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Original Article

PREPARATION AND CHARACTERIZATION OF SUMATRIPTAN-LOADED W/O/W MULTIPLE EMULSIONS

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ABSTRACT

Objective: This study aimed to develop and evaluate W/O/W multiple emulsions of Sumatriptan Succinate for enhanced drug delivery and improved bioavailability.

Methods: Multiple emulsions were prepared using a two-step emulsification method. The primary W/O emulsion was formulated using oils (olive, coconut, and mustard oil) with Span 80, followed by secondary emulsification using Tween 80 and Xanthan gum to obtain W/O/W emulsions. Preformulation studies, including organoleptic characterization, melting point, solubility, partition coefficient, FTIR, and DSC were performed to assess drug properties and compatibility. UV spectrophotometry was used for analytical method development. The formulations were evaluated for globule size, pH, viscosity, conductivity, zeta potential, phase separation, and % entrapment efficiency. *In vitro* drug release studies were carried out using a dialysis membrane, and ex-vivo permeation studies were conducted using goat intestine. Pharmacokinetic studies were performed using albino Wistar rats.

Results: The developed formulations showed appropriate physicochemical properties with nanosized globules, suitable pH, and acceptable viscosity. Optimized batches demonstrated good stability with minimal phase separation and creaming. High entrapment efficiency and controlled drug release were observed. *In vitro* and ex-vivo studies indicated enhanced drug release and permeation. Pharmacokinetic results showed improved bioavailability compared to conventional formulations.

Conclusion: The developed W/O/W multiple emulsion of Sumatriptan Succinate demonstrated improved stability, controlled release, and enhanced bioavailability, making it a promising drug delivery system.

Keywords: Multiple emulsion, Sumatriptan succinate, Drug delivery, Bioavailability, Controlled release

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INTRODUCTION

Multiple emulsions are more complex than their two-phase counterparts from the standpoint of formulation, stability, and drug release. They are useful tool in achieving sustained release drug delivery for different routes.

Multiple emulsions are novel carrier system which are complex and poly dispersed in nature where both w/o and o/w emulsion exists simultaneously in a single system. Lipophilic and hydrophilic surfactants are used for stabilizing these two emulsions, respectively. The droplets of the dispersed phase contain even smaller dispersed droplets themselves, therefore also called as “emulsions of emulsions" [1].

They are, therefore, emulsions of emulsions and can be classified as oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) types (Whitehill, 1980; Florence and Whitehill, 1982) [2].

Sumatriptan is a serotonin receptor agonist commonly used to treat migraines and sometimes cluster headaches. Sumatriptan is the first of the triptans and was made available in Europe in 1991 to treat migraines. The dose of sumatriptan varies widely by route of administration and in most cases, no more than 2 doses should be given daily [3, 4]. Multiple emulsions like W/O/W can help encapsulate hydrophilic drugs (like Sumatriptan succinate) in the internal aqueous phase, while the external aqueous phase ensures good dispersion. This approach is especially promising for nasal, transdermal, or even oral controlled-release delivery systems. The multiple barriers (oil phase and outer aqueous phase) help in slowing drug diffusion, allowing for sustained or controlled release of Sumatriptan. This may help reduce dosing frequency and prolong therapeutic effect [5, 6].

The purpose of this research was to develop and to characterize Sumatriptan W/O/W multiple emulsion to treatmigraines. Incorporating it in internal water of W/O/W emulsion improve absorption of sumatriptan succinate entrapped oil globules by lymphatic absorption. It is BCS class III drug having low permeability (Lop P – 1.07) and high solubility of 101 mg/ml in water. The bioavailability is approximately 15%, primarily due to pre-systemic metabolism and partly due to incomplete absorption [2].

MATERIALS AND METHODS

Chemicals

Sumatriptan Succinate was supplied by Niksan Pharmaceutical, Ankleshwar. Span 80, Tween 80 and Olive oil from Suvidhinath Laboratories. Coconut oil and Mustard oil from Gramodhyog Kendra, Vadodara. Xanthan Gum supplies by Loba Chemie Pvt, Ltd. Model Drug (MgSO4) from Oxford Lab Fine Chem. Ethanol from department store. Acetonitrile HPLC grade purchased from Astron India. Dialysis membrane-110 from Himedia Laboratories. All other chemicals used were of reagent grade.

Solubility

The sample was qualitatively tested for its solubility in various solvents. It was determined by taking 10 mg of drug sample in 10 ml of solvent as water, methanol, ethanol, acetonitrile, pH buffer 6.8 in small test tubes and well solubilized by shaking [7].

Melting point determination by differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC-60 instrument) was another technique used to establish the drug sample's melting point. Using a DSC-60 instrument, this method entails taking a thermogram of the drug. To carry out the DSC analysis, the drug was sealed in an aluminium pan and heated at a constant rate of 10 ºC/min over a temperature range of 30-300 ºC while purging with 30 ml/min of

nitrogen gas. The internal standard was aluminium oxide. The resulting thermogram provided information about the thermal behaviour of the drug sample, including its melting point. DSC is a common analytical technique in the pharmaceutical industry for determining the thermal characteristics of drug samples. The approach determines the differential in heat flow between a sample and a reference material as a function of temperature. It contains useful information regarding the melting point, melting range, and other thermal properties of medicinal compounds. The use of nitrogen purging during the DSC analysis helps to prevent oxidation or degradation of the drug sample during the heating process. This ensures that the thermogram obtained is an accurate representation of the thermal behaviour of the drug substance [8].

FT-IR study of pure drug

The IR spectrum of drug substance was authenticated using IR spectroscopy (FTIR spectrometer-430, IR Affinity – 1S, Shimadzu, Japan). The presence of characteristic peaks associated with specific structural characteristics of the drug molecule was noted [9].

Preparation of w/o/w multiple emulsion

W/O/W multiple emulsions were prepared by two step emulsification method. In first step W/O emulsion i. e., primary emulsion, was prepared. Different oils like Mustard oil, olive oil, coconut oil were chosen as oil phase while span 80 was selected as lipophilic emulsifier. Internal aqueous phase was made up of distilled water containing 35 mg STS. Oil phase was poured in a glass beaker and Span 80 was added and stirred at moderate speed to make homogeneous mass (High shear homogenizer-Ultra-Turrax, T-25, IKA®, WERKE, Germany and High-speed stirrer-Eurostar, T-25, IKA®, WERKE, Germany). Internal aqueous phase containing drug was slowly added in this beaker with stirring (Magnetic stirrer-Janki Impex Pvt. Ltd. Ahmedabad) at specified speed to get w/o emulsion i. e., primary emulsion. In second step primary emulsion was added in external water phase at low speed to form secondary emulsion i. e., w/o/w multiple emulsion as shown in fig. DoE software was utilized to design, generate and study main effects of formulation factors over the responses of STS multiple emulsion formulation [10, 11].

Globule size

The globule size (Microscope-UNILAB®, GE-40, India) of multiple emulsions evolves over time due to processes like coalescence and Ostwald ripening. Initially uniform after preparation, emulsion droplets can increase in size as larger droplets form through coalescence with smaller ones [12].

Entrapment efficiency of sumatriptan succinate

Entrapment efficiency (EE) was measured by centrifugation method. 5 ml of the freshly prepared Multiple emulsion were immediately centrifuged at 6500 rpm for 10 min. Then 1 ml of the aqueous phase (the lower layer) was precisely withdrawn through 2 ml hypodermic syringe [13]. The solution was filtered with a Millipore filter (0.22 µm), free drug content was analyzed on UV spectrophotometer (UV-1900, Shimadzu, Japan) at 227 nm and EE was calculated by equation (1).

$$\%EE=((T-F)/T) \times 100 \ldots\ldots. (1)$$

Where T is the total drug incorporated and F is the free (unentrapped) drug.

Stability studies

The physical stability is examined by storing MEs at room temperature as well as under stability chamber (Lab India Instruments, India). At different time intervals, samples are observed visually for phase separation and the samples showing homogeneity are evaluated by microscopy (to confirm its multiple nature and determine globule size) and drug leakage. The MEs under refrigeration are allowed to return to room temperature before observation [14].

Drug leakage is calculated by equation (2).

$$\%\ \text{Drug leakage}=(L/E) \times 100 \ldots\ldots. (2)$$

Where l is the amount of drug leaked and E is the amount of drug originally entrapped.

Organoleptic characteristics

Freshly prepared primary and multiple emulsions were investigated organoleptically (colour, appearance, odour). Organoleptic characteristics of both primary and multiple emulsions kept at different storage conditions were noted at various intervals.

pH determination

Previously calibrated pH meter (CL 54+) was used to measure pH. Prepared formulation was filled in glass beaker and pH electrode was dipped in it and allowed to stabilize. Value displayed was noted [15].

Zeta potential measurement

Zeta potential was measured to know stability of the dispersion as it indicates the kind of force acting between the nearby globules on [Malvern Zetasizers nano] [16].

Viscosity determination

Viscosity was determined using rotating spindle viscometer (Brookfield viscometer DV I Prime). Viscosity was determined at RT using Spindle No.61 at 60 rpm. The viscosity was reported as mean and standard deviation of the mean of three determinations [17].

Creaming

Creaming in multiple emulsions refers to the migration or settling of emulsion droplets towards the top or bottom of the emulsion due to differences in density or size. This phenomenon can compromise the stability [18].

***In vitro* drug release study**

Activation of dialysis membrane procedure

Dialysis membrane – 110 (LA 395) having molecular weight cut-off of 12000-14000, obtained from Himedia Laboratories Pvt. Ltd., was used for the study. Before using the dialysis membrane for drug release study, it was first activated by following steps given in product specification [19].

The drug release study was performed by dialysis method shown in fig. 1(A) *in vitro* diffusion study on dialysis membrane:

5 ml of the emulsion was placed in a dialysis tube covered by a dialysis membrane (Himedia Laboratories) at both ends that was then placed in 300 ml of dissolution media phosphate buffer pH 7.4 at 37±1 °C in a beaker. A sink condition was maintained throughout the duration of the study. Samples were withdrawn at different time intervals, and the volume of the dissolution media was kept constant by replacing it with an equal volume of fresh media. Samples were analyzed by the UV spectroscopy method at 227 nm λmax and cumulative drug release was calculated [20].

Release kinetic profile

There are five models to check the kinetics of drug release [21].

a) Zero-order kinetics

Zero-order release kinetics refers to the process of constant drug release from a drug delivery device such as oral osmotic tablets, transdermal systems, matrix tablets with low soluble drugs and other delivery systems. In its simple form, zero-order release can be represented as equation (3)

$$Q=Q_0+k_0t \ldots (3)$$

Where Q is the amount of drug released at time t, Q_0 is the initial amount of drug, and k_0 is the zero-order rate constant.

b) First-order model

Most conventional dosage forms exhibit this dissolution mechanism. Some modified release preparations, particularly prolonged release formulations, follow this type of dissolution pattern using equation (4).

$$\text{Log } Q_t=\log Q_0+(kt)/2.303 \ldots\ldots (4)$$

Where Q_t is the amount of drug remaining at time t, Q_0 is the initial amount, and k is the first-order rate constant.

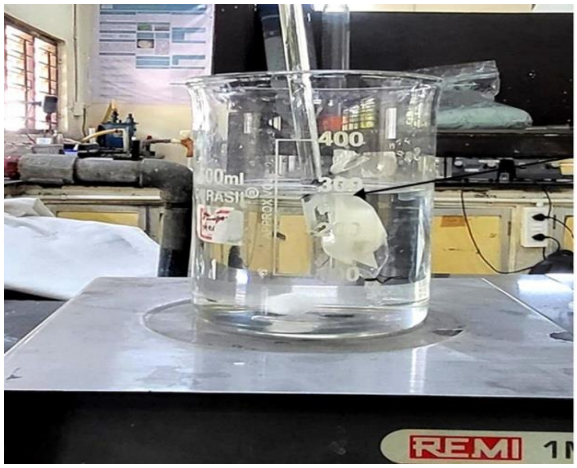

Fig. 1(A): *In vitro* diffusion study on dialysis membrane

c) Higuchi model

A large number of modified release forms contain some sort of matrix system. In such instances, the drug dissolves from this matrix. The dissolution pattern of the drug is denoted by water penetration rate (diffusion controlled) and thus the following relationship applies using equation (5)

$$M=(100-q) \times \sqrt{t} \ldots. (5)$$

whereM is the amount of drug released, *q* is the percent drug remaining, and *t* is time.

d) Korsmeyer-Peppas model

The power law describes the drug release from polymeric system in which release deviated from fickian diffusion as expressed using equation. (6)

$$M_t/M=kt^n \ldots\ldots. (6)$$

Where M_t is the amount of drug released at time *t*, *M* is the total drug released, *k* is the release rate constant, and *n* is the release exponent.

e) Hixson-crowell model

Some dosage forms contain particle of the same size and shape or their agglomerates that dissolves evenly. In such instances the cube root law can express the dissolution process using equation (7)

$$Q_0^{1/3}-Q_t^{1/3}= k_{HC}t \ldots\ldots. (7)$$

Where Q_0 and Q_t are the initial and remaining amounts of drug respectively, $k_{HC}$is the Hixson–Crowell rate constant.

***Ex-vivo* permeation study**

Ex vivo studies using MEs and a plain solution of STS on goat intestines. The intestines were promptly removed, cleaned, and immersed in PBS at a pH of 7.4. Using a 5 cm segment of the intestine, filled it with 5 ml of either the ME or the plain drug solution containing 35 mg of STS, and secured both ends with thread, as illustrated in fig. 1(B) Ex-Vivo Permeation Study In Goat Intestine. This tissue was then placed in a beaker containing 300 ml of diffusion medium (PBS, pH 7.4). To ensure proper aeration, used a pump aerator, and then maintained the temperature of the medium at 37±0.5 °C. At set time intervals, then withdraw aliquots from the diffusion medium and replaced them with an equal volume of fresh buffer. The drug content was measured spectrophotometrically at 227 nm, and it carried out the release studies for up to 8 h [22].

***In vivo* absorption study**

In this study, the selected albino wistar rats, weighing between 200 and 250 g, regardless of sex. All animal experiments received approval from the institutional animal ethics committee (Protocol No.: DDU/FOP/17/2024) at Dharmsinh Desai University in Nadiad, India, following the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals, under the Ministry of Social Justice and Empowerment, Government of India. The rats were divided into three groups, each containing three rats [23]. Then it administered a plain drug solution, along with ME1 and ME4, each equivalent to 1.0 mg/kg of Sumatriptan Succinate, using an oral feeding needle. Blood samples were collected from the retro-orbital plexus with a heparinized capillary, ensuring that no more than 1 ml was taken at intervals of 1, 3, 6, and 12 h. These samples were then analyzed for drug content at room temperature using HPLC (Agilent, India). A 100 µl** sample was combined with 100 µl** of methanol and stored in the refrigerator for an hour. After that, the mixture was centrifuged using Remi Equipment's Pvt Ltd, India (C-24 PLUS) at 4000 rpm for 20 min, and 100 µl** of the supernatant was collected for HPLC analysis. Various pharmacokinetic parameters, such as Cmax, tmax, AUC (0 – 24), and AUC (0 – ∞), were calculated [24, 25].

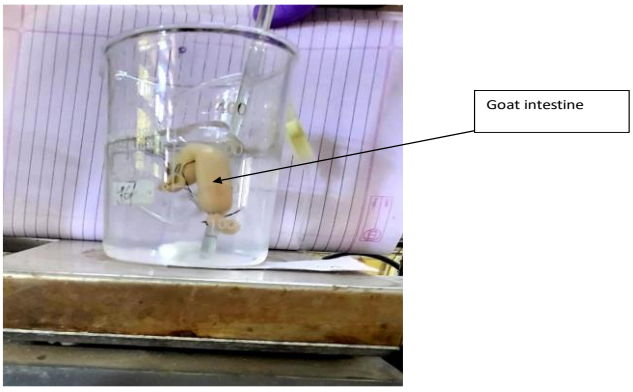

Fig. 1(B): Ex vivo permeation study in goat intestine

RESULTS AND DISCUSSION

The goal of this work is to develop and evaluate a multiple emulsion of Sumatriptan succinate. It conducted preformulation studies, including FTIR analysis, to confirm that the functional groups match those of the standard drug, and we found no interaction between the drug and the surfactant. The UV maxima of the drug were determined using a Systronic UV spectrophotometer.

Melting point

Melting point of Sumatriptan Succinate was found at 168-171 °C, indicating high purity of the drug.

Melting point determination by differential scanning calorimetry (DSC) which is shown fig. 2(A) DSC graph of STS.

FT-IR study of pure drug

The FTIR spectrum of drug substance was authenticated using IR spectroscopy. The presence of characteristic peaks associated with specific structural characteristics of the drug molecule was noted. Various peaks of the drug are shown in fig. 2 (B) FTIR Spectra of STS (sample) and (C) FTIR Spectra of STS (Reference).

Oil screening study

2-level factorial design was used for screening of oil. Three oils were taken for screening: Olive oil, Coconut oil and Mustard oil.

Formulation components for sumatriptan succinate multiple emulsion

Sumatriptan Succinate is water-soluble and is included in the innermost aqueous layer of the multiple emulsion. The middle oil phase includes oils like olive, mustard or peanut oil which entraps the inner water phase and assist in maintaining drug release. Water is used as both a solvent of drug in inner stage and continuous medium in outer stage of the emulsion. The primary water-in-oil emulsion is stabilized by using a lipophilic surfactant, span 80, which reduces interfacial tension between water and oil. A hydrophilic surfactant known as Tween 80 is used to stabilize the final water-in-oil-in-water emulsion, by decreasing interfacial tension that exists between the oil phase and the external water phase. Lastly, xanthan gum functions as a thickening agent in the outer aqueous layer, which raises the viscosity and promotes the stability of the emulsion in general. Each 5 ml of Formulation contains 35 mg of Sumatriptan Succinate equivalent to 25 mg of Sumatriptan.

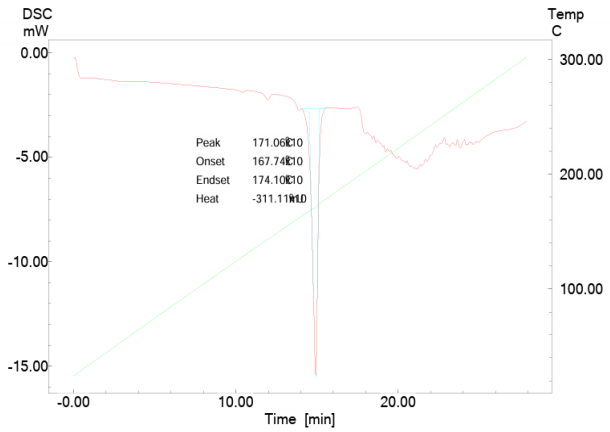

Fig. 2(A): DSC graph of STS

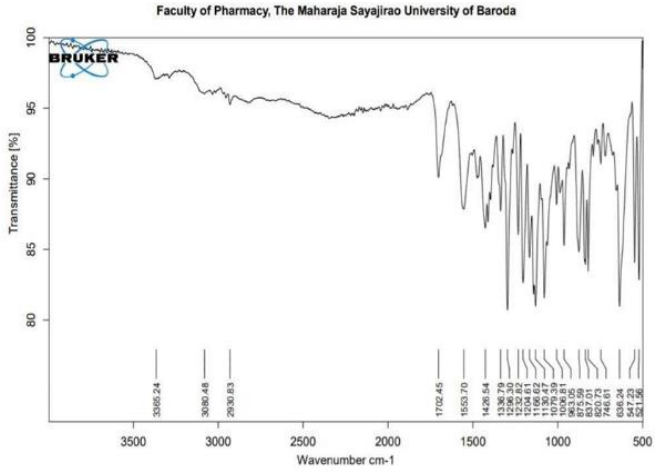

Fig. 2(B): FTIR spectra of STS (sample)

Different responses were observed for multiple emulsion of sumatriptan succinate

The multiple emulsion formulations of sumatriptan succinate depicted fairly steady properties in different runs. The size of the globule was kept within a low range of 60-58 µm, suggesting that moderately fine and relatively homogeneous droplets were formed,

which is advantageous in pharmacology to maintain the emulsions and the release of the drug in a controlled manner. Zeta potential values (−11.23 to =9.2 mV) indicated a weakly negative surface charge; the values were not very high, indicating that the system was primarily stabilized by surfactants or polymers and not only through repulsions. Entrapment efficiency was moderate at 46.39-37.61; there was a weak yet slightly declining drug encapsulation that can be further optimized to increase therapeutic efficacy and sustained release. The values of the creaming (2.72-2.45 cm) were relatively low and slightly downward, indicating good physical stability and reduced phase separation with time. The pH of the formulations was always a bit acidic (4.414.22), which is also a stable environment of the drug, although it still needed to be evaluated in terms of compatibility with the proposed administration route. Finally, viscosity rose slightly, 11.25 cps to 13.63 cps, which may be used to minimize droplet movement and creaming and enhance stability without excessive flow characteristics. Collectively, these findings indicate that the prepared multiple emulsions are fairly homogenous, fairly stable, and can be used to deliver the Sumatriptan Succinate, but the performance may be improved by increasing the entrapment efficiency and zeta potential.

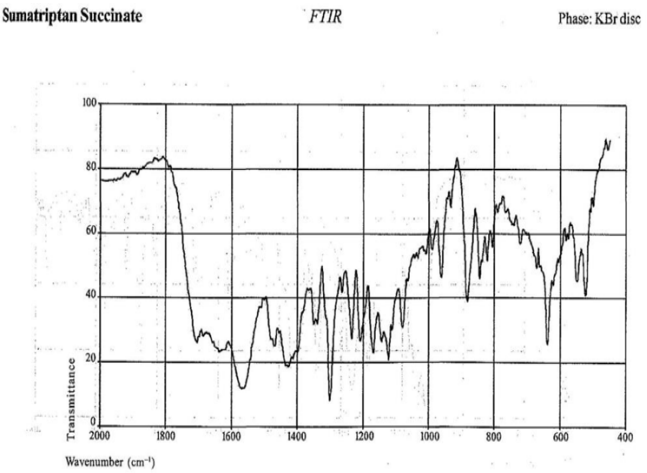

Fig. 2(C): FTIR spectra of STS (Reference)

Table 1: Responses observed of Sumatriptan succinate multiple emulsion batches formulated using central composite design

Run	Globule size (μm)	Creaming (cm)	Zeta potential (mV)	Viscosity (cps)	%EE (%)	*In vitro* diffusion (%)	Ex-vivo permeation (%)
1-8	25-22	0.3 – 0.8	-19.25--11.92	91-87	82.3737 – 83.0913	94.45 – 89.47	90.43 – 86.13

Response surface for globule size shown in fig. 3(A) Response surface for globule size:

Final equation (8) in terms of coded factors:

Globule size=+21.64+6.57A+0.3091B .... (8)

The increase in the globule size of the multiple emulsion is seen with an increase in the concentration of polymer (% of inner aqueous phase).

Contour plot for Globule size shown in fig. 3(B) Contour plot for globule size

Response surface for entrapment efficiency shown in fig. 3(C) Response surface for entrapment efficiency

Final equation (9) in terms of coded factors

Entrapment efficiency =+82.54+4.47A – 0.1761B+0.1010AB-A2+0.0045B2 .... (9)

The increase in the Entrapment efficiency of drug is seen with increase in the concentration of polymer in the innermost aqueous phase of multiple emulsion.

Contour plot for entrapment efficiency shown in fig. 3 (D) Contour plot for entrapment efficiency

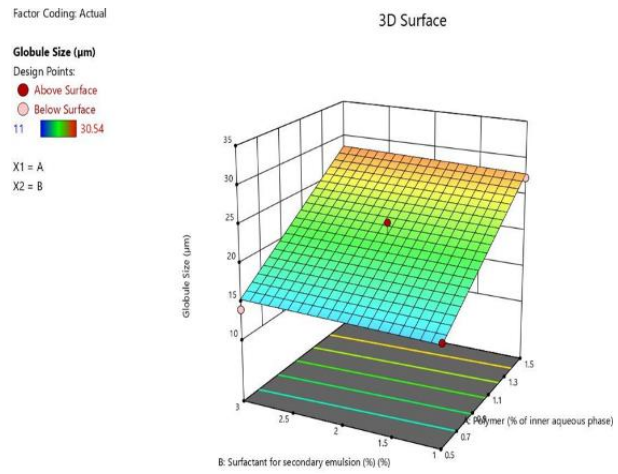

Fig. 3(A): Response surface for globule size

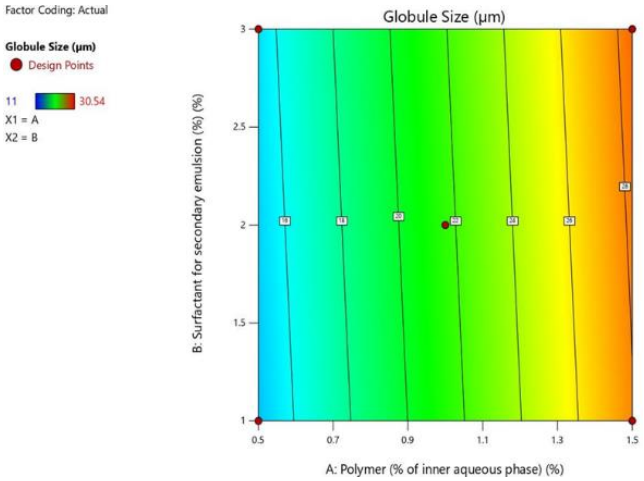

Fig. 3(B): Contour surface globule size

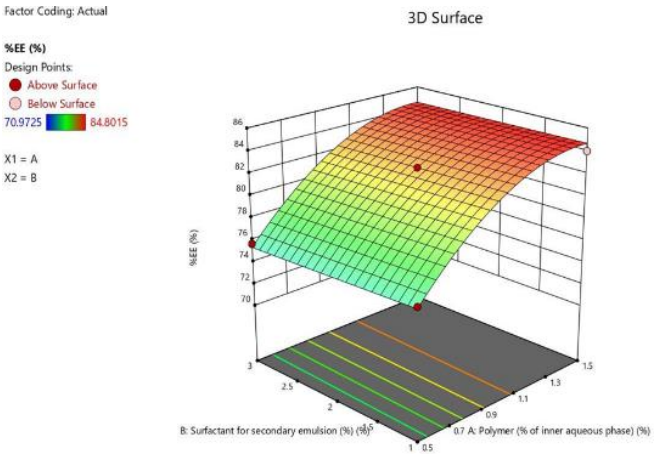

Fig. 3(C): Response surface entrapment efficiency

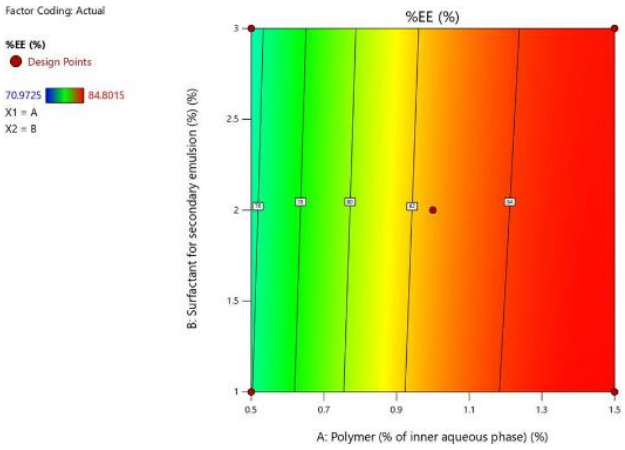

Fig. 3(D): Contour plots entrapment efficiency

Response surface for Viscosity shown in fig. 4 (A) Response surface for viscosity

Final equation (10) in terms of coded factors

$$Viscosity=+81.91+25.97A+0.8946B \ldots. (10)$$

The increase in the viscosity is seen with an increase in the polymer in inner aqueous phase.

Contour plot for viscosity shown in fig. 4 (B) contour plot for viscosity

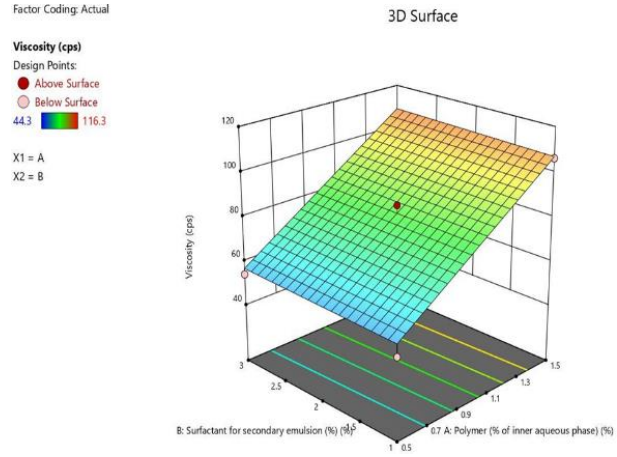

Fig. 4(A): Response surface viscosity

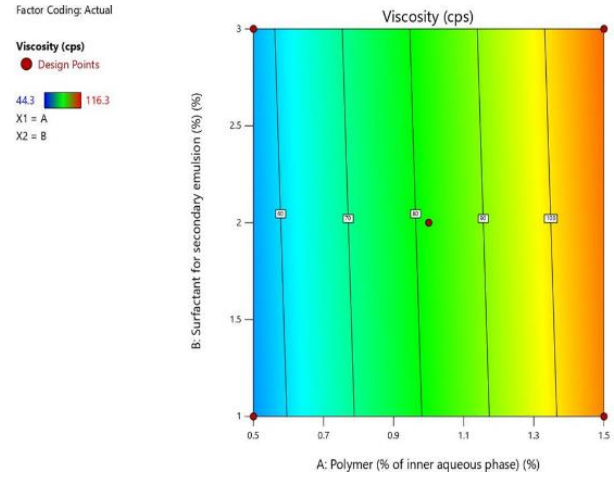

Fig. 4(B): Contour plots viscosity

Design space

An overlay plot used to create a design space is shown. To make sure the reaction stays within a specific range, we developed the design space. When there are multiple responses, it's important to find a spot that provides the best values for all the parameters. The design space is shaded grey, while areas that don't meet the optimization criteria are marked in yellow. You can add flags to an overlay plot that shows optimized variable values next to the predicted values of the intended response. Which is shown in fig. 4 (C) overlay plot for design space.

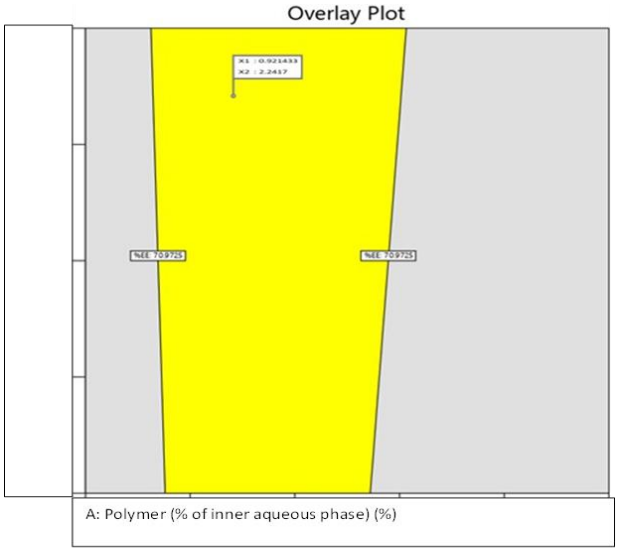

Fig. 4(C): Overlay plot for design space

Check point batch analysis

Predicted and observed batch of optimized batch

The predicted and measured values of the optimized batch of the formulation in terms of percentage of the entrapment efficiency (%EE), ex-vivo drug permeation (%CDR), and globule size. The recommended values of the percent of EE (82.89%), percent of CDR (94.07%), and globule size (11.23 nm) are within close agreement with the respective experimental values of 83.06, 93.07, and 10.64 nm, respectively. Such low deviation suggests that the applied optimization model (e. g., the response surface methodology or Design-Expert) is sound and capable of predicting the performance of the formulation. The high correlation between the predicted and actual data justifies the optimization process and justifies that the variables of the formulation and the processing conditions used can always generate the desired properties in the optimized batch.

To verify the optimization process, it conducted a checkpoint batch analysis. This analysis provides expected response values with the best variable values. It produced these checkpoint batches, along with their projected response values. The table clearly shows that the actual values and the expected values were similar. Since there was no noticeable difference between the experimental value and the observed value, it can conclude that there is no significant difference. Therefore, the central composite design was successfully used.

Table 2: The optimized batch formula and process parameter as per DOE

Ingredients	Quantity
Internal aqueous phase	7.0 ml
Span 80	0.3 ml (74.76% and 25.24%)
Tween 80	
Polymer for internal phase	1.23 %
Olive Oil	3.0 ml
RPM for primary emulsification	10000
Time required for primary emulsification	5 min
External aqueous phase	10.0 ml
Tween 80	2.64
Polymer for outer phase	1%
RPM for secondary emulsification	1500
Time required for secondary emulsification	10 min

Table 3: The results of optimized and stabilized batch

	Predicted	Observed
Globule size (µm)	12.14	10.579
Creaming (cm)	0.5	0.3
Zeta potential (mV)	-21	-35.8
Viscosity (cps)	106	116
%EE	84.69	83.11
Ex-vivo permeation (%)	93.32	91.12
In vitro diffusion (%)	96.34	95.32

Zeta potential of optimized batch

The report of zeta potential is shown in fig. 5 (A) Zeta potential

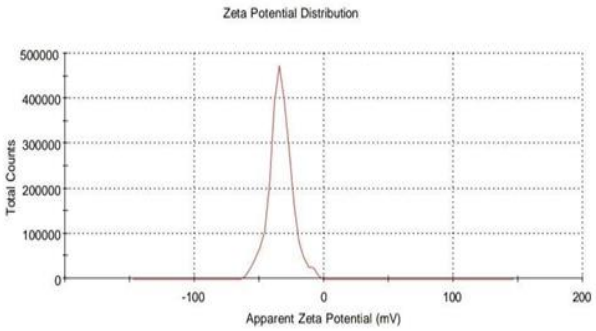

Fig. 5(A): Zeta potential of optimized batch

Organoleptic characteristics (Microscopic images of STS Multiple emulsion)

The drug (Sumatriptan Succinate) powder was examined for its organoleptic properties like colour and odour. And it was observed that Sumatriptan Succinate was white crystalline powder solid which is in shown in fig. 5(B) and (C) Microscopic images of STS multiple emulsion.

Globule size analysis of optimized batch

Globule sizes of the multiple emulsions stored under different conditions are shown in Fig.5 (D) Globule Size Analysis of Optimized Batch and photographs. You can determine the globule sizes of emulsion systems using a light microscope, laser diffraction, an electron microscope, or a Coulter counter. In this study, we used a UNILAB® microscope, model GE-40, from India. Changes in globule sizes indicate instability. Multiple droplets may merge with other oil drops. Internal aqueous droplets may be expelled one at a time, or more than one drop may be released. Internal globules may merge before being expelled, leading to the shrinkage of the internal droplets. Additionally, water may move from the external phase to the internal aqueous phase, causing the internal droplets to swell and eventually rupture completely.

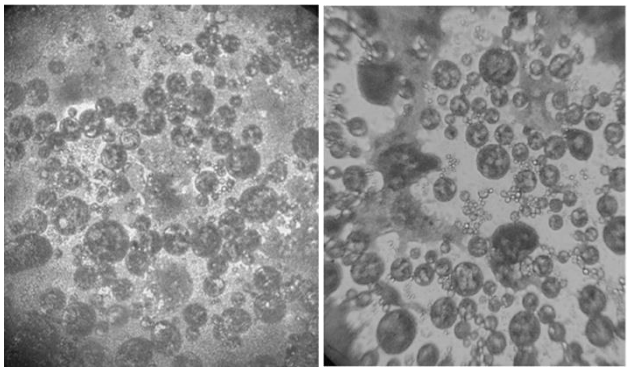

Fig. 5(B and C): Microscopic images of STS multiple emulsion

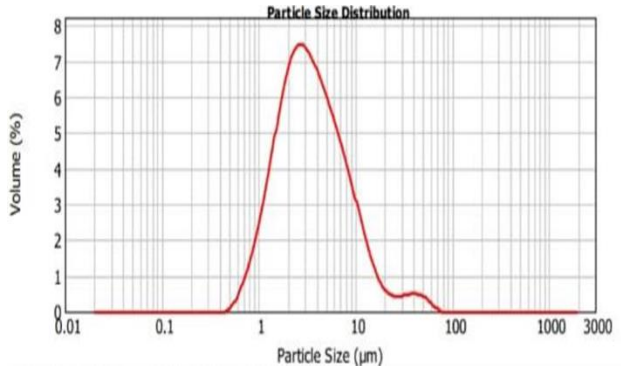

Fig. 5(D): Globule size analysis of optimized batch

***In vitro* drug release study of the optimized batch**

The release profiles of Sumatriptan Succinate from different formulations were examined using dialysis bags and goat intestine. This difference can be attributed to the larger globule size and the thicker interfacial film barrier, which leads to a smaller surface area and a thicker diffusion layer. In a w/o/w ME, the drug in the inner phase must pass through two phases before it can enter the sink solution. The release rate from the internal aqueous phase to the bulk will depend on the properties of the hydrophobic barrier, its thickness, and any interactions between the emulsifier and drug molecules. In both cases, through the dialysis bag and goat intestine, all formulations showed a quick initial release of 10 to 20 percent in the first hour, followed by a slower release of 25 to 60 percent from the second to the eighth hour. The initial phase of the release profile likely resulted from the acyclovir in the outer aqueous phase.

In vitro drug permeation study comparison, which is shown in fig. 6 (A) *in vitro* drug permeation profile

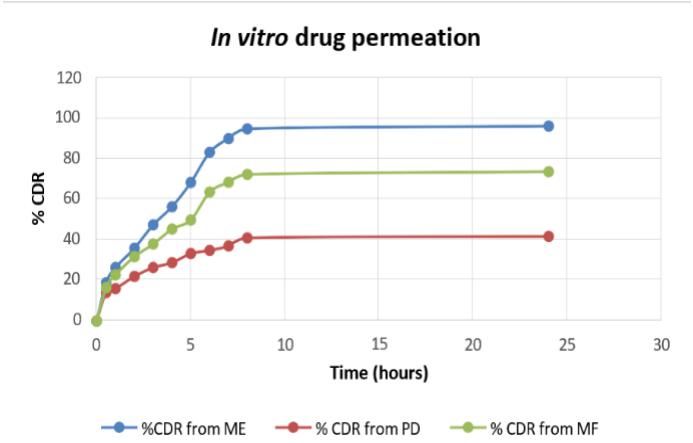

Fig. 6(A): *In vitro* drug permeation profile

***Ex-vivo* permeation study**

Ex vivo drug permeation study comparison, which is shown in fig. 6(B) Ex vivo drug permeation profile

*Sublingual tablet was triturate to powder and turn suspension.

***In vivo* absorption (permeation) study**

Flux of optimized batch at different time points

The optimized formulation shows a high initial flux (257.14 $\mu g/cm^2 \cdot h$ at 0.5 h) and permeability, indicating a burst release and rapid absorption typical of nanocarriers.

From 1–4 h, both flux and permeability decrease, suggesting a transition to controlled release as the readily available drug is depleted.

Between 4–8 h, flux stabilizes, indicating a sustained release phase with steady drug permeation.

At 24 h, flux significantly decreases, showing minimal remaining drug for absorption.

Overall, the formulation provides rapid onset followed by sustained drug release, supporting prolonged therapeutic action.

Plasma Concentration (nanogram) obtained at each time points after oral administration of STS Multiple emulsion formulation in Goat shown in fig. 6 (C) Plasma Drug Conc. Vs Time Comparison

After oral administration, the plain drug solution shows rapid absorption with peak concentration at 3 h (250.15 ng), followed by a sharp decline, indicating fast elimination and short duration of action.

The marketed formulation exhibits slower absorption but maintains higher plasma levels over time, suggesting prolonged drug retention.

The STS multiple emulsion shows the highest plasma concentrations at all time points, with a peak at 6 h (417.12 ng), indicating enhanced absorption, controlled release, and prolonged systemic retention.

Overall, the STS multiple emulsion significantly improves oral bioavailability and duration of action, potentially reducing dosing frequency.

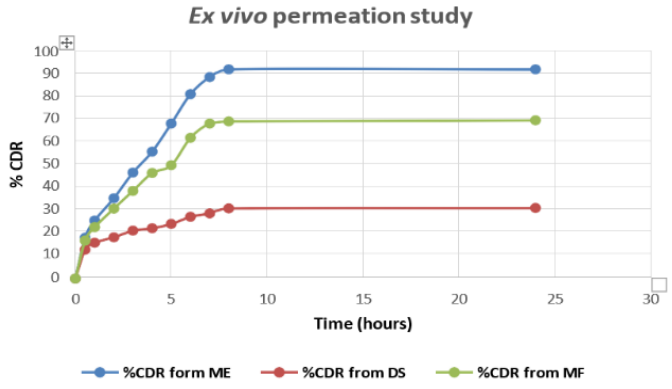

Fig. 6(B): Ex vivo drug permeation profile

Table 4: *In vitro* drug and ex vivo drug permeation study comparison

Time (Hours)	*In vitro*			*Ex-Vivo*		
	%CDR from plain drug solution	% CDR from *marketed formulation	% CDR from multiple emulsion	% CDR from plain drug solution	% CDR from marketed formulation	% CDR from multiple emulsion
0.5	14.17	16.57	19.03	12.81	16.77	18.00
1	16.09	22.93	26.56	15.88	22.72	25.67
2	22.11	31.89	36.06	18.28	30.93	35.52
3	26.49	38.12	47.69	21.15	38.80	46.87
4	28.95	45.57	56.65	22.25	46.67	56.11
5	33.40	50.09	68.97	24.16	50.09	68.56
6	35.04	63.91	83.59	27.31	62.26	81.54
7	37.16	68.76	90.43	28.88	68.56	89.07
8	41.19	72.65	95.22	31.00	67.86	92.49
24	41.95	74.02	96.59	31.21	68.54	93.17

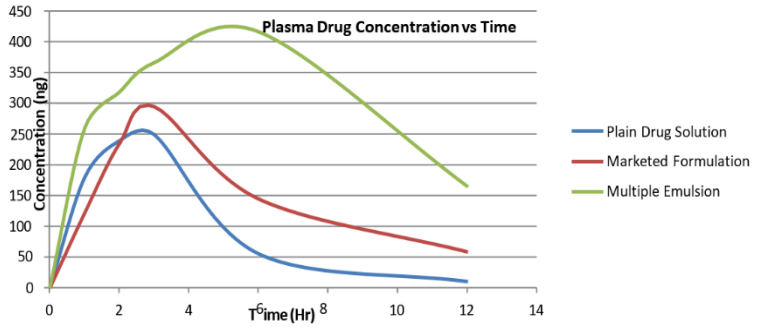

Fig. 6(B): Plasma drug conc. vs time comparison

Table 5: Pharmacokinetic parameters of plain drug solution and different STS formulations

Pharmacokinetic parameters	Plain drug solution	Marketed formulation	STS multiple emulsion
C_{max} (ng/ml)	250.15	295.02	417.12
T_{max} (h)	3	3	6
AUC (ng/ml/h)	101580.15	119864.33	169435.52
$T_{1/2}$ (hr)	2.32	3.41	6.98
Relative bioavailability	1	1.17	1.7

Stability studies at room temperature and in stability chamber

Globule size

The globule size was found to be around 10-13 µm, which indicates that product is showing consistent performance similar to initial reading and there is no change in product performance table 5.

% Entrapment efficiency

The % entrapment efficiency was found to be around 81-84 %, which indicates that product is showing consistent performance similar to initial reading and there is no change in product performance table 5.

Stability data for % entrapment efficiency, which is shown in fig. 7(B) Stability data of %EE

Table 6: Stability data of globule size and % entrapment efficiency, which is shown in fig. 7 (A) Stability data of globule size

Time	Globule size (µm)		% Entrapment efficiency	
	Initial	One month	Initial	One month
Room temperature	10.579	10.693	84.69	82.52
Accelerated temperature	12.898	13.589	83.01	81.78

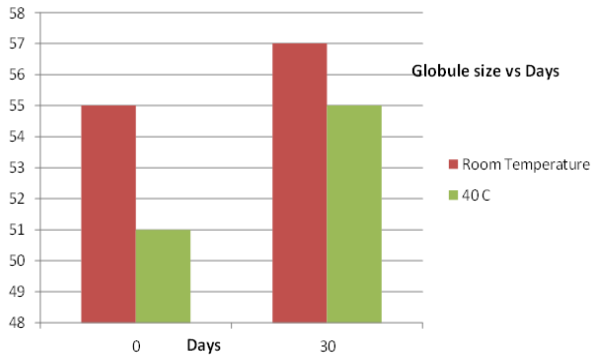

Fig. 7(A): Stability data of globule size

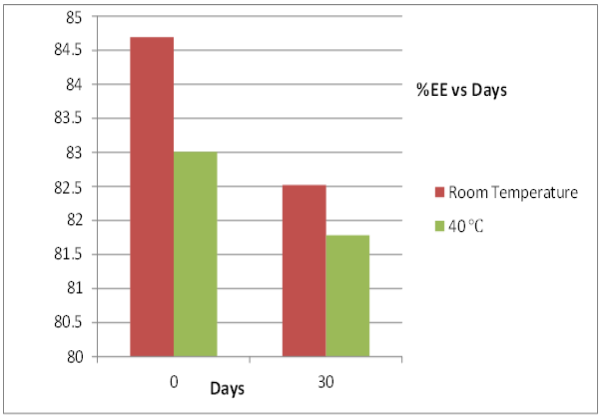

Fig. 7(B): Stability data of %EE

CONCLUSION

W/O/W multiple emulsions were successfully created for Sumatriptan Succinate using a two-step emulsification method with non-ionic surfactants. The optimized formulation showed high entrapment efficiency of about 83%, sustained drug release of up to 96% in 24 h, and improved bioavailability with a 1.7-fold increase. *In vitro* and ex-vivo studies confirmed controlled release that followed zero-order and Peppas kinetics. The formulation remained stable under various conditions, making multiple emulsions a promising system for improving the delivery and effectiveness of BCS Class III drugs like Sumatriptan Succinate.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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