Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2013, 4(3):1-7

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Formulation and evaluation of chitosan based bioadhesive transdermal drug delivery systems of lisinopril for prolonged drug delivery

Manickam Balamurugan[1&2] and Shyam S Agrawal[2]

[1]Faculty of Pharmaceutical Sciences, UCSI University, Kuala Lumpur Campus, No: 1, Jalan Menara Gading, UCSI Heights, Kuala Lumpur, Malaysia
[2]Delhi Institute of Pharmaceutical Sciences & Research, Pushp Vihar, Sec-3, New Delhi, India

---

ABSTRACT

The purpose of this work was to develop once a week matrix-type transdermal drug delivery system containing lisinopril with different concentrations of chitosan by the solvent evaporation technique without any penetration enhancers. Various concentrations of chitosan films prepared with lisinopril were evaluated. The physicochemical compatibility of the drug and the polymers was studied by Infrared spectroscopy and Differential Scanning Calorimetry. The results suggested no physicochemical incompatibility between the drug and the polymers. In-vitro permeation studies were performed in cadaver skin and rat skin by using modified Franz diffusion cells. The results followed Higuchi kinetics (R^2 = 0.98), and the mechanism of release was diffusion mediated. Based on physicochemical and in-vitro skin permeation studies, patches coded as CL-2 were taken for further studies. Accelerated stability studies performed according to ICH guidelines confirmed that there was a very low degradation rate constant was observed. In-vivo skin irritation studies confirmed there were no signs of reactions were perceived on the skin of albino rats after 7 days. The results of this study indicate that the chitosan based matrix films of lisinopril hold potential for prolonged transdermal drug delivery.

Keywords: Chitosan, Cadaver skin, Lisinopril, Skin irritation studies, Stability studies.

INTRODUCTION

The purpose of this research work was aimed to illustrate the development, characterization, evaluation and possibilities of the once a week matrix type transdermal drug delivery system for lisinopril utilizing a natural polymer i.e. chitosan as film forming and self permeation enhancing polymer. Chitosan is a tough natural biopolymer for developing films due to their non-toxicity [1], biocompatibility [2], biodegradability [3,4], film-forming ability [5,6] inherent antimicrobial properties [7] along with permeation enhancing and good adhesive properties. Chitosan can be dissolved in very many dilute organic acids such as acetic, lactic, malic, citric, ascorbic, benzoic and succinic acids and hold general antimicrobial activity can be used for this purpose [8]. In this current study, chitosan was dissolved in the biologically safe lactic acid which is a common solvent for chitosan [9].

Lisinopril is absorbed slowly and incompletely following oral administration and its absorption varies between 6 and 60% depending on the individual, but on average is 25% of the dose [10]. To overcome the problems associated with lisinopril, to improve its bioavailability and for the effective treatment of chronic hypertension, it was decided to select lisinopril in an alternative long-acting formulation like matrix type transdermal drug delivery system. Besides, in this particular work an effort has been taken to formulate a simple formulation without adding much more additives like penetration enhancer, plasticizer etc. Transdermal drug delivery is especially advantageous for drugs that require prolonged administration at controlled plasma drug concentration. The drug delivery by

transdermal route could show sustained plasma profile over a long period of time, could minimize the risk of fluctuations of drug plasma levels. [11, 12] In addition to the aforesaid advantages, it is a non-invasive technique, simple to terminate the action just by removing the patch from the applied area and on the whole it improves the patient compliance. [13-15]

MATERIALS AND METHODS

Materials

Lisinopril was received as a gift sample from Lupin labs, Ltd., Pune.India. Chitosan was obtained from Central Cochin fisheries Ltd, Cochin, India. All other ingredients used in this study were of laboratory and analytical grades.

Preparation of cadaver skin

Human skin was excised from the chest region of a male cadaver 35years of age within 12hrs of the *post-mortem*, (Department of Forensic sciences, AIIMS, New Delhi, India) then the epidermis was isolated by heat separation method [16], which was achieved by immersing the full skin in 60ºC hot water for 2 minutes and the epidermis was precisely peeled-off. [17].

Preparation of rat skin

The full thickness of rat abdominal skin was excised from albino rats. The hair and the underlying fatty tissues were clipped off by using surgical scissors. The membrane was washed thoroughly with distilled water, and then soaked in the saline solution overnight. The rat skin was then cut into appropriate size taken for the permeation studies. It was washed several times before use.

Ethical clearance was obtained from the Institutional Animal Ethical Committee (IAEC) for using the experimental animals in this study. (Protocol no. IAEC/- 16)

Fabrication and investigation of physicochemical compatibility of drug and polymer

Bioadhesive films were prepared by conventional solvent casting technique. The chitosan was accurately weighed and transferred to a beaker; containing 4 % (v/v) lactic acid (as a solvent). The beaker was kept on a magnetic stirrer to dissolve the polymer. The mixture was stirred continuously until a clear solution was obtained. The accurately weighed quantity of lisinopril was added and, the stirring was continued. The resulting solution was then transferred and casted in a petridish and dried at room temperature by covering petridish with inverted funnel for 24 hours to control the rate of evaporation of the solvent system.

Fourier Transform Infrared Spectroscopic (FTIR) analysis

FTIR spectra were obtained for lisinopril, blank chitosan films, and drug-loaded chitosan films on Perkin Elmer (1800), Japan. Spectra were acquired for all the aforementioned films directly and as well as by the potassium bromide disc method for the pure drug.

Differential Scanning Calorimetric (DSC) analysis

Required quantity of the samples (pure drug, chitosan, and drug-loaded chitosan film) were weighed and placed in isolation on the aluminium pan of DSC, which was calibrated using indium. The instrument was sealed. On the other side, an empty aluminium pan was placed as a reference standard. The sample was heated between 40-400^{o}C at the rate of 10^{o}C/min. Nitrogen gas was introduced at a pressure of 2 bars and a flow rate of 20ml.

Physicochemical characterization of lisinopril containing transdermal films

Uniformity of weight and thickness

The prepared matrices were cut into 10 pieces of equal size each and their average weight was calculated. The thickness at various bits of each patch was gauged using invert microscope (Nikkon, Japan) and the mean thickness was calculated.

Physical appearance

The polymer matrices were evaluated for their physical appearance on the basis of the following parameters: opaque/transparent/smooth/wrinkle/tough/sticky/non-sticky/flexible

Drug content uniformity

The 1cm^2 mass of transdermal patches were placed into a beaker containing 10ml of saline solution for 24hrs at room temperature with occasional stirring. The samples were filtered and then analyzed for drug content estimation using UV spectrophotometer at 258nm. (n=3)

Scanning electron microscopy (SEM)
With the aim of comprehending the drug distribution pattern and to characterize the surface morphology of transdermal patches, the SEM analysis was carried out (JSM 6100 JEOL, Tokyo, Japan) at the particular magnification and in the specific conditions.

***In-Vitro* permeation studies**
Experimental conditions
The receptor medium was normal saline solution (0.9 % w/v NaCl). The temperature of the receptor medium was maintained at 32±1°C, in view of the fact that it is very essential in the topical transdermal drug delivery experiments to maintain the skin surface temperature of 32±1°C in order to mimic the *in-vivo* situation [18].

Ex-vivo permeation studies across cadaver Skin and rat skin
Ex-vivo permeation studies were carried out in Modified Franz diffusion cell. The receptor compartment (60ml) was filled with normal saline solution (0.9 % w/v NaCl). The transdermal patches were positioned with care on the outer side of the skin (both rat and cadaver skin separately), covered with aluminium foil as backing membrane was mounted between the compartments of the Franz diffusion cell. The entire construction was kept on a magnetic stirrer with hot plate and the agitation speed of 100 rpm and 32±1ºC temperature was maintained throughout the experiment. The amount of drug permeated into the receptor compartments were quantified by withdrawing 3ml of samples at fixed time intervals up to 168hrs, were analyzed using UV double beam spectrophotometer at 258nm (n=6). The volume of receptor solution (60ml) was sustained all the time by replenishing with fresh saline solution.

***In-vivo* studies**
Primary skin irritation studies
Albino rats of both sexes, weighing 150 to 200g each were used in this study (*n*=6 in each group). A control patch (without any drug, group I) and an experimental patch (group II) were fixed firmly (dorsal side) to the shaved skin of the rats by means of adhesive tape USP. The animals were assessed for a period of 7 days and scored for the skin reactions as reported by Draize *et al.* [19].

Accelerated stability studies
The optimized CL-2 patches were wrapped in the aluminium foil and kept in the desiccators at 40±2ºC and 75±5% RH as per ICH guidelines for 6months [20]. The samples were withdrawn every month up to 6 months and analyzed for drug content analysis, *in-vitro* permeation studies in cadaver skin, FTIR analysis, DSC analysis, and Scanning Electron Microscopic (SEM) evaluations.

RESULTS AND DISCUSSION

Investigation of physicochemical compatibility of drug and polymer
The FTIR spectral analysis of the pure lisinopril showed the characteristic peaks at wave numbers 1654, 1577, 1390, 741, 732 cm^{-1} and a broad peak around 2964 cm^{-1}. In the infrared spectrum of chitosan the major peaks were observed at 3640.33, 3610, 2923, 2857, 1359.02, 1665 and 1600.08 cm^{-1}; for the drug loaded patches the peaks for lisinopril were observed at 1660, 1600.81, 1390, 746, 730 and a broad peak around 2977 cm^{-1}. There are some additional peaks were seen in the spectrum is due to the incorporation of solvent and the polymers.

Thermograms of pure drug, blank chitosan film, and drug loaded chitosan films were obtained and subjected for the analysis. The DSC thermograms of lisinopril, showed a single sharp endothermic peak at its melting point. On a scan rate of 10°C/min, the experimental peak temperature was found to be 153.03°C and the apparent heat of fusion was 81.81J/g.The DSC thermogram of blank chitosan film showed the broad endothermic peak at 187.85°C, the apparent heat of fusion was 266.1J/g, whereas in the chitosan film containing lisinopril showed peak at its melting point and the broad endothermic peak at 187.07°C with the heat of fusion was raised to 296.9J/g. This indicated that the shift in the heat of fusion might be due to the presence of the drug in the formulation. Chitosan films containing lisinopril were obtained by solvent casting method, and the chemical integrity of the drug molecule was rigid during preparation. From the above DSC and FTIR analysis the compatibility of lisinopril and chitosan was confirmed.

Physicochemical characterization of patches
A common method was adopted for formulating the various formulations. Chitosan was used as film forming polymer and was dissolved in 4 %v/v lactic acid solution prepared in distilled water. The four different formulations containing lisinopril and chitosan in varying amount coded CL-1, CL-2, CL-3 and CL-4 was fabricated by solvent casting method.

The formulations were subjected to the percent drug content analysis per cm^2 of patch and it was found that CL-1, CL-2, CL-3 and CL-4 showed 3.25, 3.36, 3.14, &2.89 mg of drug content respectively (Table-1). Among these four formulations, CL- 2 showed the maximum percentage drug content per cm^2 of the patch.

Table 1: Details of physico-chemical evaluation of the chitosan films

Formulation code	Average weight (mg)	Thickness (mm)	Appearance	Average drug content ($1cm^2$)
CL-1	10.62±0.18	0.050 ± 0.002	+	3.25±0.09mg
CL-2	15.71±0.21	0.056 ± 0.003	***	3.36±0.10mg
CL-3	17.02±0.16	0.069 ±0.006	**	3.14±0.12mg
CL-4	17.98±0.12	0.099 ± 0.005	*	2.89±0.2mg

****Thin, transparent and flexible, ** Thin, opaque and flexible,*Thick, not flexible and opaque, +sticky, thin and flexible*

The formulations were also subjected for the thickness measurement by using inverted microscope. The formulations CL-1, CL-2, CL-3 and CL-4 showed 0.05, 0.056, 0.069, &0.099 mm thickness respectively (Table-1 & Fig-1). The weight/cm^2 of the patches was calculated and it showed 10.62, 15.71, 17.02, and 17.98 mg/cm^2 for CL-1, CL-2, CL-3 and CL-4 respectively (Table-1).

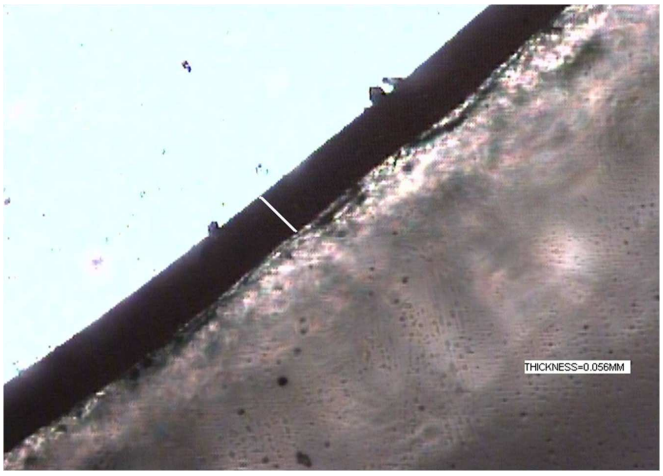

Fig. 1: Inverted microscopic photograph showing the thickness of drug loaded CL-2 film

The physical appearance of the various formulations in terms of their transparency, smoothness, flexibility, stickiness, homogeneity and opaque properties were recorded. The formulation CL-2 gave the best film with all desirable physico-chemical properties and in-vitro permeation studies results [Table-1] chosen for further evaluations.

The SEM photograph of the drug-loaded patch CL-2 confirmed the uniform drug distribution in the patch. Fig.2 shows the behavior of the polymer matrix after the release of drug molecules and it revealed that the formulation maintains its elasticity even after the release of the drug molecule without disturbing the other parts of the patch.

Permeation studies across cadaver skin

The ex-vivo permeation study was performed across cadaver skin using modified Franz-diffusion cell. It was found that the formulation coded as CL-2 showed the maximum percentage of 95.93% drug permeation at the end of 168 hours. Chitosan due to its inherent anti-microbial properties [7] protected the diffusion medium even after 168hrs of permeation studies with no microbial contamination.

From the *in-vitro* permeation profile (Fig.3 & Table-2) it is evident that kinetics of permeation from CL-2 coded transdermal film is zero order as the plots between cumulative % drug permeated versus time showed good linearity (R^2>0.9923). There is a linear relation between the amount of drug released from the patch CL-2 and the square root of time [21] according to the Higuchi equation and it was found to be 0.9905 , thus indicating that the drug release from transdermal films followed a diffusion-controlled mechanism. Flux was determined directly as the slope of the curve between the steady state values of the amount of % drug permeated versus time in hours [22] and it was found to be 6.53µg/cm^2/hr for the optimized formulation CL-2.

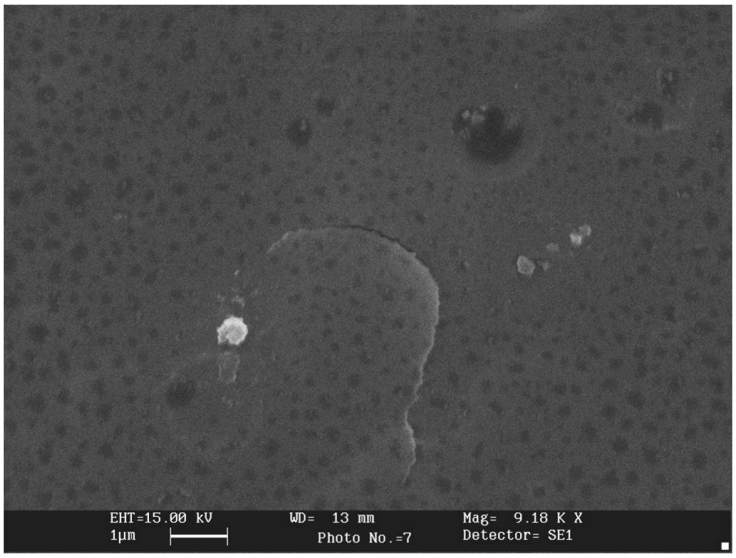

Fig. 2: SEM photograph of CL-2 patch after *in-vitro* skin permeation studies showing pores from where the drug molecules released

Table 2: Shows the regression coefficient values obtained from different kinetic plots of the formulations

Formulation code	Zero-order	First order	Higuchi's
CL-1	0.8549	0.8599	0.624
CL-2	0.9638	0.8302	0.9905
CL-3	0.974	0.9564	0.9392
CL-4	0.9601	0.9838	0.9323

Lisinopril is a water-soluble drug, adequately permeated through the cadaver skin and this fact was supported by various reasons, that the chitosan in this formulation may possibly mediates extended adhesion by means of electrostatic interaction on the cell surface of the skin. The drug diffuses passively down its concentration gradient onto the underlying skin over an extended period of time results privileged the permeation of drug [23, 24]. Chitosan itself as a self penetration enhancer may perhaps improve the permeability of the drug across the underlying tissue by its effects on epithelial tight junctions. Lactic acid (4%v/v) was used as solvent in this examination. This alpha hydroxy acid is used in various topical formulations, particularly in cosmetics, for its softening and conditioning effect on the skin. In addition to the above stated reasons, as humectants, the lactic acid might also increase the solubility of the drug, and enhanced its skin penetration.

Permeation studies across rat abdominal skin

The formulation CL-2 showed 102.85% of the percent cumulative release at 144 hour in a study carried out with rat skin for 168 hrs. The comparison of permeation of CL-2 patch across rat and the cadaver skin is shown in Fig-3, indicates the permeation of drug through rat skin was more than the cadaver skin. The permeation of drug through rat skin was high in 144hours and the release time was extended in the cadaver skin of 95.93% up to 168hours.

Accelerated stability studies

Samples from the patch coded as CL-2 were withdrawn and analyzed at 0, 1, 2, 3 and 6months for drug content spectrophotometrically, confirmed that there was no change in the drug content. The samples were also subjected for *in-vitro* permeation studies in cadaver skin and it was concluded that there were no changes seen in the rate of permeation. The SEM photograph was taken on the samples and it shows there was a little erosion on the surface was observed (Fig.4). The FTIR scan and DSC thermograms confirmed that there was no change in the formulation. Stability studies confirmed that there was a very low degradation rate constant was observed on performing the stability studies according to ICH guidelines.

***In-vivo* studies**

Skin irritation studies

Transdermal patches coded as CL-2 was subjected to skin irritation studies. No signs of erythema, oedema or ulceration were observed on the skin of albino rats after 7 days. It is very evident from the previous studies that the chitosan films prepared with lactic acid was non-allergenic and non-toxic [25]. Hence chitosan films prepared with lactic acid is more suitable for transdermal drug delivery.

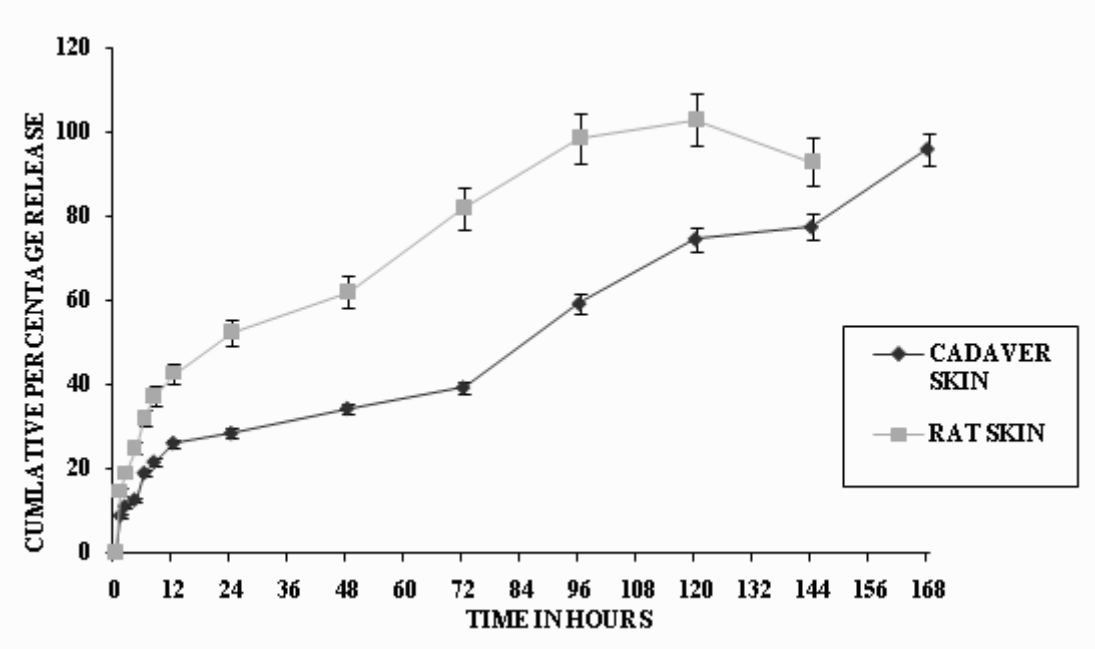

Fig. 3: Comparative in-*vitro* permeation studies of CL-2 films in rat and cadaver skin

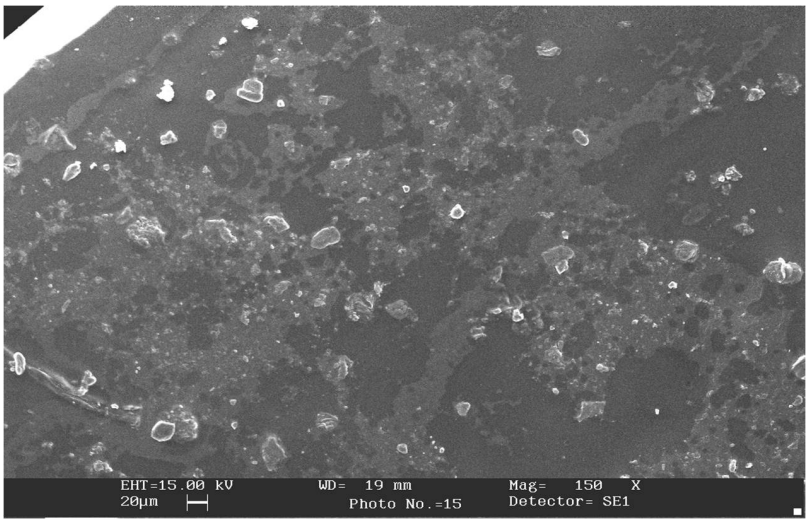

Fig. 4: SEM photograph of CL-2 films after 6 months stability studies

CONCLUSION

In the present studies, an attempt has been made to develop simple non-complex matrix-type transdermal delivery system for lisinopril without using any penetration enhancer. From the experimental results it was found that lisinopril can be delivered transdermally for an extended period of time. In the formulation of the polymeric-matrix type films single polymer, chitosan was used. The solvent used to dissolve chitosan was lactic acid which is an alpha-hydroxy acid believed to act as humectants when applied to the skin and this property may influence hydration of the *stratum corneum*. No permeation enhancers and plasticizers were added. Chitosan due to its unique properties served all the purposes. It can be stated that this formulation may be unique of its kind. The different films formulated, were evaluated and formulation CL-2, showed better *in-vitro* permeation profile and thus holds potential for transdermal delivery. A slow and controlled release of the drug is indicated by the plot of drug release versus square root of time is found to be linear. The films fabricated using chitosan gave Higuchi's release profile and maintained their release profile even after subjected for stability studies as per ICH guidelines for a period of 6 months. The FTIR, DSC, SEM analysis and *in-vitro* permeation studies in cadaver skin supported the data. The formulation CL-2 when subjected for skin irritation studies did not show any sign of skin irritation; hence the films could be used safely for application on the skin. It can be concluded that the CL-2 patch was found to the best

formulation for the delivery of lisinopril via transdermal drug delivery system as evidenced by *in-vitro* permeation studies and stability studies.

REFERENCES

[1] Hirano S, Itakura C, Seino H, Akiyama Y, Nonaka I, Kanbara N, Kanakami T, Arai K, Kinumaki T, *J Agric Food Chem*,**1990**, 38,1214-7.
[2] Muzzarelli RAA, *Biomaterials*, **1993**, 20, 7-16.
[3] Shigemasa Y, Saito K, Sashiwa H, Saimoto H, *Int J Biol Macromol*,**1994**,16,43-9.
[4] Abdul Althaf. Shaik, Umal Khair. Shaik, Praneetha P, *Der Pharmacia Sinica*, **2011**, 2,2, 375-384.
[5] B.L. Averbach, Film-forming capability of chitosan. In: R.A.A. Muzzarelli, E.R. Pariser, Proceedings of the First International conference on Chitin/Chitosan. MIT: Cambridge, MA, **1978**, 199-209.
[6] Shweta Kalyan, Pramod Kumar Sharma, Vipin Kumar Garg, Nitin Kumar, Jonish Varshney, *Der Pharmacia Sinica*, 2010, 1 (3): 195-210.
[7] Sudarshan NR, Hoover DG, Knorr D, *Food Biotechnol*, 1992, 6, 257-72.
[8] L.R. Beuchat, Food safety issues. Geneva, Switzerland: Food Safety Unit/World Health Organization, **1998**, 42.
[9] Knapczyk J, *Int J Pharm*, **1993**, 93, 233 - 237.
[10] A.C.Moffat, M.D.Osselton, B.Widdop. Clarke's Analysis of Drugs and Poisons, Pharmaceutical Press, London, **2004**. [Electronic version]
[11] Shashikant D Barhate, Mrugendra B Potdar, *Der Pharmacia Sinica*, **2011**, 2, (2), 185-189.
[12] Pintu K De1, Jibitesh Paul, Sanjoy K Dey, Subas C Dinda, Soumen Rakshit, *Der Pharmacia Sinica*, **2011**, 2 (5),98-109.
[13] B.W.Barry. Dermatological Formulations, Percutaneous Absorption, Marcel Dekker, New York, **1983**.
[14] R.H.Guy, J.Hadgraft, R.S.Hinz, K.V.Roskos, D.A.W.Bucks, In vivo evaluations of transdermal drug delivery. In: Y.W. Chien, (Ed.), Transdermal Controlled Systemic Medications. (Marcel Dekker, New York, **1987)**, 179-224.
[15] Shalu Rani, Kamal Saroha, Navneet Syan, Pooja Mathur, *Der Pharmacia Sinica*, **2011**, 2 (5),17-29.
[16] Berner B, Gerard GM, John HO, Robert JS, Juang RH, Charles DR, *J Pharm Sci*,**1989,**78,401–405.
[17] Murthy SN, Hiremath SR, Pranjothy KLK, *Int J Pharm*, **2004**, 272, 11–18.
[18] Adrian Williams. Transdermal and Topical Drug Delivery: From Theory to Clinical Practice. Pharmaceutical Press, London, **2003**.
[19] Draize JH, Woodard G, Calvery HO, *J Pharmacol Exp Ther*, **1944**,82,377–390.
[20] Singh S, *Pharm Tech*, **1999**, 23, 68–88.
[21] Higuchi T, *J Soc Cosmet Chem*, **1960**, 11, 85–93.
[22] Bonina FP, Carellii V, Cols GD, Montengro L, Nannipieri E, *Int J Pharm*, **1993,**100,41-47.
[23] Park Y, Lee Y, Lee J, Seol C, Lee S, *J Control Rel*, **2000**,67,385-394.
[24] Ramanathan S, Block L, *J Control Rel*, **2001**, 70, 109-123.
[25] Khan TA, Peh KK, Ch'ng HS, *J Pharm Pharmaceut Sci*, **2000**, 3, 303-311.