JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 10, No. 2, February 2008, p. 382 - 388

Particle size analysis of a silver–based composites for biomedical applications

N. TUDORACHI*, A. CHIRIAC
"Petru Poni"Institute of Macromolecular Chemistry, Grigore Ghica Voda Alley, 41A, Jassy-700487, Romania.

The polymer structures obtained by inclusion ionic or metallic silver (Ag^+, Ag^0) in the composition were studied due to their potential applications in medical or pharmaceutical field, to achieve biocompatible implants with antimicrobial properties, drug delivery systems, filter for drinking water. This paper presents a procedure to acquire an ionized silver-based antimicrobial polymer composition. Carboxymethyl starch synthesized by starch modification with monochloroacetic acid as matrix and silver nitrate and sodium citrate as reducing agent, were used. Due to the presence of silver ions, the composition presents bacteriostatic activity and in association with specific drugs some pharmaceutical compositions can be obtained, useful in the prevention and treatment of infections or diseases. Procaine delivery in buffer solutions pH=7.4 and pH=1.2 at 37 ^{0}C, was studied. The composition was characterized by FT-IR and UV spectroscopy, optical microscopy, particle size analysis by laser diffraction method and zeta potential measurements.

(Received September 25, 2007; accepted February 7, 2008)

Keywords: Carboxymethyl starch, Procaine, Silver ions, Phosphate buffer solution

1. Introduction

The interaction of nanoparticles with biomolecules and microorganisms is an expanding field of research. Within this field, an area that has been largely unexplored is the interaction of noble-metal nanoparticles with viruses. Among noble-metal nanomaterials, silver nanoparticles have received considerable attention due to their attractive physicochemical properties. Silver-based antibacterial materials attract much attention because of their long-term biocidal activity, low volatility and nontoxicity of active silver ion to mammalian cells [1], as well as its perfect antibacterial activity [2-6]. The surface plasmon resonance and large effective scattering cross section of individual silver nanoparticles make them ideal candidates for molecular labeling [7]. Many of these materials have been prepared through either doping silver onto the host materials [8] or compositing it with other compounds [9]. Other efforts were directed to aqueous suspension of silver-based materials. An uniform and stable emulsion is believed to be advantageous in many fields, for instance, directly coating of biomaterials, devices or medical textiles in the biologic or therapeutical applications, spraying or dispersing into the circumstance for hygienic purpose. Most of presented materials have to use additional surfactant, reductant, emulsifier or other agents which usually cause side-effects or can be toxic. Meanwhile, chitosan, is widely used as a natural polysaccharide deriving from chitin because of its excellent properties, such as nontoxicity, biodegradability, biocompatibility and antibacterial character [10-12]. Another important property of chitosan is that the amino groups and hydroxyl groups can form chelate complex with silver ion [13]. This property offers a possibility to use chitosan as both reductant and surfactant in the preparation of nano scale silver-based materials. The stable silver oxide suspension in chitosan solution was prepared from a mixture of silver nitrate and chitosan in dilute acetic acid as precursor [14]. Cotton fabrics treated by this emulsion presented remarkable antibacterial activity against *S. aureus* and *E. coli* at pH 5 and 7 with sightless colour effect and good washing fastness. The pharmaceutical compositions which are photostable and antimicrobially active comprise one or more medicinal agents and a stabilized ionized silver-based antimicrobial composition. The stabilized ionized silver-based antimicrobial composition comprises a stabilizing acyclic polyether polymer, cations, and anions present in excess with regard to the amount of cations [15]. These pharmaceutical compositions are useful in the prevention and treatment of infections and diseases.

In developing topical antimicrobial pharmaceutical compositions consisting of two different antimicrobial agents, ionized silver is the preferred agent. Silver, in its ionic state, is inherently safe and possesses a very broad spectrum of antimicrobial efficacy. Specially, ionized silver has broad antibacterial, antifungal and antiviral properties. The broad spectrum of antimicrobial activity of ionized silver is caused by the reactivity of silver ions with a variety of functional groups. Silver ions, similar to most heavy metals in their ionized state, can complex with electron-donating functional groups containing sulfur, oxygen or nitrogen. In biological systems these electron donor groups are present as functional groups such as thiols, carboxylates, phosphates, hydroxyl, amines, imidazoles and indoles, either singly or in many varied combinations. These electron donor groups are found in

great numbers in a variety of biomolecules which make up microbes. Binding of ionized silver to any of these electron donor groups causes disruption or inactivation of the biological system, resulting in microbe's death. Depending on the source of silver ions, studies indicate that silver ions kill the microbe either by attacking the cell wall and membrane producing blebs or by producing aggregation of nuclear material into filaments [16].

Carboxymethyl starch due to its excellent physico-chemical properties (water solubility, biodegradability, biocompatibility) and presence of carboxyl and hydroxyl groups in its structure can be used to obtain some chelate complexes with metal ions.

In this study, we modified starch to obtain carboxymethyl starch (CMS) by the method [17]. The stable silver ion suspension in aqueous solution of CMS was prepared from a mixture of silver nitrate and sodium citrate as reducing agent. The carboxymethyl starch suspension doped with silver ions was used for physical complexation with procaine to obtain an antibacterian composition with possible pharmaceutical applications.

The delivery of procaine *in vitro* (pH 7.4 and 1.2) at 37 ^{o}C, was studied.

2. Experimental

2.1 Materials

The pharmaceutical compositions were carried out with carboxymethyl starch synthesized in the laboratory by modification of starch with monochloroacetic acid. The raw materials used in our experiments were available as commercial products and were analytical grade reagents.

Corn starch (Amidex SA-Tg. Secuiesc, Romania), humidity 2.0 %, acidity 2.0 (determined using 0.01N NaOH solution), proteins and lipids 1.0 %, ash 0.25 %. Monochloroacetic acid, isopropanol, methanol, acetic acid, silver nitrate, sodium citrate, and sodium hydroxide (Fluka, Switzerland reactives) were used without further purifications.

The acid solution (pH=1.2) and phosphate buffer solution (pH=7.4) were obtained in the laboratory and were used for dialysis of the conjugated systems.

2.2. Methods of investigations

Fourier Transform Infrared Spectrometer (FT-IR)-spectrometer Bruker Vertex 70 on KBr pellets (5 mg sample/500 mg KBr).

Spectrofotometric analysis UV-VIS were recorded on spectrofotometer 6305-UV/VIS model JENWAY (England).

Measurement of particle dimensions was done with a Mastersizer 2000 system (version 5.31) Malvern Instruments (England). The system is constituted of an optical bank which uses laser light He-Ne 632 nm/2 mW, a dispersion unity of the sample Hydro 2000A type equipped with stirrer, recirculating pump, ultrasonics and software to record and process results on the computer. The measurement domain is between 0.020-2000 μm.

Zeta potential measurement was done with a Zetasizer Nano-ZS, ZEN-3500 model, Malvern Instruments (England). The device uses laser green light of 532 nm/50mW and can be used to measure particle dimensions between 0.6 nm-6μm and zeta potential. The dimension measurement is based on the dynamic diffusion of light emitted by laser in the presence of the particle and an optical detection system records light diffusion under angle <173 ^{o}.

Optical m*icroscopy* was used to study the morphologic aspects of the film surface. An optical microscope with light transmission Micros MCD-500 model (Austria), with video chamber incorporated and software PC, was used.

2.3. Synthesis of the compositions

Synthesis of antimicrobian polymer matrix

Starch (30 g) was dispersed by strong stirring in 400 ml isopropanol at laboratory temperature for 1h. Stirring was continued and 80 ml 30 % NaOH solution at 20 ^{0}C were gradually added. After 1h stirring, 36 g monochloroacetic acid was added. The reaction took place in a reaction vessel of 1 litre capacity covered with aluminium foil, at 55-60 ^{0}C temperature, 4 hours. The reaction product (solid) was separated from the liquid phase by decantation, mixed with methanol 70 % and the alkali excess was neutralized with acetic acid 90 % to neuter pH. The liquid phase was again decanted, and the solid product was washed with methanol and was vacuum dried at 60 ^{o}C.

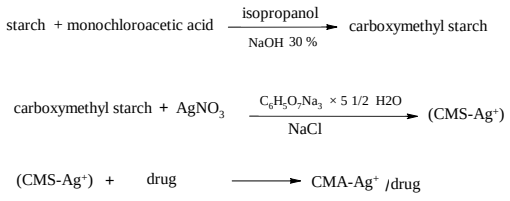
To obtain the polymer matrix with antibacterian character given by presence of Ag^{+} ions, CMS polymer (4 g) was dissolved under agitation in 150 ml bidistilled water at 65-70 ^{o}C. Then, 4 ml aqueous solution with $4{,}257 \cdot 10^{-4}$ sodium citrate moles ($C_6H_5O_7Na_3 \cdot 5\ ½\ H_2O$) as reducing agent, was added. After 30 minutes at the boiling temperature of the solution, 4 ml aqueous solution that contained $2{,}141 \cdot 10^{-4}$ $AgNO_3$ moles was gradually added and maintained under agitation 2h. Before the introduction of $AgNO_3$ solution, the reaction mixture was colourless, then it became white-spliced, and finally violet-brown indicating the formation and presence of Ag^{+} ions in the reaction mass. Carboxymethyl starch complexed with silver ions (CMS-Ag^{+}) was used to carry out the matrix/drug systems. The content of dry substance in solution was 2.33 %.

Synthesis of CMS-Ag^+/procaine complex (DDS-1)

Procaine (0.25 g) was dissolved in 2 ml aqueous solution pH=1.2, then it was gradually added in 7.82 g aqueous solution of CMS 3.84 % concentration and was maintained under intense stirring 30 minutes at laboratory temperature. In the obtained mixture 12.8 g solution CMS-Ag^+ were added under stirring and after 2 hours the solution rested over night. The mixture was used for film casting in glass molds (100×20×2 mm), and films were left at 20 ^oC in the air, 72 hours for solvent removal.

In similar conditions a second system *CMS-Ag^+/procaine (DDS-2)* was achieved, the only difference was that procaine was dissolved in phosphate buffer solution, pH 7.4. In both systems procaine concentration was 29.40 %.

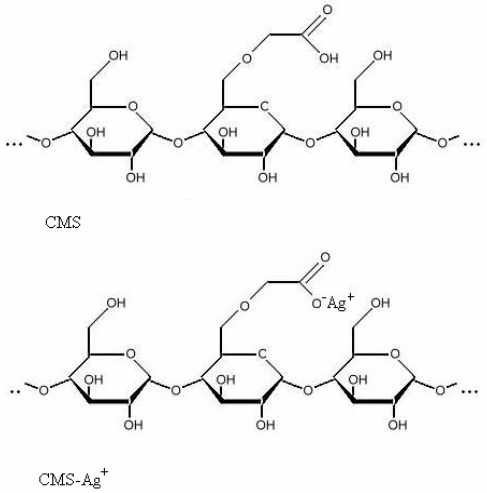
In vitro delivery of procaine – The matrix/drug systems were dialyzed in a cellophane membrane 40 μm thickness, at 37 ^0C in phosphate buffer solution pH=7.4 and acid buffer solution pH=1.2. The matrix/drug systems were introduced in 20 ml dialysis solution which was each time replaced after recording the UV absorbance at prestablished time intervals. In the case of DDS-1 system the dialysis was done in pH=1.2 solution and the absorbance was recorded at λ=272 nm wavelength, while in the case of the DDS-2 system in pH=7.4 buffer solution and absorbance was recorded at λ=288 nm. In similar conditions was done the dialysis of the polymer matrix without drug, and the UV absorbances were recorded at the same wavelengths and substracted from the respective absorbance systems to determine the quantity of procaine delivered.



Scheme 1. *The simplified scheme of matrix/drug complex synthesis.*

3. Results and discussion

The IR spectra of starch and carboxymethyl starch are presented in figure 1. In IR spectrum of starch modified with monochloroacetic acid is noticed the appearance of some vibration absorption bands νC=O at 1710 cm^{-1} characteristic to COOH groups. Also, at 1610 cm^{-1} appear high vibrations intensity of the absorption bands νC=O characteristic to COO^- groups. The absorption bands at 980-1190 cm^{-1} in starch are specific to C–O linkages present in glicosidic ring C–O–C and in C–OH groups. These bands are representative for starch. The bands are discovered also in starch modified with monochloroacetic acid but on a narrower interval 1020-1160 cm^{-1} emphasize the presence of the glicosidic structural unity in the structure of the synthesized carboxymethyl starch.



Scheme 2. The structure of CMS and CMS-Ag^+.

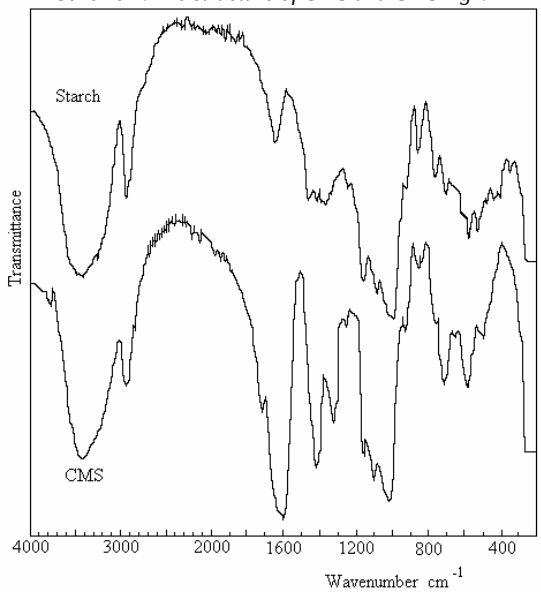


Fig. 1. IR spectra: starch and carboxymethyl starch.

In Fig. 2 is presented the IR spectrum of the CMS-Ag^+ and CMS-Ag^+/procaine system and we can notice the appearance of some additional vibration bands characteristic to functional groups of procaine. At 1020-1230 cm^{-1} appear vibration bands νC–N specific to alchil–N–$(alchil)_2$ group and at 3316-3354 cm^{-1} vibration νNH_2 characteristic to aril–NH_2 linkages. Also, at 1606 cm^{-1}, 1696 cm^{-1} and between 700-900 cm^{-1}, there are bands characteristic to the aromatic ring o,p-substituted and in the 1050-1300 cm^{-1} interval there are two peaks characteristic to the esther linkages –COOR.

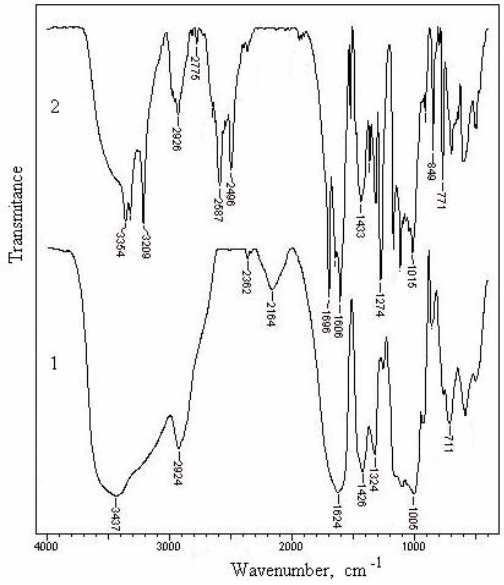
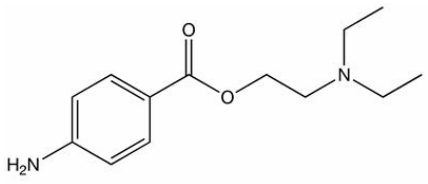


Fig. 2. IR spectra: 1- CMS-Ag$^+$; 2- CMS-Ag$^+$/procaine.



2-diethilaminoethil 4-aminobenzoate

Scheme 3. Structure of procaine.

The analysis of the dimensional distribution of polymer matrix (CMS) particles and CMS-Ag$^+$complex when the $AgNO_3$ reduction was done with NaCl and sodium citrate, and of the matrix/drug systems emphasizes that 90 % of the volume of the existent particles have diameter smaller than 470 μm. The drug release from the matrix/drug system is influenced by the dimension of the particles and specific surface area. The systems with smaller diameter of the particles release drug in a relative short time and with a higher speed. In figures 3, 4 we present the particle size distribution of matrix and CMS-Ag$^+$/procaine systems.

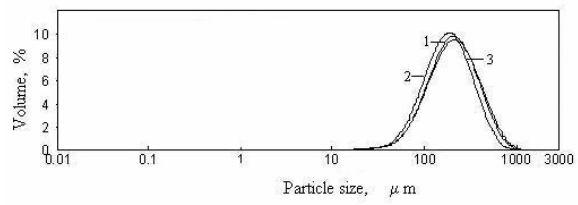


Fig. 3 Particle size distribution: 1- CMS; 2- CMS-Ag$^+$/NaCl; 3-CMS-Ag$^+$/sodium citrate.

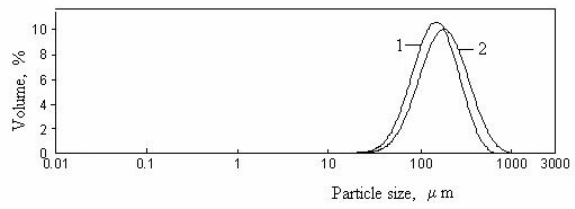


Fig. 4. Particle size distribution: 1-DDS-1, pH=1,2; 2-DDS-2, pH=7,4.

The results of measurements carried out concerning size and dimensional distribution of the particles are presented in table 1. Generally, we can notice that the diameter of the particles of matrix/drug systems are smaller than of the polymer matrix. The width of the dimensional distribution (span) has values between 1.615-1.737 in the case of polymer matrix and 1.536-1.668 in the case of the matrix/drug systems. The absolute deviation from the median value of dimension particles is almost 0.5, that means dimensional polydispersity is reduced.

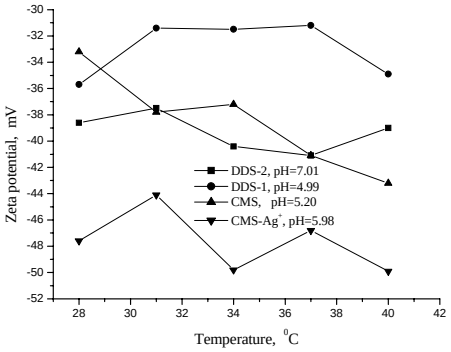


Fig. 5 Zeta potential versus temperature.

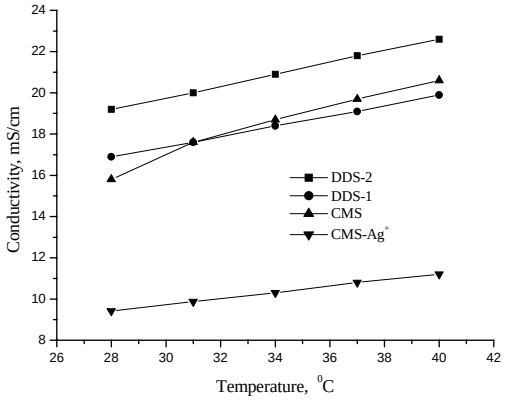


Fig. 6. Conductivity versus temperature.

In Figs. 5, 6 we present the variation of zeta potential and conductivity of the dispersions, as a function of

temperature. Zeta potential refers to the electrostatic potential generated by the accumulation of ions at the surface of a (colloidal) particle that is organized into an electrical double-layer, consisting of the Stern layer and the diffuse layer. Zeta potential of the CMS-Ag^+/procaine systems measured on the temperature domain 28-40 ^{0}C varies between -31,2 and -41,1 mV that shows that the studied dispersions are stable from colloidal point of wiev. The value of zeta potential for DDS-1 system was measured at pH=4.99, and for DDS-2 at pH=7.01. Zeta potential of CMS matrix as a function of temperature varies between –33.2 and –43.2 mV, close to of CMS/procaine systems. For the CMS-Ag^+ complex, the zeta potential has a higher absolute value (47.6 ÷ 49.9). Generally, it is known that zeta potential of the stable colloidal solutions must be situated out the domain ± 30 mV. In conclusion, matrix/drug systems achieved present a good colloidal time stability and don't deposit in time. The conductivity presents a linear growth with temperature in the case of both systems matrice/medicament, and in the case of polymer matrix.

Table 1. The results of the measurements concerning the particle size distribution.

Sample	Specific surface area (m^2/g)	d(0.1) μm	d(0.5) μm	d(0.9) μm	Surface weighted mean [D3.2] μm	Volume weighted mean D[4.3] μm	Uniformity	Residual (%)	Span
CMS	0.0346	95.650	212.35	450.53	173.505	247.921	0.521	0.330	1.671
CMS-Ag^+/ sodium citrate	0.0340	95.910	217.712	474.104	176.579	257.127	0.540	0.316	1.737
CMS-Ag^+ / NaCl	0.0386	36.838	188.29	390.92	155.602	217.948	0.502	0.305	1.615
DDS-1, pH=1.2	0.0483	70.503	148.276	298.296	124.283	169.518	0.480	0.313	1.536
DDS-2, pH=7.4	0.0396	84.042	183.211	389.648	151.427	215.381	0.524	0.294	1.668

d*(0.1); d(0.5); d(0.9)* - 10 %, 50 %, and 90 % of the sample volume are particles with a smaller diameter than specified in the table.

[*D3.2]* - medium diameter for the equivalent sphere of the same surface with that of the particles, called also *media Sauter*.

D[4.3] - medium diameter for the volume.

Uniformity - is a measure of the absolute deviation from the median value.

Residual - is a global indication of how well are fitted the information resulted from the measurements with those calculated theoretically. A good fitting must give a residue less than 1 %, if the value is higher it could be an indication that the values of the refraction index and absorbance coefficient for sample/dispersant are not correct.

Span - represents the width of the distribution. The distribution is narrower span value is smaller. Span value is calculated with the formula: [d(0.9)-d(0.1)]/d(0.5).

The morphology of the film surface was studied by light transmission optical microscopy (Fig. 7). The film obtained from CMS presents a smoth surface, homogeneous and uniform (Fig. 7a), while the film obtained from CMS-Ag^+ presents a heterogeneous aspect in which we can notice the presence and dispersion of associated silver ions microparticles (Fig. 7b). In the case of the matrix/drug systems (Fig. 7c-d) the surface is also heterogeneous due to the presence of the three species: polymer-drug-ionic silver associated. We can notice the presence of microparticles of drug obtained by precipitation, and ionic associated silver surrounded by the polymer that forms the continuous phase.

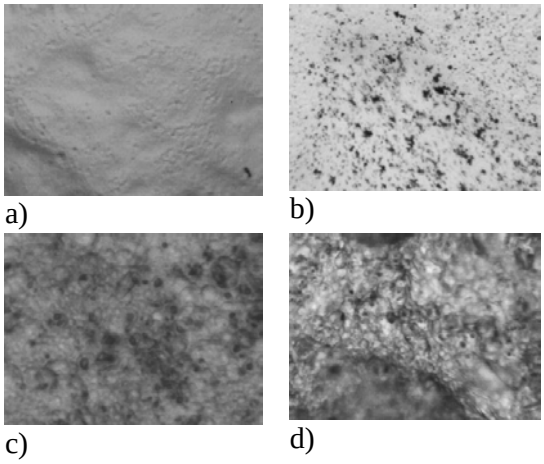


Fig. 7 Optical microscopy: a –CMS (magnitude 4×); b-CMS-Ag^+ (magnitude 10×); c- DDS-1, pH=1.2 (magnitude 40×); d– DDS-2, pH=7.4 (magnitude 20×).

The concentration of delivered procaine was determined from the UV spectra absorptions recorded after dialysis of the matrix/drug systems and from the calibrating curves of procaine (Fig. 8, 9).

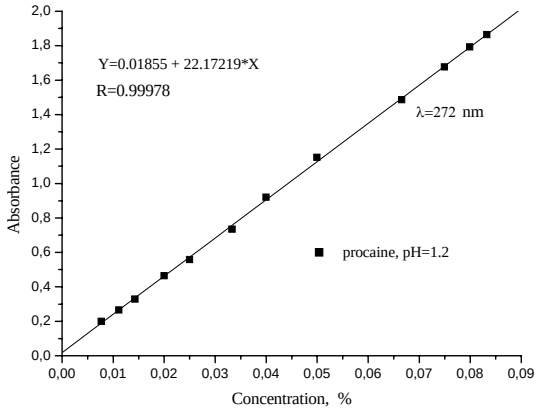


Fig. 8 Procaine calibrating curve, pH=1.2.

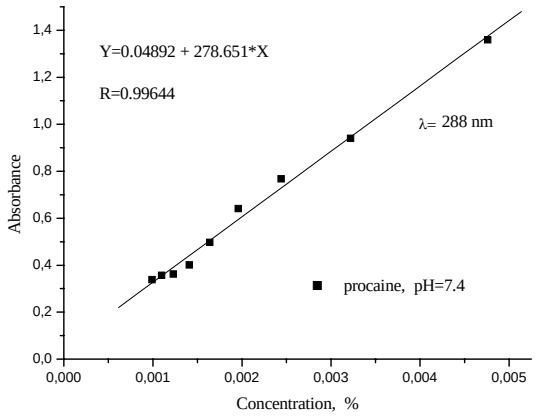


Fig. 9. Procaine calibrating curve, pH=7.4.

In Fig. 10 we present the delivery of procaine reported to the polymer. We can notice that procaine delivery in the first hours of dialysis takes place with the same speed unaffected by pH of the solutions, but in solution with pH 1.2, total delivery takes place in a shorter period of time. Procaine delivery reaches 97 % in pH=1.2 buffer solution (DDS-1) only after 24 hours of dialysis, and 94.2 % in pH=7.4 solution (DDS-2) after 88 hours (Fig. 11).

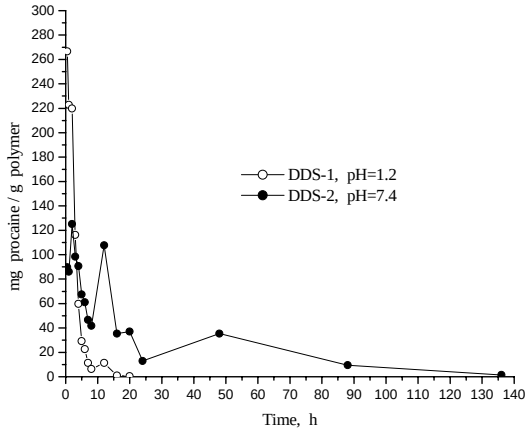


Fig. 10 Procaine delivery as a function of time at 37 ^{o}C.

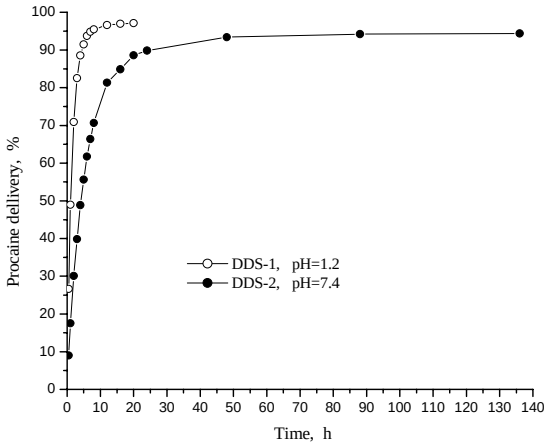


Fig. 11. Cumulated procaine delivery as a function of time at 37 ^{o}C.

4. Conclusions

The composition obtained by treating CMS with $AgNO_3$ and sodium citrate as reducing agent exhibits an antimicrobian effect on account of the presence of Ag^+ ions.

Procaine delivery from the matrix/drug systems in phosphate buffer solution pH=7.4 and acid solution pH=1.2, at 37 ^{0}C temperature was studied. The percentage and speed delivery depend on the pH of the solution, particle size and structure of the matrix. Procaine reaches a delivery degree of 97 % in buffer solution with pH=1.2 (DDS-1) only after 24 hours of dialysis, and 94.2 % in solution pH=7.4 (DDS-2) after 88 hours of dialysis.

The CMS-Ag^+ matrix was used to obtain some pharmaceutical compositions in association with drugs (procaine) that can be used in prevention and treatment of some infections or diseases

Zeta potential measurements can be a useful tool for characterizing colloidal drug delivery systems, especially when used in conjunction with other techniques, such as particle size determination procedure. They can give information about the surface properties of the carrier and therefore help to determine how the constituent molecules are organized. When a drug molecule has been incorporated, changes in zeta potential can give indications regarding its mode of association, and its release in different media.

References

[1] T. J. Berger, J. A. Spadaro, S. E. Chanpin, Antimicrob Agents Chemother **9**, 357, (1976).
[2] Q. L. Feng, J. Wu, G. Q. Chen, et al., J. Biomed. Mater. Res. **52**, 662, (2000).
[3] C. N. Kraft, M. Hansis, S. Arens, et al., J. Biomed. Mater. Res. **49**, 192, (2000).
[4] S. Y. Liau, D. C. Read, W. J. Pugh, J. R. Furr, A. D. Russell, Lett Appl Microbiol. **25**, 279, (1997).
[5] K. Nomiya, A. Yoshizawa, K. Tsukagoshi, N. C. Kasuga, S. Hirakawa, J. Watanabe, J Inorg. Biochem. **98**, 46, (2004).
[6] I. Sondi, B. J. Salopek-Sondi, Colloid. Interface. Sci. **275**, 177, (2004).
[7] S. Schultz, D. R. Smith, J. J. Mock, D. A. Schultz, Proceedings of the National Academy of Sciences of the United States of America, **97**, 996, (2000).
[8] E. Verné, S. Di Nunzio, M. Bosetti, P. Appenclino, C. Vitale-Brovarone, G. Maina, N. Cannas, Biomaterials **26**, 5111, (2005).
[9] C. J. Grunlan, K. J. Choi, L. Aklbert, biomacromolecules **6**, 1149, (2005).
[10] P. Giunchedi, et al., Biomaterials, **19**, 157, (1998).
[11] Z. Li, X. P. Zhuang, X. F. Liu, Y. L. Guan, K. D. Yao, Polymer, **43**, 1541, (2002).
[12] S. G. Hu, C. H. Jou, M. C. Yang, Biomaterials **24**, 2685, (2003).
[13] H. Z. Huang, X. R. Yang, Carbohyd. Res. **339**, 2627, (2004).
[14] H. Zhigang, Z. Jin, C. L. Wing, S. S. Yau, Mater. Res. Soc. Symp. Proc. **920**, (2006).
[15] C. C. Capelli, U S Patent 5744151
[16] N. Grier, in Disinfection, Sterilization, and Preservation, (3rd edition), S. S. Block ed., Lea & Febiger, Philadelphia, Ch. 20, p. 395, (1983).
[17] T. Heinze, T. Liebert, U. Heinzeu, K. Schwikalk, Cellulose **11** (2), 239, (2004).

*Corresponding author: ntudor@icmpp.ro