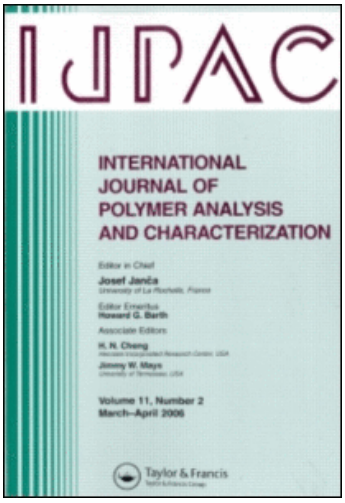

International Journal of Polymer Analysis and Characterization
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713646643

Analysis of Antibacterial Action of Polyvinyl Chloride Surface Modified with Gentian Violet

M. Sowe[a]; M. Polaskova[a]; I. Kuritka[a]; T. Sedlacek[a]; M. Merchan[a]
[a] Tomas Bata University, Zlin, Czech Republic

Online publication date: 20 November 2009

To cite this Article Sowe, M. , Polaskova, M. , Kuritka, I. , Sedlacek, T. and Merchan, M.(2009) 'Analysis of Antibacterial Action of Polyvinyl Chloride Surface Modified with Gentian Violet', International Journal of Polymer Analysis and Characterization, 14: 8, 678 — 685
To link to this Article: DOI: 10.1080/10236660903298327
URL: http://dx.doi.org/10.1080/10236660903298327

International Journal of Polymer Anal. Charact., 14: 678–685, 2009

ISSN 1023-666X print
DOI: 10.1080/10236660903298327

Analysis of Antibacterial Action of Polyvinyl Chloride Surface Modified with Gentian Violet

M. Sowe, M. Polaskova, I. Kuritka, T. Sedlacek, and M. Merchan
Tomas Bata University, Zlin, Czech Republic

Abstract: The effect of incorporation of gentian violet into medical-grade polyvinyl chloride was studied in this work. The antibacterial properties of the resulting blend were evaluated and the influence of the amount of gentian violet assessed. Dispersion of the gentian violet in the polyvinyl chloride following the blending of the two components was uniform as was demonstrated by the optical images, which is important for any further analysis. The samples of the blend were modified compared to the PVC samples that were not treated with gentian violet. This was clearly demonstrated by the scanning electron microscope images. Water contact angles reduced from 77.2° for the pure sample to lower values for the modified samples, signaling that the hydrophilicity of the modified samples was improved. All of these results correlated well with the antimicrobial investigations, which showed inhibition of *Staphylococcus aureus* action by the modified samples, but no inhibition by the pure samples. Furthermore, the inhibition zone increased with gentian violet content in the blend.

Keywords: Antibacterial properties; Gentian violet; Hydrophilicity; PVC; Surface modification

Submitted 3 July 2009; revised 30 August 2009; accepted 1 September 2009.

Presented at the 21st International Symposium on Polymer Analysis and Characterization held 22–24 June 2009 at the Tomas Bata University in Zlin, Zlin, Czech Republic. The authors would like to express their gratitude for financing of this research to the Ministry of Education, Youth and Sport of the Czech Republic (grant VZ MSM 7088352101). Grant (MPO 2A-1TP1/126).

Correspondence: M. Sowe, Tomas Bata University in Zlin, Zlin 76005, Czech Republic. E-mail: musa_sowe2002@yahoo.com

INTRODUCTION

Polymers have been applied in many areas of human activity, including biomaterials, and, over the years, they have gained increasing importance as biomedical materials because of their high versatility and excellent physical and chemical properties. Medical poly (vinyl chloride) (PVC) occupies an outstanding position within this group due to an excellent balance of properties. It offers a uniquely broad range of properties for a wide variety of applications in the medical and health care industry. It fulfills an extensive range of performance and processing requirements such as gloss, transparency, chemical resistance, scuff resistance, flexibility, and bondability. However, its surface is not always compatible with its functions as a biomedical material, and often it becomes necessary to improve its properties to make it more effective.[1]

Bacterial infection of polymer-based biomedical surfaces has long been established and is seen as an important problem in the biomedical and health care laboratories.[2] This is especially essential with regards to nosocomial infections of the biomedical material surface, which is closely related to attributes of the surface. This issue has long been studied by Lewis and Sheretz,[3] who suggested that adherence and proliferation of various strains of bacteria on surfaces of implanted polymers are among the leading causes of serious nosocomial contaminations, reportedly accounting for approximately half of all nosocomial infections. In such a situation, the treatment would be unsuccessful and the implant would have to be removed. The burden and consequences of this fact are both clinical and economic[4] due to their impact on morbidity and mortality as well as premature catheter removal. Therefore, much attention is being focused on ways to prevent or minimize medical device–related infections. As a result, the fabrication of anti-infective polymeric implants and other devices has drawn special attention in the area of biomedical production and particularly in the delivery of appropriate polymer surfaces. In their study, Camacho et al.[5] determined that the antimicrobial activity of chlorhexidine coated on catheter surfaces reduces the adherence of microorganisms to the catheters. However, the coating technique may not be the most reliable in delivering a uniform, steady, and continuous sterilized surface that will minimize bacterial infection for an extended period of time. One way to accomplish this is through blending polymers with antibacterial agents.

In this work, the antibacterial properties of polyvinyl chloride (PVC) surfaces that have been modified with gentian violet through compounding are investigated. PVC is a leading polymeric material used in medical device applications and gentian violet has antibacterial and antifungal properties. Therefore, combination of the two is proposed here to produce medical devices that will minimize the

potential for medical device–related bacterial infection. In this study, the PVC/gentian violet antimicrobial formulation blend produced is tested against *Staphylococcus aureus*, and the influence of the amount of gentian violet in the sample was assessed. *Staphylococcus aureus* is gram positive and, primarily, also coagulase positive,[6] a major human pathogen known to cause nosocomial infections.[7] It is estimated that about 20% of the healthy population are long-term carriers of *Staphylococcus aureus*,[8] and each year about half a million patients in American hospitals alone contract a staphylococcus infection.[6] In addition, about 19,000 deaths are reported from American hospitals that are due to staphylococcus infections.[9] Gram-negative bacterial strains are not proposed for this study because gentian violet is known to be ineffective against these strains.

MATERIALS AND METHODS

Materials

Commercial medical-grade polyvinyl chloride pellets (grade RB1/T3M) were acquired from Moden Plast S.P.A (Hungary) and used as received. Anhydrous ethylene glycol, diiodomethane, Triton X-100, and ethanol were obtained from Aldrich. Gentian violet was purchased from Humco (USA) and *Staphylococcus aureus* (agar used: Nutrient Agar No. 2), staphylococcus agar CCM 4516, and reference strain Staph 3953 were obtained from Czech Collection of Microorganisms (CCM) (Brno, Czech Republic).

EXPERIMENTAL SECTION

The medical-grade PVC was modified by mixing it thoroughly with gentian violet in a Haake Mini Micro-compounder at 70 rpm and 160°C. Samples were molded from this material and cut into appropriate sizes for analysis and microbiological tests. Compounding time of 10 min was sufficient to achieve constant torque of the drive in all compounded samples, suggesting a good dispersion at this time. Surfaces of the samples were characterized with surface analysis techniques, including water contact angle (WCA) measurement, scanning electron microscopy (SEM), and optical analysis of dispersion of the gentian violet in the PVC matrix. The samples were also tested for activity against *Staphylococcus aureus* in plates containing staphylococcus agar CCM 4516. The plates were incubated overnight at 37°C for 24 h, and the zones of inhibition produced around sample discs were measured and recorded as the inhibition zone.

Water Contact Angle Measurement

Wetting ability of the sample surface was evaluated by measuring the water contact angle of the samples. For this purpose deionized water, ethylene glycol, and di-iodomethane were used as the liquids for measurement. The sessile drop method was used for this purpose and 5 μL of each liquid was placed, dropwise, on a 1×4 cm piece of the PVC film, which was set up on Surface Energy Evaluation (SEE) System (Brno, Czech Republic) equipment at room temperature. For each sample, 10 drops were used for each liquid measurement to minimize statistical error, so that the reported contact angle values are the averages of 10 independent measurements. Contact angles and surface energy were determined using the acid-base model that is incorporated as a part of the evaluation system. The static contact angle was recorded 30 s after the liquid drop rested on the surface. An identical technique was utilized to determine the contact angles and surface energy of the untreated samples. The precision of the obtained angles was $\pm 3°$.

Optical Microscopy

Thin sections (1 mm × 1 mm × 30 μm) of samples were prepared and observed using an Olympus U-CMAD3 microscope (Japan) and pictures of observed areas were recorded with an Olympus CX31 (Japan) camera.

Surface Topography Examination

The surface topography of both pure and gentian violet/PVC combinations were observed using scanning electron microscopy (SEM) imaging on a VEGA II LMU (Tescan, Czech Republic). The equipment operates in the high vacuum/secondary electron imaging mode at an accelerating voltage of 20.0 kV. The samples were sputter coated with a thin layer of palladium/gold alloy and tilted at 30° for better observation. The images were taken at magnifications of 30,000×.

Microbiological Tests

Microbiological tests to investigate antibacterial activity of the PVC modified samples against *Staphylococcus aureus* were performed using standard procedures.[10] Staphylococcus agar CCM 4516 (Brno, Czech Republic) and Nutrient Agar No. 2 were used to incubate the samples, and the samples in the form of discs were placed in the agar plates containing reference strain *Staphylococcus aureus* 3953. The plates were then incubated at 37°C for 24 h. Thereafter, the zones of inhibition produced around the catheter segments were measured across the centers

of the embedded catheter segments and recorded as the diameters of inhibition.

RESULTS AND DISCUSSION

The extent of dispersion of gentian violet in the PVC is demonstrated by the optical microscope images in Figure 1, revealing reasonably good dispersion of the components, which is important for further analysis of the results.

The adherence of microorganisms to the surface and their subsequent action are determining factors in colonization of medical devices and subsequent infection.[5,11] Bacterial adhesion to surfaces is a complicated process influenced by many factors including hydrophilicity and surface energy of both the medical device and the bacteria, as well as topography of the device and environmental factors such as exposure to antibiotics.[12]

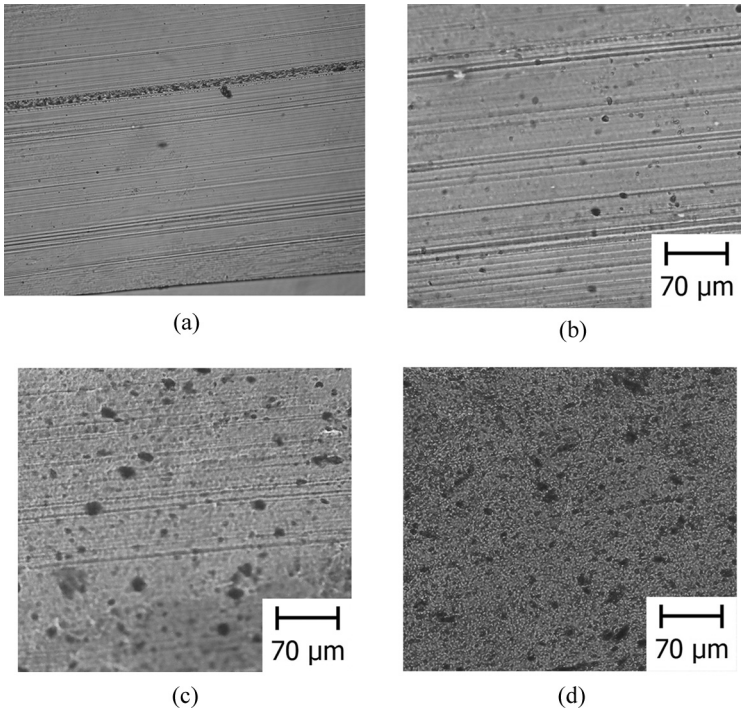

Figure 1. Optical images of samples of (a) pure PVC, (b) PVC + 2% gentian violet, (c) PVC + 5% gentian violet, and (d) PVC + 10% gentian violet.

Table I. Inhibition zone measurements of pure and modified samples against *Staphylococcus aureus*

Sample	Water contact angle (degrees)	Inhibition zone (mm)
Pure PVC	77.2	0.0
PVC + 2% gentian violet (GV)	71.7	7.3
PVC + 5% gentian violet (GV)	68.8	8.0
PVC + 10% gentian violet (GV)	64.4	9.0

In this regard the data in Table I show that the water contact angles (WCA) for the gentian violet modified PVC decreased from 77.2° for the pure sample to lower values for all three modified samples. The contact angles decreased with an increase in gentian violet content, and the sample with the highest gentian violet content showed the lowest water contact angle. The decrease in water contact angles was accompanied by corresponding increases in the surface energies of the modified samples over the pure sample and as water contact angles decreased, surface energy increased (Figure 2). These findings suggest that the hydrophilicity of the modified samples have improved over that of the pure sample. The study also illustrated that the topography of the surface of modified samples has been modified as confirmed by the scanning electron microscopy (SEM) images, which is consistent with a higher tendency towards hydrophilicity (Figures 3(a)–3(d)). The pure sample shown in Figure 3(a) demonstrates a smooth surface, unlike the surface of the samples modified with gentian violet, which showed rougher

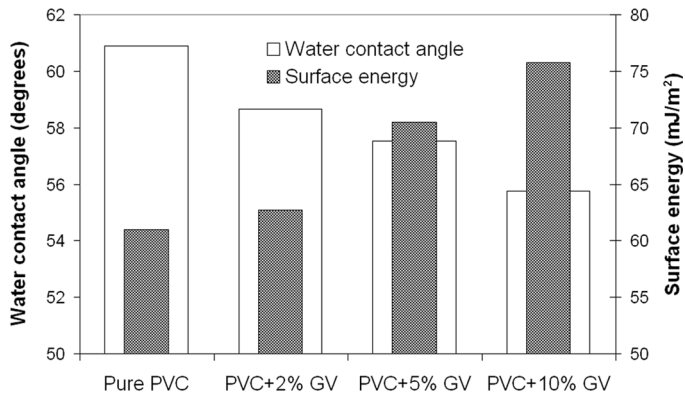

Figure 2. Water contact angle and surface energy of pure PVC and modified samples.

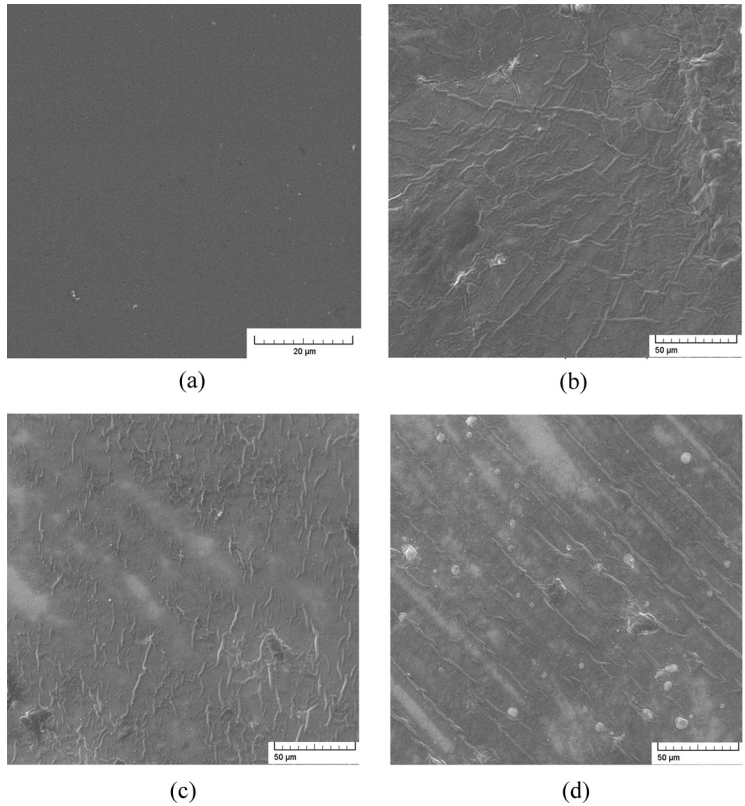

Figure 3. SEM images of (a) pure PVC, (b) PVC + 2% gentian violet, (c) PVC + 5% gentian violet, and (d) PVC + 10% gentian violet samples.

surfaces covered by ridges, indicating the extent of modification of the sample surface.

All these factors correlated well with bacterial inhibition, showing that the modified samples demonstrated inhibition against *Staphylococcus aureus* while the pure sample did not, as shown in Table I. Furthermore, the inhibition zone increased with higher gentian violet content. This fact suggests that higher gentian content in the polymer matrix is desirable for more effective results against *Staphylococcus aureus*. This may not be a major issue for applications that do not need the use of transparent devices. However, for applications that require some degree of transparency of a device, it would be necessary to balance the need for higher efficiency and transparency of the device because gentian violet is a strong colorant.

CONCLUSION

The results in this study suggest that the combination of gentian violet and polyvinyl chloride is effective against *Staphylococcus aureus* action. The antibacterial activity of this combination could, therefore, help to reduce the risk of colonization by bacteria and consequently could result in enhancing the effectiveness of polymer-based medical devices against microbial infection.

REFERENCES

[1] Braun, D. (2004). Poly(vinyl chloride) on the way from the 19th century to the 21st century. *J. Polym. Sci: Part A: Polym. Chem.* **42**, 578–586.
[2] Ong, Y. L., A. Razatos, G. Georgiou, and M. M. Sharma. (1999). Adhesion forces between bacteria and biomaterial surfaces. *Langmuir* **15**, 2719–2725.
[3] Lewis, W. J., and R. J. Sheretz. (1997). Microbial interactions with catheters. *Nutrition* **13**, 85–89.
[4] Hend, H., P. Bahna, R. Reitzel, T. Dvorak, G. Chaiban, R. Hachem, and I. Raad. (2006). Comparative in vitro efficacies and antimicrobial durabilities of novel antimicrobial central venous catheters. *Antimicrob. Agents Chemother.* **50**, 3283–3288.
[5] Camacho, D., A. Gasparetto, and T. Svidzinski. (2007). The effect of chlorhexidine and gentian violet on the adherence of *candida* spp. to urinary catheters. *Mycopathologia* **163**, 261–266.
[6] Ryan, K. J., and C. G. Ray, ed. (2004). *Sherris Medical Microbiology: An Introduction to Infectious Diseases*, 4th ed. New York: McGraw-Hill Medical, p. 99.
[7] Lowy, F. D. (1998). *Staphylococcus aureus* infections. *N. Engl. J. Med.* **339**, 520–532.
[8] Kluytmans, J., A. Van Belkum, and H. Verbrugh. (1997). Nasal carriage of *Staphylococcus aureus*: Epidemiology, underlying mechanisms, and associated risks. *Clin. Microbiol. Rev.* **10**, 505–520.
[9] Klevens, R. M., M. A. Morrison, J. Nadle, S. Petit, K. Gershman, S. Ray, L. H. Harrison, R. Lynfield, G. Dumyati, J. M. Townes, A. S. Craig, E. R. Zell, G. E. Fosheim, L. K. McDougal, R. B. Carey, and S. K. Fridkin. (2007). Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* **298**, 1763–1771.
[10] Galya, T., V. Sedlařík, I. Kuřitka, J. Sedlaříková, and P. Sáha. (2008). Characterization of antibacterial polymeric films based on poly(vinyl alcohol) and zinc nitrate for biomedical applications. *Int. J. Polym. Anal. Charact.* **13**, 241–253.
[11] Li, Z., N. Mohamed, and J. Ross. (2000). Shear stress affects the kinetics of *Staphylococcus aureus* adhesion to collagen. *Biotechnol. Prog.* **16**, 1086–1090.
[12] Missirlis, Y. F., and M. Katsikogianni. (2007). Theoretical and experimental approaches of bacteria-biomaterial interactions. *Mater.wiss. Werkst.technik* **38**, 983–994.