

Enhanced Systemic Exposure and Efficacy of Diltiazem from Novel Microemulsion Formulation: Characterization, *In vitro* Release, *In vivo* Pharmacokinetic and Efficacy Evaluation

Harsha Kamath¹ A. Sivakumar^{2*}

¹Department of Pharmaceutical Chemistry, Karnataka College of Pharmacy, #33/2, Thirumanahalli, Hegdenagar Main Road, Bengaluru, 560064, Karnataka, INDIA.

²Department of Chemistry, School of Advanced Sciences, Vellore Institute of Technology, Vellore, 632014, Tamilnadu, INDIA.

ABSTRACT

Purpose: Diltiazem, a calcium ion cellular influx inhibitor is known for its limited and variable bioavailability. This study is intended to explore the benefits of microemulsion formulation as oral drug delivery system for immediate release to improve the bioavailability and efficacy of Diltiazem.

Methods: Oil in water microemulsion was prepared using the simple water titration method. The optimized formulation was evaluated for physicochemical parameters like viscosity, pH, conductivity and accelerated stability studies. *In vitro* release, *in vivo* pharmacokinetics and *in vivo* efficacy of the optimized diltiazem microemulsion was investigated. **Results:** The optimized diltiazem microemulsion consisted of 60% water, 5% Almond oil, 35% mixture of surfactant (Tween 80) and cosurfactant (Polyethylene glycol 400) (1:8). The existence of microemulsion region was investigated using pseudoternary phase diagrams. The average particle size by dynamic light scattering technique was found to be 13.8 nm with polydispersity index of 0.474. The optimized microemulsion was found to be thermodynamically stable with *in vitro* release of 91.82% compared with that of suspension at 55.2%. The peak exposure of diltiazem was 1.23 fold higher and the extent of exposure was found to be 1.24 to 1.29 fold greater for

microemulsion compared to the reference tablet formulation when tested in rabbits. The novel formulation was found to have greater efficacy compared to conventional tablet formulation in reducing systolic blood pressure in rats. **Conclusion:** The diltiazem microemulsion greatly improves the pharmacokinetic parameters and thus improves therapeutic efficacy of diltiazem and could be a potential alternative oral dosage form in therapeutic management of hypertension.

Key words: Bioavailability, Diltiazem Efficacy, Micro emulsion, Pharmacokinetics, Release kinetics.

Correspondence:

A. Sivakumar, Department of Chemistry, School of Advanced Sciences, Vellore Institute of Technology, Vellore, 632014, Tamilnadu, INDIA.

Phone: 08124154599

Email: adi@vit.ac.in

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INTRODUCTION

Diltiazem, a benzothiazepine derivative and an antagonist of calcium channel, largely used for controlling blood pressure and angina. Although it is well absorbed across the gastrointestinal tract, it has large inter individual variation and low absolute bioavailability (30% to 40%) due to substantial pre-systemic metabolism.¹⁻⁴ Cytochrome 3A4 (CYP 3A4) and intestinal P-glycoprotein (P-gp) efflux transporter have been proposed as possible candidates for this action.^{2-5,6} The development of novel drug molecule is a time consuming, laborious and expensive process, while the development of new formulations of the existing patented drug molecules opens up vast opportunities for improvement of pharmacotherapeutic characteristics. Attempts have been made to improve its bioavailability by formulating sustained release suspensions using resin complexes and a sparingly soluble salt.^{7,8} These methods lacked simplicity in formulation and evaluation. One of the modern drug carriers which are widely researched are microemulsions. They are thermodynamically stable, isotropic liquid formulations having the advantages of optical clarity and simple preparation technique of combining oil, water and surfactant; a cosurfactant is often included in the formulation to improve the stability by reducing the particle size further. Owing to the advantages of microemulsions that include ease of preparation, thermodynamic stability and optical clarity, an attempt was made to formulate diltiazem as an oral, oil in water microemulsion formulation for immediate release. Ease of administration and flexibility in the dose adjustment are the major advantages of liquid orals. The low bioavailability of the drug was

also a key factor which attracted the development of oral microemulsion to study any potential improvement in bioavailability and efficacy. The P-gp inhibition by the excipients used in the formulation might improve the bioavailability of the drug. The study was aimed at preparing suitable oil in water microemulsion for immediate release using diltiazem free base that is water insoluble for improving bioavailability of diltiazem. The liquid oral form of the drug would be desirable for use in elderly patients with hypertension.

On literature search, we found that our study is the first attempt to formulate and characterize the pharmacokinetics of water in oil diltiazem microemulsion for oral delivery. The novelty involves in the selection of drug, preparation technique, characterization and pharmacokinetic evaluation. This study genuinely covers a new formulation development for diltiazem, a poorly bioavailable drug. Additionally, *in-vivo* pharmacokinetic parameters along with pharmacodynamic efficacy are vital information and an added value in proving the applicability of this formulation.

MATERIALS AND METHODS

Materials

Diltiazem was a gift by BalPharma (Bengaluru, Karnataka, India). Polyethylene glycol 400 (PEG 400), Polysorbate 80 (Tween 80), Isopropyl Myristate (IPM), Propylene glycol, Methanol were purchased from Merck (Schuchardh., Germany). Almond, Soyabean, Rice bran, Olive,

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Coconut, Arachis and Castor oils were obtained from standard sources. Milli-Q-water (Millipore, USA) was used throughout the process.

Pre-formulation studies

Solubility study

The components of the microemulsion were selected by solubility study using equilibration method. A known quantity of the drug was dissolved in each of the oils, surfactants and cosurfactants by vortexing in stoppered vials, followed by shaking at 100 rpm for 72 hours on an orbital shaker at $25 \pm 1^\circ\text{C}$, before centrifuging at 3000 rpm for 15 min. followed by filtration of supernatant liquid through 0.45 μm membrane. The dissolved drug after appropriate dilution was measured for its absorbance at 237nm using a double beam UV-spectrophotometer (Shimadzu UV-1700).

Drug-excipient compatibility study

Fourier Transform InfraRed analysis (FTIR) was used to study drug-excipient interaction by scanning the samples in the range of 400-4000 cm^{-1} . The pure drug was mixed with surfactant, co-surfactant and oil and this mixture was analyzed. The comparison was done with FTIR spectrum of pure drug to eliminate the possibility of important functional groups of the drug interacting with the excipients.

Formulation of Microemulsion: Construction of Pseudoternary phase diagram

Based on the solubility study, almond oil, tween 80 (HLB-15) and PEG 400 (HLB-11.6) were selected as the oil phase, surfactant and cosurfactant respectively.

Surfactant:cosurfactant (S_{mix}) were prepared at 1:0, 1:1, 1:2, 1:4, 1:8, 1:10, 1:12, 2:1, 3:1 and 4:1 ratios and oil was incorporated into each of these mixtures in the ratios oil: S_{mix} 1:1 to 1:9 and vice versa by gentle shaking and vortexing. Water was added from the burette, drop by drop to this mixture which was mixed on magnetic stirrer. The slow and continuous stirring allows equilibration between oily and aqueous phase. The resulted mixtures ranged from milky white, highly turbid, translucent and transparent liquid phase. Formation of transparent, free flowing mixtures indicated end point of titration. Other mixtures were termed unstable emulsions as they separated into two layers with time. The clear, low viscous and transparent mixtures were termed monophasic and were kept aside for few hours for visual inspection. The various ratios of oil, S_{mix} and water from the titrations that resulted in a number of mixtures were used to construct phase diagram. The three diagonals of the triangle represent aqueous phase, oil phase and S_{mix} . The shaded area represented monophasic emulsions and considered as the area of existence of stable microemulsions. All titrations were repeated twice to get reproducible results.

The drug was dissolved in oil and optimized ratios of surfactant, co-surfactant and water to get drug loaded microemulsions. These formulations were prepared with the oil concentration that dissolved the required dose of diltiazem easily. The pseudoternary phase diagrams constructed with these mixtures help to understand the effect of drug on the clear microemulsion region. The optimum formulation should have minimum S_{mix} and maximum oil concentration so as to dissolve the required dose of drug but with minimum gastrointestinal irritation effects of surfactants and co-surfactants.

Evaluation of Microemulsion System: Thermodynamic stability studies

The thermodynamic stability of diltiazem microemulsion was evaluated using following stress tests in series.

Physical stability the formulations were checked by centrifugation at 3000 rpm for 30 min. Those formulations which didn't cream, crack or separate into two layers were subjected to the next test.

The stability of the formulations at extreme temperatures were tested by storing them at 45°C and 4°C each for a period of 48 hours. This heating and cooling cycle was repeated six times. The stable formulations were taken for the next test.

The next stress test is freeze-thaw cycle that involved storing the formulations between -21°C and $+25^\circ\text{C}$ each for a minimum period of 48 hours. The formulations that passed these stress studies were considered thermodynamically stable and selected for further optimization.

Physico-chemical Evaluation

Conductivity

Drug loaded microemulsions were checked for electrical conductivity (σ) using a (Elico CM 180) conductivity meter. This helped us to ascertain if the system was oil-continuous, bi-continuous or water continuous.

pH

The pH of thermodynamically stable microemulsions, were recorded at $25 \pm 1^\circ\text{C}$ using (Mettler Toledo, pH compact 220) pH meter. The measurements were done in triplicate.

Viscosity

Viscosity measurement of the microemulsions was done in triplicates using Brookfield viscometer (DV-III +, Programmable rheometer).

Droplet size and polydispersity index measurement

The droplet size and polydispersity index of the optimized formulation was measured by dynamic light scattering (DLS) using Nanoparticle Analyzer sz-100 (Horiba Scientific, Japan).

In-vitro Release Kinetics

The drug permeability was checked using a Franz diffusion cell fitted with a 0.45 micron cellulose membrane pre-hydrated in distilled water at 25°C for 24 hours. The receptor compartment was filled with 6.8 pH phosphate buffer and the donor compartment was charged with 10 mg of pure drug or 5 mL of the microemulsions. The diffusion medium was continuously stirred at 100 rpm throughout the experiment using a magnetic stirrer (Remi 2MLH). At regular interval of 1 h, 2 mL of the sample was withdrawn from the receptor compartment for 8 h and immediately replaced with equal volume of fresh buffer. These samples were diluted with diffusion medium and the absorbance was measured spectrophotometrically at 237 nm. The percentage cumulative drug release was determined for the pure drug as well as the formulated microemulsions and data was fitted to Zero order, First order, Higuchi square root model and Koresmeyer-peppas model as per equations 1,2,3 and 4 respectively.

$$Q_t = Q_0 + k_0 t \quad (1)$$

$$Q_t = Q_0 e^{-k_1 t} \quad (2)$$

$$Q_t = k_H \sqrt{t} \quad (3)$$

$$Q_t = Q_0 + a \left(\frac{t}{r^2} \right)^n + b \left(\frac{t}{r^2} \right)^{2n} \quad (4)$$

Q_t represents the cumulative percentage of the drug released in time t (%), Q_0 the percentage of drug in formulation at 0th hour, k_0 represents

the zero order rate constant, the first order k_1 rate constant (h^{-1}) k_H Higuchi rate constant ($\% \text{h}^{-1/2}$), where r is radius of the spherical particle in cm, n is Fickian diffusion and $2n$ is case II transport which are release exponents; a and b and represent structural and geometric properties of the microparticles.^{9,10}

Accelerated stability

The accelerated stability study was conducted at $25^\circ\text{C} \pm 2^\circ\text{C}$ and $60\% \pm 5\%$ RH and $40^\circ\text{C} \pm 2^\circ\text{C} / 75\% \pm 5\%$ RH in stability chambers (Thermo labs) as per ICH guidelines. Approximately 5 mL of the formulation was stored in tightly screw capped bottle. During the 6 months duration of the study, samples were withdrawn at one, two, three and six months periods to check physical appearance, phase separation at accelerated gravitational force and drug content.

Determination of drug content

The drug was extracted from the drug loaded microemulsion using acetonitrile and drug content was measure using UV spectrophotometry after appropriate dilution using UV spectrophotometry at 238nm as well as HPLC.¹¹

The HPLC assay of diltiazem microemulsion was carried out using C18, $5 \mu\text{m}$ 250X4.6 mm column (Water'sXBridge_{TM}). A mixture of 60:40 acetonitrile : water containing 1% trifluoroacetic acid was used as eluent. It was filtered through a membrane filter and degassed in a sonicator (EQUITRON-230VAC, 50Hz) for 15 min. The isocratic flow rate for elution was 1 mL/min. Column temperature was set at 25°C , detector wavelength was set at 238 nm, injection volume of sample was 20 μL , and a total run time of 12 min per run was used. A 1 mg/mL stock solution of diltiazem in acetonitrile was prepared using the reference standard. The diltiazem was extracted from microemulsion by liquid-liquid extraction method using acetonitrile and diluted appropriately within the linearity range.

In-vivo pharmacokinetic study in rabbits

Animals

In-vivo study was conducted as per approval by Institutional animal ethics committee of Karnataka College of Pharmacy, Bangalore. (No.1564/PO/a/11/CPCSEA).

Albino rabbits of either sex, weighing between 1.35 to 1.75 Kg and aged about 12 weeks were divided into two groups each containing 6 rabbits, out of which one was used as reference standard (conventional diltiazem formulation 15 mg/kg) and the other for test formulation (Diltiazem microemulsion 15 mg/kg).

The albino rabbits used for the study were maintained at $22 \pm 2^\circ\text{C}$ with RH $50 \pm 5\%$ through a 12 h light/dark cycle, in sanitized cages on sterile paddy husk bedding. Standard pellet diet and water *ad libitum* were provided. The animals were kept under standard lab conditions for a minimum of ten days before the study was undertaken as per CPCSEA guidelines.

Food and water were withdrawn overnight prior to the examination.

Preparation of animal dose

Diltiazem dose of 15 mg/kg body weight of rabbit was selected to maintain plasma concentrations above the limit of detection, during the plasma sampling duration from 0 to 12 h in rabbits.² Diltiazem conventional tablets were crushed into powder and suspended in 0.5% sodium carboxymethyl cellulose so as to contain the required dose of 15 mg/kg and used as a reference standard. Both the groups received the respective treatments by oral gavage.

Sample preparation

The blood samples (about 0.5 to 0.8 mL) were withdrawn from a marginal ear vein using heparinized needles (24 size) into EDTA coated tubes at predetermined time intervals of 0.0 hour (pre-dose), 0.5, 1, 2, 3, 4, 6, 8 and 12 hours post dose administration. From each tube, plasma was separated after mixing and centrifuging at 5000 rpm for 5 min. The plasma was stored at -20°C until analysis. HPLC method of analysis for determination of diltiazem was used to analyze the stored plasma samples.

Determination of diltiazem in rabbit plasma by Reverse Phase HPLC

The concentration of diltiazem in the plasma samples was checked using a standardized reverse phase HPLC method with slight modification.¹² The separations were achieved on the Agilent 1120 Compact LC HPLC system with Water'sXBridge_{TM} column $5 \mu\text{m}$ 4.6X250 mm with UV detection at 237 nm. The EZ Chrome Elite software was used for acquisition, evaluation and storage of chromatographic data. The mobile phase comprised of acetonitrile: 0.01 M dibasic sodium phosphate (40:60) and triethanolamine (0.01%). The pH was adjusted to 3.0 ± 0.1 with 85% orthophosphoric acid. 50 μL Injection volume, isocratic flow of 1.2 mL/min was set and the eluting peaks were monitored at a λ_{max} of 237 nm. The HPLC method was validated for linearity range between 10 to 4,000ng/mL.

Pharmacokinetic study

The diltiazem concentrations in plasma were plotted vs time to get pharmacokinetic profile. All the estimated pharmacokinetic parameters were reported as Mean \pm SD. Determination of pharmacokinetic parameters was done using Phoenix Win Nonlin software version 6.3 (USA). The non-compartmental method was used for determination of pharmacokinetic parameters such as time to reach maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), elimination rate constant (K_{el}), area under the curve from 0 to 12 (AUC_{0-12}) and area under the curve from 0 to infinity ($\text{AUC}_{0-\infty}$). Half-life ($t_{1/2}$) was calculated as $0.693/K_{\text{el}}$.

Efficacy of Diltiazem Microemulsion: Antihypertensive Activity

Animals: Male wistar rats weighing 180-220 g were randomly divided into five groups (six per group), were used in the study. The procedure for experiments involving animals were in accordance with ethical standards of the institution, Karnataka College of Pharmacy as per Ethical approval No. 1564/PO/a/11/CPCSEA.

Group I: Rats were fed with standard rat chow (Normal Control/NC).

Group II: Fructose Rich Diet (FRD) for five weeks (FRD control)

Group III: FRD + microemulsion vehicle 0.5mLp.o/day for 5 weeks (vehicle control/VC)

Group IV: FRD + Diltiazem microemulsion (6.2mg/kg)p.o./day for 5 weeks (Test formulation/D-ME)

Group V: FRD + Diltiazem tablet formulation (6.2mg/kg)p.o./day for 5 weeks (Reference formulation/D-Ref)

The FRD contained 66% fructose, 12% fat and 22% protein.¹³ The rats were acclimatized with 12-hour light/dark (6:00-18:00 h) cycle. Additionally, they were familiarized to blood pressure measurement at the fixed time every day for at least one week. This allowed recording the blood pressure with least possible stress and restraint from animals. After the training period, rats were fed with FRD. The first few measurements were discarded and the mean six subsequent readings were recorded. Cardiovascular parameter, systolic pressure was measured once a week for five weeks by indirect non-invasive tail cuff method.¹⁴

RESULTS

Criteria for component selection

The microemulsion formulation consists of one or more surfactants in combination with cosurfactants and the drug dissolved in oil. Hence, studying the solubility of drug in all components of microemulsion system is an important parameter, particularly in oil.

The drug diltiazem free base, used for the development of microemulsion is water insoluble and the amount of oil used in the system should be sufficient to solubilize the drug and maintain it in solubilized form. This is important to know the stability and bioavailability of the drug. Almond oil showed maximum solubility for diltiazem (23.36 mg/mL). The surfactants and co-surfactants tween 80 and PEG 400 showed maximum solubility with 117.6mg/mL and 105.6mg/mL (Figure 1a, 1b).

Drug excipient compatibility studies

These are one of the important preformulation studies, where any possible interaction of diltiazem with other components in the formulation affecting its pharmacological activity is studied. The FTIR spectrum clearly indicated that none of the components interfere with the functional group of the drug. So it was concluded that there were no major interactions between the drug and excipients used in the study.

Pseudoternary phase diagrams

Conventional aqueous titration method (low energy emulsification technique) was used to construct pseudoternary phase diagrams for emulsions which were monophasic, clear, free flowing, less viscous. Almond oil as oil phase, Tween 80 and PEG 400 as S_{mix} phase and purified water as aqueous phase were selected for the construction of pseudoternary phase diagram. The shaded area indicates oil in water region and the other region in the diagram indicates turbid and conventional emulsions (Figure 2). It is clear that when surfactant and cosurfactant (S_{mix}) concentration is low the emulsion formed is biphasic and unstable. Such emulsions are turbid and milky white. As the S_{mix} ratio increases, the clear, transparent microemulsions are formed which are stable and there is no phase separation. Another important factor is the concentration of oil in the microemulsion system which is required to solubilize the maximum quantity of drug. As the oil concentration increases, phase separation occurs. To prevent phase separation, S_{mix} ratio has to be increased thus resulting in microemulsion system, which may cause gastric irritation. Such formulations are highly viscous and not free flowing. Hence the pseudoternary phase diagram gives the relevant information on the optimum ratio of oil, surfactant and cosurfactant that has to be used for preparation of a thermodynamically stable microemulsion system. To check the usefulness of the formulated microemulsion as suitable drug delivery system for Diltiazem, the drug equivalent to one dose is incorporated into the oil phase and similar phase diagrams were constructed. The phase behavior of the drug loaded system did not change. The monophasic, clear and transparent formulations were further evaluated for thermodynamic stability to optimize the best formulations presented in Table 1.

Thermodynamic stability study

The results tabulated in table 1 show that the formulations D1, D2, D3 and D4 pass the thermodynamic stability testing and could be further characterized.

Physicochemical Evaluation

Conductivity measurements

The conductivity measurements indicate that conductivity increases rapidly with increase in amount of water. The rapid increase of conductivity

continues up to 50% of aqueous phase addition and then onwards conductivity remained stagnant with further titration with water. A slight decrease in conductivity was observed on further dilution with water. These observations clearly indicate that the microemulsion prepared is water continuous type or oil in water microemulsion.

pH

The pH recorded for the microemulsion containing the drug was 5.54 ± 0.08 .

Viscosity

The viscosity of the drug loaded microemulsion was found to be 54cPs. Viscosity increases with increase in the surfactant and co-surfactant ratio. Optimum viscosity is indicative of long term stability and flow characteristics of the microemulsion.

Droplet size measurement

The globule size of the optimized formulation was measured by DLS technique that typically measures particles in the submicron region that are suspended in liquid. The globules were of 13.8 nm size with polydispersity index of 0.474 (Figure 3).

In-vitro drug release kinetics

The formulations that passed the thermodynamic stability test, were further evaluated for drug release kinetic behavior. The maximum regression coefficient (R^2 value) among all the models, concluded the best fitting model, obeyed by the formulation for drug release Kinetics. The formulation (D3) released 91.82% diltiazem in 8 hours, hence D3 was considered as the best formulation (Figure 4). The drug release from the pure drug was found to be slowest with 55.2%. The Koresmeyer-pappas model for D3 formulation was found to have R^2 value of 0.9767, which was highest amongst all the kinetic models. Hence it was concluded that D3 followed Koresmeyer-pappas Model for diltiazem release (Table 2).

Accelerated stability study

The physical examination of formulations subjected to accelerated stability study, at end of 1, 2, 3 and 6 months showed that the microemulsion can remain stable over a long period. The microemulsion remained clear and did not show any creaming or cracking, thus indicating a good stability. The drug content remained 98.32% to 99.14% over the period of six months.

Determination of drug content

The diltiazem content was found to be 98.52% in the optimized microemulsion.

In-vivo pharmacokinetic study

The plasma diltiazem concentrations for reference and the test formulation were obtained by extrapolating over standard curve. The measured diltiazem concentrations at each sampling time were fed into Phoenix WinNonlin software for calculation of pharmacokinetic parameters of diltiazem in rabbit. The pharmacokinetic parameters determined for diltiazem are presented in Table 3. The peak diltiazem concentration was found to be 98.167 and 120.833 ng/mL for reference and test formulations respectively. The extent of exposure (AUC_{0-12}) was 363.02 and 468.62 ng.hr/mL for reference and test formulations respectively. The time to peak concentration was found to be 0.5 hour for both the formulations. The observed terminal elimination half-life was 3.742 and 3.545 hour for reference and test formulations respectively. The observed

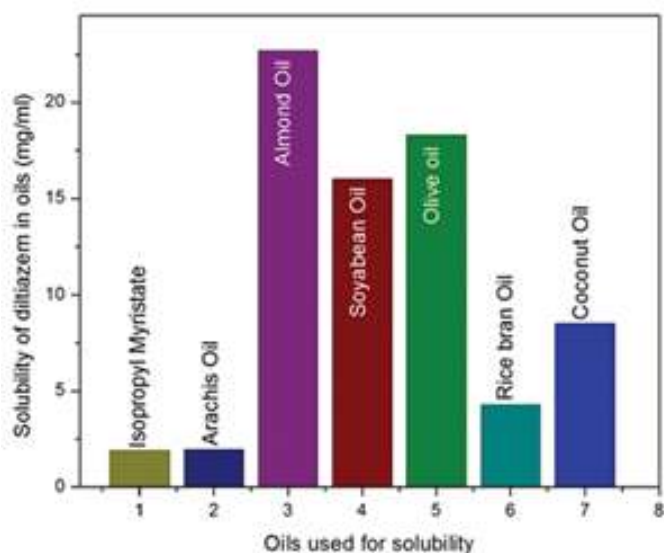


Figure 1a: Solubility of Diltiazem in various Oils

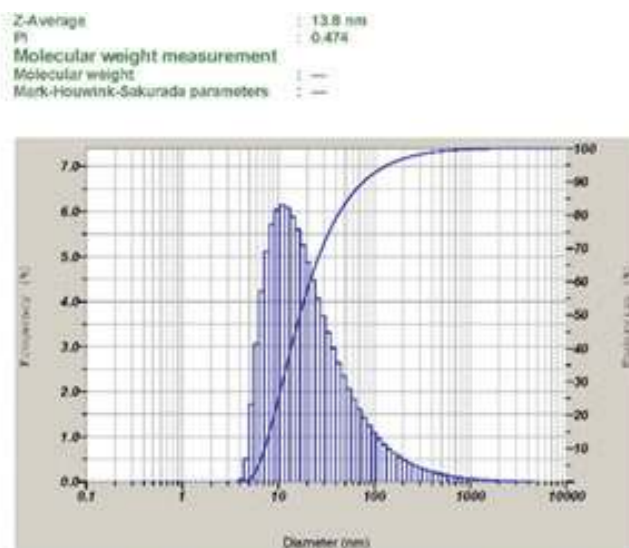


Figure 3: Droplet size measurement of optimized Microemulsion by dynamic light scattering technique

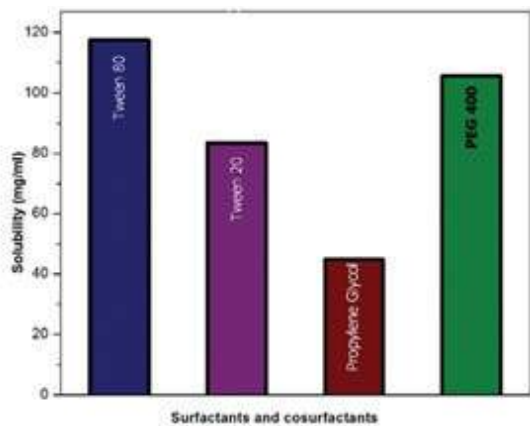


Figure 1b: Solubility of Diltiazem in surfactants and cosurfactants

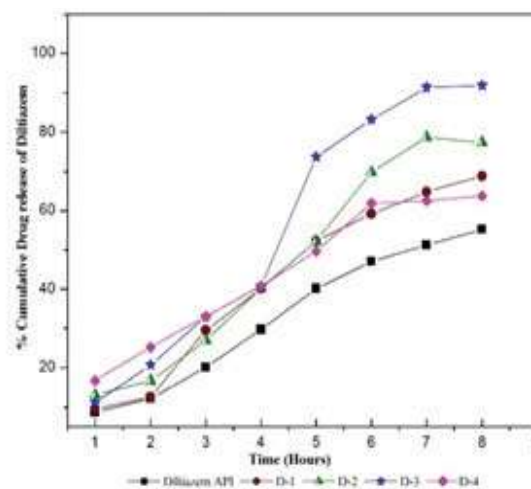


Figure 4: In-vitro release profile of diltiazem in microemulsions

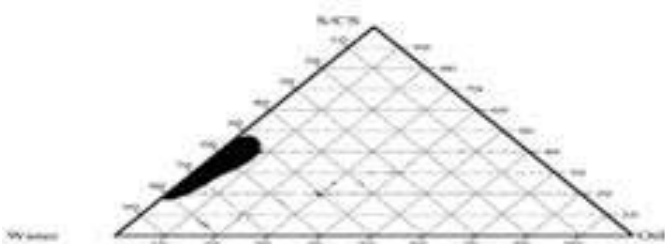


Figure 2: Pseudoternary phase diagram for diltiazem with varying ratio of oil, surfactant/cosurfactant (S/CS) mix and water; shaded area represents oil in water microemulsion region.

The diltiazem half-life was marginally higher for reference formulation. No difference was observed in time to peak concentration (T_{max}) (Figure 5).

Efficacy of Diltiazem Microemulsion

In the FRD control group, the mean value of systolic blood pressure was 98 mmHg before starting treatment, but it increased with age and was 155 mmHg on Day 36 of treatment. In the NC group the systolic blood pressure remained stable between 95 to 98 mmHg (Figure 6). In the D-ME group, systolic blood pressure was considerably lower compared to FRD Control group on Day 7 of the treatment and thereafter; D-ME significantly inhibited the increase in systolic blood pressure over 5 weeks of treatment period. D-Ref Group showed similar effectiveness in hindering the increase in systolic blood pressure. The anti-hypertensive effect of D-ME was slightly greater than that of D-Ref. Changes in mean systolic blood pressure in VC were similar to those in the FRD control group.

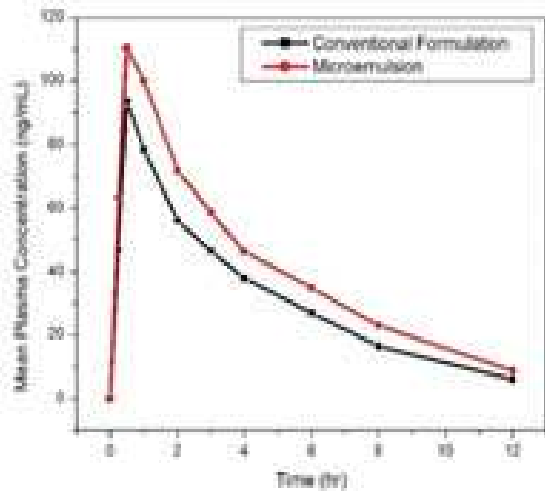


Figure 5: *In-vivo* pharmacokinetic study: Plasma concentration of Diltiazem after oral administration of Microemulsion formulation in comparison with conventional tablet formulation.

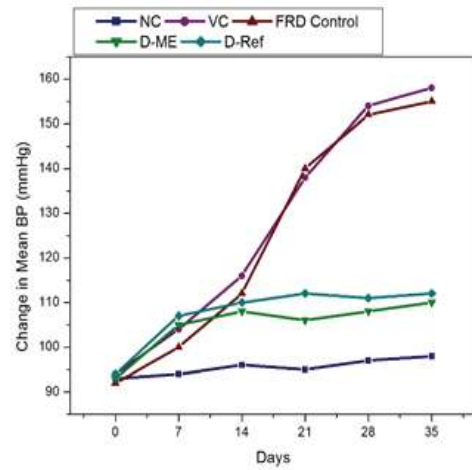


Figure 6: Evaluation of antihypertensive activity by measurement of changes in mean Systolic Blood Pressure in rats by tail cuff method.

Table 1: Composition of drug loaded formulations for thermodynamic stability testing

Formulation code	Smix %v/v	Oil (%)	Appearance	Centrifugation	Heating cooling cycle	Freeze thaw test	Inference
D2	25	5	clear and transparent	✓	✓	✓	Pass
D3	35	5	clear and transparent	✓	✓	✓	Pass
D1	45	5	clear and transparent	✓	✓	✓	Pass
D4	25	10	clear and transparent	✓	✓	✓	Pass
D5	35	10	clear and transparent	✓	✓	X	Fail
D6	45	10	clear and transparent	X	✓	X	Fail
D7	25	15	Milky white/ turbid	-	-	-	Fail
D8	35	15	Milky white/ turbid	-	-	-	Fail
D9	45	15	Clear and bluish	✓	X	X	Fail
D10	25	20	Milky white/ turbid	-	-	-	Fail
D11	35	20	Milky white/ turbid	-	-	-	Fail
D12	45	20	Milky white/ turbid	-	-	-	Fail

Table 2: *In-vitro* drug release kinetics of drug loaded formulations

Formulation	% Cumulative Drug Release at 8 hours	Regression coefficient (R ²)			
		Zero order	First Order	Higuchi	Korsmeyer-pappas
D1	68.82	0.9671	0.9397	0.9746	0.9574
D2	77.4	0.9672	0.9397	0.9449	0.957
D3	91.82	0.9496	0.9366	0.9414	0.9767
D4	63.72	0.9614	0.9633	0.9728	0.9874
Pure Drug (API)	55.2	0.9801	0.9855	0.9701	0.9747

Table 3: Pharmacokinetic parameters of diltiazem in rabbits

Parameters	Conventional formulation	Microemulsion Formulation	Relative bioavailability (F)
C _{max} (ng/mL)	98.167±10.591	120.833±3.656	1.23
t _{1/2} (h)	0.50 (0.50-1.00)	0.50 (0.50-1.00)	-
AUC _{0-t} (ng-h/mL)	363.02±61.747	468.62±63.937	1.29
AUC _{0-∞} (ng-h/mL)	433.398±86.059	539.354±61.995	1.24
T _{1/2} (h)	3.742±1.071	3.545±0.48	-
K _{el} (h ⁻¹)	0.199±0.057	0.198±0.025	-

DISCUSSION

The increased bioavailability of diltiazem from the formulated microemulsion compared to the marketed tablet formulation could be attributed to the inhibition of the P-gp efflux of Diltiazem, thereby increasing bioavailability. Diltiazem undergoes pre-systemic clearance by CYP-450 isoform CYP3A4 and P-gp. Both mechanisms synergistically work to decrease the bioavailability of Diltiazem.

This fact may be further substantiated from the work of Leu and Huang, who demonstrated improvement in etoposide bioavailability due to enhanced etoposide absorption that was caused by inhibition of intestinal P-gp by quinidine.³ Hong *et al.*, showed increased Diltiazem bioavailability in the presence of resveratrol, which is a P-gp inhibitor.⁵ Morin, an inhibitor of P-gp and CYP 3A4 improved the Diltiazem bioavailability in rats was reported by Choi and Han.¹⁵ Rege *et al.*, reported the effects of non-ionic surfactants on membrane transporters using Caco-2 cell mono layers and found that Tween 80 was effective in fluidizing the membrane and inhibited P-gp.¹⁶ Hugger *et al.*, compared the polyethoxylated pharmaceutical excipients that are commonly used to inhibit P-glycoprotein activity *in vitro*, using MDR1-MDCK and Caco-2 cells. The results showed that P-gp activity was completely inhibited by PEG-300 while Tween-80 caused only partial inhibition.⁶ Shono *et al.* have reported that tween-80 could be a useful pharmaceutical excipient for P-gp inhibition.¹⁷

CONCLUSION

A microemulsion formulation containing diltiazem was successfully prepared, which exhibited optimum thermodynamic stability with appreciably higher *in vitro* release and superior bioavailability compared to the marketed tablet formulation. The efficacy evaluation suggested superiority of the diltiazem microemulsion formulation over the conventional marketed formulation. This study provides insight into the potential advantages of diltiazem formulated as microemulsion over the existing oral formulations.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATION USED

All the abbreviations used have been described in the text.

REFERENCES

- Buckley MM, Grant SM, Goa KL, McTavish D, Sorkin EM. Diltiazem. *Drugs*. 1990;39(5):757-806. <https://doi.org/10.2165/00003495-199039050-00009>; PMID:2191851.
- Choi JS, Li X. Enhanced diltiazem bioavailability after oral administration of

- diltiazem with quercetin to rabbits. *International journal of pharmaceuticals*. 2005;297(1):1-8. <https://doi.org/10.1016/j.ijpharm.2004.12.004>; PMID:15907592.
- Leu BL, Huang JD. Inhibition of intestinal P-glycoprotein and effects on etoposide absorption. *Cancer chemotherapy and pharmacology*. 1995 ;35(5):432-6. <https://doi.org/10.1007/s002800050258>; PMID:7850926.
- Homsy W, Caillé G, Du Souich P. The site of absorption in the small intestine determines diltiazem bioavailability in the rabbit. *Pharmaceutical research*. 1995;12(11):1722-6. <https://doi.org/10.1023/A:1016217822770>; PMID:8592676.
- Hong SP, Choi DH, Choi JS. Effects of resveratrol on the pharmacokinetics of diltiazem and its major metabolite, desacetyldiltiazem, in rats. *Cardiovascular therapeutics*. 2008;26(4):269-75. <https://doi.org/10.1111/j.1755-5922.2008.00060.x>; PMID:19035878.
- Hugger ED, Novak BL, Burton PS, Audus KL, Borchardt RT. A comparison of commonly used polyethoxylated pharmaceutical excipients on their ability to inhibit P-glycoprotein activity *in vitro*. *Journal of pharmaceutical sciences*. 2002;91(9):1991-2002. <https://doi.org/10.1002/jps.10176>; PMID:12210046.
- Junyaprasert VB, Manwiwattanakul G. Release profile comparison and stability of diltiazem-resin microcapsules in sustained release suspensions. *International journal of pharmaceuticals*. 2008;352(1):81-91. <https://doi.org/10.1016/j.ijpharm.2007.10.018> PMID:18061381.
- Shah KP, Chafetz L. Use of sparingly soluble salts to prepare oral sustained release suspensions. *International journal of pharmaceuticals*. 1994;109(3):271-81. [https://doi.org/10.1016/0378-5173\(94\)90389-1](https://doi.org/10.1016/0378-5173(94)90389-1).
- Gibaud S, Al Awwadi NJ, Ducki C, Astier A. Poly (ε-caprolactone) and Eudragit® microparticles containing fludrocortisone acetate. *International journal of pharmaceuticals*. 2004;269(2):491-508. <https://doi.org/10.1016/j.ijpharm.2003.09.040>; PMID:14706260.
- Huang J, Wigent RJ, Bentzley CM, Schwartz JB. Nifedipine solid dispersion in microparticles of ammonio methacrylate copolymer and ethylcellulose binary blend for controlled drug delivery: effect of drug loading on release kinetics. *International journal of pharmaceuticals*. 2006;319(1):44-54. <https://doi.org/10.1016/j.ijpharm.2006.03.035>; PMID:16678366.
- Cumar RP, Vasudevan M, Raman D. RP-HPLC method development and validation for the estimation of diltiazem in bulk and tablet dosage forms. *Asian Journal of Pharmaceutical and Clinical Research*. 2012;5(3):62-4.
- Chaudhary RS, Gangwal SS, Avachat MK, Shah YN, Jindal KC. Determination of diltiazem hydrochloride in human serum by high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications*. 1993;614(2):261-6. [https://doi.org/10.1016/0378-4347\(93\)80317-W](https://doi.org/10.1016/0378-4347(93)80317-W).
- Reaven GM, Ho H, Hoffmann BB. Somatostatin inhibition of fructose-induced hypertension. *Hypertension*. 1989;14(2):117-20. <https://doi.org/10.1161/01.HYP.14.2.117>; PMID:2569446.
- Badyal DK, Lata H, Dadhich AP. Animal models of hypertension and effect of drugs. *Indian Journal of pharmacology*. 2003;35(6):349-62.
- Choi JS, Han HK. Pharmacokinetic interaction between diltiazem and morin, a flavonoid, in rats. *Pharmacological research*. 2005;52(5):386-91. <https://doi.org/10.1016/j.phrs.2005.05.011>; PMID:16002304.
- Rege BD, Kao JP, Polli JE. Effects of nonionic surfactants on membrane transporters in Caco-2 cell monolayers. *European journal of pharmaceutical sciences*. 2002;16(4):237-46. [https://doi.org/10.1016/S0928-0987\(02\)00055-6](https://doi.org/10.1016/S0928-0987(02)00055-6).
- Shono Y, Nishihara H, Matsuda Y, Furukawa S, Okada N, Fujita T, Yamamoto A. Modulation of intestinal P-glycoprotein function by cremophor EL and other surfactants by an *in vitro* diffusion chamber method using the isolated rat intestinal membranes. *Journal of pharmaceutical sciences*. 2004;93(4):877-85. <https://doi.org/10.1002/jps.20017>; PMID:14999725.

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