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Biomedical Approach to Developing and Characterizing Chitosan Nanoparticles Encapsulating Urapidil for Hypertension Management

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Abstract

The aim of this study was to create Chitosan Nanoparticles loaded with Urapidil to achieve controlled drug release, enhance solubility, and reduce dosing frequency to improve patient adherence to therapy for hypertension. Urapidil was formulated into nanoparticles via the ionic-gelation method using Chitosan as a polymer, Sodium tripolyphosphate as a cross-linking agent, and filled into hard gelatin capsules after lyophilization. Pre-formulation studies, including melting point analysis and determination of the absorption maximum at 268 nm, confirmed the drug and excipients' stability, safety, and effectiveness within the specified range. Urapidil-loaded chitosan nanoparticles were prepared using various concentrations of chitosan (0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.4%, and 0.5%), with sodium tripolyphosphate serving as a cross-linking agent and Tween 80 acting as a de-aggregating agent. Characterization of all seven formulations revealed a percentage yield within the range of 78.84 to 87.25% and entrapment efficiency between 83.40 and 93.15%, with higher concentrations of polymer resulting in increased entrapment efficiency. Solubility analysis showed improvement after formulation, with the solubility of formulation F5 increased to 9.4933 mg/ml in distilled water and 13.251 mg/ml in phosphate buffer pH 6.8. In vitro release studies demonstrated controlled release with formulation F5 releasing 95.03% of the drug after 12 h. This formulation was selected as the optimized one due to its higher entrapment efficiency, drug content, and prolonged drug release profile. Accelerated stability tests revealed no notable changes in the appearance, drug content, or entrapment efficiency of formulation F5 after 90 days under various storage conditions. Among all formulations, F5, with a 0.3% chitosan concentration, proved to be the most effective for achieving controlled drug release.

Keywords: Formulation, characterization, urapidil, chitosan nanoparticles, biomedical

INTRODUCTION

Over the past 50 years, researchers have extensively explored the potential of nanoparticles and nanostructured materials in biomedical and healthcare applications [1]. Nanoparticles (NPs) typically refer to tiny particles ranging from 1 to 100 nm in diameter, but larger particles up to 500 nm, such as nanorods, nanowires, and nanofibers, are also classified under this category when one dimension exceeds the nanoscale range [2]. Nanostructured materials, composed of single or multiple materials, have at least one dimension under 100 nm and are interconnected at the nanoscale [3]. These materials can consist of simple substances like metals, carbon, or polymers [4], composites such as polymer-metal or silica-metal combinations, or even core-shell structures [5–8].

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Nanomaterials are synthesized using either a bottom-up or a top-down approach. Recently, techniques such as physical and chemical vapor deposition, electrospinning, 3D printing, biological synthesis, and supercritical fluid methods have gained prominence, often in combination with other techniques to enhance efficiency [9, 10]. These materials exhibit remarkable properties, including exceptional mechanical strength, large surface area, tunable porosity, and surface functionalization capabilities, setting them apart from bulk materials [11–13]. Such features make nanomaterials ideal for biomedical applications, including tissue-engineered scaffolds (e.g., for blood vessels and bone), drug delivery systems (e.g., for gene therapy and cancer treatment), biosensors, chemical sensors, and wound dressings [14, 15]. Interestingly, historical records suggest that ancient civilizations in India, Egypt, and China used nanotechnology (e.g., metallic gold) for therapeutic purposes as early as 2500 BC [16].

Despite their advantages, the unique properties of nanomaterials can complicate the assessment of their biological effects and toxicity risks. Factors such as their chemical composition, size, shape, surface charge, surface area, and entry route into the body significantly influence their biological interactions and potential impacts [17]..

In bioimaging, fluorescent nanoparticles tailored for specific applications have shown superior sensitivity compared to traditional molecular probes [18]. Tissue-engineered nanofiber scaffolds, known for their ability to manage tissue loss and organ failure, have already benefited millions of patients globally. These polymer-based 3D scaffolds mimic the extracellular matrix with features like balanced moisture absorption, gas permeability, and organized porosity (60–90%). Nanomaterials in one or two dimensions are used for signal amplification, possess high electrical conductivity, and are compatible with drugs and biological molecules, making them valuable for early disease detection (e.g., viruses, bacteria, and cancer). Additionally, antimicrobial nanomaterials like silver (Ag), gold (Au), and copper oxide (CuO) nanoparticles are widely used in dermatology to accelerate wound healing and treat bacterial infections [19, 20].

AIMS AND OBJECTIVES

- To formulate Urapidil loaded Chitosan Nanoparticles containing Chitosan as polymer by Ionic gelation method.
- Characterizing and optimizing the formulation of Urapidil-loaded chitosan nanoparticles.
- To enclose Urapidil loaded Chitosan Nanoparticles in Hard Gelatin Capsule and their character to be evaluated.

MATERIALS AND METHODS

Materials Used in Formulation

For the formulation of Chitosan nanoparticles of Urapidil, the following materials were procured from the following manufacturer/suppliers, as given in Table 1.

METHODOLGY

Pre-formulation Studies

Pre-formulation studies entail examining the physical and chemical attributes of a drug substance independently and in conjunction with excipients. These studies represent the initial phase in the systematic design of drug dosage forms. The goal is to use biopharmaceutical principles to evaluate the drug's physicochemical properties and develop a stable, bioavailable dosage form that can be produced on a large scale. The exact data needed depends on the type of dosage form, as shown in Table 1.

Thus, the goals of the final study are:

1. To determine the physical properties.
2. To assess its compatibility with the excipients.
3. To determine kinetic rate profile.

Compatibility Studies

Characterizing and optimizing the formulation of Urapidil-loaded chitosan nanoparticles.

Physical Compatibility Study

A total of 100 mg of powdered drug, polymer, and cross-linking agent were measured. The drug by itself, the polymer (Chitosan) with sodium tripolyphosphate, and a mixture of the drug and excipients were all placed in airtight screw-cap vials. These vials were stored at room temperature and at 40ºC with a relative humidity of 75±2%. Any alteration in the color of the physical mixture was visually examined.

Chemical Compatibility Study by FTIR

Chemical compatibility studies were carried out using Fourier Transform-Infrared (FTIR) spectroscopy with a Shimadzu FTIR 8400 Spectrophotometer, covering the range of 4000 to 400 cm^{-1}. The analysis was conducted using the potassium bromide (KBr) pellet method. In this process, a small amount of finely powdered sample (drug alone, a mixture of the drug and excipients, or the optimized formulation) was thoroughly mixed with approximately 100 times its weight in powdered potassium bromide. The mixture was then subjected to high pressure (at least 25,000 psig) in a press to create thin, transparent pellets (1–2 mm thick, 1 cm in diameter). These pellets, transparent to IR radiation, were analyzed directly, as detailed in Table 2.

Determination of Melting Point

The melting point of Urapidil was measured using the capillary tube method following USP guidelines. A compact column of Urapidil powder, about 4–6 mm high, was placed in a capillary tube. The tube was placed in an electrical melting point apparatus, where the temperature was gradually raised. The melting point was determined as the temperature at which the final solid particle of Urapidil transitioned into a liquid.

Table 1. List of materials used in formulation.

S.N.	Name of the material	Procured from	Use in formulation
1.	Urapidil	Adcock Ingram, Bangalore.	Active pharmaceutical ingredient
2.	Chitosan 50 kD	Lab chemicals, Chennai.	Polymer
3.	Acetic acid	Lab chemicals, Chennai.	Solvent
4.	Sodium tripolyphosphate	Lab chemicals, Chennai.	Cross linking agent

Table 2. Composition of drug and excipients for FT-IR spectra.

S.N.	Ingredients
1	Drug
2	Drug + Chitosan
3	Drug + sodium tripolyphosphate
4	Drug + Chitosan + sodium tripolyphosphate

Determination of Lambda Max (λ_{max})

Finally, this solution was scanned at 200–400 nm in a UV-Visible spectrophotometer to determine the absorption maximum (λmax).

Standard curve for Urapidil

100 mg of Urapidil was weighed and placed in a 100 ml volumetric flask. From Stock Solution II, 2, 4, 6, 8, 10, and 12 ml were transferred to separate 100 ml volumetric flasks, and the volume in each

flask was adjusted to 100 ml with phosphate buffer (pH 6.8). The absorbance of these solutions was subsequently recorded at 268 nm using a blank as a reference.

Solubility Studies of Pure Urapidil

The solubility of pure Urapidil was evaluated in distilled water and phosphate buffer (pH 6.8). An excess amount of Urapidil was introduced into each medium, and the mixtures were stirred using a mechanical shaker at 50 rpm for 24 hours while maintaining a temperature of 37±0.5ºC. Visual inspection was conducted to confirm the presence of undissolved Urapidil solids, indicating saturation had been achieved. The mixtures were then filtered using a 0.45 µm filter, and the filtrates were properly diluted with the corresponding medium. The absorbance of the final solution was measured at 268 nm with a UV-Visible spectrophotometer.

FORMULATION DEVELOPMENT

Preparation of Chitosan Nanoparticles: Ionic Gelation Method

Chitosan nanoparticles were synthesized through ionic interaction between the positively charged Chitosan solution and the negatively charged STPP solution, both with and without the drug. The preparation process included Tween 80 as a re-suspending agent to prevent particle aggregation during stirring at room temperature. The pH of the Chitosan solution was adjusted to a range of 4.6 to 4.7. Seven different formulations (F1, F2, F3, F4, F5, F6, F7) of Urapidil-loaded Chitosan nanoparticles were developed by dissolving Urapidil in 30 ml of Chitosan at varying concentrations (0.1, 0.15, 0.2, 0.25, 0.3, 0.4, and 0.5% w/v) with 0.5% w/v Tween 80. TPP (0.1% w/v) was added dropwise under magnetic stirring at 1000 rpm. The resulting nanoparticle suspensions were then lyophilized at –40°C for 24 hours, as shown in Table 3.

Table 3. Composition of Nanoparticles.

Formulation	Polymer			Drug (mg)	Tween 80 (%w/v)	Sodium Tripolyphosphate		
	Chitosan (%w/v)	*1% Aqueous acetic acid (ml)*	*Chitosan (mg)*			*TPP (%w/v)*	*Distilled water (ml)*	*TPP (mg)*
F1	0.1%	30 ml	30	60	0.5	0.1	30	30
F2	0.15%	30 ml	45	60	0.5	0.1	30	30
F3	0.2%	30 ml	60	60	0.5	0.1	30	30
F4	0.25%	30 ml	75	60	0.5	0.1	30	30
F5	0.3%	30 ml	90	60	0.5	0.1	30	30
F6	0.4%	30 ml	120	60	0.5	0.1	30	30
F7	0.5%	30 ml	150	60	0.5	0.1	30	30

Freeze Drying

Lyophilization is an effective method for enhancing both the chemical and physical stability of substances for prolonged periods. Lyophilization is necessary to attain prolonged stability for a product containing drugs prone to hydrolysis or to create a suitable formulation for oral administration.

The Urapidil loaded Chitosan suspension is kept in the freeze dryer at –20ºC overnight and the flask was covered with parafilm sheet on the next day and perforated. After 24 h, the sample was kept inside the lyophilizer at temperature –40ºC and pressure below 15 Pa (0.1 pa) to remove the water from the samples. After lyophilization, the dried powder is used for further studies as seen in Table 4.

RESULTS AND DISCUSSION

Pre-formulation Studies

- A formulation can only be optimized after thoroughly analyzing the physicochemical properties of both the drug and excipients. Ensuring compatibility between the drug and polymer is essential for a successful formulation.

COMPATIBILITY STUDIES

Physical Compatibility Study

Inference

The study on physical compatibility was carried out over a duration of three months. No color changes were observed, indicating that the drug and excipients are physically compatible, as shown in Table 5.

Melting Point

The melting point of Urapidil was determined using the capillary tube method.

Inference

The melting point of drug was studied and tabulated, which confirms the identification of Urapidil as seen in Table 6.

Table 4. Physical compatibility study of drug and excipients.

S.N.	Drug/Excipients	Description and Condition						
		Initial	*At room temperature (in days)*			*At 40±2ºC and 75% RH ±2% (in days)*		
			I	II	III	I	II	III
1	Urapidil	White colored powder	NC	NC	NC	NC	NC	NC
2	Chitosan	Pale yellow colored powder	NC	NC	NC	NC	NC	NC
3	Sodium tripolyphosphate	White colored powder	NC	NC	NC	NC	NC	NC
4	Urapidil + Chitosan	Pale yellow colored powder	NC	NC	NC	NC	NC	NC
5	Urapidil + Sodium tripolyphosphate	White colored powder	NC	NC	NC	NC	NC	NC

**NC: No Change.*

Table 5. FT-IR spectral Interpretation of urapidil + chitosan + sodium tripolyphosphate.

Type of vibration	Wave number (cm^{-1})
Aromatic C-H	3055.02
C-N Stretching	1380.93
N-H Stretching	3209.31
C=0 stretching	1604.66
C-H Stretching	2947.01
N-C Stretching	1234.35, 1056.91
C-0-C Stretching	1350.07
C-O Stretching	1296.07
Na	570.89

Table 6. Melting point of drug.

S.N.	Drug/Excipients	Specification	Observation
1	Urapidil	157–160ºC	157–158ºC

Table 7. Saturation solubility of urapidil in phosphate buffer pH 6.8 and distilled water.

Medium	pH	Solubility (mg/ml)
Phosphate buffer pH 6.8	6.8	0.004093
Distilled water	7.0	0.000216

Table 8. Data for standard curve of urapidil in phosphate buffer pH 6.8.

S.N.	Concentration (µg/ml)	Absorbance (AU)
1	0	0
2	2	0.1153±0.000374
3	4	0.2203±0.000287
4	6	0.3445±0.000432
5	8	0.4686±0.000455
6	10	0.6035±0.000424
7	12	0.7089±0.000497

Table 9. Characterization of urapidil loaded chitosan NPs.

Formulations	Percentage yield (%)	Entrapment Efficiency (%)	Drug content (%)
F1	79.09	83.40	77.16
F2	80.53	86.08	79.98
F3	81.90	88.65	82.52
F4	85.81	90.42	86.19
F5	87.25	93.15	91.83
F6	81.16	89.59	85.62

Solubility Study

The solubility study of Urapidil in various dissolution media was conducted using the saturation solubility method, as presented in Table 7.

Inference

The drug's solubility at pH 6.8 was notably higher compared to its solubility in distilled water. Urapidil was found to be insoluble in both distilled water and phosphate buffer at pH 6.8.

Determination of Lambda Max (λ_{max}) for Urapidil

The maximum absorbance of Urapidil was determined to be at 268 nm. Therefore, a wavelength of 268 nm was chosen for the analysis of the drug in dissolution media, as shown in Table 8.

Characterization of Urapidil Loaded Chitosan NPs

The formulated Urapidil loaded Nanoparticles are characterized for Percentage yield, Drug content, Entrapment efficiency and the results were tabulated below as seen in Table 9.

Comparative *in vitro* Drug Release for All Formulations

The formulated nanoparticles' preparation containing drug and polymer were evaluated for drug release and results are tabulated, as seen in Table 10.

Table 10. *In vitro* drug release for all formulations.

Time (h)	*In Vitro* Drug Release for Nanoparticles Formulations						
	F1	*F2*	*F3*	*F4*	*F5*	*F6*	*F7*
0.5	17.25	17.45	16.04	15.09	13.19	9.71	8.32
1	20.12	19.89	19.2	18.99	16.17	11.14	10.14
2	36.4	29.13	28.83	27.02	24.02	17.35	14.14
3	44.8	38.03	37.92	36.21	34.52	31.3	29.27
4	60.2	50.08	47.22	44.1	41.23	34.02	31.73
5	72.27	63.07	59.12	53.8	50.14	36.37	32.13
6	82.29	79.02	69.03	61.78	57.71	42.13	39.92
7	97.04	89.9	78.97	73.2	64.21	50.12	43.78

8	-	96.02	89.17	86.51	73.22	62.79	59.11
9	-	-	94.18	91.52	78.01	71.23	69.24
10	-	-	-	94.47	86.22	79.13	75.17
11	-	-	-	-	91.89	83.2	81.2

SUMMARY AND CONCLUSION

The objective of this study was to develop Urapidil-loaded Chitosan nanoparticles for controlled drug release, improved solubility, and reduced dosing frequency, thereby enhancing patient compliance with the treatment. Nanoparticles were used to encapsulate Urapidil through the ionic-gelation method, utilizing Chitosan as the polymer and sodium tripolyphosphate as the cross-linking agent. Pre-formulation studies, including melting point analysis and UV absorption spectroscopy, demonstrated the stability and safety of both the drug and excipients. Physical compatibility studies demonstrated the compatibility of the drug with the excipients, while chemical compatibility was assessed using FTIR spectroscopy, indicating no interaction between the drug and polymer. Standard tables were constructed for Urapidil, showing linearity and adherence to Beer-Lambert's law.

Various formulations of Urapidil-loaded Chitosan Nanoparticles were prepared with different polymer concentrations, cross-linking agents, and de-aggregating agents. Characterization revealed satisfactory percentage yield and entrapment efficiency, with higher polymer concentrations leading to increased entrapment efficiency. Solubility analysis showed enhanced solubility profiles for the formulations compared to the pure drug. In vitro release studies revealed controlled drug release, with formulation F5 displaying the highest release percentage and a steady release pattern. Based on its entrapment efficiency, drug content, and in vitro release, formulation F5 was determined to be the optimized formulation. Further characterization through SEM analysis, particle size analysis, and zeta potential measurements revealed spherical nanoparticles with a smooth surface, a mean particle size of 188.3 nm, and stable zeta potential.

Flow property measurements showed good flow properties for Chitosan Nanoparticles compared to the pure drug, allowing for easy filling into hard gelatin capsules without the need for additional glidants. Post-formulation evaluations, including weight uniformity, disintegration testing, drug content, and in vitro drug release, all met the official specifications. Dissolution data were evaluated using various kinetic models, with formulation F5 exhibiting zero-order kinetics and anomalous diffusion. Accelerated stability studies conducted at different temperatures showed no notable changes in appearance, drug content, or entrapment efficiency for formulation F5 over 90 days. Ultimately, formulation F5, containing 0.3% Chitosan concentration, was determined to be the most effective for controlled drug release.

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