Binding studies of histamine-imprinted polymers prepared using photochemical polymerization for facile incorporation to sensing device

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ABSTRACT

Increase in histamine levels are known to be associated with food spoilage and to pathophysiological conditions linked to allergy. This study aims to prepare and evaluate histamine imprinted microspheres as recognition element for histamine sensing. The imprinted polymers and their corresponding non-imprinted versions were prepared under high-dilution photochemical polymerization using AIBN, 4% monomer containing (80:20 wt% (PCP-M80/PCP-N80) or 90:10 wt% (PCP-M90/PCP-N90) EGDMA:MAA and histamine:MAA mole ratio of 1:4 in acetonitrile at RT. These polymers with sizes ranging from 57 to 149 nm in the collapsed state were evaluated for histamine binding via frontal analysis capillary electrophoresis (FACE). PCP-M80 was found to be more selective to histamine, possessing higher number of binding sites as estimated from Freundlich affinity distribution. The selective binding capacity was also found to be higher for PCP-80 systems (N=16.0 μmol/g) compared to PCP-90 systems (N=10.1 μmol/g).

Keywords: histamine imprinted microspheres, photopolymerization, histamine

1 Introduction

Accurate determination of histamine (HTM) levels in food or in samples from biological origin (e.g., tissues, urine) is important for monitoring HTM-related food poisoning or pathophysiological conditions. We are interested in fabricating a histamine sensor using fluorophore-containing imprinted polymers as recognition element and optical fiber as susbstrate.

2 Synthesis of histamine imprinted polymers

In this study, the imprinted polymers were accessed using photochemical polymerization, where the reaction was carried out under mild temperature conditions to provide materials with superior binding performance [1,2] compared to thermally initiated polymers. Different parameters such as nature of solvent (i.e., dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF) and acetonitrile (MeCN)), amount of crosslinker (80:20 or 90:10 wt% ethylene glycol dimethacrylate (EGDMA):methacrylic acid (MAA)) and monomer feed concentration (1-10 wt %) were examined in order to prepare solution processable microspheres at 30 °C for 24 hrs. Results from the polymers prepared using 4% (80:20 or 90:10 wt% EGDMA:MAA) in MeCN were highly processable in DMSO, DMF, MeCN, THF. Hence, histamine-imprinted polymers were prepared using 4% monomer containing 80:20 (PCP-M80) or 90:10 (PCP-M90) wt% EGDMA:MAA, histamine:MAA mole ratio of 1:4 in MeCN.

2.1 Physical properties of the polymers

Four polymers were prepared and evaluated: histamine-imprinted polymers (PCP-M80 and PCP-M90) and the corresponding non-imprinted polymers (PCP-N80 and PCP-N90). The TEM micrographs of PCP-M80 and PCP-N80 after polymerization revealed that both polymers were formed as network of spherically-shaped particles. DLS measurements carried out in DMSO showed PCP-M80 (176±3 nm) and PCP-M90 (188±8 nm) were bigger compared to PCP-N80 (147±3 nm) and PCP-N90 (113±3 nm). The presence of template in PCP-M80 and PCP-M90 increases the rate of the particle growth resulting in bigger particle size. SEM micrographs (Figure 1) also confirmed that the polymers were spherical, with PCP-M80 (149±66 nm) and M90 (129±27 nm) bigger compared to that of PCP-N80 (74±20 nm) and N90 (57±9 nm) in the collapsed state.

2.2 Binding studies

Batch rebinding experiments of the polymers were carried out using 2 mg of polymer suspended in aqueous histamine solution with concentrations ranging from 0.10 to 1.0 mM bufferred at pH 7 and analyzed using frontal analysis capillary electrophoresis (FACE) [3]. Unlike HPLC, FACE method allows direct sample analysis, whereby the sample with the rebinding solution is sonicated for 10 s, equilibrated for 30 min and injected directly to the capillary without filtration. The binding characteristics of the polymers toward HTM were studied at concentrations below saturation binding (<1.0 mM), these binding isotherms have been found to conform with the Freundlich model (FI), where log plots with high regression correlation (R^2 >0.93) were obtained (Figure 2B). Freundlich isotherms define the amount template bound

(B) as a power function to the free template concentration (F) according to equation (1), which after linear transformation provided the equation (2). The linear equation (Figure 2B) yielded two fitting parameters a (y intercept) and m (slope) which were utilized to generate the affinity distribution (Figure 2C, 2D) using the equation (3) over the concentration ranges in equation (4).

$$B=aF^{m} \quad (1)$$

$$\log B= m \log F + \log \alpha \quad (2)$$

$$N(K) = 2.303am(1-m^2)K^{-m} \quad (3)$$

$$K_{min} = 1/F_{max} \text{ and } K_{max} = 1/F_{min}. \quad (4)$$

The binding results showed that binding capacity N_t for all imprinted polymers were higher than their corresponding non-imprinted conterpart, which is indicative of imprinting effect. Based from Table 1, the heterogeneity (m value) for PCP-M80 and PCP-N80 are comparable using FACE, and thus have similar heterogeneity on their binding sites. In addition, PCP-M90 and PCP-N90 also have comparable heterogeneity. However, these binding sites were more heterogenous compared to PCP-80 combination.

The AD plots using N versus log K format (Figure 2C) showed that the imprinted polymers have higher number of binding sites than the non-imprinted versions, as depicted by the area of the distribution under the curve. Moreover, PCP-M80 has more binding sites than PCP-M90. This is partly due to the higher amount of histamine incorporated in the PCP-M80 formulation than in PCP-M90 during polymerization. PCP-M80/N80 provided steeper lines indicating low ratio of high-to-low affinity binding sites (Figure 2D). Conversely, PCP-M90 and PCP-N90 provided flatter lines indicating higher concentration of high-affinity sites.

At higher analyte concentration (within the concentration range employed), the measured K_{min} values for all polymers are almost comparable (Table 2). This is reasonable because the binding sites were saturated. In addition, the corresponding N_{min} values of the four polymers showed that imprinted versions have two times more binding sites than non-imprinted counterpart as analyzed by FACE. At low analyte concentration, K_{max} and N_{max} values for PCP-M80/M90 were still higher than PCP-N80/N90. A quantitative comparison of the binding performances of the microspheres on the basis of *N*, with K = 25 mM^{-1} (i.e., log K = 1.4), the highest affinity sites across the concentration range studied showed that the values for the imprinted versions are more than twice that of the non-imprinted versions: PCP-M80 (27.4 µmol/g) and PCP-N80 (11.4 µmol/g), PCP-M90 (18.3 µmol/g) and PCP-N90 (8.20 µmol/g). Thus, the difference in binding capacities (ΔN) between imprinted versus the non-imprinted is significantly higher in PCP-80 (*N*=16.0 µmol/g) compared to PCP-90 (*N*=10.1 µmol/g).

This study showed that imprinted processable polymers prepared using photopolymerization with PCP-M80 provided better histamine binding compared to polymers prepared using PCP-M90. We are currently exploring the preparation of fluorescent imprinted PCP-80. It is also anticipated that the microspheres selective for histamine produced in this study will allow grafting of the polymers onto activated substrates for subsequent sensor fabrication.

TABLES AND ILLUSTRATIONS

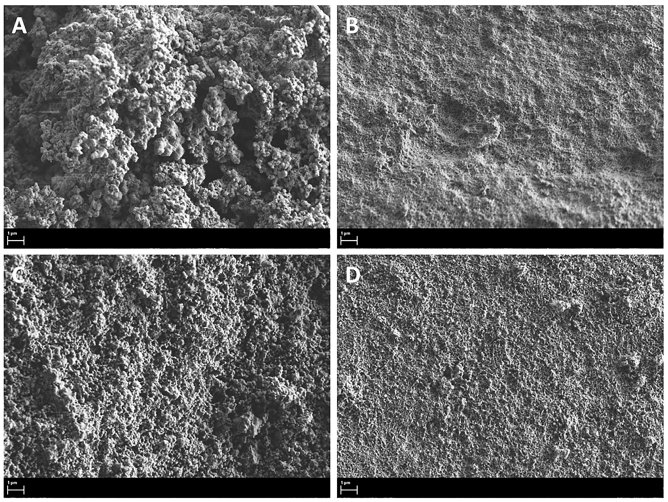

Figure 1. Scanning electron micrographs of 4% monomer containing 80 or 90 wt% EGDMA and 20 or 10 wt% MAA, and histamine:MAA mole ratio of 1:4 in MeCN using photopolymerization method at 30 °C for 24 hrs. PCP-M80 (A), PCP-N80 (B), PCP-M90 (C), PCP-N90 (D).

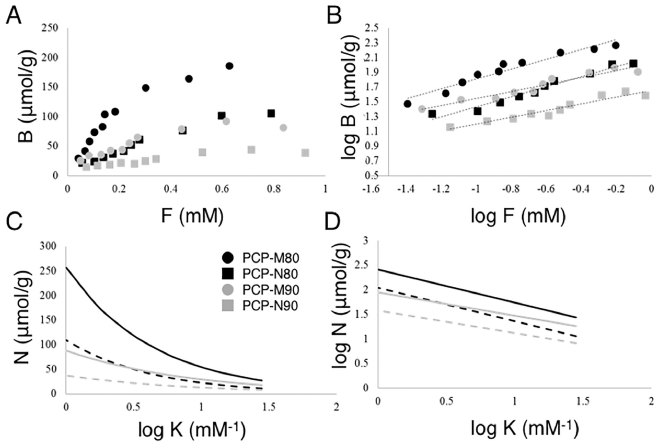

Figure 2. Binding isotherms obtained from FACE analysis of photochemically prepared PCP-M80 and M90, PCP-N80 and N90. (A) Freundlich isotherm models, (B) linearized log-log Freundlich binding isotherms, (C) Freundlich affinity distribution expressed in the N vs log K format, and (D) Freundlich linearized affinity distribution expressed in the log N vs log K format. N and K were obtained from the slope (m) and y intercept a of (B). Affinity distributions have been generated using the equation $N(K) = 2.303am(1-m^2)K^{-m}$ over concentration ranges $K_{min} = 1/F_{max}$ and $K_{max} = 1/F_{min}$.

Table 1. Binding parameters of PCP-M80 and N80, PCP-M90 and N90 expressed using Freundlich isotherms and analyzed by FACE technique

Sample	Freundlich Binding Isotherm		
	m	a= N_t(μmol/g)+K	R^2
PCP-M80	0.672	304	0.941
PCP-N80	0.680	130	0.966
PCP-M90	0.473	105	0.936
PCP-N90	0.459	45.3	0.927

a. m=slope (heterogeneity index); m=0 (heterogenous), m=1 (homogenous)
b. a= measures the binding capacity (N_t) and average binding affinity (K_o); a has a unit of (μmol/g)$(mM)^{-m}$
c. K_{min} (mM^{-1})= $1/F_{max}$; F_{max}=highest free analyte concentration; K_{max} (mM^{-1})=$1/F_{min}$; F_{min}=lowest free analyte concentration
d. N_{min} (μmol/g)=number of binding sites with K_{min}; N_{max} (μmol/g)=number of bindind sites with K_{max}

Table 2. Affinity distribution of PCP-M80 and N80, PCP-M90 and N90 analyzed by FACE technique

Sample	Affinity Distribution			
	K_{min} (mM^{-1})	N_{min} (μmol/g)	K_{max} (mM^{-1})	N_{max} (μmol/g)
PCP-M80	1.59	188	24.7	29.8
PCP-N80	1.27	93.3	17.8	15.5
PCP-M90	1.20	81.1	20.4	21.2
PCP-N90	1.08	36.4	14.1	11.2

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