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Synthesis and antimicrobial activity of polymeric guanidine and biguanidine salts

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Abstract

Polyhexamethylene guanidine hydrochloride and polyhexamethylene biguanidine hydrochloride were polycondensated in the melt of monomers. The intrinsic viscosity and $\bar{M}_n$ were determined by the viscosity of dilute polymer solution and VPO, respectively. Polyhexamethylene guanidine stearate and polyhexamethylene biguanidine stearate were synthesized using the precipitation reaction for the first time. NMR, FTIR, XPS and element analysis were used to analyze the chemical structure and element composition. The structure of the resultant polymers is coincident with the theoretic structure. The minimal inhibition concentration (MIC) was measured to characterize the antimicrobial activity of the products. The MIC is not larger than 200 $\mu g\,ml^{-1}$. The polymeric guanidine and biguanidine are effective in controlling bacteria and fungi. The TG test shows that the resultant polymers are stable to heat.

Keywords: Polymeric guanidine; Synthesis; Antimicrobial activity

1. Introduction

Since a series of substituted guanidines was reported to have antimicrobial properties in the 1930s, the guanidine derivates with antibacterial and antifungal activity have been investigated as medical, crop protection agents and antiseptics for industry products, food and other goods for daily use [1–5]. Among the guanidine derivates with antimicrobial activity, the polymeric guanidine and biguanidine salts have gained importance as the antiseptics for cosmetics and textiles. The polymeric biguanidines, produced by ICI American Inc. under the trade-names of Arlagard E. and Cosmocil CQ, are known biocides with a wide spectrum of antimicrobial activity and used as antiseptics for cosmetics [6]. Recently, Cosmocil CQ has also been used as one of the antimicrobial components for nonwoven wipes [7]. In addition, some guanidio antimicrobial agents are stable to heat, which are ideal additives for polymer materials produced in the melting process, such as melting spinning and injection [8,9]. The antimicrobial additives are dispersed throughout the polymer matrix during the molten stage and then continuously migrate to the matrix surface, which provides an effective antimicrobial activity even after a long time. Most commercial guanidine antimicrobics are soluble in water, which is environmentally friendly and convenient for use. However, if the water-soluble antimicrobics are used as additives for industrial goods and clothes, the final products are deficient in antimicrobial fastness to laundering. The antimicrobial fastness to laundering can be improved by decreasing the water-solubility of the additives [10]. It has been found that different salts have a large effect on the solubility of the guanidine compound.

In this paper, the water-soluble guanidine, polyhexamethylene guanidine hydrochloride (PHGC), and polyhexamethylene biguanidine hydrochloride (PHBGC), were polymerized in melting. The lipophilic guanidine, polyhexamethylene guanidine stearate (PHGS), and polyhexamethylene biguanidine stearate (PHBGS), were synthesized using the precipitation reaction. Meanwhile, the molecular weight, chemical structure, solubility, antimicrobial activity and heat stability were investigated in detail.

2. Experimental

2.1. Sample preparation

Equimolar amounts of guanidine hydrochloride and 1,6-hexamethylenediamine were cooligomerized for 2 h at 120°C until liberation of NH_3 was completed, and then polymerized at 160°C for 4, 5, 6 and 8 h to prepare

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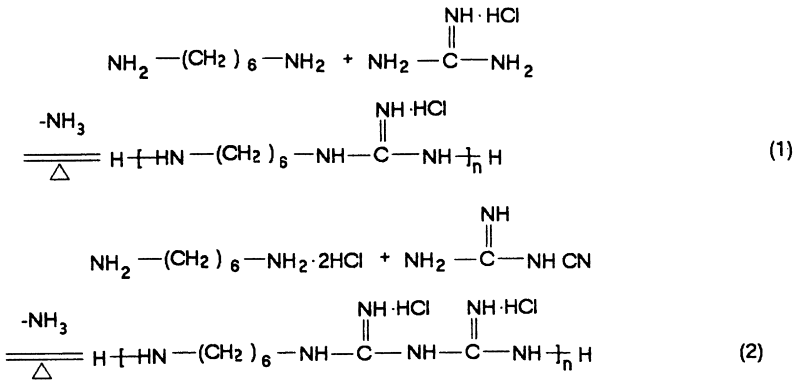


Scheme 1. Scheme of the polycondensation.

polyhexamethylene guanidine hydrochloride in the form of viscous mass which solidified after cooling.

Equamolar amounts of dicyandiamide and hexamethylenediamine hydrochloride were polycondensated at 180°C for 6, 8, 10 and 12 h in the melt of monomers and polyhexamethylene biguanidine hydrochloride was obtained.

The synthesis of lipophilic polymeric guanidine and biguanidine salts was performed by means of the precipitation reaction. Polyhexamethylene guanidine stearate (PHGS), for example, was prepared in the following steps.

PHGC and sodium stearate were dissolved in distilled water respectively. Then, the sodium stearate solution was added to the PHGC solution with stirring at 80°C, during which time the molar ratio between the sodium stearate and the repeating unit of PHGC is equal to 1:1. The precipitated PHGS was filtered, washed with boiling water and dried in vacuum.

Polyhexamethylene biguanidine stearate (PHBGS) was prepared similarly to PHGS. However, the molar ratio between the sodium stearate and the repeating unit of PHBGC is equal to 2:1.

2.2. Intrinsic viscosity

The viscosity of dilute polymer solutions were determined using an Ubbelohde viscosimeter (Φ0.44 mm) at 25 ± 0.05°C. Since PHGC and PHBGC are polycations in water, the measurements were performed in 3 g dl^{-1} aqueous NaCl solutions to suppress the polyelectrolyte effect. The measurements were made for four concentrations in the range of C = 0.75 ~ 3.0 g dl^{-1}. Using a double extrapolation of η_{SP}/C and $\ln\eta_r/C$ to infinite dilution, the intrisic viscosity (η) was determined.

2.2.1. VPO

The $\bar{M}_n$ of the polymers was determined using a Corona-117 vapor pressure osmometer. The apparatus constant was standardized with the standard sample of sucrose (M_w = 342) in the solvent of H_2O at the temperature of 60°C. The polymers were solved in H_2O at the concentration of 0.001 g ml^{-1}, and then measured at 60°C.

2.2.2. ^{1}H-NMR

The polymers were dried under vacuum at 50°C. ^{1}H-NMR spectra were recorded on an Fx-300 NMR spectrometer at 300 MHz. Deuterated methanol (δ = 4.80 ppm) and deuterated trichloromethane (δ = 7.27 ppm) was used as solvents for PHGC, PHBGC and PHGS, PHBGS, respectively, without an internal standard.

2.2.3. FTIR

FTIR were recorded on a Nicolet 170SX Fourier transform infrared spectrophotometer with the resolving power of 2 cm^{-1} and scanning times of 32.

2.2.4. XPS

The XPS spectra were obtained by means of a NP-1 X-ray photoelectron spectrometer, operated in the FAT mode, using Mg Kα X-radiation. The test temperature was 25°C and the sample chamber was pumped to about 10^{-7} Pa.

2.2.4.1. Element analysis The samples were dried under vacuum at the temperature of 50°C before measuring. The elements C, H, N were measured by a Neraeus CHN-O-rapid element analyzer and a KLY chloride micro-coulombmeter was used to measure the content of element Cl.

2.2.4.2. Solubility measurement The solubility was measured at the temperature of 25 ± 0.1°C in the solvents of distilled water, ethanol, methanol, acetone, methylbenzene and *N*,*N*-dimethylformamide (DMF).

2.2.4.3. Test of antimicrobial activity [11] The minimal inhibition concentration (MIC) of antimicrobial agents was measured using the serial dilution method. The tested samples were dissolved in methanol with the concentration from 200 ∽ 0.39 μg ml^{-1}. In addition, the samples were heated at 230, 250 and 280°C under the flow of N_2 for 15 min, respectively. Then the heat-treated samples were tested using the method of agar diffusion.

Table 1
Synthesis conditions and molecular weight of the polymers

No.	Sample	Polymerization time (h)	Intrinsic viscosity (dl g^{-1})	$\bar{M}_n$
A1	PHGC	4	0.0590	1275
A2	PHGC	5	0.0649	1842
A3	PHGC	6	0.0979	2332
A4	PHGC	8	0.1573	3520
B1	PHBGC	6	0.0101	814
B2	PHBGC	8	0.0175	1105
B3	PHBGC	10	0.0176	1127
B4	PHBGC	12	0.0192	1320

2.2.5. TGA

The TG spectra were recorded on a Perkin Elmer TAS7 apparatus at an N_2 flow rate of 40 ml min^{-1} and a heating rate of 20°C min^{-1}.

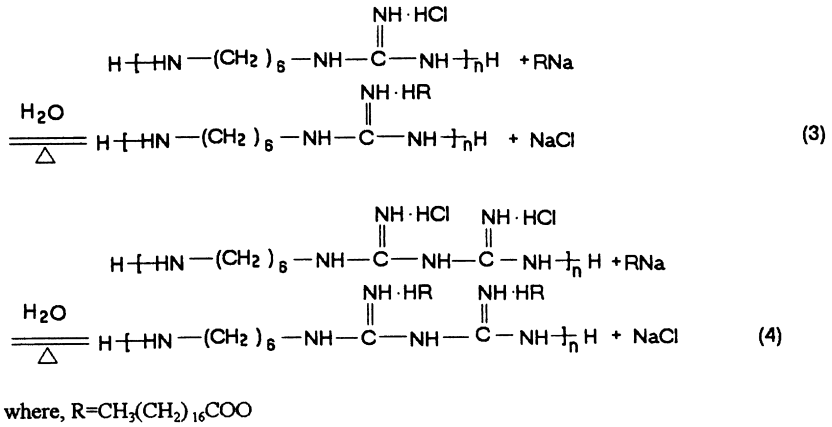
3. Results and discussion

3.1. Syntheses

There are several routes for synthesis of polymeric guanidine and biguanidine. The typical polymerization is usually performed in solution. Peter and coworkers [12,13] used the reaction of diamines and chlorine cyan (ClCN) in organic solvents such as glycerol to prepare polymeric guanidine hydrochloride. It is well-known that the monomer ClCN is toxic. In addition, sodium dicyanimide or zinc dicyanimide were also used as reactants with diamines salts in ethyl alcohol or water to prepare polymeric biguanidine [14,15]. The final products prepared in solution often have to be purified and dried. Here, the polymeric guanidine and biguanidine hydrochloride were prepared in the melt of monomers at high temperature without any solvent [16,17]. Polycondensation of hexamethylenediamine with guanidine hydrochloride gave the polyhexamethylene guanidine hydrochloride. Polycondensation of hexamethylenediamine chloride with dicyandiamide gave polyhexamethylene biguanidine hydrochloride. The reaction equations are found in Scheme 1. The resultant polymers are shown in Table 1.

Under the experimental conditions, the polymerization degree depends on the reaction time if the composition of the initial monomers and the synthesis temperature are fixed. The polymerization degree increases with increasing reaction time (shown in Table 1). It was noticed that the intrinsic viscosity and $\bar{M}_n$ were relatively low but the melt viscosity was high in the polymerization process. This is the typical character of the polyelectrolytes with the formation of ion pairs, multiplets and even clusters [18]. In this case, the functional groups were embedded within multiplets, which decreased the reactivity of the functional groups. Meanwhile, it is difficult for the small molecule NH_3 to be released during the reaction. It was also found that the molecular weight of PHBGC is lower than that of PHGC at the same reaction time. This will be discussed later. The samples used are PHGC and PHBGC, reacting at 8 h.

The conventional method of preparing guanidine salts, except for guanidine hydrochloride and guanidine nitrate, as well as guanidine carbonate, is in the following way [19]. The ethanol solution of sodium ethlate is added to the ethanol solution of guanidine hydrochloride. The resulting residue of sodium chloride is filtered out and the corresponding acid is added to the filtrate. The goal guanidine salts are obtained at last [6]. It is clear that there are several steps in the method and the resulting products are difficult to purify, especially for the salts that are easy to dissolve in



Scheme 2. Scheme of the precipitation reaction.

Table 2
Chemical shifts of different protons

Sample	Peak number	Chemical shifts δ (ppm)	Proton number	Peak description
PHGC	1	1.3 ~ 1.5	a, d, j	sharp, splitting
	3	1.5 ~ 1.7	c	sharp, splitting
	5	3.2 ~ 3.3	b	sharp, splitting
	6	6.6 ~ 6.8	e, g	weak, broadening
	7	7.1 ~ 7.2	e, g	weak, broadening
PHBGC	1	1.3 ~ 1.5	a, d, j	sharp, splitting
	3	1.5 ~ 1.7	c	sharp, splitting
	5	3.2 ~ 3.3	b	sharp, splitting
	6	6.6 ~ 6.8	e, g	weak, broadening
	7	7.1 ~ 7.2	e, g	weak, broadening
PHGS	1	0.8 ~ 0.9	a, d, j	sharp, splitting
	2	1.2 ~ 1.4	i	one peak
	3	1.4 ~ 1.6	c	broadening
	4	2.1 ~ 2.2	h	broadening
	5	3.1 ~ 3.3	b	broadening
	6	7.3 ~ 7.8	e, f, g	weak, broadening
	7	8.1 ~ 8.5	e, f, g	weak, broadening
PHBGS	1	0.8 ~ 0.9	a, d, j	sharp, splitting
	2	1.2 ~ 1.4	i	one peak
	3	1.4 ~ 1.6	c	broadening
	4	2.1 ~ 2.2	h	broadening
	5	3.0 ~ 3.2	b	broadening
	6	7.3 ~ 7.6	e, f, g	weak, broadening
	7	8.1 ~ 8.2	e, f, g	weak, broadening

ethanol. In this paper, lipophilic guanidine salts were prepared by a new method for the first time. Due to the different solubilities of different guanidine salts in water, the precipitation reaction is applied to synthesize lipophilic polymeric guanidine and biguanidine salts. Scheme 2 shows the syntheses of polyhexamethylene guanidine stearate (PHGS) and polyhexamethylene biguanidine stearate (PHBGS).

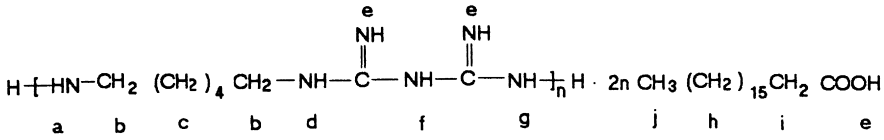
The resulting polymeric guanidine and biguanidine salts were washed with distilled water at 80°C and then dried. The final products are white solids without any odour.

The chain of the resultant polymers is composed of C and N. It is known that the proton peaks of CH_2 are often complex, even difficult to distinguish [20]. In addition, the electron density of N is close to that of C and the proton peaks of NH and CH overlap each other in some special chemical environments. Meanwhile, the exchange rate of H on NH_3^+ (or $C=NH_2^+$) with other protons is slow, which leads to coupling and splitting of the neighbor protons. In this case, the proton peaks of NH_3^+ (or $C=NH_2^+$) are broadening and even get close to the base line [20].

The chemical shifts of different protons are shown in Table 2 and the proton position responding to the peak number is shown in Scheme 3.

In Fig. 1, Peak 1 is due to the proton of C–NH–C in guanidio. It also includes the proton of CH_3 for PHGS and PHBGS. Peak 2 and Peak 4 are the peaks of the proton of βH^1 and αH^1 in the stearic ion. Peak 3 and Peak 5 are the peaks of the proton of βH^1 and αH^1 in hexamethylene. It was noticed that Peak 6 and Peak7 are weak, broadening and even level to the base line. Those are the peaks of H^1 on $C=NH_2^+$. The broadening and splitting peaks result from the relaxation of N^{14} and the slow exchange rate of the proton on $C=NH_2^+$.

Fig. 2 is the FTIR spectra of the resultant polymers. It is reported [21] that there are four characteristic peaks for guanidine compounds as following: ν_{NH} at about 3300 cm^{-1}, $\nu_{C=N}$ at 1689 ~ 1650 cm^{-1}, δ_{NH} at about 1640 cm^{-1} and ν_{C-N} at about 1300 cm^{-1}. Meanwhile, there is also a characteristic peak at 1620 ~ 1560 cm^{-1}, which means the bend vibration of NH_2^+ ($\delta_{NH_2^+}$). All the vibration peaks and the corresponding frequencies are listed in Fig. 2. The positions of characteristic peaks for guanidine and biguanidine are about the same, but the intensity is different. For example, the $\delta_{NH_2^+}$ at 1562 cm^{-1} for PHBGC



Scheme 3. Chemical structure of PHBGS.

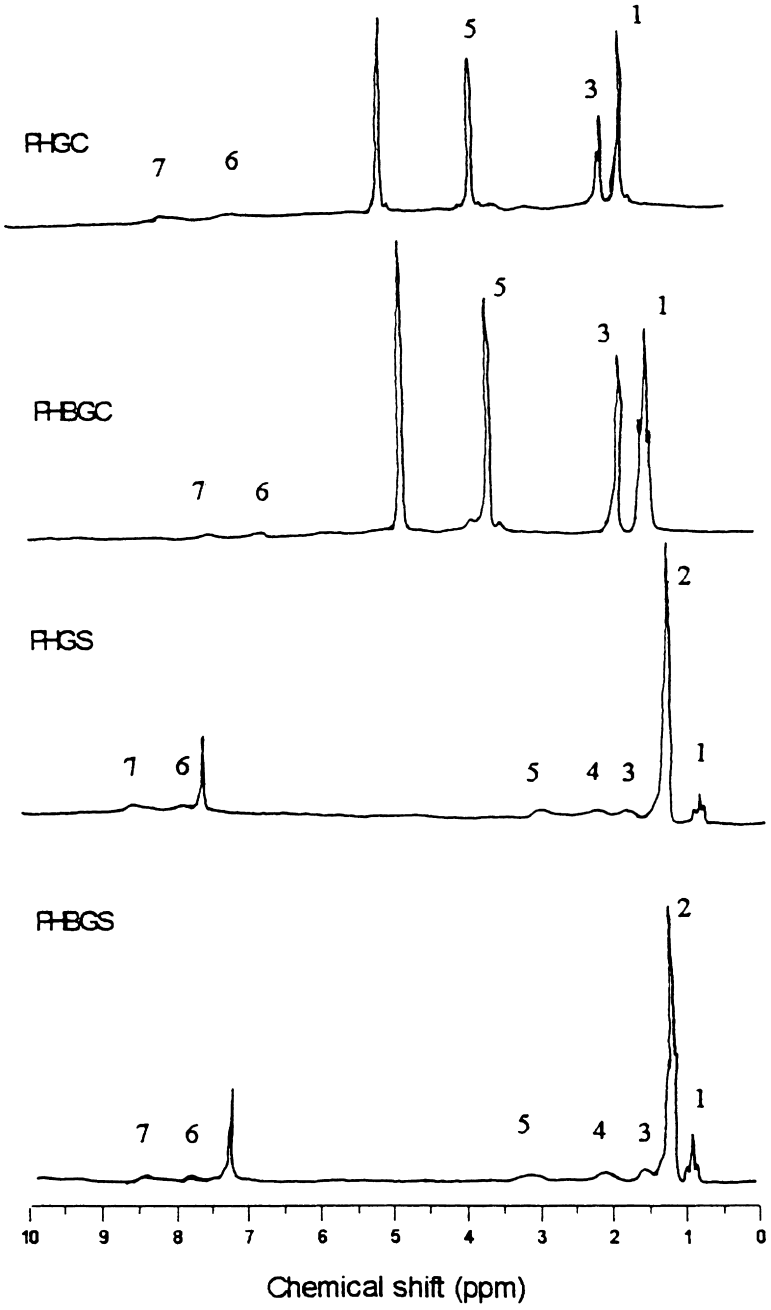


Fig. 1. NMR spectra of the polymers.

is obviously more intense than that for PHGC if the δ_{CH_2} at 1478 cm^{-1} is taken as the datum peak. In addition, there is ν_{COO^-} at about 1400 cm^{-1} for PHGS and PHBGS. It is obvious that there are clear characteristic peaks for all tested samples, which means that the resultant polymers possess the basic structure of guanidine.

The XPS spectra in Fig. 3 illustrate the element composition for the resultant polymers qualitatively. It is reported [22] that the electron binding energy of N_{1s}, C_{1s} and Cl_{2p} of guanidine hydrochloride are 400.10, 288.20 and 200.00 eV, respectively. The binding energy of the resultant polymers exhibit some deviation from the above data since the substituent on the guanidio affects the binding energy of N_{1s} and C_{1s}. The binding energy of N_{1s} is 414.5 eV. The peak of C_{1s} is broadening, which includes two peaks representing C_{1s} in CH_2 and C = N, respectively. The binding energy of O_{1s} is 500 eV. In the experimental process, the sample chamber was pumped to about 10^{-7} Pa. In fact, there is still some O_2 adsorbed on the sample surface. Therefore, the XPS spectra of PHGC and PHBGC show a few of peaks at about 500 eV, while the signal strength of PHGS and PHBGS is larger, which includes the O_{1s} in O_2 and COO^-.

Further, the content of the element is shown in Table 3. The experimental data of PHGC is coincident with

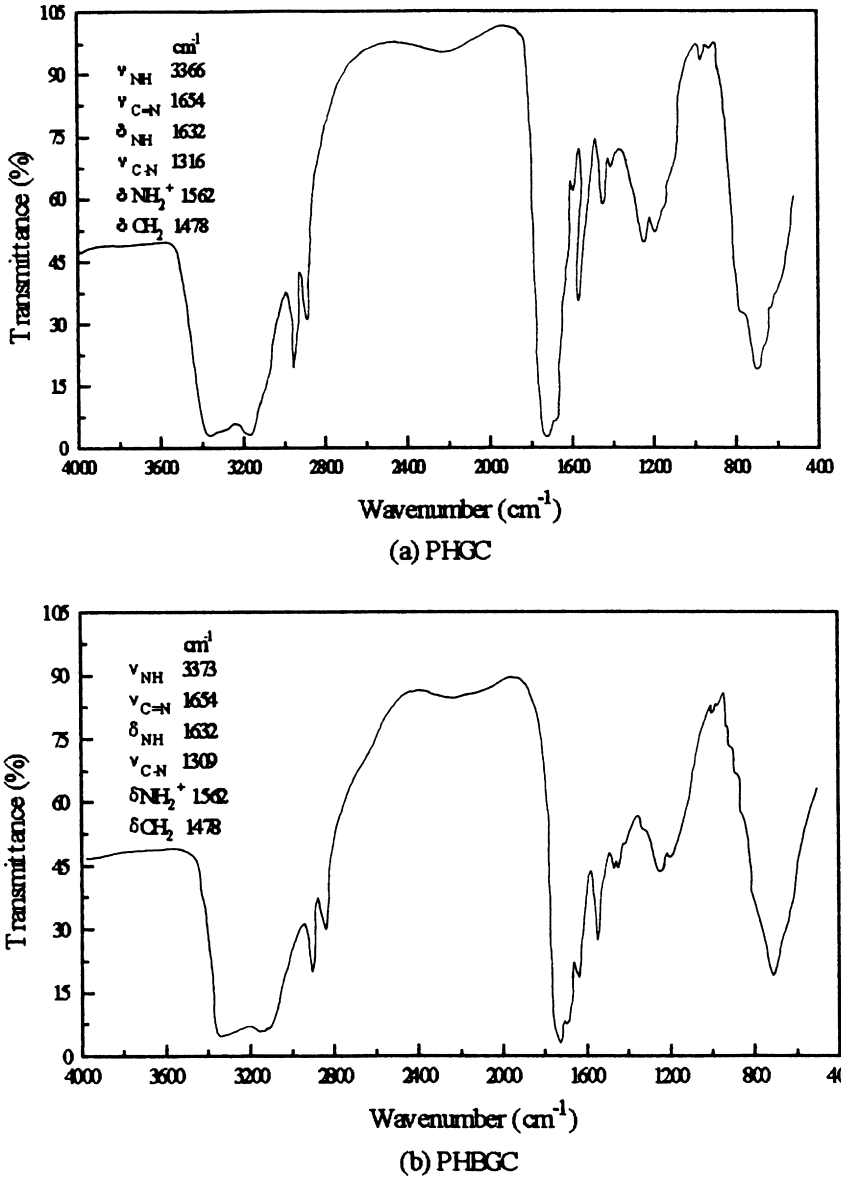
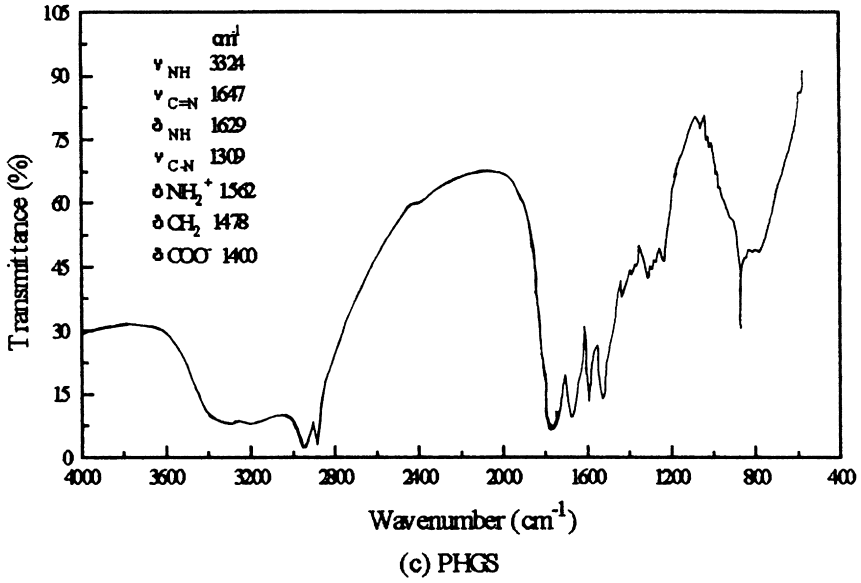


Fig. 2. FTIR spectra of the polymers.

the theoretic value, which proves that the chemical structure is in agreement with the structure shown in Eq. (1) of Scheme 1. Under the experimental conditions in this work, there is little by-reaction. The contents of element C and Cl in PHBGC are lower than the theoretic value, while the content of H and N are higher. It can be deduced that dicyandiamide is easy to self-polymerize [23] if the ratio of reactants is not exactly equal to 1:1 and the heating rate is not controlled precisely. This is one of the reasons that the polymerization degree of PHBGC is lower. For PHGS and PHBGS, the content of N is higher and the contents of C and

Table 3
Element analysis results. E = experimental value; T = theoretic value

Sample	C (%)		H (%)		N (%)		Cl (%)		O (%)	
	E	*T*	*E*	*T*	*E*	*T*	*E*	*T*	*E*	*T*
PHGC	47.23	47.32	9.07	9.01	23.64	23.66	20.05	20.00	/	/
PHBGC	36.54	37.5	7.92	7.42	30.45	27.34	25.09	27.73	/	/
PHGS	69.04	72.64	11.65	12.35	11.27	10.17	<0.3	0	/	7.75
PHBGS	68.10	70.21	11.63	11.84	11.48	9.31	<0.3	0	/	8.51



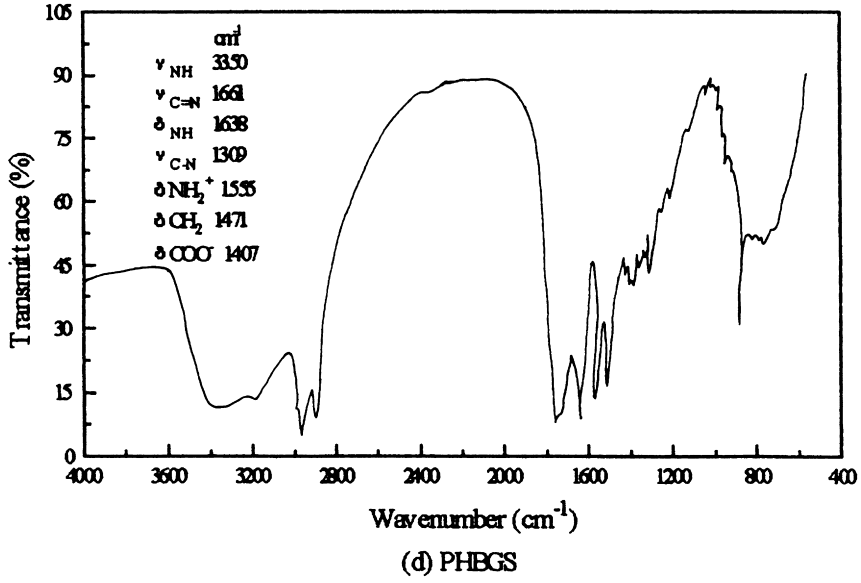


Fig. 2. (*continued*)

H are lower. In Eqs. (3) and (4) in Scheme 2, it is clear that the precipitation reaction is reversible. It is probable that a small amount of Cl^{-1} still remains in the products and is not replaced by the stearate anion. Actually, the content of Cl^{-1} can decrease by increasing the amount of stearic acid in the reaction. Obviously, that will increase the difficulty in purifying the products. In our following work, it is found that Cl^{-1} has little effect on the properties of PHGS and PHBGS, such as solubility and antimicrobial activity.

3.2. Solubility

The solubility is shown in Table 4. It is clear that

Table 4
The solubility of polymeric guanidine and biguanidine salts (g 100 g^{-1} solvent)

Samples	Water	Ethanol	Methanol	Acetone	Methylbenzene	DMF
PHGC	150	100	123	<0.01	<0.01	<0.01
PHBGC	169	105	142	<0.01	<0.01	<0.01
PHGS	<0.01	85	254	<0.01	<0.01	<0.01
PHBGS	<0.01	88	267	<0.01	<0.01	<0.01

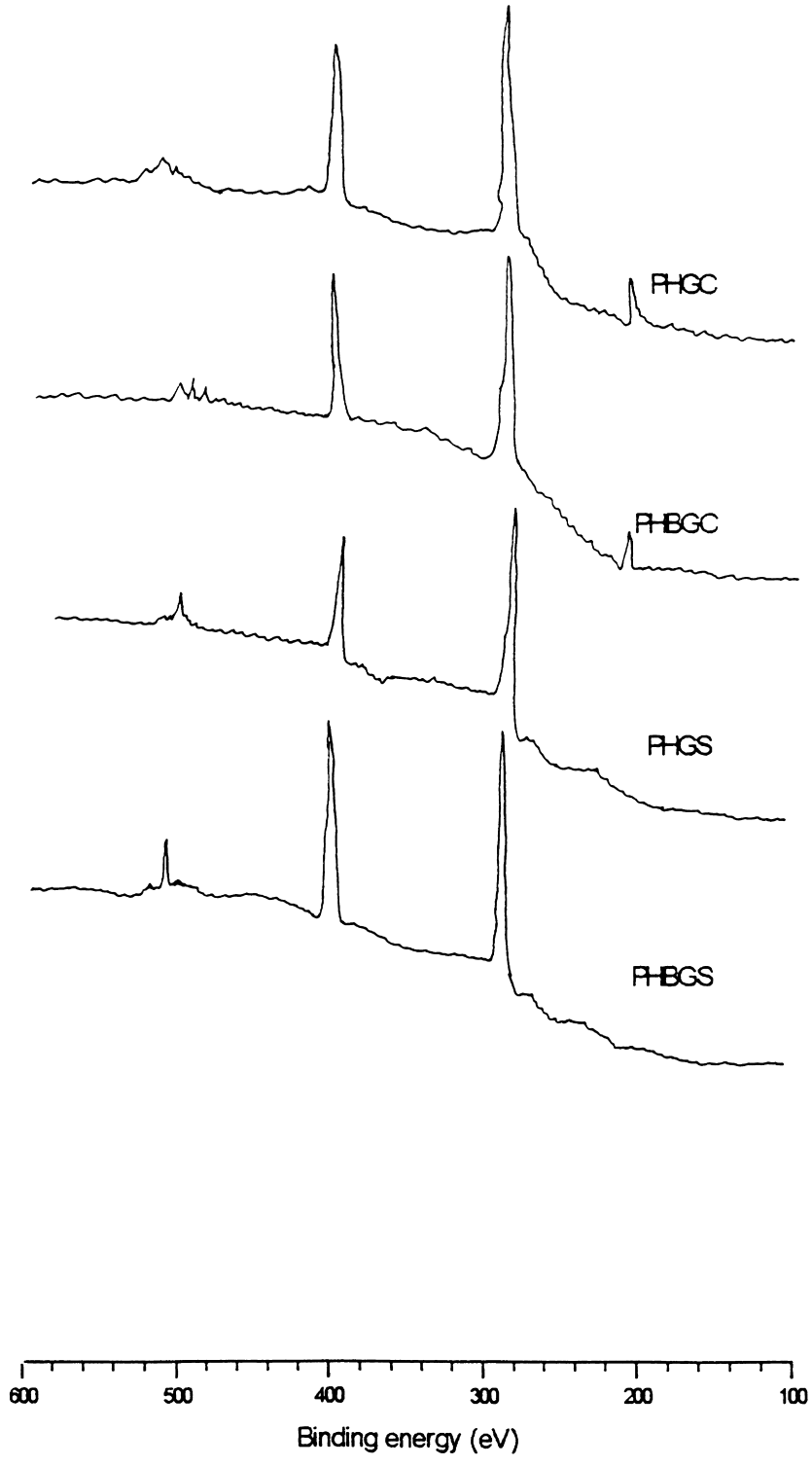


Fig. 3. XPS spectra of the polymers.

all the synthetic polymeric guanidine and biguanidine salts are soluble in ethanol and methanol but insoluble in other organic solvents. PHGC and PHBGC are water soluble, while PHGS and PHBGS are hydrophilic

3.3. MIC

The MIC of polymeric guanidine and biguanidine salts are shown in Table 5. The MIC for all polymers is not larger than 200 $\mu g\ ml^{-1}$. The resultant products are effective in

Table 5
MIC of the resulting antimicrobial agents (μg ml^{-1})

Samples	PHGC	PHGS	PHBGC	PHBGS
Bacillus subtillis	1.55	0.78	6.25	50
Sarcina	1.55	0.78	12.5	50
Staphylococcus aureus	<0.39	<0.39	12.5	0.78
Diplococci pneumomiae	1.55	0.78	25	6.25
Escherchia	12.5	25	100	200
Pseudomonas aeruginose	3.12	3.12	100	50
Rhizopus niger	0.78	3.12	12.5	12.5
Aspergillus niger	6.25	6.25	12.5	12.5
Saccharomyces cerivisiae	0.78	1.55	6.25	1.55
Candida albicans	0.39	0.78	<0.39	1.55

controlling the growth of a broad range of Gram-positive bacteria (e.g. *Staphylococcus aureus*) and Gram-negative bacteria (e.g. *Entamoeba coli*), as well as fungi and yeasts. It is also found that the MIC of different antimicrobial agents vary with the microorganism. Generally, the solubility has little influence on the antimicrobial activity for the same guanidine derivates.

3.4. Heat stability

Fig. 4 is the TGA curves for the antimicrobial agents. The beginning point of weight loss for every antimicrobial agent is higher than 250°C. Therefore, the antimicrobial agents are stable to heat and can be used as additives for polymers, such as PE, PP and PA6. It is also noticeable that there are two plateaus on the TGA curves. It can be explained that the first is caused by the degradation and reaction of the macromolecular and the second is the decomposition of the macromolecular. That can be proved by the antimicrobial test. The samples were heated at various temperatures and then the antimicrobial activity was tested. The result is shown in Table 6. The samples still have antimicrobial activity even after heating at 280°C for 15 min. It is also found that the samples are infusible and insoluble after heating at a temperature higher than 250°C.

Table 6
The antimicrobial activity of the samples after heat treatment. +indicates that the sample has antimicrobial activity

Sample	*S. aureus*				*E. coli*			
	original	230°C	250°C	280°C	original	230°C	250°C	280°C
PHGC	+	+	+	+	+	+	+	+
PHGS	+	+	+	+	+	+	+	+
PHBGC	+	+	+	+	+	+	+	+
PHBGS	+	+	+	+	+	+	+	+

4. Conclusions

The water-soluble polymeric guanidine and biguanidine were polymerized in the melt of monomers and the lipophilic polymeric guanidine and biguanidine were synthesized by precipitation reaction for the first time. NMR, FTIR, XPS and element analysis show that the structure of the resultant polymer is coincident with the theoretic structure. All polymers have good antimicrobial activity to bacteria and fungi. The MIC is not larger than 200 μg ml^{-1}. Meanwhile, the resultant polymers are stable to heat.

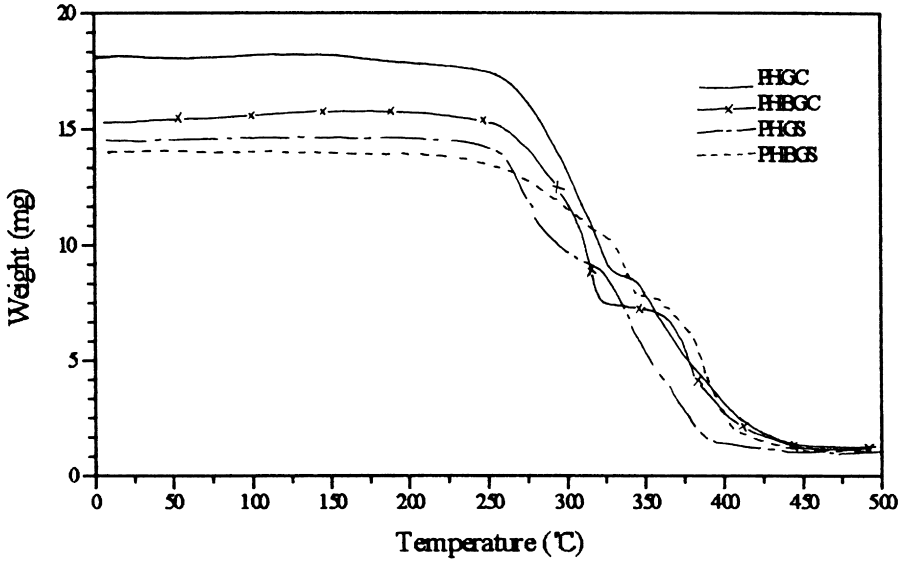


Fig. 4. TGA curves of antimicrobial agents.

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