Original Research Article

FORMULATION AND CHARACTERIZATION OF RUTIN LOADED CHITOSAN NANOPARTICLES

ABSTRACT

New drug delivery technologies are transforming drug discovery and development, as well as establishing research and development-focused pharmaceutical firms that are accelerating global progress. The bioactive rutin molecule is used in a wide range of food and medicinal goods. Its limited bioavailability and poor water solubility are major issues. Rutin is a polyphenolic natural compound with antibacterial, anticancer, antioxidant, chemopreventive, and anti-inflammatory activities. However, no research has been published to yet to improve its bioavailability and efficacy. As a result, an attempt was made in this study to load rutin into a nanoparticlulate system in order to improve its bioavailability and efficacy. Six formulations (F1-F6) of nanoparticles were prepared by solvent evaporation technique and were evaluated for particle size and shape using Zeta Sizer, Scanning Electron Microscopy (SEM) and Fourier Transform Infra-Red (FT-IR) Spectroscopy. The optimized formulation was further subjected to *in vitro* evaluation. Practical percent yield, drug entrapment efficiency and *in vitro* drug release were evaluated. Out of various formulations F1 have shown best results in particle size 80.71 (1-100 nm), particle shape (spherical nanoparticles with a smoothed surface), average size distribution (105.0), zeta potential (-20.6) percentage yield (70.83), drug entrapment efficiency (83.6 %) and drug loading (95%). Pure rutin showed incomplete dissolution of 47.69% in 330 min while Rutin loaded nanoparticles gave 94.75% release in 330 min. It is obvious from the foregoing that rutin chitosan nanoparticles were used as a novel drug delivery technology to improve therapeutic efficacy and sustained release features while overcoming issues such as poor solubility and limited bioavailability.

Keywords: Rutin, Nanotechnology, Rutin loaded chitosan nano particle, Scanning Electron Microscopy (SEM), Fourier Transform Infra-Red (FT-IR) Spectroscopy and Zeta Sizer.

1. INTRODUCTION

Many medications have been put into the market in recent years that have poor therapeutic stability due to their low water solubility. Many formulations fail to address solubility difficulties, particularly for medications classified as class II and IV by the Biopharmaceutical Classification System (BCS). If class II and IV medications are manufactured in this manner, they will have low solubility and permeability, resulting in decreased bioavailability, limited selectivity, high toxicity, and greater adverse effects. Nanoparticle-based innovative drug delivery systems offer a lot of promise and can be an effective treatment method for medications in classes II and IV. Improved bioavailability, high drug load capability, high stability, extended pharmacokinetic profile, low dosing frequency, improved site specific delivery, and feasibility of hydrophilic and hydrophobic substances of incorporation are all benefits of this technological advancement in nanoparticles [1,2]. In recent years, various approaches like micro/nano carriers, particularly nanoparticles and nanofibers, have gained much importance for enzyme immobilization. Liposomes, micelles, and phospholipid complexes are other promising novel formulations, which appear to provide longer circulation, better permeability, and resistance to metabolic processes. Nanoparticles are particles between 1 and 100 nanometers in size. In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties. Targeted drug delivery implies selective and effective localization of drug into the target at therapeutic concentrations with limited access to non-target sites. Nanomedicine can be utilized as a valid treatment because the development of the disease occurs at the cellular level. When combined with good drug carriers, nanoparticles' promising properties play a crucial role. Chitosan is a natural polymer made from the N-deacetylation of chitin, which is found in the shells of marine crustaceans, that is biocompatible, hemocompatible, biodegradable, and has a high drug loading efficiency when employed as a polymer in nanoparticle formulation [3–7]. As a result, an attempt was undertaken in this study to load rutin into a nanoparticulate structure in order to improve its bioavailability and efficacy. Flavonoids are chemicals found in plants that are consumed as fruitlets, nuts, vegetables, and derivative foods like wine and brunette. The diets of western countries are largely comprised of quercetin. Quercetin is a flavonoid that can be found in foods that contain sugars, primarily as -glycosides. The glycoside that connects the flavonol quercetin and the disaccharide rutinose is rutin, also known as rutoside. This citrus flavonoid can be found in a wide range of plant species. Rutin is a nutritional flavonoid that has gotten a lot of attention because of its pharmacological effects, which include antibacterial, anti-

inflammatory, anticancer, antidiabetic, and so on. Despite their lack of traditional nutritional value, flavonoids are being more recognised as valuable dietary elements that may act as potential defenders against human diseases such as coronary heart disease, cancer, and inflammatory bowel disease. Rutin has been discovered to be a quercetin releaser in the gut; additionally, because quercetin is widely broken down in the gut, the quercetin released from rutin and/or its colonic metabolites may play an important role [8, 9, and 10].

2. MATERIALS AND METHODS

2.1 Preparation techniques of nanoparticles

The medication may become imprisoned in the reservoir or matrix, or it may be absorbed on the particle systems' surfaces. Using appropriate techniques, the polymers are tightly shaped to nanomeric size range particles. To make rutin chitosan nanoparticles, researchers used the coacervation/ionic gelation approach.

2.1.1 Coacervation or ionic gelation method

This method was developed by Calvo and co-worker in 1997. In this method polymer solutions and polyanion solutions are mixed to form nanoparticles. The CSNPs are formed due to the electrostatic interaction between positively charged amine group of chitosan and negatively charge group of polyanion such as tripolyphosphate. This technique offers a simple and mild preparation method in the aqueous environment. First, chitosan can be dissolved in acetic acid in the absence or presence of stabilizing agent, such as poloxamer, Tween80 which can be added in the chitosan solution before or after the addition of polyanion. Polyanion such as TPP (sodium tripolyphosphate) was then added and nanoparticles were spontaneously formed under mechanical stirring at room temperature. The material undergoes transition from liquid to gel phase due to interaction. Using this method different proteins and peptides has been loaded [11].

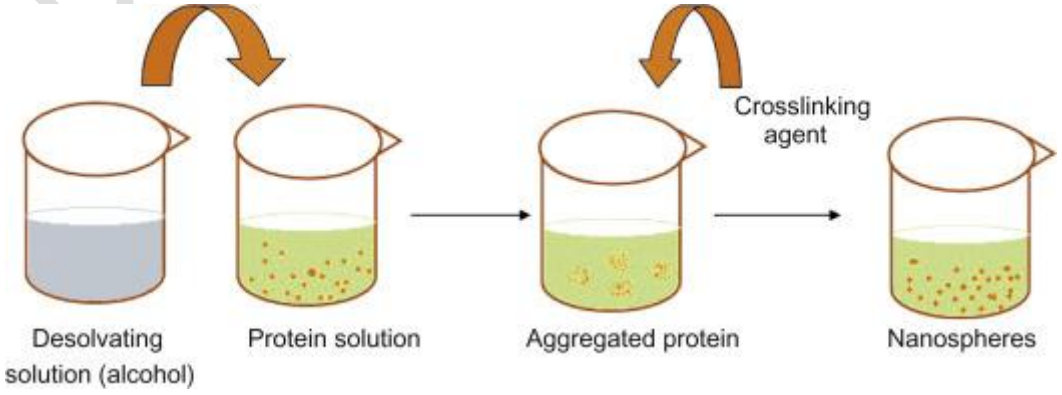

Figure 1: Coacervation or ionic gelation method

2.1.2 Need/ Scope of the research work

The purpose of this research is to focus on recent studies on various therapeutic uses of rutin, issues related with rutin delivery, and approaches to improve rutin bioavailability utilising innovative nanotechnology techniques. The following are some of the advantages of using nanotechnology:

1. Improve the ability to deliver drugs that are poorly water soluble.
2. Provide site-specific targeting to reduce drug accumulation within healthy tissue.
3. Help retain the drug in the body long enough for effective treatment.
4. Allow for the transportation of drugs across epithelial and endothelial barriers.
5. Combine therapeutic and diagnostic modalities into one agent.
6. High stability conferring long shelf lives.
7. High carrier capacity.
8. Feasibility of incorporation of both hydrophilic and hydrophobic substances.
9. Controlled drug release from the matrix.
10. Due to site specific action, reduction in quantity of drug and safe delivery to target tissue.

2.2 Preparation of Rutin nanoparticles

The nanoparticles (NPs) were produced by ionic gelation method or coacervation. In brief, chitosan solution (2 mg/mL) was prepared by dissolving chitosan in 3% (w/v) acetic acid solution and kept under stirring overnight at room temperature. Rutin was dissolved in ethanol (70%), mixed with the chitosan solution and stirred for 30 min. TPP (1mg/ mL) was added drop wise to the chitosan-rutin mixture with continuous stirring. After formation of NPs, the colloidal suspension was left for stirring for next 2 hours for particle hardening. The RCNPs (Rutin chitosan nanoparticles) were separated by centrifuging the suspension at 12,000g for 10 min. The supernatant was used to determine the entrapment efficiencies, whereas the pellet was used in the release, sizing and surface charge studies. The RCNPs pellet was suspended in water, centrifuged and freeze [12, 13, 14 15].

Table 1: Formulation of rutin loaded chitosan nanoparticles

S. No	Ingredients	Formulations					
		F1	F2	F3	F4	F5	F6

1	Rutin	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
2	Chitosan	100 mg	200 mg	300 mg	400 mg	500 mg	600 mg
3	Acetic acid	3 %	3 %	3 %	3 %	3 %	3 %
4	Sodium tripolyphosphate	1 mg/ml	1 mg/ml	1 mg/ml	1 mg/ml	1 mg/ml	1 mg/ml
5	Distilled water	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

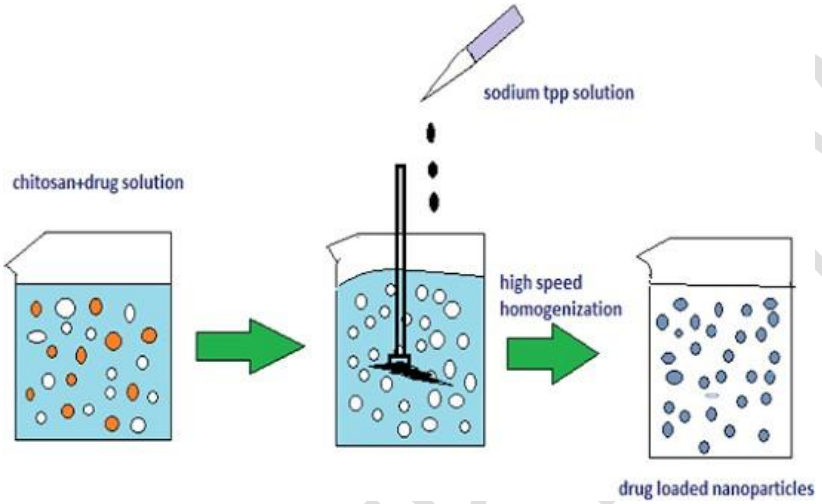

Figure 2: Preparation of nanoparticles by ionic gelation method

2.3 Evaluation of Rutin nanoparticles

The rutin nanoparticles were evaluated for particle size, shape, zeta potential, percent practical yield, drug entrapment efficiency, percent of drug loading and *in vitro* drug release.

A) Determination of Particle Size and Zeta potential

Scanning Electron Microscope was used to measure the particle size of NPs (SEM). The nanoparticles were placed on aluminium stubs for this purpose, and the surface was covered with gold particles using a sputter coater. Scanning electron microscopy (SEM) (Hitachi-S 3400 N) at 15 kV and 750 mA was used to determine the shape of the NPs. All measurements were carried out at a temperature of 25°C. A Zeta sizer was used to determine the Zeta potential of drug-loaded ceramic nanoparticles. Nanoparticle samples were diluted with water and placed in an electrophoretic cell with an electric field applied to assess the zeta potential. Each sample was examined three times. Figures 3, 4 and 5 show SEM pictures, particle size data, and Zeta potential studies.

B) Fourier Transform Infrared Spectroscopy (FT-IR) analysis

The FT-IR spectra of chitosan and NPs were recorded on a FTIR spectrometer using KBr pellets at a resolution of 4cm^{-1}. The IR absorbance were analysed between 400 to 4000 cm^{-1} for detection of any changes in the intensity of the sample peaks.

C) Determination of Percentage Practical yield

Dried nanoparticles were collected and weighed to determine practical yield (PY) from the following equation.

$$PY\% = \frac{\text{Nanoparticle weight}}{\text{Theoretical mass (Polymer + Drug + STPP)}} \text{ X } 100$$

D) Determination of Entrapment Efficiency (%EE) and Percentage of Drug loading

Drug solution of different concentrations was added to volumetric flask containing an accurately weighed amount of sugar coated ceramic core (25 mg). The flasks were stoppered and shaken vigorously at 130 rpm for 24 hrs. The particles were placed in tray containing water (room temperature) and left for about 1 hour with timely shaking. Nanoparticles were prepared. Suspension was centrifuged at 15000 rpm, 5 minutes and 12.5°C for the dispersion. Ceramic nanoparticles were separated and air dried [16].

Nanoparticles 50 mg were dissolved in 100 ml of phosphate buffer pH 6.8 and the drug amount was determined by UV analysis. The encapsulation efficiency was determined as the mass ratio of entrapped rutin in nanoparticles to the theoretical amount of the drug used in the preparation. The entrapment of the rutin nanoparticles was expressed as loading capacity.

$$EE\% = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \text{ X } 100$$

The percentage drug loading of nanoparticles was calculated by the following equation. The amount of drug in supernatant was calculated from concentration values obtained from the calibration curve of spectrophotometric analysis of the samples at 257 nm.

$$\text{Drug loading} = \frac{\text{Total rutin added in system} - \text{Drug present in supernatant}}{\text{Total amount of nanoparticle obtained}} \text{ X } 100$$

Results of Entrapment efficiency and Percentage of drug loading were reported in table 5.

E) *In-vitro* drug release studies

Dry drug loaded nanoparticles equivalent to 10 mg of pure drug were carefully weighed and transferred into empty capsules for *in vitro* release assays. *In vitro* release of rutin from optimum nanoparticles was studied by direct dissolution method. *In vitro* release studies were performed by accurately weighing dried drug loaded nanoparticles equivalent to 10 mg of pure drug, and transferring into empty capsules. Dissolution was performed using capsules as reported in USP/NF, by the use of USP type I (Basket) dissolution apparatus. At pre-determined time intervals, 5 ml of the release medium was removed and replaced with 5 ml of fresh phosphate buffer solution pH 6.8. The samples were analyzed for cumulative percent drug dissolved/released by UV spectrophotometry [17].

The capsules were disintegrated using the USP type I (Basket) dissolution apparatus, as described in the USP/NF. 5 ml of the release medium was removed and replaced with 5 ml of new phosphate buffer solution pH 6.8 at predetermined time intervals. UV spectrophotometry was used to determine the cumulative percent medication dissolved/released in the samples. Table 6 summarises the findings of *in vitro* drug release (cumulative drug release) investigations.

3. RESULTS AND DISCUSSION

3.1 EVALUATION OF NANOPARTICLES

3.1.1 Determination of Particle size and Zeta potential

The SEM images of chitosan nanoparticles showed spherical particles. The particle size was uniform and particles were mostly single (discrete), however, a few aggregates were also visible. The particle size of the rutin chitosan loaded particles was found to be within the nano range.

Table 2: Average particle size of chitosan nanoparticles

Particles	Particle size, (nm) (AM ± SD)*
Rutin chitosan loaded Nanoparticles	80.716 ± 12.404

*Each value represents the mean of 3 determinations

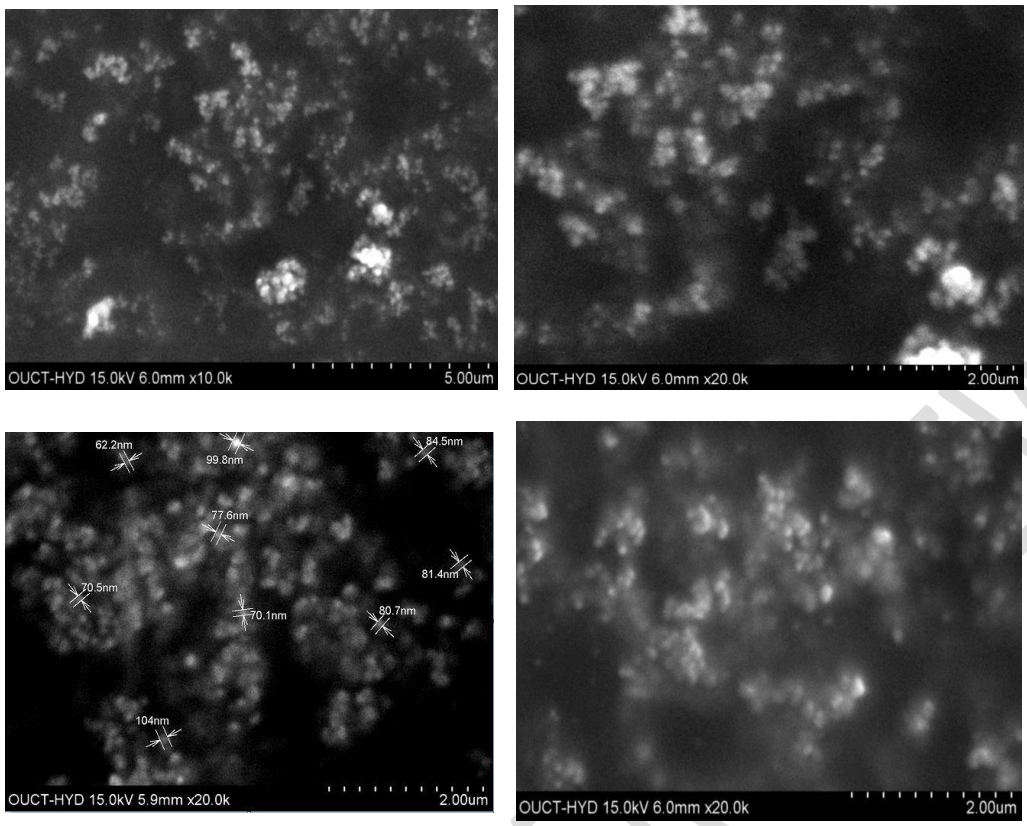

Figure 3: Scanning Electron Microscopy images of chitosan nanoparticles

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name: Nano F1
SOP Name: Nano F1.sop
General Notes: Average result created from record number(s): 70 72

File Name:	Nano F1.sop	**Dispersant Name:**	Water
Record Number:	74	**Dispersant RI:**	1.330
Material RI:	1.30	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	Wednesday, Sep 22, 2020 05:1...

System

Temperature (°C):	25.0	**Duration Used (s):**	60
Count Rate (kcps):	231.6	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	10

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	105.0	Peak 1:	288.3	73.6	194.6
PdI:	0.560	Peak 2:	15.13	24.6	3.780
Intercept:	0.906	Peak 3:	4844	1.8	767.8
Result quality	Good				

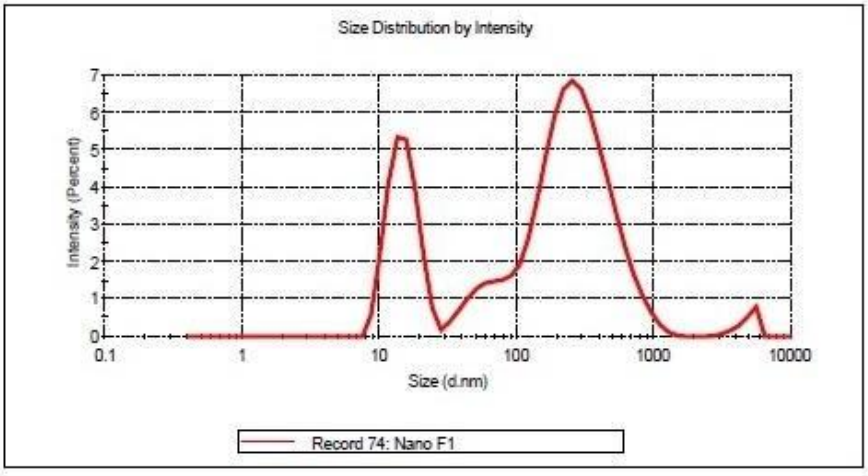

Figure 4: Size Distribution report of Nanoparticles

Zeta Potential Report

v2.3

Malvern

Sample Details

Sample Name:	Nano F1
SOP Name:	Nano F1.sop
General Notes:	Average result created from record number(s): 38 39 40

File Name:	Nano F1.sop	**Dispersant Name:**	Water
Record Number:	41	**Dispersant RI:**	1.330
Date and Time:	Wednesday, Sep 22, 2020 5:...	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	**Zeta Runs:**	20
Count Rate (kcps):	126.7	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta cell	**Attenuator:**	7

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-20.6	Peak 1:	-39.0	81.9	8.55
Zeta Deviation (mV):	59.3	**Peak 2:**	67.2	10.9	3.10
Conductivity (mS/cm):	0.0190	**Peak 3:**	92.7	5.5	2.45
Result quality	See result quality report				

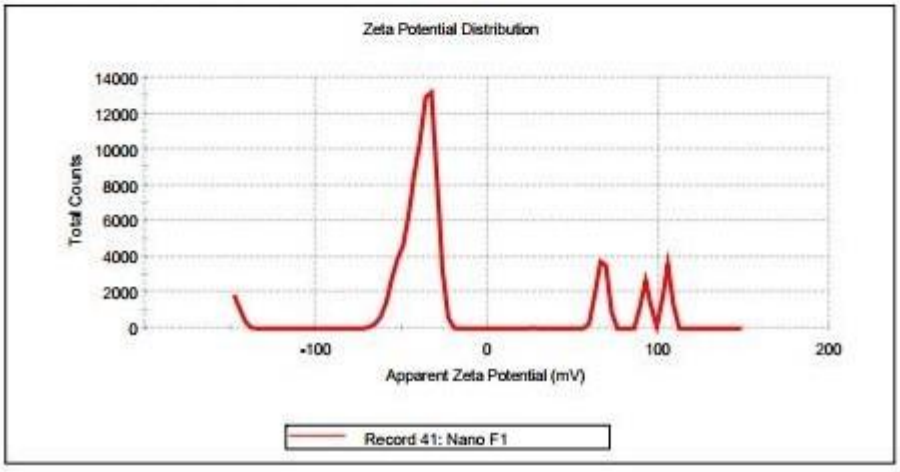

Figure 5: Zeta Potential report of Nanoparticles

3.1.2 FTIR spectroscopy analysis

KBr pellets of rutin-chitosan nanoparticles were prepared and the characteristic spectra were compared with literature values. The FTIR spectra of chitosan, Sodium TPP, Chitosan and sodium TPP and nano particles are shown in the figure 6,7and 8. The characteristic bands were reported in the Table 6 and all were found to be within the limits.

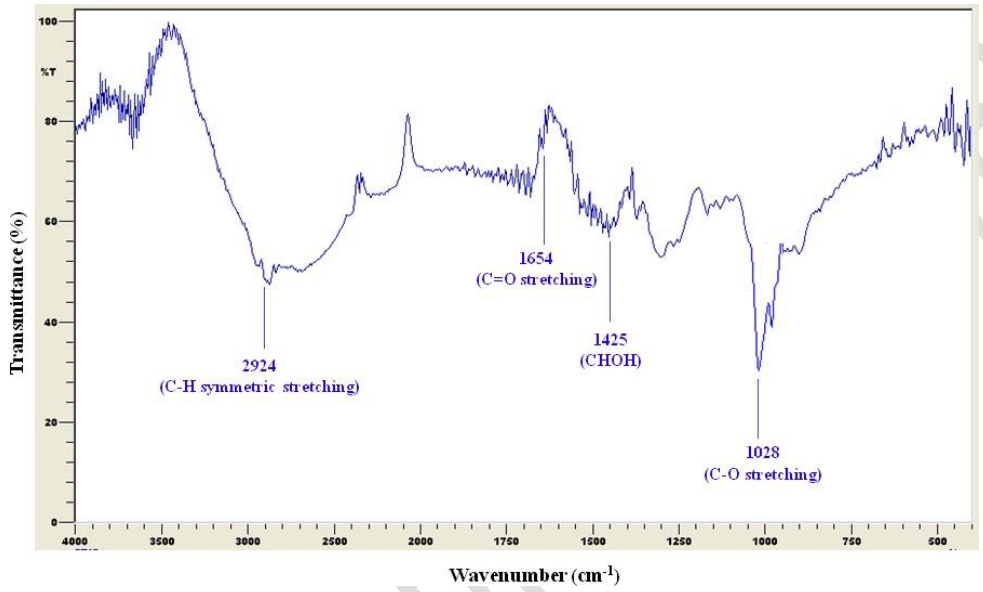

Figure 6: FTIR spectra of Chitosan

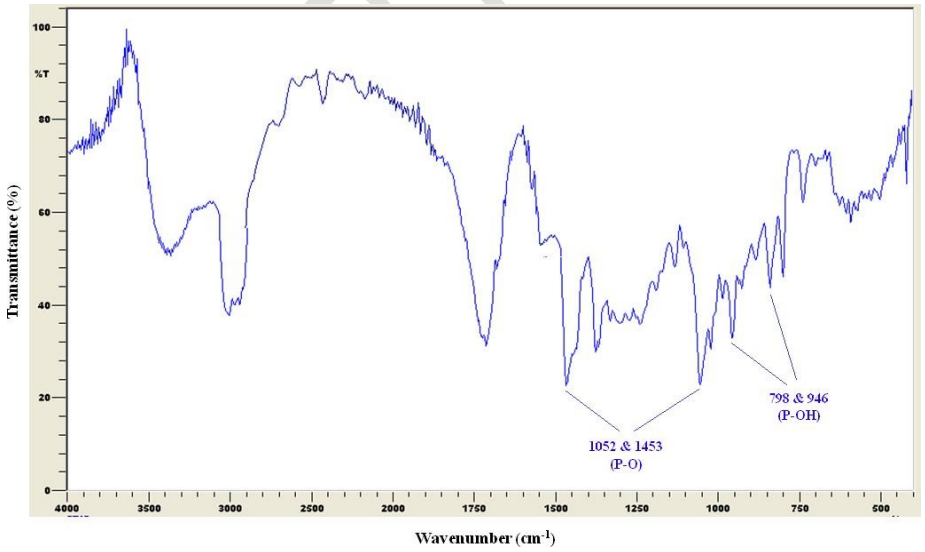

Figure 7: FTIR spectra of Sodium TPP

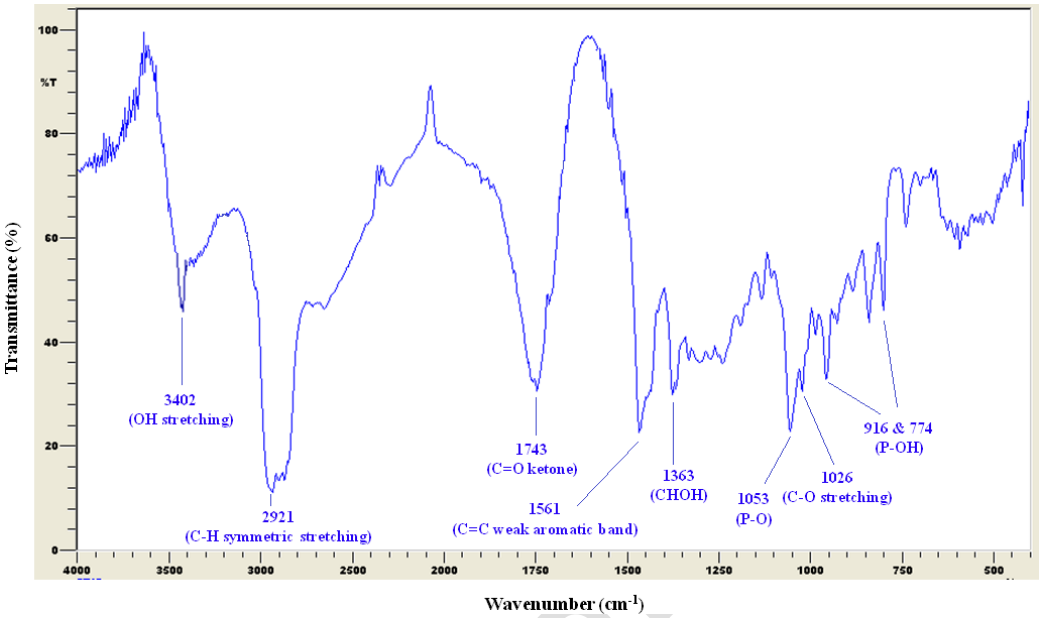

Figure 8: FTIR spectra of rutin, chitosan and sodium TPP

Table 3: Comparison of characteristic FTIR bands of final rutin nanoparticles

Characteristic bands	Literature values, cm^{-1}	Observed in this study, cm^{-1}
Rutin, chitosan, Sodium TPP		
OH stretching	3600-3200	3402
CH stretching	2950-2850	2921
C=O ketone	1745	1743
C=C weak aromatic band	1600-1400	1561
Chitosan		
C-O stretching	1066 & 1028	1028
C-H symmetric stretching	2921	2924
C=O stretching	1670-1820	1654
CHOH	1350-1480	1425
Sodium TPP		
P-O	1050-1500	1052 &1453
P-OH	450-970	798 & 946

3.1.3 Determination of Percentage Practical Yield

Percentage practical yield of different formulations of rutin chitosan nanoparticles was calculated. It was reported in Table 4 and it is found that F1 has highest percentage (70.83) yield compared to other formulations and it is carried out for further evaluation.

Table 4: Percentage yield of different formulation of rutin chitosan nanoparticles

F Code	Weight of Nanoparticles obtained (mg)	Percentage practical yield (%)
F1	**42.8**	**70.83**
F2	63.2	66.52
F3	82.4	63.38
F4	93.12	56.43
F5	103.6	51.8
F6	101.3	43.10

3.1.4 Entrapment efficiency and Drug loading

Entrapment efficiency and drug loading of rutin chitosan nanoparticles of various formulations was calculated and reported in the Table 5. F1 was said to be the best formulation in which the entrapment efficiency was found to be 83.6 % and the amount of drug loaded was about 95 %.

Table 5: Entrapment efficiency and drug loading of different rutin chitosan nanoparticle formulations

F Code	Entrapment Efficiency	Drug loading
F1	**83.6%**	**95%**
F2	76.8%	91%
F3	75.6%	87.3%
F4	74.0%	84%
F5	72.4%	78%
F6	71.2%	72.06%

3.1.5 *In vitro* drug release studies: (Cumulative percentage release)

The cumulative percentage release of different formulations of rutin chitosan nanoparticles and pure drug at 37 ± 0.5^{o}C was carried out in Phosphate buffer solution pH 6.8 and was reported in Table 6 and figure 9. Pure rutin showed incomplete dissolution of 47.69% in 330 mins. *In vitro* dissolution studies indicated that the rutin chitosan nanoparticles released the drug in a controlled manner. Rutin loaded nanoparticles (F1) gave 94.75% release in 330 min.

Table 6: Cumulative percentage release of different formulations of rutin chitosan nanoparticles and pure drug rutin

F code	Cumulative Drug release (%), (AM ± SD)					
	30 min	**90 min**	**150 min**	**210 min**	**270 min**	**330 min**
Rutin	1.162 ± 0.79	3.282 ± 0.84	12.22 ± 0.83	24.67 ± 2.43	39.72 ± 1.54	**47.69 ± 2.41**
F1	1.363 ± 0.096	7.655 ± 0.019	13.139 ± 0.02	30.109 ± 0.015	56.644 ± 0.011	**94.750 ± 0.015**
F2	1.582 ± 0.104	7.053 ± 0.117	12.920 ± 0.099	27.501 ± 0.122	53.531 ± 0.131	92.872 ± 0.144
F3	1.472 ± 0.104	7.572 ± 0.071	13.422 ± 0.119	29.790 ± 0.101	56.66 ± 0.097	90.46 ± 0.151
F4	1.839 ± 0.081	9.806 ± 0.136	15.191 ± 0.059	31.595 ± 0.081	57.702 ± 0.119	89.188 ± 0.1
F5	1.828 ± 0.09	11.165 ± 0.296	18.373 ± 0.086	36.145 ± 0.157	58.604 ± 0.136	85.613 ± 0.099
F6	2.311 ± 0.158	12.551 ± 0.15	19.759 ± 0.091	35.890 ± 0.091	55.613 ± 0.063	81.072 ± 0.107

*Each value represents the mean of 3 determinations

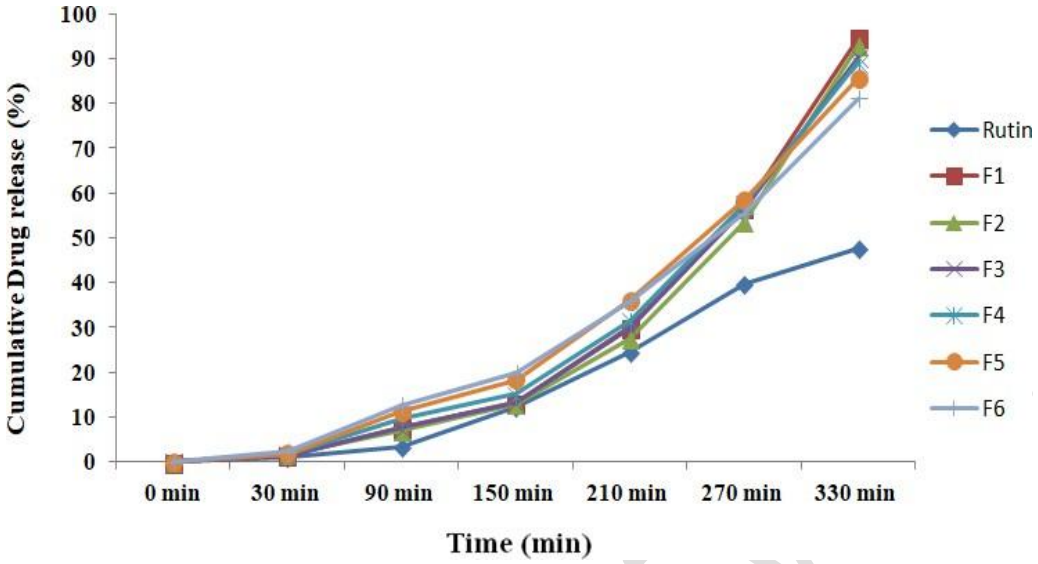

Figure 9: *In vitro* rutin release profile from pure drug and various formulations of rutinnanoparticles in Phosphate buffer solution pH 6.8

4. DISCUSSION

Rutin Chitosan Nanoparticles

Nanotechnology-based drug delivery systems improve therapeutic efficacy and reduce the negative side effects associated with conventional drugs, allowing for the development of new therapeutic classes and the re-investigation of pharmaceutically suboptimal but biologically active new molecular entities previously thought to be undevelopable [18, 19]. Six formulations of nanoparticles have been prepared and evaluated for various parameters. Normal range of particle size of nanoparticles was 1-100 nm. Obtained particle size was about 80.716 ± 12.40 (nm) (AM ± SD). This could have occurred because the rutin affected the crystalline state of the aggregated particles. Less the particle size, more will be the bioavailability and absorption of drug for producing a beneficial therapeutic effect. The average size distribution of the particles was found to be 105.0. Zeta potential is a key indicator for the determination of stability of the nanoparticles in colloidal system. Higher number of either positive or negative charge repels each other which in turn prevent the aggregation. Zeta potential of rutin loaded nano particles was found to be -20.6. A high zeta potential will confer stability of the preparation that will resist aggregation. FTIR spectroscopy can efficiently identify molecules and their surface groups

within polymer matrices [20, 21]. The preparation contains rutin, chitosan, sodium TPP, and rutin loaded nanoparticles, according to the characteristic spectra of rutin, chitosan, sodium TPP, and rutin loaded nanoparticles. The chemical properties of the material can be seen in the spectra of nanoparticles. F1 has the highest percentage yield (70.83%). F1 had an entrapment efficiency of 83.6 percent, and the amount of drug loaded was around 95 percent. In 330 minutes, pure rutin showed incomplete dissolution of 47.69 percent. The rutin chitosan nanoparticles delivered the medication in a regulated manner, according to in vitro dissolution studies. In 330 minutes, rutin-loaded nanoparticles released 94.75 percent of their rutin. As a result, nanoparticles appear to have the desired effect in the treatment of numerous cardiovascular disorders.

5. CONCLUSION

An ionic gelatine method was used to make a rutin chitosan nano formulation, which was tested for particle size, zeta potential, percent practical yield, drug entrapment effectiveness, surface morphology, percent of drug loading, and in vitro drug release. SEM morphology indicated spherical nanoparticles with a smoothed surface in rutin chitosan nanoparticles. When compared to pure rutin, the rutin chitosan nano formulation showed improved solubility. Novel drug delivery systems are now being investigated in order to improve therapeutic efficacy and long-term drug release qualities while overcoming issues such as poor solubility and oral bioavailability. Nanotechnology could thus be a viable approach enabling rutin and other poorly soluble medications to be delivered orally. Nanotechnology has a lot of potential in terms of efficiency.

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CONFLICT OF INTEREST

All authors have no conflicts of interest to declare.

REFERENCES

1. Gelperina S, Kevin K, Michael DI, Heifets L. The potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis. Am. J. Respir. Crit. Care Med. 2005;172 (12): 1487—90.
2. Desai BM. Challenges and Strategies on Nano Drug Delivery Technologies. Pharma Times. 2006;38:12–6.
3. Jia H, Zhu G, Vugrinovich B, Kataphinan W, Reneker DH, Wang P. Enzyme carrying polymeric nanofibers prepared via electrospinning for use as unique biocatalysts. Biotechnol. Prog. 2002;18:1027–32.
4. Kim J, Grate JW. Single-enzyme nanoparticles armoured by a nanometer-scale organic/inorganic network. Nano Lett. 2003;3:1219–22.
5. Lee J, Han S, Hyeon T. Synthesis of new nanoporous carbon materials using nanostructured silica materials as templates. J. Mater. Sci. 2004;14:478–86.
6. Sawicka K, Gouma P, Simon S. Electrospun biocomposite nanofibers for urea biosensing, Sens. Actuators B. 2005;108:585–8.
7. Wei Y, Xu J, Feng Q, Dong H, Lin M. Encapsulation of enzymes in mesoporous host materials via the nonsurfactant-templated sol-gel process. Mater. Lett. 2000;44:6–11.
8. Hertog MG, Hollman PC, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. Nutr Cancer. 1993; 20: 21–9.
9. Sampson L, Rimm E, Hollman PC, de Vries JH, Katan MB. Flavonol and flavone intakes in US health professionals. J Am Diet Assoc. 2002; 102: 1414–20.
10. Benavente-García O, Castilho J. Update on Uses and Properties of Citrus Flavonoids: New Findings in Anticancer, Cardiovascular, and Anti-inflammatory Activity. J Agric Food Chem. 2008;56:6185–205.
11. Calvo P, Remunan-Lopez C, Vila-Jata JL, Alonso MJ. Chitosan and chitosan: ethylene oxide- propylene oxide block copolymer nanoparticles as novel carriers for protein and vaccines. Pharm. Res. 1997;14: 1431– 143.
12. Mamillapalli V, Atmakuri AM, Khantamneni P. Nanoparticles for Herbal Extracts. Asian J Pharm. 2016;10 (2):S52-S60.
13. Sailaja AK, Siddiqua A. An overall review on polymeric nanoparticles. International

Journal of Research in Pharmacy and Pharmaceutical Sciences. 2017;2(1): 21-8.

14. Vyas SP, Khar RK. Targeted & Controlled Drug Delivery Novel Carrier Systems, CBS publishers & Distributors Pvt. Ltd. 2010; 173-243 and 331-81.
15. Pal SL, Jana U, Manna PN. Mohanta GP. Manavalan R. Nanoparticle: An overview of preparation and characterization. Journal of Applied Pharmaceutical science. 2011; 01 (06):228-34.
16. Jeyaleela GD, Vimala JR, Sheela SM, Agila A, Bharathy MS, Divya M. Biofabrication of Zinc Oxide Nanoparticles using the Isolated Flavonoid from Combretum ovalifolium and its Anti-oxidative Ability and Catalytic Degradation of Methylene Blue Dye, Oriental Journal of Chemistry. 2020;36(4):655-664.
17. Jeyaleela GD, Vimala JR, Senthil S, Manjula K. Isolation, Characterization, Molecular docking and In-vitro studies of Inhibitory effect on the Growth of Struvite crystal of Bioactive Principle Derived from *Melia dubia* leaf Extract. Asian Journal of Chemistry. 2019;31(11):2628-2634.
18. Patila AG, Jobanputraa AH. Rutin-Chitosan Nanoparticles: Fabrication, Characterization and Application in Dental Disorders. Polymer-Plastics Technology and Engineering. 2015; 54(2): 202-8.
19. Supriya A, Sundaraseelan J, Murthy BRS, Priya BM. Formulation and *In vitro* Characterization of Neomycin Loaded Chitosan Nanoparticles. Acta Scientific Pharmaceutical Sciences. 2018; 2(2): 34-40.
20. Kunjiappan S, Chowdhury A, Somasundaram B, Bhattacharjee Ch, Periyasamy S. Optimization, preparation and characterization of rutin-quercetin dual drug loaded keratin nanoparticles for biological applications. Nanomedicine J. 2016; 3(4): 253-67.
21. Zhang S, Han Y. Preparation, characterisation and antioxidant activities of rutin loaded zein-sodium caseinate nanoparticles. PLoS ONE. 2018; 13(3): e0194951.