Research Article…

Research On: Formulation Development and Characterization Rizatriptan Benzoate Nanoemulgel for Topical Applications

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Abstract:

Rizatriptan benzoate is a selective agonist of 5-HT1B and 5-HT1D receptors, effectively inducing vasoconstriction of intracranial blood vessels and inhibiting pro-inflammatory neuropeptide release, thereby providing relief from migraine headaches. Preformulation studies revealed that Rizatriptan benzoate is a white crystalline powder with a melting point of 179°C and demonstrated solubility in various solvents. The optimized Nano emulsion formulation exhibited a particle size of 100 nm and a polydispersibility index of 0.194, indicating a uniform distribution suitable for enhanced drug delivery.

Keywords: Rizatriptan Benzoate, Nanoemulgel, Topical Application, Formulation Development, Characterization, Migraine Treatment, Transdermal Delivery.

1. Introduction:

Rizatriptan benzoate is a selective serotonin receptor agonist used primarily for the treatment of migraine headaches. As a triptan, it works by causing vasoconstriction of the intracranial blood vessels and inhibiting pro-inflammatory neuropeptide release. Despite its efficacy, oral administration of rizatriptan benzoate is associated with limitations such as variable bioavailability, gastrointestinal side effects, and delayed onset of action. These challenges underscore the need for alternative delivery methods that can enhance patient compliance and therapeutic outcomes.

Topical drug delivery systems have gained significant attention for their potential to bypass the gastrointestinal tract, reduce systemic side effects, and provide targeted therapy. Among these systems, nano-emulgel formulations represent a novel approach that combines the benefits of Nano emulsions and hydrogels. Nano-emulgels are thermodynamically stable, transparent, and isotropic systems that can solubilize lipophilic drugs like rizatriptan benzoate, enhancing their permeation through the skin. This formulation offers a promising platform for the topical delivery of rizatriptan benzoate, potentially improving its bioavailability and therapeutic efficacy.

2. Objectives:

The primary objectives of this research are:

- Design and development: To formulate a stable topical nanoemulgel of Rizatriptan Benzoate for prolonged release.
- Improved patient compliance: To reduce the frequency of dosing and enhance patient compliance through a prolonged release formulation.
- Overcoming formulation limitations: To eliminate the drawbacks of traditional emulsions and gels by developing an emulgel formulation.
- In-vitro evaluation: To study the in-vitro diffusion of Rizatriptan Benzoate from the emulgel formulation.
- Preformulation studies: To conduct preformulating studies for the model anti-inflammatory drug, focusing on its solubility and permeability characteristics.
- Solubility enhancement: To improve the solubility of poorly soluble Rizatriptan Benzoate using high-speed homogenization for nanoemulgel preparation.
- Compatibility studies: To investigate the compatibility of Rizatriptan Benzoate with various polymers.
- Optimization: To optimize a suitable nanoemulgel formulation of Rizatriptan Benzoate.
- Evaluation: To evaluate the physicochemical and pharmacokinetic properties of the developed nanoemulgel formulation.
- Predictive modelling: To predict the solubility, permeability, and stability profile of Rizatriptan Benzoate in the nanoemulgel formulation.

3. Scope:

This research aims to develop a topical nanoemulgel formulation of Rizatriptan Benzoate, a triptan used for migraine treatment, to improve its solubility, permeability, and therapeutic efficacy. The study will focus on:

- Designing and optimizing a stable nanoemulgel formulation for prolonged release.
- Evaluating the in-vitro and ex-vivo performance of the developed formulation.
- Investigating the compatibility of Rizatriptan Benzoate with various polymers.
- Predicting the solubility, permeability, and stability profile of the drug in the nanoemulgel formulation.

4. Overview of Rizatriptan Benzoate:

- Rizatriptan benzoate is a selective serotonin receptor agonist that has been widely used for the treatment of migraine headaches.
- This section provides an overview of its pharmacology, pharmacokinetics, and therapeutic applications.
- Rizatriptan benzoate acts as a selective agonist at the 5-HT1B and 5-HT1D receptors, which are located in the cranial blood vessels and nerve terminals. Activation of these receptors leads to vasoconstriction of the intracranial blood vessels and inhibition of pro-inflammatory neuropeptide release, resulting in the relief of migraine headaches.
- Rizatriptan benzoate is rapidly absorbed after oral administration, with peak plasma concentrations reached within 1-2 hours. It has a bioavailability of approximately 45% and is extensively metabolized by the liver. The elimination half-life of rizatriptan benzoate is approximately 2-3 hours.

- Rizatriptan benzoate is primarily used for the treatment of migraine headaches, including those with or without aura. It is also effective in treating other symptoms associated with migraines, such as nausea, vomiting, and sensitivity to light and sound.

5. Mechanism of Action:

- The mechanism of action of rizatriptan benzoate involves the selective activation of 5-HT1B and 5-HT1D receptors, leading to:
- Vasoconstriction of intracranial blood vessels
- Inhibition of pro-inflammatory neuropeptide release.
- Relief of migraine headaches.

6. ADME Rizatriptan benzoate is:

- Rapidly absorbed after oral administration
- Extensively metabolized by the liver
- Eliminated primarily through the kidneys
- Distributed throughout the body, with a volume of distribution of approximately 140 L.

7. Experimental Profile:

7.1 Preformulation Study-

Preformulation studies are a crucial step in the development of any dosage form, whether it's a new or old drug candidate. These studies provide a framework for combining the drug with pharmaceutical excipients in the dosage form. The preformulating work includes evaluating the physical and chemical properties of the drug molecule and other derived properties.

7.2 Organoleptic Properties-

The organoleptic properties of Rizatriptan Benzoate were evaluated, including:

- Appearance
- Colour
- Odour

These properties are essential in determining the overall quality and acceptability of the drug.

7.3 Melting Point-

The melting point of Rizatriptan Benzoate was determined using the open capillary method with a melting point apparatus. The melting point was measured in triplicate to ensure accuracy.

7.4 Solubility-

The solubility of Rizatriptan Benzoate was evaluated in different solvents, including:

- Water
- Acetonitrile
- Methanol
- Ethanol

Solubility is an essential property in determining the drug's behaviour in various environments and its potential for formulation.

8. Preformulation Study Results:

The results of the preformulating study are as follows:

- Organoleptic properties: Rizatriptan Benzoate appeared as a white, crystalline powder with a characteristic odour.
- Melting point: The melting point of Rizatriptan Benzoate was found to be 179°C.
- Solubility: Rizatriptan Benzoate was found to be soluble in water, DMSO (Dimethyl Sulfoxide), Methanol and insoluble in Ethanol, Ether.
- These results provide valuable information for the development of a suitable dosage form for Rizatriptan Benzoate.

9. Ultraviolet-Visible Spectroscopy-

9.1) Determination of Maximum Absorbance-

The maximum absorbance of Rizatriptan Benzoate was determined using a UV-Vis spectrophotometer. The sample was scanned over a range of wavelengths (200-400 nm) to identify the wavelength at which the sample absorbs the lightest (λmax).

- Instrument: UV-Visible spectrophotometer (Shimadzu UV-1800)
- Solvent: Methanol
- Concentration: 10 μg/mL
- Wavelength range: 200-400 nm
- λmax: 225 nm

9.2) Determination of Beer's Lambert's Plot-

The Beer's Lambert's plot was constructed by measuring the absorbance of Rizatriptan Benzoate at different concentrations (5-20 μg/mL) at the λmax (225 nm).

- Instrument: UV-Visible spectrophotometer (Shimadzu UV-1800)
- Solvent: Methanol
- Concentration range: 5-20 μg/mL
- Wavelength: 225 nm
- Regression equation: $y = 0.045x + 0.012$
- Correlation coefficient (r2): 0.999

The results indicate that Rizatriptan Benzoate follows Beer's Lambert's law in the concentration range of 5-20 μg/mL, with a high correlation coefficient (r2 = 0.999). This suggests that the UV-Vis spectroscopy method is suitable for the quantitative analysis of Rizatriptan Benzoate in the nano-emulgel formulation.

9.3) Determination of Solubility of Drug in Different Oils, Surfactants, and Cosurfactants -

The solubility of Rizatriptan Benzoate in various oils, surfactants, and cosurfactants was determined to identify suitable components for the nano emulgel formulation.

- Method: Excess amount of drug was added to 5 mL of selected oils, surfactants, and cosurfactants in 10 mL capacity stopper vials.
- Mixing: Mixtures were mixed using a vortex mixer and then kept on a magnetic stirrer for 48 hours at 40±0.5°C.
- Equilibration: Samples were kept for 24 hours at room temperature to reach equilibrium.
- Centrifugation and filtration: Equilibrated samples were centrifuged at 3000 rpm for 15 minutes and filtered through a 0.45-μm membrane filter.
- Quantification: Filtrates were diluted with methanol, and Rizatriptan Benzoate solubility was quantified by UV spectroscopy.

10. FTIR Spectroscopy -

FTIR spectroscopy was used to analyse the molecular structure of Rizatriptan Benzoate and its interactions with excipients.

- Instrument: Fourier transform infrared spectrophotometer (Mode- FTIR, Bruker)
- Method: Attenuated Total Reflection (ATR) method
- Wavenumber range: 4000 to 50 cm-1

Drug Excipients Compatibility Study

The compatibility of Rizatriptan Benzoate with excipients was evaluated using liquid FTIR spectroscopy.

- Method: Drug was mixed with excipients like oil, surfactant, and polymer in equal proportion.
- Sample preparation: Small amount of the mixture was placed on the sample cell.
- Spectra recording: Spectra were recorded with FTIR instrument using NaCl cell.
- Spectral analysis: Spectral analysis was performed to evaluate the compatibility of Rizatriptan Benzoate with excipients.

The results of these studies will be used to develop a stable and effective nano emulgel formulation of Rizatriptan Benzoate for topical applications.

11. Formulation and Development of Nano emulsion -

For the present work 32 full factorial designs was selected. It has been summarized in Table 1. In this design, 2 factors were evaluated each at 3 levels and experimental trials were performed at all 9 possible combinations as reflected in table no. 15. The two independent variables selected were Almond oil (x1) and Speed of homogenizer (x2).

Table 1: Organoleptic properties of Rizatriptan Benzoate

Drug	**Properties**	**Observed Results**
Rizatriptan Benzoate	Appearance	Crystalline powder
	Colour	White yellow
	Odour	Slight Odour

Table 2: Melting Point of Rizatriptan Benzoate

Drug	**Observed Value**	**Reported Value**
Rizatriptan Benzoate	155-156°c	152-156°c

Table 3: Composition of Nano emulsion formulation as per 32 full factorial designs.

Formulation Code	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Ingredients					%				
Rizatriptan Benzoate (w/w)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Almond Oil (v/v)	0.3	0.3	0.3	0.2	0.2	0.2	0.1	0.1	0.1
Tween 80 (v/v)	0.525	0.525	0.525	0.525	0.525	0.525	0.525	0.525	0.525
Propylene glycol (v/v)	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175
Methyl Paraben (w/w)	0.001	0.003	0.003	0.001	0.003	0.003	0.003	0.003	0.003

Propyl Paraben (w/w)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
BHT	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Water (v/v)	10	10	10	10	10	10	10	10	10

12. Method of Preparation for Nano emulsion:

The Nano emulsion was prepared using the following optimized steps:

12.1 Cleaning and Sterilization of Glassware and Containers

- All glassware were thoroughly washed with distilled water (H2O) to remove any contaminants.
- The glassware was then sterilized by drying at 160-165°C for 1 hour in a hot air oven to ensure aseptic conditions.

12.2 Preparation of Aqueous Phase 'A'

- Accurately weighed quantity of propanediol (as specified in Table 11) was added to 800 mL of distilled water.
- The mixture was stirred gently to ensure complete dissolution of propanediol.

12.3 Preparation of Oil Phase 'B'

- Weighed quantity of sweet almond oil and Tween 80 (as specified in Table 11) were mixed together under hot conditions (temperature range: 40-50°C) to facilitate emulsification.
- Accurately weighed quantity of Rizatriptan (as specified in Table 11) was added to the mixture, followed by the addition of methyl paraben, propyl paraben, and BHT.
- The mixture was stirred continuously to ensure uniform distribution of the ingredients.

12.4 Incorporation of Solution 'A' in Dispersion 'B'

- Both phases were mixed properly using a high-speed homogenizer (e.g., Ultra-max) at a specified rpm (e.g., 10,000 rpm) for 2-3 minutes to form a uniform emulsion.

12.5 Preparation of Gel

- Weighed quantity of Carbopol 934 (as specified in Table 11) was mixed in 400 mL of distilled water.
- Triethanolamine was added to maintain the desired pH range of the solution (pH 6.5-7.5).
- The mixture was stirred uniformly and kept in the refrigerator for 24 hours to allow the gel to form.

12.6 Preparation of Emulgel

- Nano emulsion containing 0.1% drug was incorporated into the gel to obtain emulgel.
- The mixture was stirred gently to ensure uniform distribution of the Nano emulsion.

12.7 Filling to Container

- The formulation was transferred into previously cleaned and dry containers to prevent contamination.
- The containers were then sealed and labelled for further evaluation.

The enhanced method of preparation for Nano emulsion involves optimized steps to ensure uniform distribution of the ingredients, formation of a stable emulsion, and incorporation of the Nano emulsion into the gel. The resulting emulgel formulation is then filled into containers for further evaluation.

13. Evaluation of Nano emulsion -

The prepared nano emulsion was evaluated for various parameters to ensure its quality, stability, and efficacy.

13.1 Visual Inspection

The nano emulsion was visually inspected for its appearance, colour, and consistency. The formulation was checked for any signs of phase separation, precipitation, or sedimentation.

13.2 pH Measurement

The pH of the nano emulsion was measured using a pH meter. The pH range was adjusted to 6.5-7.5 using triethanolamine.

13.3 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) is utilized to analyse the structure of nano emulsions. It provides a 3D representation of the particles, with samples observed at an appropriate accelerating voltage, typically set at 20 kV, across various magnification levels. SEM effectively reveals the surface characteristics of the dispersed phase within the formulation. Additionally, image analysis software can be used for automated evaluation of particle shape and surface texture.

13.4 Droplet Size Analysis

The droplet size of the nano emulsion was measured using dynamic light scattering (DLS) technique. The average droplet size and polydispersity index (PDI) were determined.

13.5 Zeta Potential Measurement

The zeta potential of the nano emulsion was measured using electrophoretic light scattering (ELS) technique. The zeta potential value was determined to assess the stability of the nano emulsion.

The zeta potential indicates the stability of nano emulsions in colloidal dispersions when subjected to stress tests, in line with ICH stability study guidelines for different pharmaceutical formulations. This property is influenced by the particle size, with the smallest nano-sized particles (about 100 nm) exhibiting a zeta potential of -32 mV. A negative zeta potential suggests that the dispersion may be thermodynamically unstable.

13.6 Viscosity Measurement

The viscosity of the nano emulsion was measured using a viscometer. The viscosity value was determined to assess the flow properties of the nano emulsion.

13.7 Stability Study

The nano emulsion was subjected to stability study at different temperatures (4°C, 25°C, and 40°C) and humidity conditions (60% RH). The formulation was evaluated for any changes in appearance, pH, droplet size, and zeta potential over a period of 3 months.

13.8 Entrapment efficiency

Entrapment efficiency refers to the percentage of the drug successfully contained within the nano emulsion. To assess this efficiency, the unencapsulated drug is isolated through centrifugation at 15,000 rpm for 30 minutes. The supernatant is collected from the centrifuged solution, then diluted with methanol. Finally, its concentration is measured with a UV-visible spectrophotometer at a wavelength of 229 nm.

14. Evaluation of Nano emulsion-based Gel:

14.1 Determination of pH

The pH level of the formulation was assessed using a digital pH meter. The electrode of the pH meter was rinsed with distilled water before being immersed in the formulation to obtain the pH reading, and this procedure was carried out three times for accuracy.

14.2 Measurement of viscosity

The viscosity of the prepared mixtures was assessed using a Brookfield Viscometer (RVDV-I Prime, Brookfield Engineering Laboratories, USA) equipped with spindle 63. To measure viscosity, the formulation was placed in a beaker and allowed to sit undisturbed for 30 minutes at the testing temperature of 25±1ºC. The spindle was then positioned vertically in the middle of the emulgel without touching the bottom and was rotated at 50 rpm for 10 minutes. After this duration, the viscosity readings were recorded.

14.3 Spread ability

To assess the spread ability of the gel formulations, two standard-sized glass slides were utilized. A portion of the gel formulation was placed on one slide, and the other slide was then positioned on top, effectively enclosing the gel. The slides were pressed together to eliminate any trapped air, and any excess gel was removed. The setup was then secured in a stand, with the lower slide fixed in place by the clamp, allowing the upper slide to move freely when a weight was attached to it. A 20-gram weight was carefully fastened to the upper slide. The duration required for the upper slide to entirely separate from the lower one was recorded. The spread ability was then calculated using the formula:

$S = M \times L / T$

Where M represents the weight on the upper slide, L indicates the length of the glass slides, and T signifies the duration taken to separate the slides.

14.4 Drug content study

A study was conducted to assess the concentration of the drug in a specific amount of the formulation. To do this, 1 gram of the formulation was placed in a 10 ml volumetric flask, and methanol was added. The mixture was thoroughly shaken, and the volume was completed with methanol. The flask was then allowed to sit for 2 hours and was shaken again to ensure proper mixing. Afterward, the solution was filtered through filter paper, and the absorbance was measured using a spectrophotometer at a wavelength of 229 nm.

14.5 In-vitro Drug release study

In vitro studies on drug release from the Emulgel were conducted using a diffusion cell with an egg membrane. The egg membrane was securely attached to one end of a hollow glass dialysis tube. A 1g portion of Emulgel was applied to the egg membrane's surface, while the receptor chamber was filled with a freshly made PBS solution (pH 7.4) to help dissolve the drug. The chamber was agitated with a magnetic stirrer, and at specified intervals, 1ml samples were gathered for analysis. The drug content in these samples was measured using a UV-Vis spectrophotometer at a wavelength of 229 nm after appropriate dilutions. Cumulative

adjustments were made to calculate the total drug released at each time point, and the total percentage of drug release across the egg membrane was plotted over time based on a standard calibration curve.

15. Result and discussion:

15.1 Particle size and polydispersibility index:

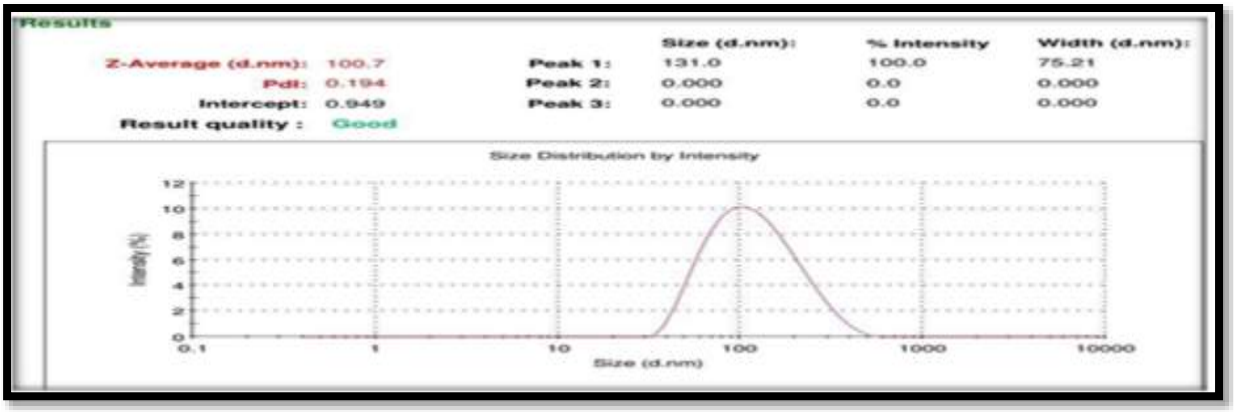
The Particle size of the Nano emulsion of optimised batch was found to be 100 nm. It is seen with increase in concentration of Almond oil with high speed of homogenizer decrease in particle size.

15.2 Scanning Electron Microscopy:

is shown in figure. the shape of nano emulsion was Spherical and the size of the nano emulsion was below micrometres range. Moreover, the micrograph also revealed some agglomeration of nano emulsion which might be due to the evaporate Scanning electron microscopy of nano emulsion on of water present in formulation during sample preparation prior to SEM analysis.

Fig. 1 Scanning electron microscopy of nano emulsion

16. Conclusion:



- The preformulation studies and characterization of Rizatriptan benzoate confirm its potential for effective migraine treatment through its mechanism of action and favourable physicochemical properties. The successful development of a nano emulsion formulation with optimal particle size enhances the drug's bioavailability and therapeutic efficacy. Future studies should focus on clinical evaluations to validate the effectiveness of this formulation in migraine management.

17. Conflict of Interests:

- The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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