Formulation and Evaluation of Amphiphilogels of Piroxicam for Enhancement of its Transdermal Drug Delivery

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***Abstract*- Piroxicam has very low permeation across skin layers due to its hydrophobicity. Amphiphilogel is a semisolid system serve as a better vehicle for this "Difficult drug". The preformulation studies were done as a means for identification of drug and gelators. Compatibility studies were done on the basis of FTIR. Solubility and partition co-efficient was measured in different solvents. Tween 80 was proved better over span 80 as a solvent. Amphiphilogels were prepared by weighing span 40 [(3% w/w), (solid gelator)]. Two groups were prepared naming as hydrophilic amphiphilogels and lypophilic amphiphilogels. The samples were found to be pale yellow in color with a minute difference in hydrophilic and lypophilic formulations. On increasing gelator concentration clusters rose in number. All formulations were easily washable and have good spredability. Piroxicam showed greater release from hydrophilic amphiphilogels as compared to lypophilic amphiphilogels. Amongst the hydrophilic amphiphilogels HF4 showed the highest permeation containing ethanol as a co-solvent followed by HF1> HF2 > HF3. Zero order as well as korsmeyer peppas kinetic model both were obeyed by all amphiphilogel formulations. Transdermal flux value were calculated from the slope which suggested that the value of transdermal flux was highest in HF4 contains ethanol as penetration enhancer. All the formulation showed increased transdermal flux then HF0 containing no penetration enhancer. Transdermal flux of hydrophilic amphiphilogels was higher than lypophilic amphiphilogels. All amphiphilogels came to gel state when the temperature was reduced to 4^0C, indicating their thermoreversibility.**

***Keywords*: Amphiphilogels, gelator, hydrophilic, lypophilic, partition co-efficient, penetration enhancer, transdermal flux, thermoreversibility.**

Introduction

Transdermal / Topical Drug Delivery

Graham *et al.*, 1987, Skin surface area is 3000 inch^2 and receivesone third of the circulating blood (Figure 1). Epidermis itself is composed of the stratum corneum, horny layer (about 10 µm thick), which is a layer of compressed, overlapping keratinized cells that form a flexible, tough, coherent membrane (Mehta *et al*., 2004; Ansel *et al*., 2006).

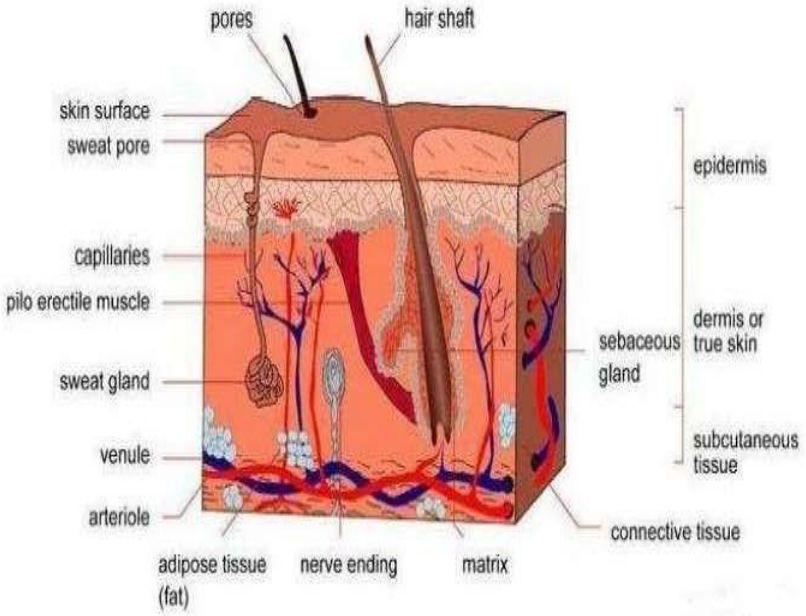


Figure 1: Structure of skin.

Produces sustained and controlled level of drug in plasma, thus reducing the chance of over or under dosing.

Organogels As Drug Delivery System

Murdan *et al*., 2005 summarised that organogels are semi-solid systems, in which an organic liquid phase is immobilized by a three-dimensional network composed of self assembled, intertwined gelator fibers (Kamble *et al*., 2011; Couffin *et al*., 2004; Vintiloiu *et al*., 2008).

Amphiphilogels

Heenan *et al*., 2004 illustrated that amphiphilogels (a subset of organogels) are topical and transdermal carriers for drugs and vaccine and can be hydrophilic. It is found that amphiphilogels are opaque thermoreversible and thixotropic by nature composed solely of nonionic surfactants. Amphiphilogel is a semisolid system or being a compound (as a surfactant) consisting of molecules having a polar water-soluble group attached to a water- insoluble hydrocarbon chain. An attempt was done to prepare an amphiphilogel system keeping in view the problems associated with poorly water-soluble nonsteroid anti-inflammatory drugpiroxicam ("Difficult Drug"), comprising of sesame oil, a nonionic surfactant tween 80, a short-chain alkanol cosurfactant (ethanol), Propylene glycol, Iso propyl myristate. To overcome side effects it is necessary to develop a newer and safer formulation of piroxicam in the form of topical drug delivery system (i.e. organogel). Objectives of the present research research include formulation and development a novel class oforganogel, where the liquid phase is a surfactant and so had termed these as amphiphilogels, to promote/increase the transdermal delivery of piroxicam by inclusion of co-solvents and reduction in drug toxicity by giving it topically.

Materials and Methods

Piroxicam- Gift sample obtained from Revenbhel Healthcare Pvt. Ltd. Jammu. Gelators e.g. Sorbitan monopalmitate, Polyoxyethylene (20), sorbitan monooleate (Polysorbate 80), Sorbitan monooleate; Co-Solvents (Penetration Enhancers): Propylene glycol, Isopropyl myristate; Chemicals (AR Grade Chemicals): Propanol, n-Butanol, n-Hexane, Methanol, Chloroform, Acetone, n-octanol, Sodium chloride, Sodium citrate, Potassium dihydrogen phosphate, Sodium dihydrogen phosphate, Sodium hydroxide.

Pre-formulation Studies

Identification of drug:

The drug sample was gifted by Revenbhel Healthcare Pvt. Ltd. (Jammu). Piroxicam was identified by spectral techniques like FTIR and UV spectroscopy. White crystalline powder

Infrared spectrum:

The pallets of KBr and drug were prepared and examined under 8400S (4000-400/cm), Shimadzu, IR spectrophotometer, Japan. Characteristics peaks attributable to functional groups present in the molecule of drug assigned to establish the identity, which are similar to reference standard (Table 1).

Table 1: Interpretation of piroxicam spectra.

S. No.	Peak cm^{-1}	Groups
1.	773.48	Ortro disubstituted phenyl
2.	1149.61	S=O stretching
3.	1181.44	SO_2-N stretching
4.	1350.96	C-N stretching
5.	1475.56	C=C aryl
6.	1525.50	C=N stretching
7.	1576.88	N-H bending
8.	1629.90	C=O stretching
9.	3338.89	N-H or O-H stretching

Determination of λ max

As per standard procedure using UV spectrophotometer (Systronics Double Beam Spectrophotometer- Model 2202) between 200–400 nm gives the absorption maxima at 334.0 nm (Figure 2).

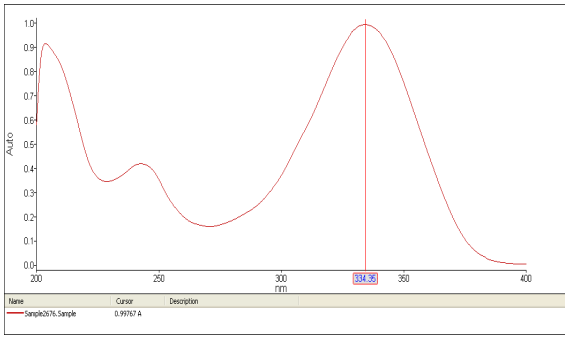


Figure 2 : UV absorption maxima of piroxicam (λ max = 334.35 nm).

Melting Point (MP) Determination

MP of the piroxicam was determined by using thieles tube method (Table 2).

Table 2: Melting point of piroxicam.

S. No.	Reported	Observed
1.	198^0C-200^0C	197^0C-200^0C

Solubility Determination/Analysis

This study was performed in different solvents like alcohol, distilled water, methanol, polyethylene glycol, tween 80 etc. Saturated solutions of drug were prepared in different solvents by adding the excess drug to the vehicles and shaking in screw capped tubes in a mechanical shaker (Hicon Products (India) Pvt. Ltd. for 48 hrs at 25^0C under constant vibration (Table 3).

Table 3: Solubility of drug in different solvents.

Solvent	Solubility
Distilled water	Soluble very slightly
Dimethyl formamide	Freely Soluble
Dimethyl sulphoxide	Soluble freely
1-octanol	Slightly soluble
Methanol	
Ethanol	
Propylene glycol	
Iso propyl myristate	
Sesame oil	
Tween 80	Soluble
Span 80	Very slightly soluble

Partition Coefficient

Partition coefficient of piroxicam was determined in pH 5.4 phosphate buffer. The content of both phase were separated. After appropriate dilutions, the aqueous phase was analyzed for piroxicam against reagent blank solution using UV spectrophotometer (Systronics double Beam Spectrophotometer- Model 2202). The partition coefficient value P was calculated by the following equation:

$$P_{o/w} = (C_{org} / C_{aq}) P_{w/o} = (C_{aq} / C_{org})$$

Same process was applied with n-octanol / phosphate buffer (ethanol 5%, w/v), n- octanol / phosphate buffer (tween-80

5%, w/v), n-octanol / phosphate buffer (span-80 5%,w/v) systems for partition coefficient determination (Table 4).

Table 4 : Partition coefficients of drug in different solvents.

S. No.	System	Log p
1.	Octanol/buffer	5.03
2.	Octanol/buffer (ethanol 5%, w/v)	4.86
3.	Octanol/buffer (Tween 80 5%, w/v)	3.13
4.	Octanol/buffer (Span 80 5%, w/v)	4.25

Compatibility Study

The drug and gelator compatibility was characterized by the means of FTIR spectroscopy. No interaction between drug and gelator observed as peaks were not changed significantly in mixture FTIR spectra and it showed similar peaks likepure drug FTIR (Table 5 -7).

Table 5: Interpretation of FTIR spectra of piroxicam and span 40.

S. No.	Peak (cm^{-1})	Groups
1.	773.48	Ortho disubstituted phenyl
2.	1187.76	C-O stretching
3.	1350.96	C-N stretching
4.	1475.56	C=C aryl
5.	1750.86	C=O stretching
6.	2889.65	C-H stretching
7.	3338.89	N-H or O-H stretching

Table 6: Interpretation of FTIR spectra of piroxicam and span 80.

S. No.	Peak (cm^{-1})	Groups
1.	773.48	Ortho disubstituted phenyl
2.	1350.96	C-N stretching
3.	1475.56	C=C aryl
4.	1750.86	C=O stretching
5.	2880.65	C-H stretching
6.	3338.89	N-H or O-H streching

Table 7: Interpretation of FTIR spectra of piroxicam and tween 80.

S. No.	Peak (cm^{-1})	Groups
1.	1187.89	C=C streching
2.	1350.96	C-N stretching
3.	1475.56	C=C aryl

4.	1750.86	C=O stretching
5.	2856.78	C-H stretching
6.	3367.78	N-H or O-H streching

Determination of Minimum Gelation Concentration

Table 8: Minimum gelation concentration of sorbitan monopalmitate(span 40) (%w/w).

S. No.	Span	Minimum Gelation concentration of Sorbitan monopalmitate (40)(%w/w)	
		Reported	Observed
1	Span 80 (Sorbitan monooleate)	25	26
2	Tween 80 (Polysorbate 80)	20	24

Preparation of Calibration Curve (CC)

Table 9: Piroxicam calibration curve in 0.1N hydrochloric acid (pH 1.2).

Concentration (µg/ml)	Absorbance (nm)
0	0.000
2	0.172
4	0.331
6	0.490
8	0.680
10	0.890

Table 10: CC of Piroxicam in buffer solution (phosphate).

Conc.(µg/ml)	Absorbance (nm)
0.0	0.000
2.0	0.143
4.0	0.312
6.0	0.472
8.0	0.654
10.0	0.865

Preparation of Formulations

Span 40 (sorbitan monopalmitate) was used as the gelator (solid component of the gel) for all the formulations, and the fluid phases, liquid tweens and liquid spans were incorporated in hydrophilic and lypophilic formulations respectively. Co-solvents (ethanol (5%w/w), propylene glycol (5%w/w), sesame oil (5%w/w), iso propyl myristate (5%w/w) were added in amphiphilogels to enhance the solubility of piroxicam (Figure 3-4).

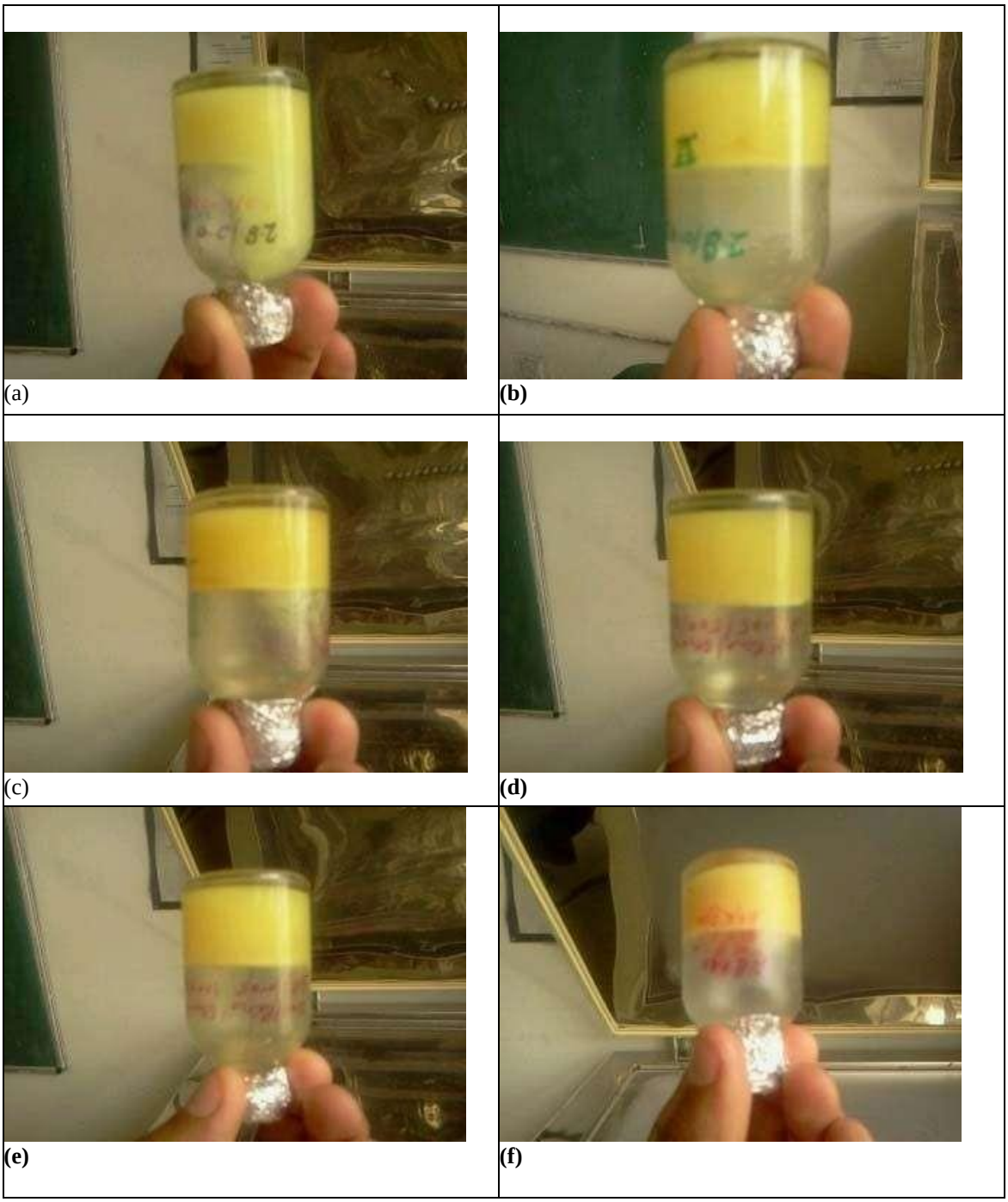


Figure 3 : Optical photographs of amphiphilogels formulations [(a)-HF0, (b)- HF-1, *(c)-HF2, (d)-HF3.*

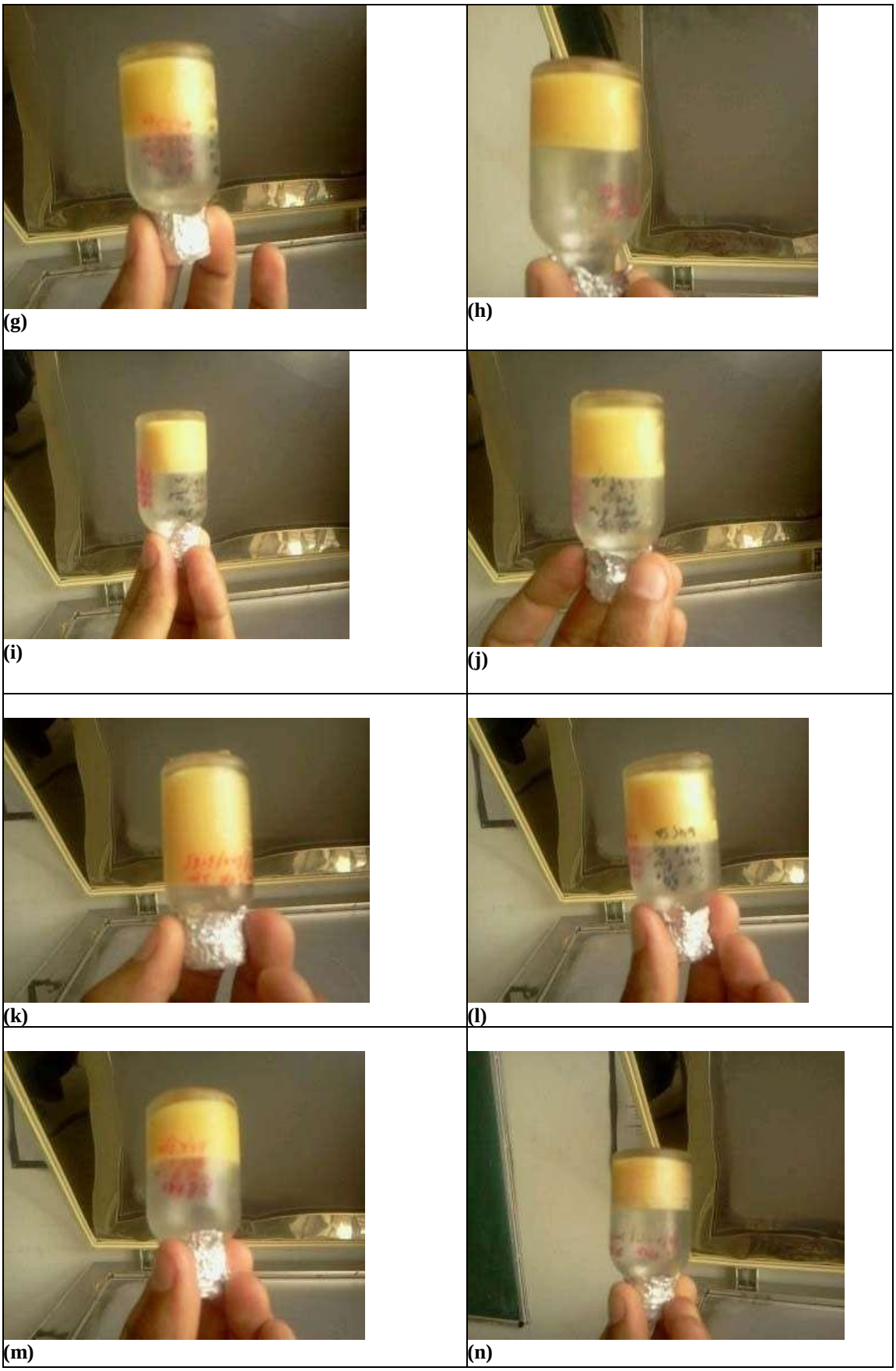


Figure 4 : Optical photographs of amphiphilogels formulations [(e)- HF4, (f)-HDC1, (g)-HGC1, (h)-LF0, *i)-LF1, (j)-LF2, (k)-LF3, (l)-LF4, (m)-HDC2, (n)-HGC2*].

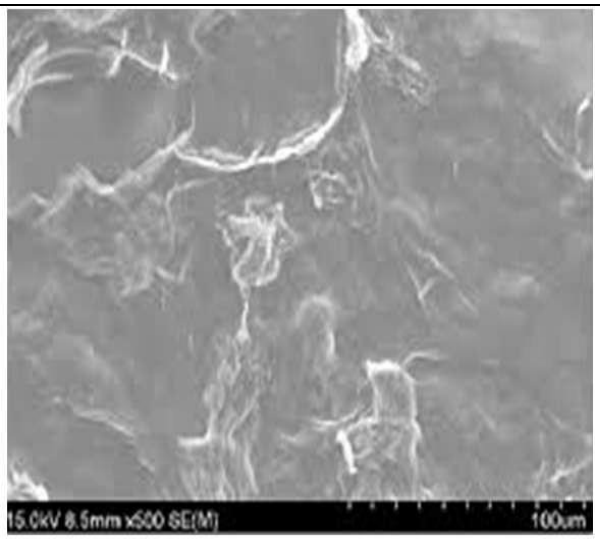


Figure 5 : SEM analysis of amphiphilogel formulation, HGC1.

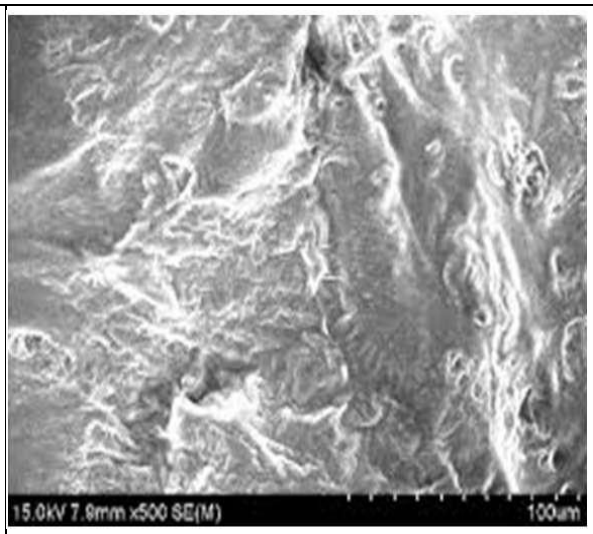


Figure 6 : SEM of HGC2.

Evaluation of Amphiphilogels

Determination of Gel Transition Temperature

Table 11: Phase transition temperature of different formulations.

Formulation code	Gel transition temperature (^{0}C)
HF0	42.8 ± 0.65
HF1	37.6 ± 0.87
HF2	35.8 ± 0.98
HF3	37.2 ± 0.65
HF4	38.5 ± 0.76
HDC1	40.8 ± 0.87
HGC1	43.0 ± 0.78
LF0	41.9 ± 0.73
LF1	36.8 ± 0.86
LF2	33.0 ± 0.99
LF3	35.1 ± 0.43
LF4	37.3 ± 0.73
HDC2	40.6 ± 0.99
HGC2	42.6 ± 0.47

Determination of Viscosity of Gel

To evaluate gel flow properties of formulations, a Brookfield viscometer were used to measure viscosity of amphiphilogel (Table 12).

Table 12: Viscosity measurement.

Formulation code	Viscosity (centipoise)
HF0	20256
HF1	17380
HF2	16284
HF3	15354
HF4	19476
HDC1	19850
HGC1	22645
LF0	18630
LF1	13498
LF2	16347
LF3	14944
LF4	13647
HDC2	17930
HGC2	20765

4.2.1. *In-Vitro* Drug Permeation

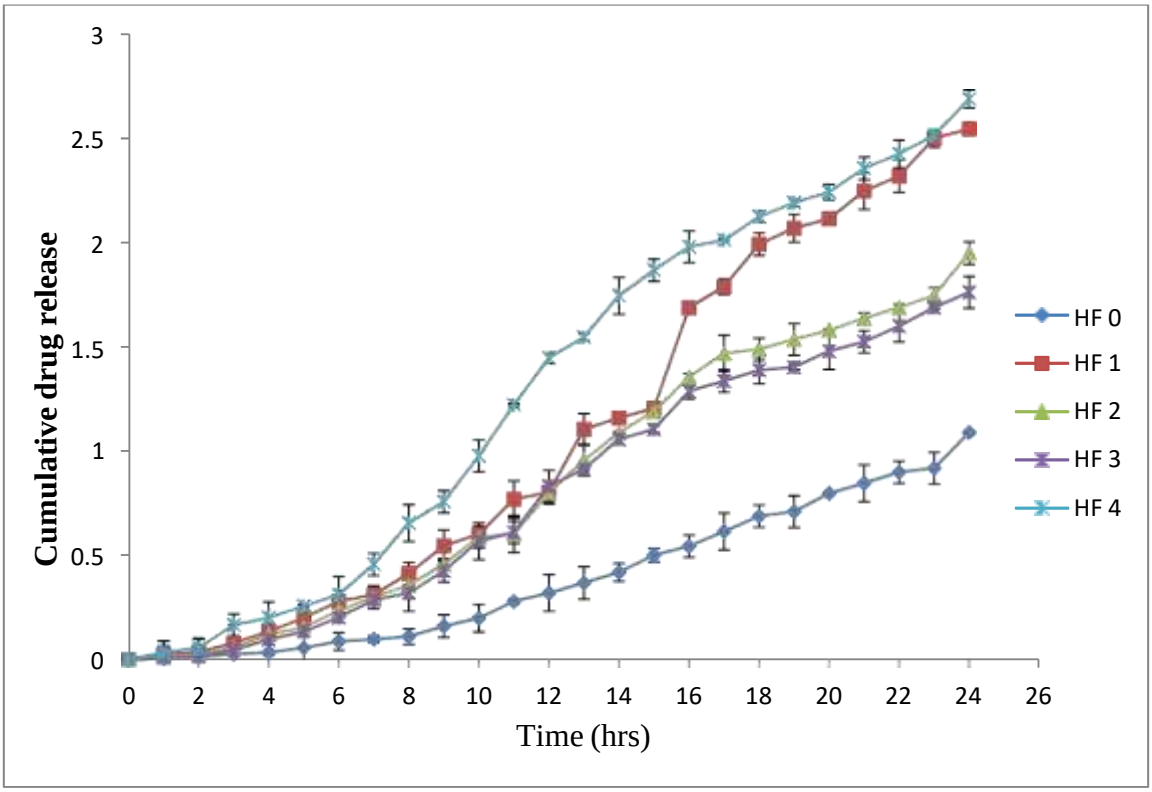


Figure 7 : Comparative cumulative release of drug (mg/cm^2) from amphiphilogelformulations (HF0, HF1, HF2, HF3, HF4).

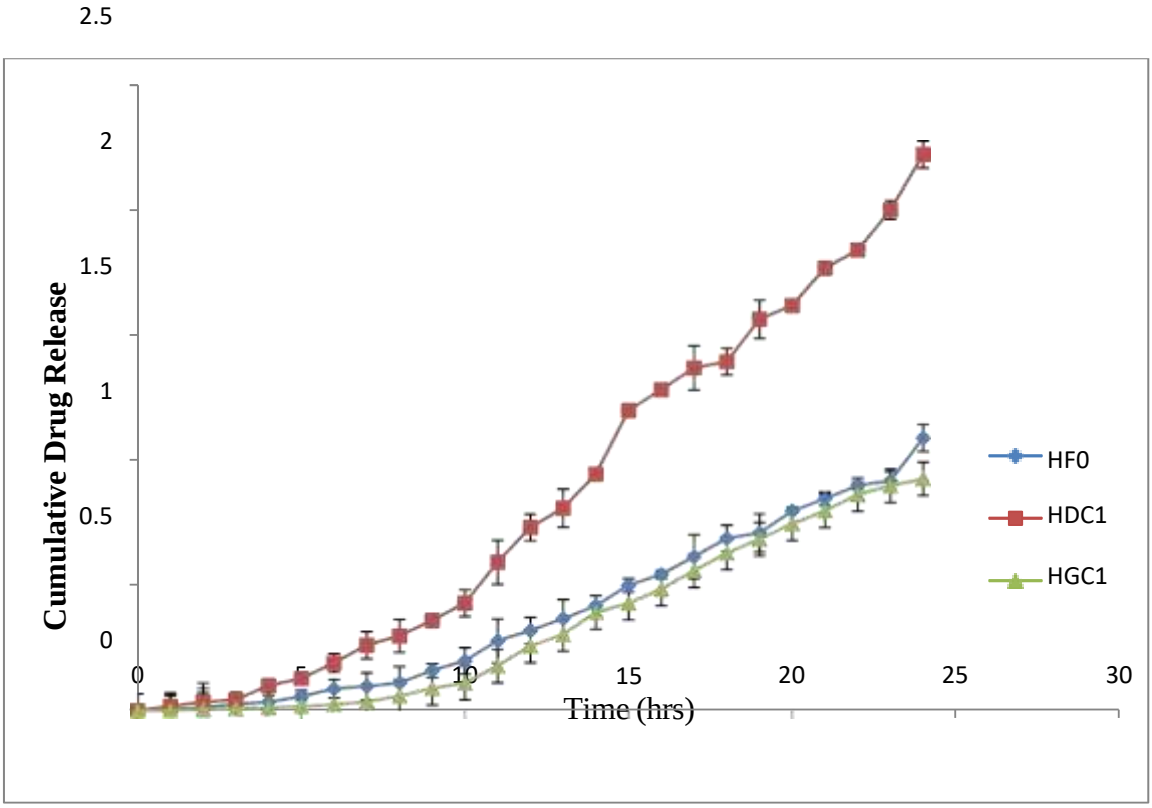


Figure 8 : Comparative cumulative release of drug (mg/cm^2) from amphiphilogelformulations (HF0, HDC1, HGC1).

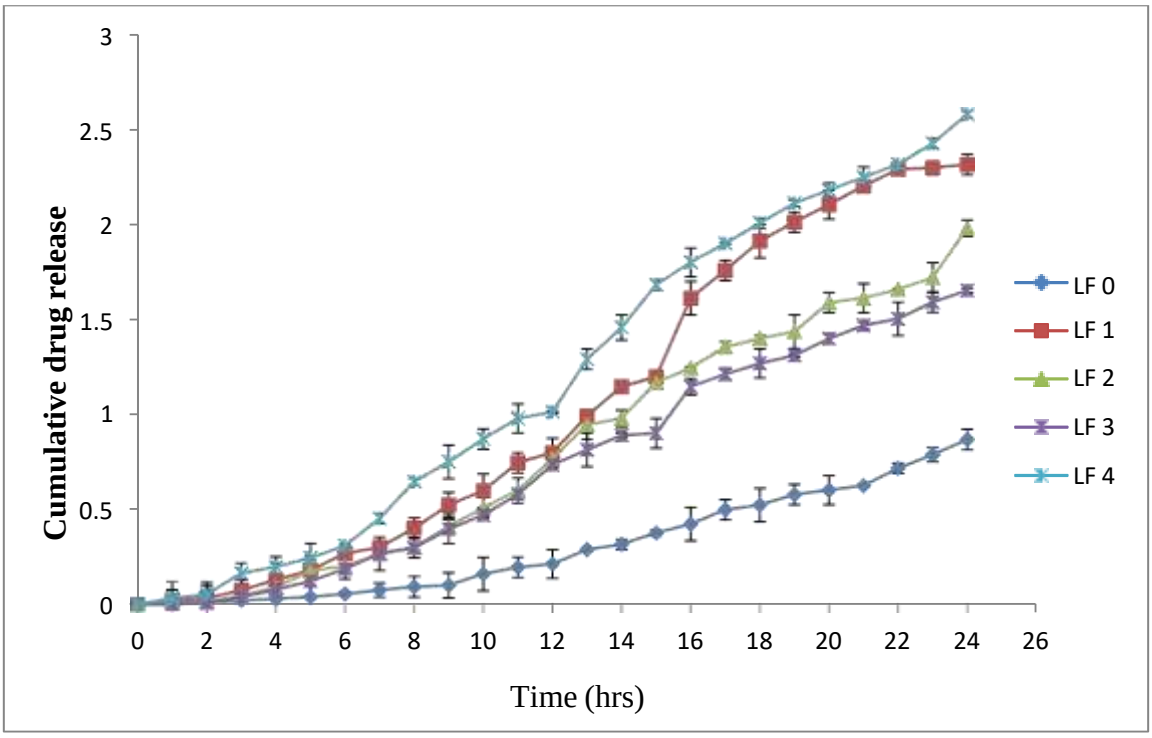


Figure 9 : Comparative cumulative release of drug (mg/cm^2) fromamphiphilogelformulations (LF0, LF1, LF2, LF3, LF4).

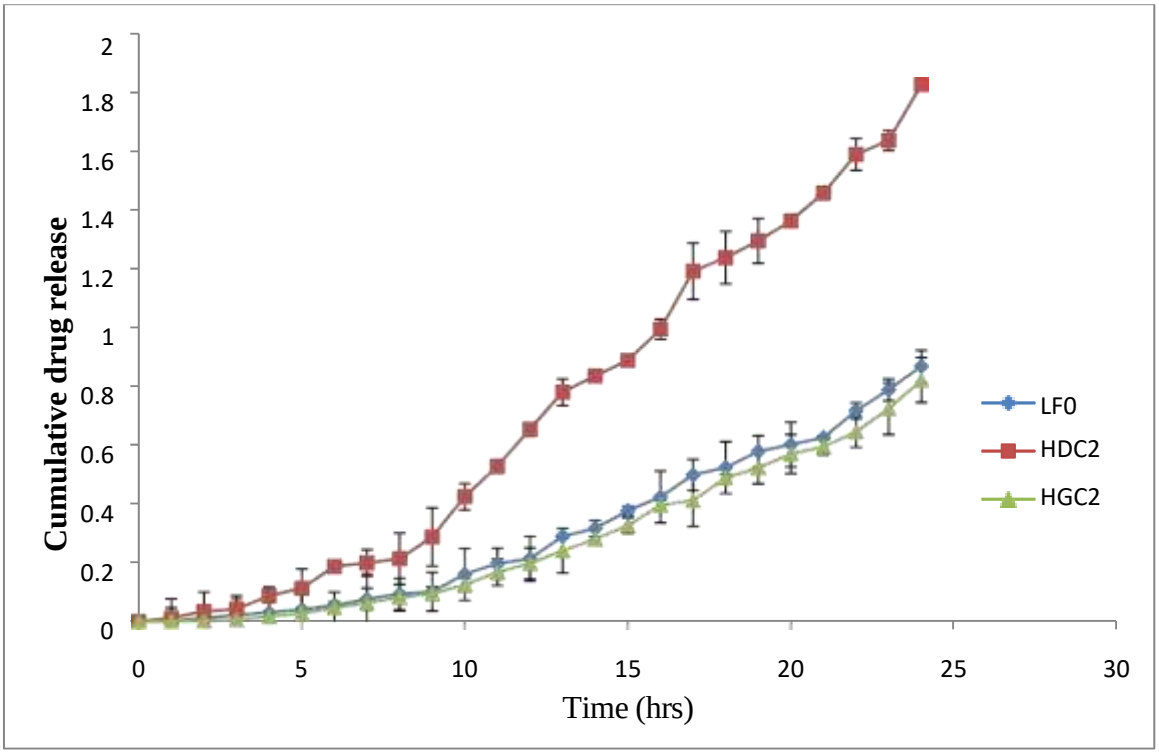


Figure 10 : Comparative cumulative release of drug (mg/cm^2) from amphiphilogelformulations (LF0, HDC2, HGC2).

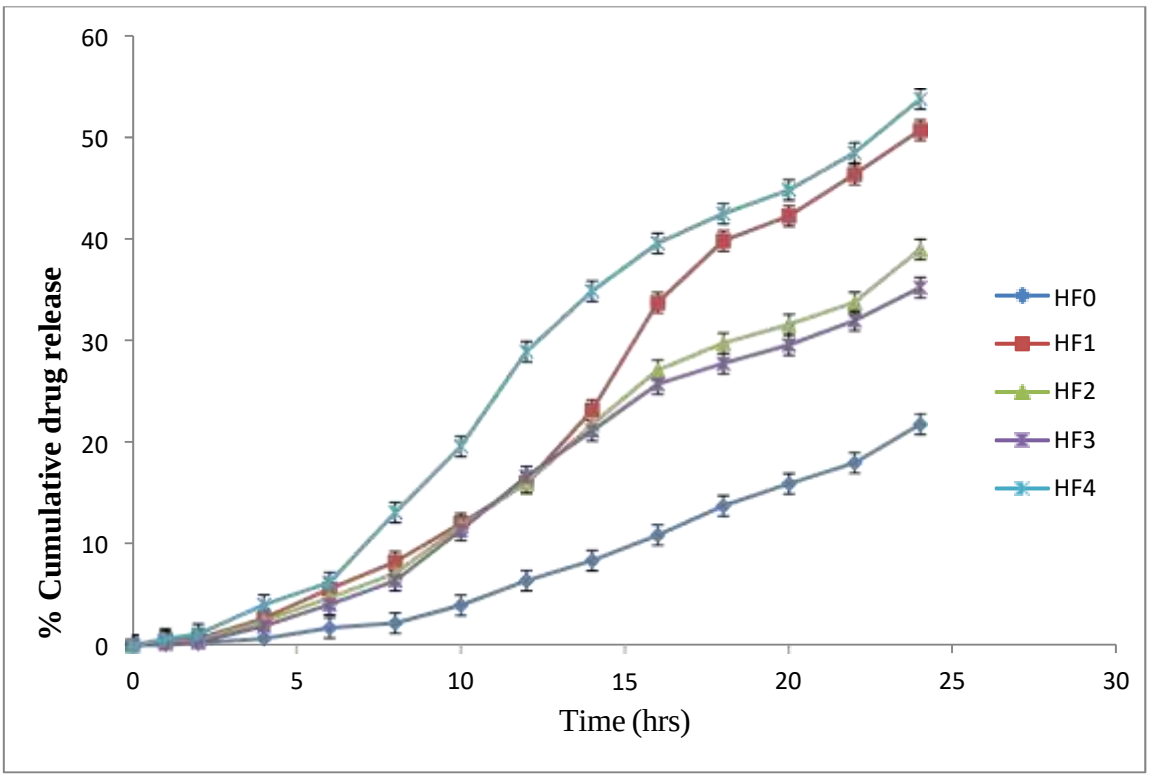


Figure 11 : Percentage cumulative release of drug (mg/cm^2) from amphiphilogelsformulations (HF0, HF1, HF2, HF3, HF4).

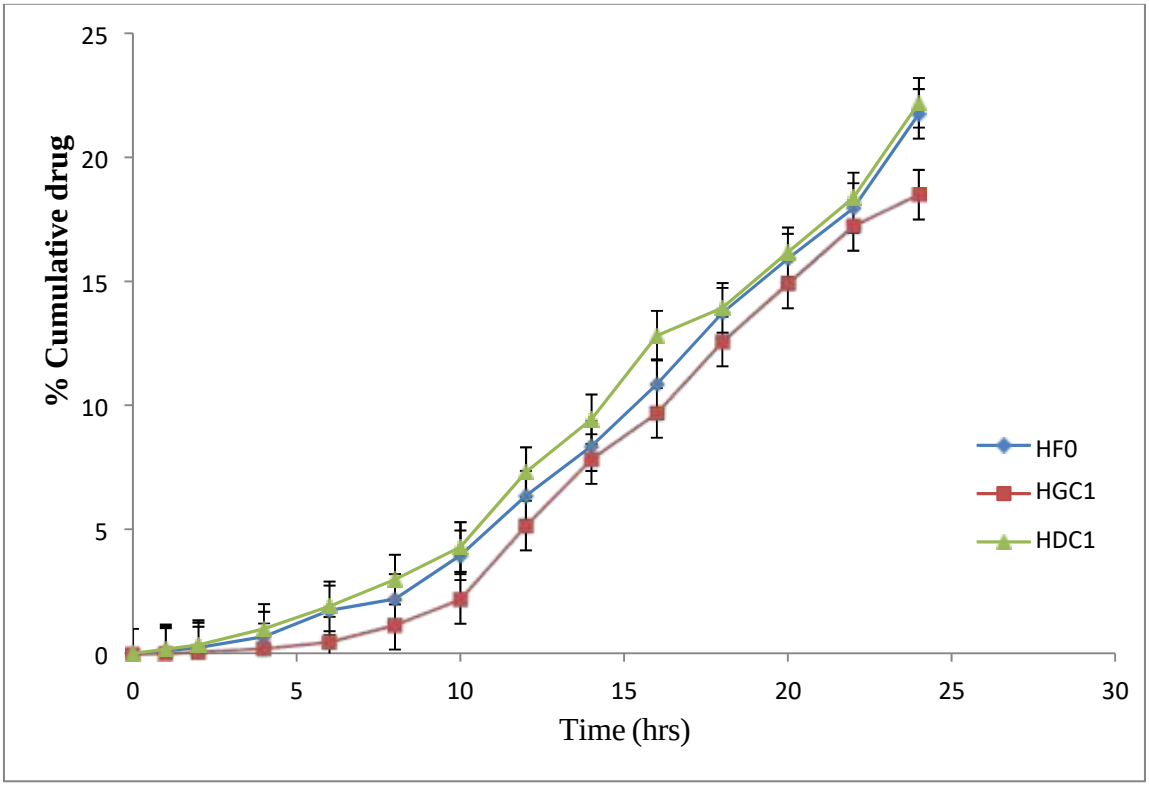


Figure 12 : Percentage cumulative release of drug (mg/cm^2) from amphiphilogelsformulations (HF0, HGC1, HDC1).

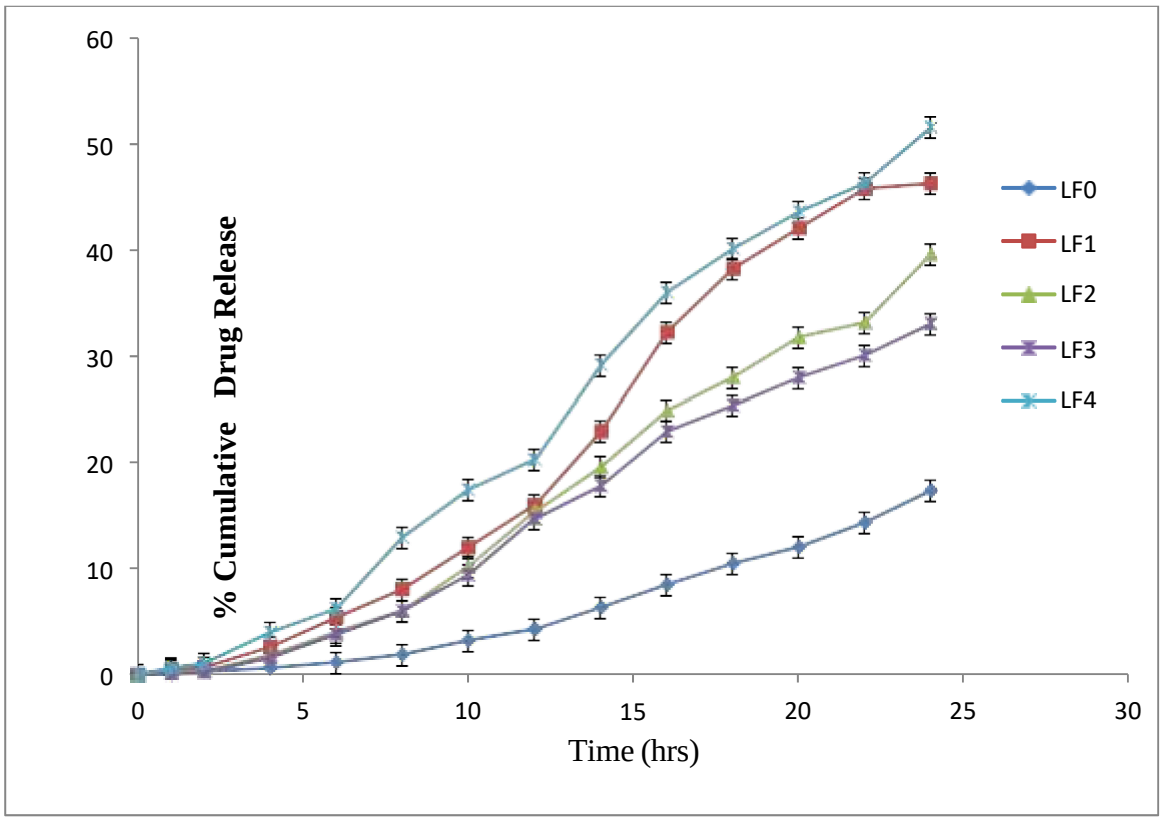


Figure 13 : Percentage cumulative release of drug (mg/cm^2) from amphiphilogelsformulations (LF0, LF1, LF2, LF3, LF4).

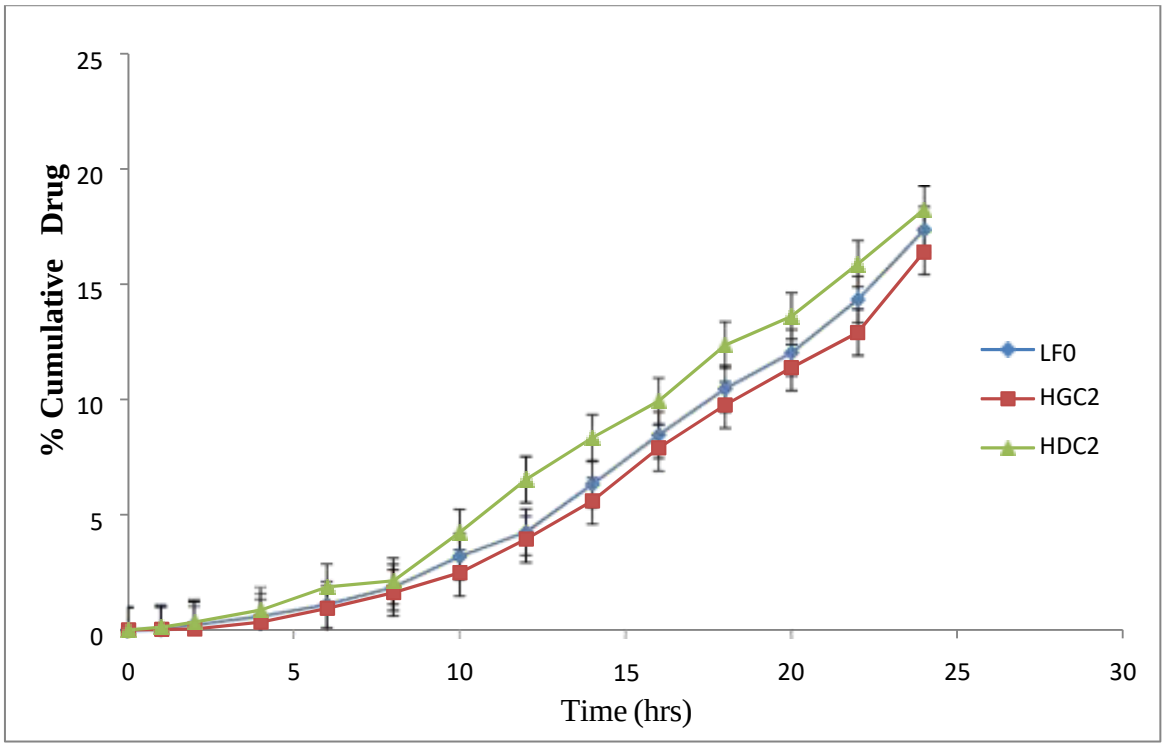
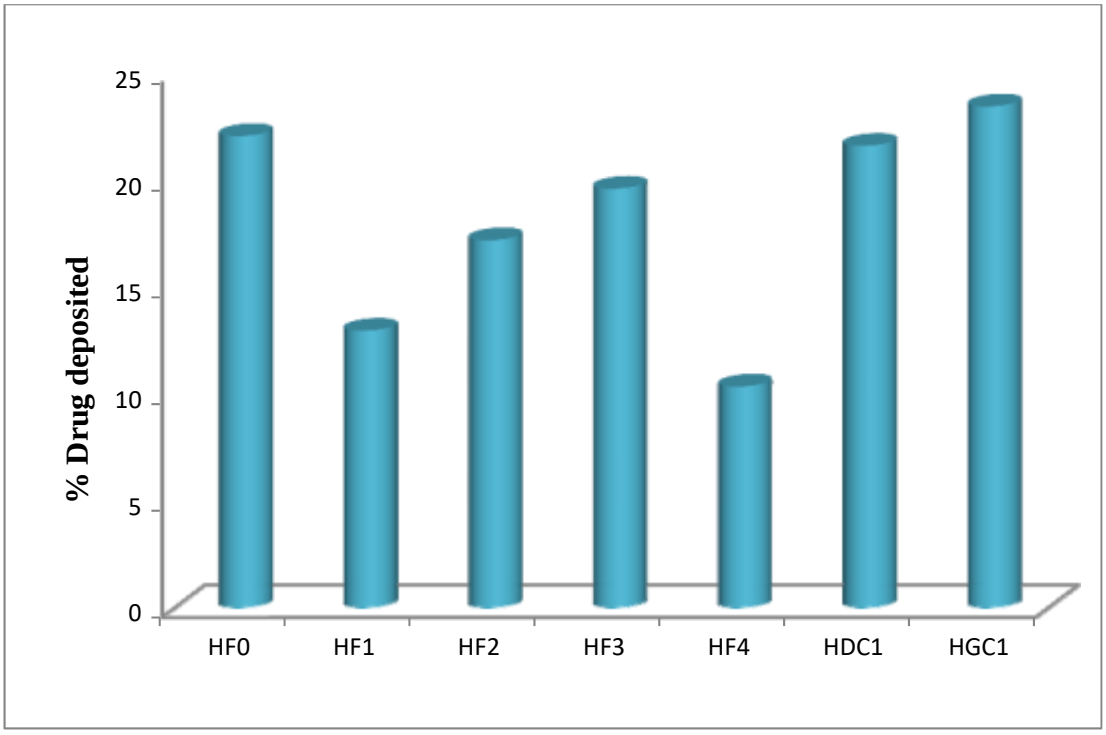


Figure 14 : Percentage cumulative release of drug (mg/cm^2) from amphiphilogelsformulations (LF0, HGC2, HDC1.

Drug Deposition Study

Table 13 : Percentage drug deposited on rat skin *in-vitro* after 12 hrs ofextraction withphophate buffer (pH-6.8) at $37^0C \pm 0.5^0C$.

S. No.	Formulation code	% Drug Deposited
1.	HF0	22.02
2.	HF1	12.92
3.	HF2	17.15
4.	HF3	19.56
5.	HF4	10.31
6.	HDC1	21.56
7.	HGC1	23.40



Formulation code

Figure 15 : Percent drug deposited on rat skin from amphiphilogelsformulations (HF0,HF1, HF2, HF3, HF4, and HDC1).

Table 14 : Drug percentage deposited on rat skin *in-vitro* after 12 hrs ofextraction withphophate buffer (pH-6.8) at 37^0C ± 0.5^0C.

S. No.	Formulation code	% Drug Deposited
1.	LF0	26.10
2.	LF1	18.13
3.	LF2	20.82
4.	LF3	24.43
5.	LF4	14.15
6.	HDC2	26.00
7.	HGC2	26.12

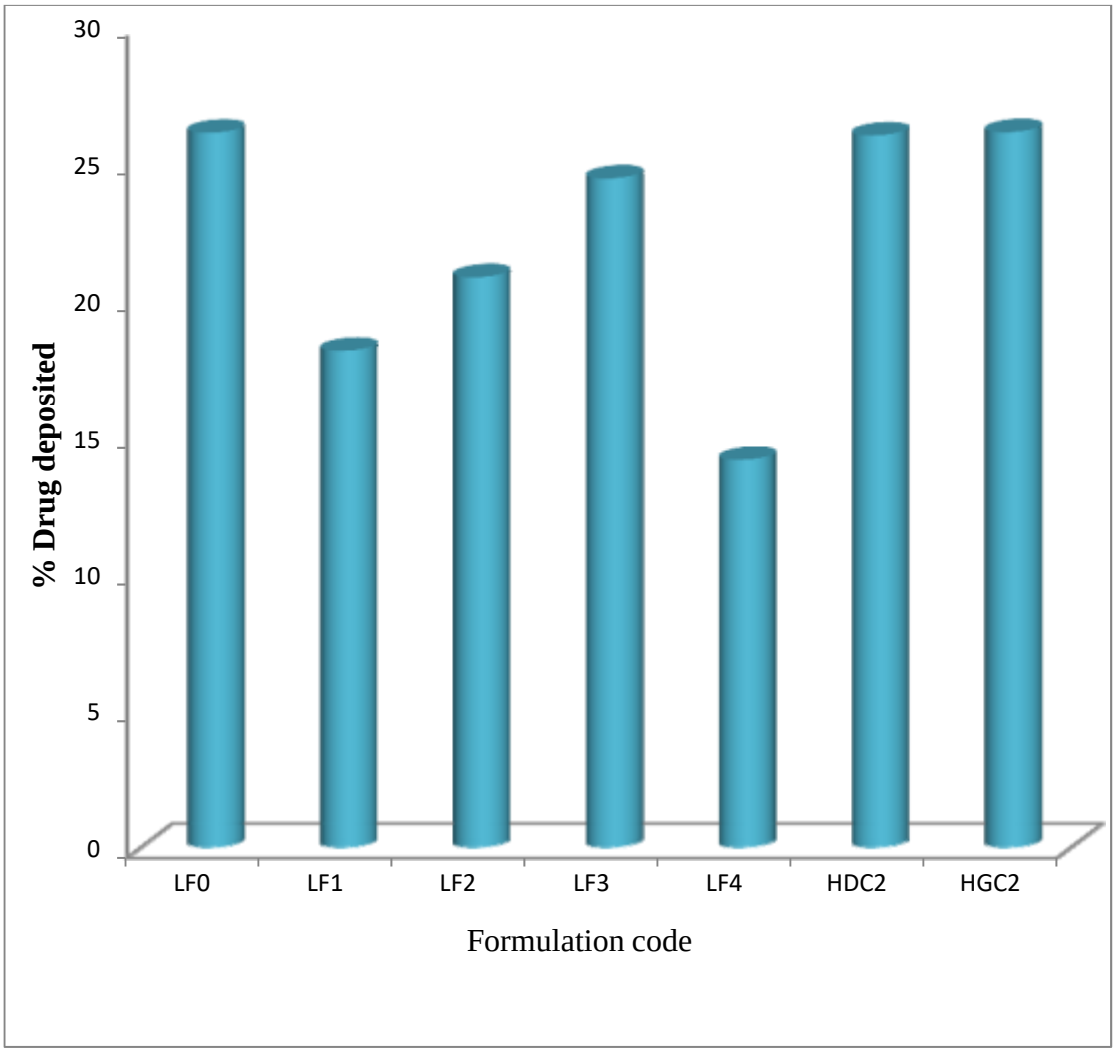


Figure 16 : Drug %age deposited on rat skin from amphiphilogels formulations(LF0,LF1, LF2, LF3, LF4, and HDC2).

Transdermal Flux

Table 15 : Skin permeation profile amphiphilogels calculated after 24 hrs.

S. No.	Formulation code	Transdermal flux(µg/cm^2/hr)	Enhancement ratio
1.	HF0	45.7	0
2.	HF1	119.3	2.61
3.	HF2	88.8	1.94
4.	HF3	83.2	1.82
5.	HF4	124.5	2.72
6.	HDC1	95.9	2.09
7.	HGC1	43.7	0.95

Stability Studies on Time Scale

Table 16 : Stability studies of formulation HF4

S. No.	Time(days)	Temperature	% Drug content	pH	Viscosity (centipoise)	Syneresis
1.	0	RT	96.9	5.6	19476	No
2.	15	4^{0}C	96.8	5.7	19398	No
3.	30	4^{0}C	96.9	5.6	19178	No
4.	45	4^{0}C	96.8	5.9	19247	No
5.	60	4^{0}C	95.2	5.6	19367	No
6.	15	RT	96.5	5.9	19498	No
7.	30	RT	96.4	6.2	19533	No
8.	45	RT	96.8	6.4	19783	No
9.	60	RT	95.3	6.3	19757	No
10.	15	40^{0}C	96.2	6.5	19899	No
11.	30	40^{0}C	95.4	6.5	19924	No
12.	45	40^{0}C	94.9	6.2	20065	No
13.	60	40^{0}C	92.6	6.5	20366	No

Results and Discussions

FTIR spectroscopy of the piroxicam. drug sample was compared with standard and absorption peaks were found similar. The Figure 4.1 represents the FTIR spectrum of piroxicam which have absorption peak at 773.48, 1149.61, 1181.44, 1525.50, 1639.90 and 3338.89 cm^{-1}. When 10 µg/ml solution of piroxicam in 0.1 M methanolic hydrochloric acid was scanned between 200–400 nm gives the absorption maxima at 334.0nm, Which is verynear to the reported value.

Piroxicam mp was 197^{0}C -200^{0}C (Table-7.2), which is very near to the reported melting point range (198-200). The solubility study of drug was performed in different solvents (alcohol, distilled water, methanol, polyethylene glycol, DMSO, tween 80 etc). Among them piroxicam shows increased solubility in propylene glycol, ethanol, tween 80. Drugwas also better soluble in iso propyl myrystate in comparison to water. The partition coefficient of drug (piroxicam) in Octanol/buffer (pH-7.4) was found to be 5.03 ± 0.20, In Oil/buffer (ethanol 5%, w/v) system it was 4.86 ± 0.15, In Oil/buffer (Tween 80 5%, w/v) it was 3.13 ± 0.20 and in Oil/buffer (Span 80 5%, w/v) was found to be 4.25 ± 0.20.

No interaction between drug and gelators observed. Peaks of functional groups were unchanged in mixture FTIR

spectra and it showed similar peaks like pure drug FTIR spectrum, with some additional peaks of surfactants (Gelators) also (FTIR spectrum of Sorbitan monopalmitate + Piroxicam), (FTIR spectra of Sorbitan monooleate + Piroxicam) and (FTIR spectrum of Polysorbate 80 + Piroxicam). Minimum gelation concentration of sorbitan monopalmitate (Span 40) (%w/w) in span 80 (Sorbitan monooleate) was found to be 26 and in tween 80 (Polysorbate 80) was 24.

Spectrophotometric method of analysis was selected to identify whether the drug (piroxtcam) obeys Beers law or not. Dilutions of the drug were prepared (1µg/ml to 10µg/ml) and absorbance was recorded at 334 nm against suitable blank using UV spectrophotometer (Systronic Double Beam Spectrophotometer- Model2202). The absorbance vs. concentration curve was plotted which yielded a straight line for a drug concentration of 1µg/ml to 10 µg/ml, showing that the dilution of drug obeyed the Beers law. The estimation of purity of drug, by plotting standard curve.

Total 14 formulations of 10 gms quantity were prepared by varying nature of liquid gelator (Span 80, Tween 80) which are lipophilic and hydrophilic in nature respectively. Co-solvents (penetrationenhancers) were added in each formulation. All formulations were homogenically uniform because no lump or any particulatewas found in any formulation. Light microscopy revealed that amphiphilogels consisted of tubules and clusters that are flower shaped or star like in shape. On increasing gelator concentration clusters rose in number. Pure tween 80 and pure span 80 were used as the representative blank. The spectras of the formulations (HF1 and LF1) and pure gelators (Tween 80) and pure Span 80 were found approximately similar. Only the change in pure gelator and formulation spectras is degree of shallowness and broadness. It was observed that on increasingdrug conc. gel transition temperature was also increased as clearly shown by the values. Viscosity of amphiphilogels was measured by Brookfield viscometer. Viscosity range of amphiphilogels was found to be within 13498-19476 centipoise. Viscosity was decreased on increasing temperature which indicated gel softening at temperatures near skin temperatures, which will facilitate easy applications.

The spreadability of amphiphilogels was measured and the values were found to be within 42.79-60.34. On increasing gelator concentration, spreadability was decreasing as formulation HGC2 is having least spreadability (42.79).

Formulations containing co- solvents were having better spreadability because less energy is needed to break 3-dimentional structures of these formulations containing liquid phase immobilized in it. Overall all formulations showed good spreadability which is very important property for an ideal gel formulation.

Formulations were almost washable, indicating easy termination or removal of the formulation. Hence all formulations were patient compliant. Drug content was ranged between 91.5-98.4 %. Piroxicam showed greater release from hydrophilic amphiphilogels as compared to lypophilic amphiphilogels. Structure of tween 80 contains ethylene oxide and a long hydrocarbon chain. This imparts both hydrophilic and lypophilic characteristics to it.

In fact, *in-vitro* drug release may influenced by the natures of both the gel matrix and the active drug, especially the drug solubility, the partition of drug molecules in the matrix, as well as the interaction between the drug molecules and other ingredients of the gel.

Amongst the hydrophilic amphiphilogels HF4 showed the highest permeation followed by HF1> HF2>HF3. All gels contained different co-solvents as HF1 contained propylene glycol, HF2 contained isopropyle myristate, HF3 contained sesame oil and HF4 contained ethanol. These co-solvents enhanced the solubility of lypophilic drug to different extent and contributed in skin drug permeation by different mechanisms. Sesame oil being thick viscous liquid might have increased the occlusive nature of the gels, which may be one of the reasons for permeation enhancement Ethanol used in HF4 interacts with lipid molecules in the polar head group region hence increased fluidity of SC lipids which was densely packed at physiological temperature. Furthermore increase in gelator concentration in HGC1 and HGC2 decreases the drug release to some extent. It should be considered that there is very little difference in the release patterns between HF0, LF0 and HGC1, HGC2.

The difference was only due to decreased amount of continuous phase available for drug to be solubilized in HGC1 and HGC2. Zero order as well as korsmeyer peppaskinetic model both were obeyed by all amphiphilogel formulations. The regression co- efficient (R^2) values were studied and results showed that korsmeyer peppas model wasthe best fit model and values of 'n' of all amphiphilogel formulations was found to be between 0.182-0.184showing simple diffusion release kinetics.

It can be clearly seen in table 7.24-7.25 that the highest drug wasdeposited in HF0 and LF0 containing no penetration enhancer while HF4 and LF4 deposited least amount of drug. The data from Table 4.22 and 4.23 suggested that the value of transdermal flux was highest in HF4 containing ethanol as penetration enhancer. All the formulation showed increased transdermal flux then HF0 containing no penetration enhancer. Transdermal flux of hydrophilic amphiphilogels was higher than lypophilic amphiphilogels. It was found that at 40^0C, there was a decrease in drug content to 1-3 % viscosity was increased upto 100-700 centipoise. There was no drastic change in pH of amphiphilogel and no syneresis occurred Furthermore the all amphiphilogels came to same state when the temperature was

Conclusions

In present research, on the basis of experimental results it has been concluded that the study reports the successful development of amphiphilogels (subset of organogels). Microscopic studies indicated that small flower-shaped clusters (size 40-50 µm) aggregated to form fibers. The pH of the organogels was in the optimum range. Studies proved that developed amphiphilogels possess enhanced release of piroxicam. The design and development of amphiphilogels (Organogel) based controlled release formulation for topical administration is to be tried and detailed studies have yet to be done on it. The mechanism of drug absorption and improvement of the absorption efficiency and the timing requirement is yet to be focused.

Incorporation of water and optimizing its concentration in amphiphilogels to improvethe 3D structures of tubules and clusters for achieving better controlled release properties.

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