

ANTIBACTERIAL CERIUM-MONTMORILLONITE AND CERIUM-VERMICULITE

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Abstract

The present work is focused on the study of antibacterial activity of cerium-montmorillonite and cerium-vermiculite. Montmorillonite (MT) and vermiculite (VT) and their modified Na-forms (NaMT and NaVT), prepared by cation exchange reaction with NaCl solution, were used. Samples with cerium were prepared by mixing of starting materials with aqueous solution of cerium nitrate. The elemental analysis revealed that VT in comparison with MT contained higher amount of cerium. The structural changes of MT and VT after modifications were characterized by X-ray diffraction (XRD) analysis. The XRD patterns of samples showed cation exchange of cerium in MT and VT. Stability of cerium in MT and VT samples in aqueous environment was also studied. It was confirmed that cerium was only slightly released from samples. Antibacterial activity was tested against Gram-positive (*Escherichia coli*) and Gram-negative (*Pseudomonas aeruginosa*) bacterial strains. The inhibitory effect of samples on bacterial growth was confirmed after 24 h of tested time with minimum inhibitory concentration of samples 10% (w/v).

Keywords: montmorillonite, vermiculite, cerium, antibacterial activity

1. INTRODUCTION

Montmorillonite and vermiculite belongs to the group of planar hydrous layered silicates (LS). Their structure consists from one octahedral sheet sandwiched between two tetrahedral sheets. The central octahedral (mainly Al³⁺ or Fe³⁺) and tetrahedral (mainly Si⁴⁺) cations can be substituted by the cations with lower valency (as Al³⁺, Fe³⁺, Fe²⁺, Mg²⁺). These substitutions resulted in the negative charge on layers which is compensated by hydrated exchangeable cations (as Mg²⁺, Ca²⁺, Na⁺, K⁺) in the interlayer space of LS [1-2]. These interlayer hydrated cations can be exchange for the other, also for Ce³⁺.

Some published works described preparation of Ce-LS by cation exchange [3-6] and study of their antibacterial properties [5-6]. Tan et al. (2008) prepared Zn²⁺ and Ce³⁺ loaded montmorillonite and studied their antibacterial activity against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Candida albicans* and *Mucor*. They found that antibacterial activity was enhanced with specific surface area and zeta potentials and observed synergistic effect of Zn²⁺ and Ce³⁺ [5]. Ouyang et al. (2009) prepared four kinds of Ce³⁺ exchanged montmorillonites and studied their antibacterial activity against *E. coli* and *S. aureus*. They found that antibacterial activity of Ce-montmorillonites increased with increasing content of cerium and 1.5 g/L of sample containing about 11 wt.% of cerium removed all the *S. aureus* and 99.9% of the *E. coli* during 24 h of contact [6].

In this work, montmorillonite and vermiculite were enriched with cerium by the cations exchange reaction. The structural characterization of prepared samples was performed by X-ray diffraction analysis, stability of cerium on clay minerals in water was determined from the amount of released cerium and antibacterial effect was evaluated against *Escherichia coli* and *Pseudomonas aeruginosa* bacteria.

2. MATERIALS AND METHODS

2.1 Starting materials

Montmorillonite (MT) from Ivančice (Czech Republic) and vermiculite (VT) from Santa Luzia (Brazil) were used as starting materials. Their fraction under 40 μm was obtained by milling in a planetary mill (Retsch 4) for 20 min and followed by sieving. Sodium chloride, NaCl was used for preparation of Na-forms. Cerium nitrate, $\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ (Sigma Aldrich) with the purity of 99,99 % was used as a source of cerium.

2.2 Preparation of samples

Na-form of MT and VT

MT and VT were mixed with aqueous solution of NaCl ($1 \text{ mol} \cdot \text{dm}^{-3}$) for 2 h at 80 °C. Afterward, the dispersions were centrifuged and washed with demineralized water until chloride ions were not present. Procedure was repeated three times, samples were dried for 24 h at 70 °C and labelled as NaMT and NaVT (Table 1).

MT and VT with Ce

VT, NaVT and MT, NaMT were mixed with aqueous solution of $\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ ($0.01 \text{ mol} \cdot \text{dm}^{-3}$, $0.1 \text{ mol} \cdot \text{dm}^{-3}$ and $0.25 \text{ mol} \cdot \text{dm}^{-3}$) for 24 h at room temperature. Afterward, the samples were centrifuged and washed with distilled water until nitrate ions were not present. The samples were dried for 24 h at 70 °C. The labelling of samples is shown in Table 1.

2.3 Stability of cerium on clay matrices in water

Stability of selected samples MT and VT with Ce was studied in an aqueous environment. Dispersions of samples in distilled water in ratio 1:10 (g/ml) were shaken for 24 h at room temperature, centrifuged and the liquid parts were analysed for cerium quantity [7]. The labelling of leached samples is shown in Table 1.

Table 1 List of prepared samples.

Sample	Description of sample preparation	Leaches in water
MT	Montmorillonite, < 40 μm	-
Ce1-MT	MT after treatment with $0,01 \text{ mol} \cdot \text{dm}^{-3} \text{ Ce}(\text{NO}_3)_3$	Ce1-MT-w
Ce2-MT	MT after treatment with $0,1 \text{ mol} \cdot \text{dm}^{-3} \text{ Ce}(\text{NO}_3)_3$	Ce2-MT-w
Ce3-MT	Ce2-MT after treatment with $0,1 \text{ mol} \cdot \text{dm}^{-3} \text{ Ce}(\text{NO}_3)_3$	-
Ce4-MT	MT after treatment with $0,25 \text{ mol} \cdot \text{dm}^{-3} \text{ Ce}(\text{NO}_3)_3$	-
NaMT	Na-form of MT prepared by cation exchange	-
Ce5-MT	Na-MT after treatment with $0,1 \text{ mol} \cdot \text{dm}^{-3} \text{ Ce}(\text{NO}_3)_3$	-
VT	Vermiculite < 40 μm	-
Ce1-VT	VT after treatment with $0,01 \text{ mol} \cdot \text{dm}^{-3} \text{ Ce}(\text{NO}_3)_3$	Ce1-VT-w
Ce2-VT	VT after treatment with $0,1 \text{ mol} \cdot \text{dm}^{-3} \text{ Ce}(\text{NO}_3)_3$	Ce2-VT-w
Ce3-VT	Ce2-VT after treatment with $0,1 \text{ mol} \cdot \text{dm}^{-3} \text{ Ce}(\text{NO}_3)_3$	-
Ce4-VT	VT after treatment with $0,25 \text{ mol} \cdot \text{dm}^{-3} \text{ Ce}(\text{NO}_3)_3$	-
NaVT	Na-form of VT prepared by cation exchange	-
Ce5-VT	NaVT after treatment with $0,1 \text{ mol} \cdot \text{dm}^{-3} \text{ Ce}(\text{NO}_3)_3$	-

2.4 Methods

The chemical composition of samples was measured on the energy dispersed X-ray fluorescence (XRF) spectrometer with Rh X-ray lamp SPECTRO X LAB. The X-ray diffraction (XRD) patterns were measured on

the X-ray diffractometer Ultima IV from Rigaku (Bragg-Brentano arrangement, CuK α radiation). The measurement was performed in ambient atmosphere under constant conditions (40 kV, 40 mA, scan step 0.05°). The Jobin-Yvon JY-24 ICP atomic emission spectrometer (ICP-AES) was used to quantify the amount of the Ce³⁺ cations which were released from samples to the water.

2.5 Antibacterial test

Antibacterial activity of the prepared samples was examined using two different bacterial strains. The Gram-negative (G⁻) strain represented the bacteria *Pseudomonas aeruginosa* (*P. aeruginosa*, CCM 1960), the Gram-positive (G⁺) strain represented the bacteria *Escherichia coli* (*E. coli*, CCM 3954).

The broth dilution method [8] was used to determine the minimum inhibitory concentration (MIC), as the lowest concentration of sample that completely stopped bacterial growth. The samples were prepared for testing as 10% (w/v) water dispersions which were further diluted to 3.33%, 1.11%, 0.37%, 0.12%, 0.041% and 0.014% (w/v). The pure glucose broth served as check test. Glucose suspensions containing the individual bacteria in concentrations: *E. coli* (1.1×10^9 cfu.ml⁻¹) and *P. aeruginosa* (1.1×10^9 cfu.ml⁻¹). Results were monitored after 30, 60, 90, 120, 180, 240 and 300 minutes. Afterwards, this procedure was repeated in 24 hours interval over six days. The detailed procedure was published by Valášková et al. [9].

3. RESULTS AND DISCUSSION

3.1 Structure characterization

The XRD pattern of pure MT (Fig. 1a) shows basal 001 reflection with interlayer distance $d = 1.479$ nm. This reflection was shifted to $d = 1.505$ nm in Ce-MT2 (Fig. 1c), $d = 1.483$ nm in Ce-MT3 (Fig. 1d) and $d = 1.568$ nm in Ce-MT4 (Fig. 1e).

The interlayer distance has slightly expanded with increasing amount of Ce³⁺ in the samples. The d -value of Ce-MT samples is similar to those of MT which contained the hydrated cations Ca²⁺ (~1.52 nm [10]) in the interlayer space. This corresponds to already published findings that Ce³⁺ is intercalated as hydrated form [Ce(H₂O)₃]³⁺ [5,6].

The XRD pattern of NaMT (Fig. 1f) shows basal 001 reflections with interlayer distance $d = 1.257$ nm which is characteristic for Na-form of MT. This value shifted to $d = 1.49$ nm in Ce5-MT which confirm the cation exchange of Ce³⁺ instead of Na⁺ in the MT interlayer space.

The XRD pattern of pure VT (Fig. 2a) shows basal 002 reflections with interlayer distances $d = 1.441$ nm, $d = 1.217$ nm and $d = 2.38$ nm which correspond to the interlayer material with two layers and one layer of water molecules and mixed-hydrate layered domains in the VT interlayer [11,12].

The XRD patterns of samples VT with Ce show only slight changes. The interlayer distances shifted to $d = 1.424$ nm and $d = 1.264$ nm in Ce-VT1 (Fig. 2b), $d = 1.479$ nm and $d = 1.278$ nm in Ce-VT2 (Fig. 2c). The interlayer distance of Ce-VT2 expanded to $d = 1.501$ nm and $d = 1.288$ nm in Ce-VT3 (Fig. 2d). This signifies that Ce-VT3 sample contain higher amount of Ce³⁺ in the interlayer then Ce-VT2, this is supported by the elemental analysis results (Tab. 1). The XRD pattern of Ce-VT4 (Fig. 1e) shows basal reflections with value $d = 2.47$ nm, $d = 1.491$ nm and $d = 1.128$ nm.

The slight changes, just like for MT samples, are due to presence of Ce³⁺ as hydrated form [Ce(H₂O)₃]³⁺ [5,6] which has the similar d -value as VT with hydrated cations Mg²⁺ or Ca²⁺ in the interlayer space.

The XRD pattern of NaVT (Fig. 2f) shows basal 002 reflections with interlayer distances $d = 1.214$ nm and $d = 1.128$ nm which are characteristic for Na-form of VT. These values shifted to $d = 1.483$ nm and $d = 1.291$ nm in Ce5-VT (Fig. 2g) and this confirms cation exchange of Ce³⁺ instead of Na⁺ in the VT interlayer space.

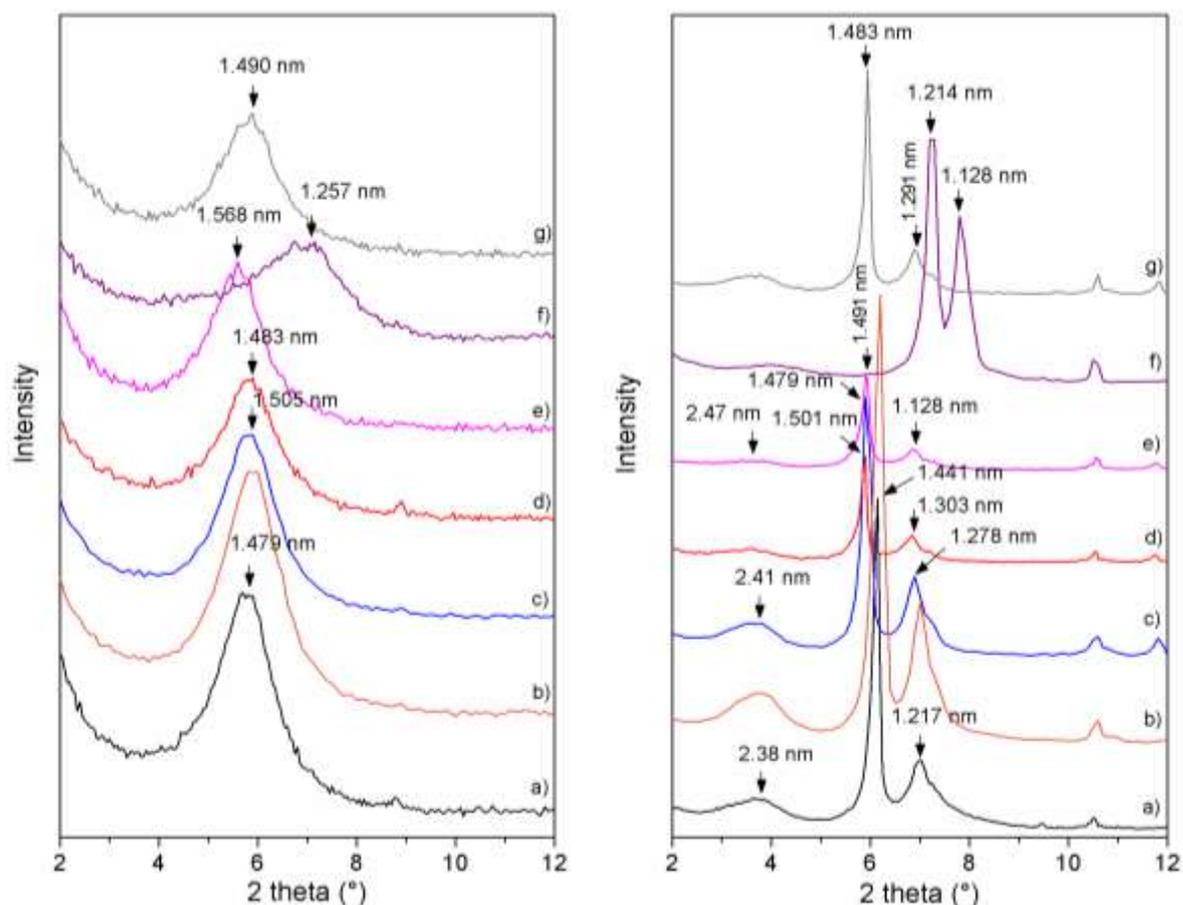


Fig. 1 XRD patterns of MT samples (left) a) MT, b) Ce1-MT, c) Ce2-MT, d) Ce3-MT, e) Ce4-MT, f) NaMT, g) Ce5-MT, and VT samples (right) a) VT, b) Ce1-VT, c) Ce2-VT, d) Ce3-VT, e) Ce4-VT, f) NaVT, g) Ce5-VT

3.2 Quantity of cerium in powder samples

The results of elemental analysis (Table 2) show that total amount of cerium in VT is higher than in MT. This finding corresponds to the higher negative layer charge and higher cation exchange capacity of VT compared to MT. The Ce3-VT (3.49 wt.%) from VT samples and Ce2-MT (2.35 wt.%) from MT samples contain the highest amount of Ce^{3+} . The samples Ce4-VT (3.09 wt.%) and Ce4-MT (2.12 wt.%) prepared from solution with the strong initial concentration (0.25 mol.dm^{-3}) contain smaller amount of Ce^{3+} than samples mentioned above. This implies that cation exchange capacity of VT and MT was already filled by lower initial concentration of the used solution. Samples Ce5-VT (3.17 wt.%) and Ce5-MT (2.08 wt.%) prepared from NaVT and NaMT contain the similar or smaller amount of Ce^{3+} compared to samples Ce2-VT (3.18 wt.%) and Ce2-MT (2.35 wt.%) from original VT and MT, thus, using of Na-forms had not positive effect on the amount of Ce^{3+} in samples.

Table 2 The amount of cerium in samples.

Sample	Ce (wt.%)	Sample	Ce (wt.%)
Ce1-MT	0.97	Ce1-VT	1.19
Ce2-MT	2.35	Ce2-VT	3.18
Ce3-MT	2.32	Ce3-VT	3.49
Ce4-MT	2.12	Ce4-VT	3.09
Ce5-MT	2.08	Ce5-VT	3.17

The amount of Ce³⁺ in leaches determined by AES-ICP (Tab. 4) shows higher quantity of cerium released from samples Ce-VT compared to Ce-MT. The amount of released cerium increased with increasing Ce³⁺ amount in sample. However, it can be concluded that cerium is stably anchored on MT and VT in distilled water. Based on the results more than 99.79 % of cerium remained in MT and VT samples.

Table 3 The amount of Ce³⁺ (mg.l⁻¹) in aqueous leachates released from samples and calculated as % release from total amount of cerium in powder samples.

	Sample			
	Ce1-MT-w	Ce2-MT-w	Ce1-VT-w	Ce2-VT-w
Ce ³⁺ (mg.l ⁻¹)	0.56	1.59	2.35	3.31
Ce ³⁺ loss (%)	0.06	0.07	0.21	0.10

3.3 Antibacterial test

The results of antibacterial test of Ce-MT and Ce-VT samples (Tables 4, 5) show no effect against G⁺ bacteria *E. coli* and G⁻ bacteria *P. aeruginosa* after 5 h. However, all tested Ce-MT samples (excluded Ce1-MT) and Ce-VT samples (excluded Ce1-VT) showed antibacterial activity against *E. coli* and *P. aeruginosa* with MIC value 10% (w/v) after 24 h. This effect of all samples was stable after 96 h and also during whole tested time for 6 days. Original MT and VT did not inhibit bacterial growth at all.

The antibacterial effect depends on the amount of Ce³⁺ in samples and also on his releasing. Samples Ce1-MT and Ce1-VT contain smaller amount of Ce³⁺, thus, they do not inhibit bacterial growth as the other samples with higher content of Ce³⁺.

The antibacterial mechanism of Ce³⁺ was ascribed to destruction of cell wall structure followed by permeability of membrane. Moreover, the Ce³⁺ could interact with some proteins inside the bacterial cell and impede the physiological activities of bacteria [13].

Table 4 Minimum inhibitory concentration (MIC) of MT and Ce-MT samples after 5 h, 24 h and 96 h of contact time with bacteria *E. coli* and *P. aeruginosa*.

sample	<i>E. coli</i> (% w/v)			<i>P. aeruginosa</i> (% w/v)		
	5 h	24 h	96 h	5 h	24 h	96 h
MT	-	-	-	-	-	-
Ce1-MT	-	10	10	-	-	-
Ce2-MT	-	10	10	-	10	10
Ce3-MT	-	10	10	-	10	10
Ce4-MT	-	10	10	-	10	10

Table 5 Minimum inhibitory concentration (MIC) of VT and Ce-VT samples after 5 h, 24 h and 96 h of contact time with bacteria *E. coli* and *P. aeruginosa*.

sample	<i>E. coli</i> (% w/v)			<i>P. aeruginosa</i> (% w/v)		
	5 h	24 h	96 h	5 h	24 h	96 h
VT	-	-	-	-	-	-
Ce1-VT	-	-	-	-	-	-
Ce2-VT	-	10	10	-	10	10
Ce3-VT	-	10	10	-	10	10
Ce4-VT	-	10	10	-	10	10

4. CONCLUSION

Cerium-montmorillonite and cerium-vermiculite were successfully prepared as antibacterial powder materials. The X-ray diffraction patterns showed that cerium was cation exchanged in the interlayer of montmorillonite and vermiculite. The amount of cerium was higher in vermiculite than montmorillonite. Stability test in aqueous environment showed that cerium is stably anchored in clay mineral matrices. Antibacterial tests against bacteria *Escherichia coli* and *Pseudomonas aeruginosa* showed good inhibited effect on bacterial growth. Antibacterial tests also showed that effect is depending on the amount of cerium in samples.

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