



## Multifunctional crosslinkable itaconic acid copolymers for enzyme immobilization



Felix Müller<sup>a,b</sup>, Bernhard Torger<sup>a</sup>, Peter J. Allertz<sup>c</sup>, Klaus Jähnichen<sup>a</sup>, Stefan Keßler<sup>a</sup>, Martin Müller<sup>a</sup>, Frank Simon<sup>a</sup>, Katrin Salchert<sup>c</sup>, Haike Mäurer<sup>d</sup>, Doris Pospiech<sup>a,\*</sup>

<sup>a</sup> Leibniz-Institut für Polymerforschung Dresden e. V., Hohe Straße 6, 01069 Dresden, Germany

<sup>b</sup> Organic Chemistry of Polymers, Technische Universität Dresden, 01069 Dresden, Germany

<sup>c</sup> Brandenburg University of Technology, Faculty of Natural Science, Universitätsplatz 1, 01968 Senftenberg, Germany

<sup>d</sup> ifn Forschungs- und Technologiezentrum GmbH, Dr. Bergius Str. 19, 06729 Elsteraue OT Tröglitz, Germany

### ARTICLE INFO

#### Keywords:

ATR-FTIR spectroscopy

Crosslinking

Enzyme-immobilization

Itaconic acid

Methacrylate copolymer

### ABSTRACT

UV-Crosslinkable itaconic copolymers are developed to provide new multifunctional materials for coatings which combine crosslinkable functionalities and the possibility to immobilize enzymes. The polymer-immobilized enzymes were used for water treatment to decompose persistent organic molecules. Introduction of suitable comonomers allows tailoring the mechanical and chemical properties for special applications. Copolymers containing MMA and itaconic anhydride were chosen because of the formation of long-term stable anhydride functionalities. These anhydride functionalities are employed to attach enzymes covalently. 4-Benzoylphenyl methacrylate is used as comonomer for UV-initiated crosslinking. Terpolymers are successfully obtained by radical copolymerization in solution. The copolymers are compared to poly(ethylene-*alt*-maleic anhydride) [P(EMA)] often used with respect to enzyme immobilization, activity and hydrolytic stability. The hydrolysis stability of the copolymers against water is studied by ATR-FTIR spectroscopy. Thin films are prepared on glass substrates in a layer-by-layer procedure by spin-coating. The layer formation is monitored by ATR-FTIR spectroscopy. UV-crosslinking of the copolymer films is performed taking the optimal irradiation dose that avoids polymer degradation. ATR-FTIR spectroscopy verifies the coupling reaction between amino groups of the enzyme and the anhydride groups on the surface of the crosslinked polymer film. The syringaldazine (4-hydroxy-3,5-dimethoxybenzaldehyde azine) test and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay demonstrate that the immobilized enzymes maintain their activities. The functional copolymers showed a significant effect in reduction of persistent organic pollutants in contaminated waste water.

### 1. Introduction

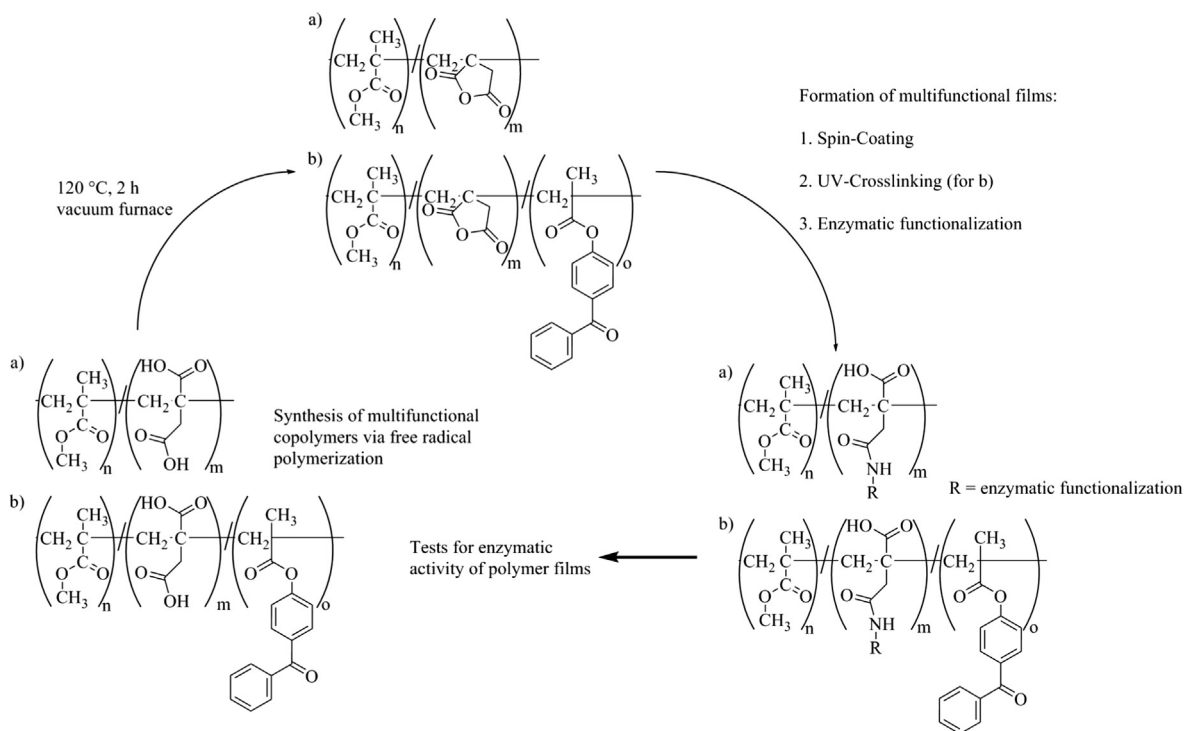
Polymer-immobilized enzymes are widely used, for water treatment to decompose persistent organic molecules [1–5]. Polymer coatings with anhydride moieties are well known as functional surfaces for immobilization of enzymes [6]. Prominent examples are alternating copolymers of maleic anhydride (MA), e.g. poly(ethene-*alt*-maleic anhydride) [P(EMA)] [7], poly(propene-*alt*-maleic anhydride) [P(PMA)] [8] and poly(octadecene-*alt*-maleic anhydride) [P(OMA)] [9–11]. Anhydride groups at the surface of a coating open the possibility to fix enzymes covalently via amide bonds. On the other hand, a significant drawback of the mentioned [P(EMA)] and [P(PMA)] polymers is their difficult handling in aqueous environment because of their rapid hydrolysis in water [12] to less reactive carboxylic acid groups. Hydrolysis affects the chemical and mechanical stability of the maleic anhydride

copolymer films drastically. Crosslinking of the copolymers by reaction of the anhydride groups with, e.g., diamines, or covalent immobilization to surfaces (for instance silica, glass, cellulose, or others) via coupling agents like 3-aminopropyltriethoxysilane (APTES) has been investigated to avoid this drawback [13,14] However, these procedures include additional preparation steps and fail sometimes.

The aim of the study reported here was directed to avoid additional preparation steps for stabilization of anhydride copolymer films and APTES interlayers. Thus, copolymers were developed to generate stable multifunctional films with anti-biofouling and enzyme immobilizing function and improved chemical stability. Copolymers of methyl methacrylate (MMA) with itaconic acid (ITA) or itaconic anhydride and additionally the UV-crosslinking comonomer, 4-benzoylphenyl methacrylate (BPMA), are presented here. BPMA, but in most cases 3-hydroxy-benzoylphenyl methacrylate, has frequently been used to

\* Corresponding author.

E-mail address: [pospiech@ipfdd.de](mailto:pospiech@ipfdd.de) (D. Pospiech).



**Scheme 1.** Statistic itaconic acid copolymers synthesized by free radical polymerization and the route to enzymatically active coatings.

crosslink and stabilize methacrylate copolymers [15,16].

MMA provides mechanical stability for copolymer coatings and chemical resistance against a number of organic solvents. Further advantages of PMMA are the good adhesion to different surfaces and the transparency of coatings [17]. These benefits were combined with the ITA functionality in the copolymers. ITA has widely been used industrially as comonomer, for instance as constituent for rubber materials, paints and colors, coatings, biologically degradable polymers or contact lenses [18,19]. P(MMA-co-ITA) copolymers [20] were expected to show reduced solubility in water compared to P(EMA). The acid groups of P(MMA-co-ITA) can be converted into anhydride groups by annealing at about 120 °C for two hours in vacuum. This functionality was used here to immobilize enzymes via the reaction of amino groups with the anhydride containing copolymer [21,22]. Laccase (LAC) was employed as model enzyme for the reduction of selected persistent organic pollutants and has also versatile applications [23–27]. The chemical structure of the multifunctional terpolymers has not been described so far and goes beyond the itaconic acid copolymers reported before. The structure and the route to multifunctional and enzymatically active coatings were illustrated in Scheme 1.

BPMA-containing copolymers were spin-coated directly on the substrate. Copolymers without crosslinking abilities (BPMA) were coated onto (3-aminopropyl)triethoxysilane (APTES) intermediate coupling layers. ATR-FTIR spectroscopy was employed to show the formation of the layers [28] and to study the experimental reactions: (1) reaction of itaconic anhydride with the APTES intermediate layer; (2) UV-crosslinking of the copolymers by BPMA; (3) immobilization of the enzyme e.g. LAC. P(MMA-co-ITA) copolymers were designed for freshwater applications. Their suitability in aqueous environment was evaluated. Thus, the behavior for the reduction of persistent organic pollutants like bisphenol A and diclofenac was investigated.

## 2. Experimental details

### 2.1. Materials

Acetone (99.5%, Sigma-Aldrich), aluminum oxide (basic, Sigma-

Aldrich), APTES (99%, Sigma-Aldrich), butyl acetate (> 99%, Sigma-Aldrich), ethanol (abs., EtOH, VWR-Chemicals), 4',6-diamino-2-phenylindole (DAPI, ≥98%, Merck), diethylether (DEE, 100%, VWR-Chemicals), *N,N*-dimethyl acetamide (DMAc, 99%, Acros Organics), *N,N*-dimethylformamide (DMF, 99.8%, Sigma-Aldrich), dimethyl sulfoxide (DMSO, 99.9%, Sigma-Aldrich), ethyl acetate (EtOAc, 99.5%, Acros Organics), *n*-hexane (≥ 98.5%, Merck), 4-hydroxybenzophenone (HBP, 98%, Acros Organics), LAC (*Trametes versicolor*, ≥10 U/mg, Sigma-Aldrich), methanol (MeOH, 99.99%, Acros Organics), methylene chloride (DCM, 99.99%, Acros Organics), methyl methacrylate (MMA, 99%, Sigma-Aldrich), phosphate-buffered saline (PBS, Fisher BioReagent), sand (50–70 mesh, Sigma-Aldrich), syringaldazine (≥99%, Sigma-Aldrich), tetrahydrofuran (THF, 99.99%, Acros Organics) were used as received. Azobis(isobutyronitrile) (AIBN, 98%, Fluka) was recrystallized twice from diethyl ether. MMA was destabilized by passing over basic aluminum oxide. All other chemicals were used as received.

### 2.2. Syntheses

BPMA was synthesized as described in [29]. 4-Hydroxybenzophenone (15 g, 0.076 mol) was introduced in a three-necked round bottom flask (1000 mL), secured for three times and dissolved under nitrogen in chloroform (300 mL). Then, triethylamine (10.495 g, 0.1064 mol) and methacryloyl chloride (10.8 g, 0.1034 mol) were added dropwise with cooling (0 °C, ice bath). The reaction was performed with stirring for 2 h at 50 °C, then the mixture was cooled down and washed two times with aqueous sodium bicarbonate solution (5 wt %) and water. The organic phase was separated and dried over magnesium sulfate. Then, the solvent was evaporated under reduced pressure. The purification procedure of the raw product was modified applying flash chromatography. The residue was adsorbed on silica gel and purified using *n*-hexane/butyl acetate mixture (90:10 vol/vol). After removing the eluent a white solid was obtained. The yield of the product was > 90%.

<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>): δ (ppm) 2.03 (t, 3H), 5.95 (quin, 1H), 6.33 (quin, 1H), 7.39 (dt, 2H), 7.58 (tt, 2H), 7.69 (tt, 1H),

Download English Version:

<https://daneshyari.com/en/article/7803736>

Download Persian Version:

<https://daneshyari.com/article/7803736>

[Daneshyari.com](https://daneshyari.com)