MEDICAL-GRADE POLYVINYL CHLORIDE MODIFIED WITH CRYSTAL VIOLET AND MONTMORILLONITE

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Bacterial colonization of polymeric medical aids causing so-called nosocomial infections is one of the main problems linked with the extensive use of polymeric devices in clinical applications. In this study, the properties of medical-grade polyvinyl chloride were modified by incorporating crystal violet into its bulk structure. Sodium montmorillonite was used as a carrier for the crystal violet in the mixture and was expected to effectively control the release rate of the crystal violet to and from the surface of the polyvinyl chloride. The extent of release of the crystal violet from such composites was determined using various leaching media.

***Keywords*: Controlled drug release; Crystal violet; Ion exchange; Montmorillonite; Polyvinyl chloride**

INTRODUCTION

Polyvinyl chloride (PVC) is widely used as a medical material in urological catheters, blood storage bags, and many other devicesJ[11] In recent years, there has been a marked interest in the design and development of medical devices with improved resistance to microbial attachment and biofilm formation, which would constitute a major advance in decreasing the morbidity of patients, especially those with long-term indwelling catheters and stents, and would contribute greatly to cost savings in medical careJ[2,3]^

It has been suggested that the success of antimicrobial chemotherapy/ modification for inhibition/reduction of microbial biofilm formation on medical devices may be markedly improved by maintaining sufficiently high concentrations of the antimicrobial agent in the vicinity of the device. To facilitate this requirement, there have been several studies in which antimicrobial agents have been directly incorporated into, or onto, biomaterialsJ[4-61]

In general, the mass of drug that can be incorporated into the medical device is limited and may often be insufficient for a prolonged antimicrobial effect in vivo. In addition, the subsequent release of the antimicrobial agent following clinical insertion of the device is rapid and relatively uncontrolled. In order to ensure sufficient concentration of bioactive agents at the surface in the long term, controlled release systems through some types of carriers represent suitable meansJ[7,8]^

In this study, medical-grade PVC is modified by incorporating crystal violet and montmorillonite into its bulk structure. In this way, the antimicrobial agent, crystal violet, is uniformly dispersed throughout the polymer, providing long-lasting activity. The montmorillonite is expected to control the extent of release of crystal violet from the surface of the polymer, thereby creating a continuous sterile surface that is less susceptible to bacterial infection. The extent of release of crystal violet from the composites employing this method is determined using different solvents to simulate environmental conditions of body fluids, e.g., gastric juice or physiological solution.

EXPERIMENTAL SECTION

Materials

Commercially available medical-grade polyvinyl chloride, RB3 grade, produced by Moden Plast, S.P.A (Italy), was used as the polymeric matrix in this study. Crystal violet (CV), supplied by PENTA, Ing. Petr Švec, was chosen as an active antibacterial agent additive. Sodium montmorillonite (MT) (92meq/100g), purchased from Southern Clay, was used as a filler for control of CV release. MT was also used as a starting material for modified montmorillonite (MMT) preparation, and its processing is described below.

Intercalation of CV into MT was carried out by conventional ion exchange reaction adopted from Wan et alJ[9]^ as follows: 25 g of MT was dispersed in a mixture of lOOOmL distilled water and 600 mL ethanol by stirring at 25°C for 1 h, and then subjected to ultrasound for 20 min to improve dispersion of the components. A solution of 7.65 g of CV in 400 mL of ethanol was added dropwise by continuous stirring. The reaction mixture was then mixed by stirring for 72 h at room temperature. At the end of the reaction, the mixture was filtered on a Buchner funnel and washed until the filtrate was clear, using ethanol/water (volume ratio of 1/2) solvent mixture. Consequently, the solid product was oven-dried at 35°C for 24 h. The dry product was then ground to a fine powder using a pestle and mortar.

Compounding

PVC pellets were melt-mixed with various amounts of additives, according to the formulation in Table I. The same ratio (3:10) between CV and the mineral part of the filler was kept in all samples. This ratio was derived from the initial composition of MMT. The melt-mixing process was performed via a Haake MiniLab II Micro Compounder at 160°C and 70rpm. The compounding time of 10 min was sufficient for achievement of constant torque of the drive motor in all compounded samples. Then, prepared PVC mixtures were compression

Table I. Composition of prepared samples

Sample label	CV	MT	MMT
Pure PVC	–	–	–
A1	0.5	–	–
A2	1.2	–	–
A3	2.3	–	–
B1	0.5	1.5	–
B2	1.2	3.8	–
B3	2.3	7.7	–
C1	–	–	2
C2	–	–	5
C3	–	–	10

molded at 160°C into 0.5 mm thick sheets used for the preparation of samples for measurements.

Leaching Test of Crystal Violet from Polymer Composite

Ethanol/water (1/2) solvent mixture (Et0H/H_20), 0.01 M hydrochloric acid (HC1), distilled water, and physiological solution, 0.9 wt.% of NaCl (NaCl/H_20), were used to determine the extent of release of the CV from the PVC composite with the highest filler content. For this purpose, samples of 1 cm diameter discs were immersed in lOmL of each liquid medium in sealed glass bottles. The bottles were treated in a Stuart Orbital Incubator SI500 at 37°C and 80 rpm for 13 days. The samples were then removed and the leachate tested for the presence of crystal violet using a Cary 300 UV-vis Absorption Spectrometer (Varian, Inc., USA).

Scanning Electron Microscopy

A VEGA II LMU (Tescan, Czech Republic) was used to provide images of the surface morphology of the pure PVC and modified samples. Before the measurements were carried out, the samples were sputter coated with a palladium- gold alloy. The samples were tilted at an angle of 30°C and measurements were carried out at an accelerating voltage of 20.0 kV under a magnification of 30,000 x.

Water Contact Measurement

Water contact angle (WAC) was determined employing the sessile drop method on Surface Energy Evaluation (SEE) System (Brno, Czech Republic) equipment. The measurements were performed at room temperature using deionized water. For each measurement, 5 JJLL of liquid were dropped on the sample surface. In order to improve precision, 10 independent measurements were made for each sample, and an average contact angle value determined. Measurements were made 30 s after the water drop rested on the surface of the sample. The estimated standard error in contact angle measurements was ±3°C.

Optical Microscopy

The structure of the prepared composites was observed using an Olympus CX31 RBSF optical microscope (Japan). Thin slices (about 40 jxm) were cut by means of a Leica RM2255 rotational microtome, and pictures of observed areas were recorded with an Olympus SP-350 camera.

Powder X-ray Diffraction

X-ray powder diffraction was performed in reflection mode using X'Pert PRO Multi-Purpose Diffractometer (PANanalytical), operating with Cu Ka monochromatic radiation. Radial scans of intensity versus diffraction angle *26* were recorded in the range of 2-12° by steps of 0.0263° and step-scan interval of 0.79 s at ambient temperature.

RESULTS AND DISCUSSION

The above described methods were used to evaluate the influence of various amounts of additives on resulting properties of PVC composites as well as the effect of ion exchange capacity (IEC) modification of MT in comparison with pristine fillers.

Modification of MT

Layered silicates are susceptible to intercalation by various cations by a simple ion exchange process. The interlayer space can be loaded even by large molecules with positive charge such as cationic dyesJ[9-121] Among them, suitable species with antimicrobial activity and long tradition in medical use can be foundJ[13,14]^ CV has been chosen for PVC modification in our study. In order to control its release from PVC, two material combinations with MT were investigated. The first is a simple co-mixing of MT and CV into PVC, which is expected to slow down and prolong the release of CV into various liquid media. The second route of taking control over the release of CV from the material is ionic bounding of the CV into the interlayer galleries of montmorillonite prior to its compounding with PVC. Thus, the release of CV into the surrounding environment is controlled by the de-intercalation process.

Comparison of powder X-ray diffraction patterns for pristine MT and MMT is shown in Figure 1. Successful intercalation of CV between MT layers was confirmed, as can be seen from the shift of position of peak maxima of MMT to lower diffraction angles (about 4.1 *20)* than that of the pure MT (about 7.2 *20).* This phenomenon clearly indicates an increase of distance of individual layers of clay due to CV intercalation.

PVC Composite Materials

The optical microscope images in Figure 2 illustrate a fairly uniform dispersion of the filler within the polymer matrix in the 10-micrometer scale. Various dispersion levels were achieved for material containing mineral filler. While the coarsest particle structure was obtained in the case of samples B (1-3), the finest structure was

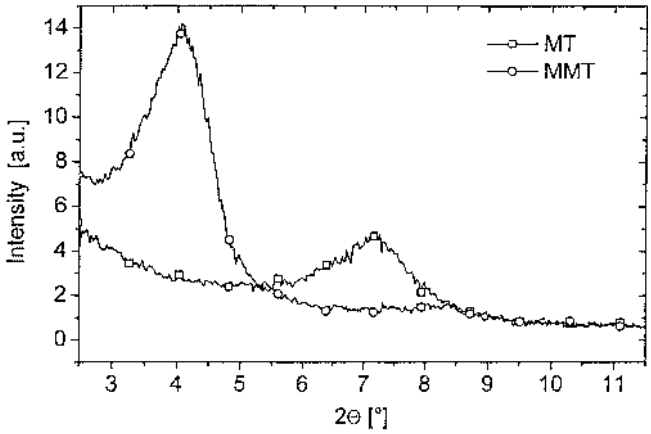

Figure 1. X-ray powder diffraction of MMT and MT.

monitored for samples C (1-3). Thus it is clear that the modification of MT through its intercalation with CV simplifies the processing of the composite, since after the same time of mixing two different states of dispersion can be obtained. As sufficient representatives, the samples with the highest loading are shown in Figure 2.

Bulk modification caused surface modification as well. Since it is a surface of the material that is in contact with host tissue, its properties strongly influence material biocompatibility. SEM images (Figure 3) show that modified PVC samples have coarser surface than pure PVC. This microrugosity significantly increases the

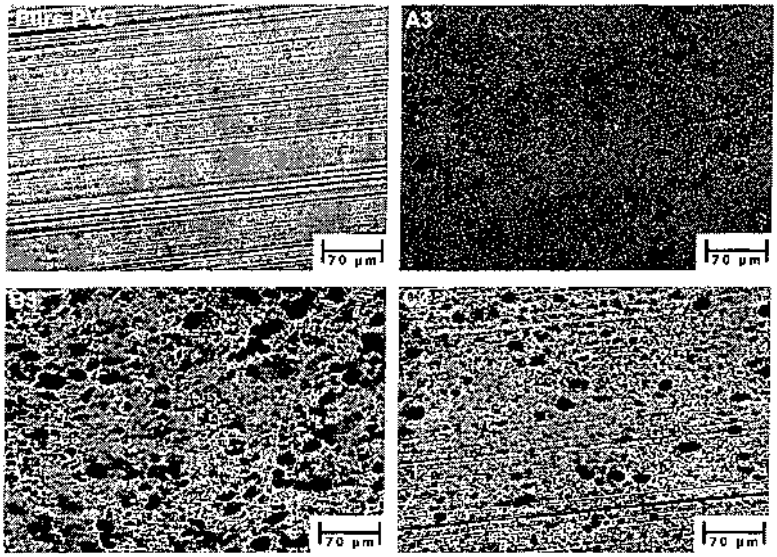

Figure 2. Optical microscope images of cross-sectional area of chosen samples (pure PVC, A3, B3, C3).

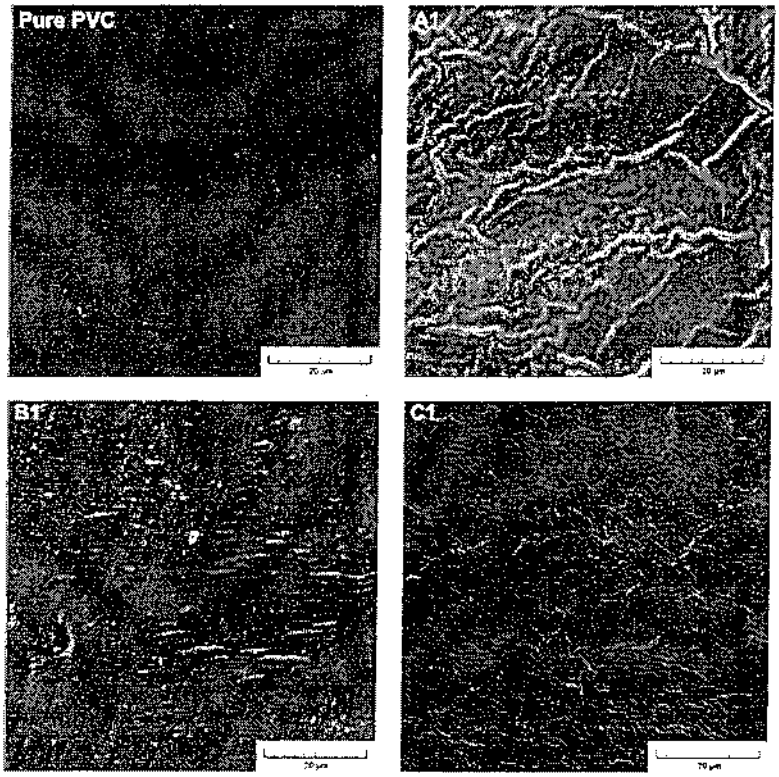

Figure 3. SEM details of surfaces of chosen samples (pure PVC, Al, Bl, Cl).

surface that is in contact with the surrounding environment (thus increasing the area of active agent release), on the other hand, it is noteworthy that a rough surface is generally more convenient for bacterial adhesion; thus these two facts have to be balanced.

Another important parameter of the surface is WCA. It is proved that the adhesion of platelets to solid substrates increases with increasing WCA, a parameter that generally correlates well with solid surface tensionJ[15]^ In other words, surface energy, physicochemical character of the material surface (i.e., hydrophobicity or hydrophilicity) can significantly influence the adherence of microorganisms.^[16]!

WCA was determined to evaluate the changes in surface nature that were caused by the addition of CV, MT, and their combination. Data in Figure 4 show that WCA decreases with increasing concentration of any filler used in this study, suggesting that the prepared composites have generally less hydrophobic surface. In biological systems, hydrophobic interactions are usually the strongest of all long-range non-covalent forces, and the adhesion to surfaces is often mediated by these types of interactions. It has been demonstrated that hydrophobicity plays an important role in a wide range of microbial infections.[117]^

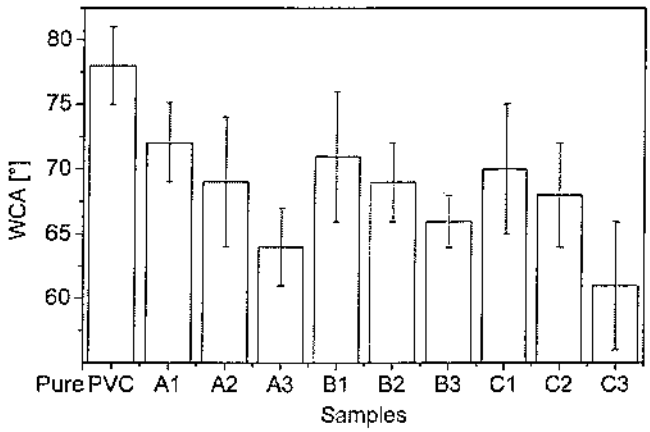

Figure 4. WCA of tested samples.

Release Study

In order to asses the effects of various compositions of the material and the influence of the leaching liquid the samples with the highest concentration of CV were chosen as they are supposed to have the most pronounced effects on leachate coloration. Pure PVC was used as a reference. Data from the releasing study for all kinds of studied materials for each leaching medium are plotted in Figure 5. The effects of medium and sample composition on the release extent, are summarized in Table II. Supposing only a weak influence of solvent on molar absorption coefficients, the effect of the leaching medium can be evaluated in the first attempt. CV is highly soluble in ethanol/water (1/2) solvent mixture, which has less polarity than water itself; it was chosen for the use in the leaching test to accelerate the release of CV from the investigated materials. Therefore, the highest absorbance value at CV's absorption maximum at wavelength 585 nm was measured in Et0H/H_20 leachates for all samples. An extraction mechanism is proposed for this system, which means that the CV cation releases from the material together with its chloride counterion. In the case of other tested media, the release of CV is governed by the ion exchange diffusion process. Release of CV^+ to the surrounding medium is balanced by counterflow diffusion of cations from the leaching medium into the material. CV releasing rate is therefore significantly affected by the chemical nature of a cation present in the leaching media. A solution of 0.01 M HC1 used for simulation of gastric juice with pH value about 2 reached much higher absorbance values than those for deionized water with several orders of magnitude lower H_30^+ concentration. H_sO^+ ions have relatively higher mobility than Na^+ in physiological solution. Physiological solution simulates osmotic pressure and ionic strength in body liquids, namely blood plasma. It is important to note that Na^+ ion has a solvatation sphere hindering its diffusion into the bulk PVC material with nonpolar character.

Moreover, all prepared samples follow a similar trend concerning CV release in dependence on the composition regardless of tested media. Namely, the highest amount of released CV was determined to be in PVC + CV composites (sample

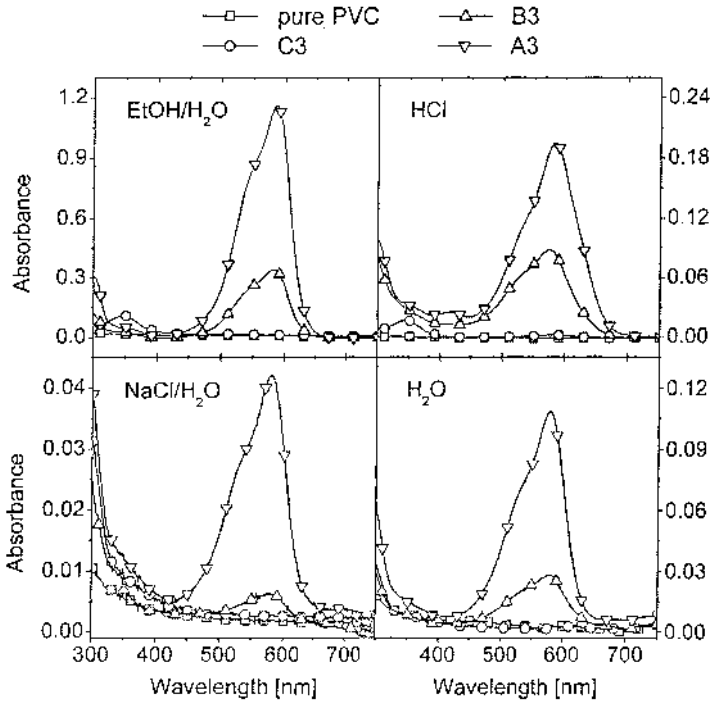

Figure 5. UV-vis absorbance spectra of leachates from the samples in tested solvents.

A3), where CV is partially dissolved in the PVC matrix and partially dispersed in the form of microscopic particles (see optical microscope details in Figure 2). Peaks of much lower intensity in absorption spectra are observable for PVC + CV + MT composites (material B3). Filler particles with high aspect ratio create a passive barrier by impeding the diffusion of CV and ions through the PVC matrix. No peak was found in the absorption spectra of PVC + MMT (material C3). These results suggest that in the case of the MMT, the diffusion barrier is increased by the effect of ion bonding interaction between CV and MT preventing CV release to the surface, unlike the case of the unmodified montmorillonite filler.

Table II. Effect of leaching medium on release extent

	Absorbance value			
Sample	$EtOH/H_2O$ $\lambda_{max} = 585$ nm	0.01 M HCl $\lambda_{max} = 584$ nm	H_2O $\lambda_{max} = 581$ nm	$NaCl/H_2O$ $\lambda_{max} = 578$ nm
Pure PVC	0.007	0.001	0.003	0.002
A3	1.162	0.195	0.076	0.042
B3	0.338	0.085	0.028	0.006
C3	0.012	0.002	0.002	0.003

CONCLUSION

The results suggest that the modification of PVC with montmorillonite and crystal violet through incorporation into the bulk of the polymer can allow effective control over release of the crystal violet from the inside of the composite to the surface. This ability could have applications in the modification of the antimicrobial effect of materials in further development of plastic devices for medical use.

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