Bioengineering Research

The study of loading and release of N-acetylcystein by chitosan nanoparticles

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Abstract

N-acetylcysteine (NAC) is derived from acetylated cysteine as an oral medication. one of the serious problem of this drug is Initial dose reduction because it is metabolized in the intestine and liver. In the present study, N-acetylcysteine encapsulated in chitosan nanoparticles by gel method with sodium polyphosphate to study oral colon cancer therapy. NAC-chitosan nanoparticles synthesized spherical shape. Zeta potential and average size using laser light scattering (DLS) 31.4 mV and 133 nm, respectively. The drug encapsulation and loading efficiency of the nanoparticles were 79.8± 1.03% and 33 ± 2% respectively. In vitro, the amount of drug release from chitosan nanoparticles in PBS buffer after 48 hours was 69.2%. It is analyzed toxicity of this drug on colon cancer cell lines by MTT method, it is shown that toxicity nanocapsulated NAC is more than free NAC. also viability of colon cancer cells treatment after 5 days decreased 92.2±2%. The results of this study could be a new way to increase the pharmacological effect of NAC particulate drug in delivery systems and reduce the dose in oral route.

Keywords: N-acetylcysteine, colon cell line, chitosan nanoparticles, encapsulation, anti-cancer activity.
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1. Introduction

N- acetylcysteine (NAC) acetylated derivative of the amino acid cysteine, which is a glutathione, has medicinal properties, and is used as antioxidant. Also NAC is known as an antidote to acetaminophen, there are a large number of other clinical applications that have been approved by scientific evidence [1,2].

The ability of this drug to support the body's antioxidant and nitric oxide during stress, infections, toxic substances and inflammatory status of clinical applications. Treatment with NAC supplementation increases the glutathione that is the main antioxidant in the body. Glutathione plays an important role in the neutralization of toxic substances, peroxide compounds. Thus, It has the protective effect on the body's cells [3,4]. NAC have antioxidant properties that can reduce stress stem cells; increase biological activity their growth, and reactive oxygen species (ROS) inhibit; and enhance the biological health of normal cells [5].

When NAC used oral, drug is metabolized in the intestines and Absorption become less in the intestine, so a large dose of the drug should be administered [6-8].

bio polymeric nanoparticles have been extensively investigated as a drug carrier owing to their ability to protect the drug from degradation, maximize the drug loading capacity and enable easy drug delivery to the target site chitosan has attracted a great attention in pharmaceutical and biomedical fields because of its advantageous biological properties, such as biodegradability, biocompatibility, and nontoxicity [9-11]. Chitosan is a cationic polysaccharide which is

Table1: Encapsulation and loading efficiency of NAC into chitosan nanoparticles, Data are expressed as mean ± SD (n = 3).

NAC.CS-NP(%)	Concentration of NAC(g)	Encapsulation efficiency (wt%)	Drug loading (wt%)
CS-TPP(3:1)NAC	0.05	70.2±1.2	10.4±1.4
CS-TPP(3:1)NAC	0.1	70.5±1.02	17±2.02
CS-TPP(3:1)NAC	0.25	79.8±1.03*	33±2*
CS-TPP(3:1)NAC	0.5	71.2±1.1	24.2±2.2

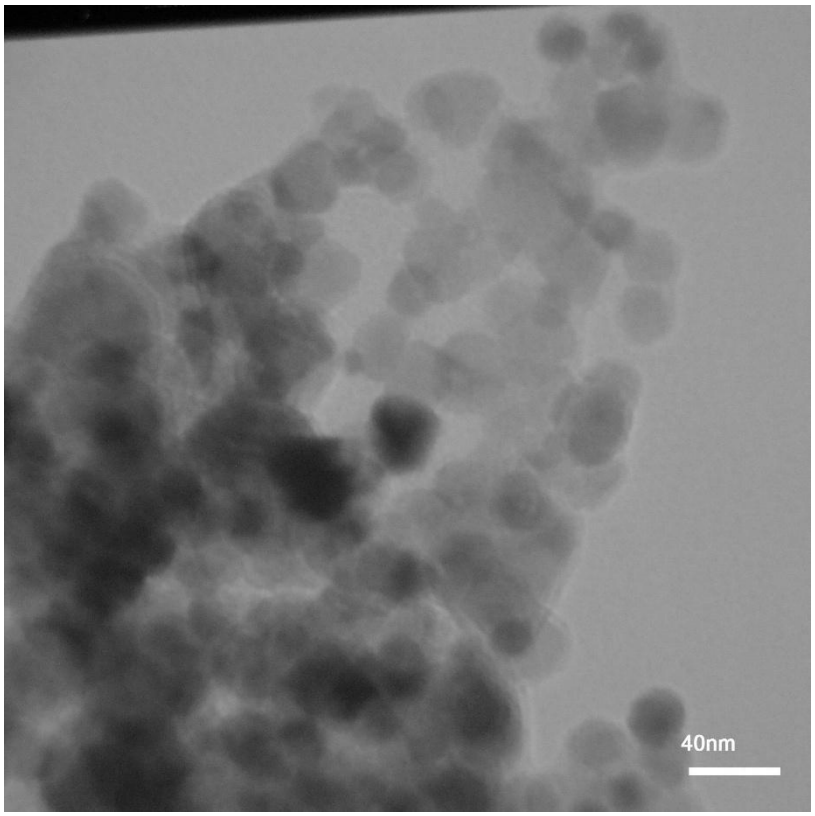


Figure 1: FE-SEM image of NAC.chitosan nanoparticles

obtained by partial deacetylation of chitin. In contrast to other polymers, chitosan is a hydrophilic polymer with positive charge, which reveals a special characteristic to chitosan from the technological point of view [12].

The amine groups(NH^{+}_{3}) of Chitosan nanoparticles make strong electrostatic bonds with the negative surface of the intestinal mucosa [13,14]. Chitosan nanoparticles control drug release, protect unstable medications, increase solubility drugs, target drug delivery, enhance drug delivery to the target tissue [15,16].

In this study, N-acetylcysteine loaded in chitosan nanoparticles and its toxicity tested on colon cancer cell lines.

2. Materials and methods

2.1. Chemicals

N-acetylcysteine(NAC), chitosan (Low molecular weight, DD: 75-85%), sodium polyphosphate was purchased from Sigma Aldrich, USA. All other chemicals used were of analytical grade.

2.2. Synthesis of NAC-chitosan nanoparticles

chitosan-NAC nanoparticles was synthesized by ionic gelation method using sodium polyphosphate as a gelating agent. Chitosan and sodium polyphosphate solution in different concentrations (0.1, 0.5, 0.75, 1g) in 100 ml acetic acid 1% was prepared, Tween-80 (0.5%v/v) added to chitosan solution. Different concentrations of N-acetylcysteine (0.05, 0.1, 0.25 and 0.5 g) dissolved in fresh solution of chitosan. The synthetic procedure of Chitosan nanoparticle was first optimized at different concentrations and was dissolved in 1% (v/v) acetic acid. The solution was continuously stirred at room temperature and the pH of the solution was adjusted to 4.6-4.8 using 1M NaOH. N-acetylcysteine was then added to the freshly prepared chitosan dispersion and stirred for 1 h. Finally, solution TPP was added drop wise to the CS-NAC solution under mild stirring. The resulting mixture was allowed to stir for 1 h to form NAC encapsulated chitosan nanoparticles. Chitosan-NAC nanoparticles was dried by freeze-drying and stored at 4ºC for further use. The amount of loading of NAC was calculated by measuring the HPLC. The quantity of loaded NAC was calculated by using the following relationships [17].

$$\text{Encapsulation efficiency \%} = \left(\frac{\text{total NAC} - \text{free NAC}}{\text{total NAC}}\right) \times 100 \quad (1)$$

$$\text{Loading capacity\%} = \left(\frac{\text{total NAC} - \text{free NAC}}{\text{chitosan} - \text{NAC.NP}}\right) \times 100 \quad (2)$$

2.3. In vitro drug release

In vitro drug release study was carried out in PBS medium according to V. Arumozhi [18]. 5ml of 20 mg/ml of NAC-Chitosan nanoparticles was resuspended in dialysis membrane bag with the molecular cut off of 10 kDa. The membrane bag was placed in 40 ml PBS (pH 7.4) under magnetic stirring at 120 rpm with the temperature maintained at 37ºC. The amount of NAC released from the dialysis bag was calculated at different time intervals (1, 2, 3, 4, 5, 6, 24, 48 h). All measurements were performed in triplicates and the release of NAC was calculated from the following equations:

$$\text{Release rate (\%)} = \left(\frac{\text{Released NAC}}{\text{Total NAC}}\right) \times 100 \quad (3)$$

2.4. Cell lines and culture conditions

The human colon cancer cell lines were purchased from Stem Cell Technology Research Center. The cells were maintained in Dulbecco's Modified Eagle's medium (DMEM) supplemented with fetal bovine serum (FBS). The cells were grown in 5% CO2 atmosphere at 37◦C and the cells should have 80–90% confluence before they are subcultured for the experiments.

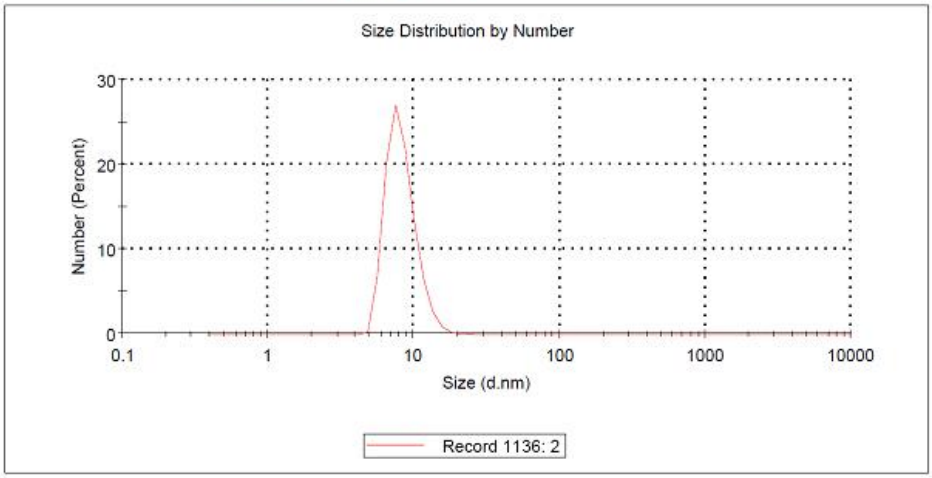
2.4.1. Cytotoxicity assay

The cytotoxicity of bare NAC and NAC- chitosan nanoparticles was investigated in colon cancer cell lines with three replications using MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5- diphenyltetrazolium bromide) assay [16]. MTT test them on the first, third and fifth days with the absorption wavelength of 570 nm using an Elisa reader and SPSS software was used to analyze the results, Values of $P < 0.05$ were indicative of significant differences. The effect of the samples on the proliferation of colon cancer cell line was expressed as the % cell viability, using the following formula:

$$\text{\% cell viability} = \left(\frac{\text{A570 of treated cells}}{\text{A570 of control cells}}\right) \times 100 \quad (4)$$

			Size (d.nm):	% Number:	St Dev (d.n...
Z-Average (d.nm):	138.2	Peak 1:	8.338	100.0	2.048
PdI:	0.941	Peak 2:	0.000	0.0	0.000
Intercept:	0.658	Peak 3:	0.000	0.0	0.000

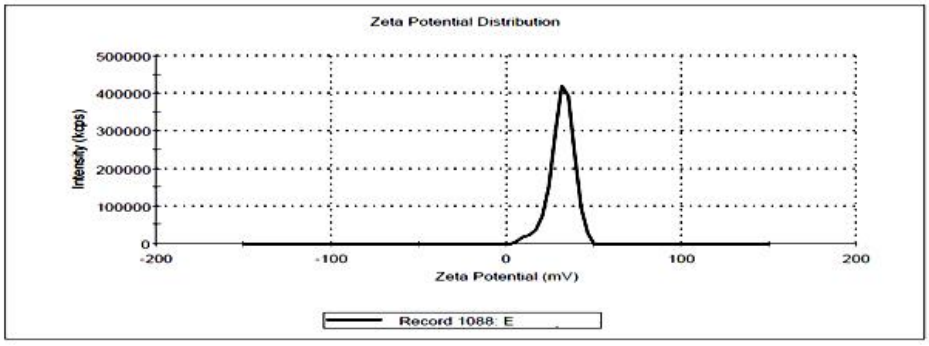
Result quality : Refer to quality report



(A)

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	31.4	Peak 1:	31.4	100.0	7.06
Zeta Deviation (mV):	7.06	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.984	Peak 3:	0.00	0.0	0.00



(B)

Figure2: (A) Particle size distribution and (B) zeta potential distribution of NAC.CS-NP.

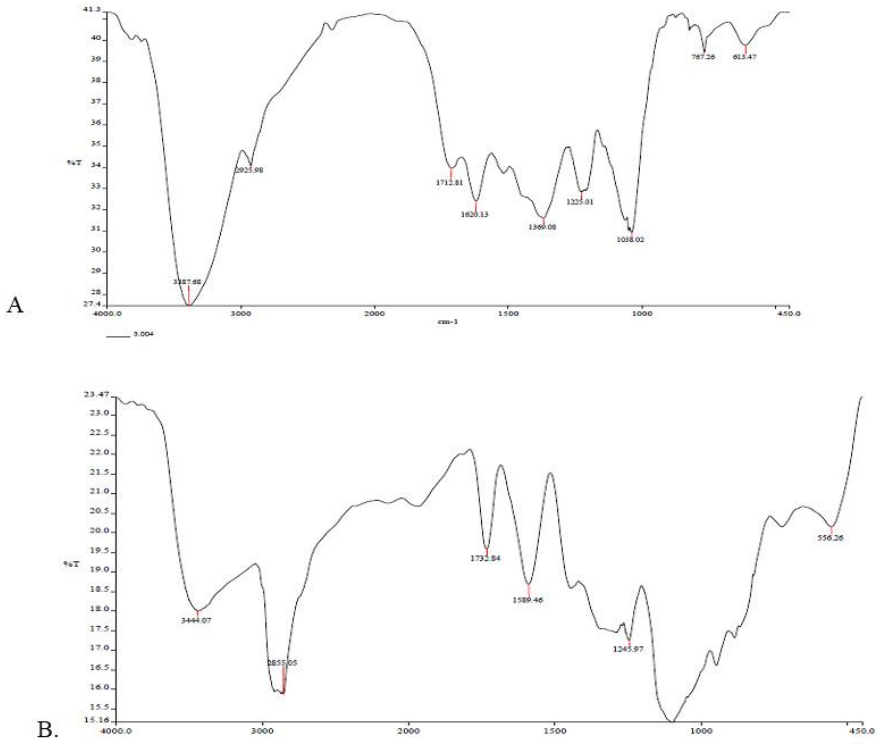


Figure 3: FT-IR spectrum of (A) CS-NP, (B) NAC-CS.NP

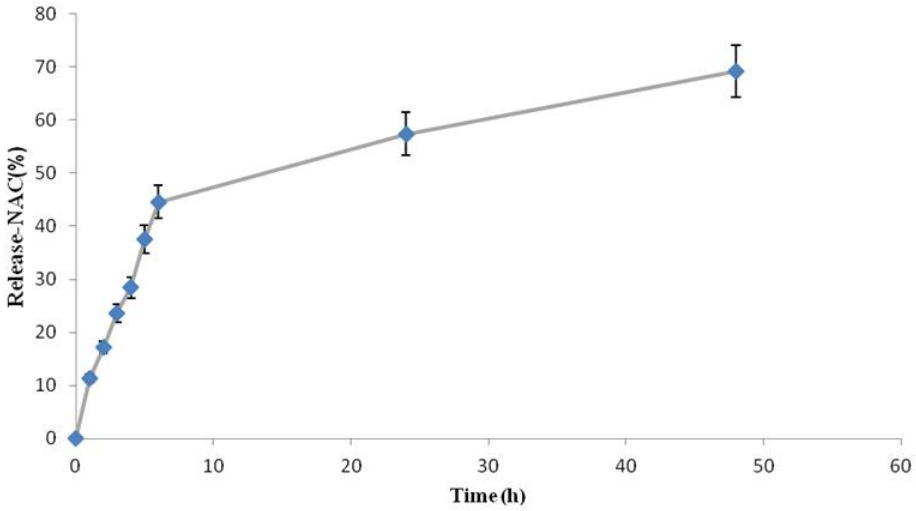


Figure 4: In vitro drug release of NAC from NAC.CS-NP (loading concentration = 0.25g) in phosphate buffer saline (pH 7.4, 120 rpm) at 37ºC. Data are represented as mean ± SD (n = 3).

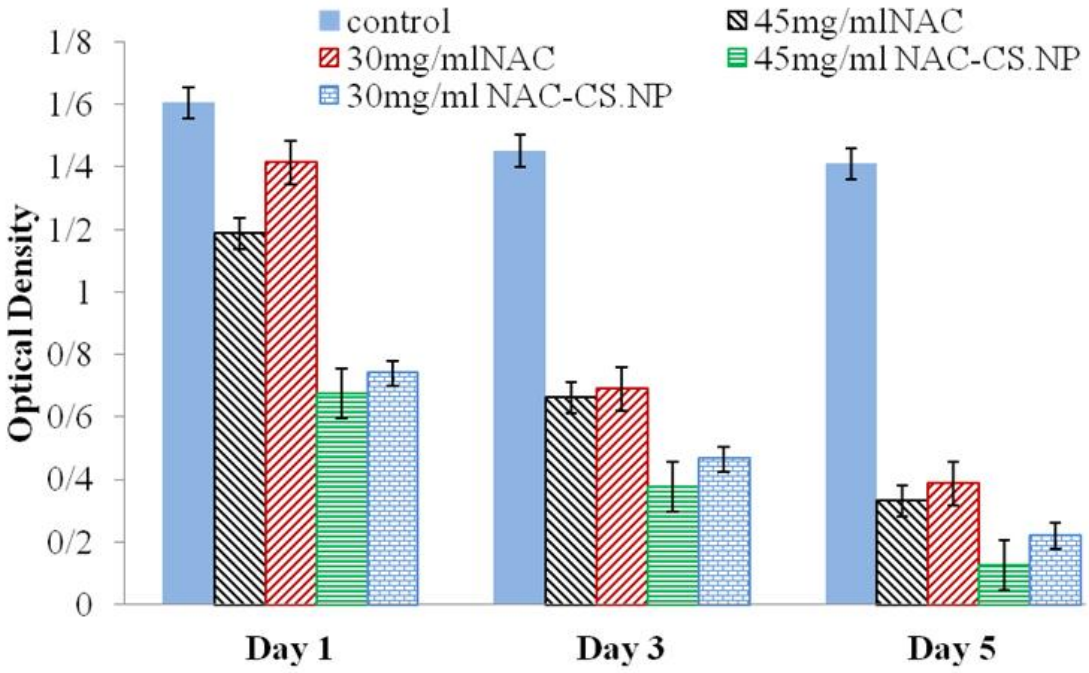


Figure 5: cells exposed to NAC and NAC-CS.NP (total volume wells 300µl), (N=3).

3. Results and discussion

Chitosan nanoparticles were synthesized by ionic gelation method with a chitosan to TPP ratio of 3:1 and were optimized chitosan concentration 0.0.25g the successful loading of NAC. Chitosan nanoparticles were optimized by varying the concentrations of NAC (0.05, 0.1, 0.25 and 0.5 g). Following the synthesis of NAC.CS-NP, their encapsulation and loading efficiency was calculated based on the HPLC studies. As shown in Table1, the maximum loading and encapsulation efficiency was determined by varying the concentration of NAC in CS_TPP ratio (3:1). The drug concentration of 0.25g NAC showed high encapsulation and loading efficiency of 79.8±1.03% and 33±2%. We also observed that increased loading concentration of NAC which dramatically decreases the encapsulation and loading efficiency of the drug. The possible reason could be that the excess drug gets loosely adsorbed onto the surface of the CS and finally gets. The FE-SEM analysis of NAC.CS-NP with size magnifications. The morphology of the NAC-chitosan nanoparticles was spherical in shape with their diameter around 133 nm, showed FE-SEM image of NAC. Chitosan nanoparticles (fig.1).

3.1. Zeta sizer and zeta potential measurement

Particle size and surface charge are the major criteria to be focused in order to have an effective drug delivery. Fig. 2(A) depicts the average size distribution of the synthesized nanoparticles using zeta sizer analysis. The average particle size of NAC.CS-NP is 133nm which almost coincide with our FE-SEM studies of NAC-CS.NP. Zeta potential measurement is an important index in determining the stability of the nanoparticle suspension. High absolute zeta potential value signifies high electric surface charge on the drug loaded nanoparticles which can cause strong repellent forces among particles to prevent it from aggregation [19]. Hence the synthesized NAC.CS-NP which is in the aggregated form can be further sonicated for several minutes in order to have an effective drug delivery. As shown in

Fig.2 (B), the zeta potential value of NAC.CS-NP was 31.4 MV. The observed decrease in the zeta potential value could be due to the adsorption of drug on the polymer surface which results in masking the free amine groups of chitosan [20].

3.2. FT-IR analysis

The FT-IR technique is one of the important tools to identify the interaction between CS and NAC which confirms the encapsulation of NAC into the nanocarrier. The FT-IR spectra of bare CS-NP and NAC.CS-NP are shown in Fig.3. The absorption spectrum of hydroxyl groups between3442-3429 cm^{-1}. The absorption spectrum of the 1114-1134 cm^{-1} is the group -C-S. Absorption spectra –C-N and -C-O are peaks of 1153 and 1080 cm-1, respectively.

3.3. In vitro drug release

In order to exploit the use of NAC loaded chitosan nanoparticles for an effective drug delivery system, we employed the in vitro drug release of NAC from NAC.CS-NP at different time intervals (1, 2, 3, 4, 5, 6, 24, 48 h) and the resulting data are shown in Fig. 4. The NAC release in the in vitro releasing medium was chosen as phosphate buffer (pH 7.4, 37ºC and 120 rpm) which simulates our body fluid [21]. Initially NAC was found to be released in a rapid rate for up to 8h which was followed by the gradual and sustained release until 48h. The observed rapid release could be due to the adsorption of NAC on the nanoparticle surface where as the sustained release is attributed to the release of the encapsulated NAC from the polymer matrix.

3.4. Cytotoxicity

The cytotoxicity of free NAC and NAC-CS.NP were initially investigated for their antiproliferative activity in human colon cancer cell line based on MTT assay. As revealed in Fig.5. Cells exposed to NAC and NAC-CS.NP after 5 days of treatment exhibited significant cytotoxicity in a dose dependent manner. Interestingly, greater degree of cytotoxicity was observed for almost all doses of NAC-CS.NP when compared to free NAC [22,23]. After five days of treatment with free N-acetylcysteine maximum inhibition of cancer cell viability 79.1%. But the drug encapsulated nanoparticles was inhibited colon cancer cell viability 92.2±2%.

4. Conclusion

We have developed a methodology for the synthesis of NAC.CS-NP by ionic gelation method using TPP. The optimized ratio of chitosan to TPP is 3:1 which act as an effective carrier with high loading efficiency almost 80%. FE-SEM analysis revealed the spherical shaped morphology and the average particle size was 71 nm. The bonding interactions between CS and NAC were studied using FT-IR. The drug is released 69.2 in phosphate buffer medium until 48 h at room temperature indicating sustained drug release pattern. Further, in vitro anticancer studies showed significant antiproliferative effect on human cancer colon cell line. These findings suggested that CS acted as a promising cancer drug carrier which enhanced the anticancer property of the N-acetyl cysteine (NAC).

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