A therapeutic TDS patch of Metformin from a HPMC-PVA blend studied with a biological membrane of fish-swim bladder: An approach for dermal application in NIDDM

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Abstract: In order to introduce an easily applicable, removable, painless and long-term drug delivery system for non-insulin dependent diabetes mellitus (NIDDM), hydroxyl propyl methyl cellulose with polyvinyl alcohol (HPMC-PVA) blend patches of metormin HCl were evaluated *in vitro* and *in vivo*. A suitable patch of metformin 800mg with HPMC-PVA blend were used, following a three cycle freeze-thaw technique. Drug release kinetic profiles were performed in both patch and swim bladder. Albino mice were artificially generated as NIDDM mice by alloxan insertion i.p and after then treated with the therapeutic patch. Blood glucose was estimated by commercially available glucose kit based on glucose oxidase method. Drug release parameters from the patch and swim bladder were typical non-Fickian diffusion and both have the same kinetic constant, revealing its possible diffusion through stratum corneum. Hypoglycemia was observed in treatment of normal mice with TDDS of metformin HCl within 4 hours i.e. 25±2.13mg/dl and within 16 hours in diabetic rats blood glucose level returned to normal level i.e. from 360±3.3mg/dl (NIDDM level) to 105±2.5 mg/dl (Normal level). The TDS-patch has got the same kinetic simulation with that of swim-bladder, which might be a prediction for *in vivo* application. Here metformin was delivered to diabetic mice and has got significant anti-diabetic effect can be considered as a kind of patch for NIDDM just like wearing and taking off a hand watch because hypoglycaemia can be removed by just taking off the patch.

Keywords: Transdermal delivery system (TDDS) Patch, tight junction, swim bladder, NIDDM, metformin.

INTRODUCTION

Transdermal drug delivery system designed for avoiding the risk and inconvenience of intravenous therapy, bypassing the liver in terms of first pass elimination, usually providing less chance of an overdose or under dose, allowing easy termination and permitting both local and systemic effects. This approach of drug delivery is more pertinent in case of chronic disorders, such as diabetes mellitus, which require long-term dosing to maintain therapeutic drug concentration.

Diabetes is one kind of silent disaster of the recent decades and mostly the type II diabetes (non-dependent insulin diabetes mellitus, NIDDM), the presence of endogenous insulin does not retaliate any regulation of blood sugar (Cline *et al*., 1991).

Metformin HCl is an orally taken anti diabetic medication of usually high amount of drug (500-900mg per tablet) used to treat type-2 (non-insulin-dependent) diabetes, has been found to be a good candidate for transdermal drug delivery. Moreover the oral bioavailability of metformin is around 50-60% and it has got a short biological half-life of 1.5-3 hours (Clifford and Robert, 1996; Karam, 1998), which in turn dictates to the frequent dosing necessary to maintain the drug within the therapeutic blood levels.

Transdermal drug delivery systems (TDDS) present a better alternative to the limitations of oral therapy (Rania *et al*., 2010; Hadgraft and Guy, 1989; Guy, 1996). Hydroxy propyl methyl cellulose (HPMC) and polyvinyl alcohol (PVA) are the polymers of choice to be formulated in the TDS-patch (Prajapati *et al*., 2011; Jalan *et al*., 2011).

The skin has been reported as a site of drug delivery almost free from *pH* effects, gastric irritation, emptying rate effects, and the most important it avoids hepatic first pass metabolism, which caused relatively reduced risk of systemic side effects. The transdermal delivery can also eliminate pulsed entry into the systemic circulation, which might often cause undesirable side effects (Garala *et al*., 2009). Stratum corneum of the skin is the major hurdle for a TDDS to diffuse through the skin (Myer and Maibach, 2013) and usually mice skins are used in Franze-type diffusion cell, which is little bit troublesome to orchestrate. So it would be desirable if there is any other alternate simulation to the stratum corneum, a semi permeable membrane of upper epidermis. And here we chose a natural tight junction enriched membrane, swim bladder of fish to simulate with that of stratum corneum. A recent study showed that swim bladder resembled to the acid collagen components with that of a skin (Sinthusamaran *et al*., 2013).

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The objective of the present work was to evaluate the release behavior of metformin from TDS patch *in vitro* and thereby *in vivo* by monitoring the blood glucose level of alloxan induced diabetic mice, wore by TDS-patch containing metformin. The originality of this work is focused on the suitability of this device for TDDS of metformin so that the people in the near future can use the patch loaded metformin like "drug reservoir of hand-watch type" to control NIDDM.

METHODS

Formulation of HPMC-PVA blend TDS- patch and drug loading

TDS-patches were formulated with the help of two polymers blend (HPMC and PVA). For the preparation of HPMC-PVA based TDS-patch, 7.44% of metformin (800mg), 4.186% of PVA, 13.95% of HPMC and 74.42% of water were used (table 1). Firstly eight hundred milligrams of Metformin HCl, polymers were accurately weighed and PVA after keeping in a beaker, which contains 8 ml distilled water was heated in a hot plate. Drug was added in the melted PVA and mingled properly with a glass rod. In the meanwhile, one thousand five hundred grade of HPMC was added in the respective formulations to formulate transdermal drug delivery system patches and placed separately in film boxes. The composition of TDS-patch is given in table 1.

Freezing and thawing process

The patches obtained in this way were cooled and introduced separately in respective Film box. Then the film boxes with HPMC-PVA based TDS-patches were subjected to three successive freezing (at-20^{o}C) for 16h followed by thawing for 8h (at room temperature). In this way three successive cycles were performed to get the perfect cross-linked hydrogel patch with good mechanical resistance, white and opaque, which proves heterogeneous structure (Peppas and Scott, 1992).

Experimental animals

A total number of 20 albino mice (local origin) were brought to carry out the experiment. The mice were about four weeks old of, either sex weighing 15-20gm. The mice were divided into 4 groups and provided with standard diet and water *ad libitum*. All mice were kept in cages with wide mesh to avoid coprophagy and maintained in a well-ventilated room under conditions of natural light and dark schedule. Male albino mice were purchased from the local company and the animal related experiment was approved by the Rajshahi University Animal Care Committee.

In vitro experimental design

Preparation of dissolution media

For the preparation of the phosphate buffer of *pH* 5.4 dissolution medium salt of potassium dihydrgen phosphate and disodium hydrogen phosphate were used. For the preparation of 1 liter 5.4 *pH* phosphate buffer, 1.76gm of disodium hydrogen phosphate and 13.61gm of potassium dihydrogen phosphate were measured in a balance and taken in a 1000ml volumetric flask. The volume was adjusted by distilled water made it 1 liter, 5.4 *pH* buffer was checked by *pH* meter.

Working curve for metformin HCl

To prepare a working curve for metformin HCl in phosphate buffer of *pH* 5.4, dilute solutions were made and UV light absorption was checked at $_{max}$ of 233 nm. Then standard curve was prepared plotting absorbance data against drug concentration with a slope of Y=0.009578 X.

Dissolution studies

It was carried out in an "Electrolab tablet dissolution tester USP XXI TDT-06". The paddle rotation was set at 50rpm and temperature was controlled at 32°C±2°C, using 900ml dissolution medium (Gennaro, 2000). A five-milliliter sample was taken at regular interval, which was immediately compensated with the same amount of fresh medium previously heated at 32°C.

Drug loading

Before dissolution studies 800mg of metformin HCl was dissolved in 10ml phosphate buffer of pH 5.4. This sample was loaded in lower part of the swim bladders, which was freshly dissected from the alive-fish (*Katla Katla*) and assembled with the paddle of a dissolution tester.

Table 1: Formulation of HPMC-PVA based TDS-patch

Ingredients	Amount
Metformin HCl	800mg
HPMC	1500mg
PVA	450mg
Water	8ml

In-vivo experimental design

Induction of diabetes mellitus in normal mice by alloxan

Mice of either sex were made diabetic with an intraperitoneal (i.p.) injection of alloxan (120mg/kg body weight) dissolved in distilled water. Animal fasted for 16 h prior to drug administration allowing access only to water. Fasting blood glucose levels were measured for a period of 28h and when the condition of diabetes was established in animals with a blood glucose level of 200 mg/dl or more they were selected for the study.

Mice were divided into four groups (n=5 per group)- Group 1: Normal; Group II: Normal + TDS-patch of metformin HCl; Group III: Diabetic control; Group IV: Diabetic + TDS-patch of metformin HCl.

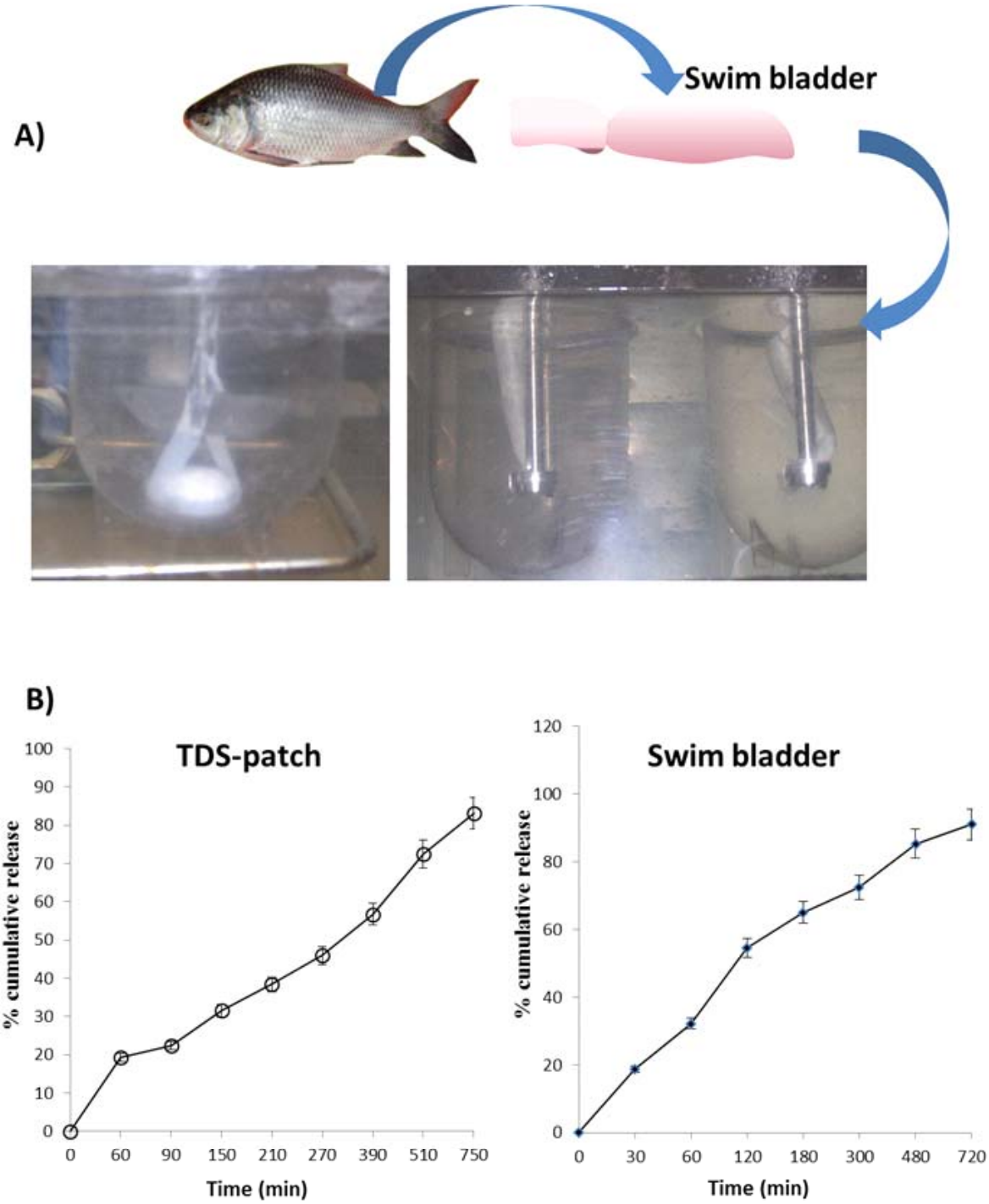

Fig. 1: *In vitro* metformin release from TDS-patch and fish swim bladder: A) Fish swim bladder and drug delivery model (left USP dissolution model for TDS-patch containing metformin and right swim bladder assembling loaded with metformin. Drug release values were calculated as mean ± SEM.

Table 2: Drug release kinetic parameters

Drug Reservoir	Drug Delivery Fashion	Kinetic Constant (k) $M_t/M_\infty \leq 0.7$ In $M_t/M_\infty = k.t^n$	Diffusion Exponent (n) $0.6 \leq M_t/M_\infty \leq 0.7$	Correlation Coefficient (r^2)
TDS-Patch	Non-Fickian Diffusion	0.015	0.6	0.99
Swim-Bladder	Non-Fickian Diffusion	0.015	0.7	0.99

TDS-patch application and blood glucose assessment of alloxan-induced diabetic mice

After alloxan treatment group: II and group: IV were subjected to TDS-patch. The patch was applied to the backside of the mice skin, which was previously shaven two days before of the application. Then blood sample was drawn time to time as before up to 16 hour and glucose level was measured at the same time of blood collection.

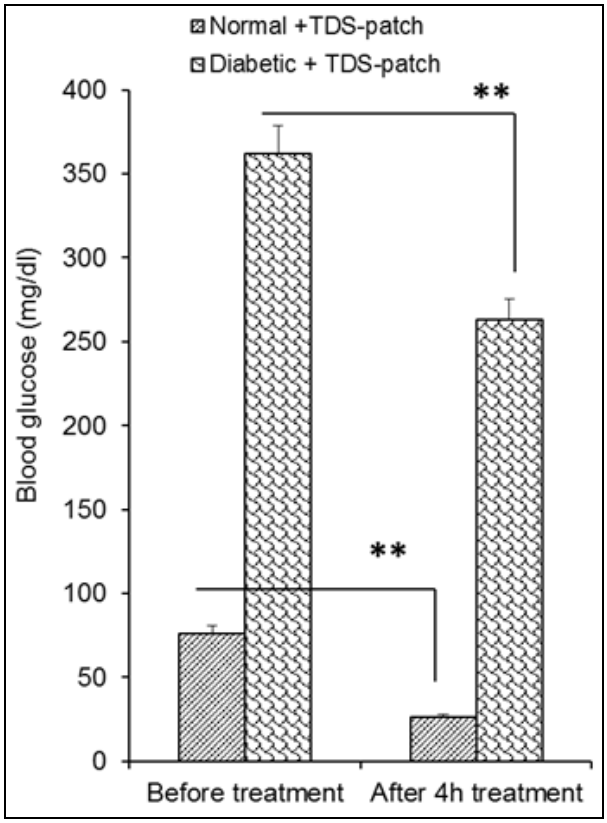

Fig. 2: Effect of transdermal drug delivery system of metformin HCl on blood glucose level in normal and alloxan induced NIDDM mice after 4h. ** P<0.01

Blood glucose estimation

Blood glucose was estimated by commercially available glucose kit based on glucose oxidize method by using One touch^{TM} Basic^{TM} plus Blood glucose meter (Life scan, Incorporation 2000, USA).

STATISTICAL ANALYSIS

All results were presented as mean ± SEM. *In vivo* data were analyzed for statistical significance by one-way ANOVA test followed by Mann-Whitney posttest.

RESULTS

Drug release kinetics from patch and swim bladder

In vitro drug release was performed for a total period of 750 min (fig. 1). Up to 70% release, kinetic parameters like diffusion exponent, kinetic constant were evaluated using the Korsmeyer and Peppas equation (Korsmeyer *et al*., 1983), $M_t/M_\infty = k.t^n$ (table2).

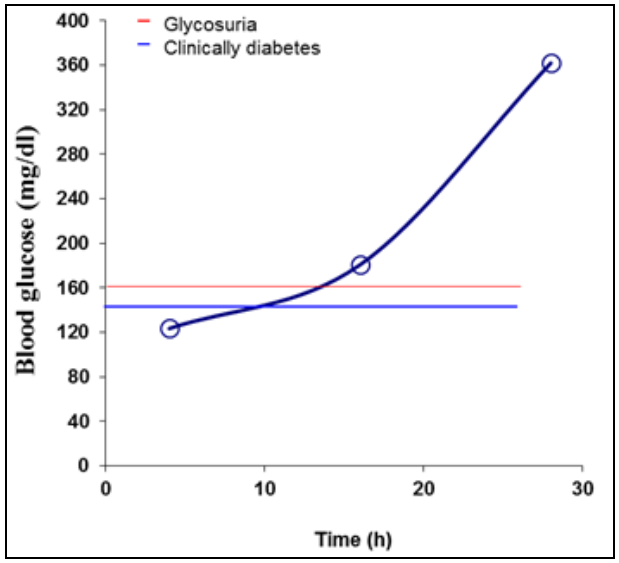

Fig. 3: NIDDM diabetic mice by alloxan induction

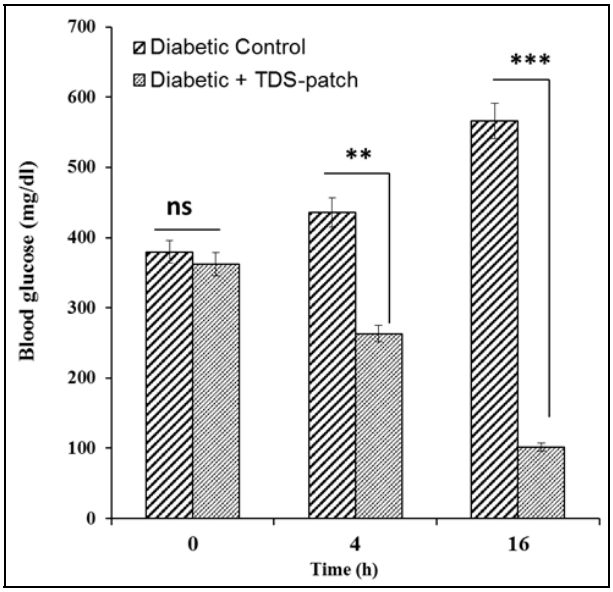

Fig. 4: Effect of transdermal drug delivery systems of metformin HCl on blood glucose level in alloxan induced diabetic mice at different time interval. **P<0.01, ***P<0.001.

Both the parameters showed almost the same values, considering the diffusion system simulated between the patch and swim bladder, which might be correlated with the stratum corneum of the skin because both the biological membranes have got the highly tight net-work junctions (Sinthusamran *et al*. 2013). Metformin release fashion was found out from the diffusion exponent (Peppas, 1985) value, which correlated with non-Fickian diffusion (Sinclair and Peppas, 1984). Drug release from swim bladder was compared with that of TDS-patch loaded with metformin.

Induction of NIDDM mice by alloxan and TDS-patch therapy

Diabetes mellitus was successfully induced in the normal mice (Group: III and IV) by alloxan administration (120 mg/kg i.p.). HPMC-PVA blend TDS-patch of metformin was previously prepared and the patch was placed on the back of the mice skin with a belt like a hand watch (Group: II and IV). On treating with metformin TDS-patch, hypoglycemia had been observed in normal mice within 4h (fig. 2). Subsequently, we induced diabetes artificially by alloxan in albino mice (local origin) (fig. 3). Clinical level of blood glucose was observed in alloxan induced diabetic mice after 16h of TDS-patch treatment (fig. 4).

In vivo patch application and NIDDM management in mice

In fig. 2, there was a significant lower blood glucose in both the normal and diabetic mice, after wearing the patch for 4h loaded with metformin. It was very significant with prolonging the time, which may deserve a promising control of NIDDM by lowering the blood glucose from the severe diabetic to normal blood sugar level (fig. 4). Here we evaluated the metformin release pattern kinetically with that of a tight junction network membrane correlated with stratum corneum of the skin (Sinthusamran *et al*., 2013), a swim bladder of *KatlaKatla* fish. And ultimately the metformin release through the skin of mice showed a promisingly normal sugar level of the esteem diabetic mice.

DISCUSSION

Ease of drug delivery to the systemic circulation to control the disease and also at the same time to minimize the untoward effects of the delivered drug are always the fundamental goal to a drug formulation scientist. Here we succeeded introducing metformin TDS Patch of HPMC-PVA blend, wearing like a watch-belt. In fig. 2, there was hypoglycimia (30mg/dl) after wearing the TDS-patch like a watch-belt for 4h, which meant that metformin HCl easily crosses across the skin to the systemic circulation and the metformin induced hypoglycaemia can also be managed just removing the watch-belt of drug loaded patch.

And in such way it can be correlated with a resemblance to the metformin diffusion from the swim bladder as a prediction of skin permeability by kinetic profiles in the table 2 (Ritger and Peppas, 1987; Shaheen and Yamaura, 2002), fig. 1 and blood glucose level after wearing the TDS-patch of metformin.

From the *in vivo* experiment (fig. 2 and fig. 4), it can be discussed that from the HPMC-PVA based TDS-patch, metformin HCl can easily pass through the skin into systemic circulation and can offer a state blood level profile, resulting in reduced systemic side effects and improved efficacy over other dosage forms. In addition metformin HCl in HPMC-PVA based TDS-patch can offer multi-day dosing and avoid first pass effect and thereby will give therapeutic effect for a long time. The great advantage of this system is that it can be taken off whenever hypoglycaemia symptom shows and comparatively the better TDDS medication for NIDDM.

CONCLUSION

We the first time so far here evaluated a TDS-patch of metformin that might be feasible to use as like a watch-belt to control NIDDM. Moreover diffusion of small molecular drug through a fish swim bladder for TDS-patch, might be an alternate approach of Franze-type diffusion cell.

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REFERENCES

Cline GW, Petersen KF and Krassak M (1991). Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. *New Engl. J. Med.,* **341**: 240.

Clifford JB and Robert CTM. (1996). Metformin, *New Engl. J. Med.*, **334**: 574-579.

Karam JH (1998). Parcreatic hormones and antidiabetic drug. *In*: Basic and clinical pharmacology. B.G. Katrurg. (ed.) Appleton lange and company. *America*, pp.684-703.

Rania El, Ali AK, Laila El, Xiaoying H and Maibacha HI (2010). Transungual drug delivery: Current status, *Int. J. Pharm.*, **384**: 1-8.

Hadgraft J and Guy RH. (Eds.) (1989). Transdermal drug delivery: Development issues and research initiatives, vol. 35, Marcel Dekker, New York, pp. 293-311.

Guy RH (1996). Current status and future prospects of transdermal, drug delivery, *Pharm. Res.,* **13**: 1765-1768.

Prajapati ST, Patel CG and Patel CN (2011). Formulation and evaluation of transdermal patch of repaglinide, *ISRN Pharm*., 651909.

Jahan L, Ferdaus R, Shaheen SM, Sultan MZ and Mazid MA (2011). *In vitro* transdermal delivery of metformin from a HPMC/ PVA based TDS-patch at different pH, *J. Sci. Res.*, **3**: 651.

Garala KC, Shinde AJ and Shah PH (2009). Formulation and *in vitro* characterization of monolithic matrix transdermal systems using HPMC/Eudragit S 100 polymer blends. *Int. J. Pharm. and Pharm. Sci.,* **1**(1): 108-120.

Myer K and Maibach IH (2013). Stratum corneum evaluation methods: Overview. *Skin Res. and Tech.*, **19**: 213-219.

Sinthusamran S, Benjakul S and Kishimura H (2013). Comparative study on molecular characteristics of acid soluble collagens from skin and swim bladder of seabass, *Food Chem.*, **138**(4): 2435-2441.

Peppas NA and Scott JE (1992). Controlled release from poly (vinyl alcohol) gels prepared by freezing-thawing processes. *J. Controlled Release*, **18**: 95-100.

Gennaro AR (2000). Remington: The science and practice of Pharmacy, 20th Edition, Philadephia College of Pharmacy and Science, pp.903-905.

Korsmeyer RW, Gurny R, Doelker E, Buri P and Peppas NA (1983). Mechanism of solute release from porous hydrophilic polymers, *Int. J. Pharm.* **15**: 25-35.

Peppas NA (1985). Analysis of fickian and non-Fickiandrugrelease from polymers, *Pharm. Acta. Helv.*, **60**(4): 110-111.

Sinclair GW and Peppas NA (1984). Analysis of non-Fickian trans-port in polymers using simplified exponential expressions, *J. Membrane Sci.*, **17**: 329-331.

Ritger PL and Peppas NA (1987). A simple equation for description of solute release I. Fickian and non-Fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs, *J. Controlled Release*, **5**: 23-36.

Shaheen SM and Yamaura K (2002). Preparation of theophylline hydrogels of atacticpoly (vinyl alcohol)/ NaCl/H O system for drug delivery system. *J. of Controlled Release*, **81**: 367-377.