Potential Biofilm Resistant Coatings

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Abstract

Five novel, biofilm resistant monomers were synthesized and formulated into a commercially available ultraviolet (UV) curable coating for metals at varying weight percents. The coatings were applied to and UV-cured on uncoated, polished stainless steel plates to a thickness of two mils. The crosshatch adhesion, pencil hardness, and chemical resistance were tested for each coating. Photo-differential scanning calorimetry (photo-DSC) was used to determine the polymerization rate of the monomers in 1,6-hexanediol diacrylate (HDODA) at the concentrations used in the formulations. A standard photoinitiator, 2,2-dimethoxy-2-phenylacetophenone (DMPA), was used for the photo-DSC studies. Finally, the resistance to biofilm formation was also examined.

1. Introduction

UV-curing is often more efficient and economic compared to traditional thermal curing methodologies. UV-curing is efficient due to its fast curing rates on the order of minutes whereas thermal curing often requires hours or days for complete cure. Thermal curing, occurring above ambient temperatures, requires a large amount of energy to equilibrate the large ovens and is also often based on solvent or water borne technologies having increasingly stringent environmental regulations. This can be very costly compared to UV-curing which uses a low amount of energy, taking place at ambient temperatures. Furthermore, UV-curable coatings can be formulated with 100% reactive solids containing no volatile organic compounds (VOCs) or hazardous air pollutants (HAPs) promoting less environmental overhead cost and more efficient material usage.[1,2]

UV-curable coatings are comprised of several components. These include a photoinitiator which begins the free-radical or cationic chain growth polymerization initiated by UV radiation.[3] Reactive oligomers are formulated into the coating to enhance the film forming

properties. Additives are often used to modify the coating's properties such as color or polymer stability.[1] Finally, a monomer must often be added controlling the viscosity and, in this paper, providing biofilm formation resistance.

Many items used in the health care industry must be free of microorganisms. Microorganism contamination (e.g. staphylococcus aureus) in hospitals, dental offices, food supplies, and water systems can cause serious infections that can lead to death.[4-6] By coating medical equipment with a biofilm resistant coating, bacterial growth should be reduced or eliminated.[4] To be effective at bacterial killing, the coating will function in one of two ways: 1) bursting the cell walls of the bacteria or 2) inhibiting critical nucleic reactions in the bacteria.[4,7] Either function should inhibit biofilm formation by reducing the bacterial concentration in solution. If these options are not possible due to the limited mobility of the antibacterial substance, an additive can be used to reduce the efficiency of bacterial attachment to the surface of the coating by having one or both of these properties: 1) unfavorable surface chemistry for bacterial accumulation and 2) ultra-smooth surface.[5,8] Originally the United States Navy coated their ship's hulls with coatings containing copper or tributyltin (TBT) additives which required costly maintenance and were harmful to the environment.[9] The benefit to the Navy of this technology causes a reduction in the drag as the ship travels through the water.[9-13]

Biofilm resistant coatings are used on a wide variety of products such as catheters, coronary stents, IV delivery systems, cell phones, food packaging, and water systems to reduce infection rates.[5,14,15] In an effort to reduce biofilm formation, an ultraviolet (UV) curable coating has been produced. Five monomers were synthesized and formulated into UV-curable coatings. The composition of each monomer was verified by infrared spectroscopy (IR) and nuclear magnetic resonance (NMR). These coatings were applied to stainless steel plates in order to determine the hardness, chemical resistance, adhesion, and impact resistance. Our research further entails the polymerization rates of each monomer's coating through Photo-DSC. Lastly, biofilm resistance against *Escherichia coli* (*E. coli*) was also investigated. These UV-curable, biofilm resistant coatings, if effective, could easily be used on dental materials (e.g. fillings), medical devices, contact lenses, and abrasion resistant surfaces (e.g. counters and tabletops).[16]

2. Experimental

2.1 Materials

The well plates and most chemicals used in the monomer syntheses and testing, including the alcoholic precursors, triethylamine (TEA), acryloyl chloride, well plates, and acetonitrile, were purchased from Sigma-Aldrich. The dichloromethane also used in the syntheses was purchased from Pharmacia. The material used for the coating formulations was obtained from Allied Photochemical and is a proprietary formulation. Cytec Specialty Chemicals provided

HDODA used in the photo-DSC. Albemarle Corporation donated the photoinitiator, 2,2-dimethoxy-2-acetophenone (DMPA). The uncoated, polished stainless steel plates were purchased from Q Panel Products. Methyl ethyl ketone (MEK) for the double rub test was purchased from The Paint Center. Pap-smear cytology fixative spray was obtained from Andwin Scientific. The *E. coli* was stained using Hema-diff solution 3:thiazine dye from Anapath.

2.2 General Synthetic Method

Each alcohol precursor was dissolved in dichloromethane after adding TEA in slight molar excess. Under a ventilation hood acryloyl chloride was added dropwise as hydrochloride gas was produced. The round bottom flask was purged with nitrogen for five seconds and stoppered to provide a nitrogen atmosphere for the reaction to take place. The reaction mixture stirred for a full day (24 hours) at room temperature using a magnetic stir bar to allow for complete reaction. TEA•HCl precipitate formed which was removed by suction filtration. Unreacted acryloyl chloride was removed by washing with five milliliters (5 ml) of deionized water five times in a separatory funnel. A rotary evaporator was used to remove any excess dichloromethane. The reaction scheme is shown below (Figure 1).

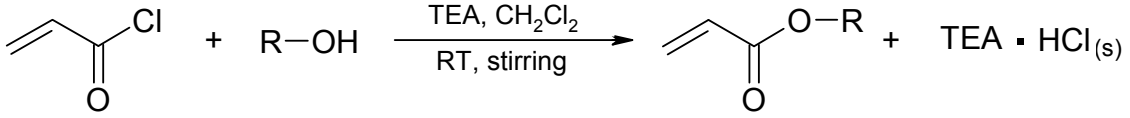

Figure 1: Monomer Synthesis

2.3 Photo-DSC

The monomers were formulated as shown in Table 1, and then two microliters (2 µl) of each formulation was measured into crimped, aluminum sample pans. After placing under the quartz window of the photo-DSC head, the light intensities were measured using black body absorbers. The calorimetric measurements were performed using a Mettler-Toledo DSC 822^e modified with a Hamamatsu Lightning Cure 200 UV-spot, equipped with a high pressure mercury lamp. The sample cell was kept at a constant 20° C by a Julabo FT 100 intercooler. The polymerization rates of each monomer were compared to that of a standard *Type II* initiated sample.

Table 1: Formulations used for Photo-DSC Testing.

Mono-/Oligomer	**Mass Run 1** (g)	**Mass Run 2** (g)	**Mass Run 3** (g)
HDODA	8.9	9.9	9.0
Monomer	1.0	0	1.0
BDK	0.1	0.1	0

2.4 Formulations

The coatings were formulated having five, ten, fifteen, and twenty weight percent of the potentially biofilm resistant monomers and applied evenly onto uncoated, polished 4" x 6" metal plates. A two mil (50 μm) thick coating was applied with a metal draw-down bar. The coatings were cured by a custom designed apparatus to provide a nitrogen atmosphere (2 minute purging prior to cure and continued purging during cure). This allowed UV radiation exposure (5 minutes) using a Sylvania medium pressure mercury arc lamp (HPL80MDX(R) 80 Watt (RQ) 0303) source which the outer casing had been removed. The intensity was 15 milliWatts/cm^2 at the top of the lid after a thirty (30) minute warm up period. The metal plate was enclosed in a screwed down lid (13" x 9" Pyrex casserole dish) with nitrogen running through it to provide an oxygen-free, nitrogen atmosphere. Determination of complete curing was based on a basic thumb twist test.

2.5 Physical Testing Procedures

All physical tests were preformed as given in standard source: pencil hardness (ASTM D3363), MEK double rub (ASTM D5402-93), and cross hatch adhesion (ASTM D 3359).[17-20]

2.6 Biological Testing

Coatings were applied to the bottom of four well plates (24 wells each) with the varying percent formulations used for the metal coatings. The coatings were cured with the curing apparatus and sterilized with 95% ethanol. *E. coli* was grown in a LB agar broth and added to the cell plates with sterile, deionized water at four concentrations, one concentration per plate. The plates were incubated for twenty-four hours at 37°C. The *E. coli* was then rinsed from the plates with sterile, deionized water and sprayed with the cytology fixative (poly(ethylene glycol)-based). After the fixative was air dried it was soaked with 95% ethanol for ten minutes for further sterilization. The plates were then rinsed with deionized water and four drops of methylene blue solution were added for four seconds. After the dye was removed, the plates were rinsed with sterile, deionized water showing residual dyed bacteria on the coating.

3. Results and Discussion

3.1 Physical Testing

The pencil hardness test showed adding the monomer to the coating increased the hardness as the concentration of the monomer increased. This is caused by the additional physical crosslinking due to increased dipole-dipole interactions from the higher concentration of monomer.

In the cross hatch adhesion test, the adhesive forces between the metal plate and the coating was not affected when the monomer was added. This shows consistent adhesive properties at varying monomer concentrations.

The MEK double rub solvent resistance test showed the association between monomer physical and chemical crosslinking with the solvent resistance. The solvent resistance generally increased when low concentrations of the monomer were added. The low concentration of the monofunctional monomer does not greatly decrease the chemical crosslinking while increasing physical crosslinking thereby leading to a high chemical resistance. Since the monofunctional monomer is highly polar, the molecular interactions (physical crosslinks) are increased in the polymer resulting in an increased solvent resistance. When the concentration of the monomer increased, the degree of chemical crosslinking decreased lowering the solvent resistant properties of the coating.

3.2 Biological Results

The outcome of the biological testing showed bacterial growth, evidenced by the blue tinting, on the control formulation as well as the coatings containing the internal control, monomer 1, and the unmodified formulation, ctrl (Figure 2).

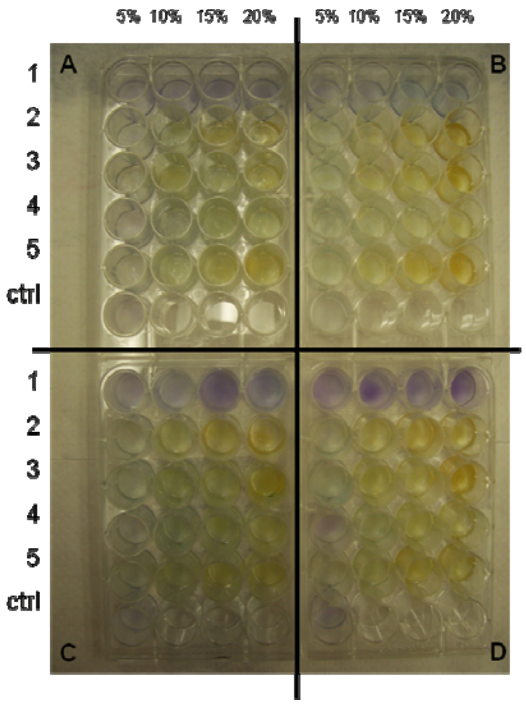

Figure 2. Biofilm resistance testing results after E. coli *growth and staining where each plate was treated with a varied concentration of the* E. coli *broth: A diluted 1:3 broth to sterile, deionized water, B was a 1:1 dilution, C was a 3:1 dilution, and D was the undiluted broth.*

The coatings containing the lowest concentrations of monomer (5 weight %) had small amounts of bacterial growth. However, when the concentration of these same monomers increased, the biofilm resistance of the coatings increased. The two highest concentrations (15 and 20 weight %) of monomers 2, 3, and 5 had excellent biofilm resistance given the lack of blue coloring. As expected, when the concentration of *E. coli* broth increased among differing plates, the bacterial growth increased. The surface of the coating was very rough as it was spread across the bottom of the well by a toothpick; thus, the biofilm resistance is most likely due to unfavorable surface chemistry for bacterial accumulation caused by the addition of the very polar monomers to the formulation.

3.3 Photo-DSC Results

The monomers were mixed in similar percentages as done with the biological assessment into 1,6-hexanediol diacrylate (HDODA) and evaluated for their activity as photoinitiating species. In each case, the active monomer did not function as a photoinitiating species as observed from the relatively low exotherm, especially when compared to that of the DMPA initiated mixture.

4.0 Conclusions

Five monomers were successfully synthesized through a standard laboratory synthesis. Each monomer was added to a compatible industrial formulation that was subsequently UV-cured onto steel plates after a two minute nitrogen purge and a five minute UV exposure in a custom apparatus. After curing, several standardized physical tests were performed including pencil hardness, solvent resistance via MEK double rubs, and crosshatch adhesion. The formulations incorporating the monomers yielded comparable solvent resistance and adhesion. The presence of the monomers increased the hardness of the coating when compared to the control formulation. Coatings incorporating monomers 2, 3, and 5 showed superb biofilm resistance with any concentration of *E. coli* broth.

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