CHITOSAN EMULSION CROSS-LINKING FOR CONTROLLED DELIVERY OF FLUNARIZINE HYDROCHLORIDE.

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ABSTRACT

Flunarizine Hydrochloride is one of the most prescribed calcium channel blockers for migraine prophylaxis. Frequent dosing is necessary due to the relatively short biological half-life, which can result in fluctuating plasma levels and compliance issues. This study deals with the development of a novel sustained-release drug delivery system using a natural bio-polymer, Chitosan, as a carrier, fabricated by a water-in-oil emulsion cross-linking method in order to improve the pharmacokinetic profile of the drug.

Chitosan microspheres were successfully prepared by dispersing an aqueous solution of Chitosan in light liquid paraffin containing Span 80 as a stabiliser. The resulting emulsion was chemically cross-linked using Glutaraldehyde to solidify the polymer droplets. Formulation variables, such as the drug-to-polymer ratio and stirring speed (optimised from 500–1500 rpm), were precisely controlled to influence particle characteristics.

The prepared microspheres were subjected to an extensive evaluation. Scanning electron microscopy confirmed their spherical morphology and showed that the average particle size depends on the stirring speed. The optimised formulation resulted in a high drug entrapment efficiency (EE) of over. FTIR and DSC studies confirmed successful drug encapsulation and integrity.

In vitro drug release studies showed a significantly sustained release up to 12 hours in 7.4 buffer, indicating controlled delivery. The release kinetics best fitted the Korsmeyer-Peppas model, suggesting an anomalous transport mechanism driven by both diffusion and polymer matrix swelling.[1]

Keywords: Flunarizine Hydrochloride, Chitosan, Microspheres, Emulsion Cross-linking, Sustained Release, Controlled Drug Delivery, Migraine Prophylaxis.

OBJECTIVES:

- Selection of appropriate polymer to prepare microspheres: studies related to drug-excipient compatibility.
- To develop various Flunarizine dihydrochloride microspheres preparations by using emulsion cross-linking with different polymer proportions.

- We can formulate microspheres of flunarizine dihydrochloride to control the release of the drug and enhance its poor solubility and bioavailability.

INTRODUCTION:

A novel drug delivery system is exclusively designed for the efficient administration of a therapeutic agent to the desired site in a controlled and sustained manner. The key objective of these systems is to achieve maximum therapeutic efficacy by delivering the optimum amount of the agent at the right time to the target tissue to minimise toxicity and side effects.[2]

THE ORAL ROUTE CHALLENGE AND NDDS SOLUTION

Oral administration is a widely used route for drug administration. However, due to pharmacokinetic problems, such as a short circulating half-life and poor absorption, traditional formulations usually require frequent administration to achieve and maintain their therapeutic effect. Well-designed controlled drug delivery systems can overcome many of the problems encountered in conventional therapy. It improves the therapeutic effectiveness of a drug and increases its bioavailability.[3]

MICROSPHERES AS DRUG CARRIERS

The different successful approaches in NDDS include the utilisation of microspheres as drug carriers.

DEFINITION: Microspheres are defined as free-flowing powders consisting of biodegradable protein or synthetic polymers and shall preferably have a particle size less than 200 µm. They are also generally termed "Monolithic spheres" and have a particle size of 1-1000nm[4]

CONTROLLED RELEASE: Microspheres are designed to maintain a consistent level of the drug in the blood. They enhance the controlled release of drugs, vaccines, antibiotics, and hormones. Controlled drug delivery with the aid of biodegradable polymers has recently been one of the important areas of research.[5]

BENEFITS: This system increases patient compliance and decreases dose and toxicity, while protecting the drug from enzymatic and photolytic cleavage. The use of biodegradable microspheres eliminates the need for surgical implantation and removal procedures, unlike large polymer implants.[6]

MATERIALS AND METHODS FOR FORMULATION OF FLUNARIZINE MICROSPHERES

Table 1: List of materials used

S.NO	CATEGORY	SOURCE
1	Flunarizine di HCL	Flamingo Laboratories Ltd
2	Glutaraldehyde	S.D. Fine Chemicals
3	Chitosan	Yarrow CHEM Products

4	Glacial acetic acid	Oxford lab fine CHEM LLP
5	Tween 80	S.D. Fine chemicals
6	Acetone	S.D. Fine chemicals

METHODS FOR MICROSPHERES FORMULATION:

The technique of preparation depends on the nature of the polymer as well as the nature of the drug and the duration of therapy. The most important physical and chemical factors that may be controlled in microsphere manufacture are

1. The particle size requirement
2. Molecular weight of polymer
3. Polymer to drug ratio
4. Stability problem
5. Reproducibility
6. Total mass of drug and polymer
7. Dispersibility in aqueous vehicle

METHODS:

1. Evaporation of the solvent
2. Single emulsion technique
3. Double Emulsion Technique
4. Phase separation coacervation technique
5. Spray drying and spray congealing
6. Solvent extraction
7. Quassi emulsion solvent diffusion
8. Emulsion crosslinking method

Emulsion crosslinking method

Encapsulation using the emulsion crosslinking method is a method that utilises cross-linking the functional group of the coating with the functional group of crosslinking agents. This method makes a water-in-oil emulsion (W/O), which emulsifies the coating solution in the oil phase. To stabilise the emulsion, it can be used. After the formation of a stable emulsion, a crosslinking agent was added to harden the emulsion droplets. The micro particles(microcapsules) formed were filtered and washed using petroleum ether, followed by hexane. With this method, the particle size can be controlled.[14]

METHODOLOGY

PRE FORMULATION STUDIES: Physical characteristics

a) Melting point determination

b) Solubility studies

a) Melting point determination:

Melting point determination is done to check the purity of a solid substance. For the determination of the melting point, a small quantity of the drug was taken in a capillary tube, and one end was closed. The capillary tube was put in an electrically operated melting point determination device, and the temperature at which the drug liquefies was recorded. This was repeated three times, and the average was noted.[15]

b) Solubility studies:

Solubility of Flunarizine: The solubility was determined by using various solvents, which was done by adding an excess of drug to various solvents and shaking on the shaker for 24 hours at 25°Ctemperature under constant speed. 1ml of solution was taken and diluted using a suitable diluent, and the solubility of Flunarizine Hydrochloride was determined using- UV-Visible spectrophotometer at an appropriate nm.[16]

EXPERIMENTAL METHODS:

Preparation of 0.1N HCL:

8.5ml of conc HCL was taken in a 1000 ml volumetric flask and diluted with distilled water to make up to 1000ml with distilled water.

Determination of λ max of Flunarizine Dihydrochloride:

Preparation of stock solution:

25mg of Flunarizine dihydrochloride was taken in a 25 ml volumetric flask, and 10 ml of methanol was added to it and shaken well to get a clear solution. Then the volume was made up to 25ml with 0.1N HCL buffer to give 1mg/1ml concentration. From the above solution, 10ml was taken in a 100ml volumetric flask, and the volume was made up to 100ml with 0.1N HCL buffer to get 100 µg / 1ml concentration. From the above solution, 1ml was taken and made up to 10ml with 0.1N HCL to give 10 µg/ml. The 10µg/ml solution was scanned between 200-400 nm using a UV-Visible Spectrophotometer.[17]

Drug-excipient compatibility studies

FTIR spectra of the drug and excipients were recorded by the KBr disc method using a Bruker Alpha Spectrophotometer with IR solution software. Drug and excipients were mixed by triturating with KBr in a glass mortar with pestle and compressed into discs in a hydraulic press. FTIR spectra of all the samples were recorded over a spectral region from 4000 to 400 cm-1.

Calibration curve:

25mg of Flunarizine di HCL was accurately weighed and dissolved in 10ml methanol and 15ml buffer (0.1N HCL) solution to get 1mg/1ml concentration (Stock-I). From the above solution (Stock I), 10ml was taken and diluted with 0.1 N HCL buffer to get a Concentration of 100µg/mL (Stock II). From the above solution (Stock -II) 0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8,0.9,1ml solutions were taken and diluted with 0.1N HCL to obtain a series of concentrations between 1-10 µg/mL. Absorbance of prepared solutions was measured by using a UV-Visible spectrophotometer at 252 nm using a 0.1 N HCL buffer as a blank.[18]

Preparation of Flunarizine-Loaded Chitosan Microspheres:

Flunarizine-loaded Chitosan microspheres were prepared using an emulsion cross-linking technique. Chitosan solution was prepared by dissolving chitosan in 2% v/v acetic acid solution, and the mixture was degassed in a sonicator for about 10 min. The drug solution was prepared by dissolving the drug in acetone. The drug solution was added to the chitosan solution and mixed thoroughly. 100 ml of light liquid paraffin containing 0.5% v/v Tween 80 was then poured into a 250 ml beaker and stirred using a propeller-type agitator. The chitosan-drug solution was added drop-wise, under stirring, to form a w/o emulsion. After 20 minutes, 25% v/v Glutaraldehyde solution (approximately 0.15ml) was added at 15-minute intervals for 5 times. Stirring was continued for about 3 hours until the microspheres were formed. They were then decanted from the paraffin by filtration and washed three times with n-hexane, followed by acetone to remove the paraffin oil.

Table 2: Formulation of microspheres of Flunarizine dihydrochloride using emulsion crosslinking method

Formulation Code	Drug: Polymer Ratio
F1	1:1
F2	1:2
F3	1:3
F4	1:4
F5	1:5

CHARACTERIZATION OF DRUG-LOADED MICROSPHERES:

Shape and size of Microspheres

The surface structure, shape, and size distribution of the chitosan microspheres were characterised from micrographs obtained with a scanning electron microscope and an optical microscope. In the SEM studies, a 100 µl aqueous suspension of microspheres was dried on a metal support in a vacuum at room temperature for 4 h, and the samples were coated with Gold. The size and size distribution of the chitosan microspheres were determined by using optical microscopy.

Encapsulation Efficiency:

Microspheres 50 mg were triturated in a mortar with pestle and then transferred to a 10 ml volumetric flask containing 0.1N HCL buffer and kept aside for 24 hrs. After 24 hrs solution was centrifuged to remove the polymeric debris, and the absorbance of the supernatant was determined at 252 nm using a UV-Visible spectrophotometer.

The encapsulation efficiency of the drug was calculated by the formula:

% Encapsulation efficiency = Actual quantity of drug determined /Theoretical quantity of drug ×100

Percent Yield:

The microspheres that had been thoroughly dried were then weighed out accurately. The % yield was calculated as the ratio between the mass of the microspheres and the mass of initially added substances, which included the drug and polymer.

Percentage yield=Weight of microspheres/Total weight of drug and polymer×100

Drug content:

The weighed microspheres equivalent to 10 mg were dissolved in 100ml of 0.1 N HCL. The solutions were filtered, and drug content was determined at 252 nm by a UV-Visible spectrophotometer.

In Vitro Drug Release Studies:

In vitro drug release studies of microspheres were carried out using a USP type II dissolution apparatus. Stirring rate was kept at 50 rpm, and 0.1N HCL buffer was used as dissolution medium. The temperature of the dissolution media was maintained at 37±0.2°C. 5ml of samples were withdrawn at regular time intervals of 15 min, 30 min, 60 min, 90 min, 120 min up to 12 hours and replaced with 5ml of fresh buffer. The samples were filtered using Millipore filters, and dilutions were made whenever necessary and were analysed for Flunarizine dihydrochloride at 252nm by using UV-visible spectrophotometry.[19]

RESULTS AND DISCUSSION:

Determination of Melting point: Using the capillary method, a melting point of 202°C was determined for Flunarizine dihydrochloride.

Solubility: Flunarizine dihydrochloride is insoluble in water and soluble in 0.1N HCL buffer, ethanol, and completely soluble in methanol.

UV SPECTRUM OF FLUNARIZINE DIHYDROCHLORIDE:

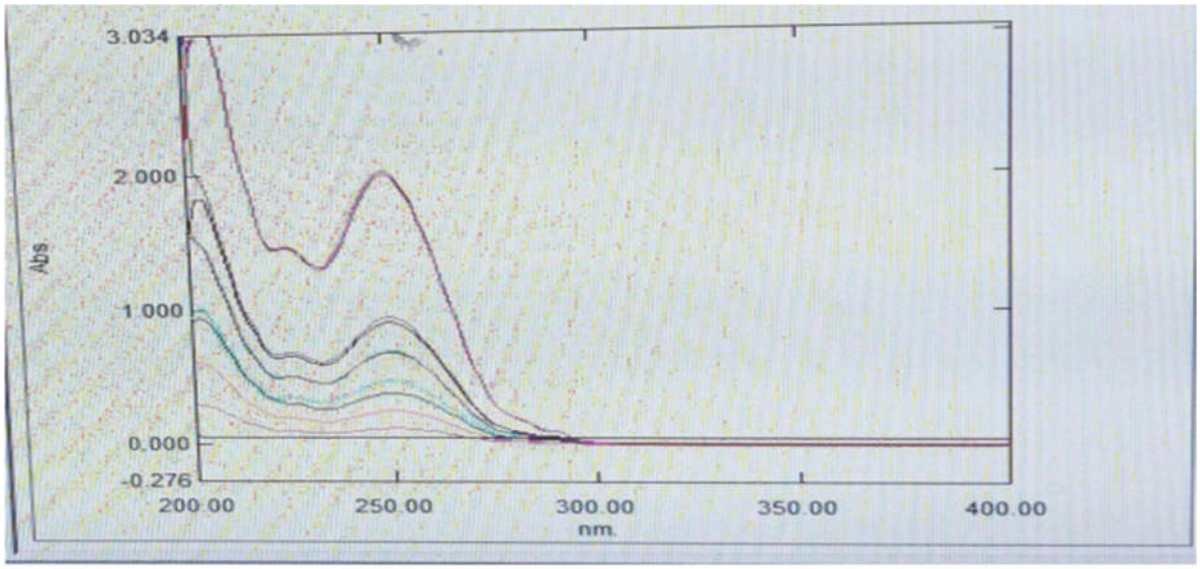

Fig 1: UV Spectrum of Flunarizine dihydrochloride

Standard Graph of Flunarizine Dihydrochloride:

Calibration Curve Data of Flunarizine Dihydrochloride

Table 3: Calibration Curve Data of Flunarizine Dihydrochloride

Concentration (ug/ml)	Absorbance
1	0.122
2	0.251
3	0.382
4	0.471
5	0.693
6	0.687
7	0.904
8	0.939
9	1.072

10	1.167

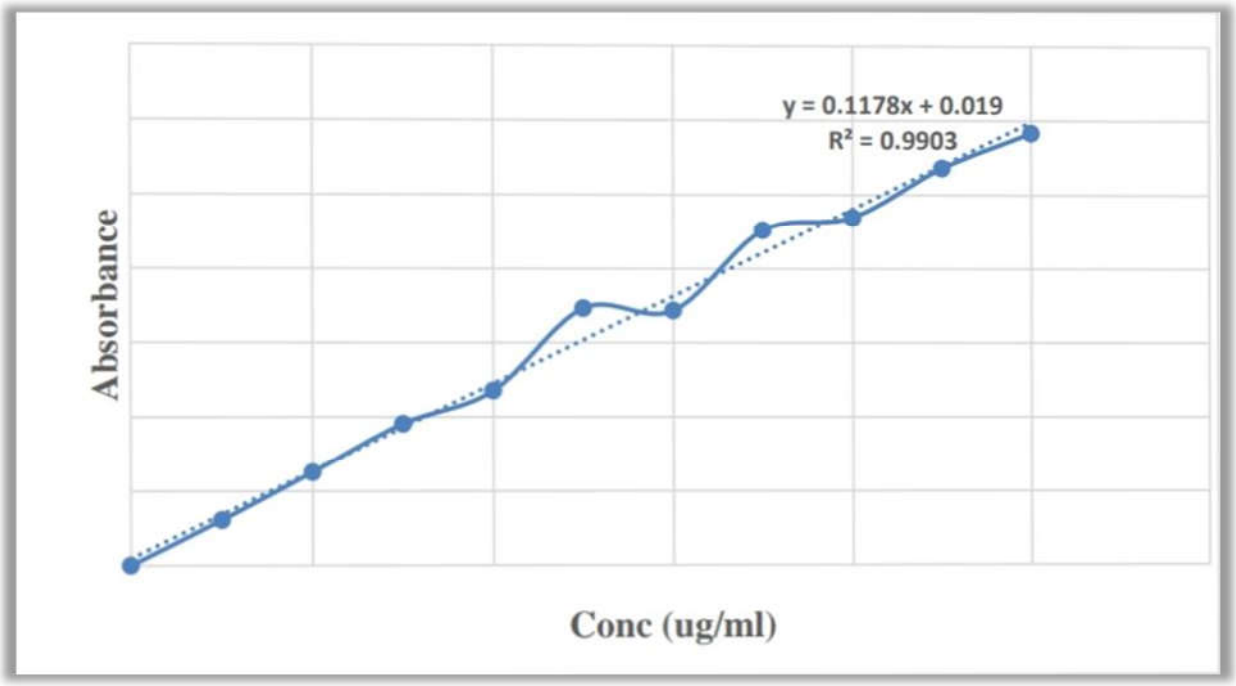

Fig 2: calibration curve of Flunarizine dihydrochloride

The calibration curve of Flunarizine dihydrochloride was found to be linear means that with the increase in the concentration of the drug, absorbance also increased. It suggests that it obeys Beer-Lambert's law.

Studies of Drug Excipient compatibility: Drug-excipient compatibility was assured by comparing the spectrum of FT-IR analysis of pure drug with that of various excipients used in the formulation of microspheres.

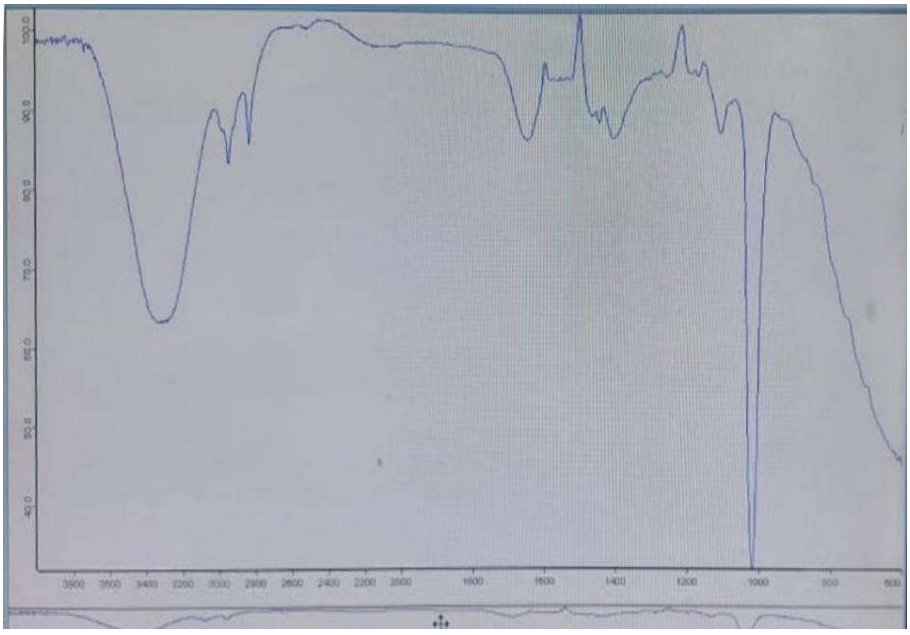

Fig 3: FT-IR Spectra of Flunarizine dihydrochloride.

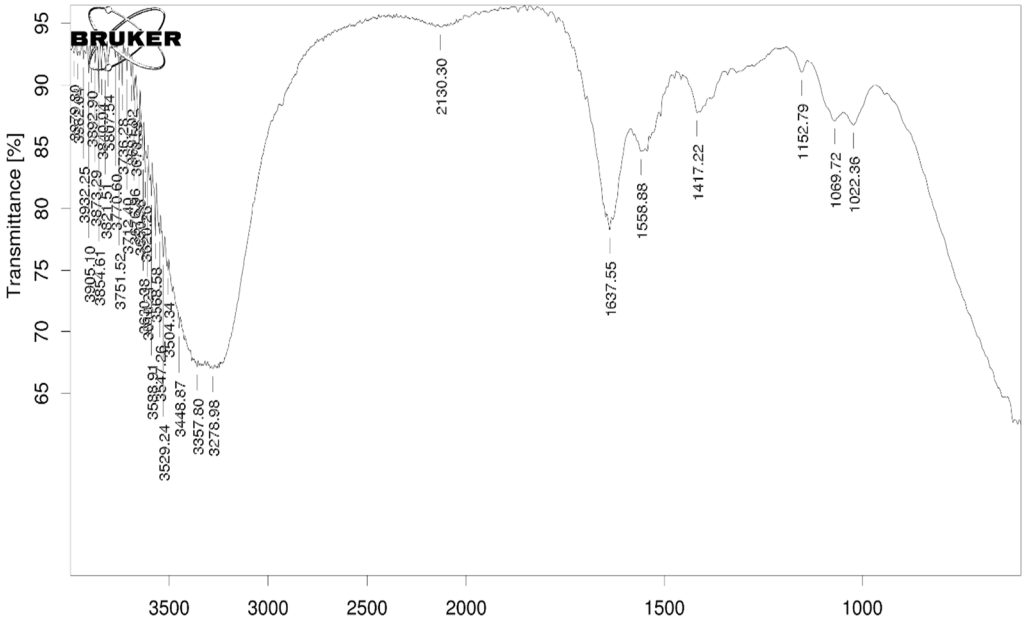

Fig 4: FT-IR Spectra of Chitosan and Glacial Acetic Acid

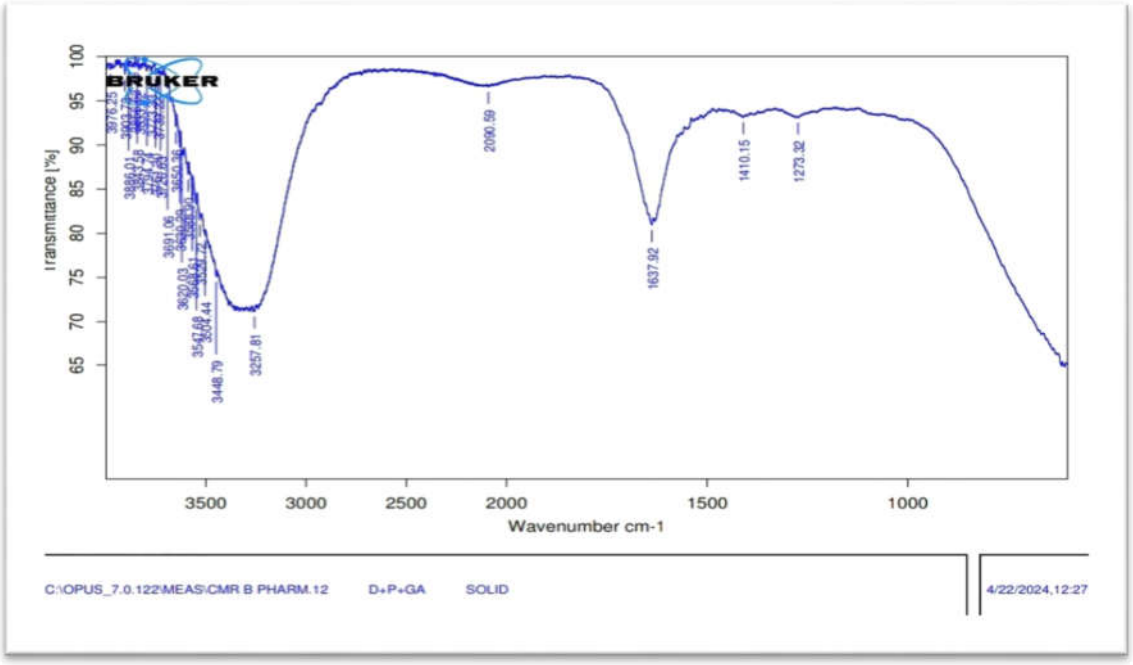

Fig 5: FT-IR Spectra of Flunarizine dihydrochloride, Chitosan and Glacial Acetic Acid

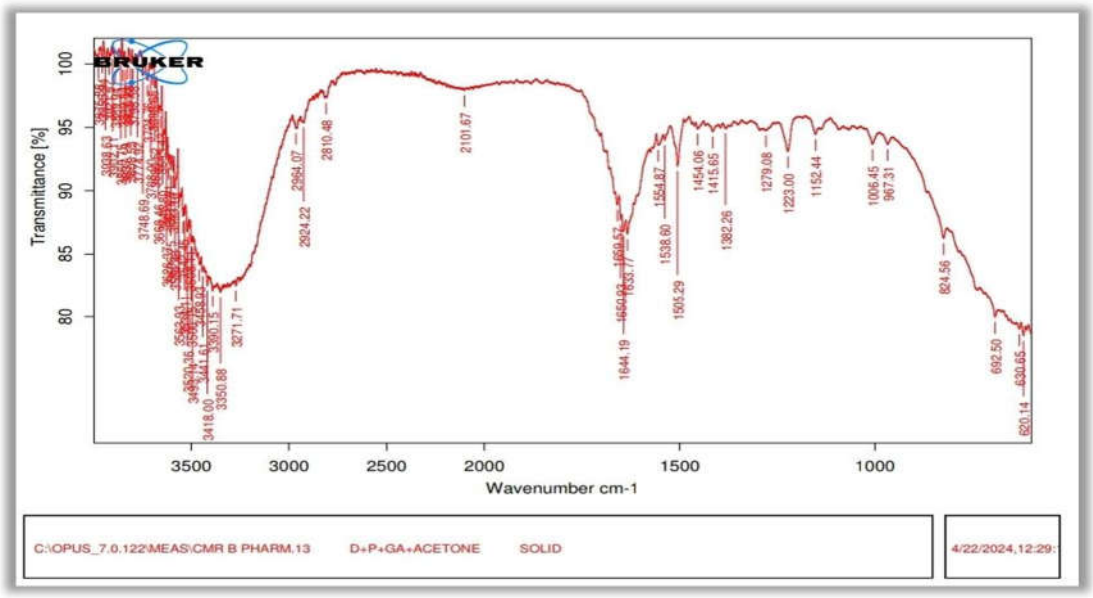

Fig 6: FT-IR Spectra of Flunarizine dihydrochloride, Chitosan, Glacial Acetic Acid and Acetone

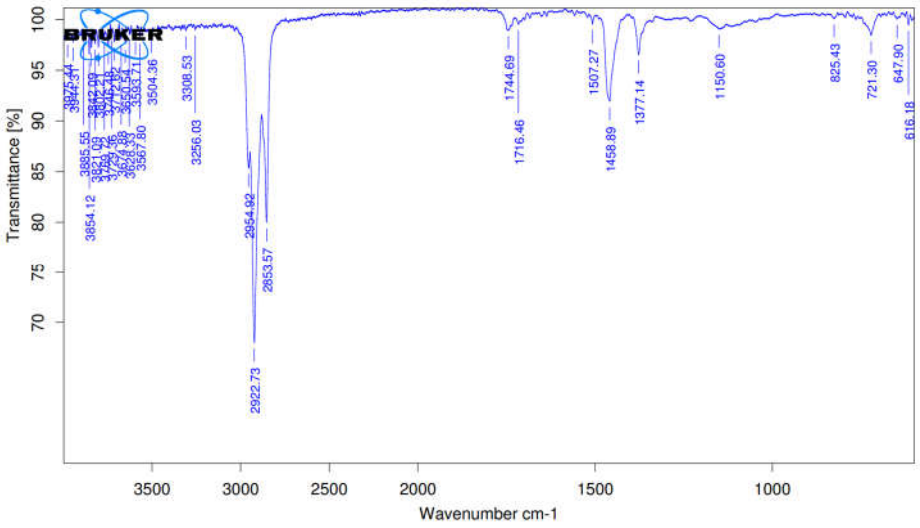

Fig 7: FTIR Spectra of microspheres formulation

The FT-IR spectra of Flunarizine dihydrochloride, chitosan and Flunarizine dihydrochloride-loaded microspheres were compared. The FTIR spectrum of Flunarizine dihydrochloride peaks at 1439.24 cm-1, which was related to C-H bending. The same peak was observed in the optimised formulation at 1458.89 cm-1. In the Flunarizine dihydrochloride spectrum, the peak at 1410.15 was related to the C-H stretch. The same peak was observed in the optimised formulation at 1458.89. The spectrum of chitosan Fig. 18 showed a peak at 1637.5 due to the amine group. The spectrum of optimised formulation showed peaks at 1744.69 and 1716.46; it may be due to the C-N vibration when the amine group of chitosan reacts with glutaraldehyde. From the FTIR spectra, it was revealed that chitosan was cross-linked with glutaraldehyde through Schiff base reaction. FTIR studies indicated that the drug and polymer are compatible with each other.

POST FORMULATION STUDIES:

Percentage yield of Microspheres

Table 4: Percentage yield of Microspheres

Formulation Code	Percentage Yield
F1	56
F2	68
F3	78
F4	84
F5	89

Percentage yield of all formulations F1 to F5 were in the range of 56 to 89. Percentage yield of formulations increased with increase in the concentration of polymer.

Mean particle size of formulation

Table 5: Mean particle size of formulation

Formulation Code	**Mean Particle Size(um)**
F1	**128**
F2	**149**
F3	**164**
F4	**193**
F5	**224**

The mean particle size of all formulations was within the range of 128 to 224 µm. Mean particle size increased with the rise in polymer content of the microspheres. When the polymer content is increased, a more viscous internal phase manifests during the process of emulsification and is poorly dispersed in the external phase. The result is large-sized microspheres.

Entrapment efficiency

Table 6: Entrapment efficiency of formulation

Formulation Code	Entrapment Efficiency
F1	43.13
F2	60.27
F3	75.31
F4	78.25
F5	70.53

Entrapment efficiency of all formulations was in the range of 43.13 to 78.25. The increase in entrapment efficiency with an increase in the polymer content of the microspheres is due to higher polymer concentrations; chitosan viscosity leads to a less diffuse matrix structure that hinders drug escape from the microsphere core. Excessively high polymer content would hinder homogeneous distribution of the added crosslinking agent (glutaraldehyde), leading to the formation of larger particles with reduced drug content and entrapment efficiency.

CUMULATIVE PERCENTAGE DRUG RELEASE:

Table 7: Cumulative percentage drug release of formulations

Time in hours	**Formulation code**				
	F1	**F2**	**F3**	**F4**	**F5**
1	**16.04**	**14.17**	**12.95**	**11.16**	**10.11**
2	**29.023**	**25.08**	**23.43**	**20.26**	**18.55**
3	**33.12**	**30.31**	**28.28**	**23.32**	**21.07**
4	**43.56**	**34.30**	**31.06**	**27.09**	**25.43**

5	**57.94**	**40.01**	**40.20**	**32.37**	**30.14**
6	**60.32**	**45.12**	**48.49**	**38.19**	**34.77**
7		**53.04**	**56.63**	**42.75**	**39.00**
8		**60.38**	**60.69**	**45.59**	**42.48**
9			**67.87**	**48.32**	**45.66**
10			**75.76**	**51.21**	**48.92**
11			**78.07**	**54.34**	**53.94**
12			**85.97**	**60.90**	**55.96**

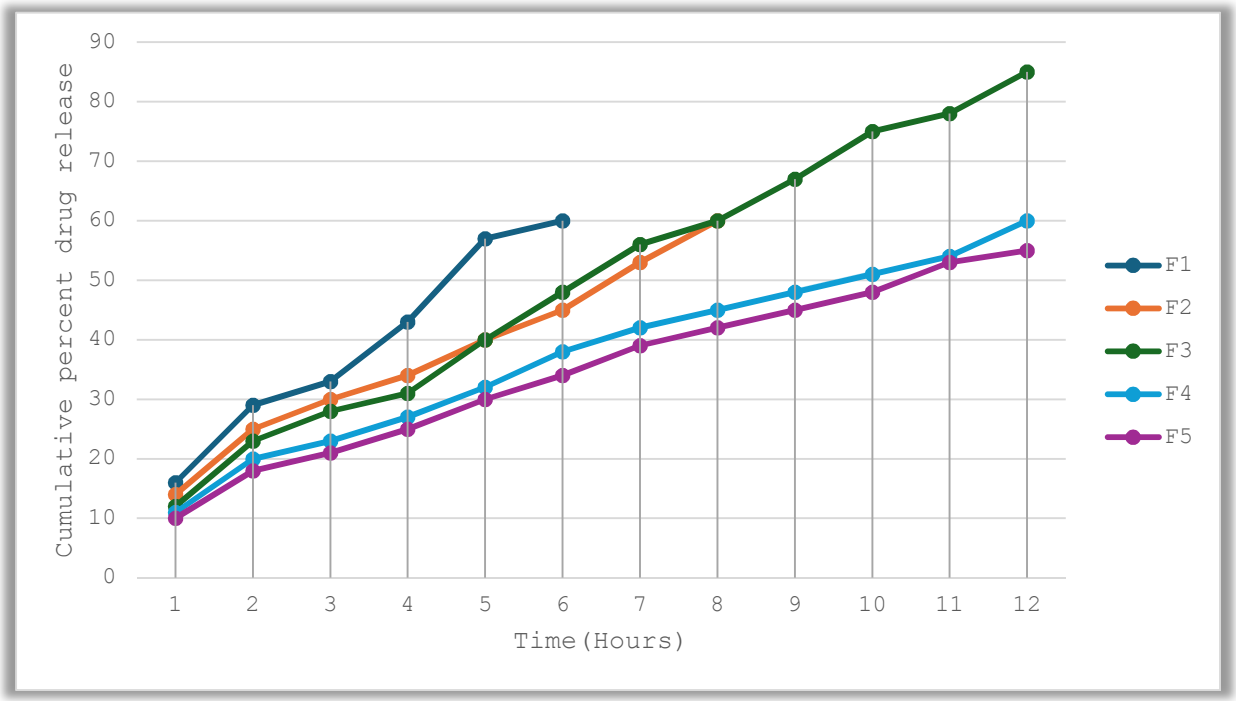

Fig 8: Cumulative percentage drug release of formulations F1 to F5

Cumulative drug release was carried out for 12 hours. F3 formulation containing a drug–polymer ratio of 1:3 presented the maximum release of 85 % at the end of 12 h. The rate of drug release from microspheres decreases with an increase in polymer concentration due to the prolongation of the diffusion of the drug.

SCANNING ELECTRON MICROSCOPY:

Scanning electron micrograph of optimised formulation F3 was studied and revealed that the surface of microspheres was porous and was almost spherical in shape.

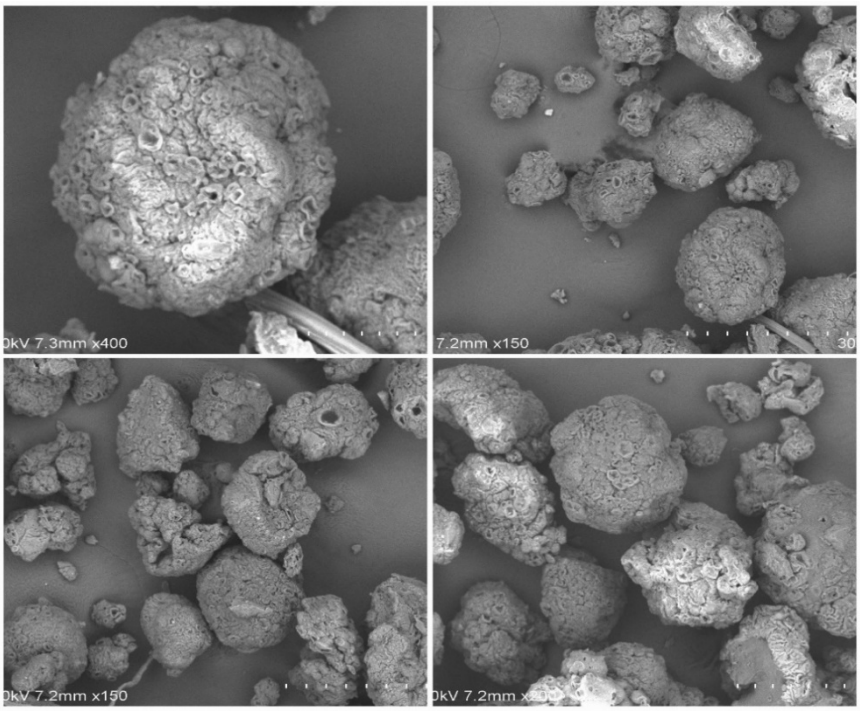

Fig 9: Scanning Electron Micrograph of formulations F3

CONCLUSION:

According to the study's findings, flunarizine dihydrochloride was successfully incorporated into chitosan microspheres that show drug-polymer compatibility and adhere to Beer-Lambert's law. Evaluation revealed that because of increased viscosity during emulsification, higher polymer concentration results in larger particle sizes and higher percentage yields. Although entrapment efficiency generally increased as polymer content increased, it eventually decreased at high concentrations. With the highest drug release of 85% over a 12-hour period, formulation F3 was determined to be optimised. The optimised formulation's SEM analysis verified that the microspheres were porous and nearly spherical in shape.

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