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## Ni(II) IMMOBILIZATION BY BIO-APATITE MATERIALS: APPRAISAL OF CHEMICAL, THERMAL AND COMBINED TREATMENTS\*

### Article Highlights

- Apatite materials derived from bovine bones were studied as Ni(II) ions sorbents
- Raw bones were compared with chemically, thermally and chemically/thermally treated samples
- Different sorption mechanisms were identified by sorption data and FT-IR spectra analysis
- Combined chemical/thermal treatment produced material with the highest sorption capacity
- Sorbed Ni(II) was very stable at low sorbent loads, while largely mobile at high loadings

### Abstract

*Animal bones are a natural and rich source of calcium hydroxyapatite (HAP), which has been found to be a good sorbent material for heavy metals and radio-nuclides. Various treatments can reduce the content of bone organic phase and improve sorption properties. In this study, sorption capacities of raw bovine bones (B) and samples obtained by chemical treatment with NaOH ( $B_{NaOH}$ ), by heating at 400 °C ( $B_{400}$ ) and by combined chemical and thermal treatment ( $B_{NaOH+400}$ ), were compared, using Ni(II) ions as sorbates. Maximum sorption capacities increased in the order  $B < B_{NaOH} < B_{400} < B_{NaOH+400}$ . Based on different sorption data and FT-IR analyses, the mechanism of Ni(II) sorption was found to be complex, with participation of both HAP and organic phase (when present). Sequential extraction analysis was applied for testing the stability of Ni(II) ions sorbed by  $B_{NaOH+400}$ . Majority of Ni(II) was found in residual phase (65%) at lower level of sorbent loading, while with the increase of sorbent saturation carbonate fraction became dominant (39%). According to the results,  $B_{NaOH+400}$  can be utilized in water purification systems. As an apatite based material with low organic content and high efficiency for Ni(II) sorption, it is also a good candidate for in situ soil remediation, particularly at lower contamination levels.*

*Keywords: bovine bones, treatments, apatite, Ni(II), sorption, sequential extraction.*

Nickel is naturally occurring heavy metal, which is in trace amounts essential for living organisms [1]. On the other hand, exposure to high nickel concentrations may cause various health effects, even death.

Single-dose oral lethality studies indicate that soluble nickel compounds are more toxic than less-soluble nickel compounds. Oral LD50 values of 46 or 39 mg Ni per kg as nickel sulfate in male and female rats [2] and 116 and 136 mg Ni per kg as nickel acetate in female rats and male mice, respectively [3] have been reported for soluble nickel compounds. In contrast, the oral LD50 values in rats for less-soluble nickel oxide and subsulfide were >3,930 and >3,665 mg Ni per kg, respectively [2]. The concentration of nickel and nickel compounds in the environment increases due to anthropogenic activity. For example, this metal is frequently found in industrial products such as

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stainless steel, metal alloys, catalysts, rechargeable batteries, and various products of common use, even jewelry [4]. Moreover, long lived radioactive isotopes  $^{59}\text{Ni}$  and  $^{63}\text{Ni}$  are frequent constituents of liquid radioactive waste [5]. Thus, the decontamination of wastewater streams containing Ni(II) ions is essential and it can be conducted using different separation processes, including sorption onto selective and high capacity materials. In addition to conventional sorbents, different waste products were considered for Ni(II) removal like red mud [6], fly ash [7], tea factory waste [8], animal bones [9], etc. It was shown that utilization of waste products as sorbent materials can be particularly a cost-effective way of wastewater purification.

Animal bones are a natural source of hydroxyapatite (HAP), which has been found to be a suitable matrix for heavy metal immobilization [10]. The studies on Ni(II) immobilization using synthetic hydroxyapatite [11,12], fluoroapatite [13] and apatite derived from fish and animal bones [9,14], have been reported so far. Thus, beside the synthetic apatites and phosphate rocks, usage of biogenic apatite forms represents one of the alternatives.

Raw animal bones contain 30-40% of organic constituents, mostly fats and proteins (collagen). Since the nanoparticles of HAP are well "packed" in the organic matrix, specific surface area of bones is extremely low [15]. Consequently, crushed, raw bones were found to be poorer sorbents, compared to synthetic apatite forms [16,17]. In order to reduce organic content, extraction of HAP has been carried out by different chemical or physical treatments. Heating in air atmosphere is one of the methods for decomposition of organic compounds. The influence of heating temperature on bone physicochemical and sorption properties has been investigated, and the optimal temperature was found to be 400 °C [15,18]. At lower temperatures organic phase was removed incompletely, while higher temperatures resulted in sintering of HAP nanoparticles and deterioration of sorption capacities.

Beside thermal decomposition processes, organic solvents such as ethanol and hexane have been applied for fat tissue removal, while collagen degradation was studied using NaOH or  $\text{H}_2\text{O}_2$  solutions [19]. The comparison of chemical agents efficiency has revealed that highest capacity sorbent was obtained using hot (60 °C) 0.1 mol L<sup>-1</sup> NaOH solution [19].

Recently, the effects of various treatment conditions on bioapatite properties were compared [20]. Using experimental design methodology, the influence of five process variables was investigated. Type

of the chemical reagent ( $\text{H}_2\text{O}_2$  or NaOH), concentration of the reagent (0.1 or 2 mol L<sup>-1</sup>), reaction temperature (20 or 60 °C), contact time (1 or 3 h) and sample annealing (without or at 400 °C), were considered. By simultaneous variation of process variables between lower and higher level, materials with different properties were obtained. Impact of treatment factors was compared by statistical analysis, and it was concluded that annealing had predominant influence on surface properties, as well as sorption capacity towards  $\text{Cd}^{2+}$  [20].

Considering the results achieved so far, this study aims to compare performances of bone sorbents obtained under conditions of chemical and thermal treatments that were found to provide the highest sorption capacities [15,18-20]. In addition, synergistic effect of both treatments, applied one after the other, was tested and the results were compared with the efficiency of raw, powdered bones. To evaluate sorption kinetics and maximum capacities, experiments were conducted in wide ranges of initial Ni(II) concentrations and contact times. Various sorption data, spectroscopic and sequential extraction analyses were considered in order to get insight into the Ni(II) sorption mechanisms.

## EXPERIMENTAL

### Preparation and characterization of the sorbents

At first, bovine femur bones, collected from the butchers shop, were cleaned from meat and cut using a circular saw. Pieces of approximate size 2-3 cm were boiled three times for about 3 h in distilled water, for the removal of fats. After drying at 80 °C, one part of the material was left for the preparation of referent (untreated) sorbent, whereas the remaining quantity was exposed to different treatments. Thermally treated sample ( $B_{400}$ ) was obtained by heating the obtained residues at 400 °C, in the electrical oven for 4 h. Chemically treated sample ( $B_{\text{NaOH}}$ ) was prepared by mixing 50 g of boiled bones with 1 L of 2 mol L<sup>-1</sup> NaOH, for 3 h, at 60 °C. The obtained suspension was filtered on the Buhner funnel. Solid residue was thoroughly rinsed with 2 L of distilled water and then dried at 80 °C. Finally, the sample denoted as  $B_{\text{NaOH}+400}$  was produced by applying previously described thermal treatment on the sample  $B_{\text{NaOH}}$ . The referent sorbent (B) and treated sorbents were powdered in an electric mill, and after sieving, the fraction with particle size 45-200 μm was used for further experiments.

Ca/P mole ratio of apatite sorbents was determined as a measure of HAP stoichiometry. Sorbents

were dissolved by the process of microwave-assisted digestion in the mixture of HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>, described previously in detail [20]. Contents of Ca and P were measured by ICP-OES (Thermo Scientific iCAP 6500 Duo ICP).

Specific surface areas (SSA) of samples B, B<sub>400</sub> and B<sub>NaOH+400</sub> have been previously reported [15,20]. Additionally, the SSA of B<sub>NaOH</sub> was determined *via* sorption-desorption isotherm of N<sub>2</sub>, at -196 °C, by the McBain gravimetric method, where the sample was firstly degassed at 100 °C and vacuumed for 24 h.

Determination of B<sub>NaOH</sub> mineral composition was performed using X-ray diffraction (XRD). The Philips PW 1050 diffractometer with CuK<sub>α1,2</sub> radiation was used, employing step/time scan mode of 0.05 °/s, and exposure time of 6 s, in the 2θ range 20–60°. Obtained diffraction peaks were compared to Powder Diffraction File database (PDF2). XRD patterns of samples B, B<sub>400</sub> and B<sub>NaOH+400</sub> have already been published [15,20].

### Sorption experiments

For the evaluation of Ni(II) sorption, separate batches were prepared in 50 mL polypropylene centrifuge tubes. Each one contained 0.1000 g of sorbent and 20 mL of solution prepared from NiCl<sub>2</sub> salt and distilled water. Initial pH values of metal solutions were fixed at 6.0±0.1 in all experiments. Adjustments of initial pH were performed by adding small aliquots of HCl or NaOH solutions. The suspensions were mixed on the rotary overhead shaker at 10 rpm.

The kinetics of Ni(II) sorption was examined using 6×10<sup>-3</sup> mol L<sup>-1</sup> Ni(II) solution, and varying contact time between 15 min and 24 h.

The effect of initial Ni(II) concentrations was investigated by varying concentrations in the range 10<sup>-4</sup>–6×10<sup>-3</sup> mol L<sup>-1</sup>, while contact time was fixed at 24 h.

After a given reaction time, liquid phases were separated from spent sorbents by centrifugation at 7000 rpm for 10 min. Equilibrium pH values were measured in clear supernatants. Determinations of residual Ni(II) concentrations, as well as the concentrations of Ca(II) ions released from bio-apatite phase,

were performed using a Perkin Elmer 3100 atomic absorption spectrometer. The amounts of Ni(II) removed from the solution were calculated as the differences between the initial and the equilibrium concentrations.

### FT-IR analysis of unloaded and Ni-loaded bio-apatites

In order to determine interactions of bio-apatite surfaces with Ni(II) ions, Fourier Transform Infrared (FT-IR) Spectroscopy was performed. Unloaded sorbents, and the solid residues obtained after equilibration of sorbents with the most concentrated Ni-solution (6×10<sup>-3</sup> mol L<sup>-1</sup>) were scanned. FTIR spectra of the samples were recorded at ambient conditions in the mid-IR region (400–4000 cm<sup>-1</sup>) with a Nicolet IS 50 FT-IR spectrometer operating in the ATR mode and using resolution of 4 cm<sup>-1</sup> with 32 scans.

Major functional groups were identified in the FT-IR spectra, and the surfaces of starting materials and fully loaded samples were compared.

### Sequential extraction of the sorbed Ni(II)

Stability of Ni(II) ions, sorbed onto the material with the highest sorption capacity (B<sub>NaOH+400</sub>), was analyzed by a sequential extraction protocol. For this purpose, the sorbent was firstly equilibrated with either 1.5×10<sup>-4</sup> mol L<sup>-1</sup> or 6×10<sup>-3</sup> mol L<sup>-1</sup> Ni(II) solution, in order to obtain samples with different degrees of saturation. Batches containing 1.000 g of B<sub>NaOH+400</sub> and 20 mL of each Ni(II) containing solution, were equilibrated for 24 h. After centrifugation, Ni(II) loaded bio-apatite samples were rinsed with 20 mL of distilled water, centrifuged again, and dried at room temperature. The sequential extraction analysis was performed according to a modified Tessier procedure [21]. Sorbed Ni(II) ions were portioned into 5 operationally defined phases: exchangeable (F<sub>1</sub>), acid soluble (F<sub>2</sub>), reducible (F<sub>3</sub>), oxidizable (F<sub>4</sub>) and residual (F<sub>5</sub>). The modification of the original Tessier protocol refers to residual phase extraction, which was performed by digestion in 6 M HCl [22,23]. The summarized procedure is presented in Table 1.

Table 1. The modified sequential extraction procedure applied in this study (mass of dry sorbent sample 1.00 g)

Phase	Fraction	Experimental procedure
F <sub>1</sub>	Exchangeable	Loaded samples were treated with 8 mL of 1 M MgCl <sub>2</sub> (pH 7.0), 20 °C, 1 h.
F <sub>2</sub>	Acid soluble	8 mL of 1 M CH <sub>3</sub> COONa (pH 5, adjusted with CH <sub>3</sub> COOH), 20 °C, 5 h
F <sub>3</sub>	Reducible	20 mL of 0.04 M NH <sub>2</sub> OH·HCl in 25 vol.% CH <sub>3</sub> COOH, 96±3 °C, 6 h
F <sub>4</sub>	Oxidizable	3 mL of 0.02 M HNO <sub>3</sub> and 5 mL of 30% H <sub>2</sub> O <sub>2</sub> (pH 2, adjusted with HNO <sub>3</sub> ), 85±2 °C, 2 h 3 mL aliquot of 30% H <sub>2</sub> O <sub>2</sub> (pH 2, adjusted with HNO <sub>3</sub> ), 85±2 °C, 3 h 5 mL of 3.2 M CH <sub>3</sub> COONH <sub>4</sub> in 20 vol.% HNO <sub>3</sub> was added and the sample was diluted to 20 mL, 20 °C, 30 min
F <sub>5</sub>	Residual	50 ml of 6 M HCl, 85±2 °C, 9 h

## RESULTS AND DISCUSSION

### Sorbents characteristics

XRD analysis of the sample  $B_{NaOH}$  is presented in Figure 1. Peaks characteristic for HAP crystalline phase (PDF2, card No. 09-0432) with the intensive background were identified.

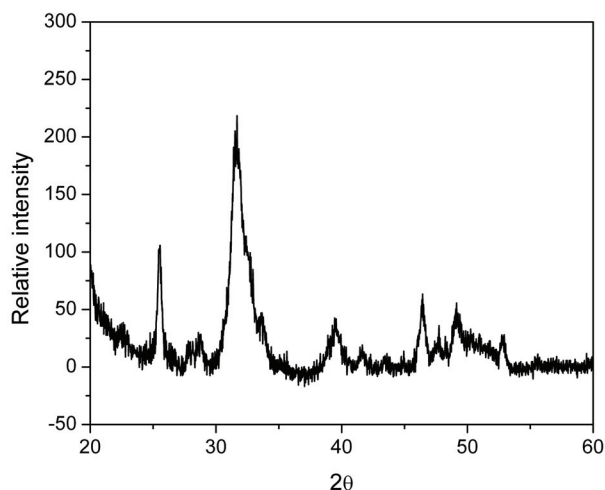


Figure 1. XRD pattern of the sample  $B_{NaOH}$ .

The reported XRD patterns of samples B,  $B_{400}$  [15] and  $B_{NaOH+400}$  [20] also showed the presence of HAP as a major crystalline phase. HAP peaks were generally of low intensities and fused, indicative of small grain size, low crystallization degree and high defectiveness of bone apatite crystals. Although the apatite samples might contain some other calcium-phosphate phases [24], their presence was not confirmed by XRD analysis. Furthermore, different intensities of the background in the XRD spectra can be ascribed to the different amounts of organic matter.

Ca/P mole ratio of the sample  $B_{NaOH+400}$  was found to be 1.23 [20], whereas molar ratios of 1.20, 1.17, and 1.16 were calculated for B,  $B_{NaOH}$  and  $B_{400}$ , respectively. Compared to the stoichiometric HAP Ca/P ratio = 1.67, considered bio-apatite samples were Ca-deficient. Determined SSA of  $B_{NaOH}$  was  $35 \text{ m}^2 \text{ g}^{-1}$ , which is much higher than SSA of raw bones ( $0.1 \text{ m}^2 \text{ g}^{-1}$  [15]), but lower than SSA of thermally treated bones ( $85 \text{ m}^2 \text{ g}^{-1}$  for  $B_{400}$  [15] and  $78 \text{ m}^2 \text{ g}^{-1}$  for  $B_{NaOH+400}$  [20]).

### Sorption kinetics

Sorption kinetics curves (Figure 2a) were of typical shape: a sharp increase at the beginning followed by slower metal uptake. With time, the active sites on the sorbent surface become increasingly occupied and Ni(II) concentration in the liquid phase

decreases; therefore, the rate of process is stabilized. Equilibrium was attained after 3h using  $B_{NaOH+400}$ , whereas approximately 24 h was required for other samples. Sorbed amounts of Ni(II) ions at equilibrium increased in the order  $B (0.22 \text{ mmol g}^{-1}) < B_{NaOH} (0.28 \text{ mmol g}^{-1}) < B_{400} (0.30 \text{ mmol g}^{-1}) < B_{NaOH+400} (0.35 \text{ mmol g}^{-1})$ .

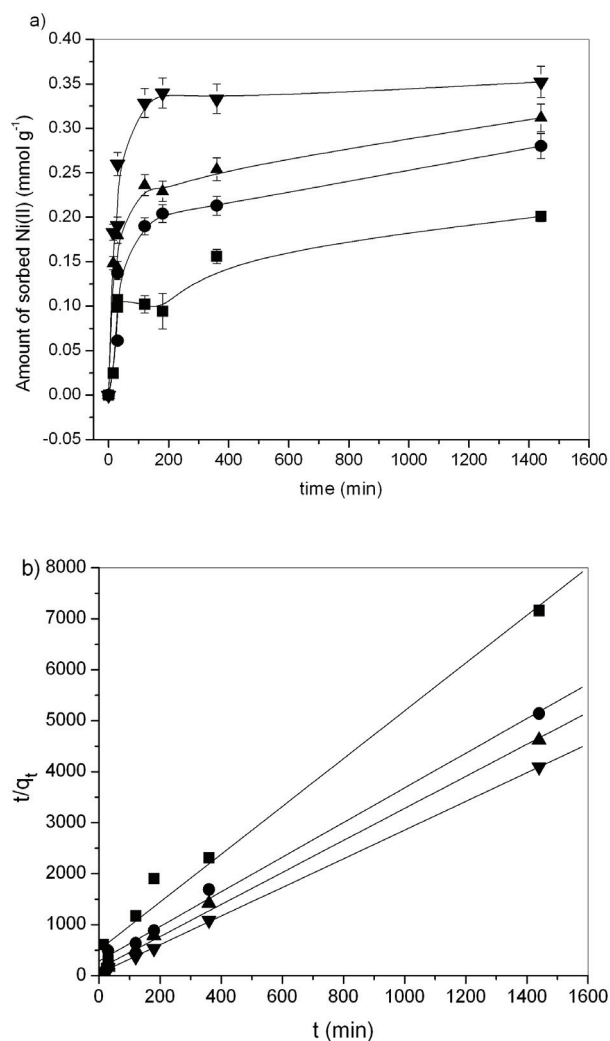


Figure 2. a) Time-dependent sorption of Ni(II) ions on different bone sorbents. b) Data fitting using pseudo-second order kinetic model. Solid/liquid ratio 1/200, initial Ni(II) concentration  $6 \times 10^{-3} \text{ mol L}^{-1}$ , initial pH 6.0. Symbols: (■) B, (●)  $B_{NaOH}$ , (▲)  $B_{400}$  and (▼)  $B_{NaOH+400}$ .

The experimental results were analyzed using pseudo-second order kinetic equation [25] which is widely used for sorption data modeling. The linear form of pseudo-second order model is given by the following equation:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (1)$$

where  $q_t$  and  $q_e$  ( $\text{mmol g}^{-1}$ ) are sorbed amounts at time  $t$  and at equilibrium, respectively,  $k_2$  ( $\text{g mmol}^{-1} \text{min}^{-1}$ ) is the pseudo-second-order rate constant. The denominator of the first member at right side is denoted as the initial sorption rate  $h$  ( $\text{mmol g}^{-1} \text{min}^{-1}$ ).

Results of data fitting are presented in Figure 2b and Table 2. Equilibrium sorbed amounts calculated by the model were 0.21, 0.29, 0.32 and  $0.36 \text{ mmol g}^{-1}$  for B,  $B_{\text{NaOH}}$ ,  $B_{400}$  and  $B_{\text{NaOH}+400}$ , respectively, closely matching the values obtained experimentally. In addition, high  $R^2$  values indicated good agreement between experimental results and mathematical model.

Table 2. Pseudo-second order model parameters for Ni(II) sorption by differently treated bones

Parameter	Sorbent			
	B	$B_{\text{NaOH}}$	$B_{400}$	$B_{\text{NaOH}+400}$
$q_e / \text{mmol g}^{-1}$	0.21	0.29	0.32	0.36
$k_2 / \text{g mmol}^{-1} \text{min}^{-1}$	0.043	0.40	0.071	0.18
$h / \text{mmol g}^{-1} \text{min}^{-1}$	0.0020	0.0035	0.0072	0.0230
$R^2$	0.991	0.998	0.999	0.999

Calculated values of  $k_2$  and  $h$  were in the range  $0.043\text{--}0.40 \text{ g mmol}^{-1} \text{min}^{-1}$  and  $0.0020\text{--}0.023 \text{ mmol g}^{-1} \text{min}^{-1}$ , respectively. Treatments have improved sorption of Ni(II) and led to an increase in the speed of sorption. Rate constant  $k_2$  increased in the order  $B < B_{400} < B_{\text{NaOH}+400} < B_{\text{NaOH}}$ , while  $h$  increased as follows:  $B < B_{\text{NaOH}} < B_{400} < B_{\text{NaOH}+400}$ .

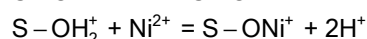
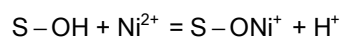
Good correlation between pseudo-second-order model and experimental data has already been reported for the sorption of divalent cations onto various sorbents:  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Sr}^{2+}$  by synthetic HAP [26],  $\text{Cr}^{3+}$  and  $\text{Sr}^{2+}$  by bone char [17,27],  $\text{Co}^{2+}$  by animal bones [15],  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  onto magnetic eggshell- $\text{Fe}_3\text{O}_4$  powder [28],  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  onto Fe(III)-modified zeolite [29], etc. Theoretically, agreement between the sorption kinetic data and mathematical models such as pseudo-second order implies that chemical reaction is the rate-controlling step [30]. However, sorption processes governed by different mechanisms (surface-complexation, dissolution/precipitation, ion-exchange, etc.) were equally well described by this model [17,31,32]. This basically means that the applicability of this model is not sufficient evidence for mechanistic interpretations, but it is suitable for mathematical description of the process, prediction of  $q_e$  values, and data comparison.

### Sorption equilibrium

The sorption isotherms of Ni(II) ions onto raw and differently treated bovine bones are presented in Figure 3a and b. The sorbed amounts of Ni(II) gen-

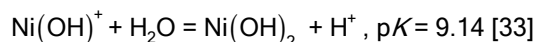
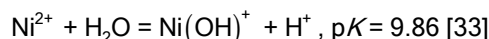
erally increased in the order  $B < B_{\text{NaOH}} < B_{400} < B_{\text{NaOH}+400}$ . Ni(II) removal from aqueous media using samples  $B_{\text{NaOH}}$  and  $B_{\text{NaOH}+400}$  was especially enhanced in the low concentration range ( $10^{-4}\text{--}5 \times 10^{-4} \text{ mol L}^{-1}$ ).

Equilibrium pH values (Figure 3c) decreased along with the increase of initial metal concentration, which may be related to the phenomenon known as specific cation sorption [29]. Specifically sorbed cations are attached strongly to the surface functional groups causing the release of  $\text{H}^+$  ions:



where S-OH and S-OH<sub>2</sub><sup>+</sup> respectively denote neutral or protonated surface functional groups of both HAP (phosphate) and organic phase, if present.

Additionally, hydrolysis of Ni ions takes place according to the reactions:



Calculation of Ni-species distribution in respect to solution pH showed that  $\text{Ni}^{2+}$  are dominant up to pH 8 [6]. Hydrolysis starts at  $\text{pH} > 8$ , reaching the maximum amount of insoluble  $\text{Ni(OH)}_2$  at pH 10. Taking this into account, large sorption of Ni(II) by  $B_{\text{NaOH}+400}$  observed in the low concentration range, can be linked to the equilibrium pH values close to the  $\text{Ni(OH)}_2$  precipitation threshold.

The sorption of Ni(II) from the solutions of different initial concentrations, was followed by almost linear increase of aqueous Ca(II) concentrations (Figure 3d). Direct linear proportionality between the amounts of sorbed and released ions points toward ion-exchange as one of the operating sorption mechanisms. The ion exchange mechanism was already recognized as the sorption mechanism characteristic for HAP phase in the case of divalent Cd and Zn sorption onto synthesized HAP [34]. Also, Cheung *et al.* [35] investigated sorption of  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  onto bone char, the heterogenous sorbent produced from the destructive distillation of dried, crushed cattle bones. Knowing that the main sorbent components are calcium hydroxyapatite,  $\text{CaCO}_3$  and carbon, the authors concluded that the main sorption mechanisms are ion-exchange in HAP lattice and chemisorption onto carbon surface. Moreover, Al-Asheh *et al.* [9] reported that the main sorption mechanism of Ni(II) ions onto raw animal bones was ion-exchange. Our study strongly supports the ion-exchange scenario. Mole ratios Ca(II):Ni(II) were less than 1:1, for all investigated sorbents, which can be explained by

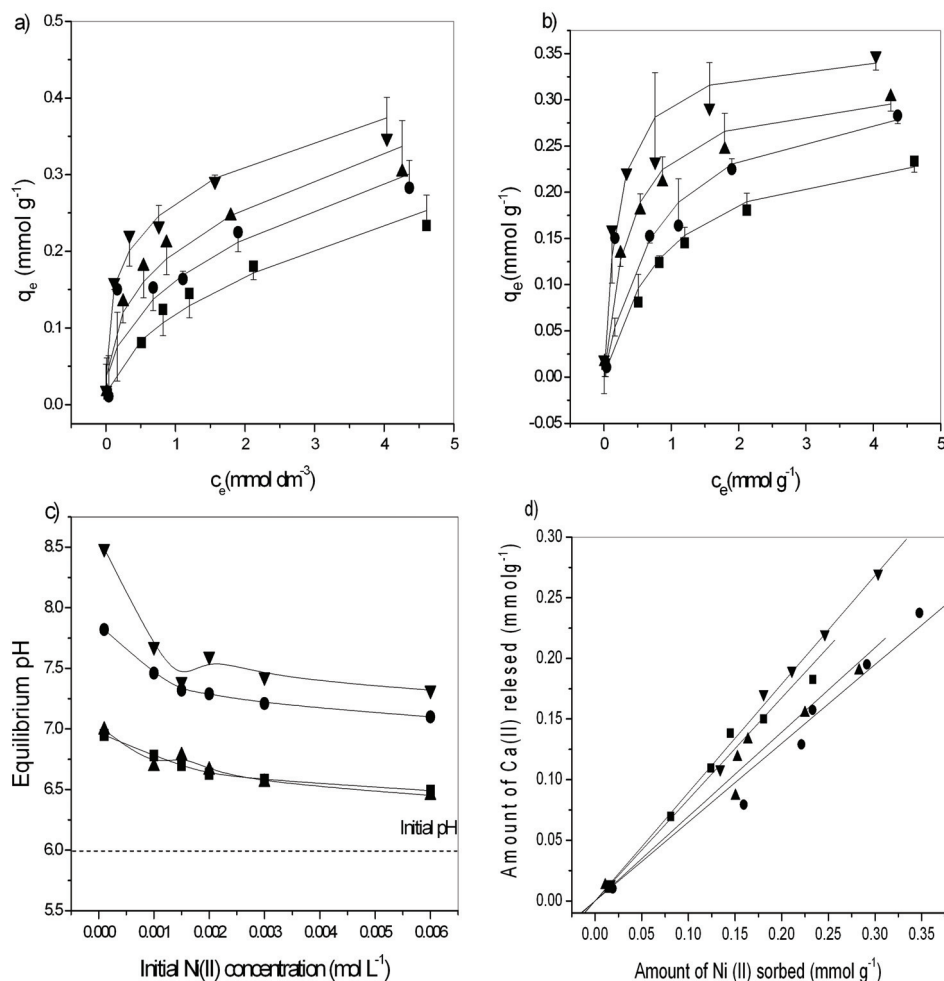


Figure 3. Ni(II) sorption isotherms: a) values predicted by Freundlich equation; b) values predicted by Langmuir model (symbols - experimental points, lines - fitting by theoretical models, error bars - deviations between experimental and predicted values); c) relationship between equilibrium pH values and initial Ni(II) concentrations; d) relationship between amounts of released Ca(II) and sorbed Ni(II) ions. Symbols: (■) B, (●)  $B_{NaOH}$ , (▲)  $B_{400}$ , and (▼)  $B_{NaOH+400}$ .

Ca-deficient bio-apatite crystal lattice [20] and the participation of other sorption mechanisms.

Sorption isotherms were described using Langmuir and Freundlich theoretical models, in the following linear forms:

$$\frac{c_e}{q_e} = \frac{c_e}{q_m} + \frac{1}{q_m K_L} \quad (2)$$

$$\ln q_e = \ln K_f + \frac{1}{n} \ln c_e \quad (3)$$

where  $c_e$  ( $\text{mmol L}^{-1}$ ) denotes the equilibrium concentrations of Ni(II) ions in the liquid phase,  $q_m$  ( $\text{mmol g}^{-1}$ ) is the maximum sorption capacity,  $K_L$  ( $\text{L g}^{-1}$ ) is the Langmuir constant related to the energy of adsorption, while  $K_f$  ( $\text{mmol}^{1-(1/n)} \cdot \text{L}^{1/n} \cdot \text{g}^{-1}$ ) and  $n$  are the Freundlich constants related to the capacity and intensity of the sorption process.

Calculated parameters are summarized in Table 3. Based on the correlation coefficients ( $R$ ), a good agreement exists between the models and experimental data. Calculated maximum sorption capacities increased in the order  $B < B_{400} < B_{NaOH} < B_{NaOH+400}$ , which is somewhat different in respect to experimentally obtained order, probably as a consequence of linearization and fitting errors. The determined  $K_L$  and  $K_f$  values increased in the same order as experimentally determined maximum sorption capacities:  $B < B_{NaOH} < B_{400} < B_{NaOH+400}$ .

From the Langmuir constant  $K_L$ , the dimensionless separation factors  $R_L$  can be calculated:

$$R_L = \frac{1}{1 + C_0 K_L} \quad (4)$$

$R_L$  is related to the nature of sorbate/sorbent attraction and isotherm type and it gives the information on whether the process is: unfavorable ( $R_L > 1$ ), linear

Table 3. Ni(II) sorption parameters calculated using Langmuir and Freundlich isotherms

Sorbent	Langmuir model			Freundlich model		
	$q_m / \text{mmol g}^{-1}$	$K_L / \text{L mmol}^{-1}$	$R^2$	$K_f / \text{mmol}^{1-(1/n)} \cdot \text{dm}^{3/n} \cdot \text{g}^{-1}$	$n$	$R^2$
B	0.274	1.06	0.974	0.118	1.78	0.993
B <sub>NaOH</sub>	0.332	1.20	0.952	0.162	1.65	0.865
B <sub>400</sub>	0.321	2.66	0.993	0.200	2.02	0.974
B <sub>NaOH+400</sub>	0.357	4.89	0.990	0.264	3.14	0.991

( $R_L = 1$ ), favorable ( $0 < R_L < 1$ ), or irreversible ( $R_L = 0$ ). The calculated  $R_L$  values increased in the order: B<sub>NaOH+400</sub> ( $R_L = 0.033$ ) < B<sub>400</sub> ( $R_L = 0.059$ ) < B ( $R_L = 0.14$ )  $\approx$  B<sub>NaOH</sub> ( $R_L = 0.12$ ) and indicated that all investigated processes were favorable.

The  $q_m$  values for commercially available synthetic HAP and HAP synthesized in the laboratory were found to be 0.184 and 0.274  $\text{mmol g}^{-1}$  [12]. Furthermore, sorption capacities of 0.039 [36] and 0.617  $\text{mmol g}^{-1}$  [37] for different nano HAP were also reported. Consequently, it can be concluded that treated bio-apatites applied in this study can be used as an alternative sorbent for synthesized hydroxyapatite.

### FT-IR analysis

Since peaks in the XRD spectra of bone samples appeared to be wide and fused, and did not provide information about the content and the composition of organic matter, FT-IR analysis was performed. The contribution of the bone organic and mineral phases to the overall FT-IR spectrum can be analyzed almost separately as their peaks occur in different regions of the spectra. FT-IR analysis of all unloaded samples showed peaks characteristic for HAP phase, whereas the content and qualitative composition of organic matter was related to the applied treatment (Figure 4).

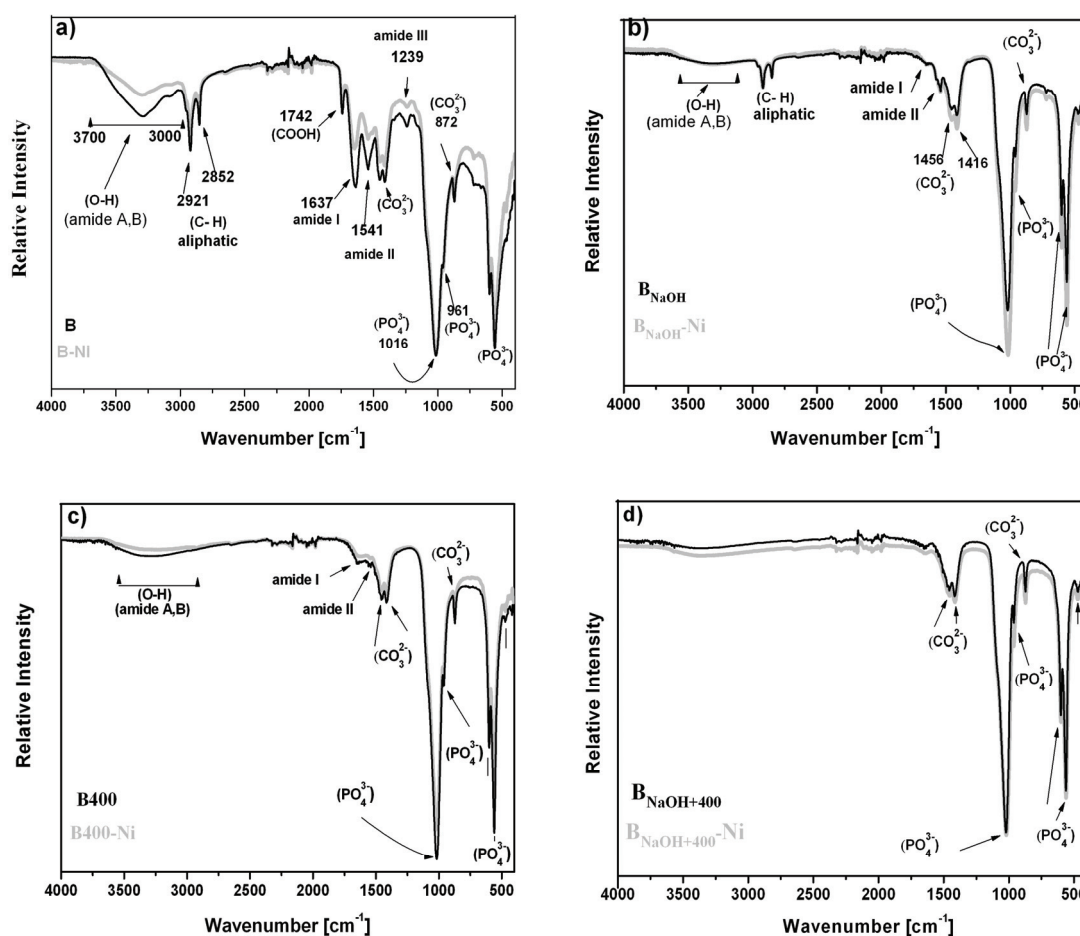


Figure 4. FT-IR analysis of investigated sorbents before (black lines) and after (gray lines) Ni(II) sorption.

The HAP phase is characterized by the most intense peaks in the spectra at about 1020, 960, 600 and 560  $\text{cm}^{-1}$ , which correspond to various modes of  $\text{PO}_4^{3-}$  vibrations. In addition, peaks at 1410, 1450 and near 870  $\text{cm}^{-1}$  can be attributed to the  $\text{CO}_3^{2-}$  group, demonstrating carbonate substitution in HAP crystal lattice [15,38]. The occurrence of  $-\text{OH}$  vibrations from different sources and Amide A and B vibrations was evident from a broad peak at 3700-3000  $\text{cm}^{-1}$  [38]. The largest number of bands characteristic for organic phase functional groups was observed in spectra of untreated bones (Figure 4a). Peaks at approximately 1640, 1540 and 1240  $\text{cm}^{-1}$  belong to amide I, II and III bands, respectively, peak at 1740  $\text{cm}^{-1}$  is characteristic for carbonyl group, and doublet at near 2920 and 2850  $\text{cm}^{-1}$  originates from  $-\text{CH}_2$  vibrations [38].

Bone treatments caused the reduction of organic phase content (Figure 4b-d). The intensity of  $-\text{OH}$  and amide A and B stretching vibrations at high wave numbers (3700-3000  $\text{cm}^{-1}$ ) was markedly reduced, and almost completely lost in the sample  $B_{\text{NaOH}+400}$ . After chemical treatment (Figure 4b)  $-\text{CH}_2$  vibrations were still visible, so as small intensity bands of Amide I and II. In the Figure 4c, only traces of amide I and II vibrations were visible, thus, the thermal treatment appeared to be more efficient than chemical, for the exclusion of bone organic phase. Higher organic content of  $B_{\text{NaOH}}$  in respect to  $B_{400}$  can be associated with its lower SSA. The spectrum of  $B_{\text{NaOH}+400}$  was organic phase free (Figure 4d), *i.e.*, it resembles the spectrum of synthetic carbonate containing HAP [38].

The greatest changes in the appearance of FT-IR spectrum before and after Ni(II) sorption were observed for sample B. Reduced intensities of absorption peaks coming from  $-\text{OH}$ , amide and carbonyl groups implies their participation in Ni(II) complexation mechanism. Similarly, removal of Ni(II) by pigeon pea pod biosorbent was attributed to the presence of C=O, C-O, O-H bonds which were identified as responsible for coordination with Ni(II) [39]. The FT-IR spectra of treated bone samples were almost unaffected by the presence of Ni(II) sorption. The fundamental apatite structure was preserved after sorption of Ni(II) ions, which is in agreement with ion-exchange mechanism.

### Sequential extraction analysis

Previous experiments have shown that  $B_{\text{NaOH}+400}$  was the most efficient sorbent, thus it was selected to investigate the stability of sorbed Ni(II) ions (Figure 5). After metal sorption from 0.15  $\text{mmol L}^{-1}$  solution,

the majority of sorbed metal was found in F5 and F2, about 65 and 17%, respectively. In F1 and F3 phase, Ni(II) ions were present with about 7.5% while almost insignificant amounts were found in F4 phase. Ni(II) distribution extremely changed with the increase of sorbent loading. After equilibration with  $6 \times 10^{-3} \text{ mol L}^{-1}$  Ni(II) solution, the percentages in F1, F2 and F3 increased up to 24, 39 and 26%, respectively, whereas content in F5 was reduced to 7%.

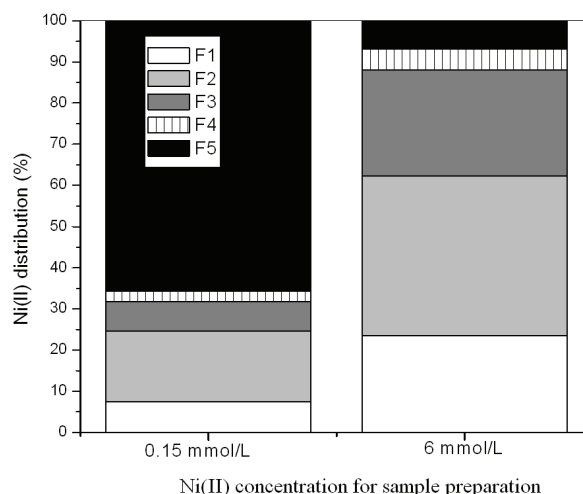


Figure 5. Effect of initial metal concentration on the distribution of Ni(II) ions sorbed by  $B_{\text{NaOH}+400}$ .

As a result of strong bonds with Ni(II) ions,  $B_{\text{NaOH}+400}$  can be recommended for utilization in water purification systems. Based on the results of sequential extraction analysis, the regeneration of the sorbent loaded with Ni(II) ions could hardly be feasible. Utilization of  $\text{Ca}^{2+}$  solutions might cause the removal of a part of exchangeable Ni(II) ions (maximum 7-24%, depending on the previously sorbed amount). On the other hand, removal of Ni(II) ions bound to phases F3-F5 requires more aggressive conditions (Table 1). Thus, the removal of at least 40-60% (depending on the sorbed amount) of Ni(II) would result in deterioration and dissolution of HAP phase. Consequently, proper disposal of spent sorbent needs to be considered. Furthermore, such high stability of Ni(II) in sorbed form is preferential for soil remediation processes [40]. Knowing that the pollutants bonded to F1 phase are considered as mobile and potentially bioavailable [40], it can be concluded that stabilization of Ni(II) ions is more efficient when lower amounts were sorbed. Thus, usage of  $B_{\text{NaOH}+400}$  as an amendment for *in situ* remediation of Ni(II) contaminated soil can be recommended especially for lower contamination levels.



## Ni(II) sorption mechanism

Experimental results indicate high complexity of Ni(II) sorption mechanism by variously treated bone sorbents. Relationships between sorbed metal quantities, equilibrium pH values, quantities of released Ca(II) ions and organic phase composition, indicated that several sorption mechanisms were operating: specific cation sorption, ion-exchange with Ca<sup>2+</sup> from HAP surface, Ni(II) hydrolysis (with possible precipitation) and complexation with organic functional groups. Also, coprecipitation of new crystal phase cannot be excluded. However, the new Ni-containing HAP phases would be hard for detection using XRD analysis due to the fact that the XRD patterns of investigated samples have intensive background and broad peaks. Ion-exchange and specific surface sorption were common mechanism for all sorbents as they relate to HAP phase. On the other hand, chemical bonding by organic functional groups was mainly detected in case of untreated bones (sample B). The presence of functional groups such as -OH, amide, carbonyl, etc., can explain relative high sorption capacity of sample B considering very low specific surface area of powdered bovine bones (0.1 m<sup>2</sup> g<sup>-1</sup> [15]). Due to various treatments, organic phase removal caused the increase of specific surface area [15,20], which resulted in improved Ni(II) sorption capacities. In addition to above, Ni(II) removal from aqueous media using sample B<sub>NaOH+400</sub> was especially enhanced in the low concentration range, due to Ni(II) hydrolysis and probably its precipitation.

## CONCLUSION

Efficiency of bovine bones towards Ni(II) sorption was compared to the performance of materials produced following chemical, thermal and combined treatments. Sorption equilibrium was satisfactorily described by Langmuir and Freundlich isotherm models, while kinetics obeyed pseudo-second order kinetics. All tested treatments improved sorption capacity toward Ni(II) ions in respect to raw bones and different synthetic HAP samples. For initial metal concentrations higher than 10<sup>-4</sup> mol L<sup>-1</sup>, sorption capacities increased in the order  $B < B_{NaOH} < B_{400} < B_{NaOH+400}$ . The material with the highest sorption capacity towards Ni(II) was obtained by synergistic effect of chemical and thermal treatments indicating great utilization possibilities in water purification systems. The mechanism of Ni(II) sorption was extremely complex, and involved inorganic (HAP) and organic phase, when present. Sequential extraction analysis of B<sub>NaOH+400</sub> loaded with lower amounts of Ni(II)

showed that metal was preferentially found in residual fraction. This implies that B<sub>NaOH+400</sub> can be considered as an additive for soil remediation, at lower levels of contamination.

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## IMOBILIZACIJA Ni(II) BIO-APATITNIM MATERIJALIMA: PROCENA EFIKASNOSTI HEMIJSKOG, TERMIČKOG I KOMBINOVANOG TRETMANA

*Životinjske kosti su prirodni i bogat izvor kalcijum-hidroksiapatita (HAP), koji predstavlja pogodan materijal za sorpciju teških metala i radionuklida. Sadržaj organske faze kostiju se može redukovati različitim tretmanima i na taj način se poboljšavaju sorpciona svojstva. U ovoj studiji, upoređeni su sorpcioni kapaciteti sirovih govedih kostiju (B) i uzoraka dobijenih hemijskim tretmanom pomoću NaOH ( $B_{NaOH}$ ), žarenjem na 400 °C ( $B_{400}$ ) i kombinovanim hemijskim i termičkim tretmanom ( $B_{NaOH + 400}$ ), korišćenjem Ni (II) jona kao sorbata. Maksimalni kapacitet sorpcije povećavao se u nizu  $B < B_{NaOH} < B_{400} < B_{NaOH + 400}$ . Na osnovu rezultata sorpcionih eksperimenata i FT-IR analize, utvrđeno je da je mehanizam sorpcije Ni(II) složen i da u njemu učestvuju i HAP i organska faze (ako je prisutna). Stabilnosti Ni (II) jona sorbovanih uzorkom  $B_{NaOH + 400}$  ispitana je primenom sekvencijalne ekstrakcije. Pri nižem opterećenju sorbenta najveća količina Ni(II) je detektovana u rezidualnoj fazi (65%), dok karbonata frakcija postaje dominantna (39%) sa porastom zasićenja sorbenta. Rezultati ukazuju na mogućnost primene  $B_{NaOH + 400}$  u prečišćavanju vode. Kao materijal na bazi apatita, sa niskim sadržajem organske materije i visokom efikasnošću sorpcije Ni(II), takode je dobar kandidat za in situ remedijaciju zemljišta, posebno pri nižim koncentracijama metala u zemljištu.*

*Ključne reči: govede kosti, tretmani, apatit, Ni (II), sorpcija, sekvencijalna ekstrakcija.*