FORMULATION AND EVALUATION OF MICROEMULSION BASED INTRANASAL DELIVERY OF AGOMELATINE.

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***ABSTRACT*:** The purpose of this study was to develop Agomelatine microemulsion for intranasal delivery. Agomelatine, an antidepressant drug, has absolute bioavailability of only 5% due to high first pass metabolism. Ternary phase diagram gave the microemulsion region and the concentration of oil; Smix and water were selected from ternary phase diagram. Based on solubility study, Capmul MCM, tween 80 and propylene glycol were selected as oil, surfactant and co surfactant respectively. Microemulsions were prepared using water titration method. 2:1% W/W ratio (Tween 80: Propylene glycol) was selected for formulation development. The prepared microemulsions were characterized for optical transparency, viscosity, measurement of globule size and in-vitro diffusion study. Formulation was optimized by considering %oil and %Smix as a independent variables and globule size, viscosity and % drug diffused as a depended variables. The optimized batch was further characterized for optical transparency, viscosity, phase separation, determination of pH, measurement of globule size, measurement of zeta potential, drug content, Ex-vivo diffusion study, stability studies.

Key word: **Depression Agomelatine, Nose to brain**

INTRODUCTION

Depression as estimated by WHO, depression shall become the second biggest illness in terms of morbidity by another decade in the world, already one out of every twelve men and five women have depression. With newer medication, and better facilities, treating depression has become easier, and most people respond very well to treatment, and return to optimum functioning very soon. Depression is a significant contributor to the global burden of disease and affects people in all communities across the world. Today, depression is estimated to affect 350 million people. The World Mental Health Survey conducted in 17 countries found that on average about 1 in 20 people reported having an episode of depression in the previous year. Depressive disorders often start at a young age; they reduce people's functioning and often are recurring. For these reasons, depression is the leading cause of disability worldwide in terms of total years lost due to disability. The demand for curbing depression and other mental health conditions is on the rise globally. Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration. Depression usually starts between the ages of 15 and 30, and is much more common in women. [1].

The word microemulsion was originally proposed by Schulman et al. (1959). They prepared a quaternary solution of water, benzene, hexanol, and k-oleate which was stable, homogenous and slightly opalescent.. Basically coarse (or macro) emulsion was prepared and the system was then titrated to clarify by adding a co-surfactant (second surface active substance). When the combination of the four components was right, the system cleared spontaneously. Most of the work reported by Schulman dealt with four component systems. Hydrocarbons (aliphatic or aromatic), ionic surfactants, co surfactants (generally 4–8 carbon chain aliphatic alcohol) and an aqueous phase. Schulman had previously published extensively in the field of monolayers and applied what he had learnt in that field to explain the formation of microemulsions. The surfactant and co- surfactant, when properly selected, form a mixed film at the oil/water interface, resulting in an interfacial pressure exceeding the initial positive interfacial tension.[2, 3]

Microemulsions are thermodynamically stable isotropic systems in which two immiscible liquids (water and oil) are mixed to form a single phase by means of an appropriate. surfactant or its mixture. The short to medium chain alcohols are generally considered as co surfactants in the microemulsion system. The presence of surfactant and co surfactant in the system makes the interfacial tension very low. Therefore microemulsions form spontaneously, with an average droplet diameter of 10 to 140 nm. Microemulsions offer several advantages like high solubilization of lipophilic drugs, stability, ease of preparation and stabilization of hydrolytically susceptible compounds.

Microemulsions provide a large surface area for better absorption of drugs due to smaller globule size. Various drugs such as Sumatriptan, Zolmitriptan, Cabergoline, Clonazepam, Nimodipine, Tacrine, and Diazepam have been successfully delivered through nasal route in the form of microemulsion and it resulted in improved drug absorption. In order to formulate a nasal formulation with desirable performance, it is advisable to focus on maximizing the residence time in the nasal mucosa and thus ensuring efficient absorption of drug. Use of mucoadhesive polymers in the nasal formulations is expected to increase the residence time and thereby enhance the absorption of the drug. [3-]

MATERIAL AND METHODS

Agomelatine was obtained as a gift sample from Enaltec Labs, Igatpuri, Nashik, India. Tween 80 and propylene glycol were purchased from Research-Lab Fine Chem. Industry – Mumbai.

Construction of pseudo ternary phase diagram

Pseudo-ternary phase diagrams were constructed using water titration method at ambient temperature (25°) to determine the ME regions. Pseudo ternary phase diagram was plotted for each Smix 1:1, 1:2 and 3:1 using the CHEMIX Software (Version 7.00). [13]

Formulation of Microemulsion

Based on the phase diagram, the optimum Smix ratio was selected and the drug loaded microemulsiom were prepared by dissolving the drug in the oil-Smix mixture, and then titrated with water on the magnetic stirrer at 150 RPM for 10 min. Agomelatine was added to the specific amount of oil then surfactant and co-surfactant with varying percentage, and then an appropriate amount of water was added to the mixture drop by drop with constant stirring on magnetic stirrer. Microemulsions containing Agomelatine were obtained spontaneously on stirring the mixtures. All microemulsions were stored at appropriate temperature. Nine formulations containing different concentration of oil, Surfactant/co-surfactant were prepared with the help of selected region area of pseudo ternary phase diagram. Each formulation was prepared according to the procedure explained above and then these formulations were evaluated. [14]

Evaluation of microemulsion

The Microemulsion was evaluated for the following characteristics: [7, 8, 9, 14,]

a. Optical Transparency-

Optical transparency of the formulation was determined by inspecting the sample in clear and transparent container under the presence of light against reflection into the eyes.

b. Viscosity Measurement-

The Viscosities of microemulsions were measured using a Brookfield rotational viscometer (LV2, Brookfield Inc., USA) at 24.9˚ at 10 rpm.

c. Phase Separation

Microemulsion system were subjected to centrifugation (Remi Motor, Mumbai) at 3000 rpm for a period of 2 h and examined for any evidence of phase separation.

d. Determination of pH

A 10% dispersion of formulation was prepared in distilled water and pH was determined by using pH meter which was prior standardized with standard buffers of pH 4 and pH 7.

e. Measurement of Globule Size

The average globule size of the microemulsions was determined by Zetasizer Nano-ZS (Malvern Instruments, UK). Measurements were carried at an angle of 90◦ at 25◦. Microemulsion was diluted with double distilled water to ensure that the light scattering intensity was within the instrument's sensitivity range. All the measurement was carried out at 25˚.The polydispersity index of the formulation was determined by the same instrument. The width of the size distribution was indicated by the polydispersity index (P.I)

f. Measurement of zeta potential-

The zeta potential was determined to verify stability of microemulsion due to charge interaction. Zeta potential was measured by using Zetasizer Nano-ZS (Malvern Instruments, UK). The measurement was performed at 25°.

g. Drug Content-

A definite volume of formulation was taken in a 10 ml volumetric flask and diluted with methanol. The resultant solution was sonicated for 3 min at ambient temperature and the absorbance of the resultant solution was measured at λmax of 230 nm against blank.

h. Ex-Vivo Permeation Study

The Ex-Vivo drug diffusion study was performed using Franz diffusion cell with a diameter of 10 mm and mucosa thickness (height) 0.2 mm. 0.5 mL of AGM solution and 0.5 mL AGM microemulsion (equivalent to 2.5 mg Agomelatine) was placed in the donor compartment along with 0.5 mL of diffusion media. Recipient compartment containing 20 mL of medium was stirred with Teflon coated magnetic stirrer. Samples from the receptor compartment were withdrawn at predetermined time intervals and analyzed using UV method. Each sample removed was replaced by an equal volume diffusion media. Each study was carried for a period of 4 hrs, during which the drug in receiver chamber (mg/mL) across the goat nasal membrane calculated at each sampling point.

i. Nasal toxicity studies-

Freshly excised goat nasal mucosa, except for the septum part was collected from the slaughter house in PBS pH 6.4. The membrane was kept in PBS pH 6.4 for 15 min. Goat nasal mucosa pieces with uniform thickness were mounted on Franz diffusion cells. One mucosa was treated with 0.5 mL pH 6.4 phosphate buffer; the other mucosa with 0.5 mL of isopropyl alcohol and the remaining with microemulsion (blank as well as drug loaded) for 1 hr. After 1 hr the mucosa were rinsed with PBS pH 4.4 and carried to the pathological laboratory in 10% formalin for the preparation of pathological slides. The goat nasal mucosa treated with pH 6.4 phosphate buffer and isopropyl alcohol were taken as positive and negative control respectively. The prepared pathological slides were studied under optical microscope for any sign of toxicity

g. Stability studies

The microemulsions were subjected to stability study at 25º C± 2 ^{0}C, 60 ±5% RH for 6 month. The samples were evaluated for transparency, drug contents, and pH and globule size for 6 month period

RESULT AND DISCUSSION

Preparation and optimization of microemulsion

Plotting of pseudo ternary phase diagram for formulation of microemulsion with Smix ratio (1:1, 2:1, 3:1) of tween 80 and propylene glycol. Pseudo ternary phase diagram shown in following fig.1

3^2 full factorial was applied to Agomelatine microemulsion % oil and %Smix was selected as a independent variables and globule size and viscosity and %drug diffused was considered as a response. Composition of various formulation and globular size, viscosity and % transmittance were given in table .1

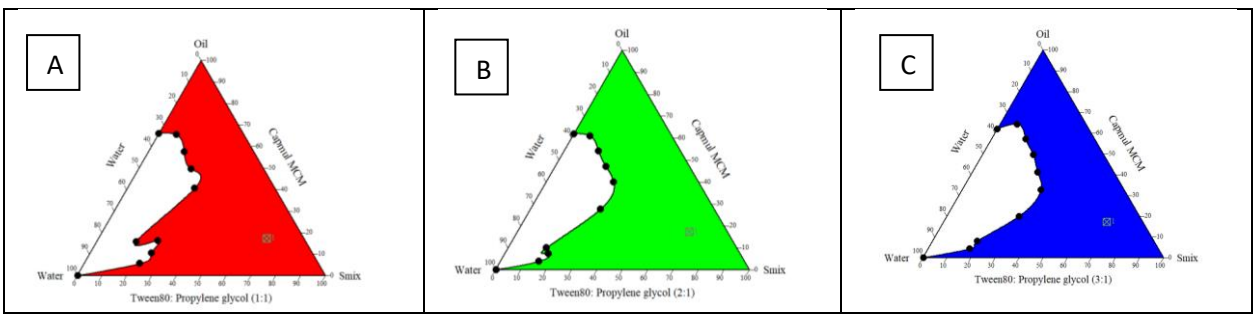

Figure 1: Pseudo ternary phase diagram

Table 1: Composition of microemulsion and characterization parameters of microemulsion

Formul-ation	% oil	% Smix	% water	Globule size (nm)	% CDR	Viscosity (cp)
ME-1	2.5	35	62.5	91.11±1.19	59.40±1.25	101.67±1.08
ME-2	2.5	45	52.5	78.63± 1.13	69.90±1.41	230.67±2.16
ME-3	2.5	55	42.5	65.60±0.54	75.43±2.47	332.67±2.68
ME-4	5	35	60	94.63± 1.36	45.37±1.04	163.33±1.08
ME-5	5	45	50	85.24±0.82	52.59±0.65	298.33±1.78
ME-6	5	55	40	76.93±1.89	56.46±0.81	393.00±1.87
ME-7	7.5	35	57.5	133.52±1.22	34.05±1.10	242.33±2.27
ME-8	7.5	45	47.5	101.94±1.41	41.07±0.83	347.00±5.79
ME-9	7.5	55	37.5	91.17±1.41	44.05±0.92	426.33±2.27

As shown in Table 1, the globule size varied from 65.60nm to 113.52nm for various factor level combinations. In order to determine the levels of factors which yield optimum particle size, mathematical relationships were generated between the dependent and independent variables using Design expert. The equations of the responses are given below.

$$\textbf{Globule Size} = 124.71 + 6.08 * [\%oil] - 0.1.42 * [\%Smix]$$

The result indicates significant effect of variables (X_1, X_2) on the globule size of Agomelatine microemulsion. As shown in table. 1 increasing the concentration of oil resulted in increased mean globule size of microemulsion. Moreover, %Smix had a negative effect on globule size. Thus, as the Smix concentration increases, the globule size decreases. This could be due to the decreased surface tension by surfactant. The role of surfactant in the formulation of microemulsion is to lower the interfacial tension which will ultimately facilitates dispersion process during the preparation of From the result of ANOVA, the concentration of oil and Smix had an individual significant effect was found (P=0.0018). Result of equation indicate that the effect of X1 (oil concentration) and X_2 (Smix concentration) both have significant .

% drug diffused is important parameter for the microemulsion for nose to brain delivery. The results of %drug diffusedis shown in table 1.

$$\textbf{\% Drug diffused} = +8.284 - 7.00 * [\%oil] + +2.77 * [\%Smix] - 0.060 * [\%oil * \%Smix]$$

$$+ 0.401 * [\%oil^2] - 0.02 * [\%Smix^2]$$

In the polynomial equation, X1, X2 , X1*X2, $X1^2$ $X2^2$ are significant model terms for the % CDR (p value < 0.05). Effect of both the formulation variables on % drig diffused can be understood by polynomial equation. The increase in % S_{mix} improved the % drug diffusion, contrary to it, increase in oil concentration was disadvantageous for the drug release. The combinations of independent variables such as % oil*% S_{mix} negative magnitude. This shows the dominance of % oil on % S_{mix} and this dominancy can be attributed to the fact that a small increment in % oil leads to significant increase in hydrodynamic diameter which eventually restricts the drug release

Viscosity of the microemulsion is another important parameter when the administration is particularly through nasal route. Very high viscosity can create breathing difficulty and directly affects patient compliance. Also

from the formulation point of view nasal spray or nasal drops requires less viscous formulation. The viscosity data is given in the following table 1. varied from 101cP to 426.33cP. The minimum viscosity 101cP was found for ME-1 batch. The equations of the responses are given below:

Viscosity = +742.81 – 52.166 * [%oil] + 26.99 * [%Smix] -0.47 * [%oil *%Smix] + 0.764* [%oil ^{2}] - 0.154* [%Smix ^{2}]

In the polynomial equation, X1, X2 and X1*X2 are significant model terms for the viscosity (p value < 0.05). While $X2^2$ and $X1^2$ are non significant model terms (p-value>0.05). These terms can be avoided from the full polynomial equation as they are not having any significant effect on viscosity of the microemulsion. Effect of both the formulation variables on viscosity can be understood by polynomial equation.

Viscosity = = +742.81 + 52.166 * [%oil] + 26.99 * [%Smix] -0.47 * [%oil *%Smix]

The result of (ANOVA) has shown that oil concentration and Smix concentration both had significant effect on the viscosity (P<0.05). Each of two factors was found to have a significant effect on the viscosity of microemulsion. Result of equation indicate that the effect of X1 (oil concentration) is more significant than X2 (Smix concentration)

SELECTION OF OPTIMIZED BATCH & ITS EVALUATION

According to our criteria of minimum globule size and minimum viscosity, ME-1 was selected as optimized formulation in (Overall desirability is 0.809). The composition for the ME-1 microemulsion was Oil 2.5%, Smix 35% and water 62.5%. The prepared optimized microemulsion was characterized for the following parameters.

EVALUATION OF PREPARED OPTIMIZED MICROEMULSION

a. Drug content:

The percentage drug content was evaluated for Agomelatine o/w microemulsion. The percentage drug content for microemulsion was found to be 98.86±1.2%.

b. Zeta potential:

Zeta potential of microemulsion was –5.22 mV, which indicate physical stability to the system. The surface charge on microscopic particles produces a different in electrical potential in mV between the surface of each particle and bulk of the suspending liquid. This difference is called zeta potential.

Zeta sizer measures the effect of electrostatic charge; this is basic force which causes electrical repulsion between adjacent particles. It should be negative or neutral. Which indicate that droplets of micro emulsion having no charge, that is system is stable. Zeta potential is essentially useful for assessing flocculation since electrical charges on particles influence the rate of flocculation.

c. Viscosity:

The microemulsion systems exhibited a simple Newtonian flow. The presence of Agomelatine did not change the flow behavior. Viscosity of microemulsion was found to be 103Cp. The viscosity of a microemulsion can be affected by the component ratio and concentration of oil, water, and surfactant.

d. pH measurement:

The pH value of microemulsion was determined using digital pH meter and it was found to be 6.12. The pH of the formulation was nearly same as the nasal pH. Hence, the formulations will not produce irritation upon instillation.

e. Globular size

The average globule size of the optimized formulation was found to be 89.06nm. It indicates that the formulated microemulsion globule size was in in the range and indicates the monodispersed stable system and could deliver the drug effectively owing to larger surface area.

f. In vitro diffusion

The permeation data obtained for Agomelatine solution and Agomelatine microemulsion are tabulated in following table no. 3. The overall %drug diffused for Agomelatine microemulsion was found to be

78.50%.The amount of drug diffused across the nasal mucosa at the end of 4 hours was found to be more for microemulsion formulation compared to simple Agomelatine solution.

Table 2: Evaluation parameters for optimized batch

Batch	Drug contain	Globular size	Viscosity	pH	Zeta potential
ME-1	98.86±1.2%.	89.06±1.45 nm	103±2.45Cp	6.12±.012	-5.22±0.01 (mV)

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

Time (min)	Root Time (min)	AGT solution (% drug diffused)	AGT microemulsion (% drug diffused)
15	3.87	2.41±0.40	8.59±.20
30	5.47	8.81±.59	14.93±.54
60	7.74	24.69±1.21	34.57±0.33
120	10.95	40.67±0.76	56.03±1.38
180	13.41	45.08±1.71	67.92±.68
240	15.49	47.20±1.31	78.50±2.02

g. Nasal ciliotoxicity study:

Nasal ciliotoxicity studies were carried out in an attempt to evaluate any potential toxic effects of excipients used in the formulation on the nasal mucosa. Thus the nasal mucosa of goat was treated with blank microemulsion to evaluate the toxic effects of excipients used in the formulation.

The figure 2. shows the nasal mucosa treated with phosphate buffer pH 6.4 (negative control) no nasociliary damage was observed and the nasal membrane remained intact. The Figure 3. show the nasal mucosa treated with isopropyl alcohol (positive control), and an extensive damage to nasal mucosa was observed coupled with loss of nasal cilia. However, with microemulsion, no damage to nasal mucosa was observed.

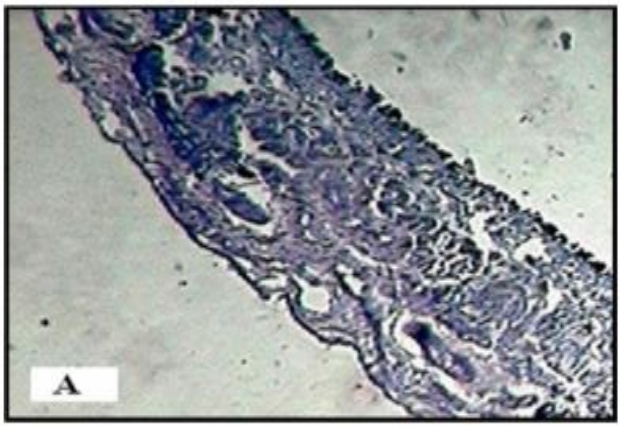

Figure 2: Nasal mucosa treated with A) Phosphate Buffer pH 6.4

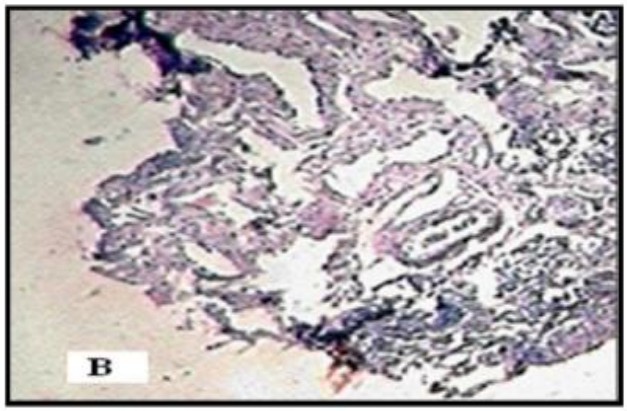

Figure 3: Nasal mucosa treated with B) IPA

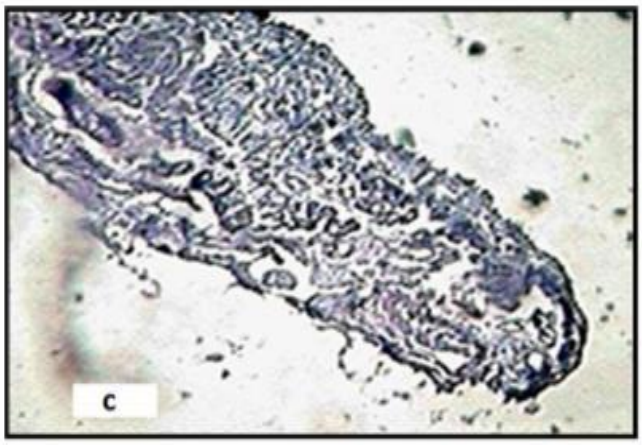

Figure 4: Nasal mucosa treated with c) Microemulsion

h. Stability Studies-

The microemulsions were subjected to stability study at 25 ± 2 °C 60±5% RH for 6 month respectively. The samples were evaluated for transparency, drug contents, pH, and globule size for 6 month period and shown in Table 4.

Table 4: Accelerated stability studies.

Sr. No.	Observation	Before Accelerated Stability Testing	After Accelerated Stability Testing (180 days)
1.	(Transparency)	Transparent	Transparent
2.	Drug content (%)	98.85 ± 0.75	96.95 ± 0.50
3.	pH	6.12±.012	6.10.± 0.52
4	Globule size	89.06±1.45nm	95.06±3.2 nm

Summery

Finally, it can be summarized that the microemulsion of Agomelatine can be one of the promising tool in improve bioavailability, increases the rate of absorption due to the small globule size and by avoiding first pass metabolism and direct transport into systemic circulation for effective and longer treatment required for antidepressant action with increased stability.

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