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Enantioselective sensor based on microgravimetric quartz crystal microbalance with molecularly imprinted polymer film

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We report a novel quartz crystal microbalance sensor that provides enantioselectivity to dansylphenylalanine enantiomers by using a molecularly imprinted polymer film as a recognition element. The polymeric recognition thin film, imprinted with chiral dansyl-L-phenylalanine, was immobilised on a gold electrode modified with a photoactive precursor monolayer *via* a self-assembly process using photopolymerisation. The fabricated sensor was able to discriminate between L- and D-dansylphenylalanine enantiomers in solution owing to the enantioselectivity of the imprinted sites. The enantiomeric composition of L- and D-enantiomeric mixtures could be quantitatively determined by the fabricated sensor. The detection limit is 5 μg mL^{-1} with a response range of 5–500 μg mL^{-1} at pH 10.0. The influence of the template concentration on the sensitivity and selectivity of the synthesised polymer membranes was investigated and optimised. The surface characteristics of the polymer coating were studied by varying the pH value of the buffer solution, and a convenient regeneration process was proposed to increase the reproducibility and reusability of the sensor by flushing with pH 2.0 buffer. The selectivity and recognition mechanism of the imprinted polymer film were studied with compounds that are structurally related to the template. The method presented in this work provides a novel means of preparing highly selective and sensitive chemical sensors *via* self-assembly and molecularly imprinting techniques.

Introduction

Achieving chiral molecular recognition of neutral molecules has become an important subject in the fields of analytical, biochemical and pharmaceutical technologies. To date, several methods have been commonly employed in the pharmaceutical industry for the determination of enantiomeric purity, including circular dichroism,[1] specific rotation,[2] separation techniques such as high-performance liquid and gas chromatography[3,4] as well as capillary electrophoresis.[5] However, they are expensive techniques in terms of reagent consumption and cost of instrumentation. Furthermore, none of these techniques are amenable to real-time analysis. Accordingly, there is considerable interest in the development of simple, rapid and economical methods that will afford the rapid analysis of enantiomeric species. Sensor-based analysis is an alternative method allowing real-time analysis, low cost instrumentation and amenability to automation, while using inexpensive reagents and producing virtually no waste.

Recently, the quartz crystal microbalance (QCM) has received significant attention in many detection needs.[6–13] Based on the piezoelectric theory, the change in resonance frequency of the QCM responds to small variations in mass adsorbed on the electrode. To increase the sensitivity and selectivity of this kind of sensor, chemical sensing films are coated onto the surface of the electrode of the transducing device to enhance the mass change induced by the analyte adsorbed on the electrode. However, the method of preparation of the coated film and the technique to increase the reproducibility of the coated sensor require further investigation. The key to sensor-based analysis of enantiomeric composition is the successful development of materials which can act as enantioselective receptors. In practice, it is quite difficult to find a simple matrix that can distinguish between enantiomers of the same molecular species because a sensor can only rely on a single partitioning or exchange event to generate the selectivity required.

Molecularly imprinted polymers (MIP) have served as practical recognition elements in a wide range of applications requiring selective ligand binding, such as in the areas of selective detection,[14–17] separation and purification.[18–21] In these techniques, complexes between imprinted molecules and functional monomers are allowed to self-assemble in solution and, subsequently, the three-dimensional architecture of these complexes is arrested by polymerisation with a high degree of crosslinking. Following the removal of the template molecules, recognition sites are left in the formed polymer that are complementary in shape and functionality to the imprinted species. The highly crosslinked polymeric nature of such imprinted matrices leads to a strong tolerance towards various external actions, such as acidic, basic and organic treatment. Recently, the combination of QCM and MIP techniques has been applied in selective sensing detection.[13–26] Kugimiya and Takeuchi[27] and Haupt *et al.*[28] have suggested that the coated film be formed by applying a slim quartz disc covering the electrode with MIP solution on its surface. We have also investigated the above-mentioned method. However, in practical operations, it is very difficult to spread the solution thoroughly and evenly when placing the disc onto the electrode surface with the template solution. As the monomer solution is subsequently polymerised in a water bath or under UV radiation, the actual film ultimately formed may not be localised in the centre of the cylinder electrode hub. Thus, the reproducibility and sensitivity of the coated films cannot be guaranteed.

We report here a novel method for preparing enantioselective QCM sensors using synthetic receptors as recognition elements. Our method consists of immobilising a molecularly imprinted polymeric thin film *via* photopolymerisation on the QCM electrode, which was modified with a vinyl group-terminated

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self-assembled monolayer. We imprinted dansyl-L-phenylalanine as the template molecule into mixtures of the crosslinker ethylene glycol dimethacrylate (EDMA) and two functional monomers, methacrylic acid (MAA) and 4-vinylpyridine (4-Vpy). The resulting imprinted polymeric thin film was chemically anchored on the gold electrodes of the QCM and formed as a circular and uniform layer, thus achieving good reproducibility. The influence of the template concentration on the sensitivity and selectivity of the synthesised polymer membranes was investigated and optimised. The surface characteristics of the polymeric film were studied by varying the pH values of the buffer solutions, and a convenient regeneration process was proposed to increase the reproducibility and reusability of the film sensor. The fabricated sensor was able to discriminate between L- and D-dansylphenylalanine enantiomers in solution owing to the enantioselectivity of the imprinted sites, thus allowing the quantitative determination of the enantiomeric composition of L- and D-enantiomeric mixtures.

Experimental

Reagents

Dansylphenylalanine enantiomers, 5-dimethylamino-1-naphthalenesulfonic acid, *N-tert*-boc-L-phenylalanine (boc, butoxycarbonyl), L-phenylalanine, *N*-dansyl-L-tryptophan and *N-tert*-boc-L-tryptophan were obtained from Sigma (St. Louis, MO, USA). Methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA), 4-vinylpyridine (4-vpy) and 2,2'-azobisisobutyronitrile (AIBN) were obtained from Aldrich (Milwaukee, WI, USA). All the monomers were distilled before use. Glycidyl methacrylate (GMA) was purchased from Fluka (Buchs, Switzerland). Thioctic acid-modified GMA and thioctic acid dodecane esters were synthesised in our laboratory and were characterised with MS and NMR. All buffer solutions were prepared with deionised water. The molecular structures of dansylphenylalanine and the other amino acids are illustrated in Fig. 5.

Equipment

The quartz crystals used were commercially available 10 MHz, AT-cut type (diameter, 13.67 mm), with polished electrodes (diameter, 5.1 mm) on both sides, and consisted of 1000 Å Au with a 50 Å Cr underlayer (International Crystal Manufacturing Co. Inc., Oklahoma City, OK, USA). The frequency changes were measured by a PZ-1001 Immuno-Biosensor system (Universal Sensors, Inc., LA, USA) attached to an AST computer.

The QCM device was placed in a home-built detection cell with one electrode exposed to a sample volume of 1.0 mL. The detection cell used for mass-sensitive measurements was temperature controlled (22 ± 0.1 °C). When contacted with the solution, the frequency response was stable within ±1.0 Hz over periods of 20 or 30 min.

Preparation of sensors

The cleaned crystal was immersed into an ethanol solution containing 0.001 M thioctic acid-modified GMA and 0.001 M thioctic acid dodecane ester for 1 h so as to introduce vinyl groups onto the gold electrode of the QCM. After the self-assembly process, this layer containing terminal vinyl groups could act as an anchor in vertically orienting the surface of the gold electrode for further polymerisation of MIP. Subsequently, the crystal was rinsed thoroughly with ethanol and then deionised water. After drying, the QCM was inserted into the detection cell.

A series of solutions of dansyl-L-phenylalanine was prepared at different concentrations in acetonitrile. MIP-1–7 contained the template molecule at 0.082, 0.164, 0.246, 0.328, 0.410, 0.492 and 0.574 mmol, respectively. Taking MIP-2 (the second one in the solution series) as an example, the procedure was as follows. To a solution of 56.4 mg (0.164 mmol) of dansyl-L-phenylalanine (template) in 2.0 mL of acetonitrile were added 69.0 mg (0.656 mmol) 4-vpy, 56.5 mg (0.656 mmol) MAA, 1.305 g (6.56 mmol) EDMA and 15 mg AIBN. An aliquot (5 μL) of this mixture was pipetted and dropped onto the surface of the electrode. Pure nitrogen gas was purged into the cell for 5 min to evacuate the air completely since the presence of oxygen would prohibit polymerisation. A slim quartz slide was subsequently placed over the inlet to the cell to maintain a nitrogen environment. Polymerisation was carried out at room temperature under UV light irradiation at 350 nm for 12 h. Removal of the template and the unpolymerised monomers was realised either by sonicating the QCM crystal in 80% ethanol solution for 2 min or simply by flushing with 0.01 M HCl solution for about 30 min. For the control experiment, a quartz crystal was also coated in a similar way without the addition of the template to the monomer solution ('blank' solution). The coated QCM was then inserted into the detection cell with only one side in contact with the buffer solution.

Buffer solution

Sodium phosphate (10 mM, pH 7.1) and a mixture of NaOH and HAc with varying pH values (from pH 2 to pH 12) were used as the background solutions for the sensor measurements.

Results and discussion

Reproducibility of the film coating procedure

A set of data on the frequency changes of QCM crystals was obtained to study the reproducibility of the MIP coating preparations. The initial frequency (F_i) was recorded when the unmodified QCM was ready for use. After the thioctic acid-modified GMA and thioctic acid dodecane ester monolayer coating process, the frequency (F_c) was measured. When the polymerisation was finished, the frequency (F_m) of the MIP-coated QCM was recorded. The final frequency (F_f) was obtained after removing the template and unreacted monomers. For comparison, all of these measurements were conducted in the presence of the same concentration of coating monomer solution. The ΔF_{i-c} value between F_i and F_c reflected the mass change brought about by the monolayer, while the ΔF_{i-f} value between F_f and F_i showed the mass change caused by the MIP coating material. Six crystals with the same amount of coating material of MIP-2 were tested. The mean ΔF_{i-c} value was 311 Hz, with a relative standard deviation (RSD) of 1.9%, and the mean ΔF_{i-f} value was 6566 Hz, with an RSD of 0.28% (Table 1). The two RSD values suggested good reproducibility of both the self-assembly and imprinting processes.

Table 1 The frequency change of the quartz crystal due to the precursor monolayer and the molecularly imprinted polymer film

Crystal	1	2	3	4	5	6	Mean	RSD (%)
ΔF_{i-c}/Hz	308	319	319	317	304	307	311	1.9
ΔF_{i-f}/Hz	6540	6576	6551	6593	6571	6567	6566	0.28

Influence of template concentration on the sensor performance

The sensitivity of the chemical sensor is mainly determined by the receptor sites on the transducer surface. Therefore, the optimum conditions for the production of the coated sensor are of significant importance in increasing its sensitivity. In the imprinted polymer film, the receptor sites are determined by the amount of template molecule. In order to study the effect of the concentration of the template on the sensitivity, six series of detection experiments against the template molecule dansyl-L-phenylalanine were performed with QCM sensors coated with different MIP films prepared with different template concentrations (Table 1). The sensor response to the analyte is illustrated in Fig. 1. As can be seen, the frequency shift does not necessarily show a direct correlation with the concentration of template in the monomer mixture. From the graph, a rough correlation for the first four points reveals that, as more template was added to the monomeric solution, more complexes between the template and polymer were formed, which subsequently resulted in more cavities left after 'washing off' the template, thus increasing the frequency shift due to the increase in template concentration. However, a higher percentage of the imprinted molecule in the polymerisation solution results in the inclusion of template clusters, causing a broadening of the size distribution of the cavities and a reduction in the sensitivity and selectivity of the polymeric films. The results in Fig. 1 suggest that the monomeric solution MIP-4, with 0.656 mmol 4-vpy, 0.656 mmol MAA, 6.56 mmol EDMA and 0.328 mmol template, seems to be a good compromise. Therefore we used this ratio in all experiments to prepare the recognition films on the QCM substrate. Since the process was conducted in the liquid phase, two different buffers (containing pH 10 NaOH–HAc and pH 7.1 sodium phosphate, respectively) were used to study the possible influence of the solvent effect. The graph indicated that the response of the QCM to the two buffers showed little difference, suggesting that the differences in viscosity and density between the two buffers have no significant effect on the frequency change.

Effect of buffer pH

In the present work, MAA and 4-vpy were chosen as the functional monomers. In a previous study,[29] it has been suggested that, in the aqueous phase, the carboxyl group of the amino acid participates in a leading interaction with pyridinyl sites on the polymer, while the role of the dimethylamino group is to provide a secondary co-operative interaction with acid sites on the polymer. As the binding ability of the sensor is mainly due to binding between the carboxyl group and pyridinyl sites, we believe that the pH value of the detection medium plays an important role in the sensor response. The sensor responses of three QCM (coated with the MIP-4 film, non-imprinted film and bare electrode, respectively) to 500 μg mL^{-1} of dansyl-L-phenylalanine at different pH values are shown in Fig. 2. It can be seen that, with increasing pH, the response for the bare electrode remains at nearly the same value, suggesting that the pH has no significant effect on the adsorption ability of the uncoated QCM. For the non-imprinted QCM, there is a slight increase in frequency response with increasing pH from 2.0 to 6.0, but it remains steady at higher pH values. At pH > 4.0, the frequency change for the imprinted QCM is much larger than that for either the non-imprinted QCM or the QCM with a bare electrode. This fact suggests that the change in pH value will not play an important role in the absence of the template molecule, which may significantly affect the surface properties of the QCM. For the imprinted QCM, when the pH value of the detection medium is lower than 3.0, the sensor response is very low, implying that the binding of the analyte molecule to the imprinted polymer film is relatively weak. With decreasing pH, an increase in the ionisation of the pyridinyl residues in the polymer may occur, thus lowering the possibility of interactions between the pyridinyl sites and the carboxyl groups and therefore reducing the binding ability. The pH value may also have an effect on interactions between dimethylamino groups and the acid sites provided by MAA in the imprinted cavities; a decreasing pH value may result in an ion exchange process which reduces the binding ability. It is revealed from the graph that, with an increase in the pH value of the detection buffer, the sensor response shows an abrupt change at around pH 4.0, and remains at a relatively high level up to a pH value of about 10.0. For the purpose of obtaining a large sensor response, all measurements of sensor response and selectivity were conducted in pH 10.0 buffer solution unless otherwise stated.

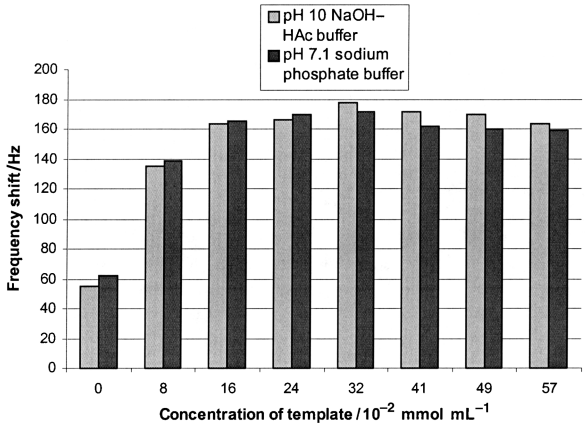

Fig. 1 Effect of template concentration in monomer formulation on sensor response. Frequency was recorded by exposure of QCM coated with different types of imprinted polymers to two buffer solutions containing 500 μg mL^{-1} of dansyl-L-phenylalanine solution.

Response time and coating regeneration

In order to obtain highly specific imprinted polymers, the formation of stable complexes between templates and their functional monomers in the reaction mixture and the preservation of these complexes in the resulting polymers are crucial. To that end, covalent bonds have most often been used for the selective cavities. However, for fast and reversible binding, the activation energies of covalent binding are often too high. Therefore, we have used 4-vpy and MAA as functional monomers to promote hydrogen bonding with the template molecule in order to obtain good selectivity and reversibility of the sensor response. The response time may be affected either by the concentration of the template or by the working buffer. Fig. 3 shows a typical temporal course of the response of the QCM sensor modified with a MIP-4 layer in aqueous buffer solution (10 mM sodium phosphate, pH 7.1). It is shown that the

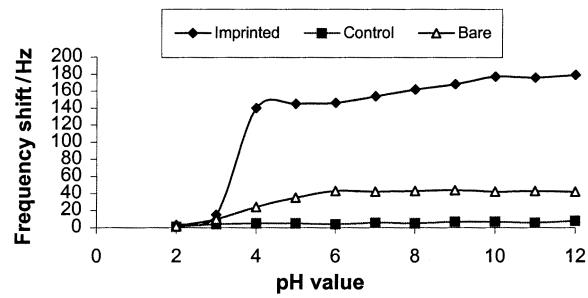

Fig. 2 Influence of pH value on the sensor response of three QCM (coated with MIP-4 film, non-imprinted film and bare electrode) to 500 μg mL^{-1} analyte (the pH value was adjusted with NaOH–HAc).

L-MIP binds the L-enantiomer more strongly than the D-enantiomer. The L-MIP coating also exhibits different binding and desorption kinetics for the L-enantiomer and D-enantiomer. The experimental results also reveal that the concentration of the template does not significantly affect the time delay. However, when using different background buffers, we found that the response time varied. The average response time in the detection medium of 0.01 M sodium phosphate (pH 7.1) is nearly three-fold higher than that in 0.01 M NaOH–HAc buffer solution (pH 10). This phenomenon could be due to the fact that the higher pH value contributes more to the ionic interaction and electrostatic force between the polymer and the analyte.

The regeneration of the coated QCM is of critical importance for the application of the sensing system. Various methods have been proposed, such as sonicating and rinsing with organic solvents to wash off the analyte and to recover the adsorbing ability of the MIP film. However, these methods are time-consuming and difficult to perform. As shown above, the binding of the analyte to the imprinted polymer film is very weak when the pH value is below 3.0 (Fig. 2). Therefore, acids with low pH values are expected to facilitate breaking of the hydrogen bond between the pyridinyl groups on the polymer coating and the amino acid. In the present study, we utilised 0.01 M HCl as the flushing solvent in the detection cell for regeneration. The frequency change during the process of flushing was recorded. Once all the analyte has been completely washed off, the frequency of the QCM in the detection medium should be nearly the same as that before adsorption. Our study reveals that the present treatment allows the sensor to be repeatedly used with good reproducibility. The usual time for the flushing process was 20–30 min. The reversibility was calculated by comparing the frequency changes obtained using the original and regenerated QCM to detect the same concentration of analyte. The regenerated QCM sensors typically recovered 97–102% of the adsorbing ability of the original QCM sensors.

Sorption characteristics of the enantioselective sensors

The sorption characteristics of the developed chemical sensors were investigated by testing a series of concentrations of enantiomers ranging from 5 to 500 μg mL^{-1}. Fig. 4 shows the calibration curves for the adsorption of L- and D-dansylphenylalanine enantiomers to the MIP-4-coated QCM. As can be seen, the signal obtained with the binding of template L-dansylphenylalanine to the imprinted membrane is about three-fold larger than that obtained with the D-dansylphenylalanine enantiomer. The sensor response increases when the analyte concentration increases. However, at concentrations of 400 μg mL^{-1}, the binding seems to approach a saturation condition. In the calibration curve obtained, the initial slope change for dansyl-L-phenylalanine is 2 Hz (μg $mL)^{-1}$, while the slope change for dansyl-D-phenylalanine is 0.3 Hz (μg $mL)^{-1}$. The enantiomeric selectivity coefficient of the fabricated sensor is 6.7. The detection limit of the L-analyte was also calculated to be 5 μg mL^{-1} from the calibration curve.

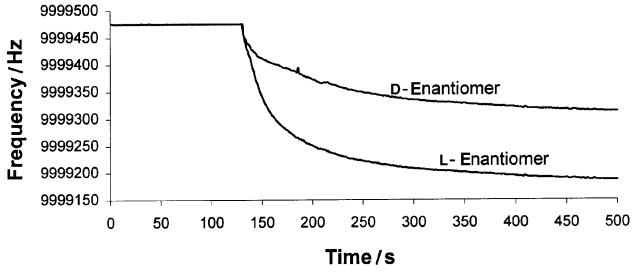

Fig. 3 Frequency change of the QCM coated with dansyl-L-phenylalanine imprinted polymer film in 500 μg mL^{-1} L- and D-enantiomer.

In control experiments, the sensor response of a crystal coated with the non-imprinted polymer film to the L- and D-dansylphenylalanine enantiomers was studied. As shown in Fig. 4, the binding of L- and D-dansylphenylalanine enantiomers on the control polymer is almost the same, which indicates no enantioselectivity of the control polymer film. The low sensor response is attributed to the non-specific binding caused by the randomly distributed carboxyl groups and pyridinyl groups.

To obtain a better understanding of the nature of the interactions between the specific binding sites of the MIP and its target molecules, and to determine the ability of this polymer to recognise the imprinted molecules, adsorption of other functionalised amino acids that have similar structures to dansylphenylalanine on the L-MIP-coated QCM was investigated. The sensor responses of the QCM with MIP-4 to these analytes are shown in Fig. 5. As illustrated in the figure, the imprinted polymer film shows the highest selectivity to the imprint molecule itself, indicating that the polymer has a better inclusion with the imprint molecule. The response for *N-tert*-boc-L-phenylalanine is the second highest. This difference may be due to the existence of the dansyl group, 5-dimethylamino-1-naphthalenesulfonic acid, which may play a steric role in the recognition process. Since the mass of the dansyl group alone is not very large, the response for the pure dansyl group (numbered 2 in the graph) is not very high. The response for l-phenylalanine is a little larger than that for the dansyl group. This is consistent with the mechanism suggested here: non-covalent interaction occurs between the recognition sites and the two functional groups of l-dansylphenylalanine. *N*-Dansyl-L-tryptophan provides both dansyl group and the same binding functional groups as that of the template molecule. However, the lower response can also be explained by the steric effect. The response for *N-tert*-boc-l-tryptophan is the lowest, which reveals that the two different groups from the template molecule result in a larger steric hindrance. It is therefore suggested that the recognition ability depends both on the ionic interaction between cavity sites and functional groups and the steric structure. In addition, non-specific binding between the different analytes and the randomly distributed pyridinyl groups and carboxyl groups may also exist. However, this contributes least to the stereoselectivity. The experimental results indicate that the application of an imprinted polymer as a chiral discrimination film, combined with a QCM as transducer, may allow the detection of the analyte enantiomeric composition from a complex matrix without separation.

Determination of enantiomeric purity/composition

After having successfully demonstrated the enantioselectivity of the MIP-coated QCM sensors, we studied further the possibility of using the fabricated chiral QCM sensor to determine the enantiomeric composition of dansyl-phenylalanine. For this purpose, the MIP-coated QCM was exposed to mixtures of different contents of dansyl-phenylalanine enantio-

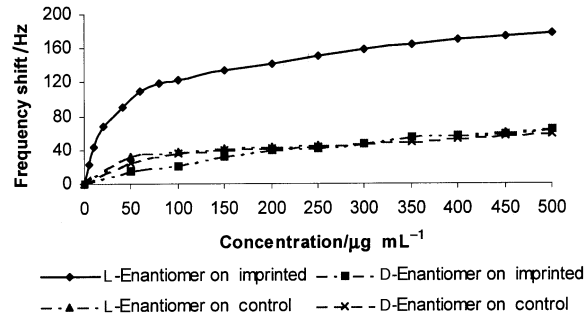

Fig. 4 Sorption characteristics of dansyl-L-phenylalanine and dansyl-D-phenylalanine on L-enantiomer imprinted polymer and control polymer film. Detection medium: 0.01 M pH 7.1 sodium phosphate buffer solution.

mers. These mixtures were prepared by mixing the two enantiomers to the desired values. From Fig. 6, it can be seen that the enantiomeric composition of mixtures of dansyl-phenylalanine can be determined only from the frequency changes. After several repetitions of the experiment, the results reveal a good linearity. We suggest that a faster and less tedious method could be employed involving a single frequency change measurement of the sample if the total analyte concentration is available.

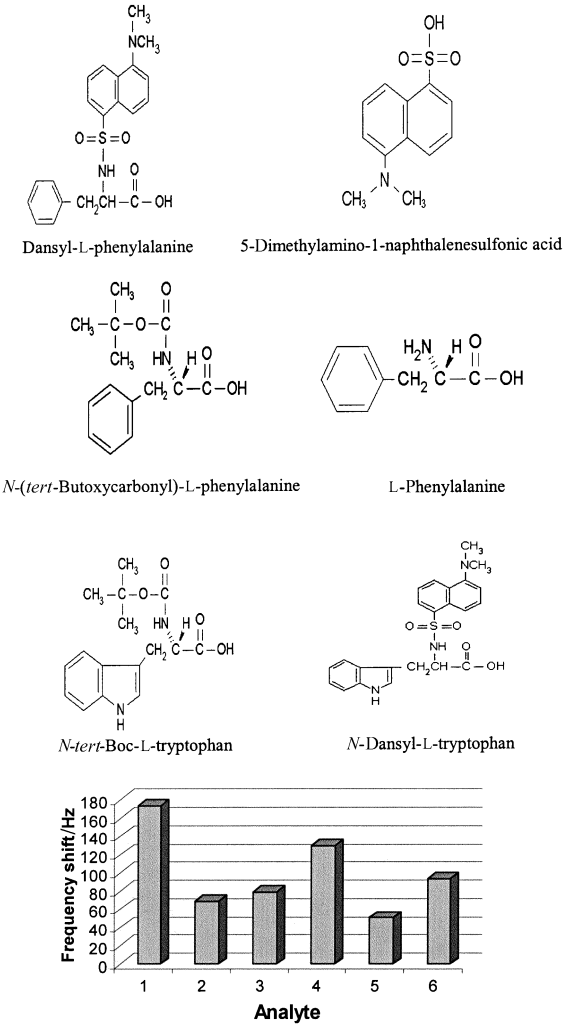

Fig. 5 Selectivity of QCM sensor coated with dansyl-L-phenylalanine imprinted polymer film. Analyte concentration: 500 μg mL^{-1}. 1, Dansyl-L-phenylalanine; 2, 5-dimethylamino-1-naphthalenesulfonic acid; 3, L-phenylalanine; 4, *N*-*tert*-boc-L-phenylalanine; 5, *N*-*tert*-boc-L-tryptophan; 6, *N*-dansyl-L-tryptophan. Structures of dansylphenylalanine enantiomers and the five other analytes are given.

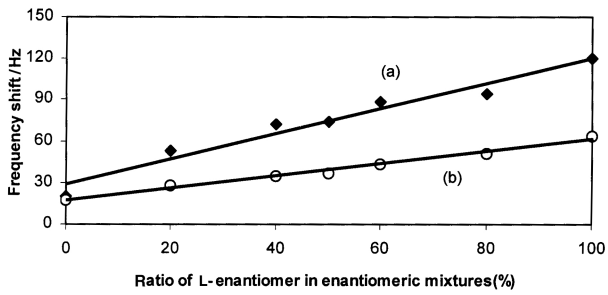

Fig. 6 Variation of frequency change of imprinted polymer-coated QCM sensor with enantiomeric composition of dansyl-L-phenylalanine and dansyl-D-phenylalanine at different concentrations: (a) 100 μg mL^{-1}; (b) 50 μg mL^{-1}

Conclusions

We have described a quartz crystal chemical sensor for the detection of enantiomeric composition using artificial recognition films prepared *via* molecularly imprinting technology. The MIP thin layers immobilised on the QCM electrodes by copolymerisation with a photoactive precursor film containing vinyl groups are insoluble, chemically resistive and highly selective, and are perfect for application in liquid media. These results demonstrate the promising feature of MIP in sensing devices. Compared to conventional methods used in enantiomeric analysis, the MIP-coated QCM sensor enjoys the advantages of *in situ* operation and simplicity. These artificial recognition systems in sensor technology provide an opportunity for the development of other acoustic wave sensors exhibiting both selectivity and stability.

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