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Abstract

Purpose – The purpose of this paper is to develop non-leaching and eco-friendly antimicrobial waterborne polyacrylates with excellent antibacterial properties by grafting antibacterial vinyl monomer, glycidyl methacrylate (GMA) modified polyhexamethylene guanidine hydrochloride (PHMG).

Design/methodology/approach – PHMG of different molecular weights were modified by GMA to synthesize antibacterial vinyl monomer, GMA-modified PHMG (GPHMG). Different content and molecular weights of GPHMG were used to synthesize antimicrobial waterborne polyacrylates through emulsion polymerization.

Findings – The addition of GPHMG gained by modifying PHMG showed little influence on thermal stability of the films, but decreased the glass transition temperature(Tg). Meanwhile, the tensile strength decreased, while the breaking elongation increased. The antibacterial properties of the antibacterial films with different GPHMG contents were studied, when GPHMG content was around 0.9 Wt.%, antibacterial films showed excellent antibacterial activity (antibacterial rate >= 99.99 per cent). When weight content of GPHMG in the films remained constant, antibacterial property of films increased first and then decreased with the increase of molecular weight of GPHMG. The structural antibacterial polymer film had more perdurable antibacterial activity than the blended one.

Research limitations/implications – The grafting efficiency of GPHMG to antimicrobial waterborne polyacrylates could be further improved.

Practical implications – Antimicrobial waterborne polyacrylates with excellent antibacterial properties can be used to antibacterial coating and adhesive.

Originality/value – The antibacterial properties of films with different molecular weight of GPHMG were studied, and the durability and stability of antibacterial properties between structural antimicrobial films and blended antimicrobial films were also investigated by ring-diffusion method.

Keywords Coatings, Polymers, Film, Emulsions, Resins, Antibacterial films, GPHMG, Molecular weight, Non-leaching, PHMG

Paper type Research paper

Introduction

As we all know, microorganisms are widely distributed in the environment, each kind of which may cause great damage to the materials we used in our life, once they adhere to the surfaces of the materials and form biofilms (Meyer, 2003; Spellberg, 2014). Besides, the actions of the microorganisms not only lead to economic problems in industry and life but also cause great harm and various infectious diseases to humans (Rudra *et al.*, 2013). For example, microbial growing on food processing equipment or food packaging materials (Mari Pau *et al.*, 2013; Severino *et al.*, 2015), to make matters worse, on medical devices and implants, undoubtedly can cause serious complications to human health (Tîlmaciu *et al.*, 2015).

Thus, the development of agents or surface coatings with antibacterial activity has gained increasing interest in recent years (Ionita *et al.*, 2011). One of the typical approaches for controlling undesirable growth of microorganisms on the

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surface of materials is to incorporate antimicrobial substances to surface coatings (Majumdar *et al.*, 2011), namely, antimicrobial peptides (Onaizi and Leong, 2011), antibiotics (Alanis, 2005), enzymes (Rodriguez *et al.*, 2004), nanoparticles (Vollmer *et al.*, 2010) and so on. The cationic antimicrobials, such as widely used quaternary ammonium compounds, and polymeric guanidines are especially prominent in combating against bacterial infections (Mereghetti *et al.*, 2000). Guanidine derivatives, particularly guanidine oligomers, have been considered as broad-spectrum antimicrobial agents (Pascal *et al.*, 1990). Furthermore, polyhexamethylene guanidine hydrochloride (PHMG), because of its high-water solubility, nontoxicity and resistance against gram-positive and gram-negative bacteria, has been commonly used as antiseptic and disinfectant in hospital (Rosin *et al.*, 2002), water treatment (Aviv *et al.*, 2016) and food industry (Oulé *et al.*, 2012).

Polymerization or copolymerization with monomers containing antimicrobial compound is a commonly used method to produce antibacterial materials. Xu *et al.* (2015) prepared antimicrobial polyethylene wax (PEW) emulsions by emulsifying PEW grafted with PHMG (PEW-g-PHMG). This not only combined the hydrophobicity of PEW and antibacterial property of PHGH but also possessed high adsorption capacity to cellolose fibers. Wang *et al.* (2013b) synthesized dual functional antibacterial and biodegradable caprolactone (CL)-based oligomers (PHMG-b-PCL) by ring-opening polymerization of caprolactone using PHMG as a macroinitiator. The hydrophilic (PHMG) and hydrophobic (PCL polyester) block made these oligomers valuable antibacterial additives for biodegradable polyesters and showed great biocompatibility.

Researchers have tried to endow PHMG with active double bond by modification, thus expanding its application in the preparation of antibacterial fibers, papers and plastics. Pan *et al.* (2016) modified PHMG oligomer via reaction with glycidyl methacrylate (GMA), and then they designed a new type of core–shell structure polymer latex containing antibacterial property. In an attempt to render the cellulose fibers highly antimicrobial, the latex was used as wet-end additive to modify the surface of natural fiber. Wang *et al.* (2013a) designed and synthesized a novel modified guanidine-based oligomer grafted with a reactive cationic surfactant (OMPAC) by a Michael addition reaction that have an enhanced antibacterial activity than PHMG, which can be used both as antibacterial agent and reactive cationic surfactant to copolymerization with other monomers. Altogether, PHMG has attracted widespread interest among researchers, and many of its application value remained to be developed.

In this work, guanidine hydrochloride and hexamethylenediamine were used as raw materials to synthesize PHMGs with various molecular weights by controlling the reaction parameters, including time, temperature, feeding molar ratio, etc. Then, GMA was used to react with PHMG to obtain a product (i.e. GPHMG), which could be applied as a functional antibacterial monomer to participate in the process of emulsion polymerization to form structural antimicrobial emulsion. Apart from GPHMG, other polymeric monomers, including butyl methacrylate (BA) and methyl methacrylate (MMA), were also used considering the sufficiently high hydrophobicity and excellent filming capability. Here, we aimed at studying the antibacterial activity that the varies dosage of GPHMG of same molecular weight and GPHMGs with different molecular weights, but of same weight content brought to antimicrobial films to find out its optimal dosage in antimicrobial films. Besides, the durability and stability of antibacterial properties between structural antimicrobial films and blended antimicrobial films is also investigated. The molecular structure and molecular weight of the obtained modified oligomer GPHMG was characterized by Fourier transform infrared (FTIR) spectroscopy and Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometer. The antibacterial properties of antimicrobial coatings were observed by plate count method and ring-diffusion method. Moreover, the influence of mechanical properties, thermal properties and water resistance of the antimicrobial films were studied when GPHMG was included.

Experimental

Materials and methods

Guanidine hydrochloride and hexamethylene diamine were purchased from Sigma-Aldrich (USA). GMA, butyl acrylate (BA) and methyl methacrylate (MMA) monomers for polymer emulsion synthesis were purchased from Sigma-Aldrich (USA). PHMG (M_n = 2000 Da, 98 per cent) were purchased from Yangzhou Brown textile company. The initiator, 2,2′-Azobis (2-methylpropion-amidine) dihydrochloride (AIBA) (assay, 98 per cent), used in preparing cationic polymerisation reaction of monomers of BA,MMA and GPHMG (PBA-co-MMA/GPHGH) latex via emulsion polymerization, and the emulsifier, cetyl trimethyl ammonium bromide (CTAB) (assay, ≥99 per cent) were also obtained from Sigma-Aldrich and used as received without further purification.

Preparation of polyhexamethylene guanidine hydrochlorides with different molecular weights

PHMG was synthesized by hexamethylenediamine and guanidine hydrochloride at elevated temperatures through thermal polycondensation (Wei *et al.*, 2013a). The two raw materials were at equivalent molar ratio, which could make the molecular weights of the products larger. The reacting temperature was 120°C, until NH_3, which was gradually generated during the reaction and absorbed by sulfuric acid latter, was fully released. Then, the temperature was increased to 160°C, at this stage, to get PHMGs with different molecular weights, the reaction time was controlled to be 4, 6, and 10 h, respectively. The solid product PHMG with the appearance of a white or milky white were dissolved in distilled water at room temperature, after being frozen in refrigerator for 48 h, the frozen PHMG solution were dried in a freeze dryer for 72 h to get pure PHMG samples. The molecular weight of PHMG was measured by MALDI-TOF mass spectrum latter.

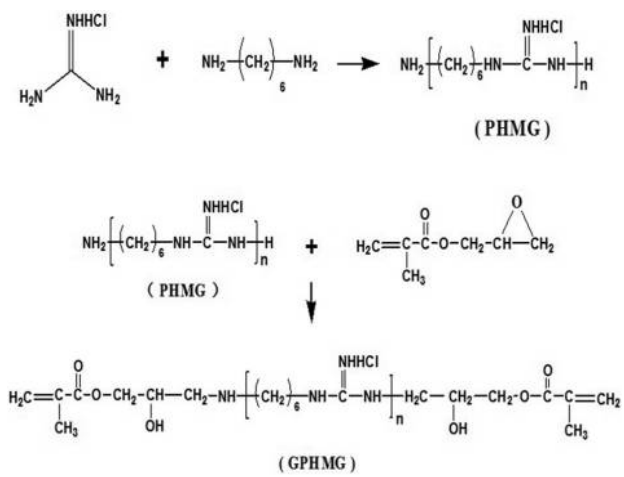
Preparation of glycidyl methacrylate-modified polyhexamethylene guanidine with different molecular weights

A certain amount of PHMG solution with a PHMG concentration of 40 Wt.% in DMSO was first prepared in a three-neck flask equipped with a magnetic stirrer and reflux condenser at 60°C, followed by the addition of a certain volume of GMA (the feeding molar ratio of GMA to PHMG was 2.0). The mixture was kept at 60°C under constant stirring for 60 h, the conversion rate was 75 per cent or more (Wei *et al.*, 2013b). To completely remove DMSO and unreacted GMA, the crude products were purified via repeated precipitation/dissolution in acetone at the assistance of sonication for three times. After being dried under vacuum at 50°C for 24 h, the purified product was obtained. The synthesis processes of PHMG and GPHMG are shown in Scheme 1.

Synthesis of cationic PBA-co-methyl methacrylate/glycidyl methacrylate-modified polyhexamethylene guanidine latex

To obtain the desired antibacterial latex, a seeded emulsion polymerization technique was adopted. The overall monomers mass and BA: MMA ratio (1.1: 1) were kept constant, while amount of GPHMG was adjusted within the range from 0.005 to 0.01 to the total amount of monomers. Typically, the monomers were first added to 80 mL distilled water along with 0.5 Wt.% of cationic emulsifier CTAB (and initiator AIBA (0.3 Wt.%) was dissolved in a glass jar to prepare the pre-emulsion. Then 5 per cent mass rate of the pre-emulsion was added to the three-neck flask containing 40 mL water solution of CTAB (0.1 Wt.% on overall monomers) along with initiator AIBA(0.2 Wt.% on overall monomers) and fitted with a reflux condenser flowed through with cooling water and a mechanical stirrer with a stirrer rate about 200 rpm and temperature at 76°C, besides nitrogen was slowly purged through the reactor to remove the oxygen for about 30 min. After that, the pre-emulsion was fed into the flask by a syringe pump at a steady rate over 2 h. Afterward, to ensure the high conversion of monomer, the emulsion polymerization was continued at 85°C for another 2 h. Finally, the acrylic latex was collected by filtering through a filter. Some of the dispersion sample was filmed in a teflon plate and dried at ambient temperature for further analysis. The chemical structures of polymer latexes are shown in Scheme 2.

Scheme 1 Preparation of PHMG and GPHMG



Characterization

Characterization of the modified guanidine oligomer

FTIR spectra were recorded by a Nicolet 5,700 FTIR spectrometer using KBr pressed disks.

MALDI-TOF-MS analysis was performed by a Bruker Reflex III apparatus equipped with a N_2 laser (λ = 337 nm) in linear mode at an acceleration voltage of 20 kV. Indole-3-acetic acid (IAA, Fluka, 99.0 per cent) was used as a matrix material. Samples were prepared with the dried droplet method from methanol solution by mixing matrix and polymer in a ratio of 20:5 (v/v) and applying approximately 1 mL to the target spot.

The particle size and zeta potential of corresponding particles were measured by MASTERIZER 2000 (Malvern Instruments Ltd, England) at 25°C.

Thermal gravimetric (TG) analysis was measured with a TA instrument SDT-Q600 thermal analyzer (USA) from 25°C to 600°C under a nitrogen atmosphere with a heating rate of 10°C/min and the injection volume was about 10 mg for each test.

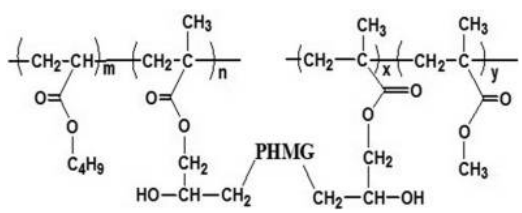
Differential scanning calorimetry (DSC) was carried out with a DSC 204F1 instrument (Netzsch, Germany) from −80°C to 150°C with a heating rate of 10°C/min and the injection volume was about 10 mg for each test. T_g was reported at the inflection point of heat capacity jump.

The following mechanical tests of antimicrobial polymer films were performed: adhesive force to glass (cross-cut test, ISO2409) and the mechanical test were conducted by INSRON 5,900 with the speed maintain 250 mm/min.

Characterization of antimicrobial activities of polymer films

To quantify the antimicrobial activities of the polymer films, *Escherichia coli* (*E. coli*, ATCC25922) and *Staphylococcus aureus* (*S. aureus*, ATCC29213), were selected as representative gram negative bacterium and gram positive bacterium, separately (Li *et al.*, 2016). Nutrient broth and phosphate-buffered saline (PBS) were prepared and sterilized according to the standard procedure. The antimicrobial activities of the polymer films were tested by plate count method according to the AATCC (American Association of Textile Chemists and Colorists) Test Method 100 (Xu *et al.*, 2013). Following are the specific procedures: *E. coli* and *S. aureus* were added to fresh nutrient broth, and were incubated on a rotary shaker with the rotation speed of 200 rpm at 37°C for 12 h. The bacterial suspensions were then diluted to a concentration of about 10^5 colony forming units (CFUs)/mL.

Scheme 2 Chemical structures of polymer latexes



Then 0.1 mL bacterial liquid was added to the surface of the polymer films which were sterilized by UV light for 30 min, to cultivate at 37°C for 4 h. After cultivation, the bacterial suspensions on the films were collected, and the films were washed three times with sterile PBS to remove residual bacterial. Then the collected bacterial suspensions were diluted to suitable concentrations. Subsequently, 0.1 mL diluted bacterial liquid was coated onto agar plates to cultivate at 37 °C for 24 hours. Then the colonies were counted and the antibacterial rate of tested sample were calculated through the following equation:

$$\text{Antibacterial rate } (\%) = \frac{(A - B)}{A} \times 100$$

where A and B are the number of the colonies observed for the control and film treated samples, respectively, under the same culturing condition. All tests were repeated at least three times and the results were averaged.

Ring diffusion method (Basri *et al.*, 2010) is a kind of semi-quantitative test, which can be used to evaluate the non-leaching properties of films. Following are the specific procedures: 0.1 mL of bacterial culture (10^6 CFUs/mL) was spread on LB agar plates before small piece of samples were plated on the surface of LB agar. The plates were placed in an incubator at 37°C for 24 h. The antimicrobial capacity and leaching characteristics can be evaluated by observing the growth of bacteria under the films and measuring the diameter of the inhibition zones.

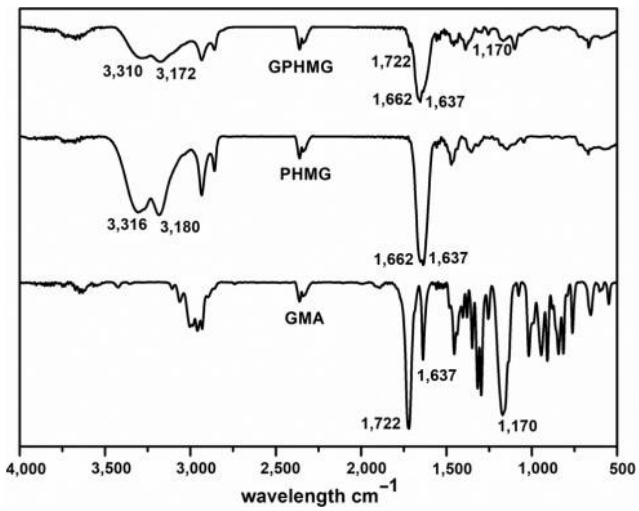
Results and discussion

It is a suitable way to synthesize PHMG by using hexamethylenediamine and guanidine hydrochloride as raw materials through thermal polycondensation at elevated temperatures to ensure a narrow molecular weight distribution, then modified antibacterial monomer GPHMG was synthesized via a ring-opening reaction between the amino group of PHMG and epoxy group of GMA (Scheme 1). The seeded emulsion polymerization technique was adopted to prepare the desired antibacterial latex (Scheme 2).

Fourier transform infrared analysis of modified guanidine oligomer

Figure 1 shows the FTIR spectra of GMA, PHMG and GPHMG. From the spectrum of GMA, it can be seen that there is a absorbance peak at 1,637 cm^{-1}, which should be assigned to the stretching vibration of C=C bonds. Besides the stretching vibrations of C=O and C-O of ester groups appeared at 1,722 and 1,170 cm^{-1}, the bands at 1,248, 907 and 804 cm^{-1} could be attributed to the characteristic bands of epoxy. As to PHMG, the vibrations of ν (NH), σ(NH), and ν(C=N) appeared at 3,316, 3,180, 1,637 and 1,662 cm^{-1}, respectively. For the spectrum of GPHMG, characteristic bands of PHMG can be seen. Furthermore, the bands of the ester group of GMA at 1,722 and 1,170 cm^{-1} appeared. Meanwhile, it is obvious that the characteristic bands of epoxy disappeared. Therefore, the occurrence of ring opening reaction of the epoxy group of GMA by PHMG was confirmed and the existence of C=C bonds in the modified PHMG was verified.

Figure 1 FTIR spectra of GMA, PHMG, and modified PHMG (GPHMG)



Polyhexamethylene guanidine hydrochloride analysis by MALDI-TOF mass spectrometry

Typical MALDI-TOF mass spectrum resulting from analysis of three PHMG samples synthesized at different conditions are shown in Figure 2. The structure of PHMG are similar to that reported by other researchers (Albert *et al.*, 2003). According to the reports (Wei *et al.*, 2013b), there are seven types of chemical structures including three linear (A, B, C) and four branched or cyclic ones (D, E, F, G). The molar contents of structure A-G in PHMG and the number average molecular weight of PHMG are shown in Table I. Among the different structures, linear structure A, B and C account for a main portion and the range of accessible products by polycondensation of a guanidinium salt and a diamine is limited to oligomers with average molecular weights between 500 to about 750 Da.

It is reported that, there are two types of functional groups in these seven species that may react with epoxy group of GMA, that is, the terminal primary amino group and the terminal amino-group on guanidine. Besides, the reaction activity of primary amino groups is much higher than that of guanidyl amino groups. However, the linear structures of PHMG contain both primary amino and guanidyl amino as end groups, which could ensure a high conversion rate when react with GMA.

Latex particle size analysis

Table II shows the influence of different weight ratios of GPHMG involved in the synthesis process of waterborne polyacrylic (WPA) emulsion on the zeta-potential and mean particle size of latex particles. From Table II, it can be seen that the particle sizes of testing samples ranged from 200 to 220 nm, which showed no direct connection with GPHMG content. Zeta potential is another representation of the emulsion stability. Obviously, the latex is cationic-charged, for the existing of cationic emulsifier CTAB and cationic monomer GPHMG. Besides, with the increasing of the content of GPHMG, the zeta-potential became obviously

Figure 2 (a) (b) (c) MALDI-TOF-MS of PHMG1,PHMG2,PHMG3

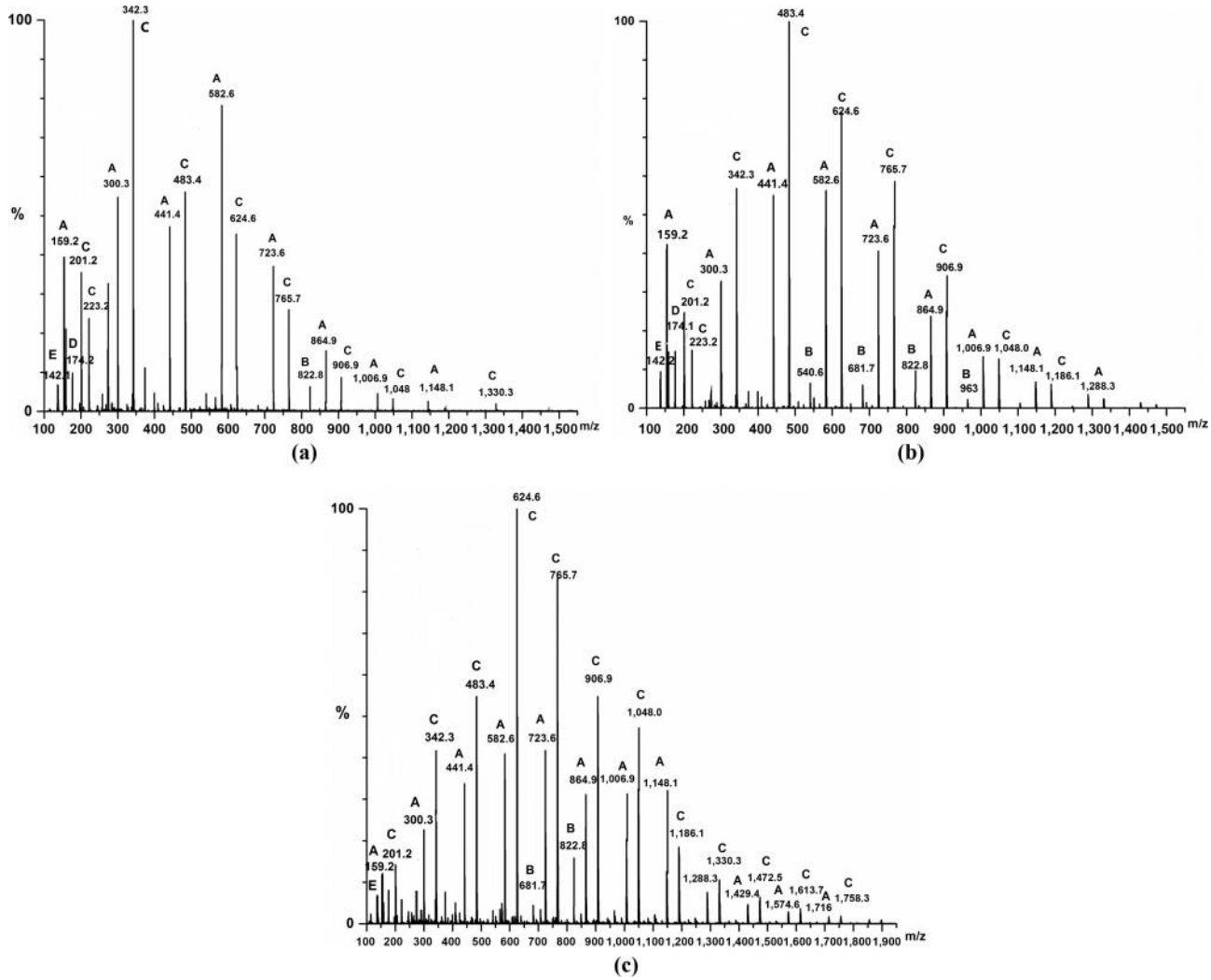


Table I Molar contents of structure A-G in PHMG and the number average molecular weights of them

Antibacterial agents	Polymerization time(h)	M_n	Liner structure (A, B, C) Content (%)	Branched or cyclic structure (D, E, F, G) Content (%)
PHMG1	6	498	88.34	11.66
PHMG2	8	584	84.54	15.46
PHMG3	12	752	81.44	18.56
PHMG4*	–	2,000	–	–

Note: *PHMG4 was purchased from Reagent Company with a molecular weight about 2,000 Da

Table II Zeta-potential and mean particle size of latex particles

GPHMG content (Wt.%) of WPA emulsion	Zeta potential (mv)	Mean particle size (nm)	PDI
0	0.505 ± 0.004	212.1 ± 8.4	0.084
0.6	1.75 ± 0.005	206.2 ± 7.2	0.098
0.7	2.59 ± 0.010	205.5 ± 7.6	0.066
0.8	3.92 ± 0.013	207.1 ± 9.1	0.095
0.9	5.85 ± 0.015	202.9 ± 8.7	0.139
1.0	7.88 ± 0.016	205.3 ± 6.9	0.035

higher, which definitely attributed to the increased number of cationic segments incorporated into the latex.

Characterization of polymer film

Thermal analysis

The TG measurement results are shown in Figure 3. The weight loss was initially plotted as a function of temperature. The result reveals that the thermal stability of GPHMG modified samples is very close to pure PA film sample. Given the low GPHMG content in PA polymers, either GPHMG is copolymerized in or blended in the polymers, this is quite understandable.

It is difficult to compare different curves in Figure 3; thus, the derivative weight loss curves (DTG, Figure 4) were plotted to clearly show the temperatures at maximum degradation. From Table III, slight differences in the temperature of maximum degradation was showed, which could almost be

Figure 3 TG curves of A, B, C, D related to Pure WPA film(A) , WPA film with 0.5wt% GPHMG (B), WPA film with 1wt% GPHMG (C), and WPA film with blended GPHMG (1wt%, D)

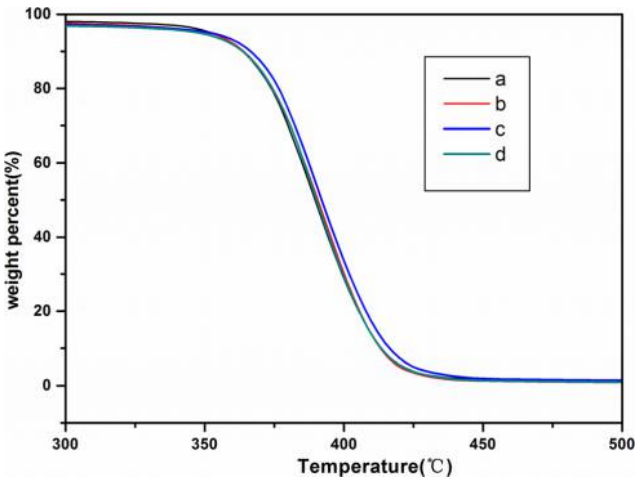


Figure 4 DTG results of Pure WPA film (A), WPA film with 0.5wt% copolymerized GPHMG (B), WPA film with 1wt% copolymerized GPHMG (C), and WPA film with 1wt% blended GPHMG (D)

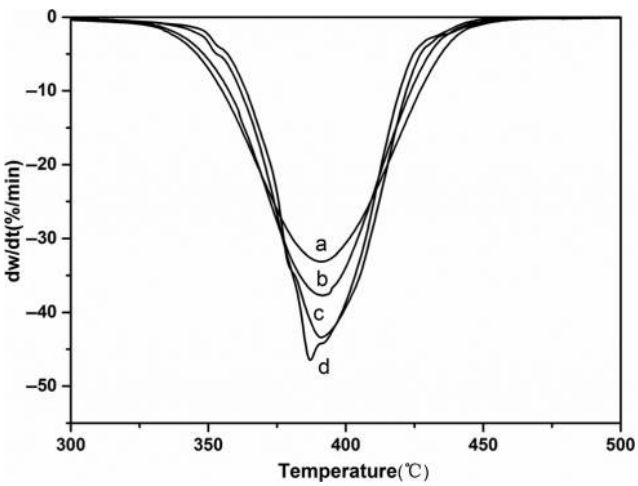


Table III TGA results for the samples

Serial no.	Sample type	Gas	Starting degradation temp. (°C)	Max degradation temp. (°C)
a	Pure PA film	Air	351.1	390.5
b	PA film with copolymerized GPHMG (0.5 Wt.%)	Air	348.1	388.5
c	PA film with copolymerized GPHMG (1 Wt.%)	Air	352.3	392.5
d	PA film with blended GPHMG (1 Wt.%)	Air	351.9	391.2

negligible. Besides noticeable trend based upon the amount of GPHMG joined the copolymerization was not revealed ether. From Figure 4, the curves A, B, C are unimodal, showing there is one kind of (co)polymer in polymerization system, which accords with the composition of (co)polymers. However, due to the blending of GPHMG to the polymer film, curve D is bimodal, and one main peak and one shoulder peak are existing. So we could ensure better antibacterial effect, while adding a small amount of GPHMG to the polymer emulsion by copolymerization; meanwhile, it did not affect the thermal stability, which greatly enhance its application value.

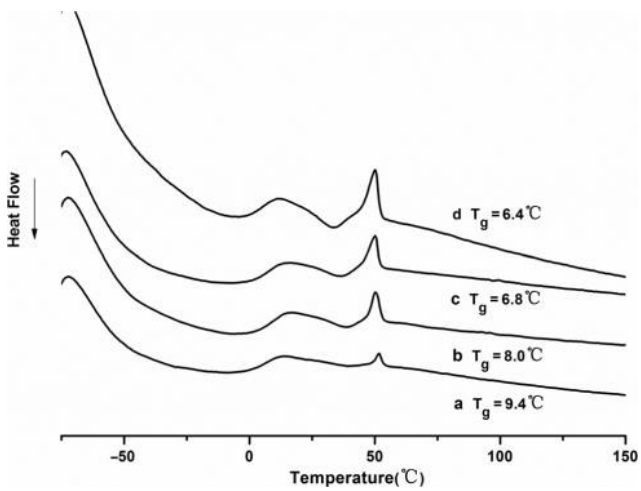
DSC analysis

DSC was also conducted on the samples. The samples were heated from −80°C to 150°Cwith a heating rate of 10°C/min, and the results are shown in Figure 5. It can be seen that whether by blending or by copolymerizing, the polymer showed a decrease in T_g with the content of GPHMG increasing. It can be deemed that the antibacterial monomer GPHMG is a soft oligomer and acting a role like internal plasticizer to make the T_g of the polymer decline. Meanwhile, considering the linear chemical structure of GPHMG, with the hydrophilic repeated guanidyl groups and the hydrophobic long-chain alkane groups of GMA, GPHMG could be a classical emulsifier undoubtedly, which may endow the polymer with lower T_g in some degree. Besides, longer polymer chain lengths give itself excellent flexibility and decrease the restriction for rotation and motion, and therefore should have a lower T_g, which could be used to explain the decrease in T_g when GPHMG was included.

Adhesion force test

The adhesion force relates to the chemical structures and surface elements on the surface of substrates. Therefore, interfacial adhesion force between the glass substrates and polymer film were measured as a part to illustrate its mechanical properties. According to the cross-cut test (ISO249) results, the adhesion force of polymer film showed

Figure 5 DSC curves of WPA films with different GPHMG contents (A, Pure WPA film, B, WPA film with copolymerised GPHMG (0.5wt%), C, WPA film with copolymerised GPHMG (1wt%), and D).WPA film with blended GPHMG (1wt%)



little relationship with the increase of GPHMG content because both of them display excellent adhesion force when coated on the surface of glass substrate and the rating is zero grade.

Mechanical characteristic

Figure 6 clearly shows the relationship between the content of GPHMG and tensile strength or breaking elongation of the WPA film. It can be seen that when the content of GPHMG increases, the tensile strength of the WPA film gradually decreases, while the breaking elongation increases. This is due to the linear structure of monomer GPHMG, which contains a lot of repeating structural units of guanidyl which endow itself excellent malleability. Therefore, when the content of GPHMG increased, the flexibility and elasticity of the WPA chains will improved, leading to the increase of the breaking elongation. On the other hand, the rigidity of GPHMG is weaker than other short-chain acrylic monomers, when its content increases, the tensile strength of the WPA film will decrease.

Water absorption rate of polymer film

The polymer film with thickness of 1 mm was made by filming the acrylic emulsion sample in a PTFE plate and dried at ambient temperature. Before soaking into the water for 24 h, the polymer film with a size of 3 × 3cm was dried to a constant mass with a weight of W_0, after the soaking process, water on the polymer surface was removed by the filter paper, then its weight was measured and recorded as W_1. The water absorption rate of polymer film was calculated by the following formula:

$$\text{Water absorption rate}(\%) = \frac{(W1 - W0)}{W0} \times 100$$

From Figure 7, it is clear that the water absorption rate gradually increased with the increasing of the content of hydrophilic GPHMG. We know that microorganism prefer to grow in somewhere warm and wet, a hydrophobic surface is more effective to prevent microorganism from growing. To endow the polymer film more excellent hydrophobic properties and retain its antibacterial properties, on one hand, the content or species of hydrophobic monomer should be

Figure 6 Effect of GPHMG content (wt%) on mechanical property of WPA film

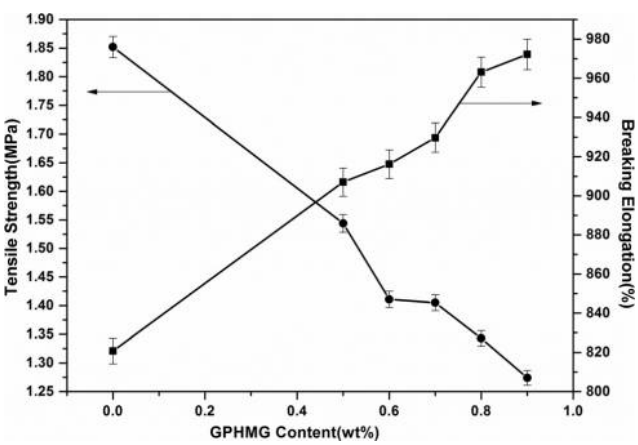
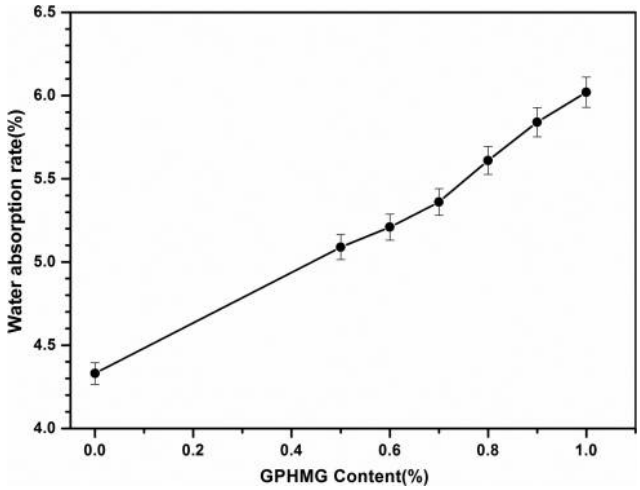


Figure 7 Water absorption rate results for the samples of different GPHMG content



increased in some degree, and, on the other hand, the amount of hydrophilic monomer, GPHMG, should be controlled to the optimum content, which will be studied later in antibacterial test part.

Antimicrobial properties of waterborne polyacrylic film

Antibacterial activity of the waterborne polyacrylic films with different glycidyl methacrylate-modified polyhexamethylene guanidine content

The antibacterial activity of the WPA films with different GPHMG (modified with PHMG3, M_n = 752) content was evaluated qualitatively by plate count method. S. *aureus* and *E. coli* were used as models in this experiment. The antibacterial photographs were obtained by culturing the same volume of bacterial solution on LB agar plate for 24 h. Figure 8 displays the bacteria survival ratios after 4 h exposure to the control sample, WPA films with GPHMG content of 0.9, 0.8, 0.7, 0.6 or 0.5 per cent, respectively. Besides the small white dots on the culture plates represented the bacterial colonies (a) *S. aureus* and (b) *E. coli*. The quantitative antimicrobial results of WPA films are presented in Figure 9.

Figure 8 reveals that plenty of bacteria colonies survived on control samples. As to photographs of *S. aureus* (a) colonies, it

Figure 8 Photographs of S. aureus (a) and E. coli (b) colonies grew on LB agar plates upon a 4 hours' exposure to film samples with different GPHMG contents

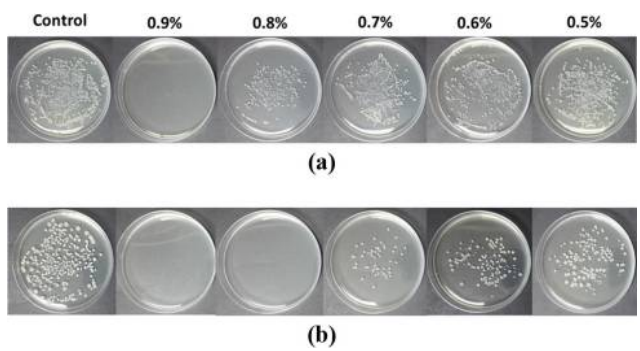
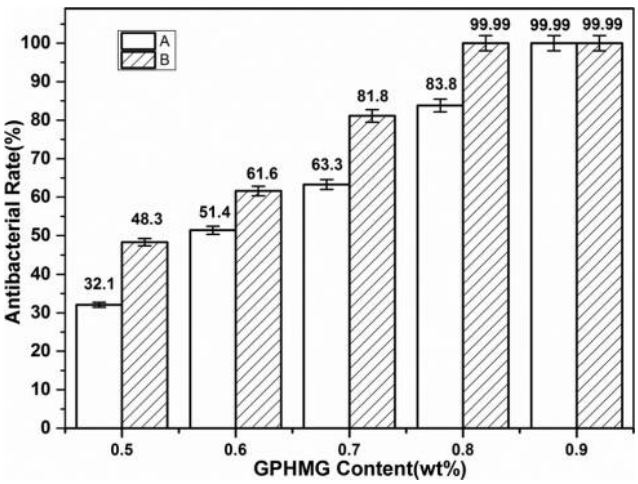


Figure 9 Antibacterial rate of antibacterial polymer films against S. aureus (A) and E. coli (B)



can be seen that when the content of GPHMG decreased to 0.8 Wt.%, the bacterial cannot be completely killed and the antibacterial rate was about 83.8 per cent (Figure 9). In contrast, the antibacterial WPA film completely killed *E. coli*, even when the content of GPHMG was as low as 0.8 Wt.% and when the amount of GPHMG was 0.7 Wt.%, the antibacterial rate was about 81.1 per cent, which demonstrates that the antibacterial polymer film possesses stronger antibacterial ability against *E. coli* than against *S. aureus*. The mechanism for the antibacterial effect of antibacterial monomer GPHMG is deemed to be the same as PHMG due to the destruction of bacterial membrane. Guanidine polymer inhibits bacterial growth by attacking them through electrostatic attraction between cationic guanidyl groups and anionic groups on the cell surface of bacteria. After attaching to bacteria cells, guanidine polymer induces bacterial membrane collapsed, then cytoplasm leaking, and finally the death of bacteria (Broxton *et al.*, 1984). A higher GPHMG content led to higher bacteria deactivating efficiency, certain amount of GPHMG content (higher than 0.9 Wt.%) is essential for sufficient antibacterial activity.

Antibacterial activity of the waterborne polyacrylic films with same glycidyl methacrylate-modified polyhexamethylene guanidine content but different molecular weights

Previously, we investigated the antibacterial activity of WPA films with different GPHMG content and found out that when the amount of GPHMG was as lower as 0.8 Wt.% or 0.7 Wt.%, *S. aureus* or *E. coli* began to appear on the LB agar plates, respectively. Then we furtherly investigated the effect of molecular weight of GPHMG brought to antibacterial properties of WPA films. The photographs are presented in Figure 10, and the relationship between antibacterial rate and molecular weight of GPHMG are shown in Figure 11. The Number 3 sample exhibited a better performance, besides antibacterial rates are 83.5 per cent against *S. aureus* and 85.4 per cent against *E. coli*, while Number 1 sample showed the weakest antibacterial property and Number 4 sample showed a moderate antibacterial property among other samples. The result showed that if weight content of GPHMG in WPA films is constant, antibacterial property of WPA film is strengthened

Figure 10 Photographs of S. aureus (a) and E. coli (b) colonies grew on LB agar plates upon 4 hours exposure to WPA film samples of same GPHMG content with different molecular weight (The number 1#, 2#, 3#, 4# related to different molecular weight of GPHMG gained by modifying PHMG, the percentage number related to the GPHMG weight content in polymer films

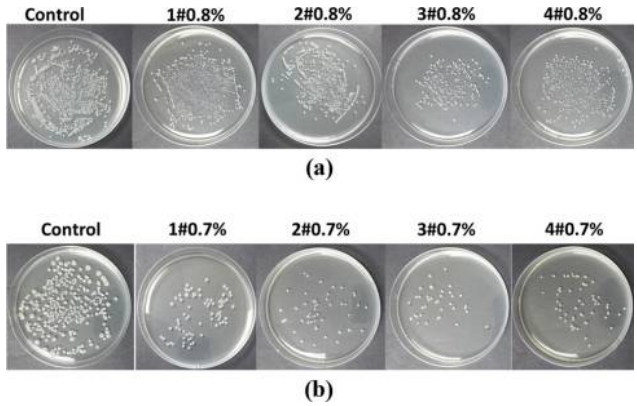
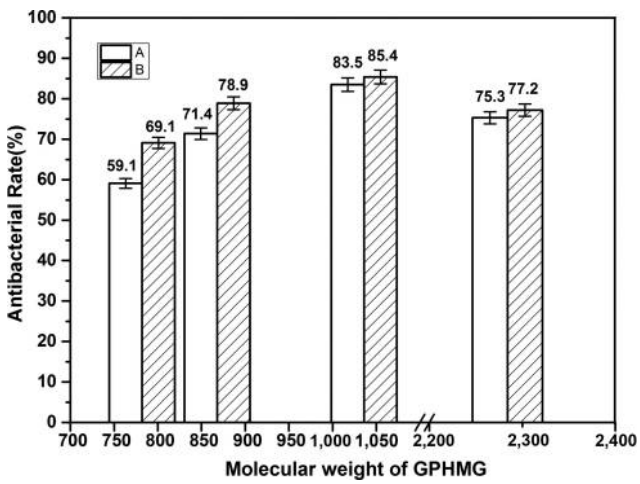


Figure 11 Antibacterial rate of WPA films with GPHMG of different molecular weight against S. aureus (A) and E. coli (B)



gradually with the increase of the molecular weight of GPHMG, until it comes to a threshold, the antibacterial property will be weakened progressively.

Due to the positive charges of GPHMG, once bacteria contacted the surface of the film, the cell membrane of bacteria could be damaged through electrostatic attraction (Zhou *et al.*, 2009). The antibacterial activity of GPHMG was closely correlative with the total charges of GPHMG molecule (Wei *et al.*, 2009), the higher the molecular weight of GPHMG, the more charges per molecular contains. When the weight content of GPHMG in the polymer is determined, so is the total charges. Therefore, the higher the molecular weight of GPHMG is, the less GPHMG molecules are. Meanwhile, we have to realize that the number of GPHMG molecules and its dispersion uniformity in the film greatly influence the spacer length (the length between the active biocidal unit and the polymer backbone) (Wang *et al.*, 2013b), further effect the charge density in unit area of film. To acquire the best

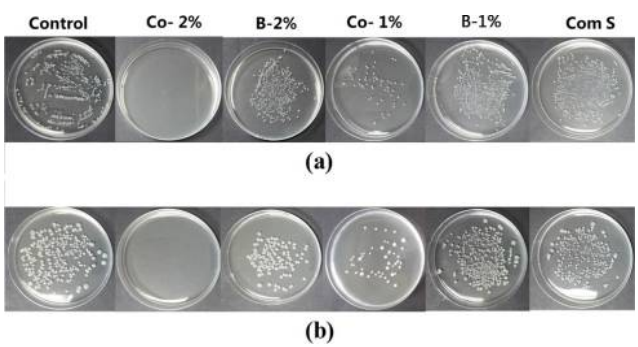
antibacterial activity, the molecule number and total charges of per GPHMG molecule should get a balance. The more the functionalized molecule is, the more functional sites to contact the bacteria are, with a suitable electrostatic attraction, which may result in the improved antibacterial efficiency (Hong *et al.*, 2010). Thence, just as the results of the experiment showed, the Number 3 series sample with a moderate molecule weight of GPHMG performed a better antibacterial activity than the others.

This give us a good reference in industrial production that at the same weight content in antibacterial products, the antimicrobial agent GPHMG or PHMG should have an appropriate molecule weight to obtain the most superior antibacterial properties, which can reduce economic costs in some degree.

Water resistance of the antibacterial waterborne polyacrylic films

We compared the antibacterial stability of the structural antibacterial WPA film and the blended one, by testing antibacterial activity of five different film samples, namely WPA film with copolymerized GPHMG (2 Wt.%,1 Wt %), WPA film with blended GPHMG (2 Wt.%, 1 Wt %) and a commercial used antimicrobial film sample synthesized by doping antibacterial agents, after soaking in DI water for 12 h. The test results are showed (Figure 12) that all the WPA films, except copolymerized 2 per cent sample, showed a diminished antibacterial properties compared to the samples without soaking. However, in contrast to other two samples prepared by doping, the WPA film with copolymerized GPHMG still displayed a very high antibacterial activity. When GPHMG came to a higher content (2 Wt %), this situation became rather apparently. The structural antibacterial WPA film we synthesized had a more stable and perdurable antibacterial properties. Meanwhile, this result made our work more meaningful and promising. The weakened antibacterial properties of structural antibacterial polymer film are mainly due to the dissolving of some antibacterial monomer GPHMG, which may not completely copolymerize with other acrylic monomers, and the copolymerization efficiency remains to be improved in our future work.

Figure 12 Photographs of S. aureus (a) and E. coli (b) colonies grew on LB agar plates upon a 4h exposure to different samples after soaking in water for 12h (Here, Control sample represents pure WPA film without GPHMG. copolymerised 2% and Blended 2% represent WPA film with copolymerized GPHMG content of 2wt%. Blended 2% represents WPA film with copolymerized GPHMG content of 2wt%, others like the same. Commercial sample represents a commercial used antimicrobial film sample synthesized by doping antibacterial agents.)



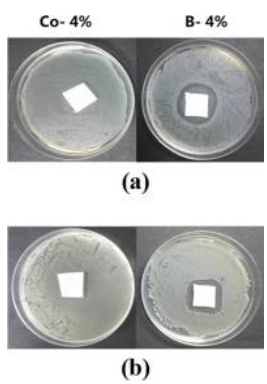
Evaluation of the non-leaching effect of waterborne polyacrylic films

The ring-diffusion method was applied to further evaluate the non-leaching effect and antimicrobial properties of the film as well. Owing to the leaching effect, the antimicrobial agents with migration ability in certain antimicrobial materials are tend to form an inhibition zone in the ring-diffusion test (Hao *et al.*, 2015). The test was performed on WPA films with a GPHMG content of 4 Wt %, but synthesized by copolymerization and blending, respectively. From Figure 13, none of the films prepared by copolymerization in this work had shown any inhibition zone, which confirmed the non-leaching effect of structural antibacterial WPA film. In contrast, the antimicrobial WPA film by doping GPHMG displayed an apparent inhibition zone with a diameter about 2 cm, demonstrating GPHMG in this film exists penetration and diffusion phenomenon. Nevertheless, the results give a further illustration that the structural antibacterial WPA film is more perdurable and more environmentally friendly.

Conclusion

A PHMG-based novel non-leaching and eco-friendly antimicrobial WPA emulsion has been prepared through emulsion polymerization. The addition of GPHMG gained by modifying PHMG showed little influence on thermal stability of the films, but decreased the glass transition temperature. Meanwhile, the tensile strength decreased, while the breaking elongation increased. The antimicrobial properties test results indicated that a relatively high GPHMG content (around 0.9 Wt.%) was essential for an excellent antibacterial activity. Besides, when the weight content of GPHMG in the films remain constant, antibacterial property increased first and then decreased with the increase of molecular weight of GPHMG. The ring-diffusion test confirmed the non-leaching effect of the prepared WPA film, and a comparison was made to prove that the structural antibacterial polymer film had more perdurable antibacterial activity than the blended one.

Figure 13 Photographs of S. aureus (a) and E. coli (b) colonies grew on LB agar plates by ring-diffusion test of the antibacterial polymer films (Here, copolymerised 4% represents polymer film with copolymerized GPHMG content of 4wt%. Blended 4% represents polymer film with copolymerized GPHMG content of 4wt%.)



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