Removal of Triolein Lipid From Aqueous System by Molecularly Imprinted Chitosan and Its Derivative

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ABSTRACT

Chitosan was molecularly imprinted to remove triolein (a model lipid triacylglyceride) from water. Molecularly-imprinted chitosan (chitosan-MIP) was synthesized by crosslinking it with glutaraldehyde in the presence of triolein as the template at 50°C for 2h. MIPs of octanoyl derivative of chitosan(Oct-MIP) were also prepared by similar method. Octanoyl chitosan was synthesized by N-acylation of chitosan using octanoyl chloride at room temperature for 12h. Contact angle measurements of water droplet on chitosan and octanoyl chitosan revealed increased hydrophobicity of octanoyl derivative of chitosan. FTIR spectroscopy was used to characterize the derivative and the MIPs. All the synthesized polymers. Oct-MIP and chitosan-MIP could imprint approximately 77% and 66% of triolein template, respectively. Binding experiments revealed that Oct-MIP exhibit higher triolein binding capacity than corresponding non-acylated polymers. In 6h, 1mg of Oct-MIP and chitosan-MIP could rebind 534.50μg and 380.35μg of triolein respectively. Non Imprinted octanoyl chitosan and chitosan bound 272μg and 198.24μg triolein respectively. Both types of MIPs could also bind a triolein analog (1,3-dioleoyl-2-palmitoyl glycerol).

Keywords: chitosan; N-acylated chitosan; molecularly imprinted polymer; triolein; lipid pollution

INTRODUCTION

Lipids are organic pollutants mainly from slaughterhouses and meat processing facilities are present at significant concentrations in wastewater. When they are present in significant quantity, they diminish the amount of dissolved oxygen in water as they need oxygen during degradation (Salam *et al.*, 2012; Campo *et al.*, 2012). Further, lipid films and emulsified lipids may interact with debris and settle to the bottom of the water body (Crump-Wiesner *et al.*, 1975). Depleted amount of dissolved oxygen has been shown to cause disruption of the aquatic ecosystem (Bucas *et al.*, 2002; Christie *et al.*, 1977; Russell *et al.*, 1978). Use of hydrolytic enzymes (Cammarota *et al.*, 2006; El-Gawad 2014), aerobic

and anaerobic biodegradation (Chipasa *et al.* 2006; Jensen *et al.*, 2015) have been identified as viable remediations for removal of lipid pollutants from water bodies. However, long-chain lipids have been found to inhibit the activity of the microorganisms responsible for anaerobic remedy (Lalman *et al.*, 2000; Lalman *et al.*, 2001). This compromises the efficiency of anaerobic biodegradation for the treatment of lipid-rich wastewater. Hence long-chain fatty acid components must be sequestered prior to anaerobic remediation. As of now there is hardly any report of using an efficient vehicle for selective sequestration of long chain lipids.

Molecularly imprinted polymers (MIPs) can be explored for the selective removal of large lipids

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by imprinting them with the desired lipid as the template. They have been utilized for selective removal of organic water pollutants such as diclofenac (Dai *et al.*, 2011), estrogenic compounds (Lucci *et al.*, 2011), polycyclic aromatic hydrocarbons (Krupadam *et al.*, 2010) and polychlorinated biphenyl analogs (Sikiti *et al.*, 2014).

One of the potential raw materials for MIP synthesis is chitosan due to its low cost and high natural abundance. Some studies have already established that chitosan MIP can efficiently remove organic pollutants from water (Zhang *et al.*, 2017; Wang *et al.*,2018). Chitosan MIPs have been also explored as absorbent of organic pollutants from various sources. including dibenzenethiophene (Aburto *et al.*, 2004; Chang *et al.*,2008), dyes(Lazaridis *et al.*, 2007) and perflurooctane sulfonate (Yu *et al.*, 2008). Though there are several studies on use of chitosan MIPs as absorbent for small molecules, the ability of chitosan MIPs and its derivatives to bind to large moieties such as triolein is yet to be explored. This study investigated the ability of MIPs of chitosan and octyl derivative of chitosan(Oct-MIP) to bind to triolein from water to explore the potential of chitosan to remove lipid pollutants from water bodies.

EXPERIMENTAL

Chemicals. Chitosan (medium molecular weight – 190-310 kDa, 200.000 cps, DDA 75-85%), octanoyl chloride (99%), triolein (≥99%), Triton X-114 and 1,3-dioleoyl-2-palmitoylglycerol (≥99%) were purchased from Sigma Aldrich, glutaraldehyde (50% aqueous solution) was obtained from Merck, The solvents absolute ethanol, glacial acetic acid, isopropanol and n-hexane (AR, RCL Labscan) were purchased from Labscan and used as received.

Instruments. *UV-visible (UV-vis) Spectroscopy.* UV-vis analyses to quantify imprinting and binding of trioelin to MIPs and NIPs were conducted using Shimadzu UV-1800 spectrophotometer and processed using UV Probe 2.35.

FTIR Spectroscopy. IR analyses of chitosan derivative and crosslinked chitosan were performed using Shimadzu FTIR – IR Affinity 1 and IR Affinity 2 at a resolution of $4.0cm^{-1}$ and with 40 scans conducted per sample. Data were processed using Shimadzu IR Solution 1.30.

Contact Angle Measurement. The change in hydrophobicity of chitosan upon acylation was observed by contact angle measurement of deionized water on chitosan and octanoyl chitosan films.

The films were produced by dissolving 0.01g of each polymer in 0.5mL of 1% acetic acid and then spin-coating 60μL of the solution onto 1cm x 1cm glass slides (Laurell WS-650Mz-23NPP spin coater). The films were washed with 0.2M NaOH and then with distilled water until they were neutral and dry. The image of water droplet on the film was captured using a digital video camera (Sony NP F300) and processed using the software ImageJ Contact angles for 3 locations on each film were measured and 3 films per polymer type was analyzed

Methodology. *Synthesis of Octanoyl Chitosan.* The acylation procedure used was adapted from the work of Remant Bahadur *et al.* (Remant Bahadur *et al.*, 2006) with some modifications. Chitosan (0.5g, 3.1 mmol) was dissolved in 50mL, 1% aqueous acetic acid solution. Octanoyl chloride (319μL, 1.86mmol) was added under constant agitation. The mixture was then diluted to 500mL with distilled water and subsequently neutralized with 0.2M NaOH every 10min for 2 hours or until the pH was constant at 7.00. The mixture was allowed to stir for 12h at room temperature. The white suspension was centrifuged and collected. This acylated product was repeatedly washed with 20mL absolute ethanol, and centrifuged to collect the final product. The product was then dried to constant weight under vacuum and characterized by FTIR and elemental analysis. The ethanol washes were subjected to FTIR analysis for detection of any unreacted octanoyl chloride and for determination of the number of washes required to completely remove the excess acylating agent.

Synthesis of Chitosan-MIP and Octanoyl Chitosan-MIP (Oct-MIP). Synthesis of chitosan-MIP was adapted from the procedure by Aburto & Le Borgne (Aburto *et al.*,2004) with some modifications. Chitosan (0.1g, 0.62 mmol), was dissolved in 8mL 1% acetic acid aqueous solution. To it, 40 μL surfactant (Triton X-114) and 0.15mL of triolein were added. The system was stirred for 30min and then 2mL of 0.031M aqueous glutaraldehyde solution was added. The reaction was allowed to proceed for 2h at 50°C.

At the end of the reaction period, 20mL of 70:30 (v/v) hexane-isopropanol was added to the viscous system and stirred for 5min. This hexane-isopropanol mixture was then recovered by centrifugation and subjected to UV-vis analysis for the quantification of unbound triolein and for the detection of the presence of unreacted glutaraldehyde by IR analysis.

The non-imprinted polymer (chitosan-NIP) was prepared using the same procedure but without the addition of triolein. The synthesis of octanoyl chitosan-MIP (oct-MIP) and its non-imprinted

counterpart (oct-NIP) also followed the same process but octanoyl chitosan was used instead of chitosan.

Triolein Removal. To generate the empty cavities inside the MIPs, the bound triolein was extracted by equilibrating the MIPs in 20mL 70:30 (v/v) hexane-isopropanol and then in hexane for 30 min and then recovering the MIPs by centrifugation. The process was repeated several times till no triolein was detected in the washing using UV-visible spectroscopy.

Triolein Binding. Triolein rebinding was studied as a function of time. Chitosan-MIP or oct-MIP (3mg) was added to a dispersion of 2.7μL triolein, in 1mL water, and subjected to constant stirring for certain time intervals. Each sample was centrifuged and the aqueous supernatant was decanted. The polymer was then given a quick wash of hexane to remove unbound or loosely bound template to the surface. The triolein in both fractions were quantified by UV-vis spectroscopy to find the amount of triolein that remained unbound to the MIP after a particular contact time.

Binding with a Triolein Analog. MIPs were allowed to bind 1,3-dioleoyl-2-palmitoylglycerol(OPO) by equilibrating the MIPs with OPO solution for 6h following the procedure that was adopted for the rebinding study of triolein.

RESULTS AND DISCUSSION

Synthesis and Characterization of Octanoyl Chitosan. Chitosan was reacted with octanoyl chloride to increase its interaction with the hydrophobic template, triolein. The reaction scheme is shown in Figure 1.
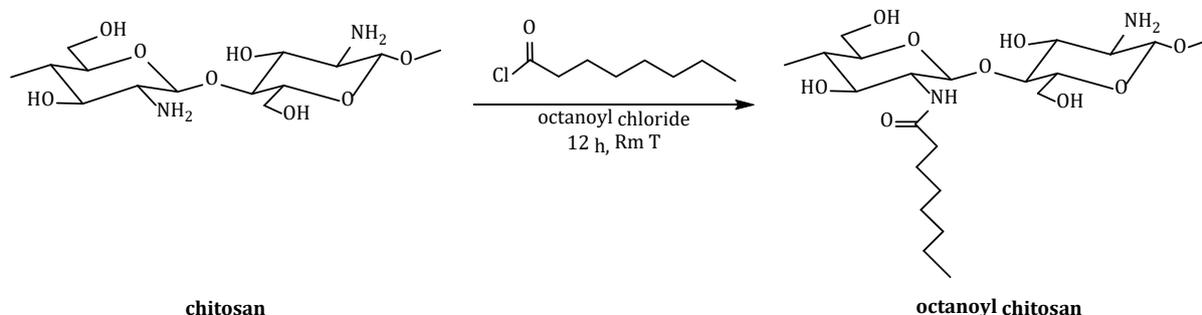

chitosan   **octanoyl chitosan**

Figure 1. N-Acylation of Chitosan Using Octanoyl Chloride.

The product of the acylation reaction for 12h was washed with absolute ethanol. FTIR analysis shows that four ethanol washes were required to remove unreacted octanoyl chloride. The IR spectrum of the 4th wash resembled that of the solvent alone.

Acylation of chitosan was confirmed using IR spectroscopy. The IR spectrum of octanoyl chitosan (Figure 2) showed increased intensity of: the alkyl stretch at 2880-2980 cm^{-1} due to the added CH$_2$ and CH$_3$ groups as compared to chitosan alone.

Contact angle measurement was performed to determine the effect of acylation on the hydrophobicity of chitosan. A drop of deionized water was placed on native chitosan and octanoyl chitosan films to measure the contact angle. As given in Table 1, higher contact angle for octanoyl chitosan indicates its enhanced hydrophobic character relative to chitosan.

Synthesis and Characterization of Chitosan-MIP and Oct-MIP. Chitosan was molecularly imprinted by crosslinking it with glutaraldehyde in the presence of the triolein. Crosslinking was confirmed by the presence of an imine (C=N) peak
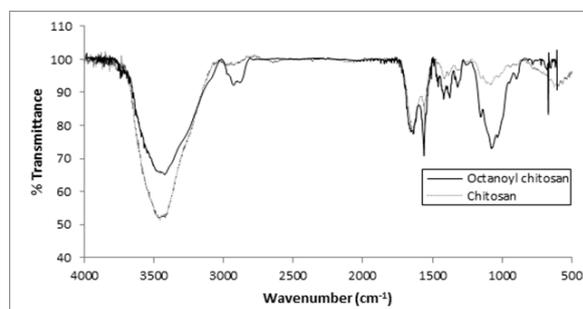

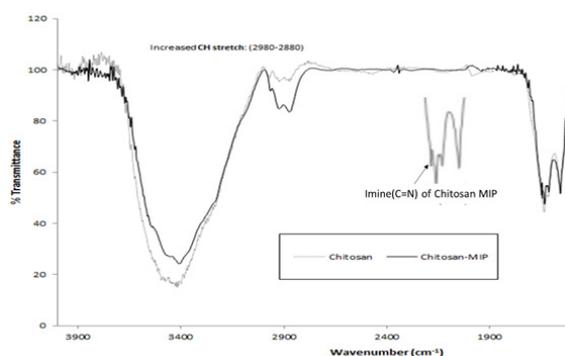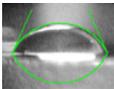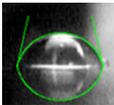*Figure 2. FTIR Spectra of Octanoyl Chitosan and Chitosan.*

Figure 3. FTIR Spectra of Chitosan and Chitosan MIP.

Table 1. Contact Angle of Water Droplet on Chitosan and Octanoyl Chitosan Film

Sample	Contact Angle (°)	Image of H₂O Drop on Film
Chitosan	57.5	
Octanoyl chitosan	76.4	

at 1655cm^{-1} in the IR spectrum of chitosan-MIP as shown in Figure 3. The FTIR spectrum of 70:30 hexane-isopropanol wash also did not show the presence of glutaraldehyde indicating completion of the crosslinking at the given reaction conditions. The imine (C=N) peak was also visible in the FTIR spectrum of Oct-MIP which has not been included as the part of the figure.

Extraction of Bound Triolein from Chitosan-MIP and Oct-MIP. To remove triolein from the MIPs, they were washed repeatedly with 70:30 hexane-isopropanol, and then with pure hexane alone. Pure hexane was not used throughout since decreasing the polarity of the solvent was found to be less efficient in removing the surfactant. The washings were subjected to UV-vis spectroscopy analysis. The peak at 277nm which corresponds to the surfactant and triolein peak was monitored for each washing. As shown in Figure 4, 16 washings were required for their removal.
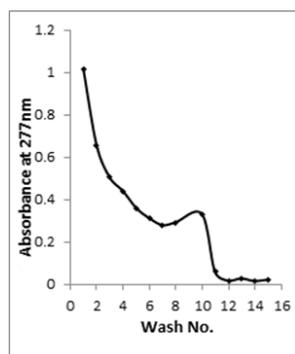

Figure 4. UV-vis Spectroscopy of Washings Used to Remove the Surfactant and Template from the MIP.

Triolein Imprinting Efficiency. As quantified from the UV-vis spectra, oct- MIP could bind 105.51±11.60mg (77.29%) of triolein whereas chitosan-MIP could bind 92.40±5.59mg (66.41%). Higher binding efficiency of oct-MIP is attributed to hydrophobic interaction between octanoyl chitosan and the hydrophobic template.

Triolein Binding. Binding of triolein to chitosan-MIP, Oct-MIP and the corresponding NIPs was performed as function of time. The binding capacity (Q) of each polymer is defined by the following equation:

$$Q = \frac{\text{mass of triolein bound to the MIP/NIP (µg)}}{\text{mass of polymer (mg)}} \quad (1)$$

The binding profiles are shown in Figure 5. Results show higher Q values for MIPs as compared to the NIPs. This is attributed to imprinting of the template that occured upon crosslinking of the polymer in the presence of triolein, creating cavities in the polymer that exhibit conformational memory for the template. Furthermore, octanoyl modification of chitosan contributed towards enhanced triolein binding. After 6h of equilibration, triolein binding of oct-NIP and oct-MIP were 26% and 41% higher than their corresponding non-acylated chitosan. Thus, affinity of a substrate for a target molecule can be increased by the formation of MIP as well as the chemical modification of the substrate.

Figure 5 also reveals that for chitosan-MIP, chitosan-NIP and Oct-NIP there was no significant increase in binding after 2h of equilibration, probably due to the saturation of hydrogels with the triolein molecules within 2h. However oct-MIP showed marked increase in binding till the equilibration time reached 6h, proving that the hydrogel got saturated at higher concentration of triolein due to its stronger affinity for the target molecule.
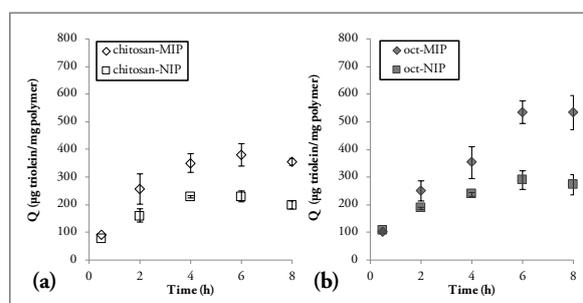

Figure 5. Triolein Bound by Chitosan MIP, Oct MIP and Their NIP Counterparts as a Function of Time.

The study by Ahmad *et al.* (Ahmad *et al.* 2005) stated that 0.5g of chitosan flakes and powder could remove 1.30g and 1.98g of lipid respectively from water, whereas this study reveals that only 0.003g of chitosan-MIP and oct-MIP could remove 1.14g/L and 1.60g/L of lipid respectively under similar initial lipid concentrations as reported by Ahmad *et al.* Thus, use of chitosan and its derivatives in the form of MIP can be considered as more efficient absorbent of lipid wastes from water bodies.

Q values were used to calculate the imprinting factor. Imprinting factor (α) is defined as following.

$$\alpha = \frac{Q_{MIP}}{Q_{NIP}} \qquad (2)$$

The imprinting factor for both types of MIP systems has been found to increase with time (Figure 6). In the case of MIPs, apart from surface adsorption, more triolein could gain access to the binding sites within the imprinted polymer over time whereas for NIPs, surface adsorption was the only binding factor. This observation further reinstates that formation of MIP aids in enhanced binding of triolein.
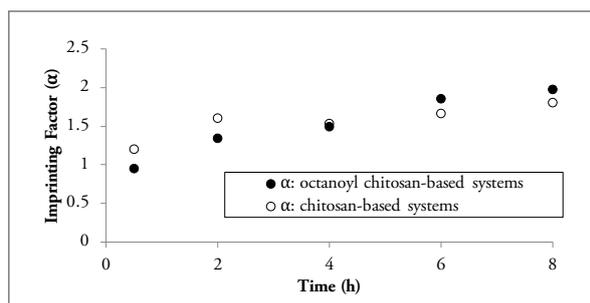

Figure 6. *Imprinting Factor as a Function of Time.*

Binding with a Triolein Analog. Triolein and 1,3-dioleoyl-2-palmitoylglycerol(OPO) differ only at the fatty acid esterified to the hydroxyl group at carbon-2 of glycerol as shown in Figure 7.

Figure 8 shows that both chitosan-MIP and oct-MIP could also bind OPO though the binding capacity towards OPO is marginally lower. This result reflects that these MIPs can not only be used for the removal of triolein but also other triacylglycerides from wastewater samples, though the binding capacity will be maximum for template specific triolein.

CONCLUSIONS

The study established that both chitosan-MIP and Oct-MIP can imprint triolein and can efficiently bind to it when the MIPs and triolein are dispersed in water. Chitosan-MIP and Oct-MIP had higher affinity for triolein than the non imprinted counterparts. Binding capacity of Oct-MIP was higher than chitosan-MIP due to the enhanced hydrophobic interaction between the MIP and the substrate. Apart from triolein the MIPs could bind to its analog 1,3-dioleoyl-2-palmitoylglycerol.

Since chitosan is one of the abundant natural polymers and has the potential to bind to lipids such as triolein and 1,3-dioleoyl-2-palmitoylglycerol in water, of which MIP is the most efficient form, chitosan and its octanoyl derivative in the form
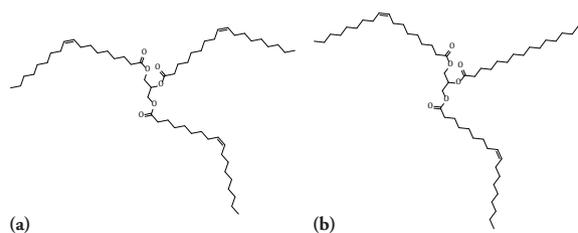

(a) **(b)**

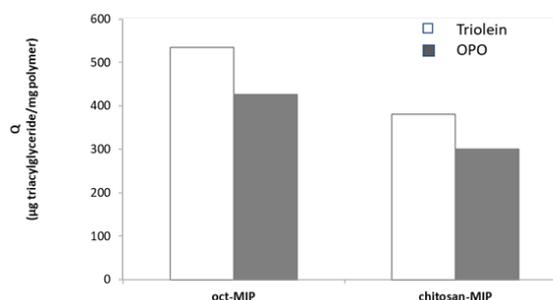Figure 7. *Structure of (a) Triolein (b) 1,3-Dioleoyl-2-palmitoylglycerol.*

Figure 8. *Binding of Triacylglycerides to Chitosan-MIP and Oct-MIP.*

of MIP can be explored further as a low-cost and efficient system to remove lipid waste from water bodies.

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