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CHARACTERIZATION AND FORMULATION CONTROLLED RELEASE AND BIOAVAILABILITY OF CHITOSAN NANOPARTICLES STABILISED WITH POLOXAMER RIVAROXABAN STRENGTHENING

Mohd. Jalaluddin*[1] Dr. Ravindra Chourasiya[1] & Mohd Shafi Dar[3]

[1]*Department of Pharmacy, Swami Vivekanand University, Sagar, Madhya Pradesh, India*

[2]*Sri Satya Sai University of Technology and Medical Sciences, Sehore, Madhya Pradesh, India*

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Corresponding Author
***M. Jalaluddin**

ABSTRACT

ORIGINAL RESEARCH ARTICLE

The present investigation is carried out for in-vivo evaluation of chitosan nanoparticles stabilized by poloxamer for controlling the release and enhancing the bioavailability of the poorly soluble drug Rivaroxaban by using Carrageenan-induced mice tail thrombosis. A total of 24 mice were randomly divided into 6 groups (n=6) and mice were randomly distributed throughout groups. Group 1 served as a control with Normal saline; Groups 3, and 4 were, respectively treated orally with 20 mg/kg and equivalent to 20 mg/kg Rivaroxaban (RVRX) and RLNPs dissolved in 20% DMSO. Groups 2 were respectively injected with 100 IU heparin sodium as positive controls i.p. One hour after test samples were administered intraperitoneally, each mouse was injected with 40 µl (1%) carrageenan dissolved in physiological saline by intraplantar administration in the right hind paw. The results of the carrageenan-induced mice tail thrombosis at different time intervals were mentioned in the above table. According to the observed results both RVRX as well as RLNPs have good anti-thrombosis activity. It was found that RVRX (63.33 %) showed significant inhibition of the thrombosis as compared to the RLNPs (68.64 %). These findings may contribute to the successful development of new pharmaceutical formulation F7 and may use as a better agent in the drug delivery system as well as can have used in the treatment area of cardiovascular diseases.

1. INTRODUCTION

Rivaroxaban (RXB) was approved for both the treatment and prevention of thromboembolic diseases **[1,2]** and is described as an anticoagulant with a reduced risk of bleeding comparing to warfarin in unprovoked venous thromboembolism (VTE) **[3].** In a fasted state, a lower RXB oral bioavailability (66 %) was found for the administration of 15 and 20 mg dosages in tablets compared to 80–100 % for a 10 mg dose that was independent of a fed or fasted state **[4].** Rivaroxaban (RXB), chemically designated as 5-Chloro-N-{(5S)-2-oxo-3-[4-(3-oxo-4- morpholinyl) phenyl]-1, 3 oxazolidin-5-yl} methyl)-2-thiophene-carboxamide is an orally active direct inhibitor of the activated serine protease Factor Xa, given as mono therapy in the treatment of venous

thromboembolism (VTE) **[5-7].** RXB is lipophilic in nature, exhibiting high permeability across gastrointestinal tract (GIT) and hence classified as a BCS class II drug. **[6]** Various formulation strategies such as amorphous co-precipitates, **[8]** co-crystals, **[9]** lipid solid dispersion, **[10]** self micro emulsifying drug delivery systems **[11]** and, mesoporous dosage form **[12]** have been explored to improve the limited solubility issues of RXB.

Nanoparticles (NPs) have received therapeutic attention and diagnosis, thanks to their unique physicochemical properties that transform treatment with extremely powerful, toxic, and intelligent effects. Nanoparticles exhibit unique physical, chemical, and biological features in Nano scale compared with their particles at higher concentrations. This condition is caused by large space compared to volume, recycling or stability in chemical process, improved mechanical strength, etc **[13].** Chitosan is a polysaccharide containing the flexible units of (1 → 4) Nacetyl glucosamine and glucosamine found in the deacetylation component of chitin. After extensive renovation of its structure, it has been reported to be an effective raw material for the production of nanoparticles with technological advantages. Chitosan NPs are rotten, stable, light, slightly toxic, compact and easy to repair. These are made from natural stainless polymer with chitosan and are approved by GRAS (Best Known for Safe by the United States Food and Drug Administration [US FDA]) of harmful solvent. NPs prepared from chitosan and their derivatives usually have a well-charged area. Chitosan also has some limitations and many of its benefits **[14].** Formulation of nanoparticles from chitosan is usually made in low concentrations as the chitosan melts in aqueous solutions at room temperature, no toxic solvents or heat are required. Chitosan nanoparticles have been extensively studied for use in the treatment of cancer. Chitosan nanoparticles can target on tumors specific organs by passive target ion also known as improved the result of permeability and retention (EPR), effective direction, and physical targeting with sensitive identification**.** Inflammation of the polymer is one of the mechanisms that control the release of drugs from chitosan nanoparticle **[15].**

Poloxamers, available also under the trademark Pluronics® (BASF), are a class of water-soluble nonionic A-B-A and B-A-B tri block copolymers, where A is poly (ethylene oxide) (PEO) and B is poly (propylene oxide) (PPO). The size and structure of poloxamer assemblies, and their adsorption properties **[16,17],** have made them useful in many applications, including, Drug delivery **[18],** nanoparticle synthesis **[19],** cosmetics **[20]** and emulsion **[21]** formulation, effective dispersants for inks/pigments **[22]** and as versatile anti-biofouling coatings **[23],** The use of poloxamers in pharmaceutical research is widely researched.

The focus of this investigation is to provide an overview of the chitosan-based nanoparticles for various non-parenteral applications and also to put a spotlight on current research including sustained release. The aim of the present study is the formulation and characterization of poloxamer stabilized chitosan nanoparticle for control release and bioavilibility enhancement of rivaroxaban which includes different parameters like pre-formulation, formulation studies and, Additionally, this study was focused on the application of Rivaroxaban-loaded chitosan NLCs using in vivo anti-thrombin model.

2. MATERIALS AND METHODS

2.1 Pre-formulation studies

The basic objective of the pre-formulation studies is to provide a rational basis for the formulation approaches, to increase the chances of success in formulating an acceptable product and ultimately to provide a basis for improving the quality and performance. Pre-formulation is defined as an investigation of the physical and chemical properties of the sustained release matrix tablet alone and when combined with an excipient. A step in time saves nine, so the pre-formulation studies of the new product can away the disaster that is disasters are prevented in advance **[24].**

2.2 Organoleptic parameter

It is the initial evaluation during preformulation studies that assesses the color, odor and taste of the substance. Appearance was examined visually to determine color, homogeneity and transparency. Appearance was tested visually for color, similarity and transparency **[25].**

2.3 Solubility Aqueous

Solubility is an important physicochemical element of a drug substance, which determines its systematic absorption and alters its therapeutic efficacy. The sample was qualitatively tested for its solubility in various solvents. It was determined by shaking 2 mg of drug sample in 5 ml of solvent given in table no. (Small test tube and observed to disappear the sample completely **[26]**.

2.4 Melting point

Melting point of drug was determined by the melting point apparatus (Tempo) and found to be 228ºC-230˚C. The melting point of a drug is one of the first and most reliable physical properties measured; can be used effectively as a guide to early detection and development of drugs **[27].**

2.5 PH

Approximately 100mg of drug was taken and dissolved in 10ml of distilled water by sonication and filtered. The pH of the filtrate was tested with a standard glass electrode.

2.6 Partition Coefficient

The partition coefficient of drug was examined in n-Octanol: Phosphate buffer pH 6.8, n-Octanol: water system. It was determined by taking 5mg of drug in three separating funnels containing, 5ml of n-Octanol and 5ml respective buffer (i.e. PBS pH 6.8, PBS pH 7.4, and water). The separating funnel was shaken for 2 hours in a wrist action shaker for equilibrium. Two phases were separated and the amount of drug in aqueous phase was analysed spectrophotometrically at 232 nm after appropriate dilution with respective buffer. The partition coefficient of drug was calculated using the following formula.

Partition coefficient, K= $Amountofdrug\ in\ organic\ phase$ / $Amountofdrug\ in\ aqueous\ phase$

2.7 Formulation of Nanoparticles

Fifteen formulations of Rivaroxaban nanoparticles (RB-NPs) were prepared by the ionic gelation method as described by Calvo et al with slight modifications **[28].** Briefly, different concentrations of chitosan and DM were dissolved in 100 ml of 1% (w/v) acetic acid solution, after that TPP was added with different rates to chitosan solution, different quantities of poloxamer 188 were added and stirred at 900 rpm with a magnetic stirrer for 60 min, at the end, the prepared DM-NPs were homogenized by IKA homogenizer (T18 basic, IKA-Werke GmbH, Germany) at different speed for different period of time as assigned by the design. The prepared NPs were washed twice with distilled water then centrifuged using Sigma Laboratory centrifuge, 3K30 (Ostrode am Harz, Germany) at 20000 rpm for 40 min and stored at -80 °C till lyophilized for 24 h using Christ lyophilizer (ALPHA 2–4 lyophilizer, Germany).

2.8 Characterization and evaluation of Nanoparticles

a) **Production yield:** The production yield of the nanoparticles is calculated for each batch by dividing the total weight of product (m) by the total expected weight of drug and polymer. Weight of nanoparticles (m).

% yield = Actual yield / Theoretical yield × 100%

b) **Determination of Drug content:** Sample containing 100 mg equivalent, nanoparticles are dissolved and the volume is made upto100ml buffer. The absorbance of resulting solution is determined using UV spectrophotometer (UV1700 Shimadzu Corporation, japan) and the drug content is estimated.

c) **Determination of Particle size:** A suitable amount of freeze-dried RB-loaded NPs was dispersed in distilled water (pH 7) by vortex then sonicated using Ultrawave Ltd., water bath sonicator (Cardif, UK). The Accepted Manuscript average of particle size and zeta potential were measured directly by dynamic light scattering (DLS) using a Zetatrac (Microtrac, Inc., PA, USA).

d) **Poly disparity index:** The size distribution and polydispersity (PDI) index of nanoparticles was determined by dynamic light scattering (DLS), using the zetasizer nano zs90 (malvern instruments, UK) instrument. Samples were analyzed in triplicate, at 25 ° c, with scattered light detected at a 90° angle**.**

e) Zeta-potential: The zeta potential depends on a variety of factors; in the present case, since the pH of the nanoparticles did not change), the change in zeta potential may be due to the rearrangement among the formulation components, notably the polymer chains. A suitable amount of freeze dried RB-loaded NPs was dispersed in distilled water (pH 7) by vortex then sonicated using Ultra wave Ltd., water bath sonicator (Cardif, UK). The Accepted Manuscript average of zeta potential were measured directly by dynamic light scattering (DLS) using a Zetatrac (Microtrac, Inc., PA, USA). Zeta potential values (in mv) were determined by electrophoresis, by triplicate analysis, at 25 ° c, using the zeta sizer zs90 instrument **[29].**

f) Entrapment Efficiency: Specified weight of RB-loaded NPs was dissolved in (80:20 CAN, water V/V) acetic acid then sonicated for 10 min and filtered, DM was measured by using UV (Agilent technologies, Germany) and the signals were monitored with UV detection at a wavelength of 249 nm" according to the previously reported method (K.-H.H. Cha, et al., 2010). The entrapment efficiency and the loading capacity expressed in percentage were calculated according to Equations

$\%EE = Actual\ drug\ content\ /\ Theoretical\ drug\ content \times 100$

% Entrapment efficiency = total amount of drug – concentration of drug / 100 Total amount of drug ×100

g) SEM: Electron microscopy scanning uses a focus beam of high-power electron to generate various signals at the surface of solid specimens. In most SEM microscopy applications, data is collected from a selected sample surface area and a two-dimensional image is generated showing specific differences in properties texture and orientation of the material and its chemical characterization.

2.9 In vivo study using Carrageenan-induced thrombosis model

Carrageenan-induced mice tail thrombosis model: Carrageenan-induced tail thrombosis model is one of the models that have been used to evaluate antithrombotic and thrombolytic agents, such as heparin and aspirin. It allows observing the progression of thrombosis visually and directly in a time-dependent manner.

A total of 24 male and female mice were randomly divided into 6 groups (n=6) and mouse were randomly distributed throughout groups. Group 1 served as control with Normal saline; Groups 3, 4 were, respectively treated orally with 20 mg/kg and equivalent to 20 mg/kg Rivaroxaban (RVRX) and RLNPs dissolved in 20% DMSO. Groups 2 was respectively injected with 100 IU heparin sodium as positive controls i.p. One hour after test samples were administered intraperitoneally, each mouse was injected with 40 µl (1%) carrageenan dissolved in physiological saline by intraplantar administration in the right hind paw. Mice were observed for the formation of thrombosis and thrombus lengths were measured and photographed at 24, 48 and 72 h.

Inbreed Swiss albino mice (30–40 g) were obtained from Experimental Animals Research Centre. Animals were maintained in a room with controlled temperature (22±2 °C) for 12 h light/12 h dark cycle with free access to food and water. Animals were obtained from Pinnacle Biomedical Research Institute. Animals were maintained in a room with controlled temperature (22±2 °C) for 12 h light/12 h dark cycle with free access to food and water.

3. RESULTS

3.1 Pre-formulation studies

Pre-formulation is defined as the investigation of physical and chemical properties of drug alone and combination with excipients. The overall objectives of pre-formulation studies are to generate information about in developing stable and bioavailable dosage form. Studies

which are included in pre-formulation studies are

a) Organoleptic parameter

The organoleptic characteristics of a drug such as color, odour, and taste were studied. The Colour of the drug was evaluated by visual observation. The taste of the drug was identified simply by taste sensation and odour by smelling. The outcomes are compiled in the below table. Table 1: Physical Appearance of Rivaroxaban

Table 1: Physical Appearance of Rivaroxaban

Drug	Physical appearance			Status
	Property	Reported	Observed	
Rivaroxaban	Color Odour Appearance	OffWhite Odourless powder	OffWhite Odourless powder	Complies

According to the present results of present study the drug Rivaroxaban is an off White in colour, observed odourless and available in powder form.

b) Solubility analysis of Rivaroxaban

The sample was qualitatively tested for its solubility in various solvents. It was determined by shaking 2 mg of drug sample in 5 ml of solvent in small test tube and observed to disappear the sample completely. The observed results are given in table no.2, 3 —

Table 2: I.P. Ranges for Solubility

Descriptive term	Parts of solvent required for Parts of soluble
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to1000
Very slightly soluble	From 1000 to 10000
Practically insoluble	10000 or more

Table 3: Solubility profile of Rivaroxaban

S.no.	Solvent	Solubility	Result
1	Acetonitrile	Freely soluble	++++
2	Methanol	Soluble	+++
3	Isopropanol	Sparingly soluble	++
4	Phosphate buffer PH 6.8	Slightly soluble	+
5	Phosphate buffer PH 7.4	Slightly soluble	+
6	Ethyl ether	Slightly insoluble	+
7	n-Hexane, Water	Practically insoluble	- - -

Freely soluble	1-10 Parts	++++
Soluble	10-30 Parts	+++
Sparingly soluble	30-100 Parts	++
Slightly soluble	100-1000 Parts	+
Practically insoluble	greater than 1000	- - -

According to the present results of present study the drug Rivaroxaban showed excellent solubility in Acetonitrile and Methanol while showed Sparingly soluble in Isopropanol, slightly soluble in Phosphate buffer PH 6.8, Phosphate buffer PH 7.4, Ethyl ether and found insoluble in n-Hexane, Water.

c) Melting point of Rivaroxaban

Melting point of Rivaroxaban was determined using Melting point apparatus (Tempo) and found to be 228°C-230°C

Table 4: Melting Point of Rivaroxaban

Drug	Melting point		Status
	Reported	Observed	
Rivaroxaban	230°C	228-230°C	Complies

According to the present results the melting point of rivaroxaban found to be 230°C

d) pH

Table 5: Determination of pH

S.NO.	Standard pH of the solution	Observed pH of the solution
1.	**7.6**	**7.6**

e) Partition Coefficient

The partition coefficient of drug was examined in n-Octanol: Phosphate buffer pH 6.8, n-Octanol: water system and analyzed spectrophotometrically at 232 nm. The partition coefficient of drug was calculated using the following formula-

Partition coefficient

$$K = \frac{Amount\ of\ drug\ in\ organic\ phase}{Amount\ of\ drug\ in\ aqueous\ phase}$$

Table 6: Partition coefficient

S.n.	Medium	K
1.	n-Octanol: PBS (pH 6.8)	1.7
2.	n-Octanol: PBS (pH 7.4)	1.9

According to the given results the partition coefficient of rivaroxaban found to be 1.7 in n-Octanol: PBS (pH 6.8) and 1.9 in n-Octanol: PBS (pH 7.4).

3.2 Formulation of Nanoparticles

Fifteen formulations of Rivaroxaban nanoparticles (RB-NPs) were prepared by the ionic gelation method as described by Calvo *et al* with slight modifications as described in section of methods**.**

Table 7: Formulation of Nanoparticles

S. No	Formulation	Drug (mg/ml)	Chitosan (%)	Tripolyphospahte (%)	Poloxamer
1	F-1	1	1	0.35	50
2	F-2	0.5	1	0.35	50
3	F-3	1	1	0.35	10
4	F-4	0.5	1	0.35	10
5	F-5	1	1	0.35	50
6	F-6	1	1	0.35	50
7	F-7	0.5	1	0.35	50
8	F-8	1	1	0.35	10

9	F-9	1	1	0.35	10
10	F-10	0.5	1	0.35	10
11	F-11	0.5	1	0.35	50
12	F-12	0.5	1	0.35	10
13	F-13	0.75	1	0.35	30
14	F-14	0.75	1	0.35	30
15	F-15	0.75	1	0.35	30
16	F-16	0.75	1	0.35	30
17	F-17	0.75	1	0.35	30

3.3 Characterization and Evaluation of Nanoparticles

For characterization and evaluation of all selected formulation different parameter study like production yield poly dispersity index, particle size, entrapment efficiency and zeta-potential were carried out. The results are given in the following table.

Table 8: Characterization and evaluation of Nanoparticles

S. No	Formulation	Production yield%	Zeta-Potential (mV)	Entrapment Efficiency%	Particle size(nm)	Poly disparity index
1	F-1	52.5	14.03	42.34	286.13±2.24	0.20
2	F-2	51.85	19.22	73.86	296.12±2.16	0.18
3	F-3	12.35	15.24	58.12	306.11±2.34	0.15
4	F-4	11.85	10.67	71.07	296.13±2.54	0.09
5	F-5	52.35	18.01	68.76	297.14±3.14	0.12
6	F-6	52.35	17.98	45.68	312.12±2.54	0.23
7	F-7	51.85	23.63	99.87	316.12±2.14	0.32
8	F-8	12.35	13.66	77.39	314.13±2.14	0.14
9	F-9	12.35	12.58	63.24	311.14±2.14	0.16
10	F-10	11.85	18.21	97.18	313.17±2.14	0.19
11	F-11	51.85	16.34	78.23	310.19±2.14	0.21
12	F-12	11.85	12.98	72.96	316.21±2.14	0.30
13	F-13	32.1	20.13	86.7	316.23±2.14	0.29
14	F-14	32.1	19.54	84.21	316.25±2.14	0.22
15	F-15	32.1	21.54	86.37	316.26±2.14	0.28
16	F-16	32.1	20.00	86.37	316.27±2.14	0.17
17	F-17	32.1	10.67	86.37	316.28±2.14	0.27

The results of the present study showed that the formulation F7 showed the significant results for all the selected parameter as compared to the other formulations. The formulation F1, F5, and F6 showed the highest production yield (52.5, 52.35, 52.35 % respectively) and F7 showed the significant production yield (51.85 %), zeta-potential (23.63 mV), entrapment efficiency (99.87), particle size (316.12±2.14 nm) and poly disparity index (0.32). These studies help in the selection of best formulation which can carried out for further pharmacological studies.

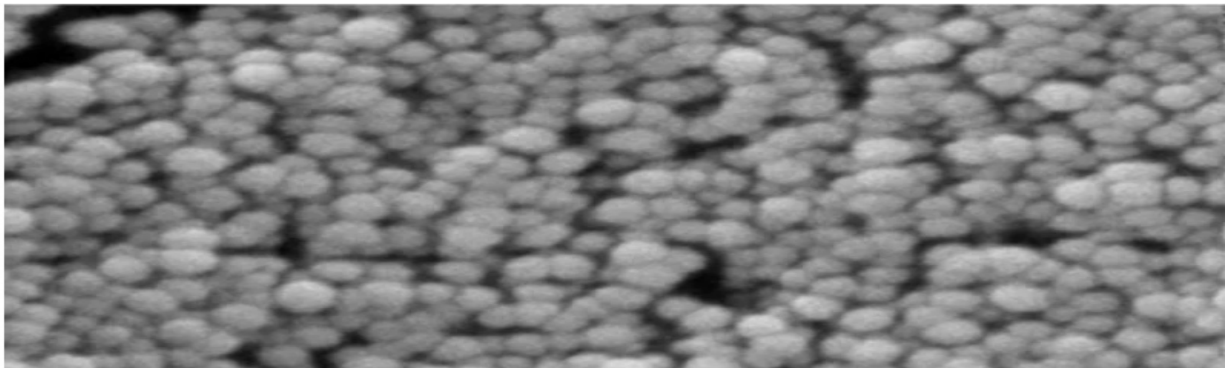


Figure 1 : SEM of formulation F7

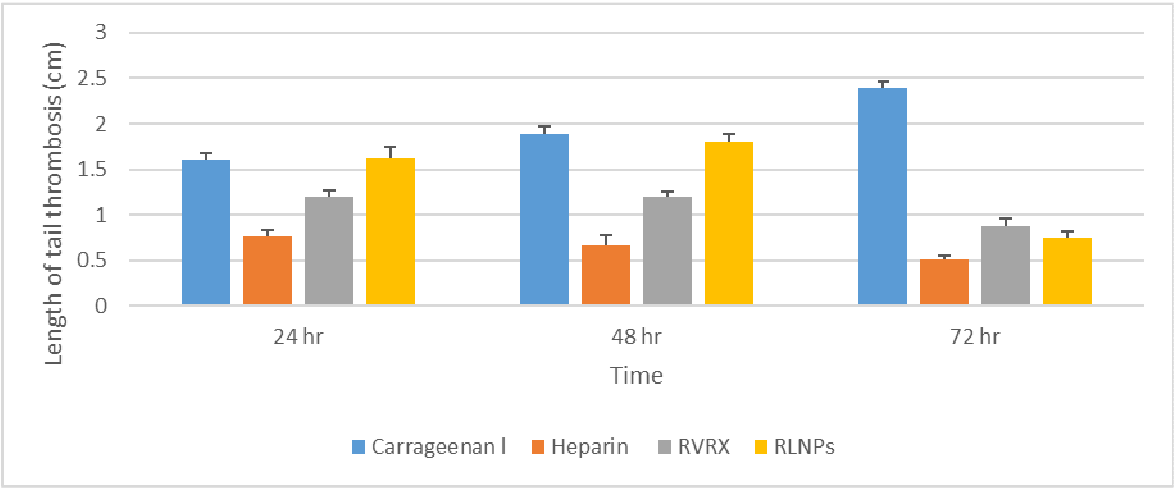
3.4 In vivo study by using carrageenan-induced mice tail thrombosis model

Table 9: Effects of RVRX, RLNPS and heparin in carrageenan-induced mice tail thrombosis at 24, 48 and 72 h.

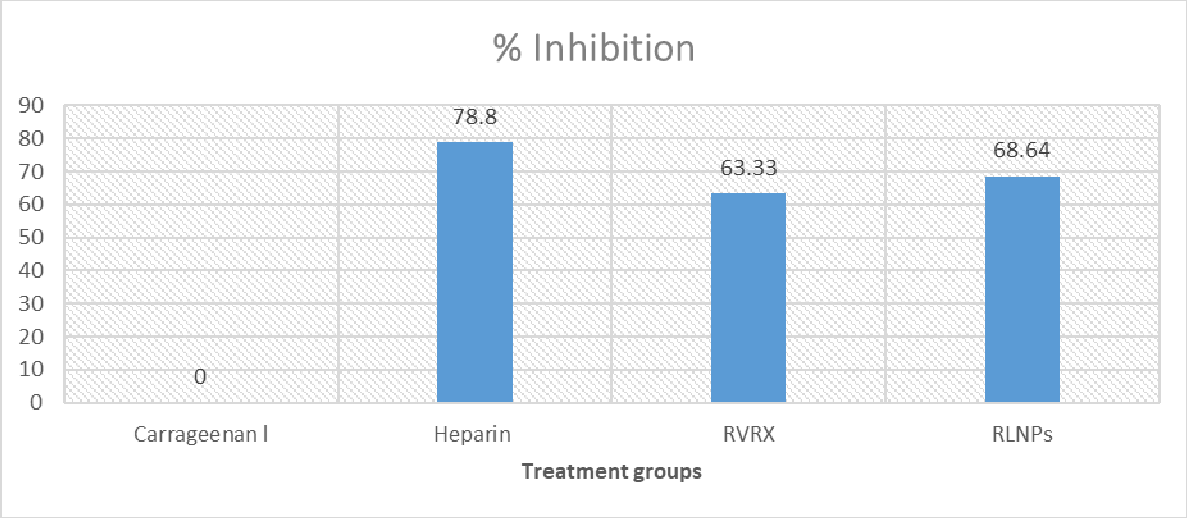
Treatment	Length of tail thrombosis (cm)			
	24 hr	**48 hr**	**72 hr**	**% Inhibition**
Carrageenan control	1.605 ± 0.066	1.892 ± 0.076	2.392 ± 0.066	-
Heparin	0.767 ± 0.069	0.670 ± 0.102	0.507 ± 0.040	78.80
RVRX	1.195 ± 0.072	1.202 ± 0.049	0.877 ± 0.090	63.33
RLNPs	1.617 ± 0.128	1.800 ± 0.082	0.750 ± 0.073	68.64

The results of the carrageenan-induced mice tail thrombosis at different time interval were mentioned in the above table. According to the observed results both RVRX as well as RLNPs having good anti- thrombosis activity. It was found that RVRX (63.33 %) showed significant inhibition of the thrombosis as compared to the RLNPs (68.64 %). Comparative representation of RVRX, RLNPS and heparin in carrageenan-induced mice tail thrombosis at different time intervals 24, 48 and 72 h and % inhibition given in the following graph.

Graph 1: Effects of RVRX, RLNPS and heparin in carrageenan-induced mice tail thrombosis at different time intervals 24, 48 and 72 h.



Graph 2: The inhibition activity of RVRX and RLNPS against carrageenan-induced tail-thrombosis in mice.



4. DISCUSSION

The rapid development of nanotechnology for biological purposes had a tremendous impact on medicine. Nanotechnology enables the manufacture and manipulation of materials on a nanometer scale, thus allowing the development of new tools for the treatment, diagnosis, monitoring, and control of biological systems. This application of nanotechnology in the field of medicine is known as Nano medicine. These NPs have special enhanced physical and chemical properties compared to their corresponding bulk materials. These properties include a high surface area-to-volume ratio and a unique quantum size effect due to specific electronic structures **[30]**. Nanoparticles (NPs) have received intensive attention in terms of therapeutics and diagnosis, because of their unique physicochemical properties that revolutionize medical treatment with more potent, less toxic, and smart outcomes. The major categories of NPs used for drug delivery and diagnosis, highlighting their fabrication techniques, characterization methods, and physicochemical properties **[31].**

The Chitosan NPs are biodegradable, more stable, simple, less toxic, biocompatible and easy to prepare. These are made of a fully biodegradable and biocompatible natural polymer chitosan and also approved by GRAS (Generally Recognized as Safe by the United States Food and Drug Administration [US FDA]) **[32].**

Nanotechnology is being explored in science for widely different applications. Polymer Nanotechnology has captivated a tremendous interest in many areas such as the pharmaceutical industry and therapeutic innovation among others. Especially, Chitosan Nanoparticles acts as are potential delivery system for hydrophilicand hydrophobic drugs due to its outstanding physicochemical and biological properties. It can control and sustain release the drug during the transportation and at the site of localization, altering dis-tribution of the drug and subsequent clearance to achieve increasein drug therapeutic efficacy and reduction in side effects. Chitosan based formulations have been used for the delivery of pharmaceutically active ingredients, nucleic acids **[33]**, protein therapeutics and antigens **[34].** After studying these reports, the present investgation was designed.

The present investigation is carried out for formulation and characterization of poloxamer stabilized Chitosan Nanoparticle for Control release and bioavailability to enhance the bioavailability of the poorly soluble drug Rivaroxaban by using different excipient which

includes pre-formulation, formulation, optimization and characterization.

Chitosan is a natural material has great attention in pharmaceutical and biomedical fields because of its advantageous biological properties, such as biodegradability, biocompatibility and nontoxicity **[35,36]**. It is a cationic polysaccharide obtained by partial deacetylation of chitin, the major component of crustacean shells. Chitosan is composed of N-acetyl-2-amino-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose linked by (1 → 4)-b-glycosidic bonds **[37]**. The use of chitosan as biomaterial is sustained primary by its biocompatibility and non-toxicityalready proven. Based on the acute toxicity test results, Rao and Sharma **[38]**, stated that chitosan is a safety material. Furthermore, it has been already approved by the Food and Drug Administration for applications as wound dressing **[39].**

Different parameters were performed during this study and results are discussed as given below. Pre-formulation study is the base of the formulation study. Pre-formulation is defined as the investigation of physical and chemical properties of drug alone and combination with excipients. The overall objectives of pre-formulation studies are to generate information about in developing stable and bioavailable dosage form. The organoleptic characteristics of a drug such as color, odour, and taste were studied. Organoleptic properties of both the drugs were evaluated using natural senses and were following the literature Pub Chem database. According to the present results of present study the drug Rivaroxaban is an off White in colour, observed odourless and available in powder form. According to the present results of present study the drug Rivaroxaban showed excellent solubility in Acetonitrile and Methanol while showed sparingly soluble in isopropanol, slightly soluble in phosphate buffer PH 6.8, phosphate buffer PH 7.4, ethyl ether and found insoluble in n-hexane, Water whereas the melting point was recorded 228°C-230°C.

The partition coefficient is defined as the ratio of un-ionized drug distributed between the organic and aqueous phases at equilibrium. It does provide a mean of characterizing the lipophilic/hydrophilic nature of the drug. It indicated that the drug was hydrophilic in nature. The partition coefficient of drug was examined in n-Octanol: Phosphate buffer pH 6.8, n-Octanol: water system. It was observed 1.7 in n-Octanol: PBS pH 6.8 and 1.9 in n-Octanol: PBS pH 7.4.

In formulation studies fifteen formulations of Rivaroxaban nanoparticles (Rivaroxaban -NPs) were prepared by the ionic gelation method as described by Calvo *et al* with slight modifications **[28]** Considerable information was provided by the statistical design to optimize the formulation after obtaining and analyzing the results. For Characterization and evaluation of all selected formulation different parameter study like production yield polydispersity index, particle size, entrapment efficiency and zeta-potential were carried out. The amount of chitosan, and TPP were optimized to prepare Rivaroxaban-NP formulation based on the responses like particle size, PDI, and zeta potential. Results showed the responses for the factors and the results acknowledge that particle size and zeta potential were increased with an increase in the concentration of TPP and chitosan. A linear increase in the polydispersity index is seen with higher concentrations of acetic acid and this was in accordance with the literature **[40,41].**

The factors temperature and stirring speed greatly affect zeta potential. The increase in stirring speed and temperature decreased the viscosity of the dispersion that leads to a decrease in zeta potential due to structural instability **[42].** The zeta-potential varied from 10.67 mV. to 21.54 Mv whereas particle size varied from 286.13±2.24 nm to 316.28±2.14 nm. Entrapment of drugs on polymeric drug carriers is primarily influenced by the nature of drug-carrier interactions. However, the release of drugs from formulations under *in vitro* as well as *in vivo* conditions largely depends on the drug binding as well as the composition of the formulations, including the excipients. The

entrapment efficiency varied from 42.34% to 99.87 %. The formulation F1, F5, and F6 showed the highest production yield (52.5, 52.35, 52.35 % respectively) and F7 showed the significant production yield (51.85 %), zeta-potential (23.63 mV), entrapment efficiency (99.87), particle size (316.12±2.14) and poly disparity index (0.32).

SEM has been used to determine the particle size distribution, surface texture and to examine the morphology of the fractured or sectioned surface. The same generally used for generating three-dimensional surface relief images derived from secondary electrons. The examination of surface of polymeric drug delivery can provide important information about the porosity and micro texture of given formulation.

As the *in vivo* study is very important to support the *in vitro* studies, in the present study *in vivo* study was carried out by using *in vivo* carrageenan induced tail model. The formation of thrombosis in cardiovascular diseases appears to be one of the leading causes of death and disability **[43]**. Intravascular arterial thrombosis causes many types of heart damage such as ischemia, myocardial infarction, and angina **[44,45]**. Antithrombotic agents have been widely used to prevent or treat thrombus formation. Today, despite the wide variety of antithrombotic drugs used in clinical practice, thrombosis-based cardiovascular diseases continue to be a major cause of illness and death. Therefore, effective treatment is still needed to address these problems.

In the present study it was found that after administration of carrageenan, a redish coloured part appears in the tail tip of the rodent and the length of the thrombosis increases with the time interval and necrosis was observed on pathogen region. As thrombus formation develops in the tail and has a clear boundary and normal component, it is possible to detect when it first appeared, the growing process, the distribution range, and the length of the formed thrombus *in vivo* **[46]**. In the present study as expected thrombosis occurred and 100% result was observed in control group animal. The results of the study showed the significant inhibition of the thrombosis by RVRX (63.33 %) as well as RLNPs (68.64 %) when compared it with standard.

5. CONCLUSION

Nanotechnology has produced an extremely important impact on Nano biomedicine and the diagnosis/treatment of disease. Nanoparticles are solid colloidal drug carriers ranging from 10—1000 nm in diameter and are composed of synthetic, natural or semi-synthetic polymers encapsulating the drug molecule. Due to its biodegradability, biocompatibility, easier formulation techniques and versatility in application aided with low toxicity chitosan offers certain advantages over others amongst the polymeric carriers for Nano particulate drug delivery. Rivaroxaban is a drug which belongs to class of anti-coagulant. Rivaroxaban is approved for the prevention of strokes and systemic embolism in atrial fibrillation. It is useful in prevention blood clot and treatment of deep venous thrombosis. These findings may contribute to the successful development of new pharmaceutical formulation F7 and may use as a better agent in the drug delivery system as well as can have used in the treatment area of cardiovascular diseases.

6. CONFLICTS OF INTEREST

The authors state that they have no conflicts of interest.

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