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Development and Evaluation of A Nose-To-Brain Drug-Loaded Microemulsion

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Abstract: The aim of this work was to make an Agomelatine microemulsion that could be given through the nose. Because it is broken down quickly in the first pass, the drug agomelatine has a low absolute absorption of only 5%. The solubility study led to the choice of Tween 80 Capmul MCM as the oil, propylene glycol as the surfactant, and tween 80 Capmul MCM as the cosurfactant. To make microemulsions, water titration was used. The 2:1% W/W mix was used to make the recipe. The microemulsions that were made were tested for visual clarity, viscosity, globule size, and an *in vitro* diffusion study to characterize them. The best mixture was found by looking at the percentage of oil and Smix as independent variables and the viscosity, globule size, and percentage of drug spread as dependent variables. To learn more about the improved batch, tests were done on its phase separation, globule size, optical clarity, pH, viscosity, zeta potential, drug content, *in vitro* diffusion, and *ex vivo* diffusion.

Keywords: Microemulsion, Intranasal delivery, Nose to brain, Depression, Agomelatine

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Introduction

The World Health Organisation (WHO) says that in ten years, sadness will be the most common illness

in the world, hitting one in twelve men and five women. It is now easier to get help for sadness

thanks to better tools and medicines (Said *et al.*, 2017). Most people respond well to therapy and quickly get back to the level of usefulness they had before. Depression affects people all over the world and is responsible for a large part of the disease load around the world (Halde *et al.*, 2019). Depressive illnesses often start early and come back, making it much harder for a person to do things. Because of these things, sadness causes more years of lost work than any other illness in the world. Around the world, more and more people need help with sadness and other mental health problems (Tomar *et al.*, 2023). People who are depressed often have low energy, trouble focusing, feelings of guilt or low self-worth, a lack of interest or pleasure, trouble sleeping or eating, and a bad mood. After the system was worked on to get rid of the large (or macro) emulsion, a co-surfactant, which is a second surface-active ingredient, was added. It cleared itself when all four parts were in the right place. Most of Schulman's written work was made up of four separate categories (Yasir *et al.*, 2018). A long time ago, Schulman wrote a lot about monolayers and used what he knew to explain how microemulsions form (Keservani *et al.*, 2016; Keservani and Gautam, 2022). When the right surfactant and co-surfactant are used, they make a mixed film at the oil/water interface. This film raises the interfacial pressure above the positive interfacial tension that was there before (Topal *et al.*, 2020).

Two liquids that do n'o mix are mixed with the right surfactant or mixture of detergents to make a single phase in microemulsions (Ahire *et al.*, 2020). These are thermodynamically stable isotropic systems. Short- to medium-chain alcohols are often thought of as cosurfactants in the microemulsion system. Because surfactant and cosurfactant are in the solution, the interfacial tension is very low (Patil *et al.*, 2023).

Because microemulsions have smaller globules, they have more surface area, which makes it easier for medicines to be absorbed (Yasir *et al.*, 2022). Focusing on making the drug stay longer in the nasal tissue while making a

nasal formulation for desired performance is suggested to ensure effective medicine absorption. It is thought that adding mucoadhesive polymers to nose formulations will make the medicine stay in the body longer and help it work better (Surana *et al.*, 2022; Komu *et al.*, 2023; Jaiswal *et al.*, 2023; Nataraja *et al.*, 2023).

Materials and Methods

Preparation of Microemulsion:

The phase diagram was used to pick the best S_{mix} ratio. To make the drug-loaded microemulsions, the drug was first soaked in the oil- S_{mix} mixture. Water was then added while the magnetic mixer was set to 150 rpm for 10 min. Agomelatine was mixed with the right amount of oil, and then surfactant and co-surfactant were added in different amounts. Lastly, the right amount of water was added drop by drop while a magnetic mixer kept stirring the mixture. The liquids were mixed on their own to make microemulsions with Agomelatine (Arora *et al.*, 2022). This made sure that all of the microemulsions stayed at the right temperature. We used a certain part of the pseudo-ternary phase diagram to make nine recipes with different amounts of oil, surfactant, and co-surfactant. Following the steps, we talked about above, we made each meal and then tried and looked over them (Nguyen and Maeng, 2022).

Evaluation of microemulsion:

The following qualities of the Microemulsion were assessed:

Optical Transparency:

By examining the sample in a clear, transparent container against light reflection into the eyes, the formulation's optical transparency was ascertained (Singh *et al.*, 2017).

Viscosity Measurement:

A rotating viscometer made by Brookfield was used to test the viscosities of the microemulsions (Kopparam *et al.*, 2020).

Phase Separation:

The microemulsion system was centrifuged for 2 h at 3000 rpm in order to look for signs of phase separation (Bhandari *et al.*, 2022).

Determination of pH:

A 10% formulation dispersion was made in distilled water, and the pH was measured with a pH metre that had previously been calibrated using standard buffers with pH values of 4 and 7 (Aher *et al.*, 2023).

Measurement of Globule Size:

Zetasizer was used to calculate the microemulsions' average globule size. At 25°, measurements were taken at a 90° angle. To make sure the light scattering intensity was within the sensitivity range of the sensor, the microemulsion was diluted with double distilled water. Every measurement was done at a temperature of 25° (Espinoza *et al.*, 2019). The same tool was used to calculate the formulation's polydispersity index. The polydispersity index (P.I) showed the breadth of the size distribution (Tekade *et al.*, 2023).

Measurement of zeta potential:

To confirm that the microemulsion's stability was caused by charge interaction, the zeta potential was calculated. Zetasizer was used to measure the zeta potential. At 25°, the measurement was made (Sonawane *et al.*, 2023).

Drug Content:

A specific amount of the formulation was collected and diluted with methanol in a 10 ml volumetric flask. After the resulting solution was sonicated for 3 min at room temperature, its absorbance at 230 nm was measured in comparison to a blank (Butani, 2018).

Ex Vivo Permeation Study:

With a mucosa thickness (height) of 0.2 mm and a diameter of 10 mm, Franz diffusion cells were used for the *ex vivo* drug diffusion investigation. The donor compartment was filled with 0.5 ml of diffusion medium, 0.5 ml of AGM solution, and 0.5 mL of AGM microemulsion. A Teflon-coated magnetic stirrer was used to agitate the 20 ml of

medium within the recipient compartment. At prearranged intervals, samples from the receptor compartment were taken out and subjected to UV analysis (Pardeshi *et al.*, 2024). Every sample withdrawn was swapped out for a diffusion medium of the same volume. The drug concentration in the receiver chamber (mg/ml) across the goat nasal membrane was measured at each sample point throughout the course of 4 h for each research (Keservani *et al.*, 2017).

Nasal toxicity studies:

Goat nasal mucosa that had just been removed, except the septum portion, was obtained from the abattoir and placed in PBS pH 6.4. The membrane spent 15 min in PBS at a pH of 6.4. Pieces of uniformly thick goat nasal mucosa were affixed to Franz diffusion cells. A pH 6.4 phosphate buffer (0.5 ml) was applied to one mucosa, isopropyl alcohol (0.5 ml) was applied to another, and a microemulsion (blank and drug loaded) was applied to the remaining mucosa for a duration of 1 h (Keservani *et al.*, 2023). The mucosa was transported to the pathology laboratory in 10% formalin for the fabrication of pathological slides after being cleaned with PBS pH 4.4 for 1 h. As a positive and negative control, the goat nasal mucosa that had been treated with pH 6.4 phosphate buffer and isopropyl alcohol, respectively, was used. Under an optical microscope, the produced pathology slides were examined for any indications of toxicity (Behera *et al.*, 2010).

Results and Discussion

Preparation and optimization of microemulsion:

The Agomelatine microemulsion percentage oil and % were subjected to a 32 complete factorial. Globule size, viscosity, and the percentage of medication dispersed were taken into consideration as responses, while Smix was chosen as an independent variable. Table 1 listed the composition of several formulations along with the globular size, viscosity, and transmittance percentage.

Table 1: The microemulsion's composition and characterization parameters

Formulation code	Percentage oil	Percentage S_{mix}	Percentage water	Globule size (nm)	Percentage CDR	Viscosity (cp)
F1	3.0	40	63	92.19±1.21	60.41±1.26	102.73±1.11
F2	3.0	50	53	79.11± 1.22	70.91±1.39	231.62±2.23
F3	3.0	60	43	66.22±0.61	76.39±2.51	333.65±2.59
F4	6.0	30	62	95.91± 1.37	46.73±1.15	164.39±1.13
F5	6.0	40	52	85.24±0.82	52.59±0.65	298.33±1.78

The globule size ranged from 66.22 nm to 95.91 nm for different factor level combinations, (Table 1). To ascertain the optimal particle size-producing factor values, mathematical correlations between the independent and dependent variables were created.

The mean globule size of the microemulsion rose as the oil content increased (Table 1). Additionally, globule size was negatively impacted by % S_{mix} . Hence, the globule size reduces as the S_{mix} concentration rises. This could be because surfactants reduce surface tension. In order to assist the dispersion process during the creation of a microemulsion, surfactants have the role of lowering the interfacial tension. The ANOVA result showed that each concentration of oil and S_{mix} had a significant impact on its own.

When administering the microemulsion, especially via the nasal route, viscosity is still another crucial factor. Extremely high viscosity has a direct impact on patient compliance and can make breathing difficult. Nasal spray or nasal drops also require a less viscous formulation when considering composition. Since these factors have no discernible impact on the microemulsion's viscosity, they may be eliminated from the entire polynomial equation.

Selection of optimized formulation and evaluation:

We selected F1 as the best formulation using our minimal viscosity and minimum globule size criteria. The composition of the F1 microemulsion comprised mix (4%), oil (3%), and water (63%).

The optimal microemulsion that was produced was characterized using the following parameters:

Characterization of optimized microemulsion formulation:

Drug content:

The proportion of drug content in Agomelatine o/w microemulsion was assessed. It was discovered that the drug content percentage for the microemulsion was 99.11±1.12%.

Zeta potential:

The microemulsion's zeta potential of 89.06 mV indicates the system's physical stability. The electrical potential difference between each particle's surface and the bulk of the suspending liquid is measured in millivolts (mV) due to the surface charge of the tiny particles. We refer to this variation as zeta potential.

Viscosity:

A basic Newtonian flow was demonstrated by the microemulsion devices. Agomelatine did not alter the flow characteristics. The microemulsion's viscosity was determined to be 5.96 Cp. The concentration and component ratio of water, oil, and surfactant can influence a microemulsion's viscosity.

pH measurement:

Using a digital pH meter, the pH of the microemulsion was estimated to be 4.98. The formulation's pH and the nasal pH were almost identical. Therefore, the compositions would not irritate skin when applied.

Table 2: Characterization of optimized formulation

Formulation	Drug Content	Zeta Potential	Globular size	Viscosity	pH (mV)
F1	99.11±1.12%	89.06±1.45 nm	102±2.61Cp	5.96±0.23	4.98±0.01

Table 3: *In vitro* diffusion of microemulsion of Agomelatine and solution

Time (min)	Root Time (min)	AGT solution (Percentage drug diffused)	AGT microemulsion (Percentage drug diffused)
30	3.93	3.11±0.50	9.90±0.30
60	6.33	9.41±0.62	15.92±0.61
90	8.2	25.13±1.23	35.77±0.43
120	11.10	41.65±0.69	57.13±1.28
150	14.24	46.01±1.69	68.21±0.90
180	16.51	46.90±1.21	79.53±2.19

Globular size:

It was discovered that the optimized formulation's average globule size was 102 Cp. Because of its higher surface area, it suggests that the formulated microemulsion globule size was within the range, indicating a monodispersed stable system that could successfully transport the medication (Table 2).

***In vitro* diffusion:**

Table 3 illustrates the permeation statistics for the Agomelatine solution and Agomelatine microemulsion. Agomelatine microemulsion was reported to have an overall drug diffusion percentage of 79.53%. After 3 h, it was discovered that the microemulsion formulation had more medication distributed over the nasal mucosa than the standard Agomelatine solution.

Nasal ciliotoxicity Evaluation:

To assess any potential harmful effects of the excipients utilized in the formulation on the nasal mucosa, investigations on nasal ciliotoxicity were conducted. Therefore, in order to assess the potentially harmful effects of the excipients employed in the formulation, goat nasal mucosa was treated with blank microemulsion.

Conclusion

In summary, the Agomelatine microemulsion showed great promise for enhancing

bioavailability, increasing absorption rate because of its small globule size, avoiding first pass metabolism, and allowing direct transport into the systemic circulation for a more effective and prolonged course of treatment that is necessary for antidepressant action with increased stability.

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