Asian Journal of Chemistry; Vol. 27, No. 12 (2015), 4369-4372

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.19124

Molecular Imprinted Polysiloxanes for Selective Removal of Leucomalachite Green

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Received: 21 February 2015; *Accepted*: 28 April 2015; *Published online*: 29 August 2015; AJC-17473

Leucomalachite green sensitive molecular imprinted polysiloxanes were synthesized by sol-gel polymerization. The imprinting of leucomalachite green in polymer matrix was investigated by FTIR studies which indicate the presence of leucomalachite green characteristic peaks. Washing imprinted polysiloxanes with deionized water leads to the removal of leucomalachite green thus, developing adapted cavities for reversible re-inclusion of analyte molecules. The recognition properties of imprinted polysiloxanes were evaluated by their rebinding affinity towards leucomalachite green. In rebinding studies, imprinted polysiloxanes showed significant binding affinity for leucomalachite green *i.e.* tested in the range of 0.04 to 0.1 mmol whereas the non-imprinted polysiloxanes exhibited much lower binding. Imprinted polysiloxanes were highly selective for leucomalachite green recognition as they showed five-fold higher binding response when compared with structurally related methyl violet dye. Finally, their reusability was evaluated which indicated that the rebinding efficiency of regenerated polysiloxanes was more than 80 % comparing to freshly prepared polymer suggesting them highly cost effective and could be used as potential recognition matrix for leucomalachite green removal from environmental samples.

Keywords: Leucomalachite green, Molecular imprinting, Polysiloxanes, Methyl violet.

INTRODUCTION

Living organisms have natural mechanism of molecular recognition found in variety of biological processes, including antibody/antigen interactions in immune system. Molecular imprinting[1] is a synthetic approach for generating artificial recognition sites in polymer matrix. Molecular imprinted polymers (MIPs) are synthesized by self-organization of pre-polymer chains around the template molecules. After curing of polymer, the template molecules are removed which results in the formation of analyte analogous adapted cavities in polymer matrix. The imprinted pockets can reversibly reincorporate the target analytes with complementary geometrical and chemical matching. The imprinted polymers can be tailored for almost all types of analytes, *e.g.* proteins[1], microorganisms[2], viruses[3] and neutral to charge-bearing species[4]. Molecular imprinted polymers have several advantages as compared to biological recognition systems including easy preparation, low cost, excellent stability and reusability without losing activity, durability and applicability in harsh environments. As an alternative to biological recognition systems, molecular imprinting has become an attractive tool for various applications *e.g.* for sensor coatings[5], solid phase extraction[6], drug delivery[7], environmental analysis[8] and clinical diagnosis[9].

Leucomalachite green (LMG) is a biotransformation[10] product of malachite green (MG) as shown in Fig. 1 largely utilizes against parasitic, fungal and bacterial infections in fish and fish eggs. On the other hand due to its potential carcinogenicity and mutagenity it is not registered in European Union[11] as veterinary medicine and therefore, banned in many countries. The use of malachite green produces environmental pollution and contaminates food chain which ultimately affects human health as the fish readily absorb malachite green into its tissues where it is rapidly metabolized into reduced form which is colourless. In literature, it has been reported that nearly 90 % of the total malachite green in fish is present in the form of leucomalachite green [12]. The high quantity of leucomalachite green in the aquatic food stuff seriously alarming for human health because structurally analogous compounds like triphenylmethane dyes cause cancers. Toxicological effect of leucomalachite green demands a reliable analyzing methodology in this regard. Various analytical techniques and procedures have been adopted for identification and quantification of leucomalachite green which includes visible light detection with

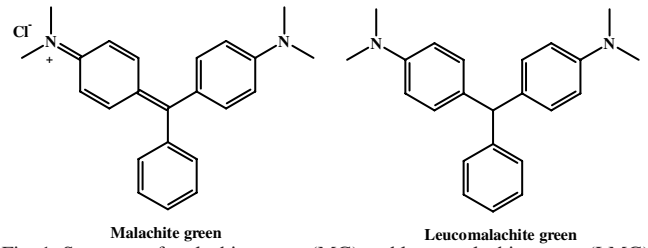

Fig. 1. Structure of malachite green (MG) and leucomalachite green (LMG)

oxidation of leucomalachite green, mass spectrometry[13,14] and electrochemical detection[15]. However, efficient separation of leucomalachite green through membrane filtration[16] or by sorption[17] is more desirable in treating environmental samples. Nonetheless, sorption is a more direct and straightforward method for removal of toxic compounds. Combining sorption with molecular imprinting could be a reliable means of selective recognition of leucomalachite green and its subsequent separation. In the present study, molecular imprinted polymers are synthesized by sol-gel method for selective sorption of leucomalachite green. The results obtained from the rebinding and reusability tests of molecular imprinted polymers strongly support that imprinted polysiloxanes are highly efficient for leucomalachite green recognition and can be used in treating effluent samples.

EXPERIMENTAL

Leucomalachite green (LMG), methyl violet and tetraethyl orthosilicate (TEOS) were obtained from Sigma-Aldrich. All the solvents and other chemicals were used as received without any further treatment. For characterization and rebinding studies different instruments were used including Shimadzu IR Prestige-21 FTIR, SPECTROstar Nano UV/visible spectrometer, Retsch Lab ultrasonic bath and XiangYi instruments centrifuge machine.

Synthesis of imprinted polysiloxanes: For preparing leucomalachite green-imprinted polysiloxanes, the reaction was initiated by the hydrolysis of tetraethyl orthosilicate (TEOS) using aqueous ammonia in ethanolic media. 20 mL of polymer precursor was mixed with 25 mL of ethanol in round bottom flask and 0.75 mmol of leucomalachite green was added into this mixture under constant stirring at 60 °C for 0.5 h. Aqueous ammonia solution was added drop wise to above mixture until precipitation completed. Reaction contents were left over for 2 h and then centrifuged at a speed of 5000 rpm for 10 min to separate imprinted sol-gel particles from the solution. The resulted precipitates were thoroughly washed with excess of deionized water to remove template and reaction impurities. The non-imprinted polysiloxanes were synthesized in exactly the same manner without adding template.

Characterization of imprinted polysiloxanes: The imprinting of leucomalachite green in polysiloxanes was investigated by FTIR before and after template removal to observe the structural changes as a result of polymer-template interactions. The supernatant of washed particles was analyzed by UV/visible to ensure complete removal of template dye.

Rebinding studies: In rebinding experiments, a stock solution of leucomalachite green was prepared from which further dilutions (from 0.1 mmol to 0.04 mmol) were made. Rebinding test for leucomalachite green was performed by taking 50 mg of imprinted particles and then adding 20 mL of standard solution of leucomalachite green in 100 mL round bottom flask. The mixture was subjected to constant stirring for 0.5 h in closed flask to avoid solvent evaporation. After that imprinted particles were separated by centrifugation at 5000 rpm and the supernatant was analyzed by UV/visible. The shift in absorbance of this solution relative to standard leucomalachite green solution of given concentration was noted. The concentration of leucomalachite green dye in solution after molecular imprinted polymer treatment was calculated. Same procedure was followed for monitoring rebinding of non-imprinted polymer with leucomalachite green.

Selectivity studies were made by exposing equimolar concentration of structurally analogous dye *i.e.* methyl violet to imprinted polysiloxanes following the same practice as mentioned above. The rebinding efficacy of regenerated molecular imprinted polymers was assessed by comparing results of regenerated polysiloxanes with freshly prepared particles. The regeneration of imprinted polysiloxanes was made by through washing of particles and then drying them in oven at 100 °C for next rounds of rebinding.

RESULTS AND DISCUSSION

FTIR characterization of imprinted polysiloxanes: FTIR spectra of leucomalachite green-imprinted polysiloxanes before and after washing were studied to investigate the imprinting effects. In Fig. 2(a) FTIR spectrum of unwashed imprinted polysiloxanes (as prepared) showed transmittance peaks around 1083.99 cm^{-1} and at 802.39 cm^{-1} representing Si–O–Si and Si–O stretching vibrations respectively which confirm the formation of sol-gel matrix.

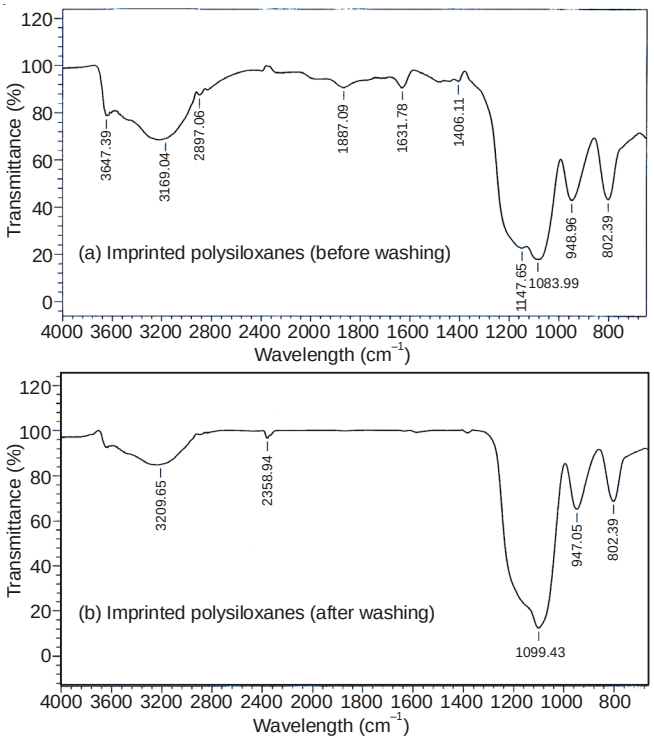

Fig. 2. FTIR spectra of imprinted polysiloxanes (a) before washing (b) after washing or template removal

While bands at 1631.78 cm^{-1} indicates about –C=C– stretching vibrations, 2897.08 cm^{-1} for C–H stretching and relatively broad band of 3169.04 cm^{-1} represents surface hydroxyl groups in polysiloxanes. The characteristic peaks of template functional groups in Fig. 2(a) represent that leucomalachite green is present in polysiloxanes matrix and therefore, successfully imprinted. After through washing of imprinted polymer with deionized water leads to removal of leucomalachite green and it can be observed as the absence of leucomalachite green characteristics peaks in Fig. 2(b). The structural features of imprinted polysiloxanes in view of Si–O–Si and Si–O stretching vibrations are similar before and after template washing.

Rebinding studies: For rebinding studies, imprinted polysiloxanes were subjected to successive washings with deionized water to ensure the complete removal of leucomalachite green from the polymer network. The thoroughly washed and dried imprinted particles of known weight were exposed to different concentrations of leucomalachite green standard solution and corresponding shift in absorbance was determined after treating with imprinted material. The range of concentrations set for rebinding studies was from 0.04 to 0.1 mmol. It can be seen from Table-1 that absorbance of leucomalachite green solution after treating with imprinted polysiloxanes decreases that indicates the adsorption of leucomalachite green in imprinted cavities of polymer network. The change in absorbance increases as the initial concentration of leucomalachite green standard solution increases.

TABLE-1
CHANGE IN ABSORBANCE OF LEUCOMALACHITE GREEN SOLUTIONS BEFORE AND AFTER TREATING WITH IMPRINTED POLYSILOXANES

S. No.	Leucomalachite green concentration (mmol)	Absorbance before treating with imprinted polysiloxanes	Absorbance after treating with imprinted polysiloxanes	Shift in absorbance
1	0.04	1.399	1.333	0.066
2	0.05	1.662	1.555	0.107
3	0.06	1.983	1.797	0.186
4	0.08	2.364	2.159	0.205
5	0.09	2.800	2.425	0.375
6	0.10	3.443	2.914	0.529

The concentrations of leucomalachite green solutions after treatment with imprinted polymer were determined from corresponding change in their absorbance values. The recognition properties of imprinted polysiloxanes can be evaluated by calculating equilibrium adsorption capacity as expressed in following equation.

$$Q = (C_i - C_f)\ V/m$$

In this equation 'C_i' and 'C_f' are initial and final concentrations respectively while 'V' is the volume of analyte solution in mL and 'm' is the mass of imprinted particles taken in gm. A graph was plotted between C_i and Q to examine the binding affinity of imprinted polymer with the increasing concentration of leucomalachite green as shown in Fig. 3. It is clearly indicated that recognition ability of molecular imprinted polysiloxanes

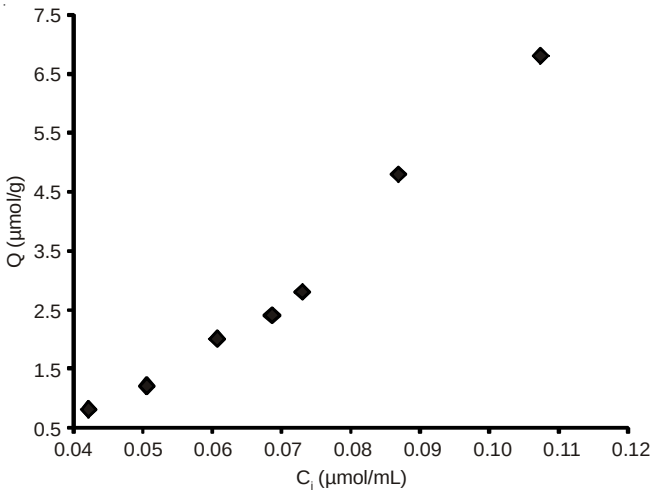

Fig. 3. Representation of equilibrium adsorption capacity for leucomalachite green imprinted polysiloxanes

increases with the increasing concentration of leucomalachite green.

Non-imprinted polymer was also treated with 0.1 mmol leucomalachite green solution and the resultant change in absorbance was calculated. The comparison of shift in absorbance for imprinted and non-imprinted polysiloxanes was made. It was observed that change in absorbance for non-imprinted polysiloxanes was 0.03 whereas for imprinted it was 0.529. This means that non-imprinted polymer has negligible binding for leucomalachite green and thus, can be taken as control material to compensate non-specific binding interactions.

Selectivity assessment: Selectivity of leucomalachite green-imprinted polysiloxanes was evaluated against a structurally related compound *i.e.* methyl violet. For selectivity studies, equal weights of imprinted particles were exposed to equimolar concentrations *i.e.* 0.05 mmol of leucomalachite green and methyl violet separately. The respective change in absorbance for leucomalachite green and methyl violet solutions were recorded and compared as shown in Fig. 4. The change in absorbance for leucomalachite green solution was 0.107 whereas for methyl violet it was 0.021 which indicates that the binding response of imprinted polysiloxanes for leucomalachite green is about 5 times higher than methyl violet despite of the fact that both compounds have similar structure. Such high selectivity is attributed to molecular imprinting effect as the cavities in imprinted polysiloxanes are tailored for leucomalachite green which was used as template therefore; polymer preferentially binds to leucomalachite green as compare to methyl violet.

Reusability of imprinted polysiloxanes: Imprinted polysiloxanes used for rebinding of leucomalachite green were regenerated through successive washings with deionized water in order to remove previously adsorbed template. The regenerated polymer particles were tested for rebinding of leucomalachite green solution of 0.05 mmol concentration and compared with rebinding results of freshly imprinted polysiloxanes. It was observed that the rebinding efficiency of regenerated polysiloxanes was more than 80 % comparing to fresh imprinted polysiloxanes thus, suggests them suitable for subsequent analyte rebinding experiments as it did not lost recognition properties on regeneration.

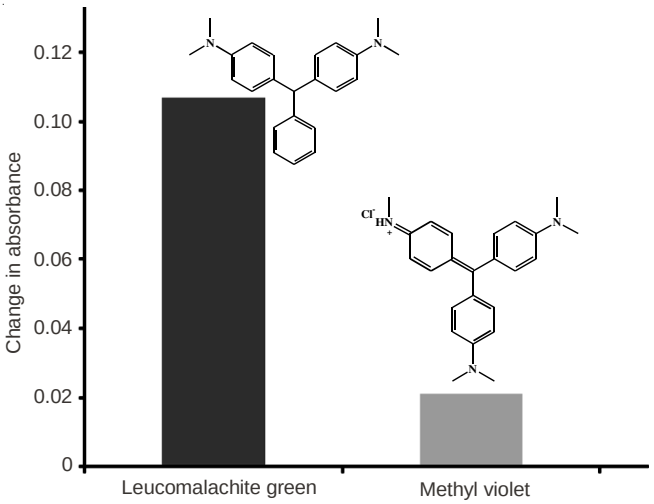

Fig. 4. Relative change in absorbance for leucomalachite green and methyl violet solutions when exposed to leucomalachite green-imprinted polysiloxanes separately

Conclusion

Molecular imprinted polysiloxanes had shown considerable potential for selective adsorption of leucomalachite green. The synthesis procedure is straightforward and template removal requires milder conditions. Imprinting character of as-prepared polymer can be accessed by FTIR. Imprinted polysiloxanes are highly sensitive and selective towards template molecules *i.e.* leucomalachite green when compared to other structurally related species. Non-imprinted material can be taken as control to evaluate non-specific template polymer binding. Finally, imprinted polysiloxanes can be regenerated and reused for subsequent binding of template which suggests that this material can be considered as a suitable recognition matrix to deal with environmental samples containing leucomalachite green.

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