Nanochitosan grafting sodium alginate improve loading and release of the antibiotic ‘streptomycin’, for drug release applications

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Abstract. Streptomycin is an antibiotic contains phenol and an amino group. It is a water-soluble and widely used in agriculture and livestock husbandry. At a high dose, streptomycin could be used in the early treatment of tuberculosis. This work aimed to use a copolymer of nanochitosan grafting Na-alginate as a carrier of streptomycin and study the kinetics of release. Chitosan and Na-alginate are natural polysaccharides, which are biocompatible and applicable in conventional pharmaceutics for drug delivery carrier. Na-alginate (1% w/v) was mixed with nanochitosan (0.13 gm.) and streptomycin (0.1 gm.) was loaded in a total concentration up to 0.1% w/v. The loaded copolymer was characterized using FT-IR spectroscopy, UV-visible, SEM/EDS analyses. Results indicated that streptomycin has successfully loaded onto Na-alginate. The release kinetics of streptomycin loaded at co-polymer nanochitosan-Na-alginate were compared with that of streptomycin loaded at nanochitosan alone. Buffer solutions with different pHs (pH 9.4, 7.4 and 1.2) at λ max 275 nm and 37 °C were applied. Results showed that the basic medium (pH 9.4) has improved the release property of streptomycin from Na-alginate more than that from nanochitosan. Thus, the co-polymer nanochitosan grafting Na-alginate is a promising candidate for drug release applications.

Keywords. release kinetic, nanochitosan, sodium alginate, streptomycin.

1. Introduction
Nanochitosan was discovered by Ohya and co-workers in 1994. Chitosan is a polysaccharide consist of α-L-guluronic acid and β-D-mannuronic acid linked by 1,4-glycosidic bonds, extracted from brown algae [1]. Chitosan nanoparticles have gained more attention as a drug carrier and as a biodegradable compound [2] due to its surface area to volume ratio, biocompatibility, efficient encapsulation, controlled release and it is less toxic [3]. In addition, it has high stability, low toxicity, simple and mild preparation method, and provide versatile routes of administration. Chitosan is insoluble in most solvents and phosphoric and sulfuric acid. However, it is soluble in most organic acidic solutions at pH less than 6.5, including formic, acetic and tartaric acid [4]. Efficient delivery and release system should have a high surface area to increase the release and delivery percentage. Therefore, rather studies have been used alginate to improve the surface area of chitosan [5], figure 1a. Sodium-alginate is a negatively
charged polysaccharide, blended hydrogel fibers. It is successfully prepared by cross-linking of Na-alginate with AlCl$_3$ followed by complexion among alginate and chitosan [8].

We aimed here to use nanochitosan grafting with Na-alginate as a drug carrier and study the release proprieties. We investigated the kinetic of loading and release of the antibiotic “streptomycin” at nanochitosan and at Na-alginate [9]. Streptomycin is a polyaromatic compound contains an amino group and phenol group [10], figure 1b. It is the choice to treat tularemia [11], tuberculous [12] interferes with translation process and thereby inhibits protein synthesis [13]. Fourier transform infrared was used to analyze the functional groups, SEM to indicate the morphology of the loaded compounds and EDx to analyze the elemental components.

2. Materials and Methods

2.1. Materials

UV-Visible spectrophotometer (CARY, 100 Conc.Austrialian), Fourier Transform (Infrared spectrophotometer, Shimadzu FT-IR 8400 series), oven up to 250 °C – Germany, vacuum pump-Germany, Nanochitosa (shaanix sangherb bio teching) Na-alginate (himedia).

2.2. Solutions and methods

For the loading study, a weight of 1.3 gm of nanochitosan was dissolved in 25 mL of 5% acetic acid and mixed well with 1gm of streptomycin (dissolved in 25 mL ethanol – purity 99%). The mixture was stirred for 15 sec. in a round bottom flask and refluxed at 60 °C. After 3 sec., 3 mL of a cross-linker (glutaraldehyde) was added to the beds of chitosan and drug, the sediment was filtered, washed with 100 mL 0.1 N NaOH, and dried using a vacuumed oven to 60 °C. The beads were prepared by ionic gelation using AlCl$_3$ as a cross-linking agent. Loading of the drug onto the polymer was carried out by the swelling equilibrium method [14].

A weight of 5%w/v of the polymer was dissolved in the deionized water under gentle heat and magnetic stirrer. The antibiotic was dissolved in an aqueous solution of the polymer, mixed homogeneously and the solution centrifuged for 30 min. The mixture was dropped via 20 gauge hypodermic needle fitted with a 50 ml syringe into 6%w/v gelling agents solution (AlCl$_3$), and stirred at 200 rpm for 10 min [15].

For release study, dried polymer samples of nanchitosan-Na-alginate after loading with streptomycin were immersed in 100 mL of a buffer solution in a certain pH (pH 1.2, 7.4 and 9.4) at 37°C. The system was continuously stirred at 80 rpm using a shaker water bath. At a certain time intervals (30 min), 4 mL of the solution was withdrawal and replaced by 4 mL of a fresh buffer solution to keep a constant volume. The collected samples were analyzed to determine the concentration of drug at $\lambda_{max}$ 275 nm for streptomycin [16]. The remnant concentration of streptomycin was determined after 24 hr which was $C_r$.

The thermodynamic parameters such as Gibbs free energy change ($\Delta G$), enthalpy ($\Delta H$) and entropy ($\Delta S$) were calculated using the entropy streptomycin from nanochitosan and streptomycin from nanochitosan-sodium alginate, and calculated Gibbs free energy and entropy using following [84]

2.3. Calibration curve of streptomycin

2.3.1. Solutions

A stock solution of streptomycin (50 E-4 mg/mL) was used to prepare a series of concentrations (9, 17, 25, 34 and 42) E-4 mg/mL. The calibration curve of streptomycin at $\lambda_{max}$ 275 is shown in Figure 1.
3. Result and Discussion

This study shed light on the binding of nanochitosan as a drug carrier for streptomycin and the kinetics of release of streptomycin under different pHs. In order to improve the release properties of streptomycin, Na-alginate has used along with nanochitosan to support the carrying of streptomycin. Results indicated that using nanochitosan grafted with Na-alginate was effective to improve the carrying properties of streptomycin since the rate of the reaction has significantly enhanced.

Table 1. Kinetic parameters of streptomycin release% from nanochitosan in pH 1.2 at 37 °C.

| No | Time (min) | Absλmax275 nm | C_t (mg/ml) | C_t/C_f | Release% |
|----|------------|----------------|-------------|---------|----------|
| 1  | 0          | 0.0101         | 0.297       | 0.172   | 17.2     |
| 2  | 30         | 0.0121         | 0.450       | 0.261   | 26.1     |
| 3  | 60         | 0.0153         | 0.695       | 0.402   | 40.2     |
| 4  | 90         | 0.0160         | 0.748       | 0.433   | 43.3     |
| 5  | 120        | 0.0181         | 0.908       | 0.526   | 52.6     |
| 6  | 1440       | 0.0288         | 1.725       | 1       | 100%     |

Table 2. Kinetic parameters of streptomycin release% from nanochitosan in pH 7.4 and 37 °C.

| No | Time (min) | Absλmax275 nm | C_t (mg/ml) | C_t/C_f | Release% |
|----|------------|----------------|-------------|---------|----------|
| 1  | 0          | 0.020          | 1.05        | 0.184   | 18.4     |
| 2  | 30         | 0.022          | 1.206       | 0.211   | 21.1     |
| 3  | 90         | 0.034          | 2.122       | 0.372   | 37.2     |
| 4  | 150        | 0.041          | 2.656       | 0.465   | 46.5     |
| 5  | 210        | 0.055          | 3.725       | 0.652   | 65.2     |
| 6  | 270        | 0.069          | 4.79        | 0.839   | 83.9     |
| 7  | 1440       | 0.081          | 5.709       | 1       | 100%     |

Table 3. Kinetic parameters of streptomycin release% from nanochitosan in pH 9.4 at 37 °C.

| No | Time (min) | Absλmax275 nm | C_t (mg/ml) | C_t/C_f | Release% |
|----|------------|----------------|-------------|---------|----------|
| 1  | 0          | 0.049          | 3.267       | 0.227   | 22.7     |
| 2  | 30         | 0.114          | 8.229       | 0.573   | 57.3     |
| 3  | 90         | 0.139          | 10.137      | 0.706   | 70.6     |
| 4  | 150        | 0.146          | 10.67       | 0.744   | 74.4     |
| 5  | 210        | 0.153          | 11.206      | 0.781   | 78.1     |
| 6  | 270        | 0.171          | 12.58       | 0.877   | 87.7     |
Table 4. The kinetic parameters of a streptomycin release % from nanochitosan-Na-alginate in pH 1.2 at 37 °C.

| No | Time (min) | $\text{Abs}_{\text{max}}$ 275 nm | $C_t$ (mg/ml) | $C_t/C_f$ | Release% |
|----|------------|---------------------------------|----------------|-----------|----------|
| 1  | 0          | 0.021                           | 1.129          | 0.48      | 48       |
| 2  | 30         | 0.023                           | 1.28           | 0.545     | 54.4     |
| 3  | 60         | 0.029                           | 1.74           | 0.75      | 75       |
| 4  | 90         | 0.030                           | 1.816          | 0.773     | 77.3     |
| 5  | 120        | 0.032                           | 1.97           | 0.86      | 86       |
| 6  | 150        | 0.036                           | 2.27           | 0.97      | 97       |
| 7  | 1440       | 0.037                           | 2.35           | 1         | 100      |

Table 5. Kinetic parameters of streptomycin release% from nanochitosan-Na-alginate in pH 9.4 at 37 °C.

| No | Time (min) | $\text{Abs}_{\text{max}}$ 275 nm | $C_t$ (mg/ml) | $C_t/C_f$ | Release% |
|----|------------|---------------------------------|----------------|-----------|----------|
| 1  | 0          | 0.366                           | 20.59          | 0.733     | 73.3     |
| 2  | 30         | 0.367                           | 21.36          | 0.761     | 76.1     |
| 3  | 90         | 0.324                           | 24.26          | 0.864     | 86.4     |
| 4  | 150        | 0.286                           | 26.55          | 0.946     | 94.6     |
| 5  | 210        | 0.354                           | 27.47          | 0.978     | 97.8     |
| 6  | 270        | 0.276                           | 27.54          | 0.981     | 98.1     |
| 7  | 1440       | 0.374                           | 28.07          | 1         | 100      |

Table 6. Kinetic parameters of streptomycin release% from nanochitosan-Na-alginate in pH 7.4 at 37 °C.

| No | Time (min) | $C_t$ | $\text{Abs}_{\text{max}}$ 275 nm | $C_t/C_f$ | Release% |
|----|------------|-------|---------------------------------|-----------|----------|
| 1  | 0          | 3.87  | 0.057                           | 0.34      | 34       |
| 2  | 30         | 5.09  | 0.073                           | 0.45      | 45       |
| 3  | 90         | 8     | 0.111                           | 0.70      | 70       |
| 4  | 150        | 9.45  | 0.130                           | 0.83      | 83       |
| 5  | 210        | 10.37 | 0.142                           | 0.91      | 91       |
| 6  | 270        | 11.28 | 0.154                           | 0.99      | 99       |
| 7  | 1440       | 11.35 | 0.155                           | 100       | 100      |

Tables 1 to 6 showed the kinetic parameters of loading and release of streptomycin, where $C_t$ is the concentration of streptomycin at different times, $C_t/C_f*100$ is the percentage of the released compound at different pHs and different grafting concentrations of nanochitosan, Na-alginate, at $\lambda_{\text{max}}$ 275 nm. Table 1 indicated that the higher release% of streptomycin from nanochitosan in pH 1.2 occurred after 120 min when the concentration of streptomycin was 0.908 mg/ml. Table 2 indicated that the higher release% of streptomycin from nanochitosan in pH 7.4 occurred after 270 min when the concentration of streptomycin was 0.839 mg/mL.

Table 3 indicated that the higher release% of streptomycin from nanochitosan in pH 9.4 occurred after 270 min when the concentration of streptomycin was 0.877 mg/mL. A copolymer resulted by mixing nanochitoan with Na-alginate was also used as a carrier of streptomycin. The release kinetic of streptomycin from nanochitoan-Na-alginate in different pHs (pH 1.2, 7.4 and 9.4) is demonstrated in Tables 4 to 6. Table 4 indicated that the higher release% of streptomycin in pH 1.2 occurred after 150
min when the concentration of streptomycin was 2.27 mg/mL. Table 5 showed that the higher release% of streptomycin from nanochitosan-Na alginate in pH 9.4 occurred after 270 min, when the concentration of streptomycin was 27.54 mg/mL.

Table 6 showed that the higher release% of streptomycin from nanochitosan-Na alginate in pH 7.4 occurred after 270 min when the concentration of streptomycin was 11.28 mg/mL. Results in Tables 1 to 6 suggested that the release of streptomycin from nanochitosan or from nanochitosan grafted Na-alginate was depending on the pH of the solution. The process of loading and release in a basic medium were higher than in the acidic medium, which could point to the selectivity of co-polymer in the medium [17]. The release kinetics indicated that release of streptomycin in pH 9.4 was 87.7% higher than that in pH 7.4 or 1.2, which may due to a high ionization degree of C=N bond in basic media than in acidic and natural media [18]. Low pH may strength the interactions between the drug molecule and functional groups on the surface of the carrier, therefore, the low release was observed [19]. At a strongly acidic medium, the amine groups of the beads (those that do not react with the crosslinker (glutaraldehyde). Moreover, the presence of more protons at low pH led to a strong competition [20].

Figure 2a-b showed the release% of streptomycin loaded at nanochitosan and streptomycin loaded at nanochitosan-Na alginate. Results indicated that the release of streptomycin from nanochitosan grafted Na-alginate was higher than that from nanochitosan only. This increase in the release% may due to the larger surface area of nanochitosan in the presence of Na-alginate.[5].

3.1. Kinetic of release
In order to determine the order of the release reaction, data were treated mathematically to indicate the zero-order reaction, R². Results in Figures 3 and 4 showed that the compounds used here as carriers for streptomycin were followed the zero-order reaction. Figure 3a-c summarized the parameters of k-P equation using the experimental data of streptomycin release from nanochitosan in different pHs (1.2, 7.4 and 9.4) at 37 °C.

Figure 3. Plots of C_t vs of time of release of streptomycin from nanochitosan beads in different pHs. a) pH 1.2, b) 7.4 and c) 9.4 at 37°C.

Figure 4 a-c summarized the parameters of k-P equation using the experimental data of streptomycin release from nanochitosan-Na alginate in different pHs at 37 °C.
Figure 4. Plots of C_t vs of time of release streptomycin from nano chitosan-Na alginate beads. a) pH 1.2, b) 7.4 and c) 9.4 at 37°C.

Figure 5 showed the kinetics used to obtain the rate of release of streptomycin from nanochitosan in different pHs. Figure 6 showed the kinetics used to obtain the rate of release of strptomycin from nanochitosan-Na-alginate in different pHs, where higher release in both cases was appeared at pH 9.4.

Figure 5. The release kinetics of streptomycin from nanochitosan beads per time (R1) and from Na-alginate-nanochitosan bead (R2). a) at pH 1.2, b) pH 7.4 and c) pH 9.4

Results in Table 7 showed that streptomycin loaded at nanochitosan-Na-alginate was better released than that loaded nanochitosan. The rate of coefficient (k) of nanochitosan and Na-alginate in different pHs (9.4, 7.4 and 1.2) at 37°C referred to an enhanced release of streptomycin, which loaded at nanochitosan-Na alginate when compared with that loaded at nanochitosan. This result could indicate that Na-alginate has improved the properties of release [16]. Additionally, the k value in both carrier types (nanochitosan) and Na-alginate-nanochitosan) was higher in pH 9.4 than other pHs.
3.2. FT-IR analysis

The FTIR spectra of nanochitosan showed broad stretching bands (NH$_2$), (OH) at 3363 and 3249 cm$^{-1}$, (C-Hal) at 2864 cm$^{-1}$ and 2910 cm$^{-1}$[21]. The FTIR spectra of streptomycin loaded at nanochitosan-Na-alginate showed a broad peak assigned in 3456 cm$^{-1}$ corresponded to O-H vibrations of Na-alginate, and a peak at 1610 cm$^{-1}$ corresponded to the carbonyl group of coo-Na [19].

The spectra of nanochitosan-Na-alginate showed no stretching vibration of NH$_2$, however, a stretching vibration of the carbonyl (amide) appeared at 1631-1638 cm$^{-1}$ and that of hydroxyl groups assigned at 3417-3432 cm$^{-1}$[22].

3.3. Scanning electron microscope-energy dispersive X-ray spectroscopy (SEM/EDX)

The SEM images in Figure 6a-d showed the surface morphology of nanochitosan, before and after loading with streptomycin. Nanochitosan appeared as un-regular blocks with different sizes before loading (Figure 6a and b). Blocks of nanochitosan after loading with streptomycin were packed closely and appeared more organized (Figure 6c) compared with blocks of Na-alginate or nanochitosan (Figure 6a and d). This change of nanochitosan shape after loading could come from the loading process. Tables (8) showed the appearance % of different elements on the surface before and after loading at nanochitosan. The carbon content of nanochitosan (48.52%) has decreased after loading with streptomycin to 26.61% and dropped down to 7.89% after loading at Na-alginate. Wherease, the oxygen content of nanochitosan (24.92%) has significantly increased after loading with streptomycin to 42.71% and to 57.27% after loading streptomycin to nanochitosan-Na-alginate. This change in carbon and oxygen content could suggest a success loading of streptomycin to nanochitosan and nanochitosan-Na-alginate [23]. The appearance of (Al) and (Cl) in (drug-nanochitosan –Na alginate) due to the presence of AlCl$_3$ in the preparation of copolymer. Content of oxygen was increased in (drug-nanochitosan) due to the presence of glutaraldehyde.

4. Conclusion

This study suggested that when streptomycin loaded at the surface of nanochitodsan and nanochitosan-Na-alginate, there was an enhance in the release percentage% from nano chitosan –Na alginate at temperature 37°C. The release in pH 9.4 after 1440 min was higher than other tested pHs (1.2, 7.4 ), which may be due to ionization of the bond C=N in the basic media.

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