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BINDING AL^{3+}, CU^{2+} JONS AND POLYELECTROLYTES WITH MACROMOLECULES IN SUGAR BEET JUICE

VEZIVANJE AL^{3+}, CU^{2+} JONA I POLIELEKTROLITA SA MAKROMOLEKULIMA SOKA ŠEĆERNE REPE

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ABSTRACT

The affinity of binding Al^{3+} and Cu^{2+} ions to non-sucrose compounds in sugar beet juice has been studied. The efficacy of ion binding was determined according to electrophoretic metod by measuring Zeta potential. Model solutions of pectin and protein preparations (0.1% w.) were treated with solutions of $CuSO_4$ and $Al_2(SO_4)_3$ at seven different concentrations. The efficacy of Al^{3+} and Cu^{2+} ion binding was expressed as a minimal concetration of $CuSO_4$ and $Al_2(SO_4)_3$ needed for charge neutralization i.e. reaching zero Zeta potential. For pectin preparation, concentrations of these salts were 82 mg/dm^3 $CuSO_4$ and 105 mg/dm^3 $Al_2(SO_4)_3$. For protein preparation the concentrations were 115 mg/dm^3 $CuSO_4$ and 122 mg/dm^3 $Al_2(SO_4)_3$. Identical experiments were performed with the addition of cationic polyelectrolyte at 3 mg/dm^3. Concentrations of $CuSO_4$ and $Al_2(SO_4)_3$ needed to change Zeta potential to zero decreased within interval from 7 to 20 mg/dm^3. When the doses of tested ions were recalculated to industrial conditions, they were much lower in relation to consumption of Ca^{2+} ions in form of CaO (1 – 3% w.) which is commonly used in rafination of sugar juice.

***Key words**: macromolecules, sugar beet, Al^{3+} and Cu^{2+} ions, polyelectrolytes.*

REZIME

U radu je proučavan afinitet vezivanja Al^{3+} i Cu^{2+} jona sa nesaharoznim materijama soka šećerne repe, pre svega pektinima i proteinima. U prisustvu ovih jona dolazi do neutralizacije naelektrisanja makromolekula čime se obezbeđuju uslovi za njihovu koagulaciju i taloženje. Izvršena su poređenja sa afinitetom vezivanja Ca^{2+} jona koji se najčešće koristi u čišćenju soka šećerne repe. Dodavanjem katjonskih polielektrolita, pored neutralizacije naelektrisanja makromolekula, ostvaruje se i mehanizam međučestičnog povezivanja. Efikasnost procesa je praćena elektroforetskom metodom, merenjem Zeta potencijala rastvora pektinskog i proteinskog preparata. Povećanjem koncentracije Al^{3+} i Cu^{2+} jona, povećava se udeo hemijskih sila (stvaranje površinskih kompleksa) u odnosu na udeo elektrostatičkih (Kulonovih) sila. Model-rastvori pektinskog i proteinskog preparata zapremine 50 cm^3 (0.1% mas.), tretirani su sa sedam različitih koncentracija rastvora $CuSO_4$ i $Al_2(SO_4)_3$ u intervalu od 30 do 210 mg/dm^3. Efikasnost vezivanja Al^{3+} i Cu^{2+} jona izražena je preko minimalnih količina $CuSO_4$ i $Al_2(SO_4)_3$ potrebnih za neutralizaciju naelektrisanja odnosno za postizanje nulte vrednosti Zeta potencijala. Koncentracije ovih soli, bez prisustva flokulanata iznose, za pektinski preparat: 82 mg/dm^3 $CuSO_4$ i 105 mg/dm^3 $Al_2(SO_4)_3$. Za proteinski preparat, ove vrednosti su: 115 mg/dm^3 $CuSO_4$ i 122 mg/dm^3 $Al_2(SO_4)_3$. Eksperimenti sa katjonskim polielektrolitom (PAM) koncentracije 3 mg/dm^3 pokazuju nešto veću efikasnost uklanjanja pektina i proteina iz ispitivanih model-rastvora. Količine $CuSO_4$ i $Al_2(SO_4)_3$ potrebne za postizanje nulte vrednosti Zeta potencijala, smanjile su se u intervalu 7 do 20 mg/dm.^{3} Količine ispitivanih jona preračunate na industrijske uslove, iznose znatno manje od potrošnje Ca^{2+} jona u obliku CaO (1 – 3% mas. računato na šećernu repu) koji se najčešće koristi u svakodnevnoj proizvodnji šećera.

***Ključne reči:** makromolekuli, šećerna repa, Al^{3+} i Cu^{2+} joni, polielektroliti.*

INTRODUCTION

Non-sucrose compounds are eliminated from raw sugar beet juice in the process of juice rafination in order to obtain final product-white sugar of better quality. The majority of sugar processing facilities in our countries employ chemical rafination of sugar beet juice using CaO in form of $Ca(OH)_2$. However, by this procedure, neither half of non-succrose compounds can be eliminated. Macromolecular compounds, above all pectins and proteins, account for around 60% of total non-sucrose compounds in sugar beet juice. The most common purification practices using milk of lime and carbon-dioxide can remove less than half of non-sucrose compounds present in sugar beet juice. The affinity of calcium to bind the undesirable macromolecules from sugar beet juice is low therefore very high doses of compound are consumed every day in sugar processing. This can negatively affect the environment because calcium can produce undesirable process of soil alcalination in the vicinity of the facility (*Haapala et al., 1996*). Double electric layer which surrounds all colloidal particles in solution, such as sugar beet juice, is consisted of two layers, the adsorbed and diffuse layers. The difference in potential between these layers is called electrokinetic or Zeta potential (*Kirby and Hasselbrink 2004; Delgado et al., 2007*). Zeta potential of sugar beet pectin and protein is mainly negative and it is strong enough to impair the coagulation and agglomeration of the macromolecules in aqueous solutions. The use of compounds with di- or threevalent cations can decrease the Zeta potential to near zero, causing the macromolecules to discharge. Consequently, the system's stability is lost, which leads to the coagulation and precipitation of agglomerated colloidal particles (*Kuljanin et al., 2008*; *Kuljanin et al., 2010; Lević et al., 2007; Schneider et al., 2011).* In the papers of *Kohn* (*1987*) and *Dronnet et al.* (*1996*), the affinity of divalent metal ions to bind with pectin and derivatives of pectin from citrus and sugar beet was investigated. In both studies, it was found that the affinity of metal ions to form complexes followed the order:

$$Cu^{2+} \sim Pb^{2+} >> Zn^{2+} > Cd^{2+} \sim Ni^{2+} \geq Ca^{2+}$$

Interaction between cations and protein macromolecules was investigated in the works of *Gaucheron et al.* (*1997*) and *Philippe et al.* (*2005*). They found the following order regarding the binding ability of cations:

$Cu^{2+} > Zn^{2+} > Ca^{2+} > Mg^{2+} > Fe^{2+}$

On the basis of selectivity scales, it is evident that Cu^{2+} ions are more efficient than Ca^{2+} ions because of marked surface complexation ability (*Garnier et al., 1994;; Kuljanin 2008a*). Among other changes, it was found that macromolecules hydration decreased in the presence of these cations which is an important prerequisite for coagulation besides charge neutralisation (*Kuljanin et al., 2010; Gaucheron et al., 1997*). Affinity of binding of threevalent cations with pectin and protein macromolecules was not investigated. However, there are many studies of applying Al^{3+} ions, especially in form of $Al_2(SO_4)_3$, in water treatment (*Duan and Gregory, 2003*). In the research of ion binding to natural organic matter, the $H^+/Me^{2+,3+}$ molar exchange ratios varied strongly with metal ion: Ca^{2+} (0.2 – 0.5); Cd^{2+} (0.5 – 1.0); Pb^{2+} (1.1 – 1.2); Cu^{2+} (1.2 – 1.7) and Al^{3+} (2.1 – 2.7) and also with free metal ion concentration (*Kinniburgh et al., 1999*). Earlier studies in application of polyelectrolytes, cationic or anionic, were related to purification of waste water. Mechanism of action of cationic polyelectrolites is more complex. Besides charge neutralization of colloidal particle, mechanism of interparticle linking exists (theory of "interparticle bridging"). (*Pefferkon, 1995*). Mechanisms of action of different types of cationic and anionic polyelectrolytes have been investigated in processing of sugar cane juices. The best results have been achieved when cationic electrolytes of small molar mass were added at doses 40 - 200 mg/dm^3 in combination with anionic flocculant of high molar mass at 3 mg/dm^3 dose (*Gorjian, 2001; Fellows et al., 2000*). It has been also studied the use of polyacrylamide flocculants in sugar beet juice puurification according to classical method. Industrial trials confirmed the technological and economical justification of this method in the phase prior to defecation (*Loseva et al., 1990; Bažel, 1980*).

MATERIAL AND METHODS

Pectin and protein preparate was extracted from cossettes obtained in the industrial processing of sugar beet (factory Žabalj). The metal salts, $CuSO_4$ and $Al_2(SO_4)_3$ in crystal hydrate form were used for preparation the studied solutions with de-ionized water. The pH of solutions with $Al_2(SO_4)_3$ was regulated at 7 before each experiment, using equivalent amount Na_2CO_3. High purity polyelectrolyte (MAGNAFLOC LT-24) was purchased from Low Moor, Bradford. It has an average molecular weight ~ 100 000 kg/kmol and 100% cationic charge density. Before extraction of pectin, freshly sliced cossettes of sugar beet were washed out with distilled water acidified with HCl to pH 5.5. Extraction of pectin was conducted at pH 3.5 and 85°C during 2.5 h. The pectin preparation was precipitated from the extract with 70% ethanol solution. Basic parameters of the pectin preparation were determined according to standard methods of AOAC (*AOAC Official Methods of Analysis*, *2000*). The isolation of protein preparation was conducted by sedimentation method at pH 3.5 and ambient temperature. Fresh and washed beet root was chopped in a kitchen mixer and juice was separated from pulp by pressing. Mechanical impurities were removed by centrifugation at 3000 r/min for 20 min. The obtained preparation was air dried and its protein content was determined according to *Kjeldahl* using a factor of 6.25 (*AOAC Official Methods of Analysis*, *2000*). In the first stage of the experiment, 0.1% (w/w) pectin and protein aqueous solutions were used whereas coagulant solutions were prepared by dissolving 1 g of these salts in 200 cm^3 of distilled water. A series of coagulant concentrations in the range of 30, 60, 90, 120, 150, 180 and 210 mg/dm^3 were prepared by adding the necessary volumes of the solution to 50 cm^3 of pectin and protein solution. Plan of the experiment and the volumes of coagulant solutions used are displayed in table 1. To accomplish the desired solution alkalinity (pH = 7), Na_2CO_3 was added to $Al_2(SO_4)_3$ in mass ratio 1/1.07, calculated on pure salt. After the coagulant was added to the tested preparations, pH was adjusted and the solution was stirred for 30 min on a high-speed magnetic stirrer (500 rpm). Then, the solution was stirred for another 5 min at low speed and left to rest another 5 min to prevent the disaggregation. In the second stage of the experiment, solution of cationic polyelectrolyte (flocculant) was used which amount was 0,001% of the quantity of solution (*Loseva et al., 1990*). Basic solution of cationic polyelectrolyte was prepared by dissolving 0.5 g of flocculants in 100 cm^3 of distilled water and left overnight to swell at room temperature. From the basic solution, flocculant concentration of 3 mg/dm^3 was prepared. After the coagulant was added and the solution was stirred on a high-speed for 30 min, flocculant was added in the solution and then was manually and slowly stirred for 5 min.

Table 1. Plan of experiment

Coagulant:	50 cm^3 flask with 0.1% (w/w) pectin or protein solution / 1 – 14: number of measurement						
	1	2	3	4	5	6	7
$CuSO_4$ (cm^3)	0.47	0.94	1.41	1.88	2.35	2.82	3.29
	8	9	10	11	12	13	14
$Al_2(SO_4)_3$ (cm^3)	0.59	1.20	1.79	2.38	2.97	3.56	4.15
Na_2CO_3 (cm^3)	0.58	1.16	1.64	2.22	2.80	3.38	3.96

Zeta potential was determined using a commercial apparatus ZETA-METER ZM 77 with the electrophoretic cell (*Riddick, 1975*). For each tested solution, measurements were repeated 3 times. An average value was used to derive the Zeta potential of colloidal particles in the tested solutions using a diagram based on the *Helmoltz-Smoluchowski* equation for electrophoretic mobility of colloidal particles. Experiments were conducted at 6-fold magnitude on a stereoscopic microscope and voltage adjusted at 200 V. Measurements were performed at room temperature for seven different solution concentrations (Table 1). Immediately before Zeta potential measurements, solution temperatures were measured. Zeta potential reading from the diagram was multiplied by the correction factor for temperature.

RESULTS AND DISCUSSION

The main parameters measured for the pectin preparation were: solid content (80.35%w/w) and degree of purity (content of galacturonic acid, 72.24%w/w). The main parameters measured for the protein preparation were: solid content (86.25%w/w) and pure protein content (56.10%w/w). These properties are in accordance with the mean content of pectin and protein found in raw sugar beet juices from diffuser reported in literature (*Kuljanin, 2008a; Kuljanin et al., 2010*). In all model-solutions, charge inversion of Zeta potential from negative to positive was observed. Changes of mean values of Zeta potential of the tested preparations after adding various quantities of coagulants $CuSO_4$ and $Al_2(SO_4)_3$ in the form of pure salts are shown in Figures 1 and 2. Changes of mean values of Zeta potential of the tested preparations after adding various quantities of coagulants $CuSO_4$, $Al_2(SO_4)_3$ in the presence of cationic polyelectrolyte (3 mg/dm^3) are shown in Figures 3 and 4. The increase in the concentration of Cu^{2+} and Al^{3+} ions results in a decrease of the absolute value of Zeta potential. This means that by controlled dosing of $CuSO_4$ or $Al_2(SO_4)_3$, the Zeta potential may be kept at the values close to 0 mV, which is the main prerequisite for the beginning of coagulation. According to the *Schulze-Hardy* rule, ions with high valence like Al^{3+} should be able to decrease Zeta potential to zero point at much lower concentration. However, from the results of readings obtained from Figure 1 and 2, it is obvious that less amount of $CuSO_4$ (82.0 mg/dm^3 for pectin and 115 mg/dm^3 for protein) compared to $Al_2(SO_4)_3$ (105 mg/dm^3 for pectin and 122 mg/dm^3 for protein) was required for lowering Zeta potential to zero point. This can be explained by higher binding ability of these ions considering that Cu^{2+} ions are first-ranged in the previously given selectivity order which describes the binding ability of divalent cations to pectin of botanical origin. Results of experiments carried out with the cationic polyelectrolyte show a slight deviation change in Zeta potential (Figure 3 and 4). Cationic flocculant (MAGNAFLOC LT-24) concentration of 3 mg/dm^3 show slightly higher removal efficiency of pectin and protein from the tested model-solution compared with metal salts without polyelectrolytes.

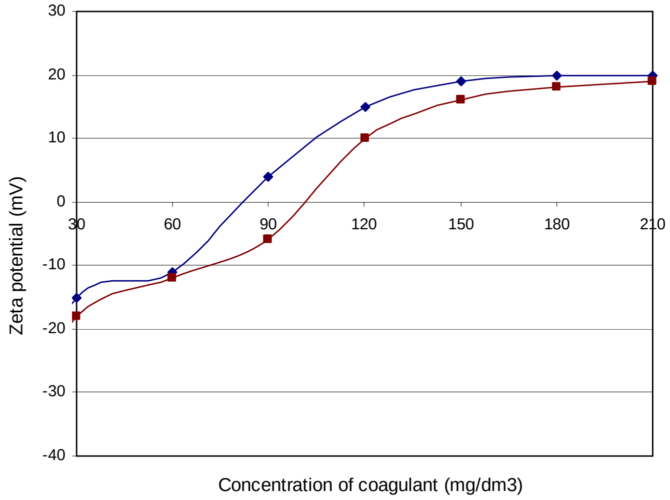

Fig. 1. Dependence of the electrokinetic potential of pectin solution on the $CuSO_4$ and $Al_2(SO_4)_3$ concentration: ♦ - $CuSO_4$; ■ – $Al_2(SO_4)_3$

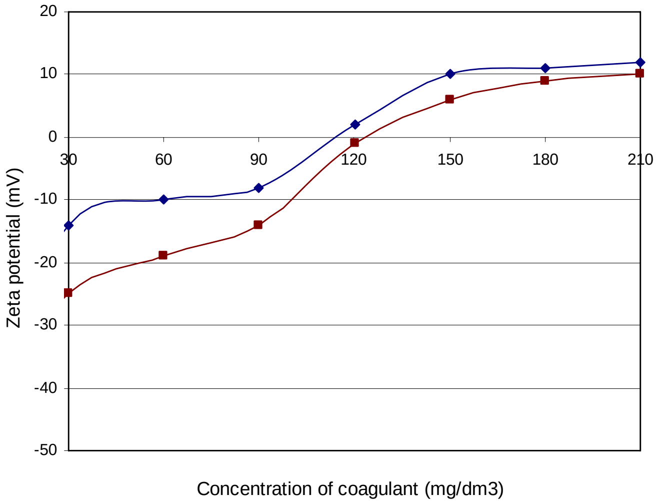

Fig. 2. Dependence of the electrokinetic potential of protein solution on the $CuSO_4$ and $Al_2(SO_4)_3$ concentration: ♦ - $CuSO_4$; ■ - $Al_2(SO_4)_3$

Seen from the electrokinetic point of view, these coagulants and flocculant compress the electrical double layer of macromolecules. This causes a decrease in the absolute value of Zeta potential of pectin and protein macromolecules and decrease the required amount of the tested coagulants in the presence of flocculant (3 mg/dm^3). After addition of cationic flocculant, the required amount of coagulant $CuSO_4$ (75.0 mg/dm^3 for pectin and 95.0 mg/dm^3 for protein) to decrease the Zeta potential to zero point is less compared to $Al_2(SO_4)_3$ (92 mg/dm^3 for pectin and 105 mg/dm^3 for protein). By adding a cationic flocculant at concentration of 3 mg/dm^3, the amount of Al^{3+} and Cu^{2+} ions required to achieve a zero value Zeta potential decreased in the range of 7 to 20 mg/dm^3. Though not extensively reported in literatures, it is highly possible that cationic polyelectrolyte provides additional charge neutralization (net charge is opposite to the anionic charge of pectin and protein macromolecules). This mechanism is associated with the mechanism of interparticle bridging where pectin and protein macromolecules are adsorbed into the high molecular weight polymers branches.

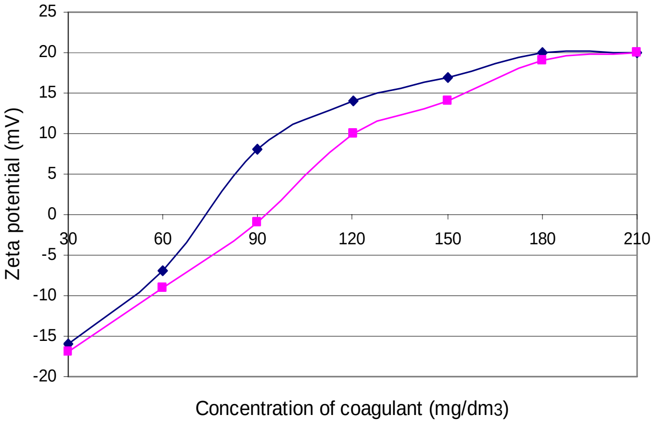

Fig. 3. Effect of $CuSO_4$ and $Al_2(SO_4)_3$ coagulants concentration on the change of Zeta potential of pectin preparation with the use of cationic polyelectrolyte: ♦ - $CuSO_4$; ■ - $Al_2(SO_4)_3$

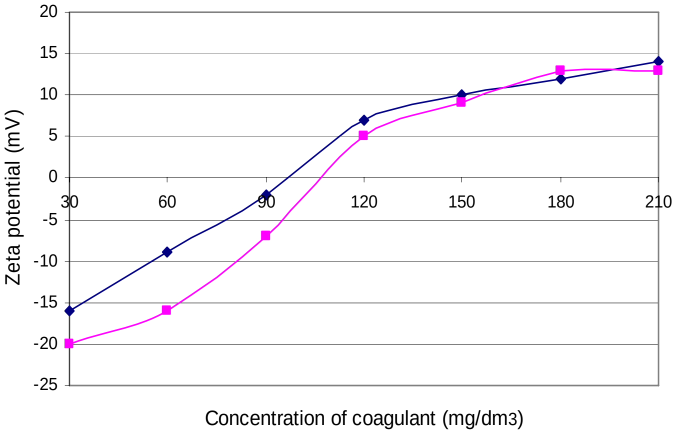

Fig. 4. Effect of $CuSO_4$ and $Al_2(SO_4)_3$ coagulants concentration on the change of Zeta potential of protein preparation with the use of cationic polyelectrolyte ♦ - $CuSO_4$; ■ - $Al_2(SO_4)_3$

CONCLUSION

In the clasical method of separation of macromolecular compounds from sugar beet juice, Ca^{2+} ions have been used. Crucial role in the attraction of these ions have weaker, electrostatic reactions. Using a method of measuring Zeta potential, the application of $CuSO_4$ and $Al_2(SO_4)_3$ salts as a source of Cu^{2+} and Al^{3-} ions in clarification of sugar beet juices was investigated. The dose of investigated cations needed to discharge pectin and protein macromolacules is much lower in comparison to Ca^{2+} ions. In this case, the stronger bonding of ions to macromolecules can be explained by formation of surface complexes. With the addition of cationic polyelectrolyte (MAGNAFLOC LT-24), these doses are even lower. Cationic polyelectrolyte provides additional charge neutralization. This mechanism is associated with the mechanism of interparticle bridging.

In comparison to Al^{+3} ions, Cu^{2+} ions showed higher bonding affinity. However, because of presumable adverse effects $CuSO_4$ might have in food processing, $Al_2(SO_4)_3$ can be recommended for partial and total substitution of classical coagulant in clarification of sugar beet juice. Aluminium salts are more favourable, not only for economic reasons, but regarding environmental concerns.

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