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Synthesis, characterization and biocidal properties of epoxy resins containing quaternary ammonium salts

Nadège Destais, Dominique Adès, Georges Sauvet

Laboratoire de Recherches sur les Macromolécules, UMR 7540, Université Paris 13, Avenue J.B. Clément, 93430 Villetaneuse, France

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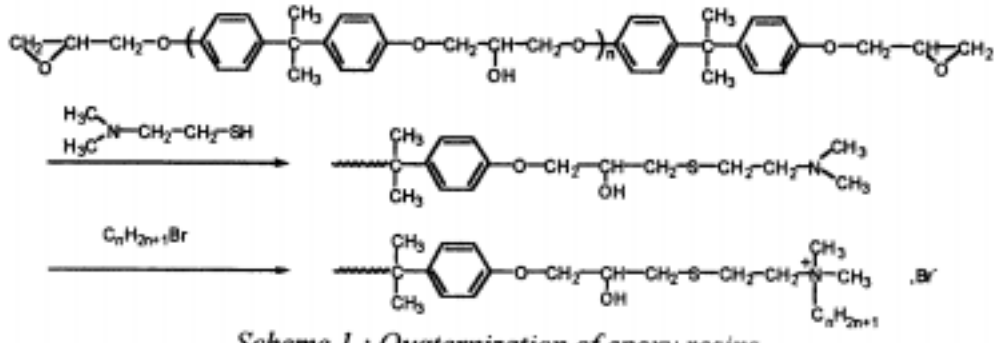
Summary

Quaternary ammonium salts (QAS) were covalently-bound to epoxy resins of different DP in two steps: addition of a N,N-dialkylaminoethanethiol followed by the quaternization of the tertiary amine by an alkylbromide ($C_8H_{17}Br$ to $C_{14}H_{29}Br$). The products were characterized by ^1H NMR spectroscopy. The QAS-containing oligomers were used as polyols to prepare polyurethane (PU) films by reaction with a triisocyanate. The films show a good bactericidal activity against *Escherichia coli*, which is preserved after 6 months of immersion in water.

Introduction

Coatings able to prevent the adhesion and growth of microorganisms are required in many domains (biomedical and food industry, outdoor paints, marine paints, etc.). Biocides incorporated in film-forming polymers are generally active by diffusion in the environment and may have negative effects. For instance, the use of tin derivatives has been the object of severe restrictions. Quaternary ammonium salts (QAS), known to be active against microorganisms by interaction with the cell membrane, offer an interesting alternative because one may expect coatings active by contact without liberation of toxic compounds. QAS-containing polymers have been the object of many investigations [1-8]. We have previously prepared QAS-modified polybutadienes [5,6] and polysiloxanes [7] and show their efficiency in terms of biocidal activity and permanency after prolonged water immersion. We report now on a new class of QAS-containing polymers based on epoxy resins, which are easily prepared and can be used as polyols in the preparation of polyurethane films (scheme 1).



Scheme 1 : Quaternization of epoxy resins

Experimental part

Materials

Two different epoxy resins (from Ciba Geigy) were used: Araldite GY250 (mainly constituted of the diglycidylether of bisphenol A or DGEBA, since the apparent DP calculated by NMR is equal to 0.08) and Araldite GT6084 (DP = 4.2).

N,N-dimethylaminoethanethiol and N,N-diethylaminoethanethiol were released from the corresponding hydrochlorides, by slowly adding a concentrated solution of sodium hydroxyde to the hydrochloride, at 150°C. The amine was distilled as soon as formed along with water and then extracted with diethylether.

Chloroform and n-hexane were distilled before use. Acetonitrile, absolute ethanol, n-alkylbromides and Tolonate were used without purification.

Addition of a N,N-dialkylaminoethanethiol to an epoxy resin

The aminothiol ($2.9 \cdot 10^{-2}$ mol) is solubilized (in 10 mL of CH_3CN for DGEBA and in 25 mL of $CHCl_3$ for Araldite GT6084) and heated at reflux of the solvent. A solution of the epoxy resin ($1.2 \cdot 10^{-2}$ mol in 10 mL of CH_3CN for DGEBA and in 5 mL of $CHCl_3$ for Araldite GT6084) is slowly added to the solution of the aminothiol. At the end of the reaction (30 min for DGEBA and 2.5 h for Araldite GT6084), the polymer is precipitated twice in hexane and dried in vacuum for 24 h.

Quaternization of the thioaminated resin

The thioaminated resin ($1.7 \cdot 10^{-3}$ mol for DGEBA and 10^{-3} mol for Araldite GT6084) and n-octylbromide (1.2 eq./amine) are dissolved (in ethanol for DGEBA and in $CHCl_3$ for Araldite GT6084) and heated at reflux during 2.5 h. The final polymer is precipitated twice in hexane and dried in vacuum for 24 h.

PU-films

The quaternary ammonium salts (QAS) were mixed with an aliphatic triisocyanate (Tolonate HDB®) in chloroform solution (Tolonate HDB is a biuret of hexamethylene diisocyanate from Rhone-Poulenc). In the case of Araldite GT6084, polypropylene glycol (M_n = 425 g. mol^{-1}) was used as a spacer (10- 30% of the total OH-groups). The two reactants were mixed and spread on a glass microscope slide by means of a handcoater (~300 µm). Curing was carried out in an oven at 70°C.

Characterization

- ^1H NMR spectra were recorded on a Bruker ACE 200 spectrometer, operating at 200 MHz. Analysis were carried out in $CDCl_3$, with tetramethylsilane as reference (0 ppm).

- Chemical titration of the epoxides [9]: about 250 mg of epoxy-containing resin are dissolved in 20 ml of pyridinium chloride in pyridine (0.2 N). The mixture is heated at reflux of pyridine for 20 minutes. Then, the flask is cooled. After adding a few grains of bromothymol blue, the solution is back titrated with a NaOH solution (0.3 N in methanol). Three blank tests (without polymer) are done in an identical manner.

- ATR-IR spectra (Attenuated Total Reflectance) were recorded on a Perkin Elmer 1600 spectrometer.

- The bactericidal properties of the PU-films were studied with *Escherichia coli* (ATCC 10536) as previously described [6]. A counting test consisted in spreading 100 µL of a

bacterial suspension containing N_o bacteria on the surface of the film (1 cm^2). After 2 h of contact at 20°C, the sample was washed with 100 mL of distilled sterile water. Tenfold dilutions in sterile water are made and filtered on 0.45 µm membranes. The membranes are placed on gelose in a Petri dish and heated for 24 h at 37°C. The surviving bacteria give birth to colonies which are counted. A test of invasion/diffusion was also performed. The surface of a nutrient medium (Counting Agar) was seeded with a uniform layer of a bacteria suspension (~10^6 bact./mL). The film was placed facing on the bacteria. If a toxic compound is liberated, a zone of inhibition is observed around the sample and measured in mm.

Results and discussion

Synthesis

1) *Ring-opening of the epoxide by an aminothiol*. Using a large excess of thiol, the addition of the aminothiol to DGEBA was quantitative in bulk, at room temperature (Exp. 1, Table 1). The relatively fast reaction observed was probably due to a catalytic effect of the aminothiol in excess since amines are known catalysts for the addition of thiol to epoxide [10]. However, thiols are expensive and the excess is difficult to remove. Conditions using a stoichiometric ratio of the reactants were studied. The opening of the epoxides by the aminothiol in acetonitrile solution was followed by ^{1}H NMR, using the signal of the CH_2 in α of the amine (2.5- 2.9 ppm) and the signal of the aromatic protons (6.85-7.1 ppm) as a reference (Fig. 1-b).

At room temperature (Exp. 2, Table 1), the reaction was very slow and a side reaction consuming the epoxides was detected by the difference between the yield calculated by chemical titration of the epoxides and the yield of amine addition determined by ^{1}H NMR. The formation of a by-product was correlated with the appearance of two new signals in the NMR spectra (a doublet at 5.1 and a multiplet at 6.3 ppm in the proportion of two to one). This is consistent with the formation of a terminal double bond (possibly an allyloxy group). In order to suppress the side reaction and to accelerate the rate of the main reaction, a catalyst was used. Lithium perchlorate is known to accelerate the addition of amines and thiols to double bonds [11]. A proportion of 10% $LiClO_4$ was necessary to obtain a real effect on the rate of the reaction, and the side reaction was not completely eliminated (Exp. 3 and 4, Table 1). Alternatively, a similar result could be obtained without catalyst by increasing the temperature to 80°C (Exp. 5, Table 1). A combination of catalyst (10%) and heating (80°C) gave a fast reaction without side-reaction (Exp. 6, Table 1). However, we found also possible to avoid the side reaction *in the absence of catalyst* by slowly adding the solution of DGEBA to the solution of thiol (20% excess) at reflux. In these conditions, the reaction was almost complete in half an hour (Exp. 7, Table 1). In the same conditions, N,N-diethylaminoethanethiol led also to a high yield of addition without side-reaction (Exp. 8, Table 1).

These last conditions were applied to Araldite GT6084. The proportion of amino groups introduced was somewhat lower (80%) than that of consumed epoxides (100%), probably because of a side reaction of the epoxide with the OH groups present in Araldite GT6084 (in agreement with a small signal appearing at 3.7 ppm corresponding to the CH_2 of the ether group). This side reaction has been shown to be more important than the eventual polymerization of the epoxide [12].

2) *Quaternization of the tertiary amine*. The second step of the synthesis was the quaternization of the tertiary amine introduced in the first step. The alkylating agents used in this study were two n-alkylbromides ($C_8H_{17}Br$ and $C_{14}H_{33}Br$), because a long alkyl chain is necessary to obtain QAS possessing good biocidal properties (at least eight carbon atoms [13]). The quaternization was followed by ^1H NMR using the signal of the CH_3 of the alkyl chain at 0.9 ppm (Fig. 1-c).

For reasons of solubility, ethanol at reflux was used in the case of DGEBA and chloroform at reflux for Araldite GT6084. The alkylbromides were used in slight excess (1.1 eq./amine). In 2.5 h, yields varying between 58 and 91% were obtained (Table 2).

The characteristics of the different QAS-containing polymers are summarized in Table 2. They are referred hereafter as Polymer-Ax-Qy where Polymer is DGEBA or Resin (for Araldite GT6084), x corresponds to the length of the substituents of the amine (1 for methyl and 2 for ethyl) and y corresponds to the length of the alkyl chain of the QAS.

Table 1 : Addition of R_2N-CH_2-CH_2-SH to DGEBA

Exp.	R	Thiol (éq.)	$LiClO_4$ (éq.)	Temp. (°C)	Time (h)	Yield (%)[a]	Yield (%)[b]	Side reactions (%)
1	Me	3[c]	/	R.T.	5	99	99	/
2	Me	1	/	R.T.	23	58	90	22.5
3	Me	1	0.01	R.T.	24	67	89	20.5
4	Me	1	0.1	R.T.	1.75	79	91	9
5	Me	1	/	80	0.5	89	88	4
6	Me	1	0.1	80	0.25	87	98	/
7	Me	1.2	/	80	0.5 [d]	97	90	/
8	Et	1.2	/	80	0.5 [d]	93	92	/

Solvent : CH_3CN

[a] Calculated from NMR spectra.

[b] Calculated from a chemical titration of epoxides

[c] In bulk

[d] Slow addition of DGEBA solution to the solution of N,N-dialkylaminoethanethiol (in 15–20 min.).

Table 2 : Quaternary Ammonium Salts based on epoxy resins.

Polymer	Aminothiol addition (%)	Quaternization (%)	**Global yield (%)**	QAS/ molecule	OH/ molecule
DGEBA-A1-Q8	93	91	**85**	1.69	1.93
DGEBA-A1-Q14	93	58	**54**	1.07	1.93
DGEBA-A2-Q8	93	79	**73**	1.46	1.93
DGEBA-A2-Q14	93	82	**76**	1.57	1.93
Resin-A1-Q8	92	78	**72**	1.44	6.04
Resin-A1-Q14	92	75	**69**	1.38	6.04

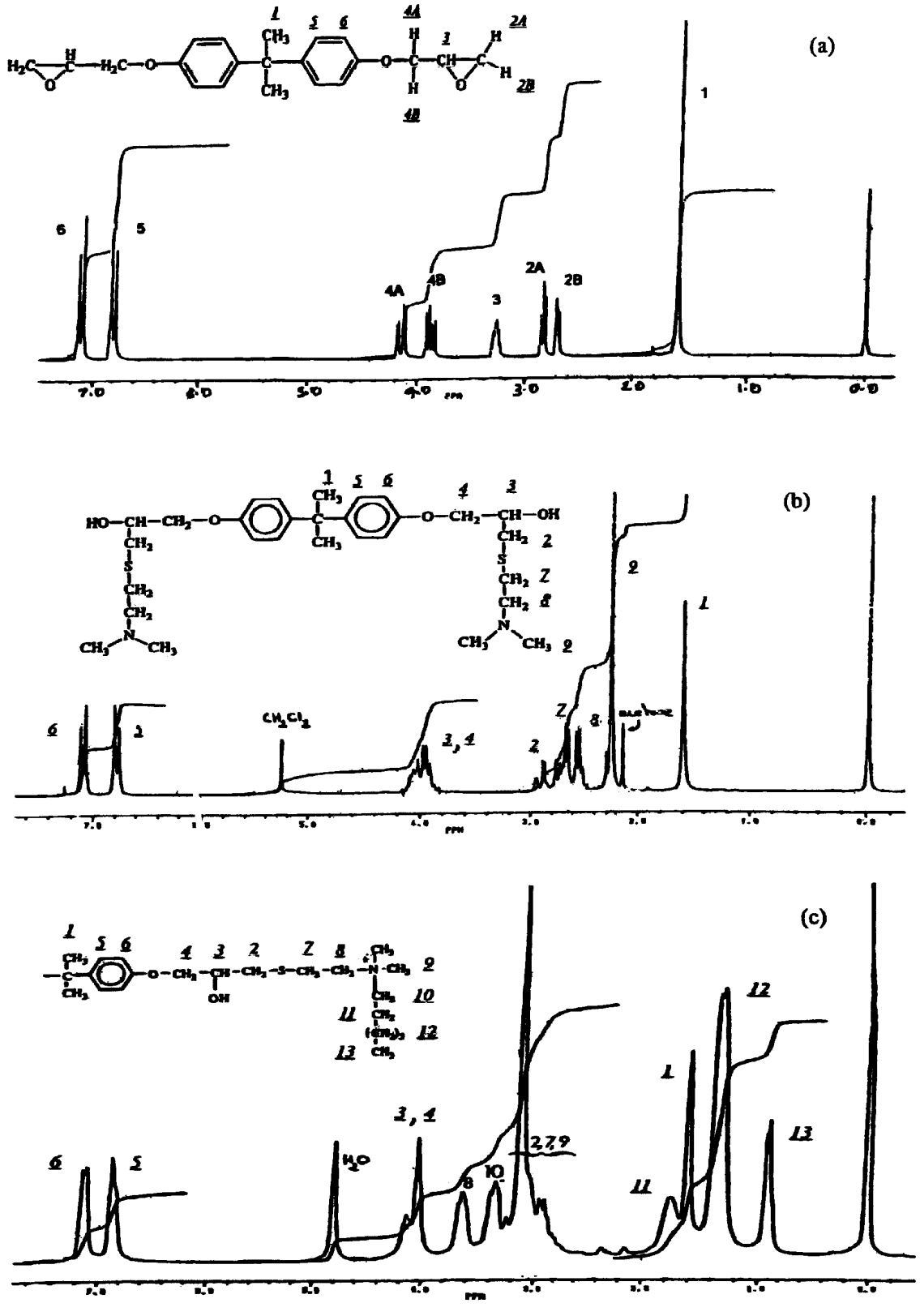


Figure 1 : ^{1}H NMR spectra (200 MHz): (a) DGEBA (Araldite GY250); (b) thioaminated DGEBA; (c) quaternized DGEBA.

3) *Polyurethane films*. For the study of the bactericidal properties, the QAS-containing polymers were incorporated in a polyurethane network by curing with the isocyanate (Tolonate HDB) at 70°C. ATR-IR was used to follow the disappearance of the NCO vibration band at 2270 cm^{-1}, using the CH_2 vibration band at 2926 cm^{-1} as an internal reference.
Different parameters were examined in the case of the quaternized compound Resin-A1-Q14, in the aim to obtain films able to withstand water contact for several months. Moreover, the network should be soft enough to let the QAS come to the surface.

The reaction with Tolonate was very slow (20% remaining NCO functions after 450 h of reaction), because of the high density of the network reached at the end of the reaction (Fig. 2). To delay the gel point, the ratio NCO/OH was tentatively lowered to 0.75 or 0.5, but no improvement was observed. The use of dibutyltindilaurate as catalyst provided some improvement (complete reaction in 300 h), but this compound was not suitable for biocidal polymers because some tin derivatives are known for their biocidal properties. To improve the reaction rate and the final yield, the best solution was to use a spacer. Three diols of different length and hydrophilicity were tested (pentanediol, poly(ethyleneglycol) (PEG, DP = 6,5) and poly(propyleneglycol) (PPG, DP = 7) in the proportion of 10 and 30% of the total OH groups. Figure 2 shows the results for 30% of spacer. Pentanediol was too short to have a real effect on the curing. PEG provided good results but its hydrophilicity led to a swelling of the films when immersed in water (water uptake ~100%). Finally, PPG appeared to be the best choice: the reaction was complete in about 100 h, and the films did not swell in water (water uptake less than 2 %).

In the case of quaternized DGEBA, the reaction with Tolonate was complete in 5 h, without spacer or catalyst.

Biocidal properties

Two different tests were carried out. A test of invasion/diffusion was used to check if a biocide not fixed to the network could be extracted from the samples. This test showed that only DGEBA quaternized in C8 presented a diffusion area during the first days of immersion in water. DGEBA quaternized in C14 and quaternized resins did not show this phenomenon.

A second test consisted in counting the surviving bacteria after a determined time of contact with the sample. The biocidal activity of the films was expressed as *the logarithmic reduction ratio*, $\log(N'_0/N)$, where N'_0 is the number of surviving bacteria on a reference film (not containing QAS) and N the number of surviving bacteria on the sample. Figure 3 presents the biocidal activity of the different polymers during the first 100 days of immersion in water. Polymers presenting a phenomenon of diffusion, such as DGEBA-A1-Q8, show a very high value of the logarithmic reduction ratio in the first days, followed by a fast decrease of this value in a few days. After 20 days, the activity remained quite constant and could be attributed to an activity by contact. It is worth to note that the same behaviour was observed for DGEBA-A1-Q14 though this compound did not show a diffusion area in invasion/diffusion test. It seems therefore that the absence of a measurable diffusion area is not an absolute proof of the absence of diffusion [(6)]. On the other hand, the quaternized polymers derived from Araldite GT6084 showed no release of active molecules (even in the case of Resin-A1-Q8) and their activity remained

constant from the beginning of the immersion. This different behaviour of DGEBA and Araldite GT6084 is probably due to their different OH-functionality. The curing of Araldite GT6084 (ca. 6 OH/mol) is probably complete while some quaternized molecules remain not linked to the network in the case of DGEBA (≈ 2 OH/mol).

Figure 2 : Influence of a spacer on the reaction of Resin-A1-Q14 with Tolonate :

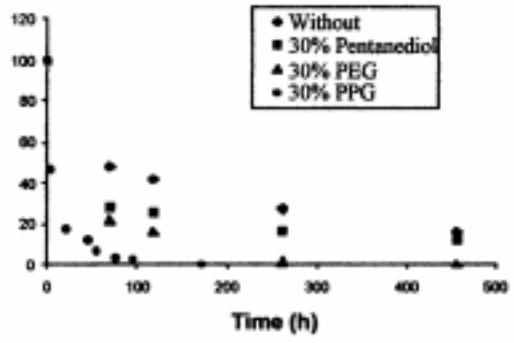
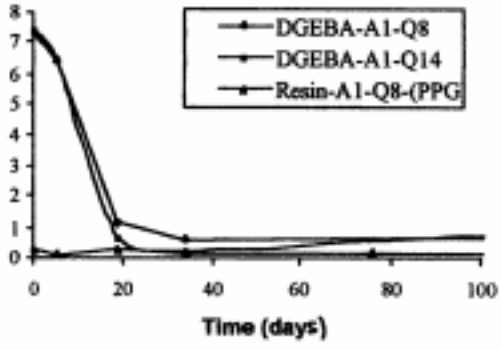


Figure 3 : Logarithmic reduction ratio of DGEBA-A1-Q8, DGEBA-A1-Q14 and Resin-A1-Q8-(PPG) as a function of the immersion time (N'_0~ 2. 10^7 bact./mL) :



Inoculum effect. The relatively low activities shown in Figure 3 were measured for high concentration of inoculum (≈ 10^7 / mL). For practical applications, it was interesting to study also the activity for much lower initial concentrations. For each polymer, we found that the activity increased when the concentration of the inoculum decreased. This effect known in bacteriology as "inoculum effect" was not mentioned in previous studies carried out on biocidal polymers carrying QAS [6]. We then decided to compare all the samples at a similar concentration N'_0 (≈ 7. 10^4 bact./mL).

Table 3 shows the logarithmic reduction ratio of the different samples after ~ 170 days (about 6 months) of immersion in water. All the samples but one (Resin-A1-Q8) still demonstrated an significant activity after this period. It was previously shown [6] that the length of the alkyl chain had an effect on the permanence of the activity of the films (Q8 leading to lower life times than Q14). This result was confirmed in the case of Araldite GT6084 (no activity for Resin-A1-Q8 compared to an activity of 0.78 for Resin-A1-Q14, whereas no clear influence was found in the case of DGEBA.

*Table 3 : Bactericidal activity of the different QAS (*Strain : *Escherichia coli)*

Polymer (QAS)	QAS / g PU	Immersion time (days)	N'_0 (bact./mL)	log N'_0/N
DGEBA-A1-Q8	$1.73 .10^{-3}$	170	$7.6 .10^4$	**2.09**
DGEBA-A1-Q14	$1.38 .10^{-3}$	170	$4.6 .10^5$	**1.15**
DGEBA-A2-Q8	$1.18 .10^{-3}$	172	$8.2 .10^4$	**0.55**
DGEBA-A2-Q14	$1.14 .10^{-3}$	172	$8.2 .10^4$	**0.89**
Resin-A1-Q8-(PPG)	$3.8 .10^{-4}$	166	$4.7 .10^4$	**0.01**
Resin-A1-Q14-(PPG)	$3.2 .10^{-4}$	179	$2.2 .10^4$	**0.78**

It was previously observed that the activity decreased with the bulkiness of the substituents of the amine (n-butyl < methyl), but that the permanency after prolonged water immersion increased (n-butyl > methyl) due to the higher hydrophobicity of the substituents [6]. In the present study, two methyl groups led also to a better activity than two ethyl ones, but no influence on the durability could be noted.

DGEBA-A1-Q14 and Resin-A1-Q14-(PPG) may be used to compare the influence of the network density because the two samples have the same QAS and differ only by the presence of the spacer PPG. In spite of the lower concentration of QAS / g PU in the case of the resin, the activities are close (Table 3). This shows that a lower density of crosslinks in the network has a beneficial effect which may compensate a lower concentration of QAS in the material.

Conclusion

Commercial diepoxy resins of bis-phenol-A (including DGEBA, the first term of the series) have been modified in two steps leading to the introduction of QAS with different substituents. The resulting compounds contain OH groups and may be used as polyols for the preparation of polyurethane films. The density of the network (and the concentration of QAS) may be varied in a large range by the incorporation of other polyols such as PPG.

The films obtained by this route do not swell in water and present a bacterical activity against *Escherichia coli* which remains practically unchanged after several months of immersion in water. Compared at a similar concentration of QAS / g PU and after a similar time of immersion, these new bacterical polymers present a behaviour which is equivalent to that of quaternized hydroxytelechelic polybutadienes previously studied [6] (e.g. $\log N'_0/N = 1.5$ after 178 days of immersion in water for $N'_0 = 0.9 \cdot 10^4$ bact./mL). However, the reactants used in the present syntheses and the conditions of synthesis present a considerable economic advantage with respect to the QAS-modified polybutadienes.

These compounds can be incorporated in differents types of coatings to give them a permanent antimicrobial / antifungal protection without the hazard linked to the release of toxic compounds which is a major drawback of current biocidal coatings.

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