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Surfactant-modified clinoptilolite as a salicylate carrier, salicylate kinetic release and its antibacterial activity

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ABSTRACT

The cationic surfactant benzalkonium chloride (BC) was used for modification of the natural clinoptilolite surface in order to prepare a suitable carrier for the salicylate anion. The salicylate-containing clinoptilolite composite was prepared by adsorption from an aqueous aspirin solution. The composite releases salicylate in two stages: about 50% of the adsorbed salicylate is delivered in the first 15 min and an additional 30% is released slowly within the next 5 h. The Korsmeyer–Peppas model best describes the release profile. Both the BC-modified clinoptilolite and the salicylate-containing product were tested against *Escherichia coli* and *Staphylococcus aureus*. The results indicate that the salicylate-containing material may represent an alternative drug having simultaneous antibacterial and anti-inflammatory effects.

1. Introduction

Medical and pharmaceutical applications of natural zeolite have emerged as a promising field over the last decade, due to good performance of this material in ion exchange, adsorptive and biocatalytic processes [1]. The modified forms of natural zeolite have been evaluated as a gastric antacid [2], antidiarrheic [3], and a potential adjuvant in anticancer therapy [4,5]. One of the prospective pharmacological applications of zeolites is the adsorption of different drugs and their subsequent controlled release [6–8]. Conventional drug administration results in a rapid concentration change which is especially problematic for drugs with a narrow range of therapeutic concentrations. After intake, the drug level continually rises above the effective range, potentially inducing adverse side effects, and then falls below the minimum effective concentration. The controlled drug release allows for a constant drug level in the therapeutic range for a prolonged time.

Clinoptilolite is the most abundant natural zeolite. There are several deposits rich with clinoptilolite in Serbia among which the Zlatokop mine is the largest one with a high-quality clinoptilolite-rich zeolitic tuff. For this clinoptilolite it has been shown that it is not toxic and can be used in human and veterinary medicine [9].

Aspirin (ASA), the trade name of acetylsalicylic acid, acts as analgesic, antipyretic and anti-inflammatory agent. In water solution ASA hydrolyses to yield salicylic and acetic acid [10]. Considering that clinoptilolite exhibits an antacid ability [2], only a few studies have been reported regarding the theoretical possibility of physical adsorption of aspirin on the natural clinoptilolite [11] and a preliminary characterization of the surfactant-modified clinoptilolite as an aspirin carrier [12].

A negatively charged framework and its hydrophilic properties cause the clinoptilolite to be inappropriate for binding of anionic and hydrophobic species [13]. However, adsorption of surfactants can modify the properties of the clinoptilolite surface [7]. Taking into account that the literature data on the use of zeolite-surfactant composites as drug carriers are relatively scarce [6–8, 14–17], we have investigated the surfactant-modified clinoptilolite as a model system for the salicylate ion carrier. Namely, an aqueous solution of ASA contains mainly salicylate ion (SA). To modify the clinoptilolite surface we have used benzalkonium chloride (BC) since it is safe for human use. Moreover, since the BC has been known as a fast-acting biocidal agent we have also studied the antibacterial activity of the BC-modified clinoptilolite and its SA-composite.

2. Materials and methods

2.1. Characteristics of the natural zeolite

The zeolite used in this study is a clinoptilolite-rich tuff from the Zlatokop mine (Vranjska Banja, Serbia). The as-received sample was sieved to yield particles in the 0.063 to 0.1 mm size range.

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According to a detailed X-ray powder diffraction analysis, clinoptilolite is the most abundant phase (up to 73%), whereas feldspar and quartz are the major impurities [18]. The chemical composition of the clinoptilolite phase was as follows (in wt.%): 65.7 SiO_2, 13.0 Al_2O_3, 1.48 Fe_2O_3, 3.08 CaO, 1.41 MgO, 1.33 K_2O, 0.95 Na_2O, the ignition loss being 12.86% [19]. The total cation-exchange capacity (CEC) of the starting material was 139 mmol M^+/100 g, while its external cation-exchange capacity (ECEC) was 10 mmol M^+/100 g [20].

2.2. Preparation of the surfactant-modified zeolite

The sample (1.0 g) of the natural zeolite (NZ) was pretreated with 100 cm^3 of 1 mol dm^{-3} solution of NaCl (p.a., Carlo Erba) in order to improve the tuff's exchange capacity [18]. The suspension was magnetically stirred for 24 h at room temperature. The obtained sample (NaNZ) was then washed by distilled water and dried in oven at 105 °C. The NZ surface was modified using the cationic surfactant benzalkonium chloride (Fluka, >95.0%). For the preparation of the surfactant modified zeolite, 1.0 g of the NaNZ was treated for 24 h at room temperature with 100 cm^3 of the BC aqueous solution equivalents of 100%, 200% and 500% of its ECEC. The obtained products, denoted as NZ-BC_{100}, NZ-BC_{200}, NZ-BC_{500}, were centrifuged and dried at 60 °C.

2.3. Adsorption and release experiments

The adsorption experiment was performed using the ASA aqueous solution (high purity standard provided by pharmaceutical company Hemofarm, a member of STADA Group, Serbia). NZ-BC_{500} (0.5 g) was treated with 50.0 cm^3 of the ASA aqueous solution of appropriate concentration (500 or 1000 μg cm^{-3}). The experiments took place at room temperature under agitation at 600 rpm with a magnetic stirrer. The system was agitated and equilibrated for 4 h and then centrifuged. The obtained composites NZ-BC_{500}-SA were then dried at room temperature.

Using the liquid chromatography–mass spectrometry it was determined that only the negatively charged ion *m/z* 137, corresponding to deprotonated SA, was present in solution, and not the *m/z* 180 from deprotonated ASA. The amount of the adsorbed SA was determined by measuring the concentration in the aqueous phase before and after adsorption using the high performance liquid chromatography–ultraviolet spectroscopy (HPLC–UV). The SA desorption was performed by soaking 0.1 g of the composite NZ-BC_{500}-SA in 50.0 cm^3 of the phosphate buffer (pH 7.0) under constant stirring at 100 rpm and 37 °C in a thermostated water bath (Memmert WPE 45). At predetermined time intervals, 0.5 cm^3 of the sample was withdrawn and replaced with an equivalent volume of the fresh buffer solution. All data-points were determined as the average value for three independent measurements. The percentage of the released SA was determined as a ratio of SA concentration in the buffer solution to the SA loaded on the NZ-BC_{500}-SA.

All the experiments were carried out under controlled conditions: the temperature in the thermostated bath was maintained constant to within ±0.1 °C, the clinoptilolite sample was weighted to a four-digit accuracy, and the solution concentrations were determined with a four-digit accuracy.

2.4. Antibacterial activity

The antibacterial activity of NZ-BC_{500} (containing 85 mg BC g^{-1}) and NZ-BC_{500}-SA (containing 85 mg BC g^{-1} and 19 mg SA g^{-1}) was tested against pure bacterial cultures of Gram-negative bacteria *Escherichia coli* (strain DSM No. 498) and Gram-positive bacteria *Staphylococcus aureus* (strain DSM No. 799), obtained from the Deutsche Sammlung von Microorganismen und Zellkulturen GmbH. The bacteria were pre-grown on Luria Bertani (LB) agar for 16 h at 37.0 ± 0.1 °C. The bacterial biomass was then suspended in the sterile 0.05 mol dm^{-3} NaCl solution. One cm^3 of the suspended biomass of *E. coli* or *S. aureus* was inoculated into Schott bottles which contained 100 cm^3 of autoclaved LB medium of pH 7.0 ± 0.2, giving the initial number of colony forming units (CFU) of 10^6–10^7 cm^{-3}. The bottles contained 0.01–1.0 g of the NZ-BC_{500}, NZ-BC_{500}-SA or the BC and ASA solutions giving a final concentration which is equivalent to the amounts loaded onto the NZ-BC_{500} and NZ-BC_{500}-SA. The control bottles were left without addition of the zeolites or BC and ASA. The bottles were sealed and incubated in darkness for 24 h in a water bath (Memmert, WNB22) at 37.0 ± 0.5 °C with shaking at 70 rpm.

The number of the *E. coli* and *S. aureus* viable cells was determined at the beginning of the experiment and after 1, 3, 5 and 24 h of exposure. In experiments with BC the numbers of viable cells were determined after 1–60 min of exposure. Gram staining followed by light microscopy (Olympus, CX21) was performed in order to estimate the range of the high or low bacterial numbers in the bottles and the immobilization of bacteria onto the zeolites. The immobilization of bacteria onto zeolites was confirmed by the scanning electron microscopy. For the determination of high bacterial numbers, 1 cm^3 of the suspension was vigorously shaken by a mechanical shaker (40 Hz/3 min, Kartell, TK3S) and serially diluted (10^{-1}–10^{-9}) in triplicate in a sterile 0.05 mol dm^{-3} NaCl and volumes of 0.1 cm^3 were aseptically inoculated onto the LB agar plates (spread plate method). For the determination of low bacterial numbers, 10, 20 and 30 cm^3 of the suspension were filtered through 0.20 μm Sartorius sterile nitrocellulose filters and the filters were aseptically placed onto the LB agar. The LB agar plates were incubated at 37.0 ± 0.1 °C for 24 h. After the incubation period, the bacterial colonies were counted and the number of viable cells was reported as CFU cm^{-3}.

Statistical analyses were carried out using Statistica Software 9.1 (StatSoft, Tulsa, USA). The numbers of viable bacterial cells were logarithmically transformed beforehand to normalize distribution and to equalize variances of the measured parameters. Comparisons between log CFU were done using the one-way analysis of variance (ANOVA) and subsequently the post hoc Duncan test was performed for calculations concerning pair-wise comparisons. Statistical decisions were made at a significance level of $p < 0.05$.

2.5. Characterization and analytical methods

XRPD patterns of the NaZ and NZ-BC_{500} were recorded using Ital Structure APD2000 diffractometer operating with CuK radiation (λ = 0.15418 nm) in the 2θ range 5–50. The surface areas were determined by the BET method, using an automated gas adsorption analyzer TriStar 3000 at relative pressures from 0.05 to 0.25. Fourier transformed infrared (FTIR) spectra were recorded in the 4000–400 cm^{-1} range on a Digilab-FTS-80 spectrophotometer, using the KBr wafer technique. Thermogravimetric analysis (TGA) was performed in order to quantify the amount of surfactant adsorbed to the zeolite surface. The samples were measured using a SDT Q-600 simultaneous DSC-TGA instrument (TA Instruments). They were heated from the room temperature to 800 °C at a heating rate of 10 °C min^{-1} under air with a flow rate of 0.1 dm^3 min^{-1}. Amounts of carbon, hydrogen and nitrogen in NZ-BC samples were determined by a standard CHN analyzer. The scanning electron microscopy (Jeol JSM 6460LV) was used to examine the zeolite surface.

The amount of the adsorbed SA was determined by HPLC–UV. Surveyor HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) was used with a reverse-phase Zorbax Eclipse® XDB-C18

column, 75 mm × 4.6 mm i.d. and 3.5 μm particle size (Agilent Technologies, Santa Clara, CA, USA). The mobile phase consisted of methanol and deionized water mixed at the ratio of 10:90. The flow rate of the mobile phase was 0.8 ml min^{-1}. The sample injection volume was 10 μl. UV spectra were obtained by a Surveyor photo-diode array detector (Thermo Fisher Scientific). The UV spectrum displays three absorption maxima at 205 nm, 230 nm and 295 nm. The absorption maximum at 205 nm was used for the quantification.

3. Results and discussion

3.1. Adsorption and release of salicylate

The negatively charged zeolitic surface exhibits a negligible affinity for anionic species, such as the salicylic anion formed by the hydrolysis of ASA. Accordingly, it is necessary that the clinoptilolite surface be chemically changed. In this work the modification was performed with BC which is considered to be safe for human use and is widely employed in pharmaceutical industry as preservative for drugs [6]. Also, XRPD analysis of the BC-modified zeolite (not given) confirmed that the sorption of the BC does not affect the clinoptilolite crystal structure. Since the dimensions of the BC head groups exceed the diameter of largest ten-membered clinoptilolite channel (the latter being approx. 0.7 nm) and the BC does not enter the clinoptilolite framework, the BC quaternary amine ions can replace only the positively charged counter ions (Na^+) at the external surface. The amount of the sorbed BC depends on the initial concentration. At higher BC concentrations which exceed the ECEC of the clinoptilolite, the hydrophobic tails of the BC associate to form a bilayer [21]. The results obtained by standard CHN analysis are shown in Table 1. The amounts of carbon and nitrogen found in NZ-BC_{500} are doubled in comparison with NZ-BC_{100}, indicating the presence of a bilayer. Moreover, it is evident that for the NZ-BC_{500}-SA composite the amounts of carbon and hydrogen were higher than in the NZ-BC_{500} sample, whereas the amounts of nitrogen were the same. This confirms the presence of SA in NZ-BC_{500}-SA.

NZ and NZ-BC_{500} were further studied using FTIR spectroscopy (Fig. 1). The bands at 3620 and 3420 cm^{-1}, present in both IR spectra, are characteristic of the clinoptilolite lattice and are related to the acidic hydroxyls Si–O(H)–Al and to the vibration of the hydrogen-bonding hydroxyl groups, respectively [22]. A band at 1640 cm^{-1} is associated with the deformation vibration of the absorbed water, whereas the band at 1020 cm^{-1} is related to the asymmetric valence vibration in the SiO_4 tetrahedra. No variations in the frequency of these bands in the spectra of NZ and NZ-BC_{500} samples were observed, confirming that BC does not affect the zeolite structure. The spectrum of NZ-BC_{500} displays three additional bands corresponding to BC: the bands at 2925 and 2855 cm^{-1} are assigned to the C–H stretching vibrations of the hydrocarbon chain, whereas the band at 1465 cm^{-1} corresponds to the C–H bending of the methyl and methylene groups [17].

The specific surface areas of the NZ and NZ-BC_{500}, measured by the BET method, were found to be 35 $m^2 g^{-1}$ and 12 $m^2 g^{-1}$, respectively. This indicates that the sorbed BC reduces the clinoptilolite specific surface area. Also, the co-adsorption of SA does not change the specific surface area of NZ-BC_{500}, confirming that SA interacts only with BC.

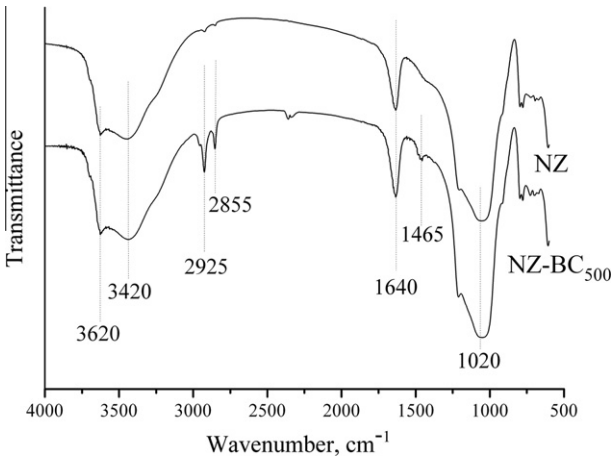

Fig. 1. FTIR spectra of the natural zeolite (NZ) and the surfactant-modified zeolite (NZ-BC_{500}).

Fig. 2 shows the TG/DTG curves for the NZ-BC_{100}, NZ-BC_{500} and NZ-BC_{500}-SA. The first mass loss (4.5%) accompanied by a DTG maximum at 80 °C, present in all TG-curves, is attributed to the unbound (physisorbed) water. DTG curves of NZ-BC_{500} and NZ-BC_{500}-SA show two well resolved DTG maxima at higher temperatures, indicating that the BC decomposition proceeds in two steps; this suggests that BC present at the zeolite surface is bound in different manners. This could be due to the presence of BC in the form of mono- or bilayer [13]. The latter interacts with the monolayer via hydrophobic–hydrophobic interactions and BC that belongs to the bilayer decomposes at a lower temperature than in the monolayer. Accordingly, the mass loss (7.6%) between 160 and 370 °C accompanied by a derivative curve (DTG) peak centred at 217 °C could be ascribed to the BC in the bilayer. The absence of this peak in the DTG curve of NZ-BC_{100} could be an indication that there is only a monolayer present in this sample. The ammonium cation head groups of the BC and the negatively charged zeolite surface interact via strong electrostatic interactions and the second loss (4.9%) with the DTG peak cantered at 427 °C, present in all DTG curves, could correspond to the loss of BC in the monolayer. The total mass loss in the temperature range of 160–800 °C is in accordance with the amount of BC obtained by the CHN analysis. The TG/DTG curves of the NZ-BC_{500}-SA composite display similar features as NZ-BC_{500}. It can be seen that only the second peak is shifted to a higher temperature, 242 °C, which could be explained by co-adsorption of SA at the NZ-BC_{500} surface. In order to get an insight into the SA-composite interaction, solid state ^1H–^{13}C CPMAS NMR analysis was performed (not shown). Only the peak corresponding to the BC was evident. It seems that the concentration of aspirin was not high enough to enable a detailed ^1H–^{13}C CPMAS NMR analysis.

Adsorption of SA on NZ-BC_{500} was carried out starting from two ASA concentrations (500 and 1000 μg cm^{-3}). The amounts of the adsorbed SA determined by HPLC–UV method show that the SA-loading on NZ-BC_{500} increases with the increase of initial ASA concentration. The NZ-BC_{500}-SA obtained from the lower ASA concentration contains 13 mg SA g^{-1}, whereas 19 mg SA g^{-1} were obtained from the ASA solution with the higher concentration.

The release of the SA was performed from the NZ-BC_{500}-SA composite with 19 mg SA g^{-1}. The results are presented in Fig. 3 showing the percentage of SA released from the NZ-BC_{500}-SA composite as a function of time. SA delivery from the composite into solution occurs in two stages. The first one takes place rather

Table 1
The results of CHN analysis of the NZ-BC composites.

Sample	%N	%C	%H	Mass of BC, mg BC g^{-1}
NZ-BC_{100}	0.17	3.11	1.52	41
NZ-BC_{200}	0.23	4.48	1.70	60
NZ-BC_{500}	0.33	6.21	1.94	85

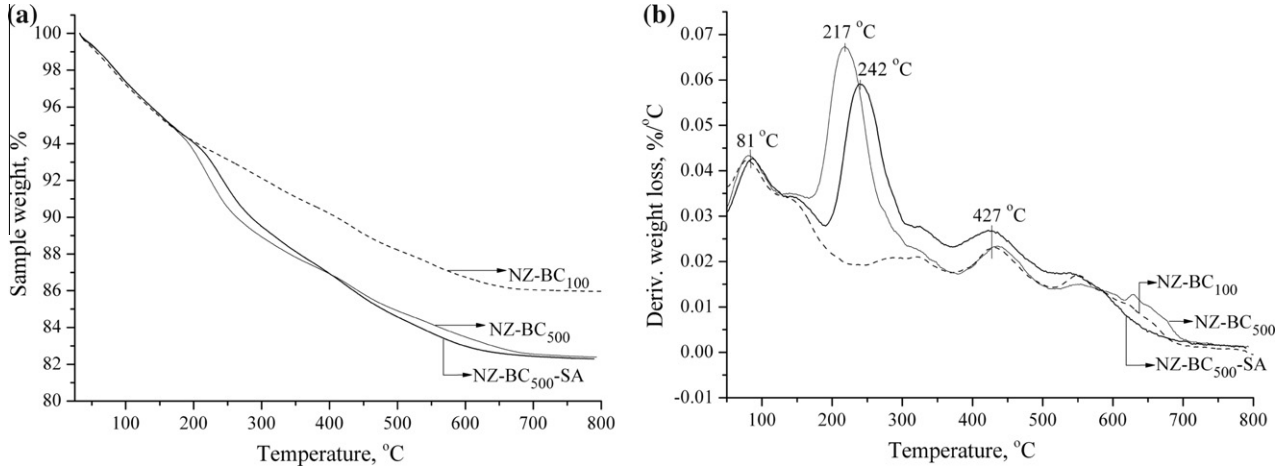

Fig. 2. TG (a) and DTG curves (b) of NZ-BC_{100}, NZ-BC_{500} and NZ-BC_{500}-SA.

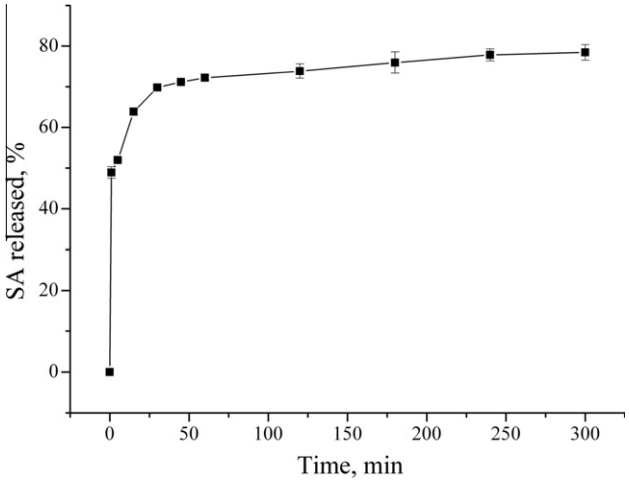

Fig. 3. Percentage of SA released from the NZ-BC_{500}-SA composite.

Table 2
Numbers of *E. coli* or *S. aureus* and final pH values in control bottles and bottles containing the BC, ASA or BC and ASA in concentration equivalent to the amounts loaded onto 1.0 g of NZ-BC_{500}-SA in 100 cm^3 of LB medium during 24 h of contact. Mean values of triplicate measurements are expressed. Initial CFU cm^{-3}: $1.65 \pm 0.35 \times 10^6$ *E. coli*; $1.75 \pm 0.05 \times 10^7$ *S. aureus*.

Time/pH	Control	BC	ASA	BC + ASA
Log CFU E. coli cm^{-3}				
1 min	6.22	0.00*	6.23	0.00*
1 h	6.45	0.00*	6.47	0.00*
3 h	7.99	0.00*	7.94	0.00*
5 h	8.97	0.00*	8.98	0.00*
24 h	9.42	0.00*	9.39	0.00*
Final pH	7.04	7.10*	6.94*	7.12*
Log CFU S. aureus cm^{-3}				
1 min	7.25	0.00*	7.24	0.00*
1 h	7.37	0.00*	7.28	0.00*
3 h	8.16	0.00*	8.17	0.00*
5 h	8.96	0.00*	8.88	0.00*
24 h	9.61	0.00*	9.57	0.00*
Final pH	6.98	7.09*	6.80*	6.79*

* Significantly different as compared to the corresponding control.

Table 3
Numbers of *E. coli* or *S. aureus* and final pH values in control bottles and bottles containing the different amounts (0.01–1.0 g/100 cm^3) of NZ-BC_{500} during 24 h of contact. Mean values of triplicate measurements is expressed. Initial CFU cm^{-3}: $1.67 \pm 0.34 \cdot 10^7$ *E. coli*; $4.45 \pm 0.45 \cdot 10^6$ *S. aureus*.

NZ-BC_{500} (g/100 cm^3)/time (h)	1 h	3 h	5 h	24 h	Final pH
Log CFU E. coli cm^{-3}					
0 (control)	7.18	8.12	9.07	9.27	6.98
0.01	6.77*	8.01	8.80*	9.23	6.78*
0.05	6.56*	7.22*	8.17*	8.86*	6.75*
0.1	5.88*	5.02*	4.68*	8.39*	6.72*
0.5	0.00*	0.00*	0.00*	0.00*	7.11*
1.0	0.00*	0.00*	0.00*	0.00*	7.15*
Log CFU S. aureus cm^{-3}					
0 (control)	6.88	8.19	8.97	9.21	6.73
0.01	6.80	6.45*	6.02*	5.94*	7.02*
0.05	6.55*	3.61*	2.90*	0.71*	6.98*
0.1	3.98*	2.40*	2.38*	0.00*	6.97*
0.5	0.00*	0.00*	0.00*	0.00*	7.13*
1.0	0.00*	0.00*	0.00*	0.00*	7.14*

* Significantly different as compared to the corresponding control.

Table 4
Numbers of *E. coli* or *S. aureus* and final pH values in control bottles and bottles containing the different amounts (0.01–1.0 g/100 cm^3) of NZ-BC_{500}-SA during 24 h of contact. Mean values of triplicate measurements is expressed. Initial CFU cm^{-3}: $1.67 \pm 0.34 \cdot 10^7$ *E. coli*; $4.45 \pm 0.45 \cdot 10^6$ *S. aureus*.

NZ-BC_{500}-SA (g/100 cm^3)/time (h)	1 h	3 h	5 h	24 h	Final pH
Log CFU E. coli cm^{-3}					
0 (control)	7.18	8.12	9.07	9.27	6.98
0.01	6.56*	8.04	9.06	9.13*	6.81*
0.05	6.39*	6.65*	7.34*	8.74*	6.71*
0.1	5.95*	4.52*	3.40*	8.42*	6.60*
0.5	0.00*	0.00*	0.00*	0.00*	6.99
1.0	0.00*	0.00*	0.00*	0.00*	6.92*
log CFU S. aureus cm^{-3}					
0 (control)	6.88	8.19	8.97	9.21	6.73
0.01	6.63*	6.33*	6.27*	6.29*	7.00*
0.05	5.69*	3.98*	3.56*	0.36*	6.99*
0.1	3.98*	1.98*	0.04*	0.00*	6.98*
0.5	0.00*	0.00*	0.00*	0.00*	7.01*
1.0	0.00*	0.00*	0.00*	0.00*	6.95*

* Significantly different as compared to the corresponding control.

sharply within the first 15 min. Afterwards, the release proceeds more gradually. About 50% of the immobilized SA is delivered in the first stage and then the release slows down to a maximum value of 80% within 5 h. This suggests that in the first 15 min the delivery rate is controlled by a diffusion process, whereas the electrostatic interactions between the carboxylic groups of SA and

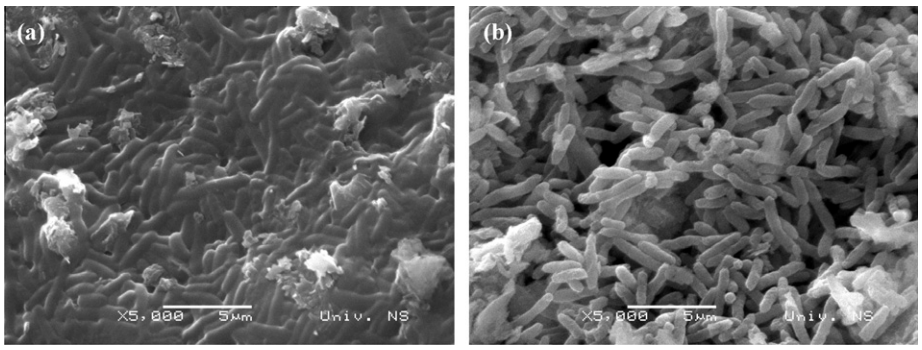

Fig. 4. Immobilized cells of *E. coli* onto NZ-BC_{500}-SA used in concentration (per 100 cm^3) of: 0.1 g (a) and 0.01 (b).

ammonium cation head groups of the BC are predominant in the second stage.

The release profile was analyzed using a set of generally recommended mathematical models [23,24]. It was found that the obtained results were in good agreement ($r^2 > 0.98$) with the Korsmeyer–Peppas model given by the equation:

$$F = kt^n$$

where F is the fractional release of SA, k is the kinetic release constant, t is the elapsed time and n is the release exponent describing the drug release mechanism [23]. The obtained value for n was 0.1 indicating that a diffusion of SA is the predominant release mechanism [16].

3.2. Antibacterial activity

The results for the antibacterial activity of the solution of BC, ASA or BC and ASA in concentrations equivalent to the amounts loaded to 1.0 g of zeolite in 100 cm^3 of LB medium are shown in Table 2. In the control bottles without BC, ASA or BC and ASA the *E. coli* and *S. aureus* showed progressive growth during 24 h of experiment. In bottles with ASA no significant changes in bacterial numbers were observed as compared to the corresponding controls, suggesting that ASA did not display the antibacterial activity towards the investigated bacteria. In bottles with BC or BC and ASA no viable bacteria were detected after only 1 min of contact. This suggests a strong antibacterial activity of BC, which is consistent with the reported antimicrobial activity against different bacteria [25,26]. Although significantly different, the difference in final pH among control and experimental bottles was not higher than 0.19 pH units. Therefore, changes in pH values were not the reason for the reduction in the bacterial numbers.

The results of antibacterial activity of NZ-BC_{500} in the dose range of 0.01–1.0 g per 100 cm^3 of solution are shown in Table 3. At the concentration of 0.01 g of NZ-BC_{500} per 100 cm^3 a slight inhibitory effect was observed against *E. coli*, and a more pronounced one against *S. aureus*. Tests with 0.05 and 0.1 g of NZ-BC_{500} per 100 cm^3 resulted in a significant decrease of the bacterial numbers as compared to the corresponding control systems, where after 24 h of contact the population of *E. coli* recovered and *S. aureus* did not. Tests with 0.5 and 1.0 g of NZ-BC_{500} per 100 cm^3 caused a complete decay of *E. coli* and *S. aureus* after 1 h of exposure. *S. aureus* was more susceptible to the antibacterial activity of NZ-BC_{500} than *E. coli*. The difference in the final pH among control and experimental bottles was not higher than 0.41 pH units.

The results of antibacterial activity of NZ-BC_{500}-SA in the dose range of 0.01–1.0 g per 100 cm^3 are shown in Table 4. Antibacterial activity of NZ-BC_{500}-SA against *E. coli* and *S. aureus* had trends very similar to those of NZ-BC_{500}. The difference in final pH among control and experimental bottles was not higher than 0.38 pH units. According to the results of the experiments with solution of BC, ASA or BC and ASA (Table 2), NZ-BC_{500}-SA exhibits antibacterial activity which could be ascribed only to BC bonded to the NZ. Moreover, it has been reported [27,28] that salicylate ions decrease production of extracellular substances in some Gram-negative and Gram-positive bacteria and thus diminish the possibility of infection.

SEM photos show that the immobilized cells of *E. coli* and *S. aureus* are found on the surfaces of NZ-BC_{500} and NZ-BC_{500}-SA. At the surface of NZ-BC_{500}-SA used in the concentration of 0.05 and 0.1 g per 100 cm^3 the cells of *E. coli* that are strongly adsorbed and that adhere to one another by extracellular substances were found (Fig. 4a). At the NZ-BC_{500}-SA used in concentration of 0.01 g per 100 cm^3 no extracellular substances were seen (Fig. 4b). It seems that the production of extracellular substances by *E. coli* is responsible for its survival in the presence of BC toxicant. The *S. aureus* was not able to produce the extracellular substances.

Although the ASA itself does not display antibacterial activity, salicylate ion can reduce production of the bacterial virulence factors and thus can act beneficially in the antimicrobial therapy [29]. On the other hand, salicylate ion can increase the mutation frequency in bacteria which are responsible for multiple antibiotic resistances [29]. The effects of the salicylate ion bound to the BC-modified clinoptilolite will be investigated in detail.

4. Conclusions

The cationic surfactant benzalkonium chloride was adsorbed onto the natural clinoptilolite with the aim to use the clinoptilolite-surfactant product as a carrier for salicylate ion. Adsorption of the salicylate ion was performed from an aqueous solution of aspirin. The salicylate ion delivery from the clinoptilolite composite into an aqueous solution occurs in two stages. About 50% of the adsorbed salicylate is delivered in the first 15 min whereas the remaining 30% is released slowly within the next 5 h. The release kinetics is best described by Korsmeyer–Peppas model. The composite (NZ-BC_{500}-SA) displayed antibacterial activity against *E. coli* and *S. aureus* in the dose of 0.5 g /100 cm^3 within 1 h of contact only due to the presence of BC. The presence of the co-adsorbed SA does not influence the bacterial activity.

All obtained results indicate that the salicylate-containing clinoptilolite composite may represent an alternative drug with simultaneous antibacterial and anti-inflammatory effects. However, its effectiveness and safety need be validated in clinical trials.

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