Mechanistic approach for the development of ultrafine oil-water emulsions using monoglyceride and blends of medium and long chain triglycerides: enhancement of the solubility and bioavailability of Perphenazine.

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Original Article

ABSTRACT

A kinetically stable ultra-fine oil-water (o/w) emulsion containing Perphenazine and blends of long and medium chain triglycerides was prepared. The purpose of the ultra-fine emulsion was to increase the oral bioavailability of Perphenazine. The formulations were prepared using a low energy emulsification phase titration method. The optimized formulations consisted of blends of linseed oil and Sefsol 218 (1:1) (oil phase), polysorbate 40 (surfactant), polyethylene glycol 400 (cosurfactant) and distilled water (dispersion medium). Characterization of viscosity, refractive index, particle size distribution, spectral transmittance and surface morphology of the formulations was performed. The release rate of Perphenazine from the formulations was quantified using the everted gut sac of rat intestinal mucosa. The *ex vivo* release data demonstrated that the formulated nanoemulsions increased significantly the permeation rate of Perphenazine when compared with a suspension. Following oral administration of selected nanoemulsions in Wistar rats, the AUC and C_{max} of Perphenazine increased by 2.9 and 2.54-fold respectively compared with the Perphenazine suspension. The observed increase in bioavailability may be due to the increase in the dissolution rate from the molecularly dissolved drug in the oil phase and an increased rate of dispersion of the drug in the gastrointestinal (GI) tract which leads to greater absorption into the blood.

KEY WORDS: Perphenazine, nanoemulsion, phase diagram, pharmacokinetic

INTRODUCTION

Perphenazine (PPZ) is an antipsychotic drug (Figure 1) used in the treatment of schizophrenia and severe emesis. The systemic availability of PPZ via the oral route seems to be variable and often poor (1) due to an extensive hepatic first pass metabolism and poor aqueous solubility (28.5 μg/ml) (2). The present study was undertaken with the objective of increasing the oral bioavailability of Perphenazine by formulating it as a nano-emulsion.

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The effect of particle size, related to the rate of dissolution and bioavailability, has been documented by many researchers (3-8). Different approaches, such as, drug nanosizing, complexation and solid dispersion have been used to increase the rate of drug dissolution. When diluted by gastric and/or intestinal fluids, drug precipitation from the complexes and a tendency to agglomerate in order to reduce the interfacial surface area, remains a big challenge with drug complexation and drug particle nano sizing (9, 10). Formulating drugs as nano-emulsions provides a versatile technology that has the potential to increase bioavailability. The internal (dispersed) phase of nanoemulsions can be used to molecularly solubilize hydrophilic and/or lipophilic drugs and therefore improve the bioavailability of BCS Class II and BCS Class IV drugs. The nanometer sized droplets yield a very large interfacial area, thereby increasing the rate of release of the drug into the external phase. Provided that a judicious choice of (self emulsifying) excipients is made and because of the presence of significant barriers (surfactants) to phase separation, nano-emulsions containing a selfemulsifying oil, surfactant and co-surfactant ingredients require negligible energy input during their preparation thereby facilitating scale up (11-13). Perphenazine was formulated into
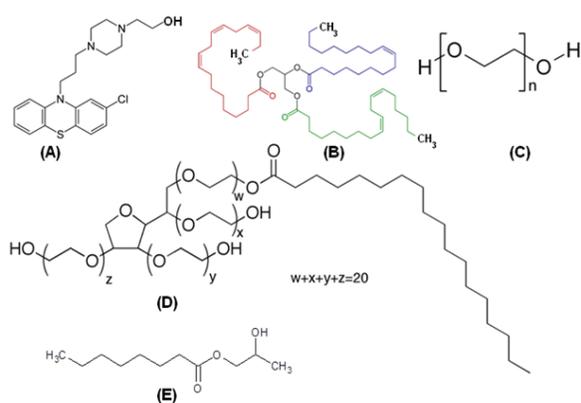
nanoemulsions together with lipidic components in an attempt to increase oral bioavailability. Medium and long chain trigly-cerides have been shown to increase oral bioavailability of hydrophobic drugs and to self emulsify in the presence of surfactants and co-surfactants (14-16). Such excipients were therefore chosen as solubilizers for Perphenazine.

MATERIALS AND METHODS

Materials

Perphenazine (98.0-102%, USP) was a gift from Xylopia Ltd (Ahmadabad, India). Oleoyl macrogol-6 glycerides NF (Labrafil®) and diethylene glycol monoethyl ether EP/NF (Transcutol® P) were gifts from Gattefosse (Lyon, France). Propylene glycol monocaprylic ester (Sefsol 218®) was a gift from Nikko Chemicals (Tokyo, Japan). Triacetin (Glycerol triacetate), Polyoxyethylene glycol sorbitan monooleate (Tween® 80), Polyoxyethylene sorbitan monolaurate (Tween® 20), Polyoxyethylene sorbitan monopalmitate (Tween® 40), Polyethylene glycol (PEG® 400) and Sodium carboxymethylcellulose (Calbiochem®, Low viscosity grade, 25-75 mPas (2%) at 25°C) were purchased from Merck (Schuchardh, Hokenbrunn, Germany). Water (Milli®Q, 18.2 MΩ.cm^{-1} at 25°C) was obtained from Milli-Q water purification system (Millipore, MA, USA). Dialysis membrane (MWCO=12,000 Da) was obtained from Sigma Aldrich (Batch no. 3110 (D9652), Sigma-Aldrich Corp. St. Louis, MO, USA). All other chemicals were of analytical reagent grade. The structures of the different excipients used for the preparation of nanoemulsions including that of Perphemnazine are shown in Figure 1.

Analytical method

The RP-HPLC-PDA method for the determination of Perphenazine was performed using a Waters Alliance e2695 separating module (Waters Co., MA, USA) with a photo diode array detector (Waters 2998), an

Figure 1 The structure of Perphenazine (A) and different excipients including Linseed oil (B), Polyethylene glycol 400 (C), Polyoxyethylene (20) Sorbitan monopalmitate (D) and Propylene Glycol monocaprylate (E) used for the preparation of the nanoemulsion.

autosampler and column oven. The instrument was controlled by the Empower software installed with the equipment for data collection and acquisition. Separation was achieved using a C18 reverse phase column (25 x 4.6 mm, particle size 5.0 µm, Merck, Germany) maintained at room temperature. The mobile phase, composed of 80% methanol (phase A) and 20% of 0.1% aqueous solution of orthophosphoric acid (H_3PO_4) (phase B), was injected with a flow rate set at 1 ml/min. The detection was performed at 256 nm and Perphenazine eluted at 3.03 minutes with a total run time of 7 minutes. The calibration curve was linear with a correlation coefficient of > 0.999. Limit of detection (LOD) and limit of quantification (LOQ) of the method were set at signal to noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentrations.

Solubility studies

Orally administered medium and long chain triglyceride containing oils are preferentially absorbed into the blood (13). The solubility of Perphenazine was determined in various oils with medium and long chain fatty acid constituents. Briefly an excess amount of drug was added to 1 ml of the oil, surfactant or cosurfactant in a 10 ml vial. The vials were tightly closed and were continuously stirred for 72 hours at 37°C ±0.5. Samples were centrifuged (REMI International, Mumbai, India) at 1957 x g for 20 minutes and the supernatant was analyzed after filtration through a membrane filter (MicroSolve®, 0.45 µm) (17, 18). The concentration of the drug was determined using UV-spectro-photometry (Shimadzu UV-1601, Tokyo, Japan) at 257.6 nm using the corresponding oil, surfactant or co-surfactant as a reference.

Construction of pseudoternary phase diagrams

Pseudoternary phase diagrams were constructed using aqueous titration to determine regions where nanoemulsions formed. The surfactant (Tween 40) and the co-surfactant (PEG 400) were mixed (designated as S_{mix}) in different volume ratios (1:0, 1:1, 1:2, 1:3, 2:1, 3:1, 4:1). These S_{mix} ratios were chosen to reflect permutations that would encompass as large a nanoemulsion area in the phase diagram as possible. A mixture of linseed oil and Sefsol 218 (1:1) was chosen as the oil phase based on the solubility study. For each phase diagram, the oil phase and a given S_{mix} ratio were mixed in different volume ratios ranging from 1:9 to 9:1 in different glass vials. Sixteen different combinations of oil and S_{mix} (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3.5, 1:3, 1:2.33, 1:2, 1:1.5, 1:1, 1:0.66, 1:0.43, and 1:0.25) were prepared to maximize the precision of the phase boundary delineation. The mixture of the selected oil and surfactant/co-surfactant mix were titrated against distilled water. After every 5% addition of aqueous phase to the oil and s_{mix} mixture, the contents were examined visually.

The percentage of water, oil and surfactant/co-surfactant mix at which there was visual evidence of the formation of a nanoemulsion, were plotted on the ternary phase diagram with the axes representing the aqueous phase, the oil and the S_{mix}.

Selection of formulation

Different formulations were selected from the nanoemulsion region of the constructed phase diagrams. Those compositions from the nanoemulsion regions that contained the least amounts of oil and S_{mix} capable of solubilizing the required amount of drug were selected. The selection criteria minimized surfactant related toxicity including colon epithelial proliferation.

Physical stability

Selected formulations were subjected to accelerated physical stresses.

Centrifugation

The prepared formulations were centrifuged at 1957 x g for 20 minutes and visually observed for phase separation, creaming or cracking.

Heating–cooling and freeze–thaw cycle (accelerated ageing)

Six cycles between 4 and 40°C with storage at each temperature for not less than 48 hours were performed. Similarly, the formulations were subjected to three freeze– thaw cycles at temperatures between –21 and +25°C with storage at each temperature for not less than 48 hours.

Dispersibility

The effect of dilution on the PPZ loaded oral nanoemulsion (NE) was assessed. 2 ml of the nanoemulsion was added to 250 ml of distilled water in a beaker while stirring. The *in vitro* performance of the NE formulations was visually assessed using the grading system, Grade A: formation of a transparent nanoemulsion within a minute of dilution, Grade B: formation of a bluish-white nanoemulsion (indicative of a larger particle size) within a minute of dilution, Grade C: a milky-white emulsion formed within 2 minutes of dilution, Grade D: gradually formed (> 2 minutes of dilution) oily emulsion with a grayish-white appearance and Grade E: no formation of emulsion, rather the appearance of large oil globules on the surface of the dilution medium indicative of phase separation.

In vitro release study

In vitro release studies were carried out to compare the release of Perphenazine from Perphenazine loaded NE formulations (PZ 3, PZ 7 & PZ 21) to that from a PPZ suspension. Perphenazine was mixed with a 2% sodium carboxymethyl cellulose solution in a mortar until the mixture became uniform. The volume was made up with water to prepare a 2 mg/ml of suspension. The dialysis bag was pretreated as per instructions. 1 ml of the NE (2 mg/ml) and suspension was filled in separate treated dialysis bags which were closed using a nylon thread. The release study was performed in 500 ml of phosphate buffer (pH 6.8, 0.05 M) using a basket type USP apparatus with a stirring speed of 50 RPM with the temperature maintained at 37°C ± 0.5 (Hanson Research SR8 plus, California, USA). The dialysis bag was kept inside the basket. A 1 ml sample was withdrawn at predetermined time intervals and an equivalent amount of phosphate buffer was replaced in order to maintain sink conditions (11, 18). The samples were analyzed for the drug content spectrophotometrically at 255.6 nm. The release of the drug from the nanoemulsion was compared with its release from the PPZ suspension (2 mg/ml). The data obtained from *in vitro* drug release was fitted to various release models to determine the mechanism of drug release from the optimized formulation.

Ex vivo release modeling

The drug release study is one of the most critical parameters in formulation development. Therefore, the *in vitro* release data was further authenticated by an *ex vivo* study using rat duodenum. All animal experiments were carried out after approval of the protocol by Jamia Hamdard, Institutional Animal Ethics Committee, New Delhi. Rat duodenum was washed with saline to remove any excretory products. After preparing the rat duodenum, the *ex vivo* study was performed as per the *in vitro* protocol stated previously.

Characterization

Percent transmittance

Percent transmittance of the prepared nanoemulsion was determined spectro-photometrically. 1 ml of the formulation was diluted 100 times using water and analyzed at 630 nm using the dispersion medium as a reference.

Viscosity

The viscosity of the prepared nanoemulsion was determined without dilution using an R/S CPS Plus Rheometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) at 25°C ±0.5. The software used was RHEO3000. 1 ml of the formulation was used

for viscosity determination. The speed of the spindle was adjusted to 70 RPM and a single run was performed. Shear rate applied was 413 per minute using a spindle with a 50 mm diameter.

Particle size distribution

The size of the nanoemulsion droplet was analyzed by dynamic light scattering using Zetasizer Nano ZS (PCS, Zetasizer-1000 HAS, Malvern Instruments, UK). The apparatus is equipped with a 4mW He/Ne laser emitting at 633 nm, measurement cell, photomultiplier and correlator. Prior to measurement, the samples were diluted (1:400) into a distilled water ultra-filtrate. The samples were placed in a vertical disposable cuvet (DTS0012). The scattering intensity was measured at a scattering angle of 90° relative to the source, at 25°C ±0.5. Intensity autocorrelation functions were analyzed using a General Purpose Algorithm (integrated in the Malvern Zetasizer software) in order to determine the distribution of translational z-averaged diffusion coefficient of the particles, D_T ($m^2 s^{-1}$). The D_T parameter and the hydrodynamic radius (Rh) of particles are related through the Stokes-Einstein equation: $D_T = kBT/6\pi\eta$. Dispersed particles undergo Brownian motion, which causes emission of light from the small particles because their diameter (10 nm) is an order of magnitude smaller than the incident light wavelength (633 nm). This causes a fluctuation in intensity as a function of time, the rate of which is droplet size dependent. Droplet size was obtained from the autocorrelation function calculated by the instrument. The refractive index (RI) and absorbance of dispersion medium (water) was taken to be 1.334 and 0.01 at 25°C respectively (19). The measurements were performed in triplicate .

Surface morphology

Surface morphology of the formulations was performed using a Morgagni 268D transmission electron microscope (TEM) (FEI, Netherlands) operating at 70 KV. Prior to the analysis, the self-nanoemulsifying mixture

(SNEDDS) samples were diluted 1000 times with water to form an emulsion. In order to perform the TEM observations, a drop of nanoemulsion was stained with 2% phosphotungstic acid (PTA) for 30 seconds and applied on a 400-mesh carbon coated copper grid. The dried coated grid was mounted on a slide and examined.

Pharmacokinetic studies

Approval to carry out pharmacokinetic studies, was obtained from the Jamia Hamdard Institutional Animal Ethics Committee (IAEC), New Delhi (173/CPCSEA, 28 Jan 2000; Form No 833). Albino Wistar rats (average weight range of 350-400 gm) were used for the pharmacokinetic study and were divided into two groups with six animals in each group. PPZ suspension and PPZ nanoemulsion was administered to the control group and the test group respectively. The rats were housed under standard conditions of temperature, relative humidity and light, and had free access to standard rodent diet (Lipton feed, Mumbai, India) and water before the experiment. Rats were fasted overnight prior to drug dosing. The formulations were administered orally using an 18-gauge oral feeding needle. Dose for the rats was calculated after taking into consideration surface area ratio of a rat to that of a human body (20, 21). Blood was withdrawn from the tail vein into heparinized centrifuge tubes at 0 (pre-dose), 0.5, 1, 1.5 2.5, 3, 12, and 24 hours after oral dosing. Blood samples were centrifuged at 1957 x g for 20 minutes to separate the plasma and stored at −20°C. The plasma concentrations of PPZ were determined after adding 10 μl of Fluphenazine (FPZ) as an internal standard (100 ng/ml) using the methods described above after extracting with ethyl acetate. Pharmacokinetic parameters (C_{max}, T_{max}, AUC_{0-128} and $AUMC_{0-128}$) were calculated using Kinetica (version 4.10, Innaphase, Philadelphia, PA, USA) and the Graph Pad instat software3 trial version. The relative bioavailability (F) of PPZ (PPZ nanoemulsion and PPZ suspension) was calculated from their AUC values.

Statistical Analysis

Data was expressed as mean ± SD. The PK data for the different formulations was compared for statistical significance by one way ANOVA followed by Dunnett test (GraphPad Software Inc trial version., CA, USA). A p value <0.05 was considered as statistically significant. A p value of <0.001 was considered statistically significant for the *in vivo* bioavailability study.

RESULTS AND DISCUSSION

Solubility studies

For hydrophobic APIs, the oil represents one of the most vital excipients in the formulation of nanoemulsions. The API must be sufficiently soluble in the oil to compensate for this phase constituting a minor part of the formulation. Similarly, the selection of surfactant is an important criterion because of toxicity concerns. In general, non-ionic surfactants are considered to be less toxic than ionic ones. It was found that PPZ exhibited maximum solubility in Sefsol 218 (78.77±3.47 mg/ml) and Linseed oil (88.26±3.21 mg/ml) (Table 1A). Among the various combination mixtures tested, a mixture of Linseed oil, a triester (triglyceride) derived of linoleic acid, alpha-linolenic acid, and oleic acid and Sefsol 218 a 2-hydroxypropyl ester (1:1) achieved a PPZ solubility of 148.09±7.43 mg/ml. Linseed oil is a long chain triglyceride.

Miscibility of the oil with the surfactant and co-surfactant determines the area of the nanoemulsion region in the ternary phase diagram. The miscibility of Linseed oil and Sefsol was tested with different surfactants and co-surfactants (Table 1B). Although the drug was the most soluble in Tween 20, this surfactant was not miscible with the oil phase. Based on solubility and miscibility considerations, Tween 40 and PEG 400 were selected as the surfactant and cosurfactant respectively.

Table 1 Solubility of PPZ (Part A) and miscibility study (Part B) in various oils and surfactants

Part A (Solubility study)	
Name of oil	**Solubility of PPZ (mg/ml) (Mean±SD)**
Captex 8000	12.32 ± 0.02
Corn oil	6.51 ± 0.02
Olive oil	31.85 ± 0.04
Caprol 10G100	9.25 ± 0.02
Soyabean oil	36.40 ± 0.03
Peanut oil	22.45 ± 0.03
Labrafil	56.31 ± 0.03
Oleic acid	66.25 ± 0.021
Sefsol 218	78.77 ± 0.03
Linseed oil	88.26 ± 0.03
Olive oil+Soyabean oil	66.15 ± 0.02
Soyabean oil+ Peanut oil	58.71 ± 0.02
Linseed oil + Sefsol 218	148.09 ± 0.04

Part B (Oils-surfactant miscibility study)			
Surfactants	**HLB value**	**PPZ Solubility(mg/ml) Mean±SD**	**Miscibility with linseed oil & sefsol 218**
Tween 20 (S)	16.7	5.45 ± 0.03	X
Tween 40 (S)	15.6	5.43 ± 0.04	√
Tween 60 (S)	14.9	2.35 ± 0.02	√
Tween 80 (S)	15	5.21 ± 0.03	√
PEG 200 (Cos)	12.7	4.67 ± 0.04	√
PEG 400 (Cos)	11.4	5.15 ± 0.02	√
PEG 600 (Cos)	14.6	4.51 ± 0.04	√
Carbitol (Cos)	8.3	5.10 ± 0.01	X

S= surfactant, Cos= Co-surfactant, n=3

Construction of the pseudoternary phase diagrams

Pseudoternary phase diagrams were constructed using the aqueous titration method. The phase diagram delineates the phase boundary within which a nanoemulsion exists. Observations were made carefully to separate metastable systems from phase boundaries. The relationship between the phase behavior of a mixture and its composition can be selected with the aid of a phase diagram. Linseed oil, Sefsol 218 (oil phase), Tween 40 (surfactant), PEG 400 (co-surfactant) and water were used as constituents to construct the phase diagrams. The systems were observed for visual clarity and flow ability characteristics. Those which did not show a change in the meniscus after tilting to an angle of 90° were classified as nanogels, a metastable system and were therefore not selected. Pseudoternary phase diagrams were constructed based on the observations marked
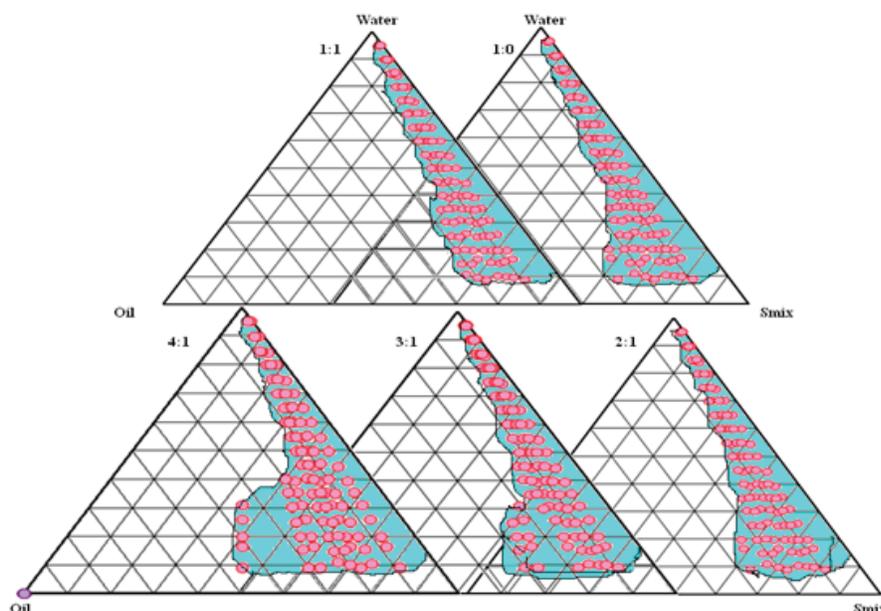
Figure 2 A pseudoternary phase diagram constructed by aqueous titration method to delineate the nanoemulsion region using blends of Sefsol 218 and Linseed oil (1:1, v/v) as an oil phase and surfactant-cosurfactant mixture of Tween 40 and PEG 400 (S_{mix} 1:0, 1:1, 2:1, 3:1 and 4:1).

during titration. Phase diagrams were constructed separately for each ratio of S_{mix} (1:0, 1:1, 2:1, 3:1 and 4:1), so that o/w nanoemulsion regions could be identified. In the phase diagrams o/w nanoemulsion regions are shown. Formulations that provided the required dosage of the drug were selected from different points in the nanoemulsion regions of the phase diagram (dotted regions in Figure 2).

When Tween 40 was used without any co-surfactant in the S_{mix} (1:0), the nanoemulsion region represented a very small portion of the phase diagram. The maximum amount of oil that could be emulsified was found to be 13.7% (v/v) using 41% (v/v) of S_{mix} (1:0) and 13.5% (v/v) using 45% of S_{mix} (1:1). This indicated that the co-surfactant did not contribute significantly to an increase in the nanoemulsion area of the phase diagram. When the surfactant concentration was increased and the co-surfactant was included in the S_{mix} (surfactant:cosurfactant) 1:1, 2:1 and 3:1, the area of the phase diagram occupied by the

nanoemulsion region increased considerably. Adding more surfactant in the S_{mix} (ratio 4:1), the nanoemulsion region did not increase proportionately. Increasing the concentration of co-surfactant in the S_{mix} from 1:2, 1:3 and 1:4 did not produce nanoemulsions. Nano-emulsification is specific to the nature of the oil and surfactant pair, the surfactant concentration and oil/surfactant ratio (10). The minimum concentration of surfactant or S_{mix} that resulted in a kinetically stable nanoemulsion was considered to have potential for oral drug delivery.

Selection of the formulation

From the phase diagrams constructed with S_{mix} ratios of 4:1, 3:1 and 2:1, different formulations were selected from the nanoemulsion region so that sufficient amount of the API could be incorporated into the oil phase (Table 2). The following criteria determined the selection of different formulations from the phase diagrams:

Table 2 Physical stress and dispersibility tests

CODE	% COMPOSITION			SMIX RATIO (v/v)	PHYSICAL STRESS			DISPERSIBILITY STUDIES
	Oil	S_{mix}	Water		Freez-Thaw	Heating-Cooling	Centrifugation	Distilled water
PZ 3	10.11	38.89	51		√	√	√	Grade A
PZ 5	8.89	31.11	60		√	√	√	Grade B
PZ 7	10	40	50	4:1	√	√	√	Grade A
PZ 9	7.02	28.07	64.91		X	-	-	-
PZ 11	5.8	28.99	65.22		√	√	X	-
PZ 14	11.24	33.71	55.60		√	√	√	Grade B
PZ 21	10	40	50		√	√	√	Grade A
PZ 23	8	32	60	3:1	√	√	√	Grade C
PZ 24	7.02	28.07	64.91		√	√	X	-
PZ 25	7.49	37.45	55		√	√	X	-
PZ 26	5.8	28.99	65.22		X	-	-	-
PZ 27	9.09	45.45	45.45	2:1	√	√	√	Grade B
PZ 28	6.67	33.33	60		√	√	X	-

Oil phase used was a 1:1 weight ratio of linseed oil and Sesfol 218

Table 3 Release performance and characterization of the optimized formulations

CODE	PPZ SUSPENSION % Composition				DRUG RELEASE PERFORMANCE (24 h) (Mean ± S.D)		CHARACTERIZATION OF THE PHASE CONSTRUCTED NanoemulsionS (Mean ± S.D)				
	Oil	S_{mix}	Water	S_{mix} ratio	In vitro	Ex vivo	%T	RI	η (cps)	Δdm	PDI
PZ 3	10	39	51		85.85±4.3	86.56±4.5	93.82±5.37	1.312±0.021	558.45±0.99	100.2	0.21
PZ 7	10	40	50	4:1	93.75±3.5	95.18±2.6	97.21±8.28	1.426±0.013	386.76±0.1	46.48	0.16
PZ 21	10	40	50	3:1	76.14±2.4	76.5±2.6	95.29±11.18	1.383±0.011	516.98±0.91	78.28	0.208
	PPZ suspension				55.9±4.3	57.12±7.82	NP	NP	NP	NP	NP

Note: %T= Percentage transmittance, RI = Refractive index, η= Viscosity, Δdm=Particle size, PDI=polydispersity index, NP = not performed

1. The oil concentration is such that the amount of Perphenazine (equivalent to the oral dose of 2 mg) is significantly lesser than its saturated solubility in the oil.

2. The minimum concentration of S_{mix} is administered.

Physical stability study

Nanoemulsions (NE) are considered kinetically stable systems which are produced at a particular concentration of oil, surfactant/co-surfactant and water. NE formulations selected from the phase diagram (Table 2) were subjected to stress stability testing such as centrifugation, freeze-thaw cycles and heating cooling cycles. Formulations that did not show phase separation, creaming, cracking, coalescence and phase inversion during stress stability tests, were selected for further testing (Table 2).

Dispersibility

The formulations that passed the dispersibility test (assigned grade A in Table 2) were considered to pass the dispersibility test in the GIT. Selected formulations were subjected to *vitro* drug release study.

Release study

Three formulations that demonstrated acceptable physical stability and dispersibility were selected for *in vitro* release studies (Table 3). The *in vitro* release studies were performed to compare the release of PPZ from the drug loaded NE (PZ 3, PZ 7, and PZ 21) with PPZ suspension (Figure 3A, Table 3). In the selection of the formulation for *in vitro* release
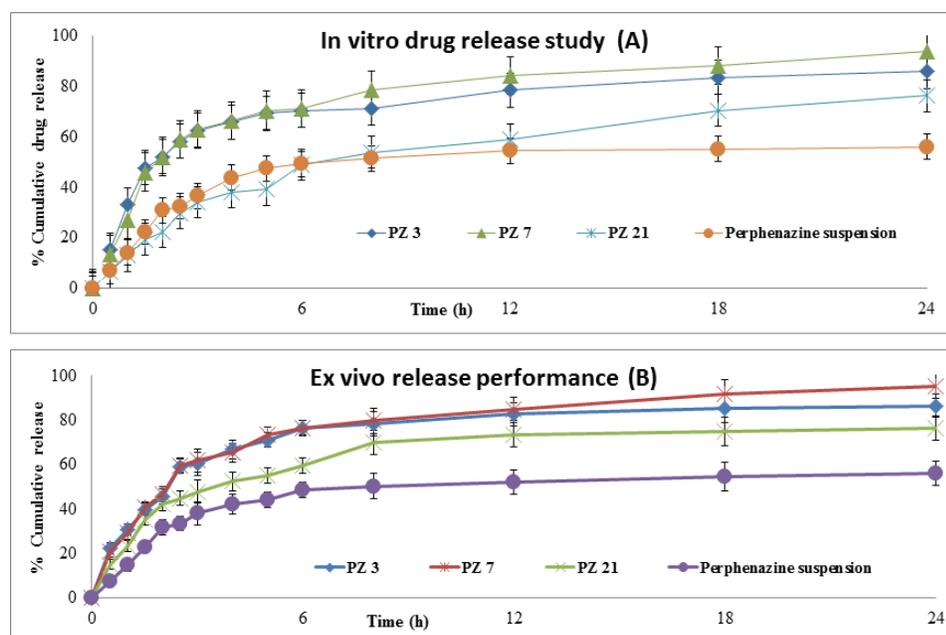
Figure 3 Comparative *in vitro* (A) and *ex vivo* (B) drug release profile of the selected formulations.

study, the content of oil phase was kept constant (10%) while the content of S_{mix} was varied from 35-40%. Percentage cumulative release after 24 hours from PZ 3, PZ 7 and PZ 21 was found to be 85.85, 93.75 and 76.14 % respectively. The concentration of S_{mix} in these formulations was 38.89, 40 and 40 % with 31.11(7.78), 32(8) and 30(10) % of surfactant(co-surfacatant) respectively. A significant increase in percentage drug release was achieved in the case of NEs as compared to the suspension. PZ 7 contained the highest amount of surfac-tant (32%) amongst the selected formulations and showed the greatest release of 93.75%. The presence of co-surfactant in the formulation did not play a significant role in enhancement of the release of PPZ. This is clear from the comparison of release profile of PZ 7 & PZ 21 (S_{mix} 40%). This indicated that the co-surfactant did not assist in the release of Perphenazine from these nanoemulsions. A smaller globule size may also partly have contributed to the greater release rate of PZ 7 (Table 3).

Substantially similar results were obtained in the *ex vivo* release studies. PZ 7 which contained the greatest amount of the surfactant (32%) showed the greatest improvement in the release profile (Table 3, Figure 3B). Surfactants cause a change in the organization of the lipid bilayer of the GIT epithelium. Because this layer presents a rate limiting step toward drug permeability and absorption, a larger surfactant concentration may increase absorption primarily via this bilayer disrupting mechanism (22, 23) Thus based on these studies, PZ 7 showed the greatest improvement of the *in vitro* and *ex vivo* release profiles. Consequently, this formulation was used for characterization and for the *in vivo* study.

In vitro release modeling

The formulation the PZ 7 that demonstrated an acceptable *in vitro/ex-vivo* release profile was selected as a final formulation. The release of Perphenazine from this optimized nanoemulsion followed first order release kinetics ($R^2 > 0.92$).

Characterization

Formulation compositions which were assigned an 'A' grade in the dispersibility test were further characterized (Table 3). Their globule size distribution, refractive index and viscosity were measured.

Percent transmittance

The percentage transmittance of the optimized formulations was determined. The results are shown in Table 3. Amongst the selected formulations, the percent transmittance of formulation PZ 7 was significantly greater than for the other formulations.

Viscosity

The values of viscosity for the optimized formulations are shown in Table 3. The viscosity of formulation PZ 7 was significantly lower.

Particle size distribution

Table 3 shows the droplet size and polydispersity indices of the nanoemulsions. The oil phase in all nanoemulsions comprised of droplets in the range of 40–150 nm (Figure 4). The poly dispersity index showed that all the nanoemulsions had a narrow size distribution. Table 3 shows that the globule size of formulation PZ 7 was the smallest (46.48±8.24 nm) which was significantly smaller compared

to other formulations in the group. Since the diameter of the dispersed oil droplets of the nanoemulsion PZ 7 was much smaller than the smallest blood capillary (400 nm), the probability of mechanical capillary blockage during transport of the droplets is expected to be minimal. The size and size distribution of the droplets is also conducive toward a greater circulation time after in vivo administration.

Surface morphology

Transmission electron microscopy (TEM) is the most important technique for the study of microstructures, because it produces images at high resolution and can capture any coexistent structures and microstructure transitions (17, 20). The morphology and size of particles of nanoemulsion were determined by TEM images. A combination of bright field imaging at increasing magnification and diffraction modes was used to reveal the form and size of the nanoemulsions. The TEM micrographs revealed that the particles of optimized nanoemulsion were spherical in shape with sizes in the range of 78 - 112 nm (Figure 4). The droplet sizes were in agreement with the results obtained using photon correlation spectroscopy.

Bioavailability studies

The *in vivo* study was performed to quantify PPZ after oral administration of PPZ loaded NE and PPZ suspension. The calculated dose
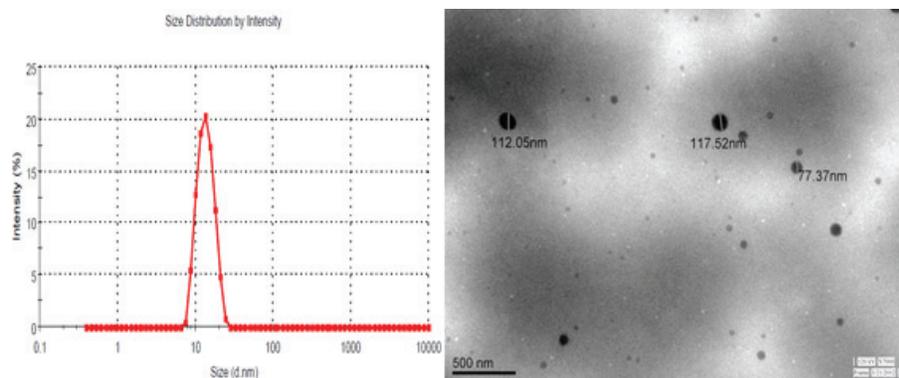

Figure 4 Particle size distribution and Transmission Electron Microscopy of a Perphnazine encapsulated nano emulsion.

Table 4 Mean pharmacokinetic parameters (mean ± S.D. n = 6) of PPZ from formulation PZ 7, and PPZ suspension.

FORMULATION	T_{max}[a] (h)	C_{max}[b] (ng/mL)	$T_{0.5}$ (h^{-1})	$AUC_{0\rightarrow24}$[c] (ng.h/mL)	$AUMC_{0\rightarrow24}$[d] (ng.h/mL)	Rel. B.A.[e] (%)
PPZ Suspension	2.5±0.12	1.3±0.15	9.39±1.56	9.46±1.87	59.65±2.76	100
PZ 7 (PPZ NE)	1.5±0.08	3.89±0.20	9.08±1.35	24.05±3.54	180.78±21.9	254.17

[a] Time of peak plasma concentration.
[b] Peak plasma concentration.
[c] Area under the plasma concentration versus time curve until the last observation.
[d] Area under the moment curve computed upto last observation.
[e] Relative bioavailability.

of Perphenazine administered in rats was 0.137 mg/kg body weight which was equivalent to 2 mg oral dose for adult human being. The plasma samples were analyzed using validated HPLC method described earlier. The LOD and LOQ of Perphenazine using this method were found to be 0.0345 and 1 ng/ml respectively. The plasma profiles of PPZ in adult Albino Wistar rats following oral administration of nanoemulsions and PPZ suspensions were compared. It can be seen that the plasma concentration time profile of the PPZ nanoemulsion represented greater improvement of drug absorption than the PPZ suspension (Figure 5).

Table 4 shows that the T_{max} and C_{max} of the nanoemusion (PZ 7) were 1.5±0.08 hours and 3.89 ± 0.20 ng/ml respectively. These were significantly lesser and greater (respectively) as compared to the PPZ suspension whose values were 2.5±0.12 hours and 1.3 ± 0.15 ng/ml respectively. It was also observed that $AUC_{0\rightarrow24}$ of the PZ 7 formulation (24.0555 ±3.54 ng.h/ml) was significantly greater than that of the PPZ suspension 9.464 ± 1.87 ng.h/ml. Similarly, $AUMC_{0\rightarrow24}$ for the nanoemulsion PZ 7 (180.789 ± 21.98) was significantly greater than that for the PPZ suspension 59.651±2.76 ng.h/ml. The bioavailability of PZ 7 relative to that of the Perphenazine suspension was 254.17%. The enhanced bioavailability is probably due to the increase in solubility and a faster dispersion in, and greater absorption from, the GI tract, which leads to greater absorption into the blood (12).
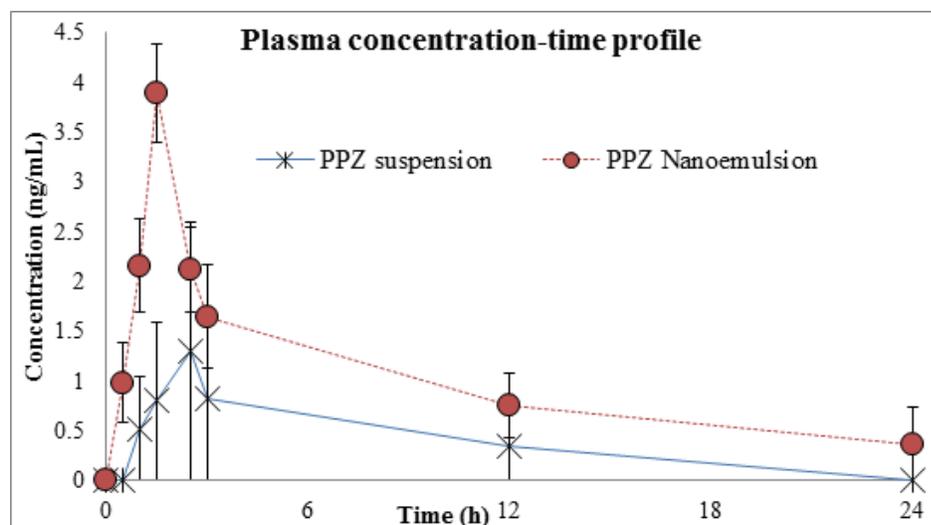

Figure 5 A comparative plasma concentration-time profile of Perphenazine after oral administration of the Perphenazine nanoemulsion and suspension to adult Wistar rats (n = 6 and dose = 0.137 mg/kg body weight).

CONCLUSION

An oral o/w nanoemulsion of Perphenazine was formulated using a blend of long and medium chain triglycerides for the oil phase. *In vivo* studies demonstrated significantly greater absorption of the API from the nanoemulsion than from the conventional drug suspension. Compared to the conventional suspension, the absorption of Perphenazine from the nanoemulsion resulted in a 2.54 fold increase in bioavailability. Part of the increased bioavailability could be attributed to the physical state of the drug in the two formulations.

The nanoemulsion contained molecularly solubilized drug inside the oil phase while the suspension contained the drug in the solid crystalline phase. The nanometer sized globules in the emulsion were postulated to release the drug at a faster rate than its corresponding release rate from the suspended solid and the presence of surfactants in the formulation may have disrupted the organization of the bilayer epithelial cells of the GIT to partially circumvent this rate limiting absorption mechanism. Thus form the above findings, it can be concluded that a physically stable PPZ loaded NE improved the drug permeability and increased its oral bioavailability.

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