Preparation and Evaluation of Liquid and Solid Self-Microemulsifying Drug Delivery System of Mebendazole

Ahmed A. Hussein[*,1]

[*]Department of Pharmaceutics, College of Pharmacy, University of Baghdad,Baghdad, Iraq

Abstract

The aim of present study was to develop solid and liquid self-microemulsifying drug delivery system of poorly water soluble drug mebendazole using Aerosil 200 as solid carrier. Microemulsions are clear, stable, isotropic liquid mixtures of oil, water and surfactant, frequently in combination with a co-surfactant having droplet size range usually in the range of 20-250 nm. Oleic acid, tween 80 and polypropylene glycol were selected as oil, surfactant and co-surfactant respectively and for preparation of stable SMEDDS, micro emulsion region was identified by constructing pseudo ternary phase diagram containing different proportion of surfactant: co-surfactant (1:1, 2:1 and 3:1), oil and water. In brief S/ CoS mix means surfactant to co-surfactant and oil were mixed at ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 manner. To the resultant mixtures, water was added drop wise till the first sign of turbidity in order to identify the end point and after equilibrium; if the system became clear then the water addition was continued. Prepared optimised formula of microemulsion was evaluated for SEM, particle size analysis, polydispersity index, phase separation, viscosity determination, zeta potential, *in- vitro* dissolution study and *in-vivo* studies. The optimized microemulsion was converted into solid form by Spray Drying technique by using Aerosil 200 as solid carrier. Prepared SMEDDS was characterized for same parameters as that of microemulsion. Solid SMEDDS of mebendazole prepared using Aerosil 200 by spray drying technique showed good drug content uniformity. After reconstitution it formed microemulsion with micrometric range. *In-vitro* drug release and *in-vivo* plasma drug concentration of microemulsion and SMEDDS was much higher than that of marketed praparation. Hence lipid based drug delivery system may efficiently formulate microemulsion and it can be solidified easily by spray drying technique which enhances dissolution rate and thus concomitantly bioavailability. In conclusion ,self micro emulsifying drug delivery system has become promising tool to overcome shortcomings associated with conventional delivery.

Kew words: Self-microemulsifying drug delivery system, Microemulsion, Mebendazole.

تحضير وتقييم نظام لايصال الدواء على شكل سائل وصلب ذاتي الاستحلاب الى المبيندازول

احمد عباس حسين [*،1]

* فرع الصيدلانيات، كلية الصيدلة،جامعة بغداد،بغداد،العراق.

الخلاصة

الهدف من الدراسه المقدمة هو تطوير نظام صلب وسائل ذاتي الاستحلاب للعقار القليل الذوبانية المبيندازول بأستخدام الايروزيل كناقل. المستحلب المايكروي شفاف ومستقر ومزيج موحد من الزيت والماء والسيرفكتانت وغالبا مايستخدم مع كوسيرفكتانت له حجم قطره يتراوح من ٢٠-٢٥٠ نانوميتر. الاوليك اسد ,التوين ٨٠ والبروبلين كلايكول يستخدمون كزيت وسيرفكتانت وكوسيرفكتانت بالتعاقب وتم تعيين المستحلب المايكروي باستخدام مخطط ثلاثي الحالة يحتوي نسب مختلفه من السيرفكتانت والكوسيرفكتانت.

للمزيج الناتج يضاف الماء تدريجيا لحين اول منطقه من التعكر لكي تعيين نقطة النهاية بعد التوازن اذا اصبح النظام واضح يستمر بعد ذلك اضافة الماء. الصيغه الامثل المحضرة من المستحلب المايكروي قيم لمسح المجهر الالكتروني, حجم الجزيئه, مؤشر التشتت المتعدد , حالة الانفصال، وقيمة اللزوجة، زيتا المحتملة، في دراسة حل التجارب المختبرية والدراسات المجراة. المستحلب المايكروي الامثل تم تحويله الى صلب بواسطة بخاخ تقنية التجفيف باستخدام الايروسيل ٢٠٠ كناقل صلب. المستحلب المايكروي الصلب يقييم لنفس العوامل للمستحلب المايكروي السائل. المستحلب المايكروي الصلب للمبيناديزول المحضر باستخدام ايروسيل ٢٠٠ يظهر محتوى جيد للمحتوى.

بعد اعادة الذوبانيه المسحلب المايكروي تكون بالمدى المايكروي. معدل تحرير العقار داخل وخارج الجسم للمستحلب المايكروي اعلى من المحضر تجاريا. بالتالي وجد ان نظام العقار المستند للدهون يمكن ان يكون مؤثر كمستحلب مايكروي ويمكن ان يتصلب بسهولة بواسطة تقنية تجفيف الرذاذ والذي يزيد من معدل تحرر العقار ولذلك تزداد التوافر البايولوجي. النظام الذاتي المستحلب للعقار اصبح كتقنيه واعده لتجاوز الانظمه التقليديه.

الكلمات المفتاحية :نظام دوائي ذاتي الاستحلاب ، مستحلب مايكروي ،مبيندازول .

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[1]Corresponding author E-mail: ahmed_abbas201383@yahoo.com
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Introduction

Microemulsions are clear, stable, isotropic liquid mixtures of oil, water and surfactant, frequently in combination with a co-surfactant having droplet size range usually in the range of 20-250 nm. Its aqueous phase may contain salt and/or other ingredients, and the "oil" may actually be a complex mixture of different hydrocarbons and olefins. If we compare it with 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 emulsions [(1)] .

There are two basic types of microemulsions one is direct (oil dispersed in water, o/w) and other is reversed (water dispersed in oil, w/o). In ternary systems such as microemulsions, where two immiscible phases (water and oil) are present with a surfactant, the surfactant molecules 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 [(2)].

As in the binary systems (water and surfactant or oil and surfactant), self-assembled structures of different types can be formed. A self - microemulsifying drug delivery system (SMEDDS) typically comprises a mixture of surfactant, oil and drug which when introduced in the body to form droplet of approximately the same size range as those observed in the microemulsion system [(3)].

The release of the drug compound from SMEDDS takes place upon its partitioning into the intestinal fluids during droplet transport and disintegration along the gastrointestinal tract. It was proposed that two main factors, small particle size and polarity of the resulting oil droplets determine the efficient release of the drug compound from SMEDDS. In o/w microemulsions, however, the impact of the polarity of the oil droplets is not very significant because the drug compound reaches the capillaries incorporated within the oil droplets [(3)].

Many studies carried out in animals for the assessment of the oral bioavailability of hydrophobic drugs formulated in o/w emulsions indicated better absorption profiles but, the use of these systems is limited due to their poor physical stability and the large volumes needed. Thus, SMEDDS may be a promising alternative to orally administered emulsions because of their relatively high physical stability and ability to be delivered in standard soft gelatine capsule [(4)].

Mechanism of self emulsification

Self-emulsification takes place when the entropy change favoring dispersion is greater than the energy required to increase the surface area of the dispersion. The free energy of a conventional emulsion formulation is a direct function of the energy required to create a new surface between the oil and water phases. The two phases of the emulsion tend to separate with time to reduce the interfacial area and thus the free energy of the systems [(5)].

The conventional emulsifying agents stabilize emulsions resulting from aqueous dilution by forming a monolayer around the emulsion droplets, reducing the interfacial energy and forming a barrier to coalescence. On the other hand, emulsification occurs spontaneously with SEDDS because the free energy required to form the emulsion is either low and positive or negative. It is necessary for the interfacial structure to show no resistance against surface shearing in order for emulsification to take place [(4)].

The ease of emulsification was suggested to be related to the ease of water penetration into the various liquid crystal (LC) or gel phases formed on the surface of the droplet. The interface between the oil and aqueous continuous phases is formed upon addition of a binary mixture (oil/non-ionic surfactant) to water. This is followed by the solubilisation of water within the oil phase as a result of aqueous penetration through the interface. This will occur until the solubilisation limit is reached close to the interphase. Further aqueous penetration will lead to the formation of the dispersed LC phase [(3)].

In the end, everything that is in close proximity with the interface will be LC, the actual amount of which depends on the surfactant concentration in the binary mixture. Thus, following gentle agitation of the self-emulsifying system, water will rapidly penetrate into the aqueous cores and lead to interface disruption and droplet formation [(3)].

According to Reiss, the energy required to increase the surface area of the dispersion for self emulsification process bear less importance when compared to the entropy change that favours dispersion.

Self microemulsification is related to the free energy. That is free energy of conventional emulsion is direct function of the energy essential to create a new surface between the oil and water phases and can be described by equation [(4)],

$$DG = S N p r 2s$$

Where, DG is the free energy related to the process, N is the number of droplets of radius, r and s represents the interfacial energy. The

emulsifying agent forms a monolayer of emulsion droplets , and hence reduces the interfacial energy, and providing the barrier to avoiding coalescence. In case of self emulsification , the free energy required to form the microemulsion is either very low or positive or negative. Emulsification requires very low energy involves destabilisation through contraction of local interfacial regions [1].

The aim of present study was to develop SMEDDS of poorly water soluble drug mebendazole using Aerosil 200 as solid carrier. Microemulsion was prepared using oleic acid, tween 80 and propylene glycol as oil, surfactant and co-surfactant respectively and was converted to solid SMEDDS by adsorbing it on Aerosil 200. Prepared optimised formula of microemulsion was evaluated for SEM, particle size analysis, polydispersity index, phase separation, viscosity determination, zeta potential, *in-vitro* dissolution study and *in - vivo* studies.

Materials and Methods

Materials

The following materials were used : Mebendazole (Ciron Pharma Ltd. Mumbai). Oleic acid, Tween-80 ,Propylene glycol, Dimethyl sulfoxide, Methanol (Research Lab Fine Chem Industry, Mumbai). Dialysis membrane (12000 Da) (Hi media), Sodium hydroxide and Acetonitril (Sd Fine Chem).

Methods

Solubility study

The solubility of mebendazole in various oils, surfactant, and cosurfactant was determined by: An excess amount of mebendazole powder was added to 2 ml of vehicle (oil, surfactant, cosurfactant), shaken in a mechanical shaker at 37^0 C for 48 hrs, and centrifuged at 5,000 rpm for 15 min. Supernatant was diluted with methanol for the quantification of mebendazole and analysed by UV Spectrometer [2].

Preparation of microemulsion

Construction of pseudo-ternary phase diagram

Oleic acid, tween 80 and polypropylene glycol were selected as oil, surfactant and co-surfactant respectively and for preparation of stable SMEDDS, microemulsion region was identified by constructing pseudo ternary phase diagram containing different proportion of surfactant: co-surfactant (1:1, 2:1 and 3:1), oil and water. In brief S/ CoS mix means surfactant to co-surfactant and oil were mixed at ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 manner. To the resultant mixtures, water was added drop wise till the first sign of turbidity in order to identify the end point and after equilibrium; if the system became clear then the water addition was continued [6-8].

Selection of optimised ratio

All ratio of S/Co gives region in pseudo ternary phase diagram and compared among them to select optimized ratio.

Preparation of mebendazole microemulsion

Mebendazole was added to the mixtures of oil, surfactant, and co-surfactant with varying percentage as described in table 1 and then an appropriate amount of water was added to the mixture drop by drop with constant stirring on magnetic stirrer. Microemulsions containing mebendazole was obtained spontaneously on stirring the mixtures. All the formulations were stored at appropriate temperature.

Table(1)Composition of microemulsion formulation at 2:1 (Oil to S/CoS)

Batch Code	Mebendazole(mg)	Oleic acid (mg)	Tween-80 (mg)	Propylene glycol (mg)	Water (ml)
B1	5.18	1.5	20	10	68.5
B2	5.18	2.5	20	10	67.5
B3	5.18	3	20	10	67
B4	5.18	4	20	10	66
B5	5.18	5.5	20	10	64.5
B6	5.18	6.5	20	10	63.5
B7	5.18	8.5	20	10	61.5
B8	5.18	10.5	20	10	59.5
B9	5.18	12.5	20	10	57.5

Evaluation of microemulsion

The optimised formulations were evaluated for the following characteristics:

Optical transparency

Optical transparency of the formulas was determined by inspecting the sample in clear and transparent container under the presence of good light against reflection into the eyes, and viewed against black and white illuminated background.

Phase separation

Microemulsion system were subjected to centrifugation at 15,000 rpm for a period of 15

minute and examined for any change in phase separation [9,10] .

Emulsification test

Self emulsification ability of surfactant was assessed to select the best surfactant from a large pool of surfactant. Selected oil and surfactant were mixed in 1:3 ratio heated at 40-50 ^{0} C and vortexed to form a homogenous mixture at room temp (25 ± 1^0 C). 500 mg of oil- surfactant mixture was dispersed in 500ml double distilled water in a glass beaker, was prepared under gentle stirring. Visual test was used to assess self emulsification of surfactant in terms of dispersibility, ease of emulsification and appearance using a grading system [11].

Measurement of globule size

The average globule size was measured using a Nanophox (NX0088), Cross corrélation. The measurement was performed at 25°C [12].

Polydispersity Index

Polydispersibility which determines size range of particles in the system, it is expressed in terms of polydispersibility index (PDI). An ideal SMEDDS should be widely distributed with particles less than 100 nm and so PDI should be less than 0.3 or in other words particles having size more than 100 nm should be maximum up to 23% [13].

(D0.9 - D0.1) / D0.5

i. e. (X90 - X10) / X50

Viscosity measurement

The viscosity of microemulsion was measured using a Brookfield viscometer equipped with the spindle no. 64. The measurement was performed at ambient temperature of a single formula or for all formulas and it reflects interdroplet interaction [14].

Zeta potential

The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. If all the particles have a large negative or positive zeta potential they will repel each other and there is dispersion stability. If the particles have low zeta potential values then there is no force to prevent the particles coming together and there is dispersion instability. Zeta potential is determined by using Zetasizer [13].

SEM analysis

The morphology of mebendazole loaded SMEDDS (Globules) prepared under the optimum condition was observed under scanning electron microscope.

In- vitro study

Dialysis membrane was used to carry out mebendazole release from its suspension. Mebendazole suspension containing 10 mg of drug was placed into dialysis bag (HiMedia, molecular weight cut off 12000 da). *In- vitro* drug release of all formulas was carried out using USP- type II dissolution apparatus (paddle type). The dissolution medium, 900 ml 0.1 N HCl and 1% SLS was placed into the dissolution flask maintaining the temperature at 37 ± 0.5^{0}C and speed of 100 rpm. Dissolution studies were carried out for 2 hr. 5 ml of aliquot was withdrawn at an interval of 5, 10, 20, 40, 60, 80, 100, 120 min. After collecting the sample, the dissolution medium was replenished with the same volume of fresh medium, and the sample was filtered.The samples were then analyzed at 300nm by UV-visible spectrophotometer [15,16].

Release kinetic modelling

To analyze the *in-vitro* release data, various kinetic models were used to describe the release kinetics. Zero order rate equation (Equation-1) describes drug release rate is independent of its concentration in the systems. The first order equation (Equation-2) describes the release from system where release rate is concentration dependent. Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion (Equation-3).

The Hixson-Crowell cube root law (Eqution-4) describes the drug release from systems where there is a change in surface area and diameter of particles or tablets (Hixson and Crowell, 1931). Korsmeyer et al (1983) derived a simple relationship which described drug release from a polymeric system (Eqution-5).

$C = k_0 t$ (1)

Where, K_0 is zero-order rate constant expressed in units of concentration/time and t is the time.

$\text{Log } C = \text{Log } C_0 - kt / 2.303$ (2)

Where, C_0 is the initial concentration of drug and K is first order constant.

$Q = K^{t\,1/2}$ (3)

Where, K is the constant reflecting the design variables of the system.

$Q_0^{1/3} - Q_t^{1/3} = K_{HC}^{t}$ (4)

Where, Q_t is the amount of drug released in time t, Q_0 is the initial amount of the drug in tablet and K_{HC}^{t} is the rate constant for Hixson-Crowell rate equation.

$Mt / M_\infty = Kt^n$ (5)

Where Mt / M_∞ is fraction of drug released at time t, k is the rate constant and n is the release exponent [7].

Plots were made cumulative % drug release vs. time for zero order kinetic model, log cumulative of % drug remaining vs. time for first order kinetic model, cumulative %

drug release vs. square root of time for Higuchi model, log cumulative % drug release vs. log time for korsmeyer model and cube root of cumulative % drug release vs. time Hixson-crowell cube root.

In - vivo study

HPLC specification

An analysis was carried on Jasco HPLC instrument equipped with quaternary gradient pump PU-2089 Plus, Photo Diode Array detector- MD-2018 Plus and Hi-Q-Sil C_{18} column (250 mm X 4.6mm, 5µ, particle size [17].

Prepartion of stock solution

25 mg of pure mebendazole was transferred in 25 ml of volumetric flask and dissolve in 15 ml of DMF, sonicated for 15 min. And then finally volume made upto mark by DMF to form stock soln of 1000µ g/ml. The further dilutions made by the developed mobile phase i.e (Acetonitril : Phosphate buffer 55:45 at pH 6.5 adjusted with Ortho-Phosphoric acid) to get concentration range of 10-60 µ/ml by taking appropriate aliquots from the above stock solution.

In- vivo bioavailability measurement

The *in-vivo* studies of optimised formulations of mebendazole, an microemulsion (test) marketed formulation (standard), normal saline (control) was performed in rats. All animals care and procedures were conducted according to the guiding principles in the use of animals in toxicology. Albino Wistar rats weighing 200 - 250 ± 20 g were fasted for 10–12 hours prior to the experiments but were allowed free access to water [18].

Each groups comprises of 6 rats. The rats in each group were administered orally with 2.5 mg of mebendazole preparations (liquid SMEDDS and marketed preparation) as test and standard respectively. Then, 0.25 ml of blood was collected from the right or left Retro-orbital cavity using 1-ml needle at predetermined time intervals and 0.1 ml of plasma was separated by centrifugation of blood samples at 10,000 rpm for 15 min .

Plasma samples were stored at -20 ^{0}C until further analysis. The plasma was then deproteinized with 0.9 ml of acetonitrile and was vortex- mixed for 5 min and then centrifuged at 8000g for 2 min. The residue was reconstituted with 100 µl of acetonitrile and 50 µl of the resulting solution was analyzed by HPLC as mentioned above [19].

Stability studies of microemulsion

Optimised formula of liquid microemulsion were subjected to accelerated stability study at 25°C ± 2°C and 60% RH, 30°C ± 2°C and 65% RH, 40°C ± 2°C and 75% RH respectively in stability chamber (Biotech India) for 1, 2 and 3 months. After completion of time, samples were drawn and analyzed for physical parameters.

Preparation of solid SMEDDS by spray drying

Aerosil 200 (500 mg) was suspended in 200 ml methanol by magnetic stirring. The liquid SMEDDS (12 ml) was then added with constant stirring, and the solution was kept stirring at room temperature for 15 min to obtain a good suspension of Aerosil 200 [20]. The suspension was spray dried by using a spray dryer (Labultima model No.LU-222-Advanced) and following condition were maintained.

Evaluation of solid SMEDDS

IR spectroscopy

The IR spectra of SMEDDS containing solid carrier (Aerosil 200) were recorded using fourier transform infra-red spectrophotometer (Shimadzu 8400-S) with diffuse reflectance principle. Sample preparation involved, drying of potassium bromide (KBr), drug and excipients in the oven to get rid of any moisture content then mixing the sample with KBr by triturating in glass mortar. Finally preparing of pellet and placing in the sample holder. The spectrum was scanned over a frequency range 4000 – 400 cm^{-1} [21].

Dilution study by visual observation

Dilution study was done to observe the effect of dilution on solid microemulsion, because dilution may better mimic the condition of stomach after oral administration. In this method, solid SMEDDS (100 mg) was introduced into 100 ml double distilled water in a glass beaker that was maintained at 37^0 C and the contents mixed gently using a magnetic stirrer. The emulsification ability of SMEDDS judged qualitatively "good" when clear microemulsion formed and "bad" when there was turbid or milky white emulsion formed after stopping of stirring [22]

Droplet size determination

Optimised formulation of solid microemulsion is dispersed in 100 ml of water and kept it on magnetic stirrer for 1 min. Sample were allowed to stand for 10 min so that colloidal silicon dioxide particle could sediment. The supernatant was filtered through coarse filter (Whatman paper) and the filtrate was used for globule size analysis. The average globule size was measured using a Nanophox (NX0088), Cross corrélation. The measurement was performed at 25°C [23].

Polydispersity index

Polydispersibility which determines size range of particles in the system, it is expressed in terms of polydispersibility index (PDI). An

ideal SMEDDS should be widely distributed with particles less than 100 nm and so PDI should be less than 0.3 or in other words particles having size more than 100 nm should be maximum up to 23%.

(D0.9 - D0.1) / D0.5

i.e (X90 - X10) / X50

Drug content

Drug content was estimated by extracting mebendazole from solid microemulsion. In brief solid microemulsion was dissolved in sufficient quantity of methanol. Solution was bath sonicated for 10-15 min for extraction of the mebendazole in methanol and filtered. The absorbance of filtrate was read at 300 nm on UV- visible spectrophotometer [24]

In- vitro drug release studies

Dialysis membrane was used to carry out release of mebendazole suspension. Mebendazole SMEDDS containing 10 mg of drug was placed into dialysis bag (Himedia, molecular weight cut off 12000 da). *In- vitro* drug release of all formulas was carried out using USP- type II dissolution apparatus (paddle type). The dissolution medium, 900 ml 0.1 N HCl and 1% SLS was placed into the dissolution flask maintaining the temperature at 37 ± 0.5^0C and speed of 100 rpm. Dissolution studies were carried out for 2 hours. 5 ml of aliquot is withdrawn at an interval of 5, 10, 20, 40, 60, 80, 100, 120 min. After collecting the sample, the dissolution medium was replenished with the same volume of fresh medium, and the sample was filtered. The samples were then analyzed at 300nm by UV- visible spectrophotometer.

In - vivo Study

HPLC specification

Procedure same as that in evaluation of microemulsion.

In - vivo bioavailability measurement

Procedure same as that in evaluation of microemulsion.

Result and Dicussion

Solubility studies

One important consideration when formulating a self emulsifying formulations is avoiding precipitation of drug on dilution in the gut lumen *in-vivo*. Therefore the component used in the system should have high solubilisation capacity for the drug, ensuring the solubilisation of the drug in the resultant d9-ispersion. Oleic acid shows the highest solubilisation capacity than other oil for mebendazole (5.1816 mg/ml) , followed by tween 80 (9.7579 mg/ml) and propylene glycol (1.9250 mg/ml) . Thus for further study Oleic acid as oil and tween 80 and propylene glycol as surfactant and co-surfactant respectively was selected.

Construction of phase diagram

The pseudo ternary phase diagrams for different surfactant: co-surfactant ratios are as shown in figure 1. All diagrams showed the maximum amount of surfactant, cosurfactant required to form a microemulsion. Highlighted part in each phase diagram shows the region in which two immiscible phases exist. Whereas all plotted points indicate the instantaneous formation of microemulsions for respective oil to water ratios with specific amount of surfactant and cosurfactant. From diagrams it can be concluded that with at ratio of surfactant: cosurfactant 2:1 the large microemulsion region was observed compared to 1:1 and 3:1. Therefore 2:1 ratio of surfactant and co-surfactant was selected for preparation of microemulsion.

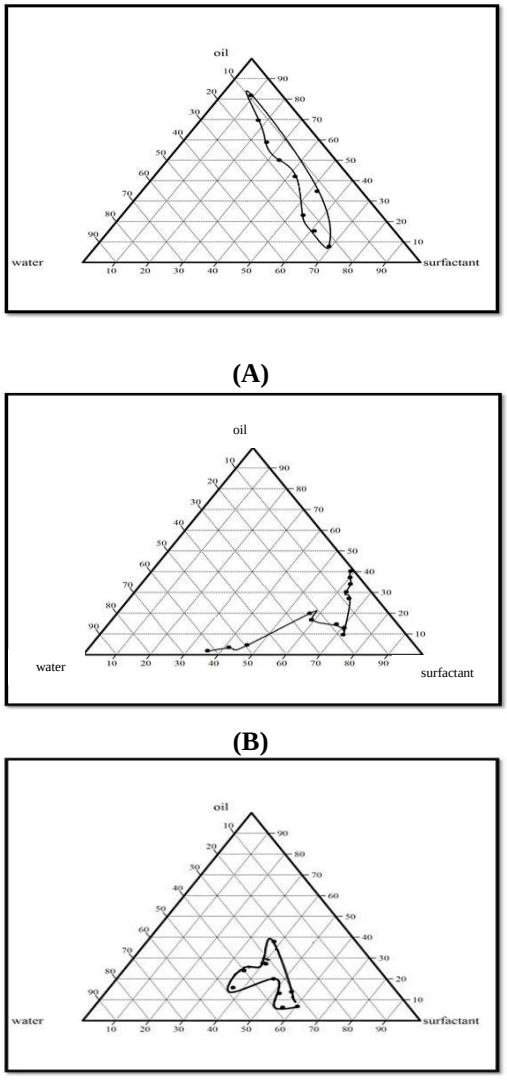

Figure 1: Pseudoternary phase diagram (A) ratio 1:1 (B) ratio 2:1 (C) 3:1.

Evaluation of microemulsion

Optical transparency

All formulas of liquid SMEDDS were transparent .

Phase separation study

None of the microemulsion formulation showed signs of phase separation on centrifugation at 15,000 rpm for 15 minutes. This result provided a rapid and full proof identification of the system as micro - emulsion, and which is sign of stability of microemulsion [25,26].

Emulsification test

A visual test was carried out to assess self emulsification of liquid SMEDDS in 100 mi of distilled water at 37 ^{0} C under gentle agitation. All solid SMEDDS formulations showed spontaneous microemulsification (< 1 min) and there was no sign of phase separation of microemulsification.

Determination of globule size

An increase in ratio of oil phase resulted in the proportional increase in particle size. Formula B1 shows the least globule size (Figure2) as compare to the all six micro-emulsion formulas. It is well known that the addition of surfactants to the microemulsion system causes the interfacial film to stabilize and condense, while the addition of cosurfactant causes the film to expand thus the relative proportion of surfactant has varied effect on the droplet size [27].

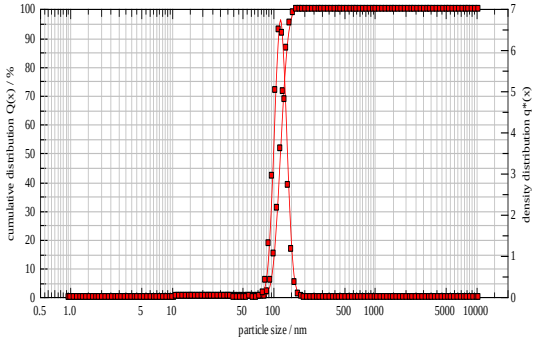

Figure 2: Droplet size distribution of microemulsion. (Batch no B1)

Polydispersity index

Table 2 showed polydispersity index of microemulsion.

Table (2) Polydispersity index of microemulsion

Batch	PDI
B1	0.360
B2	0.280
B3	0.291
B4	0.311
B5	0.320
B6	0.281

Viscosity measurement

It was observed that there is increase in viscosity as increasing oil concentration. Formula B9 which contains higher amounts of oil therefore it shows highest viscosity(155 cps) as compared to all formulas. Formula B1 shows least viscosity (78 cps) so it contain less oil in formula [28].

Zeta potential determination

A dividing line between stable and unstable aqueous dispersions is generally taken at either +30 or -30 mV. Particles with zeta potentials more positive than +30 mV are normally considered stable. Particles with zeta potentials more negative than -30 mV are normally considered stable [13]. Figure 3 given that zeta potential was 31.5 mean result microemulsion normally stable.

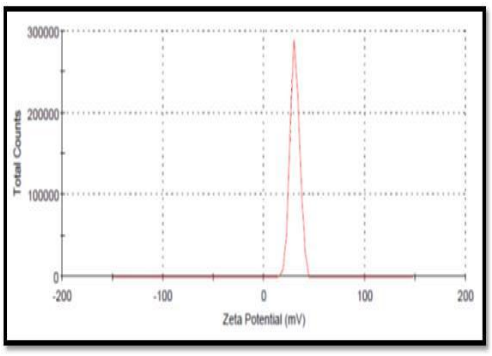

Figure 3: Zeta potential of microemulsion

SEM photomicrograph of microemulsion

Figure 4 showed SEM of microemulsion (Batch B1).

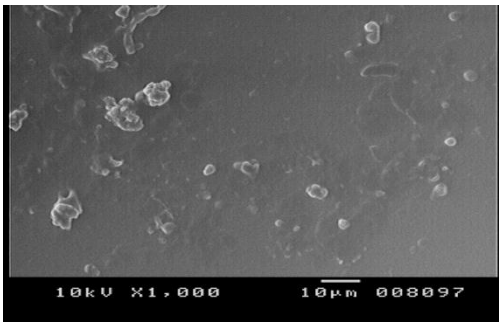

Figure 4. SEM of microemulsion (Batch B1)

In - vitro dissolution study

In-vitro drug release profile of mebendazole was carried out for developed formulation (microemulsion) and marketed formulation. After 2 hr, the amount of drug released from marketed formulation was 66.95% ± 0.04562 and for microemulsion was 101.1 % ± 0.213 as showen in figure 5.

The best fit model was found to be Koresmayer Peppas kinetic model equation plot (r2 = 0.9985 microemulsion) indicating the dissolution rate limited drug release from a SMEDD formulation. The n value (0.1818) indicate the mechanism of drug release was super case II transport.

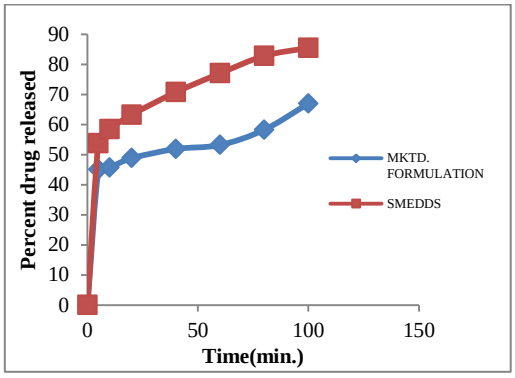

Figure 5: Percentage drug release of microemulsion and marketed formulation

***In - vivo* studies**

Figure 6 shows the plasma concentration profiles of mebendazole after oral administration of various formulations to rats. The AUC of mebendazole in optimised formula increased 2 fold compared with that of mebendazole in the orally administrated marketed preparation (10.1464 vs. 5.7758 µg/mL* h). The C_{max} was higher 3 fold in microemulsion with that of the orally administrated suspension (3.6954 vs 0.2445). Thus, the oral bioavailability of mebendazole increases 2 fold in microemulsion as compared to marketed preparation. The improved oral bioavailability of mebendazole in the microemulsions could be explained by the combination of the following effects: (1) significantly improved solubility of mebendazole by microemulsions which could keep the drug as the soluble form during the gastrointestinal dilution and permeation process; (2) the synergistic effect of oil and surfactants as absorption enhancers; (3) the inhibition of P-gp efflux [29].

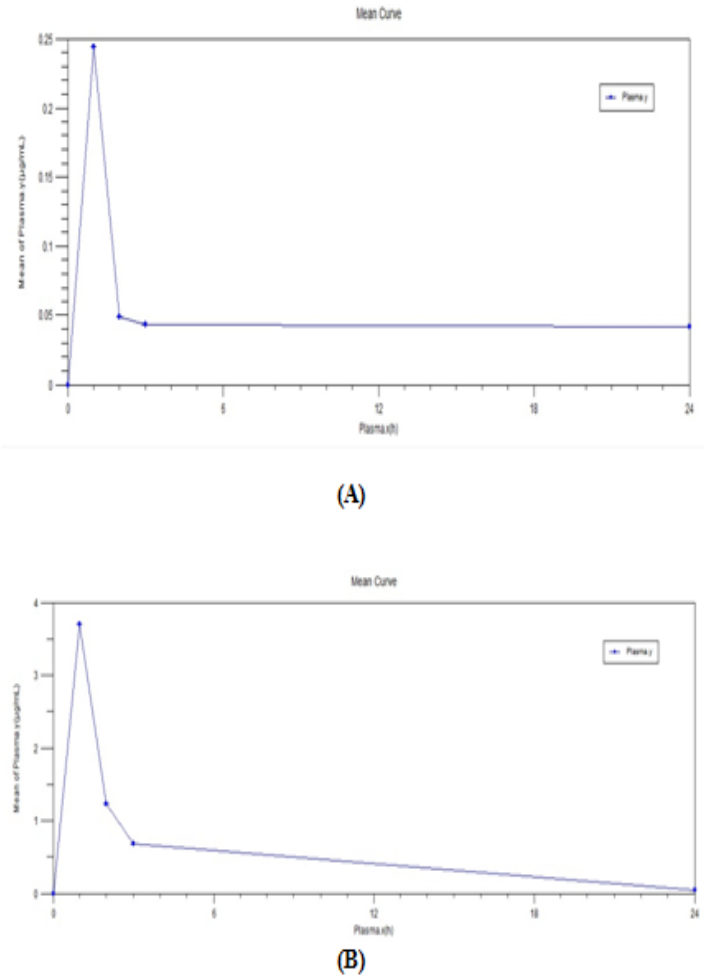

Figure 6: (A) Plasma Drug Concentration of Marketed preparation (B) Plasma drug concentration of microemulsion Stability studies

There are no phase separation found after optimised formulations of microemulsion were subjected to accelerated stability study at 25°C ± 2°C and 60% RH, 30°C ± 2°C and 65% RH, 40°C ± 2°C and 75% RH respectively in stability chamber (Biotech India) for 1, 2 and 3 months.

Evaluation of solid SMEDDS

IR spectrum

The FTIR absorption spectrum of mebendazole and its solid SMEDDS is shown in figure 7. FTIR spectrum of mebendazole showed all the peaks corresponding to the functional groups present in the structure of mebendazole. The band at 1720 cm^{-1} is due to C = O Amide stretching, 1650 cm^{-1} is due to C=O stretch, 2795 cm^{-1} is due to C-H stretch, 3415 cm^{-1} is due to N-H stretching.

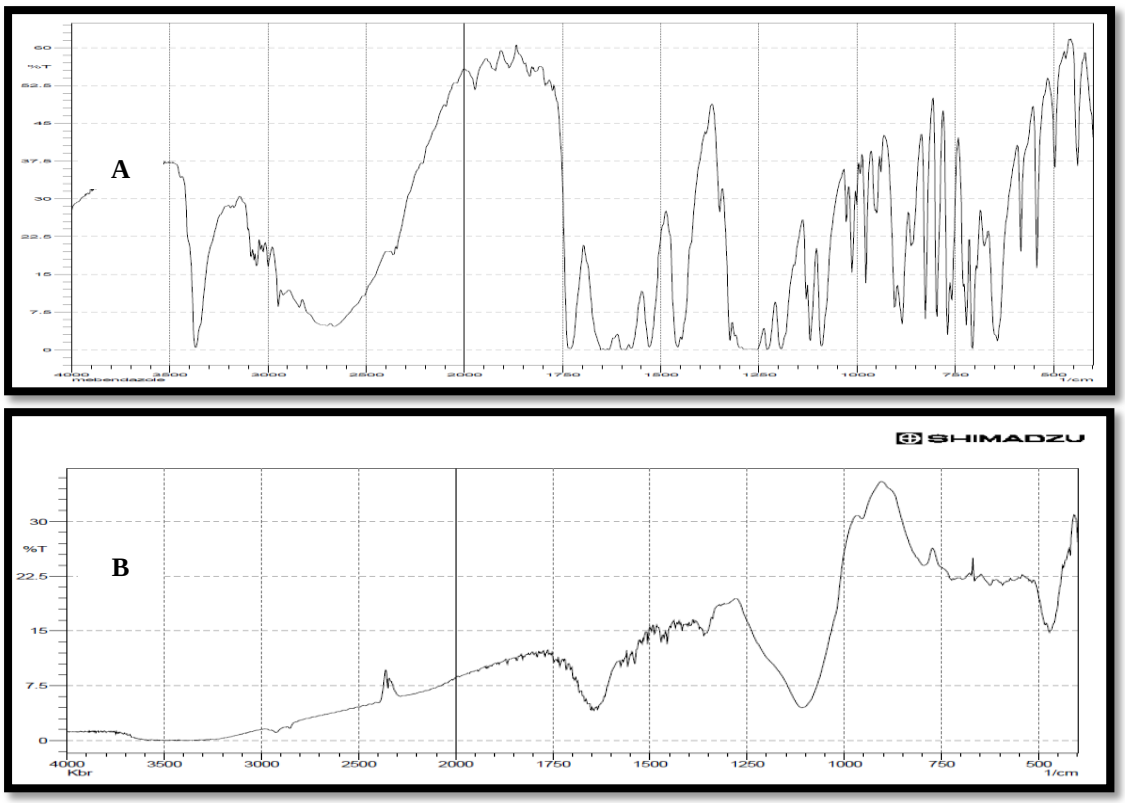

Figure 7: IR spectrum of (A) mebendazole (B) solid SMEDDS

Dilution study

Dilution study was done to observe the effect of dilution on SMEDDS, because dilution may better mimic the condition of stomach after oral administration. In this method, SMEDDS (100 mg) was introduced into 100 ml double distilled water in a glass beaker that was maintained at 37^0 C and the contents mixed gently using a magnetic stirrer. The emulsification ability of SMEDDS judged qualitatively " good " when clear microemulsion formed and "bad" when there was turbid or milky white emulsion formed after stopping of stirring [30, 31].

Droplet size measurement

The droplet size (Figure 8) of selected formula of solid SMEDDS (Batch B1) was 401 nm.

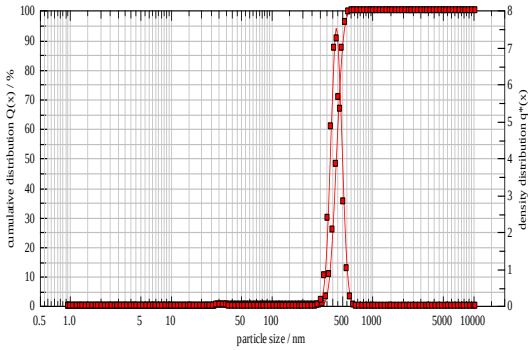

Figure 8. Droplet size distribution of SMEDDS formulation

Polydispersity index

Polydispersity index of selected formula of solid SMEDDS (Batch B1) was 0.281.

Drug content

Percentage of drug content in selected formula of solid SMEDDS (Batch B1) was within USP limit (97.22 %).

***In - vitro* dissolution studies**

In-vitro drug release profile of mebendazole was carried out for developed formulation (SMEDDS). After 2 hr, the amount of drug released from the SMEDDS was 85.47% ± 0.1614 as showen in figure 9.The best fit model was found to be Koresmayer Peppas kinetic model equation (r^2 = 0.9985) indicating the dissolution rate limited drug release from a solid SMEDD formulation. The n value (n = 0.1496) indicate the mechanism of drug release was super case II transport.

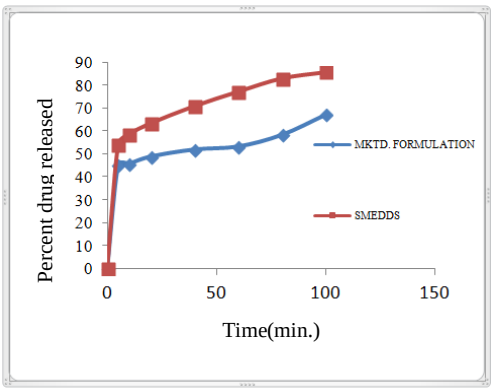

Figure 9: Percentage drug release of SMEDDS and marketed formulation

In - *vivo* study

Figure 10 shows the plasma concentration profiles of mebendazole after oral administration of various formulations to rats. The AUC of mebendazole in optimised formulation increased 1.8 fold compared with that of mebendazole in the orally administrated marketed preparation (8.3145 vs. 5.7758 μg/mL* h). The C_{max} was 6 fold higher in SMEDDS as compared with that of the orally administrated suspension (6.2015 vs 0.2445). Thus, the oral bioavailability of mebendazole was 1.8 fold higher in (SMEDDS) as compared to marketed preparation.

The improved oral bioavailability of mebendazole in the microemulsions could be explained by the combination of the following effects: (1) significantly improved solubility of mebendazole by microemulsions which could keep the drug as the soluble form during the gastrointestinal dilution and permeation process; (2) the synergistic effect of oil and surfactants as absorption enhancers; (3) the inhibition of P-gp efflux [29].

Conclusion

Self micro emulsifying drug delivery system has become promising tool to overcome shortcomings associated with conventional delivery. With several advantages like increased bioavailability, faster drug release, reduced dose, reduced dosing frequency, and better patient compliance, gives wide scope in research for lipid based drug delivery system.

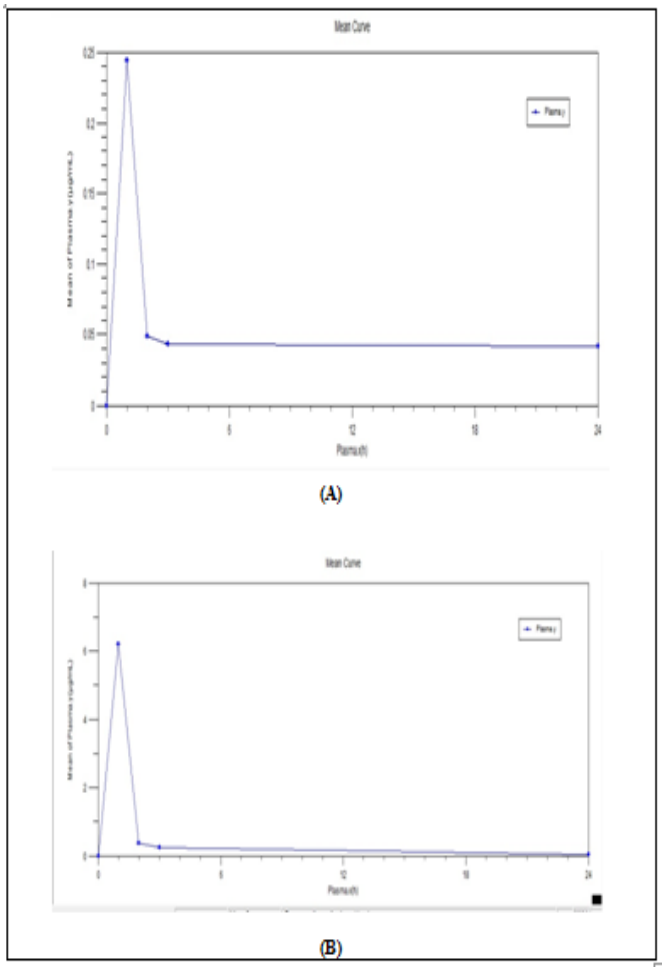

Figure 10. Plasma drug concentration of (A) marketed formulation (B) Plasma drug concentration of SMEDD

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