Available online at www.sciencedirect.com
ScienceDirect
European Polymer Journal 42 (2006) 3383–3389
EUROPEAN POLYMER JOURNAL
www.elsevier.com/locate/europolj

Polyethylene compounds with antimicrobial surface properties

Gerhard Seyfriedsberger [a], Karl Rametsteiner [b], Wolfgang Kern [c,*]

[a] *Polymer Competence Center Leoben GmbH, A-8700 Leoben, Austria*
[b] *Ke Kelit Kunststoffwerk GmbH, A-4017 Linz, Austria*
[c] *Institute for Chemistry and Technology of Organic Materials, Graz University of Technology, Stremayrgasse 16, A-8010 Graz, Austria*

Received 20 December 2005; received in revised form 3 July 2006; accepted 28 July 2006
Available online 12 October 2006

Abstract

The present study aims at antimicrobial polyethylene surfaces. To achieve this, LLDPE was compounded with the polymeric biocide poly(2-*tert*-butylaminoethyl) methacrylate TBAM (bulk modification with 1.5–5.0 wt.% of TBAM). Surfaces of these polymer compounds were then subjected to microbial assays. Using standard methods the colony forming units (CFU) for *Escherichia coli* and *Staphylococcus aureus* were determined on these surfaces. In all cases, polyethylene surfaces with highly antimicrobial properties were achieved. An average reduction of 10^4 CFU ml^{-1} compared to neat LLDPE was achieved. The surfaces of these LLDPE/TBAM compounds were assessed by electrokinetic (zeta potential) measurements. The results indicate a relation between the antimicrobial activity and the zeta-potential of the polymer compounds. Moreover, the antimicrobial compounds were investigated towards biofilm formation. Compared to pristine LLDPE, the surfaces of the polymer compounds showed less adhering biofilm after a testing period of 16 weeks.

Keywords: Polyethylene; Antibacterial; Biocide; Zeta potential; Biofilm formation

1. Introduction

Because of the growing demand for healthy living there is an increasing interest in materials capable of killing harmful microorganisms. Potential fields of application include, for example, water treatment, food processing and inclusion in medical devices. Over the past decades, low-molecular-weight biocides such as chlorinated phenols, derivatives of isothiazolone, chlorine releasing *N*-halamines as well as salts and complexes of metals (typically Zn and Ag) have been applied preferentially. Such agents act efficiently by affecting cell metabolism but may be disadvantageous with respect to toxicity and the formation of bacterial resistance. In many cases it is possible to substitute low-molecular-weight substances for macromolecular antimicrobial agents. Polycationic substances are well established nowadays [1–5]. They provide the advantage of reduced toxicity and do not cause bacterial resistance. Polycationic substances act by inducing a phase-separation of charged and uncharged lipides inside the cytoplasma membrane of bacteria [6,7]. Finally, the cytoplasma membrane

* Corresponding author. Tel.: +43 316 873 8458; fax: +43 316 873 8951.
E-mail address: W.Kern@TUGraz.at (W. Kern).

0014-3057/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.eurpolymj.2006.07.026

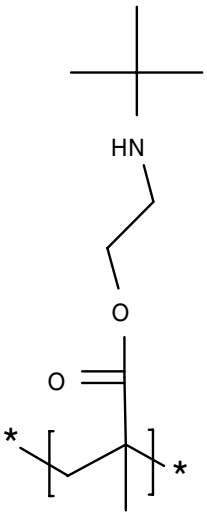

Fig. 1. Structure of the amino functionalized polymer poly(2-*tert*-butylaminoethyl) methacrylate (TBAM).

disintegrates (lysis) which causes the death of the microorganism (apoptosis). In a similar fashion, polymers functionalized with pendant amino groups display high antimicrobial activity [8,9]. As reported recently, the polymer poly(2-*tert*-butylaminoethyl) methacrylate (TBAM; see Fig. 1) acts as a very efficient contact biocide [9]. In this case, the mere contact of bacteria with the polymer surface is sufficient to provide the biocidal effect. It has been reported that the solubility of TBAM in water (at neutral pH = 7) is lower than 3.0 mg per liter. This renders this biocide especially useful for construction materials designed to be in contact with water, since a very low leachability of TBAM from polymer blends and compounds can be expected [10].

The aim of this work was to confer antimicrobial properties onto polyethylene. To achieve this, linear low-density polyethylene (LLDPE) was compounded with TBAM and surfaces of these compounds were then subjected to microbiological investigations (colony forming units, biofilm formation). In addition to this, elektrokinetic (zeta-potential) measurements where carried out to investigate relations between antimicrobial activity and surface charge of the active polymer surfaces.

2. Experimental

2.1. Preparation of antimicrobial polyethylene compounds

Commercial linear low-density polyethylene (LLDPE; Dowlex 2344-115) and poly(2-*tert*-butylaminoethyl) methacrylate (TBAM; research sample supplied by Degussa Creavis, Germany) were compounded with a counter rotating twin-screw extruder at a maximum temperature of 215 °C. The compounder was a Thermo Electron PRISM TSE 24HC model. The polymer melt was extruded into a water bath, cut into pellets and dried for 3 h at 80 °C and 30% relative humidity. From these pellets, plates with the size of 200 × 200 mm (thickness 2 mm) were prepared by recasting at 160 °C. For this process, a vacuum-press (Collin P 200 PV) was applied. The plates were then cut to the size of 40 mm × 40 mm. By this procedure, LLDPE test plates, containing 0, 1.5, 3.0 and 5.0 wt.% of TBAM, were prepared. For mechanical testing, shouldered test bars were produced by injection moulding. The dimensions of the test bars were chosen according to EN ISO 527-2 (type 1A), injection molding of the test bars was carried out according to the standard EN ISO 3167. Tensile testing was carried out according to ISO 527-1 using a Zwick/Roell Z020 tensile testing machine.

2.2. Electron microscopy

Transmission electron microscopy (TEM) was carried out at the Institute of Electron Microscopy at TU Graz. Samples of the polyethylene compounds were cut using kryomicrotomy. The TEM samples were stained with OsO_4 and investigated using a Philips CM 20 electron microscope.

2.3. Electrokinetic measurements

The zeta (ζ) potential of the sample surfaces was determined by the streaming potential method, using a clamping cell connected with an EKA electrokinetic analyzer (Anton Paar GmbH, Graz, Austria). In this cell the sample is pressed against a PMMA spacer with seven channels. The measurements were performed with a KCl electrolyte solution (10^{-3} M, 500 ml). The pH was adjusted to about 10 by adding NaOH (0.1 M, 2.5 ml) and was then decreased stepwise (0.3–0.4 units) by titration with HCl (0.1 M), until pH = 3 was reached. Using the Clamping Cell a pressure ramp from 0 to 400 mbar was employed to force the electrolyte solution through the cell. Streaming potentials were converted to zeta potentials using the Helmholtz–Smoluchowski equation [11] and the Fairbrother–

Mastin approach [12]. Each value of the zeta potential at a given pH value represents an average value over at least three individual measurements.

2.4. Testing of antimicrobial properties

Prior to this investigation, the polymer sample plates were sterilized using gamma irradiation (dose rate 0.12 kGy h^{-1}, overall dose 27 kGy). Antibacterial testing was carried out at the Federal Institute for Material Research and Testing (BAM Berlin, Germany) according to the Japanese Industrial Standard JIS Z 2801:2000. In this test a bacterial cell suspension is held in intimate contact with the (active) surface using a sterile cover in humid conditions. After a set contact time of 24 h the size of the residual bacterial population is compared to an appropriate control probe. The antibacterial test plates and pure LLDPE as control were tested against *Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 6528P). The amount of micro-organisms is expressed in colony forming units per milliliter (CFU ml^{-1}).

2.5. Testing of biofilm formation

The biofilm formation on LLDPE surfaces was assessed following the (preliminary) standard CEN TC164/WG3/AHG3. The tests were carried out at the Austrian Research Institute for Chemistry and Technology (OFI, Vienna). Representative samples of the material to be tested are incubated in tap water containing specified inorganic supplements and inoculated with a mixture of naturally occurring micro-organisms derived from river water. These product samples are incubated for a period up to 16 weeks at a constant surface to volume ratio of 0.16 cm^{-1}. The test water is replaced at a frequency of once a week. Formation of biomass on the product surface (biofilm) is determined with *adenosine tri-phosphate* (*ATP*) measurements after 8, 12 and 16 weeks of incubation. The ATP concentration is used as a measure for the presence of active microbial biomass, and the biomass production per unit surface is calculated from the concentration of attached and suspended biomass. Validation of the results is achieved by testing glass controls and reference materials (negative control: glass; positive control: plasticized PVC) in parallel with the materials under test.

3. Results and discussion

3.1. Preparation of antimicrobial LLDPE compounds

Linear low-density polyethylene (LLDPE) and TBAM were compounded and LLDPE sample plates containing 0, 1.5, 3.0 and 5.0 wt.% of TBAM were prepared (see experimental section). Fig. 2 displays a representative transmission electron micrograph (TEM) of the LLDPE compound containing 5 wt.% of TBAM. In the TEM micrograph particles of TBAM dispersed in the polyethylene matrix can be discerned. The diameter of these particles is in the range from 0.05 to 0.5 μm. For the compounds containing less than 5.0 wt.% of TBAM, a similar situation was found.

In a series of experiments, a better dispersion of TBAM in the LLDPE matrix was attempted by use of several commercial compatibilizers (block copolymers of ethylene and methylacrylate, polyethylene grafted with maleic anhydride). However, TEM investigations did not reveal significant differences to compounds without compatibilizers. Consequently, all further investigations were performed with standard TBAM/LLDPE compounds (no compatibilizers added).

With respect to practical applications also the mechanical properties of LLDPE and its compounds are of interest. Pristine LLDPE and

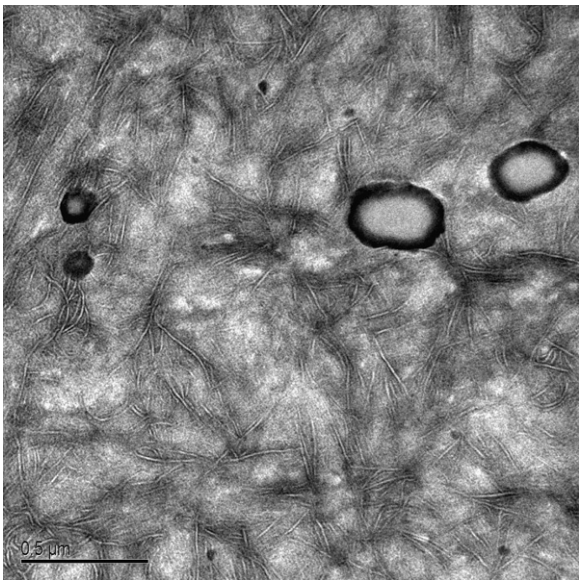

Fig. 2. TEM micrograph of an LLDPE compound containing 5.0 wt.% of TBAM (dark areas). The magnification is 1:51,000, the length of the bar corresponds to 0.5 μm.

Table 1
Tensile testing of LLDPE/TBAM compounds

Composition [wt.% of TBAM]	E[a] [MPa]	σ_y[b] [MPa]	ε_y[c] [%]	σ_B[d] [MPa]
0	465	18.6	46	16.3
1.5	465	18.4	46	16.1
3.0	490	18.2	45	15.3
5.0	520	17.2	43	14.9

[a] E: Modulus of elasticity in tension in MPa.
[b] σ_y: Yield stress in MPa.
[c] ε_y: Yield strain in %.
[d] σ_B: Tensile stress at break in MPa.

compounds of LLDPE with TBAM were subjected to tensile testing according to standardized procedures, see experimental section. Table 1 contains data on E (modulus of elasticity in tension), σ_y (yield stress), ε_y (yield strain) and σ_B (tensile stress at break) that were derived from stress–strain curves.

From the data in Table 1 is becomes apparent that the modulus E increases with increasing amount of TBAM additive (from 464 MPa for 0 wt.% of TBAM to 520 MPa at 5.0 wt.% of TBAM. Also the values for σ_y, ε_y and σ_B change slightly when TBAM is added to LLDPE. In conclusion, no dramatic deterioration in the mechanical properties of LLDPE is observed upon the addition of up to 5.0 wt.% TBAM.

3.2. Elektrokinetic measurements

While electron microscopy reveals that the polymeric additive is heterogeneously dispersed in the LLDPE matrix, additional techniques are required to obtain information on the surface properties of the compounds. Surface properties are crucial since the added polymer TBAM acts as a contact biocide [8,9]. Consequently, the antibacterial properties of the compounds, as prepared in this work, are expected to depend on the amount of TBAM present at the sample surface. Elektrokinetic (zeta potential) measurements are generally useful to detect dissociable groups such as carboxylic acids and amino groups at surfaces, see e.g. reference [13].

Zeta potential versus pH curves were recorded with an electrokinetic analyzer (EKA, from Anton Paar, Austria) using a clamping cell as described in the experimental section. The zeta potential of the samples obtained with this clamping cell is no absolute value since the grooved PMMA spacer of the measuring cell contributes one measured surface

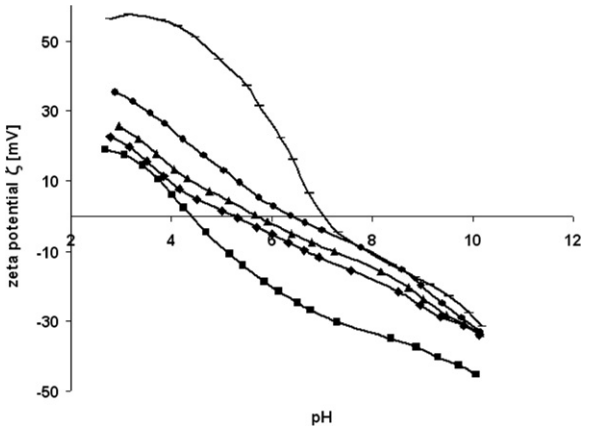

Fig. 3. Elektrokinetic measurements of LLDPE/TBAM compounds. Zeta potential versus pH curves for (■) pristine LLDPE, (♦) LLDPE with 1.5 wt.% TBAM, (▲) LLDPE with 3.0 wt.% TBAM, (●) LLDPE with 5.0 wt.% TBAM, (–) film of neat TBAM.

while the other is the sample surface. Anyway, changes in the zeta potential of LLDPE depending on the surface composition can be detected and interpreted quite well. Fig. 3 displays the zeta potential versus pH curves of pure LLDPE and LLDPE compounds containing 1.5, 3.0 and 5.0 wt.% of TBAM. For comparison, the data for a film of neat TBAM (spin coated on a glass plate) are also presented in Fig. 3.

The isoelectric point (IEP) of LLDPE is around pH = 4.3 and the shape of the curve is typical of polymers bearing no dissociating groups. Due to the hydrophobic character of the unmodified polyethylene, preferred adsorption of hydroxide anions is observed which gives a negative zeta potential in the range from pH = 4 to pH = 10. Compared to pure LLDPE, all samples containing TBAM show a marked shift of the isoelectric point towards higher pH and a plateau occurring at low pH values. The shift of the isoelectric point can be explained by the protonation of the amino functionlized side-chains of TBAM. At the same time, the zeta potential (at a given pH value) increases with the amount of TBAM added which is also typical of basic groups present at the surface. The zeta potential versus pH curves of the polymer compounds are positioned between the curves obtained for neat LLDPE and neat TBAM. From these investigations it is concluded that the physico-chemical surface properties (such as the elektrokinetic potential) of the system LLDPE/TBAM strongly depend on the relative amounts of the two

components at the surface of the polymer compound. It must be taken into account that – depending on the processing conditions – the relative amounts of LLDPE and TBAM at the surface of the compound may differ from the bulk composition. In any case, zeta potential measurements can be useful to obtain information on the amount of TBAM present at the surface of a given compound.

3.3. Antimicrobial properties

Test plates of the antibacterial compounds (1.5%, 3.0% and 5.0 wt.% TBAM) and of pristine LLDPE where sterilized using gamma radiation. Antimicrobial testing was carried out following the Japanese Industrial Standard JIS Z 2801:2000. For this assessment, gram-positive bacteria *S. aureaus* as well as gram-negative bacteria *E. coli* were used. Pristine LLDPE was applied as control sample in all experiments.

For *S. aureaus*, the number of colony forming units per ml (CFU/ml) was reduced to zero after a contact time of 24 h between the bacteria and the polymer surface. This was observed for all LLDPE compounds containing TBAM. For the LLDPE control surface, only a slight reduction of bacteria from their starting concentration of 1.0×10^6 CFU ml^{-1} to 6.6×10^4 CFU ml^{-1} was observed. These results are displayed on a logarithmic scale in Fig. 4.

The number of *E. coli* bacteria was also strongly reduced during contact with the antimicrobial LLDPE surfaces. The compound containing 5 wt.% TBAM reduced the amount of bacteria to

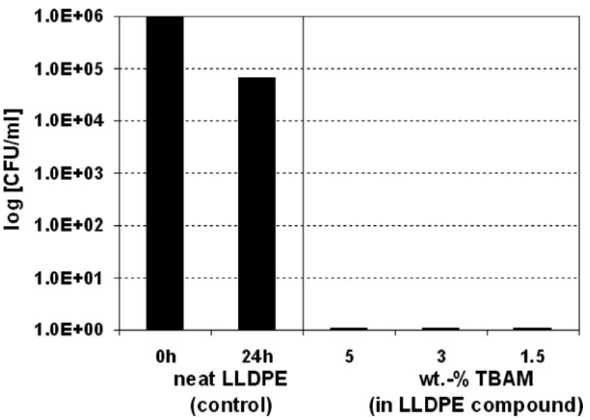

Fig. 4. Antibacterial activity of surfaces of LLDPE/TBAM compounds against *Staphylococcus aureus* (after 24 h of testing according to JIS Z2801:2000).

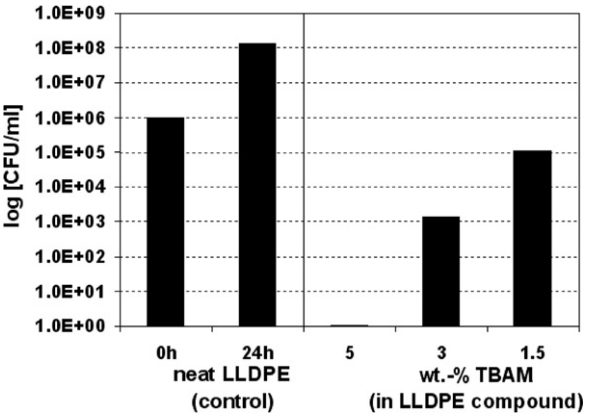

Fig. 5. Antibacterial activity of surfaces of LLDPE/TBAM compounds against *Escherichia coli* (after 24 h of testing according to JIS Z2801:2000).

0 CFU ml^{-1}, while LLDPE compounds with 3.0 and 1.5 wt.% TBAM gave a reduction to 1.4×10^3 and 1.2×10^5 CFU ml^{-1}, respectively (Fig. 5). In contrast to this, contact of *E. coli* bacteria with pristine LLDPE surfaces resulted in an increase from the starting concentration (1.0×10^6 CFU ml^{-1}) to 1.4×10^8 CFU ml^{-1} after 24 h.

Summing up, the compounding of polyethylene with the amino-functionalized polymer p*oly(2-tert-butylaminoethyl) methacrylate* resulted in surfaces with high antimicrobial activity against *E. coli* and *S. aureus*. This is indicated by an average reduction of 10^4 CFU ml^{-1} compared to neat LLDPE.

3.4. Biofilm formation

The antimicrobial activity of agents (or contact surfaces), which results in the killing of microorganisms, is of general importance for hygienic surfaces. It should not be overlooked that the formation of biofilms on surfaces is another issue. Biofilms are described as a deposition of biomass at surfaces. Such films are mainly consisting of water, polysaccharides, cell fragments, dead microorganisms and other components [14]. Since these mucous biofilms are important habitats for bacteria, both the formation and the adherence of biofilms to plastic substrates are undesired in numerous applications (e.g. water tubing). However, antimicrobial properties of surfaces do not necessarily reduce the formation of biofilms at these surfaces. Besides the ability of the surface to kill microorganisms, also the surface roughness, the polarity and hydrophilicity of

a substrate must be taken into account when considering the deposition of biofilms.

We wished to see if the addition of the antimicrobial polymer TBAM to LLDPE also influences the deposition of biofilms. The biofilm formation potential of modified LLDPE surfaces was assessed following the (preliminary) standard CEN TC164/WG3/AHG3. With this method, the formation of biomass (adhering biofilm) on a test substrate is determined by adenosine tri-phosphate (ATP) measurements after 8, 12 and 16 weeks of incubation in water. ATP measurements give evidence about the amount of living microorganisms present in a biofilm adherent to a sample surface. Due to the high sensitivity of the ATP measurements, this testing procedure is much more sensitive than conventional standard methods which rely on the determination of the mucous volume (e.g. the German standard DVGW W270). For the investigation of biofilm formation, glass was employed as negative control (low biofilm formation), while plasticized PVC plates served as positive control (strong biofilm formation).

After 8 weeks of testing, no adhering biofilm was detectable on any of the investigated LLDPE surfaces. After 12 and 16 weeks of incubation, biofilm formation became detectable by ATP measurements. Fig. 6 displays the results obtained after 16 weeks of incubation. In this diagram the amount of adhering biomass is expressed in pg of ATP per cm^{-2}. In comparison to soft PVC (21,000 pg ATP cm^{-2}), biofilm formation on pristine *LLDPE* is comparably low (145 pg ATP cm^{-2}). The addition of the antimicrobial polymer TBAM to LLDPE then leads to a further reduction of biofilm formation to approx. 70 pg ATP cm^{-2}. However, these values are still higher than those recorded for glass plates (negative control; 32 pg ATP cm^{-2}). Summing up, compounds of LLDPE with TBAM not only lead to killing of bacteria but also lead to a significant reduction of biofilm formation. According to this test procedure, this is evidenced by a decrease in the number of living microorganisms in the adhering biofilm.

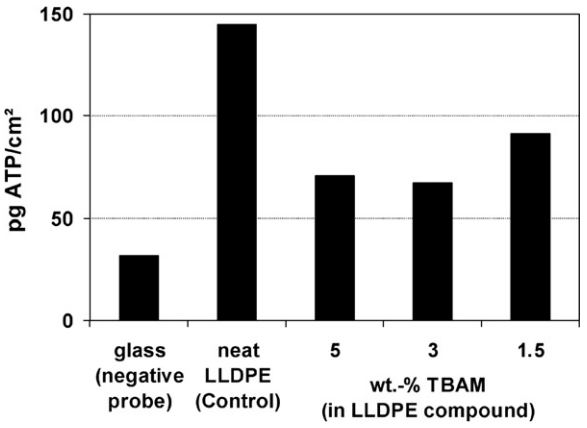

Fig. 6. Biofilm adhering to surfaces of LLDPE/TBAM compounds after 16 weeks of testing as expressed by ATP formation.

4. Conclusions

Polyethylene with antimicrobial properties can be prepared easily by compounding with the polymer-based biocide TBAM. The mechanical properties of the antibacterial LLDPE compounds are comparable to those of pristine LLDPE, when the content of TBAM is between 1.5 and 5.0 wt.%. TEM measurements of properly produced LLDPE/ TBAM compounds showed that the size of TBAM particles in the LLDPE matrix ranges between 0.05 and 0.5 μm. Compatibilizers do not seem to exert a significant influence on the dispersion of TBAM.

Surfaces of LLDPE/TBAM compounds containing 1.5–5.0 wt.% of TBAM display strong antimicrobial activity against *E. coli* and *S. aureus*. An average reduction of 10^4 CFU ml^{-1} compared to neat LLDPE was achieved. In addition to antimicrobial properties, the presence of TBAM also reduces the amount of biofilm on the polyethylene surface by a factor of two.

Electrokinetic investigations (zeta potential measurements) showed that the surface properties of the compounds change gradually when the content of the antibacterial polymer TBAM in the compound is increased from 0 to 5.0 wt.%. Due the fact, that the antimicrobial activity of LLDPE/TBAM compounds increases with increasing content of TBAM, a relation between the electrokinetic surface properties of the compounds and their antimicrobial activity can be established. Such a relation has also been demonstrated for other amino-functionalized polymers compounded with LLDPE (this will be published in a separate contribution). Thus, for a specific system a prediction of the antimicrobial activity can be made on the basis of zeta potential measurements. In combination with microbiological assays, relations of this kind may helpful when a screening (pre-testing) of various materials for their antimicrobial properties is required. This is also important when considering that as a result of melt processing of compounds (e.g. extrusion, injection

moulding, recasting) an enrichment of additives at the polymer surface can occur. Clearly, different processing techniques and parameters can lead to different surface properties and – in this specific case – to variations in antibacterial activity.

Acknowledgements

This study was performed at the Polymer Competence Center Leoben GmbH (PCCL, Austria) within the framework of the K_{plus}-program of the Austrian Ministry of Traffic, Innovation and Technology with contributions of Graz University of Technology (TU Graz), KeKelit Kunststoffwerk GmbH (Linz) and Anton Paar GmbH (Graz). PCCL is funded by the Austrian Government and the State Governments of Styria and Upper Austria. The authors with to thank Degussa Creavis (Marl, Germany) for supplying research samples of the antimicrobial polymer TBAM, and Anton Paar GmbH (Graz; Dr. T. Luxbacher) for providing the EKA instrument and valuable support during the zeta potential measurements. We also thank Dr. Ina Stephan (BAM, Berlin) and Dr. Christa Hametner (OFI Vienna) for carrying out the microbiological assays. Further thanks go to Dr. E. Ingolic (TU Graz; electron microscopy), Dr. W. Stadlbauer (Upper Austrian Research UAR; compounding) and to Dr. G. Riess (University of Leoben; polymer recasting). Finally, the cooperation of S. Temmel during the zeta potential measurements is gratefully acknowledged.

References

[1] Schmidt O, Schmidt A, Toptchiev D. Patent WO0185676; 2001.
[2] Cornish A, McGeechan P, Hodge D, Sinawati E. Patent WO20028952; 2002.
[3] Payne J, Kudner D, Pierce J, Lee P. International Nonwovens Conference, Crystal City, 18.1–18.9; 1996.
[4] Tiller J, Liao C, Lewis K, Klibanov A. Proc Natl Acad Sci 2001;98(11):5981.
[5] Thorsteinsson T, Másson M. J Med Chem 2003;46(19): 4173–81.
[6] Ikeda T, Tazuke S, Watanabe M. Biochem Biophys Acta 1984;735:380–6.
[7] Gelman MA, Weisblum B, Lynn DM, Gellmann SH. Org Lett 2004;6(4):557–60.
[8] Ottersbach P, Sosna F. Patent DE10022453, 2001.
[9] Ottersbach P, Kossmann B. GIT Labor Fachzeitschrift 2002;46(4):452–6.
[10] Buranasompob A. Kinetics of the inactivation of microorganisms by water insoluble polymers with antimicrobial activity. PhD thesis. Institute of Food Technology and Food Chemistry, TU Berlin 2005.
[11] Smoluchowski M. Handbook of Electricity and Magnetism Vol. 2. Barth, Leipzig 1921.
[12] Fairbrother F, Mastin H. J Chem Soc 1924;75:2318.
[13] Hunter RJ. Zeta potential in colloid science. London: Academic Press; 1981.
[14] Flemming H-C. Biofilms as a particular form of microbial life. In: Flemming H-C, Geesey GG, editors. Biofouling and biocorrosion in industrial water systems. Heidelberg: Springer; 1991.