# Formulation And Evaluation of Nanoparticles Loaded Transdermal Drug Delivery System of Ramipril.

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## Abstract:

Ramipril is an Angiotensin Converting Enzyme (ACE) inhibitor, it belongs to the BCS class II. The current study encompasses the formulation of novel nanoparticles loaded transdermal patches of Ramipril with the aid of Box Behnken Design.

Pre-formulation studies such as identification of the drug by FT-IR, the estimation of the drug by UV spectroscopy and the melting point determination were performed. The nanoparticles were formulated by solvent emulsification evaporation method using Compritol 888 ATO as lipid, Span 60 and Tween 80 as surfactants to improve the physicochemical properties of Ramipril. The prepared nanoparticles were evaluated for particle size, polydispersity index, zeta potential, entrapment efficiency, drug content and in-vitro drug release studies and the evaluated data was used to carry out numerical optimization to obtain the best formulation with the optimum concentration of excipients. The optimized concentration for independent variables Ramipril (X1), Compritol 888 ATO (X2), and Span 60 (X3) were found to be 75mg, 2000mg, and 60mg respectively, with the desirability of 0.916.The optimized formulation of Nanoparticles were then loaded into a HPMC-E50-based transdermal patch by solvent casting method to overcome the restricted bioavailability of Ramipril. The thickness, folding endurance, weight uniformity, drug content and in-vitro drug release of the patch were evaluated. The drug release from the patch was found to be greater than the release from the solid lipid nanoparticles. The reason could be due to the intimate contact of the patch to the skin and the use of a penetration enhancer in the patch formulation. In conclusion, the results obtained suggest that a patch formulation containing nanoparticulate system is a potentially promising formulation for the efficient delivery of Ramipril through skin.

**Keywords: Nanoparticles; Ramipril; Compritol 888 ATO; Span 60; Tween 80; HPMC-E50; transdermal.**

## Introduction:

Ramipril is a potent anti-hypertensive drug. It is an ACE inhibitor and a class II drug with limited solubility which represent a rate-limiting step in its absorption thus, Ramipril faces a decreased bioavailability (28-35%) which could limit its therapeutic activity[1].

Nanoparticles (NPs) are solid colloidal particles ranging from 10 to 1000nm in size, where the active pharmaceutical ingredient(s) are dissolved, entrapped, encapsulated, adsorbed, or attached to a nanoparticle matrix. Nanoparticles have special characteristics such as small particle size, large surface areas and the capability of changing their surface properties makes them good and advantageous candidates for drug delivery compared to other drug delivery systems.

In recent years nanotechnology has offered a suitable means for delivery of small molecular weight drugs as well as macromolecules such as proteins, peptides or genes to cells and prevent them against enzymatic degradation. Nanoparticles as drug delivery systems are biodegradable, non-toxic, and capable of being stored for longer periods as they are more stable.[2]

Transdermal drug delivery system delivers the drugs through the skin portal to systemic circulation at apredetermined rate and maintains clinically the effective concentrations over a prolonged period of time. The main merits of transdermal drug delivery are non-invasiveness, sustained release, self-administration and enhanced therapeutic efficacy and specificity to the target.Stratum corneum acts as a natural barrier and limits the transport of drug through the skin. Nanocarriers are able to alter the stratum corneum due to their shape, size, surface chargesand show an enormous potential for transdermal delivery. The nanocarriers can be classified into polymer based and lipid-based delivery carriers. The lipid based nanocarriers show great properties in terms of safety, physical stability, biocompatibility, efficacy scale-up, ease of preparation, better entrapment of lipid soluble drugs, prolonged drug release of formulation. These may improve the solubility and bioavailability of active pharmaceutical ingredients

(API), guard these compounds from external objects such as light or oxygen thereby facilitating drug targeting. Since Ramipril is a lipophilic drug with low aqueous solubility, solid lipid nanocarriers were selected for this study[3]. Myriad studies have shown Ramipril's distribution and bioavailability have been improved employing nanoparticle formulations for the drug by using different polymers and lipids[4–7].

On this basis, the aim of this study is to formulate and evaluate solid lipid nanoparticles of Ramipril using Compritol 888 ATO as lipid, Span 60 and Tween 80 as surfactants and to load the formulated nanoparticles into a HPMC-E50 based transdermal patch in order to improve its bioavailability by by-passing its extensive first pass metabolism associated with its oral administration, also to improve its permeation through the skin by the use of nanocarriers.

# Materials and Methods.

## Material

Ramipril was purchasedfrom Triveni Interchem Pvt.Ltd, Gujarat, Compritol 888 ATO was obtained as gift sample from Gattefosse India Pvt.Ltd, Mumbai, Span 60 and Hydroxyl Propyl Methyl Cellulose (E50) were obtained from Micro Lab, Mumbai, Tween 80 from Merck Ltd, Mumbai and all other chemicals used were of analytical grade.

## Methods

## Pre-formulation studies: Determination of UV absorption maxima of Ramipril:

To determine the wavelength of maximum absorption (λmax), Ramipril standard solution (10µg/ml) was prepared in Phosphate buffer solution pH 7.4 containing 10% methanol, scanned in UV wavelength range of 200-400nm using phosphate buffer pH 7.4 as a blank. The absorption maxima obtained in the graph was considered as λmax for the pure drug solution in the respective buffer.

## Preparation of calibration curve of Ramipril in pH 7.4 phosphate buffer

**Stock-1 solution:** 100mg of Ramipril was weighed accurately and dissolved in methanol using a 100ml volumetric flask(1000µg/ml).
**Stock-2 solution:** 2.5ml from stock-1 solution was pipetted out and diluted to 25ml with phosphate buffer pH 7.4 (100µg/ml). From this solution 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1 and 2.4ml was pipetted out and diluted to 10 ml using pH 7.4 phosphate buffer to get aliquots of 3, 6, 9, 12, 15, 18, 21 and 24µg/ml respectively.pH 7.4 phosphate buffer was used as blank solution. The absorbance was measured at 209nm using UV- Visible Spectrophotometer. The standard graph was repeated three times (n=3). The mean data was plotted by taking concentration on X-axis and absorbance on Y-axis.

## Identification of drug by FTIR

Weighed amount of drug (Ramipril) was mixed with IR grade potassium bromide KBr (1:10) and compressed under 10-tons pressure in a hydraulic press to form a transparent pellet. The pellet was scanned by IR spectrophotometer over a frequency of 4000 $cm^{-1}$ to 400 $cm^{-1}$ range.[1]

### Drug-excipients compatibility study

Physical mixture of drug (Ramipril) and excipients (Compritol 888 ATO, Span 60, Tween 80, HPMC-E50) was mixed with IR grade KBr (1:10) and compressed under 10-ton pressure in a hydraulic press to form a transparent pellet. The pellet was scanned by IR spectrophotometer over a frequency of 4000 $cm^{-1}$ to 400 $cm^{-1}$range.

## Melting point determination

Fine powder of Ramipril was filled in glass capillary tube one end of which was previously sealed. The capillary tube was inserted into the melting point apparatus which contained liquid paraffin and heated. The temperature t which the drug began to melt was observed by using the thermometer which was already immersed into the liquid paraffin in the apparatus.

## Formulation studies:

## Preparation of Nanoparticles:

Compritol was selected as the solid lipid for preparing the SLN. The lipid phase containing Compritol ATO 888, and Span 60 was melted to 80℃. The Ramipril was dissolved in methanol and added to the melted lipid phase. The aqueous phase was prepared by dissolving the Tween 80 in distilled water and heated it up to the same temperature of the lipidphase.The lipid phase was poured into the aqueous phase and stirred for 6 hours using magnetic stirrer until all the methanol evaporated. Nanoparticles thus produced were evaluated for different parameters such as particle size, zeta potential, polydispersity index, entrapment efficiency, drug content and drug release.[8-9]

**Design of experiment:** Use of experimental design allows for simultaneous testing of factors and precludes the use of a large number of independent runs. Systematic optimization procedures are carried out by selecting an objective function, finding the contributing factor(s) and investigating the relationship between response(s) and factor(s) by the response surface methodology.[10] The Objective for the present study was maximizing entrapment efficiency, while minimizing particle size. Box–Behnken design was used to statistically optimize formulation parameters and evaluate the main effects, interaction effects, and quadratic effects of the lipids, surfactant, and drug on the responses listed above. The three-factor two-level design was used to explore quadratic response surfaces and construct second-order polynomial models using Design Expert software version 13. The Box-Behnken design was constructed where the amounts of COMPRITOL 888 ATO, SPAN 60 and RAMIPRIL were selected as independent variables. The levels of the 3 variables were selected based on preliminary trials carried out before implementing the experimental design.

**Table 1: Factors of Box Behnken Design**

| Factors | Name | Units | Low | High |
|---|---|---|---|---|
| A | COMPRITOL 888 ATO | MG | 1000 | 2000 |
| B | SPAN 60 | MG | 30 | 60 |
| C | TWEEN 80 | MG | 75 | 150 |

**Table 2: Formulation chart of Ramipril loaded nanoparticles (F1 to F13):**

| Formulation Code | Ramipril (mg) | Compritol 888 ATO (mg) | Span 60 (mg) | Tween 80 (mg) | Distilled water (ml) |
|---|---|---|---|---|---|
| F1 | 150 | 1000 | 45 | 45 | 30 |
| F2 | 112.5 | 2000 | 30 | 45 | 30 |
| F3 | 112.5 | 1500 | 45 | 45 | 30 |
| F4 | 75 | 1000 | 45 | 45 | 30 |
| F5 | 150 | 1500 | 30 | 45 | 30 |
| F6 | 75 | 1500 | 60 | 45 | 30 |
| F7 | 112.5 | 1000 | 60 | 45 | 30 |
| F8 | 150 | 2000 | 45 | 45 | 30 |
| F9 | 112.5 | 2000 | 60 | 45 | 30 |
| F10 | 150 | 1500 | 60 | 45 | 30 |
| F11 | 112.5 | 1000 | 30 | 45 | 30 |
| F12 | 75 | 2000 | 45 | 45 | 30 |
| F13 | 75 | 1500 | 30 | 45 | 30 |

Box–Behnken design was specifically selected, because it requires fewer runs than a central composite design, with same number of variables. A design matrix that consists of 13 experimental runs was constructed. All the batches were evaluated, and different polynomial equations were derived for particle size, zeta potential, % entrapment efficiency and % drug release. The statistical analysis of the factorial design batches was performed by ANOVA using Design-Expert software. A statistical model incorporating interactive and polynomial terms were used to evaluate the response.

$Y = b_0 + b_1 A + b_2B + b_3 AB + b_4 A^2 + b_5B^2$

In this equation, Y is the response, and b0 is the arithmetic mean response of 13 runs. b1, b2, b3, b4, and b5 are the estimated coefficient for the factors A and B. The polynomial equations were generated by linear multiple regression that quantitatively explain the effect of the different variables on the attributes.[11]

## Evaluation of Nanoparticles

## Particle size and Polydispersity index

The Particle size of nanoparticles plays an especially significant role in drug permeation through the skin. Polydispersity index describes the particle size distribution of nanoparticles. It helps to know the average uniformity of particles size of thesample. High poly dispersity index value describes the high degree of non-uniformity of a distribution of the particles in the nanoparticle formulation. By using the dynamic light scattering (DLS) technology, the average particle size and the poly dispersity index were evaluated in Horiba Scientific (Nano-partica) SZ-100 at an angle of 90˚ at 25℃. For the measurement, 1ml of the sample diluted to 10ml with double distilled water and sonicated for 15 minutes and vortexed, kept in polystyrene cuvettes for the evaluation of the particle size and poly dispersity index(n=3).[1]

## Zeta potential

Zeta Potential (ZP) is the electric charge on the particle surface. It helps to predict the storage stability of the colloidal dispersion. At higher zeta potential, less particle aggregation occurs due to electrical repulsions. Zeta potential was measured using Horiba Scientific (Nano-partica) SZ-100. 1ml of formulated SLNs was diluted to 10ml with double distilled water and sonicated for 15 minutes [12]. Then the sample was transferred into the cuvette which contains electrode and kept inside the instrument and zeta potential of sample measured (n=3).

## Drug content

Formulation equivalent to 5mg of drug was taken, 1ml of chloroform was added and then the volume was made up to 10ml with methanol. The resultant solution was then centrifuged at 6000 RPM for 1 hour after which the supernatant layer was filtered. 1ml was taken from the filtered supernatant and the volume made up to 10ml [12]. Absorbance of the sample was then measured at 209nm, and the drug content determined using the following formula:

Drug Content in mg = absorbance/slope *dilution factor*1/1000.

## Entrapment efficiency

The Entrapment efficiency of prepared sample was determined by measuring the concentration of the drug in the dispersion medium. The free drug Ramipril was determined by adding formulation equivalent to 5mg of Ramipril in a volumetric flask and the volume was made up to 10ml with ethanol and then transferred to centrifuge tubes and then this dispersion was centrifuged at 6000 rpm for 1 hour. The supernatant was filtered and diluted with buffer and measured spectrophotometrically at 209nm (n=3). The amount of drug in supernatant ($W_{free}$) was then subtracted from the total amount of drug of the preparation ($W_{total}$) [1].The entrapment efficiency was calculated using the following equation.

$$\text{Entrapment Efficacy (EE\%)} = \frac{(W_{total} - W_{free}) \times 100}{W_{total}}$$

## Diffusion study of Ramipril from SLNs

In vitro release of Ramipril from nanoparticle formulation was studied using dialysis bag method in phosphate buffer pH 7.4. The dialysis bag procured from HIMEDIA having molecular weight cut off between 12000 to 14000 Daltons and pore size is 2.4nm. 7cm of the dialysis bag was soaked in distilled water for 24 hrs before the

experiment. One end of the bag was tied with a thread and Ramipril nanoparticles sample (equivalent to 5mg of drug) was added into the dialysis bag from another end and tied to the burette stand with a thread. The dialysis bag containing nanoparticle sample was placed in 1000ml of dissolution medium (glass rod was used to immerse the dialysis bag in order to avoid the floating of the bag) which was continuously stirred at 100 rpm at 37℃ using magnetic stirrer. Aliquots (5ml) were withdrawn at predetermined time intervals (1 hour) and the same volume of fresh dissolution medium was added to maintain a sink condition. The samples withdrawn were analyzed spectrophotometrically at 209nm (n=3).[1]

## Preparation of Transdermal Patch of Ramipril

The matrix-type transdermal patch containing Ramipril was prepared by solvent casting method using hydroxyl propyl methyl cellulose (HPMC E50) polymer. The total weight of polymer used was 350mg. Accurately weighed amount of polymer was dissolved in distilled water with continuous stirring. Polyethylene glycol 400 was added as plasticizer and dimethyl sulfoxide as penetration enhancer.[13] Both plasticizer and penetration enhancer were used at 10% w/w of total polymeric concentration. An area of4$cm^2$ was desired to contain 5mg of Ramipril. Hence, the film casting area of 25$cm^2$would require the quantity of nanoparticle formulation equivalent to 31.25mgof drug. The nanoparticle formulation was added to polymer solution. The resultant formulation was sonicated for 5 minutes to remove air bubbles. The solution was then poured into a Petri dish using aluminum foil as backing membrane. The solvent was then allowed to dry in the hot air oven at a temperature of 45°C for 24 hours. The patch obtained was carefully peeled off and evaluated.

**Table 3: Formulation chart of Ramipril nanoparticles loaded transdermal patch.**

| s/no | Ingredients | Quantities |
|---|---|---|
| 1 | Ramipril (mg) | 31.25 |
| 2 | HPMC-E50 (mg) | 350 |
| 3 | Polyethylene glycol(mg) (10%w/w of total polymer weight) | 35 |
| 4 | Dimethyl sulfoxidemg (10%w/w of total polymer weight) | 35 |
| 5 | Distilled water (ml) | 20 |

## Evaluation of patch:[14]

**Physical appearance:** The patch was physically inspected for color, clarity, uniformity, flexibility and smoothness.

**Determination of thickness:** Vernier calipers were used to determine the thickness of randomly selected patches. The patch was measured at different points and the average of three readings was taken.

**Folding endurance:** Folding endurance of patch was measured manually by folding the patch at one place a number of times till it breaks. This gives a value of folding endurance.

**Weight variation:** Weight variation was studied by individually weighing 3 randomly selected patches. The mean value was calculated.

**Drug content:** A prepared patch was added to 10ml methanol and stirred vigorously for 2 hours using a magnetic stirrer. The solution was Filtered and 1ml was diluted to 10ml with pH 7.4 phosphate buffer and Ramipril was estimated spectrophotometrically at wavelength of 209 nm.

## Diffusion study of Patch:

In- vitro drug release studies were performed by a Franz diffusion cell. Drug release from patch formulation was compared with nanoparticle formulation. Dialysis membranehas moleculae weight cut off between 12000to 14000Da and having pore size 2.4 nm (HI MEDIA) was employed for the determination of drug diffusion. The receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4 and the patch or the nanoparticle formulation was placed in the donor compartment. The recipient compartment was constantly and continuously

stirred using magnetic bead and the temperature was maintained at 37±0.5°C. The samples were withdrawn at time intervals of 1 hour and analyzed for drug content spectrophotometrically.

## Results and Discussion

### Pre-formulation studies:

### Determination of UV absorption maxima of Ramipril:

Ramipril standard solution (10µg/ml) was scanned in UV-Spectrophotometer with a wavelength range of 200-400nm. The absorption maxima were found to be 209nm. So, the standard curve was developed at this wavelength.

### Preparation of Calibration Curve of Ramipril in PH 7.4 phosphate buffer:

The calibration curve was linear between 3-24 µg/ml concentration range. The standard calibration curve of Ramipril was constructed in pH 7.4 phosphate buffer by plotting mean absorbance vs concentration at 209nm (n=3). The $R^2$ and slope values were found to be 0.999 and 0.0366 respectively.

### Identification of drug by FTIR and Drug-excipients compatibility study:

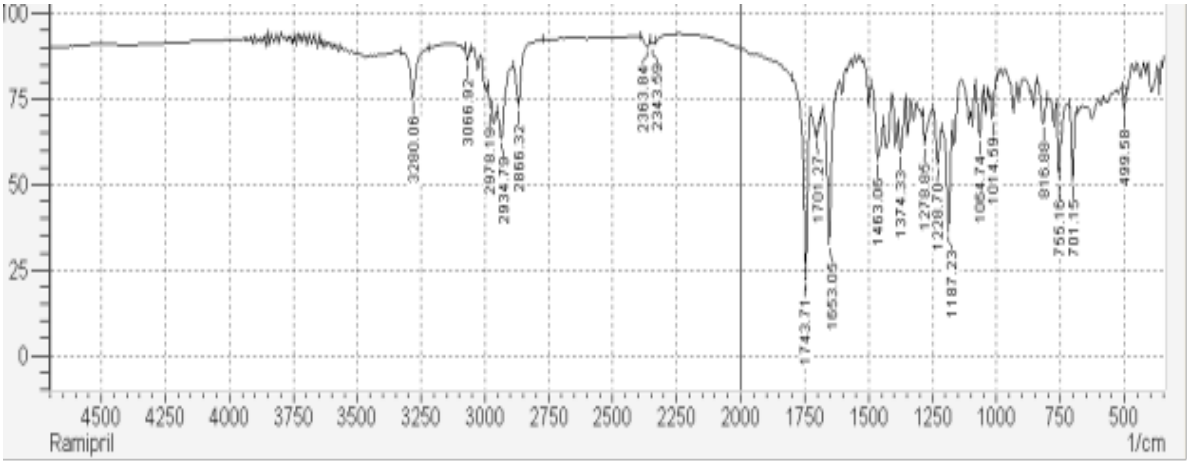


Fig 1: FTIR spectra of Ramipril.

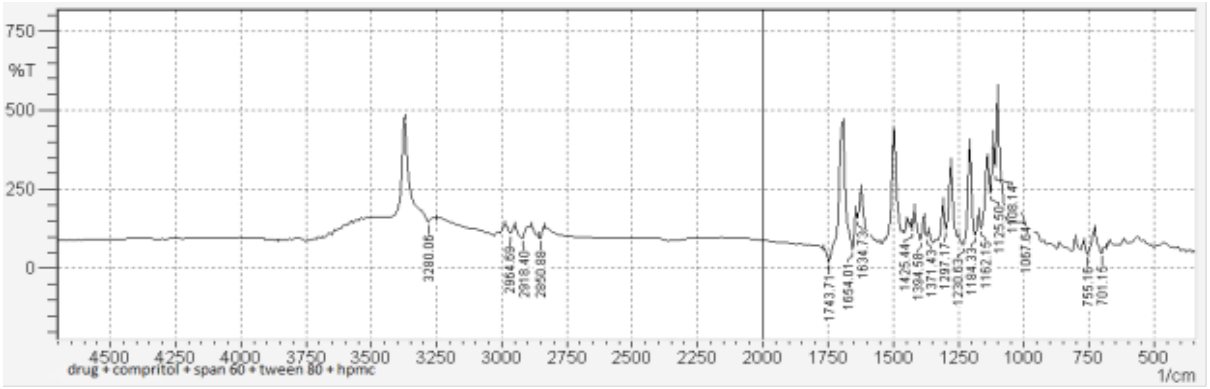


Fig 2: FTIR spectra of Ramipril + Compritol + Span 60 + Tween 80 + HPMC.

Identification study was performed using FTIR spectrophotometer. The characteristic absorbance peaks of Ramipril were obtained at different wave numbers. The peaks obtained in the spectra of pure drug correlates with the peaks of official spectrum of standard reference which confirms the purity of the drug.

The compatibility study was carried out using FTIR spectrophotometer. The FTIR spectrum of pure drug and physical mixture of drug and excipient was studied.The FTIR spectra of Ramipril indicates the characteristic peaks at 3280 cm¯1 due to N-H Stretching, at 3066 $cm^{-1}$ due to O-H Stretching, at 2934 $cm^{-1}$due to C-H Stretching, at 1773 $cm^{-1}$ due to C=O Stretching, at 1278 $cm^{-1}$due to C-N Stretching and at 1228 $cm^{-1}$ due to C-O Stretching. It was observed that there is no chemical interaction between the drug and the excipients used in the formulations as indicated by the presence of all characteristic peaks of the drug which indicates that the drug was compatible with the formulation components.

## Melting Point

The melting point of Ramipril was determined by capillary method. The melting point was found to be 109℃. The melting point of Ramipril is 105-112℃ as per literature thus indicating the purity of the drug.[1]

## Evaluation of Nanoparticles of Ramipril

**Table 4: Results of evaluated parameters of Ramipril Nanoparticles.**

| Formulation | Particle Size (nm) | Zeta Potential (mv) | Drug Content (%) | Entrapment Efficiency (%) | *In-vitro* drug Release at 6th hour (%) |
|---|---|---|---|---|---|
| **F1** | 106.7 | 34.5 | 95.8 | 52.0 | 91.6 |
| **F2** | 149.2 | 24.0 | 95.6 | 89.2 | 57.1 |
| **F3** | 99.7 | 30.5 | 93.6 | 77.9 | 82.9 |
| **F4** | 33.9 | 30.6 | 99.0 | 80.0 | 88.8 |
| **F5** | 180 | 27.4 | 94.2 | 63.8 | 81.9 |
| **F6** | 37.3 | -31.4 | 94.0 | 92.0 | 90.5 |
| **F7** | 18.9 | 44.1 | 88.8 | 65.2 | 98.3 |
| **F8** | 187.4 | 23.9 | 94.2 | 77.7 | 80.3 |
| **F9** | 31.5 | 14.8 | 93.0 | 87.2 | 81.1 |
| **F10** | 89.3 | 15.4 | 95.0 | 66.9 | 91.7 |
| **F11** | 127.7 | -38.7 | 90.4 | 66.6 | 86.2 |
| **F12** | 79.7 | 22.0 | 96.2 | 92.8 | 77.3 |
| **F13** | 160.6 | 31.1 | 91.6 | 92.3 | 81.8 |

## Particle size:

The particle size of obtained nanoparticles ranged from 31.5nm to 180nm.

The mean particle size of colloidal particles is an important characteristic of solid lipid nanoparticles (SLNs) which is measured using Nanoparticle analyzer Horiba SZ-100. Mean particle size and polydispersity index of the nanoparticles were obtained. The results showed that all the SLNs are in the nano-sized range and had low polydispersity index which indicates relative narrow size distribution. Sizes ranged from 18.9nm to 180.7nm and the polydispersity index ranged from 0.3 to 3.0 respectively. The ANOVA (Analysis of Varience) for linear model was used to analyze the particle size of the formulated nanoparticles. The P value and $R^2$value was found to be 0.0003 and 0.8631 respectively, showing the model is significant.

The final equation in terms of Coded Factors

Particle size = +100.08 + 32.01 A + 19.55 B – 55.06 C

Were,

A = Ramipril, B = Compritol 888 ATO and C = Span 60

According to the coded factor equation of particle size, the Ramipril has a positive effect on particle size. As concentration of Ramipril increases, particle size also increases and vice-versa. Compritol 888 ATO has the same effect as Ramipril on particle size. Whereas the concentration of Span 60 shows a negative effect on particle size, as concentration of Span 60 increases, the particle size decreases. This is because higher surfactant concentrations effectively stabilized the particles by forming a steric barrier on the particle surface and thereby protect smaller particles and prevent their coalescence into bigger ones. High surfactant concentrations might decrease particle size due to effective reduction in interfacial tension between the aqueous and lipid phases leading to formation of emulsion droplets of smaller size.[15]A possible explanation for the influence of Compritol on SLNs may be due to its high melting point (65-77 °C) and the long chain hydrocarbon chain of Behenic acid (C22) which represents 85% of the acids esterifying glycerol in Compritol. High melting point results in an increase in viscosity and leads to an insufficient homogenization which causes an inefficient reduction of the particle sizes. Moreover, the long chain hydrocarbons of Behenic acid in Compritol result in bulkier molecules that are less susceptible to packaging into small particles.[16]The type and concentration of surfactants used are helpful in preventing aggregation of the particles, thus maintaining a low polydispersity index.

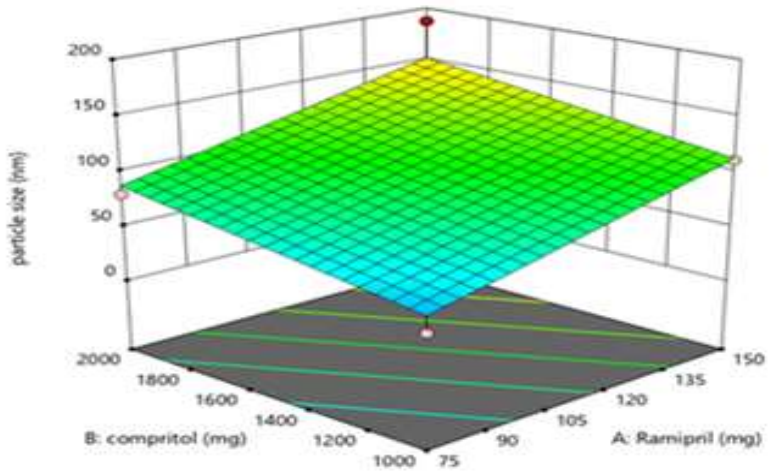


Fig.3: 3-D graph of particle size.

## Zeta potential

The zeta potential of obtained nanoparticles ranged from +24.0mv to +44.1mv. No model was found to be sifnificant for statistical evaluation of zetapotential.

The ANOVA (Analysis of Variance) for 2FI model was used to analyze the zeta potential of the prepared nanoparticles. The p-value and $R^2$value were found to be 0.6329 and 0.4281 respectively, indicating the model was not significant.

According to the DLVO theory, a system is regarded as stable if the electrostatic repulsion dominates the attractive van der Waals forces. The particles have to overcome an energy barrier of electrostatic repulsion to approach closely

and form agglomerates. If their velocity or kinetic energy is high enough, they will collide. Higher temperatures as well as light increase the kinetic energy of a system, in combination with a reduced zeta potential this leads to SLN aggregation.[17] A zeta potential value in the range of -30 mV to +30 mV is generally considered as the standard value in which particles shows the sufficient repulsive force to attain better physical colloidal stability.[18] Except a few formulations (F1,F7,F11), all formulations showed zeta potential between -30mv to +30mv.

## Entrapment Efficiency

The results showed that the entrapment efficiency of Ramipril loaded solid lipid nanoparticles ranged from 52 to 92.8%.ANOVA for the linear model of entrapment efficiency showed the p-value of <0.0001 and an $R^2$value of 0.9585 indication the model is significant.
Final equation in terms of Coded Factors:

Entrapment Efficiency = +77.20 – 12.09 A + 10.39 B – 0.0750 C

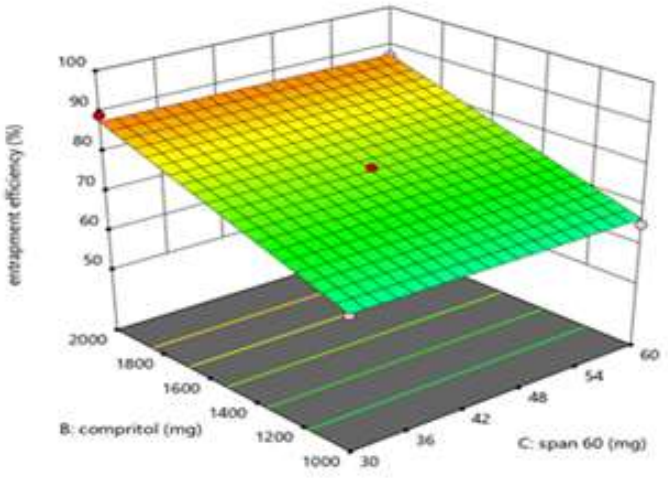


Fig.4: 3-D graph of entrapment efficiency

According to this equation, an increase in Ramipril concentration decreases entrapment efficiency while Compritol 888 ATO has a positive effect on entrapment efficiency. As concentration of Compritol increases, entrapment efficiency increases. Increased concentration of compritol increases the entrapment efficiency this is because compritol is a complex lipid made of mono-, di- and triglycerides which form fewer perfect crystals with many imperfections offering space to accommodate the drugs thereby increasing the entrapment efficiency. Also, the high entrapment efficiency observed with compritol can be attributed to the high hydrophobicity due to long chain fatty acids attached to the triglycerides resulting in the increased accommodation of lipophilic drugs.[17][19-20]

Span 60 has a negative effect on entrapment efficiency. As concentration of Span 60 increases, the entrapment efficiency decreases. High surfactant levels in the external phase might increase the partition of the drug from the internal to the external phase of the medium. This increased partition is due to the increased solubilization of the drug in the external aqueous phase such that more volumes of the drug can disperse and dissolve in it.[15][21]

## In-vitro drug release study using dialysis membrane

In-vitro drug release of Ramipril SLNs was performed in Phosphate buffer (pH 7.4) using a dialysis membrane technique. The % drug release at 6$^{th}$ hour from the prepared formulation is given in the table 4.

ANOVA (Analysis of Variance) for the linear model of in-vitro drug release showed the p-value and $R^2$value of drug release at $6^{th}$ hour are 0.0011 and 0.8185 respectively.

Equation in Terms of Coded Factor:

Drug release at $6^{th}$ hour = +83.81 + 0.8875 A – 8.64 B + 6.83 C

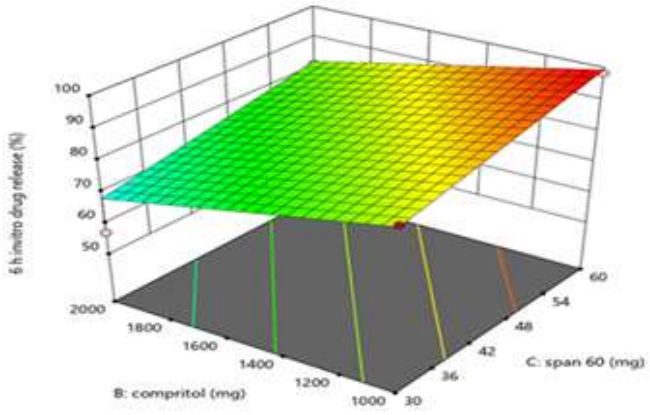


Fig.5: 3-D graph of in-vitro drug release.

According to the equations above, Ramipril shows a positive effect on the drug release. As the concentration of Ramipril increases, the drug release increases. Compritol 888 ATO shows a negative effect. As the concentration of Compritol increases, the drug release decreases. Increased concentration of lipid results in higher drug entrapment and significantly prolong drug release. This may be related to the triglycerides with lipophilic long chain fatty acids which prolong the release of lipophilic drug. Increase in lipid concentration can also lead to prolonged drug release due to reduced drug partition in the outer phase and consequently its release in the receiver media. In other words, as the lipid concentration in the SLN preparation was increased, the thickness of the lipid coating increased thereby increasing the length of diffusion resulting in decrease in the drug release. Compritol ATO 888 produces less ordered lipid crystals leading to lower drug expulsion from the imperfect lattice, contributing to the prolonged release of the lipophilic drug.

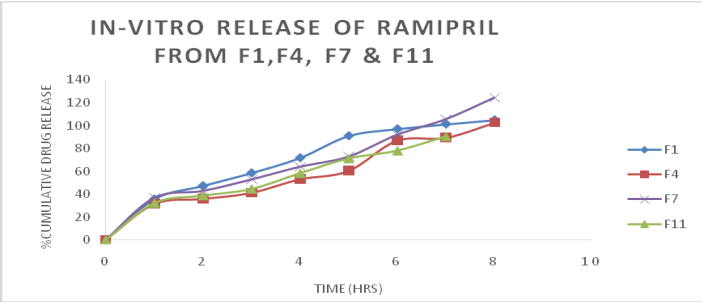


Fig 6: In-vitro drug release of Ramipril solid lipid nanoparticles (F1, F4, F7 and F11):

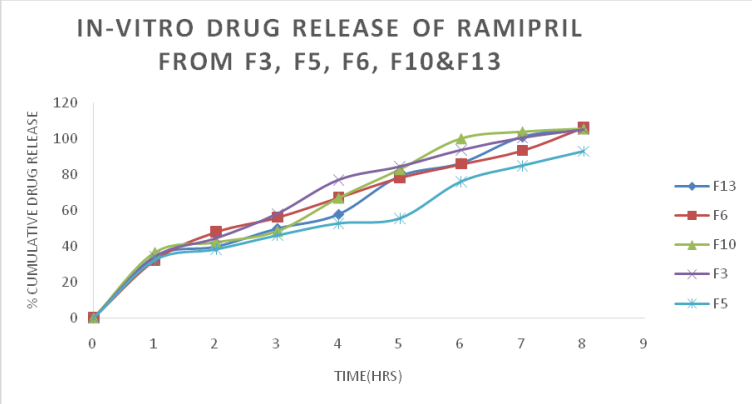


Fig 7: In-vitro drug release of Ramipril solid lipid nanoparticles (F3, F5, F6, F10 and F13)

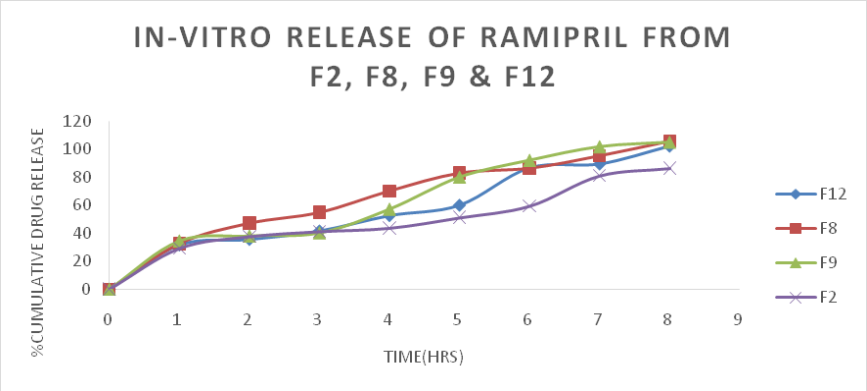


Fig 8: In-vitro drug release of Ramipril solid lipid nanoparticles (F2, F8, F9 and F12)

The concentration of the lipid, Compritol 888 ATO was kept constant at 1000mg while concentration of Span 60 was varied (Fig 6). Similarly the lipid was kept constant at 1500mg in Fig 7 and at 2000 mg in fig 8 respectively. It was observed that as the concentration of Span 60 increased, %drug release increased.

Increasing the concentration of Span 60 increases drug release from the SLNs. Increased concentration of surfactant increased in-vitro drug release due to decreased particle size hence increased surface area with improved solubilization in the recipient media. The fast or rapid release and higher release efficiency noticed at higher surfactant concentration could be explained by the partitioning effects of the drug between the melted lipid phase and the aqueous surfactant phase during particle production. During particle production by the solvent emulsification technique, drug partitions from the liquid oil phase to the aqueous water phase. The amount of drug partitioning to the water phase will increase with the increase of the drug solubility in the water phase, which means with increasing temperature of the aqueous phase and increasing surfactant concentration. The higher the temperature and surfactant concentration, the greater is the solubility of the drug in the water phase so the amount of drug in the outer shell increased and released in a relatively rapid way. Surfactants can increase the drug release due to a high HLB value and its ability to reduce the interfacial tension between SLN and the dissolution media and decrease the aggregation of particles and enhances the dissolution of drug.[15]

Table 5: In-vitro drug release parameters of formulations F1-F13

| Formulation | Zero | First | Higuchi plot | kosermeyerPeppas | | Mechanism Of drug |
|---|---|---|---|---|---|---|
| | $R^2$ | $R^2$ | $R^2$ | $R^2$ | n value | |
| **F1** | 0.985 | 0.821 | 0.979 | 0.983 | 0.626 | Non-Fickian |
| **F2** | 0.985 | 0.818 | 0.979 | 0.972 | 0.733 | Non-Fickian |
| **F3** | 0.983 | 0.852 | 0.989 | 0.970 | 0.633 | Non-Fickian |
| **F4** | 0.982 | 0.919 | 0.992 | 0.969 | 0.608 | Non-Fickian |
| **F5** | 0.975 | 0.813 | 0.997 | 0.967 | 0.627 | Non-Fickian |
| **F6** | 0.978 | 0.756 | 0.989 | 0.869 | 0.732 | Non-Fickian |
| **F7** | 0.970 | 0.803 | 0.996 | 0.966 | 0.663 | Non-Fickian |
| **F8** | 0.974 | 0.894 | 0.997 | 0.965 | 0.641 | Non-Fickian |
| **F9** | 0.984 | 0.962 | 0.980 | 0.972 | 0.763 | Non-Fickian |
| **F10** | 0.972 | 0.797 | 0.997 | 0.969 | 0.720 | Non-Fickian |
| **F11** | 0.996 | 0.776 | 0.982 | 0.986 | 0.678 | Non-Fickian |
| **F12** | 0.972 | 0.688 | 0.982 | 0.963 | 0.666 | Non-Fickian |
| **F13** | 0.996 | 0.675 | 0.996 | 0.967 | 0.677 | Non-Fickian |

## Numerical Optimization

Numerical optimization technique was carried out using Design Expert software version 13 to obtain optimal concentrations of independent variables in order to minimize particle size, to maximize entrapment efficiency and to enhance the % drug release which are basis for selecting an optimized formulation. The optimized formulation suggested the composition as follows.

Ramipril (X1) = 75mg, compritol 888 ATO (X2) = 2000mg and Span 60 (X3) = 60mg with a desirability of 0.916. With these concentrations, the suggestedresponses were 32.55nm for particle size, 12.89mv for zeta potential, 99% for entrapment efficiency, 48.6% for drug release at 6th hour and 70.8% fordrug release at 6th hour. The practical results of the optimized formulation were closely related to the predicted results. The optimized formulation was loaded into the transdermal patch.

## Evaluation of Ramipril Nanoparticles loaded Transdermal Patch:

**Table 6: Evaluation of Ramipril SLNs loaded transdermal patch:**

| Formulation | Thickness(mm) | Folding endurance | Weight Variation (mg) | Drug Content(%) |
|---|---|---|---|---|
| **Patch** | 0.099±0.005 | 189±0.03 | 96.90±0.002 | 98.90 |

**Thickness:** The thickness of the patch was found to be 0.099±0.05mm.

**Folding Endurance:**The folding endurance of the patch was found to be 189±0.03 indicating that the patch did not break easily and maintained its integrity with general skin folding when applied and it had the ability to withstand rapture.

**Weight uniformity:**The patch was subjected to weight variation by individually weighing dried patches of 4.0 $cm^2$**.** The weight variation was found to be 96.90±0.002mg.

## Drug Content:

The drug content of the patch was found to be 98.90%.
The thickness and weight of the patch depends on the concentration of polymer used in the formulation. An excessive increase in the concentration of the polymer could lead to an increase in weight and thickness of the patch. However, high concentration of polymer decreases the drug loading capacity of the patch, hence, the concentration of polymer used has to be carefully considered.

## Diffusion study of Patch:

The drug release from the patches was compared to the release of the drug from the SLNs. Release from the patches was found to be more compared to that from the SLNs. The drug release from the patch followed zero order kinetics and the mechanism of release was non-Fickian. The release of drug from the patch was found to be greater than the release from the nanoparticle formulation. The reasons could be as follows:

1. The intimate contact the patch has with the diffusion membrane aids the natural diffusion of the drug into the diffusion media.

2. The use of DMSO as penetration enhancer in the patch formulation could have increased diffusion of drug through the diffusion membrane.

So it can be concluded that the drug release from patch was comparatively more than solid lipid nanoparticles and follows zero order release kinetics and non fickian diffusion mechanism.

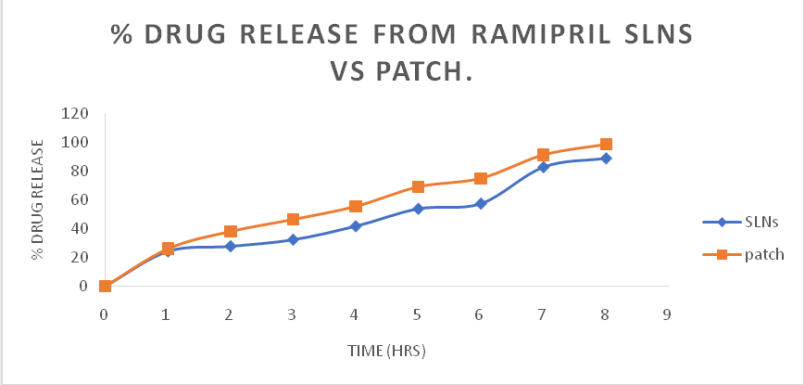


Fig.9**:** % Drug release from Ramipril loaded SLNs in comparison to the % drug release from SLNs loaded patch.

**Table 7: kinetic values of drug diffusion from patch formulation:**

| Formulation Code | Zero order Plot | First order plot | Higuchi plot | Kosermeyer Peppasplot | | Mechanism Of drug release |
|---|---|---|---|---|---|---|
| | $R^2$ | $R^2$ | $R^2$ | $R^2$ | n-value | |
| patch | 0.988 | 0.766 | 0.920 | 0.878 | 0.640 | Non-Fickian |

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