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RESEARCH ARTICLE

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Sodium Alginate Nanoparticles of Isoniazid: Preparation and Evaluation

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ABSTRACT

Fabrication and evaluation of the Isoniazid loaded sodium alginate nanoparticles (NPs) was main objective of current investigation. These NPs were engineered using ionotropic gelation technique. The NPs fabricated, were evaluated for average particle size, encapsulation efficiency, drug loading, and FTIR spectroscopy along with *in vitro* drug release. The particle size, drug loading and encapsulation efficiency of fabricated nanoparticles were ranging from 230.7 to 532.1 nm, 5.88% to 11.37% and 30.29% to 59.70% respectively. Amongst all batches studied formulation F-8 showed the best sustained release of drug at the end of 24 hours.

Keywords: Isoniazid, sodium alginate nanoparticles, *in vitro* release.

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INTRODUCTION

Mycobacterium tuberculosis is second, next to HIV, in causing mortality from infectious disorder which affects 1/3 of the total world population. [1] The biopharmaceutical and pharmacokinetics properties of antitubercular drug necessitate the daily intake of these for tuberculosis management. A pronounced incidence of adverse effects, daily dosing and low patient compliance may result in discontinuation of therapy and increase in multidrug-resistant strains of Mycobacterium tuberculosis. [2-3] Hence, the major objectives of tuberculosis research are to minimize the dose and dosing frequency. By using various drug delivery systems the pharmacokinetics of antitubercular drugs can be enhanced, that may lead to an improvement in the therapeutic strategy for management of tuberculosis. During the last decade, several researches were carried out with some modifications in drug delivery systems to enhance the pharmacokinetics of antitubercular drugs. These drug delivery systems with some modifications have been combined with some disadvantages such as increased consumption of polymer [4], low drug encapsulation [5] and use of organic solvents [6] etc. This investigation was designed for the development of nanoparticles drug delivery system using polymer of natural origin. This developed novel drug delivery system would have the potential to overcome the drawbacks of modified

drug delivery systems mentioned above. The properties like biocompatibility, biodegradability and non-toxicity of Sodium alginate made it suitable for its use in the field of drug delivery. The United States Food and Drug Administration has recommended Sodium alginate (SA) for oral use [7-8] and is enriched with the property of hydrophilicity (which protect it from fast uptake by the reticuloendothelial system). It is a natural polysaccharide, rich in carboxyl group and is easy to bind with positive charge cations such as Ca^{2+}. It is of low toxicity, good biocompatibility and relatively low cost, therefore it is widely used in medicine and food industry. [9-10] Therefore, it was employed to fabricate nanoparticles entrapping antitubercular drugs. [11] Further, research will look into designing of novel drug delivery systems with drug targeting to lungs.

Our research group has explored the fabrication of Sodium alginate nanoparticles by simple and fast ionotropic gelation between the SA (polymer) and its counter ion calcium chloride which exhibit a significant ability in protein association (about 90%), along with peptide absorption improvement through numerous epithelia, like nasal, intestinal and ocular. [12] Recently, the fabrication of sodium alginate nanoparticles as carriers for doxorubicin to increase efficacy of active moiety has been reported. [13] In view of the above, it was planned to prepare and characterize sodium alginate nanoparticles encapsulating Isoniazid, followed by evaluation for the sustained release of the drug.

MATERIALS AND METHODS

Materials

Isoniazid was procured from High Purity Laboratory Chemicals (HPLC), Mumbai (India). Calcium chloride was procured from SD Fine-Chem Ltd, Mumbai (India). Sodium Alginate was obtained from Himedia laboratories Pvt. Ltd., Mumbai (India). All other chemicals and reagents were of analytical grade.

Experimental design

The optimization technique was applied to obtain an appropriate formulation design in order to minimize the number of experiment. The experimental design of sodium alginate nanoparticles as described in Table 1.

Table 1: Experimental design of Sodium alginate nanoparticles

Independent variables	Levels		
	Low (-1)	Medium (0)	High (1)
A Sodium alginate (%w/v)	2	2.5	3
B Calcium chloride (%w/v)	1	1.5	2

Method

Preparation of nanoparticles

Ionotropic gelation technique with some modifications was used to fabricate Isoniazid loaded sodium alginate nanoparticles. Sodium alginate was used at various concentrations and dissolved in distilled water using magnetic stirrer and allowed to stand for 30 minutes. Then the drug was suspended in above mentioned sodium alginate solution along with stirring. Various concentrations of calcium chloride solution were added in drop wise manner in prepared solution of drug and polymer and suspension of Isoniazid - sodium alginate - calcium chloride nanoparticles was formed. Calcium chloride was added as a cross linking agent for the sodium alginate nanoparticles to achieve sustained drug release. It was kept for sonication for 25 minutes. After sonication, the centrifugation of nanoparticles suspension was carried out at 10,000 rpm (Remi, Mumbai) for 30 minutes & supernatant was discarded. The pellet was collected and redispersed in de-ionized water followed by sonification, centrifugation and lyophilisation. [13-14]

Characterization of sodium alginate nanoparticles

Particle size

Freeze-dried nanoparticles were dispersed into HPLC grade water. The particle size of the Isoniazid loaded sodium alginate nanoparticles were evaluated by Particle Size Analyzer (Malvern Instruments Ltd, Malvern, UK). The particle sizes of various fabricated nanoparticle formulations are shown in table 2.

Determination of Encapsulation Efficiency (EE) of nanoparticles

10 ml suspension of nanoparticles was centrifuged at 10,000 rpm (Remi, Mumbai) for 90 minutes at 10°C. After centrifugation, the clear supernatant procured was diluted 10 times with double distilled water to quantify the amount of unbound Isoniazid using UV-Visible spectrophotometer at λ_{max}= 263 nm. Encapsulation Efficiency & drug loading of nanoparticles were determined using equation given below. [15]

$$Encapsulation\ effeciency = \frac{Total\ amount\ of\ isoniazid - Amount\ of\ free\ isoniazid}{Total\ amount\ of\ isoniazid} \times 100$$

$$Drugloading = \frac{Total\ amount\ of\ isoniazid - Amount\ of\ free\ isoniazid}{Total\ weight\ of\ nanoparticles} \times 100$$

Fourier transform infrared spectroscopy (FTIR)

The infrared spectrum of pure Isoniazid, sodium alginate and physical mixture (sodium alginate and isoniazid) was carried out to advocate drug excipients interaction. The peaks of individual pure Isoniazid drug, sodium alginate polymer and peak of drug-polymer combination were compared to find out the interactions. [16] FTIR spectrum of pure isoniazid drug and drug polymer physical mixture were obtained in KBR pellets method.

In vitro drug release study of sodium alginate nanoparticles

The drug release studies were carried out using dissolution medium (250 ml of 7.4 pH phosphate buffer) at 50 rpm at 37 ± 0.5°C temperature. The nanoparticles containing drug equivalent to 50 mg were taken in a dialysis bag and put into flask containing dissolution medium. 5 ml aliquots of samples were withdrawn at specific interval i.e. 1, 2, 4, 6, 12, 14, 16, 18, 20, 22 & 24 hours. After suitable dilutions, absorbance of the samples was determined

by UV-Visible Spectrophotometer at λ_{max} 263 nm. Absorbance for the samples withdrawn was recorded and percentage drug release at different time intervals was plotted against time.

RESULTS AND DISCUSSION

Optimization using 3^2 factorial design

The factorial design was applied for the determination of appropriate amount of sodium alginate and calcium chloride on the basis of loading capacity, encapsulation efficiency and average particle diameter measurements. 13 formulations were fabricated as per experimental design and influence of 2 factors, i.e. SA (A) and calcium chloride (B) was examined on 3 responses viz. loading capacity, encapsulation efficiency and average particle diameter. The loading capacity and encapsulation efficiency of all fabricated formulations was found to be in the range of 5.88% to 11.37% and 30.29% to 59.70%, respectively, whereas mean particle size was observed between 230.7 to 532.1 nm. The obtained polynomial equations were employed for the calculation of the variance and responses were evaluated for parameters such as degree of freedom, F value, sum of squares & mean sum of squares applying the software. The polynomial equations were obtained through regression analysis for responses are as follows

Loading capacity = +9.51-1.08*A+1.27*B (1)

Encapsulation efficiency = +49.49+5.82*A+6.81*B (2)

Particle size = +324.40+96.63*A+25.72*B (3)

Response surface curve depicting the combined effect of both factors on loading capacity, encapsulation efficiency and particle size of nanoparticles is given below in Figures 1, 2 and 3, respectively.

The importance was designated to mean particle size, loading capacity and encapsulation efficiency. The variables according to Design Expert software for optimized nanoformulation were sodium alginate 3.0% w/v and calcium chloride 2% w/v which led to formulation of nanoparticles with average particle diameter of 245.5 nm.

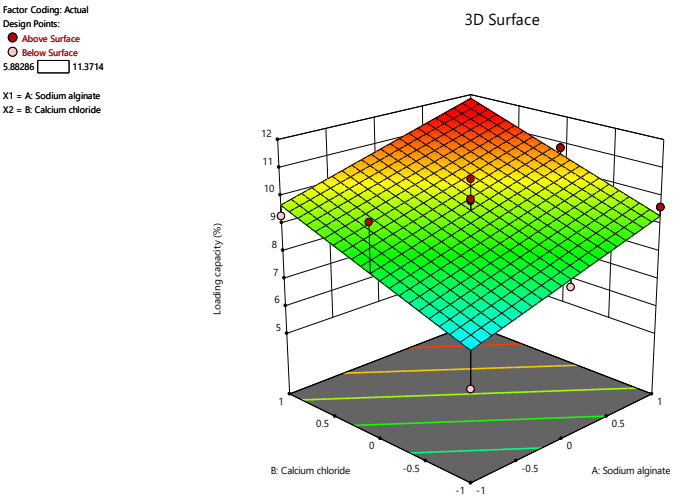

Fig. 1: Response surface plot depicting the combined effect of sodium alginate & calcium chloride on loading capacity (%) of Isoniazid nanoparticles

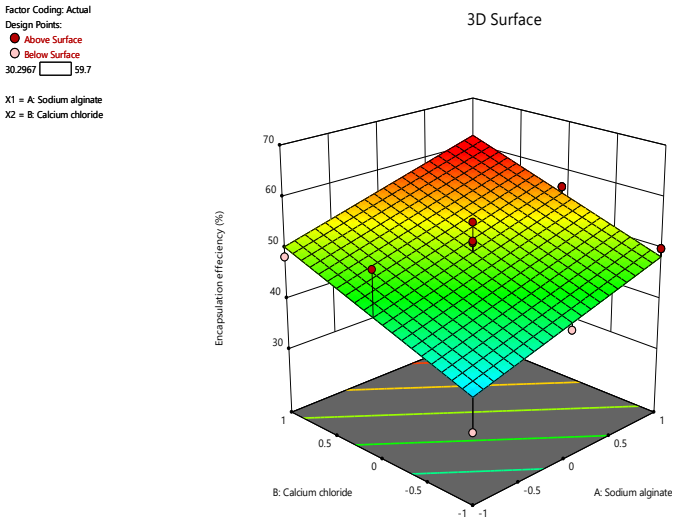

Fig. 2: Response surface plot depicting the combined effect of sodium alginate & calcium chloride on encapsulation efficiency (%) of Isoniazid nanoparticles

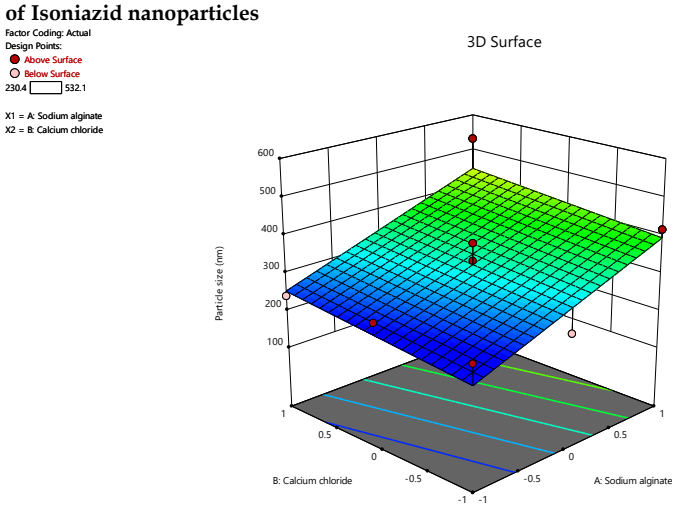

Fig. 3: Response surface plot depicting the combined effect of sodium alginate & calcium chloride on particle size of Isoniazid nanoparticles

		Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 245.5	Peak 1:	229.8	79.9	67.47
PdI: 0.525	Peak 2:	74.30	14.7	17.69
Intercept: 0.988	Peak 3:	11.14	5.4	1.798
Result quality : Refer to quality report				

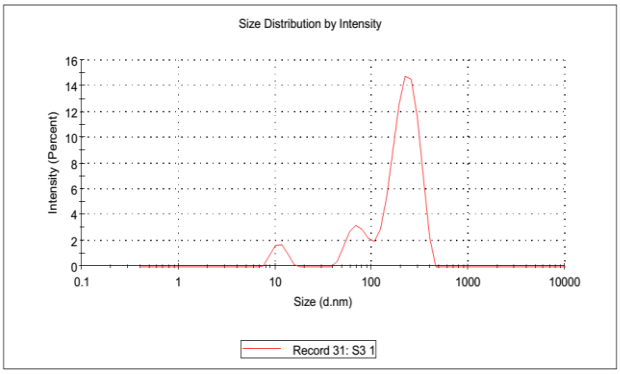

Fig. 4: Particle size distribution curve of the Isoniazid loaded sodium alginate nanoparticles (F-8).

Particle size

Particle size of the prepared formulations is shown in Table 2. It was observed that particle size varied from 230.7 and 532.1 nm for sodium alginate nanoparticles and particle size of optimized formulation (F-8) was

shown in Figure 4. For SA nanoparticles, an increase in concentration of the calcium chloride led to increase in average size of nanoparticles. Quantity of calcium chloride has significant role in the protection of nanoparticles because it hinders the cluster formation of nanoparticles.

Table 2: Experimental Design for Isoniazid nanoparticles and results for the measured responses

Std	Run	Factor 1 Sodium alginate	Factor 2 Calcium chloride	Response 1 Drug loading (% w/w) ± SD	Response 2 Entrapment efficiency (%) ± SD	Response 3 Average particle size (nm)
7	1	0	-1	7.97 ± 0.18	41.25 ± 0.08	230.7
6	2	1	0	10.82 ± 0.19	56.53 ± 0.20	386.8
8	3	0	1	10.40 ± 0.13	54.34 ± 0.29	284.2
9	4	0	0	8.99 ± 0.13	46.78 ± 0.19	231.5
13	5	0	0	9.91 ± 0.10	51.55 ± 0.82	285.3
1	6	-1	-1	5.88 ± 0.09	30.29 ± 0.25	253.7
3	7	-1	1	9.31 ± 0.10	48.43 ± 0.26	240.9
4	8	1	1	11.37 ± 0.11	59.70 ± 0.15	245.5
2	9	1	-1	9.62 ± 0.05	50.06 ± 0.07	416.3
5	10	-1	0	10.17 ± 0.15	52.63 ± 0.18	532.1
10	11	0	0	10.62 ± 0.09	55.26 ± 0.17	334.4
11	12	0	0	9.84 ± 0.10	51.21 ± 0.13	382.6
12	13	0	0	8.70 ± 0.10	45.26 ± 0.08	381.4

Determination of Encapsulation Efficiency (EE) of nanoparticles

The drug loading and encapsulation efficiency of fabricated nanoparticles is shown in Table 2. It was observed that with increase in the concentration of sodium alginate, encapsulation efficiency and drug loading were also found to increase, owing to increase in viscosity of the aqueous medium. But the encapsulation efficacy increased with increasing in calcium chloride concentration. When quantity of calcium chloride was increased, it promoted the solubilisation of drug in the aqueous phase.

Fourier transform infrared spectroscopy (FTIR)

Drug compatibility studies using FTIR were conducted for the pure drug, sodium alginate and the physical mixture. The spectral data are given in Figure 5-7. The results indicated no chemical incompatibilities between Isoniazid and sodium alginate used in nanoparticles.

In vitro drug release studies

The pattern of drug release from nanoformulation displayed cumulative drug release in the range 66.56%–83.53% as shown in Figure 8.

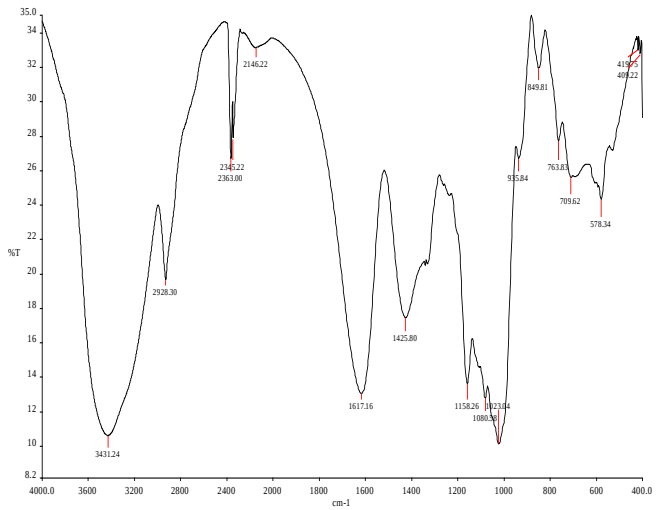

Fig. 5: FTIR spectrum of the sodium alginate

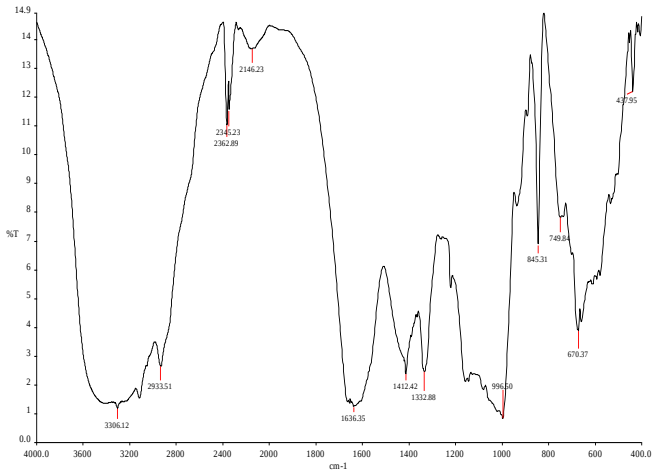

Fig. 6: FTIR spectrum of physical mixture of Isoniazid and sodium alginate

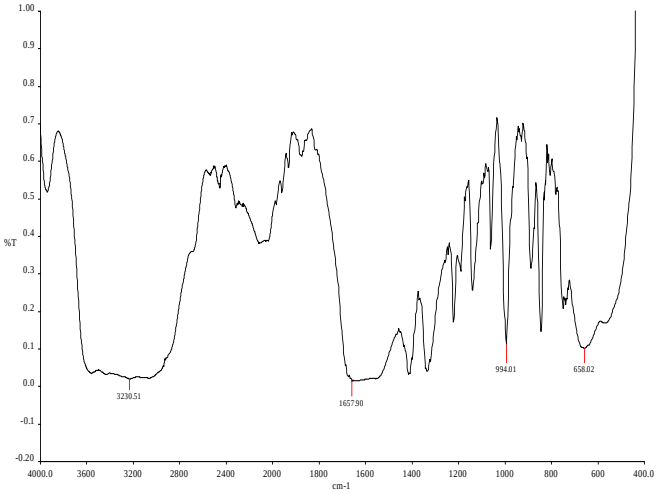

Fig. 7: FTIR spectrum of Isoniazid

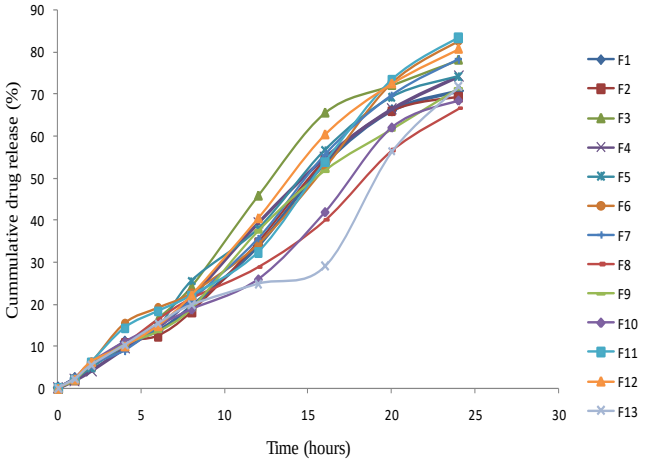

Fig. 8: *In vitro* drug release profiles of Isoniazid loaded sodium alginate nanoparticles (F1 -F13)

The Isoniazid loaded nanoparticles showed a biphasic drug release profile initially with outburst release of Isoniazid followed by sustained release of Isoniazid. The initially outburst release may be due to association of Isoniazid with surface of nanoparticles. Initial release of Isoniazid is linked with those Isoniazid moieties dispersing from near the nanoparticles surface.

The drug release may depend upon the sodium alginate amount. In optimized formulation (F-8) sodium alginate showed drug release of 66.56% within

24 hours showing a sustained release profile, during the first hour nanoparticles formulation gave outburst release and after that it showed a sustained. Literature reports suggest that macrophages take 2 h to achieve their maximum engulfment capacity. Therefore, it can be deduced that the majority of drug would be released inside the cell following endocytosis of the carrier system. [17-18]

The objective of current investigation was fabrication and evaluation of Isoniazid loaded sodium alginate nanoformulations. The Isoniazid-loaded sodium alginate nanoformulations were evaluated by particle size analyzer and Fourier transform infrared spectroscopy. Existence of Isoniazid in loaded nanoparticles was confirmed by Fourier transform infrared spectroscopy studies. Sodium alginate nanoparticles with properties like biodegradability, biocompatibility, more stability, low toxicity, convenient and simple preparation technique, offers an important and valuable tool for the Isoniazid delivery through novel drug delivery system. The sodium alginate & calcium chloride concentration role on loading capacity, encapsulation efficiency and particle size was evaluated by optimization. The observed parameters for formulation F-8 were significant as compared to software predicted values given by design expert. The *in vitro* drug release for the optimized formulation was 66.56% in 24 h. The development of this Isoniazid loaded sodium alginate nanoformulation has the potential to provide enhanced efficacy of Isoniazid.

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REFERENCES

1. Dye C, Watt CJ, Bleed DM, Hosseini SM, Raviglione MC. Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence, and deaths globally. JAMA 2005; 293(22):2767-2775.
2. Burman WJ, Cohn DL, Rietmeijer CA, Judson FN, Sbarbaro JA, Reves RR. Noncompliance with directly observed therapy for tuberculosis. Epidemiology and effect on the outcome of treatment. Chest 1997; 111:1168–1173.
3. Pandey R, Khuller GK. Antitubercular inhaled therapy: opportunities, progress and challenges. J Antimicrob Chemother 2005; 55: 430–435.
4. Ain Q, Sharma S, Khuller GK, Garg SK. Alginate-based oral drug delivery system for tuberculosis: pharmacokinetics and therapeutic effects. J Antimicrob Chemother 2003; 51:931–938.
5. Labana S, Pandey R, Sharma S, Khuller GK. Chemotherapeutic activity against murine tuberculosis of once weekly administered drugs (isoniazid and rifampicin) encapsulated in liposomes. Int J Antimicrob Agents 2002; 20:301-304.
6. Bala I, Hariharan S, Kumar MN. PLGA nanoparticles in drug delivery: the state of the art. Crit Rev Ther Drug Carrier Syst 2004; 21: 387-422.
7. Tonnesen HH, Karlsn J. Alginate in drug delivery systems. Drug Dev Ind Pharm 2002; 28:621–630.
8. Pandey R, Khuller GK. Chemotherapeutic potential of alginate–chitosan microspheres as antitubercular drug carriers. J Antimicrob Chemother 2004; 53:635-640.
9. Jain D, Bar-Shalom D. Alginate drug delivery systems: application in context of pharmaceutical and biomedical research. Drug Dev Ind Pharm. 2014; 40:1576–1584.
10. Zhan XP, Wu GH. Characteristics of sodium alginate and its application in food. Food Eng 2011; 1:7–9.
11. Ahmad Z, Pandey R, Sharma S, Khuller GK. Pharmacokinetic and pharmacodynamic behaviour of antitubercular drugs encapsulated in alginate nanoparticles at two doses. Int J Antimicrob Agents 2006; 27(5):409-416.
12. Dornish M, Hagen A, Hansson E, Peucheur C, Vedier F, Skaugrud O. Safety of ProtasanTM: ultrapure chitosan salts for biomedical and pharmaceutical use, in: A. Domard, G.A.F. Roberts, K.M. Varum (Eds.), Advances in Chitin Science, Jacques Andre Publisher, Lyon, 1997: 664–670.
13. Chen Y, Ye Q, Gong T, Kuang J, Li S. Preparation of Doxorubicin-loading Sodium Alginate Nanoparticles and its Anticancer Activity in HepG2 cells. J Pharm Biomed Sci 2019; 8(5):79-83.
14. Sailaja AK, Amareshwar P, Chakravarty P. Different techniques used for the preparation of nanoparticles using natural polymers and their application. Int J Pharm Pharm Sci. 2011; 3(2):45-50.
15. Rajaram S, Natham R. Influence of Formulation and Process Variables on the Formation of Rifampicin Nanoparticles by Ionic Gelation Technique. RJPBCS 2013; 4: 820-832.
16. Gaikwad A, Tamizhrasi S, Sorti A, Gavali P, Mehare G. Formulation and *in vitro* characterization of polymethacrylic acid nanoparticle containing frusemide. Int J Pharmtech Res 2010; 2: 300-304.
17. Bathool A, Vishakante GD, Khan MS, Shivakumar HG. Development and Characterization of Atorvastatin Calcium Loaded Chitosan Nanoparticles For Sustain Drug Delivery. Adv Mat Lett 2012; 3 (6): 466-470
18. Nagajyothi B, Babu MK. Design & development of glipizide fast dissolving tablets using natural gum superdisintegrant. AJPCR 2014; 7:144-148.

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