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## RELATIONSHIPS BETWEEN BONE TREATMENT CONDITIONS AND CO<sup>2+</sup> SORPTION CAPACITIES

Marija Šljivić-Ivanović<sup>1</sup>, Ivana Smičiklas<sup>1</sup>, Aleksandra Milenković<sup>1</sup>, Slavko Dimović<sup>1</sup>

<sup>1</sup> University of Belgrade, Vinča Institute of Nuclear Sciences, P.O.B. 522, 11000, Belgrade, Serbia

**Abstract.** <sup>60</sup>Co is an important radionuclide in spent nuclear fuel and liquid radioactive wastes. For the purification of water containing Co<sup>2+</sup> ions sorption on hydroxyapatite (Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>) can be applied. The process is particularly cost-effective if biological apatite from animal bones is utilized. In this study, dependence between bovine bone treatment conditions and Co<sup>2+</sup> sorption was investigated as a function of initial metal concentration. Eight sorbents were previously prepared using fractional factorial design, by simultaneous variations of five process variables between two levels: factor A-the type of the chemical reagent (H<sub>2</sub>O<sub>2</sub> or NaOH), factor B-reagent concentration (0.1 mol/L or 2 mol/L), factor C-reaction temperature (20°C or 60°C), factor D-contact time (1h or 3h) and factor E-sample annealing (without or at 400°C). At this point, the effects of treatment factors on Co<sup>2+</sup> sorption efficiency were evaluated using statistical analysis. Amounts of Co<sup>2+</sup> sorbed, amounts of released Ca<sup>2+</sup> ions and final pH values were considered as system responses. The results showed that the impact of various treatment factors was different for different starting concentrations of Co<sup>2+</sup>. Consequently, no statistically important relations could be established between treatment conditions and Co<sup>2+</sup> sorption from 10<sup>-4</sup> and 5•10<sup>-4</sup> mol/L solutions, whereas thermal treatment at 400°C was the only statistically significant factor influencing sorption from the most concentrated solution (5•10<sup>-3</sup> mol/L). Depending on initial Co<sup>2+</sup> concentration, various factors had statistically significant effect on equilibrium pH values, whereas no relation was found between bone treatment conditions and the amounts of Ca<sup>2+</sup> released during the sorption.

**Key words:** design of experiments, bioapatite, Co<sup>2+</sup>, sorption

### 1. INTRODUCTION

<sup>60</sup>Co is a radionuclide with relatively short half-life of 5.3 years, which is widely used in research, industrial and medical applications. As a corrosion product, <sup>60</sup>Co is also present in liquid wastes from pressurized water nuclear power reactors. Furthermore, inactive Co is a transition heavy metal which is very toxic in elevated quantities. Because of high negative impact onto human health, the wastewaters containing cobalt isotopes have to be purified, and for that purpose sorption process onto different materials can be used.

Hydroxyapatite (HAP) based materials represent suitable matrix for the immobilization of heavy metals and radionuclides from aqueous solutions, ground water and soil [1-4], due to low solubility, buffering properties and high tendency for cationic and anionic substitutions inside the crystal lattice [5]. Among various heavy metals and radionuclides, less attention has been devoted to investigation of Co<sup>2+</sup> sorption onto apatite materials. Animal bones are composite material consisted of HAP phase and organic matter, mainly collagen. Knowing that non-stoichiometric HAP represents approximately 60-70% of bones, they were recognized as low-cost raw material for HAP extraction. In order to remove organic phase from bones, chemical and thermal treatments can be applied.

Commercially available product - Apatite II™, derived from fish bones (US Patent #6,217,775), was tested for Cd, Cu, Co, Ni and Hg removal from wastewater [6]. Based on the results of column study, this material was characterized as a suitable filling for permeable reactive barriers. Commercial bone char, produced by cattle bones calcinations, was also found to be a promising immobilization matrix for Co<sup>2+</sup> ions [7]. Following detailed kinetic study, the main sorption mechanisms were suggested.

The systematic study on the influence of heating temperature (400-1000°C) onto bovine bone physico-chemical and sorption properties, showed that removal of Co<sup>2+</sup> [8] and Sr<sup>2+</sup> [9] ions was positively correlated with the sorbents specific surface area. Maximum sorption capacities were determined for the sample heated at 400°C, due to its highest specific surface area.

In our previous study, the effects of various chemical and thermal treatment factors on the bone properties and Cd<sup>2+</sup> sorption capacities were studied [10]. For that purpose, Design of Experiments (DOE) methodology was applied. The effects of chemical reagent type, concentration, reaction time and temperature, and the influence of annealing, were investigated and compared by fractional factorial design. The relationships between treatment conditions and sorbents properties (specific surface area, loss of ignition, point of zero charge, Cd<sup>2+</sup> sorption capacities, etc.) were established by statistical analysis.

In this study, previously prepared and characterized bone samples [10] were tested for  $\text{Co}^{2+}$  immobilization. The main aim was to explore and compare sorption of  $\text{Co}^{2+}$  ions by investigated materials using different initial concentrations of the pollutant, and to find out which treatment factors significantly affected  $\text{Co}^{2+}$  removal. The possible mechanisms of  $\text{Co}^{2+}$  sorption were also discussed.

## 2. METHODS AND EXPERIMENTS

### 2.1 Design of Experiments (DOE)

Design of Experiment (DOE) is a method for experiment planning, which is widely applied to make the experiments more efficient [11]. The strategy of DOE method is simultaneous variation of several factors in one experiment. This approach is often used for process "screening", when the influence of a large number of process variables is investigated. The process variables are commonly varied between two levels: lower and higher. Furthermore, the effect of each variable is determined using the Yates algorithm [12], and, on the basis of calculated effects, it can be concluded whether the change of variable influences the system response. Major DOE output is the information - which process variable significantly influences the response of a chosen system.

### 2.2 Sorbents preparation and properties

Apatite materials were prepared using femur bovine bones. The procedure of sorbents preparation was given in details previously [10]. In general, five process variables were simultaneously varied between two levels according to the fractional factorial design matrix (Table 1). Process variables were denoted as:

- A- type of chemical reagent,
- B - concentration of chemical reagent,
- C - reaction temperature,
- D - contact time, and
- E - sample annealing at 400°C.

According to the results of chemical and instrumental analyses, samples with higher amounts of organic content were B3, B5, B6 and B8 [10]. Specific surface area (SSA) determination showed that obtained samples were non-porous (B5, B6 and B8), mesoporous (B1, B2, B3 and B4) and meso- and microporous (B7). Moreover, all materials exhibited buffering properties in the range of initial pH 4 – 10, whereas the determined points of zero charge ( $\text{pH}_{\text{pzc}}$ ) values were in the range 6.67 - 7.58 [10].

### 2.3 Sorption experiments

The  $\text{Co}^{2+}$  solutions of different initial concentrations ( $10^{-4}$ ,  $5 \cdot 10^{-4}$  and  $5 \cdot 10^{-3}$  mol/L) were prepared by dissolution of  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  in deionized water. The initial pH values were adjusted to 5.0, using small quantities of  $10^{-3}$  mol/L KOH or

$\text{HNO}_3$  solutions. This particular pH was selected in order to prevent HAP phase dissolution at lower pH and  $\text{Co}(\text{OH})_2$  precipitation at equilibrium pH  $\geq 8$ .

Sorption experiments were conducted by mixing 0.10 g of sorbent and 20 mL of various  $\text{Co}^{2+}$  solutions, on a horizontal shaker (120 rpm), for 24 h to achieve equilibrium. After equilibration, the suspensions were filtrated through blue-band filter paper (pore size 2-3  $\mu\text{m}$ ), and  $\text{Co}^{2+}$  concentrations in filtrates were measured together with the final pH values. The amounts of  $\text{Ca}^{2+}$  ions released to the solutions were determined as well, in order to evaluate the participation of ion-exchange mechanism.

For determination of exact initial and equilibrium cation concentrations, flame Atomic Absorption Spectrometer (AAS) Perkin Elmer 3100 was used. Certified Perkin Elmer 1000 mg/L standards for  $\text{Co}^{2+}$  and  $\text{Ca}^{2+}$  were used for instrument calibration. The detection limits for  $\text{Co}^{2+}$  and  $\text{Ca}^{2+}$  ions were 0.04 mg/L and 0.03 mg/L, respectively. Sorbed amounts of  $\text{Co}^{2+}$  ions were calculated as differences between starting and equilibrium concentrations.

Table 1 Factorial design matrix - the combination of factor levels for each experimental run [10]

Run	Sample	Coded values of variables and their levels				
		(A)	(B) mol/L	(C) °C	(D) h	(E)
1	B1	NaOH	2	60	3	Yes
2	B2	NaOH	0.1	60	1	Yes
3	B3	NaOH	2	20	3	No
4	B4	$\text{H}_2\text{O}_2$	2	20	1	Yes
5	B5	$\text{H}_2\text{O}_2$	2	60	1	No
6	B6	$\text{H}_2\text{O}_2$	0.1	60	3	No
7	B7	$\text{H}_2\text{O}_2$	0.1	20	3	Yes
8	B8	NaOH	0.1	20	1	No

### 2.4 Statistical analysis

To assure reproducibility and accuracy of experimental data, sorption experiments were conducted in duplicate. Mean values of measured parameters were used as system responses for data interpretation and statistical analysis. Statistical software MINITAB, which was used to create matrix for bone treatment conditions, was also applied for  $\text{Co}^{2+}$  sorption data analysis, the regression calculations and graphical interpretations.

## 3. RESULTS AND DISCUSSION

### 3.1 $\text{Co}^{2+}$ sorption by different bioapatite samples

The amounts of sorbed  $\text{Co}^{2+}$  and released  $\text{Ca}^{2+}$  ions, as well as the pH values obtained after equilibration, are given in Figure 1. It is evident that sorption from  $10^{-4}$  mol/L solution was almost constant for all bioapatite samples, while with initial  $\text{Co}^{2+}$  concentration increase, sorption capacities drastically changed and became dependant on used

sorbents. Sorbed amounts of  $\text{Co}^{2+}$  ions from the solution of  $10^{-4}$  mol/L were in the range of 0.0171 - 0.0198 mmol/g, and for the concentration of  $5 \cdot 10^{-4}$  mol/L in the range of 0.0526 - 0.096 mmol/g. Considering the highest initial metal concentration, capacities varied from 0.257 mmol/g (B3) to 0.525 mmol/g (B7). Samples B7, B4, B2 and B1 revealed the highest sorption efficiencies.

In general, the majority of investigated eight samples showed better  $\text{Co}^{2+}$  sorption capacities in respect to values obtained for different apatite materials. Maximum sorption capacities of synthetic HAP, reported by Suzuki et al. (1982) [13] and Smičiklas et al. (2006) [14], were 0.300 mmol/g and 0.354 mmol/g, respectively. For comparison, untreated bovine bones and bones after  $\text{H}_2\text{O}_2$  treatment showed maximum sorption capacities of 0.248 mmol/g and 0.469 mmol/g, respectively [8], whereas bones exposed solely to thermal treatment showed variable capacities depending on the annealing temperature: 0.495 mmol/g at  $400^\circ\text{C}$ , 0.342 mmol/g at  $600^\circ\text{C}$ , 0.195 mmol/g  $800^\circ\text{C}$  and only 0.078 mmol/g at  $1000^\circ\text{C}$  [8].

As a measure of ion-exchange and specific cation sorption mechanism, the amounts of released  $\text{Ca}^{2+}$  ions and pH change were measured.

Final pH values varied between 6.6 - 7.6, 6 - 7.5, and 5.7 - 6.8 for initial  $\text{Co}^{2+}$  concentrations of  $10^{-4}$  mol/L,  $5 \cdot 10^{-4}$  mol/L and  $5 \cdot 10^{-3}$  mol/L, respectively (Fig. 1b). Equilibrium pH values obtained after sorption from  $10^{-4}$  mol/L solution, were close to  $\text{pH}_{\text{PZC}}$  values of investigated materials [10]. With the increase of metal concentration, decrease of final pH values was detected, suggesting that specific cation sorption is involved in  $\text{Co}^{2+}$  sorption. The differences between  $\text{pH}_{\text{PZC}}$  values and equilibrium pH obtained in  $5 \cdot 10^{-3}$  mol/L solution were in the range of 0.63 - 1.11 and decreased in the following order:  $\text{B6} > \text{B5} \approx \text{B7} > \text{B3} > \text{B1} \approx \text{B8} > \text{B4} \approx \text{B2}$ .

The amounts of  $\text{Ca}^{2+}$  ions, released from the sorbents surfaces into the solution, varied in the wide range and were strongly dependent on initial metal concentration and used sorbent (Fig. 1c). The relationships between sorbed  $\text{Co}^{2+}$  and released  $\text{Ca}^{2+}$  ions are given in Figure 2. The obtained linear dependencies indicate that ion-exchange is another operating mechanism in  $\text{Co}^{2+}$  sorption. Calculated  $\text{Co}^{2+}/\text{Ca}^{2+}$  ratios were in the range of 0.31 (B5) and around 0.62 (B1, B4). Generally, the highest contribution of ion exchange ( $>0.5$ ) was observed when the annealed samples were used.

For synthetic, stoichiometric HAP,  $\text{Co}^{2+}/\text{Ca}^{2+}$  molar ratio was determined to be close to 1 [14], i.e.  $\text{Co}^{2+}$  was incorporated inside the HAP crystal lattice by ion-exchange mechanism. Ca-deficiency of bone derived samples is one of the explanations for such low ion-exchange ratio. Investigated sorbents exhibited Ca/P ratios in the range 1.16 - 1.23 [10], which were very low compared to the value of 1.67 characteristic for stoichiometric HAP. On the other hand, Ca-deficient HAP crystal lattice is highly defective, which elevates the tendency for cation sorption.

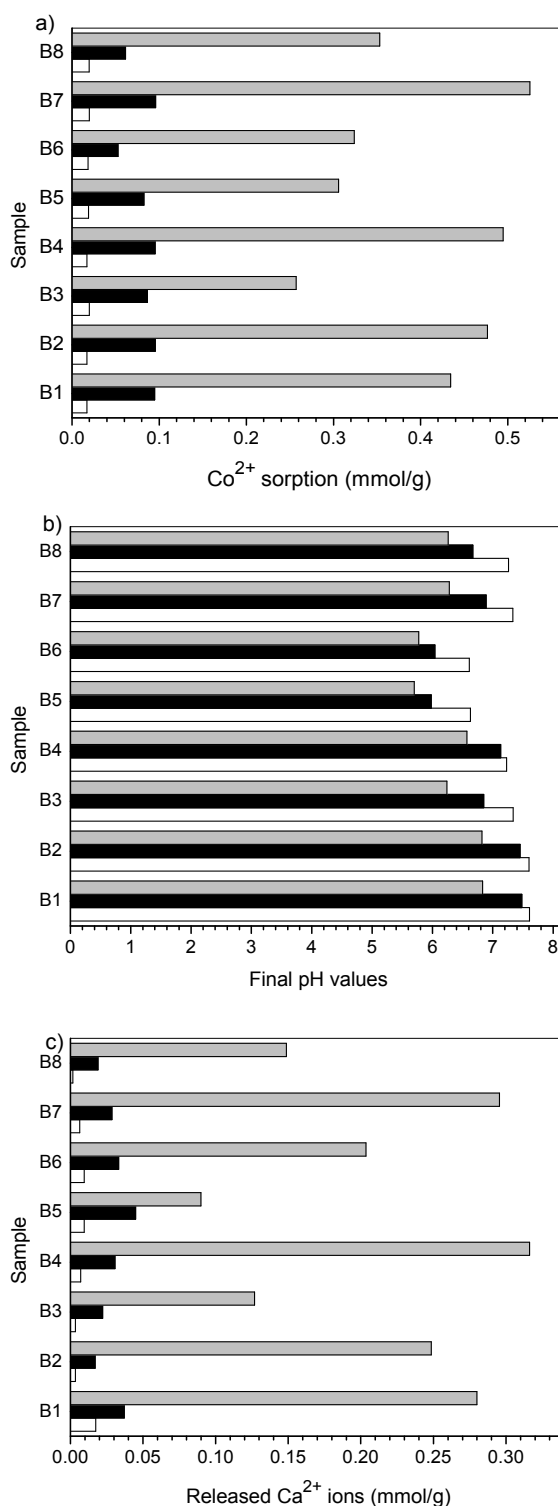
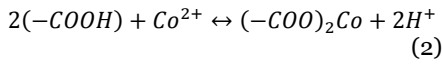
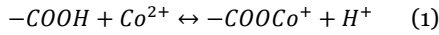


Fig 1. The absolute effects of process variables onto bone properties: a) sorbed  $\text{Co}^{2+}$  amounts, b) final pH values, and c) released  $\text{Ca}^{2+}$  amounts. Initial  $\text{Co}^{2+}$  concentration:  $10^{-4}$  mol/L (white bars),  $5 \cdot 10^{-4}$  mol/L (black bars) and c)  $5 \cdot 10^{-3}$  mol/L (light grey bars)

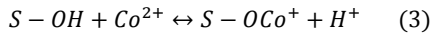
Samples B5, B6, and B8, exposed solely to chemical treatments, were characterized as non-



porous, with very low specific surface area [10]. Higher sorption capacities of these samples in respect to the sorption capacity of raw animal bones [8] can be attributed to higher number of surface functional groups, obtained by chemical treatment. Organic phase detected in these samples, is able to bond metal ions from the solution in complex compounds on the sorbent surface. For example, the reactions between  $\text{Co}^{2+}$  ions and carboxylic surface functional group can be described as follows:



The occurrence of chemical reactions (1) and (2) using samples B5, B6 and B8, explains detected release of  $\text{H}^+$  ions during  $\text{Co}^{2+}$  sorption, i.e. the decrease of equilibrium pH in respect to  $\text{pH}_{\text{PZC}}$ . Sample B3 exhibited lower organic content and higher sorption capacity, which can be associated with higher specific surface area of this sample [10]. Furthermore, specific cation sorption on HAP phase occurs, as well:



where, S –denotes HAP surface. Reaction (3) illustrates one of the important sorption mechanisms characteristic for annealed samples, which contained no organic components.

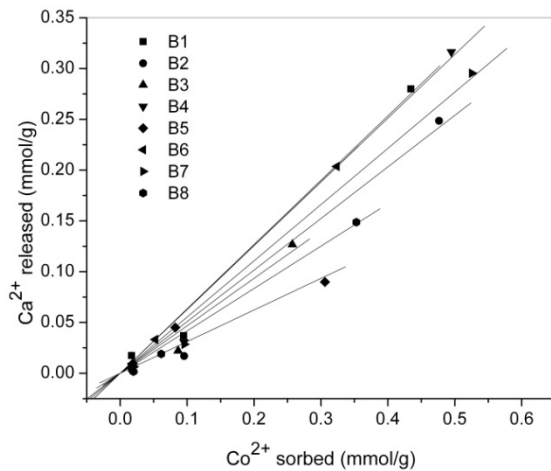


Fig. 2.  $\text{Ca}^{2+}/\text{Co}^{2+}$  ratios obtained for sorption onto different samples

### 3.2. Results of statistical analysis

Considering sorbed  $\text{Co}^{2+}$ , released  $\text{Ca}^{2+}$  amounts and final pH values as system responses, the Pareto graphs were constructed (Fig. 3 and Fig. 4). Pareto charts are consisted of bars which length is proportional to standardized effects, produced by variation of a given factor between lower and higher level [10]. The values of absolute effects are given in decreasing order, while the reference vertical line represents the border line of statistical significance. The influence of bone treatment factors onto chosen

responses was investigated at a confidence interval of 95% ( $\alpha = 0.05$ ). The factors which bar lengths exceed reference line are statistically significant (i.e.  $p < 0.05$ ).

For lower initial  $\text{Co}^{2+}$  concentrations ( $10^{-4}$  and  $5 \cdot 10^{-4}$  mol/L), although the factor E exhibited largest effect, no statistically significant process variable could be found (Fig. 3). With the further increase of  $\text{Co}^{2+}$  concentration, the significance of factor E became statistically important. This is in agreement with the outcomes of a previous study conducted using  $\text{Cd}^{2+}$  cation [10]. The results from both the previous and the present study imply that varying the factors of chemical treatments, in the wide ranges defined by experimental matrix, had much less effect of bone sorption properties when compared to thermal treatment at  $400^\circ\text{C}$ .

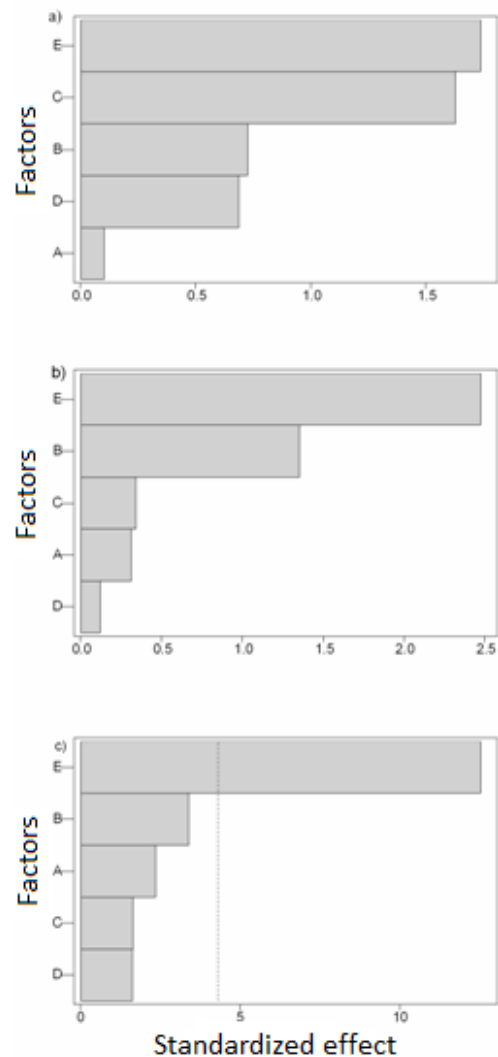


Fig. 3. Pareto plots for sorbed  $\text{Co}^{2+}$  amounts as the system response. Initial  $\text{Co}^{2+}$  concentration: a)  $10^{-4}$  mol/L, b)  $5 \cdot 10^{-4}$  mol/L and c)  $5 \cdot 10^{-3}$  mol/L

The effects of process variables onto final pH values are presented in Fig. 4. It can be noticed that two or three process variables significantly influenced final pH values. The highest effect was produced by varying factors A and E, whereas factor

C was important only considering the lowest initial  $\text{Co}^{2+}$  concentration. The significant influence of A and E factors was also noticed for  $\text{Cd}^{2+}$  sorption onto these materials [10].

Finally, the variation of treatment factors produced no statistically significant influence onto released quantities of  $\text{Ca}^{2+}$  (results not shown).

The behavior of the system can be expressed by multiple regression model given by the following equation:

$$Y_{\text{predicted}} = b_0 + \sum b_i x_i \quad (4)$$

where Y is predicted response,  $b_0$  is the value of fitted response at the center point of design,  $b_i$  are regression coefficients and  $x_i$  variables which can be expressed as main effects (A, B...) or the interaction between two (A•B) or more (A•B•C, A•B•C•D, etc.) process variables [12]. The highest coefficients are obtained for terms which have highest influence on the system responses. Also, signs in front of the coefficients indicate whether the increase of process variable increases (positive sign) or decreases (negative sign) system response.

When sorbed amounts of  $\text{Co}^{2+}$  from  $5 \cdot 10^{-4}$  mol/L solution were chosen as system response, the following equation was obtained using MINITAB software:

$$Y = 0.39639 + 0.08639E \quad (5)$$

If final pH values were used as system response, the following equations were obtained for solutions containing  $10^{-4}$  mol/L,  $5 \cdot 10^{-4}$  mol/L or  $5 \cdot 10^{-3}$  mol/L of  $\text{Co}^{2+}$ , respectively:

$$Y = 7.20125 + 0.25125A + 0.08875C + 0.24125E \quad (6)$$

$$Y = 6.81125 + 0.30125A + 0.42625E \quad (7)$$

$$Y = 6.30875 + 0.2287A + 0.31625E \quad (8)$$

Using equations 5-8, the system responses can be predicted.

Positive regression coefficient figuring with factor E (Eq. 5) implies that annealing of bone samples at  $400^\circ\text{C}$  increases their affinity toward  $\text{Co}^{2+}$  ions. This can be connected with the observed porosity of annealed samples and their high surface area. These characteristic were also majorly influenced by factor E [10]. In addition, utilization of NaOH as a chemical reagent and annealing the samples at  $400^\circ\text{C}$  provoked higher equilibrium pH values.

#### 4. CONCLUSION

Results from the present study demonstrate that high capacity sorbents for  $\text{Co}^{2+}$  ions can be produced from animal bones. Comparing various factors of chemical and thermal treatments, it was observed that the most efficient sorbents were those exposed to heating at  $400^\circ\text{C}$ . Sorbed amounts of  $\text{Co}^{2+}$ , final pH values and amounts of released  $\text{Ca}^{2+}$  ions were chosen as system response for statistical analysis. Sorption efficiency was significantly influenced only by the thermal treatment. The final pH values were

influenced by reagent type and annealing for all investigated sorbate concentrations, whereas significant influence of chosen process variables was not observed for released  $\text{Ca}^{2+}$  amounts. Ion-exchange and surface complex formation were detected as main operating mechanisms, but, due to diverse physicochemical properties of obtained sorbents, their participation was different.

Synergic effect of chemical and thermal treatments produced materials with highest sorption capacity toward  $\text{Co}^{2+}$  ions. In the same time, due to the loss of organic components, these samples are environmental friendly and safer for handling and storage.

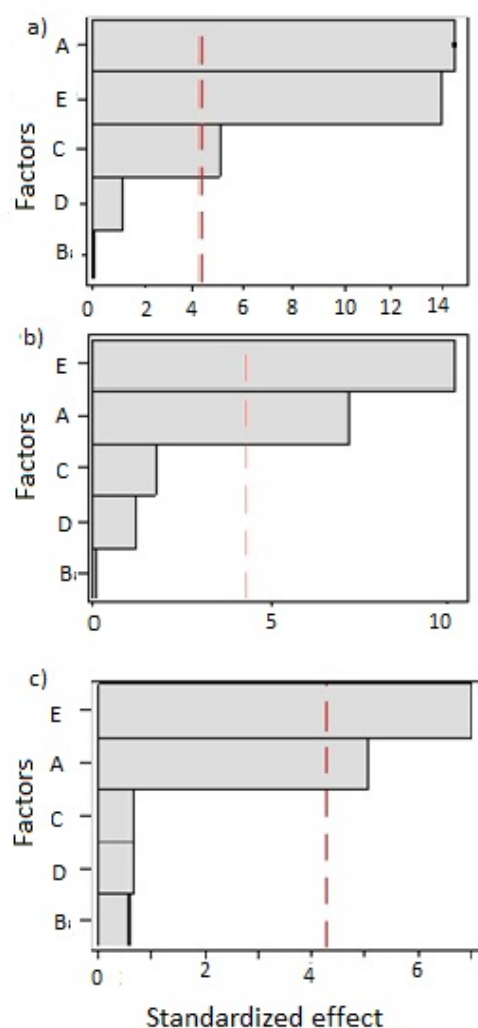


Fig. 4. Pareto plots for final pH values as the system response. Initial  $\text{Co}^{2+}$  concentration a)  $10^{-4}$  mol/L, b)  $5 \cdot 10^{-4}$  mol/L and c)  $5 \cdot 10^{-3}$  mol/L

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