The β-cyclodextrin-chitosan inclusion complex: Characterization and application in the removal of pesticides in wastewater

Zoheb Karim* and Rohana Adnan

School of Chemical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia
*To whom correspondences should be addressed
E-mail: zoheb.karim@gmail.com

Abstract

In the present study, the interaction of chitosan with β-cyclodextrin producing a complex in the solution was carried out by freeze drying method. An apparent stability constant of 370 M^{-1} and a 1:1 stoichiometric ratio were observed for the complex formed by using the phase solubility method. The complex was characterized by UV-Vis, FT-IR, AFM, and XRD techniques. The comparative study of chitosan and β-CDC complex has been investigated for the treatment of wastewater contaminated with pesticides. The maximum removal efficiency of parathion of 65% was found when using 200 mg β-CDC complex in sodium acetate buffer (pH 5.5) at 40°C within 24 h. Under the same experimental conditions, pure chitosan showed low percentage of removal efficiency (25%). The removal efficiencies of parathion, chlorpyrifos, dichlofenthion, phosmet, and azinophos-methyl by the complex were found to be 65%, 82%, 75%, 51%, and 40%, respectively. In the mixture of all the five pesticides, the percentages of removal efficiency by β-CDC complex and chitosan were 89% and 57%, respectively. In the batch study, the removal efficiency of parathion, chlorpyrifos, dichlofenthion, phosmet, and azinophos-methyl were 74%, 90%, 83%, 66%, and 59%, respectively. Batch process was also performed to remove the pesticides in the mixture and complete removal of pesticides in the mixture was observed for β-CDC complex but only 77% removal for pure chitosan.

1. Introduction

Cyclodextrins (CDs) are cyclic compounds consisting of six, seven, or eight α-D-glucopyranose units connected by α-(1-4) linkages which are commonly referred to as α-, β-, and γ-CDs, respectively [1]. CDs possess a characteristic toroidal shape with a well-defined hydrophobic cavity and lipophilic exterior that is suitable for inclusion and binding of appropriate sized guest compounds. CDs are of interest because of their ability to form stable inclusion complexes in aqueous solution as well as in solvents [2]. These macromolecules (CDs), which can be spatially represented as a torus with wide and narrow openings corresponding to secondary and primary hydroxyl groups, respectively, can encapsulate a large variety of compounds due to the hydrophobic characteristic of their internal cavity [3]. This outstanding property has long been utilized in pharmaceutical, food, cosmetic, and textile industries and has also found its applications in the field of catalysis, environmental remediation, chemical sensing, and enantiomeric separations [1,4]. Certain applications require the immobilization of CDs or CDs derivatives onto an insoluble supports. Many studies have been carried out using organic materials [5,6], metal surfaces [4] or inorganic oxide supports.

Chitosan [poly (1,4-b-D-glycopyrano-amine)], which is a highly biocompatible and natural material, can be considered as a suitable support for CDs immobilization. Because of its high biocompatibility and easy biodegradability, chitosan has been used as a raw material in the medical applications such as a matrix for drug delivery systems [7]. Chitosan has also been used as a matrix for immobilization of several enzymes [8,9] since it possesses several reactive functional groups. Immobilization to chitosan also has the advantages of high stability, recoverability and reutilization [10].

The enormous use of pesticides by human beings in agriculture as well as in the other sphere of life has added these compounds to the environment as pollutants [11]. The pesticides or insect killers are among the most extensively used chemicals and they are also among the most hazardous compounds for the human

beings [12,13]. Population based studies have shown the association between certain types of pesticide related to certain cancers and suppress the immune system [14,15].

An increasing attention has been focused on adsorption technique in the removal of pollutant from aqueous solution [16, 17]. A large number of low-cost materials including industrial and agricultural wastes have been used in the removal of different pesticides from the aqueous solution. Activated carbon is one of the most widely used materials for the removal of organic pollutants. The sources of activated carbon includes tea leaves [18], chickpea husk, rice bran, rice husk, baggasse fly ash [19], date stone [20], bio-waste, coconut tree male flower and Jute fiber [21-24].

In this study, the preparation of β-CD-chitosan complex was carried out by freeze drying method and the characterization of this complex was determined by UV-Visible, FTIR, AFM, and XRD. The phase solubility diagram of chitosan in the presence of different β-CD concentrations was reported and a linear increase in the solubility of chitosan was observed. A comparative study was performed to remove the contaminants from model wastewater containing five types of pesticides; parathion, chlorpyrifos, dichlofenthion, phosmet and azinophos-methyl as well as the mixture of these pesticides by pure chitosan and β-CD-chitosan (β-CDC) complex. Various experimental parameters were evaluated to standardize the maximum removal efficiency of the pesticides by chitosan and complex from model wastewater. The removal of the pesticides was also performed in the batch study with the aim to apply this immobilization preparation at industrial level.

2. Materials and Methods

2.1. Materials

The pesticides: parathion, chlorpyrifos, dichlofenthion, phosmet and azinophos-methyl, chitosan, dimethyl sulfoxide (DMSO) and β-cyclodextrin, were obtained from Sigma-Aldrich Inc. (Germany). Other chemicals and reagents were of analytical grade.

2.2. Phase Solubility and Preparation of Inclusion Complex

The phase solubility studies were performed according to the method reported by Villaverde et al. [25] with some minor modifications. Chitosan (5 mg) was added to the sodium acetate buffer, pH 5.5 (10 ml) containing different concentrations of β-CD (0, 2, 4, 6, 8, 10 and 12 mM). The experiment was carried out in triplicate. Solutions were shaken at room temperature for 24 h. The solution was filtered through a 0.22-µm Millipore glass fiber membrane filter paper and the concentration of chitosan was determined. The apparent stability constant K_c was calculated from the straight line obtained in the phase solubility diagram following the equation proposed by Higuchi and Connors [26].

$$K_c = \text{slope}/S_o \, (1\text{-slope}) \qquad (1)$$

where S_o is the chitosan equilibrium concentration in aqueous solution in the absence of β-CD and the slope was obtained from the phase solubility diagram. The 1:1 stoichiometric ratio employed for the preparation of the solid complex was deduced from the phase solubility diagram. Finally the solution which has the most binding capability of chitosan with β-CD was centrifuged and freeze dried using a vacuum freeze-dryer (LABCONCO freeze dry/shell freeze system, model freezone 12, U.S.A) at -41°C under a pressure of 5.33 Pa for 12 h. After freeze drying, this solid complex was stored in the desiccator for further use.

2.3. Characterization of Inclusion Complex

UV-visible spectra

UV-visible spectra were recorded by Perkin Elmer, Lambda 25 spectrophotometer. The measurements were done in a 10 mm quartz cell.

FT-IR spectra

The Fourier transform infrared (FT-IR) spectral studies were performed using KBr pelleting technique with Perkin Elmer System 2000 instrument in range of 400-4000 cm^{-1}. FT-IR analysis was carried out to determine the variation of the functional groups present in the native compounds and in the prepared complex.

AFM analysis

Atomic force microscopy (AFM) analysis of chitosan and β-CDC complex was performed using commercially etched silicon tips as AFM probes with typical resonance frequency of ca.

300 Hz (RTESP, Veeco). The samples were placed drop wise on a mica wafer, air dried at room temperature for 12 h and the images were recorded with a Veeco Innova nanoscope II AFM. AFM scans were carried out on several surface positions to check the surface uniformity.

X-ray diffraction

X-ray diffraction (XRD) was obtained with a D\max-2200 X-ray diffractometer by using graphite-monochromatized CuKα radiation (k = 1.54178 A_o).

2.4. Effects of Dosage, Time, Temperature and pH of Chitosan and β-CDC Complex on the Removal of Parathion

Parathion containing synthetic wastewater (200 ppm, 10 ml) prepared in 10% dimethyl sulfoxide (DMSO) was incubated with increasing amount of chitosan and β-CDC complex (50, 100, 200, 400, 600, 800 and 1000 mg) in 100 mM sodium acetate buffer, pH 5.5, at 40℃ for 24 h.

An amount of 200 mg chitosan and the complex prepared were used for the removal of parathion from synthetic wastewater with increasing time interval (12, 24, 36 and 48 h) in 100 mM sodium acetate buffer. The percentage of removal efficiency was calculated by taking untreated parathion containing wastewater as control (100%).

Water containing parathion was incubated with chitosan and β-CDC complex (200 mg) in 100 mM sodium acetate buffer, pH 5.5 at various temperatures (20–80℃) for 24 h.

Model wastewater containing pesticides was treated with chitosan and complex (200 mg) in the buffers of different pH (2.0–10.0). The buffers used were glycine–HCl (pH 2.0-3.5), sodium acetate (pH 4.0-5.5), sodium phosphate (6.0–8.5), and Tris–HCl (pH 9.0-10.0). The molarity of each buffer was 100 mM.

2.5. General Procedure for the Removal of Pesticides

All the five pesticides model wastewater prepared in 10% DMSO (200 ppm, 10 ml each) such as; parathion, chlorpyrifos, dichlofenthion, phosmet and azinophos-methyl were incubated with chitosan and β-CDC complex (200 mg) in 100 mM sodium acetate buffer, pH 5.5 at 40℃ for 24 h. Reaction mixtures without β-CDC complex and chitosan were used as a control and buffer solution having 10% DMSO without the complex and chitosan was used as blank to measure the percentage of removal of the mentioned pesticides. After centrifugation at 3000 rpm for 15 min, the decrease in absorbance at specific $λ_{max}$ was monitored spectrophotometrically.

Percent removal of pesticides was calculated as:

$$\text{pesticides removal} (\%) = (A_o - A_t/A_o) \times 100 \quad (2)$$

where A_o is the absorbance of untreated pesticides and A_t is the absorbance of treated pesticides.

2.6. Treatment of Mixture of Pesticides

The mixture of pesticides was prepared in 100 mM sodium acetate buffer, pH 5.5 to a final concentration of 200 ppm, by taking each pesticide in equal proportion. The mixture of pesticide was incubated with chitosan and β-CDC complex (200 mg) for 24 h at 40℃. After incubation period of 24 h, the solution was centrifuged at 3000 rpm for 15 min. The decrease in the absorbance of the mixture at their respective $λ_{max}$ was monitored. Similarly, the percentage of removal efficiency was calculated by taking untreated mixture as control (100%).

2.7. Monitoring of Absorption Spectra of Parathion and the Mixture

The absorption spectra of treated and untreated parathion and the mixture of pesticides polluted water were recorded on UV-visible spectrophotometer.

2.8. Batch Adsorption Study

Batch adsorption experiments were conducted using individual pesticide solutions and the prepared mixture (200 ppm, 500 ml) in 10% DMSO solution, incubated with chitosan and β-CDC complex adsorbents (600 mg). Adsorption data were determined for the period of 24 h at 40℃. All of the adsorption experiments were conducted in triplicate and the concentrations of pesticide in the solution were determined by taking the model wastewater without treatment with chitosan or complex as control (100%). The remaining pesticide concentrations were monitored spectrophotometrically.

2.9. Statistical Analysis

Each value represents the mean of the three independent experiments performed in duplicates, with average deviations of < 5%. Data obtained in various studies was plotted using Sigma Plot-10.0 and Microsoft Excel 2003. P-values of < 0.05 were considered statistically significant.

3. Results

3.1. Phase Solubility

The phase solubility diagram of chitosan in the presence of different β-CD concentrations is shown in Fig. 1. A linear increase in the chitosan solubility is observed when increasing β-CD concentration. A solubility limit is not obtained in the range of β-CD concentrations used which is in agreement with the AL classification according to Higuchi and Connors [26]. The diagram is a straight line with a slope of less than unity and it may be ascribed to the formation of a 1:1 stoichiometry of the complex in the solution. The apparent formation constant (K_c) was calculated according to Eq. (1) (Fig. 1). A value of $K_c = 370$ M^{-1} was obtained in this study.

Figure 1. Phase solubility diagram of β-CD and chitosan
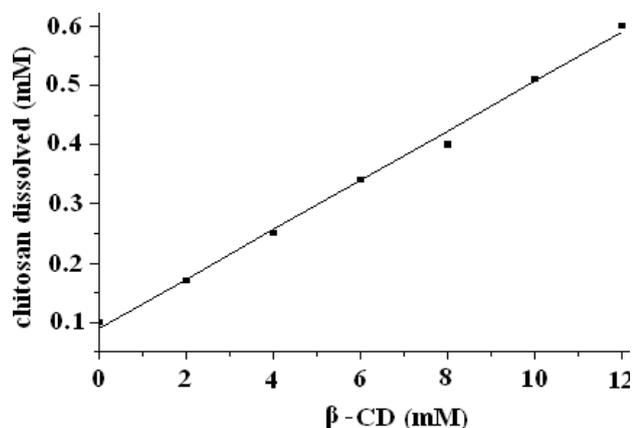
3.2. UV-Visible Analysis

Figure 2 shows the UV-Vis spectra of β-CDC complex. The maximum absorbance (λ_{max}) of this inclusion complex was recorded at 360 nm (Fig. 2b). The λ_{max} of β-CD and chitosan were 260 nm (Fig. 2a) and 420 nm (Fig. 2c), respectively. The change in the λ_{max} further confirmed the formation of the β-CDC complex.

3.3. FT-IR Spectra

The FT-IR spectra of β-CD and complex are given in Fig. 3. The existence of β-CD-chitosan complex formation could be demonstrated by spectral comparison among β-CD, and β-CD-chitosan complex. The β-CD spectrum shows α-pyranyl vibration at the wavenumber of 942cm^{-1} while chitosan exhibits β-pyranyl vibration at 922 cm^{-1} (Fig. 3a). For the inclusion complex, α-pyranyl vibration appeared at 946 cm^{-1} indicating the presence of β-CD and while the β-pyranyl vibration of chitosan might be covered by that of β-CD (Fig. 3b). Moreover, as for β-CD, a strong band at 3430 cm^{-1} was ascribed to the stretching vibration of hydroxyl group and a broader band at 3295 cm^{-1} observed in the FTIR spectrum of chitosan were attributed to OH and NH$_2$ groups. For the complex, the peak at 3286 cm^{-1} was broader due to the presence of a large quantity of hydroxyl groups introduced through β-CD (Fig. 3b).

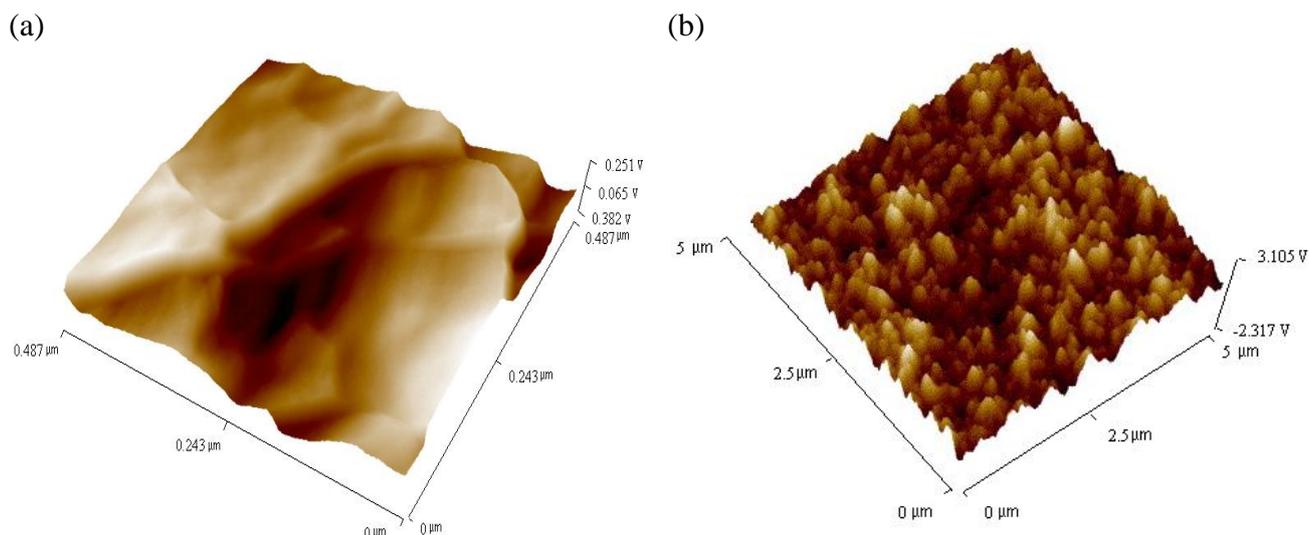**Figure 2**. UV-Visible spectra of β-CD, β-CDC complex and chitosan alone
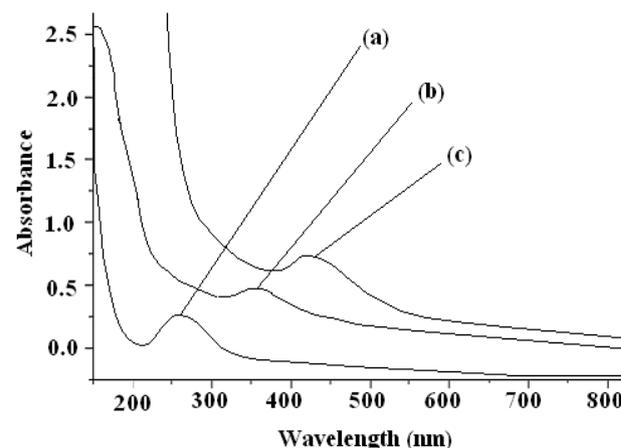
Figure 3. FTIR spectra of β-CD and β-CDC complex
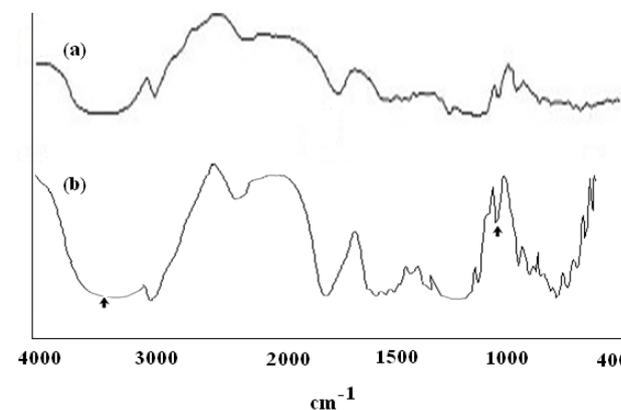
3.4. AFM Analysis

Visualization of surface topography of chitosan and chitosan encapsulated within β-CD; i.e. β-CDC complex, with AFM analysis revealed a significant change in the morphology of the surface when chitosan molecules formed complex with β-CD (Fig. 4b). The peak-to-valley distance in these images wa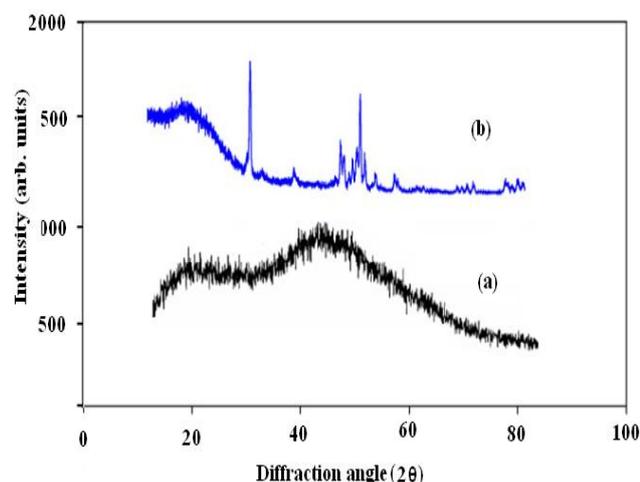s used as an indicator of the surface roughness. The surface of chitosan was smooth (Fig. 4a) as compared to the β-CD-chitosan complex surfaces. It is evident that as the encapsulation progressed the roughness of support surface increases, which could be seen from the increase of peak-to-valley value (Fig. 4b). The roughness of the sample surface is an important feature and plays an important role in affecting enzyme activity [26].

Figure 4. AFM analysis of chitosan and β-CDC complex

(a)

(b)

3.5. X-Ray Diffraction

X-ray diffraction can give useful information about the composition and crystallinity of the β-CD-chitosan complex. The XRD pattern of pure chitosan (Fig. 5a) indicates that the reflection peaks are less pronounced as compared to the inclusion complex. Meanwhile, the inclusion complex of β-CDC has one major peak at 2θ = 28° indicating the existence of a crystalline structure (Fig. 5b).

Figure 5. XRD spectral analysis of chitosan and β-CDC complex

3.6. Effect of Dosage on the Removal of Parathion

The adsorption and removal of the pesticide efficiency of parathion increased with increasing dosage of chitosan and β-CDC inclusion complex and attained the maximum of 25% and 65%, respectively, in the presence of 200 mg of the chitosan/complex (Table 1). Further increase in the amount of chitosan/complex showed no increase in the percentage of removal efficiency of parathion. The β-CDC complex was more effective in the removal of parathion as compared to pure chitosan in all the dosages studied.

3.7. Effect of Contact Time on the Removal of Pesticide

The effect of contact time on the removal of parathion by chitosan and complex was determined. The removal of pesticide increases continuously with time in both experiments. The maximum removal of parathion was within 24 h of incubation time (Table 2). Further increase in the incubation time had no significant effect on the removal of parathion. Under similar experimental conditions, the removal

efficiencies of parathion were 25% and 65%, respectively, by chitosan and β-CDC complex.

Table 1. Effect of chitosan and β-CDC inclusion complex dose on the removal of parathion

Dose of chitosan or β-CDC complex (mg/10 ml)	Parathion removal (%)	
	Chitosan only	β-CDC complex
50	7	35
100	12	46
200	25	65
400	25	66
600	25	65
800	26	67
1000	25	68

Parathion model wastewater (200 ppm, 10.0 ml) prepared in 10% DMSO was incubated with increasing amount of chitosan and β-CDC complex (50, 100, 200, 400, 600, 800, and 1000 mg per 10 ml of parathion) in 100 mM sodium acetate buffer, pH 5.5 at 40°C for 24 h. The percent removal of pesticide was calculated as described by equation 2 in the text.

Table 2. Effect of time on the removal of parathion

| Time in h	Parathion removal (%)	
	Chitosan only	β-CDC complex
12	11	31
24	25	65
36	26	67
48	25	66

The effect of time on removal of parathion by chitosan and complex was determined. 200 mg/10ml of chitosan and β-CDC complex was used to remove the parathion. The reaction was incubated upto 24 h at 40°C. The percent removal of parathion was calculated by taking untreated parathion as control (100%).

3.8. Effect of pH and Temperature

Figure 6 shows the effect of pH on the removal of parathion by chitosan and β-CD-chitosan inclusion complex. Most of the pesticides studied were removed in the pH ranges of 5.0–8.0. Maximum removal efficiency of parathion was found at pH 5.5 by pure as well as the modified chitosan. Lower or higher than this pH value, a decrease in the percentage of removal efficiency was detected.

The effect of temperature on the removal of pesticide by chitosan and the complex was illustrated in Fig. 7. Maximum removal was observed in the temperature ranges of 40-50°C and there was a decrease in the removal efficiency in the lower or higher of this temperature ranges by both adsorbents.

Figure 6. Effect of pH on the removal of parathion from model wastewater.
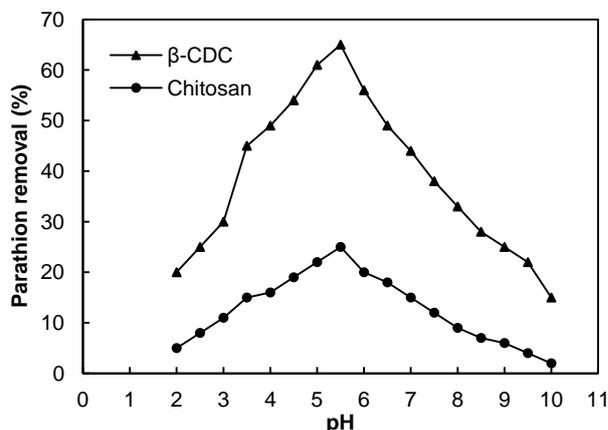
Figure 7. Effect of temperature on the removal of parathion from model wastewater.
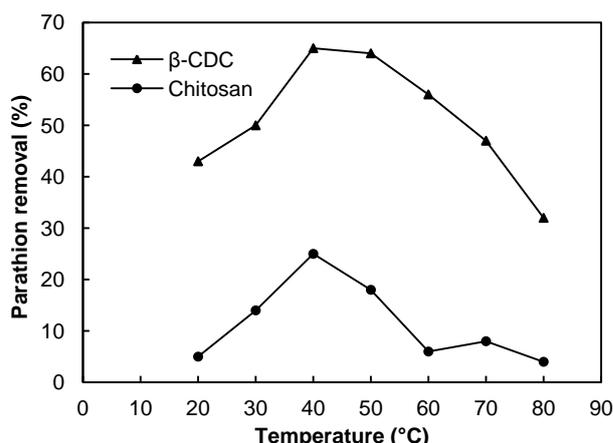
3.9. Removal of Various Pesticides and the Mixture

Table 3 shows the removal efficiency of five different pesticides with chitosan and the complex. Parathion, chlorpyrifos, dichlofenthion, phosmet and azinophos-methyl were removed up to 65%, 82%, 75%, 51% and 40%, respectively, by the complex. Maximum removal efficiency of 50% was obtained by pure chitosan for chlorpyrifos. In the case of pesticides mixture, 89% removal efficiency of pesticides by β-CD-chitosan complex was observed. However, the removal by pure chitosan was less effective as the percentage of removal efficiency was only 57%.

3.10. Removal of Pesticides and the Mixture in Batch Study

Table 3 depicts the removal of pesticides by chitosan and inclusion complex in batch study. It was observed that inclusion complex could remove only 74% of parathion within 24 h of incubation. There was 90%, 83%, 66% and 59% removal of chlorpyrifos, dichlofenthion, phosmet and azinophos-methyl, respectively, in the batch process. Complete removal of pesticides from the mixture of model wastewater was observed within the specific time. Table 3 also shows the removal efficiencies of the five pesticides and the pesticide mixture by pure chitosan. The maximum removal efficiency of 63% was observed for chlorpyrifos and the lowest removal efficiency was found for azinophos-methyl by pure chitosan.

**Table 3**. Removal of different pesticides and mixture

Name of pesticides	λ_{max}	Removal (%)	Removal (%)	Removal (%) in batch process	
		Chitosan only	β-CDC complex	Chitosan only	β-CDC complex
Parathion	275	31	65	46	74
Chlorpyrifos	255	50	82	63	90
Dichlofenthion	290	49	75	55	83
Phosmet	300	22	51	40	66
Azinophos-methyl	280	19	40	34	59
Mixture	330	57	89	77	100

All five pesticides model wastewater prepared in 10% DMSO and the mixture were incubated with 200 mg/10ml and 600 mg/500 ml of chitosan and β-CDC complex as mentioned in the text, in 100 mM sodium acetate buffer, pH 5.5 at 40°C for 24 h. Reaction mixture without chitosan and complex was used as a control. After centrifugation at 3000 rpm for 15 min, the decrease in absorbance at specific λ_{max} was monitored. The percent removal was calculated by taking untreated pesticides wastewater as control (100%).

3.11. UV Absorbance Spectra of Parathion and Mixture

The UV absorbance spectra of parathion and pesticide mixture have been taken before and after treatment by β-CDC complex (Fig. 8). The diminution in the peaks of parathion and mixture in the UV region took place due to the removal of the pesticide. However, the peaks decrease by more than 50% for both parathion and the mixture.

Figure 8. Control and treated UV visible spectra of parathion and mixture
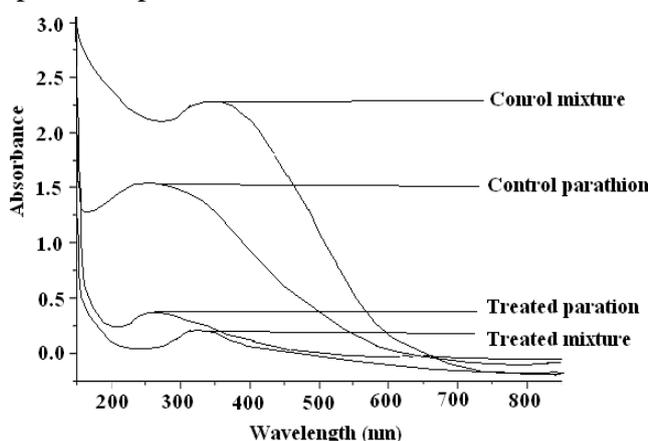
4. Discussion

The phase solubility diagram for complex formation between β-CD and chitosan is shown in Fig. 1. The plot shows a typical AL-type solubility. The phase solubility diagram indicates the feasibility of obtaining solid inclusion complex by freeze-drying soluble chitosan in β-CD solution by using 10% DMSO. The drying of solubilized chitosan in β-CD solution (1:1 mol ratio) yielded a crystalline product with the presence of small spherical particles distinguished by the formation of aggregates as observed by AFM analysis. Recently Filipovic-Grcic et al. [27] illustrated the formation of the chitosan microsphere with hydrocortisone acetate and hydroxypropyl-β-cyclodextrin and the physical mixture incorporated into chitosan microsphere by spray-drying. The 1:1 mol ratio of β-CD with drug was reported in the given study. The results in this study were also agreed with recently published work related to the formation of the inclusion complex in aqueous solution containing β-CD based urethane copolymers.

The copolymers studied were prepared at the β-CD:linker ratio 1:1, 1:2, 1:3, respectively and the best decolorization studies provided estimates of the 1:1 binding constant (K_1) for the formation of the inclusion complex [28].

At different chitosan: β-CD ratios the characteristic absorbance of inclusion complex shows gradual blue shifts from lower to higher contents of β-CD. Fig. 2 shows this effect of the formation of inclusion complex at 24 h. Huang and Yang [29] demonstrated that high amount of chitosan act as a reducing agent besides being a stabilizer or a protecting agent. Thus, the presence of high contents of chitosan would make the inclusion complex more unstable.

Another significant observation is the change in the λ_{max} as compared to β-CD and chitosan which indicates the formation of inclusion complex. Similar observation was also observed by Wang et al. [30], in which the change in the absorbance with time was observed. In another study, the formation of β-CD-polyaniline inclusion complex was confirmed by UV-visible spectral analysis. It was reported that the complex has absorption band at about 630 nm, while the pure β-CD has no absorbance between 400-900 nm.

FT-IR technique has been used to detect the formation of inclusion complex in solid phase. This technique is also used to indicate the implication of the different functional groups of the guest and host molecules in the inclusion process. It can be done by studying the changes in the shape and position of the absorbance bands of the guest, cyclodextrin, its physical mixture and that of inclusion complexes [31]. Recently, N-maleoyl was used as polymer to prepare inclusion complex with chitosan [32]. The FT–IR spectrum showed a strong band due to ν (C=O) at 1717 cm^{-1} and ν (C=C) at 1636 cm^{-1}. The structure of complex was verified from the following bands: ν (C=O) 1650 cm^{-1} (amide I), β (N–H) 1540 cm^{-1} (amide II) as shown in this study. This study suggests that there are chemisorptions between the β-CD molecules and chitosan via the hydroxyl group. Sorption of β-CD might be due to the binding of chitosan to hydroxyl group at position C_2 or C_3. The oxyanion thus formed is less nucleophilic than the non-deprotonated hydroxyl at position C_6 and therefore it is more likely to bind to the chitosan.

Figure 4 shows the surface morphology of chitosan and β-CDC complex by atomic force microscopy (AFM). A relatively smooth morphological characteristic with granular structure was observed in this figure. Similar observation was also reported by Yap et al. [33]. This morphology is mostly related to the physical property of the outmost chitosan component. The high intrinsic chain stiffness [34] and high molecular weight for the chitosan molecules affect its diffusion ability during the preparation process. Most recently, Nosal et al. [35] carried out the characterization of chitosan film by AFM technique. The surface roughness was studied from an isometric view. The image has a one micron square area and z-axis scale of 20 nm between tick marks seen in the upper left corner. It was also observed from the AFM tapping force that the tip has a low adhesion force when contacted to the modified surface as compared to unmodified chitosan. In non-technical terms, the modified chitosan is noticeably less sticky upon AFM tip contact than the bare unmodified chitosan.

Further evidence for the complex formation was obtained by X-ray power diffraction, as shown in Fig. 5. The diffraction patterns display crystallinity, whereas an amorphous pattern lacking crystalline peaks is observed for the pure chitosan. Similarly Filipovic-Grcic and co-workers [27] also reported the same change in crystallinity in the case of inclusion complex of β-CD with hydrocortisone.

The pesticides under study were selected by taking into account several factors: (a) the usage of each pesticide and how often they are applied to the crops, (b) the lifetime of each pesticide, (c) the mutual compatibility within the group of selected pesticides in order to avoid the chemical reaction or degradation between them in the same solution or application, and (d) the chemical structure of these pesticides. Adsorption is a process highly dependent on the solubility, polarity and molecule sizes, which all depend on the chemical structure of solute or the solvent, the pesticide and the polymer, respectively. To understand all these given factors, some researchers used plastic films for the removal of some pesticides from wastewaters. It was understood that the removal efficiency was completely dependent on the

solubility, polarity and the size of pesticides [36].

The 200 mg of inclusion complex was sufficient for the maximum removal of parathion fromsynthetic wastewater (Table 1). Recently various inclusion complexes were prepared with different polymers. Chatterjee et al. [37] have studied the application of chitosan beads with cetyl-trimethyl ammonium bromide in the adsorption of Congo red (CR) from aqueous solutions and found that the 373 mg/g beads were sufficient for the maximum removal of CR from aqueous solution. There were many studies related to adsorbents, such as activated carbon obtained from coir pith (6.70 mg/g), [38] neem leaf powder (41.20 mg/g), [39] acid activated red mud (7.08 mg/g), [40] bagasse fly ash (11.89 mg/g), [41] and *Aspergillus niger* biomass (8.19 mg/g) [42] etc., towards CR which has indicated that the adsorption capacity of chitosan hydrogel beads was very high. Chitosan has a charged head group, therefore an increase in chitosan concentration will increase the adsorption of pesticides molecules due to enhanced electrostatic interaction. However, when the chitosan concentration exceeded a certain maximum value, the adsorption capacities remains constant. This indicates that the concentration of chitosan in the complex has reached the saturation limit. Thus the adsorption of pesticides becomes constant after some limit.

The incubation time of parathion with inclusion complex recorded a 65% removal of parathion within 24 h (Table 2). Chiu et al. [43] had compared the time of incubation of different adsorbents for the removal of cholesterol from egg yolk and had observed that 6 h was sufficient for 3 mg/g removal of cholesterol by alumina/methane adsorbent. The difference in the time course for the removal of organophosphorus pesticide might be attributed to the structural barrier and the electron localization in them [44].

The maximum parathion removal was observed in the buffer solution of pH 5.5 by β-CD-chitosan inclusion complex (Fig. 4). This observation was in agreement with the earlier work by Wang et al. [45] where chitosan microspheres were used to select an optimum condition for the preparation of insulin-loaded chitosan microsphere. In another report, chitosan beads with cetyl trimethyl ammonium bromide were used for the removal a dye, the maximum adsorption of dye was obtained in acidic solution, however, the desorption procedure was done in alkaline solution [37]. There are various immobilized as well as soluble enzymatic processes used for the oxidative degradation and polymerization of many organic pollutants present in the wastewater at different pHs [44, 46-49].

It is confirmed that pH is an important parameter for the adsorption of pesticides in aqueous solution because it affects the solubility of pesticides, concentrations of the counter ions on the functional groups of the adsorbent and the degree of ionization of the adsorbate during reaction. Pesticides have been found to possess relatively high mobility in the aquifer environment because their sorption to mineral surfaces was usually minimal. Soil organic matter was found to be a dominant factor which influences the fate of organic contaminants in soils and aquifer material. Therefore the sorption of pesticides by β-CDC complex was mainly contributed to chitosan. At low pH (below 5.5), the amine group on chitosan is protonated to certain degree but some pesticides are not dissociated because of their weak acid properties. Physical and chemical adsorptions were known as sorption. The results indicated at pH 5.5 the chemical affinity between the OH and NH_2 groups in the structure of chitosan and the OH group in the structure of pesticides is higher. Non-polar portions play an important role in hydrophobic interactions. The mechanism involved in the association of pesticides with β-CDC is proton transfer, hydrogen bonding and van der Waals forces because there are OH and NH_2 groups in the chitosan chain [50].

The pesticide removal efficiency by inclusion complex prepared increased with increasing temperature. The maximum temperature for pesticide removal by the prepared inclusion complex was at 40°C (Fig. 7). In a study for the immobilization of chitosan with β-CD for the removal of cholesterol from egg yolk, the maximum removal of cholesterol was reported at 25°C [43].

The adsorption data of pesticides in the batch study was shown in Table 2. Most recent research study regarding the removal of ten pesticides from polluted water was

demonstrated by Liu et al. [51] and these ten different pesticides were removed in batch process by cyclodextrin polymer. Maximum removal was obtained for butenfipronil by immobilized preparation.

The main purpose to design this experiment is to aim at applying this technique to the industry. The batch process uses information, knowledge and experience from various areas, e.g. thermodynamics, chemical kinetics, fluid mechanics, mass and heat transfer, and economics. According to the Pavko [52] there are various factors that enhance the removal of pollutants in batch process, such as molecular motion and interaction, heat transfer, mass transfer as well as surface tension also.

In order to confirm the removal of parathion and pesticides mixture from the wastewater, spectral analysis using UV-Vis can be used to detect the removal of pesticides after treatment with inclusion complex. The decrease in the absorbance peaks in UV region provides a strong evidence for the removal of these pollutants from polluted water (Fig. 8). The disappearance of absorption peak in the UV region was due to the removal of parathion and the mixture from the synthetic wastewater.

5. Conclusion

In this study, the application of chitosan and β-CD-chitosan complex in the removal of organophosphorus pesticides from synthetic wastewater was investigate. The complex was prepared using freeze drying method and the characterization was carried out by using UV-Visible, FT-IR, AFM and XRD analysis. This simple procedure for the preparation of the inclusion complex would be highly useful not only in the removal of pesticides from the polluted water but also could be applied for the removal of many other hazardous aromatic pollutants that are present in wastewater. These findings suggested that the use of this preparation procedure could be extended to the large scale treatment of many contaminants and other related aromatic pollutants in industries. Due to the highly active, effective and stable binding of β-CD with chitosan, this prepared complex can be used as a matrix for the bioaffinity immobilization of enzymes in the future.

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