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Research

Preparation and evaluation of dorzolamide hydrochloride polymeric nanoparticles by emulsification sonication method

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Abstract

In this contemporary work we intended to develop Nanoparticle of Dorzolamide hydrochloride.The proposed Dorzolamide hydrochloride polymer loaded with nanoparticles was prepared by emulsification sonication method.Nanoparticles represent a promising drug delivery system of sustained and targeted drug release. They are specially designed to release the drug in the vicinity of target tissue. The aim of this study was to prepare and evaluate polymer loaded nanoparticles containing Dorzolamide hydrochloride in different drug to polymer ratio. SEM indicated that nanoparticles have a discrete spherical structure. FT-IR studies indicated that there was no chemical interaction between drug and polymer and stability of drug. The *in vitro* release behavior from all the drug loaded batches was found to be Peppas release and provided sustained release over a period of 48 hours. The developed formulation overcome and alleviates the drawbacks and limitations of Dorzolamide hydrochloride sustained release formulations and could possibility be advantageous in terms of increased bioavailability of Dorzolamide hydrochloride.

Keywords: Dorzolamide hydrochloride, Nanoparticles. Eudragit RS 100, emulsification sonication method

INTRODUCTION

Recent years it has become more evident that the development of new drugs alone is not sufficient to ensure progress in drug therapy. I*n vitro* obtained are more exciting but very often followed by disappointing results *in vivo*. To overcome these biopharmaceutical challenges, versatile formulation approaches are required which will accommodate the physicochemical properties of the individual drug while simultaneously exploiting the physiological environment. Solid lipid nanoparticles have been reported as an alternative drug delivery device to traditional polymeric nanoparticles. SLNs are in submicron size range (50-1000nm) and are composed

of physiologically tolerated lipid components[1-6]. At room temperature the particles are in solid state. These are made of biocompatible and biodegradable materials capable of incorporating lipophilic and hydrophilic drugs.

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then to maintain the desired drug concentration. That is, the drug delivery system should deliver drug at a rate dictated by the needs of the body over a specified period of treatment. This idealized objective points to the two aspects most important to drug delivery namely spatial placement and temporal delivery of a drug. Spatial placement relates to targeting of drug to a specific organ or tissue, while temporal delivery refers to controlling the rate of drug delivery to the target tissue.

An appropriately designed controlled release drug-delivery system can be a major advance towards solving these two problems[7-15]. It is for this reason that the science and technology responsible for development of controlled-release pharmaceuticals has been, and continues to be the focus of a great deal of attention in both industrial and academic laboratories.

MATERIALS AND METHODS

The materials used in this current research work are procured from reliable sources of Dorzolamide hydrochloride, SURA LABS, Dilsukhnagar, Eudragit RS 100Gattefosse Pvt. Ltd., MumbaiChitosan, Ethyl cellulose from Merck Limited, Mumbai (India), Tween 80SD Fine- Chem Limited, Mumbai, Span 60Loba Chemie Pvt Ltd. (Mumbai, India). All other reagents used I this research work are procured from most reliable vendors.

Analytical Method Development- Determination of absorption maxima

Absorption maxima are the wavelength at which maximum absorption takes place. For accurate analytical work, it is important to determine the absorption maxima of the substance under study.

Preparation of calibration curve

It is soluble in Methanol; hence Methanol was used for solubilizing the drug. Stock solution (1 mg/mL) of Dorzolamide hydrochloride was prepared in Methanol and subsequent working standards (5, 10, 15, 20 and 25 µg/mL) were prepared by dilution with phosphate buffer of pH-5.5. These solutions were used for the estimation Dorzolamide hydrochloride by UV method. The whole procedure was repeated three times and average peak area was calculated[16-23]. Calibration plot was drawn between concentrations and peak area. Calibration equation and R^2 value are reported.

Preparation of nanoparticles

Preparation of Dorzolamide hydrochloride loaded nanoparticles

Dorzolamide hydrochloride loaded Nanoparticle was prepared by previously reported emulsification sonication method. Dorzolamide hydrochloride was dissolved in organic solvent (10 ml, methanol). Polymers in different concentrations were dissolved in water. The organic phase was added drop wise into the polymeric solution for emulsification. Then the dispersion was sonicated (20 min) with the application of ultra-probe sonication (60 W/cm^3, Hielscher, Ultra-sonics, Germany). The formulation was stirred at 1500 rpm for 6 h using a magnetic stirrer to evaporate the organic solvent[24-29]. The prepared NPs were centrifuged at 15,000 rpm for 20 min at 4 °C (Remi, Mumbai, India). The formulations with code are shown in the table 1.

Table 1: Composition of nanoparticles formulations (F1 to F9)

Excipients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Dorzolamide hydrochloride (%)	2	2	2	2	2	2	2	2	2
Eudragit RS 100 (mg)	100	150	200	-	-	-	-	-	-
Chitosan (mg)	-	-	-	100	150	200	-	-	-
Ethyl cellulose	-	-	-	-	-	-	100	150	200
Tween 80 (mL)	0.5	1	1.5	2	-	-	-	-	1
Span 60 (mL)	-	-	-	-	0.5	1	1.5	2	1
Distilled water (ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Dichloromethane (ml)	15	15	15	15	15	15	15	15	15
Methanol	10	10	10	10	10	10	10	10	10

Characterization of nanoparticles

Particle Sizes, PDI, Zeta Potential

The mean particle length and polydispersity index (PDI), that's a degree of the distribution of nanoparticles population, was decided the usage of dynamic light scattering (Delta Nano C, Beckman counter), and Zeta capability becomes anticipated on the premise of electrophoretic mobility under an electric powered

field, the use of zeta Sizer Nano ZS (Malvern Instruments, UK). Samples had been diluted with the distilled water before measurement and measure at a hard and fast angle of 1650c for the particle size and poly dispersity index (PDI) analysis[30-36]. For the Zeta ability measurement, Samples have been diluted as 1;40 ratio with filtered water (v/v) before analysis. Average particle size, PDI, and zeta potential have been then measured in triplicate.

Percent amount of drug release from semi permeable membrane

Franz diffusion cell was used for the in vitro drug release studies. Semi permeable membrane was placed between donar and receptor chamber of diffusion cell. Receptor chamber was filled with freshly prepared 30ml 5.5 PH phosphate buffer. SLN gel equivalent to 1gm was placed on semi permeable membrane. The Franz diffusion cell was placed over magnetic stirrer (REMI 1ML) with 500rpm and temperature was maintained at 37 ± 1^0C. 5ml of samples were withdrawn periodically and replaced with fresh buffer as shown in the figure 1. The withdrawn samples were periodically diluted and analysed for drug content using UV visible spectrophotometer (Lab India 3200) at 252 nm.

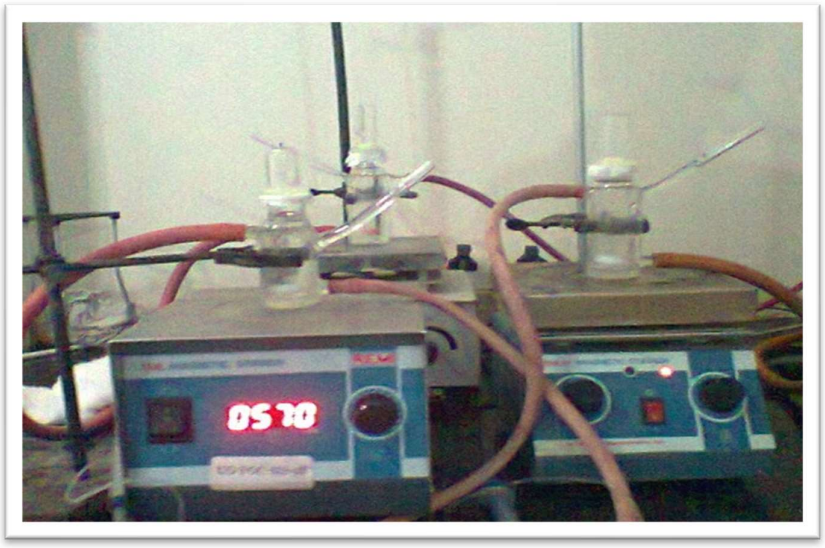

Fig 1: Drug release from semi permeable membrane

Powder X-ray Diffraction (PXRD) Studies

The prepared mixtures were also analyzed using X-ray powder diffractometer (PXRD) which confirms the formation of the new solid phases. This technique detects changes in the crystal lattice and is therefore a powerful tool for studying polymorphism, pharmaceutical salts, and cocrystalline phases[37-42]. Spectra of PXRD were taken on a sample stage Spinner PW3064. The samples were exposed to nickel filtrate Cukœ radiations (40 KV, 30 mA) and were scanned from 10° to 40°, 2Ɵ at a step size of 0.045° and step time of 0.5 s.

***In Vitro* Release Studies**

Drug release was determined by dialysis method; two ml of each formulation (test and control) were poured into dialysis bags and put into 25 ml phosphate buffer (pH 6.8) and stirred (100 rpm, room temperature). At predetermined time intervals, 2 ml of phosphate buffer was taken and then substituted by fresh phosphate buffer. Finally, the amounts of released Simvastatin in phosphate buffer were measured by spectrophotometer at 235 nm. Aliquots withdrawn were assayed at each time interval for the drug released at λ max of 235 nm using UV-Visible spectrophotometer by keeping phosphate buffer pH 6.8 as blank and the amount of released drug was estimated by the standard curve.

Fourier Transform Infrared (FTIR) spectroscopy

The formulations were subjected to FTIR studies to find out the possible interaction between the drug and the excipients during the time of preparation. FT IR analysis of the pure drug and optimized formulation were carried out using an FT IR spectrophotometer (Bruker FT-IR - GERMANY).

Differential Scanning Calorimetry

The possibility of any interaction between the drug and the Excipients during preparation of SLN was assessed by carrying out thermal analysis of optimised formulation using DSC. DSC analysis was performed

using Hitachi DSC 7020, on 5 to 15 mg samples. Samples were heated in sealed aluminum pan at a rate of 10°C/min conducted over a temperature range of 30 to 350°C under a nitrogen flow of 50 mL/min.

SEM (Scanning Electron microscope) studies

The surface morphology of the layered sample was examined by using SEM (Hitachi, Japan). The small amount of powder was manually dispersed onto a carbon tab (double adhesive carbon coated tape) adhered to an aluminum stubs. These sample stubs were coated with a thin layer (30Å) of gold by employing POLARON-E 3000 sputter coater[43-47]. The samples were examined by SEM and photographed under various magnifications with direct data capture of the images onto a computer.

RESULTS AND DISCUSSION

Calibration plot of Dorzolamide Hydrochloride

A standard graph of Dorzolamide Hydrochloride in phosphate buffer of pH-5.5 was plotted using Absorbance and concentration as shown in Table and Fig. Equation for linearity curve and R^2 were calculated as Y=0.06X+0.003 and R^2 =0.999 as represented in the figure 2.

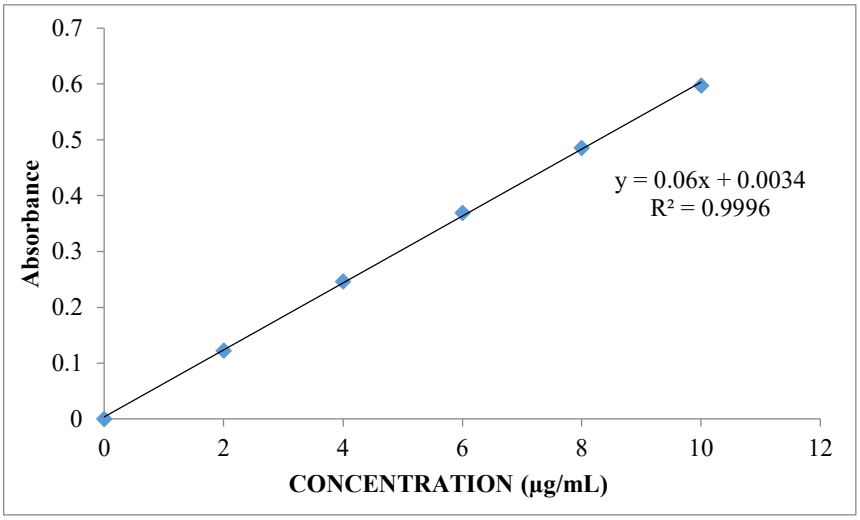

Fig 2: Calibration curve of Dorzolamide Hydrochloride in phosphate buffer pH 5.5

Characterization of nanoparticles

Percentage yield of formulations F1 to F9 by varying drug to polymer was determined and is presented in Table. Highest drug content, Highest Entrapment efficiency observed for F4 formulation. The Percentage yield, Drug Content and Entrapment Efficiency of nanoparticles has been shown in the figure 3,4&5.

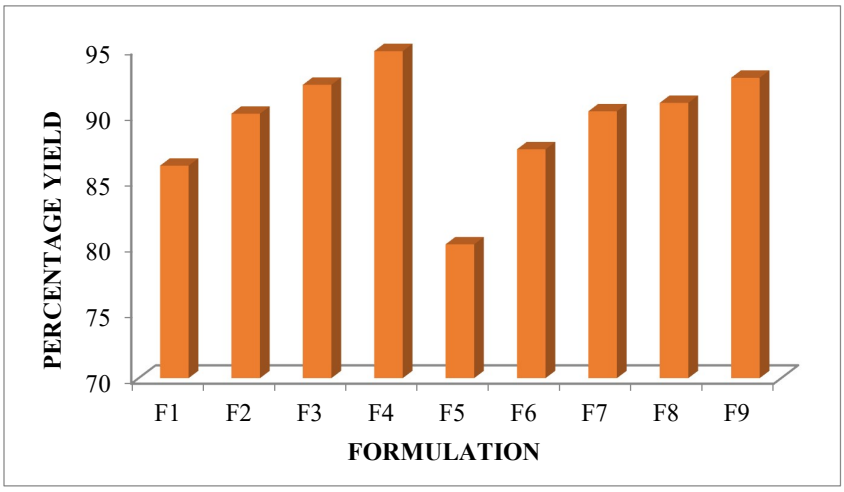

Fig 3: Percentage yield of all nanoparticles

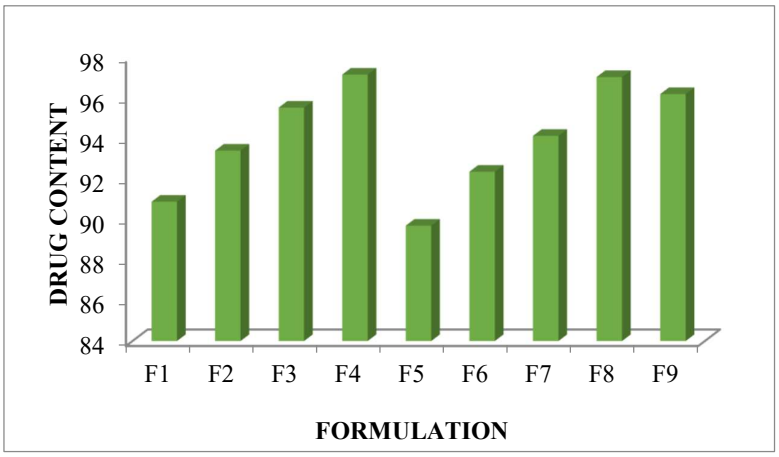

Fig 4: Drug Content yield of all nanoparticles

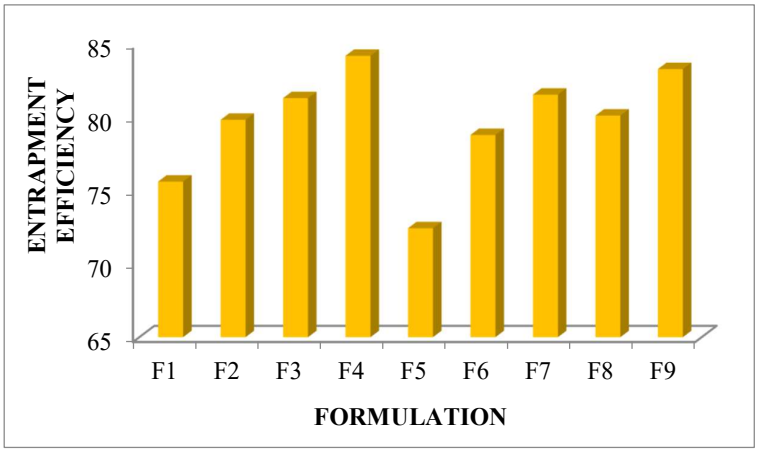

Fig 5: Entrapment Efficiency of all nanoparticles

The particle size of the all formulations was observed in the range of 412.9 to 1212.1. The less particle size, PDI observed in the F4 formulation i.e., 0.126 nm respectively. The Zeta potential range from -20.55 mV to -36.25 mV to all the formulations. The F4 formulations Zeta potential, Differential Scanning Microscopy, Scanning Electron Microscopy and X-RD images are as shown in the figure 6,7,8 & 9.

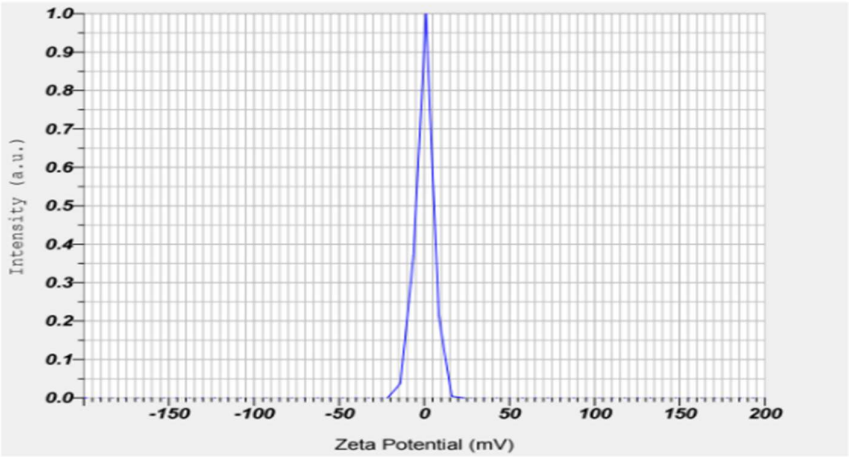

Fig 6: Zeta potential of F4 Formulation

Differential Scanning Microscopy

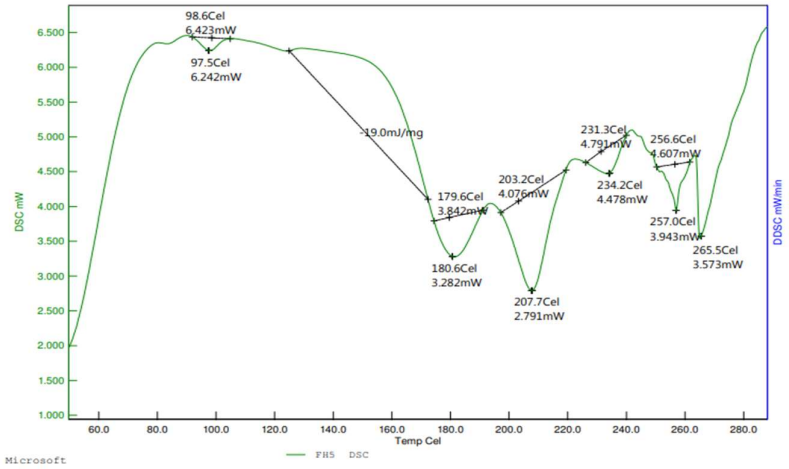

Fig 7: Dorzolamide Hydrochloride Pure

Scanning Electron Microscopy

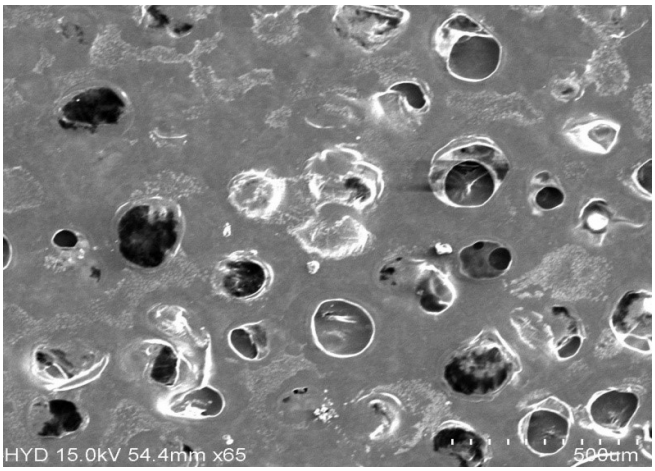

Fig 8: Dorzolamide Hydrochloride F4 optimized nanoparticles

SEM studies showed that the Dorzolamide Hydrochloride- loaded nanoparticleshad a spherical shape with a smooth surface as shown in Figure.

X-Ray Diffraction

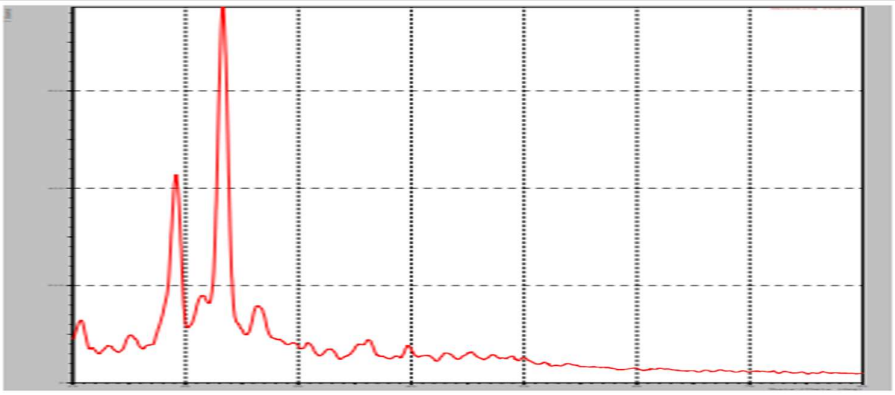

Fig 9: XRDDorzolamide Hydrochloride F4 nanoparticles

***Invitro* Drug Release study**

In vitro drug release study of the selected nanoparticles (F1, F2, F3, F4, F5, F6, F7, F8 and F9) was carried out. The nanoparticles exhibited 48 hours sustained release pattern. Forty nine of the incorporated amounts of drugs were found to be released during the first 2 hours, followed by a slowed release of 99.19 % of the drug up to 48 hours. The Dorzolamide Hydrochloride -loaded nanoparticles F4 showed a better release profile of 99.19% by 48 hours as shown in the figure 10.

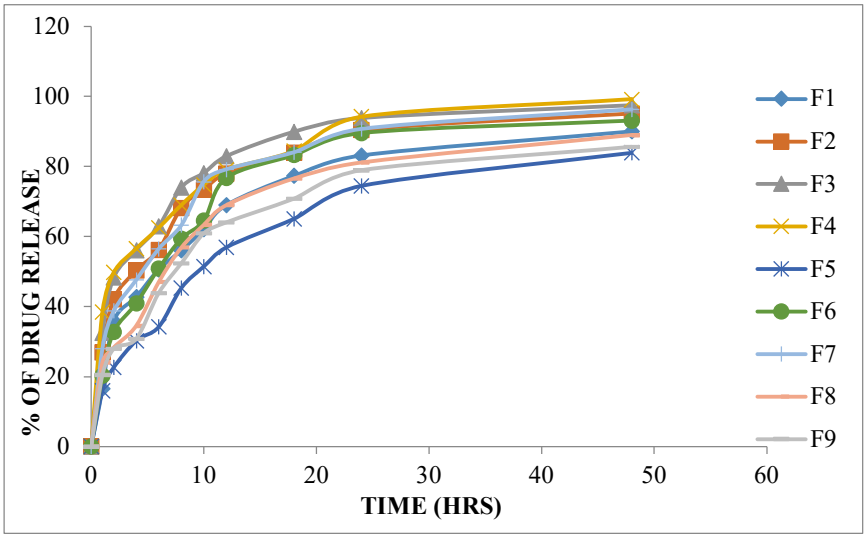

Fig 10: *In vitro* dissolution studies of F1-F9 nanoparticles formulations in percentage

Release Kinetics

To analyze the drug release mechanism the *in vitro* release was fitted into various release equations and kinetic models first order, zero order, Higuchi and Korsmeyer-peppas. The release kinetics of optimized formulation F4 (Chitosan) is shown in Table and in following Figure 11.

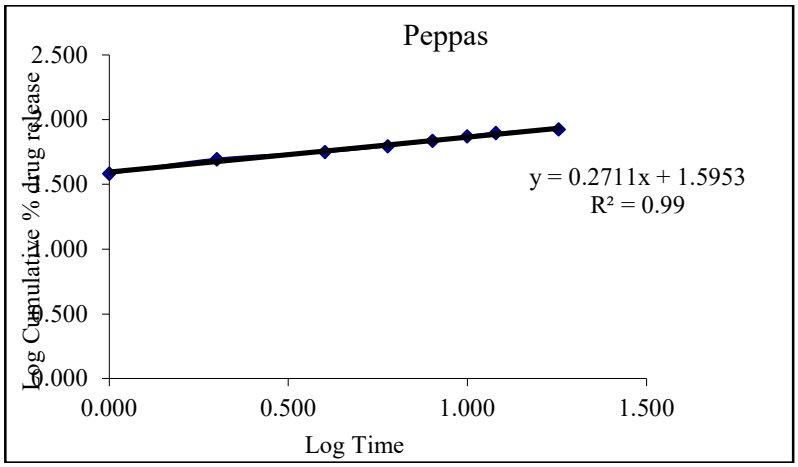

Fig 11: Peppas release kinetics

The prepared F4 optimisedChitosan nanoparticles were subjected to the drug release kinetics and release mechanism. The formulations were studied by fitting the drug release time profile with the various equations such as Zero order, First order, Higuchi and Korsmeyer pappas. The optimised formulation F4 optimisedChitosan nanoparticles was analyzed for the drug release mechanism. The best correlation coefficient value (0.99) indicates the best release mechanism (Peppas release).

FTIR studies

Infrared studies were carried out to confirm the compatibility between the polymer, drug, and selected excipients. From the spectra it was observed that there was no major shifting, as well as, no loss of functional

peaks between the spectra of the drug and drug-loaded nanoparticles. This indicated no interaction between the drug and other excipients as represented in the figure 12 & 13.

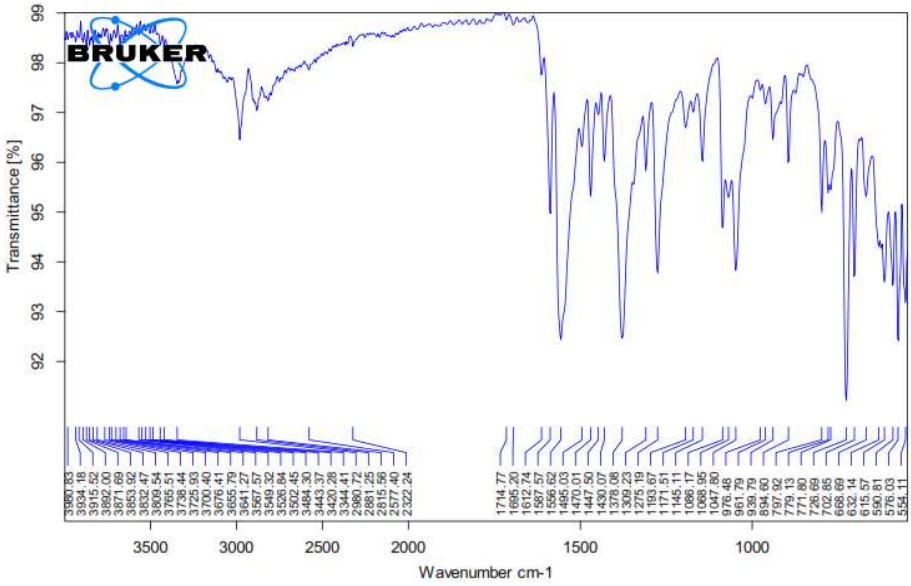

Fig 12: Dorzolamide Hydrochloride Pure drug FTIR

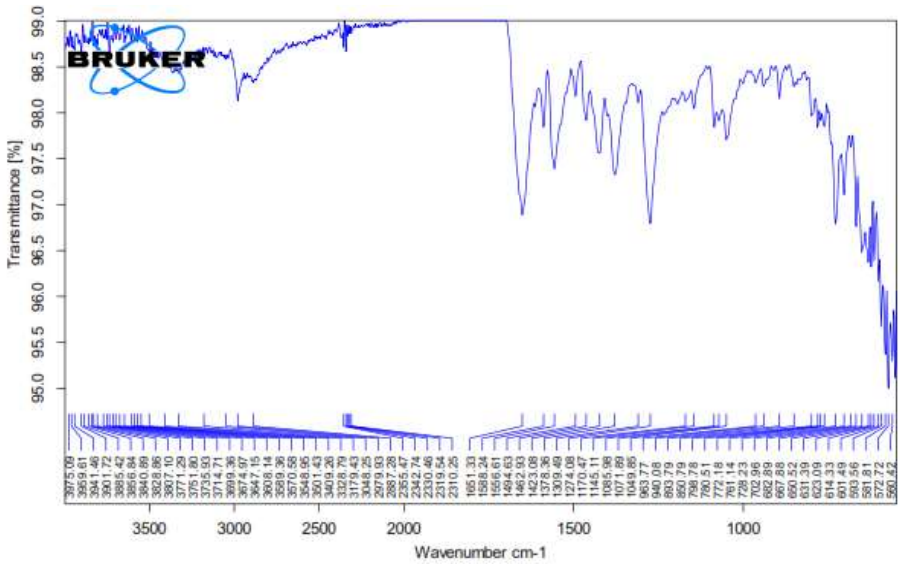

Fig 13: Dorzolamide Hydrochloride F4 optimized

CONCLUSION

In this research work, Dorzolamide hydrochloride loaded nanoparticles were successfully formulated and loaded. Drug and excipient compatibility were studied by FTIR, and no incompatibility was observed.Evaluation parameters revealed that the percentage of polymer have significant effects on the particle size, drug content, entrapment efficiency and *invitro* release from the nanoparticle's formulation. Nanoparticles formulation F4 was the most effective formulation with optimum particle size, high entrapment efficiency and improved release profile.The optimized Dorzolamide hydrochloride polymer loaded nanoparticles formulations (F4) were in nano size range (412.9 nm) with high drug release (99.19%) adequate encapsulating efficiency exhibiting a homogenousand effective and hence the emulsification sonication method is very conducive method for the preparation of nanoparticle containing Dorzolamide hydrochloride has been established.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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