Research Article

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N-octyl chitosan derivatives as amphiphilic carrier agents for herbicide formulations

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Abstract: This study investigates the potential of *N*-octyl chitosan derivatives, namely *N*-octyl-*O*-sulfate chitosan (NOOSC), *N*-octyl-*N*-succinyl chitosan (NONSC) and *N*-octyl-*O*-glycol chitosan (NOOGC) as amphiphilic carrier agents for atrazine in water-insoluble herbicide formulations. The *N*-octyl chitosan derivatives were characterised using several analytical instruments such as Fourier Transform Infrared (FTIR) Spectrometer, CHNS-O Elemental Analyser (CHNS-O), Transmission Electron Microscope (TEM), Thermogravimetric Analyser (TGA), Differential Scanning Calorimeter (DSC) and Fluorescence Spectrometer. The encapsulation of atrazine by *N*-octyl chitosan derivatives was studied using a High Performance Liquid Chromatography (HPLC). The FTIR spectra of *N*-octyl chitosan derivatives confirmed the presence of hydrophobic and hydrophilic groups on chitosan backbone. TEM images revealed that *N*-octyl chitosan derivatives have formed self-aggregates with a spherical shape. The CMC values for *N*-octyl chitosan derivatives were between 0.06 and 0.09 mg/mL. The encapsulation efficiency (EE) values for amphiphilic chitosan were greater than 90%. The release profiles showed different release behaviour of pure herbicide in solution as compared to atrazine-loaded *N*-octyl chitosan derivatives. Results suggest that the chitosan derivatives offer promising characteristics that enable them to act as effective carrier agents for atrazine. In conclusion, the application of *N*-octyl chitosan derivatives could reduce the use of organic solvents in herbicide formulations by 37.5%.

Keywords: chitosan; amphiphilic; carrier agent; atrazine; herbicide formulations.

1 Introduction

Invasive weeds are one of the common problems faced by farmers causing degradation of crop quality. It was estimated that around 8,000 species of weeds were accountable for the loss of global crop production [1]. Therefore, the application of herbicides in agriculture practice is inevitable. Atrazine has moderate toxicity and widely used as a selective pre- or post-emergence herbicide worldwide, especially in the United States [2-5]. The herbicide targets the chloroplast membrane of the weeds by inhibiting the photosynthesis activity [5,6]. However, atrazine has poor solubility in water, which is about 0.028 mg/mL [7]. To overcome this issue, manufacturer often use large quantity of organic solvent to dissolve the herbicide [7]. In addition, the herbicide atrazine has high soil mobility [8]. This scenario may lead to environmental problems such as groundwater and volatile organic compounds (VOCs) contamination [8-10].

In recent years, efforts have been made in finding a good carrier system for the development of more environmentally friendly pesticide formulations [11,12]. Among them, polymeric micelles especially amphiphilic chitosan derivatives, have attracted enormous interest as potential carrier agents due to their unique properties [13-15]. The amphiphilic chitosan derivatives have ability to self-assemble into micelles and load a water-insoluble compound into their hydrophobic core [16]. The application of amphiphilic chitosan derivatives could increase the solubility of hydrophobic pesticide in water while controlling the release of the pesticide [17,18].

In this work, amphiphilic chitosan derivatives comprised of hydrophobic octyl group and three different types of hydrophilic groups, namely sulfate, succinyl and glycol, were synthesised and loaded with atrazine. The amphiphilic chitosan derivatives were characterised using several analytical instruments to evaluate their physicochemical properties. The ability of the *N*-octyl

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chitosan derivatives to encapsulate and control the release of the hydrophobic herbicide was investigated. The influence of different types of hydrophilic groups on the encapsulation of atrazine was also evaluated. The release mechanism of atrazine from *N*-octyl chitosan derivatives micelles was modelled using the semi-empirical Korsmeyer-Peppas kinetic equation.

2 Experimental

2.1 Materials

Chitosan with molecular weight of 600,000 to 800,000 Da and deacetylation degree of 92% and *N,N*-dimethylformamide (DMF) were obtained from Acros Organics. Acetic acid, chlorosulfonic acid, 2-chloroethanol, hydrochloric acid, sodium borohydride, sodium hydroxide and succinic anhydride were purchased from Merck. Atrazine, octaldehyde and pyrene were supplied by Sigma-Aldrich while phosphate buffered saline (PBS) solution was obtained from Santa Cruz Biotechnology. The solvents used in the work, namely acetone, acetonitrile (HPLC-grade) and methanol were purchased from HmbG Reagent Chemicals. All chemicals in this study were of analytical grade and deionised water was used throughout this study.

2.2 Synthesis of *N*-octyl chitosan derivatives

N-octyl chitosan derivatives were prepared by using *N*-octyl chitosan as an intermediate. The *N*-octyl chitosan (NOC) was synthesised according to the approach proposed by Zhang et al. [19]. The *N*-octyl-*O*-sulfate chitosan (NOOSC) was synthesised according to methods outlined by Ronghua et al. [20] and Zhang et al. [19], with some modifications. NOC suspension was prepared by suspending 1 g of NOC in 20 mL of DMF. Meanwhile, 10 mL of chlorosulfonic acid was added in dropwise into 40 mL of DMF, and the solution was agitated at a temperature of 0°C. After 1 hr, the NOC suspension was added and the mixture reacted at 40°C for 24 hrs. Then, an abundant amount of cold acetone was poured to precipitate the solution. The precipitate was filtered and re-dissolved in 50 mL of deionised water. The solution pH was adjusted to pH 7.0 and dialysed in a dialysis bag with molecular weight cut off (MWCO) of 14,000 Da against the deionised water, followed by lyophilisation.

N-octyl-*N*-succinyl chitosan (NONSC) was synthesised by the method proposed by Kajjari et al. [21], with some modifications. Briefly, 50 mL of 1% (v/v) of the acetic acid solution was used to dissolve 1 g of NOC and the resulting solution was diluted with 50 mL of methanol. Then, succinic anhydride solution (0.5 g in 5 mL of acetone) was slowly poured into beaker containing the NOC solution. The resulting solution was continuously stirred for 24 hrs at room temperature, before was neutralised and precipitated with cold acetone. The precipitate was re-dissolved in 50 mL deionised water. The re-dissolved NONSC solution was then dialysed (MWCO 14,000) against deionised water and lyophilised.

N-octyl-*O*-glycol chitosan (NOOGC) was synthesised using a procedure described by Shen et al. [22], with some modifications. An exact amount of NOC (1g) was suspended in 50 mL of 14% (w/v) NaOH. Then, 2-chloroethanol (2 mL) was added slowly to the NOC solution and reacted under continuous agitation for 24 hrs. The solution was neutralised and dialysed (MWCO 14,000) against deionised water and followed by lyophilisation.

2.3 Preparation of atrazine-loaded micelles

Atrazine-loaded *N*-octyl chitosan derivatives micelles were prepared by the reverse micelles method [12]. The atrazine solution with a concentration of 100 mg/L was prepared by dissolving an appropriate amount of atrazine in methanol. Each of the amphiphilic chitosan derivatives was then added to the atrazine solution at a weight ratio of 1:100 (w/w) of atrazine to *N*-octyl chitosan derivatives. Deionised water that was five-times higher than the initial volume of atrazine solution was added dropwise to the mixture solution and stirred for 2 hrs at room temperature. Then, the mixture solution was centrifuged at 1,000 rpm for 10 minutes and filtered with a 0.45 µm pore-size membrane.

The amount of encapsulated atrazine was determined by an Agilent 1200 Infinity High Performance Liquid Chromatography (HPLC). The mobile phase used in the analysis was the mixture of acetonitrile (HPLC grade) and water at a volume ratio of 70:30 (acetonitrile:water). The analysis was carried out using an Agilent Poroshell 120 C18 (50 mm x 4.6 mm, 2.7 µm) column at 40°C and a flow rate of 1.0 mL/min. The sample was injected at a volume of 15.0 µL, and the analysis was conducted at a wavelength of 220 nm. Equations 1 and 2 were applied to calculate the encapsulation efficiency (EE) and loading capacity (LC) of atrazine in the *N*-octyl chitosan derivatives micelles, respectively [23]:

$$EE = \frac{\text{Amount of atrazine in } N\text{-octyl chitosan derivatives}}{\text{Total amount of atrazine}} \times 100 \quad (1)$$

$$LC = \frac{\text{Weight of atrazine in } N\text{-octyl chitosan derivatives}}{\text{Weight of } N\text{-octyl chitosan derivatives}} \times 100 \quad (2)$$

2.4 Characterisation study

The FTIR analysis was conducted on a Thermo Nicolet 6700 ATR-FTIR Spectrometer. The FTIR spectra of chitosan and *N*-octyl chitosan derivatives were recorded at a resolution of 4 cm⁻¹ in the wavenumber range from 4000 to 500 cm⁻¹ with over 32 cumulative scans.

The ¹H NMR spectra of chitosan and *N*-octyl chitosan derivatives were recorded at 500 MHz using a Jeol JNM-ECX-500 Nuclear Magnetic Resonance Spectrometer. The solvent used to dissolve the samples in NMR analysis was acetic acid-d (CD₃COOD, 99.5%).

A CHNS-O Flash EA 1112 Series Elemental Analyser was used to determine the composition (%) of C, H, N, O and S of chitosan and *N*-octyl chitosan derivatives. The analysis was conducted by transferring approximately 2 mg of sample in a tin capsule to combustion reactor. The standard for this analysis was acetanilide while the oxidant and carrier gasses used were oxygen and helium, respectively.

The internal morphology of NOOSC, NONSC and NOOGC was observed using a Hitachi SU 8020 UHR Field Emission Scanning Electron Microscope. The sample was placed on a 300-mesh-carbon coated copper grid, and the analysis was performed at an acceleration voltage of 20 kV.

A TGA/DSC 1 Mettler Toledo Analyser was used to perform thermogravimetric and differential scanning calorimetry analyses. Approximately 17 mg of samples were heated from 25 to 900°C under argon gas at a flow rate of 20 mL/min and a heating rate of 10 °C/min.

The solubility trends of chitosan and *N*-octyl chitosan derivatives in a series of aqueous solution at various pH values (1.0 to 13.0) were determined by the percentage of transmittance [24]. The experiment was conducted by dissolving each sample in 2% (v/v) HCl to obtain a sample solution with concentration of 2 mg/mL. The pH of the sample solution was altered using NaOH solutions (0.1-4.0 mol/L), and the percentage of transmittance was recorded using an Agilent Cary 60 UV-Visible Spectrophotometer at 600 nm.

2.5 Critical micelles concentration (CMC) study

The critical micelles concentration (CMC) of the *N*-octyl chitosan derivatives was determined using an Agilent Cary Eclipse Fluorescence Spectrometer [25]. In this study, pyrene was applied as a hydrophobic probe. Briefly, 1 mL of pyrene in methanol (1.25 x 10⁻³ mg/mL) was added into a beaker and thereafter the methanol was evaporated. Then, 6 mL of the *N*-octyl chitosan derivatives solution with the concentration varies from 1.0 x 10⁻⁴ to 1.0 mg/mL was added individually in a beaker containing methanol-free pyrene. The *N*-octyl chitosan derivatives and pyrene mixture solution was homogenised by using a sonicator for 10 minutes. The pyrene emission was determined from 344 to 700 nm with excitation wavelength set at 334 nm. Both excitation and emission slit openings of the instrument were set at 5 nm. The ratios of (I₃=373 nm) and (I₃=392 nm) against logarithms concentration of the *N*-octyl chitosan derivatives were then plotted to obtain the CMC value of the chitosan derivatives.

2.6 In vitro release study

The *in vitro* release study of atrazine from *N*-octyl chitosan derivatives micelles were carried out through the dialysis method under sink conditions [23]. The *in vitro* release study was carried out by dissolving by approximately 5 mg of each atrazine-loaded *N*-octyl chitosan derivative in 3 mL of PBS solution (0.1 M, pH 7.4). The atrazine-loaded *N*-octyl chitosan derivative solution or pure atrazine solution was subsequently placed in a dialysis bag (MWCO 14,000 Da) and immersed in a beaker containing 250 mL of PBS solution. The PBS solution was continuously agitated (100 rpm) at room temperature. At the predetermined time interval, the amount of atrazine released from the dialysis bag was measured by collecting 3 mL of PBS solution from the beaker and replacing it with 3 mL of fresh PBS. The measurement was conducted by means of an Agilent Cary 60 UV-Visible Spectrophotometer at 220 nm. The release study for each sample was performed in three replicates.

The release mechanism of atrazine from *N*-octyl chitosan derivative micelles was determined by fitting the release data of atrazine to semi-empirical Korsmeyer-Peppas kinetic model (equation 3) [26,27]:

$$\frac{M_t}{M_\infty} = kt^n \quad (3)$$

Figure 1: The synthetic scheme of *N*-octyl chitosan derivatives.

where M_t indicates the amount of atrazine released at time t and M_∞ represents the maximum amount of atrazine released. The k is the constant that incorporates the characteristic of the polymeric carrier and atrazine, while n indicates the release mechanism. A linear graph of $\ln(M_t/M_\infty)$ versus $\ln(t)$ can be used to determine the value of release constant (k), release mechanism (n) and correlation coefficient (R^2).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

3.1 Synthesis and characterisation of *N*-octyl chitosan derivatives

The *N*-octyl chitosan derivatives were prepared by introducing the hydrophobic group (octyl) and followed by hydrophilic groups (sulfate, succinyl and glycol). The synthetic scheme for the syntheses of *N*-octyl chitosan derivatives are summarised in Figure 1. As shown in Figure 1, the hydrophobic octyl group was introduced by a

Schiff base reaction between the amino group of chitosan and octaldehyde. The NOOSC, NONSC and NOOGC were synthesised by covalently linking the sulfate, succinyl and glycol groups on the chitosan backbone.

The chemical structures of chitosan and the *N*-octyl chitosan derivatives were confirmed by FTIR analysis. The FTIR spectra of chitosan, NOOSC, NONSC and NOOGC are shown in Figure 2. Based on Figure 2a, basic absorption bands for chitosan appeared at 3440 cm^{-1} ($-\text{OH}$ and $-\text{NH}$ stretches), 2919 and 2870 cm^{-1} ($-\text{CH}$ stretches) and 1646 and 1596 cm^{-1} (NH_2 scissoring vibration) [28,29]. Two absorption peaks at 1421 and 1383 cm^{-1} are attributed to C-N stretching vibration [28,29]. The characteristic frequencies of C-O stretching vibration for secondary and primary alcohol groups appeared at 1153 and 1081 cm^{-1} [28,29].

Following the alkylation process with octaldehyde, the intensity of the absorption band related to $-\text{CH}$ stretching vibration in the FTIR spectra of NOOSC, NONSC and NOOGC was increased significantly (Figures 2b, 2c and 2d). This scenario could be due to the addition of octyl group to amine group of chitosan [30,31]. The appearance of new peaks positioned at 1243 and 1212 cm^{-1} in the FTIR spectrum of NOOSC (Figure 2b), could be attributed to the sulfate group [32,33]. Moreover, the conjugation

Figure 2: FTIR spectra of (a) chitosan, (b) NOOSC, (c) NONSC and (d) NOOGC.

of the sulfate group has shifted the absorption band corresponding to -OH stretch from 3440 to 3278 cm^{-1} and C-O of primary alcohol from 1081 to 989 cm^{-1} [32,33].

The absorption intensity of NH_2 scissoring vibration of chitosan at 1646 and 1596 cm^{-1} (Figure 2a) increased prominently and shifted to new wavenumbers at 1639 and 1544 cm^{-1} (Figure 2c), which could be due to addition of a C=O stretching vibration from succinyl groups conjugated at amine group of chitosan [34,35]. Furthermore, the changes were also observed in the intensity and wavenumbers of absorption band related to

C-N and C-O stretching vibrations following conjugation of carboxyl group of the succinyl group [36,37]. The shifted wavenumbers related to -OH and C-O stretching vibrations in the FTIR spectrum of NOOGC (Figure 2d) may be related to the incorporation of the glycol group at 6-OH of chitosan [30,38]. Presumably, the aforementioned changes suggest that the NOOSC, NONSC and NOOGC were successfully synthesised.

The chemical structures of chitosan and *N*-octyl chitosan derivatives were further confirmed by ^1H NMR analysis. The peak at a chemical shift of 2.02 ppm , which

Figure 3: ^1H NMR spectra of (a) chitosan and (b) NOC in acetic acid-d (99.5%).

corresponded to solvent, was used as a reference to interpret the NMR spectrum. Figure 3 shows the ^1H NMR spectra of chitosan and NOC. The ^1H NMR spectrum of chitosan (Figure 3a) shows signals at chemical shifts of 3.32 to 4.05 ppm and 5.08 ppm, which correspond to the proton at 2-H to 6-H and 1-H, respectively [30]. As compared to the chitosan spectrum, the ^1H NMR spectrum of *N*-octyl chitosan (Figure 3b) shows that the signals correspond to the proton of octyl group at chemical shifts of 0.79 ppm ($-\text{NHCH}_2(\text{CH}_2)_6\text{CH}_3$), 2.31 ppm ($-\text{NHCH}_2(\text{CH}_2)_6\text{CH}_3$) and 3.38 ppm ($-\text{NHCH}_2(\text{CH}_2)_6\text{CH}_3$) [31,32].

Based on the ^1H NMR spectrum of *N*-octyl chitosan, the signal related to the octyl group can be observed in the ^1H NMR spectrum of NOOSC (Figure 4a), which suggest that NOOSC was successfully synthesised [33]. In addition, the appearance of peaks at 2.76 and 2.88 ppm in the ^1H NMR spectrum of NONSC (Figure 4b) could be corresponded to the proton of the succinyl group [36,37]. The overlap peaks observed in the ^1H NMR spectrum of NOOGC (Figure 4c) at

a chemical shift of 3.29 to 3.92 ppm could be related to the proton of the glycol group and chitosan [30].

The results for the elemental composition of chitosan and *N*-octyl chitosan derivatives are listed in Table 1. It is clear that the experimental results for the composition of C, H, N, O and S of the samples are close to the theoretical values. For instance, the weight percentage of C, H, N, O and S for NOOSC was determined as 46.88 ± 1.3 , 7.38 ± 0.8 , 4.50 ± 0.5 , 32.82 ± 0.9 and $8.42 \pm 0.7\%$, respectively. Meanwhile, the theoretical weight percentage of C, H, N, O and S for NOOSC was calculated as 47.32, 7.64, 4.42, 32.44 and 8.23%, respectively. Based on the elemental analysis data, the degree of substitution (DS) of octyl group on chitosan was $6.81 \pm 4.16\%$. Therefore, the elemental composition results suggest that the chitosan derivatives were successfully synthesised in this study.

TEM micrographs of NOOSC, NONSC and NOOGC micelles at 100,000x magnifications are presented in Figure 5. The TEM images revealed that the *N*-octyl

(a)

(b)

(c)

Figure 4: ^1H NMR spectra of (a) NOOSC, (b) NONSC and (c) NOOGC in acetic acid-d (99.5%).

Table 1: The elemental composition of chitosan, NOC, NOOSC, NONSC and NOOGC.

Sampel	Chemical formula	Molecular weight (g/mol)	Weight percent (%)				
			C	H	N	O	S
Chitosan	(C ₆ H ₁₁ NO ₄) _n	161.16	44.08 ± 0.2 (44.72)	7.94 ± 0.8 (6.88)	6.92 ± 0.1 (8.69)	41.06± 0.5 (39.71)	-
NOC	(C ₂₀ H ₃₈ N ₂ O ₈) _n	434.52	54.17 ± 0.3 (55.28)	9.83± 0.4 (8.81)	8.32± 0.6 (6.45)	28.68 ± 0.8 (29.46)	-
NOOSC	(C ₃₄ H ₆₅ N ₃ O ₁₈ S ₂) _n	868.02	46.88 ± 1.3 (47.05)	7.38 ± 0.8 (7.55)	4.50 ± 0.5 (4.84)	32.82 ± 0.9 (33.18)	8.42 ± 0.7 (7.39)
NONSC	(C ₃₀ H ₅₃ N ₃ O ₁₅) _n	695.75	54.05 ± 0.5 (51.79)	8.10 ± 0.8 (7.68)	5.27 ± 0.7 (6.04)	32.58 ± 0.3 (34.49)	-
NOOGC	(C ₃₈ H ₇₃ N ₃ O ₁₄) _n	796.00	59.72± 0.4 (57.34)	7.85 ± 0.3 (9.24)	6.68 ± 0.7 (5.28)	25.75 ± 0.1 (28.14)	-

Theoretical values are in brackets. Values represent mean of three replicates ± standard deviation.

Figure 5: TEM images of (a) NOOSC, (b) NONSC and (c) NOOGC at 100,000x magnifications.

chitosan derivatives micelles were capable to form self-aggregates in aqueous solution. It is apparent that *N*-octyl chitosan derivatives exhibit nearly spherical shape micelles. From Figure 5a, the size at dry state of the NOOSC micelles ranged from 47.6 to 101 nm. Meanwhile, the NONSC (Figure 5b) and NOOGC (Figure 5c) exhibit the

micelles size ranged from 65.5 to 177 nm and 41.7 to 85.3 nm, respectively. The NONSC micelles show the largest aggregation size range followed by NOOSC and NOOGC. Figure 6 displays the TGA thermograms of chitosan, NOOSC, NONSC and NOOGC. Chitosan exhibits two decomposition stages at 54°C and 328°C. The first

Figure 6: TGA thermograms of chitosan, NOOSC, NONSC and NOOGC.

decomposition stage was due to the vaporisation of moisture content while the second was related to the dehydration of the polysaccharide ring and decomposition of chitosan polymer [39]. Two weight loss stages were observed during thermal decomposition of NOOSC. The first weight loss occurred at 53 °C with a weight loss of 21%, which was due to the loss of moisture content bound to the polymer [31,33]. The second weight loss reached its maximum at 335 °C with a weight loss of 46%, which corresponds to the decomposition of NOOSC [31,33].

The thermal decomposition of NONSC occurred at two different temperatures, namely 69 °C and 395 °C. The initial weight loss (23%) was related to water desorption from the polymer, while the second weight loss (45%) corresponds to the degradation of the polymer and succinic acid group [21,34]. The thermal decomposition of NOOGC involved three major steps of weight loss at 57 °C, 284 °C and 470 °C. The weight loss steps were attributed to the desorption of the water content, followed by the degradation of the low molecular polymer and finally by the decomposition of the high molecular weight polymer and glycolidic bond

[31,40]. The addition of hydrophobic and hydrophilic groups altered the thermal stability of the chitosan.

The DSC thermograms of the chitosan and the *N*-octyl chitosan derivatives are presented in Figure 7. Chitosan and *N*-octyl chitosan derivatives show characteristics of an endothermic peak at temperatures below 100 °C, which attributed to the loss of water associated with the hydrophilic segments of the polymers [21,41,42]. The exothermic peak observed at 306 °C of the DSC thermogram of chitosan (Figure 7a) could be related to the degradation of the saccharide structure in the chitosan [43,44]. As shown in Figure 7b, the appearance of the two endothermic peaks on the NOOSC curve at 276 °C and 453 °C corresponds to melting and the dissociation of the chitosan derivatives [19,43].

The exothermic peaks at 294 °C and 775 °C of NONSC (Figure 7c) are associated with degradation of the polymer and succinic acid [21]. Meanwhile, the curve of NOOGC shows an exothermic peak at 292 °C and an endothermic peak at 479 °C which may be due to polymer decomposition and dissociation of glycol chitosan [44,45].

Figure 7: DSC thermograms of (a) chitosan, (b) NOOSC, (c) NONSC and (d) NOOGC.

Results obtained from thermal analysis suggest that the insertion of hydrophobic and hydrophilic groups to the chitosan backbone improved its thermal stability.

The solubility properties of chitosan, NOOSC, NONSC and NOOGC are shown in Figure 8. Chitosan was soluble at a pH less than 7.0 due to the protonation of the amine group in the acidic medium [46]. On the other hand, chitosan was insoluble in both neutral and basic media attributed to the rigid crystalline structure of chitosan that has a strong hydrogen bond. In contrast to chitosan, all *N*-octyl chitosan derivatives exhibited excellent solubility properties at pH values of 1.0 to 13.0. The percentage of transmittance (%) for NOOSC, NONSC and NOOGC were above 80%. The presence of –OH and –COOH functional groups from sulfate, succinyl and glycol groups, has endorsed the formation of hydrogen bonds between the chitosan and hydrogen atom in water. However, the NONSC was slightly insoluble (78%) at pH 5.0 due to the isoelectric point of equimolar co-existing of NH_3^+ and COO^- [47]. Overall, NOOSC, NONSC and NOOGC endow the characteristics of excellent carrier agents for atrazine.

3.2 Critical micelle concentration (CMC) of *N*-octyl chitosan derivatives micelles

The fluorescence probe technique was applied to determine the critical micelle concentration (CMC) value of the *N*-octyl chitosan derivatives. The CMC was described as the threshold concentration in which self-aggregation of polymeric micelle can form via intra- and/or intermolecular association [25]. Pyrene was used as a hydrophobic probe due to its strong hydrophobic properties and self-quenching property in aqueous solutions. Were the micelle to present in the aqueous solution, the pyrene would automatically localise inside the micelle hydrophobic core and strongly emit fluorescence intensity [25].

Figure 9a displays one of the examples of fluorescence emission spectra of pyrene in the NOOGC micelles solution at concentration of 1.0×10^{-4} to 1.0 mg/mL. The intensity of fluorescence emission spectra exhibits an increasing trend as the concentration of NOOGC increases until a certain concentration is reached. The emission intensity ratio of the first peak (I_{373}) and third peak (I_{392}) was used to determine the aggregation behaviour of the *N*-octyl chitosan derivatives as it was highly sensitive to

Figure 8: Solubility of chitosan, NOOSC, NONSC and NOOGC at pH 1.0 to 13.0. Values represent mean of three replicates $\pm$ standard deviation.

Table 2: The encapsulation efficiency and loading capacity of NOOSC, NONSC and NOOGC.

Samples	Encapsulation efficiency (%)	Loading capacity (%)
NOOSC	91.15 $\pm$ 0.42	0.09 $\pm$ 0.01
NONSC	93.80 $\pm$ 0.51	0.09 $\pm$ 0.05
NOOGC	92.13 $\pm$ 0.24	0.09 $\pm$ 0.02

Values represent mean of three replicates $\pm$ standard deviation.

the change in the surrounding environment [44]. The CMC value of NOOSC, NONSC and NOOGC can be determined from the crossover point of the intensity ratio of I_{373}/I_{392} versus logarithm concentration of the chitosan derivatives [25].

Figure 9(b) shows as an example a graph of the intensity ratio of I_{373}/I_{392} of the pyrene emission spectra versus logarithm concentration of NOOGC. The CMC value of NOOSC, NONSC and NOOGC was determined as 0.062, 0.074 and 0.089 mg/mL, respectively. Overall, the NOOSC had the lowest CMC value, followed closely by NONSC and NOOGC. The CMC values determined for *N*-octyl chitosan derivatives were found to be lower than that of the low molecular surfactant sodium dodecyl sulfate (2.3 mg/mL) [48] and poloxamer (1.0–24 mg/mL) [49]. Moreover, the CMC values of *N*-octyl chitosan derivatives could be considered comparable with other polymeric micelles such

as deoxycholic acid chitosan-grafted poly(ethylene glycol) methyl ether (0.0657 mg/mL) [50] and carboxymethyl pachyman-deoxycholic acid conjugates (5.89×10^{-3} to 1.55×10^{-2} mg/mL) [51]. Results from this experiment suggest that the NOOSC, NONSC and NOOGC micelles can form stable self-aggregation in an aqueous solution even after dilution [25].

3.3 Encapsulation efficiency and loading capacity of atrazine-loaded *N*-octyl chitosan derivatives

The encapsulation efficiency (EE%) and loading capacity (LC%) of atrazine into each of the *N*-octyl chitosan derivatives micelles are presented in Table 2. From Table 2, the *N*-octyl chitosan derivatives micelles can encapsulate more than 90% of atrazine in its micelles. Moreover, the loading capacity of the atrazine-loaded *N*-octyl chitosan derivatives micelles was around 0.09%. It was observed that there was no significant difference in the amount of atrazine that could be encapsulated by NOOSC, NONSC and NOOGC. It was apparent that the types of hydrophilic segments did not significantly influence the encapsulation of atrazine. This may be due to the *N*-octyl chitosan derivatives having the same hydrophobic moieties (octyl group) as the entrapment of hydrophobic molecules which usually occur through the interaction

Figure 9: (a) The fluorescence spectra of pyrene (1.25×10^{-3} mg/mL) in NOOGC solution (1.0, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 and 0.0001 mg/mL). (b) The intensity ratio (I_{373}/I_{392}) of pyrene versus the logarithm concentration (Log. C) of NOOGC.

with hydrophobic moieties of the micelles [30]. High encapsulation efficiencies achieved by NOOSC, NONSC and NOOGC indicated that the chitosan derivatives have good affinity towards atrazine, a hydrophobic herbicide [30].

3.4 *In vitro* release of atrazine-loaded *N*-octyl chitosan derivatives

The release profile of atrazine from NOOSC, NONSC and NOOGC micelles in PBS solution are shown in Figure 10. The release profile of all atrazine-loaded *N*-octyl chitosan derivatives showed an initial burst release during the first 10 hrs of the *in vitro* study, followed by controlled

release behaviour. The burst release profile recorded at the beginning of the *in vitro* study could be based on the dissolution of the herbicide adsorbed on the surface of *N*-octyl chitosan derivatives or entrapped in between hydrophilic moieties of the chitosan derivatives [52]. After 10 hrs, the release of atrazine came from the hydrophobic core of the chitosan derivatives [52]. It is evident that the NOOSC, NONSC and NOOGC could prolong the release of atrazine up to 57, 60 and 58 hrs, respectively. In comparison, the pure atrazine solution underwent a rapid release profile whereby around 50% of the herbicide was released from a dialysis bag within 8 hrs and completely released at about 29 hrs. According to Hu et al. [53], the release rate of a hydrophobic compound from core micelles is mainly affected by its interaction with hydrophobic moieties of the

Figure 10: *In vitro* release of atrazine and atrazine-loaded *N*-octyl chitosan derivatives micelles in PBS solution at room temperature. Values represent mean of three replicates $\pm$ standard deviation.

polymer. As the *N*-octyl chitosan derivatives comprised of the same hydrophobic group, the release of atrazine from its micelles mainly influenced by the amount of atrazine entrapped in the chitosan derivatives micelles.

The release profile data of atrazine were fitted to the Korsmeyer-Peppas kinetic model to obtain further information related to the release of the atrazine from the *N*-octyl chitosan derivatives. The parameters of the the Korsmeyer-Peppas kinetic model are shown in Table 3. Based on the correlation coefficient value (R^2), the release data of atrazine fitted well with the Korsmeyer-Peppas model ($R^2 > 0.96$). The value of the release exponent (n) of NOOSC, NONSC and NOOGC, which indicates the type of release mechanism involved, was higher than 0.85. These findings suggest that the release of the atrazine from

Table 3: Parameters of Korsmeyer-Peppas kinetic model for *in vitro* controlled release of atrazine-loaded *N*-octyl chitosan micelles in PBS solution at room temperature and pH 7.4.

Samples	k (hr^{-1})	n	R^2
NOOSC	0.043	0.852	0.977
NONSC	0.030	0.918	0.969
NOOGC	0.039	0.882	0.976

the *N*-octyl chitosan derivative micelles follow the case II transport, which associate with stress and the state transition of the hydrophilic group in polymer (relaxation of polymer chain) in water and biological fluids [26,41]. Based on the release constant (k) values presented in Table

3, NONSC has the lowest *k* value which demonstrates the slowest atrazine release, followed by NOOGC and NOOSC. These data were in accordance with the experimental data for the release of atrazine from the *in-vitro* released study.

4 Conclusions

In this research, the amphiphilic chitosan derivatives NOOSC, NONSC and NOOGC were successfully synthesised and characterised. Their key property is the presence of hydrophilic segments (sulfate, succinyl and glycol) and hydrophobic region (octyl) that enabled the formation of micelles in aqueous solution. The NOOSC, NONSC and NOOGC micelles have a good affinity towards atrazine, thus able to encapsulate the herbicide up to 93.80%. The *N*-octyl chitosan derivatives enhanced the solubility of atrazine 3.4% than pure atrazine in water. By introducing the *N*-octyl chitosan derivatives as carrier agents in herbicide formulations, the application of an organic solvent could be reduced by 37.5%. The ability of NOOSC, NONSC and NOOGC to encapsulate and control the release of atrazine would be an advantage in formulating environmentally friendly agrochemical products.

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References

- [1] Zhang W., Jiang F., Ou J., Global pesticide consumption and pollution: with China as a focus, *Proc. Int. Acad. Ecol. Environ. Sci.*, 2011, 1, 125-144.
- [2] Kah M., Machinski P., Koerner P., Tiede K., Grillo R., Fraceto L.F., et al., Analysing the fate of nanopesticides in soil and the applicability of regulatory protocols using a polymer-based nanoformulation of atrazine, *Environ. Sci. Pollut. Res.*, 2014, 21, 11699-11707.
- [3] Lobo F.A., de Aguirre C.L., Silva M.S., Grillo R., de Melo N.F.S., de Oliveira L.K., et al., Poly (hydroxybutyrate-co-hydroxyvalerate) microspheres loaded with atrazine herbicide: screening of conditions for preparation, physico-chemical characterization, and *in vitro* release studies, *Polym. Bull.*, 2011, 67, 479-495.
- [4] Mitchell P.D., Market-level assessment of the economic benefits of atrazine in the United States., *Pest Manag. Sci.*, 2014, 70, 1684-1696.
- [5] Marutescu L., Chifiriuc M.C., Molecular mechanisms of pesticides toxicity, In: Grumezescu A.M. (Ed.), *New Pesticides and Soil Sensors*, Academic Press, 2017, 393-435.
- [6] Venceslau A.d.F.A., dos Santos F.E., de Fátima Silva A., Rocha D.A., de Abreu A.J., Jaime C., et al., Cyclodextrins as effective tools to reduce the toxicity of atrazine, *Energ. Ecol. Environ.*, 2018, 3, 81-86.
- [7] Brand R.M., Mueller C., Transdermal penetration of atrazine, alachlor, and trifluralin: effect of formulation, *Toxicol. Sci.*, 2002, 68, 18-23.
- [8] Grillo R., de Melo N.F.S., de Lima R., Lourenço R.W., Rosa A.H., Fraceto L.F., Characterization of atrazine-loaded biodegradable poly (hydroxybutyrate-co-hydroxyvalerate) microspheres, *J. Polym. Environ.*, 2010, 18, 26-32.
- [9] Capello C., Wernet G., Sutter J., Hellweg S., Hungerbühler K., A comprehensive environmental assessment of petrochemical solvent production, *Int. J. Life Cycle Assess.*, 2009, 14, 467-479.
- [10] Jessop P.G., Ahmadpour F., Buczynski M.A., Burns T.J., Green Ii N.B., Korwin R., et al, Opportunities for greener alternatives in chemical formulations, *Green Chem.*, 2015, 17, 2664-2678.
- [11] Hazra D.K., Recent advancement in pesticide formulations for user and environment friendly pest management, *Int. J. Res. Rev.*, 2015, 2, 35-40.
- [12] Lao S.B., Zhang Z.X., Xu H.H., Jiang G.B., Novel amphiphilic chitosan derivatives: Synthesis, characterization and micellar solubilization of rotenone, *Carbohydr. Polym.*, 2010, 82, 1136-1142.
- [13] Feng B., Ashraf M.A., Peng L., Characterization of particle shape, zeta potential, loading efficiency and outdoor stability for chitosan-ricinoleic acid loaded with rotenone, *Open Life Sci.*, 2016, 11, 380-386.
- [14] Lucia A., Toloza A.C., Guzmán E., Ortega F., Rubio R.G., Novel polymeric micelles for insect pest control: encapsulation of essential oil monoterpenes inside a triblock copolymer shell for head lice control, *PeerJ*, 2017, 5, e3171.
- [15] Mei X.D., Liang Y.H., Zhang T., Ning J., Wang Z.Y., An amphiphilic chitosan-poly lactide graft copolymer and its nanoparticles as fungicide carriers, *Adv. Mater. Res.*, 2014, 1051, 21-28.
- [16] Li Y., Zhang S., Meng X., Chen X., Ren G., The preparation and characterization of a novel amphiphilic oleoyl-carboxymethyl chitosan self-assembled nanoparticles. *Carbohydr. Polym.*, 2011, 83, 130-136.
- [17] Kuskov A.N., Kulikov P.P., Goryachaya A.V., Tzatzarakis M.N., Tsatsakis A.M., Velonia K., et al., Self-assembled amphiphilic poly-*N*-vinylpyrrolidone nanoparticles as carriers for hydrophobic drugs: Stability aspects, *J. App. Polym. Sci.*, 2018, 135, 45637.
- [18] Liu G., Gan J., Chen A., Liu Q., Zhao X., Synthesis and characterization of an amphiphilic chitosan bearing octyl and

- methoxy polyethylene glycol groups, *Nat. Sci.*, 2010, 2, 707-712.
- [19] Zhang C., Qineng P., Zhang H., Self-assembly and characterization of paclitaxel-loaded *N*-octyl-*O*-sulfate chitosan micellar system, *Colloids Surf. B Biointerfaces*, 2004, 39, 69-75.
 - [20] Huang R., Du Y., Yang J., Preparation and anticoagulant activity of carboxybutyrylated hydroxyethyl chitosan sulfates, *Carbohydr. Polym.*, 2003, 51, 431-438.
 - [21] Kajjari P.B., Manjeshwar L.S., Aminabhavi T.M., Novel blend microspheres of poly(vinyl alcohol) and succinyl chitosan for controlled release of nifedipine, *Polym. Bull.*, 2013, 70, 3387-3406.
 - [22] Shen C.R., Liu C.L., Lee H.P., Chen J.K., The identification and characterization of chitotriosidase activity in pancreatin from porcine pancreas, *Molecules*, 2013, 18, 2978-2987.
 - [23] Zhang J., Li M., Fan T., Xu Q., Wu Y., Chen C., et al., Construction of novel amphiphilic chitosan copolymer nanoparticles for chlorpyrifos delivery, *J. Polym. Res.*, 2013, 20, 107.
 - [24] Kubota N., Tatsumoto N., Sano T., Toya K., A simple preparation of half *N*-acetylated chitosan highly soluble in water and aqueous organic solvents, *Carbohydr. Res.*, 2000, 324, 268-274.
 - [25] Wang F., Zhang D., Duan C., Jia L., Feng F., Liu Y., et al., Preparation and characterizations of a novel deoxycholic acid-*O*-carboxymethylated chitosan-folic acid conjugates and self-aggregates, *Carbohydr. Polym.*, 2011, 84, 1192-1200.
 - [26] Korsmeyer R.W., Gurny R., Doelker E., Buri P., Peppas N.A., Mechanisms of solute release from porous hydrophilic polymers, *Int. J. Pharm.*, 1983, 15, 25-35.
 - [27] Pereira A.E.S., Grillo R., Mello N.F.S., Rosa A.H., Fraceto L.F., Application of poly(epsilon-caprolactone) nanoparticles containing atrazine herbicide as an alternative technique to control weeds and reduce damage to the environment, *J. Hazard. Mater.*, 2014, 268, 207-215.
 - [28] He L., Wang H., Xia G., Sun J., Song R., Chitosan/graphene oxide nanocomposite films with enhanced interfacial interaction and their electrochemical applications, *Appl. Surf. Sci.*, 2014, 314, 510-515.
 - [29] Zuo P.P., Feng H.F., Xu Z.Z., Zhang L.F., Zhang Y.L., Xia W., et al., Fabrication of biocompatible and mechanically reinforced graphene oxide-chitosan nanocomposite films, *Chem. Cent. J.*, 2013, 7, 39.
 - [30] Huo M., Zhang Y., Zhou J., Zou A., Yu D., Wu Y., et al., Synthesis and characterization of low-toxic amphiphilic chitosan derivatives and their application as micelle carrier for antitumor drug, *Int. J. Pharm.*, 2010, 394, 162-173.
 - [31] Qu D., Lin H., Zhang N., Xue J., Zhang C., In vitro evaluation on novel modified chitosan for targeted antitumor drug delivery, *Carbohydr. Polym.*, 2013, 92, 545-554.
 - [32] Roldo M., Power K., Smith J.R. Cox P.A., Papagelis K., Bouropoulos N., et al., *N*-octyl-*O*-sulfate chitosan stabilises single wall carbon nanotubes in aqueous media and bestows biocompatibility, *Nanoscale*, 2009, 1, 366-373.
 - [33] Zhang C. Ping Q., Zhang H., Shen J., Preparation of *N*-alkyl-*O*-sulfate chitosan derivatives and micellar solubilization of taxol, *Carbohydr. Polym.*, 2003, 54, 137-141.
 - [34] Jain N., Rajoriya V., Jain P.K., Jain A.K., Lactosaminated-*N*-succinyl chitosan nanoparticles for hepatocyte-targeted delivery of acyclovir, *J. Nanopart. Res.*, 2014, 16, 2136.
 - [35] Hou Z., Han J., Zhan C., Zhou C., Hu Q., Zhang Q., Synthesis and evaluation of *N*-succinyl-chitosan nanoparticles toward local hydroxycamptothecin delivery, *Carbohydr. Polym.*, 2010, 81, 765-768.
 - [36] Lin Z.T., Song K., Bin J.P., Liao Y.L., Jiang G.B., Characterization of polymer micelles with hemocompatibility based on *N*-succinyl-chitosan grafting with long chain hydrophobic groups and loading aspirin, *J. Mater. Chem.*, 2011, 21, 19153-19165.
 - [37] Sui W., Wang Y., Dong S., Chen Y., Preparation and properties of an amphiphilic derivative of succinyl-chitosan, *Colloids Surf. A Physicochem. Eng. Asp.*, 2008, 316, 171-175.
 - [38] Cho I.S., Park C.G., Huh B.K. Cho M.O., Khatun Z., Li Z., et al., Thermosensitive hexanoyl glycol chitosan-based ocular delivery system for glaucoma therapy, *Acta Biomater.*, 2016, 39, 124-132.
 - [39] Liu Y., Sun Y., He S., Zhu Y., Ao M., Li J., et al., Synthesis and characterization of gibberellin-chitosan conjugate for controlled-release applications, *Int. J. Biol. Macromol.*, 2013, 57, 213-217.
 - [40] Mishra S.K., Kannan S., Development, mechanical evaluation and surface characteristics of chitosan/polyvinyl alcohol based polymer composite coatings on titanium metal, *J. Mech. Behav. Biomed. Mater.*, 2014, 40, 314-324.
 - [41] Silva M.d.S., Cocenza D.S., Grillo R., de Melo N.F.S., Tonello P.S. de Oliveira L.C., et al., Paraquat-loaded alginate/chitosan nanoparticles: Preparation, characterization and soil sorption studies, *J. Hazard. Mater.*, 2011, 190, 366-374.
 - [42] Fei X., Yu M., Zhang B., Cao L., Yu L., Jia G., et al., The fluorescent interactions between amphiphilic chitosan derivatives and water-soluble quantum dots, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, 2016, 152, 343-351.
 - [43] Shelma R., Sharma C.P., Development of lauroyl sulfated chitosan for enhancing hemocompatibility of chitosan, *Colloids Surf. B Biointerfaces*, 2011, 84, 561-570.
 - [44] Sukamporn P., Baek S.J., Gritsanapan W., Chirachanchai S., Nualsanit T., Rojanapanthu P., Self-assembled nanomicelles of damncanthal-loaded amphiphilic modified chitosan: Preparation, characterization and cytotoxicity study, *Mater. Sci. Eng. C*, 2017, 77, 1068-1077.
 - [45] Quiñones J.P., Gothelf K.V., Kjems J., Caballero Á.M.H., Schmidt C., Covas C.P., Self-assembled nanoparticles of glycol chitosan - Ergocalciferol succinate conjugate, for controlled release, *Carbohydr. Polym.*, 2012, 88, 1373-1377.
 - [46] Mourya V.K., Inamdar N.N., Tiwari A., Carboxymethyl chitosan and its applications, *Adv. Mater. Lett.*, 2010, 1, 11-33.
 - [47] Bashir S., Teo Y.Y., Ramesh S., Ramesh K., Khan A.A., *N*-succinyl chitosan preparation, characterization, properties and biomedical applications: a state of the art review, *Rev. Chem. Eng.*, 2015, 31, 563-597.
 - [48] Rahman A., Brown C.W., Effect of pH on the critical micelle concentration of sodium dodecyl sulphate, *J. App. Polym. Sci.*, 2003, 28, 1331-1334.
 - [49] Prasad K.N., Luong T.T., Paris A.T.F., Vaution C., Seiller M., Puisieux F., Surface activity and association of ABA polyoxyethylene-polyoxypropylene block copolymers in aqueous solution, *J. Colloid Interface Sci.*, 1979, 69, 225-232.
 - [50] Zhang X.R., Shi N.Q., Zhao Y., Zhu H.Y., Guan J., Jin Y., Deoxycholic acid-grafted PEGylated chitosan micelles for the

delivery of mitomycin C, *Drug Dev. Ind. Pharm.*, 2015, 41, 916-926.

- [51] Hu Y., He X., Lei L., Liang S., Qiu G., Hu X., Preparation and characterization of self-assembled nanoparticles of the novel carboxymethyl pachyman-deoxycholic acid conjugates, *Carbohydr. Polym.*, 2008, 74, 220-227.
- [52] Farhangi M., Kobarfard F., Mahboubi A. Vatanara A., Mortazavi S.A., Preparation of an optimized ciprofloxacin-loaded chitosan nanomicelle with enhanced antibacterial activity, *Drug Dev. Ind. Pharm.*, 2018, 44, 1273-1284.
- [53] Hu F.Q., Ren G.F., Yuan H., Du Y.Z., Zeng S., Shell cross-linked stearic acid grafted chitosan oligosaccharide self-aggregated micelles for controlled release of paclitaxel, *Colloids Surf. B Biointerfaces*, 2006, 50, 97-103.