Rheological behavior and viscosity measurement

The performance of topical formulation is monitored by its rheological behavior, which govern its flowability, spreadability and release of drug. The rheological evaluation of pharmaceutical semisolids is useful since it provides a method of quality control during and after the manufacturing process and information about the structure of the phase's information present in a product and the influence of various agents used in the formulation. Nanoemulgel was subjected to rheological examination. Farrow's constant (N) of the formulation BG6 was found to be 1.52. A value of $N>1$ indicates pseudoplastic flow or shear thinning and $N<1$ indicates dilatant or shear thickening flow [Figure 5]. The results confirmed that the nanoemulgel exhibited pseudoplastic behavior. This pseudoplasticity results from colloidal network structure that aligns itself in the direction of shear, thereby decreasing the viscosity as the shear rate increases. The pseudoplastic flow performance justifies that the developed system will require some force to expel.

It can be observed [Table 3] that increase in surfactant concentration leads to increase in viscosity of the nanoemulgel. Tween 80, used as surfactant here, was more soluble in the external aqueous phase. This is due to the concentration of water soluble surfactant in the system increased, the self-association of these amphiphilic molecules increased and formed different sizes and shape of micellar aggregates.[32] As the concentration in external phase increased, the network will form between the surfactants molecules, micelles and oil droplets. The denser the network the closer the distance between the dispersed phase, the higher the viscosity.

Skin permeation studies

The *ex vivo* skin permeation studies were carried out to confirm as well as to compare the permeation potential of the nanoemulgel formulations (BG1-BG9) [Table 4]. Nanoemulgel, in particular, was known to enhance permeation rates in deep skin layers and decrease lag time when compared to conventional formulations. It was well-reported in previous works that nanoemulgel could perform as drug reservoir where drug was released from inner phase to outer phase and then further into the skin. The enhanced transdermal drug delivery might had resulted due to different mechanisms, which include the permeation enhancement potential of different components of nanoemulgel.

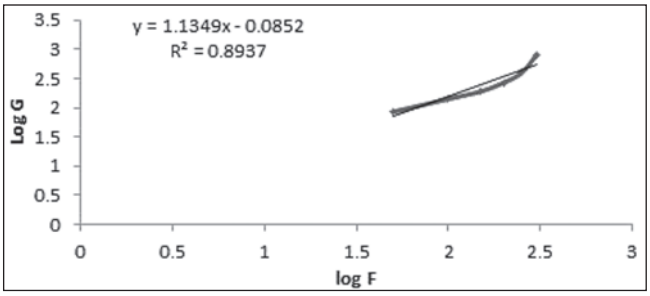

Figure 5: Rheogram depicting pseudoplastic behavior of nanoemulgel

In the present study, oleic acid (oil phase) was employed as an integral component which was widely known for increasing permeation the results revealed as the oleic acid in the formulation was increased from 5% w/w to 10% w/w, the flux (rate of permeation) was increased. This may be due to that oleic acid permeate within lipid bilayers of the stratum corneum by disrupting their order and management. Oleic acid had been commonly used as a powerful enhancer for many drugs,[33] including piroxicam.[34,35] The enhancing mechanism by oleic acid involves the increased fluidity of lipid portion of the stratum corneum.[36] [Figure 6] it was also observed that when the oleic acid concentration was further increased to 15% w/w, the flux gets decreased. This may well be correlated with aggregation of oil due to its higher concentration. Thus, formulation containing 10% w/w oil (BG4-BG6) attributed maximum permeation and less lag time in comparison to other nanoemulgel formulations.

Results also indicated that in nanoemulgel formulations (BG4-BG6) as the surfactant co-surfactant mixture concentration was decreased from 55% to 35% the skin permeation rate was increased to two-fold. The reason attributed to the situation

Table 4: Permeation parameters of various nanoemulgel formulations

Formulation code	CADP (mg/cm²)	Flux (mg/cm²/h)	Lag time (h)	Drug retained (mg)
BG1	0.78±0.03	0.033±0.001	0.53±0.01	1.12±0.03
BG2	0.80±0.06	0.034±0.002	0.51±0.01	1.04±0.02
BG3	0.82±0.04	0.035±0.002	0.48±0.03	0.94±0.04
BG4	0.87±0.06	0.036±0.003	0.46±0.02	1.69±0.05
BG5	0.95±0.05	0.040±0.002	0.45±0.01	0.73±0.01
BG6	1.00±0.04	0.042±0.003	0.41±0.03	0.09±0.001
BG7	0.77±0.03	0.032±0.004	0.51±0.02	1.22±0.03
BG8	0.79±0.05	0.034±0.002	0.54±0.04	1.12±0.06
BG9	0.81±0.04	0.035±0.003	0.55±0.03	1.03±0.08
BS1	0.61±0.04*	0.020±0.002*	0.77±0.04*	1.79±0.05*
BS2	0.64±0.02*	0.024±0.003*	0.74±0.02*	1.72±0.04*
BS3	0.68±0.05*	0.027±0.004*	0.71±0.03*	1.68±0.03*

CADP: Cumulative amount of drug permeated. *$P < 0.05$, Student unpaired t-test was used to compare BS batch (formulation prepared without co-surfactant) with BG6 (optimized formulation) for CADP, flux and lag time

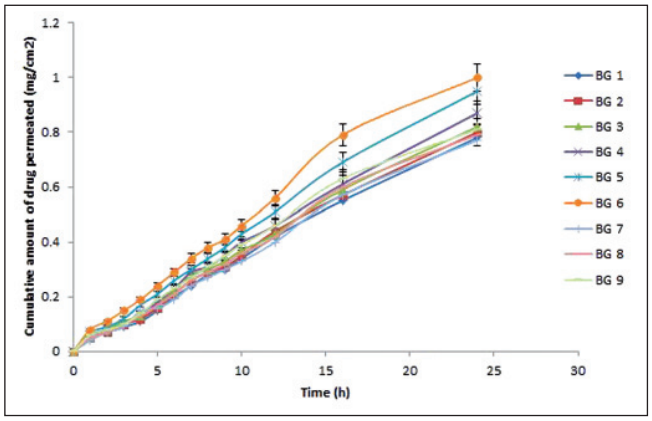

Figure 6: Permeation profile of nanoemulgel formulations (BG1-BG9)

could be an increase in thermodynamic activity of drug in nanoemulgel at lower content of surfactant.[15] It was well said that the thermodynamic activity of drug in the formulation was a sufficient driving force for the release and permeation of drug in to the skin.[5] The formulations (BG4-BG6) were also compared on the basis of concentration of aqueous phase, as water was the hydrophilic domain of nanoemulgel. When water content was increased, cumulative amount of drug permeated increased substantially. Furthermore, the flux calculated for formulation BG6 containing 55% of water was found to be significantly higher ($P < 0.05$, *t*-test) than formulation BG4 containing 35% water. The plausible rationale might be the entrance of aqueous fluid of nanoemulgel in polar pathway which increases interlamellar volume of lipid bilayer of stratum corneum, resulting in disruption of its interfacial structure and thereby, enhancing permeation.[3]

The cumulative amount of drug permeated, flux, enhancement ratio, lag time, skin retention and LAE were calculated for each formulation of nanoemulgel. The formulation BG6 having oil content 10% w/w, surfactant cosurfactant mixture 35% w/w, aqueous phase as 55% w/w showed highest CADP (1.0 ± 0.04 mg/cm^2) as compared to other formulations. Also a comparatively higher flux (0.042 ± 0.003) was observed for this formulation. In addition, lower lag time (0.41 ± 0.03 h) and less skin retention (0.09 ± 0.001 mg/cm^2) of BG6 than the other formulations tested, made it considerable for being selected as the optimized formulation.

Effect of ethanol as permeation enhancer

In order to investigate the effect of ethanol on the permeation of drug through skin, three batches (BS1-BS3) were prepared without the use of cosurfactant in the same composition as of BG4, BG5 and BG6. The BS1, BS2 and BS3 formulations showed significantly ($P < 0.05$) less permeation when compared with BG6 (optimized formulation containing cosurfactant) [Figure 7].

The lag time and drug retained was significantly higher in case of BS batch compared to BG6 formulation which explained that BS formulations showed less permeation as more amount of the drug was retained in the skin and took more time to permeate through the skin. The results confirmed that ethanol (cosurfactant) itself acting as permeation enhancer. The presence of ethanol decreased the ending stress of interface and made the interfacial film sufficiently flexible to take up different curvatures required to form nanoemulsions over a wide range.[37]

Comparison of permeation studies of marketed formulation, optimized nanoemulgel, plain nanoemulsion, plain drug gel and drug solution

The nanoemulgel formulation had higher flux (0.042 ± 0.003 mg/cm/h), than conventional marketed formulation (0.040 ± 0.02 mg/cm/h), plain drug gel (0.037 ± 0.02 mg/cm/h) and drug solution (0.032 ± 0.01 mg/cm/h) depicted in [Table 5]. The lower LAE of nanoemulgel which was found to be 0.09 elucidated the lower retention of drug in the skin and confirmed that the maximum amount of the drug has been permeated through skin. The LAE for drug solution was found to be highest, i.e., 1.10, showing that drug had permeated through skin to a negligible extent and retained in skin only.

Furthermore, low droplet size also accounts for high permeation potential of nanoemulgel. The number of droplets that could interact on fixed area of stratum corneum would increase as droplet size decrease. Also small droplets might embed into the stratum corneum without a transfer through hydrophilic phase of nanoemulsions and drug molecules were easily permeate into the skin. In the present study, nanoemulgel was also compared with plain nanoemulsion. It was observed that the flux of nanoemulgel was lower than nanoemulsion, which may be due to higher

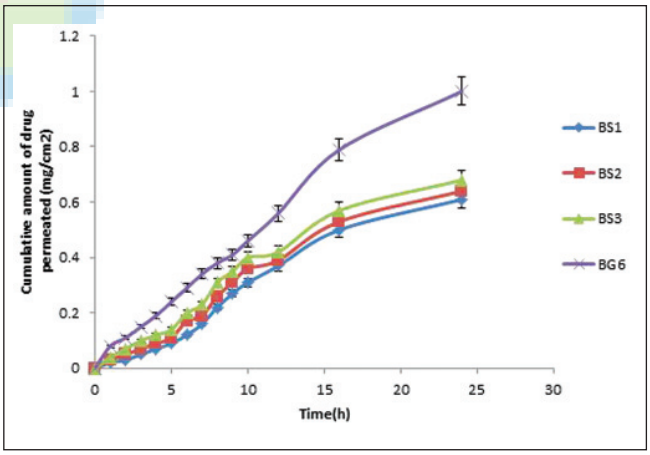

Figure 7: Comparison of permeation profile of BS (formulations without co-surfactant)

Table 5: Comparative permeation parameters of different formulations

Formulation code	CADP	Drug permeated %	Flux	Lag time	E_{pen}	Drug retained (mg)	LAE
Drug solution	0.83±0.02	81.5	0.032±0.01	0.79±0.11	1	0.92±0.02	1.10
Plain drug gel	0.93±0.03	91.3	0.037±0.02	0.72±0.25	1.15	0.43±0.03	0.46
Marketed formulation	0.96±0.05	94.2	0.040±0.02**	0.55±0.09	1.25	0.28±0.02	0.29
BP6	1.01±0.06**	99.1	0.044±0.04**	0.57±0.05	1.37**	0.04±0.02	0.03
BG6	1.0±0.04*	98.2	0.042±0.003*	0.41±0.03*	1.31*	0.09±0.001*	0.09*

CADP: Cumulative amount of drug permeated, E_{pen}: Enhancement ratio, LAE: Local accumulation efficiency = piroxicam retained into skin/piroxicam permeated through skin. *$P < 0.05$, Student *t*-test was used to compare B6 (optimized formulation) with drug solution, plain drug gel; **$P > 0.05$, Student *t*-test was also used to compare BP6 (plain nanoemulsion) with BG6 and marketed formulation, no significant difference was found

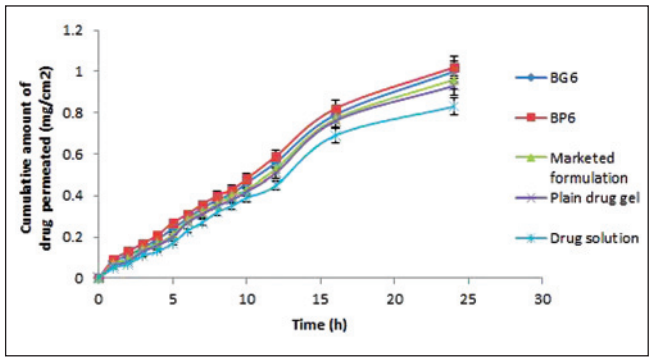

Figure 8: Comparison of permeation profile of optimized nanoemulgel BG6 with marketed formulation and plain nanoemulsion

viscosity of the formulation [Figures 8 and 9]. When the flux of plain nanoemulsion (BP6) was compared using unpaired Student *t*-test, no significant ($P > 0.05$) difference was observed [Table 5] though, the nanoemulgel had lower flux, it could be favored over the nanoemulsion, due to prolonged effect and increased viscosity from viewpoint of its applicability on skin.

Investigation of release kinetics

The release kinetics was studied by plotting *ex vivo* permeation data in various kinetic models. The best fit with highest r^2 value was found shown by zero order permeation for all nanoemulgel formulations. To analyze the release mechanism of drug from nanoemulgel, the data was fit to Korsmeyers peppas model, *n* value was obtained close to 0.5 suggesting that piroxicam was released from nanoemulgel through aqueous channels of gel matrix by Fickian diffusion release model.

Stability studies

Nanoemulgel formulations when centrifuged showed no phase separation or drug precipitation, indicating that prepared nanoemulgel were physically stable. They showed no signs of breaking or cracking even when subjected to freeze thaw cycles. Nanoemulgels remained clear even after a period of 3 months at temperature 25 ± 2°C, 40 ± 0.1°C and 4 ± 0.2°C. All the formulations were found to be consistent with respect to their pH values, drug content, phase separation and transparency during the stability study. Carbomer 934 in nanoemulgel resulted in high viscosity and oil droplets might be distributed in gel network, which might contribute to enhancement of stability of droplets in nanoemulsion.[38]

DISCUSSION

Nanoemulgel was proposed as a carrier for transdermal delivery of piroxicam due to its high solubilizing ability and permeation enhancing properties. Apart from this its transdermal route has the potential to bypass the problems associated with chronic oral delivery of piroxicam. The novel nanoemulgel system is drug loaded multi-component system containing oil, surfactant cosurfactant mixture, aqueous phase and gel base.

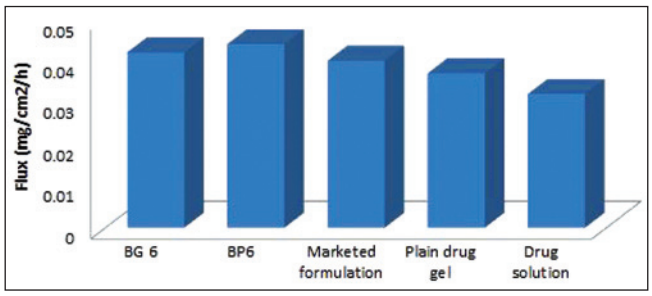

Figure 9: Comparative profile of flux for different formulations

The nanoemulsions were prepared by figuring out the concentration range of components.[5] All 9 nanoemulsions (B1-B9) formed were optimized for morphological structure, droplet size, viscosity and conductivity. The TEM image revealed spherical structure and all formulations were in nano size range (<200 nm) with low PDI value indicating uniformity of droplet size in formulation. The high conductivitry values confirmed the o/w structure of nanoemulsions. The viscosity values were found to be low which was not suitable for application on to the skin. Thus all nanoemulsions were incorporated in a gel base, carbomer 934 (0.5%), resulted in nanoemulgel. The formulations were subjected to drug content determination which showed that drug loss during formulation occurred within the limits. The pH was near to pH of the skin which revealed non-irritating nature of the formulation. The spreadability of all formulations exhibited slips and drag phenomenon with higher diameters. The viscosity was increased and rheogram displayed pseudoplastic behavior which ensures that the developed system will not flow by itself and when filled into a container viz. collapsible tube it will require some yield value for ejection.[22]

All nanoemulgels were subjected to permeation studies, nanoemulgel formulation showed significantly higher permeation which can be well correlated with the concentration of components. As the oil content was increased from 5% to 15% and content of surfactant cosurfactant mixture was decreased from 55% to 35% the permeation is enhanced. This synergistic effect was may be due to the oleic acid permeate within lipid bilayers of stratum corneum by disrupting their order and management and increase in thermodynamic activity of the drug in skin at lower content of surfactant.[18] The effect of cosurfactant (ethanol) as permeation enhancer was also established by comparing the BG6 (optimized formulation) with BS1-BS3 (formulations prepared without cosurfactant) which showed BS formulations had significantly lower cumulative amount of drug permeated with lower flux. Furthermore, significantly higher lag time and skin retention than BG6, proved the permeation enhancing effect of cosurfactant. Furthermore, the optimized formulation (BG6) was compared for various permeation parameters with plain nanoemulsion (BP6), marketed formulation (Feldene®), plain drug gel and drug solution. Results clearly indicated that BG6 had higher cumulative amount of drug permeated, flux, enhancement ratio and lower skin retention, LAE than marketed

formulation, plain drug gel and drug solution. However, BP6 showed higher flux than BG6, but not with significant difference ($P > 0.05$, *t*-test). Though the nanoemulgel has lower flux, it can be considered as superlative option over the nanoemulsion, because the prololnged effect of nanoemulgel can be expected. Moreover, pseudoplastic flow behavior imparted by gel makes naoemulgel superior in terms of ease of applicability.[36]

The formulated nanoemulgel system was found to possess good permeation potential without incorporation of any chemical enhancers which are habitually irritants.[7] Hence, the novelty of this system lies here, as the components (oil, surfactant and especially cosurfactant) of nanoemulgel their selves acted as permeation enhancers. The stability studies were carried out at room temperature and refrigerator temperature, indicating that the formulation is stable and no change in drug content and pH was observed. Thus, the nanoemulgel formulation could be beneficial in improving bioavailability and permeation of piroxicam for transdermal fungal infections without the use of chemical permeation enhancers.

CONCLUSION

The novel nanoemulgel of piroxicam with suitable viscosity was successfully formulated for transdermal application. Nanoemulgel was formulated by addition of carbomer 934 into nanoemulsion, which resulted in increase in viscosity and had no significant influence on penetration of piroxicam. The contact of the nanoemulgel with skin and effect of oleic acid and ethanol with fine permeation enhancing potential acted as key role for permeation of drug through skin. The optimized formulation was compared with conventional marketed formulation and showed higher permeation rate and significantly less drug retained which justifies the nanoemulgel system to be a promising surrogate carrier for transdermal delivery of piroxicam.

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