Antibacterial Polyvinyl Chloride/Antibiotic Films: The Effect of Solvent on Morphology, Antibacterial Activity, and Release Kinetics

Martha Merchan,[1] Jana Sedlarikova,[2] Vladimir Sedlarik,[1,2] Michal Machovsky,[1] Jitka Svobodova,[1] Petr Saha[1]

[1]*Faculty of technology, Polymer Centre, Tomas Bata University in Zlin, Nam. T. G. Masaryka 275, Zlin 762 72, Czech Republic*
[2]*Innovation Centre, University Institute, Tomas Bata University in Zlin, Nad Ovcirnou 3685, Zlin 760 01, Czech Republic*

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ABSTRACT: Medical-grade polyvinyl chloride was modified with sodium ampicillin in a concentration range from 0 to 5 wt % by solvent casting technique using cyclohexanone and *N,N*-dimethylformamide. The obtained polymeric systems were characterized by optical microscopy, tensile test, and scanning electron microscopy. In addition, *in vitro* antibacterial activity against Gram-negative and Gram-positive bacteria was determined by an agar diffusion test. Antibiotic release experiments were performed in distilled water and physiological saline solution, which were monitored by UV-vis spectroscopy. The results showed a crucial role of the solvent on the morphology, antibacterial activity, and releasing characteristic of the ampicillin. Furthermore, a mathematical model was applied to data obtained from release study, to characterize the release kinetics of the ampicillin from the polyvinyl chloride-antibiotic systems.
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Key words: antibacterial properties; antibiotic; polyvinyl chloride; solvent cast; release kinetics

INTRODUCTION

Nowadays, polymeric medical devices occupy a decisive place in hospital settings. Polyvinyl chloride (PVC) has an outstanding position within the group of materials used for biomedical applications due to its high versatility, mechanical and chemical resistance, inertness against biological fluids, and wide range of processing possibilities.[1,2] There are numerous PVC medical articles, including packages, blood bags, syringes, pump sacks, hoses, intravascular, and urinary catheters.[1]

However, the application of such devices is often related to the risk of infections caused by bacteria attacking the material surfaces. In fact, medical devices specially indwelling catheters are often colonized by microorganisms forming so called biofilm, which consists of surface-adhering microorganisms encased in a hydrated matrix of polysaccharides and proteins. Consequently, morbidity, mortality rate, and health care costs increase significantly.[3,4]

Prevention of colonization of polymer devices by the modification of their surfaces and/or structures has become the subject of many research articles in the past years. Several methods of surface modification, such as plasma treatment, corona discharge, chemical grafting, or direct chemical modification by oxidation, hydrolysis, etc. have been reported.[1,5–9] Also, coating and immersion of the device surface in antimicrobial agent solutions, provide an alternate approach to minimize bacterial adherence.[10–15] Because of these methods, microbial colonization and biofilm formation are inhibited by the elution of adsorbed antimicrobial from the device. However, this approach suffers from a number of limiting conditions, for example a rapid release of the adsorbed antibiotic resulting in a relatively short persistence of antimicrobial action.[4,16] Longer-term protection can be achieved by the introduction of a bonding cationic surfactant, tridodecylmethyl ammonium chloride. Polymers precoated with this surfactant are then coated with an anionic antibiotic or antiseptic agent that slowly releases from the material due to the ion-exchange process.[17,18] The ability of polymers to absorb higher amount of antibiotics was increased by introducing specific functional groups, which can interact with drugs, to the side chain of polymers. In

Correspondence to: V. Sedlarik (sedlarik@ft.utb.cz).
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particular, β-lactam antibiotics were bound to the device surface using surfactant agents.[19,20]

The promising solution in the development of a polymer-antibiotic system with long-term antibacterial activity is the incorporation of antimicrobial drugs within the polymer matrix.[15,16,21,22] Polymer matrices with antimicrobial agents can be used directly to construct a device or to be applied as a thin layer of coating on a medical device surface. This can be carried out by using a liquid coating composed of specific polymers, antimicrobial agents, and solvents. After the liquid coating is applied to a medical device surface, solvents are allowed to evaporate, leaving a thin film consisting of the polymers with uniformly dispersed antimicrobial agent. The thin polymer film acts as a reservoir for a sustained release of antimicrobial substances over an appropriate period at an effective concentration. Antimicrobial agents in the matrices are not chemically bonded to the polymer matrix and retain their activity. Selecting suitable antimicrobial agents and polymer matrices influence on the desired physico-chemical properties of polymeric device.[23]

It has been reported that the solvent cast technique is an approach to prepare long-lasting systems with antibacterial activity.[24] According to this method; the films can be prepared with extremely high quality requirements. The advantages of this technology include uniform thickness distribution and extremely low haze.[25] Several biocompatible polymer coatings that actively release antibiotics prepared by the solvent casting technique were studied.[24,26–29] The effectiveness of such coatings is strongly dependent on the antibiotic release profile from the polymer, due to the chemical similarity between the drug and the polymer matrix.[26]

Sodium ampicillin belongs to the group of β-lactam antibiotics and is used to treat urinary infections, salmonellosis, *Listeria* meningitis, periodontitis, etc.[30] It was selected due to its antibacterial property to penetrate both gram-positive and some gram-negative bacteria and its previous use in drug release polymer systems.[31–34]

In this work, antibacterial medical-grade PVC films containing sodium ampicillin (Fig. 1) were prepared, using the solvent casting technique by using various solvents (cyclohexanone and *N,N*-dimethylformamide). The main attention was paid to the investigation of solvent effect on the morphology, mechanical properties, antibacterial activity, and release kinetics of the incorporated antibiotic into resulted polymeric films.

EXPERIMENTAL PART

Materials

Medical-grade thermoplastic plasticized polyvinyl chloride RB1 (PVC) was supplied by Modenplast, Italy. Cyclohexanone (CYH), *N,N*-dimethylformamide (DMF), and sodium chloride (analytical grade) were purchased from IPL, Czech Republic. Sodium Ampicillin (7-(2-amino-2-phenyl-acetyl)amino-3,3-dimethyl-6-oxo-2-thia-5-azabicyclo[3.2.0]heptane-4 carboxylic acid, sodium salt) (AMP) was supplied by Pharmos, A.S., Czech Republic under the trade name Ampicillin 1,0 Biotika. (Register No. 15/548/92-s/c). Bacterial strains *Escherichia coli* 3954, *Staphylococcus aureus* 3953, *Klebsiella pneumonie* 4415, and *Pseudomonas aeruginosa* 3955 were obtained from the Czech Collection of Microorganisms, Czech Republic.

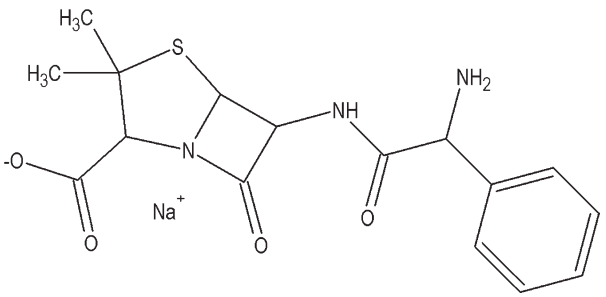

Figure 1 Structure of sodium ampicillin.

Methods

Sample preparation

PVC/solvent/AMP films were prepared by a solvent casting technique. PVC granules were dissolved in DMF (24 h) and/or CYH (6 h) with a weight ratio of 1 : 10 at room temperature under continuous stirring. Once, the PVC solution was prepared, varied quantities of AMP (0 to 5 wt %, related to PVC) were added and further stirred until dissolution (6 h with DMF, 4 h with CYH). The samples were then poured into glass dishes and the solvent was allowed to evaporate (at 35°C with DMF followed for further 24 h and at room temperature from CYH during one week). The PVC control sample was prepared through the previous procedure without the AMP incorporation. The conditions of the films preparation were chosen on the basis of practical laboratory experience to achieve the smoothest fine films of comparable quality. The thickness of resultant films was about 500 μm.

Optical microscopy

The mixing nature of the sample's cross sections were investigated by using OLYMPUS CX 31, optical microscope equipped with OLYMPUS DP 750 camera set with the software OLYMPUS PHOTO QUICK

CAMERA 2.0. The specimens were cut by LEICA RM2255 rotary microtome with a thickness of 40 μm.

Tensile measurements

The effects of various AMP concentrations added into PVC matrix on the mechanical properties were studied by using tensile test. The specimens (initial length 20 mm, width 15 mm, thickness about 500 μm) were tested on tensile testing machine T2000 (Alpha Technologies) at 25°C according to the standard ČSN EN 527 1–3. The speed of the moving clamp was 100 mm min^{-1}. Tensile modulus, E, was determined by the slope of the initial part of the stress vs. strain curve (5% strain). Tensile stress and tensile strain were other studied parameters. Ten specimens were tested in each case.

In vitro antibacterial activity

The antibacterial properties of the PVC/solvent/AMP films were assessed by using the agar diffusion test.[35–37] Round specimens (8 mm in diameter) were placed on the surface of an individual nutrient agar plate, where bacterial solution (4×10^8 CFU mL^{-1}) of chosen microorganisms (*Escherichia coli* 3954, *Staphylococcus aureus* 3953, *Klebsiella pneumoniae* 4415, and *Pseudomonas aeruginosa* 3955) had been swabbed uniformly. After 24 h incubation at 37°C, the dimensions of the inhibitions zones were measured in four directions, and the average values were used to calculate the circle zone inhibition area.

Antibiotic release studies

To measure the drug release, round shaped PVC/solvent/AMP samples (15 mm in diameter) were washed and dried, till constant weight, then immersed into 10 mL distilled water and/or NaCl 0.9 wt % solution (physiological solution) at 37°C with continuous shaking (100 rpm). After defined periods of time, the samples were transferred into the fresh medium to reach perfect sink conditions. The released AMP in the elutions was detected by means of UV-vis spectrophotometer (Thermo Scientific, Heλios Gamma) at wavelength of 210 nm. Calibration dependences of the absorbance (A) on AMP concentration (C_{AMP} in μg L^{-1}) for release in distilled water ($A = 0.0528C_{AMP} + 0.0436$, $R^2 = 0.9984$) and physiological solution (AMP $= 0.0468C_{AMP} + 0.0592$, $R^2 = 0.9939$) were determined prior to the release investigation. Afterward, the cumulative mass was calculated. The measurements were performed by triplicate.

The observed data of the cumulative mass of the released AMP related to 1 g of the sample material were evaluated by using first-order kinetics [eq. (1)] and regression processed by the least squared method, applying the Solver subprogram of Microsoft Excel 2003.

$$C_{REL} = C_{MAX} \times (1 - e^{-kt}) \quad (1)$$

where C_{REL} (μg/g) is the experimental concentration of antibiotic that was released at time t, C_{MAX} (μg/g), means the maximal theoretical concentration of the AMP released from 1 g of the sample, and $-k$ (h^{-1}) represents the rate constant i.e., time needed to reach C_{MAX}.

Scanning electron microscopy

To visualize the effect of loaded AMP and further release from the PVC matrix; cast films were studied by thermionic-emission scanning electron microscopy TESCAN VEGA/LMU, Czech Republic. The surfaces were prepared by cryogenic fracturing in liquid nitrogen and then coated with a thin layer of Au/Pd. The microscope was operated in high vacuum mode at acceleration voltage 5 kV.

RESULTS AND DISCUSSION

Optical microscopy

Unmodified PVC films cast from CYH (PVC/CYH/AMP 0 wt %) [Fig. 2(a)] are colorless and transparent flat in appearance, in comparison with yellowish PVC/CYH/AMP films [Fig. 2(b)]. As can be seen, the mixing nature of the PVC/CYH/AMP 5 wt % shows a complete AMP uniform distribution within the PVC matrix. On the other hand, PVC/DMF/AMP films [Fig. 3(b)] exhibit all AMP crystalline particles in the top of the film (left side). The pure PVC cast from DMF, (PVC/DMF/AMP 0 wt %) [Fig. 3(a)] looks resemble to its equivalent obtained from CYH.

The reason of such a significant variation of the resulting films morphology can be found in the nature of the used solvents. Both CYH and DMF are strong electron donors, which do not have significant steric interference to approach their electrons to the polymer. However, the bulky cyclic CYH can increase the distance of PVC chains in polymer solution. This effect may cause a reduction of the intermolecular forces, allowing better arrangements of AMP crystals in the matrix. AMP can also act as electron donor toward suitable acceptors;[38] which could achieve a higher interaction with the PVC chains. More hydrophilic DMF creates a large electron donor contribution, but it is deficient in bulk.[39] As it has been also reported, more compact structures as cyclic substances (CYH) have more efficient van der Waals interaction between molecules. However, the solvency power of DMF can be ascribed to

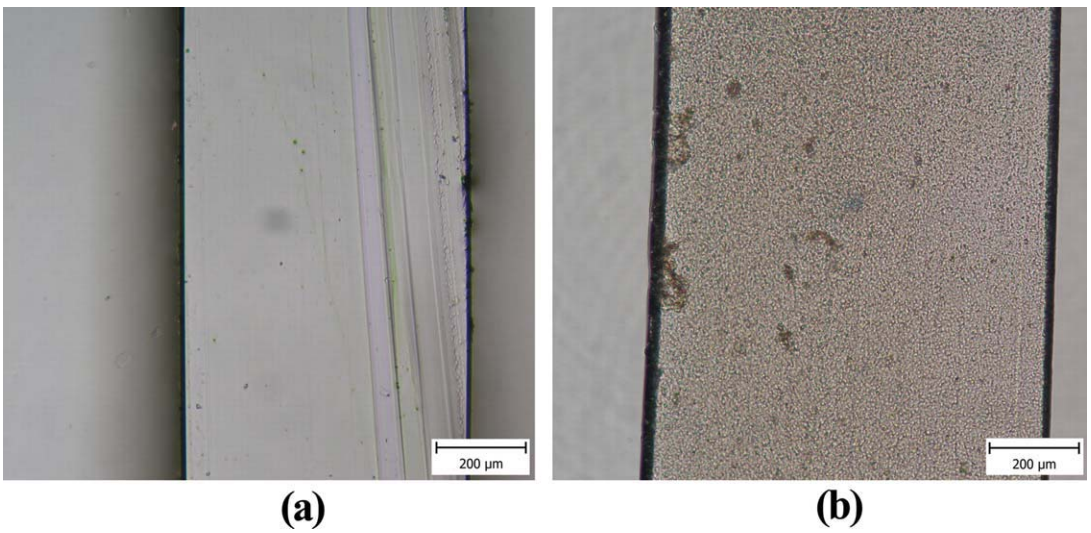

Figure 2 Optical micrographs of PVC/CYH/AMP samples (a) PVC/CYH/AMP 0 wt %, (b) PVC/CYH/AMP 5 wt %. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

its high electric constant, large dipole moment and electron donor capacity ($\mu = 3.24$ deybe and $\varepsilon = 36.71$ at 298.15 K); the presence of two electron repelling –methyl groups make the alone pair of electrons at nitrogen still more perceptible toward donation.[40] Subsequently, other unlike aspects, regarding physical properties as density, solubility parameter, vapor pressure, amongst others, could explain the migration of AMP in the upper part (polymer solution-atmosphere interface) of PVC/DMF/AMP systems. In addition, this explanation could be also supported by the close solubility parameter of PVC $\delta = 9.6–9.7$ (cal cm^{-3})^{½} and CYH $\delta = 9.9$ (cal cm^{-3})^{½} (DMF $\delta = 12.1$ (cal cm^{-3})^{½}) proposed by Small.[40] The solubility parameters for good PVC solvents fall in the range 8.6–13 (cal cm^{-3})^{½}. The solubility parameter for AMP was calculated as 12.19 (cal cm^{-3})^{½}, according to the AMP structure by using the group molar attraction constants proposed by Small.[41] Moreover, Hansen's solubility parameter, based on his statement about concept of interaction radius, catalogs good solvents for PVC giving 0 for nonsolvents and 1 for solvents. Solvent with 1 are: chlorobenzene, CYH, cyclopentanone, DMF, methylethyl ketone, tetrahydrofuran, nitrobenzene amongst others. There is a distance from the radius to DMF and dimethyl sulfoxide; however, these are considered as exceptions that dissolve the PVC.[42]

Tensile properties

The influence of the PVC modification with AMP on mechanical properties of the resulting material is

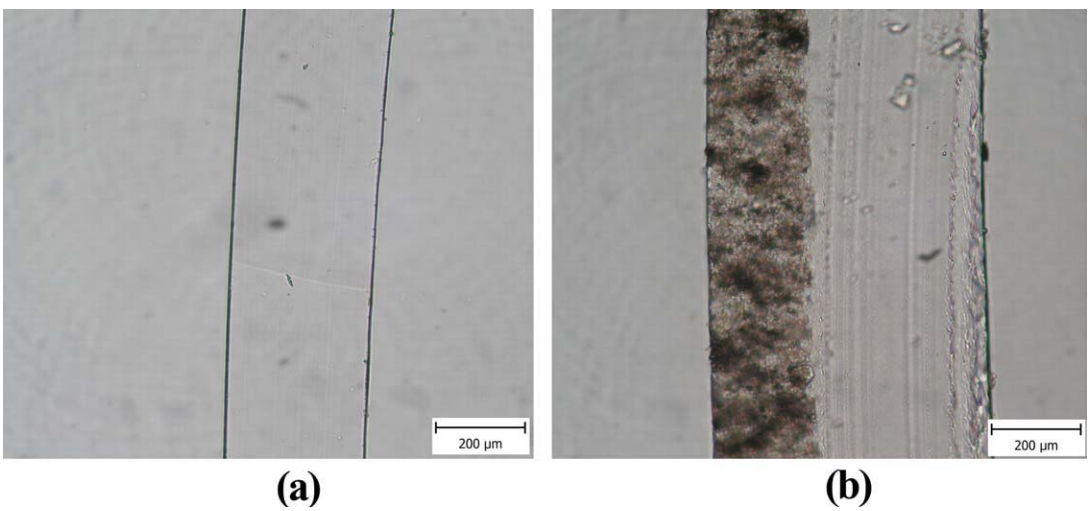

Figure 3 Optical micrographs of PVC/DMF/AMP samples (a) PVC/DMF/AMP 0 wt %, (b) PVC/DMF/AMP 5 wt % (Top – left side). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I
Mechanical Properties of PVC/CYH/AMP Systems

Sample	E modulus (MPa)	Tensile strain (%)	Tensile stress (MPa)
PVC/CYH/AMP 0 wt %	0.63 ± 0.03	299.42 ± 61.80	11.16 ± 2.15
PVC/CYH/AMP 1 wt %	0.67 ± 0.14	315.63 ± 33.43	12.92 ± 2.44
PVC/CYH/AMP 2 wt %	0.62 ± 0.18	335.20 ± 16.30	14.55 ± 1.52
PVC/CYH/AMP 3 wt %	0.54 ± 0.07	335.68 ± 33.01	13.94 ± 0.82
PVC/CYH/AMP 4 wt %	0.67 ± 0.16	333.65 ± 47.51	13.74 ± 0.97
PVC/CYH/AMP 5 wt %	1.01 ± 0.01	315.69 ± 29.08	15.33 ± 0.15

expressed as the dependences of E modulus, break strain, and break stress on AMP concentration in Table I for PVC/CYH/AMP and Table II for PVC/DMF/AMP systems, respectively. The effect of the used solvent on the investigated parameters can be clearly noticed in case of unmodified PVC films. Although PVC/CYH/AMP 0 wt % show E modulus 0.63 MPa, tensile strain 299.42%, and tensile strength 11.16 MPa, PVC/DMF/AMP 0 wt % prove generally higher values of all characteristics (E modulus 1.38 MPa, tensile strain 324.18%, and tensile strength 19.55 MPa). The rising content of AMP causes an enhancement of the tensile properties in case of cast films from CYH. For instance, PVC/CYH/AMP 5 wt % show an increase of E modulus about 60% and tensile strength about 37%. On the other hand, the presence of AMP in the films cast from DMF has an opposite effect on the tensile strain and tensile stress of the samples. The increasing content of AMP enhanced E modulus of the films (about 32% in the case of PVC/DMF/AMP 5 wt %). However, these modifications decrease the observed values of tensile strength (-40%) and tensile strain (-32%) of the material containing the same amount of the modifier in comparison with unmodified PVC film.

The obtained results are the consequence of various natures of the used solvents, which strongly influences the morphology of the films. Although CYH provides the smooth films with uniform distribution of the modifier (see Fig. 2), the films cast from DMF are typical by the AMP accumulation by the surface (Fig. 3). This irregularity of the AMP distribution seems to be a crucial factor that affects the resulting mechanical properties of the films, and their antibacterial and release characteristics, as further discussed.

In vitro antibacterial activity

The results of the antibacterial activity studies, which were proceeded by using agar diffusion test method, are presented in Figure 4 (PVC/CYH/AMP) and Figure 5 (PVC/DMF/AMP), respectively, in form of the dependences of the diameter of the growth inhibition zone against the concentration of the antibiotics in the polymer film. As it is previously mentioned, our experiment was focused on the antibacterial action of the polymer/antibiotic films against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*) bacterial strains.

Figure 4 shows that PVC/CYH/AMP systems merely undergo their antibacterial activity against Gram-positive *Staphylococcus aureus*. The significant inhibition growth (inhibition zone diameter) appeared at the lowest AMP content (1 wt %), and succeeding increases with rising amount of the antibiotic in the film. The other tested bacterial strains are not inhibited in this case.

On the other hand, the PVC/DMF/AMP systems significantly inhibit the growth of all studied bacterial strains (see Fig. 5). The investigated films are most effective against *Staphylococcus aureus*, followed by Gram-negative *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, respectively. However, the representatives of the Gram-negative strains sustain approximately half as large inhibition zones as Gram-positive *Staphylococcus aureus*.

TABLE II
Mechanical Properties of PVC/DMF/AMP Systems

Sample	E modulus (MPa)	Tensile strain (%)	Tensile stress (MPa)
PVC/DMF/AMP 0 wt %	1.38 ± 0.22	324.18 ± 12.80	19.55 ± 2.06
PVC/DMF/AMP 1 wt %	1.33 ± 0.35	263.87 ± 40.75	15.28 ± 2.10
PVC/DMF/AMP 2 wt %	1.10 ± 0.14	238.55 ± 37.64	12.22 ± 1.87
PVC/DMF/AMP 3 wt %	1.08 ± 0.18	190.61 ± 41.97	6.17 ± 1.12
PVC/DMF/AMP 4 wt %	1.63 ± 0.76	292.73 ± 24.94	15.48 ± 3.56
PVC/DMF/AMP 5 wt %	1.84 ± 0.20	219.70 ± 52.75	11.85 ± 2.55

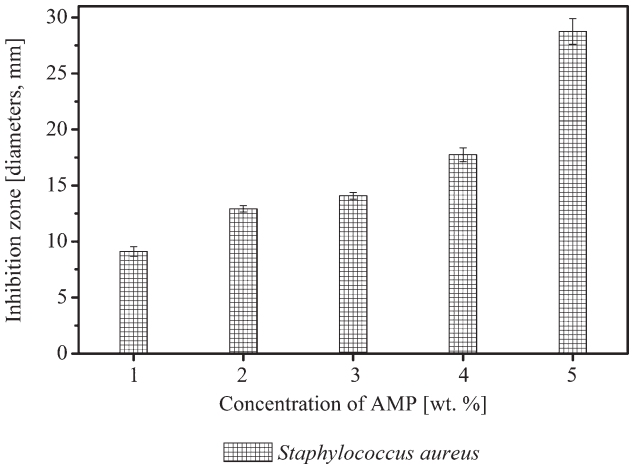

Figure 4 Antibacterial activity of PVC/CYH/AMP systems.

The reason of these results dissimilarities is given by the features of the individual bacteria. The most noticeable is the cell wall structure variance between Gram-positive and Gram-negative bacteria. It is known that Gram-negative bacteria have more complex cell walls, which may provide a better protection against external impacts, including a chemical substance effect.[43] This assumption can be also adopted in case of the AMP used as the antibacterial agent in this work.

However, the morphology of the prepared films cannot be omitted here. The distribution of the modifier, AMP, within the PVC matrix plays a crucial role in this study. The uniform distribution of AMP was formed in the case of PVC/CYH/AMP unlike PVC/DMF/AMP systems where the accumulation of the modifier occurred at the top part of the films (see Figs. 2 and 3). While the former case represents a situation when the antibacterial agent is entrapped inside of the polymer, that means its

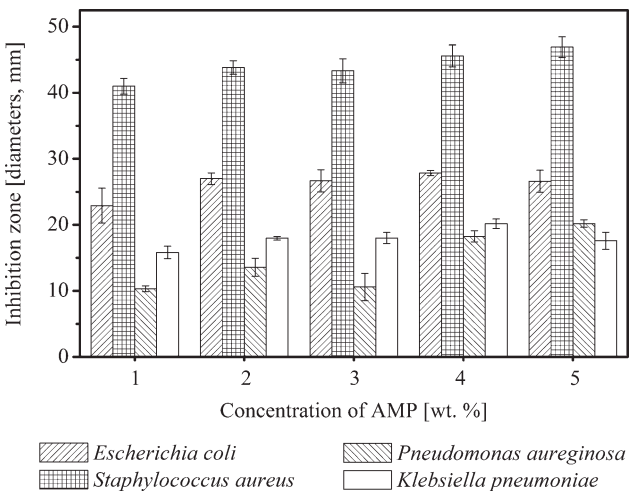

Figure 5 Antibacterial activity of PVC/DMF/AMP systems.

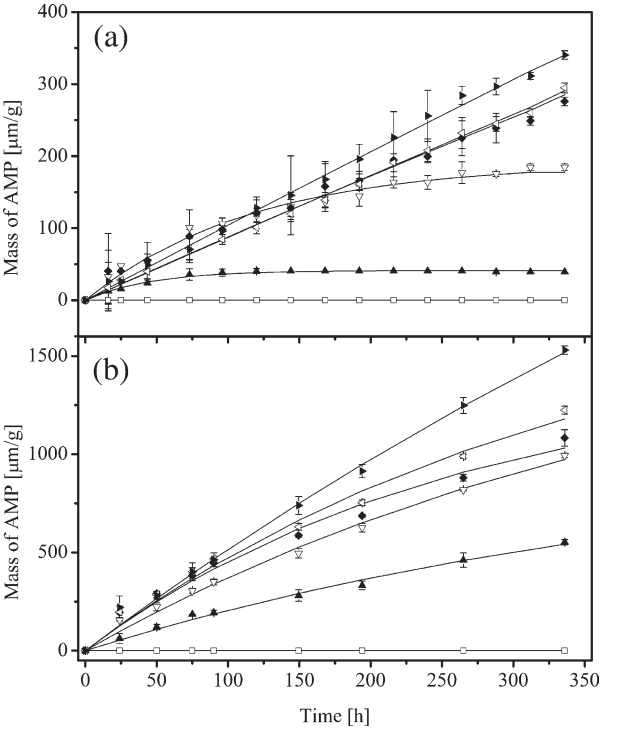

Figure 6 Release studies in: (a) distilled water, (b) physiological solution of PVC/CYH/AMP systems. Lines represent fitted model according eq. (1) □ PVC/CYH/AMP 0 wt/ %, ▲ PVC/CYH/AMP 1 wt %, ▽ PVC/CYH/AMP 2 wt %, ◆ PVC/CYH/AMP 3wt %, ◁ PVC/CYH/AMP 4 wt %, ▶ PVC/CYH/AMP 5 wt %.

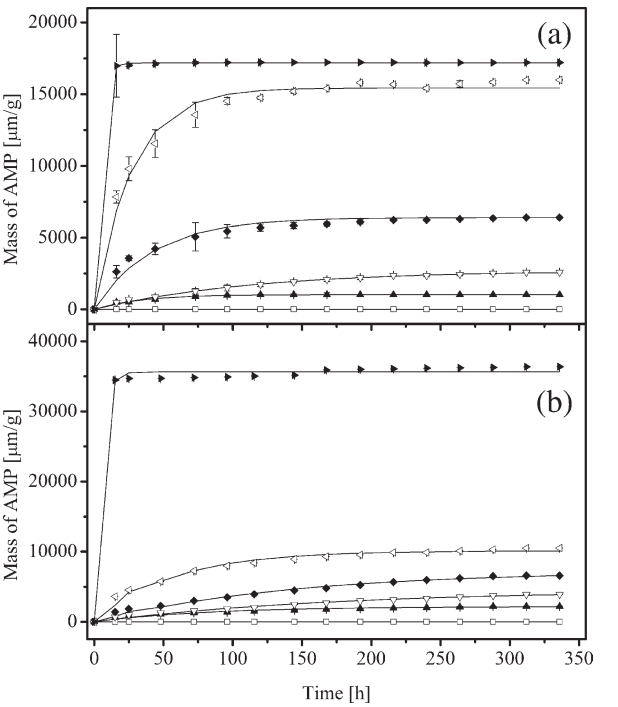

Figure 7 Release studies in (a) distilled water, (b) physiological solution of PVC/DMF/AMP systems. Lines represent fitted model according eq. (1) □ PVC/DMF/AMP 0 wt %, ▲ PVC/DMF/AMP 1 wt %, ▽ PVC/DMF/AMP 2 wt %, ◆ PVC/DMF/AMP 3wt %, ◁ PVC/DMF/AMP 4 wt %, ▶ PVC/DMF/AMP 5 wt %.

TABLE III
Constants of eq. (1) Describing the Release Studies of AMP from PVC/CYH/AMP Systems

	Distilled water			Physiological solution		
% AMP	C_{MAX} (μg/g)	$-k \times 10^{-3}$ (1/h)	r^2	C_{MAX} (μg/g)	$-k \times 10^{-3}$ (1/h)	r^2
1	41.29	22.12	0.98	1025.05	2.24	0.99
2	190.89	8.54	0.99	1800.49	2.32	0.99
3	2249.61	0.39	0.99	1842.18	3.84	0.98
4	5310.77	0.2	0.99	1916.97	2.85	0.98
5	5310.97	0.17	0.99	4727.86	1.15	0.99

release is restricted, the latter system provides a relatively high AMP amount, available to be distributed into a surrounding (aqueous or moist enough) environment.

Antibiotic release studies

The mean cumulative mass of AMP released from PVC/CYH/AMP and PVC/DMF/AMP systems into distilled water and physiological solution as a function of elution time are shown in the Figures 6 and 7. The calculated constant of the eq. (1) are summarized in Tables III and IV.

The release profiles from PVC/CYH/AMP systems presented in Figure 6 show that increasing content of the antibiotic corresponds to rising amount of the detected AMP in the release medium. However, there is a significant effect of the ionic strength of the medium on the AMP mass released. Generally, the content of the disengaged AMP is ~ ten times lower in distilled water [Fig. 6(a)] than in physiological solution [Fig. 6(b)]. This can be also clearly noticed in Table III from the values of the C_{MAX} parameters; and the higher rate constants ($-k$) from PVC/CYH/AMP 3 wt % in physiological solution. It means that AMP (sodium salt) is released favorably in the environment with higher ionic strength and osmotic pressure. It corresponds to literature sources presenting the high solubility of the AMP in isotonic solutions.[44,45]

The PVC/DMF/AMP systems show totally different behavior by comparison with PVC/CYH/AMP. The release profiles presented in Figure 7 are typical by the fast release of the AMP within the first 50–100 h of the experiment, when the maximal concentration C_{MAX} is reached. Further testing does not evidence an increase in cumulative concentration of AMP released. This phenomenon called "burst effect", corresponds to the morphological arrangement of the AMP within the PVC matrix, as discussed above. The effect of the release medium can be observed as well. Table IV shows that the kinetics constant C_{MAX} [eq. (1)] is generally higher for the physiological solution release medium. However, the difference by contrast to distilled water is just twofold. In addition, rate constants, $-k$, are higher in the case of distilled water. It could mean that the morphological factors (accumulation of the AMP close to the surface of the films) surpass the higher solubility of the AMP in the isotonic environment during the initial stage of the release procedure.

Scanning electron microscopy

Scanning electron microscopy (SEM) analysis was applied to support the assumption of the morphological effect of the prepared films (effect of used solvent) on AMP release behavior. The SEM images of the cross sections of the PVC/CYH/AMP 5 wt % samples, before and after releasing studies (300 h in physiological solution), are shown in Figure 8. As can be seen, the exposure of the specimen in the physiological solution does not cause significant morphological changes. On the other hand, the noticeable effect of releasing medium can be seen in Figure 9, where SEM images of PVC/DMF/AMP 5 wt % are presented.

TABLE IV
Constants of eq. (1) Describing the Release Studies of AMP from PVC/DMF/AMP Systems

	Distilled water			Physiological solution		
% AMP	C_{MAX} (μg/g)	$-k \times 10^{-3}$ (1/h)	r^2	C_{MAX} (μg/g)	$-k \times 10^{-3}$ (1/h)	r^2
1	1026.34	30.44	0.98	2187.62	11.97	0.97
2	2730.48	8.64	0.99	4530.63	5.74	0.98
3	6400.02	23.81	0.98	7278.74	7.08	0.99
4	15438.55	36.91	0.98	10113.64	17.67	0.98
5	17197.77	268.27	0.99	35645.16	219.28	0.99

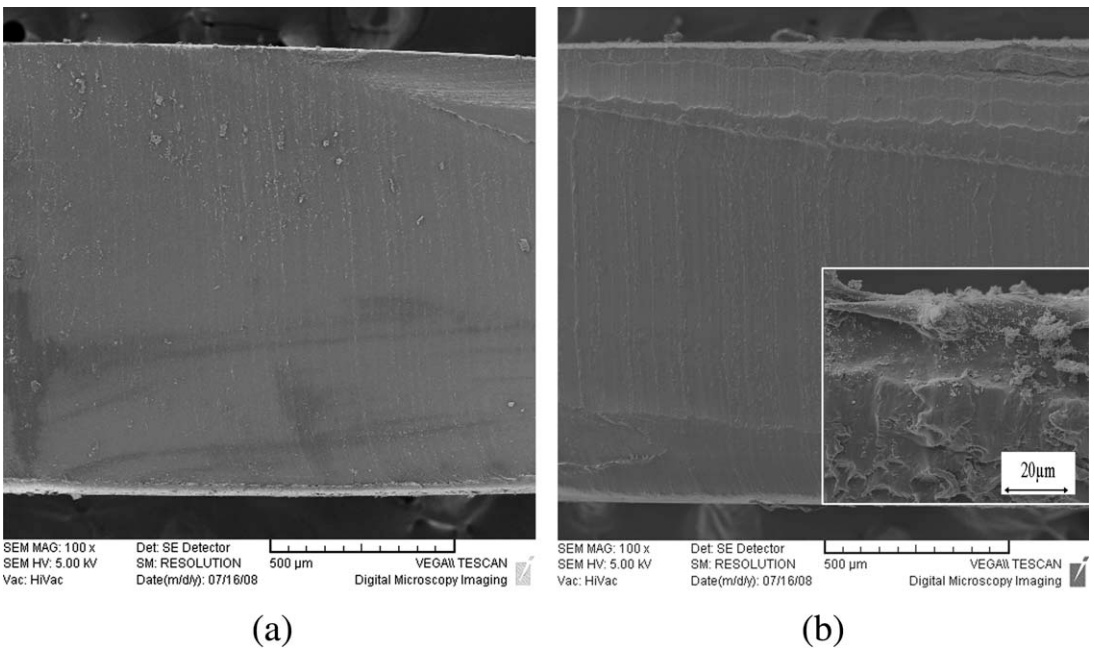

Figure 8 SEM micrographs of PVC/CYH/AMP 5 wt %: (a) before and (b) after the release studies.

From the SEM observations, cross-section of PVC/CYH/AMP sample show uniform AMP distribution [Fig. 8(a)]. After the release studies no negligible change is noticed [Fig. 8(b)]. In the case of PVC/DMF/AMP in the top of the micrograph is visible a rough surface due to the presence of AMP crystals [Fig. 9(a)]. Once the release study was consumed, a disturbed surface is obtained due to the spaces left from AMP during the release studies [Fig. 9(b)]. These observations are in agreement with the proposed statements above. The seen cavities clearly show a massive accumulation of AMP in the upper part of the film and its subsequent release from the PVC matrix.

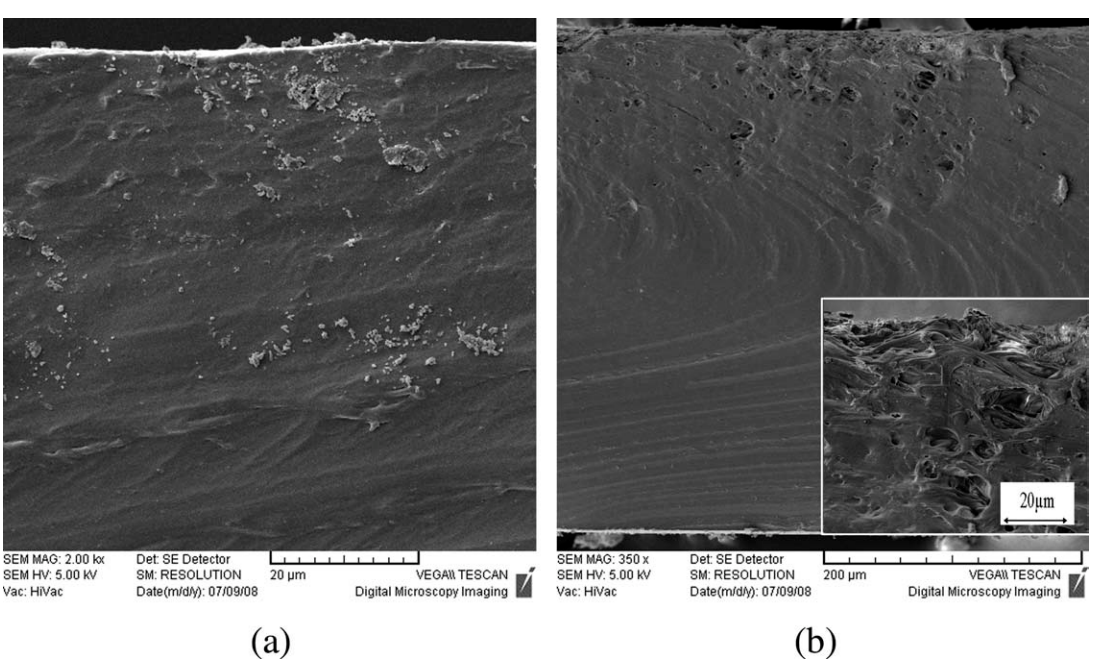

Figure 9 SEM micrographs of PVC/DMF/AMP 5 wt %: (a) before and (b) after the release studies.

CONCLUSIONS

The antibacterial polymeric films based on PVC and sodium ampicillin (AMP) (0–5 wt %) were prepared by solvent cast technique. This work was aimed at the investigation of the used solvent [cyclohexanone (CYH) and *N*,*N*-dimethylformamide (DMF)] on the resulting morphology, mechanical properties, anti bacterial activity, and release kinetics of the AMP incorporated into the PVC matrix.

The results revealed an important role of the structural characteristics of the solvents in all observed characteristics. While CYH provides the film with uniform distribution of the AMP particles within the PVC matrix, the separation process was observed (AMP accumulated in the surface upper part of the film) in the case of DMF. The morphological arrangement of the studied systems was characterized by both optical and scanning electron microscopy. The higher uniformity of modifier distribution enhanced tensile properties of the samples. In the case of the films cast from CYH, the mechanical properties (E modulus, tensile strain, and tensile strength) were enhanced with rising content of AMP in the system. The significant reduction of tensile strain and tensile strength was observed for the film cast from DMF with presence of the modifier.

The antibacterial activity of the prepared films, studied by agar diffusion test, showed that it is also affected by morphological point of view. The PVC/CYH/AMP films proved inhibition growth activity only against Gram-positive *Staphylococcus aureus* due to the restricted ability of AMP to release from the matrix. On the contrary, the PVC/DMF/AMP systems showed strong antibacterial activity against both Gram-positive and Gram-negative (*Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*) bacterial strains due to the easily availability of the antibiotic.

This trend was also observed during AMP release studies, which were preceded in distilled water and physiological solution and detected by UV-vis spectrometry. The rapid AMP release was observed in the case of PVC/DMF/AMP films, unlike the PVC/CYH/AMP samples, where continuous and long-lasting release can be expected. The effect of ionic strength (distilled water vs. physiological solution) on release profile was also studied in this work. The results reveal significantly higher amount of released AMP into physiological solution in both cases, which is in agreement with the reported affinity of used antibacterial agent, AMP, toward isotonic solutions. The applied mathematical model to obtained release data, found out that first-order kinetics fits relatively well to the experimental data and the calculated kinetics constants sufficiently characterize the release process.

References

1. Herrero, M.; Navarro, R.; Reinecke, H.; Mijangos, C. Polym Degrad Stab 2006, 91, 1915.
2. Blass, C. R.; Jones, C.; Courtney, J. M. Int J Artif Organs 1992, 15, 200.
3. Ruggeri, V.; Francolini, I.; Donelli, G.; Piozzi, A. J Biomed Mater Res Part A 2006, A81, 287.
4. Francolini, I.; Donelli, G.; Stoodley, P. Environ Sci Technol 2003, 2, 307.
5. Zhang, W.; Chu, P. K.; Ji, J.; Zhang, Y.; Liu, X.; Fu, R. K. Y.; Ha, P. C. T.; Yan, Q. Biomaterials 2006, 27, 44.
6. Balazs, D. J.; Triandafillu, K.; Chevolot, Y.; Aronsson, B.-O.; Harms, H.; Descouts, P.; Mathieu, H. J. Surf Interface Anal 2003, 35, 301.
7. Triandafillu, K.; Balazs, D. J.; Aronsson, B.-O.; Descouts, P.; Tu Quoc, P.; Van Delden, C.; Mathieu, H. J.; Harms, H. Biomaterials 2003, 24, 1507.
8. McCubbin, P. J.; Forbes, E.; Gow, M. M.; Gorham, S. D. J Appl Polym Sci 2006, 100, 538.
9. Lehocký, M.; Drnovká, H.; Lapčiková, B.; Barros-Timmons, A. M.; Trindade, T.; Zembala, M.; Lapčik, L., Jr. Colloids Surf A 2003, 222, 125.
10. Bower, C. K.; Parker, J. E.; Higgins, A. Z.; Oest, M. E.; Wilson, J. T.; Valentine, B. A.; Bothwell, M. K.; Mcguire, J. Colloids Surf B 2002, 25, 81.
11. Darouiche, R. O.; Smith, J. A.; Hanna, H.; Dhubuwala, C. B.; Steiner, M. S.; Babaian, R. J.; Boone, T. B.; Scardino, P. T.; Thornby, J. I.; Raad, I. I. Urology 1996, 54, 976.
12. Donelli, G.; Francolini, I. Chemotherapy 2001, 13, 595.
13. Bach, A.; Böhrer, H.; Motsch, J.; Martin, E.; Geiss, H. K.; Sonntag, H. G. J Antimicrob Chemother 1994, 33, 969.
14. Boelens, J. J.; Wee-Fu Tan; Dankert, J.; Zaat, S. A. J. J Antimicrob Chemother 2000, 45, 221.
15. Kohnen, W.; Kolbenschlag, C.; Teske-Keiser, S.; Jansen, B. Biomaterials 2003, 24, 4865.
16. Shintani, H. Trends Biomater Artif Organs 2004, 18, 1.
17. Donetz, A. P.; Harvey, R. A.; Greco, R. S. J Clin Microbiol 1984, 19, 1.
18. Raad, I. I.; Darouiche, R.; Hachem, R.; Mansouri, M.; Bodey, G. P. J Infect Dis 1996, 173, 418.
19. Donelli, G.; Francolini, I.; Piozzi, A.; Di Rosa, R.; Marconi, W. Chemotherapy 2002, 14, 501.
20. Marconi, W.; Francolini, I.; Piozzi, A.; Di Rosa, R. J Bioact Compat Polym 2001, 16, 393.
21. Piozzi, A.; Francolini, I.; Ochiaperti, L.; Venditti, M.; Marconi, W. Int J Pharm 2004, 280, 173.
22. Schierholz, J. M.; Fleck, C.; Beuth, J.; Pulverer, G. J Antimicrob Chemother 2000, 46, 45.
23. Danese, P. N. Chem Biol 2002, 9, 873.
24. Liang, X.; Wang, A.; Cao, T.; Tang, H.; Mcallister, J. P., II; Salley, S. O.; Simon Ng, K. Y. J Biomed Mater Res 2006, 76, 580.
25. Siemann, U. Prog Colloid Polym Sci 2005, 130, 1.
26. Schierholz, J. M.; Steinhauser, H.; Rump, A. F. E.; Berkels, R.; Pulverer, G. Biomaterials 1997, 18, 839.
27. Rossi, S.; Azghani, A. O.; Omri, A. J Antimicrob Chemother 2004, 54, 1013.
28. Kim, J.; Kim, S.; Lee, S.; Lee, Ch.; Kim, D. Int J Pharm 2000, 201, 29.
29. Jones, D.; Djokic, J.; Gorman, S. P. Biomaterials 2005, 26, 2013.
30. Patel, J. S.; Patel, S. V.; Talpada, N. P.; Patel, H. A. Angew Makromol Chem 1999, 271, 24.
31. Fontana, G.; Pitarresi, G.; Tomarchio, V.; Carlisi, B.; San Biagio, P. L. Biomaterials 1998, 19, 1009.

32. Morck, D. W.; Lam, K.; Mckay, S. G.; Olson, M. E.; Prosser, B.; Ellis, B. D.; Cleeland, R.; Costerton, J. W. Int J Antimicrob Agents 1994, 4, S2.
33. Giunchedi, P.; Genta, I.; Conti, B.; Muzarelli, R. A. A.; Conte, U. Biomaterials 1997, 19, 157.
34. Queiroz, A. C.; Santos, J. D.; Monteiro, F. J.; Gibson, I. R.; Knowles, J. C. Biomaterials 2001, 22, 1393.
35. Galya, T.; Sedlařik, V.; Kuřitka, I.; Novotný, R.; Sedlarikova, J.; Saha, P. J Appl Polym Sci 2008, 110, 3178.
36. Galya, T.; Sedlařik, V.; Kuřitka, I.; Sedlarikova, J.; Saha, P. Int J Polym Anal Charat 2008, 13, 241.
37. Sedlařik, V.; Galya, T.; Sedlarikova, J.; Valasek, P.; Saha, P. Polym Degrad Stab 2009, doi: 10.1016/j.polymdegradstab. 2009.11.017
38. Milojevic, D.; Molnar, J.; Gutmann, F.; Wong, D. Anal Chim Acta 1996, 319, 353.
39. Aldeman, R.; Klein, I. M. J Polym Sci 1958, 31, 77.
40. Lee, K. H.; Kim, H. Y.; La, Y. M.; Lee, D. R.; Sung, N. H. J Polym Sci Part B: Polym Phys 2002, 40, 2259.
41. Small, P. A. J Appl Chem 1953, 3, 71.
42. Hansen, C. M. Hansen Solubility Parameters A User's Handbook; CRC Taylor & Francis Group: Boca Raton, 2007.
43. Peake, R. C.; James, D. A.; Susman, M.; Kennedy, C. A.; Dursan, M. J.; Schenke, S. Medical Microbiology The University of Texas Medical Branch at Galveston; Baron, S., Ed. The University of Texas Medical Branch at Galveston: Galveston Texas, 1996; Section 1.
44. The United States pharmacopeia–The national formulary; USP 29th revison (January 1, 2006), NF 24th ed; (January 1, 2006).
45. The United States pharmacopeia. Material safety data sheet; Ampicillin Sodium USP (January 25, 2008).