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Communications

Molecular Recognition by Hydroquinidine-Imprinted Polymers

Jong-Man Kim, Byong-Oh Chong, and Kwang-Duk Ahn*

Division of Polymer Research, Korea Institute of Science and Technology
P.O. Box 131, Cheongryang, Seoul 130-650, Korea
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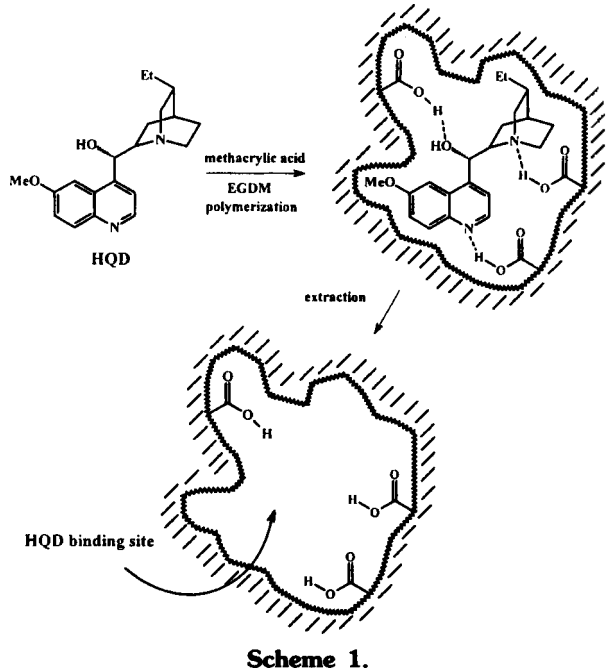
Nature builds three major biopolymers, polypeptides, nucleic acids (DNA, RNA), and polysaccharides to carry out specific cellular functions such as biocatalysis, signal transduction, and information storage. Recently, enormous efforts have driven to develop synthetic polymers which mimic the function of natural biopolymers. Due to the relative ease of handling and avoid of costly production, biomimetic synthetic polymers have gained great attention as natural biopolymer substitutes. Molecular imprinting is one of the areas which take advantage of novel properties from both natural biopolymers and synthetic polymers.[1] Molecular imprinting technique involves polymerization of functional monomers in the presence of the template (print) molecule and cross-linking reagent. After removal of the template molecule, the resultant polymer provides specific binding cavities complementary to the template molecules within the polymer matrices. Application of the molecular imprinting technique ranges widely from polymer catalysts[2] to sensor design,[3] artificial antibodies,[4] and HPLC stationary phase[5] for chiral resolution.

Hydroquinidine (HQD) and its derivatives have been used in the resolution of racemic acids as well as in the preparation of enantiomerically pure or enriched compounds.[6] We have been interested in the design of biomimetic polymers which can be eventually used as tailor-made separation materials for a racemic resolution. As an initial effort, we have investigated molecular imprinting and measured rebinding properties of HQD in highly cross-linked polymers. The molecular imprinting technique used here is based on noncovalent complementary interactions between HQD and polymerizable monomers (Scheme 1). The carboxylic acid group of methacrylic acid (MAA) is expected to form ionic interactions with the amino group and hydrogen bonds with the polar groups of HQD. These interactions would create complementary binding cavities in the polymer after removal of HQD.

Mixing of methacrylic acid (16 mol%) and HQD (4 mol%) followed by AIBN initiated copolymerization with ethylene glycol dimethacrylate (EGDM, 80 mol%) at 65 °C for overnight provided highly cross-linked network polymers. Several different polymerization conditions were employed to provide polymers $\mathbf{P_1}$-$\mathbf{P_6}$ (Table 1). Since these pol-



Scheme 1.

Table 1. Preparation of HQD-imprinted and nonimprinted polymers

Polymer	Template monomer	mol% template	mol% MAA	mol% EGDM	Solvent	Yield
P_1	none	none	20	80	$CHCl_3$	87
P_2	HQD	4	16	80	$CHCl_3$	99
P_3	none	none	20	80	*	100
P_4	HQD	4	16	80	*	94
P_5	none	none	20	80	none	74
P_6	HQD	4	16	80	none	82

*$CHCl_3$: toluene=9 : 1 (v/v)

ymers are highly cross-linked, they are practically insoluble in any solvents. Therefore, we were unable to obtain information on the molecular weights of the polymers. In the case of the polymers P_3 and P_4, toluene was introduced as a porogen. P_1, P_3, and P_5 are prepared as control polymers which do not have the template molecule. In order to obtain the template recognition sites the polymers were ground and extracted with 10% CH_3COOH-MeOH. The amount of the template molecule extracted out was calculated by ^1H NMR analysis. Since the amount of HQD in the polymer matrix before extraction is known, it is relatively straightforward to determine the number of available binding sites after removal of the template. Approximately, 60% of available HQD was removed by the extensive extraction.

Rebinding experiments were performed in chloroform. A typical rebinding experiment is as follows. The required amount of polymers weighed was placed in a 5 mL vial. A solution (2 mL) of $CDCl_3$ containing HQD and benzoin methyl ether (internal standard) was added. The resulting suspension was placed in a shaker overnight at room temperature. After removing the polymers by filtering, the filtrate was analyzed by ^1H NMR spectrometer to determine the amount of HQD remaining in solution. Binding experiments with both the non-imprinted polymers P_1, P_3 and P_5 and the HQD-imprinted polymers without extraction

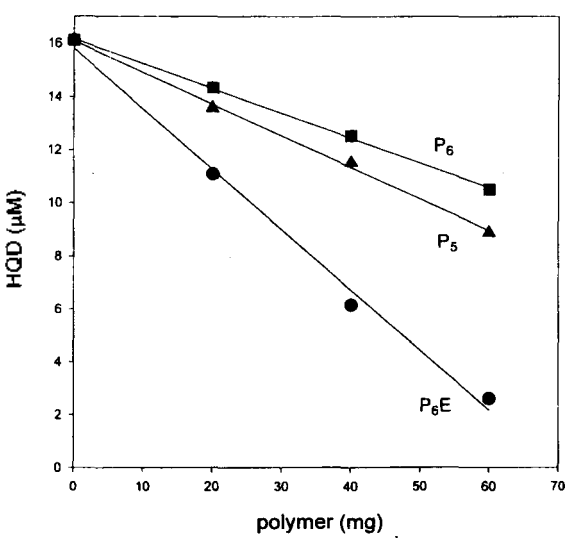
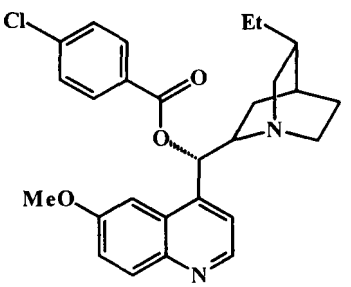


Figure 1. Results of equilibrium binding experiments with HQD: The concentration of HQD in solution as a function of added polymer: P_5 (nonimprinted polymer), P_6 (HQD-imprinted and unextracted polymer), P_6E (HQD-imprinted and extracted polymer).



HQD-CB

were also carried out. Among the polymers tested, polymers P_5 and P_6 prepared in bulk provided the most consistent results.

The results of HQD rebinding experiments obtained with the non-imprinted polymer P_5 and HQD-imprinted polymer P_6 are shown in Figure 1. The vertical axis indicates the concentration of HQD measured in the supernatant and the horizontal axis represents the amount of polymer added. The polymer obtained from P_6 by the extensive extraction with a mixture of 10% CH_3COOH-MeOH is given as P_6E. As the polymers absorb HQD from the solution, the amount of HQD remaining in the solution decreases. The degree of decrease in the concentration of HQD with the imprinted-and extracted polymer P_6E is relatively large compare with those of the control polymers, P_5 and P_6. About 60 mg of P_6E was shown to bind more than 80% of HQD initially present (the concentration of HQD decreases from 16 to 2 μM). Control polymers P_5 (nonimprinted) and P_6 (HQD-imprinted and unextracted) also absorb HQD from the solution. It is believed that the free carboxylic acid groups on the surface of the polymers P_5 and P_6 form hydrogen bonding with HQD. The nonimprinted polymer P_5 shows a slightly larger uptake of HQD than the imprinted-unextraced polymer P_6 under similar conditions. This is presumably because the polymer P_5 has more free carboxylic groups on the surface than the polymer P_6. Since HQD in the polymer P_6 already forms hydrogen bonding with the carboxylic acids, available free acids for nonspecific binding with HQD from the solution is limited.

In order to investigate substrate specificity, binding of hydroquinidine 4-chlorobenzoate (**HQD-CB**), a derivative of HQD, was measured with P_6E. It is expected that addition of phenolic group to HQD would reduce capability of hydrogen bonding with carboxylic group and disrupt the ligand fit in the recognition site in the polymer matrix. In fact, the binding affinity of HQD-CB with P_6E was much less than that by HQD (Figure 2).

In summary, we have prepared several HQD-imprinted polymers and investigated their binding with HQD and HQD-CB. Incubation of HQD with the polymer P_6E resulted in a decrease of HQD in solution with increasing of the amount of polymer. The different binding affinity between HQD and HQD-CB to the polymer P_6E would allow the imprinted polymers to use as useful separation materials in chromatography. Based on the above preliminary results, efforts toward efficient molecularly-imprinted polymeric systems for racemic resolution are currently underway.

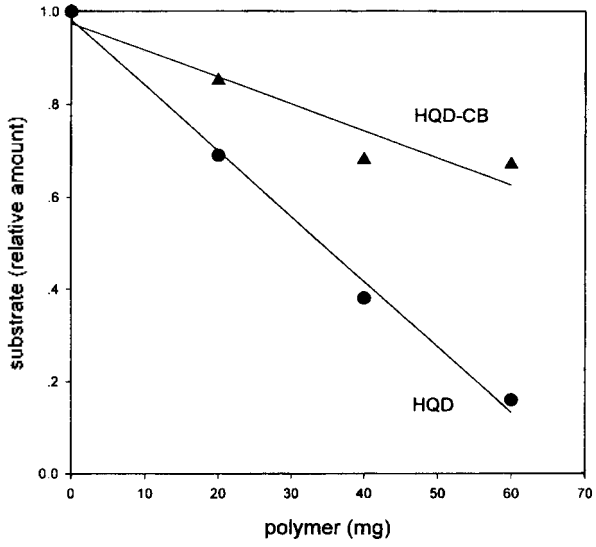


Figure 2. Equilibrium binding experiments with HQD (●) and its structural analog HQD-CB (▲). Substrate remained in solution as a function of the mass of added polymer P_6E is presented.

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New Nozzle System of the Corona Excited Supersonic Expansion

Sang Kuk Lee

Department of Chemistry, College of Natural Sciences, Pusan National University, Pusan 609-735, Korea
Received September 22, 1997

The supersonic free jet expansion of molecules in the gas phase has become an important spectroscopic tool for the observation of rotationally cooled vibronic spectra of large molecules at low temperature.[1-3] Stable molecules having appreciable vapor pressures in the gas mixture of high pressure carrier gas are expanded into the vacuum side through small hole to obtain low rotational temperature and reduced Doppler broadening as well as van der Waals cluster. This technique has been routinely employed for the spectroscopic study of stable molecules and clusters generated from stable molecules. Methods have been also developed for the transient molecules such as radicals and ions in free jet.

Engelking[4] has developed the nozzle system of the corona excited supersonic expansion, in which the transient molecules at the excited state were produced in free jet from stable precursor by electron impact. The nozzle was made by a simple method. A small glass tube was closed down at one end by flame heating and then ground back until the nozzle opening of the appropriate dimension is formed. The metal anode sits just behind the nozzle opening on the high pressure side by 3-5 nozzle diameter, thus allowing expansion after excitation of the molecules. Also, the efficient collisional vibrational cooling at the nozzle throat simplifies the vibronic emission spectrum by reducing the intensity of hot bands originating from the vibrationally excited states of the excited electronic state. Thus, the emission spectrum is similar to the dispersed fluorescence spectra observed by exciting the origin band. This type of nozzle has been widely used for the observation of vibronic emission spectra of stable molecules5 and unstable species.[6-11] However, this substantially deteriorates the stability of discharge when heavy organic compounds were used as precursors. The messy fragments generated from the precursor by electron impact easily clog the small throat of the nozzle. Thus, with this type of nozzle, it was extremely difficult to obtain the vibronic emission spectra of large molecules showing the well-resolved rotational contours.

In the spectroscopic studies of vibronic transition, the rotational constants and symmetry of large molecules at a given state can be determined from the analysis of rotational contour of each vibronic band.[8,12,13] In order to observe the vibronic emission spectrum showing the well-resolved rotational contour, highly stable discharge condition should be maintained in a corona excited supersonic expansion, since the ratio of signal to noise of the spectrum is usually limited by the fluctuation of the discharge current which is also affected by the amount of the gas flow through the throat of nozzle.

Recently, we have improved the stability of the discharge