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Chitosan Curcumin Film As A Sensor For Detection of O-Nitrophenol and Fluoride Ion Using Fluoresce Quenching Technique

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Curcumin was immobilized in chitosan films fabricated by solvent casting method. The amount of curcumin immobilization was more when methanol was used as a solvent to dissolve curcumin than the butanol solvent. The maximum amount of curcumin immobilized per gram of chitosan film was 0.023 g. Immobilized curcumin was not released back in water even after prolong contact of the films with water. Fluorescence intensity of the films got quenched when these films were in contact with an aqueous solution of o-nitrophenol (ONP) and sodium fluoride (NaF). The extent of quenching depended on the concentration of these attributes. Fluorescence intensity was highly pronounced even when the concentration of ONP and fluoride (FL) was as low as 2.0 x 10^{-6} M and 2.5 x 10^{-5} M, respectively. UV-vis spectroscopy could not detect 2.5 x 10^{-6} M ONP; similarly, ion chromatography was not sensitive towards 2.5 x 10^{-5} M FL. Since the extent of quenching varies linearly with the concentration of ONP and FL in aqueous solution, the Stern-Volmer equation can be used for quantification of these.

Keywords: chitosan, curcumin, fluoride, immobilization, o-nitrophenol, sensor

INTRODUCTION

Nitrophenols and fluorides are considered as priority water pollutants along with several other chemicals. Nitrophenols are extensive use for pesticide, paint, dye, plastic, and rubber production (Hartter 1985). Their inappropriate disposal in industrial water is causing is contaminating the water bodies. The restricted concentration of nitrophenol in water is specified as less than 7 x 10^{-8} mol/L (Musilová *et al.* 2011).

The toxicological effect of nitrophenols such as headache, drowsiness, nausea, and cyanosis are well-documented (Pham *et al.* 2016). Hence, there is a dire necessity of the development of detection methods to detect nitrophenol in the water at a very low concentration in a cost-effective way. Several instrumental techniques are routinely utilized for the determination of nitrophenol – for instance, high-performance liquid chromatography, capillary zone electrophoresis and electrochemical techniques, and UV-vis and fluorescence spectroscopies (Galeano-Diaz *et al.* 2000; Niazi and Yazdanipour 2007; Nistor *et al.* 2001; Zhou *et al.* 2019; Liu *et al.* 2009; Kafi and Chen 2009).

FL, on the other hand, is used for dental and skeletal improvement. Due to its strengthening effect on bones and teeth, FL is added to water for human consumption. However, FL is not easily digested and is instead retained in the body leading to its accumulation, causing skeletal fluorosis and bone fracture (Li *et al.* 2020). Ion chromatography is one of the most common method used for FL detection (Agnieszka and Agnieszka 2019).

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Although there are several existing methods of detection for FL and nitrophenol, the exploration of environment-friendly, cost-effective, and efficient detectors is still on.

Curcumin [1,7-bis(4-hydroxy-3methoxyphenyl)-1,6-heptadiene-3,5-dione] is extracted from the root of turmeric plant (*Curcuma longa*) and is used as a cooking ingredient. Its antioxidant property is well-established (Abrahams *et al.* 2019). Hence, it is found in a wide range of applications in food, drug, and cosmetic formulations (Abrahams *et al.* 2019; Tuba and Gülçin 2008; Rafiee *et al.* 2019; Sundaramoorthy *et al.* 2019; Jutkova *et al.* 2019; Batra *et al.* 2019). Curcumin is also a natural fluorophore, and this property has been exploited for chemosensing of molecules and ions (Pang *et al.* 2017; Khorasani *et al.* 2019; Madhu and Sivakumar 2019).

Curcumin's fluorescence could be further studied in relation to its capacity to detect pollutants. It has been proven that the fluorescence intensity of curcumin alters significantly when it interacts with nitrophenol and FL (Wang *et al.* 1997; Wu *et al.* 2010). Thus, this behavior can be explored for the development of a curcumin-based sensor for the detection of the above pollutants.

Chitosan is one of the most abundant, natural polymer that has been widely explored as an immobilizing matrix for biomolecules (Thirumavalavan and Lee 2015; Urrutia *et al.* 2018). Curcumin has also been deposited in the chitosan film to improve its antimicrobial property (Liu *et al.* 2015).

Our research aimed toward the formulation of an environment-friendly sensor from natural resources that could detect and quantify the presence of ONP and FL in water, even when they are present in minute quantities. Although there are several studies on using curcumin in immobilized synthetic films such as starch (Boonkanon *et al.* 2020), nano cellulose (Naghdi *et al.* 2019), and tara gum / poly(vinyl alcohol) (Ma *et al.* 2017) for detection of attributes, the use of curcumin immobilized chitosan film for detection of ONP and FL through fluorescence has not yet been reported. This study could usher the development of an economical, eco-friendly sensor, which not only can detect the abovementioned attributes but can also be safely disposed of for biodegradation after use.

Curcumin was immobilized in the chitosan film and its fluorescence intensity was compared before and after it was exposed to an aqueous solution of ONP and FL to quantify the amount of these species present in water.

MATERIALS AND METHODS

Materials

Medium-molecular-weight chitosan ($C_{56}H_{103}N_9O_{39}$), curcumin ($C_{21}H_{20}O_6$), NaF, and ONP were purchased from Sigma Aldrich (St. Louis, MO, USA).

Instruments

Fluorescence values of the films were measured using the FLUOstar Optima Fluorometer at excitation and emission wavelengths of 420 and 520 nm, respectively. UV-Vis Shimadzu 1800 PC Spectrophotometer was used to determine the concentration of curcumin in methanol and n-butanol and ONP concentration in water. Shimadzu LC-10 Ion Chrom was used to determine the concentration of FL in water.

Synthesis of Chitosan Film

To fabricate the film, 0.20 g medium-molecular-weight chitosan powder was dissolved in 10.0 mL 1% acetic acid. This chitosan solution was transferred to a Petri dish and then partially dried in an oven at 50 °C for 1 h, resulting in the formation of a film. This film was then immersed in 20 mL 0.18 M NaOH solution for 4 h to neutralize the film. It was then kept immersed in 20 mL deionized water for 12 h to remove excess NaOH. After 12 h, the film was transferred to an oven maintained at 40 ˚C and dried until a constant weight was reached. The dry film was stored in a desiccator.

Immobilization of Curcumin in Chitosan Film

Curcumin was dissolved in 30 mL methanol as the amount of curcumin was varied from 0.0030–0.1200 g to form the solution. A chitosan film of 0.1 g with a surface area of 34.2 cm^2 was immersed in each methanolic solution of curcumin for 2 h and then oven-dried at 40 ˚C until a constant weight was reached. The amount of curcumin absorbed in the film was determined by computing the difference in the curcumin concentration in methanol before and after the films were immersed in curcumin solution using a UV-vis spectrophotometer. A similar procedure was used to immobilize curcumin in chitosan films by immersing the films in butanolic solution curcumin where methanol was replaced by n-butanol.

Leaching of Curcumin from Chitosan Film in Aqueous Environment

The curcumin-chitosan (cur-chi) films were immersed in 30.0 mL deionized (DI) water for 12 h, after which the water was subjected to UV-vis spectrophotometry analysis to check the presence of curcumin in it.

Detection of ONP and FL by Fluorescence Quenching of Cur-Chi Film

ONP and NaF solutions of various concentrations were prepared separately by dissolving these compounds in 20 mL DI water. After the dissolution of the compounds, one cur-chi film was placed in each beaker and the systems were allowed to equilibrate for 5 min. Afterward, the films were removed from the beakers, pat-dried, and was monitored for fluorescence intensity using a fluorometer with excitation set at 420 nm and emission at 520 nm. The reading was subtracted from the fluorescence intensity of cur-chi film alone to obtain the quenched fluorescence of curcumin-ONP or curcumin-FL complex. The fluorescence data were used to quantify the amount of ONP and FL.

RESULTS AND DISCUSSION

Chitosan and Cur-Chi Film

Chitosan was chosen as the immobilization matrix as it can form a film of high strength, retains dimensional stability in an aqueous medium, and shows no fluorescence activity of its own. Immobilization of curcumin in chitosan film can be attributed to the hydrogen between them. The oxygen of hydroxyl on the benzene ring of curcumin binds with either the hydroxyl or the amino group of chitosan as documented by Liu *et al.* (2015).

Curcumin is soluble in methanol and butanol; likewise, chitosan is stable in these solvents. Since the solubility of immobilized material and stability of the immobilizing matrix are the absolute requirements for efficient immobilization from the solution, methanol and butanol were used as the solvents for immobilization. Chitosan films formed by the solution casting method were

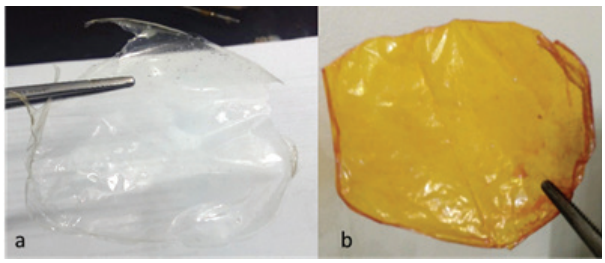

Figure 1. a) Chitosan film; b) cur-chi film.

transparent with a smooth texture. Cur-chi films formed by immobilization of the chitosan films in curcumin solution were likewise transparent and smooth. The films are shown in Figure 1.

The solubility of curcumin in methanol, ethanol, propanol, and n-butanol was tested and it was found that curcumin dissolves in all these solvents. Methanol and n-butanol were further explored as the solvents to dissolve curcumin for immobilization. These solvents were selected as both differ markedly in their behavior and a pronounced difference in their interaction with curcumin was anticipated. The amount of curcumin

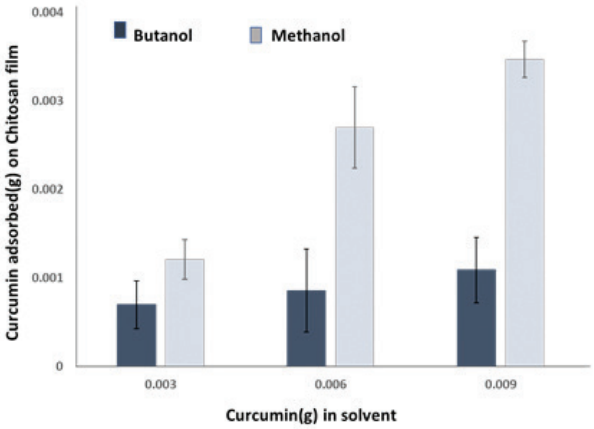

Figure 2. Effect of solvent and curcumin concentration on immobilization of curcumin on chitosan film (each data point represents averages of three trials).

immobilized in chitosan film was quantified by finding out the concentration of curcumin in the solvents before and after immersion of the films in the solutions. Figure 2 shows the effect of solvent and curcumin concentration on immobilization.

The amount of curcumin absorbed by the films was more when methanol was used to make the curcumin solution. Moreover, curcumin immobilization increased significantly with the increase in the concentration of curcumin in methanol, whereas no marked increase in immobilization was observed when butanol was used as the solvent. Improved immobilization of curcumin in the film when methanol was used as the solvent can be attributed to better swelling of chitosan in methanol than in butanol.

In order to determine the maximum amount of curcumin that could be immobilized on chitosan film, chitosan films were immersed in methanolic curcumin solution of different concentrations and the amount of curcumin absorbed by each film as the function of curcumin concentration was determined by UV-vis spectrophotometry.

As seen in Figure 3, the amount of curcumin absorbed per gram of chitosan increased as the curcumin content increased. However, no significant increase in absorption of curcumin was noticed when the curcumin concentration exceeded 20.0 x 10^{-4} g/mL, indicating saturation of the film with curcumin in the films at this concentration.

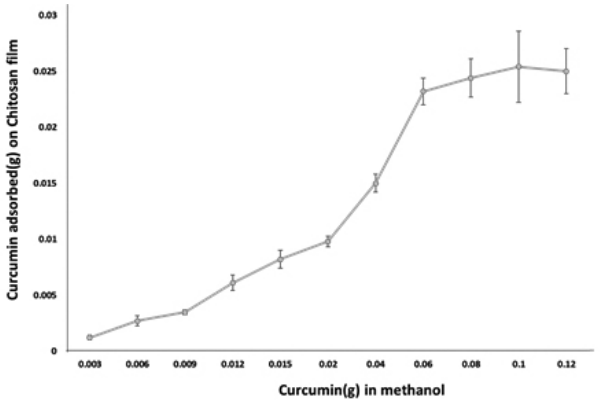

Figure 3. Amount of curcumin absorbed by chitosan film as function of curcumin concentration (each data point represents averages of three trials).

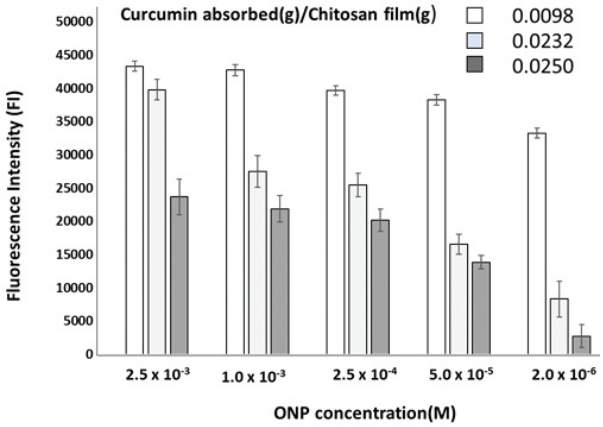

Figure 4. Effect of ONP concentration in water on the fluorescence intensity of cur-chi films (each data point represents averages of three trials).

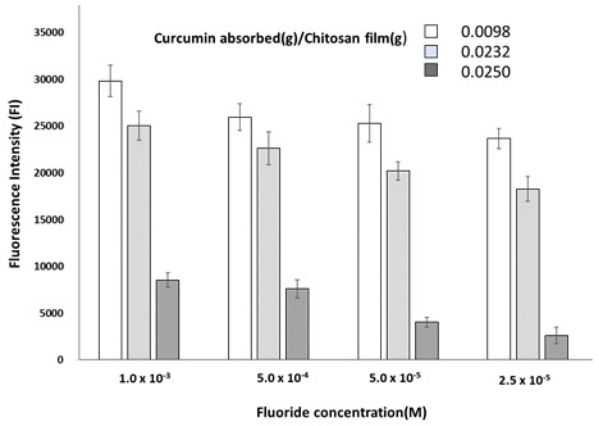

Figure 5. Effect of FL concentration in water on the fluorescence intensity of cur-chi films (each data point represents averages of three trials).

Leaching of Curcumin from Chitosan Film in Aqueous Environment

It is critical that curcumin should not get released in water due to swelling of chitosan while the film is in use as the sensor. When the curcumin loaded film was kept immersed in water for 12 h, no trace of curcumin was observed in water by UV-vis analysis after the film was removed. This implies that curcumin remains immobilized in chitosan film even when the film remains exposed to an aqueous environment for an extended period. The strong interaction of chitosan and curcumin can be attributed to the hydrogen bonding of chitosan NH_2 and OH groups with curcumin OH groups.

Detection of Presence of ONP and FL Ion in Water Using Cu-Ch Films as the Sensor

Curcumin is a natural fluorophore; however, in the presence of ONP or FL, it forms a complex with curcumin and quenches its fluorescence activity. The extent of quenching depends on the concentration of the quencher. Thus, if a water body contains a pollutant such as ONP or FL, then the comparison of fluorescence of curcumin film before after it is dipped in the water body should be able to quantify the amount of pollutant in the form of ONP or FL.

In order to gauge the sensitivity of this system, the fluorescence intensity (FI) of cur-chi films immersed in an aqueous solution of various concentrations of ONP and FL was determined using the following equation.

$$FI = F_0\text{-}F \qquad (1)$$

Where F_o is the fluorescence intensity of the sensor without quencher and F is the fluorescence intensity with a quencher.

Figures 4 and 5 manifest the effect of ONP and FL on the fluorescence intensity of cur-chi film.

As seen in Figures 4 and 5, the fluorescence intensity of curcumin in cur-chi films got impacted when they came in contact with ONP and FL in water. The fluorescence intensity diminished as the concentration of the above attributes increased. ONP and FL form complexes with curcumin through hydrogen bonding that are not fluorescence active, thus quenching the fluorescence intensity of curcumin. The figures reveal that the extent of quenching depends on the concentration of the quencher as well as the concentration of curcumin in the film. Strong FI was recorded both for ONP and FL even at very low concentrations of 2.0 x 10^{-6} M and 2.5 x 10^{-5} M, respectively. The FI was more pronounced when the concentration of curcumin was 0.02 g compared to 0.06 g and 0.12 g in the film. Most probably, curcumin-curcumin interaction is stronger than curcumin-ONP/FL interaction. At lower concentrations, the self-interaction of curcumin predominated, making F_0 much higher than F and, thus,

stronger FI. However, further investigation is required on curcumin-curcumin and curcumin-ONP/FL interaction to prove this hypothesis. Since the films show strong fluorescence response when in contact with ONP and FL even when they are in minuscule amount, these cur-chi films can serve as a sensor for the detection of ONP and FL in water bodies.

Quantification of ONP and FL

The sensor can be used not only to detect the presence of ONP and FL in water in minuscule amount but also to quantify them using the Stern-Volmer equation (Equation 2)

$$\frac{Fo}{F} = 1 + Ka[Q] \qquad (2)$$

Where F_o is the fluorescence intensity of the sensor without quencher, F is the fluorescence intensity with a quencher, K_a is the association constant (slope), and [Q] is the quencher concentration.

In order to determine K_a for ONP, an aqueous solution of ONP of known concentrations was prepared. The fluorescence intensity of cur-chi film with a surface area of 34.2 cm^2 was determined after immersing the film in ONP solution (F). The fluorescence intensity of cur-chi film was also determined without its contact with the quencher (F_0). Plotting F_o / F against the concentration of the quencher gave a linear curve, with K_a determined as the slope of the plot. A similar procedure was followed to determine K_a for FL. Figures 6 and 7 show plots and corresponding K_a values for ONP and FL, respectively.

Once the K_a values are known for a given surface area of the film, these films can be dipped in an aqueous solution of ONP or FL of unknown concentrations, and their concentrations can be determined from the fluorescence intensity of the films before and after immersion in the quencher solutions.

Comparison of Limit of Detection of Fluorescence of Cur-Chi Film with Other Detection Methods

UV-vis spectroscopy is often used to determine the concentration of ONP in water. Hence, to determine the limit of detection of ONP by UV-vis spectroscopy, ONP aqueous solutions of various concentrations were prepared and subjected to UV-vis spectroscopy. When the concentration of ONP reached as low as 2.0 x 10^{-6} M, no UV-vis peak was detected at 278 nm (λ max), as shown in Figure 8.

Similarly, the limit of detection of FL by ion chromatography (IC) was compared with that of the reported sensor. An aqueous solution of NaF of various concentrations was prepared and subjected to IC analysis. The lowest concentration of FL that IC could detect was 2.6 x 10^{-5} M, which was above the concentration of F detected through the fluorescence of cur-chi film.

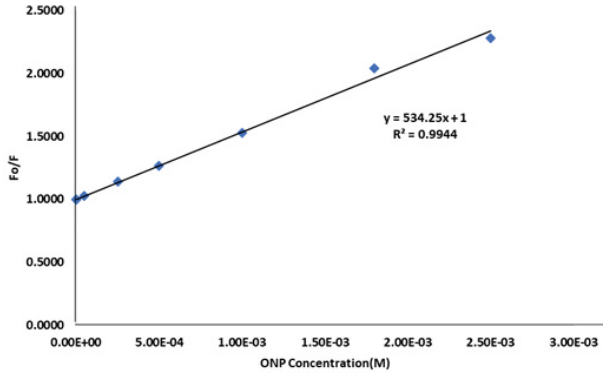

Figure 6. F_0/F as function of ONP concentration to determine K_a value.

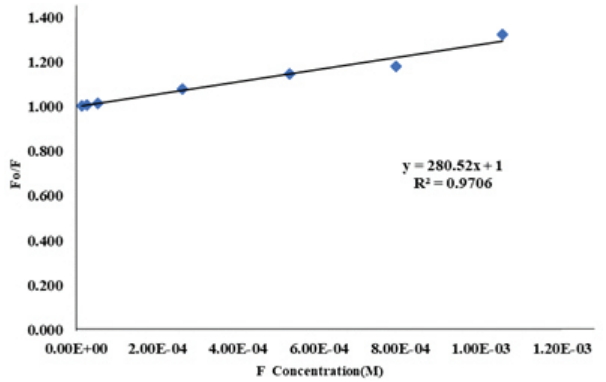

Figure 7. F_0/F as function of FL concentration to determine K_a value.

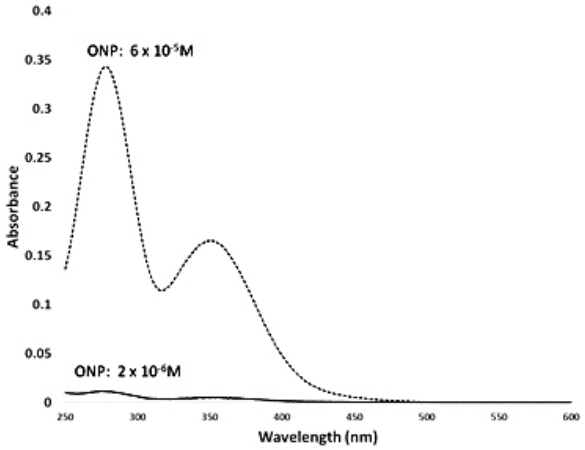

Figure 8. UV-vis spectroscopy of aqueous solution of ONP.

CONCLUSION

Transparent chitosan film fabricated by solvent casting method can be used for the immobilization of curcumin. The study established that the presence of ONP and FL in water can be detected and quantified by monitoring the quenching of fluorescence of this curcumin immobilized chitosan film. The limit of detection of ONP and FL by this method is much higher than that by UV-vis spectroscopy and IC for ONP and FL, respectively. Hence, the system has the potential to serve as an efficient sensor for the detection of ONP and FL from water bodies. This sensor can be explored further for the detection of other attributes that serve as water pollutants and can quench the fluorescence intensity of curcumin.

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