Effect of olive oil on transdermal penetration of flurbiprofen from topical gel as enhancer

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Abstract: The present study was conducted to formulate and evaluate flurbiprofen transdermal gel. A standard calibration curve was constructed to obtain a regression line equation to be used for finding out the concentration of drug in samples. Olive oil was used as penetration enhancer and was added in different concentrations to some selected formulations to see its enhancement effect on *in vitro* drug release profiles. The prepared gels were evaluated for several physico-chemical parameters to justify their suitability for topical use. The *in vitro* drug release studies were carried out by using Franz cell diffusion apparatus across both synthetic membrane and excised albino rabbit skin. In order to investigate the drug release mechanism a kinetic approach was made by employing Korsmeyer kinetic model to the *in vitro* drug release profiles of flurbiprofen. The flurbiprofen topical gels were successfully prepared and could be beneficial for topical use.

Keywords: Flurbiprofen, olive oil, transdermal gel, *in vitro* release kinetics.

INTRODUCTION

The delivery of medicinal agents via the topical route can be dated thousands of years back. In ancient times the Greeks used to apply a mixture of water, olive oil and lead oxide to the skin as a balm. Lead oxide is used as astringent and olive oil is used in combination with drug because it could act as a barrier and it also moisturizes the skin. It was thought up until the end of 19th century that the skin was impermeable for many elements to be penetrated to the systemic circulation even to the penetration of gases. But later on in 20th century it was suggested that skin is permeable but the outer layers of skin being acts as barrier and later epidermis was much debated to be responsible for the permeability barrier (Morrow *et al.,* 2007). The transdermal route for drug delivery is often comprised by low bioavailability of the drug because of the skin barrier functions (Francesco *et al.,* 2005), but this route can provide constant drug release and avoiding the first past metabolism by GIT. In transdermal therapies the amount of drug needed for therapeutic effect is less bio available as compared to oral route of administration, but the absorbed drug appears to be adequate for therapeutic uses (Syed NHS *et al.,* 2006). Several diseases can be treated via topical route but are often limited by the poor transdermal penetration through the human skin. The fact that topical formulations like gels, ointments and creams can improve the delivery of NSAID's, could be of great importance in succeeding transdermal therapeutic approaches. The way to improve the penetration of certain drugs via transdermal route could be achieved by the use of penetration enhancers (Donatella *et al.,* 2002). The physico-chemical properties of the drug could presume the poor absorption of drug via transdermal route, but the penetration of drug could be enhanced by modifying the medicinal chemists and the nature of physiological barriers. Great investigative studies have been carried out so far by many scientists to overcome the physiological barriers and came to know that certain natural and synthetic compounds could enhance the penetration properties of drugs via transdermal route (Syed NHS *et al.,* 2006). An alternative method is the use of a system which has greater chemical potential then those of corresponding saturated solutions and which can promote the penetration of the drug from the vehicle to the skin. The efficacies of such studies were proven by transdermal patches. Drug loaded monolayer transdermal patches could be easily obtained by fast solvent evaporation (Francesco *et al.,* 2005).

MATERIALS AND METHODS

Flurbiprofen (Leeds Pharma, Islamabad, Pakistan), Carboxy poly methylene (Sigma Chemicals, USA), Triethanolamine, PVP K30 (Merck, Germany), Ethyl cellulose, Eudragit, Polyethylene glycol 400, Potassium dihydrogen phosphate, Sodium hydroxide (Merck, Germany), Ethanol, Olive oil (Sigma Aldrich, Germany), Magnetic stirrer, pH meter, Weighing balance, UV-Visible Spectrophotometer (Shimadzu, Japan), Particle size analyzer (Horiba LA 300), Franz diffusion cell apparatus (Perm Gear, USA).

Standard calibration curve of flurbiprofen

The standard calibration curve of flurbiprofen was constructed by taking 20 mg flurbiprofen in a 100 ml

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volumetric flask and 100 ml solvent (Hydro alcoholic solvent) was added to the flask. This stock solution was stirred with the help of magnetic stirrer for 1-2 hrs until the complete dissolution of the drug in solvent. From the stock solution different dilutions were prepared such as 0.025 mg/ml, 0.0125 mg/ml, 0.0062 mg/ml, 0.0031 mg/ml and 0.0015 mg/ml. The dilutions were analyzed for drug absorbance through UV Visible spectrophotometer (UVIDEC 1601 Shimadzu, Japan) at 247 nm.

Table 1: Concentration versus absorbance of flurbiprofen in phosphate buffer pH 7.4

S. No.	Concentration	Absorbance
1	0.000mg/ml	0.000
2	0.025 mg/ml	1.884
3	0.0125 mg/ml	0.913
4	0.0062 mg/ml	0.474
5	0.0031 mg/ml	0.241
6	0.0015 mg/ml	0.121

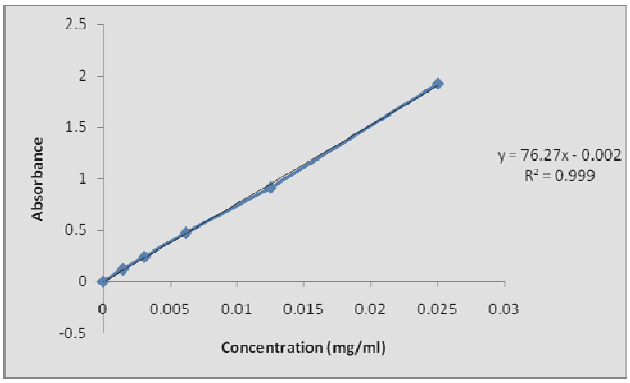

Fig 1: Standard calibration curve of flurbiprofen

Development of flurbiprofen gel

Flurbiprofen topical gel was prepared in laboratory to investigate the penetration of flurbiprofen into systemic circulation from gel and to investigate the effect of olive oil on penetration enhancement of flurbiprofen. The penetration of flurbiprofen from topical gel was investigated across both synthetic membrane as well as albino rabbit skin. Flurbiprofen topical gel was developed by dispensing 1 gm carboxy poly methylene in 50 ml distilled water and was stirred with the help of magnetic stirrer up till a homogeneous dispersion was obtained. In another step 1 gm flurbiprofen was dispensed in 10 ml ethanol and was stirred to get a homogenous dispersion. The drug solution was added to carboxy poly methylene solution drop-wise with the help of micro pipette and was stirred continuously. To some selected gel formulations enhancer was added in different concentrations so that 7 different formulations were obtained, one being blank (without enhancer) and the others having different concentrations of enhancer i.e. 0.5%, 1%, 1.5%, 2%, 2.5% and 3% (0.5% increase in each) respectively. To the final solution 1 ml of triethanolamine was added and mixed well. The final volume was made upto 100 ml by adding sufficient quantity of distilled water and again stirred until a homogenous transparent gel was obtained.

Physico-chemical evaluation of transdermal gel

Flurbiprofen topical gel was evaluated for various physico-chemical parameters to check its suitability for transdermal use. These parameters are as follows:

pH

The pH of different gel formulations was checked by using digital pH meter (Shivhere *et al.,* 2009).

Spreadability

The spreadability of flurbiprofen topical gels was checked by using wooden block and a fixed glass slide. A small pan was attached to another glass slide which was movable. To determine the spreadability, approximately 20 mg gel was placed on the fixed slide. The movable slide with an attached pan was placed over the fixed slide in such a manner that the gel was sandwiched between the two glass slides for 4-5 min. After the specified time interval a 50 gm weight was placed in the pan attached to movable slide. Now the time taken by movable slide to separate completely from fixed slide was noted and the spreadability was determined by the following formula:

$$S = ML/T$$

Where S is spreadability in g.cm/sec, M is the mass in grams, L is length and T is time in sec. (Shivhare *et al.,* 2009).

Consistency

Consistency of flurbiprofen gel was evaluated by dropping cone method. In this method a cone attached to

Table 2: Formulation of 1% (w/v) flurbiprofen transdermal gel

S. No.	Flurbiprofen	CPM	TEA	Ethanol	Olive oil (Enhancer)	Distilled water
1	1 gm	1 gm	1 ml	10 ml	-	QS to 100 ml
2	1 gm	1 gm	1 ml	10 ml	(1 ml)	QS to 100 ml
3	1 gm	1 gm	1 ml	10 ml	(2 ml)	QS to 100 ml
4	1 gm	1 gm	1 ml	10 ml	(3 ml)	QS to 100 ml
5	1 gm	1 gm	1 ml	10 ml	(4 ml)	QS to 100 ml
6	1 gm	1 gm	1 ml	10 ml	(5 ml)	QS to 100 ml
7	1 gm	1 gm	1 ml	10 ml	(6 ml)	QS to 100 ml

a holding rod was dropped from a distance of 10 cm in the center of a cup filled completely with gel. The distance inside the gel travelled by the cone was noted and after 10 sec. the distance was noted again and the consistency was determined (Shivhere *et al.,* 2009).

Homogeneity

The homogeneity of flurbiprofen gels were checked by visual inspection. In this regard the gels were filled into narrow transparent glass tubes and were checked in light for the presence of any particulate or lump (Shivhere *et al.,* 2009).

Drug uniformity

For the drug content of flurbiprofen in respected gels, approximately 100 mg gel was dispensed in 100 ml hydroalcoholic solvent and was stirred with the help of a magnetic stirrer for 2-3 hrs until the complete dissolution of the gel. The solution was then filtered through a membrane filter (0.45µm) and was analyzed by using UV Visible spectrophotometer at 247 nm for drug concentration (Shivhere *et al.,* 2009).

Skin irritation test

Skin irritation tests were performed for flurbiprofen topical gel on human volunteers to find out any irritation problems which could reject its suitability for topical use. Skin irritation tests for each gel were performed on three volunteers. Approximately 1 gm gel was topically applied to the hand near the wrist to a 2 square inch area and were observed for any lesions or irritation followed by redness (Shivhere *et al.,* 2009).

Extrudeability

For extrudeability tests of flurbiprofen gel, a specific amount of gel was filled in a collapsible tube with one end being closed. The tube was firmly pressed at the crimp end and the cap was removed, the gel inside the tube was extruded at a pressure until the internal pressure of the tube was dissipated (Gupta *et al.,* 2009).

Rabbit skin preparation for *ex-vivo* studies

Albino rabbit skin was used for *ex vivo* studies of flurbiprofen. The rabbit was given anesthesia with chloroform and hairs from its dorsal area were shaved carefully with the help of electric razor. After shaving the rabbit was scarified and the skin was excised carefully with the help of sharp surgical blade. The epidermis was separated by dipping the skin in hot water of approximately 60°C and then the epidermis was teased from the dermis and the two layers were thus separated and were covered with aluminum foil for protection.

***In vitro* study protocol**

Franz cell apparatus (Perm Gear, USA) was used for *in vitro* diffusion studies of flurbiprofen across synthetic membrane and rabbit skin. The synthetic membrane/ albino rabbit skin was fixed in between the donor and the receptor compartments of Franz cell apparatus. Phosphate buffer pH 7.4 was used as a receptor solvent and 5 ml of it was filled in the receptor compartment. The donor compartments were filled with flurbiprofen gels, one being blank (without enhancer) and the others with 0.5%, 1%, 1.5%, 2%, 2.5% and 3% concentration of penetration enhancer respectively and were labeled. The temperature of the solvent was maintained at a constant temperature of 37°C by circulating water. Samples of each 2 ml were withdrawn from receptor solvent at specific time intervals (0.5, 1, 1.5, 2, 3, 4, 8, 12, 16, 20 and 24 hrs) and were immediately replaced with fresh solvent having the same temperature. The collected samples were filtered and the drug concentration present in the solvent was analyzed by UV Visible spectrophotometer at 247 nm.

***In vitro* release kinetics**

To the *in vitro* drug penetration profiles of flurbiprofen gels and patches, Korsmeyer Pappas kinetic model was applied in order to find out the drug release mechanism. The *in vitro* drug release mechanism of flurbiprofen was determined by putting the *in vitro* penetration values in Korsmeyer Pappas equation:

$$M_t / M\infty = K\, t^n$$

Where $M_t / M\infty$ represent the fractional drug release from formulation into the receptor solvent, K is a drug delivery constant and (n) is diffusion coefficient and its value in equation indicates the release mechanism of the drug in solvent. The value of (n) if equal to 0.5 indicates Quasi-Fickian diffusion mechanism, if (n>0.5) then anomalous or non-Fickian diffusion mechanism exists and if its value is (=1) then Zero order release mechanism exists.

RESULTS

Physico-chemical evaluation of transdermal gel

The developed gels of flurbiprofen were evaluated for physico-chemical tests including pH, spreadability, consistency, homogeneity, skin irritation and lesions, drug content and extrude ability as shown in table 3. The pH of all the gel formulations ranges from 6.7 to 6.9 which were in normal pH range. The spreadability were calculated for flurbiprofen gels to find out whether the gels could be spread easily or not, hence, the values varies from 5.4 to 6.2 g.cm/s indicating that the gels were easily spreadable with a little shear stress. Good homogeneity was found in gel formulations with no visual particles and lumps. After the gel application to skin of human volunteers no signs and symptoms of lesions, redness and itching were found. The drug content of all the prepared gels ranges from 97.5% to 99.1 indicating good content uniformity. The values of extrudeability calculated from collapsible tube method ranges from 185 to 195 g. The values of all the physical parameters applied to flurbiprofen gels indicate that the gels were suitable for topical applications.

Table 3: Physico-chemical evaluation tests applied to flurbiprofen gel

S. No	Parameters	Flurbiprofen gel (Blank)	Flurbiprofen gel with Olive oil at different concentrations					
			0.5%	1%	1.5%	2%	2.5%	3%
1	pH	6.7	6.8	6.9	6.9	6.8	6.7	6.9
2	Spreadability (g.cm/sec)	6.2	5.8	6.0	5.7	5.9	5.4	5.4
3	Consistency (60 sec)	7mm	6mm	6mm	7mm	6mm	6mm	6mm
4	Homogeneity	Good	Good	Good	Good	Good	Good	Good
5	Skin irritation test	No	No	No	No	No	No	No
6	Drug content (%)	97.5	97.9	99.1	98.4	98.9	97.8	98.7
7	Extrudeability (grams)	191	195	194	188	185	190	192

In vitro drug penetration studies

The percent drug release of flurbiprofen from topical gel across synthetic membrane is shown in fig. 2, it could be observed that the percent drug release from gel formulation without penetration enhancer was very small and only upto 24% drug was penetrated across synthetic membrane into the receptor solvent in 24 hrs but an increased drug penetration was observed from formulations having olive oil as penetration enhancer. The percent penetration was reached to a maximum 48% by the addition of 0.5% enhancer to flurbiprofen gel formulation and the penetration of drug was increased by increasing the concentration of enhancer and a maximum 67% drug penetration was observed from the formulation having 3% olive oil as penetration enhancer implying the ability of olive oil to increase the drug diffusion by modifying the barrier properties of stratum corneum. Similar results were found in an investigative study (Naseem *et al.,* 2008) when the amount of flurbiprofen penetrated was increased when the concentration of penetration enhancer was increased which could suggest that by increasing the amount/concentration of penetration enhancer in a flurbiprofen topical formulation significantly increase the penetration. CPM was used in gel formulations as a rate controlling polymer which was observed to be effective by controlling the release rate of drug upto 24 hrs of time period.

In order to study the effect of olive oil as penetration enhancer on flurbiprofen permeation through albino rabbit skin, the flurbiprofen penetration from formulations with different concentrations of penetration enhancer (0.5%, 1%, 1.5%, 2%, 2.5% and 3%) was determined by excised albino rabbit skin. The flurbiprofen penetration across rabbit skin as a function of time upto 24 hrs is shown in fig. 3, it could be observed from the fig. that the penetration of flurbiprofen across rabbit skin without enhancer was small but was gradually increased by increasing the amount of enhancer to the formulations and a maximum 70% drug was penetrated by increasing the amount of enhancer from 0.5% to 3%.

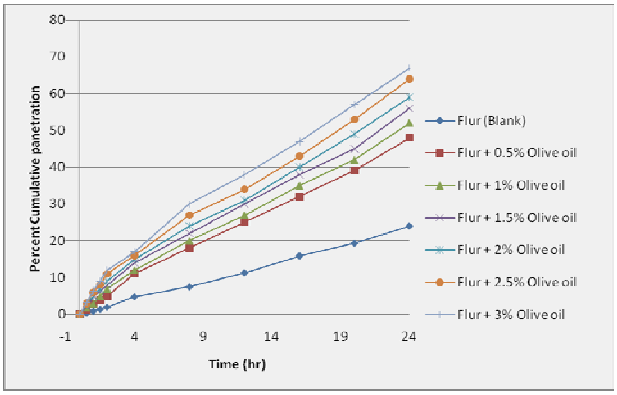

Fig 2: Release curves of flurbiprofen across synthetic membrane in phosphate buffer pH 7.4

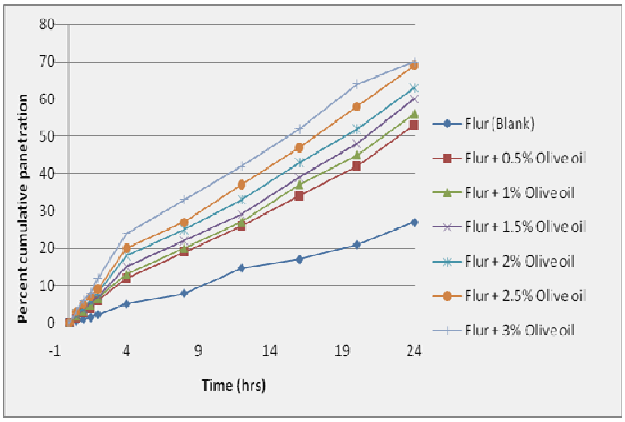

Fig 3: Release curves of flurbiprofen across albino rabbit skin in phosphate buffer pH 7.4.

DISCUSSION

Drug release kinetics

In order to investigate the mechanism involved in the release of flurbiprofen from topical gel, a kinetic approach was made by employing Korsmeyer Pappas kinetic model to the *in vitro* drug release profiles. As discussed earlier an (n) value in Korsmeyer Pappas

equation represents the way by which the drug is released from the gel formulation across synthetic membrane/ rabbit skin into the receptor solvent. By employing the kinetic model the values of (n) were (1<n>0.5) indicating that two release mechanism were involved in the release of flurbiprofen from transdermal gel, non-Fickian (anomalous) and super case II transport (Khan and Zhu, 1999).

However, the penetration of flurbiprofen across rabbit skin was significantly more than that penetrated across synthetic membrane which could be due to the reason that synthetic membrane used in this study was thicker as compared to rabbit skin which allow more drug to be penetrated. It could be also due to the reason that natural skin (rabbit skin) contains a large number of hair follicles and it is suggested that the penetration of drug occur mainly through these follicles (Beatrice *et al.*, 2001).

The comparative effect of olive oil as penetration enhancer on *in vitro* drug penetration of flurbiprofen across synthetic membrane and rabbit skin could be seen from the fig. 4.

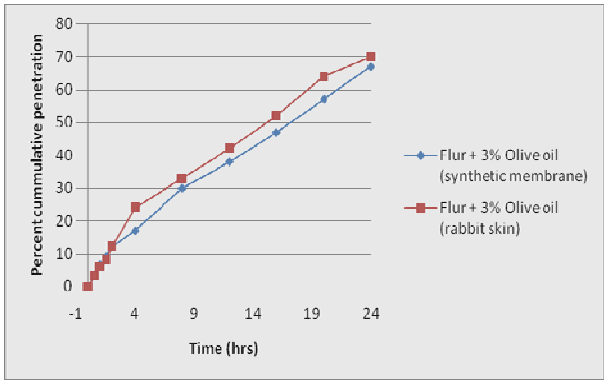

Fig 4: Comparative release curves of flurbiprofen across synthetic membrane and rabbit skin in phosphate buffer pH 7.4

CONCLUSION

Flurbiprofen transdermal gel was successfully prepared and evaluated with a high *in vitro* penetration rate. From the above results olive oil was found to be effective enhancer for the *in vitro* skin permeation of flurbiprofen. It was also observed that high concentration of olive oil was more effective by enhancing penetration of drug through stratum corneum. The transdermal flurbiprofen gel will be further investigated for *in vivo* studies in laboratory animals.

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