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Macroporous monoliths for biodegradation study of polymer particles considered as drug delivery systems

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ABSTRACT

Nanostructures based on biodegradable polymers are often considered as drug delivery systems. The properties of these nanomaterails towards *in vitro* biodegradation are very important and usually are studied using the model physiological conditions. In this work the novel approach based on application of monolithic immobilized enzyme reactors (IMERs) as the systems for biodegradation study of the nanoobjects of different nature and morphology was suggested. Rigid nanospheres based on poly(lactic acid) and self-assembled nanoobjects formed from block-copolymer of glutamic acid and phenylalanine were applied as model nanomaterials. For that, two enzymes, namely, esterase and papain were chosen for preparation of the monolithic IMERs. The properties of immobilized enzymes were compared to those obtained for soluble biocatalysts in the reaction of poly(lactic acid) and poly(glutamic acid) degradation. The monitoring of substrate destruction process was carried out using different HPLC modes (anion-exchange, cation-exchange or precipitation-redissolution based process) also based on application of the same modern stationary phase, namely, macroporous monoliths (CIM disks and lab-made column). Finally, the applicability of monolithic immobilized enzyme reactors for degradation of polyester and polypetide-based particles was demonstrated and compared to the process observed in human blood plasma.

1. Introduction

Nowadays, polymeric nanostructures are often considered as drug delivery systems for the treatment of a variety of diseases [1–3]. It is well known that the properties of medical materials strongly depend on their stability. The stability of polymeric nanoobjects can be governed *via* the choice of polymer nature (non-degradable or biodegradable), varying of polymer composition (homopolymer or copolymer), as well as the way of particle preparation that influences on particle morphology. Among the diversity of existing polymers, the biocompatible and biodegradable macromolecules with controllable lifetime have deserved much attention as biomedical and environmentally compatible materials [4,5]. The controlled and predictable time of polymer degradation is a key issue to make the best selection for biomedical applications.

In general, the newly developed materials before their *in vivo* application are studied *in vitro* regarding to their biodegradation properties using the enzymatic systems and model physiological conditions [6–8]. This approach is very useful to compare the material properties when the copolymer composition, polymer molecular weight or the way of material preparation were changed. However, native enzymes are sensitive and fragile to temperature, pH and the presence of organic solvents and may rapidly undergo to inactivation, what limits their applications [9]. One of the most common methods that can help to stabilize a biocatalyst is its immobilization on a solid support [10–12]. Furthermore, the enzyme immobilization allows the simple elimination of reaction products from biocatalyst, its reusability and combination of catalytic processes with analytical techniques in a single *on line* step [9,13].

The successful realization of counted preferences of immobilized enzymes depends on the nature of solid phase, its properties and the way of enzyme attachment. Several immobilization techniques are already well documented and can be generally divided into following categories: adsorption on solid matrix [14,15], inclusion into organic or inorganic gel (entrapment) [16,17], and chemical attachment of enzyme macromolecule to a solid support [18,19]. However, only chemical immobilization can guarantee the reliable binding of enzyme to the sorbent surface [20].

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Macroporous monoliths, designed initially as HPLC stationary phases [21,22], have gained rapidly the popularity in different processes based on interphase mass transfer, e.g. gas chromatography [23], capillary electrochromatography [24], thin-layer chromatography [25], solid-phase extraction [26,27], as well as the supports for the immobilization of enzymes and application in different processes of flow-through degradation of water-soluble biopolymers [28–30].

The achievements in the fabrication and application of monolithic supports as flow-through immobilized enzymatic reactors have been described in details in numerous original articles and reviews [30–34]. Such elevated interest to the monolithic materials has been caused by their numerous advantages. In particular, the macroporous structure of monolithic materials favors to the high flow rates and low backpressure, as well as to the absence of diffusion limitations to normal mass transfer due to the convection mechanism [35]. Moreover, the diversity of surface functional groups allows wide modification and, consequently, application of macroporous monolith in many fields.

The goal of presented work was the investigation of applicability of monolithic materials for biodegradation study of nanosized polymeric materials. Two kinds of nanoparticles differed with polymer nature and morphology were in the focus of this research. Particularly, the rigid nanospheres made of poly(L-lactic acid) and self-assembled nanoparticles of poly(L-glutamic acid)-*b*-poly(L-phenylalanine) were the objects. The first polymer represents the polyester, whereas the second one is related to polypeptides. Both polymers are biodegradable and biocompatible and widely considered for the fabrication of modern biomaterials including drug delivery systems [36–39].

The degradation of poly(lactic acid) in organisms occurs with participation of different enzymes of esterase's subclass. In turn, polypeptides are structurally similar to proteins and are degraded by enzymes of protease's subclass. Thus, in current work two kinds of monolithic bioreactors bearing immobilized enzymes from subclasses of esterases and proteases were prepared, characterized and applied for degradation of corresponding nanoparticles. The reactions with soluble enzymes were also used as a control. Both model processes were compared to *in vitro* degradation in human blood serum. Moreover, the monitoring of degradation process was carried out by HPLC method with the application of same kind of materials, namely, macroporous monolithic columns of different functionality (DEAE, SO_3, C12) and design (disk, stack of disks or rod).

2. Materials and methods

2.1. Reagents and supplements

Alkaline protease from *Bacillus licheniformis* (MW 27,000), papain (MW 23,400), lipase B from *Candida rugosa* (MW 62,000), lipase from bovine pancreas (MW 63,000) and esterase from porcine liver (MW 162,000) were the products of Sigma-Aldrich (Taufkirchen, Germany). Analytical grade sodium dihydrogen phosphate dihydrate, disodium hydrogen phosphate dodecahydrate, sodium tetraborate decahydrate, tris(hydroxymethyl)aminomethane, ethylenediaminetetraacetic acid disodium salt (EDTA), sodium chloride, sodium hydroxide, sodium borohydride, periodic acid, as well as 25% aqueous ammonium solution and hydrochloric acid were purchased from Neva-Reactiv Ltd. (St. Petersburg, Russia). The buffer solutions were prepared by dissolving salts in bidistilled water and filtered through a 0.45 μm membrane Millipore filter (Merck, Germany).

Water-soluble polymer of 2-deoxy-*N*-methacryloylamido-D-glucose (PMAG), MW 25,000, was synthesized by free-radical polymerization, characterized and oxidized to form reactive aldehyde groups following previously developed protocol [40]. The oxidized PMAG (ox. PMAG) containing 28 mol% of aldehyde groups was applied as a spacer for enzyme immobilization. Poly(L-lactic acid) (PLA), poly(L-glutamic acid), poly(glutamic acid)-*b*-polyphenylalanine (PGlu-*b*-PPhe) and the particles on their basis were prepared as described in [36] and in [7]. In the case of amphiphilic PGlu-*b*-PPhe the approximate content of polymer was $PGlu_{117}$-*b*-$PPhe_{81}$. The characteristics of polymers and particles used for biodegradation study are summarized in Table 1.

Stainless steel tubes of 50 mm length and 4.6 mm i.d. used for monolithic column preparation were purchased from Supelco (Bellefonte, PA, USA). The synthesis of lauryl methacrylate-*co*-ethylene dimethacrylate monolithic column has been described earlier [41]. Commercially available CIM Epoxy, CIM QA, CIM DEAE disks (12 mm i.d. × 3 mm) used both for enzyme immobilization and consequent chromatographic analysis of the resulting products of catalytic reactions were purchased from BIA Separations (Ajdovscina, Slovenia).

2.2. Instruments

The chromatographic and bioconversion experiments were carried out using the HPLC Shimadzu system consisting of two pumps LC-10AD VP and LC-20AD, scanning UV detector SPD-10AV VP, system controller SCL-10A VP and degasser pump DGU-14A, all purchased from Shimadzu USA Manufacturing, Inc (Canby, OR, USA). Column temperature was controlled using column thermostat Eppendorf TC-50 (Hamburg, Germany). To perform the bioconversion experiments in recirculation mode, the single-channel peristaltic pump LKB Pharmacia P-1 (Uppsala, Sweden) was applied. The centrifuge Sigma 2-16P (Osterode am Harz, Germany) was used for ultrafiltration of reaction mixture to remove enzyme. For the incubation of reaction solutions, air thermostat LF-25/350-VS2 LOIP (Moscow, Russia) was used.

2.3. Methods

2.3.1. Immobilization of enzymes

Immobilization processes were fulfilled by the reaction of enzymes with aldehyde-bearing polymer spacer preliminary bound to monolith's surface and included several steps, such as: (1) amination of epoxy groups of monolithic material; (2) covalent

Table 1
Polymers and polymer nanoparticles used for biodegradation study.

Object	Polymer molecular weight (M_w)	PDI of polymers (M_w/M_n)	Particle size (DLS), nm	PDI of nanoparticles	Morphology of particles
Soluble polymers					
PLA	30,000	1.5	–	–	–
PGlu	15,000	1.2	–	–	–
Polymer particles					
PLA	30,000	1.5	350	0.25	dense nanospheres
PGlu-*b*-PPhe	27,000	1.4	220	0.15	polymersomes