

Influence of Marich (Piperine) Micro-Emulsion on In Vitro & Ex Vivo Permeation Study

*Sampada Vasantrao Bende, **Arati Dubewar

Introduction

Microemulsions are clear, thermodynamically stable, isotropic liquid mixtures of oil, water and surfactant, frequently in combination with a co-surfactant. The aqueous phase may contain salts and /or other ingredients, and the oil may actually be a complex mixture of different hydrocarbons and olefins. In contrast to ordinary emulsions, microemulsions form upon simple mixing of the components and do not require the high shear conditions generally used in the formation of ordinary emulsion. The three basic types of microemulsions are direct (oil dispersed in water, o/w), reversed (water dispersed in oil, w/o) and bicontinuous. The diameter of droplet may be in the range of 100A to 1000A.

In ternary systems such as microemulsions, where two immiscible phases (water and oil) are present with a surfactant, the surfactant molecule may form a monolayer at the interface between the oil and water, with the hydrophobic tails of the surfactant molecules dissolved in the oil phase and the hydrophilic head groups in the aqueous phase.

History and terminology

The term microemulsion was first used by T.P. Hoar and J.H. Shulman, professors of chemistry at Cambridge University, in 1943. Alternative names for these system are often used, such as transparent emulsion, swollen micelle, micellar solution, solubilized oil. More confusingly still the term microemulsion can refer to the single isotropic phase that is a mixture of oil, water and surfactant or to one that is in equilibrium with coexisting predominantly oil and / or aqueous phases, or even to other non-isotropic phases, as in the binary system (water/surfactant or oil/surfactant), self-assembled structures of different types can be formed, ranging, for example, from (inverted) spherical and cylindrical micelles to laminar phases and bicontinuous microemulsions, which may coexist with predominantly oil or aqueous phases.⁹

Phase diagrams

Microemulsion domains are usually characterized by constructing ternary phase diagrams. Three components are the basic requirements to form microemulsion: two immiscible liquids and a surfactant. The majority of microemulsion use oil and water as immiscible liquid pairs. If a cosurfactant is used, it may sometimes be represented at a fixed ratio to surfactant as a single component, and treated as a single “pseudo-component.” The relative amounts of these three components can be represented in a ternary phase diagram. Gibbs phase diagram can be used to show the influence of changes in the volume fractions of the different phases on the phase behavior of the system. Stabilizers and Emulsifiers lowers interfacial tension of two immiscible liquids.

The three components composing the system are each found at an apex of the triangle, where their corresponding volume fraction is 100%. Moving away from that corner reduces the volume fraction of that specific component and increases the volume fraction of one or both of the two other components. Each point within the triangle represents a possible composition of a mixture of three components or pseudo-components, which may consist of one, two or three phases. These points combine to form regions with boundaries between them, which represent the “phase behavior” of the system at constant temperature and pressure.

*MD (Ayurved), Ph.D (Scholar.), Rasashastra and Bhaishajya kalpna department, Dr. D. Y. Patil college of ayurved and research center, Pimpri, Pune. ** HOD of Rasashastra and Bhaishajya kalpna department, Dr. D. Y. Patil college of ayurved and research center, Pimpri, Pune.

The gibbs phase diagram, however, is an empirical visual observation of the state of the system and may, or may not express the true number of phases within a given composition. Apparently clear single phase formulations can still consist of multiple isotropic phases. Since these system can be in equilibrium with other phases, many systems, especially those with high volume fractions of both the two immiscible phases, can be easily destabilized by anything that changes this equilibrium e.g. high or low temperature or addition of surface tension modifying agents.

Material and method

Method	Emulsion titration / dilution method
Material	1) Piperine drug = 1mg/ml (from Sinhgad college of Pharmacy, Pune)
	2) Capmul oil = 4% (from Sinhgad college of Pharmacy, Pune)
	3) Labrasol (surfactant) = 32% (from Sinhgad college of Pharmacy, Pune)
	4) Transcutol (co-surfactant)= 8% (from Sinhgad college of Pharmacy, Pune)
	5) Double distilled water = 56% (from Sinhgad college of Pharmacy, Pune)

Preparation of microemulsion:

Preparation of surfactant mixture

This step of formulation involves the preparation of mixture of the surfactant and cosurfactant in an appropriate ratio. The surfactant and co-surfactant mixture was made homogenous with continuous stirring at 550 rpm.

Addition of oil to surfactant mixture

The second step involves the addition of an oil phase containing drug to the surfactant mixture with vigorous stirring at high speed (900 rpm) in an appropriate ratio.

Addition of water to oil and surfactant mixture

Then the addition of water in a drop wise manner at a rate of 1ml/min to the oil and surfactants mixture was done under vigorous stirring (900 rpm).

Preparation of mucoadhesive microemulsion

The formulation of mucoadhesive microemulsion involves the addition of 18% aqueous solution of poloxomer 407 (made by mixing of poloxomer 407 with water and soaked for 24 hrs, then used for preparation) and 0.4% aqueous solution of HPMC K4M.

This aqueous solution of poloxomer 407 and HPMC K4M was added to the mixture of oil and surfactants instead of plane water.^{13,14}

Characterization of microemulsion of Piperine:

Clarity

Clarity was observed visually.

Centrifugation

The microemulsion system was centrifuged at 3000 rpm for 15 min to determine whether the system shows signs of creaming or phase separation (Ghosh, et al 2006). The system was observed microscopically for appearance.

Thermodynamic stability study

In order to assess the physical stability of the selected formulations the effect of the following stresses were analyzed:

Centrifugation test: the formulation were centrifuged at 5000 rpm for 30 min and observed for phase separation, creaming or cracking.

Heating-cooling cycle: the stable samples after centrifugation test were stored at refrigerated temperature 4°C and 40°C (six cycles each) for 48 hrs at each temperature.

Freeze-thaw cycle: carefully selected formulation were thus subjected to three freeze-thaw cycles between -20°C and at 25°C, the storage time at each temperature being 48hrs.

Dispersibility test

This test was performed to evaluate the efficiency of self-emulsification of the formulated microemulsions. The study was performed by mixing 1 ml of the formulated microemulsions in 500 ml of water, maintained at 37°C ± 0.5°C using a standard USP XXII dissolution apparatus with the paddle rotating at a speed of 50 rpm for gentle mixing. The results of the in vitro dispersion of the formulations were evaluated visually as per table. Formulation qualifying the dispersibility test in Grade-A were selected for further studies.³

Observation	Grade
Rapidly forming microemulsion (within 1 min) (producing a clear or slight bluish appearance)	A
Rapidly forming, slightly less clear microemulsion (having bluish appearance)	B
Fine milky emulsion that formed within 2 min	C
Dull, grayish white emulsion having slightly oily appearance (slow to emulsify longer than 2 min)	D
Formulation exhibiting either poor or minimal emulsion with large oil globules present on the surface	E

Transmittance

The prepared formulation was diluted 10 times with water and the effect of dilution percentage transmittance was checked. The percentage transmittance of the developed formulation was measured at 650 nm using UV- visible spectrophotometer keeping distilled water as a blank.^{4,5} (Rudree et al, 2014)

Results

Characterization of microemulsion

Clarity

Clarity of formulated microemulsion was observed visually. All microemulsion formulations F1 to F5 was found to be clear and whitish yellow in appearance.

Thermodynamic Stability

Centrifugation

The selected microemulsions were centrifuged at 5000 rpm for 30 minutes to determine whether the system shows signs of creaming or phase separation. After centrifugation formulations F3, F5 showed phase separation while formulations F1, F2, F4 did not show any sign of phase separation.

Heating and cooling cycle

The formulations were found to be stable after centrifugation were further tested for heating and cooling cycles. The formulations were stored at refrigerated temperature (4 °C) and (40° C) (six cycles each) for 48 h at each temperature. The F1, F2, F4 did not show any sign of phase separation.

Freeze- thaw cycle

The above sample F1, F2, F4 were further subjected to three freeze-thaw cycles between -20° C and at 25° C, the storage time at each temperature being of 48 h. All four samples were found to be stable.

Dispersibility test

This test was performed to evaluate the efficiency of self-emulsification of the formulated microemulsions. The formulations F1, F2, F4 were found to be Grade A.

The thermodynamic stability and dispersibility test were done to find out the metastable formulations. Formulations (F1, F2, F4) that passed the centrifugation and dispersibility tests were subjected to further characterization.

Effect of formulation variables on Transmittance (%T)

Transmittance of the microemulsion was measured against distilled water with a UV- visible spectrophotometer at 650 nm. Formulation with higher % T was selected as it is indicative of isotropic and clear formulations.

The counter plot of % T showed that an increase in the concentration of oil phase (Capmul MCM) resulted in a proportional increase in %T, because of the simultaneous decrease in the Smix proportion.

Refractive index

The refractive index of the optimized piperine microemulsions (1.446 ± 0.12) was found to be similar to the placebo and were closer to that of Capmul MCM (1.449 ± 0.15) although these optical values were found to be directly proportional to the oil content of the formulations. This proved isotropic nature of prepared formulation.

Viscosity

Rheological properties of optimized microemulsion is of much scientific importance since it is well known that viscosity increases with increase in concentration of oil owing to increase in interfacial tension with water. We have observed that optimized formulation exhibited a minimum viscosity of 40.283 ± 0.148 cps. Viscosity value of our formulation is certainly advantageous from patient compliance and manufacturing point of view.

pH

pH of optimized formulation was found to be 5.56. The observed pH was well within the physiological pH range and signifies that formulation will not cause any irritation to

nasal mucosal membrane.⁷

Drug content

The Piperine content (%w/v) of optimized formulation was analysed by validated UV method. The drug content was found to be in the range of 98.73 ± 0.75 .¹

The assay value is well within the acceptable limits (95-105 %) of labeled claim.

In Vitro Drug Release

The results of drug release studies from marich-churna, piperine-microemulsion, and marich-solution are shown in Fig. 1. The drug release behavior was studied in simulated nasal electrolyte solution.⁶ Incorporation of drug in microemulsion based system improved drug solubilization and in vitro release, as compared

(2006) prepared clonazepam mucoadhesive microemulsion, they found the rapid and larger extend of transport of clonazepam to brain trough nasal route.¹⁰

Ex vivo permeation studies

The permeation of marich churna, piperine in situ gelling microemulsion and marich-solution across the nasal mucosa is shown in Fig. 2. The drug solution in water showed lowest permeability. Drug permeation improved in microemulsion based system, which may be attributed to the presence of surfactants and co-surfactants, which helps in solubilization. Rudree et al., (2014) also found

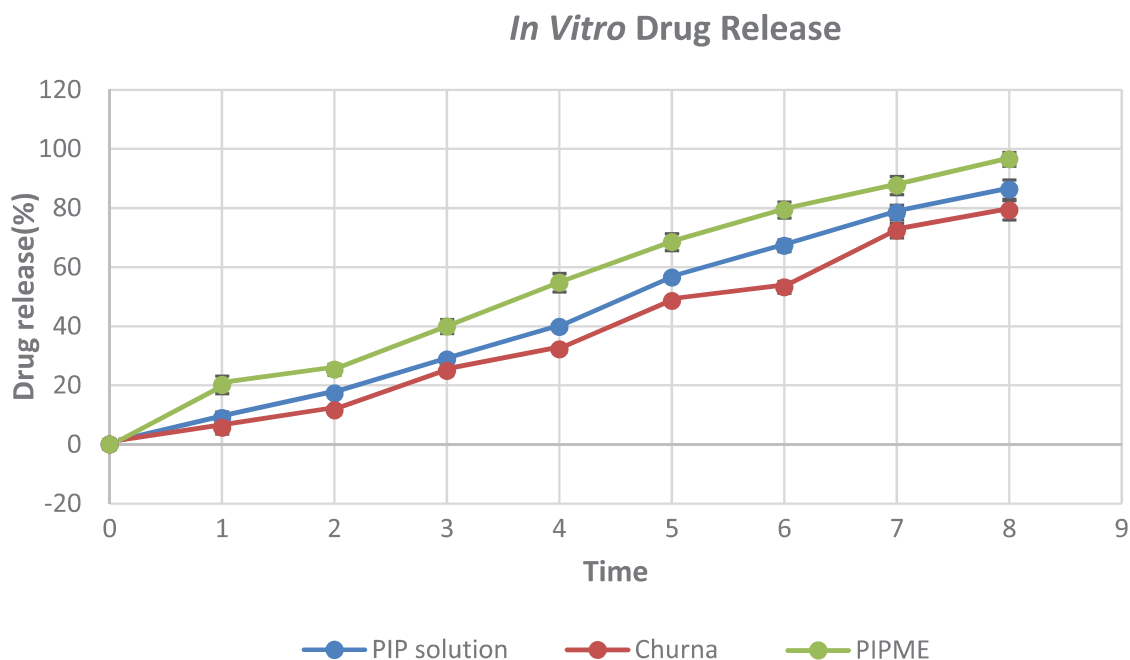


Fig. 1 : In vitro drug release of piperine from different formulations by HPLC. Data are expressed as mean $\pm$ SD (n=3).

to drug suspension. In situ gelling microemulsion based system showed a high initial burst. The drug release at later stages was hampered due to extensive cross linking of Pluronic F127 and F68 at higher temperature (temperature - dependent gelling). The higher initial burst release is desirable in case of immediate therapeutic intervention as in the case of patients suffering psychosis. Vyas et al.,

the same results when they prepared the mucoadhesive microemulsion of nimodipine using a blend of Pluronic F127 and Pluronic F68 as gelling agent and 0.3 % w/w Carbopol as a mucoadhesive agent.¹¹

Ex vivo permeation studies

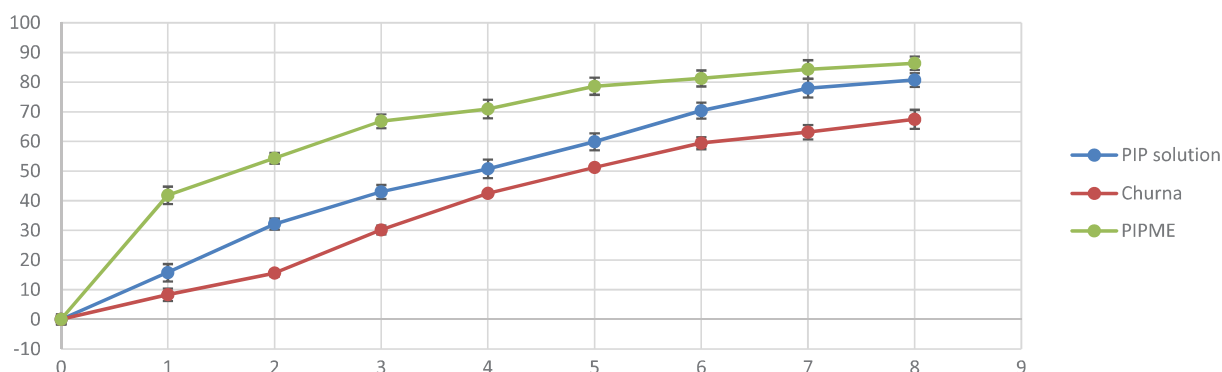


Fig.2 : ex vivo drug release of piperine from different formulations. Data are expressed as mean $\pm$ SD (n=3).

Conclusion

The optimum microemulsion formulation contained Capmul MCM (7%), Labrasol, Cremophor RH40 and Transcutol P (Smix 46%), and double distilled water (56%), which was a transparent and less viscous system.

The pH of the formulation (5.56) was found to be in range of nasal pH, this pH was suitable to ensure stability of drug.

The morphology of optimum microemulsion revealed presence of nearly monodispersed, spherical oil globules of nanometer size without visible drug crystal in the field.

The optimum microemulsion formulation showed the good in vitro diffusion $95.65 \pm 4.20\%$ compared to drug solution ($73.54 \pm 1.35\%$) and marich-churna formulation ($85.96 \pm 2.91\%$). This enhanced release from formulation was due to presence of drug in lipidic nano form.

The optimum microemulsion formulation showed the good ex vivo drug permeation $86.36 \pm 4.20\%$ compared to drug solution ($64.08 \pm 1.35\%$) and marich-churna formulation ($78.36 \pm 2.91\%$). This enhanced release from formulation was due to presence of drug in lipidic nano form.

The prepared microemulsion was found to be stable at temperature $40^\circ\text{C} \pm 75\%$ RH for 3 months.

In conclusion, microemulsion system is a promising approach for the brain targeting of piperine in Alzheimers, Parkinson's and other neurodegenerative disorders.^{15,16}

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