Facile transdermal delivery of upconversion nanoparticle by iontophoresis-responsive magneto-upconversion oleogel  

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Keywords: upconversion, white emitting NaYF₄, oleogel, reactive oxygen species, skin-permeation enhancement.

Abstract
The effective application of upconversion nanoparticle (UCNP) as a photo-medicine in skin cancers critically depends on a facile transdermal delivery process through topical route. Herein, combining two non-invasive techniques, i.e. skin permeation enhancement and alternating current (AC) induced iontophoresis, we report a controlled transdermal delivery of UCNP with a time advantage. We have synthesized a series of soybean oil-based oleogels termed as magneto-upconversion (MU) gels by incorporating a fixed amount of UCNP and different proportions of magnetic nanoparticle (MNP) using stearic acid-based gelator as a skin permeation enhancing agent. The microstructures of the synthesized MU gels were characterized by microscopy, X-Ray diffraction and vibrational spectroscopy. A detailed analysis of the electrical properties revealed a gradual increase in the electrical conductance in the MU gel series with increasing proportion of MNP. Such trend of conductance imparted proportional iontophoretic response within the respective MU gels, validated through the release of ciprofloxacin hydrochloride as a model drug preloaded within the oleogels. Through a series of skin permeation experiment using pig ear skin as animal model, we established that the UCNP was able to permeate the whole thickness of the skin within as little as 3 h, only when the two conditions, i.e. the presence of skin permeation enhancer and iontophoresis were met. Within the same time, UCNP permeation was enhanced by the presence of MNP in the MU gels up to 2 folds. Our study developed a rational method for the transdermal delivery of any electrically non-conducting nanoparticle in a faster and tunable way.

1. Introduction

In recent times, near infra-red (NIR) triggered theranostics mediated by upconversion nanoparticle have emerged as a promising modality for minimally invasive photo-medicine [1–5]. Owing to the superior tissue penetration ability of NIR laser source (e.g. 808 nm, 980 nm, etc), success in non-invasive photochemical tissue bonding and tumor-induced wound healing has been achieved [6–8]. Such reports indicate the enormous potential of using NIR driven upconversion nanoparticle (UCNP) in non-invasive treatment of sub-cutaneous cancer and skin diseases. Surprisingly, the application of UCNP in the context of dermatology is extremely limited [9]. The major reason behind this limitation is the inability to localize the UCNP at a precise sub-cutaneous location up to a therapeutically significant extent. Conventional oral and parenteral routes fail in this context due to several clinical and pharmacokinetic disadvantages [10, 11]. Although the transdermal route offers an attractive viable option for the delivery of UCNP based photo-medicines, overcoming the stratum corneum (SC) barrier of skin poses an insurmountable challenge [12–14]. Thus, the wide application of UCNP...
as a non-invasive photo-medicine for skin diseases is critically dependent on the success of the transdermal delivery of the same.

Considerable research since ancient times has been concentrated on the transdermal delivery of medicines. Focused solely on topical application, the delivery of low-molecular-weight, low dose lipophilic drugs across the skin constitute first-generation transdermal delivery system (TDS) [15]. The restrictions regarding molecular weight, lipophilicity and dosage of drugs associated with first-generation TDS have led to the development of second-generation TDS, which utilizes the skin permeability enhancement strategy. Reversible disruption of SC layer either by chemical permeation enhancing agents or by iontophoresis or via non-cavitational ultrasound, serves as the key factor behind this strategy [15]. Although this approach demonstrates transdermal delivery of lipophilic and lipophobic drugs at a considerably higher dosage, it appears ineffective in delivering macromolecules across the skin [15]. While the third-generation TDS exhibits promise in effectively delivering molecules of a varying size range, it usually involves much stronger disruption of SC utilizing more aggressive techniques such as combination of chemical and biochemical enhancers, electroporation, microneedles, cavitational ultrasound, thermal ablation and microdermabrasion [15]. Moreover, majority of these third-generation techniques rely on minimally invasive clinical settings, which may invoke higher costs and cannot be self-administered [15]. Thus, it is a significant challenge to develop of second-generation TDS capable of delivering UCNP (average diameter ∼50 nm) across the skin, without causing any severe disruption of SC in a mild and non-invasive way, which can be potentially self-administered.

UCNP incorporated in soybean oil-based oleogel has been recently reported to overcome SC barrier through proof-of-concept experiments via topical route [9]. The major driving force for such UCNP permeation was reported to be the formation of transient pores in SC layer due to the presence of skin permeation enhancers present in the oleogel. However, the movement of UCNP across the whole skin sample turned out to be extremely slow (∼24 h), leading to potential problem in clinical application. Interestingly, it has been observed that in the presence of skin permeation enhancers, application of iontophoresis led to the more pronounced transient pore formation in the epidermis layer [16]. Such observation indicates that faster transdermal delivery of UCNP could be facilitated using permeation enhancer containing oleogels that simultaneously respond to electric field induced iontophoresis [17]. Imparting iontophoretic response within UCNP incorporated oleogel is a major hurdle since both the soybean oil-based oleogels and UCNP are expected to show high electrical impedance.

In this context, we report the development of UCNP and magnetic nanoparticle (MNP) incorporated soybean oil-based oleogel. Since, MNP incorporated oleogels (magnetogels) has already been reported to show alternating current (AC) induced iontophoretic behavior, assimilation of both UCNP and MNP in the oleogel is expected to make a final formulation capable of exhibiting faster transdermal delivery of UCNP under the dual action of both skin permeation enhancing and iontophoretic effects. Herein, we aimed to demonstrate that the combined effect of external AC field-induced iontophoresis and the presence of skin permeation enhancers leads to rapid permeation of UCNP along with the drug molecules across the full thickness of a porcine skin model through the development of MNP, UCNP and ciprofloxacin hydrochloride (model drug) incorporated oleogels. To the best of our knowledge, this is the first report of accelerated skin permeation of any electrically non-conducting nanoparticle through a mild, non-invasive approach involving techniques pertaining to second-generation TDS.

2. Materials and methods

2.1. Materials

Yttrium (III) oxide (Y₂O₃, 99.9%), Ytterbium oxide (Yb₂O₃, 99.9%), Thulium oxide (Tm₂O₃, 99.9%), sodium trifluoroacetate (NaCOOCF₃, 98%), Iron (III) acetylacetonate (Fe(acac)₃), 1-octadecene (ODE, 90%), and oleylamine (OL, 90%) were purchased from Sigma-Aldrich (reagent grade). The chemicals were used as received. Trifluoroacetic acid (TFA, 95%) was purchased from Finar and used as received. Stearic acid and chloroform were purchased from HiMedia. Ethanol was procured from CSS and used as received. Ciprofloxacin hydrochloride was received as a gift from Aristo Pharmaceuticals Ltd, India. Commercially available edible soybean oil (Nature Fresh, Cargill India Pvt. Ltd) was purchased from the local market. Deionized (DI) water (Millipore Co., USA; 18.2 MΩ.cm resistivity at 25 °C) water was used wherever necessary.

2.2. Methods

2.2.1. Oleylamine coated upconversion nanoparticles (OA-UCNP)

Oleylamine coated NaYF₄ nanoparticles doped with Yb³⁺ (20%) and Tm³⁺ (0.5%) have been synthesized by thermal decomposition method. 0.795 mmol of Y₂O₃, 0.20 mmol of Yb₂O₃, and 0.005 mmol of Tm₂O₃ were
dissolved in 10 ml (50% v/v) aqueous TFA in a 100 ml two-necked round bottom flask. The detailed synthetic procedure has been described in our earlier report [9].

2.2.2. Oleylamine coated magnetite (Fe₃O₄) nanoparticles (OA-MNP)
We used the same sample of nanoparticles, the detailed synthesis of which has been reported earlier [17].

2.2.3. OA-UCNP and OA-MNP incorporated oleogels (MU gels)
OA-UCNP and OA-MNP incorporated oleogels have been prepared by a two-step heat cool method. In the first step, the calculated amount of OA-UCNP and OA-MNP were sonicated in soybean oil to form a uniform suspension. In the next step, stearic acid was added and kept in a water bath at 70 °C to form a clear homogeneous solution. It was then subjected to vortex to form MU gel. A control oleogel without any nanoparticle was also prepared.

2.2.4. Drug loaded MU gels
First, ciprofloxacin hydrochloride (1% w/w) was suspended in soybean oil through vigorous stirring. Then, a specific amount of OA-UCNP and OA-MNP was added into the suspension and sonicated to form a dispersion. The procedure described in section 2.2.3. was followed to complete the gelation.

2.2.5. Synthesis of Rose Bengal (RB) conjugated UCNP (UCNP-RB)
In order to achieve this, OA-UCNP was conjugated with Rose Bengal (RB) using a two-step method to form UCNP-RB nanoparticles using a method reported in literature [18]. In the first step, OA-UCNP was converted into water soluble UCNP using a ligand exchange method. For this purpose, 200.0 mg of AEP was first dispersed in a 10.0 ml mixture of 3:2 (v/v) DI water and ethanol. To this solution, nanoparticle dispersion (20.0 mg in 5.0 ml) was added dropwise and kept for stirring at room temperature for 48 h. The solution was centrifuged at 6000 rpm for 5 min to obtain the hydrophilic nanoparticles. In the next step, 20.0 mg of RB was first activated in the presence of 200.0 mg of EDC and 200.0 mg of NHS in DI water. This solution was kept for stirring at room temperature for 4 h. To this solution, an aqueous dispersion of hydrophilic nanoparticles (100.0 mg in 2.0 ml) was added. This mixture was kept for stirring at room temperature for 24 h to get UCNP-RB nanoparticles. These UCNP-RB nanoparticles were thoroughly washed with water to remove any unconjugated RB molecules.

2.3. Characterization
2.3.1. Characterization of OA-UCNP
Powder x-ray diffraction studies (PXRD) was performed using x-ray diffractometer (Rigaku x-ray diffraction system, USA) having a copper target (λ = 1.54). Transmission Electron Microscopy (TEM) was performed using CM 12 Philips TEM operated at 100–300 kV. The sample was prepared by drying a chloroform solution of OA-UCNP on a copper grid. Fourier Transform Infrared (FTIR) was performed on OA-UCNPs using an FTIR spectrophotometer (Alpha-E, Bruker, USA), operated in Attenuated Total Reflectance (ATR) mode. The pure sample was directly placed on the Zinc Selenide (ZnSe) crystal and the scanning was done in the IR wavenumber range of 400–4,000 cm⁻¹. Photoluminescence studies (PL) were carried out on OA-UCNPs using Horiba JobinYvon, USA/Fluoromax 4P Fluorescence spectrometer fitted with an external NIR laser source.

2.3.2. Characterization of MU gel
X-ray diffraction studies were performed using an x-ray diffractometer (Rigaku x-ray diffraction system, USA) with a copper target (λ = 1.54). The microstructures of the MU gels were analyzed under a bright field microscope (LEICA-750, Germany). The confocal analysis of MU gels was performed on Leica TCS SP8, Germany using 0.01% (w/w) fluorescent yellow 088 dye solution. The surface morphology of the oleogels was studied under ESEM (Quanta 250; FEI, USA). Infra-red analysis of the UCNP-oleogels was carried out using an FTIR spectrophotometer (Alpha-E, Bruker, USA), operated in ATR mode, and the scanning was done in the IR wavenumber range of 400–4,000 cm⁻¹. PL studies were carried out on MU gels to study the fluorescence properties using Horiba JobinYvon, USA/Fluoromax 4P Fluorescence spectrometer fitted with an external NIR laser source. An in-house developed impedance analyzer was used to study the electrical properties of the oleogels. Current (6.25 μA) was injected by varying the frequency from 10 kHz to 60 kHz and the Vmax output was recorded.

2.3.3. In vitro drug release studies
The in vitro drug release studies were carried out using the iontophoresis method for a time period of 3 h. This was carried out using an in-house developed experimental set up. The method followed was the same as described in our previous report [17]. First, the drug loaded MU gel (1.5 g) was loaded in a donor cell and the
open end of the cell was covered with dialysis membrane (pre-activated). The receptor cell was filled with DI water (100 ml, approximately). Both the cells were connected to stainless steel electrodes with approximate surface area of 2 cm². A current of 6.25 μA was applied across the electrodes using a resistance of 120 kΩ at a frequency of 50 kHz. The whole experimental set-up was kept for stirring at 100 rpm, maintaining the temperature at 37 °C. At definite time intervals, specific amount of water was replaced from the receptor cell. The amount of drug released was analyzed from the collected water samples using UV–vis spectroscopy, measured at 271 nm.

2.3.4. Pig ear skin permeation experiment
Skin samples were obtained from the pigs that had been sacrificed for human consumption on commercial basis in a local butcher shop. The breed of the pigs used was ‘harsuk’, an indigenous breed developed by Indian Council of Agricultural Research (ICAR) for safe and commercial consumption in the local region. As per the guidelines of ICAR, the pigs that were selected weighed between 60–80 kg at their recommended slaughter age between 8–10 months. Pig ears were collected post-sacrifice without subjecting the carcass to any pre-treatment to ensure that the integrity of the skin was not compromised. A standard dermatoming procedure was followed to obtain the whole skin from the underlying cartilage of the pig ears [19, 20]. A skin integrity test was performed to check the suitability of the skin for permeation experiment as per our published protocol [9]. The details about the skin integrity test has been discussed in supporting information, section S1.1 is available online at stacks.iop.org/NANOX/1/010012/mmedia. The skin sample (with exposed surface area of approximately 1.0 cm²) was mounted on a modified Franz cell. The receiver cell was filled with phosphate buffer saline (PBS) having physiological pH of 7.4. Next, the exposed surface of the skin was smeared with approximately 50.0 mg of drug-loaded MU gel. Both the donor cell and the receiver cell were connected with electrodes, and 0.42 mA of current was passed through the electrodes. This experiment was continued for 3 h, and at fixed intervals of time, specific volume of buffer was replaced from the receiver cell. The same sets of experiments were also performed under passive conditions. UV-visible spectroscopy was used to analyze the amount of drug present in the collected buffer samples at the end of the experiment. All these experiments were conducted in triplicates at an average ambient temperature of 25 °C since all the ‘proof-of-concept’ experiments were conducted at the same temperature [9].

In order to study the UCNP permeation ability of the oleogels through the skin tissue, fluorescence was also measured for each of the above-collected samples. Then, using a standard curve, the amount of UCNP present in the receptor solution was quantified. A standard curve was drawn by dissolving known concentrations of OA-UCNP in chloroform and recording the PL intensities at 475 nm (figure S8, supporting information). Control skin experiments were also set up where the exposed surface of the skin was coated with 12.5 mg of OA-UCNP, as 50.0 mg of MU gel contains this amount of OA-UCNP. All the experiments were performed in triplicates.

2.3.5. Imaging of pig ear skin through skin permeation experiment
In order to qualitatively analyze the permeation of UCNP into the inner skin structure, the skin samples were visualized under a phase-contrast microscope. For this purpose, the skin samples were dismounted from the Franz cell, and the exposed surface of the skin was thoroughly washed to remove any remnants of oleogel or nanoparticles present on the surface. Further, thin sections of the pig ear skin were taken and observed under a phase-contrast microscope. Photographs of the same were taken using an external camera before and after torching the skin with a 980 nm laser.

In order to track the permeation of OA-UCNP into the pig ear skin, the imaging of the pig ear skin was done following the skin permeation experiment. For this purpose, UCNP-RB nanoparticles were synthesized as described in section 2.2.5. Then, this UCNP-RB nanoparticles were incorporated into the oleogel using the same method as described in section 2.2.3. This oleogel was applied on pig ear skin following the same procedure as described in section 2.3.4. The skin samples were kept for 3 h under active condition and then images were taken using confocal microscope under red channel.

3. Results and discussion
3.1. Incorporation of OA-MNP and OA-UCNP into oleogels
Upconversion materials based on NaYF₄ host is one of the most widely used UCNP. Its synthesis using the thermal decomposition method has been reported to yield bright photoluminescent UCNP with high crystalline phase purity. Instead of selecting green-emitting NaYF₄:Yb³⁺/Er³⁺ as in the case of UCNP incorporated oleogel reported earlier, we have selected blue-emitting NaYF₄:Yb³⁺/Tm³⁺ in this study as UCNP. The purpose of such selection was to avoid the quenching of NIR induced green emission (λmax = 500 to 540 nm) from NaYF₄:Yb³⁺/Er³⁺ based UCNP by Fe₃O₄ nanoparticles [21]. The Tm³⁺ based blue-emitting UCNP...
synthesized here as already been reported for extensive theranostic applications [22]. The target of our synthetic method was to generate oleylamine coated blue-emitting UCNP, so that the particles can be readily dispersed in organic or lipophilic phase. The physical characterization confirmed the expected crystalline nature of OA-UCNP (figure 1(A)) by PXRD. The presence of oleylamine capping on the surface of the nanoparticles was confirmed through the typical FTIR spectra reported earlier (figure 1(B)) [17]. The morphology of OA-UCNP was analyzed using TEM (figure 1(C)), where the nanoparticles exhibited near-spherical shape with average diameter around 25 nm as obtained from ImageJ analysis (figure S1 is available online at stacks.iop.org/NANOX/1/010012/mmedia, supporting information). Under ambient light, OA-UCNP dispersed in chloroform did not show any luminescence (figure 1(D)), but when excited by 980 nm laser, it emitted bright blue luminescence (figure 1(E)). The PL spectra of OA-UCNP in chloroform revealed a major upconversion emission peak with maxima at 475 nm and three minor peaks at around 360 nm, 460 nm, and 650 nm under 980 nm laser excitation (figure 1(F)). We used the same OA-MNP sample that was synthesized and characterized in our earlier report, as the source of MNP in this study [17].

Next, six different oleogel compositions were formulated by varying the amount of OA-MNP, while keeping the amount of OA-UCNP constant (table 1). A control sample was also prepared (S0) where, no nanoparticles were incorporated. The weight ratio of soybean oil and stearic acid was taken to be 85:15, respectively. This ratio was selected based on our previous studies, where it formed a well-characterized stable gel [17]. On a 2.0 g scale, the actual masses of OA-MNP incorporated into the respective oleogels were 0.0 mg, 0.1 mg, 1.0 mg, 10.0 mg, 100.0 mg, and 500.0 mg from UCMN0 to UCMN500. The mass of OA-UCNP incorporated into the oleogels was 500.0 mg in all the samples. Since on gelation, the oleogels became opaque and dense on adding high concentration of OA-MNP (figure S2, supporting information), the mass of OA-UCNP needed to be chosen in such a way that the PL due to upconversion process is not masked and can be optically detected.

3.2. Microscopic characterization of the MU gels
The bright-field micrographs of the MU gels suggested the appearance of predominantly fibrous network like structures (figures 2(A1)–(G1)). The fiber-like structures were present throughout that formed a network-like architecture. We observed short, hyper-branched and non-interconnected fibers of stearic acid in S0, the control sample that was free of any nanoparticles. In UCMN0, with the introduction of OA-UCNP, we observed
enormous growth in the length as well as the hyper-branching of the fibers (figure 2(B1)), that was similar to the trend observed in case of UCMN-oleogels [9]. This indicated the improved synergistic interactions amongst the nanoparticles and the stearic acid molecules. On further addition of OA-MNP into the oleogels, an increase in the length of the fibers was apparent to a great extent in the case of UCMN0.1 and UCMN1. Also, the fiber-like structures seemed to form an interconnected mesh-like structure (figures 2(C1), (D1)). Upon addition of OA-MNP in the sample UCMN10, we observed a shortening in the length of the fibers, and an enhancement in the hyper-branching of the stearic acid molecules (figure 2(E1)). No conclusive observation on the fiber length was found in the case of UCMN100 and UCMN500 from this bright field microscopy (figures 2(F1) and (G1)).

The confocal micrographs of the MU gels gave us a deeper understanding of the microstructure of the oleogels (figures 2(A2)–(G2)). With the addition of nanoparticles, there was an increase in the length of the fibers till UCMN1. With further increase in the concentration of OA-MNP in UCMN10, we noticed a drastic decrease

| Sample  | Stearic acid (%) | Soybean oil (%) | OA-UCNP (%) | OA-MNP (%) |
|---------|-----------------|-----------------|-------------|------------|
| S0      | 15              | 85              | 0           | 0          |
| UCMN0   | 12              | 68              | 20          | 0          |
| UCMN0.1 | 11.99952        | 67.99728        | 19.9992     | 0.0004     |
| UCMN1   | 11.995202       | 67.97281        | 19.992      | 0.0399     |
| UCMN10  | 11.952191       | 67.72908        | 19.92032    | 0.3984     |
| UCMN100 | 11.538462       | 65.38461        | 19.23077    | 3.8461     |
| UCMN500 | 10              | 56.66666        | 16.66667    | 16.6667    |
in the length of the fibers. This observation was also visible in the bright field micrographs (figure 2(E1)). We also found that the fibers showed a continuous branching at the tips and sides. Thereafter, with an increase in OA-MNP, an increase in the length of the fibers in UCMN100 and UCMN500 became evident (figures 2(F2) and (G2)). We also observed numerous branching of the fibers at the sides and tips in UCMN500.

ESEM microscopy studies of the MU gels revealed the presence of plate-like as well as some fiber-like structures in all the MU gels (figure S3, supporting information). The micrograph of UCMN500 oleogel revealed an extensive mesh-like structure, which may be due to the hyper-branching of the fibers (figure S3(G), supporting information). These observations were in accordance with the findings from bright field microscopy and confocal microscopy (figures 2(G1) and (G2)). Such characterizations implied some significant alterations in the oleogel gelation network due to the incorporation of the nanoparticles. Thus, a synergistic interaction amongst the stearic acid molecules and the nanoparticles in these formulated oleogels, as observed in other cases, can be estimated [17].

3.3. Photoluminescence (PL) studies
The photographs of the MU gels under 980 nm laser excitation have been presented in figure 3(A). The control sample (S0) did not show any PL under 980 nm laser excitation due to the absence of OA-UCNP in the oleogel (figure 3(A(a))). Instead, we observed the thermal pattern of the IR laser captured by the sensor of the digital camera. The oleogel UCMN0 exhibited bright bluish-purple PL under 980 nm excitation (figure 3(A(b))). The corresponding PL spectra of that sample showed the major emission peak at 475 nm and the minor peaks at 460 and 650 nm, which accounted for the overall bluish-purple visible colour (figure 3(B)). The intensity of the emitted bluish-purple light got gradually diminished from sample UCMN0.1 to UCMN500 as apparent from the set of photographs itself (figures 3(a)–(g)). The same observation was further supported by the corresponding PL spectra of the samples (figure 3(B)). Such attenuation of observed visible PL was expected, as an increased proportion of OA-MNP in oleogel gradually rendered the samples opaque (figure S2, supporting
information) and thereby prevented the transmission of the optical signals. The emission maxima ($\lambda_{\text{max}} = 540 \text{ nm}$) of the OA-UCNP did not change in the fabricated oleogels in both the absence and presence of OA-MNP (figure 3(B)).

3.4. Molecular interaction studies
The x-ray diffraction studies of the MU gels gave us an insight into the type of interactions occurring between the nanoparticles and the matrix of the oleogel (figures 4(A)–(G)). The analysis of the XRD peaks for the control sample (S0) showed peaks at 4.88°, 7.14°, 9.40°, 11.70°, 20.60°, 21.66° and 24.01° 2$\theta$ (figure S4(A), supporting information). The intensity of the long spacing peaks was higher in S0, which does not contain any nanoparticles. On addition of nanoparticles, some alterations in the intensity of the long spacing as well as short spacing peaks were observed. These observations indicated that the incorporation of the nanoparticles led to the significant alterations in the molecular packing of stearic acid. An analysis of the long spacing peaks and short spacing peaks were carried out by deconvoluting the most intense crystalline peak ($\sim 7.14° 2\theta$) and the amorphous peaks ($\sim 20.60°, \sim 22.66°$ and $24.01° 2\theta$), respectively. We have calculated the d-spacing, crystallite size and lattice strain following the same procedure as mentioned earlier [17].

The deconvolution of the peak at $\sim 7.14° 2\theta$ showed the occurrence of three different peaks with different intensities and full width at half maxima (FWHM) (figure S5 and table S1, supporting information). This may be due to the combination of three different types of crystallites present at that position. On addition of OA-UCNP in UCMN0, the d-spacing of the crystallites increased slightly for both long-range and short-range peaks (figure 4(A)). On further addition of OA-MNP, the d-spacing values increased gradually. Overall, a slight increase in the d-spacing values was observed in both the cases (figure 6 and table S2, supporting information). The analysis of the crystallite size showed that with the addition of OA-UCNP, the crystal growth was prominent which may be due to the decreased lattice strain (figures 4(B), (C)). Thereafter, with the incorporation of OA-MNP, an increase in the crystallite size till UCMN100 was apparent in case of the long-range peaks. The addition of higher amount of OA-MNP in UCMN500 led to a decrease in the crystallite size that may be attributed to the increase in the lattice strain. In the case of short-spacing peaks, the crystallite size increased till UCMN10 due to
the decreased lattice strain. In UCMN100, a decrease in the crystallite size was observed, but thereafter the crystallite size increased in UCMN500. The lattice strain of the corresponding oleogel also decreased.

The changes in the crystallinity of the oleogels with the incorporation of the nanoparticles were determined by calculating the crystallinity index (\(I_c\)) equation (1) (table S3, supporting information).

\[
I_c = \frac{I_a - I_b}{I_a} \times 100
\]

Where, \(I_c\) is the intensity of the peak at 7.14° 2θ as the representative crystalline peak and \(I_b\) is the intensity of the amorphous region at 20.60° 2θ of the oleogels.

With the incorporation of OA-UCNP into the oleogel UCMN0, there was a drop in the crystallinity of the oleogel and made it amorphous in nature. Thereafter, with the addition of OA-MNP, the crystallinity of the oleogels increased till UCMN500 except in UCMN10. Overall, there was an increase in the crystallinity with the increase in the OA-MNP concentration. Hence it can be concluded that, the crystallinity of the oleogels increases with the addition of OA-MNP and makes them predominantly crystalline in nature.

The FTIR spectra of the oleogels showed the characteristic peaks of stearic acid (figure 4(D)). The peaks at ~1746 cm\(^{-1}\) and ~967 cm\(^{-1}\) were assigned to the C-O stretching frequency and the bending vibration of OH–H group, respectively. On addition of OA-UCNP, a shift in these peaks occurred towards higher wavenumbers (~1746 cm\(^{-1}\) and ~970 cm\(^{-1}\)), respectively. Such type of shift indicated a variation in the chemical environment of the stearic acid molecules. With the addition of nanoparticles, some interaction is believed to occur between the oleylamine groups of the nanoparticles and the stearic acid molecules during recrystallization process. This interaction was the most likely factor that resulted the changes in the stearic acid lamellar structure causing a shift in the peaks.

3.5. Electrical studies

The electrical properties of the MU gels were analyzed by measuring the impedance profiles in the range of 10 kHz to 60 kHz (figure 5(A)). The oleogel sample containing UCNP only (UCMN0) exhibited higher impedance than that of control (S0), which had no nanoparticles. With the addition of OA-MNP, the impedance of the oleogels was found to be gradually decreasing. A uniform decrease in the impedance profiles of the oleogels was observed with the increase in the MNP concentration (figure 5(A)). The sample containing highest proportion of MNP (UCMN500) showed the least impedance. This decrease in impedance profile may be attributed to the electro-conducting nature of the magnetic nanoparticles [17]. The impedance profiles were modeled using RQ(Q) electrical model via non-linear curve fitting (figure 5(B)) so as to have an in-depth understanding of the electrical properties. The component ‘Q’ is constant phase element and is often regarded as non-homogeneous capacitor. The reactance \(X_Q\) is mathematically represented by equation (2).

\[
X_Q = (j\omega)^{n-1} \times Q
\]

Where, \(X_Q\) is the reactance and Q is the constant phase element.

It was observed that the bulk resistance (R) of the oleogel UCMN0 increased significantly as compared to control (S0). With the increase in the concentration of OA-MNP, there was increase in bulk resistance value till UCMN10. Thereafter with an increase in the OA-MNP, the bulk resistance value decreased. The constant phase element Q1 as well as n1 values for all the oleogels, were constant. A significant variation was also observed in the bulk capacitive component (Q). The addition of OA-UCNP in UCMN0 oleogel increased the bulk capacitive component drastically. Further, with addition of OA-MNP, the capacitive component decreased till UCMN10. Thereafter, the capacitive component increased with the increase in concentration of OA-MNP. Among the OA-MNP incorporated oleogels, UCMN500 exhibited the highest bulk capacitive component. Such behavior was further confirmed by the exponential constant (n). With the addition of OA-UCNP, a decrease in the exponential constant was recorded. On further addition of OA-MNP, there was increase in the exponential constant. The oleogel UCMN500 showed the highest exponential constant value. This is in correlation with the microscopic studies where UCMN500 showed an extensive mesh-like structure. Hence, impedance studies also suggested a significant alteration in the micro-architecture of the oleogels with the addition of the nanoparticles.

The Nyquist plot of the corresponding electrical data was also determined (figure 5(C)). All the oleogels showed a semi-circular region in the high-frequency zone and a spike in the low-frequency region. The spike in the low-frequency zone could be associated with the grain boundary effect among the gelator molecules, and/or gelator and the nanoparticles, and/or between the nanoparticles. Interestingly, the impedance of only OA-UCNP incorporated oleogel (UCMN0) was found to be higher than that of S0 (nanoparticle free oleogel). This confirmed the inherently poor electrical conductance of UCNP. Our results showed that the incorporation of MNP drastically improved the conductance of the same oleogels incorporated with UCNP. Thus, these...
formulations of MU gels are expected to be suitable candidates responding to an AC field-induced iontophoresis.

3.6. In vitro drug release studies
In order to understand the iontophoretic behavior of MU gels, in vitro drug release studies were carried out. Asymmetric AC field-induced iontophoretic drug release has been found to have a direct relationship with the electrical conductance through the oleogel formulations [17]. For this purpose, ciprofloxacin hydrochloride was used as a probe to estimate the iontophoretic capability of the oleogels. These studies were conducted under both active and passive conditions. Iontophoresis is an active drug delivery mode where, the drug release profile is studied by applying low electric current between a set of electrodes. On the other hand, in the passive condition, no electric current was applied. Drug release studies were conducted for all the samples. The cumulative drug release profile of ciprofloxacin hydrochloride from the drug-loaded oleogels is shown in figures 6(A), (B). Under both passive and active conditions, the oleogels S0 and UCMN0 showed similar drug release profile. Under passive condition, the percentage drug release increased with the subsequent increase in the MNP concentration. UCMN500 showed the highest drug release percentage. This increased drug release in case of UCMN500 may be due to the altered micro-architecture of the oleogels due to the presence of OA-MNPs. Under the active conditions, there was an increased drug release for all the oleogels as compared to the passive condition. The enhanced release of the drugs under active conditions as compared to passive condition might be due to the increased electrostatic repulsion between the drug molecules. Interestingly under active conditions, a 3 fold increase in the drug release between the oleogels S0 and UCMN500 was found. This profound increase in release of drugs observed in the case of UCMN500 can be attributed to the presence of OA-MNPs. The enhancement in the drug release by the addition of OA-MNP may be due a combination of various plausible factors. Dielectrophoresis associated with asymmetric AC field and AC induced variation of the magnetic moments of the iron oxide nanoparticles which increases the flexibility of the polymeric network may be the causes behind the enhanced drug release [17].

Korsmeyer–Peppas (KP) model (equation (3)) and Peppas-Sahlin (PS) model (equation (4)) were used to study the release mechanism of the drugs from the oleogels. For this purpose, the 60% of release data were fitted into these models [23, 24].
Where, CPDR is the percentage drug released at time \( t \), \( k \) is the release rate constant and \( n \) is the diffusion exponent.

\[
CPDR = k \times t^n
\]  

(3)

Where, CPDR is the fraction of drug released at time \( t \), \( a_1 t^m \) is the contribution of Fickian diffusion, \( a_2 t^{2m} \) is the contribution of case II relaxation mechanism, \( a_1 \) and \( a_2 \) are diffusion constants and \( m \) is the diffusion exponent.

The release parameters have been tabulated in table S5, supporting information. All the oleogels showed good fit towards KP model with correlation coefficient values greater than 0.99. A gradual increase in the 'k' values was observed with the increase in the concentration of OA-MNPs. The value of 'k' was found to be highest for UCMN500 in passive condition. This can be attributed to the presence of OA-MNP. Similar observations were found in UCMN500 oleogel under active conditions. The value of 'n' was found to be similar for S0 and UCMN0 oleogels under both passive and active conditions. The value of 'n' was found to be in the range of 0.45–0.89 for all the oleogels. This value of 'n' suggested that the diffusion of the drug molecules might have

Figure 6. In vitro drug release profile of the oleogels (A) passive condition, and (B) active condition. All the experiments have been carried out in triplicates (\( n = 3 \)). The error bars (standard deviation) have been included in the given data. Korsmeyer-Peppas model fitting of (C) passive condition, and (D) active condition. Peppas-Sahlin model fitting of (E) passive condition, and (F) active condition.
occurred due to anomalous diffusion. This type of diffusion usually occurs when Fickian diffusion as well as diffusion due to gelator network relaxation, occurs simultaneously.

In order to get further insight into the diffusion mechanism, the drug release data were fitted to PS model. This model helps to predict the extent of diffusion of the drug molecules due to Fickian diffusion as well as case II relaxation mechanism. The data showed a good fit with the model having correlation coefficient values greater than 0.99. The PS model suggested that the release of the drug molecules may be mostly due to Fickian diffusion as the value of $K_2$ was equal to zero. This was found to be consistent with magnetic nanoparticle incorporated oleogels, where drug release mechanism was found to be due to Fickian diffusion only [17].

### 3.7. Permeation of drug and UCNP through pig ear skin

Pig ear skin serves as a model animal skin for in vitro permeation studies due to its anatomical similarity with human skin [19, 25, 26]. We first analyzed the drug permeation across the whole thickness of the skin samples in the absence (passive) and presence (active) of iontophoresis for all the oleogel samples up to a period of 3 h. For clarity of presentation, we have included the results for four samples (S0, UCMN0, UCMN1, and UCMN500) in figures 7(A), (B), while the complete results of the entire set of samples have been provided in figure S7 (supporting information). Under passive condition, no detectable amount of drug could be traced in the receptacle of Franz cell corresponding to any of the samples (figures 7(A) and S7(A)). On the contrary, the same study under the active condition revealed a gradual increase in drug accumulation from sample S0 to UCMN500 (figures 7(B) and S7(B)). Notably, a relatively higher AC frequency, which was used in all the iontophoretic experiments has been reported to prevent skin burns or electrolysis and helps in prolonged transport of drug molecules due to low skin polarization effect [27–29].

Next, the skin permeation study of UCNP was presented in figure 7(C). The active mode of transdermal delivery of UCNP was carried out for 3 h; since the time duration of 2–5 h have been typically reported in most of the iontophoresis experiments involving skin permeation studies (including in vivo) [30–39]. In the context of developing a potential dermatological patch technology under active condition, such time period of application is expected to meet the requirement of patient compliance and clinical applicability [40]. Moreover, the above...
time period was justified in this case as the in vitro drug release experiments (without skin) already exhibited a significant difference between the passive and the active conditions. Under the passive condition, no detectable amount of UCNP could be found permeating the skin sample after a period of 3 h in all the cases. Under the active condition, there was a gradual increase in the concentrations of UCNP, which penetrated the skin samples after the same time period. As observed in the case of drug permeation across the skin, with increasing MNP amount, permeation of UCNP got increased proportionally (figures 7(C) and S9). Interestingly, a detectable amount of UCNP was recorded to permeate in case of the sample UCMN0. This permeated amount was enhanced to 1.5 fold and 2 fold using UCMN1 and UCMN500, respectively (figure 7(C)). In order to ascertain that the role of permeation-enhancing agents is imperative in addition to the application of iontophoresis; we conducted a control experiment, where adequate amount of OA-UCNP was subjected to the same skin permeation experiment under both passive and active iontophoresetic condition for a period of 3 h (figure S10, supporting information). No detectable amount of UCNP was recorded in the receptacle in the Franz cell under both these conditions (figure S10, supporting information). The results (figures 7(A)–(C)) indicated that both the drug and the UCNP permeation across the whole skin samples induced by iontophoresis were facilitated by the presence of increasing amount of MNP in the oleogels, the trend of which was reflected in the electrical properties reported earlier (figure 5). We selected the 3 h time point for iontophotic experiments on skin permeation, since through a separate control experiment we established that it took a minimum of 2.5 h for the nanoparticles to permeate through the whole skin thickness and a significant change in the permeation patterns started to appear at 3 h (figure S11, supporting information). Such data also indicated that the amount of UCNP that can be delivered from a particular MU gel composition, was tunable with respect to the time duration of iontophoresis in excess of 2.5 h. It is noteworthy that under the active condition along with UCNP, some MNP might have permeated through the skin samples as expected from the mechanism of transport [9]. In this report, we did not focus on MNP permeation as our aim was to validate faster permeation of UCNP through optical detection for potential theranostic application.

To obtain conclusive proof of the permeation of UCNP across porcine skin samples, we conducted a confocal imaging study involving a sample composition corresponding to UCMN100 as a representative of the samples. The image shown in figure 7(D) represented the cross-section of the pig ear skin sample, where the marked surface had been smeared with UCMN100 containing fluorescent-labeled UCNP. The confocal image, imaged right after the application of iontophoresis for 3 h, exhibited permeation of nanoparticles through the whole skin that reached the bottom layers of the skin. The demarcation of layers of the skin has been provided in supporting information, figure S12.

We conducted a few imaging experiments based on 980 nm laser-induced PL of UCNP. After completion of the skin permeation experiments, we washed the top surface the sample containing UCMN100 (fluorescent label-free). Under 980 nm laser excitation the bluish-purple PL was photographed (figure 7(E)). In the same experiment, when performed with the skin sample containing OA-UCNP as control, no PL except thermal profile of the laser could be photographed (figure 7(F)). This confirmed that washing the top surface removed all the UCNP containing materials from the top surface and the PL recorded in case of figure 7(E) only came from the bottom layer of the skin as observed earlier. Further, small portions of both the skin segments were observed under phase contrast microscope. The sample skin and the control skin were observed through the microscope under ambient light in the absence of any laser (figures 7(G), (H)). Under 980 nm laser excitation, the control skin did not show any PL (figure 7(J)), whereas the sample skin showed bluish-purple PL under laser excitation (figure 7(I)). The imaging experiments confirmed that the UCNP even after being delivered to the inner skin structure using MU gels were capable of showing the desired PL output when non-invasively excited by the 980 nm laser. Such PL output can be utilized for photo-theranostic applications in targeting diseases beneath the skin.

4. Conclusion

To develop a faster, transdermal delivery method of UCNP in a tunable fashion, we have designed a novel magnetic-upconversion oleogel by combining both the skin permeation enhancement as well as the iontophoresis-based strategy. We have incorporated both UCNP and MNP in soybean oil-based oleogel in the presence of stearic acid as a gelator to form MU gel, which acquired the necessary electrical properties to exhibit iontophotic behavior under an asymmetric AC field. Using a model drug, ciprofloxacin hydrochloride, we have validated the MU gel’s ability to show drug release behavior under iontophoresis in vitro. By varying the amount of MNP incorporation in MU gels, a varied rate of drug release was achieved. Experiments on animal skin model revealed that both drug and UCNP were able to penetrate the whole thickness of the skin, only when the drug-loaded MU gels were subjected to iontophoresis. We also categorically proved that for the whole skin permeation of UCNP within a time period as little as 3 h, both the combined effects of skin permeation...
enhancement and iontophoresis are absolutely necessary. Within a time period of 3 h, the efficacy of both drug delivery and UCNP permeation was shown to be enhanced by 3 fold and 2 fold, respectively, due to the maximum presence of the MNP in the oleogels under iontophoretic condition. The ability of these oleogels to deliver drugs and UCNP in a tunable manner indicated the development of a multimodal TDS triggered by an alternating AC field as stimulus. These results also established a rational way of delivering an electrically non-conducting nanoparticle across the SC layer with undisputed time advantage. Since, the method is based on the components of a second-generation TDS, the associated mild, non-aggressive approach is expected to pave the future way to fabricate a non-invasive therapeutic platform for skin diseases as a self-administered and cost-effective alternative.

Acknowledgments

The authors acknowledge and thank the financial support obtained from DST Women’s Scientist Scheme A (Kiran Division) (Grant No. SR/WOS-A/CS-1012/2015 (G)).

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