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Influence of Formulation and Process Variables on the Formation of Rifampicin Nanoparticles by Ionic Gelation Technique

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ABSTRACT

Chitosan, a natural biodegradable polymer is recommended for development of nanoparticles intended for chronic infection such as tuberculosis. Formulation and processing variables play a critical role in the formation of nanoparticles prepared with chitosan. The present study investigated the influence of polymer (chitosan) molecular weight, stabilizing agent (tween 80) concentration and stirring speed on rifampicin nanoparticles prepared by ionic gelation of chitosan with sodium tripolyphosphate. FTIR and DSC analysis revealed no chemical interaction between rifampicin and chitosan. DSC thermogram showed amorphous formation of the drug and polymer.Chitosan molecular weight, tween 80 concentration and stirring speed did not affect morphology of nanoparticles; however influenced the particle size, zeta potential, polydispersity index, drug encapsulation and loading efficiency and release of rifampicin nanoparticles. Our findings suggest that chitosan molecular weight, tween 80 concentration and stirring speed are important factors that need to be optimized to meet the desired qualities of rifampicin loaded chitosan nanoparticles.

Keywords: Nanoparticles, Chitosan, Ionic gelation, Rifampicin, Variables

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INTRODUCTION

Occurrence of treatment failure in tuberculosis (TB) is due to the development of resistant to individual drugs such as rifampicin (RIF), isoniazid (INH), pyrazinamide (PYZ) and ethambutol (ETH). To overcome the treatment failure and improve the patient compliance the World Health Organization (WHO) and international union against tuberculosis and lung disease (IUATLD) recommended the use of four drug fixed dose combination (FDC) of first-line drugs containing rifampicin, isoniazid, pyrazinamide, ethambutol. However a major concern has been expressed on the varied plasma profiles and unacceptable poor bioavailability of rifampicin from fixed dose combination (FDC) products containing first line drugs. This variable bioavailability is considered as a major obstacle in the effective implementation of FDCs in national TB programs and thus successful treatment of the same (Blomberg B et al., 2001). The WHO and IUATLD issued a joint statement in 1994 pointing out that anti-TB FDC products should only be issued if the bioavailability of at least the rifampicin component has been demonstrated (IUATLD/WHO,1994).

Recently several pharmaceutical approaches were attempted to improve the bio availability of rifampicin. Controlled drug delivery systems, nanoparticles, liposomes, and microspheres were developed for the sustained drug delivery of anti-TB drugs that have demonstrated better chemotherapeutic efficacy when investigated in animal models (Falk R et al., 1997). Nanoparticulate delivery of anti- TB drugs has assumed significance recently. Nanoparticles are stable solid colloidal particles consisting of biodegradable polymers or lipid materials and range in the size from 10 to 1000 nm. Drugs can be adsorbed onto the particle surface, entrapped inside the polymer/lipid or dissolved within the polymer matrix (Kreuter J, 1999). Oral nanoparticle based anti- TB drug therapy can allow for reduction in dosing frequency for better management of TB from a study conducted in animal model (Lee Guellec C et al., 1997).

In earlier studies, Dalencon et al reported the loading of rifabutin in nanocapsules, and the preparation was evaluated in experimental toxoplasmosis (Dalencon F et al., 1997). Lopes et al reported the encapsulation of ethionamide, a second- line antitubercular drug (Lopes et al., 2000).Evaluation of three front-line antituberculosis drugs (RIF, isoniazid , pyrazinamide) co encapsulated in PLGA NPs was carried out by Pandey et al (Pandey R et al., 2003). Anti tuberculosis drug-loaded solid lipid NPs have produced encouraging results (Pandey R et al., 2005)

Various polymers have been recommended for preparation of nanoparticles. Biodegradable polymers are preferred as they are eliminated from the body and therefore polymer induced toxicity is unlikely. As TB requires long term treatment, naturally occurring bio degradable polymers are recommended for chronic infections (Lifeng Q I et al., 2004). Among various polymers chitosan has shown favorable biocompatibility and membrane permeability both in vitro and in vivo (Yan Wu et al., 2005). It is a naturally occurring bio compatible cationic polysaccharide obtained from the deacetylation of chitin. Compared to other natural polymers chitosan has a positive charge and is mucoadhesive and it is used extensively in drug delivery

applications (Yongmei X et al., 2003). It has the capacity to protect sensitive bioactive macromolecules from enzymatic and chemical degradation during storage (Mao HQ et al., 2001). Chitosan has many advantages particularly for developing micro/nanoparticles. These include its ability to control the release of active agents; it avoids the use of hazardous organic solvents while fabricating particle since it is soluble in aqueous acidic solution (Tejraj M A et al., 2004). There is a growing interest in producing nanoparticles containing antituberculous drugs using natural polymer such as chitosan. (Bivas-Benita M et al., 2004)

Physicochemical properties play a major role to generate pharmaceutically acceptable nanoparticulate system. To obtain biocompatible nanoparticle system, molecular weight of polymers, concentration of surfactants and their influence on particle size and release need to be considered. In nanoparticle system particle size becomes increasingly importance since small particle have high absorption area, therefore they penetrate cell membrane more efficiently (Mehta S K et al., 2007). This may depend on the polymer molecular weight, properties of drug and device characteristics (preparation conditions, particle size, morphology and drug loading) (Shelesh Jain et al., 2009). Surfactants are used to stabilize nanoparticles by hindering their growth. The increase in the surfactant amount in colloidal dispersions may contribute to the reduction of mean particle size because of the surface active properties of surfactants. (Park K M et al., 1999).They improve the stability of system through static electricity repulsive forces,steric hindrance, and Van der waals force by absorbing on to the surface of nanomaterials (Kvitek L et al., 2008, Zhou X et al., 2007).

In this study, rifampicin loaded chitosan nanoparticles were prepared by ionic gelation with sodium tripolyphosphate. The effect of variables such as polymer (chitosan) molecular weight, stirring speed and surfactant (tween 80) concentration on the physic-chemical and invitro release characteristics of rifampicin nanoparticles were studied. To our knowledge no study has been reported on this line of work earlier.

MATERIALS AND METHODS

Rifampicin was obtained as gift sample from Astha Laboratories Pvt ltd, Hyderabad, India. Biodegradable Chitosan (deacetylation degree 85%), low MW (150KDa), medium MW (300 KDa), high MW (600KDa) were gifted by Central Institute of Fisheries Technology, Cochin, India. HPLC solvents were obtained from Merck, India. Deionized water was used throughout the experiments. All other chemicals used were of reagent grade.

Preparation of rifampicin loaded chitosan nanoparticles

Rifampicin loaded chitosan nanoparticles were prepared according to the procedure first reported by Calvo et al (1997b) based on the ionic gelation of chitosan with sodium tri polyphosphate anions (STPP) in the presence of tween 80 as a suspending agent to prevent aggregation, at ambient temperature. Rifampicin and chitosan with different molecular weight (150, 300, 600KDa) were dissolved in acetic acid in aqueous solution under magnetic stirring at room temperature in the presence of tween 80 at various concentrations (0.25, 0.5,1.0%).

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Nanoparticles were prepared by adding STPP aqueous solution dropwise to chitosan solution under magnetic stirring with different speed (400,800,1200rpm) at room temperature for 45 min. The nanosuspensions were cold centrifuged at 12000g, in a glucose bed for 30 min using Remi centrifuge (R4C-DX, USA). The supernatant liquid was analyzed by spectrophotometer at 475 nm to calculate the percentage drug entrapment and drug loading (Shrutidevi A et al 2004). The final suspensions were then frozen and lyophilized at 0.4 mbar and -40 ºC for 5 hrs using sucrose and glucose (1:1) as cryoprotective agents. The lyophilized nanoparticles were stored in desiccators at 4ºC. The formula for preparation of nanoparticles is given in Table 1

Table 1: Formulation of rifampicin loaded chitosan nanoparticles

Formula code		**Composition**	
	Chitosan Mwt(KDa)	(%) Tween 80	Stirring speed(rpm)
F1		0.25	400
F2		0.5	400
F3		1.0	400
F4	150 (Low)	0.25	800
F5		0.5	800
F6		1.0	800
F7		0.25	1200
F8		0.5	1200
F9		1.0	1200
F10		0.25	400
F11		0.5	400
F12		1.0	400
F13		0.25	800
F14	300 (Medium)	0.5	800
F15		1.0	800
F16		0.25	1200
F17		0.5	1200
F18		1.0	1200
F19		0.25	400
F20		0.5	400
F21		1.0	400
F22		0.25	800
F23	600 (High)	0.5	800
F24		1.0	800
F25		0.25	1200
F26		0.5	1200
F27		1.0	1200

Physicochemical characterization of nanoparticles

Drug and carrier interaction by Fourier Transform Infra Red Spectroscopy

FTIR Spectroscopy (Perkin Elmer RX1) was performed on pure drug, polymer and nanoparticles. The pellets were prepared by gently mixing 1mg sample with 200mg potassium bromide at high compaction pressure. The scanning range was 450 to 4000 cm^{-1} and the resolution was 4 cm^{-1}. The pellets thus prepared were examined and the spectra of all the samples were compared.

Thermal analysis by differential scanning calorimetry (DSC)

Differential scanning calorimetric measurement of nanoparticles was carried out by using a thermal analysis instrument (DSC CA 60 Shimadzu. Japan) equipped with liquid nitrogen sub ambient accessory. Samples were accurately weighed in aluminum pans thematically sealed and heated at a rate of 10ºC min^{-1} in a 30 to 300ºC temperature under nitrogen flow of 40 ml / min.

Morphology by scanning electron microscopy

The morphology of nanoparticles was analyzed by scanning electron microscope (JEOL MODEL JSM 6400). The nanoparticles were mounted directly on the SEM stub, using double – sided, sticking tape and coated with platinum and scanned in a high vacuum chamber with a focused electron beam. Secondary electrons, emitted from the samples were detected and the image formed.

Surface characteristics by zetasizer

The particle size, and particle size distribution of nanoparticles were measured with a malvern instrument (Zetasizer 3000 HS U.K).The particle size distribution is reported as poly dispersity index. The samples were placed in the analyzer chamber and readings were performed at 25°c with a detected angle of 90 degrees. The zeta potential of nanoparticles was measured with a malvern instrument (Zetasizer 3000 HS U.K). The samples were diluted with pH 7.4 buffer, and placed in eletrophoretic cell and measured in the automatic mode.

Rifampicin encapsulation efficiency and loading capacity of nanoparticles

The encapsulation efficiency and loading capacity of nanoparticles were determined by the separation of nanoparticles from the supernatant liquid containing non associated rifampicin obtained after cold centrifugation at 12000g for 30 minutes .The amount of free rifampicin in the supernatant liquid was measured by spectrophotometer at 475 nm. The rifampicin encapsulation efficiency (EE) and loading capacity (LC) of the nanoparticles were calculated from the following equations (De Campos A et al., 2001).

$$\text{Encapsulation efficiency} = \frac{\text{Total amount of rifampicin} - \text{Free rifampicin}}{\text{Total amount of rifampicin}} \times 100$$

$$\text{Loading capacity} = \frac{\text{Total amount of rifampicin} - \text{Free rifampicin}}{\text{Weight of nanoparticles}} \times 100$$

***In-vitro* diffusion study**

The study was performed on rifampicin and rifampicin nanoparticles in 0.1 N HCL. Samples equivalent to 100mg of rifampicin were redispersed in 10ml 0.1N HCL solution and placed in a dialysis membrane bag with a molecular cut-off of (MWCO 12,000-15,000Da, Himedia, India) which acts as a donor compartment, tied and placed into 10 ml 0.1N HCL solution in a beaker which acts as a receptor compartment .The entire system was kept at 37ºC±0.1°C with continuous magnetic stirring at 50 rpm. At appropriate time intervals (15, 30, 45, 60min) 1 ml of the release medium was removed through 0.1µm membrane filter immediately and 1 ml fresh 0.1N HCL solution was added in to the system. The amount of rifampicin in the release medium was evaluated by spectrophotometer at 475 nm and the percentage release of rifampicin recorded. The experiment was run in triplicate and the mean values were recorded as percent release of rifampicin (Gaurav K S et al., 2010).

RESULTS AND DISCUSSION

Preparation of rifampicin nanoparticles

The present study followed ionic gelation method for preparation of rifampicin nanoparticles using the natural and biodegradable polymer chitosan of different molecular weights. Natural polymers are more desirable for nanoparticles used in chronic infections like tuberculosis as they are free from polymer induced toxicity (Walls T et al., 2004) and therefore chitosan was chosen in the study. Ionic gelation is simple to process and nanoparticles of desirable size are easily obtained. Different grades of chitosan were used in order to examine their influence on the physicochemical and release characteristics of drug. Lyophillization was followed for preparation of nanoparticles using sucrose and glucose (1:1) as cryoprotective agent to yield stable amorphous solid with the desired property such as high redispersion speed, acceptable storage stability and also residual moisture content.

Physicochemical characterization of nanoparticles

The FTIR spectra of drug, polymer and nanoparticles are shown in Figures 1a-1j. There are three characteristic peaks of chitosan of different grades; 3318 cm^{-1} of υ(OH),1078cm^{-1} of υ(C-O-C) and 1621cm^{-1} of υ(NH_2) for low molecular weight chitosan; 3267 cm^{-1} of υ(OH),1078cm^{-1} of υ(C-O-C) and 1675cm^{-1} of υ(NH_2) for medium molecular weight chitosan;

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3324 cm^{-1} of υ(OH),1317cm^{-1} of υ(C-O-C) and 1578cm^{-1} of υ(NH_2) for high molecular weight chitosan.

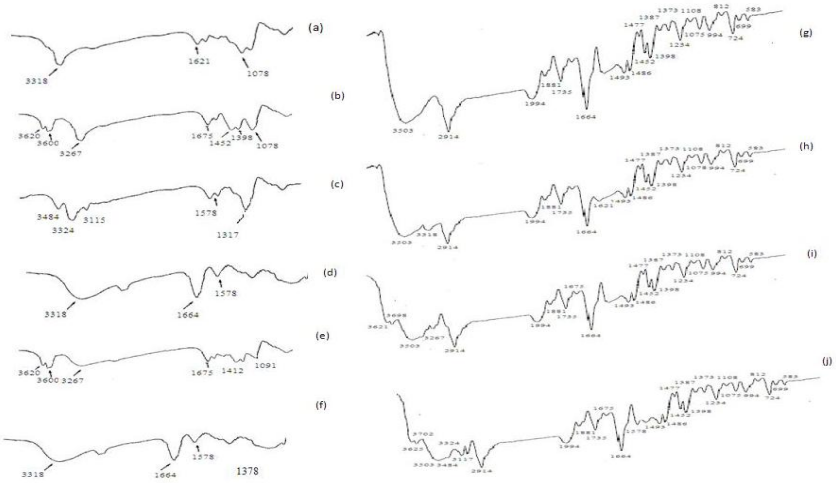

Figure1: FTIR of Low(a), medium(b), high(c) molecular weight chitosan, chitosan low(d), medium(e), high(f) molecular weight-TPP nanopracticle, rifampicin(g), and rifampicin loaded low(h), medium(i), high(j) molecular weight chitosan nanopraticle

The peaks of chitosan at 1621cm^{-1} (low molecular weight), 1675cm^{-1} (medium molecular weight), 1578cm^{-1} (high molecular weight) disappeared and new sharp peaks at 1664 cm^{-1}, 1421cm^{-1},1378 cm^{-1} were observed in the chitosan-TPP nanoparticles. These changes indicate that amino groups of chitosan were cross linked with tripolyphosphate groups of TPP in the nanoparticles. Our findings are consistent with earlier report (Knaul J Z et al., 1999). Rifampicin showed characteristic peaks at 1719cm^{-1} (acetoxyl C=O) 1746cm^{-1}(furanone C=O) and 2898cm^{-1}(amyl NH-C=O). These characteristic peaks of rifampicin were found in the rifampicin loaded chitosan nanoparticles (Figs.1h, 1i, 1j) indicating the absence of chemical interaction between the drug and polymer and thus safe use of ionic gelation method for development of rifampicin nanoparticles using chitosan as polymer.

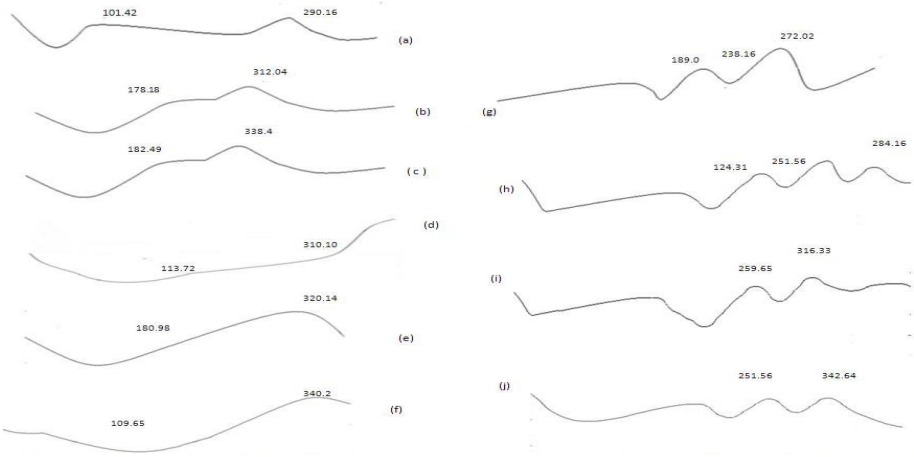

Figure 2: DSC of Low(a), medium(b), high(c) molecular weight chitosan, chitosan low(d), medium(e), high(f) molecular weight-rifampicin, rifampicin(g) and rifampicin loaded low(h), medium(i), high(j) molecular weight chitosan nanopraticle

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Fig.2.shows the physical state of rifampicin in the nanoparticles, as it could influence the release characteristics of the drug from the system. The drug and the polymer may co-exist in the polymeric carrier as a) amorphous drug either in amorphous or in crystalline polymer or b) crystalline drug either in amorphous or in crystalline polymer. The DSC thermogram of polymer showed characteristic endothermic peaks at 101.42^{0}C and 290.03^{o}C for low molecular weight (Figure.2a), at 178.18^{0}C and 312.04^{0}C for medium molecular weight (Figure.2b), and at 182.49^{0}C and 338.4^{0}C for high molecular weight (Figure.2c) chitosan. The DSC of rifampicin showed endothermic peaks at 189.0^{0}C, 238.16^{0}C and 272.02^{0}C (Figure.2g). The characteristic peaks of chitosan as well as rifampicin were shifted. The peaks of chitosan of 290.03^{o}C (low Mwt), 312.04^{0}C (medium Mwt), 338.4^{0}C (high Mwt) were shifted to 310.12 ^{o}C, 320.14 ^{o}C, 340.21 ^{o}C respectively in physical mixture (Figures. 2 d, e, f) similarly the endothermic peaks of rifampicin at 238.16^{0}C and 272.02^{0}C were shifted to 284.16 ^{o}C, 316.33 ^{o}C, and 342.64 ^{o}C (Figures. 2 h, i, j) in the nanoparticles. These findings indicate that physical state of rifampicin and chitosan has changed from crystallinity to amorphous in nanoparticle that helps improved dissolution of the drug in the environment.

Influence of polymer molecular weight, tween 80 concentration, and stirring speed on the physicochemical properties of rifampicin loaded chitosan nanoparticles

Fig.3 shows the morphology of nanoparticles. All nanoparticle formulations prepared with different grades of chitosan at variable stirring speed and concentration of tween 80 were spherical with smooth surfaces and solid dense and showed no aggregation due to the result of stable zeta potential on the surface of the nanoparticles that prevent the agglomeration process. The morphology of all formulations was uniform and not affected by the molecular weight of polymer, concentration of tween 80 and stirring speed.

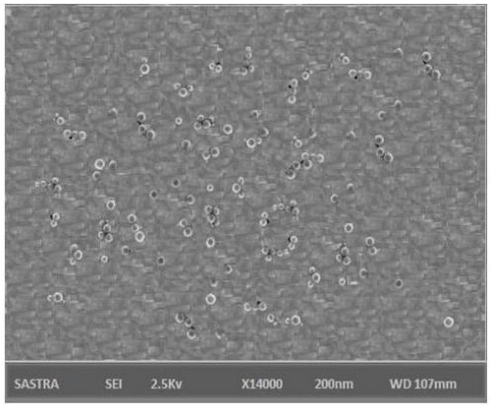

Figure 3: Scanning electron microscopy of rifampicin nanopraticles

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Table 2: Influence of molecular weight, concentration of tween 80, and stirring speed on the physicochemical properties nanoparticles

Formula Code	(%) Encapsulation Efficiency± S.D	(%) Loading Capacity± S.D	Particle size (nm)	Zeta potential (mV)	PDI
F1	49.82±0.34	25.96±0.45	423	+37	0.429
F2	56.96±0.35	29.80±0.51	410	+37	0.431
F3	57.85±0.25	31.76±0.52	412	+35	0.495
F4	62.85±0.32	35.91±0.32	325	+37	0.302
F5	64.64±0.41	36.78±0.44	302	+38	0.285
F6	70.19±0.36	39.69±0.29	308	+39	0.301
F7	77.67±0.24	43.93±0.34	215	+40	0.285
F8	80.71±0.33	45.65±0.35	202	+42	0.225
F9	85.00±0.32	48.57±0.62	225	+39	0.285
F10	50.89±0.15	27.66±0.35	460	+38	0.452
F11	57.85±0.45	30.56±0.42	442	+37	0.441
F12	59.10±0.23	32.13±0.49	496	+32	0.510
F13	66.25±0.31	35.67±0.43	359	+37	0.325
F14	66.96±0.12	37.87±0.22	334	+41	0.285
F15	72.50±0.54	41.01±0.35	334	+39	0.323
F16	78.92±0.22	44.64±0.62	229	+40	0.292
F17	82.14±0.31	46.23±0.32	212	+42	0.231
F18	85.71±0.52	48.87±0.34	240	+39	0.273
F19	53.92±0.11	28.49±0.47	490	+38	0.494
F20	59.28±0.32	31.32±0.51	453	+37	0.451
F21	61.07±0.26	33.20±0.19	513	+28	0.523
F22	73.92±0.24	39.42±0.35	380	+37	0.325
F23	68.75±0.31	38.89±0.21	345	+41	0.292
F24	75.17±0.21	42.52±0.69	360	+38	0.335
F25	80.00±0.24	45.25±0.23	302	+40	0.230
F26	83.57±0.23	47.27±0.22	214	+42	0.235
F27	87.14±0.26	49.39±0.57	250	+39	0.310

Table 2 shows the zeta potential of the nanoparticles. Commonly, zeta potential is an index of the stability of the nanoparticles. Under most conditions, the higher the absolute value of the zeta potential of the nanoparticles, the larger the charge on their surface, leading to stronger repulsive interaction between the dispersed nanoparticles and higher stability and more uniform size (Muller R H, 1999). A high potential value of above ±25mV ensures a high energy barrier that stabilizes the nano suspension (Mora C H et al., 2010). The zeta potential of all formulations was between +32mV to +42mV. All nanoparticles showed a positive surface charge possibly due to the presence of amino groups in the polymer and zeta potential of all the formulations was above+25mV, the limit for the stability of nanoparticles. The zeta potential of nanoparticles was affected by molecular weight of chitosan, stirring speed, concentrations of tween 80. The zeta potential decreased significantly with increase in molecular weight of chitosan; conversely, the zeta potential of nanoparticles increased with increase in stirring speed and tween 80 concentration. The possible mechanism may be formation of fine particles by increased stirring speed that may facilitate greater interaction between the drug and the polymer and therefore increased zeta potential of the nanoparticles.

These formed nanoparticles with higher zeta potential are better stabilized with higher concentration of tween 80. Higher molecular weight chitosan may probably hinder fine particles formation by ionic gelation method and thus reduced zeta potential. Zeta potential values obtained in the present study allow predicting good colloidal stability due to high energy barrier between particles. These findings suggest chitosan molecular weight, stirring speed, and tween 80 concentration need to be optimized for the development of stable nanoparticles.

Poly dispersity index is another factor that represents the dispersion homogeneity; the range for the PDI is from 0-1.Values close to 0 indicates the homogenous dispersion and those greater than 0.5 indicate high heterogeneity. The PDI for all formulations was between 0.2 - 0.5 which indicates a relative homogenous dispersion (Fernandez-U R et al., 1999). Further, the homogeneity of the dispersion was influenced by the molecular weight of polymer, stirring speed and concentration of the stabilizing agent; however maintained homogeneous dispersion with PDI at or lower than 0.5.

Particle size plays a critical role in influencing the physicochemical and biological characteristics of nanoparticles. Smaller particles less than 400nm are most preferred in pharmaceutical product development. In the present study the particle size of all formulations ranged from 202nm-513nm, and increased significantly with increase in molecular weight of polymer. The increase in particle size is possibly due to the increased solubility of low molecular weight chitosan may aid in the colloidal solubility of nanoparticles in the solution.[20] Furthermore, higher molecular weight chitosan is less soluble, and as a result an increase in particle diameter or even aggregation may be obtained.

Tween 80 as a stabilizing agent is adsorbed on to the surface of the nanoparticles, thereby slowing down the growth of crystal phases by reducing the surface free energy. It was found in the study that the nanoparticles prepared in the presence of tween 80 are much smaller, spherical particles, however the size of these particles decreased significantly as the concentration of tween 80 increased. Our finding is consistent with earlier report that higher concentration of tween 80 reduces the surface free energy to a great extent and hence stabilization of the smaller particles formed (Alanso J L et al., 1999). Besides, stirring speed also has produced the same effect on the particle size of nanoparticles in our study. As the stirring speed increased the particle size of the nanoparticles decreased significantly. This effect is due to the occurrence of external energy and thus the shear stress causing droplet breakdown increased with increased stirring speed (Thagele R et al., 2011).

The drug encapsulation efficiency and loading capacity of the nanoparticles depend upon the molecular weight of the polymer, the interaction between the drug and the polymer, the stirring speed used and also the concentration of the stabilizing agent added in the development of nanoparticles. Table 3 shows that the increase in the molecular weight of chitosan, the stirring speed and the concentration of tween 80 (stabilizing agent), all resulted increase in the encapsulation efficiency and loading capacity significantly. Rifampicin having carboxyl group resulted in electrostatic interaction with the amino group of chitosan and therefore influenced the encapsulation efficiency and loading capacity of nanoparticles. High

molecular weight chitosan has more reaction sites and therefore more electrostatic interaction with rifampicin. Further increasing stirring speed or concentration of tween 80 brings about breaking down of the particles to smaller size caused by increased shearing stress and consequently stabilization of the formed nanoparticles by higher concentration of the stabilizing agent. These findings are consistent with earlier observation that longer chains of high molecular weight chitosan can entrap greater amount of drug when gelated with tripolyphosphate (Yongmei X et al., 2007).

Table 3:Influence of molecular weight, concentration of tween 80, and stirring speed on release of drug from nanoparticle (n=3, mean±SD).

Formula Code	(%)Diffusion ± S.D	Formula Code	(%) Diffusion ± S.D	Formula Code	(%) Diffusion ± S.D
F1	28.75 ±0.33	F10	26.01±0.62	F19	21.39±0.19
F2	29.93±0.16	F11	28.30±0.25	F20	23.11±0.30
F3	30.06±0.48	F12	29.63±0.30	F21	23.98±0.29
F4	30.81±0.11	F13	29.98±0.24	F22	25.15±0.17
F5	32.60±0.32	F14	30.46±0.19	F23	28.60±0.20
F6	33.01±0.18	F15	31.09±0.55	F24	30.34±0.24
F7	40.05±0.15	F16	32.64±0.51	F25	30.83±0.06
F8	44.16±0.25	F17	35.71±0.22	F26	33.98±0.16
F9	45.09±0.33	F18	36.43±0.11	F27	34.67±0.27

In-vitro diffusion

The results of in vitro diffusion are shown in Figure 4. The size of the nanoparticles is the most important factor that influences diffusion of the drug through the biological membrane. It is an established fact that formulation as well as processing variables in the preparation of nanoparticles greatly influence the physicochemical characteristics, more importantly, the particle size that influence the diffusion of the drug across the membrane.

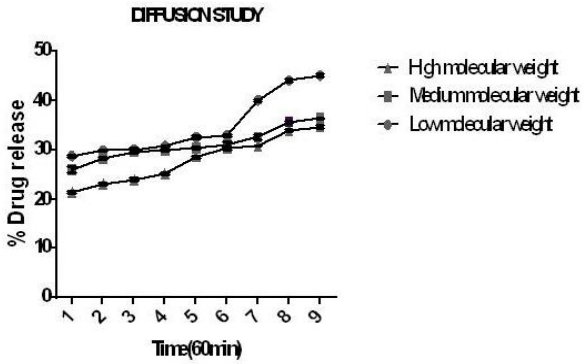

Figure 4: Influence of molecular weight on %release of drug

We observed in our study that chitosan molecular weight, tween 80 concentrations and stirring speed, all influenced the particle size and therefore the percent diffusion of the drug. Maximum percent drug diffusion (44.16%) was observed with low molecular weight chitosan

including 0.5% tween 80 and stirring speed 1200 rpm. As the molecular weight of the chitosan increased, the particle size and diffusion of the drug decreased irrespective of the concentration of tween 80 or of the stirring speed. The effect of tween 80 concentration on the particle size of nanoparticles at a fixed stirring speed was also examined. It was observed that as the concentration of tween 80 increased, from 0.25% to 0.5%, the particle size decreased and beyond 0.5% tween 80 concentration, the particle size showed increasing trend. Such finding was observed with all the three grades of chitosan. However, beyond these two factors such as concentration of tween 80 and stirring speed, the molecular weight of chitosan appears predominantly influencing the particle size as well as the diffusion of the drug. Our findings clearly suggest that low molecular weight chitosan improves the percentage diffusion of the drug possibly due to the fact that low molecular weight chitosan is more soluble that may aid in the formation of colloidal nanoparticles with enhanced diffusion, whereas medium or high molecular weight chitosan is less soluble resulting in aggregation of the particles with increase in particle size of the nanoparticles, and therefore decrease in the diffusion of the drug.

CONCLUSION

The findings of the present study demonstrate that molecular weight of chitosan, stirring speed, and concentration of tween 80 are important factors that need to be considered for development of rifampicin nanoparticles by ionic gelation method with desirable physico-chemical and release characteristics.

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