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Comparison of vegetable and volatile oils as skin permeation enhancers for transdermal delivery of losartan potassium

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ABSTRACT

The present work comprises the formulation and evaluation delivery system of transdermal drug with a view of developing and preparing a losartan potassium releasing system utilizing natural oils as permeation enhancers for transdermal applications. Matrix systems were prepared by using polyvinyl pyrrolidone and ethyl cellulose (EC) polymers by incorporating dibutyl phthalate as plasticizer and chloroform as solvent. The physicochemical and *in vitro* drug release studies indicated that formulation containing PVP and EC in the ratio of 3:2 was better than other combination of polymers. Penetration enhancing potential of vegetable oils (jojoba oil, sunflower oil, sesame oil) and volatile oils (clove oil, peppermint oil, eucalyptus oil) was determined by incorporating oils in different concentration in optimized transdermal patch and *in vitro* permeation of losartan potassium across goat skin was studied. Maximum transdermal flux of 0.29 mg/cm²/h was obtained with formulation containing 10 % jojoba oil (vegetable oil) as permeation enhancer while in case of volatile oil maximum transdermal flux of 0.27 mg/cm²/h was obtained with 10% peppermint oil as permeation enhancer respectively. The results of permeation fluxes supported the data for prolongation of drug release characteristics of formulated transdermal films. The release kinetics revealed that the release was sustained and it follows zero-order kinetics. Stability studies of optimized formulation were stable at 45° C & 75% RH with respect to their physical parameters and *in vitro* drug release. This suggests the transdermal application of losartan potassium holds promising for improved bioavailability and better alternative to oral dosage form in hypertensive patients.

Keywords: Jojoba oil, sunflower oil, sesame oil, clove oil, peppermint oil, eucalyptus oil, Permeation

INTRODUCTION

Losartan potassium is an angiotensin-receptor blocker that may be used alone or with other agents to treat hypertension, isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy. It may also be used as an alternative agent for the treatment of systolic dysfunction, myocardial infarction, coronary artery disease, and heart failure. Losartan potassium is not rapidly and completely absorbed after oral administration. The presence of food does alter the extent of absorption of losartan potassium. It shows the plasma protein binding up to 98% but has plasma half life of 2-2.5 hours and shows bioavailability only up to 32 %. The usual dose of drug ranges from 50-200 mg per day. Due to the less plasma half life of drug, it is necessary to repeat its dose twice in the day to maintain its effect [1]. So it is not easy to deliver an adequate amount of losartan for effective treatment of cardiac disorders, particularly sustained delivery over a period of time. Hence an alternative route of drug delivery is required for losartan potassium as therapeutic outcomes require not only proper drug selection but also effective drug delivery [2].

Transdermal drug delivery methods offer substantial clinical advantages, including reduced dosing frequency therefore, improved patient compliance in long therapy, minimized fluctuation of the concentrations and maintenance of blood levels within a desired range, localized drug delivery and potential for reduced adverse effects and making it possible to terminate drug therapy when needed. There is still no transdermal losartan potassium

delivery system of a convenient size that may be applicable on a patient by him over a period of days which can deliver a flux adequate for therapeutic effect. As this drug has an important role to play in the treatment of cardiac disorders, it is desired to develop low dose maintenance therapy of losartan potassium, that specializes in phasing the drug administration so that the optimum amount of drug is provided to control the disease conditions and may result in lower adverse effect (i.e) that seen with oral delivery.

Based on this hypothesis, the main aim of the present investigation is to develop transdermal drug delivery system (TDDS) of losartan potassium which can minimize the risk of major oral side effects and address the problems of poor compliance in patients. Furthermore, this transdermal patch can allow a more steady sustained delivery than doses taken orally at different time intervals hours [3, 4]. This delivery system can lead to cost effectiveness of health care treatment for long term management of the disease.

For successful development of TDDS, chemical permeation enhancers are widely used that reduce barrier properties of skin by acting on different components of skin such as lipids and proteins. Recently, natural permeation enhancers that are nontoxic, nonallergic, and compatible with drug and excipients have received increasing attention of researchers. Vegetable oils and volatile oils are used in cosmetics and medicine, are safe to use, metabolized in body, and are easily available [5]. We have also reported in recent report that natural oils are effective penetration enhancers[6,7]. In the present study, different vegetable oils *viz.* jojoba oil, sunflower oil, and sesame oil and volatile oils *viz.* clove oil, peppermint oil, eucalyptus oil were investigated to find out their effect on rate of permeation of losartan potassium through TDDS.

MATERIALS AND METHODS

Materials

Losartan potassium with 99.5% purity was provided by Ranbaxy Laboratory Limited Baddi, H.P, India. Oils were obtained from Rajesh Biological, Mumbai and other materials used in this study were of analytical grade.

Formulation of transdermal patches

Transdermal patches of Losartan potassium was prepared by solvent casting technique in a glass mould fabricated locally. To determine the optimum combination of polymers, plasticizer and solvent, placebo patches were formulated. On the basis of preliminary studies, the optimized polymers polyvinyl pyrrolidone (PVP) and ethyl cellulose (EC) in different ratios were mixed to a total weight of 500 mg and dissolved in 10 ml of chloroform solvent system using magnetic stirrer. Polymers and drug were weighed accurately and drug (20 % w/w of polymer weight) was added slowly to the polymer solution and mixed thoroughly to obtain a homogenous solution and dibutyl phthalate (DBP) was used as plasticizer. Natural permeation enhancers *i.e.* vegetable oils (sesame oil, sunflower oil, jojoba oil,) and volatile oils (clove oil, peppermint oil, eucalyptus oil) were added in three different concentrations *i.e.* 1%, 5% and 10% w/w of polymer weight for each. The resulting polymeric solution was stirred and casted into glass moulds (5×3 cm²) and dried at room temperature for 24 hr for solvent evaporation. The dried films were carefully removed and checked for any imperfection or air bubbles. The films were packed in an aluminium foil and stored in an air tight glass container to maintain the integrity and elasticity of the patches [8].

Investigation of drug polymer compatibility

FTIR spectra of pure drug losartan potassium and mixture of drug with both the polymers *i.e.* polyvinyl pyrrolidone (PVP), ethyl cellulose (EC), were taken using Perkin Elmer FTIR spectrophotometer (RXIFT-IR system). Sample was prepared with potassium bromide and data were collected, indicating compatibility of excipients with drug. The samples were scanned from 400cm⁻¹ to 4000cm⁻¹.

Characterization of transdermal patches

Physical Appearance, Weight and Thickness: All the transdermal films were visually inspected for colour, uniformity and smoothness. Weight variation was studied by individually weighing 10 randomly selected patches and film thickness was determined by micrometer at random points on the film of all batches [9].

Drug content determination: An accurately weighed portion of the film (100 mg) was dissolved in 100 ml of chloroform, and then the solution was shaken continuously for 24 h in shaker incubator. After sonicating and filtering, concentration of drug was estimated spectrophotometrically (at 234 nm) by appropriate dilution.

Flatness: For flatness determination, one strip was cut from the centre and two from each side of patches. The length of each strip was measured and variation in length was measured by determining percent constriction. Zero percent constriction is equal to 100% flatness [10].

Folding endurance and tensile strength: Folding endurance of the film was determined repeatedly folding the film at the same place until it break. The number of times the film could be folded at the same place without breaking was the folding endurance value [11]. Tensile strength was determined by weight pulley method [12].

***In vitro* drug release studies**

The *in vitro* drug release of Losartan potassium from the prepared transdermal patch of $2.5 \times 2 \text{ cm}^2$ were performed by using a modified USP type II dissolution apparatus using 900 ml of PBS 7.4 as dissolution medium. A patch with an diameter of $2.5 \times 2 \text{ cm}^2$ was used for the study and a stainless steel ring was employed to sink the patch at bottom of dissolution apparatus. All dissolution studies were performed at $32 \pm 0.5 \text{ }^\circ\text{C}$ (temperature of skin) at 100 rpm. After different time intervals, 3 ml sample was withdrawn, filtered through a whattman filter paper and assayed spectrophotometrically at λ_{max} 234 nm. Immediately after each sample withdrawal, a similar volume of phosphate buffer pH 7.4 was added to the release medium to maintain the volume in the vessel constant [13].

Release kinetics

To study the release kinetics, data obtained from *in vitro* drug release studies were fitted in various kinetic models: zero order as cumulative percent of drug released *vs.* time, first order as log cumulative percentage of drug remaining *vs.* time and Higuchi's model as cumulative percent drug released *vs.* square root of time. To determine the mechanism of drug release, the data were fitted into Korsmeyer and Peppas equation as log cumulative percentage of drug released *vs.* log time, and the exponent *n* was calculated from slope of the straight line. For slab matrix, if exponent is 0.5, then diffusion mechanism is fickian; if $0.5 < n < 1.0$, mechanism is non-fickian; if *n* is 1.0, mechanism is zero order and if $n > 1.0$, then it is super case II transport[14].¹

***In vitro* Permeation studies**

The *in vitro* permeation studies were carried out in a modified Franz diffusion cell with the capacity of 20 ml, using goat skin. Goat skin was obtained from slauke house.

Prereration of skin

Hairless animal skin and human cadaver skin are used for permeation studies. Human cadaver skin may be a logical choice as the skin model because the final product will be used in humans. But it is not easily available. So, in the present study, hairless Wistar rat abdominal skin was used. Rats were sacrificed by excess ether inhalation. Hairs on dorsal skin of animal were removed with animal hair clipper, subcutaneous tissue was surgically removed and dermis side was wiped with isopropyl alcohol to remove residual adhering fat. The skin was washed with phosphate saline buffer (PBS) pH 7.4. The skin so prepared was wrapped in aluminum foil and stored in a deep freezer at $-20 \text{ }^\circ\text{C}$ till further use. The skin was defrosted at room temperature when required. The *in vitro* permeation studies were carried out in vertical Franz diffusion cell with a capacity of 20 ml, using goat skin [15]. The patch was placed on the skin with the drug matrix side towards the donor side and backing membrane on the upper side. PBS 7.4 was used as receptor fluid as in release studies. The receptor fluid was agitated at 100 rpm by magnetic stirrer and temperature was maintained at $32 \pm 0.5 \text{ }^\circ\text{C}$. The samples were withdrawn at different time intervals and replaced with equal amounts of dissolution media. Samples were analyzed for its drug content. The drug permeated per cm^2 of patch was calculated and plotted against time and the flux was calculated as drug permeated per cm^2 per hour. The steady state flux was determined from the slope of the linear portion of a cumulative amount permeated versus time plot. The lag time (T_{lag}) was determined by extrapolating the linear portion of the cumulative amount permeated versus time curve to the abscissa. Enhancement ratio of the flux (E_{pen}) was calculated as:

$$E_{\text{pen}} = P_{\text{treatment}} / P_{\text{control}}$$

Where $P_{\text{treatment}}$ is flux of formulation containing enhancer and P_{control} is flux of control group (Without permeation enhancer)

Comparison of best batches of Vegetable oils and volatile oils of losartan potassium transdermal patch

On the basis of results obtained from permeation study, *in-vitro* release studies comparison of the best batches of vegetable oils and volatile oils was done.

Stability studies

The stability studies are conducted to investigate the influence of temperature and relative humidity on the drug content in different formulations. The optimized transdermal formulations were subjected to stability studies for 3 months using storage conditions 45°C & 75% RH as per ICH guidelines. Throughout the course of aging study, triplicate samples were taken at three sampling times (*i.e.* 0, 1 month and 3 month) and evaluated for physical texture, drug content and *in vitro* permeation studies as the indicators.

Statistical Analysis

Graph pad prism 5 was used for statistical analysis. All studies were done in triplicates unless specified and data represent the mean \pm SD. The statistical analysis was performed using student t-test. A difference below the probability level was considered statistical significant.

RESULTS AND DISCUSSION

Good, transparent and uniform films were obtained by using mixture of PVP and EC. However, they were found to be brittle without plasticizer. Then dibutylphthalate(10% w/w of polymer weight) was added as plasticizer to decrease the brittleness, which formed the films with good elasticity as reported in earlier studies. It was observed that when oils were added as permeation enhancer, only 5% w/w of plasticizer was sufficient to produce films with good elasticity. This may be done to that oils itself acted as plasticizer. The average weights ranged between 176 to 183 mg, which indicates that different batches were relatively similar in weights. The thickness of the films was measured by micrometer and film thickness was found to lie between 0.59 to 0.67 mm. Accepted uniformity of drug content among the batches was observed with all formulations and ranged from 95 to 99%. The results indicate that the process employed to prepare transdermal patches in this study was capable of producing formulations with uniform drug content and minimal patch variability. The flatness study showed that all the formulations had the same strip length before and after their cuts, indicating 100% flatness [16]. Thus, no constriction was observed indicating all patches had a smooth and flat surface. Tensile strength was observed from 0.31 to 0.42 kg/mm² shown in Table 1. Folding endurance test results indicated that the patches would not break and would maintain their integrity with general skin folding when applied [17]. Drug polymer interaction study was carried out to eliminate the possibility of interaction between drug and polymer used with analytical method of drug estimation. IR spectra of pure drug losartan potassium showed sharp peaks at 11424 cm⁻¹, 11649 cm⁻¹, 1458 cm⁻¹, 11189 cm⁻¹ that confirmed the presence of (C-H Stretch), C=C, bend and C-O-C respectively. It was seen that the major peaks of the pure drug were conserved in all the scans taken in combination with the polymer complex (Figure 1).

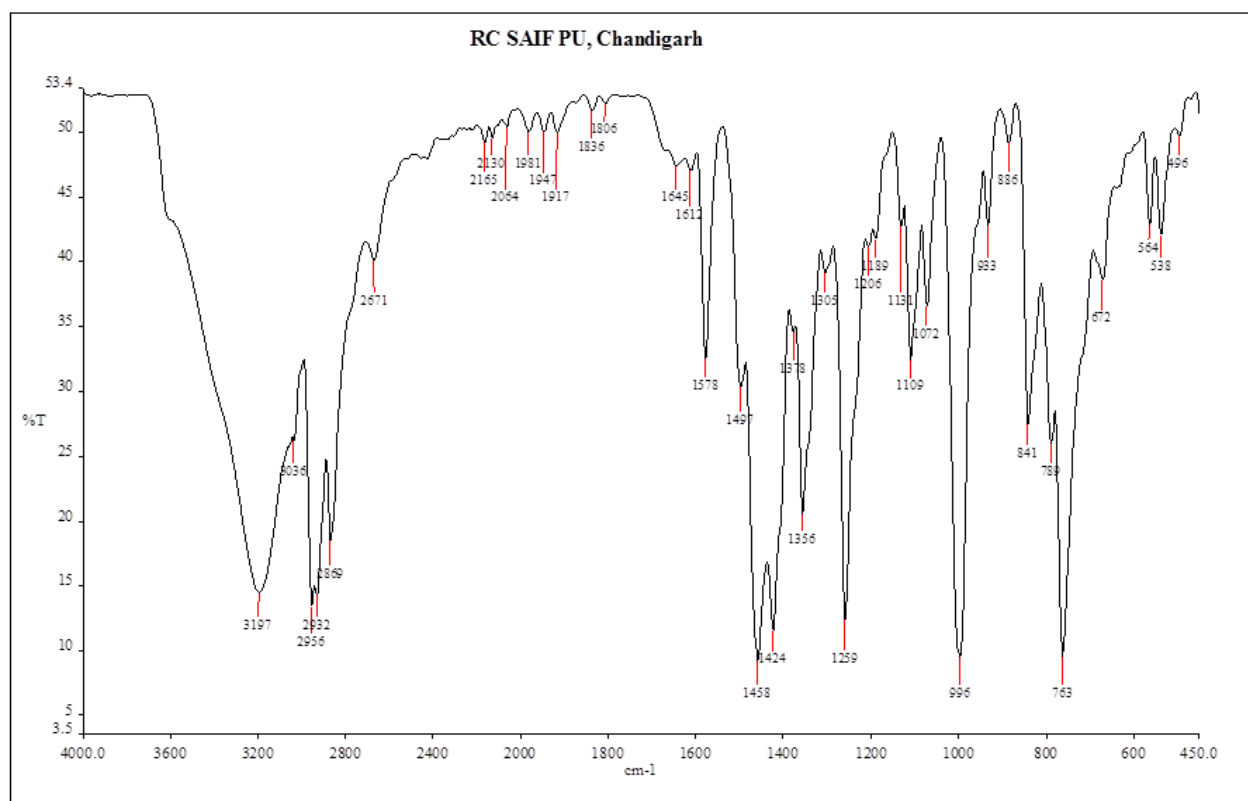


Fig 1: FTIR spectra of Losartan potassium

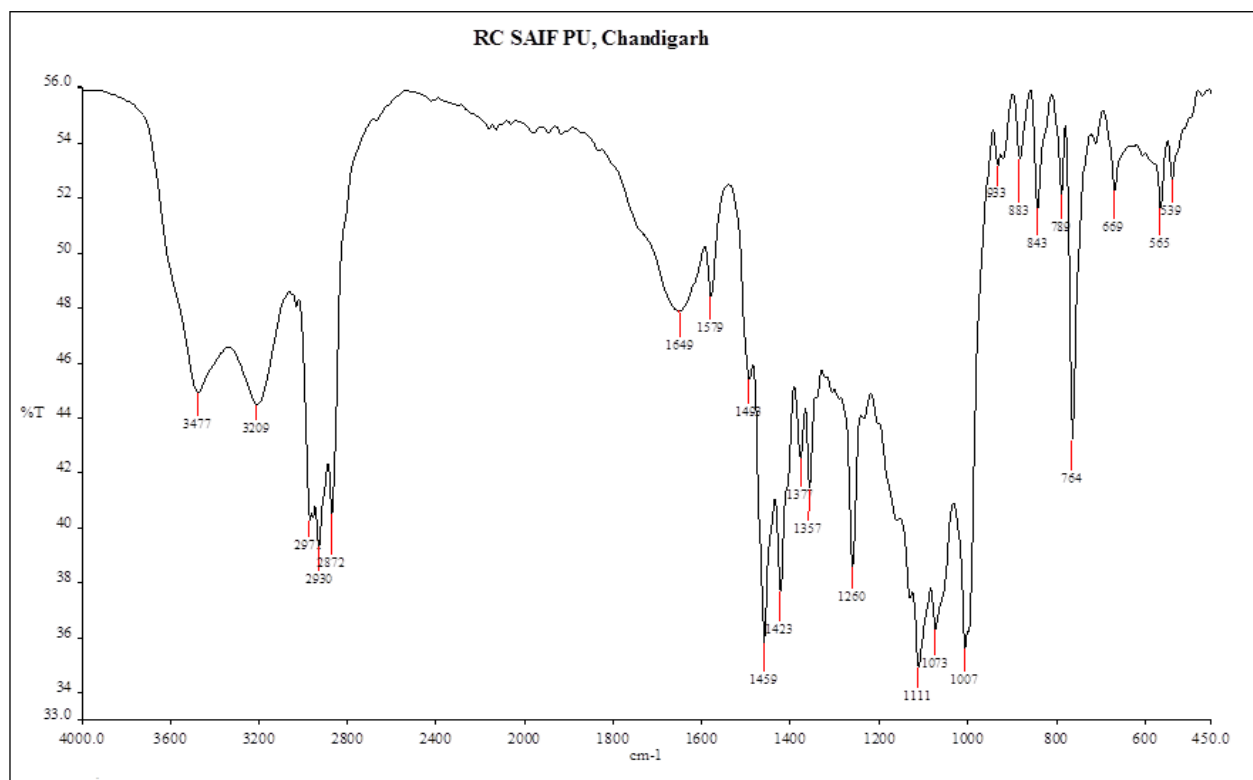


Fig 2: FTIR spectra of Ethyl cellulose, polyvinyl pyrrolidone and Losartan potassium (1:1:1)

Table 1: Composition and physicochemical characteristics of prepared formulations

Code	PVP:EC (500mg)	Permeation enhancer (% w/w of polymer weight)	Average Weight variation (mg)*	Thickness (mm)	Drug content (%)	Folding endurance	Flatness (%)	Tensile strength (kg/mm ²)
A1	0:1	-	176.67 ± 0.15	0.61 ± 0.02	96.43 ± 0.47	146 ± 10	100 ± 0.00	0.325 ± 0.02
A2	1:2	-	177.29 ± 0.27	0.59 ± 0.02	95.88 ± 0.21	145 ± 07	100 ± 0.00	0.315 ± 0.03
A3	1:3	-	179.07 ± 0.13	0.62 ± 0.01	98.07 ± 0.33	147 ± 09	100 ± 0.00	0.429 ± 0.03
A4	2:3	-	178.55 ± 0.29	0.63 ± 0.01	97.07 ± 0.33	151 ± 10	100 ± 0.00	0.349 ± 0.06
A5	3:2	-	179.83 ± 0.27	0.63 ± 0.01	99.54 ± 0.22	156 ± 15	100 ± 0.00	0.425 ± 0.04
A6	2:1	-	176.35 ± 0.42	0.60 ± 0.03	95.18 ± 0.21	143 ± 08	100 ± 0.00	0.398 ± 0.03
A7	1:0	-	177.76 ± 0.54	0.61 ± 0.01	99.87 ± 0.46	144 ± 09	100 ± 0.00	0.421 ± 0.04
B1	3:2	Jjoba oil (1%)	178.42 ± 0.25	0.62 ± 0.005	97.29 ± 0.06	161 ± 10	100 ± 0.00	0.383 ± 0.03
B2	3:2	Jjoba oil (5%)	181.93 ± 0.10	0.62 ± 0.015	97.28 ± 0.33	163 ± 07	100 ± 0.00	0.358 ± 0.03
B3	3:2	Jjoba oil (10%)	183.47 ± 0.14	0.63 ± 0.011	98.1 ± 0.79	163 ± 14	100 ± 0.00	0.361 ± 0.02
C1	3:2	sunflower oil 1%	181.36 ± 0.33	0.65 ± 0.017	97.13 ± 1.33	162 ± 10	100 ± 0.00	0.326 ± 0.03
C2	3:2	sunflower oil (5%)	181.80 ± 0.23	0.64 ± 0.017	97.88 ± 0.39	163 ± 12	100 ± 0.00	0.323 ± 0.04
C3	3:2	sunflower oil (10%)	183.53 ± 0.44	0.63 ± 0.02	96.30 ± 0.31	162 ± 08	100 ± 0.00	0.332 ± 0.02
D1	3:2	sesame oil (1%)	179.67 ± 0.09	0.67 ± 0.01	97.35 ± 1.04	166 ± 10	100 ± 0.00	0.390 ± 0.07
D2	3:2	sesame oil (5%)	180.18 ± 0.32	0.66 ± 0.17	97.79 ± 0.72	165 ± 15	100 ± 0.00	0.403 ± 0.02
D3	3:2	sesame oil (10%)	183.91 ± 0.17	0.64 ± 0.15	97.56 ± 0.41	166 ± 11	100 ± 0.00	0.415 ± 0.05
E1	3:2	clove oil (1%)	178.79 ± 0.17	0.65 ± 0.05	97.73 ± 1.17	164 ± 07	100 ± 0.00	0.393 ± 0.02
E2	3:2	clove oil (5%)	179.17 ± 0.16	0.64 ± 0.03	97.58 ± 0.51	164 ± 10	100 ± 0.00	0.382 ± 0.01
E3	3:2	clove oil (10%)	182.59 ± 0.16	0.61 ± 0.05	98.5 ± 0.06	163 ± 06	100 ± 0.00	0.363 ± 0.02
F1	3:2	peppermint oil (1%)	179.71 ± 0.26	0.64 ± 0.03	98.77 ± 0.10	165 ± 10	100 ± 0.00	0.338 ± 0.02
F2	3:2	peppermint oil (5%)	180.66 ± 0.28	0.66 ± 0.02	98.45 ± 1.14	166 ± 09	100 ± 0.00	0.342 ± 0.04
F3	3:2	peppermint oil (10%)	181.91 ± 0.07	0.63 ± 0.05	98.29 ± 0.22	164 ± 15	100 ± 0.00	0.348 ± 0.02
G1	3:2	eucalyptus oil (1%)	181.51 ± 0.22	0.61 ± 0.05	99.33 ± 0.12	165 ± 07	100 ± 0.00	0.3740 ± 0.03
G2	3:2	eucalyptus oil (5%)	182.64 ± 0.12	0.61 ± 0.05	98.35 ± 0.88	165 ± 10	100 ± 0.00	0.366 ± 0.02
G3	3:2	eucalyptus oil (10%)	183.46 ± 0.11	0.61 ± 0.05	98.68 ± 1.01	163 ± 05	100 ± 0.00	0.363 ± 0.01

Concentration of drug (20% w/w of polymer weight) was kept constant in all formulations; EC is ethyl cellulose and PVP is polyvinyl pyrrolidone n = 10 for weight;

IN VITRO RELEASE STUDIES

In vitro release studies were conducted as per USP procedure using phosphate buffer pH 7.4 as dissolution medium. As illustrated in Figure 3, among the different ratios of polyvinyl pyrrolidone (PVP) and ethyl cellulose (EC), the formulations A1 to A4 showed the less release as compared to other batches because the concentration of ethyl cellulose in these batches is relatively high. As EC is a hydrophobic polymer and this property make dissolution as rate limiting step which reduce the release rate of drug [17]. While the formulations A6 and A7 showed burst release within 2 hrs of dissolution which may be attributed by the high concentration of PVP (hydrophilic polymer) in the respective formulations. Formulation A5 (PVP:EC,3:2) was optimized as best formulation as it showed consistent and sustained release of drug which may be due to optimized composition of hydrophilic (PVP) and hydrophobic (EC) polymer. The hydrophilic nature of PVP which increases release rate and it attributed by the leaching of soluble compounds which leads to the formation of pores and thus decreases the mean diffusion path length of drug molecule to release in dissolution medium which results in higher dissolution rate and at the same time ethyl cellulose restrict the burst release of drug by its hydrophobic property. But the cumulative % drug release from mixed polymers without permeation enhancer was found to be only 43% to 56% at 24 h, which was very less. So it is decided to for incorporate permeation enhancers in formulation A5 (Figure 3) [18].

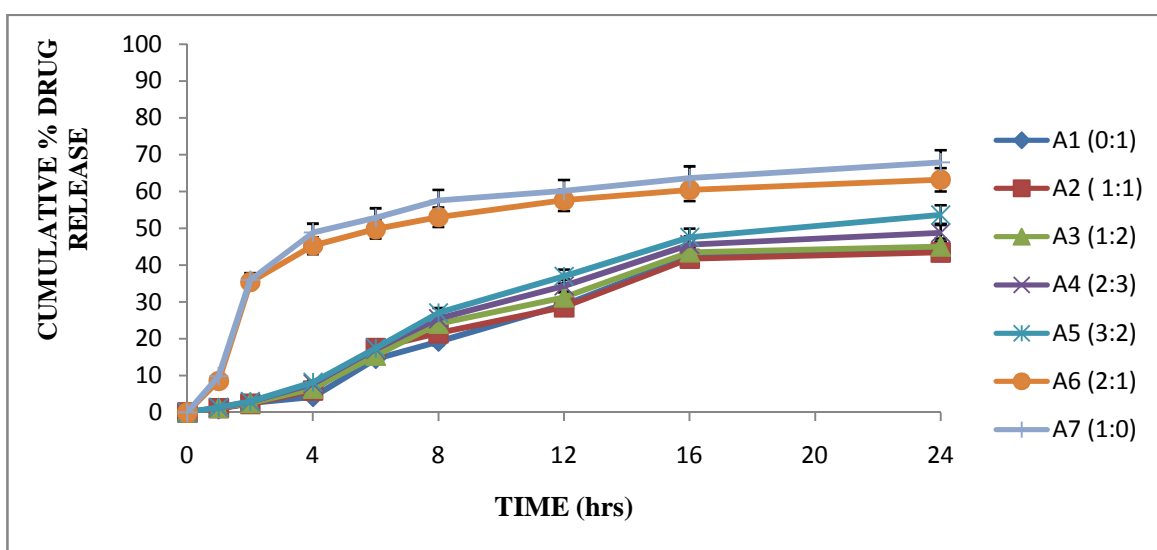


Figure 3 : *In vitro* release profile of Losartan potassium TDDS without permeation enhancers

Effect of permeation enhancers on *in vitro* release of losartan potassium**Effect of vegetable oils**

Release was found to increase with increasing in concentration of vegetable oils, which may be attributed to presence of fatty acids in vegetable oils. Presence of oil cannot increase solubility of drug significantly in release media [19]. Studies revealed that sunflower oil, sesame oil and jojoba oil in concentration of 10 % w/w of polymer weight exhibited enhancing effects on losartan potassium release from transdermal patches, with the cumulative amount of drug release being $98.08 \pm 0.24\%$, $96.32 \pm 0.54\%$ and 91.42 ± 1.07 respectively (Figure 4-6). When the jojoba oil as permeation enhancer was compared for 1%, 5% & 10% concentration, it was found the release from 10% concentration was significantly ($p < 0.05$, t- test) more than the other concentration. (Figure 4) When sunflower oil and sesame oil were compared for different concentrations of oil as permeation enhancer, the release with 5% oil was insignificantly ($p > 0.05$, t- test) lesser than 10% oil. (Figure 5 & 6) It may be due to the maximum solubility of drug was with 5 % oils. After that, there may be saturation and solubility is not increasing after increasing the concentration of oils. So the order of release observed with oil was sunflower oil 5% (C2) > sesame oil 5% (D2) > jojoba oil 10% (B3). It was observed that as the amount of vegetable oils was increased (from 1% to 10%), release rate also increased and rate of release with vegetable oil depends upon the amount of fatty acids in oils. More release with sunflower oil may be due to more oleic acid constituent (fatty acids) present in sunflower oil [6].

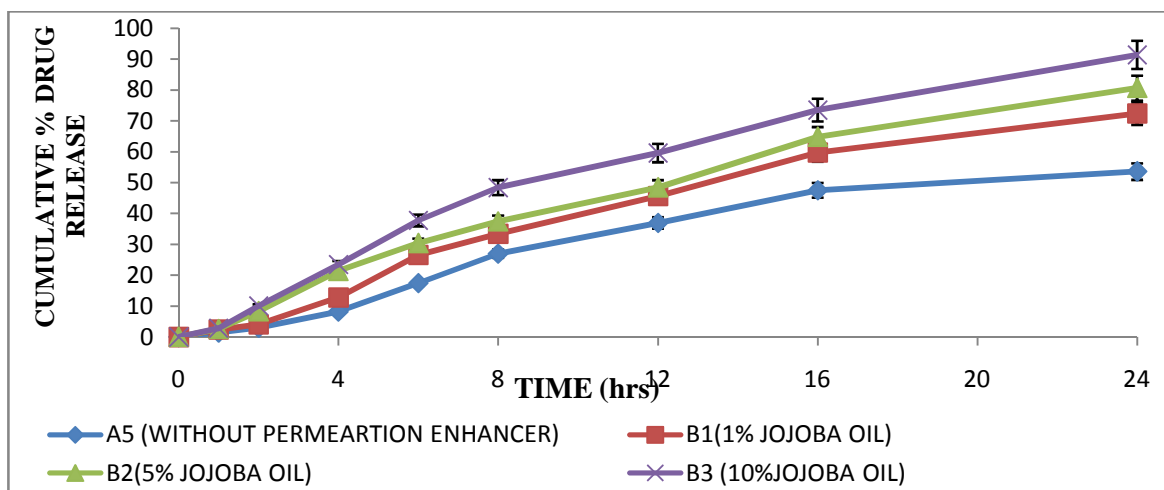


Figure 4: *In vitro* release profile of losartan potassium TDDS with jojoba oil as permeation enhancer

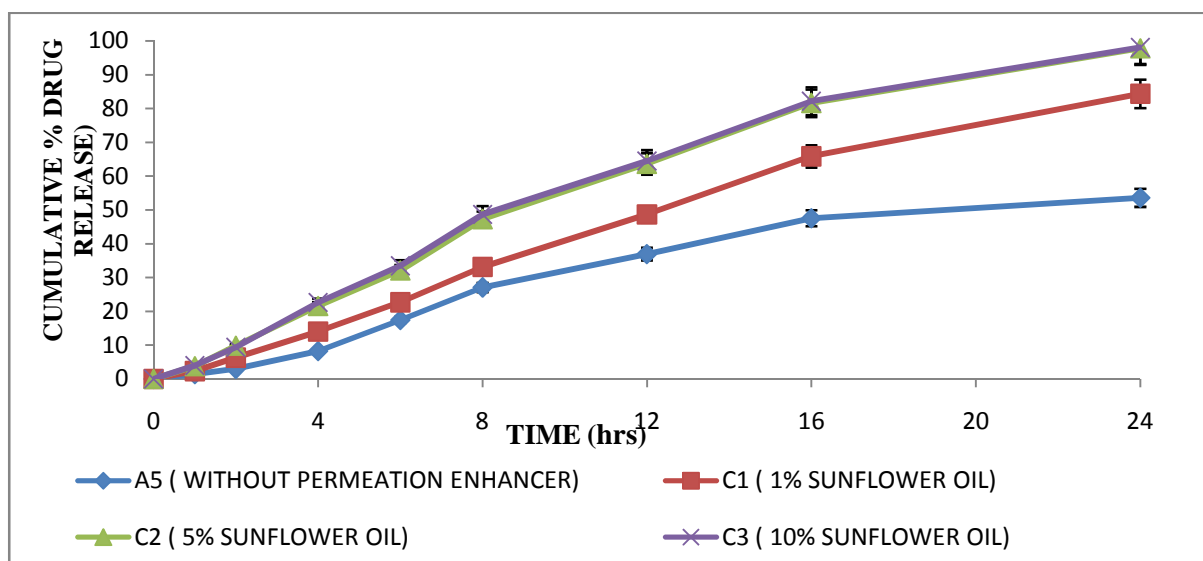


Figure 5: *In vitro* release profile of losartan potassium TDDS with sunflower oil as permeation enhancer

Effect of volatile oils

Release was found to increase with increasing in concentration of volatile oils as in case of vegetable oils, which may be attributed to presence of terpene constituents in volatile oils. Studies also revealed that, peppermint oil in the concentration of 5 % w/w, clove oil and eucalyptus oil in the concentration of 10% w/w of polymer weight exhibited maximum enhancing effects on losartan potassium release from transdermal patches, with the cumulative amount of drug release being $96.09 \pm 0.68\%$, $93.08 \pm 0.04\%$, and $92.49 \pm 0.78\%$ respectively (Figure 7-9). Degree of release with various oils was in the following order clove oil > eucalyptus oil > peppermint oil. It was observed that the rate of release with volatile oil depends upon the amount of terpenes constituents in oils. More release with peppermint oil may be due to more to more cineole (terpene constituent) present in peppermint oil(Figure 7). While eucalyptus oil at 10% w/w concentration was insignificantly ($p > 0.05$; t-test) higher than peppermint oils (5%w/w)(Figure 8). This again may be due to saturated solubility of drug at 5% w/w of peppermint oil. The higher release with clove oil and eucalyptus oil is due to presence of eugenin in clove oil which is the type of terpene constituent and cineole in eucalyptus oil(Figure 9) [20].

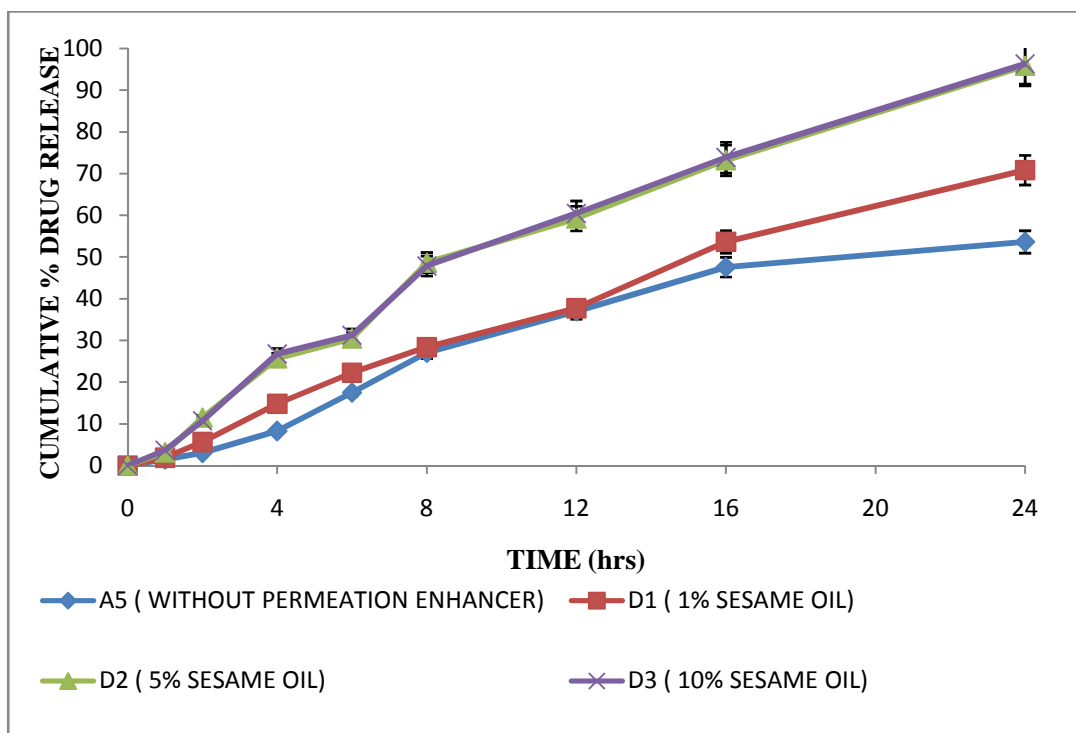


Figure 6: *In vitro* release profile of losartan potassium TDDS with sesame oil as permeation enhancer

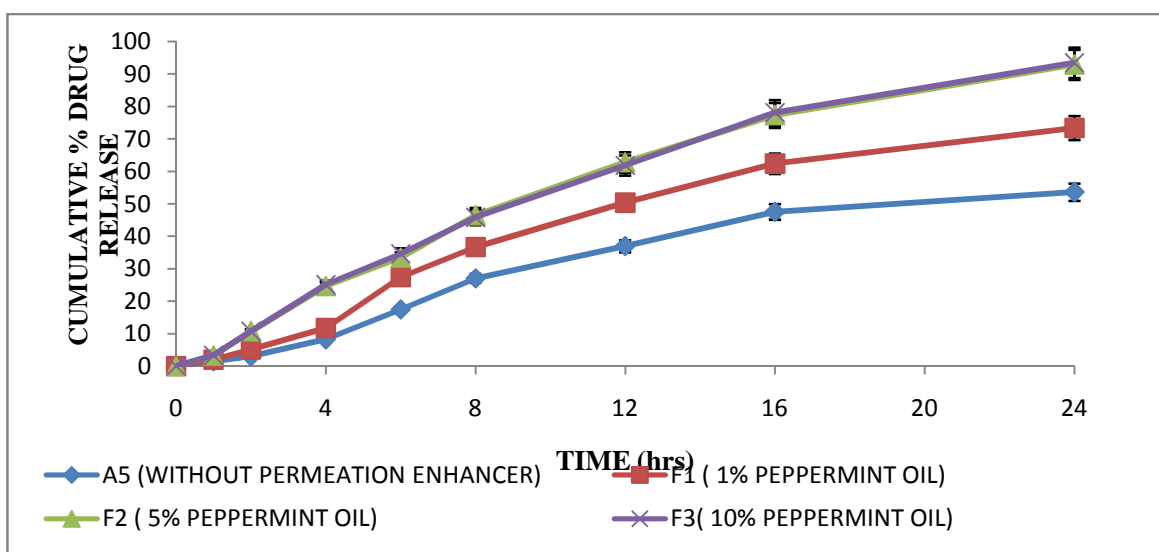


Figure 7: *In vitro* release profile of losartan potassium TDDS with peppermint oil as permeation enhancer

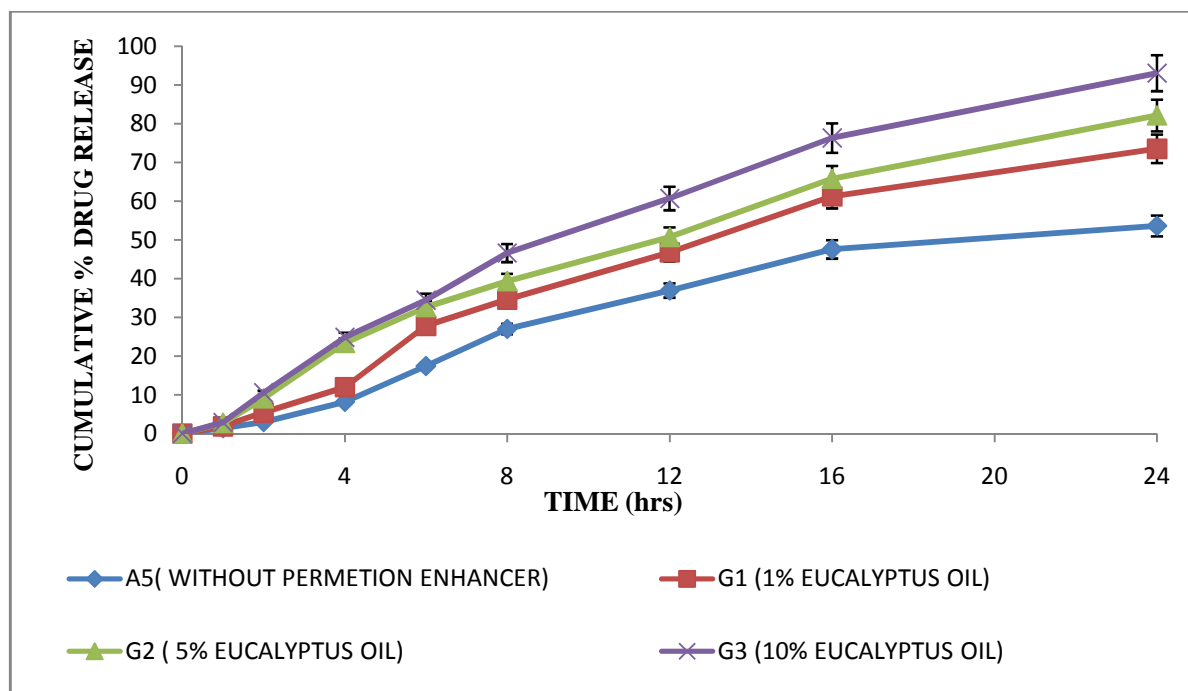


Figure 8: *In vitro* release profile of losartan potassium TDDS with eucalyptus oil as permeation enhancer

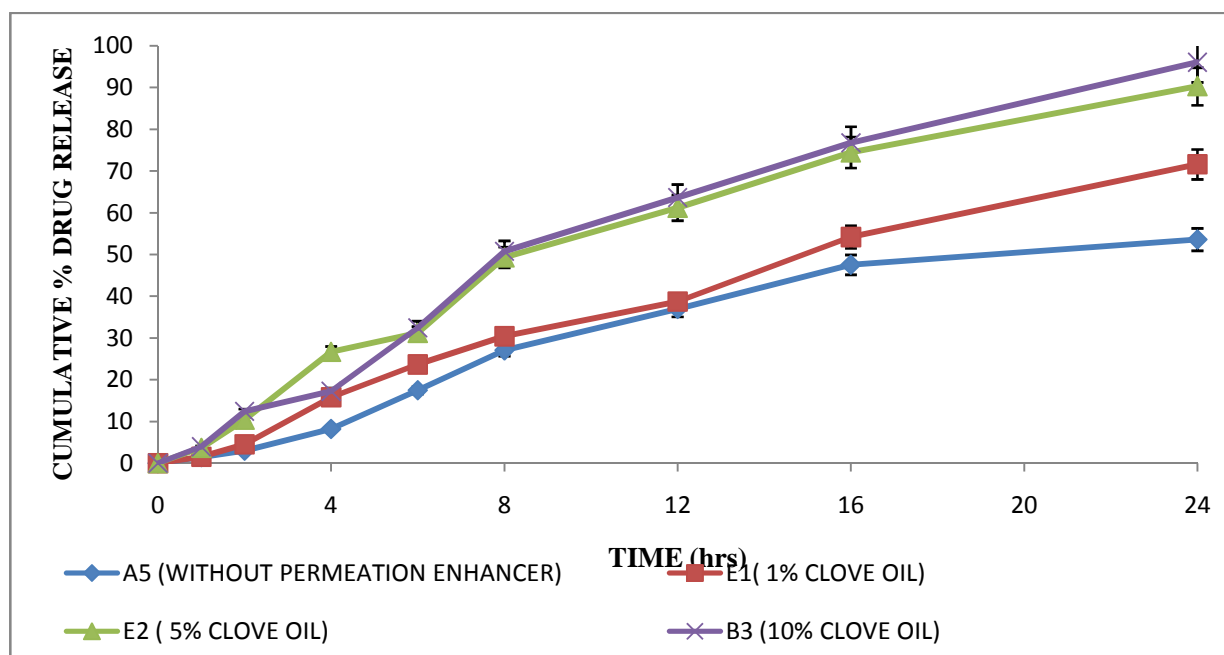


Figure 9 : *In vitro* release profile of losartan potassium TDDS with clove oil as permeation enhancer

Investigation of drug release kinetics

The selected formulations from all batches were subjected to release kinetics study. Results confirmed that the *in vitro* drug release of losartan potassium TDDS without permeation enhancers was best explained by first order equation. In case of transdermal formulations prepared with vegetable oils (jojoba oil, sunflower oil and sesame oil) and volatile oils (clove oil peppermint oil and eucalyptus oil) release pattern was again best fitted for zero order release kinetics. Based on Korsmeyer Peppas model of these formulations, the best fitting was obtained with $n > 1$, indicating super case II transport, which means relaxation process is very slow as compared to diffusion for these formulations [21]. On the basis of these considerations, it is clear that drug release from these formulations is controlled by diffusion.(Table-2)

Table no 2: Release kinetics of losartan potassium formulations

S.no	Batch no.	Zero order r ²	First order r ²	Higuchi model r ²	Korsmeyer-Peppas model (n)	Release order & main transport mechanism
1	A5	0.9614	0.9798	0.7922	1.43	First order, Non-fickian
2	B3	0.9924	0.9665	0.8556	1.51	Zero order, Non-fickian
3	C3	0.9749	0.9235	0.8923	1.36	Zero order, Non-fickian
4	D3	0.9871	0.9104	0.8932	1.21	Zero order, Non-fickian
5	E3	0.9835	0.9779	0.9075	1.36	Zero order, Non-fickian
6	F3	0.9749	0.9313	0.9597	1.37	Zero order, Non-fickian
7	G3	0.9879	0.9529	0.9509	1.15	Zero order, Non-fickian

***In vitro* permeation studies**

In vitro permeation studies are predictive of an *in vivo* performance of a drug. Permeation studies were conducted on formulation A5 (without permeation enhancer) and with all formulation prepared with different concentration of oils. The results of *in vitro* skin permeation of losartan potassium transdermal patches showed that flux was significantly enhanced with the addition of permeation enhancers than the formulation containing no permeation enhancer [22]. The results of release studies and permeation studies were found to be different in case of jojoba oil and peppermint oil. Although the percentage release of drug with jojoba oil and peppermint oil was less, but the amount of drug permeated was more with these oils than other oils which showed more *in vitro* release. So it can be concluded that rate of release depends mainly on the solubility of drug in dissolution media, but rate of permeation depends on the mechanism of penetration of drug through skin membrane. All the vegetable oils (jojoba oil, sunflower oil and sesame oil) and volatile oils (clove oil, peppermint oil and eucalyptus oil) enhanced the permeation of drug in the concentration of 10% w/w of polymers through skin maximally. This shows that as the amount of oil as skin permeation enhancer was increased they enhance permeation of drug. This may be due to the presence of terpenes and fatty acids in the oil. The results of permeation studies were found to be different when different oils were used

All the permeation enhancers enhanced permeation of losartan potassium through excised goat skin than control group (without permeation enhancer). But the formulations containing jojoba oil as permeation enhancer (area of 2.5×2 cm²) (*i.e* B3) exhibited the highest % cumulative amount of drug permeated 91.34% ± 0.07) (Table 3; Figure 10), which was significant (p<0.05; t-test) than formulation containing no permeation enhancer *i.e.* A5 (44.17% ± 0.03 mg/cm²) in 24 h. Jojoba oil in the concentration of 10% exhibited highest permeation as it contains fatty acids like eicosenoic, docosenoic and oleic acid. It also contains myristic acid has a similar in composition to that of the skin's own oils, so it is quickly absorbed and which may contribute to faster permeation of drug. It has already been reported that oleic acid may increase the epidermal permeability through a mechanism involving the perturbation of stratum corneum lipid bilayers and lacunae formation by virtue of which It gets incorporated into skin lipid, disrupt molecular packing and alter the level of hydration and allow drug penetrates faster to enhance transdermal drug delivery [23].

Sunflower oil in the concentration of 10% exhibited second highest %age cumulative amount of drug permeated 89.25% ± 0.06 mg/cm²) (Table 3; Figure 11) as it contains monounsaturated fatty acid /polyunsaturated fatty acid mixture of mostly oleic acid linoleic acid. A large number of fatty acids and their esters have already been used as permeation enhancers. A general trend has been seen that unsaturated fatty acids are more effective in enhancing percutaneous absorption of drugs than their saturated counterparts [24]. Fatty acids such as oleic acid have been studied as a skin penetration enhancer for topically applied medications, primarily via its action mainly on the stratum corneum lipid structure. It has been found to increase the epidermal permeability through a mechanism involving the stratum corneum lipid membrane. It is incorporated into skin lipid, disrupt molecular packing and alter the level of hydration and allow drug penetrates faster [25]. Although not as good as jojoba oil and sunflower oil, other oils of this category *i.e* sesame oil and volatile oils (clove oil, peppermint oil, eucalyptus oil) also exhibited good enhancing effects, with %age cumulative amounts being, 84.92 ± 0.05, 83.43 ± 0.08, 87.76 ± 0.05 and 85.97 ± 0.04 mg/cm² after 24 h respectively showed in table 3. Permeation enhancers in concentration of 1% exhibited only a minor enhancing effect. A poor drug release from batch A5 (without permeation enhancers) might be attributed to a tough barrier *i.e.* stratum corneum, which contributes to low diffusivity. Results indicated that drug permeated from patches without permeation enhancer was less than the required amount for therapeutic effect. Hence other formulations prepared with permeation enhancers were found to be release and permeate drug as required to produce therapeutic effect.

Results revealed that addition of permeation enhancers enhanced the flux of formulation significantly

($p < 0.05$, $p < 0.001$, t-test) than the formulation without permeation enhancers. The data from Table 5.21 manifests that the steady state flux values for formulations containing permeation enhancer is in the following decreasing order: jojoba oil > sunflower oil > peppermint oil > eucalyptus oil > sesame oil > clove oil.

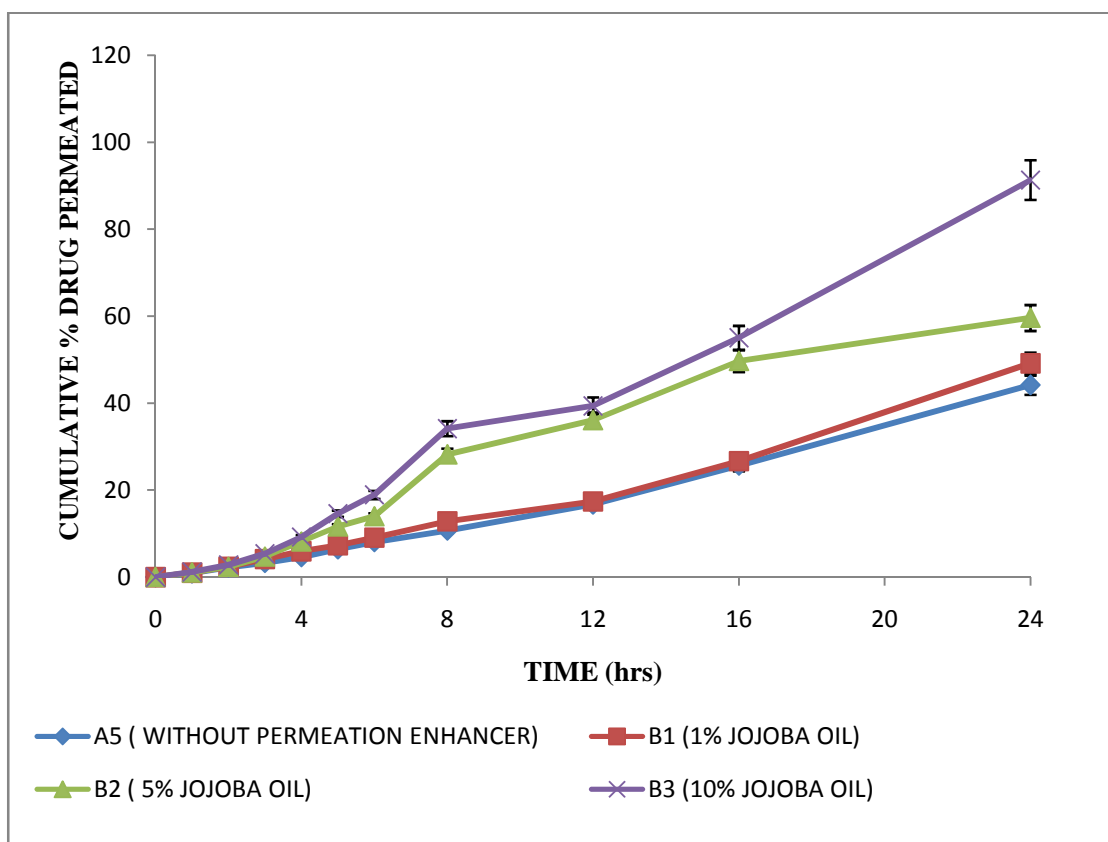


Figure 10 : *In vitro* permeation profile of losartan potassium TDDS without permeation enhancer along with jojoba oil as permeation enhancer

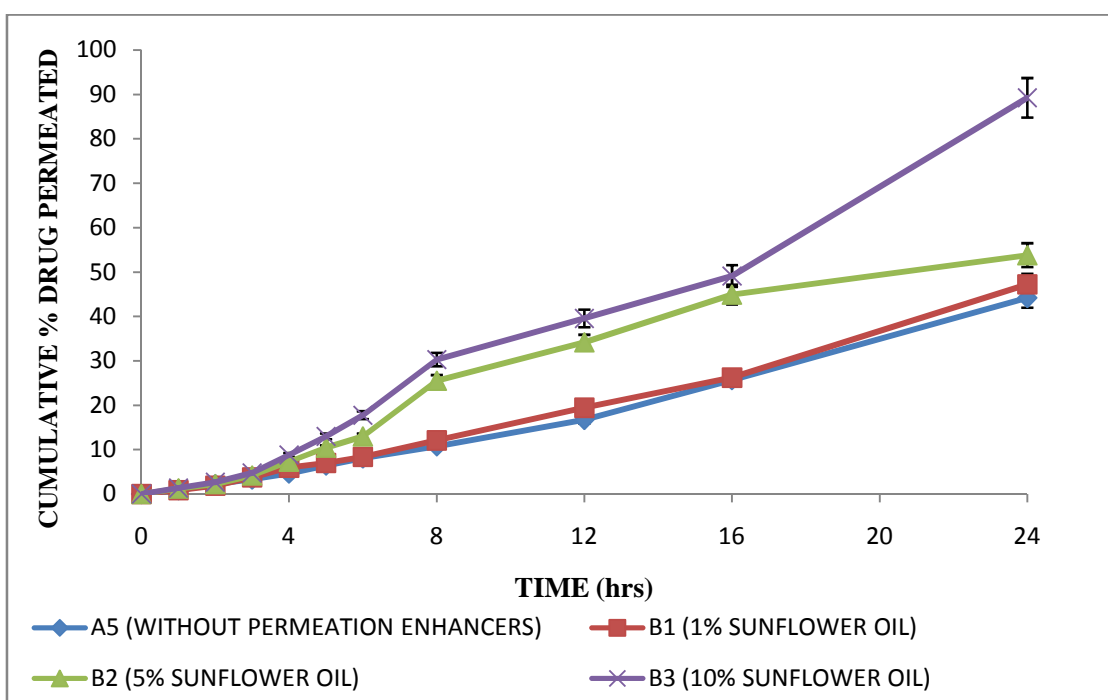


Figure 11: *In vitro* permeation profile of losartan potassium TDDS with sunflower oil as permeation enhancer

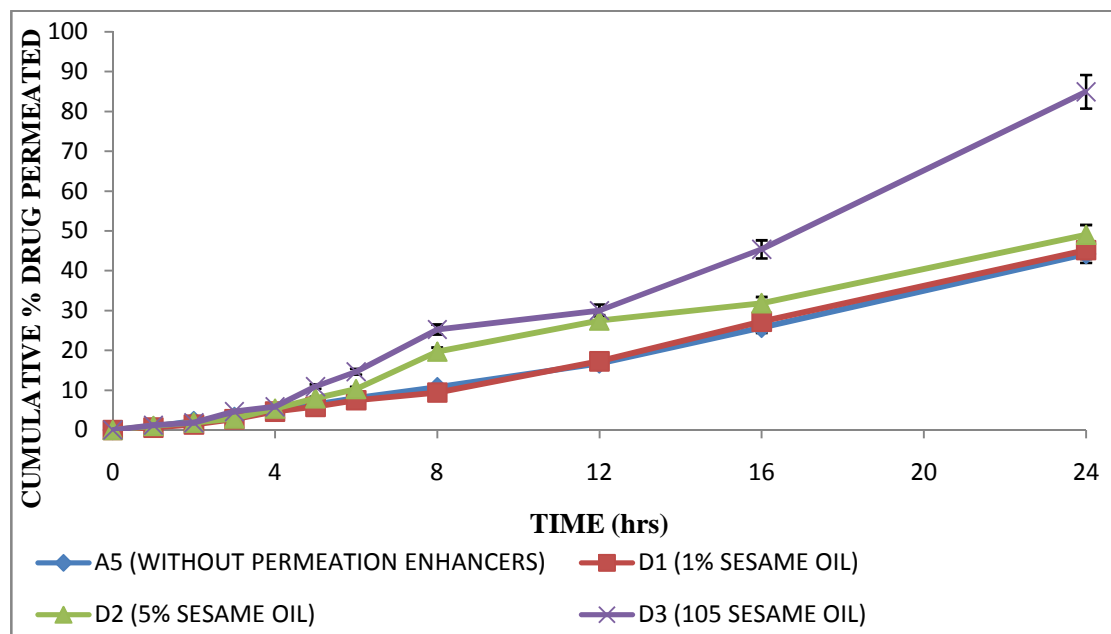


Figure 12: *In vitro* permeation profile of losartan potassium TDDS with sesame oil as permeation enhancer

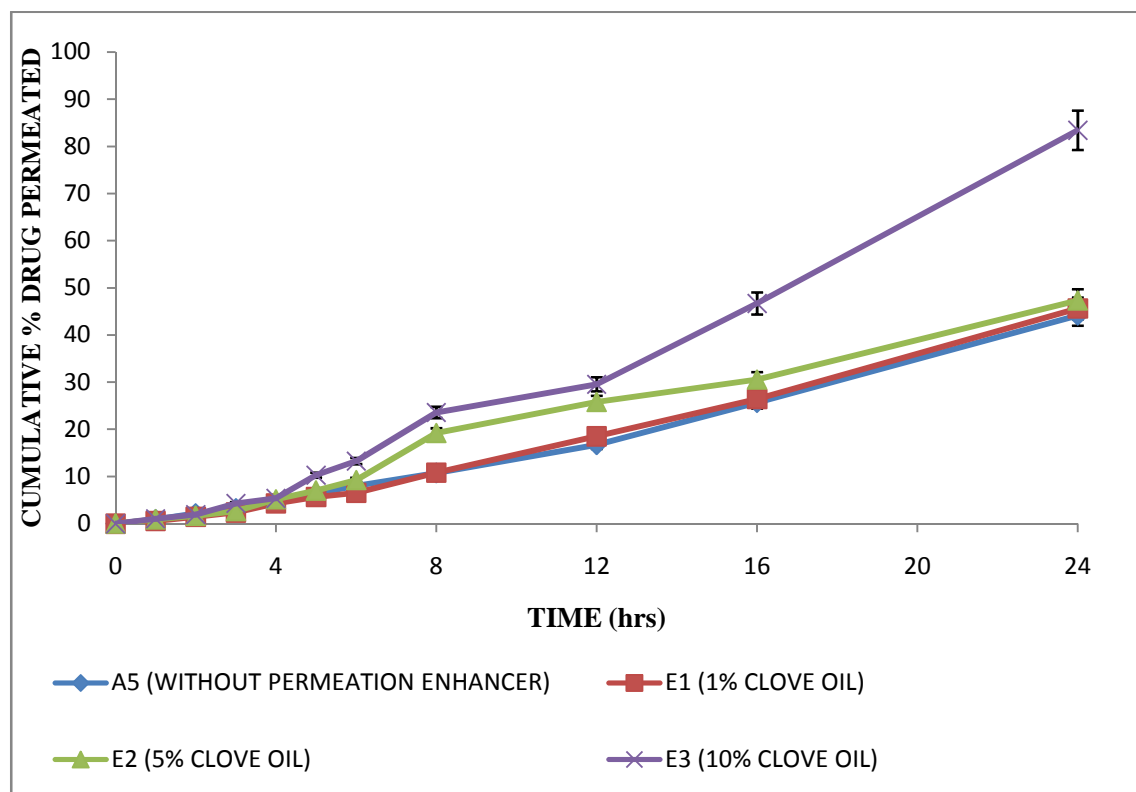


Figure 13: *In vitro* permeation profile of losartan potassium TDDS with clove oil as permeation enhancer

While addition of permeation enhancers decreased lag time with minimum lag time of the formulations containing, jojoba oil, sunflower oil and peppermint oil, which can be attributed to decrease in diffusional path length of molecule due to changes in stratum corneum by permeation enhancers as already observed in literature.^[11] Thus transdermal patches prepared with 10% jojoba and sunflower oil as permeation enhancer showed maximum cumulative amount of drug permeated through excised goat skin and maximum cumulative amount of drug release in dissolution media. All the oils in the concentration of 10% enhanced permeation of drug more than three times as compared to controlled formulation.(A5)

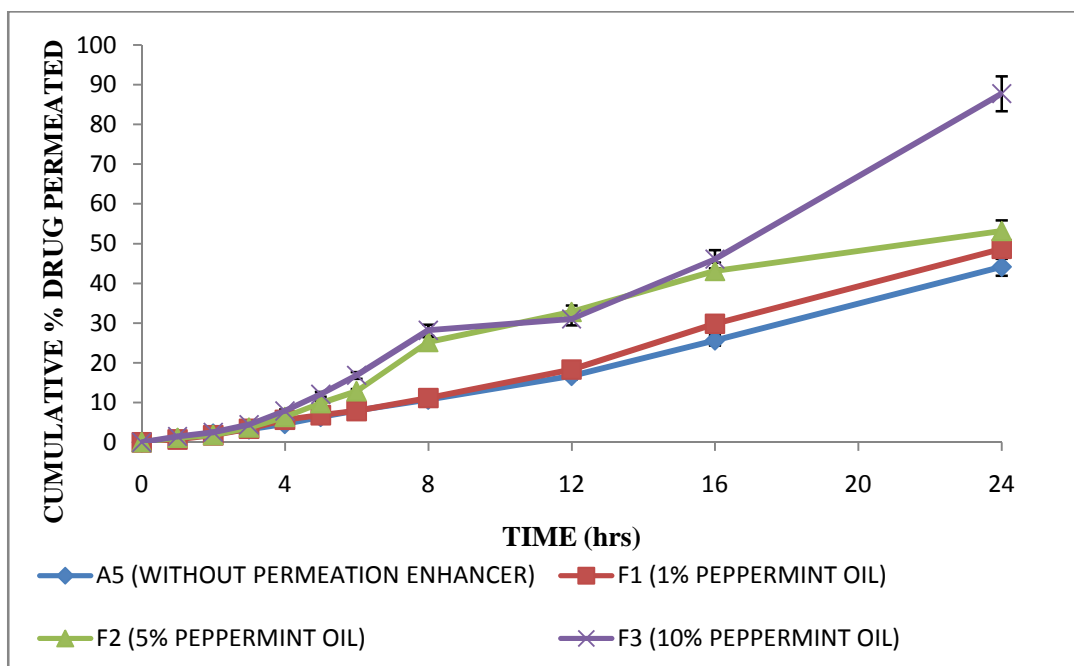


Figure 14: *In vitro* permeation profile of losartan potassium TDDS with peppermint oil as permeation enhancer

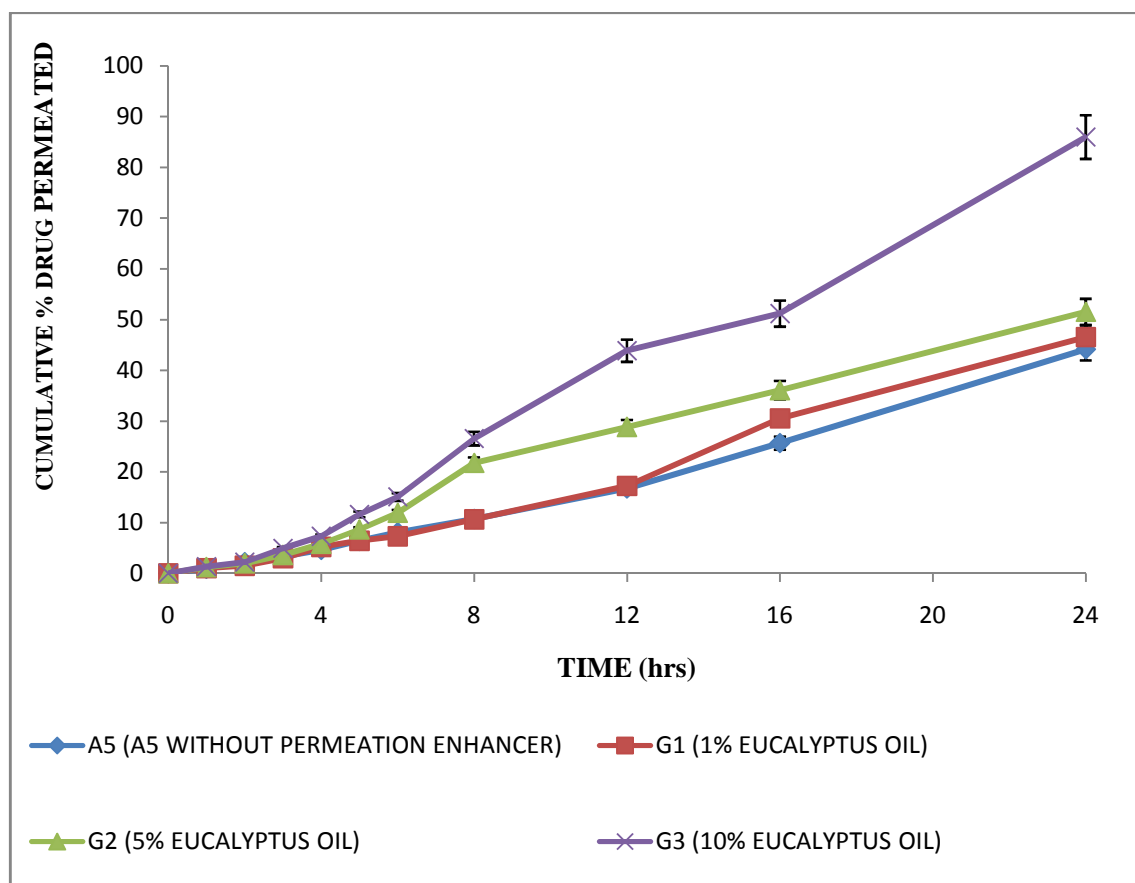


Figure 15: *In vitro* permeation profile of losartan potassium TDDS with eucalyptus oil as permeation enhancer

Comparison of vegetable oils and volatile oils for their permeation ability

When vegetable oils and volatile oils were compared for their ability to enhance the permeation, vegetable oils (jojoba oil, sunflower oil) and volatile oil (peppermint oil) showed the significantly higher flux ($p < 0.05, p < 0.001, t$ -test) with decreased lag time and increased E_{pen} as compared to formulation without permeation enhancers and other permeation enhancers. Among these oils jojoba oil; a vegetable oil was found to

have maximum effect on the permeability enhancement of losartan potassium. But when jojoba oil was compared with peppermint oil (volatile oil); the CADP and flux was insignificantly ($p > 0.05$, t-test) higher for jojoba oil. So it can be concluded that among vegetable oils, jojoba oil and among volatile oils, peppermint oil can enhance the skin permeation of drug when it is given by transdermal

Table no 3: Comparison of vegetable oils and volatile oils for their in vitro release and permeation studies

Formulation code	Cumulative % drug release	Cumulative %drug permeated	Flux (mg/cm ² /h)	Lag time (h)	E _{pen}
A5	53.63 ± 1.06	44.17 ± 0.03	0.082 ± 0.04	1.42 ± 0.03	1
B3	91.45 ± 1.07	91.34 ± 0.87	0.29 ± 0.01	0.27* ± 0.05	3.53
C3	98.08 ± 0.75	89.25 ± 0.36	0.28 ± 0.03	0.29* ± 0.02	3.39
D3	96.32 ± 0.09	84.92 ± 0.35	0.26 ± 0.07	1.12 ± 0.15	3.17
E3	96.09 ± 0.65	83.43 ± 0.85	0.25 ± 0.02	0.32* ± 0.1	2.99
F3	93.49 ± 0.35	87.76 ± 0.58	0.27 ± 0.03	0.31* ± 0.07	3.35
G3	93.08 ± 0.04	85.97 ± 0.44	0.26 ± 0.02	0.37* ± 0.10	3.17

* $p < 0.05$, Student unpaired t-test was applied to compare flux and lag time of B3, C3, D3 E3, F3 and G3 with control group (A5)

Stability studies

Stability studies of selected formulations (B3 and F3) were carried out according to ICH guidelines to establish the structural integrity of matrix transdermal patch. The results revealed no changes in the physical appearance of the formulations after 3 months study. The drug content was found to be 97.10% and 98.26% after 3 months respectively. So there was no significant ($P > 0.05$; t-test) change in drug content after storage of the formulations. In vitro permeation studies confirmed that all the batches were same after three month storage in different conditions of temperature and humidity.

CONCLUSION

Losartan potassium is an antihypertensive which is highly effective and safer in the treatment of hypertension. Low maintenance dose therapy of this antihypertensive drug has the capability to minimize the side effects, overall costs and compatibility issues. Thus optimized transdermal formulations of losartan potassium using polymers such as PVP and EC with jojoba oil among vegetable oils and peppermint oil among volatile oils as permeation enhancers demonstrated their ability to give sustained release, because of excellent release and permeation of drug. The developed formulation of this antihypertensive are expected to improve the patient compliance, form better dosage regimen and provide maintenance therapy to hypertensive patients. These promising results showed the feasibility of delivering antihypertensive (losartan potassium) through transdermal drug delivery system. The developed TDDS of this antihypertensive may prove to be a better alternative to oral dosage form in hypertensive patients.

REFERENCES

- [1] Aviskar DT; Parik VB; Gupta HN; Maniyar AH; Jain DK. *PDA J Pharma Sci Technol*, **2012**,66, 2, 126-35.
- [2] Shams MS; Alam MI; Ali A; Sultana Y; Agil M. *Drug Dev Ind Pharm*, **2010**,36(4), 385-92
- [3] Scheindlin. *Molecular Interventions*, **2004**, 4(6), 308-12.
- [4] Aggarwal G; Garg A; Dhawan S. *Ind J Pharm Educ Res*, **2009**, 43(3), 251-9.
- [5] Charoo N A; Anwar A; Kohli K; Pillai K K; Rahman Z. *Pharm Dev Technol*, **2005**, 10(3), 343 – 51.
- [6] Aggarwal G; Dhawan S; Harikumar SL. *Curr Drug Deliv*, **2012**, 9, 1-10.
- [7] Higo N. *Yakugaku Zasshi*, **2007**, 127(4), 655-62.
- [8] Aggarwal G; Dhawan S; Harikumar SL. *Drug Dev Ind Pharm*, **2013**, 39(1),1–10.
- [9] De P; Damodharan N; Mallick S; Mukherjee B. *PDA J Pharm Sci Technol*, **2009**, 63, 537-46.
- [10] Chandak AR; Prasad Verma PR. *Pharm Dev Technol*, **2010**, 15, 296-304.
- [11] Chandak AR; Verma PR. *Res Reg Affairs*, **2008**, 25, 13-30.
- [12] Mittal A; Parmar S; Singh B. *Curr Drug Deliv*, **2009**, 5, 511-9.
- [13] Setty CM; Jawarkar; Y Pathan. *Acta Pharmaceut Scientia*, **2010**, 52, 159-68.
- [14] Alam M I; Baboota S; Kohli K; Ali J; Ahuja A. *PDA J Pharm Sci Tech*, **2009**, 63(5), 429-37.
- [15] Jain R; Aqil M; Ahad A; Ali A ; Khar RK. *Drug Dev Ind Pharm*, **2008**, 34, 384-9.
- [16] Chandra A; Sharma PK. *Yakugaku Zasshi*, **2009**, 129(3), 373-9.
- [17] Ubaidulla U; Reddy MVS; Ruckmani K; Ahmad FJ; Khar RK. *AAPS Pharm SciTech*, **2007**, 8, E1-8.
- [18] Mittal A; Sara US; Ali A Mohammed. *Pharm Dev Technol*, **2009**,14(4), 422-34.
- [19] Gullick DR; Pugh WJ; Ingram MJ; Cox PA; Moss GP. *Drug Dev Ind Pharm*, **2009**, 36(8), 926-32.
- [20] Charoo NA; Shamsher AA; Kohli K; Pillai K; Rahman Z. *Colloids Surf B Biointerfaces*, **2008**, 65, 300-7.
- [21] Devareddy sandeep; D Krishnarajan; R Manivannan; N Senthis kumar. **2012**, 3(6), 159-164.

[22] Wang LH; Wang C C; Kuo SC. *J Cosmet Sci*, **2007**, 58, 245-54.

[23] Aggarwal G; Dhawan S; Harikumar SL. *Pharm Dev Technol*, **2011**, 1-10.

[24] Sanap GS; Dama GY; Hande AS; Karpe SP; Nalawade SV; Kakade RS; Jadhav UY. *Int J Green Pharmacy*, **2008**, 2,129-33.

[25] Sinha VR; Kaur MP. *Drug Dev Ind Pharm* **2000**, 26, 1131-40.