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Formulation and Evaluation of Idebenone Microemulsion as a Potential Approach for the Transmucosal Drug Delivery Systems

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Abstract

Idebenone, a synthetic analogue of ubiquinone (Co-Q10) is used for the treatment of Leiber's hereditary optic neuropathy. Although it is almost completely absorbed from GIT, its extensive rate of metabolism makes its oral bioavailability less than 1%. This study aims to formulate the poorly water-soluble drug as microemulsion (ME) to increase drug solubility as well as transmucosal permeation and circumventing hepatic biodegradation by using transmucosal routes of administration such as intranasal route to enhance drug bioavailability. Microemulsion components were selected through the screening of the preferential solubility of IDB in several oils and emulsifying agents then subsequent formulation optimization through screening for their best miscibility through a phase study. Lemongrass oil showed superior characteristics to represent the oil phase in which the drug was dissolved while Cremophor EL® and Transcutol-P® represented the surfactant system. The method of spontaneous-emulsification was used to prepare twelve ME formulas (F1-F12) which were subjected to several characterization tests. Out of four successful formulas, F-11 showed the best globule size (42nm), PDI (0.172) zeta-potential (-6.4 mV), low viscosity and suiTable pH (5.6). Using a sheep nasal mucosa as model, (F-11) was subjected to ex-vivo transmucosal permeation study against plain drug oil dispersion with pronounced enhanced permeation due to formulation as ME. Permeation kinetic parameters were obtained such as permeation rate 40.9 µg/h/cm^2, lag time 22min, permeability coefficient 40.9x10^{-4} cm/h and enhancement ratio 37.7 times. The outcome of this study indicates the possibility of incorporation of this formula into a suiTable carrier and can be optimized to be administrated as non-enteral dosage form with enhanced IDB bioavailability.

Keywords: Bioavailability, Idebenone, Microemulsion, Permeation, Solubility.

تصييغ وتقييم مستحلب الايدبنون المايكروي كوسيلة محتملة لايصال الدواء عبر الاغشية المخاطية

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الخلاصة

الايدبنون هو مثيل صناعي لليوبيكينون (كو-كيو ١٠) يستخدم لعلاج اعتلال العصب البصري الوراثي نوع ليبر . بالرغم انه يمتص كاملا تقريبا من القناة الهضمية الا ان معدل استقلابه العالي يجعل من توافره الحيوي اقل من ١ %. تهدف هذه الدراسة لتصييغ الدواء قليل الذوبانية كمستحلب مايكروي لزيادة ذوبانيته وقابلية تعابره عبر الاغشية المخاطية وتلافي التأيض الكبدي عن طريق اعطائه عبر الاغشية المخاطية كالانف لزيادة توافره الحيوي.

مكونات المستحلب المايكروي تم اختيارها عن طريق اجراء مسح لذوبانية الدواء في عدد من الزيوت والمستحلبات تليها تدقيق تلك النتائج عن طريق اجراء فحص قابلية الامتزاج مع بعضها ودراسة الاطوار. زيت عشبة الليمون اظهر خواص متقدمة ليمثل الطور الزيتي والذي سيتم اذابة الدواء فيه في حين ان مستحلب الكريموفور أي ال مع الترانسكيوتول مثلا نظام الاستحلاب. تم استخدام طريقة الاستحلاب الذاتي لتصييغ اثني عشر تركيبة (من ١-١٢) والتي خضعت لعدة فحوصات تقييمية. من مجموع اربع تركيبات ناجحة اظهرت التركيبة رقم ١١ افضل حجم للقطيرات ٤٢ نانو متر, مؤشر تعدد الانتشار ٠,١٧٢ وفرق جهد زيتا -٦,٤ ,لزوجة قليلة واس هيدروجيني مناسب ٥,٦. تم باستخدام غشاء مخاطي مستأصل من الخراف اجراء دراسة العبور خلال الاغشية المخاطية للأنف وباستخدام المحلول الزيتي للمادة كمقارن وأظهرت الدراسة قيم عالية نتيجة لتحضير المادة كمستحلب مايكروي وتم دراسة مخرجات الدراسة الحركية كمعدل العبور والذي بلغ ٤٠,٩ مايكرو جرام/س/سم مربع ومعامل العبور ٤٠,٩ x ١٠-٤ ووقت التأخر الذي يساوي ٢٢ دقيقة ومعدل الحث على الامتصاص =٣٧,٣ مرة ان هذه النتائج تؤشر إمكانية اكمال تصييغ الدواء كشكل صيدلاني لا فموي مع زيادة توافر الدواء حيويا.

الكلمات المفتاحية: ايدبنون ، تعابر، توافر حيوي، ذوبانية، مستحلب مايكروي.

Introduction

Transmucosal drug permeation to circulation bypassing hepatic metabolism is an approach that is frequently proposed to protect drug substances that are extensively metabolized by the liver. It can be achieved through several routes of which nasal administration is an attractive one. Besides being accessible, non-invasive route of administration , there are other advantages that characterize nasal route such as a suiTable surface area and high blood perfusion. Microemulsions are one of the most important pharmaceutical approaches that can mediate drug delivery systems. They are either o/w or w/o colloids of globule size between (10-100nm) [(1)].

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Microemulsions can be loaded with different lipophilic or hydrophilic drugs. Microemulsions can increase drug bioavailability through increasing drug solubility and through the enhancement of permeation process. In addition, MEs can be formulated as oral or non-enteral dosage form like (nasal, transdermal, ophthalmic and parenteral dosage form) to overcome the problem of extensive hepatic metabolism of the drug[2].

Idebenone (IDB) is a quinone-like drug with shorter side chain relative to the natural ubiquinone (Co-Q10)[3], an enzyme that is involved in the respiratory chain of the mitochondria. Idebenone has potent antioxidant activity and therefore it acts as free radical scavenger that can protect cellular and mitochondrial membranes against oxidative stress leading to prolong their integrity and viability over time [4]. In 2015, IDB was approved by EMA to treat Leiber's hereditary optic neuropathy (LHON)[5], which is an autosomal recessive neurodegenerative disease of mitochondrial origin characterized by gradual loss of vision acuity ending with complete blindness with no cure other than IDB[6]. To attain effective therapeutic level of the drug in the circulation, IDB is given in an oral dose of 900mg/day [8]. This relatively high dose is mandatory to overcome the extensive hepatic metabolism and to adequately reach blood circulation. According to BCS system of classification, IDB belongs to class II with high permeability and low solubility (8mg/L)[7]. Microemulsions have higher drug loading capacity comparing to other drug carrier such as solid lipid nanoparticles and liposomes and can protect against direct drug exposure[8]. Therefore, after taking these two reasons into account it is favorable to use ME as solubility enhancing system for IDB with subsequent incorporation into a nasal drug carrier such as sprays taken into consideration the low dosage volume of most nasal sprayers that do not exceed 200µL/actuation.

Materials and Methods

Materials

Idebenone was purchased from (XI'An Geekee Biotech Co, Ltd, China). Corn oil, sun flower oil, safflower oil, castor oil, codliver oil, lemongrass oil (LGO), olive oil, and cinnamon oil were purchased from (Shaanxi Guangie technology Co.,Ltd, China). Tween 20® (Tw20), tween 60® (Tw60) and tween 80®(Tw80) were purchased from (Hefei TNJ chemical industry Co., Ltd, China). Cremophor EL® (CrEL) was purchased from (Shanghai Taijie chemical Co., Ltd, China). Transcutol P® (TC) was purchased from (Dayhang Chemicals Co., Ltd hangzou, China). Propylene glycol (PG), ethylene glycol (EG), polyethylene glycol 400 (PEG 400) and triethanolamine (TEA) were purchased from (Thomas Baker chemicals PVT, Ltd, India). Absolute ethanol was purchased from (Merk KGaA Germany). NaCl, KCL, $CaCl_2$, Na_2HPO_4 and KH_2PO_4 were purchased from (Green land chemical comp.U.K).

Methods

Determination of saturated solubility

Saturated thermodynamic solubility of IDB was calculated using shake-flask method by the addition of an excess amount of IDB to 5 ml of each oil, surfactant, co-surfactant and both simulated nasal saline (SNF) and phosphate buffer saline (PBS) in 10 ml flask, mixed by vortex shaker and kept in thermostat-controlled water bath with 50 rpm shaker at 25°C for 72h [9]. Saturated solubility of IDB at 25°C was screened in many essential oils (corn oil, sun flower oil, safflower oil, castor oil, codliver oil, lemongrass oil, olive oil, and cinnamon oil, surfactants (Tw 20, Tw 60, Tw 80, CrEL), and co-surfactants (TC ,PG, EG ,PEG 400 and TEA). The solvents and media used were absolute ethanol, SNF pH=6.4 with 1% Tw80 and PBS pH=7.4 with 1% Tw80. The mixtures were centrifuged at 6000 rpm for 15 minutes and the supernatants were carefully withdrawn, filtered using syringe filter 0.45µm, appropriately diluted with ethanol and the concentration could be calculated after determining the sample absorbance using UV-visible spectrophotometer at wavelength equals 279nm. The same procedure was followed to measure the drug solubility at 34°C for the selected components.

Selection of the best emulsifying system and co-surfactant

Several emulsifying system mixtures (Smix) were prepared by mixing the selected surfactant of the highest drug solubility with different candidate co-surfactants. In this study, LGO with CrEL was mixed with (TC, PG, EG, PEG 400 and TEA) at different ratios. For each prepared Smix, a ternary plot was established by phase inversion composition (PIC) method [10] which include carefully titrating 10g of incremental Smix:oil ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) with DIW under continuous magnetic stirring at (300 rpm) with careful visual and electrical conductivity monitoring by mean of digital conductometer[11]. Results were fed into an online plot software (ternaryplot.com) and the ternary plots were obtained. The best co-surfactant can be validated by the ternary plot of the highest shaded area of single phase that represents the ratio of most sTable ME system.

Preparation of IDB-loaded ME

The method of spontaneous-emulsification was followed to prepare the drug-loaded MEs formulas. The method involves separately preparing of both oil and aqueous phases. The oily phase was prepared by dissolving the drug substance in the oil phase at 50°C, while the aqueous phase has been made by the addition of water-soluble surfactant/co-surfactant (CrEL/TC) to the de-ionized water followed by warming up the mixture up to 50°C.

The oily phase was then added slowly to the aqueous phase under vortexing to produce a clear and transparent ME. The prepared MEs were left to cool down at room temperature, labeled and stored in tightly closed containers.

Characterization of the IDB-loaded MEs

Determination of ME type

Emulsions, whether w/o or o/w can be distinguished by dye test utilizing either water soluble dye (e.g., methylene blue and amaranth). These dyes could be added to the prepared MEs with gentle shaking then subsequent examination under light microscopy for the phase at which the dye was occupying would reveal ME type [12]. Fast and easy dispersion of methylene blue without any aggregations or flocculation in the continuous phase indicates o/w emulsion.

Thermodynamic stability tests

Samples were consequently subjected to the following conditions to prove stability of MEs before conducting any further tests. First, centrifugation test of MEs was carried out at 4000 rpm for 15 min[13] and any sample with signs of phase separation, cracking or creaming were excluded. Then, the heating-cooling test was done in which the MEs samples were subjected to six rounds of consecutive heating to 45°C followed by cooling to 4°C within 48h. Finally, the samples were subjected to three cycles of freezing thawing between -20°C and 25°C[14] with continuous observation of the integrity of MEs physical state such as separation or cracking.

Dilution test

The dilution test was carried on where a specified amount equals to 0.1g from each of the prepared MEs was diluted with external phase (DIW) in two ratios ,1:50 and 1:100. These diluted samples were set aside for subsequent visual inspection for any precipitation or turbidity that indicates instability issue[15].

Determination of average globule diameter and poly dispersity index (PDI)

Mean globule diameter of the prepared MEs was determined using Zetasizer (Nano ZS red label, Malvern, UK) operated at 25°C, the angle of the incident light was 173° to decrease back scattering and it was equipped with optical filter[16]. Zetasizer relies on light diffraction technique. It depends on the measurement of the fluctuation intensity of an incident laser beam through colloidal dispersion and analyzing the diffraction pattern by using certain algorithms that compare the obtained pattern to an already built-in data. Samples of MEs were diluted 100 times to avoid multiple diffraction by using ultra purified water and were introduced by disposable polystyrene cell to zetasizer. Analysis report generated by software 2.0.1.1 revealed Z-average in nm in addition to the PDI which is a scale from 0 to 1 that represents the size distribution or homogeneity of the globules size within the sample[17].

Determination of Zeta potential

Using DTS 1070 zeta-cell, samples prepared as in the previous section were introduced into the zetasizer to measure the potential of the diffused layer (zetapotential). It is an important parameter in defining the stability of MEs. Particles with zeta potentials of ±30 mV are generally considered sTable[18]

Drug content uniformity

An equivalent weight to 10 mg of IDB from the prepared ME was withdrawn, diluted adequately by absolute ethanol, and drug content was calculated based on sample absorbance using UV-visible spectroscopy at maximum absorbance at 279nm versus absolute ethanol as blank and drug content uniformity was obtained using a previously performed calibration curve. Measurements were taken as mean value of triplicate measurements and the standard deviation was calculated for each formula.

Viscosity

At room temperature, the viscosity of the prepared MEs was tested using rotatory disc type digital viscometer NDJ-5S, China, supplied with spindles 1-4 for different viscosity ranges. Spindle No. 1 was chosen according to its viscosity limits and was changed with spindle No. 2 whenever necessary to maintain effective torque ratio between (15-85%) as per instrument manual. The shear force was increased at 4 pre-defined speed (12, 30, 60, 100) and the correspondent dynamic viscosity was measured in units of (mPa.sec) [19].

Light transmittance

Using the UV-visible spectrophotometer with wavelength that is already set at 600 nm and after the dilution of each sample 100 times[20] the percent of the light transmittance could be obtained for the prepared MEs. Light transmittance is an indication of ME clarity, and it is assigned as clear when transmittance exceeds 99%[21].

Ex-vivo permeation study

Permeation of the prepared MEs through excised nasal mucosa was tested using the anterior turbinate mucosa of a recently sacrificed sheep. A lateral incision into the skull was made to expose the inner anatomy of the sheep nasal cavity, the turbinate was extracted, and the mucosa was excised carefully from the underlying bone and tissues. The collected tissue was cut, washed with PBS of pH 7.4 and preserved cooled at 4°C until mounted in 20-ml vertical Franz-diffusion cell that has an effective permeation surface area of 3.14 cm^2. The obtained mucosa thickness was measured using Mitutoyo digital thickness meter and the surface area obtained was more than 3.14 cm^2. The Franz cell was kept in

thermoregulated water bath at 34°C during the experiment to simulate the temperature of the nasal cavity. The lower receptor chamber was filled with (PBS with 1% Tw80, pH7.4±0.1) without any entrapped air bubbles and stirred at 600 rpm using magnetic stirrer. The excised mucosa was fixed in such a way that the upper part was facing the donor chamber and was left for 30 min for equilibrium. A defined amount of ME that is equivalent to 10 mg IDB was applied to the donor chamber. Aliquots of 1ml from receptor chamber were withdrawn at a predetermined points of time (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5 and 3.0h), filtered using 0.45µm syringe filter and its IDB concentration was measured. The media in the receptor chamber was replenished with the same amount of fresh PBS solution each time to preserve sink condition[22].

Permeation test data analysis

The permeation profile was constructed by plotting the cumulative amount of IDB permeated per unit area (Q,µg/cm^2) across the nasal mucosa on the Y-axis against its corresponding time (t, h) on the X-axis. This plot was used to obtain:

Permeation rate or transmucosal flux of IDB at the steady state (Jss, µg/cm^2/h), which was obtained from the slope of straight linear portion of regression line as given in the following equation:

$$J_{ss} = dQ / dt \quad \text{……. (Equation 1)}$$

Lag time (Tlag) was obtained from the intercept of regression straight steady state line with time axis.

Permeability coefficient (PC, cm/h), which was obtained by dividing the ratio of drug flux (Jss) by the initial concentration (C_0) of IDB in the tested ME formula as shown in the following equation:

$$PC = Jss / C_0 \quad \text{…. (Equation 2)}$$

Enhancement ratio (Er), which was obtained by dividing the permeation rate or flux (Jss) of IDB from each tested ME formula by the flux (Jss) of pure IDB oily dispersion (control) as given in the following equation:

Er = Jss of IDB formulation / Jss of IDB control …. (Equation 3)[23,24]

Fourier-transform infrared spectroscopy (FTIR) of the selected formula

For chemical stability test of the selected formula, with best particle size, PDI, zeta-potential, dissolution profile and pH, it was submitted to Fourier transform-Infra red spectroscopy (FT-IR) (Shimadzu, Japan) covering the range (4000-400 cm^{-1}). Formula F-11 ingredients were tested individually and then their physical mixtures. The compatibility of each component was determined by comparing FT-IR spectra for the presence or absence of specific bands that are corresponding to chemical structural variation before and after formulation [25].

Results and Discussions

Selection of ME components

Components selection of ME is generally based on their safety profile, quality, efficacy, and stability. Regarding the safety, all the selected materials were chosen from GRAS lists according to US-FDA CFR [26]. The results of the thermodynamic saturated solubility are shown in Figure (1) in which LGO oil showed superiority over both cinnamon oil and castor oil in solubilizing maximum quantity of the drug. Lemongrass is found in herbal remedies for coughs, ophthalmic, pneumonia and vascular disorders. Furthermore, it has certain properties which are essential for any formulation stability e.g., antioxidant, antiseptic, astringent, bactericidal, fungicidal, and tolerability during storage. As surfactants, CrEL had the best drug solubility over Tw 20, Tw 60 and Tw 80. In-vitro studies has shown that CrEL has the ability to modulate p-glycoprotein, a carrier enzyme in the cell membrane involves in drug resistance through efflux mechanism[27]. TC is 2-(2-Ethoxyethoxy) ethanol, a widely used molecule in pharmaceutical preparations as solvent, cosolvent and emulsifying agent, has the higher drug solubility as co-surfactants and follow the order in which TC>PEG 400> EG> PG.

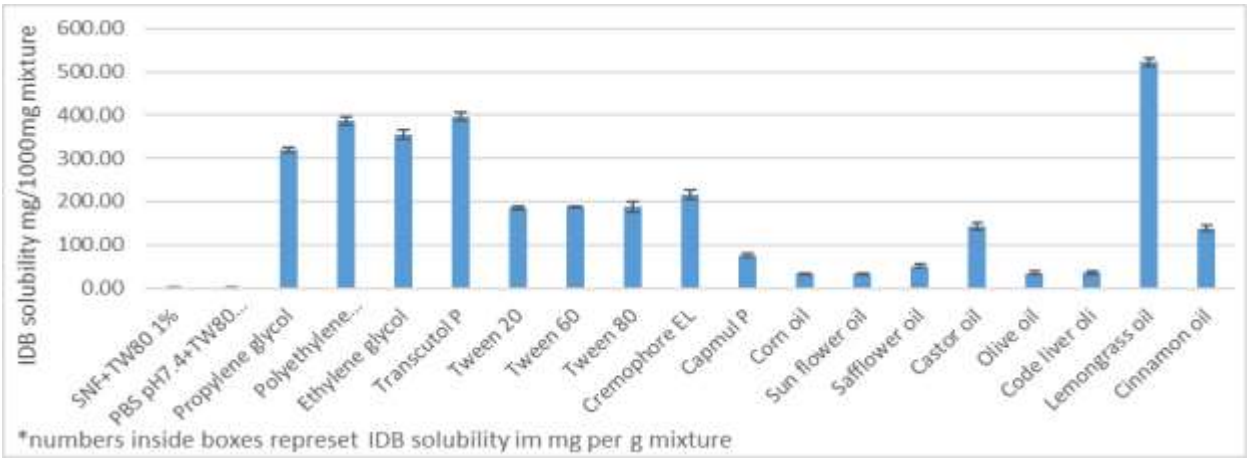

Figure 1. Saturated solubility of IDB at 25°C expressed as milligram weight of the drug per one gram of each component

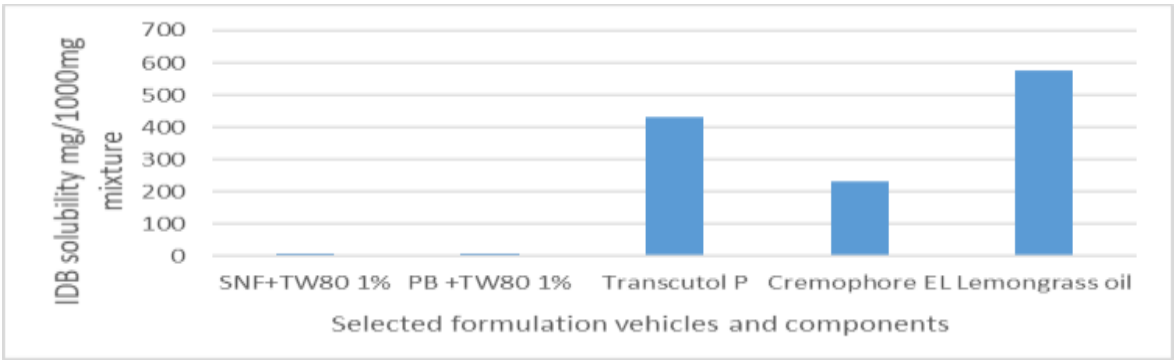

Figure 2. Saturated solubility of IDB at 34°C expressed as milligram weight of drug per one gram of the selected components

All the surfactants that are used in this study have high HLB value and therefore tend to form o/w MEs(28). Cremophor EL has lower HLB value=12-14 among other surfactants used in this study therefore it was able to form the most stabilized ME [29] Figure (1) revealed the solubility of IDB in the selected formulation components and vehicles.

Selection of the best emulsifying system, co-surfactant and establishment of blank ternary phase diagrams

In this study, LGO with CrEL were chosen as emulsifying system for their higher drug solubilizing capacity while the co-surfactants that were tested were (TC, PG, EG, PEG 400). The best result in term of highest ME shaded area in ternary phase plot was obtained with TC. It was noticed that increasing the CrEL:TC ratio from 1:1 to 8:1 gives CrEL the best emulsification capacity with optimum system stability as in Figure (3). This is attributed to the decrease in the interfacial tension to the limit that enables the formation of stabilized drug-oil droplet. These results also were obtained in previous work [30]. The constructed ternary phase diagrams were used to map the optimal drug-loaded formulas that are shown in Table (1).

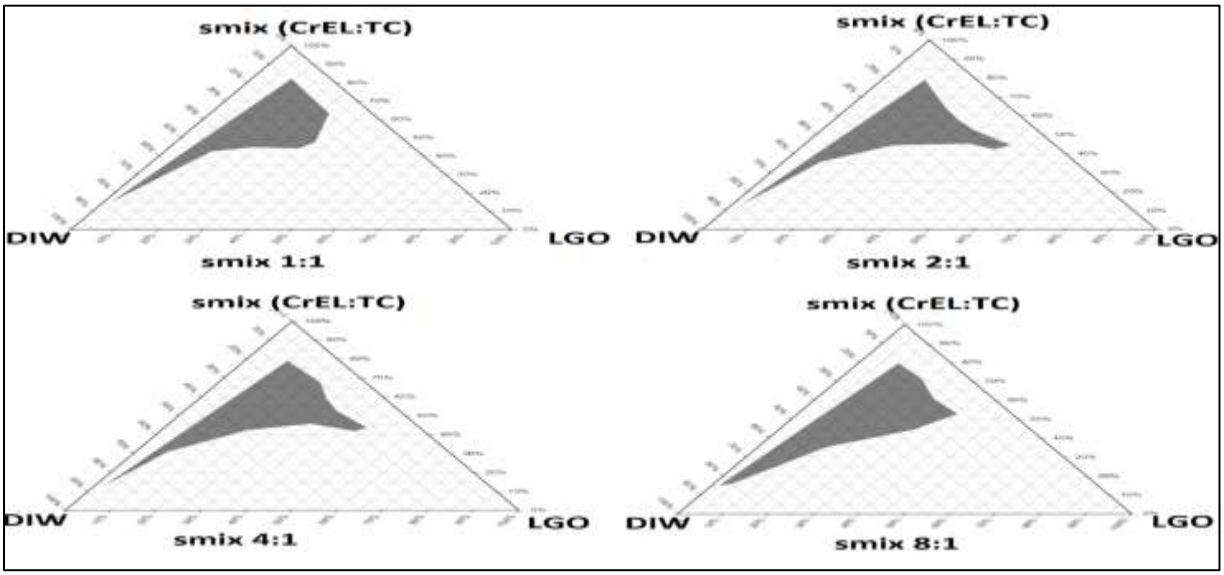

Figure 3. Ternary phase plots of different Smix ratios.

Preparation of ME formulas

Twelve drug loaded formulas that have shown in (Table 1) (F1-F12) were prepared using the self-emulsification method already discussed. Self-emulsification is one of the low energy methods in which emulsification energy is obtained from physical interaction between molecules. All formulas were inspected visually, and no formula exhibited any kind of turbidity, phase separation, creaming or cracking. The obtained MEs were labeled and loaded in tightly closed glass jars for further investigations. However, changes in phase behavior should be monitored carefully before and after the addition of the drug. In this study the amount of the drug-loaded oil was fixed to 20% in all formulas to ensure that the amount of the drug was fixed as well. In this context, the outcomes of the saturated solubility of the drug in LGO should be respected even though the other ME components can contribute to the total drug solubility. Any extra

amount of the drug beyond solubilizing capacity could be liable for precipitation especially upon ME dilution because of changing dielectric constant[31]. However, in this study drug loading wasn't exceeding LGO solubilizing potential in both 25 and 34°C.

Table 1. Formulas of IDB-loaded MEs

Quantities represented as parts (gram)					
	Code	**Smix (CrEL:TC)**	**10:10 IDB-LGO**	**DIW**	**Total wt.**
Smix 1:1	F-1	45 (22.5:22.5)	20	35	100
	F-2	55 (27.5:27.5)	20	25	100
	F-3	65 (32.5:32.5)	20	15	100
Smix 2:1	F-4	45 (30:15)	20	35	100
	F-5	55 (36.6:18.3)	20	25	100
	F-6	65 (43.3:21.7)	20	15	100
Smix 4:1	F 7	45 (36:9)	20	35	100
	F-8	55 (44:11)	20	25	100
	F-9	65 (52:13)	20	15	100
Smix 8:1	F-10	45(40:5)	20	35	100
	F-11	55 (48.9:6.1)	20	25	100
	F-12	65 (57.7:7.3)	20	15	100

Characterization of the prepared MEs

Thermodynamic stability

Thermodynamic stress test reflects the dispersibility of the ME components and hence, its stability. No formula showed any kind of creaming or phase separation after these procedures. This result indicates the pronounced stability is imparted by the non-ionic surfactant (CrEL) to the MEs[32]

Determination of ME type

Dye test with methylene blue (MB) was followed to determine the type of the prepared MEs. Microscopically, all formulas revealed the dispersion of MB dye in the background without any aggregations or flocculation indicating that all the prepared formulas were of (o/w) type. However, the method of spontaneous emulsification used to prepare MEs does not involve phase inversion[33]

Determination of pH

All prepared MEs showed pH value within the range of the (5.5-6.0) as shown in Table (2). Extremely high or low pH value causes discomfort and patient noncompliance especially when used in sensitive route of administration like ophthalmic or nasal route.

Dilution test and Light transmittance

Dilution test is an important indication that MEs can withstand dilution with body fluids, further formulation steps. Some characterization tests rely on the ability of ME formula to withstand dilution several tenth times or more for example Zeta-average, Zeta-potential, and light-transmittance test and in-vitro dissolution procedures[34]. Only F-6, F8, F9, F11 and F-12 passed this test without any sign of turbidity or separation. According to Bancroft's rule, the surfactants molecules are found preferentially in the bulk liquid in which they are more soluble[35] . Increasing surfactant concentration above the critical micelle concentration leading to the formation of the swollen micelles that is the primary mechanism for emulsification. These surfactant monomers in swollen micelles are found in an equilibrium state with surfactant monomers in the bulk and any dilution could lead to their movement from micelles toward the bulk leading to ME destabilization[36]. Formula F-6, F8, F9, F11 and F-12 have reasonably high surfactant concentration that could maintain surfactant concentration above the critical micelle concentration that could stabilize ME upon dilution as shown in Table (2).

Using UV-visible spectrophotometer at λ=600nm act as turbidimeter revealed that F-1, F-2,F-3, F-4, F-7 and F-10 were showing low transmittance values as shown in Table (2) due to the turbidity that is related to ME separation as a consequence of dilution test. This indicates the instability of these formulations against dilution at these ratios therefore they were excluded from further tests

Determination of average globule diameter and poly dispersity index (PDI).

Results obtained from laser diffraction technique using Malvern Nano–ZS are listed in Table (2) Data showed that the mean diameter of oil globule were within nanoscale ranged from (42-56.5 nm) F8, F9, F11 and F-12. These results mimic the claims that these formulas which have the higher surfactant concentration would be of the finest globule size.[37]

Determination of Zeta potential

Values generated by Zetasizer (Nano–ZS Malvern UK) Varied from (0.7 to -6.4mV) which are considerably low to reach electrostatic stabilization of ME. These results, shown in Table (2), are attributed to the non-ionic nature of the CrEL

used in this study and the use of DIW as external aqueous phase. However, stabilization imparted by non-ionic surfactants is mainly of steric type that results from geometrical configuration of the side chains which maintain the distancing between oil globules in a range beyond the range of the effective intermolecular attractive forces thus preventing Ostwald's ripening and maintain ME stability[38].

Drug content uniformity

Drug content variation between (98-99%) were satisfactory with narrow SD values between (0.5-1.5) This indicates that the used method of preparation is of high precision and accuracy and the parameters of the process were met like the mixing time and efficiency as shown in Table (2).

Table 2. Results of dilution test, light transmittance test, globule size, PDI, zeta potential, pH and drug content

Formula code	Dilution test 50 X	Dilution test 100 X	Light transmittance	ME globules size	PDI	Zeta potential (mV)	pH	Drug contents % ±SD
F-1	FAIL	FAIL	0.87	324.48	0.43	0.65	5.7	99.5±0.15
F-2	FAIL	FAIL	0.89	471.9	0.57	0.86	5.6	99.1±0.04
F-3	FAIL	FAIL	0.84	377.14	0.52	1.2	6.0	99.7±0.09
F-4	FAIL	FAIL	0.82	302.54	0.47	0.6	5.6	99.0±0.12
F-5	PASS	FAIL	0.97	112.38	0.29	-2.4	5.7	98.8±0.02
F-6	PASS	PASS	0.96	129.97	0.24	-2.8	5.7	99.3±0.05
F-7	PASS	FAIL	0.85	212.69	0.43	0.8	5.8	98.9±0.1
F-8	PASS	PASS	0.98	53.94	0.11	-3.1	5.8	99.1±0.03
F-9	PASS	PASS	0.98	56.54	0.1	-3.2	5.5	99.6±0.04
F-10	PASS	FAIL	0.85	298.43	0.4	0.76	5.6	99.4±0.1
F-11	PASS	PASS	0.98	42	0.172	-6.4	5.6	99.5±0.09
F-12	PASS	PASS	0.98	45.63	0.19	-2.8	5.5	99.6±0.06

Viscosity

Generally, MEs are freely flowing liquids resulting from the dispersion of ultra-fine oil globules inside the continuous aqueous phase. The viscosity profile revealed non-Newtonian behavior related to shear thinning type of flow that is seen in the liquids containing high concentration of long chain molecules. This kind of molecules aligned with the direction of the flow thus lowering shear force that is required cause flow as shown in Figure (4).

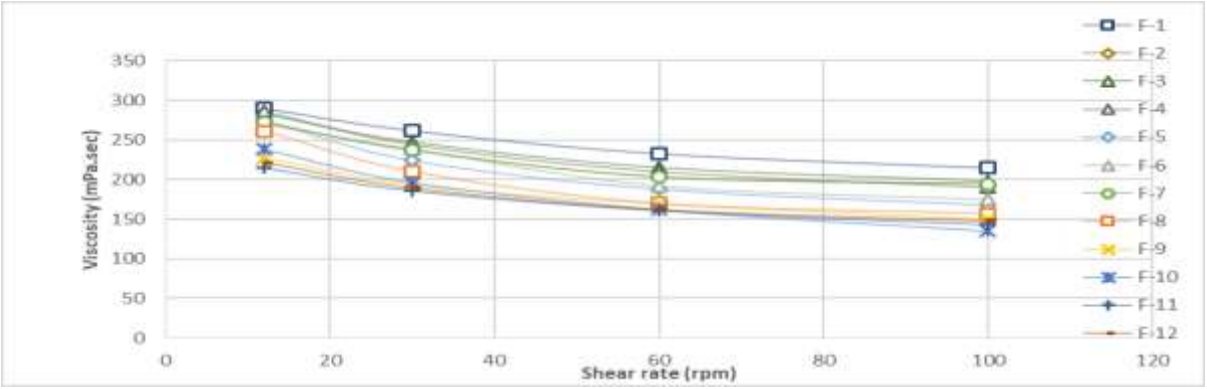

Figure 4. Viscosity of different IDB MEs formulas

Determination of ex-vivo permeation kinetic parameters

Ex-vivo permeation parameters for F-11 showed significant drug flux into the receptor chamber of the Franz-cell compared to results obtained from reference oil dispersion of the drug as shown in Figure (5). Permeation rate was 40.9µg/h/cm2, Permeation coefficient $40.9x10^{-4}$ cm/h while the lag time equals 0.37h**.** Furthermore, a total enhancement ratio obtained from ME comparing to oil dispersion of IDB was 37.3 times. These results are attributed to several reasons among which is the tremendous increase in the total surface area of the dispersed oil phase which can profoundly increase the soluble species of the drug that is liable for diffusion across epithelial membrane. In addition, the ultra-fine drug-loaded oil droplets that are formed by the action of the emulsifying agent and due to their size and nature can easily permeate through the cellular membrane and cross tight junctions. Another mechanism that could be offered by ME is the permeation

enhancement effect of the surfactant that contribute to the higher diffusion rate[39].

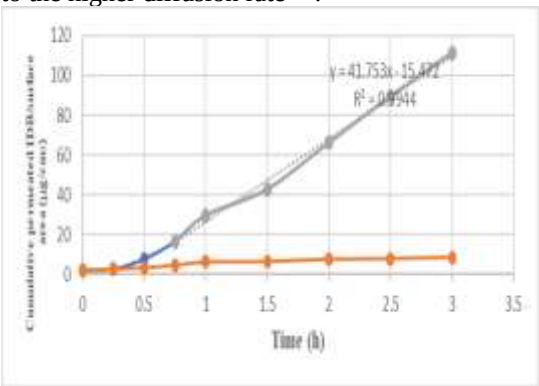

Figure 5. Cumulative permeated drug quantity against its correspondent time profile

Stability determination by FTIR

The idebenone FTIR spectrum showed several characteristic bands for the O-H, C-H, C=O, C=C ring stretching, and C-C long chain rocking vibrations at 3569, 2923, 2846, 1656, 1610 and 746 cm-1, respectively. which were obtained from a previous work(40). The obtained FTIR spectrogram of F-11 shown in Figure (6) revealed the corresponding peaks 3471, 2924, 2862,1728, 1651and cm^{-1} as well as the long tail C-C rocking at 728cm^{-1} which indicate chemical stability of the drug during formulation.

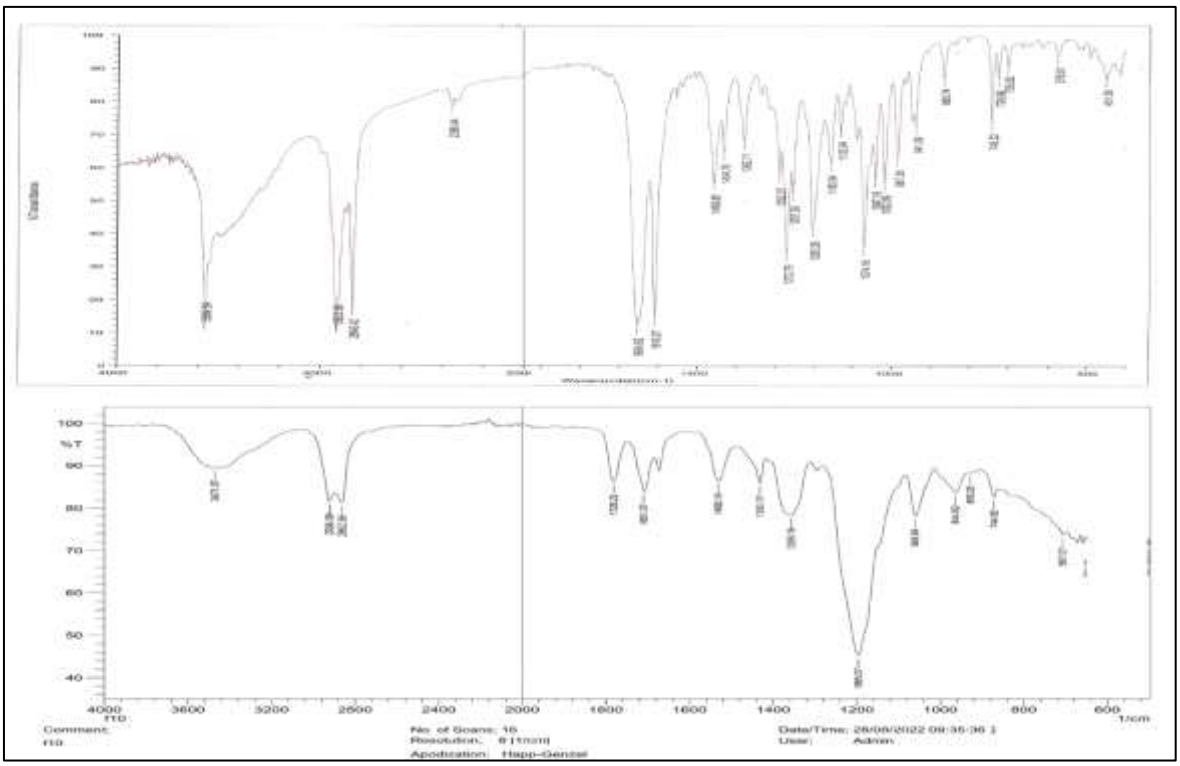

Figure 6. FT-IR spectrograph of IDB before (upper) and after the incorporation(lower) in F-11

Conclusions

In case of the practically insoluble IDB, the selection of ME approach for transmucosal drug delivery systems meet several demands for efficient drug delivery. The tremendous increase in IDB solubility of several hundred times and the reduction in globule size to nanoscale enable better drug permeation through the mucous membrane toward the blood circulation. These formulations can offer a good solution for the lipophilic drug IDB that is susceptible for extensive hepatic biotransformation but after further formulation optimization. Transmucosal routes are secured routes of administration against hepatic metabolism and ultimately their bioavailability will be guaranteed. Nevertheless, formulation of poorly soluble drug as ME can offer higher drug loading capacity comparing to other nano-sized carriers thus a starting point for developing low volume drug delivery systems such as ophthalmic, nasal, transdermal patches, microneedles and other non-enteral dosage forms.

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Conflict of Interest and Funding

We (authors) hereby declare that there is no conflict of interest issues and the work has not received any external fundings.

Ethics Statements

No human subjects nor living animals were used in this study thus no consents were required.

Author Contribution

Hussein J. Kadhim: MSc Student Khalid K Al-Kinani: Supervisor

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