The effect of the crosslinking agent on the performance of propranolol imprinted polymers

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ARTICLE INFO

ABSTRACT

Molecularly imprinted polymers (MIPs) of propranolol were synthesized by bulk polymerization. Methacrylic acid (MAA) was selected as the functional monomer and symmetrical dimethacrylate crosslinkers of different length between the double bound as well as an asymmetrical crosslinker containing an allylic and a methacrylic double bond were investigated in this study. The rebinding experiments were carried by radioligand experiments in presence of [^{3}H]-(S)-propranolol. The microstructure of MIPs (morphology, crosslinking density and surface properties) were analysed by B.E.T. surface, SEM images and swelling experiments. MIPs containing 1,6 hexanediol dimethacrylate (HDDMA) and allyl methacrylate (AMA) improved the binding results of MIPs having the most common crosslinking agent ethylene glycol dimethacrylate (EGDMA).

In addition, methyl methacrylate (MMA) was included in the formulation to decrease the crosslinking density. The binding specificity decreased except for MIPs containing AMA as crosslinking agent, which showed a similar specificity to MIPs without MMA.

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1. Introduction

Molecularly imprinted polymers (MIPs) are solid materials with high molecular recognition capacity of a template molecule. MIPs are synthesized in presence of a functional monomer, a crosslinking agent and a target molecule (template). MIPs are based on the strength of the interaction between the functional monomer and the template molecule and they are classified upon the type of this interaction. MIPs are synthesized by two different approaches based on the binding between the functional monomer and the template. The covalent approach developed by Wulff and coworkers in early 1970s [1], is based on covalently bonding the functional monomer and

template prior polymerization. After polymerization, the covalent bond has to be broken during the extraction and the removal of the template exposes the recognition sites. The stability and reversibility of the covalent linkage is a necessary parameter in the creation of sites having recognition selectivity. The covalent approach brought a large improvement on the development of the synthetic materials that mimicked the behavior of enzymes, but the complex synthetic procedure and the single specificity of each template with the recognition sites were tedious aspects to overcome. The work of Mosbach [2] was essential on the study of the monomer-template interactions; ionic bonds, hydrophobic or Van der Waals interactions and π–π interactions, to open the path to non-covalent interaction between the functional monomer and the template. Even if these types of non-covalent interactions are weaker than the covalent bonds, templates of biochemical, pharmaceutical and environmental interest have polar groups such as hydroxyl, carboxyl, amino, and amide that

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are well suited for the non-covalent approach. Hydrogen bonding is a well-tailored interaction for a precise molecular recognition. In addition Sellergren and Andersson [3] and Whitcombe et al. [4] reported an intermediate approach that combined the advantages of the covalent and non-covalent approaches. This approach combined the creation of a covalent complex of functional monomer and template during the prepolymerization, while the recognition was carried out by non-covalent interactions.

In the last years, the interest on MIPs has increased enormously as it is demonstrated by the increasing number of articles that are published in this field. Most of these articles present MIPs synthesized by bulk polymerization, the most common procedure used in MIPs synthesis. An easy attachment between functional monomer and template, in conjunction with a cheap and easy synthetic procedure has led to the development of an unimaginable sort of applications such as chromatographical detectors, chiral molecules separations [5–7], membranes [8–10], reaction catalysts [11,12], anti-doping test molecule detectors [13,14], environmentally interesting molecule detectors [14] and even blood group detectors [15]. The simplicity of the procedure together with good rebinding results has made bulk polymerization to be the technique most frequently used to produce MIPs.

In terms of this work, Propranolol was selected as template to imprint MIPs by bulk polymerization. Propranolol is a non-selective β-blocker mainly used in hypertension treatment and more recently, in post-traumatic stress disorder treatment [16]. Because of key side effects associated with β-blockers, they are heavily regulated by government agencies worldwide. More recently, many β-blockers have been included in the list of banned substances by the World Anti-Doping Agency (WADA). Therefore, it is necessary to accurately detect β-blockers. One of the pioneering works that synthesize MIPs to detect β-blockers like propranolol was the work developed by Andersson [17]. Usually MIPs are synthesized using methacrylic acid (MAA) as functional monomer. The interaction between MAA and propranolol occurs through hydrogen bond donor and acceptor interactions between the alcohol and ether groups on the propranolol molecule and the carboxylic acid groups of MAA. Proton transfer and subsequent ionic interactions may also occur through proton donation from the carboxylic acid residue of MAA to the basic secondary amine of the propranolol molecule.

In spite of being the major component in the formulations to produce MIPs, the number of works that have studied the effect of the type of crosslinker and the effect of the crosslinking density on the recognition capabilities of MIPs is scarce [17–19]. Garcinuño et al. [18] suggested that the crosslinking density in MIPs may have importance on cavities disposition. Wulff [19] did also note that the choice of the type and amount of crosslinker might have a strong impact on the selectivity of MIPs. Furthermore, it is necessary a compromise between an inflexible arrangement of the polymer chains to give high selectivity and an appropriate degree of flexibility for good accessibility to the cavities and rapid attainment of binding equilibrium. In addition other authors studied the effect of the crosslinking density on the recognition capacity of MIPs

[20,21]; in general, increasing the crosslinking density of the MIP recognition capacity increased, however Biffis et al. [21] found that the optimum amount of crosslinker was in the range 50–80% depending on the functional monomer used.

In this work the effect of the structure and reactivity of different crosslinkers (dimethacrylates with ethylen glycol spacers of different sizes and crosslinkers with double bonds of different reactivities) employed in the synthesis of propranolol MIPs synthesized by bulk polymerization on the rebinding performance is investigated. Furthermore, for each crosslinker analyzed the effect of the crosslinking density on the rebinding performance of the propranolol MIPs is also studied by decreasing the concentration of the crosslinker adding a monovinyl monomer like methyl methacrylate in the formulation. The microstructure of the polymer networks is analyzed in detail to understand the differences found in the rebinding performances of the MIPs.

2. Experimental

2.1. Chemicals and materials

Methacrylic acid (MAA), methyl methacrylate (MMA), allyl methacrylate (AMA), 1,6 hexanediol dimethacrylate (HDDMA), ethylene glycol dimethacrylate (EGDMA), polyethylene glycol dimethacrylate (200) (PEGDMA$_{200}$) (the structure of the crosslinkers is included in Fig. 1), and toluene were used as received (all from Aldrich). R,S-propranolol (Aldrich) was converted to its free base form by
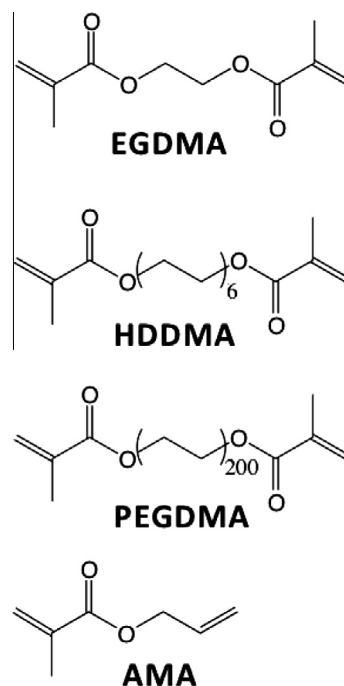
Fig. 1. Structure of ethylene glycol dimethacrylate (EGDMA), 1,6-hexanediol dimethacrylate (HDDMA), polyethylene glycol dimethacrylate (n = 200) (PEGDMA) and allyl methacrylate (AMA) crosslinkers.