

Molecularly Imprinted Polymers with Bi-functional Monomers of Polymerizable Cyclodextrin Derivatives and 2-(Diethylamino)-ethyl Methacrylate for Recognition of Norfloxacin in Aqueous Media

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Abstract: A molecularly imprinted polymer was synthesized using 2-(diethylamino)ethylmethacrylate (DEM) and bismethacryloyl- β -cyclodextrin (BMA- β -CD) as bi-functional monomers and norfloxacin (NOF) as a template. The results of equilibrium binding experiments indicated that the polymer has affinity and specificity for NOF in aqueous media, and that its selective recognition ability for the template was higher than that of the imprinted polymers synthesized with a single functional monomer (BMA- β -CD or DEM).

Keywords: Molecular imprinting, norfloxacin, β -cyclodextrin, binding specificity.

Molecular imprinting technique is one of the most promising methodologies for synthesizing artificial receptors and has already demonstrated their potential for the separation and analytical application¹⁻⁶. In the case of molecularly imprinted polymers prepared by non-covalent interactions, hydrogen bonding is the most commonly used interaction for the pre-organization of templates and functional monomers. Exploiting hydrogen bonding as the main interactions, molecularly imprinted polymers of morin and bisphenol A have been prepared by our group^{7,8}. They can recognize their templates efficiently in the lower polar organic solvents. Hydrogen bonds interactions between template and functional monomers are easily destroyed in protonic solvents, because the protonic solvents can competition with the template for the functional monomers. So, it was difficulty to achieve recognition in aqueous media. When the templates are soluble in polar solvents, the result was the same. In order to overcome this problem, different methods have been investigated during the past few years. Recently, the polymerizable β -cyclodextrin derivatives were used as a functional monomer to utilize molecular imprinting in aqueous solution⁹⁻¹¹. In the present study, a norfloxacin-imprinted polymer was prepared using 2-(diethylamino)ethylmethacrylate (DEM) and bismuthacryloyl- β -cyclodextrin (BMA- β -CD) as functional monomers. The results of binding experiments indicated that the polymer can selective binding the target molecule in aqueous media.

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Experimental

The BMA- β -CD was synthesized according to S. A. Piletsky, *et al.*¹⁰. The product is a mixture of substitutional isomers with 2.0 methacryloyl substitutions per β -cyclodextrin determined from the elemental analysis (Anal. Calcd. for BMA- β -CD: C 46.09, H 5.99; found: C 46.31, H 5.67%). The IR spectrum showed a characteristic α,β -unstaturated carbonyl absorbance at 1726 cm^{-1} . The 1:1 BMA- β -CD-NOF complex (1.59 g containing 1.0 mmol β -CD unit) was prepared according to the ref.¹².

A series of molecularly imprinted polymers (P) and non-imprinted polymers (NP) were prepared (**Table 1**). The procedure for the synthesis of the standard polymer P₁ was as follows: BMA- β -CD-NOF complex and DEM were dissolved in dimethyl sulfoxide(DMSO) in a 50 mL glass ampoule, ethylene dimethacrylate(EDMA) and 2, 2'-azobis(isobutyronitrile)(AIBN)were added. After nitrogen gas was sparged into the solution for 5.0 min, the ampoule was sealed in vacuum and the mixture was kept in a water bath at 60.0°C for 24 h. The resultant rigid polymer was grounded and passed through a 90 μm sieve. The obtained particles were Soxhlet extracted with a mixture of methanol-acetic acid (9:1, v/v) for 72 h. Then the particles were washed with methanol and dried in vacuum at 60.0°C. Anal. Calcd. for P₁ and NP₁(calculated according to the chemical composition for making these polymers): C 55.76, H 5.46, N 0.419%; P₁ Found: C 55.2, H 6.47, N 0.40%; NP₁ Found: C 54.7, H 6.45, N 0.43%. IR (KBr) ν (cm^{-1}): P₁ 3600-3000, 2956, 2922, 1728, 1637, 1456, 1389, 1258, 1156, 1052, 1025, 757; NP₁ 3600-3000, 2956, 2923, 1729, 1637, 1455, 1381, 1257, 1156, 1052, 756. The thermogravimetric analysis (TGA) showed that P₁ and NP₁ start to decompose at about 256°C and 258°C, respectively.

Equilibrium binding experiments were carried out in methanol/water (1:1, v:v) solution(3.0 mL) with 1.0 mmol/L substrate concentration at 30°C. The polymer particles (20.0 mg) were mixed with the solution in a conical flask. The conical flask was shaken for 4 h and then the mixture was filtrated through a 0.45 μm filter. The concentration of free substrate in the solution was determined using a spectrophotometer at λ_{max} (276 nm). The amount of substrate bound to the polymer was calculated by subtracting the concentration of free substrate from initial substrate concentration.

Table 1 The chemical composition for making polymers

Polymer	BMA- β -CD-NOF ^a	NOF ^a	BMA - β -CD ^a	DEM ^a	EDMA ^a	DMSO ^b
P ₁	0.63	—	—	0.63	6.0	10
NP ₁	—	—	0.63	0.63	6.0	10
P ₂	0.63	—	—	—	3.0	7
NP ₂	—	—	0.63	—	3.0	7
P ₃	—	0.63	—	0.63	3.0	6
NP ₃	—	—	—	0.63	3.0	6

Unit: ^a mmol, ^b mL

Result and Discussion

Binding specificity of the polymers

The affinity and specificity of the polymer were estimated by the distribution coefficient (K_d) of NOF between the polymer and solution. The distribution coefficient (K_d) is defined as: $K_d = C_p / C_i$, where C_p is the concentration of the substrate bound in the polymer (umol/g polymer) and C_i is its concentration in solution (umol/mL)¹³. The obtained distribution coefficients are list in **Table 2**.

The data show that the imprinted polymer used bi-functional monomers (P_1) obviously exhibited high binding affinity and specificity for NOF, while the imprinted polymer used DEM as the functional monomers (P_3) showed much less binding specificity for NOF, the polymer used BMA- β -CD as the functional monomers (P_2) showed no binding specificity for NOF.

Selectivities of P_1 and NP_1

The selectivity test of P_1 and NP_1 were performed using a series of structurally related substrates. Their amounts bound to the polymers were determined by equilibrium binding method. The distribution coefficients of the substrates are listed in **Table 3**.

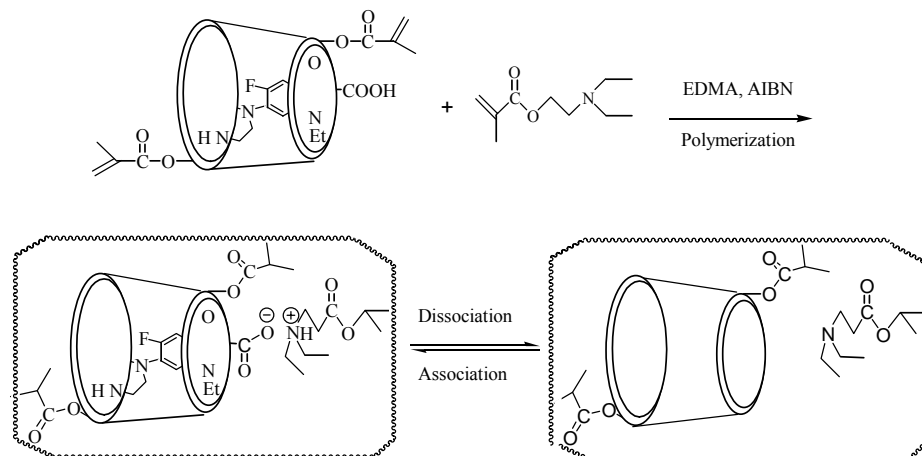
The data presented in **Table 3** show that P_1 exhibit highly selective binding for NOF, and it has little or no affinity for the other compounds. Cyclodextrin and modified cyclodextrins are the most interesting hosts in host-guest chemistry, they can form inclusion compounds with guests in water through hydrophobic interactions. In this study, BMA- β -CD and NOF can form 1:1 inclusion complex in aqueous media¹². The carboxyl group of NOF can form strong ionic interactions with the basic functional group of 2-DEM in aqueous media. Thus, hydrophobic and electrostatic interactions are combined in molecular imprinting (**Figure 1**). The equilibrium binding experiments have demonstrated that the affinity and specificity for the guest has been efficiently improved compared to the imprinted polymers using single functional monomer.

Table 2 K_d of NOF on the polymers under equilibrium binding conditions

P_1	NP_1	P_2	NP_2	P_3	NP_3
38.13	20.20	10.34	9.68	29.45	20.06

Table 3 K_d of the substrates on P_1 and NP_1 under equilibrium binding conditions

	Ofloxacin	Ibuprofen	Alizarin	Cefalexin	NOF
P_1	7.37	21.64	1.78	9.46	38.13
NP_1	7.06	16.78	1.67	7.85	20.20

Figure 1 Schematic illustration of the molecular imprinting procedure

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