European Polymer Journal 75 (2016) 423–430

Contents lists available at ScienceDirect

European Polymer Journal

journal homepage: www.elsevier.com/locate/europolj

Macromolecular Nanotechnology

The effect of crosslinking density on molecularly imprinted polymer morphology and recognition

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ARTICLE INFO

Article history:
Received 27 October 2015
Received in revised form 17 December 2015
Accepted 1 January 2016
Available online 2 January 2016

Keywords:
Molecularly imprinted polymer
Molecular dynamics
Molecular recognition
Molecular imprinting
Morphology
Crosslinking

ABSTRACT

In this report, the crosslinking density of bupivacaine molecularly imprinted methacrylic acid (MAA)–ethylene glycol dimethacrylate (EGDMA) copolymers was investigated through replacement of EGDMA by methyl methacrylate (MMA). The effects were examined using a series of full-scale MD simulations of pre-polymerization mixtures, equilibrium rebinding studies on the corresponding synthesized polymers and morphology characterization through nitrogen sorption measurements. While the extent of hydrogen bonding between the functional monomer MAA and bupivacaine observed in the MD pre-polymerization mixtures was comparable in each of the systems studied, the decrease in degree of crosslinking impacted directly on polymer morphology as observed in BET and BJH studies of surface area and porosity. Further, decreases in the crosslinking density induced reductions in template rebinding capacity as seen from a series of radio-ligand binding studies, demonstrating the importance of crosslinking on the performance of molecularly imprinted MAA–EGDMA copolymers, the polymer system most commonly used in molecular imprinting science and technology.

1. Introduction

The molecular imprinting technique [1,2] is widely used for generating polymeric recognition materials with molecular recognition properties that in some cases can be compared with those of antibodies and enzymes [3–5]. The physical and chemical stabilities of molecularly imprinted polymers (MIPs) [6] make them suitable for use in a growing number of application areas; *e.g.* biomimetic assays [7,8], separation materials [9,10], sensor recognition elements [11–14] and therapeutic strategies [2,15].

The basis for the recognition properties of MIPs prepared using non-covalent strategies is generally accepted to arise from the fixing of template–monomer complexes present in the pre-polymerization stage through crosslinking [16–18]. Our understanding of the mechanisms underlying template-functional monomer interactions has driven the development of novel monomer systems and an improved understanding of the mechanisms of MIP–ligand interactions. A far less studied area is the role of crosslinking on recognition and its impact on morphology, and the impact of morphology on recognition. In the present study we have undertaken a comprehensive analysis of the impact of crosslinking on the morphology and recognition properties of a series of bupivacaine-imprinted methacrylic acid (MAA)–ethylene glycol dimethacrylate (EGDMA) copolymers. The strategy used was the replacement of EGDMA with two equivalents of methyl methacrylate (MMA), Fig. 1.

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http://dx.doi.org/10.1016/j.eurpolymj.2016.01.008
0014-3057/

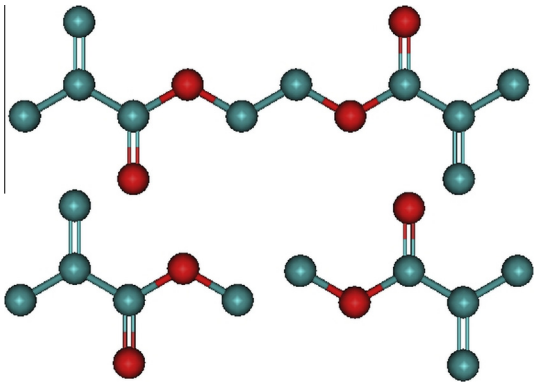

Fig. 1. The crosslinking density in the polymers was reduced by replacing EGDMA (top structure) with two equivalents of MMA (bottom structures).

This substitution was envisaged to provide change in the degree of crosslinking without influencing either the number of heteroatoms available for hydrogen bonding interactions or the number of alkene functionalities.

MAA–template interactions have previously been identified to account for the formation of high affinity binding sites in bupivacaine imprinted MAA–EGDMA copolymers [19]. Here it was shown that replacement of EGDMA with MMA affects the extent of hydrogen bonding interactions between MAA and bupivacaine in the pre-polymerization mixtures to only a minor extent. Moreover, the total frequency of hydrogen bonding to the template by EGDMA and MMA interactions in each system was found to be constant, justifying the use of MMA to investigate the influence of EGDMA-crosslinking. In the present study, varying the degree of crosslinking was used to examine the affect of crosslinking on polymer morphology and polymer-template recognition.

2. Experimental section

2.1. Chemicals

[^{3}H]-(*R*,*S*)-bupivacaine (specific activity 2.7 Ci/mmol) was purchased from Moravek Biochemicals Inc. (California, USA). (*R*, *S*)-bupivacaine hydrochloride, methyl methacrylate (MMA) and methacrylic acid (MAA) were obtained from Sigma–Aldrich (Steinheim, Germany). Toluene was purchased from Merck (Solna, Sweden). Ethylene glycol dimethacrylate (EGDMA) was obtained from Fluka (Buchs, Switzerland) and 2,2′-azobis-(2-methylpropionitrile) (AIBN) from Janssen Chimica (Geel, Belgium). All chemicals were of analytical grade and the water used was of Millipore quality (Millipore AB, Sweden).

2.2. Molecular dynamics simulations

All-atom MD simulations were performed using the AMBER [20,21] (v.10.0 UCSF, San Francisco, CA) platform of programs. Five molecularly imprinted and corresponding non-imprinted reference pre-polymerization mixtures differing in crosslinking density were simulated. The number of EGDMA molecules was decreased and substituted with twice the number of MMA molecules while the other components were held constant (Table 1). All systems were simulated in quintuplicate, each covering 10 ns of recorded trajectory data for each mixture (in total 50 ns).

The molecular compositions and molar ratios of the monomers in the simulated pre-polymerization mixtures were representative of prepared bulk-polymers. The simulated systems were built using the PACKMOL [22,23] software to obtain random initial starting configurations and parameterized using the Amber99 [24] and GAFF [25] force fields. Partial atomic

Table 1
Composition of simulated molecularly imprinted (MIP) pre-polymerization mixtures.[a]

System	Number of molecules						Molar ratio					
	Bupivacaine	MAA	MMA	EGDMA	AIBN	Toluene	Bupivacaine	MAA	MMA	EGDMA	AIBN[b]	Toluene[c]
S1	10	144	0	557	16	1122	1	14.4	0	55.7	1.3	1.6
S2	10	144	288	413	16	1122	1	14.4	28.8	41.3	1.3	1.6
S3	10	144	576	269	16	1122	1	14.4	57.6	26.9	1.3	1.6
S4	10	144	864	125	16	1122	1	14.4	86.4	12.5	1.3	1.6
S5	10	144	1114	0	16	1122	1	14.4	111.4	0	1.3	1.6

[a] Non-imprinted reference systems (REF) were simulated under similar conditions as the MIP-systems.
[b] Mol% of the total amount of polymerizable methacrylate units present in the mixtures.
[c] Times the total number of the monomers present in S1.