

Investigation of removal possibilities of chromium(III) oxide from water solution in the presence of albumins

K. Szewczuk-Karpisz · M. Wiśniewska

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Abstract System destabilization is a highly desirable phenomenon during colloidal impurity removal from sewages and wastewaters. Therefore, in this study, the stability of chromium(III) oxide suspension in the absence and presence of albumins [bovine serum albumin (BSA), ovalbumin (OVA), human serum albumin (HSA)] was investigated. Based on the analysis of experimental results, i.e., measurements of adsorption amount, electrokinetic potential, and metal oxide surface charge density as well as system stability, the mechanism of the suspension stabilization/destabilization was proposed. The examined system without the albumins is relatively stable at pH 3, 4.6, and 9 (TSI = 34.8, 36.6, 34.22, respectively), which is associated with the electrostatic stabilization phenomenon. In turn, the least stable is the suspension at pH 7.6 (TSI = 55.43). This is the result of the adsorbent zero surface charge. Regardless of solution pH, the BSA, OVA, or HSA adsorption causes an increase in the system stability ($17.55 < \text{TSI} < 30$). Probably, the steric stabilization is involved in this phenomenon, which results from the mutual repulsion of the adsorption layers formed on the solid surface. Thus, it can be concluded that the albumin presence in the industrial wastewaters impedes the chromium(III) oxide removal.

Keywords Albumin adsorption · Colloids · Electrostatic and steric stabilization · Zeta potential

Introduction

Stable suspension is characterized by no macroscopic processes, such as observable separation of suspended particles and water. This means that even after a long time, the particles sedimentation will not be observed. High suspension stability is highly undesirable in wastewater treatment, especially during colloidal impurity removal. Then, settling tanks cannot effectively perform their task (Nowicki 2005). The polymer addition often changes the system stability. The most preferred phenomenon is the suspension destabilization. Macromolecular compound adsorption on colloidal particle surface changes the nature of interactions between solid particles. The low suspension stability is equivalent to the formation of large particle aggregates which fall on the reservoir bottom (Wiśniewska and Szewczuk-Karpisz 2013).

Contaminant removal from aqueous solutions is a research subject of many scientists (Gupta et al. 2012a). Of particular interest are methods for various dye removal, i.e., chrysoidine Y (Mittal et al. 2010), amaranth (Mittal et al. 2005; Gupta et al. 2012d), erythrosine (Gupta et al. 2006b), brilliant blue FCF (Gupta et al. 2006a), reactofix golden yellow (Gupta et al. 2007d), reactofix red (Gupta et al. 2007c), carmoisine A (Gupta et al. 2009b), blue 113 (Gupta et al. 2011c), metanil yellow (Mittal et al. 2008), safranin T (Gupta et al. 2007b), crystal violet (Gupta et al. 2010a), phenol red (Gupta et al. 2009a), and triaryl methane (Gupta et al. 2010b). Waste (Gupta et al. 2007a; Jain et al. 2004, 2003), activated carbon (Gupta et al. 1998; Karthikeyan et al. 2012), carbon nanotubes (Gupta et al. 2011a, b), and specific columns (Gupta et al. 2012b) are used in many technologies.

In this paper, the influence of biopolymers, such as albumins: bovine serum albumin (BSA), ovalbumin (OVA),

K. Szewczuk-Karpisz (✉) · M. Wiśniewska
Department of Radiochemistry and Colloid Chemistry, Faculty of Chemistry, Maria Curie Skłodowska University, M. Curie Skłodowska Sq. 3, 20-031 Lublin, Poland
e-mail: k.szewczuk-karpisz@wp.pl

and human serum albumin (HSA), on the stability of the chromium(III) oxide suspension was examined. Cr_2O_3 was used in the experiments because of its wide industry application. This is a dark green, amphoteric, sparingly water-soluble solid, as well as the most stable green dye used in glass and ceramic industries, construction, painting, etc. (Barnhart 1997; Gattens and Stout 1966; Patnaik 2002). In recent years, Cr_2O_3 is tested for the catalytic properties (Kantserova et al. 2009) as well as its ability to increase the collagen stability (Sangeetha et al. 2012). Due to the adverse effects of chromium(III) oxide on organisms in aquatic ecosystems, the search for effective methods of its removal from aqueous solutions is fully justified. Cr_2O_3 contributes to reducing the efficiency of photosynthesis and thus other vital processes occurring in water (Świdarska-Bróz 1993). Several methods of chromium and other metal removal from aqueous solutions have been described in the literature (Gupta et al. 2010c, 2012c; Gupta and Sharma 2003; Saleh et al. 2011; Gupta and Rastogi 2009).

Albumins were selected for the study due to their important functions in organisms and wide use in many scientific areas. Bovine serum albumin is a protein composed of 583 amino acids, obtained on a large scale from cow blood. BSA is used in molecular biology and biotechnology as a stabilizing agent in buffers, protein for a standard curve as well as nutrient in cell cultures. The described protein does not possess enzymatic properties (Yamane et al. 1975; Wright and Thompson 1975). Ovalbumin is the main protein component of egg yolk. It consists of 385 amino acids. Due to its high availability, OVA is used in the studies of protein structure and properties as well as in immunology as a factor stimulating allergic reaction (Huntington and Stein 2001; Baynes and Dominiczak 2005). Human serum albumin is the predominant protein in human serum, consisting of 585 amino acids. It is produced in the kidney and performs an important function in the human body, inter alia, it maintains the osmotic pressure on a constant level and is involved in the transport of hormones, fatty acids, and drugs. Because of remarkable HSA role and its wide use in clinical cases, the method of HSA synthetic equivalent preparation is being developed (Karlsson et al. 2010; Roche et al. 2008). Perhaps, the results presented in this study could be helpful in achieving the above objective.

As it was mentioned above, in this paper, the stability mechanism of chromium(III) oxide suspension in the absence and presence of albumins was proposed. The main aim of the study was to determine whether the selected biopolymers cause suitable changes in the Cr_2O_3 suspension stability. The research team wanted to test whether the albumin presence helps or hinders the metal oxide removal from aqueous solutions. The studies on efficient removal of colorful contaminants from water and wastewater are

particularly important because of the constant population problem with access to drinking water. Thus, the stability mechanism of the Cr_2O_3 suspension in the presence of various polymers, i.e., ionic polyamino acids (Ostolska and Wiśniewska 2014), *Sinorhizobium meliloti* 1021 exopolysaccharide (Szewczuk-Karpisz et al. 2014), and polyacrylic acid (Wiśniewska and Szewczuk-Karpisz 2013), has been investigated.

It should be also noted that the examination of metal oxide suspension stability in the protein presence can certainly be regarded as innovative. There are few studies on this subject in the literature. Wells et al. (2011) described the stability of iron(III) and zinc oxide nanoparticle suspensions in the serum protein presence, and Szekeres et al. (2013) determined the modified magnetite suspension stability. However, their main purpose was to check the possibility of medical applications. Moreover, the method used for stability measurements of the Cr_2O_3 and Cr_2O_3 –albumin systems is very modern. The calculated Turbiscan Stability Index (TSI) parameter accurately determines the suspension stability.

Materials and methods

Experiments design and sampling preparation

Chromium(III) oxide (*POCH*) of the specific surface area equal to $7.12 \text{ m}^2/\text{g}$ was used as the adsorbent in the experiments (Table 1). The metal oxide-specific surface area as well as average pore diameter was determined based on the analysis of nitrogen adsorption–desorption isotherms (BET method). The average size of the Cr_2O_3 particles was measured using a Zetasizer (Szewczuk-Karpisz and Wiśniewska 2014). The adsorbent was washed out from inorganic ions using redistilled water to the conductivity $<3 \text{ mS/cm}$. Then, it was dried and crushed in a porcelain crucible.

Bovine serum albumin (BSA), ovalbumin (OVA), and human serum albumin (HSA) (Sigma-Aldrich) were used in the experiments as adsorbates (Table 2). As reported in the literature, the BSA molecular weight is 66.43 kDa (Hirayama et al. 1990) and its isoelectric point (pI) is in the range 4.7–4.9 (Dawson et al. 1993; Malamud and Drysdale 1978). In turn, the HSA molecular weight is 66.248 (Putnam 1975) or 66.437 kDa (Meloun et al. 1975), and its isoelectric point is equal to 4.7 (Putnam 1975). Due to high amino acid sequence similarity (76 %), bovine serum albumin and human serum albumin are considered as homologues. BSA and HSA molecules are approximately heart-shaped in the pH range 4–8 (Carter and Ho 1994). Ovalbumin is a protein with the mass of 42.7 kDa (Nisbet et al. 1981) and the isoelectric point in the range 4.43–4.9

Table 1 Chromium(III) oxide parameters

Adsorbent		S_{BET} (m ² /g)	D (nm)	D_p (Å)
Chromium(III) oxide	Cr ₂ O ₃	7.12	265	93.25



S_{BET} specific surface area, D average particle size, D_p average pore diameter

Table 2 Albumin characteristics

Adsorbates	M (kDa)	pI
Bovine serum albumin (BSA)	66.43	4.7–4.9
Human serum albumin (HSA)	66.248 or 66.437	4.7
Ovalbumin (OVA)	42.7	4.43–4.9

(Beeley et al. 1972; Kidwai et al. 1976). Due to the OVA amino acid sequence and spatial structure, it was classified as serpin (Gettins 2002).

The chromium(III) oxide suspensions for stability measurements were prepared by adding 0.02 g of the adsorbent to 20 ml of electrolyte solution (0.01 M NaCl). On the other hand, suspensions containing adsorbates were prepared by adding the identical portion of the solid to the solution containing 0.01 M NaCl. After albumin addition (100 ppm) and adjusting the suspension pH (3, 4.6, 7.6 or 9), the examined system was transferred to a glass vial and the measurement was started in a constant temperature chamber (25 °C).

The samples for zeta potential measurements were prepared by adding 0.03 g of the solid to 500 ml of 0.01 M NaCl solution. Suspensions containing biopolymers were prepared by the addition of identical metal oxide portion to the solution containing 0.01 M NaCl and 10, 50, 100, 300, or 500 ppm of albumin. After 3-min sonication, the samples were poured into five Erlenmeyer flasks and their pH values (3, 4.6, 6, 7.6 and 9 ± 0.05) were adjusted using 0.01 M HCl and 0.01 M NaOH. The measurement system was washed twice with distilled water before each measurement.

Analytical methods

Stability measurements of the chromium(III) oxide suspension in the absence and presence of albumin were performed by using a turbidimeter Turbiscan Lab^{Expert} with the cooling module TLab Cooler. The results were obtained as the curves of transmission and backscattering of light ($\lambda = 880$ nm) passing through the sample and the TSI values. A single stability measurement lasted 3 h, during which relevant data were recorded every 5 min. The

TSI value was calculated by the computer software working with the turbidimeter on the basis of the following formula:

$$\text{TSI} = \sqrt{\frac{\sum_{i=1}^n (x_i - x_{\text{BS}})^2}{n - 1}} \quad (1)$$

where x_i average backscatter for every minute of measurement, x_{BS} average x_i , n number of scans.

The electrokinetic potential of the chromium(III) oxide particles in the absence and presence of albumin was measured by a zetameter Zetasizer 3000 from Malvern Instruments. The above apparatus determines the zeta potential of colloidal particles moving in electric field on the steady level of electrophoretic cell. The potential reading is made automatically when the particle movement is compensated for by the applied voltage. According to Smoluchowski, the speed (u) of the colloidal particles moving in the electric field is associated to the zeta potential (ζ) by the equation:

$$\zeta = \frac{\alpha u}{DF} \eta \quad (2)$$

where α factor depending on the particle shape (for spherical particles $\alpha = 6\pi$ for cylindrical $\alpha = 4\pi$); D dielectric constant; F electric field strength; η viscosity.

Statistical analysis

One result of zeta potential was the average of five measurements. The measurement error was calculated as the standard deviation. The obtained values did not exceed 5 %.

Results and discussion

Stability of the chromium(III) oxide suspension in the absence and presence of albumin

The stability of the chromium(III) oxide suspension in the absence and presence of albumin was measured using a turbidimeter. The results were obtained in the form of the curves of transmission and backscattering of light beam



passing through the sample as well as the TSI values. The graphs for the Cr_2O_3 and Cr_2O_3 –BSA systems at pH 3 and 7.6 are shown in Figs. 1 and 2. The results obtained for OVA and HSA are not presented because they are very similar to those for BSA. The TSI values are summarized in Table 3.

Based on the obtained results, it was stated that the Cr_2O_3 suspension stability depends on the solution pH (Ostolska and Wiśniewska 2014; Wiśniewska and Szewczuk-Karpisz 2013). Thus, among the examined systems, the metal oxide suspension is characterized by the highest stability at pH 3 and 9. This is evidenced by the relatively low TSI values (34.8 and 34.22, respectively) and also low, in comparison with other systems, transmission level of light (in both cases approximately 25 % in the last minutes of the measurement). At pH 4.6, the system is slightly less stable than at pH 3 and 9 (TSI = 36.6). On the other hand, the Cr_2O_3 suspension has the lowest stability at pH 7.6. The proof is the high TSI value (55.43) and high transmission

level (52 % in the last minutes of measurement). The presented results correspond to the data published by Ostolska and Wiśniewska (2014). Moreover, they are similar to those obtained during the stability examination of Cr_2O_3 suspension in the PAA presence (Wiśniewska and Szewczuk-Karpisz 2013).

The albumin addition, regardless of its type and solution pH, increases the chromium(III) oxide suspension stability. This phenomenon is equivalent to the drop in the TSI value (for example, for BSA at pH 3–25.9), the backscatter level increase (for BSA at pH 3 from 10 to 18 % in the last minutes of the measurement) and the decrease in transmission level (for BSA at pH 3 from 25 to 12 % in the last minutes of the measurement). A similar serum protein effect was observed during the study of inorganic compounds, i.e., TiO_2 (Allouni et al. 2009; Meißner et al. 2009; Vamanu et al. 2008), Fe_2O_3 and ZnO (Wells et al. 2011), WC (Meißner et al. 2009), and fullerene (Deguchi et al. 2007). On the other hand, Flynn et al. (2012) claimed that

Fig. 1 Transmission and backscatter curves for the chromium(III) oxide suspensions as a function of solution pH: **a** pH 3, **b** pH 7.6. $C_{\text{NaCl}} = 0.01 \text{ M}$

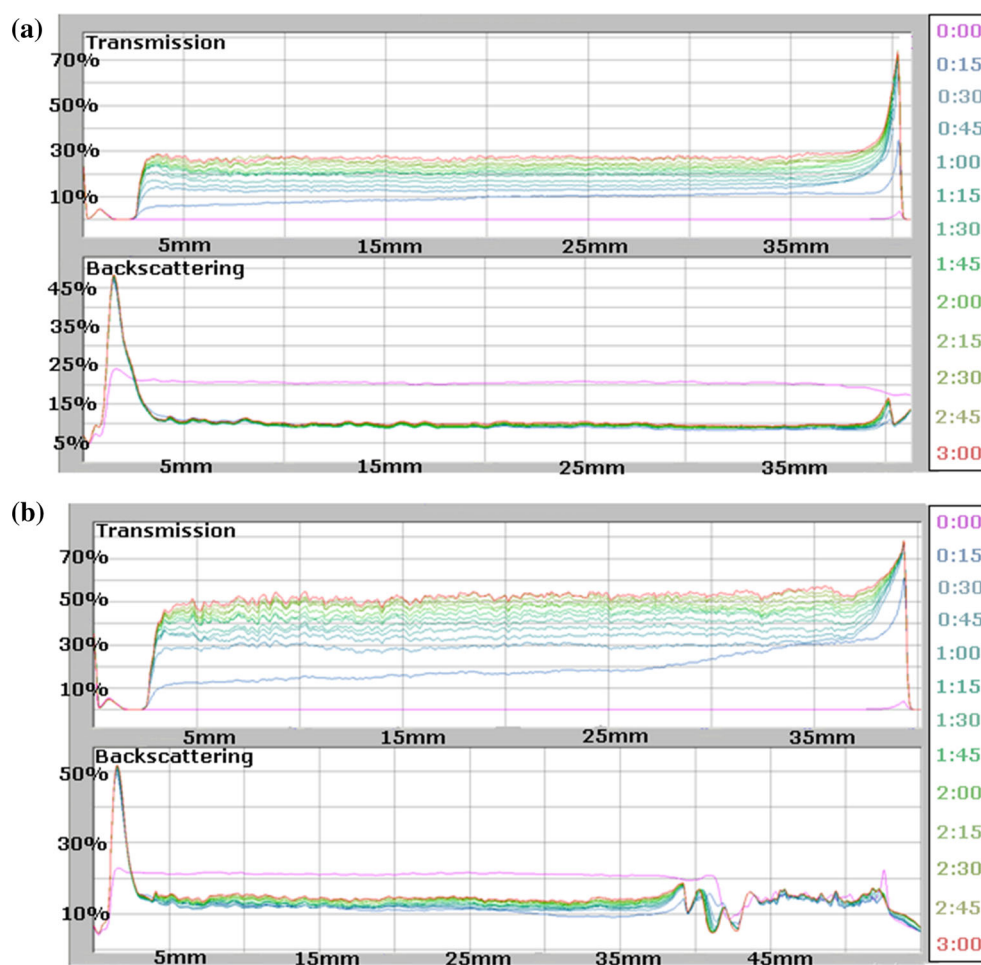


Fig. 2 Transmission and backscatter curves for the systems: **a** Cr₂O₃–BSA at pH 3, **b** Cr₂O₃–BSA at pH 7.6. C_{BSA} = 100 ppm, C_{NaCl} = 0.01 M

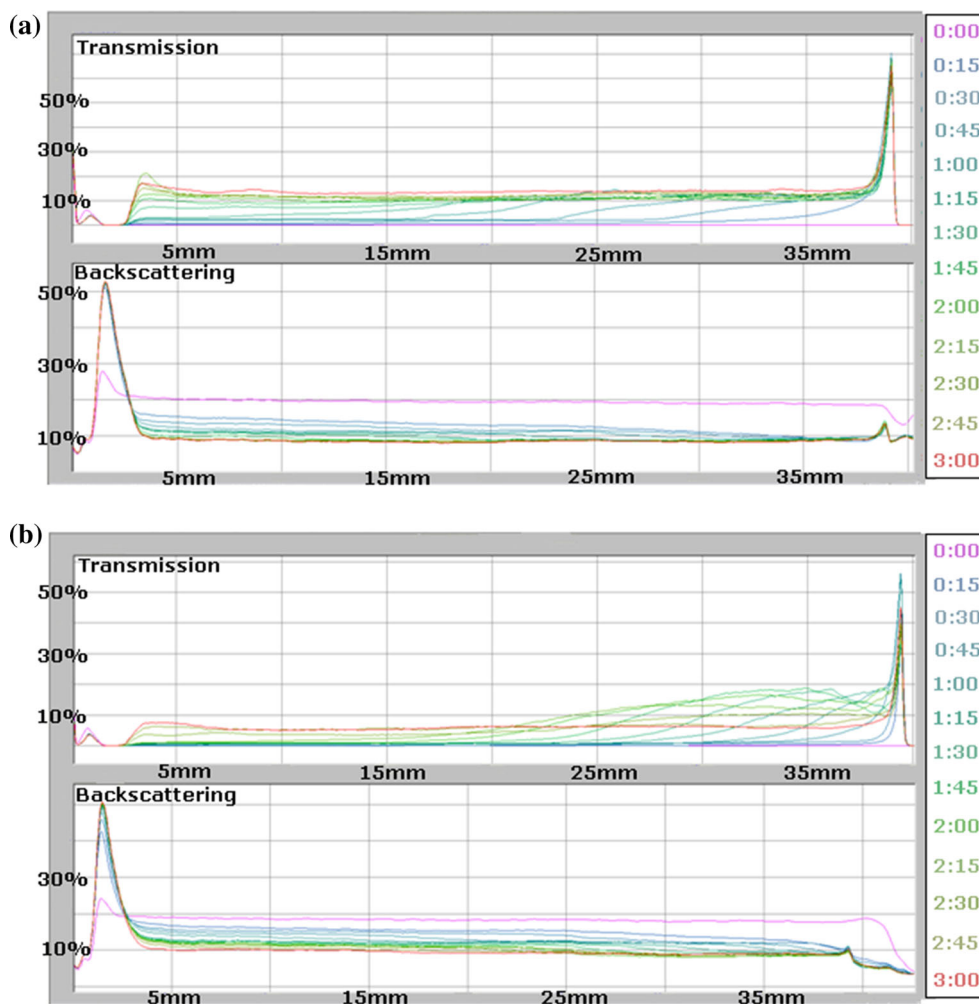


Table 3 Turbiscan Stability Index (TSI) values for chromium(III) oxide suspension in the absence and presence of albumins (100 ppm) calculated on the basis of data obtained at 15 h using Eq. (1)

System	Turbiscan Stability Index (TSI)			
	pH 3	pH 4.6	pH 7.6	pH 9
Cr ₂ O ₃	34.8	36.6	55.43	34.22
BSA–Cr ₂ O ₃	25.91	24.55	18.82	17.55
OVA–Cr ₂ O ₃	30.0	25.27	22.11	17.56
HSA–Cr ₂ O ₃	19.8	24.09	14.33	18.82

BSA presence slightly reduces electrostatic repulsion between colloid particles, which is equivalent to a slight stability decrease.

Electrokinetic potential of the chromium(III) oxide particles in the absence and presence of albumin

The electrokinetic potential (ζ) of the chromium(III) oxide particles was measured in the absence and presence of

albumin. The obtained values are shown in Figs. 3 and 4. The graphs for OVA and HSA are not presented due to a high similarity to the BSA one.

The result analysis showed that the isoelectric point (pH_{iep}) of chromium(III) oxide is approximately six. At pH values above this value, negatively charged groups dominate in the slip plane of the metal oxide, whereas below this value—positively charged groups. The albumin presence, regardless of its type and concentration, shifts the Cr₂O₃ pH_{iep} point to a more acidic value, i.e., about five, which corresponds to the adsorbate isoelectric point. Thus, it can be assumed that at specific albumin concentrations (10–500 ppm), the Cr₂O₃ particles are completely covered with the albumin macromolecules and the adsorbent surface charge is totally masked. Then, the adsorbed biopolymer macromolecules give the Cr₂O₃ surface such chemical properties which are characteristic of the albumin. The described albumin adsorption influence on the metal oxide pH_{iep} value correspond to the data published by Rezwan et al. (2004, 2005) working on BSA and

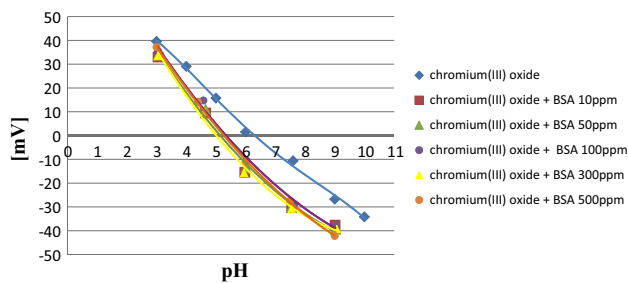


Fig. 3 Electrokinetic potential of the Cr_2O_3 particles in the absence and presence of BSA. The results for OVA and HSA were similar

lysozyme. Moreover, Hindber et al. (1996) observed that also the adsorption of a small molecule, i.e., citric acid, shifts the alumina pH_{iep} point to the pK value of citric acid.

The albumin adsorption amount on the Cr_2O_3 surface was measured on the basis of differences in the protein concentration in solution before and after the process (Szewczuk-Karpisz and Wiśniewska 2014). The results are shown in Fig. 5. Their analysis showed that the adsorption amount varies with the solution pH. This is associated with the conformation changes of the biopolymer macromolecules. At the isoelectric point, albumins have the most compact coil structure (in the case of BSA the α -helix content is equal to 55 % Foster 1977), which enables the adsorption of the largest macromolecule number on the unit adsorbent surface. Moving away from the pI value is equivalent to gradual expansion of their conformation. At pH 3, macromolecules adopt the most expanded structure (for BSA the α -helix content is about 35 % Foster 1977), which contributes to the lowest adsorption level under these conditions. However, the obtained electrokinetic potential values showed that despite the differences in the albumin conformation at different solution pH values, in each test case, the adsorbent surface is completely covered with the albumin macromolecules. This is evidenced by the Cr_2O_3 pH_{iep} value in the albumin presence which is close to the pI value of pure adsorbates.

The analysis of the zeta potential values also showed that the albumin adsorption on the metal oxide surface reduces the solid particle zeta potential in the whole pH range. In the interpretation of electrokinetic properties of the synthetic polymer/metal oxide system, this decrease is explained by the slipping plane offset by the adsorbed macromolecules (Wiśniewska and Szewczuk-Karpisz 2013). Perhaps, the adsorption of the biopolymer macromolecules, such as albumin, is also related to a similar phenomenon. Furthermore, the dissociated functional

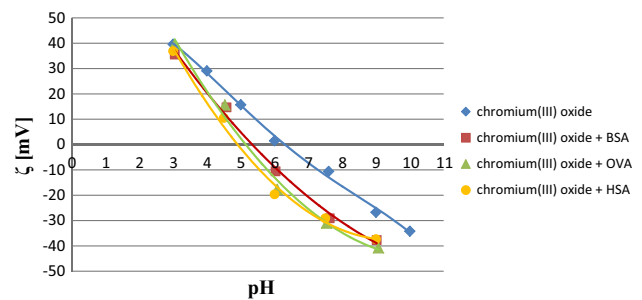


Fig. 4 Electrokinetic potential of the Cr_2O_3 particles in the absence and presence of albumins with the concentration of 100 ppm

carboxylic groups derived from acidic amino acids present in the albumin macromolecules may be responsible for the electrokinetic potential reduction. They are mainly located in the parts that do not directly interact with the solid surface. Charge distribution in the BSA and HSA macromolecules was presented by Rezwan et al. (2004).

It should be also noted that the reduction of the Cr_2O_3 electrokinetic potential is very similar for the three studied albumins (BSA, OVA and HSA) (Fig. 4). This is probably associated with similar properties of the adsorbates, including their acidic nature and approximated values of the isoelectric point.

Stabilization/destabilization mechanism of the chromium(III) oxide suspension in the absence and presence of albumin

Knowledge of the albumin adsorption amount on the chromium(III) oxide surface, the stability, and electrokinetic properties of the examined systems makes determination of the probable stability mechanism of the Cr_2O_3 suspension in the absence and presence of albumin possible.

Thus, the chromium(III) oxide suspension without albumin is the most stable at pH 3 and 9, which correspond to the results obtained by Ostolska and Wiśniewska (2014). This is mainly due to the electrostatic repulsion occurring between the solid particles, which are surrounded by the ions of the supporting electrolyte (NaCl) charged oppositely to the solid surface. The previous measurements of the surface charge density of chromium(III) oxide carried out using the potentiometric titration method showed that the pH_{pzc} (the point of zero charge) of the metal oxide is about 7.6 (Szewczuk-Karpisz and Wiśniewska 2014). Thus, at pH 3, the solid particles are positively charged and the sheath around them is formed by chloride ions. In contrast, at pH 9, the

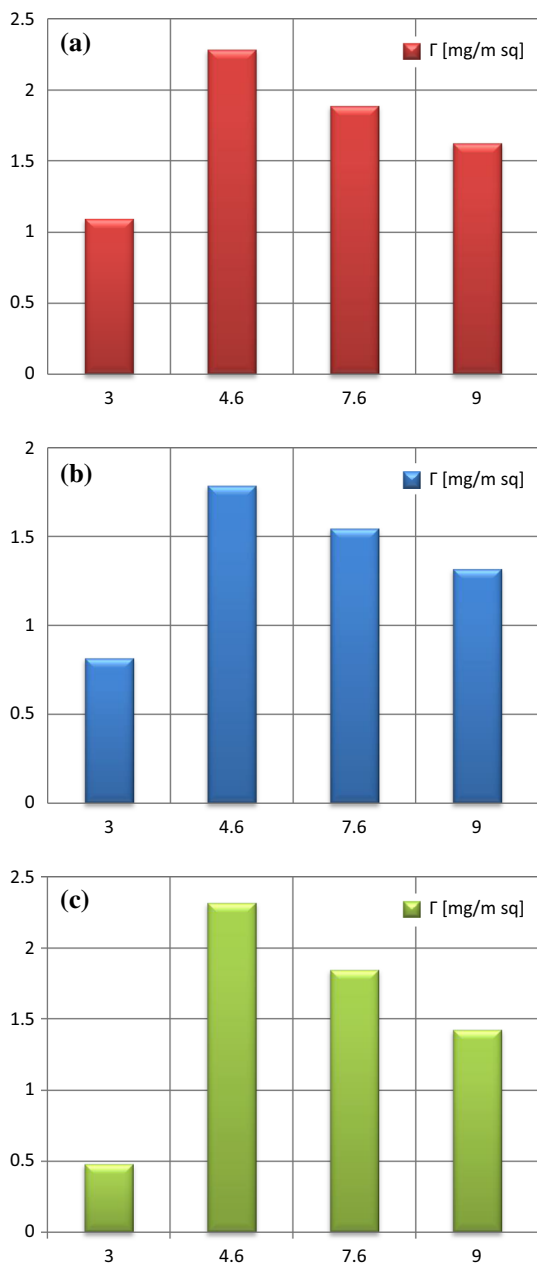


Fig. 5 The albumin amount adsorbed on the Cr₂O₃ particles as a function of solution pH: **a** BSA, **b** OVA, **c** HSA. C_{albumin (initial)} = 250 ppm

Cr₂O₃ particles are negative and the sheath around them is made by sodium ions. The observed values of the solid electrokinetic potential at pH 3 and 9 (40 and -22 mV, respectively) are the result of this phenomena. Smaller stability of the system at pH 4.6 as compared to pH 3 is associated with the smaller zeta potential value of the

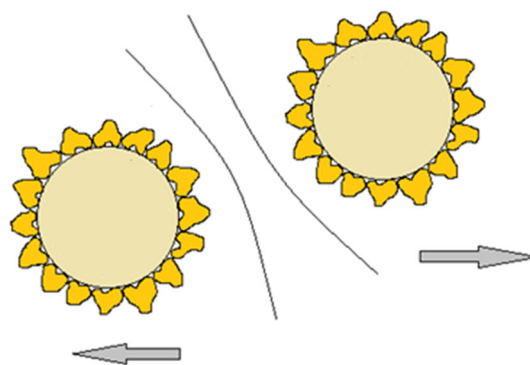


Fig. 6 Stabilization mechanism of the Cr₂O₃ suspension by albumins

metal oxide (about 20 mV) and thus with the weaker electrostatic repulsion between the particles. Thus, it can be concluded that the relatively high stability of the suspension at pH 3, 4.6, and 9 is related with the electrostatic stabilization phenomenon, which limits the particle collision and the formation of solid aggregates.

The chromium(III) oxide suspension without albumin is the least stable at pH 7.6. The low stability of the system under these conditions is primarily due to the zero surface charge of the metal oxide, and thus, no electrostatic repulsion between the particles is found.

The albumin addition, regardless of their type and solution pH, increases the Cr₂O₃ suspension stability. Biopolymer macromolecules adsorb onto the metal oxide surface in each test solution pH, the greatest amount adsorbed at pH 4.6 (a value close to the isoelectric point of the adsorbate) and the lowest at pH 3. The increase in the suspension stability in the albumin presence is evidenced by both the TSI values and the increase in backscatter and the decrease in transmission of light passing through the examined systems. During the analysis of the obtained adsorption amount and the Cr₂O₃ zeta potential values, it was found that in the whole pH range, the solid particle surface is completely covered with the biopolymer macromolecules. Thus, the most likely mechanism of stabilization suspension, which contributes to increase in stability of the tested systems, is the steric stabilization (Kraynov and Muller 2011). It is due to the mutual repulsion of the adsorption layers formed on the solid surface. Increase in the Cr₂O₃ suspension stability in the albumin presence is equivalent to even more difficult formation of solid particle aggregates, which is highly undesirable in the procedure of waste-waters treatment.

The proposed mechanism of the chromium(III) oxide suspension stabilization in the albumin presence is shown

schematically in Fig. 6 (for BSA at pH 7.6 using the model developed by Rezwan et al. 2004).

Conclusion

The pooled result analysis allowed to draw the following conclusions: (1) the isoelectric point (pH_{iep}) of chromium(III) oxide is about 6, (2) the albumin presence, regardless of their type, in the concentration range of 10–500 ppm, shifts the Cr_2O_3 pH_{iep} point to 5, (3) the isoelectric point of chromium(III) oxide in the albumin presence is close to the isoelectric points of the pure adsorbates, which shows that the adsorbent surface is fully coated with the biopolymer macromolecules, (4) the Cr_2O_3 suspension is relatively stable at pH 3, 4.6, and 9, which is connected with the electrostatic stabilization phenomenon, (5) the metal oxide suspension is the least stable at pH 7.6, which is associated with the adsorbent zero surface charge, and (6) the albumin presence causes the increase in the Cr_2O_3 suspension stability in the whole pH range, which is connected with the steric stabilization phenomenon.

Thus, the presence of albumin in the industrial wastewaters is highly undesirable as it hinders the removal of chromium(III) oxide and probably other particles of colloidal dimensions.

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