nevertheless, this polysaccharide does not have an effect in the sorption of the selected model molecules (1-naphthol or phenolphthalein) and its networks behave as a physical support for the cyclodextrin moieties. Xanthan-based matrices show a considerably higher swelling capacity. For 1-naphthol sorption, the process is more efficient for high sorbate concentrations due to the gum component. On the other hand, for low sorbate concentrations, the β -cyclodextrin moieties are responsible for the sorption. Finally, locust bean gum does not present a good affinity for β -cyclodextrin and citric acid, as reflected by the low reaction yields and poor amounts of cyclodextrin available found. In addition to this, for high concentrations of 1-naphthol, the sorption by the β CD-free crosslinked gum can be as efficient as that of the CTR- β CD matrices. Obviously, it is expected that other sorbates will present their own peculiar sorption behaviors in these versatile green sorbents. In order to assess the influence of hydrophobicity, inclusional and specific interactions (such as hydrogen bonding) in the sorption behavior of cyclodextrin-functionalized polymers more model compounds need to be studied. Thus, the potential applications of these carbohydrate matrices will be explored in future works.

CRediT authorship contribution statement

Max Petitjean: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing- Original draft, Visualization. José Ramón Isasi: Conceptualization, Supervision, Writing- Reviewing and Editing, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Characterization data of polysaccharides; scheme of ternary plot with mixtures; ternary plots with reaction yields; FTIR spectra and curve fittings; Langmuir, Freundlich and Hill isotherms and fitting values; Hill equation parameters. (PDF). Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijbiomac.2021.03.098>.

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