Polym. Bull. (2011) 67:997–1016
DOI 10.1007/s00289-011-0474-3

ORIGINAL PAPER

Thermoplastic modification of medical grade polyvinyl chloride with various antibiotics: effect of antibiotic chemical structure on mechanical, antibacterial properties, and release activity

Martha Merchan · Jana Sedlarikova · Miha Friedrich · Vladimir Sedlarik · Petr Saha

Received: 23 July 2010 / Revised: 9 February 2011 / Accepted: 13 March 2011 /
Published online: 20 March 2011

Abstract Novel polymeric systems with antibacterial properties based on medical-grade polyvinyl chloride and antibiotics (Ampicillin, Minocycline, and Rifampicin) in the concentrations up to 1 wt% were prepared by thermoplastic compounding. Thermal stability of the antibiotics was corroborated by IR spectroscopy, X-ray diffraction, differential scanning calorimetry, and ^{1}H nuclear magnetic resonance spectroscopy. The effect of chemical structure on mechanical properties of the prepared systems was determined by tensile testing measurements and correlated with optical microscopy observations. In vitro antibacterial properties of samples were determined by agar diffusion and adhesion test against Gram-negative and Gram-positive bacteria. Antibiotic release experiments in distilled water and physiological saline solution and follow-up detecting by Ultraviolet–Visible spectroscopy were carried out. A mathematical model was applied to evaluate the release kinetics of the antibiotics from prepared polymeric systems. The results show significant influence of antibiotic's chemical structure on all

Electronic supplementary material The online version of this article (doi: 10.1007/s00289-011-0474-3) contains supplementary material, which is available to authorized users.

M. Merchan · V. Sedlarik · P. Saha
Centre of Polymer Systems, Polymer Centre, Tomas Bata University in Zlin, nam. T. G.Masaryka 5555, 760 01 Zlín, Czech Republic

J. Sedlarikova (✉)
Department of Fat, Tenside and Cosmetics Technology, Tomas Bata University in Zlin, nam. T. G. Masaryka 275, 762 72 Zlin, Czech Republic
e-mail: sedlarikova@uni.utb.cz

M. Friedrich
EN-FIST Center of Excellence, 1000 Ljubljana, Slovenia

V. Sedlarik
Jozef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia

studied characteristics of the systems due to the various interactions with polyvinyl chloride matrix.

Keywords Antibacterial modifications · Antibiotics · Polyvinyl chloride · Release activity · Mechanical properties · Thermal stability · Thermoplastic compounding

Introduction

In the last years the use of polymeric medical devices, such as intravascular catheters, endotracheal tubes, central venous catheters, and cerebrospinal fluid shunts, has become a routine in health care units, even if their implantation frequently causes nosocomial infections resulting in increased morbidity and hospital costs [1–4]. The nosocomial infections are because of colonization of microorganisms on device surfaces forming a slimy layer called "biofilm" that is believed responsible for the increased resistance to antibiotics of the consequent infections [5]. A biofilm has been defined as a multicellular consortium of microbial cells that is irreversibly associated with a surface and enclosed in a self-produced extracellular matrix composed primarily of polysaccharides [6–8]. Eradication of the biofilm is rarely possible without removal of the infected device, followed by long lasting antimicrobial chemotherapy, transient external drainage, and implantation of new device [1, 9, 10].

To limit infections on medical devices, several interventions have been reported. These include maximal sterile barriers, topical antibiotics, or antimicrobial flush solutions, tunnelled catheters, subcutaneous ports, and subcutaneous catheters cuffs [11, 12]. Most of these interventions have resulted in a significant reduction of infections. However, medical device-related infection still occurs more often with more resistant organism [11]. In consequence, the application of effective doses of antibiotics and their continuous release from polymeric surfaces seems to be promising approach [13, 14]. Therefore, several strategies to prevent devices-related infections are main objects of current investigations [15–25].

Coating of the medical devices with antimicrobial agents is in the vanguard of the biofilm prevention techniques because of noteworthy microbial restrain by their control elution from the device. To insure the slow release of the antibiotic from the material due to the ion-exchange process, a common method of coating catheters involves the use of cationic surfactants that binds to the polymer at one end and to anionic antimicrobials at the other [4, 11, 12, 26–28]. The incorporation of either acid or basic groups in the polymeric matrix, which work as binding places for the antibiotics is another possible strategy. Whereby, the interaction between polymer and drug is increased allowing the matrix to absorb higher amount of antibiotics [5, 9–11].

The local delivery of antibiotics to the infected site offers major advantages over traditional intravenous therapy and the effectiveness of such coatings is strongly dependent on the antibiotic release profile from the polymer [29].Therefore, as an approach to obtain a functional material close to the production in a big scale; polyvinyl chloride (PVC), one of the most frequently used thermoplastic polymers

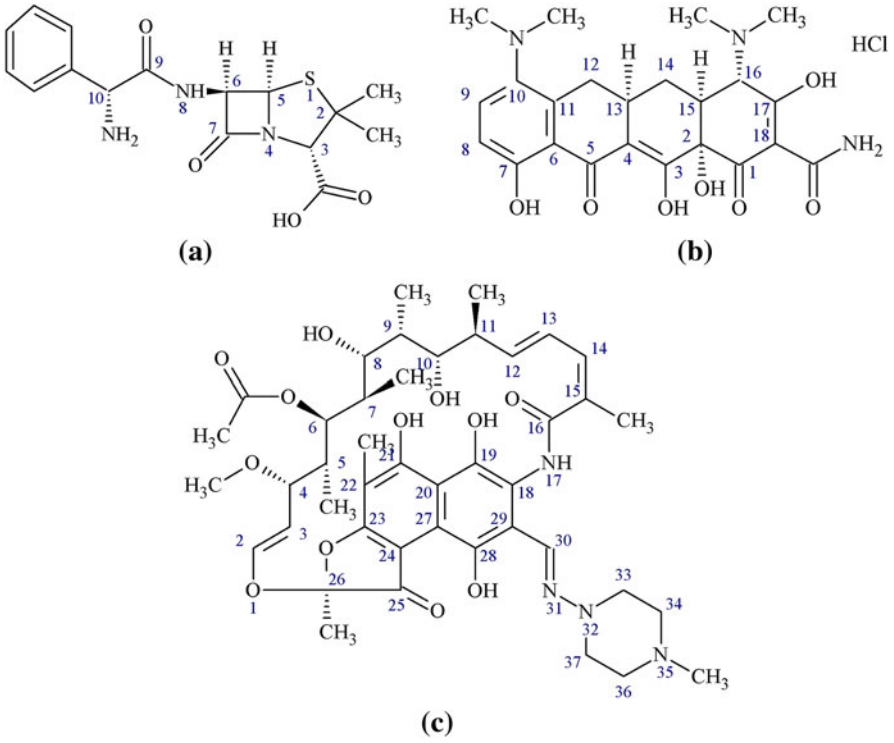

Fig. 1 Structural formula of the used antibiotics: **a** ampicillin (AMP), **b** minocycline (MIN), **c** rifampicin (RIF)

for biomedical disposable devices [30, 31], was compounded with three different antibiotics (sodium ampicillin, minocycline, and rifampicin) (AMP, MIN, and RIF) with the aim to get polymeric systems with antibacterial properties. The selection of antibiotics was affected by their broad spectrum action, their functional groups (Fig. 1) as well as their thermal stability. The main attention was paid to the physicochemical relation between the used antibiotics and the polymeric matrix. Mechanical, optical properties, and antibacterial activity as well as release kinetics of the incorporated antibiotics were investigated.

Experimental part

Materials

Commercially available medical-grade thermoplastic, free from phthalates, plasticized polyvinyl chloride RB1 (PVC) with a density of 1.23 g/cm^3, hardness values is 81 Shore A/15″ (according ISO 868) was supplied by Modenplast, Italy. Sodium ampicillin (7-(2-amino-2-phenyl-acetyl)amino-3,3-dimethyl-6-oxo-2-thia-5-azabicyclo[3.2.0]heptane-4carboxylic acid, sodium salt) (AMP) (Fig. 1a)) was supplied by Pharmos,a.s.,

Czech Republic; under the trade name Ampicillin 1,0 Biotika. (Register No. 15/548/92-s/c). Minocycline hydrochloride, 4,7-Bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11,-dioxo-2-naphthacenecarboxamide monohydrochloride (MIN) (Fig. 1b)) was purchased from STADApharm GmbH, Germany; with the trade name Minocyclin 50 STADA® (Register No. 15/289/00-C). Rifampicin, 3-[[(4-methyl-1-piperazinyl)imino]methyl]-5,6,9,17,19,21-Hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-[*N*-4(-methyl-1-piperazinyl) formimidoyl]-2,7-(epoxypentadecal [1, 11, 13] trienimino)naphtha[2,1-*b*]furan-1,11-(2*H*)-dione21-acetate (RIF) (Fig. 1c)) was acquired from BELUPO, s.r.o. Bratislava, Slovak Republic; the trade name is Arficin 300 (Register No. 15/829/92-B/C). The structures of used antibiotics are shown in Fig. 1, which includes the numbering used for each molecule for better orientation in results of thermal stability testing. Bacterial strains *Escherichia coli* 3954, *Staphylococcus aureus* 3953, were obtained from Czech Collection of Microorganisms, Brno, Czech Republic.

Methods

Compounding of PVC and antibiotics

To obtain various polymeric systems, medical-grade polyvinyl chloride pellets were thermoplastically compounded with three different antibiotics (AMP, MIN, and RIF) (from 0 to 1 wt%) in a Brabender Plasti-corder kneader. The volume of the chamber was 50 cm^3. The melt mixing was carried out at 160 °C for 10 min and 30 rpm. The obtained samples were then compression molded at 160 °C for 5 min in a manual press into thin films (up to 1 mm thickness) and subsequently cooled under the pressure of 10 MPa. A blank sample of PVC, which was used in all experimental testing for the comparison, was prepared by the same procedure without the addition of antibiotics.

Optical microscopy

The mixing nature of the samples' cross-sections was investigated by optical microscopy in the microscope and stereoscope STM, equipped with USB camera DCM 310 set with the software Scope photo, version 3.0. The specimens were cut by LEICA RM2255 rotary microtome with 40 μm of thickness before optical microscopy investigation.

Thermal stability

Regarding the fact that antibiotics undergo high-temperatures during the processing, their thermal stability was tested. Defined amount of antibiotics were annealed at 160 °C for 10 min with further analysis by IR spectroscopy, X-ray diffraction (XRD), and differential scanning calorimetry (DSC). Nuclear magnetic resonance (^{1}H NMR) spectroscopy, as the most important form of spectroscopic methods, was also carried out to supplement the thermal stability testing [32–35]. Data before and after the thermal treatment were compared to estimate possible changes or

decomposition. The physico-chemical identification was done by NICOLET 320 FTIR equipped with attenuated total reflectance accessory (ATR) and OMNIC software package over the wavelength range of 4,000–750 cm^{-1} at the room temperature. XRD measurements were carried out on Xpert PRO diffractometer equipped with a rotation anode using Cu Kα radiation. Radial scans of intensity versus diffraction angle 2θ were recorded in the range of 5–60° at ambient temperature. DSC measurements were performed on a Perkin-Elmer 1, calibrated in temperature and heat flow using indium. Both annealed and not annealed antibiotics were heated in the range from 0 to 250 °C (heating rate of 10 °C/min, 20 mL/min, under nitrogen atmosphere). ^{1}H NMR spectra were recorded on Varian Unity Inova 300 MHz NMR spectrometer equipped with ID/PFG probe at 25 °C. Samples RIF and AMP were dissolved in D_2O, whereas MIN was dissolved in $CDCl_3$. All sample concentrations were 40 mg/mL. Chemical shifts were referred to TMS at δ 0.0 ppm.

Tensile measurements

The mechanical properties of prepared PVC/antibiotics systems were studied 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, tensile stress, and tensile strain were determined parameters. Ten specimens (initial length 80 mm, width 15 mm, thickness 1 mm) were tested in each case. The specimens were stored in silica gel containing dissicator for 1 week before their analysis.

In vitro antibacterial activity and biofilm formation (adhesion test)

An agar diffusion test was carried out to determine in vitro antibacterial activity of PVC/antibiotic samples. Round-shape samples (8 mm in diameter) were placed in Petri dishes containing Nutrient agar seeded with 10^8 CFU/mL of *Escherichia coli* 3954 and/or *Staphylococcus aureus* 3953. After the incubation at 37 °C for 24 h, the inhibition zone of bacterial growth was measured in four directions and the average values were used to evaluate the antibacterial properties.

Bacterial adhesion and biofilm experiments were performed using *Staphylococcus aureus* 3953 and *Escherichia coli* 3954. The circular shape specimens ($d \approx 15$ mm) were cut from the pristine and PVC/antibiotics 1 wt% samples before further investigation. 10 mL of sterile water solution of nutrient broth were inoculated with given bacterial strain to reach $\approx 10^8$ CFU/mL and left at room temperature for 30 min. Then, the specimens were inserted into the test tubes. After 24 h of incubation at 37 °C, the test tubes were opened and the specimens were carefully removed from the medium, rinsed with sterile distilled water to remove loosely adhered bacteria and placed into other test tubes containing 2 mL of sterile deionized water. The bacteria adhered on the surface of the specimens were removed by vigorous shaking of the test tube at 2000 rpm for 30 s and quantified by serial dilutions and spread plate technique. A 1 mL aliquot of the suspension was diluted decimally and from each dilution, 0.1 mL was transferred to a nutrient agar

plate and the surviving bacteria were counted after 24 h of cultivation at 37 °C reported as CFU/cm^2. Each experiment was repeated in triplicate.

Antibiotic release studies

The release profiles of antibiotics from PVC matrix were studied in distilled water and/or NaCl 0.9 wt% solution (physiological solution). Round-shape PVC/antibiotic specimens (15 mm in diameter) were washed and dried (25 °C) till constant weight (approximately 24 h). Then, they were immersed in 10 mL of medium, maintained at 37 °C with continuous shaking (100 rpm). Samples were transferred at each sampling time to fresh medium to reach perfect sink conditions. The aliquots of 4.5 mL (old medium) were withdrawn at defined time intervals and analyzed by UV–Vis spectrophotometer (Thermo Scientific Heλios Gamma) at wavelength of 210, 244, 257 nm for AMP, MIN, and RIF, respectively.

Table 1 (see supplementary information) shows the calibration dependences of the absorbance (A) and given antibiotic concentration (c), which were determined before release investigation for (I) distilled water and (II) physiological solution. Each measurement was performed in triplicate.

The cumulative mass of the released antibiotics was determined and the data were then recalculated to 1 g of the sample material. The first-order kinetics (Eq. 1) and regression by the least squared method was applied using the Solver subprogram of Microsoft Excel 2003.

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

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

Results and discussion

Optical microscopy

The efficiency of compounding process was substantiated by optical microscopy. Cross-section illustrated details of PVC/antibiotics in the ratio of 1 wt% as well as pristine PVC can be seen in Fig. 2. Films from pure PVC were colorless, transparent, and flat in appearance, compared with light yellow PVC/AMP films, yellow-green color of PVC/MIN films, and red-brown color of PVC/RIF. In the systems filled with AMP and MIN agglomerates with an average size of 50 μm are visible. This tendency might be related to the difference between the processing conditions and melting point. For the particular case, the chosen processing temperature 160 °C, remains far from the melting points of AMP and MIN, which were reported at 215 and 217 °C, respectively, [36, 37]. This means that the antibiotics were not completely incorporated in the polymer matrix, which could

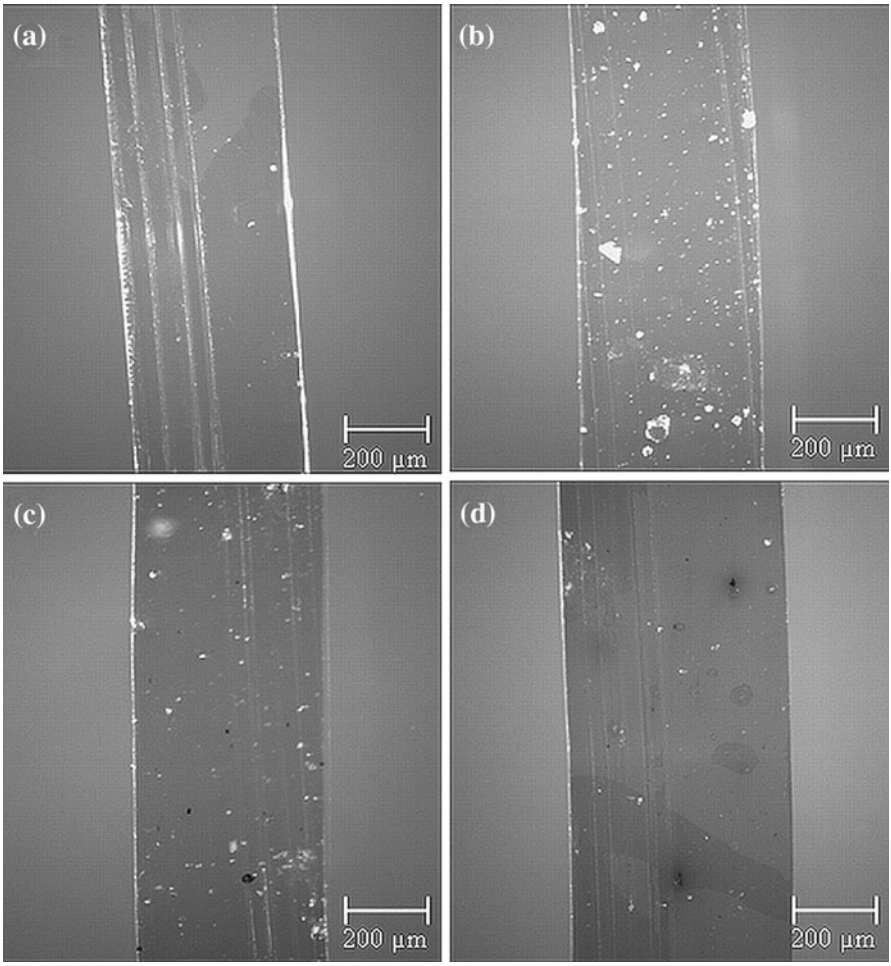

Fig. 2 Optical micrographs of **a** pure PVC and PVC systems with **b** AMP, **c** MIN, and **d** RIF 1 wt%

result in their low mutual adhesion influencing the ultimate polymer-antibiotic interface. The mixing intensity (level of homogenization) plays predominant role here. A better distribution of the antibiotics in the PVC matrix can be expected when, for instance, the twin-screw extruder or its combination with a single extruder in a multi-step process is used for compounding [38–40]. On the other hand, with RIF, which has the melting point in the range of 138–188 °C [41], the films with uniform distribution of the filler were obtained.

Thermal stability

In order to estimate the stability of the antibiotics during the processing technique used in this study, IR spectroscopy, X-ray diffraction, DSC, and ^{1}H NMR spectroscopy

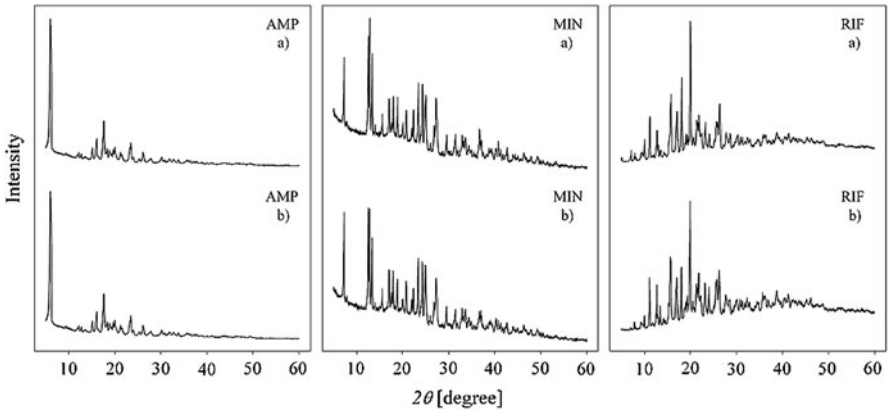

Fig. 3 Comparison of XRD antibiotic patterns **a** before and **b** after annealing at 160 °C

were performed with annealed antibiotics at the same temperature (160 °C for 10 min) like in the melt-mixing process for the obtaining the PVC/antibiotic systems. IR spectra (are not shown here) displayed characteristics bands without remarkable shifting and XRD patterns (Fig. 3) of the antibiotics before and after thermal treatment (annealing) did not show significant discrepancies. AMP patterns resemble the peaks before and after the annealing (before: 5.81°, 15.07°, 16.07°, 17.61°, and 23.43°), (after: 5.87°, 15.06°, 16.01°, 17.63°, and 23.31°) in the same way is in the case of MIN; (before: 7.28°, 12.58°, 12.87°, 13.36°, 13.98°, 15.59°, 17.09°, 17.97°, 18.89°, 20.07°, 20.83°, 21.99°, 22.33°, 23.41°, 24.34°, 25.00°, 27.26°, 29.55°, 31.45°), (after: 7.26°, 12.55°, 12.84°, 13.32°, 13.97°, 15.55°, 17.06°, 15.93°, 18.83°, 20.00°, 20.81°, 31.92°, 22.32°, 23.37°, 24.31°, 24,98°, 27.21°, 29.55°, and 31.46°). MIN as well as RIF patterns show characteristics peaks for the crystalline polymorphism form II (before: 11.08°, 19.98°, 15.89°, and 12.79°), (after: 11.08°, 19.98°, 15.69°, and 12.70°) [42, 43]. The crystalline network of each antibiotic after the treatment was not interrupted. These results are in agreement of the DSC curves (are not shown here) where AMP presented a sharp endothermic peak at 227.46 °C (before) and 227.29 °C (after) with further exothermic peak (decomposition) at 235.24 °C. The MIN thermogram showed an endothermic peak at 235.02 °C (before) and 234. 91 °C (after) but no exothermic peak was detected in this measurement. RIF exhibited an endothermic peak at 192.80 °C (before) and 192.34 °C (after) but no exothermic peak was detected in this measurement, thermograms are comparable with reports to form II. Since no significant changes, decomposition or hydrolytic reactions were observed, the antibiotics were able to be melt-mixed with the PVC in order to obtain various polymeric systems. Another evidence of the antibiotic thermal stability is presented in Figs. 4, 5, and 6 where ^{1}H NMR spectra of thermally treated and non-treated antibiotics are shown. The observed spectra provide relevant information regarding the structure of antibiotics. As can be seen, no differences between given counterparts were revealed. The following peaks were observed in measured spectra (related to numbering indicated on the structure in Fig. 1): AMP ^{1}H NMR (D_2O, 25 °C): δ 1.45 (2–CH_3, s, 3H), 1.52 (2–CH_3, s, 3H), 4.19 (H3, s, 1H), 4.66 (H10, s, 1H),

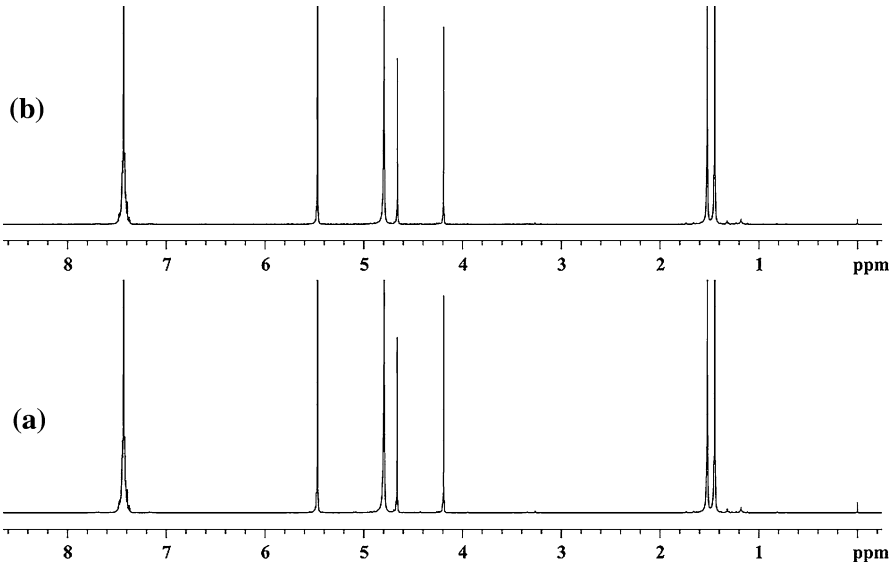

Fig. 4 ^{1}H NMR spectra of ampicillin **a** before and **b** after annealing at 160 °C

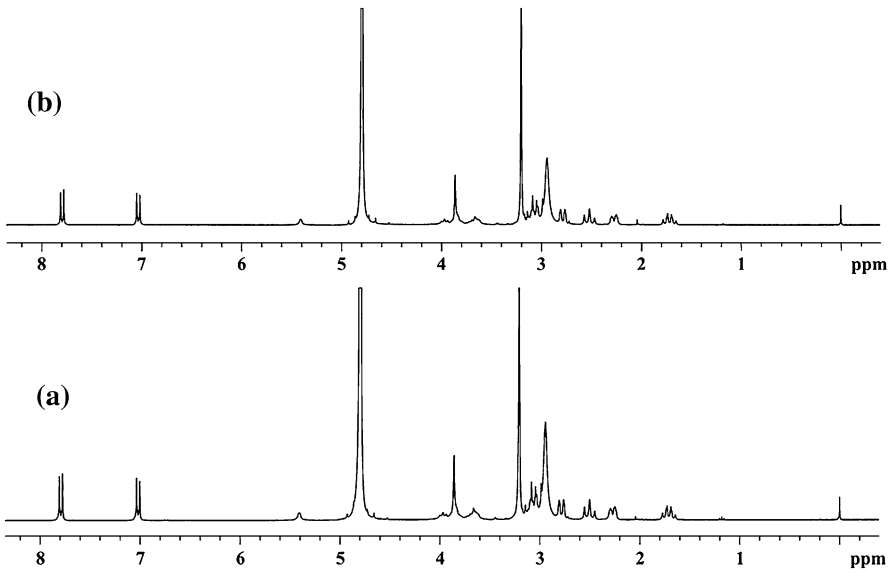

Fig. 5 ^{1}H NMR spectra of minocycline **a** before and **b** after annealing at 160 °C

5.47 (H5, H6, s, 2H), 7.42–7.44 (Ph, m, 5H) ppm; MIN ^{1}H NMR (D_2O, 25 °C): δ 1.71 (H14a, m, 1H), 2.27 (H14b, m, 1H), 2.50 (H12a, *t*, 1H), 2.78 (H15, d, 1H), 2.8–3.3 (H12b, H13, 10-N$(CH_3)_2$, 16-N$(CH_3)_2$, m, 14H), 3.86 (H16, m, 1H), 7.02 (H9, d, 1H), 7.79 (H8, d, 1H) ppm; RIF ^{1}H NMR (D_2O, 25 °C): δ −0.31 (5–CH_3, d, 3H),

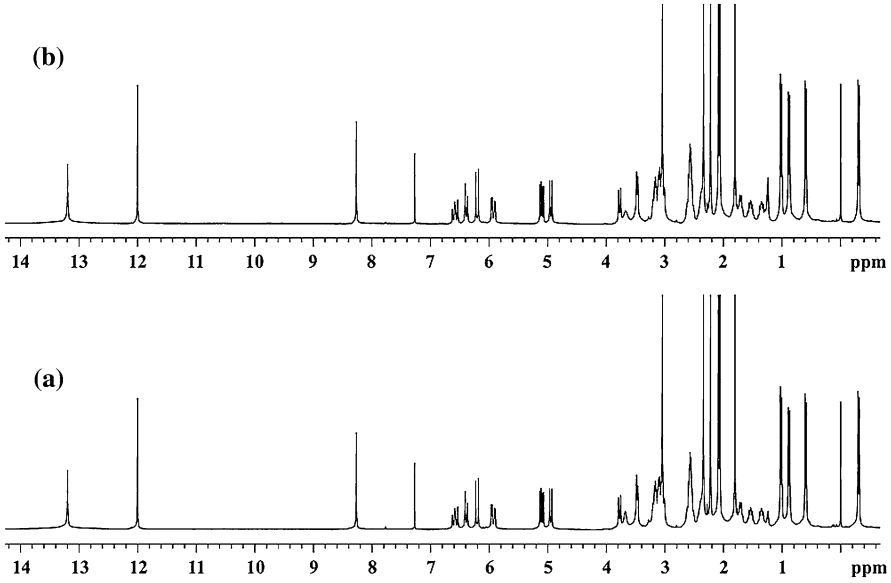

FIg. 6 ^{1}H NMR spectra of rifampicin **a** before and **b** after annealing at 160 °C

0.60 (7–CH_3, d, 3H), 0.88 (11–CH_3, d, 3H), 1.02 (9–CH_3, d, 3H), 1.35 (H5, m, 1H), 1.53 (H7, m, 1H), 1.74 (H9, m, 1H), 1.80 (26–CH_3, s, 3H), 2.0–2.4 (15–CH_3, 22–CH_3, 35–CH_3, 6–$COCH_3$, m, 12H), 2.56 (H11, H34, H36, m, 3H), 3.0–3.2 (H4, H8, H33a, H37a, 4–OCH_3, m, 7H), 3.47 (H33b, H37b, m, 2H), 3.77 (H10, d, 1H), 4.95 (H6, d, 1H), 5.10 (H3, dd, 1H), 5.93 (H12, dd, 1H), 6.21 (H2, d, 1H), 6.39 (H14, d, 1H), 6.58 (H13, m, 1H), 8.27 (H30, s, 1H) ppm.

Tensile measurements

The influence of biomaterials modifications on resulting mechanical properties of the material is one of the key factors, which can subsequently limit the potential applicability of the product. The modifications which lead to significant reduction of mechanical properties are not acceptable for practical use. In case of medical PVC, the crucial attention should be paid to saving of the material flexibility and tenacity as it is expected from the materials used for the production of various catheters and so forth.

The values of the observed tensile characteristics of the unmodified PVC and PVC films containing various concentrations of antibiotics are shown in Tables 2, 3, 4 (see supplementary information). As can be noticed there, unmodified PVC is characteristic by relatively low Young's modulus (0.93 MPa) and high-maximal elongation (over 354%). Tensile strength is 20.2 MPa. These properties correspond to highly plasticized PVC matrix (over 45%, estimated according to the hardness versus plasticizer content in PVC dependence) [44]. So, it could be expected that the effect of plasticizer will be predominant at the lower concentrations of a modifier (filler).

In case of AMP (Table 2), the effect of its presence in the systems on Young's modulus (E) is hardly noticeable in the investigated range of the antibiotic (up to

1 wt%). The E values of the modified PVC/AMP films fluctuate around the value of pure PVC. However, the presence of AMP influences tensile strain and tensile strength significantly. Table 2 shows that the former mentioned tensile characteristic is reduced about 16.1% at 1 wt% presence of AMP. The latter was reduced about almost 19.8%. Similar trend was found in case of MIN as well (Table 3). Young's modulus, expressing material stiffness, is not affected significantly due to the presence of MIN in the system. The reduction of tensile strain and tensile strength for the samples containing 1 wt% MIN was 17.2 and 19.3%, respectively. Rather different behavior was observed for PVC/RIF system. The data presented in Table 4 indicates the enhancement of Young's modulus (about 29% at 1 wt% RIF). On the other hand, reduction of other studied characteristics occurred as well as in previous two cases. Maximal elongation was decreased about 25.4% and tensile strength about 22.8% at 1 wt% of RIF (all reductions are related to mechanical properties of unmodified PVC specimen).

The results presented above, more or less correspond to the observations achieved by optical microscopy (Fig. 2b, c). The formation of the aggregated antibiotics (AMP and MIN) domains within the PVC matrix can be clearly noticed there. These morphological irregularities are not significant enough to affect Young's modulus of the material due to high level of matrix plasticization (see above). However, they can cause reductions of toughness and ductility. On the other hand, optical micrograph of the PVC/RIF (Fig. 2d) sample does not reveal noticeable agglomerates, which leads to the assumption of higher affinity (hydrophobicity) of the antibiotic (Fig. 1c) and polymer/plasticizer systems. Moreover RIF has closer solubility parameter ($\delta = 10.3(\text{cal/cm}^3)^{1/2}$) to PVC ($\delta = 9.6$–$9.7\ (\text{cal/cm}^3)^{1/2}$) than the other antibiotics, AMP $\delta = 12.1\ (\text{cal/cm}^3)^{1/2}$ and MIN $\delta = 2.5(\text{cal/cm}^3)^{1/2}$ [45, 46]. This could cause better distribution of the modifier within the PVC matrix and subsequent enhancement of stiffness (Young's modulus).

As mentioned above, the affinity between the polymer matrix and the antimicrobial modifier plays an important role in subsequent mechanical properties of the resulting system. The dependence of tensile strain reduction on the antibiotic's concentration is depicted in Fig. 7a. From the chemical structure point of view, three types of modifiers were used. β-lactam AMP, which could also act as electron donor toward suitable acceptors, a tetracycline antibiotic MIN, flat, planar molecule with a little molecular dynamicism, and macrolide RIF, which is particularly formed with 4-methyl-1-piperazinaminyl group (Fig. 1). Their structure as well as their physicochemical properties are assumed to be crucial for both material integrity (represented by mechanical properties here) and release kinetics (afterwards mentioned). The results in Fig. 7 show that AMP significantly reduces maximal elongation up to 0.5 wt%. Further additions of AMP are followed by a plateau. However, it is supposed to increase with rising AMP content. The modifiers with more complex chemical structure including several cycles (MIN and RIF) are characteristic by lower reduction of tensile strain at low contents. On the other hand, the reduction increases with rising concentration of MIN and RIF in the system and it exceeds the reduction level obtained in case of AMP.

Tensile strength reduction versus the antibiotic's concentration is shown in Fig. 7b. Here, the structural configuration of the individual antibiotics effect on

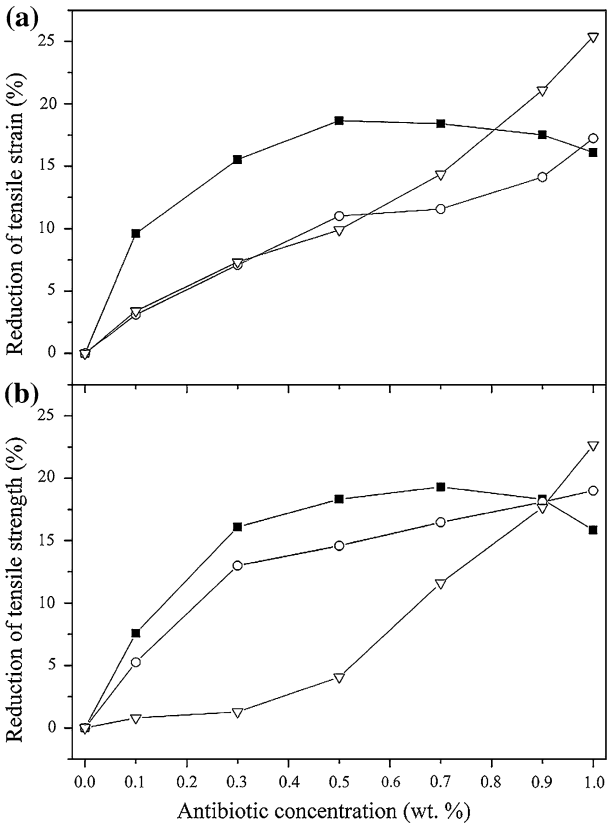

Fig. 7 Reduction of **a** tensile strain and **b** tensile strength versus concentration of antibiotics in PVC matrix: *filled square* AMP, *open circle* MIN, *open inverted triangle* RIF

mechanical properties of the PVC/antibiotic systems is more noticeable and it corresponds to optical microscopy results (Fig. 2). It can be seen that both AMP and MIN follows relatively same pattern of tensile strength reduction behavior; i.e., significant increase up to 0.3 wt% followed by sight reduction ratio rise with further additions. The obtained dependence has a concave like shape. On the contrary, RIF proves a convex like shape of the tensile strength versus RIF concentration dependence. It means that RIF influences the tensile properties in PVC/RIF systems less than AMP and MIN at low concentrations. The reason could lie in already discussed better affinity between an antibiotic and polymer matrix.

In vitro antibacterial activity and biofilm formation (adhesion test)

The antibacterial properties of each polymeric system were evaluated by an agar diffusion test (Fig. 8). The dependences of the diameter of the growth inhibition zone against the antibiotic concentration in the polymer film are shown in Table 5 (see supplementary information). As it has been mentioned above, the experiment

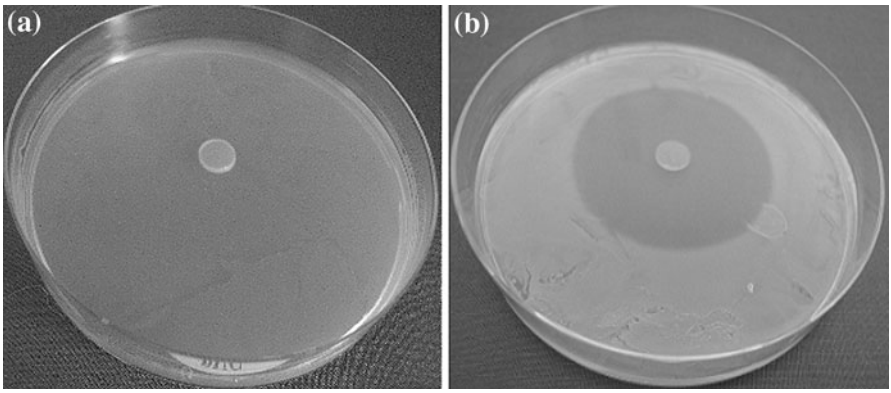

Fig. 8 Example of agar diffusion test against *Staphylococcus aureus* **a** PVC, **b** PVC/AMP 1 wt%

was focused on the antibacterial action of the PVC/antibiotic films against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria that are the most frequently occurred strains in nosocomial infections. All polymeric systems showed to be effective against the Gram-positive strain even from the lowest antibiotic concentration. Pure PVC film did not have any antibacterial action. PVC/AMP proved to be the most efficient system with the biggest inhibition zone. In case of *Escherichia coli*, its growth was inhibited only by PVC/AMP and PVC/MIN systems from 0.5 wt%, whereas PVC/RIF samples showed to be ineffective against this strain.

The number of viable adhered bacteria (CFU/cm^2) of *Staphylococcus aureus* and *Escherichia coli* revealed a restrain of adherence of cells in contrast with the pristine PVC. The percentage of reduction of adherence is included in the Table 6 (see supplementary information). As it can be seen from the obtained data after 24 h of incubation, the CFU after the adhesion test corresponds to the data performed by agar diffusion test. The obtained results suggest the capability of the antibacterial systems to hamper the adhesion of bacterial cell mainly in the Gram-positive strain.

The antibacterial properties of the obtained systems could be attributed to the variance between the cell walls of the tested strains. The higher complexity of Gram-negative cell wall makes the action of an antibacterial agent more difficult. AMP belongs among the broad spectrum β-lactam antibiotics that are able to penetrate the outer membrane of bacteria thanks to its α-amino group, but the mechanism of its action is unknown. It is used in the same manner like tetracycline antibiotics (including MIN), which was confirmed by the resulting antibacterial activity of PVC/AMP and PVC/MIN systems against both Gram-positive and Gram-negative strain. In case of MIN, the strong binding properties are suggested and its antibacterial action may consist in an ability to remove essential metal ions and chelated compounds, like magnesium ions that are related to molecular processes carried out in the bacteria. On the other hand, RIF is a transcription inhibitor that inhibits DNA-dependent RNA-polymerase with a very good activity against mainly the Gram-positive microorganisms.

Nevertheless, the relation between antibiotic and PVC matrix plays an important role in this study. The presence of aggregates of AMP and MIN even in the surface of the films may also influence the antibacterial properties. In case of RIF, which seems to be entrapped inside the matrix, its migration from the polymer is limited.

Antibiotic release studies

Figures 9, 10, 11 demonstrate the cumulative amounts of antibiotics released from PVC/AMP, PVC/MIN, and PVC/RIF systems into distilled water and physiological solution as a function of elution time. The calculated constants from the Eq. 1 are summarized in Tables 7, 8, and 9 (see supplementary information). The earliest samples were taken at 5 min, 15 min, and 30 min. Then, the sampling intervals were prolonged to every hour (up to 12 h) and every 24 h up to 316 h.

It is known that the systems based on hydrophobic polymer matrix, where an active agent is directly incorporated into the bulk have relatively low efficiency from the ratio of released/incorporated amount of the agent point of view [47]. The active agent (antibiotics in this case) is entrapped within the polymer matrix where its transport from the bulk to the surface and surrounding environment is limited or

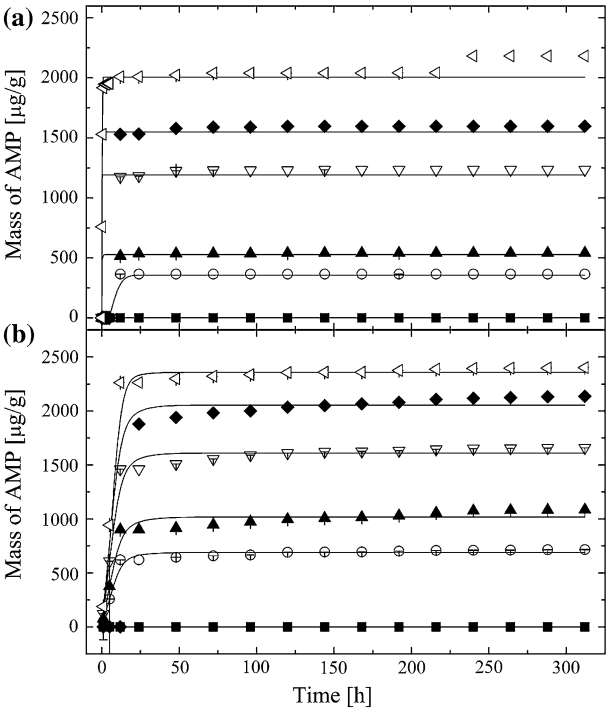

Fig. 9 Release studies in: **a** distilled water, **b** physiological solution of PVC/AMP systems. *Lines* represent fitted model according Eq. 1. *filled square* PVC/AMP 0 wt%, *open circle* PVC/AMP 0.3 wt%, *filled triangle* PVC/AMP 0.5 wt%, *open inverted triangle* PVC/AMP 0.7 wt%, *filled diamond* PVC/AMP 0.9 wt%, *left pointing triangle* PVC/AMP 1 wt%

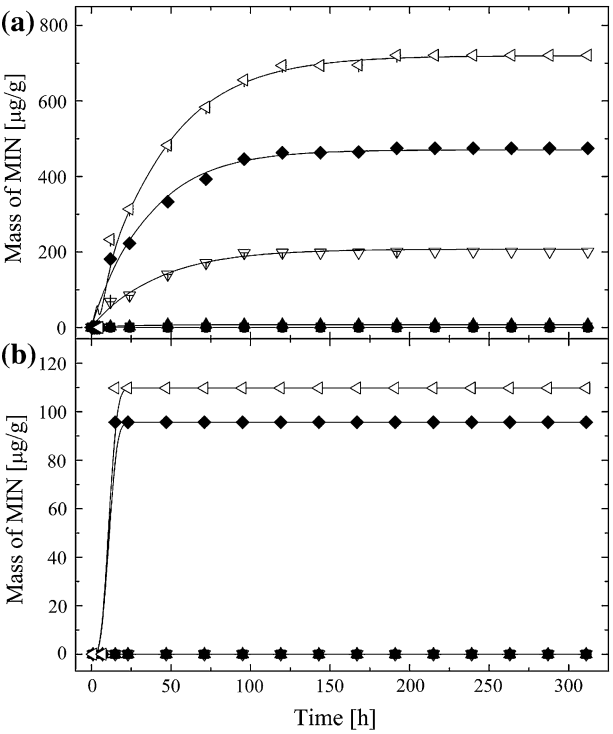

Fig. 10 Release studies in: **a** distilled water, **b** physiological solution of PVC/MIN systems. *Lines* represent fitted model according Eq. 1. *filled square* PVC/MIN 0 wt%, *open circle* PVC/MIN 0.3 wt%, *filled triangle* PVC/MIN 0.5 wt%, *open inverted triangle* PVC/MIN 0.7 wt%, *filled diamond* PVC/MIN 0.9 wt%, *left pointing triangle* PVC/AMP 1 wt%

even restrained. Thus, the agent occurring on the surface or very close can be released. This phenomenon was noticed in the case. Comparing the values C_{MAX} (Tables 7, 8, 9), it can be observed that the amount of the released antibiotic from the 1 g of PVC containing 1 wt% of antibiotic is 2.00 mg/g for AMP, 0.72 mg/g for MIN, and 0.15 mg/g for RIF (release in distilled water). It means only 0.200, 0.072, and 0.015 wt% from the initially incorporated AMP, MIN, and RIF, respectively. On the other hand, it is required to mention that the release effect is governed by diffusion; i.e., the amount of the released antibiotic is dependent on concentration of the antibiotic available on the surface or very close to it (see increasing C_{MAX} with rising concentration of antibiotic in the system in Tables 7, 8, 9) but also on the area of the polymer surface-surrounding environment interface. On the basics of the experimental settings it is easy to recalculate the amount of the released antibiotic on cm^2 of the sample. In practice, thermoplastically prepared systems containing directly incorporated active agents are supposed to be used as a coating of various medical devices. Since concentration of the releasable antibiotic is limited, area factor will be predominant. Thus, apparently non-effective system becomes extremely useful when the authors add the simplicity of the preparation process, which can be done by using conventional polymer processing methods.

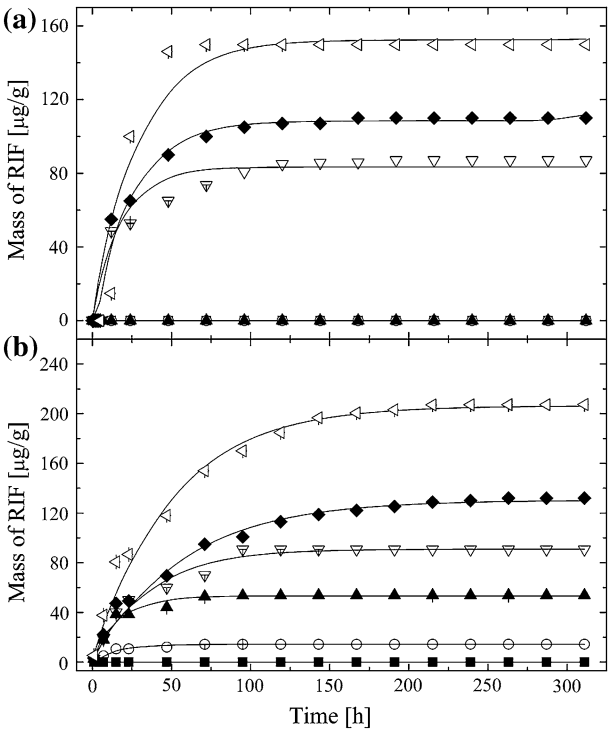

Fig. 11 Release studies in: **a** distilled water, **b** physiological solution of PVC/RIF systems. *Lines* represent fitted model according Eq. 1. *filled square* PVC/RIF 0 wt%, *open circle* PVC/RIF 0.3 wt%, *filled triangle* PVC/RIF 0.5 wt%, *open inverted triangle* PVC/RIF 0.7 wt%, *filled diamond* PVC/RIF 0.9 wt%, *left pointing triangle* PVC/RIF 1 wt%

Figure 9 showing the release kinetics profile of PVC/AMP system reveals that the maximum concentration of antibiotic (C_{MAX}) is reached immediately after the first sampling and detection. This concentration is not increasing even after further testing. The C_{MAX} was firstly reached in distilled water, which could be seen from higher values of $-k$, compared with physiological solution. This phenomenon called "burst effect" can be attributed to different lipophilicity between PVC and the antibiotic, as well as the morphological arrangement of AMP in the polymeric matrix, as it is discussed above. Moreover, AMP (sodium salt) possesses high solubility in isotonic solutions, which favors its release in the higher ionic strength medium [38, 45, 48].

In case of MIN (Fig. 10), the kinetic constants C_{MAX} are higher with lower values of $-k$ (0.02) in distilled water, which gives an explanation for long lasting release of MIN from the films with later burst effect. Currently, MIN is slightly soluble in water and for this reason it is used in a hydrochloride form; it could mean that the polar environment is more suitable for MIN release than the ionic medium, which is also supported from the amphoteric nature of MIN.

The lowest values for C_{MAX} and $-k$ can be seen in the release studies of the macrolide antibiotic RIF (Fig. 11). The differences between these two kinetic parameters are not so far, which means that both mediums are suitable for the release. RIF is slightly soluble in water, which is in agreement with the obtained results. Nevertheless, this antibiotic has ketone and lactone groups (electron donor groups) in its structure that might have stronger interaction either with the PVC chains or with the plasticizer that could limit the release as indicated above.

Conclusions

The antibacterial polymeric films based on polyvinyl chloride (PVC) and antibiotics, sodium ampicillin (AMP), minocycline (MIN), and rifampicin (RIF) were thermoplastically compounded in a kneader. The aim of this study was to study the effect of PVC modification on the resulting morphology, mechanical properties, antibacterial activity, and release profiles of the PVC/AMP, PVC/MIN, and PVC/RIF systems.

The characterization of antibiotics by IR spectroscopy, XRD, DSC, and ^{1}H NMR spectroscopy confirmed the thermal stability which was an indicator for their further processing. The nature and structural properties of each antibiotic influenced the results of all the tests. The differences in hydrophilicity, melting point, and processing temperature and homogenization intensity, might explain the formation of aggregates in PVC/AMP and PVC/MIN systems, which were possible to observe from the optical micrographs. On the other hand, the uniform distribution of RIF in PVC gives the evidence of the affinity between this macrolide antibiotic and polymer matrix. It was confirmed by the various tensile strain and tensile strength reduction level occurring due to the presence of the antibiotics.

The antibacterial properties, evaluated by an agar diffusion test and adhesion test revealed the inhibition activity of AMP and MIN against both used strains (*Staphylococcus aureus* and *Escherichia coli*) because of their mechanism of action as well as the availability of the antibiotics in the film. PVC/RIF sample, which had the best distribution in optical pictures, was only effective against the Gram-positive bacteria due to the restriction of the release from the matrix. The effect of the features of individual antibiotics and the arrangement in the matrix on antibacterial activity was confirmed.

The same tendency was observed during the release studies, which were carried out in distilled water and physiological solution and detected by UV–Vis spectrometry. The rapid release of AMP from PVC/AMP and MIN from PVC/MIN sample in physiological solution as well as the limited release of RIF in both mediums corresponds to the physicochemical behavior of each antibiotic related with the polymeric matrix, which also involves the affinity with the release medium. The release process was successfully characterized by the mathematical model using the first-order kinetics that sufficiently fitted to the experimental data.

Release activity of antibiotics was limited by their low ability to diffuse through polymer matrix and not more than 0.2 wt% of the antibiotic (AMP, distilled water) was detected. Despite that polymer-active agent entrapped systems are useful when

the simplicity of the preparation process, which can be done by using conventional polymer processing methods, is considered.

Acknowledgments Authors are grateful to the Ministry of Education, Youth and Sports of the Czech Republic (Grant no. MSM7088352101) and Ministry of Industry and Trade of the Czech Republic (Grant no. 2A-1TP1/126) for providing the financial support covering the research work. Authors also gratefully acknowledge Slovenian NMR centre at National Institute of Chemistry, Ljubljana for their assistance in acquiring and processing of NMR spectra. This article was created with support of Operational Program Research and Development for Innovations co-funded by the European Regional Development Fund (ERDF) and national budget of Czech Republic, within the framework of project Centre of Polymer Systems (reg. number: CZ.1.05/2.1.00/03.0111).

References

1. Schierholz JM, Steinhauser H, Rump AFE, Berkels R, Pulverer G (1997) Controlled release of antibiotics from biomedical polyurethanes: morphological and structural features. Biomaterials 18:839–844
2. Schierholz JM, Fleck C, Beuth J, Pulverer GJ (2000) The antimicrobial efficacy of a new central venous catheter with long-term broad-spectrum activity. Antimicrob Chemoth 46:45–50
3. Hanna H, Bahna P, Reitzel R, Dvorak T, Chaiban G, Hachem R, Raad I (2006) Comparative in vitro efficacies and antimicrobial durabilities of novel antimicrobial central venous catheter. Antimicrob Agents Ch 50:3283–3288
4. Ruggeri V, Francolini I, Donelli G, Piozzi A (2006) Synthesis, characterization, and in vitro activity of antibiotic releasing polyurethanes to prevent bacterial resistance. J Biomed Mater Rev Part A 81:287–298
5. Piozzi A, Francolini I, Occhiaperti L, Venditti M, Marconi W (2004) Antimicrobial activity of polyurethanes coated with antibiotics: a new approach to the realization of medical devices exempt from microbial colonization. Int J Pharm 280:173–183
6. Padera RF (2006) Infection in ventricular assist devices: the role of biofilm. Cardiovasc Pathol 15:264–270
7. Francolini I, Donelli G, Stoodley P (2003) Polymer designs to control biofilm growth on medical devices. Environ Sci Technol 2:307–319
8. Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8:881–890
9. Schierholz JM (1997) Physico-chemical properties of a rifampicin-releasing polydimethyl-siloxane shunt. Biomaterials 18:635–641
10. Schierholz J, Jansen B, Jaenicke L, Pulverer G (1994) In vitro efficacy of an antibiotic releasing silicone ventricle catheter to prevent shunt infection. Biomaterials 15:996–1000
11. Raad I, Darouiche R, Hachem R, Mansouri M, Bodey GP (1996) The broad-spectrum activity and efficacy of catheters coated with minocycline and rifampin. J Infect Dis 173:418–424
12. Raad I, Darouiche R, Dupuis J, Abi-Said D, Gabrielli A, Hachem R, Wall M, Harris R, Jones J, Buzaid A, Obertson C, Shenaq S, Curling P, Burke T, Ericsson C (1997) Central venous catheters coated with minocycline and ifampin for the prevention of catheter-related colonization nd bloodstream infections. A randomized, double-blind trial Texas Medical Center Catheter Study Group. Ann Intern Ed 127:267–274
13. Jones DS, McGovern JG, Woolfson AD, Adair CG, Gorman SP (2002) Physicochemical characterization of hexatidine-impregnated endotracheal tube poly(vynil Chloride) and resistance to adhere of respiratory bacterial pathogens. Pharm Res 19:818–824
14. Kohnen W, Kolbenschlag C, Teske-Keiser S, Jansen B (2003) Development of a long-lasting ventricular catheter impregnated with a combination of antibiotics. Biomaterials 24:4865–4869
15. Junkar I, Cvelbar U, Vesel A, Hauptman N, Mozetic M (2009) The role of crystallinity on polymer interaction with oxygen plasma. Plasma Process Polym 6:667–675
16. Junkar I, Vesel A, Cvelbar U, Mozetic M, Strnad S (2009) Influence of oxygen and nitrogen plasma treatment on polyethylene terephthalate (PET) polymers. Vacuum 84:83–85

17. Vesel A, Junkar I, Cvelbar U, Kovac J, Mozetic M (2008) Surface modification of polyester by oxygen- and nitrogen-plasma treatment. Surf Interface Anal 40:1444–1453
18. Vesel A, Mozetic M, Zalar A (2008) XPS characterization of PTFE after treatment with RF oxygen and nitrogen plasma. Surf Interface Anal 40:661–663
19. Galya T, Sedlarik V, Kurika I, Novotny R, Sedlarikova J, Saha P (2008) Antibacterial poly(vinyl alcohol) film containing silver nanoparticles: preparation and characterization. J Appl Polym Sci 110:3178–3185
20. Galya T, Sedlarik V, Kuritka I, Sedlarikova J, Saha P (2008) Characterization of antibacterial polymeric films based on poly(vinyl alcohol) and zinc nitrate for biomedical applications. Int J Polym Anal Ch 13:241–253
21. Galya T, Sedlarik V, Sedlarikova J, Valasek P, Saha P (2010) The effect of preparation temperature on the mechanical and antibacterial properties of poly(vinyl alcohol)/silver nitrate films. Polym Degrad Stabil 95:399–404
22. Sedlarik V, Galya T, Sedlarikova J, Valasek P, Saha P (2010) The effect of hydrolysis degree on the properties of antibacterial polymeric films based on poly(vinyl alcohol) and zinc sulphate for biomedical applications. J Biomater Sci Polym Ed 21:1421–1440
23. Asadinezhad A, Novak I, Lehocky M, Bilek F, Vesel A, Junkar I, Saha P, Popelka A (2010) Polysaccharides coatings on medical-grade PVC: a probe into surface characteristics and the extent of bacterial adhesion. Molecules 15:1007–1027
24. Sowe M, Polaskova M, Kuritka I, Sedlacek T, Merchan M (2009) Analysis of antibacterial action of polyvinyl chloride surface modified with gentianviolet. Int J Polym Anal Ch 14:678–685
25. Sedlarik V, Saha N, Kuritka I, Saha P (2006) Preparation and characterization of poly (vinyl alcohol)/lactic acid compounded polymeric films. Int J Polym Anal Ch 11:253–270
26. Crisante F, Francolini I, Bellusci M, Martinelli A, D'Ilario L, Piozzi A (2009) Antibiotic delivery polyurethanes containing albumin and polyallylamine nanoparticles. Eur J Pharm Sci 36:555–564
27. Francolini I, Norris P, Piozzi A, Donelli G, Stoodley P (2004) Usnic acid antimicrobial agent able to inhibit bacterial biofilm formation on polymer surfaces. Antimicrob Agent Ch 48:4360–4365
28. Danese PN (2002) Antibiofilm approaches: prevention of catheter colonization. Chem Biol 9:873–880
29. Shintani H (2004) Modification of medical device surface to attain anti-infection. Trends Biomater Artif Organs 18:1–8
30. Donetz AP, Harvey RA, Greco RS (1984) Stability of antibiotics bound to polytetrafluoroethylene with cationic surfactants. J Clin Microbiol 19:1–3
31. Li LC, Deng J, Stephens D (2002) Polyanhydride implant for antibiotic delivery-from the bench to the clinic. Adv Drug Deliver Rev 54:963–986
32. Shamsipur M, Talebpour Z, Bijanzadeh HR, Tabatabaei S (2002) Monitoring of ampicillin and its related substances by NMR. J Pharm Biomed Anal 30:1075–1085
33. Makuc D, Albrecht M, Plavec J (2010) Conformational changes of functionalized indole receptors upon their interaction with anions. Supramol Chem 22:603–611
34. Trajkovski M, Sket P, Plavec J (2009) Cation localization and movement within DNA thrombin binding aptamer in solution. Org Biomol Chem 7:4677–4684
35. Makuc D, Triyanti, Albrecht M, Plavec J, Rissanen K, Valkonen A, Schalley CA (2009) The halide behaviour of 2-Carbamoyl-7-ureido-1H-indoles: conformational aspects. Eur J Org Chem 28:4854–4866
36. The United States pharmacopeia. Material safety data sheet; Ampicillin Sodium USP (25 Jan 2008)
37. The United States pharmacopeia. Material safety data sheet; Minocycline hydrochloride USP (14 Feb 2007)
38. Leadbitter J, Day JA, Ryan JL (1997) PVC: compounds, processing and applications. Rapra, Shawbury
39. Giles HF, Wagner JR, Mount EM (2005) Extrusion: the definitive processing guide and handbook, vol 1. Plastics design library, Norwich
40. Chanda M, Roy SK (2007) Plastics technology handbook. CRC Press, Boca Raton
41. Gallo G, Radaelli P (1976) Analytical profiles of drug substances. Academic Press, New York
42. Henwood SQ, de Villiers MM, Liebenberg W, Lötter AP (2000) Solubility and dissolution properties of generic rifampicin raw materials. Dug DevInd Pharm 26:403–408
43. Thangadurai S, Abraham JT, Srivastava AK, Moorthy MN, Shukla SK, Anjaneyulu Y (2005) X-Ray powder diffraction patterns for certain β-lactam, tetracycline and macrolide antibiotic drugs. Anal Sci 21:833–838

44. Wypych G (2004) Handbook of plasticizers. William Andrew Publishing, Norwich
45. Merchan M, Sedlarikova J, Sedlarik V, Machovsky M, Saha P (2010) J Appl Polym Sci 118: 2010–2369
46. Small PA (1953) Some factors affecting the solubility of polymers. J Appl Chem 3:71–80
47. Tiwari S, Murthy T, Raveendra M, Mehta R, Chowday P (2003) Controlled release formulation of tramadol hydrochloride using hydrophilic and hydrophobic matrix system. Pharm Sci Tech 4:1–6
48. The United States pharmacopeia. The national formulary. USP 29th revison. NF 24th edn (1 Jan 2006)