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Transdermal delivery of anticancer drug caffeine from water-in-oil nanoemulsions

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ABSTRACT

Recently caffeine has been investigated for the treatment of various types of cancers upon oral administration. There is also some evidence that dermally applied caffeine can protect the skin from skin cancer caused by sun exposure. Therefore nanoemulsion formulation of caffeine for transdermal drug delivery was developed and evaluated in the present investigation. Different w/o nanoemulsion formulations of caffeine were prepared by oil phase titration method. Thermodynamically stable nanoemulsions were characterized for morphology, droplet size, viscosity and refractive index. The *in vitro* skin permeation studies were performed on Franz diffusion cell using rat skin as permeation membrane. The *in vitro* skin permeation profile of optimized formulation was compared with aqueous solution of caffeine. Significant increase in permeability parameters was observed in nanoemulsion formulations ($P < 0.05$) as compared to aqueous solution of caffeine. The steady-state flux (J_{ss}) and permeability coefficient (K_p) for optimized nanoemulsion formulation (C12) were found to be 147.55 ± 8.21 μg/cm^2/h and $1.475 \times 10^{-2} \pm 0.031 \times 10^{-2}$ cm/h, respectively. Enhancement ratio (E_r) was found to be 17.37 in optimized formulation C12 compared with other formulations. Overall these results suggested that w/o nanoemulsions are good carriers for transdermal delivery of caffeine.

1. Introduction

Chemically caffeine is 1,3,7-trimethylhanthine and found in tea leaves, coffee, cocoa, guarana and kola nuts [1]. Recently some reports indicated potential anticancer effects of caffeine upon oral administration [2–4]. There are also some reports that indicated that orally applied caffeine could protect the skin from ultraviolet (UV) light induced skin cancer [5,6]. Transdermally applied caffeine could be suitable for local as well as for systemic treatment of skin cancer. Therefore transdermal w/o nanoemulsion formulation was designed and evaluated in the present investigation.

Transdermal drug delivery represents the successful and innovative area of research in drug delivery and known to enhance therapeutic efficacy, bioavailability and to avoid any adverse effects [7,8]. Different approaches such as chemical enhancers [9], iontophoresis [10], sonophoresis [11], transdermal gels [12] and nanoemulsions [7,8,13–15] have been used to enhance skin permeation and bioavailability of drugs. There has been increased interest during recent years in the use of nano or microemulsions that could modify drug permeation through the skin [7,8,13–15]. Nanoemulsion is one of the most promising technique for transdermal delivery of drugs and present many advantages like higher storage stability, lower preparation cost, good production feasibility, thermodynamic stability, absence of organic solvents and no need of intensive sonication [14–16]. Nanoemulsions are thermodynamically stable transparent or translucent dispersions of oil and water stabilized by an interfacial film of surfactant usually in combination with cosurfactant having the droplet size less than 100 nm [17–19]. Nanoemulsions have also shown improved transdermal permeation of many drugs over the conventional topical formulations such as emulsions [20] and gels [13–15]. Aqueous phase titration or spontaneous emulsification method has been successfully investigated for the preparation of oil-in-water (o/w) nanoemulsions of many lipophilic drugs in our previous articles [13–15,17–19]. Caffeine is hydrophilic drug and aqueous phase titration method is not suitable for preparation of water-in-oil (w/o) nanoemulsions of caffeine. Therefore different w/o nanoemulsions of caffeine were prepared using oil phase titration method. Oil phase titration method has not been used extensively for the preparation of nanoemulsions. Moreover transdermal or dermal delivery of caffeine has not been investigated using nanoemulsion technique. Therefore the aim of this article was to investigate the potential of w/o nanoemulsion system for transdermal delivery of anticancer drug caffeine using nonirritant, pharmaceutically acceptable ingredients without using additional chemical enhancers as components of nanoemulsions themselves act as permeation enhancers. All these chemicals are nonirritant, nontoxic and safe to the skin and fall under generally

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regarded as safe (GRAS) category and are pharmaceutically acceptable.

2. Materials and methods

2.1. Materials

Caffeine was extracted from tea leaves using sublimation method. Caprylic/capric triglyceride polyethylene glycol-4 complex (Labrafac), caprylo caproyl macrogol-8-glyceride (Labrasol), jojoba oil, oleoyl macroglycerides EP (Labrafil), Lauroglycol-90, Lauroglycol-FCC and diethylene glycol monoethyl ether (Transcutol-HP) were kind gift samples from Gattefossé (France). Isopropyl alcohol (IPA), glycerol triacetate (Triacetin) and olive oil were purchased from E-Merck, Germany. Polyoxy-35-castor oil (Cremophor-EL), Tween-80 and Tween-85 were purchased form Sigma–Aldrich, USA. All other chemicals used in the study were of analytical reagent (AR) grade.

2.2. Screening of oils and water

The solubility of caffeine in various oils (Triacetin, Labrafac, olive oil, Labrafil, Lauroglycol-90, Lauroglycol-FCC and jojoba oil) was determined by dissolving excess amount of caffeine in 2 ml of each of the selected oils in 5 ml capacity stoppered vials separately. Excess amount of caffeine was added to each 5 ml capacity stoppered vial and mixed for 10 min using a vortex mixer. The mixture vials were then kept at $37 \pm 1.0\,^{\circ}C$ in an isothermal memmert shaker bath (Memmert, Germany) for 72 h to get equilibrium. The equilibrated samples were removed from shaker and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a 0.45 μm membrane filter. The concentration of caffeine was determined in each oil and distilled water by UV spectrophotometer at the wavelength of 273 nm [21].

2.3. Screening of surfactants

Six types of surfactants were tried for w/o nanoemulsion formulation, which included Labrasol, Cremophor EL, Tween 80, Tween 85, Transcutol-HP and Plurol oleique. In water, 2.5 ml of 15% (w/w) of surfactant solution was prepared and 4 μl of oil was added with vigorous vortexing. If a one-phase clear solution was obtained, the addition of the oil was repeated until the solution became cloudy.

2.4. Screening of cosurfactants

Transcutol-HP was combined with five types of solubilizers as cosurfactants, namely, ethanol, isopropyl alcohol (IPA), n-butanol, PEG 400 and propylene glycol. At a fixed S_{mix} ratio of 1:1, the pseudoternary phase diagrams were constructed. Twelve different combinations in different weight ratios of oil and S_{mix}, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 6:4 (1:0.7), 7:3 (1:0.43), and 9:1, were taken so that maximum ratios were covered to delineate the boundaries of phases precisely formed in the phase diagrams.

2.5. Effect of surfactant and cosurfactant mass ratio on nanoemulsion formation

Surfactant (Transcutol-HP) was blended with cosurfactant (IPA) in the weight ratios of 3:1, 2:1, 1:1, 1:0, 1:2, and 1:3. These S_{mix} ratios were chosen in decreasing concentration of surfactant with respect to cosurfactant and increasing concentration of cosurfactant with respect to surfactant for detailed study of the phase diagrams. Oil phase titration method was used for the construction of the pseudoternary phase diagrams, which involves stepwise addition of oil phase to each weight ratio of water and surfactants, and then mixing the components with the help of vortex mixer at 25 °C. The nanoemulsion phase was identified as the region in the phase diagram where clear, easily flowable, and transparent formulations were obtained based on the visual observation. Twelve different combinations in different weight ratios of water and S_{mix}, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 6:4 (1:0.7), 112 7:3 (1:0.43), and 9:1, were taken. One axis of the pseudo-three-component phase diagram represented the aqueous phase, the other represented the oil phase, and the third represented a mixture of surfactant and cosurfactant at a fixed weight ratio (S_{mix}).

2.6. Preparation of caffeine nanoemulsion formulations

From each phase diagram constructed, different formulae were selected from nanoemulsion region for incorporation of drug into the aqueous phase. 1% (w/w) of caffeine was dissolved in aqueous phase (distilled water) of all selected nanoemulsion formulation. Then selected quantity of S_{mix} was added in aqueous solution of drug and stirred for 2 min. The oil phase (Lauroglycol-90) was added slowly with continuous stirring. Selected formulations were subjected to different dispersion stability tests.

2.7. Dispersion stability studies

In order to overcome the problem of metastable and unstable formulations, dispersion stability tests were performed. Selected formulations were subjected for three dispersion stability tests like centrifugation, heating & cooling cycles and freeze thaw cycle test using procedure as described in our previously published articles [17–19]. The formulations which showed no phase separation, creaming, coalescence or phase inversion upon these tests were selected for characterization.

2.8. Characterization of nanoemulsions

Morphology and shape of the drug loaded nanoemulsion (C12) were studied using transmission electron microscopy [TEM] (Philips CM-10, USA).

Droplet size distribution of the nanoemulsion was determined by photon correlation spectroscopy (PCS) using a Zetasizer 1000 HS (Malvern Instruments, UK). These studies were performed at refractive index of 1.392 because the refractive index for all formulation was within this range. The viscosity and dielectric constant of the medium were set at 1.05 cp and 79.4, respectively.

The viscosity of the formulations was determined using Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories, Inc, Middleboro, MA) using spindle # CPE40 at $25 \pm 0.5\,^{\circ}C$.

Refractive index of placebo and drug-loaded formulations was determined using an Abbes type refractometer (Precision Standard Testing Equipment Corporation, Germany).

2.9. In vitro skin permeation studies

In vitro skin permeation of caffeine from selected nanoemulsions (C1–C12) and aqueous solution of caffeine all containing same quantity of caffeine (1%, w/w) was performed on Franz diffusion cell with an effective diffusional area of 2.54 cm^2 and 20 ml of receiver chamber capacity using rat abdominal skin as permeation membrane. The full thickness rat skin was excised from abdominal region and hairs were removed with the help of an electric clipper. The skin was prepared properly and stored in the deep freezer at $-21\,^{\circ}C$ till further use [13–15]. On the day of experiment the skin was brought to room temperature and mounted between