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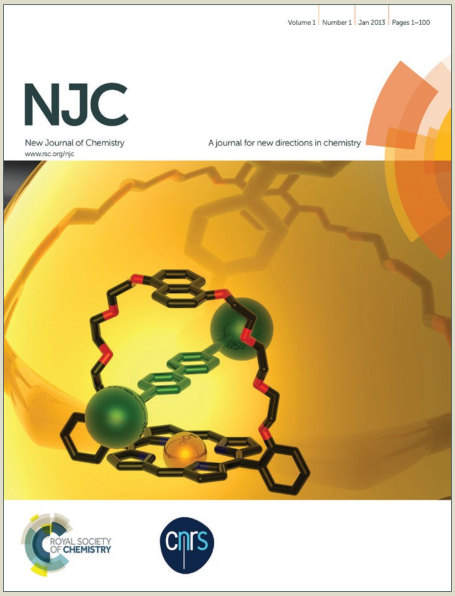

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Cross-linking of highly methoxylated pectin with copper: The specific anion influence[‡]

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The aim of this work was to investigate the influence of specific anions on the cross-linking process of highly methoxylated pectin using the following copper salts $CuSO_4$; $Cu(C_2H_3O_2)_2$; $CuCl_2$; $Cu(NO_3)_2$ wherein the initial salt concentrations were varied from 0.5 up to 10 g/l . It was found that the anions affected the sorption capacity wherein the Cu^{2+} sorption capacity from the sulphate solution was the highest, while it decreased in the presence of CH_3COO^-, Cl^- and NO_3^- ions, respectively. This difference was mostly pronounced at the higher initial salt concentrations ($c_0(Cu^{2+})>2$ g/l). The obtained beads were characterized by FTIR, AAS, SEM/EDS microanalysis and mechanical compression tests up to 80% of deformation.The sorption data were applied to the Langmuir and Freundlich isotherm models and various calculated parameters confirmed the sulphate anion supportive nature in metal ion binding.The value of the K_f parameter for the cross-linking process of pectin in the presence of acetate, chloride and nitrate was approximately the same ($K_f \approx 0.027$ g/g), while it was higher in the presence of sulphate anions by more than 20% ($K_f=0.0352$ g/g). The predicted 1/n values ($1/n<1$, $1/n>1$, $1/n=1$ for the sulphate, nitrate and acetate, and chloride anions, respectively) were quantitative confirmation of the specific interactions involved in the cross-linking mechanism caused by different anions. The established anion influence was in accordance with the typical ion-specific influence on macromolecules in aqueous systems proposed by Hofmeister.

1 Introduction

Natural polymers are very interesting materials for the removal of transition and heavy metals from wastewater with significant advantages such as low-cost, availability, profitability, the ease of use and good efficiency.[1-3] Pectins are complex polysaccharides extracted from plant cell walls, characterized by a backbone of α-(1 → 4) linked galacturonic acid residues, which are partly methylesterified.[4] Native pectins are highly methoxylated (HM) (>50% esterified), while low methoxylated (LM) pectins (<50% esterified) are generally obtained by the controlled acid de-esterification of HM pectins. The presence of carboxyl groups and methyl esters has a determinant influence on the physico-chemical properties of pectins, mainly in terms of complexation and gel formation.[5] The ester groups eliminate negative charges and may sterically hinder the formation of chain aggregates.[6] The extent of this effect depends mainly on the cation and also on the origin of the pectin sample.[7] Pectin isolated from natural sources has a very high content of ester groups and any further treatment in order to reduce the degree of esterification (DE) leads to additional costs. Thus, regardless of the application area, it is favourable for pectin to be used without further modification.

A large number of reported studies have dealt with the affinity of pectin towards different metal cations and the sorption mechanisms involved.[8,9] The cross-linking process includes electrostatic interactions between the cations and negatively charged cavities formed by the polymer chains. In addition to electrostatic interactions, two neighbouring chains are stabilized by Van der Waals interactions and hydrogen bonds.[10] Different radii of the metal ions force the polysaccharide chain conformation to adapt in order to form junction zones. This suggests that the complexes may be governed by different rules, especially the varying coordination arrangements of transition elements. Comparing a range of divalent cations, Cu binds the most strongly to pectin and Ca the most weakly.[7,11] This behaviour may be explained as the result of the binding conformation of Cu to pectin. Namely, hydrated Cu gels form inner sphere

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[‡] *Electronic supplementary information (ESI) available: Fig. S1. The comparison of sorption capacities of pectin on the initial concentration of Cu^{2+} with and without the addition of equimolar concentrations of K_2SO_4; Fig.S2. EDS spectra of the pectin beads surface cross-linked using: a) $CuSO_4$; b) $Cu(CH_3COO)_2$ c) $Cu(NO_3)_2$ and d) $CuCl_2$; Fig.S3. EDS spectra of interior of the pectin beads cross-linked using: a) $CuSO_4$; b) $Cu(CH_3COO)_2$; c) $Cu(NO_3)_2$ and d) $CuCl_2$.*

carboxylate complexes, wherein Cu is bound covalently to two carboxyl groups acting as unidentate ligands.[6,12] Escandar and Sala have shown that interactions between Cu and galacturonic acid, the main component of the pectin chain, involved both the carboxylate and the endocyclic sugar oxygen.[13] In contribution to this, the gelation of pectin with a DE of 94% was still possible with Cu ions, but not with Ca ions. This is very important from the standpoint of pectin application as well as for the understanding of the specific Cu-pectin interaction.[6]

Still, the deeper insights explaining the influence of numerous important factors that could affect the cation binding tendency are relatively rare.[9,22] To the best of our knowledge, there are no scientific data regarding the influence of the anion species on the pectin-metal binding interactions. The specific anion influence has been reported only within a few studies dealing with metal sorption, whereby the reasons for such occurrences stayed unrevealed.[20,22] The reoccurring trend, called the Hofmeister series, ranks the relative influence of ions on the physical behaviour of macromolecules in aqueous solutions and could be a guideline for the establishment of a new theoretical and practical approach concerning these systems.

The typical ordering of the anions from the Hofmeister series is as follows: $F^- \approx SO_4^{2-} > HPO_4^{2->} CH3COO^- > Cl^- > NO_3^- > Br^- > ClO_3^- > I^- > ClO_4^- > SCN^-$. The anions found on the right side of Cl^- are known as chaotropes (weakly hydrated, “water structure breakers”, with destabilising and salting in effects on proteins and macromolecules), while the ones situated on the left side of Cl^- are known as kosmotropes (strongly hydrated, “water structure makers”, with stabilising and salting out effects on proteins and macromolecules).[25] Similarly, in the case of cations, a representative series is: $(CH_3)_4N^+ > NH_4^+ > CS^+ > Rb^+ > K^+ > Na^+ > Li^+ > Mg^{2+} > Ca^{2+}$, wherein the cations found on the left side of K^+ are reported as chaotropes and the ones on the right side as kosmotropes.[34] The significant role of the Hofmeister effect in a wide range of biological and physico-chemical phenomena (from the cloud point of polymer and surfactant solutions to the action of ions on ion-channels in biological membranes) has been confirmed in extensive experiments over the last century, but still, the precise origin of the action of anions is unclarified.[36,37]

Our objective was to investigate if there was any influence of specific anions (Cl^-, NO_3^-, CH_3COO^-, SO_4^{2-}) on the cross-linking process of highly methoxylated pectin by applying the following methods of characterisation: FTIR spectroscopy, atomic absorption spectroscopy, mechanical testing, scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS).A multidisciplinary approach in the results analysis paved the way for the establishment of an analogy with the Hofmeister phenomenon. So, beside the scientific benefits related to the deeper understanding of the interactions involved in the cross-linking of pectin via Cu^{2+} ions, the obtained results may be a step forward in the clarification of the molecular-level mechanisms by which anions operate.

2 Experimental

2.1 Chemicals

Pectin from apples with a 70–75% DE was purchased from the Sigma-Aldrich Company (USA). The following salts were used for the cross-linking of pectin (all supplied by Merck, Germany): $CuSO_4x5H_2O$, K_2SO_4; $Cu(C_2H_3O_2)_2xH_2O$; $CuCl_2x2H_2O$; $Cu(NO_3)_2x3H_20$.

Cu(II) stock solutions (1000 mg/L) were prepared by dissolving the appropriate amount of copper salts in 1000 mL of water. Solutions with different concentrations were obtained by dilution of the stock solutions. All other reagents were of analytical grade. Demineralised water (3 μS/cm) was used in the preparation of all solutions.

2.2 Preparation and characterisation methods

2.2.1 Preparation of the pectin beads. Pectin solution of 2% by weight was prepared by dissolving HM pectin (70-75% DE) in demineralised water. The pectin beads were obtained by the drop-wise addition of 2 g of the resultant pectin solution into each aqueous copper salt solution under gentle magnetic stirring (150rpm). The diameter of the injector was 0.45 mm. All the cross-linking experiments were performed at room temperature. The so produced beads were separated from the solution immediately after cross-linking and were washed 3 times with distilled water. Based on the average value for ten samples, a single hydrogel bead measured approximately 2.8-3.2 mm in diameter. Optical microscopy was used to determine the particle size.

2.2.2 Sorption experiments and pH. The concentration of copper ions in solution, before and after the cross-linking process (which occurred momentarily) was determined by a Perkin-Elmer Analyst 700 AAS. All the sorption experiments were performed at room temperature.

All the experiments were conducted under the same pH value of 4. Namely, pectin dissolved in water has a high content of ionisable COOH groups, which makes it very liable to the influence of pH.[9] The uptake of free ionic copper also depends on the solution pH and increases with the increase in pH from 2.0 to 5.0. Above pH 5.0, insoluble copper hydroxide starts to precipitate from the solution, making sorption studies impossible.[18] At pH values lower than 3.0, Cu(II) removal is interrupted, probably by competition with hydrogen ions for the same sorption sites. Furthermore, H^+ ions may restrict the approach of metal cations as a consequence of the repulsive force between the two types of ions. An increase in solution pH above 3 leads to an increased negative charge density of the pectin chain, so attractive interactions toward metal cations are promoted.[19]

Considering the ionisation of COOH groups of the pectin chain and the solubility of metal ions, it was estimated that pH 4 provided the most adequate conditions for the examination of the cross-linking phenomenon.

2.2.3 Freeze-drying. Prior to freeze-drying, the as prepared beads were frozen for 48h at -20°C. The freeze-drying was conducted for 24h, under a pressure of 0.370 mbar at -30 °C, using a Martin Christ Freeze-dryer Alpha 1-2 LDplus.

2.2.4 Fourier-transform infrared spectroscopy (FTIR). Infrared spectra of pure pectin and cross-linked beads in the freeze-dried state were recorded in transmission mode on a FTIR spectrophotometer (Bomem MB100) in the mid IR region between 4000 and 400 cm^{-1} from KBr pellets.

2.2.5 Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS). SEM analysis was performed using a Jeol JSM 5800 scanning electron microscope with an acceleration voltage of 20 kV. The compositional analysis of the samples was performed by energy dispersive spectroscopy (EDS), model Oxford Inca 3.2, coupled with the scanning electron microscope. Prior to SEM and EDS analysis, the beads were freeze-died in order to conserve the structure without collapsing. The samples were fractured by cutting in half in the frozen state. The bead surfaces and cross-sections were covered with platinum vapour in a LEICA SCD005 nebuliser before the analysis.

2.2.6 Mechanical testing. Room-temperature compressive tests of wet samples were carried out using a 1185 Instron testing machine at a strain rate of 1 mm per minute. Compression was guided up to 80% of particle deformation. The mechanical measurements were repeated three times for each sample.

3 Results and Discussion

The pectin beads were obtained via cross-linking in four copper salt solutions (($CuSO_4$; $Cu(C_2H_3O_2)_2$; $CuCl_2$; $Cu(NO_3)_2$) of different initial concentration (from 0.5 up to 10 g/l). The influence of the variation of the type of anion and their concentration was examined in detail by FTIR and SEM/EDS analysis, as well as sorption investigations and mechanical compression tests.

FTIR spectroscopy was employed to provide the preliminary qualitative analysis of the major functional groups of pectin after cross-linking in copper salt solutions (Fig. 1). The broad and intense absorption peak at 3400 cm^{-1} corresponds to the stretching of -OH groups due to the inter- and intramolecular hydrogen bonding of pectin. The peaks at 1610 cm^{-1} and 1440 cm^{-1}, in the pure pectin spectrum, were assigned to the asymmetric and symmetric stretching vibrations of the carboxylate anion (COO−), while the peak at 1010 cm^{-1} indicates the stretching vibration of C-OH of alcoholic groups and carboxylic acids.[14] The peak observed at 1736 cm^{-1} corresponds to the stretching of the C=O bond due to non-ionic carboxyl groups (–COOH, $–COOCH_3$), and may be assigned to carboxylic acids or their esters.[15]

Noticeably, the FTIR spectrum of pure pectin shows that carboxyl and hydroxyl groups were present in abundance, which could be very indicative for the interpretation of the pectin-copper interaction, hence deprotonated hydroxyl and carboxyl groups are involved in the coordination with metal ions.[16]

After cross-linking via Cu^{2+} ions , the peaks observed at 3400, 1610, 1440 and 1010 cm^{-1} in the spectra of pure pectin had shifted to 3436-3440, 1617-1625, 1418-1420 and 1015-1017 cm^{-1}, respectively (Figure 1). These shifts may be attributed to changes in the counter ions associated with the carboxylate and hydroxylate anions, confirming that carboxyl and hydroxyl groups are predominant contributors in metal ion uptake.[16] Nevertheless, the magnitude of the observed shifts were unsubstantial to be associated with different anion type. After the copper uptake, the peak expected at 1736 cm^{-1} was significantly reduced, which could be explained by the substitution of the H(I) from the COOH by Cu(II) and the formation of a COO-Cu complex.[17]

Still, the FTIR spectrum did not provide any relevant information concerning the influence of anions on the structure of the pectin-copper complex. There was no major shifting or new peaks to confirm the presence of specific anions, probably because of their insufficient concentration to be analysed by FTIR.

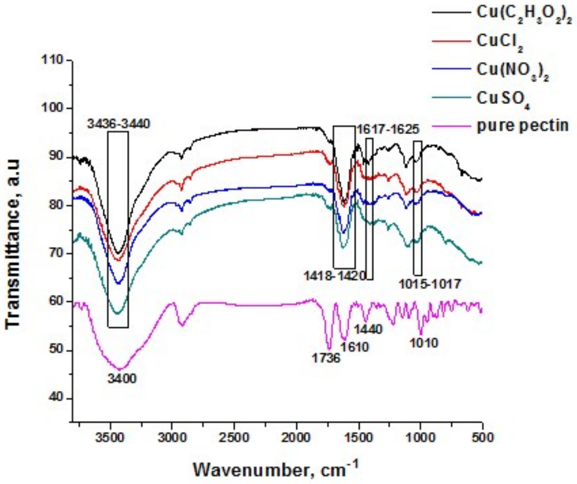

Fig. 1.FTIR spectra of pure pectin and pectin cross-linked in aqueous copper salt solutions.

The cross-linking of pectin via Cu^{2+} ions was governed by a sorption mechanism and quantified by atomic absorption spectroscopy. The equilibrium sorption capacity (q_e) was calculated according to the following equation:

$$q_e = \frac{(c_0 - c_e)V}{w} \qquad (1)$$

where q_e is the equilibrium adsorption capacity (g/g), c_0 and c_e are the initial and equilibrium liquid phase solute concentration (g/L), respectively; V is the liquid phase volume (L) and w is the weight of sorbent (g).

Regarding the dissociation of the used copper salts, it was obvious that the molar concentration of sulphate anions present in solution was different from the concentration of the other anions considered within this study (acetate, chloride, and nitrate). In order to establish the concentration equality of all anions, the concentration of sulphate ions was adjusted by the addition of potassium sulphate in an equimolar ratio. The sorption curves obtained for the cross-linking process of pectin, both with and without the addition of K_2SO_4, are presented in Figure S1 (see Supplementary Information). The amounts of Cu^{2+} ions required for the cross-linking process of pectin in aqueous solutions of $CuSO_4$ and $CuSO_4/K_2SO_4$ were almost the same, so it could be taken that the sorption capacity was not affected by the considered difference in SO_4^{2-} concentration. Fig. 2 shows the cumulative dependencies of the sorption capacities of pectin beads on the initial concentrations of different copper salts.

Generally, the increase in initial concentration of the copper salt solution ($CuSO_4$; $Cu(C_2H_3O_2)_2$; $CuCl_2$; $Cu(NO_3)_2$) from 0.5 up to 10 g/l resulted in the increased values of sorption capacity, which implied greater exhaustion of the copper ions involved in the cross-linking process. All the curves presented in Fig 2 overlap at low initial solution concentrations ($c_0(Cu^{2+})<2$ g/l), so the influence of the anion species could be considered as negligible in this concentration range. Further increase in the initial solution concentration affected the sorption capacity toward Cu^{2+} ions, wherein the maximum sorption capacity was achieved by cross-linking pectin in an aqueous solution of copper sulphate. The sorption capacity, i.e. the amount of Cu^{2+} ions required for the cross-linking process of pectin in aqueous solutions of different copper salts decreased in the following order: $CuSO_4>Cu(C_2H_3O_2)_2>CuCl_2>Cu(NO_3)_2$.

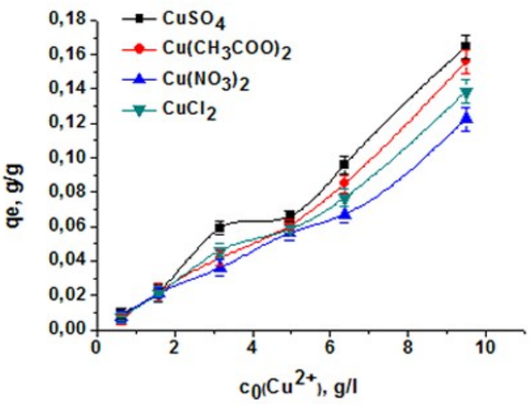

Fig. 2.Cumulative dependencies of sorption capacities of pectin on the initial concentration of Cu^{2+} in aqueous copper salt solutions.

Since all the copper salt solutions were prepared under the same conditions, it was assumed that the effect of the cation would be the same, so, any difference would be ascribed to the different anion. Obviously sulphate, with a higher charge than the other investigated anions, might be more effective in charge compensation and ionic binding.[20,21] Furthermore, sulphate can act as a hydrogen acceptor in moderately strong hydrogen bonds, so the sulphate anion was the most supportive in metal ion binding.[20] Chen et al. have found similar behaviour for copper sorption onto chitosan-based materials.[22] They investigated the sorption capacities of epichlorohydrin-crosslinked chitosan and pure chitosan towards copper (II) cations from an aqueous Cu^{2+} solution of sulphate and chloride, at the same pH. The obtained results have shown that higher values of sorption capacities were attained in sulphate solution.

The differences in sorption capacity toward the same metal cation could also be explained by the influence of the specific anion proposed by Hofmeister.[23] All the anions considered in our research, belong to the Hofmeister order ($F^-\approx SO_4^{2-}>HPO_4^{2-}>CH3COO^->Cl^->NO_3^->Br^->ClO_3^->I^->ClO_4^->SCN^-$).

As already mentioned, the Hofmeister series predicts the ranking of anions according to its specific influence, wherein Cl^- is usually considered to be an indifferent ion or a reference point. The species found on its left are referred to as strongly hydrated/structure-makers (SO_4^{2-} and CH_3COO^- in this study), and the ones one its right as weakly hydrated/ structure-breakers (NO_3^- in this study).[3] With respect to the influence caused by cation species in solution, K^+ is also considered to be without influence.[34] Therefore, the K^+ cation derived from the dissociation of potassium sulphate was taken as neutral in the investigated pectin/Cu^{2+}/SO_4^{2-} system.

The basic characteristics of the anions relevant for the present study including size in solution, the number of water molecules in the hydration layer, polarisability, and molar surface tension increment are listed in Table 1.[26,27]

Table 1. Anion radius – r; polarisability – α, molar surface tension increment – σ, partial molar volume – ν of anions for 1 M aqueous solutions of the studied anions.

Anion	r (pm) [28]	α (Å³) [27]	σ (mN L m⁻¹ mol⁻¹) [23]	ν (cm³ mol⁻¹) [27]
SO_4^{2-}	258	6.3 ±0.4	2.74	11.62
CH_3COO^-	162	/	0.93	39.23
Cl^-	181	3.76	1.63	16.5
NO_3^-	206	4.47	1.18	27.8

Copper salts solvated in water quickly separate into anions and cations and disturb the natural hydrogen bond network of water molecules. The breaking of hydrogen bonds is energetically unfavourable, however, depending on the kind of ion, the loss of hydrogen bonds between water molecules can be compensated.[24] With regard to the Hofmeister theory, at low initial solution concentrations ($c_0(Cu^{2+})<2$ g/l), the influence of anions was negligible since the interactions between ions and water molecules were not strong enough to disrupt the natural hydrogen bond structure (Fig .2).[25] According to the data from Table 1 and the sorption results (Fig. 2), it was obvious that the anions with lower values of polarisability (Cl^-, NO_3^-) had a destabilizing effect, which resulted in decreased values of the sorption capacity at higher concentrations of copper salts. On the contrary, highly polarisable ions, such as sulphate and acetate, were more effective in limiting the electrostatic repulsion, thereby contributing to higher sorption capacities. The effects of a specific anion were more pronounced at higher initial salt concentration, which was clearly shown by differences in the sorption capacity. Since protons may restrict the approach of copper cations, the sulphate anion had the most stabilising effect in terms of reducing the electrostatic repulsion between ions of the same charge.

It is important to emphasize that the given explanation related to the Hofmeister effect was associated with the interaction between specific anions and water molecules, which is the most studied and controversial theory at the same time.[38]

The experimental data obtained for the sorption of Cu^{2+} cations exhausted in the cross-linking process (Fig. 2) were further analyzsed by the well-known models given by Langmuir and Freundlich.

The Freundlich isotherm model assumes neither homogeneous site energies nor limited levels of sorption. It is the earliest known empirical equation and has shown to be consistent with the exponential distribution of active centers, characteristic of heterogeneous surfaces.[29] It is given as:

$$q_e = K_f C_e^{\,n} \qquad (2)$$

Where K_f and n are characteristic Freundlich constants indicating the sorption capacity and sorption intensity, respectively. The linear form of equation (2) is given by equation (3):

$$\ln q_e = \ln K_f + \frac{1}{n}\ln C_e \qquad (3)$$

The K_f constant represents the quantity of sorbed metal ions for a unit equilibrium concentration. The slope 1/n is a measure of the sorption intensity or surface heterogeneity.[30] For 1/n=1, the partition between the two phases is independent of the concentration. The situation where 1/n > 1 is indicative for cooperative sorption, which involves strong interactions between the pectin chains and metal ions.[31] If the value of 1/n is below one, the sorption is normal.[32,33]

The Langmuir equation, which is valid for monolayer sorption onto a completely homogeneous surface with a finite number of identical sites and with negligible interaction between the sorbed molecules, is given by the following equation (4):

$$q_e = \frac{q_{\max} K_l C_e}{1 + K_l C_e} \qquad (4)$$

The sorption parameters were determined by transforming the Langmuir equation (4) into the linear form (5):

$$\frac{1}{q_e} = \frac{1}{q_{\max}} + \frac{1}{q_{\max} K_l}\frac{1}{C_e} \qquad (5)$$

where C_e is the equilibrium concentration (g/L), q_e the amount of sorbed material at equilibrium (g/g), K_l the affinity parameter or Langmuir constant (L/g), and q_{max} the maximal monolayer sorption capacity (g/g). The collected sorption data, fitted to the Freundlich and Langmuir models, are presented in Figs. 3 and Fig. 4, respectively.

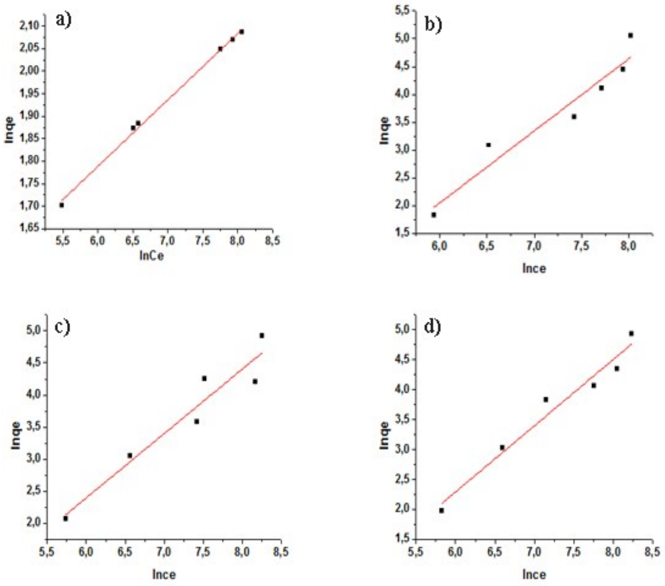

Fig. 3. Freundlich sorption isotherms for the removal of Cu^{2+} ions from aqueous copper salt solutions: a) $CuSO_4$; b) $Cu(CH_3COO)_2$; c) $CuCl_2$ and d) $Cu(NO_3)_2$.

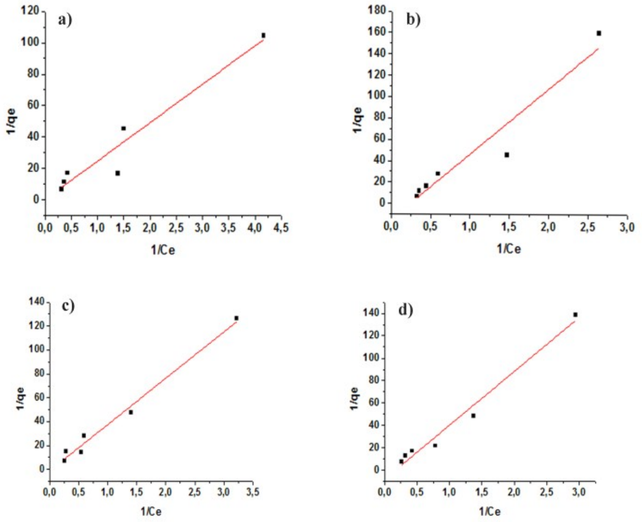

Fig. 4. Langmuir sorption isotherms for the removal of Cu^{2+} ions from aqueous copper salt solutions: a) $CuSO_4$; b) $Cu(CH_3COO)_2$; c)$CuCl_2$ and d) $Cu(NO_3)_2$.

The parameters calculated according to these models are presented in Table 2.

Table 2. Parameters of the Freundlich and Langmuir isotherms for the sorption of Cu^{2+}ions onto highly esterified pectin from different copper salt solutions.

Anion	Freundlich isotherm parameters		
	R^2	1/n	K_f(g/g)
SO_4^{2-}	0.94	0.97	0.0352
CH_3COO^-	0.92	1.30	0.0252
Cl^-	0.91	1.00	0.0270
NO_3^-	0.95	1.10	0.0273
	Langmuir isotherm parameters		
	R^2	q_{max}(g/g)	K_l(l/g)
SO_4^{2-}	0.92	3.28	0.0125

Based on the presented results, it could be assumed that both sorption isotherms were applicable only in the case of pectin cross-linked in copper sulphate solution. For all the other considered cases the system could not be described by the Langmuir isotherm due to the negative values of the intercept ($1/q_{max}$) obtained using this model (Fig. 4). Since the sorption mechanisms predicted by the Langmuir and Freundlich isotherms are completely different, it was obvious that the sulphate anion interacted differently compared to the CH_3COO^-, Cl^- and NO_3^- anions.

Analysing the 1/n values (Table 2), it could be concluded that the cross-linking of pectin in the presence of different anions was governed by different rules. As already mentioned, $1/n > 1$ supports cooperative sorption, so it could be considered that cross-linking in the presence of acetate and nitrate anions was governed by this mechanism. According to a 1/n value equal to 1, the chloride anion induced the concentration independence of the cross-linking process, while the presence of the sulphate anion ($1/n < 1$) resulted in favourable sorption.[31]

The acetate, chloride and nitrate anion did not provide any significant difference related to the K_f parameter, attributable to the sorption capacity. The only difference was associated with the sulphate anion (K_f=0.0352 g/g), which was another indication of the specific interactions induced by this anion.

The surface and inner morphology of the beads was investigated using SEM analysis. SEM studies were performed on freeze-dried beads previously cross-linked in aqueous copper salt solutions of the same concentration (0.1 mol dm^{-3}), prepared as described in the Experimental section. The micrographs of whole beads (a1, b1, c1, d1), their surfaces (a2, b2, c2, d2) and corresponding cross-sections (a3, b3, c3, d3) are presented in Fig. 5.

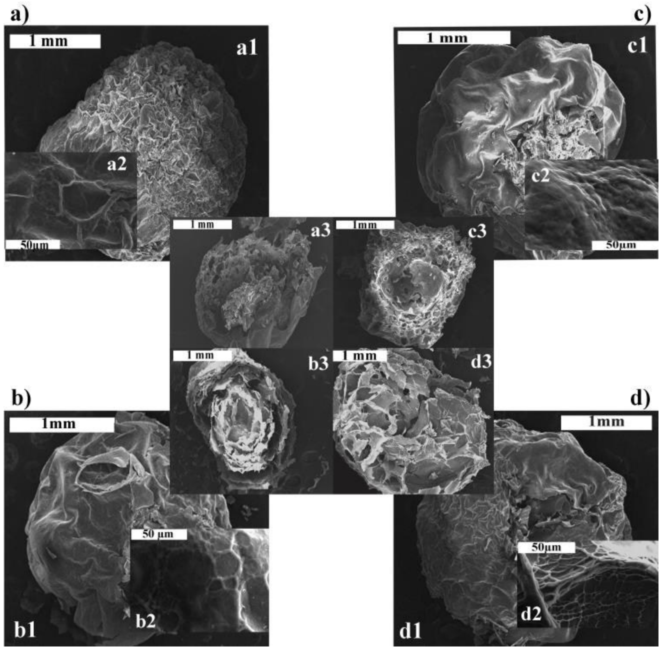

Fig. 5. SEM micrographs of freeze-dried pectin beads cross-linked using: a) $CuSO_4$; b) $Cu(CH_3COO)_2$; c) $Cu(NO_3)_2$ and d) $CuCl_2$.

Although the pectin was cross-linked using the same metal, there were very obvious differences that could be observed both in the bead surfaces (Fig 5, a1, b1, c1, d1) and cross-sections (Fig. 5, a3, b3, c3, d3). The surfaces of all samples were rough and covered with characteristic wrinkles of different size and density (Fig 5, a1, b1, c1, d1). The Cu^{2+} sorption capacity from sulphate solution was the largest, so the surface of the sample obtained from this solution was the most wrinkled (Fig 5, a1). The wrinkle density was decreased with a decrease in the amount of copper ions exhausted in the cross-linking process and this kind of surface morphology may be associated with the shrinkage of beads at higher metal concentrations.[38] The micrographs of the cross-sections (a3, b3, c3, d3) illustrate the ''spongy''-like inner structure with a large number of voids and channels, possibly formed as a result of the migration of water molecules.[39] The size of the voids could be correlated with the cross-linking density, accordingly to the copper content involved in the cross-linking process. So, the cross-section of the bead obtained from copper nitrate solution was dominated by large voids (Fig5, d3). The network-like structures observed at higher magnification (Fig. 5, a2, b2, b3, c3) were the most pronounced on the surface of the bead obtained from copper sulphate solution (Fig. 5, a2), while they were less prominent in the case of beads cross-linked in copper nitrate solution (Fig. 5, d2). Since the sorption capacity and therefore, the cross-linking density decreased in the following order $SO4^{2-}$ $>CH3COO^->Cl^->NO_3^-$, it was expected for the network-like structure of each sample to be present accordingly to this ranking. Fig. 5 confirmed this finding.

SEM analysis was coupled with EDS measurements, which allowed the obtaining of semi-quantitative results on the metal content both on the surface and in the interior of the beads (see Figure S2 and S3, Supplementary Information). For all considered samples, a higher amount of copper was concentrated in the interior of the beads, compared to the bead surface (Table 3). The highest internal content of copper was found at beads cross-linked in copper sulphate solution and decreased in the following order CH_3COO^-, Cl^-, NO_3^-. These results were in accordance with the obtained sorption data. The values obtained for the external copper content in the case of beads cross-linked in copper-sulphate solution were 6-10 times lower compared to the other three anions. This could be one more indication of the specific interaction induced by the presence of sulphate anions. A similar study performed by Ouwerx and co-worker has shown that copper-alginate beads are not uniformly complexed in the bulk as the result of a the specific cross-linking mechanism.[40] Such an explanation could also be acceptable for copper-pectin beads.

Table 3. Distribution of atomic copper content on the surface and interior of pectin beads provided by EDS analysis and mechanical compression test results

Copper salt	Average bead diameter (mm)	Average compressive strength σ(N/mm^2)	Average atomic copper content, %	
			Surface	Interior
$Cu(SO_4)_2$	2.77±0.02	0.101±0.01	0.16±0.07	16.73±0.12
$Cu(CH_3COO)_2$	2.87±0.08	0.099±0.02	1.39±0.09	11.8±0.09
$CuCl_2$	2.91±0.05	0.082±0.02	1.01±0.09	4.46±0.11
$Cu(NO_3)_2$	3.15±0.08	0.072±0.01	1.75±0.08	3.51±0.10

The mechanical analysis of the beads was performed on wet beads, previously cross-linked in 0.1 mol dm^{-3} aqueous copper salt solutions (Table 3). At this initial concentration, the differences between the amounts of copper ions exhausted in the cross-linking process were very pronounced (Fig. 2). Hence, it was expected that the mechanical response of the beads to the applied compression force was directly correlated with the copper ions involved in the cross-linking process. The samples with the highest internal copper content had the largest compression strength at the same deformation. Depending on the salt solution used for the cross-linking of highly methoxylated pectin, the average compressive strength of the beads decreased in the following order $CuSO_4 > Cu(C_2H_3O_2)_2 > CuCl_2 > Cu(NO_3)_2$.

Another experimental result which supported the assumption of the anion influence was the difference in bead average diameter. Due to its asymmetry, H_2O interacts with the anions (through hydrogens) causing small but detectable changes of the surface tension, which certainly affects the cross-linking process on the polymer/salt solution interface. The water surface tension increment as a function of the anion effective polarisability in solution decreases in the following order: $SO4^{2-} > CH_3COO^- > Cl^- > NO_3^-$.[35] The reduction of surface tension as a result of the interaction of water molecules with a particular anion, led to an increase in the contact surface between pectin drops and copper salt solutions. The size of the formed beads was inversely proportional to the surface tension increment, so the beads obtained from copper sulphate solution had the smallest diameter, while the maximum bead diameter was attained from copper nitrate solution.

4 Conclusions

The main goal of this research was to investigate the effect of specific anions on the cross-linking process of pectin. The pectin beads were obtained by cross-linking pectin using a varying initial concentration (from 0.5 up to 10 g/L) of copper salts ($CuSO_4$; $Cu(C_2H_3O_2)_2$; $CuCl_2$; $Cu(NO_3)_2$. Firstly, the binding of copper to pectin chains and the formation of a COO-Cu complex was confirmed using FTIR spectroscopy. Still, this method did not provide enough information to interpret the anion influence. The cross-linking process of pectin was quantified by employing sorption experiments. It was found that the anions affected the sorption capacity wherein the Cu^{2+} sorption capacity from sulphate solution was the largest, while it decreased in the presence of CH_3COO^-, Cl^- and NO_3^- ions, respectively. This difference was mostly pronounced at the higher initial salt concentrations ($c_0(Cu^{2+}) < 2$ g/l). Furthermore, it was demonstrated that the anion influence found in our study was in accordance with the typical ion-specific influence on macromolecules in aqueous systems proposed by Hofmeister, which represents a completely new approach in the interpretation of pectin-metal interactions. The results obtained from the isotherm modelling indicated good fitting to the Freundlich isotherm equation under the considered concentration range, while the Langmuir equation was applicable only in the case of pectin cross-linked in copper sulphate solution. The different values of the 1/n parameter obtained for the cross-linking of pectin in the presence of the investigated anions ($1/n<1$, $1/n>1$, $1/n=1$ for the sulphate, nitrate and acetate, and chloride anions, respectively) were another confirmation of the specific interactions involved in the cross-linking mechanism using the same metal ion. Therefore, in order to explain the cross-linking mechanisms, it is necessary to take into account the direct interactions of the Cu^{2+} with the polymer, as well as the interactions between the anions and water molecules. A wrinkled surface morphology of the beds was found by SEM analysis, wherein the size and density of the wrinkles varied depending on the Cu^{2+} content exhausted in the cross-linking process. EDS analysis showed the different distribution of atomic copper content on the surface and in the interior of the pectin beads, which affected their mechanical properties. The largest compression strength at the same deformation was exhibited by samples with the highest internal copper content (the beads cross-linked in copper sulphate solution) and vice versa.

Though the anion influence on the investigated process was emphatic and clearly presented, further clarifications are needed to determine whether our findings could be applied to other systems (different cations, anions, solution pH, ionic strength, etc.).

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Highly methoxylated pectin was cross-linked using different copper salts. The dependence between anion type, sorption capacity, mechanical and structural properties of obtained beads was found and correlated with Hofmeister theory.