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Research paper

# Folic acid loaded lipid nanocarriers with promoted skin antiaging and antioxidant efficacy

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ABSTRACT

Ageing is the progressive deterioration of physiological functions in organisms. Folic acid (FA) can be used as antiaging. The objective of this research is the feasibility of topical delivery of FA using nanostructured lipid carriers (NLCs) to offer sustained antioxidant and antiaging effects. FA NLCS were prepared by hot high pressure homogenization. Encapsulation efficiency (EE), *in vitro* release, *ex vivo* permeation and skin targeting were evaluated. Characterization of selected formulations was assessed, together with stability and water occlusion. Further, histology and antioxidant potential were evaluated. EE exceeded 95%. Formulations with 30% oil showed maximum skin targeting, water occlusion and high efficiency of hydration and antioxidant potential. Incorporation of oil into solid lipids causes more imperfections in the crystal lattice, providing high drug encapsulation. The small particle size leads to forming a depot in the skin providing efficient skin targeting, water occlusion, antiaging and antioxidant effects especially for the 30% oil containing formula; F11. The results emphasize the success of topical application of FA NLCs. F11 represents a promising, stable preparation, achieving remarkable long acting skin hydration, *in vitro* and *ex vivo* antioxidant potential lasting for >4 days.



## 1. Introduction

Ageing is defined as the progressive deterioration of physiological functions in organisms, eventually leading to senescence and death. Ageing can be divided into two categories: intrinsic (chronological ageing) and extrinsic (premature or photo-ageing) [1]. Intrinsic ageing is a natural occurrence in which numerous simultaneous mechanisms occur. Collagen and elastin fibres make up the dermal matrix and give our skin the ability to bounce back into its original position. Their production slows down as we age, where skin cell turnover slows down leaving excess dead skin cells remaining on the surface. The skin becomes thinner, the dermal–epidermal junction compresses and the dermal structure begins to collapse [1]. The visual results of dermal intrinsic ageing are: dry/flaky skin, fine lines, wrinkles and sagging/lax skin. Extrinsic ageing is caused by outside factors such as cigarette smoke, exhaust and pollution but the most common culprit is over exposure to UV radiation. Continual sun exposure not only hinders the skin's ability to repair itself but continues to break down and debilitate the synthesis of new collagen [1]. UV radiation can lead to degradation of elastin fibres causing the premature decrease in skin flexibility. The perceivable results of photo-ageing are: hyperpigmentation, leathery appearance, dry skin and deep wrinkles [1].

UV radiation is a potent initiator of reactive oxygen species (ROS) generation in the skin. UV radiation leads to lipid peroxidation which leads to mitochondrial damage [2]. UV radiation also causes damage of DNA within the epidermis which is a leading cause of ageing [3].

Folic acid (FA) is a member of the B-complex family of vitamins and works in concert with vitamin $B_{12}$. FA functions primarily as a methyl donor involved in many important body processes, including DNA synthesis [4]. FA can be used as an antiaging constituent [5]. FA plays an important role in mitotically active tissues thus it can induce skin cell regenerating properties by supplementing necessary micronutrients. There are thus many

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possibilities to use it in personal care products and cosmetics. Small amounts of this vitamin have been only observed in cosmetic preparations based on algae [5]. FA deficiency is quickly observed at those who take sunbaths or solaria due to engagement of FA in the repair of skin damage. UV-radiation cause genetic damage and together with FA deficiency may cause skin cell death. It seems that FA improves viability of UV-damaged skin cells by modulation of DNA-repair mechanism [5].

Topical drug application has been introduced since a long time. Several problems have been reported with conventional preparations as, low uptake due to the barrier function of the stratum corneum and absorption to the systemic circulation [6]. Nanoparticles based on lipid systems are most commonly studied for topical application [6]. They have been investigated as delivery systems because of their advantageous features as, occlusive properties, increase in skin hydration, modified release profile, increase of skin penetration associated with targeting effect and avoidance of systemic uptake [6]. SLNs and NLCs can be synthesized with an active ingredient in the centre to delay release [7]. Due to their unique size and composition dependent properties, lipid nanoparticles pose the ability penetrate through anatomical barriers and release their contents, encouraging applications in the field of controlled drug delivery [8]. Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are composed of physiological and biodegradable lipids that show low toxicity [9]. NLCs are produced using blends of solid lipids and liquid lipids (oils). The mean particle size of NLCs is in the submicron rage, ranging from about 40 nm to 1000 nm [10].

The objective of this research addresses the feasibility of topical delivery of FA using nanostructured lipid carriers to provide controlled delivery of the drug to the skin. Optimization of FA NLCs through the proper selection of components will be investigated through the performance of comprehensive, integrated *in vitro* as well as *ex vivo* studies will be undertaken to characterize the properties and the biological performance of this new drug delivery system to offer sustained antioxidant and antiaging effects on the skin.

## 2. Materials and methods

### 2.1. Materials

Folic acid obtained from Hebi. Jihng., China.; was kindly donated from El-Nil Company, El Amirya City, Cairo, Egypt. Apifil® (PEG-8 Beeswax), Monosteol™ (Propylene glycol monopalmitostearate EP), Geleol™ pellets (Glycerol monostearate type I EP) Capryol™ 90 (Propylene glycol monocaprylate type II NF) and Labrafil® M 2130 CS (Lauroyl macrogol - 6- glycerides EP) were kindly donated by Gattefosse, SAINT-PRIEST, Cedex, France. Captex 355 EP/NF (triglycerides of caprylic/capric acid) was kindly donated by ABITEC Corporation, USA. DPPH (1,1-diphenylpicryl-hydrazyl) was purchased from Sigma Chem. Co., St. Louis, MO, USA. Trichloroacetic acid (TCA) A.R., Fischer Scientific UK Limited, Loughborough, UK. Thiobarbituric acid, (TBA), 4,6-dihydroxy-2-mercaptopyrimidine, 98% was obtained from Acros Organics, Jeel, Belgium. All other chemicals were of analytical grade.

### 2.2. Methods

#### 2.2.1. Establishment of spectra and calibration curves of FA in different solvents

A stock solution of 100 μg/ml FA was prepared in each of methanol and 10% methanol/phosphate buffers pH 5.5 and 7.4. Spectra were obtained for each solvent and calibration curves plotted at the respective λmax values.

#### 2.2.2. Assessment of solubility of FA in different excipients

Solid lipids; Monosteol™, Geleol™, Apifil® were investigated. The melting points of selected lipids were 36.5, 57.5 and 67.5 °C, respectively [11]. Repeatedly, weighed amounts of FA were added to 1 g molten solid lipid using a hot water bath until a saturated solution of FA in lipid was formed [12]. Liquid lipids; Captex 355, Imwitor 742, Capmul MCM – C 8 and Capryol™ 90 and surfactants; Tween 80 and Labrafil® M2130 CS were investigated by adding known excess amounts of FA to 5 ml of each excipient, vortex mixing, shaking at room temperature in an isothermal shaker, GFL 3032, Germany, at 100 rpm, for 48 h. Afterwards, samples were centrifuged at 5000 rpm for 20 min, the clear supernatants were diluted with methanol and the drug concentration was determined spectrophotometrically [12,13] at 285 nm. Triplicate evaluations were considered.

#### 2.2.3. Preparation of FA NLCs

Preparation of NLCs was conducted by hot high pressure homogenization. The solid lipid was molten at 5–10 °C above its melting point together with Labrafil® and FA (0.5%) for 20 min. Water and Tween 80 were heated to the same temperature added to the lipid mixture and homogenized for 5 min at 20,000 rpm using a Silent Crusher M Homogenizer Heidolph Instruments, No: 595-06000-00-2, Germany. This was followed by ultra sonication for 30 min at the same temperature, using the bath sonicator ULTRAsonik® Model 28x, Ney Dental, Yucaipa, California, USA. The preparations were kept at −4 °C for 24 h before use [5,14]. Lipids and oils were mixed at different weight ratios; 9:1, 8:2, 7:3. The lipid: aqueous phase ratio was maintained at 1:1. The composition of prepared NLCs is presented in Table 1.

#### 2.2.4. Determination of encapsulation efficiency in NLCs

The encapsulation efficiency (EE) of FA in NLCs was determined by centrifugation of 1 g preparation with 10 ml methanol for 1 h at 7000 rpm and −4 °C [15]. Further, the supernatant was filtered and diluted with methanol. The free drug was determined spectrophotometrically and subtracted from the total drug added at the start of the experiment, to determine the EE [12,16]. Experiments were conducted in triplicate.

#### 2.2.5. Release study of FA from NLCs

*In vitro* release study was performed across a dialysis tubing non-rate limiting cellulose membrane (molecular weight cutoff, 12 000 g/mol). One g of FA NLCs was placed in the clean dialysis bag, placed into the dissolution apparatus containing 10% methanol/phosphate buffer (pH 5.5), at 32 ± 0.5 °C and stirred at 50 rpm. After 1, 2, 3, 4, 5 and 6 h, 5 ml aliquots were sampled and replaced with fresh medium [17]. Samples were analysed for FA spectrophotometrically, at the 281.5 nm. Three replicates were performed.

#### 2.2.6. Ex-vivo permeation study

The *ex-vivo* study was performed using female albino rats [15]. Skin was excised, depilated, extraneous fats removed, then washed with distilled water and examined for integrity. Excised rat skins were stored at −21 °C till further use. Permeation experiments were performed using vertical Franz diffusion cells [18]. The skin was clamped between the donor and receptor chambers with the stratum corneum (SC) facing upwards [19–21]. The receptor chamber was filled with 10% methanol/phosphate buffer (pH 7.4), kept at 37 ± 0.5 °C and stirred at 600 rpm [15]. Half a gram of FA NLCs was applied onto the SC. Five ml aliquots were withdrawn after 1, 2, 3, 4, 5, 6 h [22] and replenished with an equal volume of methanolic buffer which was equilibrated at 37 ± 0.5 °C. Samples were analysed for permeated FA spectrophotometrically [23–26], at 286.5 nm. Triplicate tests were conducted.