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Original Article

Development of oil-in-water Microemulsions for the oral delivery of Pioglitazone HCl.

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Abstract

Poor aqueous solubility of Pioglitazone (PGZ) results in the delayed onset of action as a result sub-therapeutic plasma drug levels may lead to therapeutic failure. In present study, oil-in-water (O/W) microemulsions (MEs) were developed and characterized as oral delivery systems for PGZ. Based on pseudo ternary phase diagrams, PGZ-loaded MEs with mean droplet sizes about 30 nm were successfully produced. Both the ultra filtration and dialysis studies revealed that the release of 80% of PGZ was released from the microemulsion within 12 hrs in vitro. The bioavailability studies for F-PGZ2, MF, and PDZ-CD carried out using Wistar rats. Though there was no significant difference in C_{max} (9.03 ± 0.98 µg/ml, 11.796 ± 1.23 µg/ml, and 10.02 ± 0.96 µg/ml) of the formulations, a significant difference ($P < 0.01$) in t_{max} values (4.0 hours for F-PGZ2 and MF and 0.793 hour for PGZ-CD) was observed. The decrease in t_{max} values indicates faster absorption of the drug from PGZ-CD formulation.

Keywords: Pioglitazone HCl, microemulsion, biological activity.

1. Introduction

Pioglitazone (PGZ) is an oral hypoglycemic agent used in the treatment of type II diabetes which acts by decreasing insulin resistance. PGZ free base and its hydrochloride salt have very low aqueous solubility, and the hydrochloride salt (PGZ-HCl) is used in the pharmaceutical formulations. The aqueous solubility of PGZ free base were investigated by in water[1], surfactant containing solutions, and as a function of pH in buffer solutions. They reported solubility in water, 0.039 mM; 58.00 mM sodium dodecyl sulfate (SDS), 1.171 mM; 51.00 mM cetyl triethylammonium bromide (CTAB), 0.232 mM; 51 mM polysorbate 80 (PS80), 0.252 mM; SDS + PS80, 1.588 mM; and CTAB + PS80, 0.498 mM. IUPC name is, 5-[4-[2-(5-Ethyl-2-pyridinyl) ethoxy] benzyl] thiazolidine-2,4-dione, is a thiazolidinedione derivative with insulin-sensitizing effect that acts as agonist of the peroxisome proliferator-activated receptor subtype gamma in type II diabetes. PGZ-HCl has biological half-life of 4 to 7 hours with excellent oral bioavailability (83%)[2]. Although at steady state, the maximum plasma drug concentrations (C_{max}) were reported as 0.7 (for 15 mg/day dose) and 1.2 mg/l (for 30 mg/day dose), the t_{max} were reported to be 4.8 and 3.7 hours, respectively. This delayed t_{max} may be due to the poor aqueous solubility of PGZ (solubility of 0.015 mg/ ml) and may result in the delayed onset of action which leads to sub therapeutic plasma drug levels and finally to therapeutic failure[3]. This low solubility of PGZ (0.015 mg/) ml leads to low bioavailability as well as membrane permeability, which hinder the development of formulations for the oral route that is the most convenient and acceptable route for patients. Recently, lipid-based formulations have been extensively investigated as a suitable approach to improve the bioavailability of poorly soluble drugs after oral administration[4]. When incorporated into these systems, the active molecules are being regarded to remain in solution throughout its residence in the gastrointestinal tract[5].

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Additionally, the absorption of the drug could be increased by the presence of lipids as a result of stimulation of biliary and pancreatic secretions by the gallbladder, an increase in the gastric residence time and others[6]. Microemulsions, as drug delivery systems, have several merits such as enhanced drug solubility, high stability, and ease of manufacturing[7]. Since microemulsions (MEs) are able to entrap a wide range of drug molecules, improving their solubilization and bioavailability, and even reduce their toxicity, they are emerging delivery systems for oral administration of lipophilic molecules, such as PGZ-HCl[8]. Therefore, the aim of this work was to formulate, categorize and oil-in-water (O/W) MEs based on long- and medium-chain triglycerides in order to increase the solubility of pioglitazone HCl (PGZ-HCl) and render capable its use by the oral route.

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals

Pioglitazone HCl was received from Ranbaxy laboratories Ltd as a gift sample. Sodium hydroxide (NaOH), chloride acid, pioglitazone (HCl and HPLC grade methanol were purchased from Sigma–Aldrich (Saint Quentin Fallavier, France).

2.1.2. Surfactants

Span®20, Span®80, Span®85, Tween®20, Tween®80 andTween®85 were, Labrafac CC purchased from Sigma–Aldrich (Saint Quentin Fallavier, France).

2.1.3. Lipids

Labrafac CC (LC), Labrafil M 1944CS (LM), Labrafac®PG (LPG); Capryol®90 (C90), were kindly supplied by Gattefossé S.A. (Saint-Priest, France).

Corn oil and olive oil, Isopropyl myristate were obtained from Sigma–Aldrich (Saint Quentin Fallavier, France).

2.2. Methods

2.2.1. Selection of oil and hydrophilic surfactant

Nonionic surfactants of the Tween® series (Tween®20, 80 and85) and the lipid mentioned in Section 2.1.3 were weighed and put into a series of screw cap test tubes in the ratios of 0.1:0.9, 0.2:0.8,0.3:0.7, 0.4:0.6, and 0.5:0.5 (w/w) g of 1 g per batch, mixed together, and vortexed thoroughly. Afterwards, 100 µL of distilled water was added to each oil–surfactant mixture in 20–25 µL drops using a micropipette. After each drop of water was added, the system was vortexed for 15 s at room temperature. Visual observations were made, and the clarity or turbidity of each sample was recorded. The surfactant forming most clear systems was selected as the hydrophilic surfactant that best matched the tested lipid.

2.2.2 Selection of surfactant blends

The individual nonionic hydrophilic surfactant chosen in Section2.2.1 was blended with the lipophilic surfactants of the Span® series(Span®20, 80 and 85) in ratios of 3:2, 7:3, 4:1, and 9:1 (w/w) to produce blends of surfactants with various hydrophilic-lipophilic balances (HLBs) in the range of 9.7–14.4 (Table 1). The solubilization capacities of the blends of surfactants were studied using the same method as that used to study the other surfactants individually. The blend of surfactants forming a clear system at most of the ratios was selected as the blend that best matched the HLB of the tested lipid

Table 1. Composition of the surfactant blends and their final HLB values.

Surfactant blend	Surfactants		Weight ratio	HLB
	Tween® 80	Span® 20		
S1	Tween® 80	Span® 20	3:2	12.4
S2	Tween® 80	Span® 20	7:3	13.1
S3	Tween® 80	Span® 20	4:1	13.7
S4	Tween® 80	Span® 80	9:1	14.4

S5	Tween® 80	Span® 80	3:2	10.7
S6	Tween® 80	Span® 80	7:3	11.8
S7	Tween® 80	Span® 80	4:1	12.9
S8	Tween® 80	Span® 80	9:1	13.9
S9	Tween® 80	Span® 85	3:2	9.7
S10	Tween® 80	Span® 85	7:3	11.0
S11	Tween® 80	Span® 85	4:1	12.4
S12	Tween® 80	Span® 85	9:1	13.7

2.2.3. Construction of pseudoternary phase diagrams

After selection of the most suitable surfactant blend, The oil phase was mixed with the surfactant phase in the ratios (volume basis) of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. A water titration technique was employed for the preparation of the pseudo ternary phase diagrams. Distilled water was added drop by drop to the mixture of oil/surfactant phase at room temperature pseu-doternary phase diagrams were constructed based on the types of systems formed when the mixtures of lipids and surfactant blend were serially titrated by water followed by sonication. The systems were characterized by visual observation such as(1) transparent or translucent and can flow easily i,e Microemulsion (ME), (2) Transparent or translucent non flowable when inverted 90◦ liquid crystal(LE),(3) Milky or cloudy and can flow easily i,e emulsion (EM) (4) Milky or cloudy non flowable when inverted 90◦ i,e emollient gel or cream (EG or EC) ,(5)More than one type of dispersion existing in the mixture, as indicated by the presence of more than one abbreviation of dispersions i,e bicontinuous phase (BP) as described by[9]. The systems were also assessed regarding their isotropy by polarized light microscopy as described in above section.

3. Preparation of microemulsions

Based on the pseudoternary phase diagrams, the most suitable ratios of oil, surfactant blend and water for the production of O/W microemulsions were selected. The lipid was mixed with the surfactant blend in the weight ratios of 1:9 and 2:8 and 5 mL of water. The surfactant blend selected for all the formulations comprised a mixture of Tween® 80: Span® 80 in the weight ratio of 9:1.F1, F2, F3 and F4 are PGZ-unloaded formulations while F- PGZ 1, F- PGZ 2, F-PGZ 3 and F-PGZ 4 are PGZ loaded formulations. All formulations prepared have 82.2% of water, 1.8% of oil and 16% of surfactant blend for F1, F3, F-PGZ 1 and F-PGZ 3, 3.6% of oil and 14.2% of surfactant blend for F2, F4, F-PGZ 2 and F-PGZ 4. The PGZ containing formulations have 0.10% of PGZ (Table 2). The mixture was vortexed and subjected to sonication at 140 V for 60 s (Digital Sonifier, model 450, Branson Ultrasonic SA, France). Various batches of microemulsions were prepared by water titration method and optimization was done in terms of clarity, % transmittance, conductance particle size, viscosity, % drug content and % drug release.

Table 2. Composition of the PGZ -loaded and PGZ -unloaded microemulsions.

Formulation	Oil	Oil: surfactant weight ratio	PGZ
F1	Capryol®90	1:9	-
F2	Capryol®90	2:8	-
F3	Labrafil M 19444 CS	1:9	-
F4	Labrafil M 19444 CS	2:8	-
F-PGZ1	Capryol®90	1:9	+
F-PGZ2	Capryol®90	2:8	+
F-PGZ3	Labrafil M 19444 CS	1:9	+
F-PGZ4	Labrafil M 19444 CS	2:8	+

PGZ –unloaded=F1-F4, PGZ –loaded= F-PGZ1-4

3.1. Drug incorporation

An excess of PGZ was added to the blank MEs, and the systems were vortexed for 2 min. After stirring, the mixtures were left for 10, 30 and 60 min under magnetic stirring at pH 11 in order to evaluate the time necessary for the incorporation of PGZ into the systems at 25 ± 0.1◦C. Thereafter, the pH was neutralized. The MEs were centrifuged at 10,000 × g in a Hitachi Himac CP-80 Ultracentrifuge (USA) for 15 min to remove the excess of drug. The supernatant was recovered and carefully filtered using a 0.22µm membrane. The filtrate was diluted and dissolved in methanol for the quantitative analysis of the PGZ by HPLC.

3.2 HPLC assay of Pioglitazone

The HPLC system consisted a Shimadzu Model SPD-M 20A variable-wavelength UV detector (Shimadzu Corporation, Kyoto, Japan) governed by a microcomputer running Millennium® version 32 software, and vortex mixer (Scientific Industries Inc., New York, USA). The detector wavelength was set at 269 nm. Mobile phase was prepared with Acetonitrile, 0.1 N Ammonium acetate, Glacial acetic acid (25:25:1). HPLC was then performed and retention time was noted. To determine the linearity of the method, different concentrations of PGZ in the 50-300µg/ml were prepared and analyzed.

3.4. Microemulsion characterization

3.4.1. Percent drug content

The drug content of the microemulsion formulation was determined by dissolving 1 ml (equivalent to 10mg drug) of the formulation in 10 ml of methanol. After suitable dilutions with methanol, absorbance was determined using the UV spectrophotometer (AU-2701 Systronic, Mumbai, India) keeping blank microemulsion as control at wavelength 269 nm**.**

3.4.2. Determination of pH

The pH values of the samples were measured by a pH meter (Digital Systronics, Mumbai, India) at ambient temperature with glass electrode**.**

3.4.3. Thermodynamic stability

Microemulsions are thermodynamically stable formulations and are formed at particular concentration of oil, surfactant and water, with no phase separation, creaming and cracking. It is the thermo stability that differentiates microemulsions from emulsions that have kinetic stability and eventually phase separate. Thus, the selected formulations were subjected to different thermodynamic stability by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests[10].

3.4.4. Dispersibility test[11]

The thermodynamically stable microemulsions were further taken for the dispersibility test for visual assessment and were assessed using following grading system

Grade A: Rapidly forming (within 1 min) microemulsion, having a clear or bluish appearance.

Grade B: Rapidly forming, slightly less clear microemulsion, having a bluish white appearance.

Grade C: Fine milky microemulsion that formed within 2 min.

3.4.5. Robustness to dilution

Microemulsions resulting from dilution with dissolution media must be robust to all dilutions and should not show any separation even after 24 hours of storage.

3.4.6. Percent transmittance

Transparency of microemulsion formulation was determined by measuring the percentage transmittance at 650 nm with purified water taken as blank through UV spectrophotometer.

3.4.7. Conductance

Electrical conductivity (s) has been traditionally used as a standard technique to study the phase behavior. The underlying principle for phase determination by conductivity is the ability of water to conduct an electric current, which is measured in Scm^{-1} or μScm^{-1}. The conductive measurements were taken by a conductivity meter. The microemulsion prepared with addition of water was measured after thorough mixing and temperature equilibration at 25°C, the electrode was dipped in the microemulsion sample until equilibrium

was reached, and reading becomes stable. Reproducibility was checked for certain samples and no significant differences were observed.

3.4.8. Mean droplet size and distribution

The droplet size and distribution of the microemulsions loaded with PGZ-HCL was measured by an electrophoretic light-scattering spectrophotometer (ELS-8000, OTSUKA Electronics Co. Ltd., Japan). The microemulsions were transferred to a standard quartz cuvette, and the droplet size and polydispersity index of the microemulsions were determined via dynamic He–Ne laser (10 mW) light-scattering at an angle of 90° at 25 °C. Data analysis was conducted using a software package (ELS-8000 software) supplied by the manufacturer.

3.4.9. TEM

The morphologies of the microemulsions were examined by an Energy-Filtering Transmission electron microscopy (TEM) (LIBRA 120, Carl Zeiss, Germany) with a 80 kV accelerating voltage with the aid of A.I.M.S New Delhi. The microemulsions were negatively stained by 2% phosphotungstic acid PTA) and placed on carbon-coated 400 mesh copper grids followed by drying at room temperature before measurements.

3. 4.10. Rheological behavior

The rheological properties of PGZ-loaded and unloaded MEs were determined using a using Brookfield's viscometer (Brookfield DV-2+ pro viscometer) at single mode using spindle # CPE40 at 32 ± 0.5°C. The analyses were carried out with a shear rate in the range of 10^{-3}–$10^{5}s^{-1}$. All rheological determinations were carried out in triplicate for all samples and at 25.0 ± 0.2◦C.

3.5. In vitro release of PGZ using ultra filtration and dialysis methods

The in vitro release of PGZ from microemulsions was conducted by both the ultrafiltration and dialysis methods. For the ultrafiltration method, an aliquot of each PGZ microemulsion (200 µL) was placed in 900 mL of release medium (PBS, pH 7.4) containing 0.1% Tween 80 (w/v) to maintain sink condition. While stirring the release medium using the magnetic stirrer at 100 rpm at 37±0.5°C, aliquots of dissolution medium (0.5 mL) were withdrawn at predetermined time intervals for 12 h, and were refilled with the equal volume of fresh medium. The samples (0.3 mL) were centrifuged at 7000× g for 10 min in an Ultracel YM-3 ultrafiltration tube (MWCO: 3,000, Millipore Corporation, MA, USA). The concentration of PGZ-HCL in the filtrate was determined by HPLC after appropriate dilution with methanol. For the dialysis method, aliquot of each PGZ-HCL microemulsion was placed in the mini dialysis kits (MWCO 6–8 kDa) (Kfar-Hanagid, Israel), and was immersed in 900 mL of release medium (PBS, pH 7.4) containing 0.1% Tween 80 (w/v). Aliquots of dissolution media (0.5 mL) were withdrawn, and the concentration of PGZ-HCL was determined by HPLC after appropriate dilution with methanol. The percent cumulative amount of PGZ released from microemulsions was calculated as a function of time.

4. In vivo studies

The bioavailability studies for tablets with F-PGZ2, Plain drug solution, and MF (PioglitR) were carried out using male Wistar rats (200-250 g). The animals were maintained in a clean room at a temperature between 20 – 25°C with 12-hour light and dark cycles and controlled relative humidity. The animals were fasted for 12 hours prior to commencement of the study as well as during the study and had access to water ad libitum. The institutional animal ethical clearance (vide letter no. Protocol No. IAEC/CCP/12/PR-016) was obtained before conducting the studies. They were divided into four groups (six in each group); group I served as a control group whereas other three groups were treated with tablet formulation containing F-PGZ2, MF, and plain drug solution, respectively. Tablets with a dose of 10 mg/kg body weight of rats were administered by dispersing in distilled water through oral feeding pipe[12]. Blood samples were collected through the lateral tail vein[13] of rats at 0, 5, 10, 15, 20, 30, 40, 50, and 60 minutes followed by 3, 8, 12, and 24 hours after administration. The blood samples were centrifuged at 10,000 rpm for 10 minutes. After centrifugation, plasma was transferred into clean, fresh eppendorf tubes and frozen at

20°C until assayed. The plasma concentration of drug was determined by High Performance Liquid Chromatography (HPLC)[14] (Shimadzu LC 10 AT VP pumps; SPD-10 A detector), using Merck C-18 (250 mm × 4.6 mm, 5 µm) column and 0.05M potassium dihydrogen phosphate and methanol (35:65) as mobile phase at 269 nm. The results obtained were analyzed for various non-compartmental pharmacokinetic parameters using Kinetica 2000 software.

5. Statistical analysis

Statistical analysis of the raw data was conducted using one-way analysis of variance (ANOVA) in Graph Pad Prism (Graph Pad software for Mac v. 6.0, San Diego, CA). Differences between different groups were considered statistically significant at $p < 0.05$.

6. Acknowledgement and conflict of interest

The authors are grateful to Madhu Chitkara, Vice Chancellor, Dr. Sandeep Arora, Chitkara University for providing excellent infrastructure facility for literature review. I appreciate the co-operation extended by the AIIMS, New Delhi for providing me TEM facilities. There is no conflict of interest what so ever.

Results and Discussion

Selection of surfactant and surfactant blendsC90

Sunflower oil is a monounsaturated (MUFA)/polyunsaturated (PUFA) mixture of mostly oleic acid (omega-9)-linoleic acid (omega-6) group of oils. Labrafil M 1944 CS (polyoxyethylated oleic glycerides), labrafac CC & (C90) (caprylic/capric glycerides). The major unsaturated fatty acids in soybean oil triglycerides are the poly-unsaturates, alpha-linolenic acid (C-18:3), 7-10%, and linoleic acid (C-18:2), 51%; and the mono-unsaturate, oleic acid (C-18:1), 23%.It also contains the saturated fatty acids, stearic acid, (C-18:0), 4%, and palmitic acid, (c-16:0), 10%. An important parameter take into account to develop MEs, is the HLB of the surfactant or surfactant blend[15]. It is related to the contribution of both hydrophilic and liophillic fragments of a surfactant molecule. Ideally, surfactants with HLB values between 8 and 20 are able to form O/W MEs, while W/O MEs are formed when the HLB range is 4–7[16]. Tween®80 was shown to be the hydrophilic surfactant with the highest solubilization capacity when compared with Tween®20and Tween®85. These emulsifiers have the same polar head, but different hydrophobic tails (lauric, oleic and oleic acid, respectively in Tween 20, 80 and 85). The length of these hydrophobic chains determines the interactions with the oil phase[17]. In our experiments, we obtained clear mixtures of water and oil at the highest weight ratios for Labrafil M 19444 CS(LM), Labrafac CC(LC) and , Capryol®90 (C90), For the other oils tested, no clear mixture was obtained. Therefore, these were not used in further studies. It is possible that the low HLBs of olive, Labrafac®PG (LPG, sunflower oil and soyabean oil respectively, were responsible for the incompatibility between these lipids and the hydrophilic surfactants. It has also been stated that inter-actions between surfactants at an oil–water interphase are known to be highly dependent on the nature of the oil[18]. Soybean and olive oils are composed of long-chain triglycerides and probably showed a weak interaction with the surfactant from the same fatty acid derivative, as stated in the literature[19]. Moreover solubility studies conducted in as shown in Table 3 for PGZ in different oils also support the fact stated above for selection of oil.

Table 3. Solubility of Pioglitazone HCl in various Vehicles.

S. No.	Vehicles	Solubility (mg/ml ± SD)
1	Sunflower oil	25.9 ± 2.3
2	Isopropyl Myristate	35.9 ± 1.6
3	Capryol 90	91.1 ± 1.2
4	Labrafil M 1944CS	58.7 ± 1.4

5	Olive oil	8.5 ± 1.3
6	Labrafac CC	27.5 ± 2.8
7	Tween 20	5.53 ± 0.8
8	Span20	11.7 ± 1.3
9	Span80	29.2 ± 1.2
10	Span 85	23.6 ± 1.7
11	Tween 80	8.23 ± 1.5

Construction of pseudoternary phase diagrams

It is known that a single surfactant is not sufficient to form single-phase microemulsions and an adequate mixture of surfactants may be required to optimize the microemulsion formation[20]. The use of mixtures of nonionic surfactants is an interesting approach from the pharmaceutical point of view, since such surfactants are generally regarded as having low toxicity and irritancy and therefore, considered to be acceptable for oral administration. Additionally, the use of mixtures allows the individual concentration of each surfactant to be decreased, which may increase the biocompatibility of the final formulations[21]. Therefore, Tween®80 was mixed with the hydrophobic surfactants of the Span® series to provide surfactant blends in order to screen and select the best surfactant mixture to prepare oil-in-water microemulsions. The results obtained for the solubilization power of the surfactant blytends revealed two mixtures as having the highest capacities:Tween®80/Span®20 7:3 (v/v) (S2) and Tween®80/Span®80 9:1(v/v) (S8), the HLB values of the two blends being 13.1 and 13.9 and 14.1 respectively. Thus, these two surfactant blends were selected to study the phase diagram behavior of Labrafil M 19444 CS, Labrafac CC and , Capryol®90 (C90). According to the pseudoternary phase diagrams, several types of dispersions could be produced by mixing Labrafil M 19444 CS, Labrafac CC and , Capryol®90 (C90) with the surfactant blends S2 and S8 followed by titration with water. For instance, large areas of emulsions, microemulsions and some considerable areas of liquid crystal could be detected, as well as smaller areas of bicontinuous phase, cream and gel Figure (1 and 2). Both the surfactant blends M2 and M8 were able to produce some microemulsion-forming regions for the three lipids tested. This seems to be coherent with the HLB values of surfactants or mixture of surfactants reported to be optimal for the preparation of microemulsions, since S2 and S8 presented very similar HLB values: 13.1 and 13.9, respectively. However, it was evident that both C90 and Labrafil M 19444 CS were able to produce larger areas of O/W emulsions than Labrafac CC. Thus, propylene glycol esters of caprylic acid seem to be more appropriate for the preparation of O/W emulsions and microemulsions than propylene glycol esters of lauric acid. Furthermore, the phase diagram behavior of those lipids was not only affected by the HLB value of the surfactant, but also by the structure of the co-surfactant. Previous studies have observed that in general the most stable emulsions are formed when the two emulsifying agents have the same hydrocarbon chain length, such as the combination between Tween betweenTween®80 and Span®80, because of their similar chemical structure[22]. Mahdi and coworkers stated that high solubilization capacity can be obtained when surfactants with the lowest and highest HLB values are mixed. In our case, we believe that the three chains of oleic acid esters in the molecule of Span®85 hinder the interaction with Tween®80. On the other hand, the interaction between Span®80 and Tween®80 (S8) proved to be more effective in reducing the oil-water interfacial tension and producing MEs. These formulations were used for further studies.

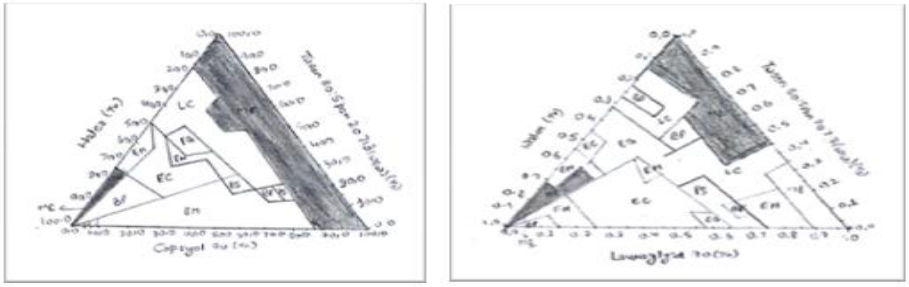

Fig.1. (a), (b):-Pseudoternary phase diagrams formed by (a) C90, (b) L90 as the oil phase and Tween 80: Span 20 7:3 (w/w) as the surfactant blend and water. ME, microemulsions; LC, liquid crystal; EM, emulsion; EG, emollient gel; EC, emollient cream; BP, bicontinous phase; PS, phase separation.

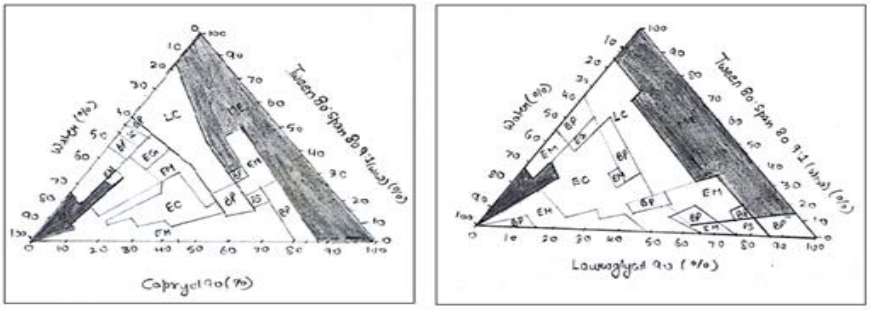

Fig. 2. (a), (b):-Pseudoternary phase diagrams formed by (a) C90, (b) L90as the oil phase and Tween®80: Span®80 9:1 (w/w) as the surfactant blend and water. ME, microemulsions; LC, liquid crystal; EM, emulsion; EG, emollient gel; EC, emollient cream; BP, bicontinous phase; PS, phase separation.

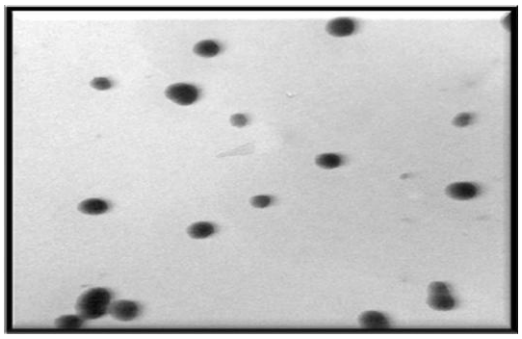

Fig. 3. TEM (Transmission electron microscopy) of optimized formulation F-PGZ2.

Table 4. Evaluation parameters of prepared microemulsion.

Formulation code	Viscosity (cP)	% Transmittance	Particle size (nm)
F-PGZ1	27.32	97.21	28.27
F-PGZ2	36.43	96.29	36.72
F-PGZ3	29.32	95.49	45.60
F-PGZ4	38.74	94.67	162.43
F5	41.35	93.33	115.49

Microemulsions could be diluted by water in the gastrointestinal tract upon oral administration, which could lead to drug precipitation. Microemulsions of PGZ exhibited satisfactory solubilization capacity for at least 24 h.

Rheological behavior

The physicochemical characterization of delivery systems is an essential step in the pre-formulation process to predict the feasibility of the final products. Among the parameters for the characterization of MEs, rheology is a fundamental approach to investigate structural properties and acquire helpful information not only on the stability of such systems, but also on the handling, storage and pipeline transportation of MEs[23]. Although the rheological analysis indicated that the viscosity was very low for all the samples, it appeared to decrease at low shear rates between $10^{-3}$and 10 $s^{-1}$and remained constant at higher shear rates than 10 s^{-1}. However, the flow curves revealed that all the ME systems showed a linear relation-ship between the shear stress and shear rate, which is a feature of Newtonian flow materials[24] (Fig. 4a and b). These results confirm that our samples are discontinuous MEs. As reported by previous studies, discontinuous MEs show constant viscosity over a wider range of shear rates than bicontinuous MEs[25]. As a consequence of their low viscosity, such systems are considered suitable for oral delivery32q[26]. The influence of PGZ on the micro-organization of the MEs was investigated. No change in the linear profile of the flow curves was observed (Fig. 4b), indicating that the drug did not influence the flow properties of the system.

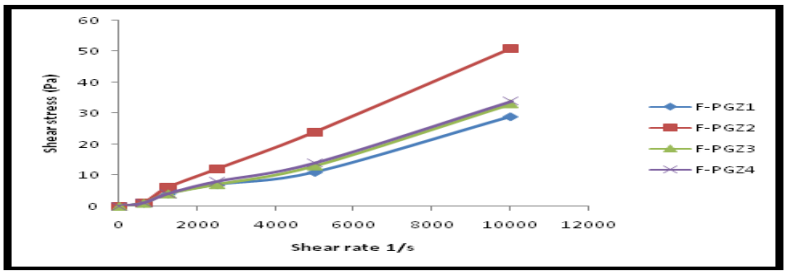

Fig. 4(a). Rheological Behavior of loaded Formulation.

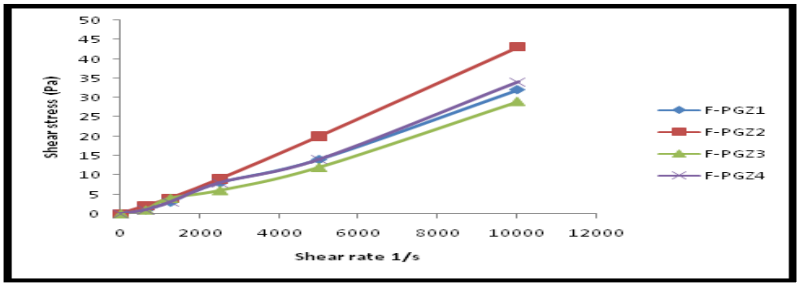

Fig. 4 (b). Rheological Behavior of un-loaded Formulation.

In vitro release study

The in vitro release of PGZ from the microemulsions is shown in Fig. 5. Ultrafiltration study was conducted where no significant difference among the three microemulsions was observed (Fig. 5a). Dialysis studies also showed no significant difference among the three microemulsions: no initial burst release was observed and about 80% of PGZ was released within 12 h. However, the initial release of PGZ from drug solution was faster than that from the microemulsion within 1 h, after which that of the former slowed down (Fig. 5b) due to the decrease in drug solubility in diluted conditions

(Fig. 3). A study on phenytoin has been reported where drug dissolution study showed about 90% release within 10 min when the microemulsion is released from hard gelatin capsules[27]. In this case, the emulsion could disperse quickly in the release media once the gelatin capsule was dissolved. However, in the ultrafiltration study, the actual drug released into the media is determined. Moreover, in the dialysis experiment, membrane was used to eliminate the possibility of the microemulsions being dispersed instantaneously in the release media, thereby ensuring more accurate dissolution profiles of the drug itself from the microemulsions. Since the molecular cut off was 6–8 kDa, it is unlikely that the microemulsion itself could penetrate the dialysis membrane or would it work as the rate-determining step of the drug release. Thus, both the ultrafiltration and the dialysis methods seem to reflect the actual release profile of PGZ from the microemulsion into the release media.

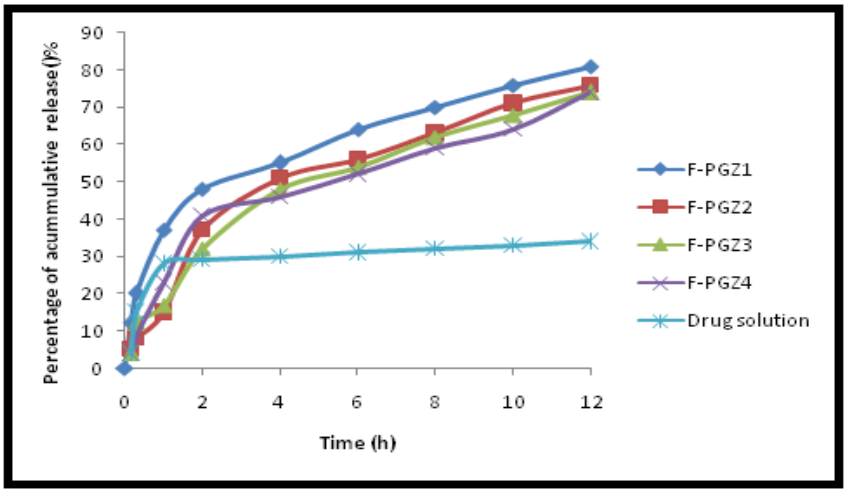

Fig. 5(a). In vitro release comparison through % dialysis method.

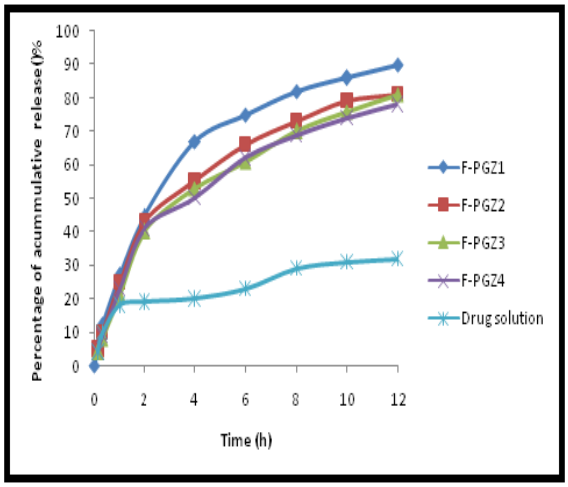

Fig. 5(b). In vitro release comparison through % release Ultracentrifugation method

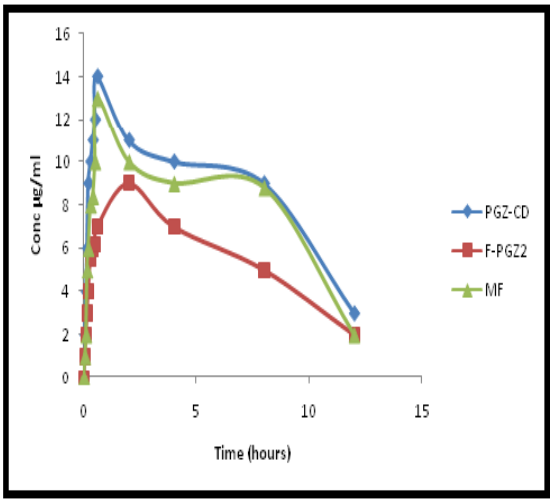

Fig. 6. In vivo comparison Graph between PGZ-CD, F-PGZ2 and Marketed formulation.

Table 5. Pharmacokinetics of Microemulsion of Pioglitazone Hydrochloride.

Pharmacokinetic parameter	F-PGZ2	PGZ-CD	MF
C_{max} µg/ml	9.03 ± 0.98 µg/ml	11.796 ± 1.23 µg/ml	10.02 ± 0.96
t_{max} (hr)	4.0± 0.00	0. 998± 0.00	4.00±0.00
$t^{1/2}$(hr)	8.24±1.09	7.86±0.77	8.59± 0.87
$K_E(hr^{-1})$	0.113±0.016	0.121±0.014	0.095±0.022
AUC µg/ml*hr	144.81±24.42	180.90±29.48	141.61±26.38
AMUC µg/ml*hr	1705.48±449.67	1820.81±513.38	1591.18±533.57
MRT(hr)	20.59±1.45	18.29±1.15	19.27±1.42

In Vivo Studies

The plasma drug level curve for the formulation F-PGZ2, MF and PDZ-CD is shown in Figure 6. The various pharmacokinetic parameters were calculated using Kinetica 2000 software, and results are shown in Table 5. The bioavailability studies for F-PGZ2, MF, and PDZ-CD carried out using Wistar rats. Pharmacokinetic Profile of F- PGZ2, MF was compared by one way ANOVA followed by Dunnett Post Hoc multiple comparison test. Though there was no significant difference in C_{max} (9.03 ± 0.98 µg/ml, 11.796 ± 1.23 µg/ml, and 10.02 ± 0.96 µg/ml) of the formulations, a significant difference ($P < 0.01$) in t_{max} values (4.0 hours for F-PGZ2 and MF and 0.793 hour for PGZ-CD) was observed. The decrease in t_{max} values indicates faster absorption of the drug from F-PGZ2 formulation. This would be particularly beneficial when PGZ-CD is administered at bed time after food.

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