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ENHANCEMENT OF ORAL BIOAVAILABILITY OF REPAGLINIDE BY SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM

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ABSTRACT

Repaglinide is considered the drug of choice for diabetic patients with impaired kidney function as it is excreted mainly in the bile. Unfortunately, it possesses low oral bioavailability of approximately 56 %. Therefore, nano-sized globules containing the drug are expected to enhance its bioavailability and sustain its glucose lowering action. Self nano-emulsifying drug delivery systems (SNEDDS) of repaglinide have been prepared for improving the water solubility and oral bioavailability of the drug. Various compositions of SNEDDS were prepared using four types of oils (oleic acid, isopropyl myristate IPM. Labrafil 1944 and 2125), surfactants (chromophore El35, chromophore RH 40, Labrasol and Span 20) and a variety of co-surfacatnts. Low energy emulsification was adopted as the method of preparation for its feasibility and low cost. The prepared nano-emulsions showed small average droplet size (13.5-20 nm) and low polydispersity index (0.10 - 0.30). *In-vitro* dissolution studies indicated that the drug release from some of the prepared nanoemulsion droplets reached 75 % within the first 30 minutes. The i*n-vivo* data demonstrated that repaglinide in the nano-emulsion formulations F8 (IPM, Cremophor EL35 and Propylene glycol) and F16 (Oleic acid, Cremophor RH40 and Lauroglycol FCC) lowered the plasma glucose level (< 110 mg/dL) of experimental rabbits in a similar trend to that of the commercial product (Novonorm®), moreover, it caused an excess reduction in blood glucose level at the end of the 24 hrs period by virtue of its long circulation time compared to the marketed formula.

Keywords: Diabetes, Oral, SNEDDS, Repaglinide.

INTRODUCTION

Self-emulsifying drug delivery systems (SEDDS) are considered excellent alternatives to conventional dosage forms by virtue of their high solubilization and permeation attributes suited for poorly soluble and slowly absorbable drugs. SEDDS are isotropic mixtures of oils, surfactants/solvents and co-solvents/co-surfactants. They can be specially designed to improve the oral absorption of highly lipophilic drugs [1]. Depending on the method of preparation; either a self-microemulsifying drug delivery system (SMEDDS) or a self-nanoemulsifyingdrug delivery system (SNEDDS) is obtained. Both systems have common characteristics to the extent that sometimes makes it difficult to differentiate between them. However, nano-emulsions have some special advantages over micro-emulsionssuch as being transparent or translucent in color, having smaller droplet size (50-200 nm) andpossessinglong term physical-stability against sedimentation or creaming[2]. Identification of nano-emulsion formation can be done using ternary/pseudoternary phase diagrams[3]. Repaglinide is an oral blood glucose-lowering drug which belongs to themeglitinide class. It has been used in the treatment of type 2 diabetes. It exerts its action by stimulation of beta cells for insulin secretion in the pancreatic islets[4]. It has a short elimination half-life (1 hour) and a low oral bioavailability which reach about 56 % [5]. However, it is considered the drug of choice for diabetic patients with impaired kidney function as it is excreted mainly in the bile [6]. Therefore, combining the benefits of SNEDDS with those of repaglinide, a delivery system overcoming the drawbacks of the drug and capable of offering an effective andprolonged control of blood sugar levels will be developed. This study will focus on optimization, physicochemical characterization, *in vitro* and *in vivo* assessment of the developed formulation.

MATERIALS AND METHODS

Materials

Repaglinide was a gift from The Egyptian International Pharmaceutical Industries Co. "EIPICO", Cairo, Egypt. Oleic acid, ispropylmyristate (IPM)and Span 20 (sorbitanmonolaurate) were purchased from Sigma Aldrich, USA. Labrafil 1944 (oleoyl polyoxyl-6 glycerides) andLabrafil 2125 (linoleoyl polyoxyl-6 glycerides)were gifts fromGattefosse, Saint-Priest Cedex, France. Cromophor El 35 (polyoxyl 35 castor oil), Cremophor RH 40 (polyoxyl 40 hydrogenated castor oil), ethanol and Propylene glycol were purchased from El Gomhoria Pharm. Chem. Co., Egypt. Labrasol(caprylocaproyl polyoxyl-8 glycerides), Lauroglycol FCC (propylene glycol monolaurate) were gifts from Gattefosse, Saint-Priest Cedex, France. The commercial product; Novonorm 0.5 mg tablets, NovoNordisc, Bagsvaerd Denmark, was purchased from the Egyptian Company for medicinal trades, Cairo, Egypt.

Components screening

The solubility of repaglinide in different nano-emulsion components including oils, surfactants and co-surfactants was evaluated by dissolving a known amount of repaglinide (10 mg) in 2 ml of each of oil, surfactant andcosurfactants using 5-mlcapacity vials. The vials were then sealed carefully with rubber stoppers and mixed using a vortex (Nickel- Electro Ltd, Oldmixon Crescent, UK). The vials were then placed in an isothermal shaker (Nirmal International. New Delhi, India) operated at 40 ºC till equilibrium. The mixtures were removed from the shaker and the contents transferred to 5 ml centrifuge tubes followed by centrifugation at 5000 rpm for 30 minutes using refrigerated large capacity centrifuge(Union 32R, Korea). The supernatant was then separated, filtered using a 0.45-µm membrane filter and its contentdetermined using UV-Spectrophotometer (UV-240 1PC, Shimadzu, Japan) at 283.2 nm.

Construction of pseudoternary phase diagrams

Pseudoternary phase diagrams of oil, surfactant/cosurfactant mixture (S-mix.) and aqueous phase were constructed using the aqueous titration method [7]. The boundaries of phase diagrams designated the system's three components; one axis representing the aqueous phase, the second for the oil, and the third representing the S-mix[8]. Selected nano-emulsion components based on the

solubility study were incorporated in the final formulations using varieties of weight ratios. The surfactant/cosurfactant mixtures were prepared in ten weight ratios, then the oil was added to previous mixtures of surfactant/cosurfactantin the ratio of oil:S-mix; 1:9, 2:8, 3:7, 4:6 and 5:5. The aqueous phase (distilled water) was added portion wise to the previous mixtures of oils and S-mix up to a final volume of 10 milliliters. The area of nanoemulsion formation was identified for the respective system in which transparent and easily flowable oil-in-water (o/w) nanoemulsions with desired globule size were obtained.

Characterization of SNEDDS

Morphology

Samples of the nano-emulsion formulations were examined at high magnification using transmission electron microscopy (TEM)[9], (JEOL-JSM-6510LA Analytical Scanning Microscope, JEOL Ltd., Japan) to determine the particles morphology.

Evaluation of particle size and zeta potential

Evaluation of average particle size, size distribution and zeta potential of each sample was carried out using Malvern master sizer, Hydro 2000S, UK.

In-vitro release studies

The release of repaglinide from the prepared formulations was determined using USP Dissolution Apparatus 2 – Paddle type (37°C). In this method 1 ml of the preparednano-emulsion base (mixture of oil, surfactant and co-surfactant) containing 0.50 mg repaglinide were filled into hard shell capsules (size 1) then dropped in a dissolution medium of 1000 ml 0.1N HCL. Samples of 5 mls from the dissolution medium were withdrawn after specific time intervals (15, 30, 60, 90, 120,150 and 180 minutes) and replaced with fresh 0.1N HCl. Samples were assessed spectrophotometrically.

In-vivo studies

Samples of the prepared nano-emulsion base (mixture of oil, surfactant and co-surfactant) containingrepaglinide were filled into hard shell capsules (size 1). The content of each capsule was determined based on animal dosing calculated according to Paget and Barnes calculations [10]. In this experiment 6 male albino rabbits weighing (2.5-3.0 kg) were selected for the test.

The animals fasted overnight prior to oral dosing of the test samples. Randomized cross-over design was adopted for comparing the pharmacology of both SNEDDS formulations and the marketed product (Novonorm® 0.5). Samples of animal blood (one drop) were withdrawn at zero time and at time interverals (30 min and 1,2,3,4,5,6,8,10,12,18,24 hrs) directly onto the strip of an automatic blood glucose checking apparatus (Accu-Chek Active blood glucose meter, ROCHE, Germany). The results were taken in triplicate for both the control and treatment groups (Fig. 4).

RESULTS AND DISCUSSION

Components screening

The components of SNEDDS and their concentration have profound effects on the various characteristics of nanoemulsions, such as droplet size, polydispersity index, self-nanoemulsification time and *in vitro* drug release. Hence, it is important to optimize the quantities of the SNEDDS components after initial selection. The initial selection of the components can be on the basis of their ability to solubilize the drug of interest. Solubility of repaglinide in various SNEDDS components is presented in table 1.

The results indicated that among the tested oils,repaglinideshowed a very high solubility in isopropyl myristate (IPM) where 180 μg/ml dissolved after 72 hours. The evaluated solubility of repaglinide in different surfactants demonstrated the superiority of Cremophor EL35 and Cremophor RH40 over others and hence they were employed in most formulations. Ethanol, Lauroglycol FCC and Labrasolwere selected as cosurfactants governed mainly by both their emulsification efficiency more than their ability to solubilize repaglinide.

Table 1: Solubility of repaglinide in various nano-emulsion components

Component	Total Solubility (μg/mL)		
	24 hrs	48 hrs	72 hrs
Oleic acid	11.34	21.22	28.29
Olyl alcohol	13.17	17.15	43.53
IPM	147.07	163.65	180.73
Labrafil 2125	----	----	14.14
Labrafil 1944	----	----	15.85
CremophorEL35	----	----	161.34
Cremophor RH40	----	----	103.53
Span 20	40.85	58.17	136.7

Phase study

Fourty systems were prepared with the oils, surfactant/cosurfactant (S/CoS) combinations listed in table 1.

Each system was prepared with S/CoS weight ratios from 1:9 to 9:1. Pseudoternary phase diagrams were constructed using water titration method to identify transparent nanoemulsions at a temperature of 25±1ºC, which corresponds to common conditions of preparation, storage and application of pharmaceutical nanoemulsions. The nanoemulsion region appeared on the phase diagram as shaded areas (data not shown). The rest of the regions on the phase diagrams represent turbid and conventional emulsions based on visual observations. No liquid crystalline structures were observed under light microscope. Within the grey areas, the nanoemulsions were formed with only gentle vortexing. This is possible as surfactant strongly localized on the surface of the emulsion droplets reduces interfacial free energy and provides a mechanical barrier to coalescence resulting in a thermomechanically spontaneous dispersion.

It was observed that the nanoemulsion existence areas obtained with more oils to S/CoS ratios were obtained for IPM compared to oleic acid and Labrafil® 1944 and 2125. This finding is expected as the phase behaviour is strongly influenced by the size of the molecule of the oil used [11]. Depending on the chain length and on the volume of the molecules, penetration of the surfactant into the hydrocarbon tails is expected to change the hydrocarbon chain volume of the surfactant molecule and thus, the effective critical packing parameter (CPP)[12], Since oleic acid and Labrafil®1944 and 2125 have large molecular size compared to that of IPM and to that of the surfactants used, low degree of oil penetration was expected to take place in the interfacial surfactant layer.

A distinct central core, which greatly disrupts the packing of the surfactant molecules in this region, could be formed causing destabilization of the nanoemulsion with consequent reduction in its existence with different oil: S/CoS ratios. From the results obtained as well. It was also evident that CremophoreEL35/Labrasol and Cremophor RH40/Labrasol S-mix has larger nanoemulsification range as compared to other S-mix. Composition of selected self nanoemulsifying systems from all prepared formulations is presented in table 2.

Droplet size and zeta potential

The nano-emulsion droplets were evaluated for average diameter and zeta potential as shown in table 3. All nanoemulsion preparations were in the defined nanoemulsion droplet diameter (13.51 ± 0.08 - 19.68 ± 0.36 nm). The small average diameters of nanoemulsion droplets is mainly due tocosurfactant molecules penetrating the surfactant film, lowering the fluidity and surface viscosity of the interfacial film, decreasing the radius of curvature of the microdroplets and forming transparent nanosized systems [13]

The smallest average size (13.51 nm) was obtained from Formula S5 composed of IPM/CremophoreEL35/Labrasol (10/20/70 % w/w). Formula S8 composed of IPM/Cremophore EL35/propylene glycol(10/90/0 % w/w) showed the highest average droplet size;19.68 nm. Since both formulations are prepared using the same oil and surfactant, the difference in particle size might be mainly

attributed to the cosurfactant effect; no clear mechanism was found to elucidate this influence. PDI was less than 0.35 and zeta potential values were sufficient for maintaining stable nanoemulsion preparations.

Stabilizers presented as surfactants and cosurfactants are meant to wet the surfaces of the particles and retard Ostwald ripening and agglomeration to increase the stability of the preparation by providing a steric barrier.

Table 2: Composition of selected nanoemulsionformualtions

Formula	Repaglinide (mg)	Oil (0.1-0.9 mL)	S-mix (0.9-0.1 mL)		S-mix ratio	Oil: S-mix ratio	Aqueous phase (mL)
			Surfactant (mL)	Cosurfactant (mL)			
S5	0.50	IPM	Cremophor El-35	Labrasol	1/9 2/8 3/7 4/6	0.1/0.9	Water to 10 mL
S8	0.50	IPM	Cremophor El-35	Propylene glycol	10/0	0.1/0.9	
S9	0.50	IPM	Labrasol	Ethanol	8/2 9/1	0.1/0.9	
S10	0.50	IPM	Cremophor RH-40	Labrasol	1/9 2/8 3/7 4/6 5/5	0.1/0.9	
					5/5 6/4	0.2/0.8	
S16	0.50	Oleic acid	Cremophor RH-40	Lauroglycol FCC	9/1 10/0	0.1/0.9	
S39	0.50	Labrafil 1944	Cremophor El-35	Ethanol	7/3	0.2/0.8	
S40	0.50	Labrafil 2125	Cremophor El-35	Ethanol	8/2	0.1/0.9	

Characterization

Morphology

Transmission electron microscopy (TEM) was utilized for elucidating morphology of the prepared nanoemulsions. Photographs of TEM (Figs. 1-3) demonstrate clearly the spherical outlines of nanoemulsion droplets.
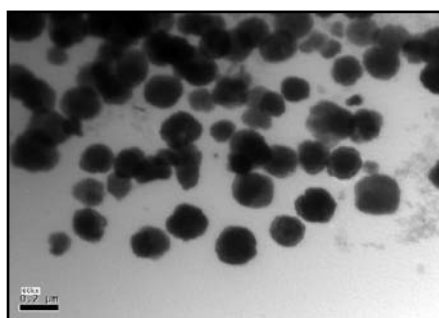

Fig. 1: Scanning electron micrographs of nano-emulsion globules (Formulation S8)

In-vitro release studies

The *in vitro* drug release study represents a very important parameter in the prediction of drug bioavailability form different formulations. Comparing the release profile of repaglinide from the selectednano-emulsion formulations to that of Novonorm® tablets (0.5 mg), Formula S8 composed of IPM/Cremophore EL35/ propylene glycol (10/90/0 % w/w) was found promising where it showed a biphasic zero order release profile with a rapid initial burst that lasted for about 30 min at which approximately 93% of the drug content was released from the SNEDDS this was followed by a slower and more gradual drug release pattern reaching approximately 100% at 3 h (table 4), a comparable release pattern was achieved with S16 as well.

In-vivo studies

Randomized cross-over design was adopted for comparing the pharmacology of both SNEDDS formulations and the marketed product (Novonorm® 0.5). The anti-diabetic activity of the promising nanoformulations S8 (IPM/CremophoreEL35/Labrasol) and S16 (Oleic acid/Cremophore RH40/Lauroglycol FCC) *vs* a control as well as a repaglinide market product showed that both repaglinide SNEDDS formulations showed percentage reduction in blood glucose level comparable to the reference drug with S16 showing lower glucose levels at certain points during and till the end of the 24 hrs. period (Fig. 4).
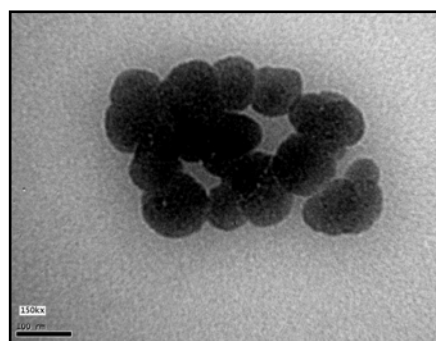

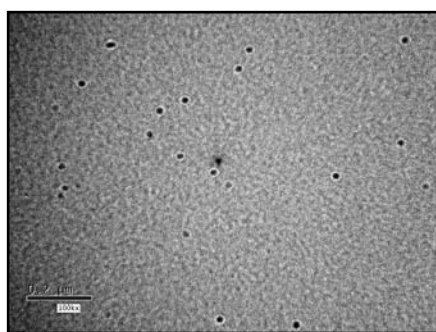**Fig. 2: Scanning electron micrographs of nano-emulsion globules (Formulation S40)**

Fig. 3: Scanningelectron micrographs of nano-emulsionglobules (Formulation S16)

Table 3: Average size, size distribution and zeta-potential of prepared repaglinidenano-emulsions

Formula	Specific surface area (m²/g)	Average P. size (nm S. D)	Mean diameter (nm)			Average polydispersity index S. D	Average Zeta pot. (mV) ± S. D
			D (10 %)	D (50 %)	D (90 %)		
S5	66.40	13.51 ± 0.08	64	94	138	0.165 ± 0.02	-8.68 ± 2.16
S8	32.90	19.68 ± 0.36	157	182	220	0.317 ± 0.03	-3.80 ± 0.18
S9	35.40	21.04 ± 0.08	128	173	240	0.167 ± 0.01	-28.7 ± 0.92
S10	22.42	23.46 ± 0.91	1697	2564	4323	0.230 ± 0.01	-29.35 ± 1.67
S16	21.50	15.62 ± 0.02	173	228	3447	0.119 ± 0.02	-3.20 ± 0.01
S40	22.40	18.65 ± 0.04	174	267	584	0.214 ± 0.01	-3.52 ± 0.56

Table 4: Percentage repaglinide released from prepared nano-emulsion formulations and the marketed product

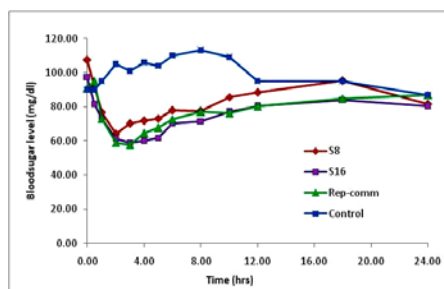| Formulation	pH	% released after Q (hours)		
		$Q_{\frac{1}{2}hr}$	$Q_{1\frac{1}{2}hr}$	Q_{3hr}
S5	4.76	12.20	85.51	101.64
S8	4.01	93.52	108.78	102.39
S16	4.58	88.49	104.01	95.51
S40	3.86	24.32	73.38	99.19
Novonorm® 0.5	---	52.91	61.64	111.54

Fig. 4: *In-vivo*study

CONCLUSION

SNEDDS of repaglinide showed satisfactory physicomechanical characteristics. The selected formulations S8 and S16 of repaglinideSNEDDS gave a reasonable *invitro*release profile compared to the market product. Combining the benefits of SNEDDS with the effectiveness of repaglinide, a promising antidiabetic drug delivery system could be achieved.

CONFLICT OF INTERESTS

Declared None

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