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MODEL ANTIMICROBIAL POLYMER SYSTEM BASED ON POLY(VINYL CHLORIDE) AND CRYSTAL VIOLET

MODEL PROTIMIKROBNEGA POLIMERNEGA SISTEMA NA OSNOVI POLIVINILKLORIDA IN KRISTAL VIOLETA

Jiří Klofáč[1,2], Ivo Kuřitka[1,2], Pavel Bažant[1,2], Kristýna Jedličková[1,2], Jakub Sedlák[1,2]

[1]Polymer Centre, Faculty of Technology, Tomas Bata University in Zlin, Nam. T. G. Masaryka 275, 762 72 Zlin, Czech Republic
[2]Centre of Polymer Systems, University Institute, Tomas Bata University in Zlin, Nad Ovcirnou 3685, 760 01 Zlin, Czech Republic
j_klofac@ft.utb.cz

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The development of novel antimicrobial materials for biomedical application in indwelling devices such as catheters is very important as the resistance of the pathogens responsible for nosocomial infections towards recent systems has been emerging with an increasing rate. The work presented here is focused on the preparation and characterization of an antimicrobial polymeric system composed of poly(vinyl chloride) in combination with crystal violet as a model compound of organic ionic active species. The antimicrobial activity of the system against gram-negative bacteria *Escherichia coli*, gram-positive bacteria *Staphylococcus aureus* and yeasts *Candida albicans* as the representative microorganisms were evaluated with a disk-diffusion test. The release profile of the active substance was observed with UV-VIS spectrometry. The mechanical properties of the prepared material were tested to verify that they were not altered with respect to the original medical-grade polymer matrix.

Keywords: poly(vinyl chloride), crystal violet, antibacterial, antimicrobial, release

Pomemben je razvoj novega protimikrobnega materiala za biomedicinsko uporabo notranjih pripomočkov, kot so katetri, ker se vedno pogosteje pojavlja odpornost patogenov, odgovornih za bolnišnične okužbe. Predstavljeno delo je osredinjeno na pripravo in karakterizacijo protimikrobnega polimernega sistema, ki ga sestavlja polivinilklorid v kombinaciji s kristal violetom kot model za spojine organskih ionskih aktivnih vrst. S ploščinskim difuzijskim preizkusom je bila ocenjena protimikrobna dejavnost sistema proti predstavnikom mikroorganizmov: gramnegativni bakteriji *Escherichia coli*, grampozitivni bakteriji *Staphylococcus aureus* in kvasovkam *Candida albicans*. Profil sprostitve aktivnih snovi je bil opazovan z UV-VIS-spektrometrijo. Mehanske lastnosti pripravljenega materiala so bile preizkušene, da bi potrdili, da niso drugačne od navadnega medicinskega polimera.

Ključne besede: poli(vinil) klorid, kristal violet, protibakterijski, protimikrobni, sproščanje

1 INTRODUCTION

Polymers are known for their high versatility and excellent physical-chemical properties and, in some cases, they are suitable as biomaterials for the medical sector and packaging industry. As a candidate for these applications, the third most common polymer, poly(vinyl chloride) (PVC), can be considered due to its high mechanical and chemical resistance, inertness against biological fluids and a wide range of processing possibilities.[1–3]Among many products, urinal catheters, blood bags and cardiovascular implants are typical items used in the medical sector; nevertheless, they exhibit a vulnerability towards surface bacterial colonization.[2,4] Therefore, it is important to enhance their antimicrobial properties by modifying the surfaces of such materials with a plasma treatment, corona discharge and chemical grafting.[5,6] Another promising strategy of modifying PVC is to incorporate an antimicrobial substance within the polymer matrix.[4,7,8] Such modifications have long-term effects and are relatively easy to perform, showing high rates of success. The solvent-cast technique allowing a preparation of the films with extremely high quality requirements and great uniformity of the thickness is often employed due to its advantage of easy blending of the films with the active molecular compounds soluble in the used solvent system.[9,10] The effectiveness of the antimicrobial properties of such films is strongly dependent on the release profile of the antimicrobial substance from the polymer matrix.[11]

Organic substances migrate, over time, out of the polymer matrix and onto the polymer surface and are then released into the surrounding liquids. Migration occurs as the organic molecules follow down a concentration gradient and exit the plastic. The migration is driven by the inherent compatibility differences between the organic antimicrobials and the polymer substrates in which they are dispersed. The losses of the organic molecules into the environment are replenished by the additives within the substrate volume. The benefit of this mode of action is that it can have a very high activity rate, and the migratory molecules can very quickly interact with large numbers of microbes. This does, however, affect the lifespan of the activity, as the additives leach out over time, emptying the polymer's reservoir. The concentration and choice of the respective organic additive depend on the level of efficacy required and the duration of the action needed.[12]

An antimicrobial agent release that is too fast can be harmful under certain conditions and perceived as undesirable. Besides inorganic fillers, ionic organic compounds can provide a real option in the material design having long-lasting mild effects due to their relatively slow migration rates. An organic salt structure comprised of a large bulky organic cation and a small inorganic anion can be considered to have the migration rates in a polyolefinic matrix slow enough in comparison with the molecular organic antimicrobial additives. As a well-known model representative for this class of compounds crystal violet (CV) can be chosen. It is a triarylmethane dye formerly used in medicine due to its antibacterial, antifungal, anthelmintic and antiseptic properties.[13–15] CV was generally considered to be safe for a long time in the history of medicine; however, many studies have reported that CV has potentially mutagenic and carcinogenic effects on humans and animals.[16–19] In spite of this, CV is an excellent model compound for the release-profile studies. This dye can be easily mixed with polymers; it has a very good and broad antimicrobial activity, giving deep violet colour to its solutions; hence, its concentration can be easily monitored with an UV-VIS absorption spectrometer.

This study focuses on the modification of a medical-grade PVC with a crystal-violet (CV) addition, using the solvent-casting technique resulting in a model organic antimicrobial polymer system. Its composition, morphology, mechanical properties, antimicrobial tests and the kinetics of the CV release from a polymeric matrix in water and physiological solution used as model liquids, were investigated in the presented work.

2 EXPERIMENTAL WORK

2.1 Materials

Medical-grade thermoplastic plasticized poly(vinyl chloride) (PVC) compound RB3 was purchased from Modenplast Medical (Italy). This material is in compliance with the European Pharmacopeia and biocompatible according to ISO 10993, USP, Class VI. Crystal violet $C_{25}N_3H_{30}Cl$ (CV) and cyklohexanone $C_6H_{10}O$ (CYH) were purchased from PENTA (Czech Republic). All the chemicals were of the analytical grade and used as received without further purification. Demineralized water was used for all of these experiments.

2.2 Sample preparation

PVC/CYH/CV films were prepared with the solvent-casting technique. In the first step a solution of CV in CYH was prepared: 0.2522 g of CV was added to 250 mL CYH to get a concentration of 1 g/L. 20.005 raw PVC in the form of granules was dissolved in 300 mL of CYH during a period 16 h at the room temperature under continuous stirring. The amount of 250 mL of the CV solution was added and this blend was left to mix for another 8 h. Finally, the mixing was finished with a sonication of the solution for 15 min in an ultrasonic bath. The solution was then poured into glass dishes and the solvent was allowed to evaporate at the laboratory temperature for 10 d. The PVC/CYH control sample was prepared with the same procedure, but without incorporating the CV. The conditions for the film preparation were chosen on the basis of practical laboratory experience, with the aim to achieve the smoothest fine films of comparable quality. The thickness of the resultant films was about 500 μm.

2.3 Characterization

2.3.1 Infrared absorption spectroscopy

A FTIR analysis was used to compare the PVC pellets, PVC/CYH and PVC/CYH/CV films. All the measurements were performed with a Nicolet 6700 spectrophotometer (Nicolet, Czech Republic) with the ATR accessory and the Ge crystal for the attenuated-total-reflection method.

2.3.2 SEM analysis

The micrographs of the prepared materials were taken with a Vega II LMU scanning electron microscope (Tescan, Czech Republic). The freeze fracture surfaces were obtained with liquid nitrogen and observed after the coating with a thin layer of gold/palladium by an SC 7640 sputter coater (Quorum Technologies Ltd, UK).

2.3.3 Tensile tests

The effects of the CV added to the PVC matrix on the mechanical properties were studied using a tensile test. The specimens for the test were cut from the prepared film samples as rectangular stripes with the width of 5 mm and the length of 36 mm. The specimens were tested on a tensile testing machine Testometric M350-5CP (LABOR machine, Ltd.) at 25 °C according to standard ISO 37:2005. The speed of the moving clamp was 500 mm/min. The Young´s modulus, the stress at break and the strain at break were determined. All the samples were measured in 5 replicates and standard deviations were estimated.

2.3.4 Antimicrobial tests

The antimicrobial properties of the PVC/CYH/CV films were assessed using the agar-diffusion test. Round specimens (8 mm in diameter) were placed on Petri dishes with the nutrient agar inoculated with the dispersion of microorganisms (a concentration of CFU 1.0×10^7 mL^{-1}). The samples were tested against gram-negative *Escherichia coli* (EC) 4517, gram-positive *Staphylococcus aureus* (SA) 4516, and yeast *Candida albicans* (CA) CCN 8215. After a incubation 72 h at 23 °C for the yeast and a incubation 24 h at 37 °C for bacteria, the dimensions of the inhibition zones were measured in four directions, and the average values were used to calculate the diameter of the circle-zone inhibi-

tion area and its standard deviation. All the tests were done in triplicates.

2.3.5 Release of CV and plasticizers from the PVC matrix

Round specimens with a diameter of 12.7 mm were cut from the PVC/CYH/CV samples to be used in the release-profile study of CV in the water and physiological-solution environment. One specimen was always placed in a beaker with 50 mL of elution liquid and the beaker was shaken at 60 r/min to ensure a good homogenization of the liquid media. The measurement of the CV release was performed with a UV-VIS spectrophotometer Cary 300 (VARIAN, USA) equipped with a sipper (a peristaltic pump) and a flow cell (a cuvette). The whole spectral range (200–800 nm) was monitored and the spectra were recorded in the preselected time intervals covering representatively the full time range of each individual release experiment. The same procedure was used for obtaining the reference leachate for the specimens cut from the neat PVC sample with the thickness of 0.5 mm obtained by hot pressing at 170 °C for 5 min to evaluate the release of the plasticizers after three days, which was done to investigate the influence of either the CV addition or the preparation process on the release of the plasticizers from the PVC matrix. The absorbance value at the wavelength of 580 nm was chosen for a quantitative evaluation of the observed release profile of CV because this is the position of the absorption maximum of CV in the elution medium. The data were then converted to the concentration of the released CV using calibration curves. The data were fitted with a non-linear fitting procedure using the Lavenberg-Marquart algorithm incorporated in the Origin 7.0 software.

3 RESULTS AND DISCUSSION

3.1 Infrared absorption spectroscopy

The aim of the FTIR analysis was to study a possible modification of the PVC material with the preparation process and an addition of CV. In **Figure 1**, the FTIR spectra of the neat PVC (pellets), the processed PVC/CYH sample and the modified PVC/CYH/CV material are plotted. The infrared absorption spectrum of an unplasticized polyvinyl chloride contains the bands typical for the aliphatic CH groups at their most typical positions, except that, due to the CH_2 deformation vibration, a band is shifted by about 30 cm^{-1} to the lower wavenumbers, nearly to 1430 cm^{-1} as typically observed for PVC. In addition to the aliphatic CH bands, the spectra of PVC contain contributions due to the C-Cl vibrations that can be found as a weak band at 1425 cm^{-1} and as a medium-intensity band at 959 cm^{-1}. The most intense and significant band for the C-Cl vibration at 610 cm^{-1} cannot be observed due to the range of measurement. In general, the spectrum of the neat PVC

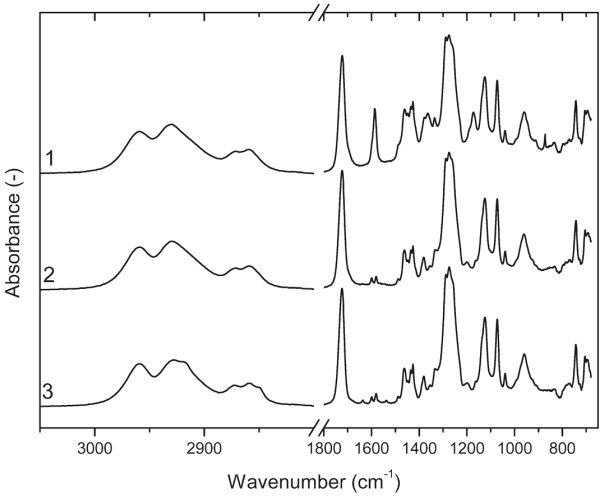

Figure 1: FTIR ATR spectra of neat PVC pellets (curve 3), PVC/CYH film (curve 2) and PVC/CYH/CV film (curve 1) samples. The region between 2800–1800 cm^{-1} is hidden in the graph as no absorption peaks were manifested.

Slika 1: FTIR ATR-spektri vzorcev gladkih PVC-pelet (krivulja 3), PVC/CYH-plast (krivulja 2) in PVC/CYH/CV-plast (krivulja 1). Področje 2800–1800 cm^{-1} je skrito v grafu, ker tam ni bilo izrazitih absorpcijskih vrhov.

material is greatly affected by the presence of plasticizers and dominated by their absorption bands. The manifestation of the polymer matrix is, therefore, quite weak. The most prominent band at 1725 cm^{-1} can be assigned to the carbonyl group (C=O) stretching mode typically observed for the plasticizers. It can be expected that the medical-grade PVC contains the additives circumventing the crucial plasticizer migration problem associated with the softened PVC. The position of this peak is too low for the aliphatic low-molecular plasticizers such as dioctyl-sebacate, citrate or adipate esters. On the other hand, a common phthalic ester plasticizer with a typical manifestation of the carbonyl group at 1720 cm^{-1} cannot be successfully used as a medical material intended for modern indwelling applications. A careful analysis of the wavenumber region between 1500 cm^{-1} and 1650 cm^{-1} revealed that there is a quadruplet of peaks at positions (1540, 1580, 1600 and 1637) cm^{-1}, while the phthalic ester plasticizers only display doublets at 1580 cm^{-1} and 1600 cm^{-1}. Alkyde (based on vegetable fatty acids) polyanhydrides were found as the highest scoring records in the available IR spectra database[20]; however, the exact identification was impossible. There is virtually no difference between the spectra recorded for the neat material and for the PVC/CYH sample, proving that the RB3 composition did not change during the solution-casting process and that no solvent residuals were manifested.

The IR absorption spectrum of the PVC/CYH/CV sample displays all the characteristic peaks of CV in addition to the aforementioned spectral features of the plasticized medical-grade PVC; namely, 1587 cm^{-1} due to the C=C stretching in phenyl rings, 1365 cm^{-1} due to

the C-H deformation vibrations in methyl groups, 1174 cm^{-1} due to the C-H in-plane deformation in 1,3,5 substituted aromatic ring, and 1128 cm^{-1} due to the C-N stretching vibration in trisubstituted aromatic amines.

3.2 SEM analysis

SEM images were obtained for the freeze-fracture surfaces of the films prepared with CV. The PVC/CYH/CV film morphology before the immersion into the liquid media is shown in **Figure 2a** and the morphology of the PVC/CYH/CV film after the release-profile measurement is shown in **Figure 2b**. First, there are no observable crystals of CV in the polymer matrix, and second, there is no observable change in the material after the release test.

3.3 Tensile tests

The influence of the PVC modification with CV on the mechanical properties of the material prepared by casting from a CYH solution can be seen in **Table 1** where the measured values with their standard deviations are summarized. The mechanical properties of the PVC/CYH film and the PVC/CYH/CV film are very similar. The Young's modulus of PVC/CYH and PVC/CYH/CV is about 5 MPa and the tensile stress at break is about 11–13 MPa. The only property showing a slight difference between the samples is the strain at break. The PVC with CV shows a higher deformation (elongation) ability than the pure PVC sample, which can be considered as an advantage of the material with the additive. Moreover, the obtained result is in accordance with the microscopic observation, both testifying a good dispersion (blending) of CV in the material.

Table 1: Selected mechanical properties of the PVC/CYH and PVC/CYH/CV samples and their standard deviations
Tabela 1: Izbrane mehanske lastnosti vzorcev PVC/CYH in PVC/CYH/CV in njihov standardni odmik

Sample	Young's modulus (MPa)	Strain at break (%)	Tensile stress at break (MPa)
PVC/CYH	5.0 ± 0.6	490 ± 40	11 ± 3
PVC/CYH/CV	4.8 ± 0.4	620 ± 50	13 ± 3

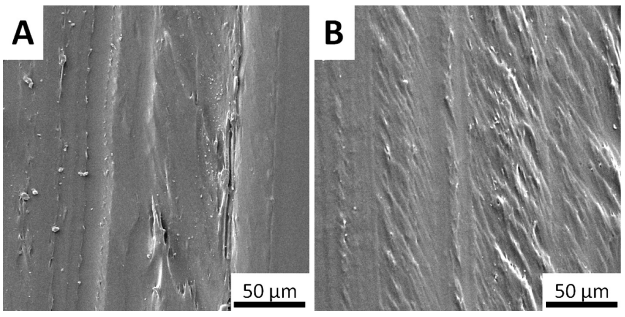

Figure 2: Microphotographs of: a) PVC/CYH/CV before immersion and b) after 3 d in the liquid
Slika 2: Posnetka: a) PVC/CYH/CV pred potopitvijo v tekočino in b) po 3 d

3.4 Antimicrobial activity

The results of the antimicrobial-activity halo-zone test against *S. aureus, E. coli* and *C. albicans*, performed with the agar-diffusion test method, are presented in **Table 2**, while **Figure 3** demonstrates the inhibition zones around the samples of the antimicrobial material (PVC/CYH/CV) studied with the agar-diffusion test. The obtained values show that the pure PVC material has no antimicrobial properties, but the PVC with CV shows an activity against all the tested microorganisms.

Table 2: Antimicrobial activity expressed as inhibition-zone diameters and their standard deviations for PVC/CYH and PVC/CYH/CV
Tabela 2: Protimikrobna aktivnost, izražena kot premer področja zaviranja in njegov standardni odmik za PVC/CYH in PVC/CYH/CV

SAMPLE	*SA*	*EC*	*CA*
PVC/CYH (mm)	0	0	0
PVC/CYH/CV (mm)	14.8 ± 0.9	10.3 ± 1.0	15 ± 3

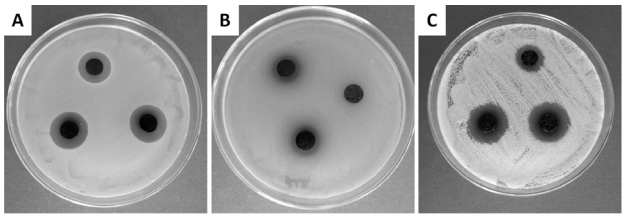

Figure 3: Photographs of Petri dishes after cultivation in the agar-test diffusion zone against: a) *S. aureus*, b) *E. coli,* c) *C. albicans*
Slika 3: Posnetki petrijevke po kultiviranju v difuzijski coni preizkusa z agarjem proti: a) S. *aureus*, b) *E. colli*, c) *C. albicans*

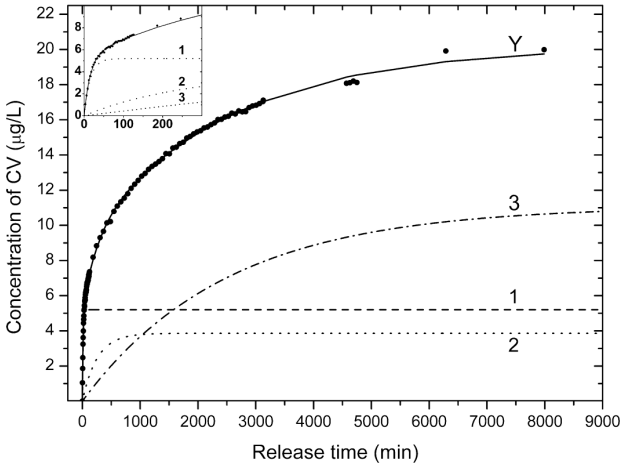

Figure 4: Release profile of CV from PVC/CYH/CV in the demineralised water. The experimental data points are represented by full-circle symbols; curve Y represents equation (3) fitted into the data; curves 1, 2 and 3 represent the single-term contributions to curve Y, respectively. The inset graph shows detailed data from the initial stage of the experiment.
Slika 4: Profil sproščanja CV iz PVC/CYH/CV v demineralizirani vodi. Eksperimentalni podatki so prikazani s polnimi krogci, krivulja Y ponazarja enačbo (3), urejeno s podatki; krivulje, označene z 1, 2 in 3 so posamezni prispevki h krivulji Y. Vstavljeni diagram prikazuje podrobne podatke iz začetka preizkusa.

3.5 Release profile of CV from PVC/CYH/CV films

The obtained release profiles are shown in **Figures 4** and **5** where the dependences of the CV concentration in the elution liquids are plotted in dependence on the release time for demineralised water and physiological solution, respectively. According to the literature, the first-order kinetic model can be a suitable formal kinetic description of the process of a water-soluble compound release from an insoluble polymer matrix to the liquid medium although it cannot be straightforwardly related to the sample geometry and it is difficult to conceptualize this mechanism on a theoretical basis.[21]

The release rate of the model compound (CV in our case) that obeys first-order kinetics can be expressed with the following equation:

$$\frac{\mathrm{d}c}{\mathrm{d}t} = -\frac{1}{\tau}c \tag{1}$$

where c is the concentration of the model compound in the elution media, the expression on the left side of the equation is the release rate defined as the concentration increase rate in the elution medium (directly obtained from absorbance, which is the observable quantity in this study), t is the release time, τ^{-1} is the first-order release-rate constant. Equation (1) can be integrated into the following form:

$$c = C_{\max}\left(1 - exp\left(-\frac{t}{\tau}\right)\right) \tag{2}$$

where parameter $C_{\max}$ is the integration constant representing the maximum achievable concentration of the model compound in the elution media for the infinite time. With respect to the second boundary condition, it is assumed that the initial concentration of the model compound is zero in the liquid media at the beginning of all the experiments. The rest of the variables and constants have the same meanings as in equation (1).

It can be expected that this formal description only relates to a limited concentration range and to certain boundary conditions. According to our observations, several mechanisms can be active at different time scales during the release process and, thus, it is reasonable to extend the kinetic description by one or two more terms for the first-order processes if they differ significantly in their rate constants, i.e., by orders of magnitude. The following equation represents the extension of equation (2) for three formally independent and additive contributions to the release process:

$$c = C_{\max} - C_1 \cdot exp\left(-\frac{t}{\tau_1}\right) - C_2 \cdot exp\left(-\frac{t}{\tau_2}\right) - C_3 \cdot exp\left(-\frac{t}{\tau_3}\right) \tag{3}$$

where C_1, C_2, C_3 represent the maximum contribution of each process to the infinite time $C_{\max}$ concentration. The rest of the variables and constants have the same meanings as in equation (2) with the indexes showing their relations to the respective process. It is obvious that:

$$C_1 + C_2 + C_3 = C_{\max} \tag{4}$$

For the two processes involved in the release, equation (3) can be simplified by omitting the third term.

Obtained equation (3) was fitted into the experimental data as can be seen in **Figure 4** representing the water and **Figure 5** representing the physiological solution where the two-term variant was used. The contributions of each process are plotted separately with simulated curves for a better clarity. The obtained parameters are summarized in **Table 3**. This mathematical analysis was performed with a full awareness of the fact that the terms in equation (3) are not sequential but running parallel as

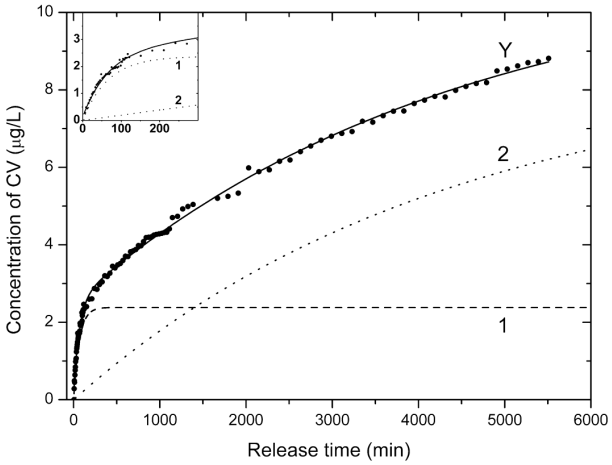

Figure 5: Release profile of CV from PVC/CYH/CV in the physiological solution. The experimental data points are represented by full-circle symbols, curve Y represents simplified equation (3) without the third term fitted into the data; curves 1 and 2 represent the single-term contributions to curve Y, respectively. The inset graph shows detailed data from the initial stage of the experiment.

Slika 5: Profil sproščanja CV iz PVC/CYH/CV v fiziološki raztopini. Eksperimentalni podatki so prikazani kot simboli polnega kroga, krivulja Y ponazarja poenostavljeno enačbo (3) brez vključitve tretjega izraza v podatke; krivulji z oznako 1 in 2 pomenita posamezen prispevek h krivulji Y. Vstavljeni diagram prikazuje podrobne podatke iz začetka preizkusa.

Table 3: Fitting-equation parameters and their standard errors describing the release profile of CV from the polymer matrix in the PVC/CYH/CV sample

Tabela 3: Parametri urejanja enačb in njihove standardne napake, ki opisujejo profil sproščanja CV iz polimerne osnove v vzorcu PVC/CYH/CV

Environment	Water	Physiological solution
$C_{\max}$ /(μg/L)	20.19 ± 0.14	11.15 ± 0.24
C_1/(μg/L)	5.20 ± 0.12	2.38 ± 0.06
T_1/min	15.2 ± 0.7	66 ± 3
C_2/(μg/L)	3.86 ± 0.16	8.63 ± 0.21
T_2/min	256 ± 24	4340 ± 220
C_3 /(μg/L)	11.09 ± 0.14	n. a.
T_3/min	2488 ± 98	n. a.

they use the common time and start at $t = 0$. However, the differences in the rate-constant magnitude separate them to an acceptable level resulting in a good approximation. Each process (term) dominates its own time-scale window and relies on its specific concentration range as it can be seen from the graphs.

The first exponential component (the term with the shortest time constant, τ_1) probably represents the release of CV from the matrix surface, because this process is the shortest and could only be limited by the CV solubility in water that is 10 g/L as indicated by the supplier. It is evident, that even the highest CV concentrations in the elution medium are far from approaching this limit. In the case of the physiological solution that shares a chloride anion with CV the solubility must be lower due to the solubility-product limitation; however, even here the solubility is more than several orders in magnitude higher than the observed concentrations. The value of the solubility product is $K_s = 6 \times 10^{-4}$ mol^2 dm^{-6} estimated roughly from the CV solubility in water. The solubility in the physiological solution can be derived by solving the following equation:

$$(x + 0.154 \text{ mol/L})x = K_s \quad (5)$$

where x is the maximum CV concentration and 0.154 mol/L is the chloride concentration in the physiological solution. The equation gives only one positive root, x = 0.0038 mol/L, which corresponds to the CV concentration of 1.55 g/L. This value is about six and a half times lower than the limitation for the sample in distilled water.

The second phase of the release process is slower because the CV readily available from the matrix surface is already depleted and the CV from the subsurface layers of the film needs to cross an energetic barrier before being released into the demineralised water.

The third phase is characterized by a further significant decrease-release rate that can be ascribed to the diminishing of the gradient between the film surface and the solution layer in its proximity and to the depletion of the extractable CV in the subsurface of the film. According to the macroscopic observation, the material changed neither its colour nor any other property after its immersion into the liquid for several days. No dimension or significant mass changes were observed which confirms there was no swelling or matrix-component dissolution. Therefore, we believe that the CV located in the deeper layers of the material is not released into the solution in the relevant time horizon.

These three phases were observed for the sample immersed in the water. The sample in the demineralised water has a higher saturation value (C_{max}) than the sample in the physiological solution. In general, this might be caused by the omnipresence of chloride anions with a relatively high concentration diminishing all the gradients discussed above in the case of pure water.

The first and second processes of the sample in the physiological (saline) solution were slow and the third process was not observed at all. In this case, the solubility of CV is influenced by the presence of the chloride anion, which is commonly shared between CV and the physiological solution. Moreover, the CV molecule can leave the polymer matrix as a CV^+ cation and a Cl^- anion, always in a pair, i.e., in the ratio of 1 : 1 due to the electroneutrality condition that must always be kept. This condition is obviously satisfied in the case of water, whereas in the case of the physiological solution this pair would be released into a medium with a high concentration of chloride anions. Alternatively, a lone CV^+ cation can be released into the liquid with a concurrent counter transport of a Na^+ cation to the matrix. Both options can be considered for significantly slowing and limiting the diffusion process, so only the first two phases were observed within the time scale of several days.

3.6 Plasticizer role in the release of CV from PVC/CYH/CV films

Although the neat PVC material has been approved for medical use and can be considered as safe from the point of view of the release of the contained plasticizer or plasticizers, it must be re-evaluated after being mixed with CV as the eventual synergic effects cannot be excluded and the release of the plasticizer could be enhanced by adding other species to the compound. A simplified test was performed analysing the absorption spectra in the wavelength region where both the plasticizer and CV absorb light.

The graph in **Figure 6** shows the UV absorption spectra of the leachates obtained for the PVC/CYH/CV and neat PVC samples after a three-day elution in water. The third curve represents the absorption spectrum of CV in water with the same concentration as that in the liquid media collected for PVC/CYH/CV. It can be

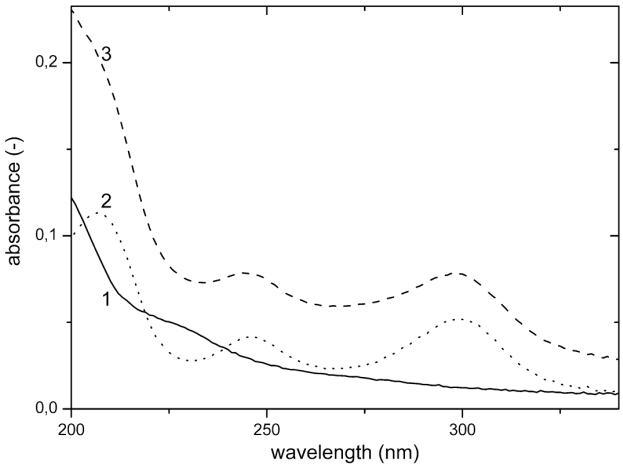

Figure 6: UV absorption spectra recorded for the CV solution in water (curve 2), leachate from the neat PVC specimen (curve 1) and leachate obtained for the PVC/CYH/CV specimen (curve 3). For details, see the text.

Slika 6: Posneta absorpcija UV-spektra za raztapljanje CV v vodi (krivulja 2), izcedna voda iz čistega PVC-vzorca (krivulja 1) in izcedna voda iz PVC/CYH/CV-vzorca (krivulja 3). Za podrobnosti glej tekst.

clearly seen, that there is no enhancement of the plasticizer release and that only a simple additivity of the signals takes place as the absorption spectrum recorded for PVC/CYH/CV is approximately the sum of the spectra of the plasticizer released from the neat PVC sample into the liquid medium and the CV solution. The test for the physiological solution showed the same result, but it is not shown here for the sake of brevity.

4 CONCLUSIONS

A model organic antimicrobial polymeric PVC/CYH/CV system based on medical-grade poly(vinyl chloride) and crystal violet was prepared with the solvent-casting technique. The work was focused on investigating the effects of the used technique for preparing and entering substances and it was shown that the CYH solvent and the model CV-active substance did not have any adverse influence on the chemical structure, morphology and mechanical properties.

The prepared solvent-cast materials can be used in the form of a film, as a volume material or as an additive for further compounding but, preferentially, we aim at various coatings and thin-film applications on the surfaces of medical devices or other plastic articles wherever this technique allows hopes for a good adhesion and compatibility with the substrate material, especially when coated on the plastic articles made of the same neat PVC resin.

The antimicrobial activity was investigated using the agar-diffusion test method and the PVC/CYH/CV material manifested a good antimicrobial activity against gram-positive *S. aureus,* gram-negative *E. coli* and yeast *C. albicans*. Although the material is an organic-doped antimicrobial polymer system, the release profile of CV, as the representative model compound with a large organic cation and halide anion, to the demineralised water and physiological solution simulating body liquids is appropriately slow allowing a long-lasting mild delivery effect of the active species on the closest proximity of the place of insertion or application. Next, no adverse effect of either the CV addition to the PVC matrix or the preparation process on the release of the plasticizers from the PVC matrix was observed.

These results suggest that the prepared model material has a potential in medical plastic industries and the obtained knowledge can be generalised to a certain degree, without losing its relevance, covering the whole class of modelled compounds and used for a further development of the materials or coatings for PVC medical devices and hygienic products.

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