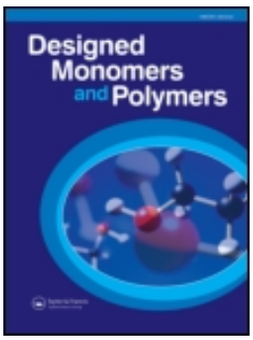

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Groundnut oil based emulsion gels for passive and iontophoretic delivery of therapeutics

Vinay K. Singh[a], Indu Yadav[a], Senthilguru Kulanthaivel[a], Bibhas Roy[b], Supratim Giri[c], Tapas K. Maiti[b], Indranil Banerjee[a] and Kunal Pal[a]

[a]Department of Biotechnology & Medical Engineering, National Institute of Technology, Rourkela, India; [b]Department of Biotechnology, Indian Institute of Technology, Kharagpur, India; [c]Department of Chemistry, National Institute of Technology, Rourkela, India

ABSTRACT

There is a persistent demand for an efficient drug delivery system, suitable for the delivery of both hydrophilic and lipophilic drugs. This study explores groundnut oil-based emulsion gels for the above-mentioned application. On the basis of stability, two representative gels OG5-80 (low oil content) and OG7-45 (high oil content) were studied further. Analysis of microarchitecture by ESEM and confocal microscopy, in conjugation with fluorescence recovery after photobleaching, confirmed the conversion of the dispersion phase from oil-continuous (OG7-45) to bicontinuous (OG5-80) with increasing water proportion. The gels were viscoelastic with unique stress relaxation properties. Passive and active (iontophoretic) release kinetics of the drugs showed differential release patterns. Mathematical modeling elucidated composition-dependent temporal variation in the drug release and stress relaxation patterns. *In vitro* cell viability study, cell cycle analysis, and immunocytochemistry divulged compatibility of the gels to human skin cells (keratinocytes). Drug-loaded gels were found active against *B. subtilis* and *E. coli*. Hence, groundnut oil-based emulsion gels can be an efficient and stable multimodal carrier system for the passive and the active delivery of both hydrophilic and lipophilic drugs.

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Groundnut oil; sorbitan monopalmitate; emulgel; biocompatibility; drug delivery

1. Introduction

In recent years, organogels have evolved as a novel therapeutic delivery system. Organogel can be defined as semi-solid formulation consisting of gelators [low-molecular-weight organic gelator (LMOG) or polymeric gelator] and an apolar solvent as the continuum phase. [1] The gelator mesh network is formed by self-assembly of the gelator molecules during organogel preparation. Stability of such self-assembled LMOG-based organogel (often referred as a physical gel) depends mostly on weak inter-chain interactions, such as, hydrogen bonding, van der Waals forces, and π–π stacking. The molecular interactions can be modulated by adding appropriate amount of water. In an early report, Scartazzini et al. [2] described gelation of lecithin on the addition of small quantities of water. Later, different kinds of organogels, capable of delivering therapeutics in a controlled manner were reported by various groups.[3]

Over the past few years, a few reports have been published on the topical application of organogels as drug delivery systems. Bhatia et al. [4] reported tamoxifen-loaded lecithin organogel for topical application. Balata et al. [5] investigated propolis organogel as a novel topical delivery system for treating wounds. Topical delivery systems are employed not only to treat skin related problems, but also for complications associated with eyes,[6] nose,[7], vagina [8], or rectum [9] etc. where patients compliance and satisfaction are essential. The fundamental advantage of the biphasic organogels is that it can deliver both hydrophilic and lipophilic drugs and enhance penetration of the drugs into the skin as a topical delivery system.[10] Despite its promising attributes, the number of organogel formulations reported till date is not significant and in many cases, its physicochemical properties and therapeutics delivery potentials are not well characterized. Also, they are often associated with poor physical stability that limits their practical application.

Keeping the aforementioned perspective in mind, in the present study, we have reported the preparation, physicochemical characterization, and drug delivery applications of a new set of organogels prepared using groundnut oil (GNO) and sorbitan monopalmitate (SMP). Water was added to modulate the physical stability of the prepared organogels to form emulgels (emulsion gels). For drug release study, two different drugs, namely, metronidazole (hydrophilic) and ciprofloxacin (lipophilic) were used.

CONTACT Indranil Kunal Banerjee Pal banerjeei@nitrkl.ac.in; palk@nitrkl.ac.in, pal.kunal@yahoo.com; kpal.nitrkl@gmail.com
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Sorbitan monopalmitate, commercially known as span 40, is a non-ionic emulsifier having hydrophilic-lipophilic balance value of 6.7. It has been extensively used in semi-solid formulations to immobilize apolar liquid component, but its application in pharmaceutical organogel or emulgel synthesis is not explored yet.

Groundnut oil is an edible vegetable oil obtained from the seed kernels of the groundnut. It has been used to develop formulations for drug delivery applications.[11] Oleic and linoleic acids make up for the 80% of the total fatty acids in groundnut oil. The oil also contains 6–8% of arachidic acid, arachidonic acid, behenic acid, lignoceric acid, and other fatty acids.[12] It also contains natural antioxidants (e.g. tocopherol and tocotrionols) and sterols (e.g. β-sitosterol), which are of nutritional importance.[13] Groundnut oil-based organogels were developed using aluminum monopalmitate for the delivery of interferon-α.[14] Barbeau et al. [15] used groundnut oil for making emulgels for extracting proteins from green leaves (alfalfa).

The study was carried out with an objective to find out groundnut oil-sorbitan monopalmitate water-based stable emulgels. For this purpose, numbers of different formulations were prepared in combinatorial fashion. Among the stable emulgels, two characteristically different formulations were chosen. Their physicochemical properties were investigated by organoleptic, microscopy, mechanical, thermal, and electrical property studies. Biocompatibility, especially skin cell compatibility, and therapeutics release were also tested in-depth. For drug release study, metronidazole (hydrophilic drug) and ciprofloxacin (lipophilic drug) was chosen as model drugs. Metronidazole (MZ), a synthetic nitroimidazole derivative, has been reported to be effective in treating topical complications such as chronic periodontitis,[16] bacterial vaginosis,[17] papules and pustules of rosacea [18], etc. Ciprofloxacin (CP) is a second-generation fluoroquinolone and is used in the treatment of topical complications such as conjunctival biota,[19] periodontitis [20] and myringosclerosis [21], etc.

2. Experimental section

2.1. Materials

Sorbitan monopalmitate was purchased from Loba Chemie, (Mumbai, India). Groundnut oil was obtained from Adani Wilmar Ltd. (Gujrat, India). Fetal bovine serum (FBS), Dulbecco's Modified Eagle's Medium (DMEM), FBS, 0.25% trypsin-EDTA, MTT, Rhodamine B and dialysis tubing (MW cutoff: 60 kDa) were procured from Himedia (Mumbai, India). HaCaT cell line was obtained from NCCS (Pune, India). Metronidazole was a gift from Aarti Drugs (Mumbai, India). Ciprofloxacin was purchased from Fluka (China).

The microbial cultures of *Bacillus subtilis* (NCIM 2699) and *Escheratia coli* (NCIM 2563) were obtained from NCIM (Pune, India). All experimental studies were carried out using double distilled water.

2.2. Methods

2.2.1. Preparation of organogels and drug-loaded organogels

Sorbitan monopalmitate was first dissolved in groundnut oil (60 °C, 500 rpm) maintaining an accurate w/w ratio. Water (60 °C) was added dropwise to the solution and the mixture (60 °C) was stirred for 30 min. The hot emulsion, so formed, was cooled down to room temperature (25 °C). Formulations of different compositions were prepared by varying sorbitan monopalmitate: groundnut oil: water ratio. The triphasic formulations either formed a semi-solid gel or remained as liquid emulsions. The concentration of the gelator (SMP) was varied from 2 to 15% (w/w) and the proportion of water was varied from 20 to 80% (w/w) to determine the critical gelation composition. The formation of the gels was confirmed by the inverted tube method. The gels were subjected to accelerated stability study by alternatively incubating at 70 °C (in a water bath) and at 4 °C (refrigerator) for 30 min for 7 cycles. The stability of the gels was checked by inverted tube method. Gels which withstood at least five cycles of thermocycling were considered as stable gels.

Metronidazole (model hydrophilic drug) and ciprofloxacin (model lipophilic drug) were incorporated in the optimized gel formulations. The method of preparation of the drug-loaded gels was similar to the blank gels. Drugs dispersed in groundnut oil were used for the preparation of the drug-loaded formulations. The final concentration of the drug in the formulations was 1% (w/w). Metronidazole and ciprofloxacin-loaded gels were designated as OG5-80M, OG7-45M and OG5-80C, and OG7-45C, respectively.

The long-term stability of the gels was studied as per the International conference on Harmonization guidelines. The gels were incubated at 25 ± 2 °C and 60% RH ± 5% RH for 12 months and checked at regular intervals. Syneresis or change in the color of the product was considered as signs of destabilization.

2.2.2. Physicochemical characterization of organogels

A detailed analysis of the physicochemical properties of the gels was done using different analytical techniques. Preliminary microarchitecture analysis of the gels was carried out by light microscope (Carl Zeiss, HBO 50, Germany). The microstructure was further investigated by environmental scanning electron microscope (Quanta 200, FEI, Netherlands) and confocal laser scanning microscope

(FluoView1000, Olympus, Hamburg, Germany). To examine the mobility of the solute molecules entrapped within the gels, fluorescence recovery after photobleaching (FRAP) analysis was employed.[22]

Mechanical properties of the gels were evaluated on the basis of the stress relaxation study using a static mechanical tester (Stable Microsystems, TA-HD plus, UK). The details of the instrumental parameters set for the mechanical property studies have been summarized in (Table SI of Supplementary Information).

2.2.3. Biocompatibility Study

Skin cell cytocompatibility of the organogels was evaluated using HaCaT cells (human skin keratinocyte). The effect of the releasate of the gel and the gel itself on the viability of the HaCat cells were tested *in vitro*. The details of the study have been provided as Supplementary Information.

2.2.4. In vitro drug release studies

2.2.4.1. Passive drug delivery. A two-compartment modified Franz's cell was used for the study. The compartments (donor and receptor) were separated by a dialysis membrane (MW cut-off – 60 kDa). The donor compartment contained 1.0 g of the drug-loaded gels while the receptor compartment contained 50 ml of water. The temperature of the dissolution media was maintained at 37 °C and stirred at 100 rpm. Water in the receptor compartment was completely replaced with fresh 50 ml of water at an interval of 15 min for first 1 h, 30 min for next 2 h and on an hourly basis for the next 9 h. Collected samples were analyzed at 321 nm and 271 nm for the determination of metronidazole and ciprofloxacin, respectively, using UV–visible spectrophotometer (UV 3200 double beam, Labindia).

2.2.4.2. Active drug delivery. An *in-house* developed iontophoretic device was used to perform the *in vitro* drug release (active and passive form) studies of metronidazole-loaded gels. During the experiment, care was taken to keep the current density below 0.5 mA/cm^2 as per the FDA regulations. A microprocessor (SPEEDY-33, National Instruments, USA, controlled LabVIEW 8.6 software) was used to generate a sinusoidal signal of 440 Hz (amplitude of 3.1 V). The system ensured 0.048 μA/cm^2 current density into the iontophoretic diffusion cell. 10.0 g of metronidazole-loaded gels were taken in the donor compartment (attached with an active electrode) separated from the receptor by a dialysis membrane (MW cut-off – 60 kDa). The receptor compartment contained 130 ml of water as the dissolution media, kept on stirring (100 rpm) at 37 °C. Samples were collected at regular intervals for 2 h. Three milliliters of the samples were drawn from the receptor and was subsequently replenished with 3 ml of fresh water. The releasate were analyzed at 321 nm using UV–visible spectrophotometer (UV 3200 double beam, Labindia).[23]

2.2.5. Statistical analysis

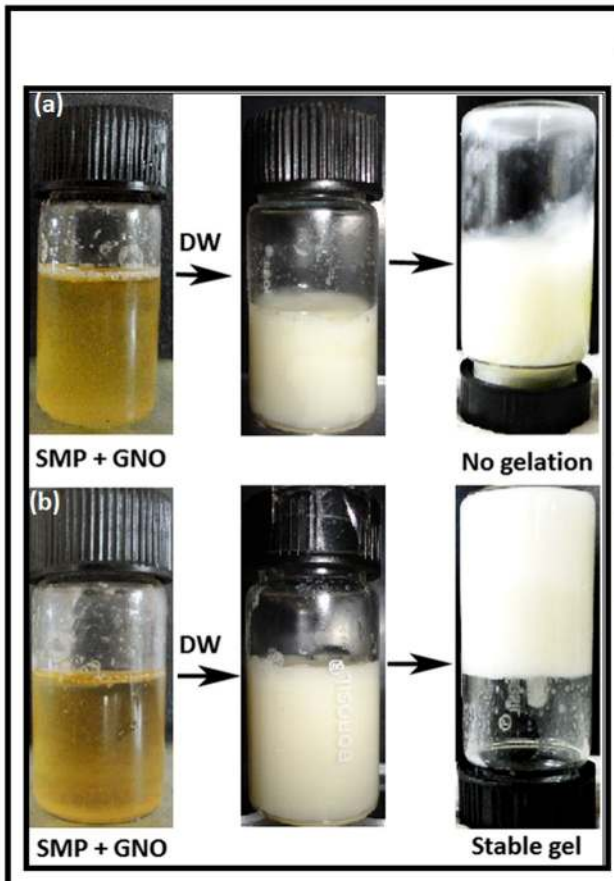
All the measurements were done in triplicate and represented as mean ± SD. Least significant difference test and single-way ANOVA was applied to determine significant differences among the means at $p \leq 0.05$.

3. Results and discussion

3.1. Preparation of emulgels

Preparation of emulgels was done by varying the proportions of the gelator, oil, and water. During the formation, transparent solution of sorbitan monopalmitate in groundnut oil turned into a white turbid emulsion upon addition of water at 60 °C (shown in table embedded in Figure 1). As the temperature of the solution was lowered to room temperature (25 °C), the solution either remained as liquid or formed a semi-solid formulation depending upon the composition (Figure 1(a) and (b)). The critical gelation concentration for the groundnut oil: sorbitan monopalmitate: water system was found to be 3:37:60 w/w. When the gelator concentration was <3% w/w, the liquid mixture failed to form gels. At 3% w/w of sorbitan monopalmitate, the gelation of groundnut oil occurred only when the proportion of water was 60% w/w (Figure 1(c)). As the gelator proportion was increased, the gelation was achieved even at lower proportions of water. With the increase in the relative proportion of water, there was an increase in the viscosity of the gels. The gels were white/off-white in color, greasy to touch, and had smooth textures.

The major concern about the commercial use of monophasic organogels as a drug delivery vehicle is the syneresis of the oil from the gelled structure, associated with the lower thermal stability of the formulations. The term 'stability' in case of these formulations implies the on-shelf stability as well as the stability under exaggerated environmental condition (referred as accelerated stability). In the present investigation, accelerated stability test has been chosen as a criterion to select the composition of the gels for further study. The red outlined box in Figure 1(d) identify the gel formulations which initially formed gel, but got destabilized during the accelerated stability test. The results showed that most of the gels, having gelator concentrations of 3, 4, and 5% w/w failed to retain their structural integrity during the test. Based on the results of the accelerated stability test, two gels were selected for further studies. The composition of the first gel (OG5-80) was 5% w/w of sorbitan monopalmitate, 15% w/w of groundnut oil and 80% w/w of water, whereas, the composition of the other gel (OG7-45) was 7% w/w of sorbitan



(c) Optimization of gel formulations

SMP (% w/w)	DW (% w/w)						
	20	40	45	50	60	70	80
15	X	√	√	√	√	√	√
10	X	√	√	√	√	√	√
8	X	X	√	√	√	√	√
7	X	X	√	√	√	√	√
6	X	X	X	√	√	√	√
5	X	X	X	√	√	√	√
4	X	X	X	X	√	√	√
3	X	X	X	X	√	X	X
2	X	X	X	X	X	X	X

(d) Selection of formulation on the basis of accelerated stability study

SMP (% w/w)	DW (% w/w)						
	20	40	45	50	60	70	80
15	X	√	√	√	√	√	√
10	X	√	√	√	√	√	√
8	X	X	√	√	√	√	√
7	X	X	√	√	√	√	√
6	X	X	X	√	√	√	√
5	X	X	X	X	X	X	√
4	X	X	X	X	X	X	X
3	X	X	X	X	X	X	X
2	X	X	X	X	X	X	X

Figure 1. Pictorial representation of the formation of emulgels (a) composition failed to form gel, (b) composition formed gel evident from tube inversion study, (c) tabular representation of optimization of the gels, and (d) selection of gels on the basis of accelerated stability study.

monopalmitate, 48% w/w of groundnut oil, and 45% w/w of water. OG5-80 was selected as it was having the minimum gelator concentration among all the samples that passed the accelerated stability test. Since OG5-80 had higher proportion of water (80% w/w), there was a chance of phase inversion during the formation of the gelled structure. This could have resulted in the formation of hydrogel-based emulgel. OG7-45 was selected since it was expected to be an organogel-based emulgel, due to the presence of higher proportions of oil (48% w/w of groundnut oil). It was further observed that the gels, which passed the accelerated stability test, were stable even after 12 months of storage at 30 ± 2 °C and 75 ± 5% RH.

Formation of physical emulgels depends on the delicate balance of different non-specific intermolecular interactions like electrostatic interaction, van der Waals interaction and π–π interactions among the gelator, lipid and water molecules. Therefore, any variation in the relative composition has a profound effect on the stability of the emulgel. In the present study, it was observed that the formation of the emulgel depends typically on the concentration of the gelator molecules. None of the compositions with gelator concentration <3% w/w resulted in the formation of stable gels. It is important to mention that an appropriate amount of gelator (5% w/w) can provide a structural mesh, capable of holding even 80% w/w water of the total composition, as was observed in case of OG5-80. However, most of these gels, when subjected for thermocycling (accelerated stability test), lost their structural rigidity. Interestingly, it was observed that such stability is also associated with the gelator concentration. FTIR study (Figure S1) indicated formation of intermolecular hydrogen bonding among the gelator and the polar phase, which could be the reason for such kind of gelator concentration dependent stability of the emulgel. Behera et al. [24] has reported similar type of intermolecular hydrogen bonding in span 80–Tween 80-based fluid-filled organogels. However, 'intermolecular hydrogen bonding' alone cannot explain the collapse of the rigid gel structure during thermocycling (accelerated stability study). Such

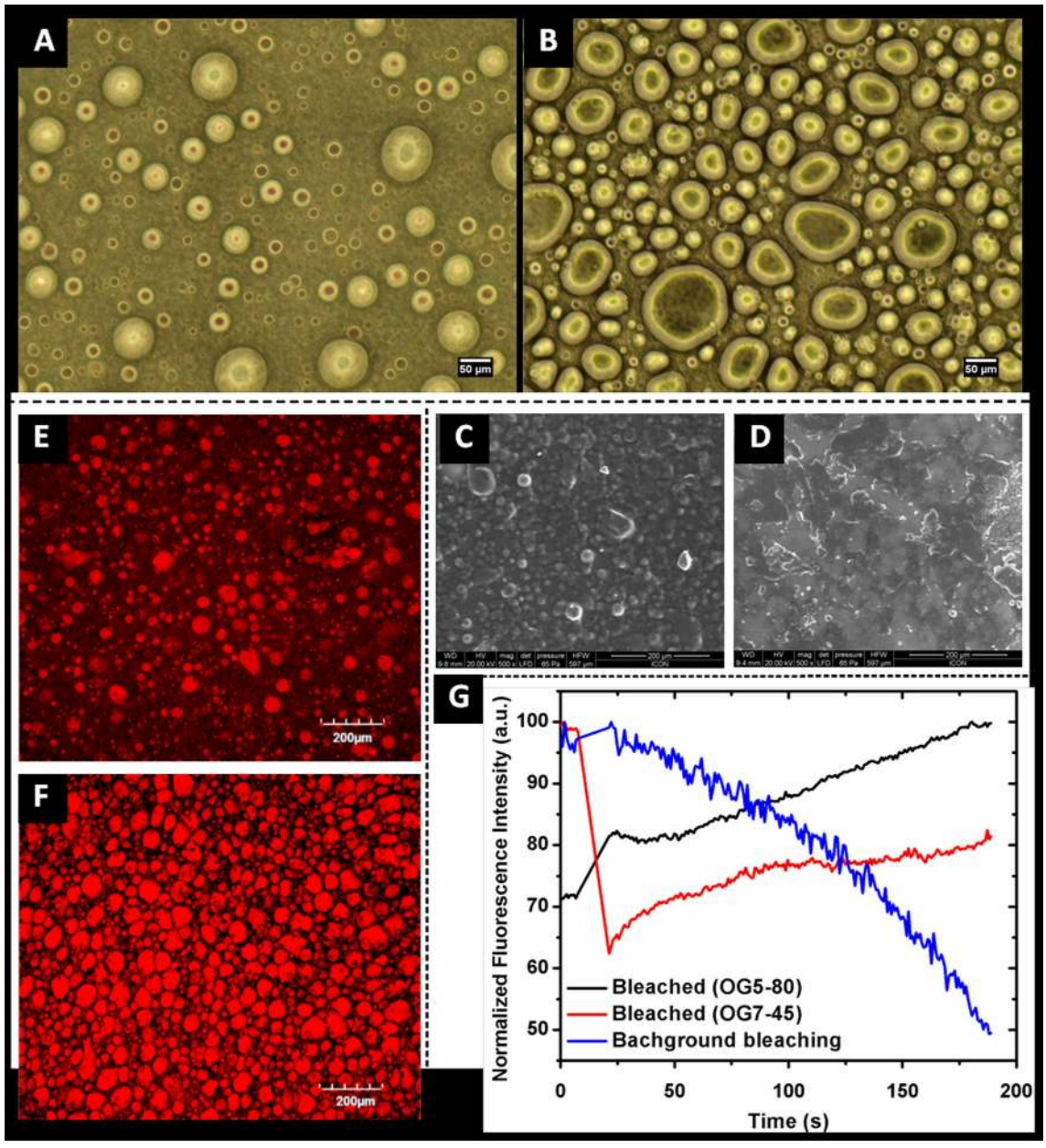


Figure 2. Light microscopy of the gels: (A) OG5-80, (B) OG7-45; environmental scanning electron microscopy of the gels: (C) OG5-80 and (D) OG7-45; confocal laser scanning micrographs of the gels: (E) OG5-80, (F) OG7-45, (G) FRAP study.

phenomena can be deciphered by considering hydrophobic interactions inside the gel.

3.2. Physicochemical characterization of organogels

3.2.1. Microarchitecture analysis

Analysis of the microarchitecture of the freshly prepared gels by light microscopy showed uniformly dispersed globules throughout a continuum phase (Figure 2(A) and (B)). The droplets appeared as double layered indicating the existence of the gelator molecules at the interface of the two phases. There were a lesser number of droplets and the droplet size distribution was less uniform in OG5-80 as compared to OG7-45. Environmental scanning electron microscopy showed that OG5-80 possessed heterogeneous matrix structure along with few spherical structural moieties, whereas, OG7-45 was having evenly distributed spherical globules throughout the matrix (Figure 2(C) and (D)). Although light microscopy gave an indication of the presence of two phases, i.e. water and groundnut oil separated from each other probably by sorbitan monopalmitate, but it failed to explain whether water exist as the inner phase or continuum phase. To clarify this point, water soluble fluorophore (rhodamine B) was incorporated in the aqueous phase of the gels and further analyzed by

Table 1. Mechanical properties of the formulations.

Model	Parameters	Formulations		
		OG5-80	OG7-45	Metrogyl
–	F_0	8.307	33.789	34.001
	F_r	3.282	13.770	15.228
	%SR	60.493	59.248	55.213
Kohlrausch	σ_∞/σ_0	0.356	0.358	0.426
	σ_1/σ_0	0.644	0.642	0.574
	T	1.479	1.479	1.426
	B	0.447	0.447	0.684
	R^2	0.959	0.954	0.938
Weichert	P_0	0.356	0.358	0.426
	P_1	0.300	0.312	0.359
	τ_1	0.434	0.352	0.498
	P_2	0.335	0.320	0.200
	τ_2	7.207	7.870	8.699
	R^2	0.992	0.997	0.997

confocal laser scanning microscopy. The confocal micrographs (Figure 2(E) and (F)) showed the existence of fluorescence in the dispersed droplets of OG5-80. This implied that the internal phase was aqueous in nature in OG5-80. This is typically the microarchitecture of the emulsion organogels. OG5-80 also showed feeble fluorescence in the continuum phase, suggesting bicontinuum dispersion phase. In case of OG7-45, the fluorescence was only found in dispersed droplets that confirmed oil continuous dispersion phase. Mobility of solutes inside an emulsion gel depends on the mesh structure of the gel and the nature of the emulsion (i.e. relative abundance of different phases). [25] However, mesh size plays a significant contribution only when the size of the solute molecule is comparable to the mesh dimension. In this present study, a small molecular weight fluorophore was used to avoid the complications described above. In such case, mobility of the solutes (in terms of recovery) would directly correspond to the microenvironment of the gels.[26] FRAP analysis showed that in OG7-45, the solute molecules were experiencing certain transportational limitations. The graph showed a typical FRAP profile where the recovery of the fluorophore at the bleached region of interest is limited and attained only 50% of its initial intensity (Figure 2(G)). In case of OG5-80, an anomalous behavior was obtained pertaining to more than 100% recovery at the region of interest. A plausible explanation could be the existence of bicontinuous dispersion phase- or heat-induced rupture (during photobleaching) of the microstructure that facilitates the solute transport at that site. Our earlier confocal study showed a dispersed fluorescence in OG5-80. Hence, the anomalous dye transport inside OG5-80 may happen because of the bicontinuous nature of the organogels.

The confocal study showed that both the gels have water as the dispersed phase. During thermocycling, crystallization of water took place in a dynamic hydrophobic environment which altered the interfacial surface energy, therefore, changing the droplet size. As a consequence, hydrophobic interactions between the droplets change with every thermal cycle. This render the formulations unstable, until and unless enough intermolecular hydrogen bonding is there to retain the structural rigidity. In this regard, a critical analysis of the microarchitecture of the thermocycled samples (after accelerated stability study) revealed that the size distribution of the droplets (dispersed phase) became narrower after thermocycling (Figure S2), which supports the arguments. Moreover, decrease in the average droplet size was more in OG7-45 (45% water) in comparison to OG5-80 (80% water), which further confirms the role of hydrophobic interaction during thermocycling.

3.2.2. Mechanical properties

Detailed analysis of the viscoelastic properties (stress relaxation) of the organogels was done using static mechanical tester and results are reported in Table 1. For comparison, commercial gel formulation Metrogyl® (available in market) was used as the control. Stress relaxation is a test wherein the samples are subjected to strain followed by the monitoring of the stress (or force values) on the samples for a definite period of time. The maximum force achieved when a definite strain is achieved is regarded as F_0. F_0 provide information about the firmness of the formulations. Analysis of the results (Table 1, Figure 3) suggested that OG5-80 had lowest firmness as compared to OG7-45 and Metrogyl®, which had similar F_0 values. This implies that OG5-80 has lower mechanical strength as compared to OG7-45 and Metrogyl®. The results also indicate a decrease in the mechanical strength with an increase in the water content of the gels. The force rapidly exponentially decreased to a residual value, F_r. Percent stress relaxation (%SR) was calculated from F_0 and F_r values (Equation 1). Interestingly, the %SR values of OG5-80 and OG7-45 were similar (~60%) and were higher than the Metrogyl®. This indicated viscoelastic nature of the formulations. It can further be predicted that either the molecular rearrangement of the molecules

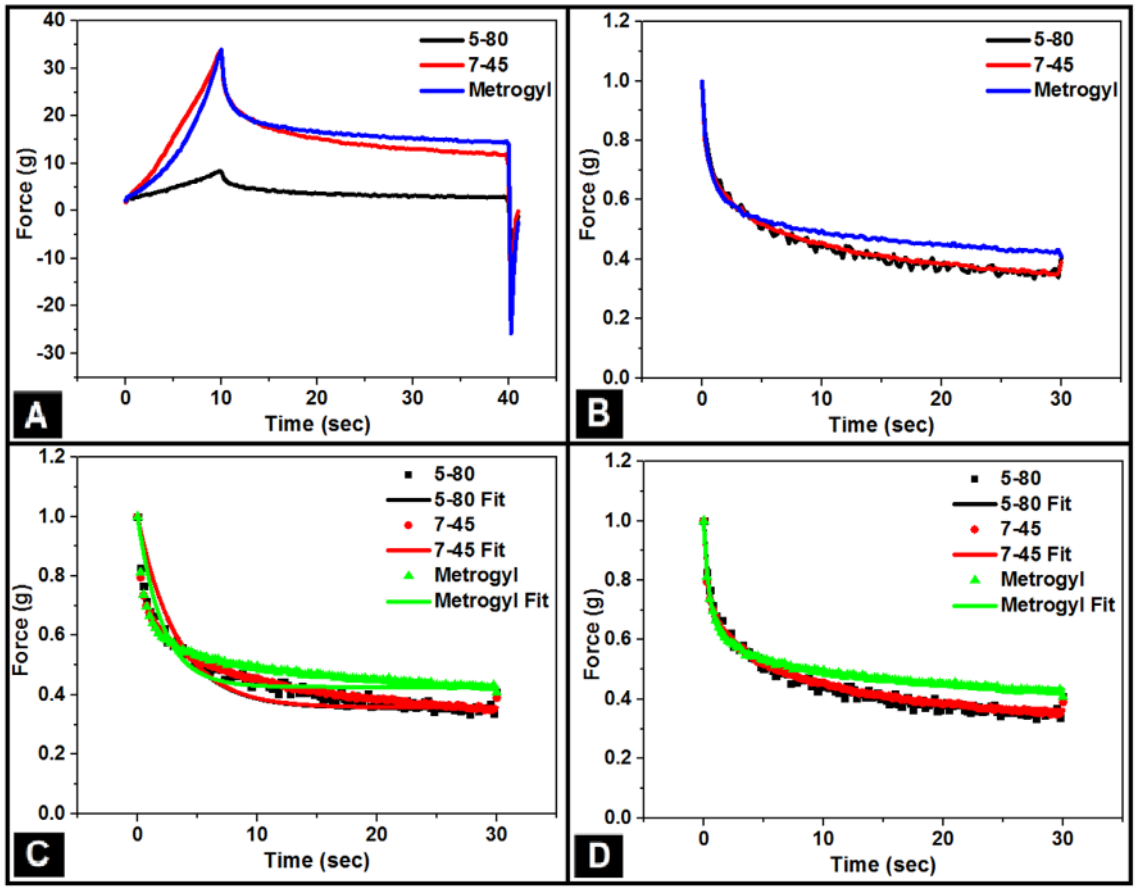


Figure 3. Mechanical property of the gels. (A) stress relaxation profiles, (B) normalized stress relaxation profiles, (C) Kohlrausch model fitting of the normalized profiles, and (D) Weichert model fitting of the normalized profiles.

in OG5-80 and OG7-45 were better than Metrogyl® or the network structure of OG5-80 and OG7-45 was compromised very easily. The relaxation profiles were normalized and fitted to Kohlrausch (Equation 2) and Weichert (Equation 3) models of viscoelasticity. The model parameters were calculated by nonlinear least sum of squared difference method. Limiting stress (σ_∞/σ_0), transient stress (σ_1/σ_0) and relaxation time (τ) were calculated from the Kohlrausch model.[27] Limiting stress is a marker of the inherent stability (elasticity) of the formulations. From the results, it was found that Metrogyl® had better inherent stability as compared to OG5-80 and OG7-45. The transient stress is an indicator of fluidic component within the formulations. Quite expectedly, the fluidic component of OG5-80 and OG7-45 was found to be higher. Even though there was a marked difference in the limiting stress and transient stress values of OG5-80 and OG7-45 as compared to Metrogyl®, the relaxation time of all the formulations was nearly equal. Like the limiting stress value (from Kohlrausch model) was equal to the P_0 values (from Weichert model, Equation 3).[28] This is because both limiting stress and P_0 values are the marker of the inherent elastic component of the formulations. P_0 of an ideal elastic body is 1, whereas, it is 0 for an ideal liquid. Any value in between 0 and 1 suggests the viscoelastic nature of the formulations. In this study, P_0 was in the range of 0.35 and 0.45; suggesting a viscoelastic fluidic nature of the formulations and Metrogyl®.[29] Instantaneous (τ_1) and delayed (τ_2) relaxation times were also calculated from the Weichert model. Instantaneous relaxation time is due to the molecular rearrangement within the formulations. The instantaneous relaxation time was in the order of OG7-45 < OG5-80 < Metrogyl®. Hence, it can be predicted that the molecular rearrangement was far superior in OG7-45, followed by OG5-80 and Metrogyl®, respectively. The delayed relaxation time, associated with the breakage of the intermolecular associations, was in the order of OG5-80 < OG7-45 < Metrogyl®. This suggested that even though the intermolecular rearrangement of OG7-45 was superior than OG5-80, the disruption of the intermolecular interactions occurred relatively quickly in OG5-80.

$$\%SR = \frac{F_0 - F_r}{F_0} \times 100 \quad (1)$$

where, F_0 = peak force at a target distance of 10 mm from a trigger force of 5 g; and F_r = final force after holding the probe for 30 s.

$$\frac{\sigma_t}{\sigma_0} = \frac{\sigma_\infty}{\sigma_0} + \frac{\sigma_1}{\sigma_0} \exp\left[-\left(\frac{t}{\tau}\right)\beta\right] \quad (2)$$

where, σ_t/σ_0 = normalized stress or force profile, σ_∞/σ_0 = limiting stress, σ_1/σ_0 = transient stress, t = time, τ = relaxation time, and β = spreading function.

$$P_{(t)} = P_0 + P_1 \cdot e^{-t/\tau_1} + P_2 \cdot e^{-t/\tau_2} \quad (3)$$

where, P_0, P_1, and P_2 are the pre-exponential factors; and τ_1 and τ_2 are the relaxation times of the dashpots.

3.2.3. Skin cell compatibility test

Epidermal keratinocytes are the first cells that get exposed to any topical formulations *in vivo*. Therefore, understanding the effect of a topical formulation on skin keratinocytes is paramount. So far, many oil-based topical formulations were reported.[30] Groundnut oil is extensively used for edible purposes. From the 'test on extract' study, it was observed that the releasate of OG5-80, when applied to the cells in concentrations of 10% v/v of total media, showed no cytotoxicity. But in case of OG7-45, a slight decrease in cell viability was observed with respect to control (0.25 fold) and OG5-80 (0.1-fold). Cell cycle analysis showed that the population of cells in G1 phase (57.3%) in OG7-45-treated cells was higher in comparison to OG5-80-treated cells (49.0%) and control (48.95%). The S phase for OG5-80 was 35%, whereas, the same was 29 and 36% for OG7-45 and control, respectively. Cell viability and cell cycle data implied that the organogel releasate of OG7-45 and OG5-80 has no significant toxic effect on human skin keratinocyte.

To study the effect of the gel formulations on the skin keratinocytes, cells were incubated with the gels of different concentration (100 ng/ml–100 µg/ml) for 24 h following the protocol described by Kimura et al. [31]. MTT assay (Figure 4(A)) showed that both the formulations did not affect the viability over a range of 100 ng/ml–100 µg/ml. However, a little decrease in the viability (11%) was observed at a higher concentration (100 µg/ml) for OG 5-80-treated cells. It is important to mention that relatively less cell viability was observed in the case of OG7-45 when releasate was used. It may possible that in the presence of extracellular enzymes and reactive oxygen species OG5-80 got degraded at a faster rate and produced more free lipid molecules and surfactants that damaged the cell membrane. To confirm this point, LDH assay-based evaluation of cell membrane integrity was carried out (Figure 4(B)). For both the samples, LDH activity was not more than 15% with respect to the control. This implied that formulations were cytocompatible. Further analysis showed that LDH activity in the supernatant of OG5-80 was 1.1-fold higher than OG7-45. This supports the MTT assay result. Flow cytometry-based cell cycle analysis (Figure 4(C)) showed that there was no significant variation in the cell cycle among the three sets (control, OG5-80, OG7-45). Cell populations in the G1 phase were 52.0, 52.5, and 49% for control, OG7-45 and OG5-80, respectively. However, a small pro- G_0/G1 cell population (5% approx.) was observed in the case of OG5-80 which could be considered as apoptotic population. This cell cycle results corresponds to the earlier findings (MTT assay and LDH assay). Immunocytochemistry (Figure 4(D)) showed that these organogels have no detrimental effect on the cytoskeletal organization. In all cases, cells were well adhered to the matrix and showed a characteristic F-actin distribution.

3.2.4. In vitro drug release studies

3.2.4.1. Passive drug delivery. *In vitro* drug release study was done to predict the drug release pattern and release kinetics. The study helps in correlating the drug release mechanism when administered in the physiological system. The release of a drug from the formulations depends upon its solubility and partition coefficient. The cumulative percentage drug release (CPDR) profile of metronidazole from OG5-80M, OG7-45M, and Metrogyl® has been shown in Figure 5(A). Metrogyl® has shown almost complete release of the drug from the formulation in 8 h, whereas, for OG5-80M it was 7 h. Interestingly, OG7-45M has shown approximately 81% w/w drug release in 8 h and nearly complete release at 12 h. This suggests that the variation in the gel structure may lead to different type of release kinetics that could be exploited for various drug delivery applications.

CPDR profile of ciprofloxacin (lipophilic drug) has been shown in Figure 5(B). The results suggested that the CPDR from the drug-loaded formulations were 9.05 and 9.85% (w/w) for OG5-80C and OG7-45C, respectively, at the end of 12 h. It may be associated with the hydrophobic nature of the drug, which resulted in the release of the lower amount of the drug from the formulations. The release profile suggested that the organogels may be a suitable carrier for sustained release of lipophillic drug for longer time periods.

Korsmeyer-Peppas model was used to examine the release pattern of the drugs from the developed formulations.[32] The diffusion parameters (release rate constant: *K*; and release exponent: *n*) of the drugs were estimated using Korsmeyer-Peppas model (Equation 4). Among the metronidazole containing formulations, the release rate constant was highest in Metrogyl® followed by OG5-80M and OG7-45M, respectively (Table 2). This suggested that the diffusion of the drug within the formulation matrix was in the order of Metrogyl® > OG5-80M > OG7-45M. This can explain the faster release of the drug from Metrogyl® and OG5-80M as compared to OG7-45M. There was not much

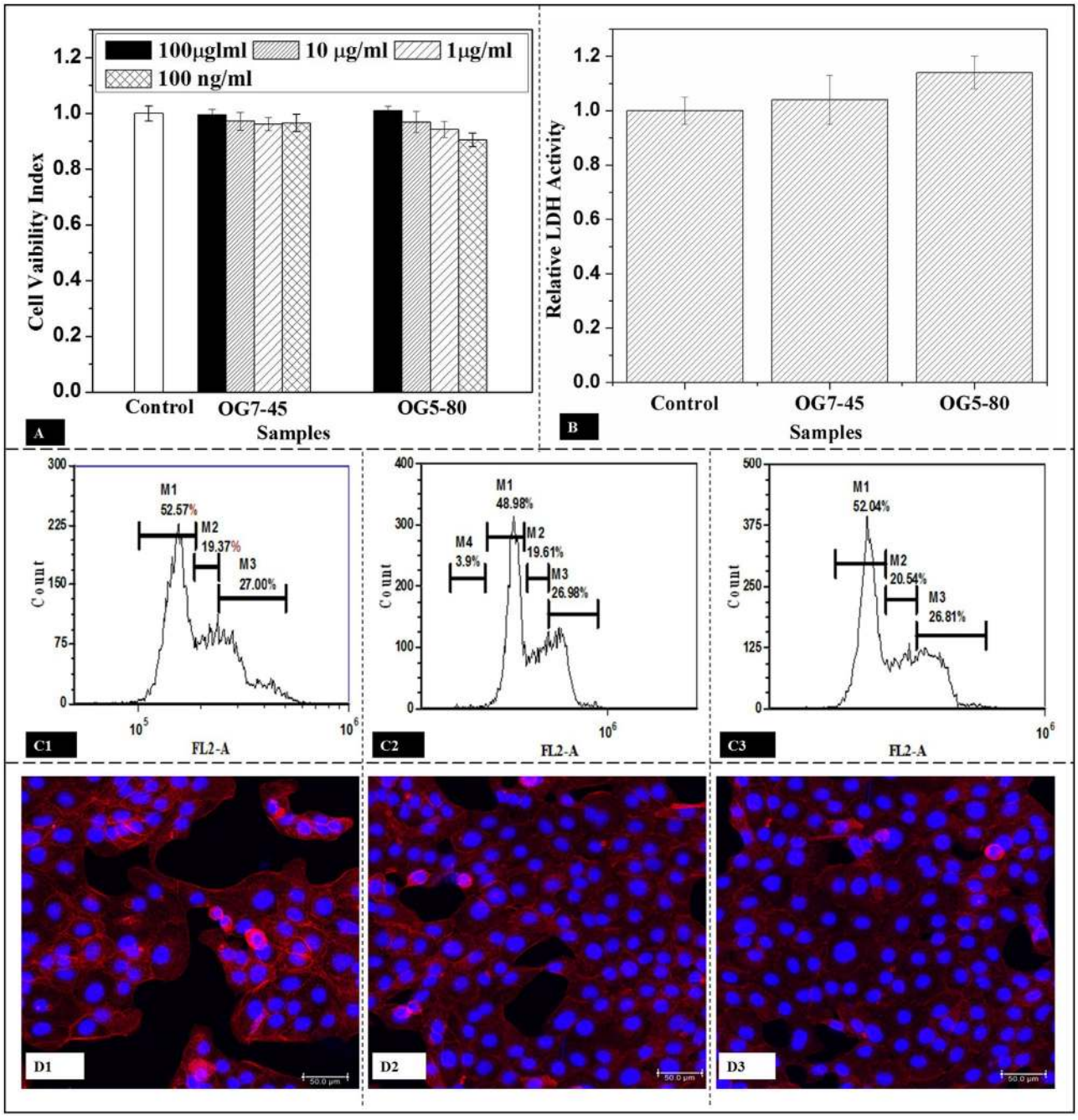


Figure 4. Cytocompatibility study using organogels of different concentrations. (A) Cell viability study. (Data were expressed as mean ± SD, $n = 6$; statistical significance was tested using ANOVA). (B) LDH assay (C1-3) Cell cycle analysis of HaCaT cells treated with organogels (C1: control, C2: OG5-80, C3: OG7-45). In all histograms first peak corresponds to G1 phase followed by S phase and G2/M phase. (D1-3) Immunocytochemistry analysis of HaCaT cell morphology (D1: control, D2: OG5-80, D3: OG7-45). F actin is stained with TRITC Phalloidin (Red) and nucleus is stained with Hoechst (blue). For LDH assay, cell cycle analysis and immunocytochemistry, organogels were used at a concentration 100 µg/ml.

difference in the release rate constant of ciprofloxacin. This can explain the near similar release pattern of ciprofloxacin from OG5-80C and OG7-45C. The release exponent (*n*-value) was found to be in the range of 0.45 and 0.89. This suggested anomalous diffusion was predominant during the release of the drugs from the formulations. A further in-depth analysis of the release profiles using Peppas–Sahlin model (Equation 5) suggested that the release of the drug was purely Fickian diffusion with no contribution of the polymer relaxation process ($k_2 = 0$) (Equations 6 and 7).[33] This suggested that the anomalous diffusion, as predicted from the Korsmeyer–Peppas model, may be due to the erosion of the gel structure during the release process in association with the Fickian diffusion.

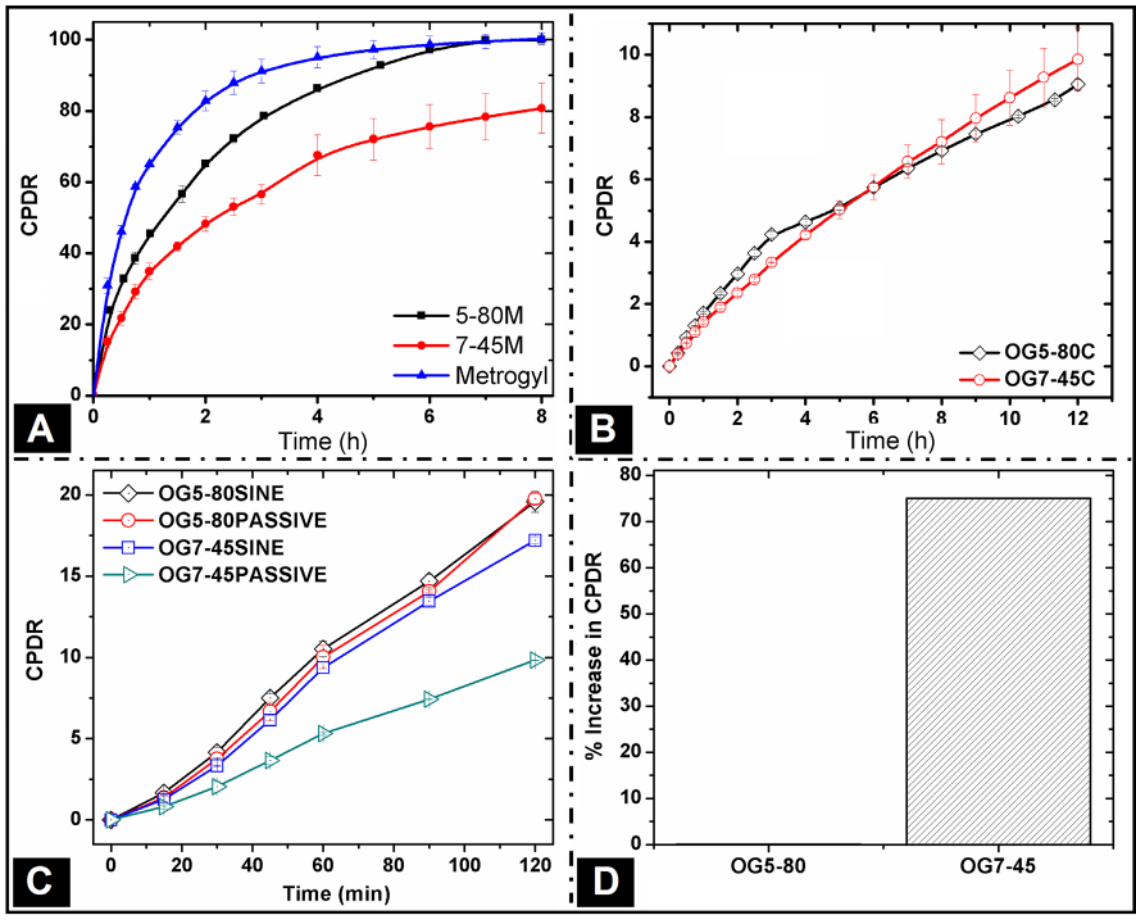


Figure 5. Drug release studies. Cumulative percent drug release (CPDR) of: (A) metronidazole, (B) ciprofloxacin from Metrogyl® and the prepared organogels, and (C) Iontophoretic release of metronidazole from the gels by active and passive mechanisms, and (D) % increase in CPDR after iontophoresis.

Table 2. Drug release kinetics parameters.

		Formulations				
Model	Parameters	OG5-80M	OG7-45M	Metrogyl	OG5-80C	OG7-45C
Korsmeyer–Peppas	K	45.87	33.28	69.41	1.86	1.39
	n	0.49	0.51	0.59	0.64	0.79
	R^2	0.99	0.99	0.99	0.99	0.99
Peppas–Sahlin	k_1	45.24	33.38	62.13	1.78	1.34
	k_2	0.00	0.00	0.00	0.00	0.00
	m	0.49	0.49	0.35	0.69	0.82
	R^2	0.99	0.99	0.99	0.99	0.99

$$\text{CPDR} = K \cdot t^n \tag{4}$$

where, CPDR = % drug released at time (t), K = diffusion rate constant of the drug within the polymer matrix, and n = diffusion exponent.

$$\text{CPDR} = k_1 \times t^m + k_2 \times t^{2m} \tag{5}$$

where, CPDR = % drug released at time (t), k_1 and k_2 = diffusion rate constant of the drug due to Fickian diffusion and polymer relaxation, and m = diffusion exponent.

$$F = \frac{1}{1 + \frac{k_2}{k_1} \times t^m} \tag{6}$$

where, F = contribution of Fickian diffusion during drug release.

$$R = \frac{k_2}{k_1} \times t^m \times F \tag{7}$$

where, R = contribution of polymer relaxation associated diffusion during drug release.

The fundamental advantage of emulgel as pharmaceutical formulation is its ability to deliver both hydrophilic and lipophilic drugs. In this present study, analysis of release

kinetics of metronidazole (model hydrophilic drug) and ciprofloxacin (model lipophilic drug) revealed that the rate of release of the lipophilic drug from both the formulation is almost 10-fold slower than the hydrophilic drug. This is expected because of the slower transport of the drug molecules from oil phase to the aqueous phase of the dissolution medium. Interestingly, at the early phase of drug release (within 4 h), the release of ciprofloxacin was higher in OG5-80 in comparison to OG7-45. This might be because of the biphasic nature of the OG5-80 gel which facilitates transport of lipophilic drug from oil phase to the release medium. It is important to mention that the FRAP study also indicated about the existence of similar kind of auxiliary transport phenomena in OG5-80. In case of metronidazole, OG7-45 has shown less release of drug in comparison to OG5-80. This is because of low water content within the formulation which resulted in restricted diffusion. FRAP analysis also showed a limited diffusion of solute in OG7-45.

3.2.4.2. Active drug delivery. Iontophoretic drug delivery is based on the principle of 'like charges repel each other.' This technique involves the application of an electric field to the charged drug molecules, which pushes the drug molecules out of the matrix by electrostatic repulsion and leads them into the systemic circulation. The preliminary experimentations were carried out using metronidazole-loaded gels. Dialysis membrane was used as a semi-permeable membrane. The release of metronidazole from OG5-80M was equal (~20%) under both active and passive conditions over a period of 2 h (Figure 5(C)). On the other hand, the release of metronidazole from OG7-45M was ~17 and ~10% during active and passive release studies, respectively, over a period of 2 h. The results suggested that in the presence of the electrical field, there was an increase in the rate of release of the drug. This effect was not seen in the release pattern of the drug from OG5-80M, but was prominent in OG7-45M (Figure 5(D)). This may have happened because of the presence of the water in higher proportions in OG5-80M which nullified the effect of the externally applied electrical field during the release study. The release results showed that the release of metronidazole from the gels followed zero-order kinetics, thereby, suggesting a concentration-independent release behavior. This study implied that OG7-45 may be used as carriers for iontophoretic drug delivery.

4. Conclusion

The present study delineates the development and characterization of emulgels of distinct features (bicontinuous and biphasic) and its potential application for drug delivery. The initial part of the investigation precisely reported that the accelerated stability study could be used as an early screening protocol for finding out an emulgel of desired stability. Subsequent efforts lead to the development of two stable and biocompatible emulgels (OG5-80 and OG7-45) that can be used as sustained as well as stimuli-responsive (iontophoretic) drug release system. A detailed analysis gives a clear indication that controlled maneuverasion in relative proportion of gelator and water content can lead to emulgels of desired physicochemical properties. Discrepancies in mechanical properties, solute mobility profile (as evident from FRAP studies) and drug release kinetics of the two gels clearly speaks for such possibilities. Moreover, the difference in the release profiles of hydrophobic and hydrophilic drugs from the emulgel highlights the possibility of using it as matrix for combination therapy. Skin cell cytocompatibility of the developed emulgels, especially OG5-80, implies its potential application in topical delivery. However, *in vivo* delivery in the animal model is required to confirm it.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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