UNIVERSITI PUTRA MALAYSIA

PREPARATION, CHARACTERISATION AND EVALUATION OF POLYMERIC THIN FILM FROM KOJIC MONOOLEATE NANOEMULSION FOR COSMETIC APPLICATION

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By

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January 2021

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A derivative of kojic acid, kojic monooleate (KMO) contains tyrosinase inhibitor and exhibits strong antioxidant activity makes it a good candidate to be incorporated into a formulation for cosmetic application. Recent developed formulation containing KMO formed a sticky, fragile and easy to fall-off thin film on the skin, in order to counter the problem, thin film system (TFS) was introduced and incorporated into the formulation. In this study, an oil in water (O/W) nanoemulsion formulation in thin film system (TFS) containing KMO as a sole active ingredient was developed. TFS was chosen to ensure better hydration effect and penetration of KMO into the skin. TFS was developed by adding the best plasticiser and solvent at optimum percentage. The nanoemulsion was developed by using high and low energy emulsification technique. Response surface methodology (RSM) was used to optimise and analyse the effect of three variables on droplet size as a response. The optimised KMO nanoemulsion in TFS with desirable criteria was PVA (27.61% w/w), PG (1.05% w/w), and shear rate (8656.17 rpm) with predicted droplet size (110.21 nm) and actual droplet size (105.93 nm) with a residual standard error (RSE) of < 2.0% was obtained. The statistical analysis was performed by ANOVA which indicated good correlation of experimental parameters. The physicochemical properties (Zeta potential -37.37 mV, PDI 0.13, pH 4.74, viscosity 0.1058 Pa.s and heterogenous distribution) and its stability of the KMO nanoemulsion in TFS under centrifugal force and stability study for 28 days at 4 different conditions (4°C, 25°C exposed to sulight, 25°C protected from sunlight and at 45°C) showed physical properties for cosmeceutical applications. The optimised KMO nanoemulsion in TFS also shows the desired criteria of TFS with flexible and peelable characteristics, non-sticky, and short drying time of approximately 8 ± 0.13 min and this shows suitability for topical application as peeling mask. KMO nanoemulsion in TFS also disports 79.99 ± 2.53 % released of KMO across the cellulose acetate membrane after 180 min of study time. In addition, the KMO nanoemulsion in TFS was proven to be less toxic as the LC_{50} of KMO

nanoemulsion in TFS was found to be at 50.12 mg/mL at 72hpf and 0.63 mg/mL at 96hpf and 120hpf. The results of the hydration effect of KMO nanoemulsion in TFS towards human volunteer's skin suggesting that the KMO nanoemulsion in TFS does increase hydration of skin by 12.33% due to occlusive effect of KMO. In short, KMO nanoemulsion in TFS shows an increment of water content on skin and the penetration rate of KMO.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENYEDIAAN, PENCIRIAN DAN PENILAIAN SISTEM FILEM NIPIS POLIMER PNANOEMULSI DARI KOJIK MONOOLEAT NANOEMULSI UNTUK APLIKASI KOSMETIK

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Kojik asid derivatif, iaitu kojik monooleat (KMO) yang mengandungi aktiviti penghindar enzim tyrosinase dan menunjukkan aktiviti antioksidan yang tinggi menjadikan ia sebagai bahan aktif yang sesuai di dalam formulasi untuk rawatan kosmetik. Melalui kajian terbaru yang mencipta formulasi yang mengandungi KMO membentuk filem nipis yang melekit, rapuh dan mudah lekang dari kulit, oleh itu, untuk mengatasi masalah ini, system filem nipis telah diperkenalkan dan digabungkan ke dalam formulasi ini. Dalam kajian ini, nanoemulsi formulasi minyak dalam air dalam sistem filem nipis yang mengandungi KMO sebagai bahan aktif utama telah dicipta. Sistem filem nipis telah dipilih untuk meningkatkan kesan kelembapan kulit dan penembusan KMO ke dalam kulit. Sistem filem nipis dicipta dengan menambah pemplastik dan pelarut terbaik pada kadar peratus yang optimum. Sistem nanoemulsi dihasilkan menggunakan teknik emulsifikasi tenaga tinggi dan rendah. Pengoptimuman kaedah gerak balas permukaan digunakan untuk mengoptimumkan formulasi dan menganalisa impak kesan tiga parameter terhadap saiz partikel sebagai hasil tindak balas. Nanoemulsi KMO dalam sistem filem nipis yang telah dioptimumkan dengan kriteria yang diingini mengandungi PVA (27.61% w/w), PG (1.05% w/w), dan kadar ricih (8656.17 rpm) dengan saiz partikel ramalan (110.21 nm) dan saiz partikel sebenar (105.93 nm) dengan nilai RSE <2.0% diperolehi. Analisis statistik (ANOVA) menunjukkan korelasi yang baik bagi setiap parameter eksperimen. Ciri fizikokimia (potensi Zeta -37.37 mV, PDI 0.13 dan pH 4.74, kelikatan 0.1058 Pa.s dan pengedaran pelbagai) dan kestabilan nanoemulsi KMO dalam sistem filem nipis di bawah daya pengemparan dan kajian kestabilan dilakukan selama 28 hari pada 4 keadaan yang berbeza (4°C, 25°C terdedah kepada cahaya matahari, 25°C terlindung dari cahaya matahari dan pada suhu 45°C) menunjukkan sifat fizikal yang sesuai untuk aplikasi kosmetik. Nanoemulsi KMO dalam sistem filem nipis optimum juga menunjukkan kriteria sistem filem nipis yang dikehendaki iaitu fleksibel, mudah ditanggalkan, tidak melekit, dan masa pengeringan yang pendek iaitu 8 ± 0.13 minit dan ini

menunjukkan kesesuaian formulasi ini untuk aplikasi topikal sebagai topeng pengelupas. Nanoemulsi KMO dalam sistem filem nipis juga menunjukkan kadar penembusan KMO sebanyak 79.99 ± 2.53 % melalui selulosa asetat membran selepas 180 minit masa kajian. Selain dari itu, nanoemulsi KMO dalam sistem filem nipis juga dibuktikan kurang toksik apabila kajian LC_{50} didapati berada pada tahap 50.12 mg/mL pada 72hpf dan 0.63 mg/mL pada 96hpf dan 120hpf. Kajian kelembapan kulit oleh nanoemulsi KMO dalam sistem filem ke atas kulit sukarelawan manusia menyarankan bahawa nanoemulsi KMO dalam sistem filem mampu meningkatkan kelembapan kulit sebanyak 12.33% disebabkan oleh sifat oklusif KMO. Secara ringkasnya, nanoemulsi KMO dalam sistem filem menunjukkan peningkatan kandungan air pada kulit dan meningkatkan kadar penyerapan KMO.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research and the writing of this thesis were done under our supervision;
- supervisory responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2015-2016) are adhered to.

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LIST OF ABBREVIATIONS

KMO	Kojic monooleate
TFS	Thin film system
CO	Castor oil
PVA	Polyvinyl alcohol
PG	Propylene glycol
Tween 80	Polyoxyethylene sorbitan monooleate
XG	Xantham gum
HPMC	Hydroxypropyl methyl cellulose
PVP	Polyvinyl pyrrolidone
SC	Stratum corneum
DOE	Design of Experiments
RSM	Response Surface Methodology
TEM	Transmission Electron Microscopy
TPC	Total phenolic content
TFC	Total flavonoid content
DPPH	2,2-diphenyl-1-picrylhydrazyl
C	Control formulation
M	Marketed formulation

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Every year, the cosmetic industry is getting bigger and one of the most growing products are the brightening product. At the end of 2017, the global brightening product market was valued at USD 5,283.82 million and was expected to reach USD 8,479.08 million in 2022 (Zion Market Research, 2017; Business Wire, 2017; Persistence Market Research, 2016). The current trend in the cosmetic industry is recurring to traditional natural ingredients to develop novel formulations, with less side-effects towards the consumers (Cosmetics Europe, 2017).

Skincare products in the market are mostly used to reduce dryness, eczema, acne, aging, hyperpigmentation and to protect skin from free radicals of sunlight (Rodrigues *et al*., 2019). Thin film is the type of dosage form which is gently applied onto the facial skin surface and is peeled off after a few minutes of its application. It is used as a remedy to treat facial skin related problems such as hyperpigmentation, wrinkles, aging, and acne. Its main role is to stimulate the metabolism due to its occlusive effect (Kathe and Kathpalia, 2017). Thin film entraps moisture beneath the film lead to the increment of skin hydration, thus, ease the penetration of substance into the skin within a short period of time.

Synthesis and distribution of melanin affects the coloration of skin, hair, and eyes (Wang *et al*., 2011). Hyperpigmentation and melasma are the results of abnormal production of melanin throughout the skin (Lajis *et al*., 2012; Heo *et al*., 2010; Panic *et al*., 2010). Melanin is synthesised via the melanogenesis process in melanoma and melanocytes (Lajis *et al*., 2012; Huang *et al*., 2008; Hurst *et al*., 2003). Tyrosinase is a rate-limiting enzyme in the biosynthesis of melanin, therefore making it the key target to inhibit the synthesis of melanin (Wang *et al*., 2011; Kim *et al*., 2008; Slominski *et al*., 2005). Prolong exposure of UV radiations triggers the production of reactive oxygen species (ROS) which leads to photo-aging, sunburn, wrinkles, photosensitivity and DNA damage (Imam *et al*., 2015; Karthika *et al*., 2013). Antioxidant protects the skin from oxidative stress as it scavenges the free radicals (Damle *et al*., 2016; Heberle *et al*., 2012). Therefore, antioxidant activity is very useful in cosmetic formulations (Damle *et al*., 2016).

A potent tyrosinase inhibitor, kojic acid (5-hydroxymethyl)-1, 4-pyrone) is a metabolite produced by fungi species such as *Aspergillus, Acetobacter* and *Penicilium* (Norddin *et al*., 2017; Ashari *et al*., 2009; Uber *et al*., 2000). Kojic monooleate (KMO) is a derivative of kojic acid that improves the storage instability, oil-solubility and toxicity of kojic acid (Norddin *et al*., 2017; Lajis *et al*.,

2012; Ashari *et al*., 2009). KMO also found to be a better tyrosinase inhibitor compared to kojic acid and exhibits strong antioxidant activity (Lajis *et al*., 2012). The objectives of this study were to develop KMO nanoemulsion formulation in thin film system (TFS) and analyse the cytotoxicity, antioxidant activity and skin hydration effect of the formulation.

1.2 Problem statements

KMO possessed significant antioxidant and depigmenting activity, which makes it a good candidate to be incorporated into a formulation to treat hyperpigmentation. A nanoemulsion formulation containing KMO has been developed in recent study by Azhar *et al*., (2018), however, the formulation developed formed a sticky, fragile, and easy to fall-off thin film on the skin which makes it impossible to stay on the skin for a long period of time. The developed formulation was intended to function as serum product which requires prolong exposure towards the skin to allow KMO penetrate deep into the epidermis layer and react with melanin and tyrosinase in order for KMO to work effectively on human skin. However, to counter the problem with texture of formulation formed on the skin and the penetration rate, thin film system (TFS) was introduced and incorporated into the formulation.

Latest technology, thin film system (TFS) is chosen to ensure better texture, better ability of formulation to stay on skin for intended period of time and better penetration of KMO into the skin layer. The challenging part of developing a thin film system is to choose a suitable polymer (plasticiser and solvent) that compatible with the KMO formulation and maintain the nanosized range of 20 - 200 nm. Therefore, there may be some challenges in maintaining the required droplet size and stability of the nanoemulsion.

A study on the effect of polymer towards the size of nanoemulsion is required to obtain an optimised formulation with a droplet size of 20 - 200 nm. The conventional method is time consuming, require a higher number of experimental run and prediction of actual droplet size of nanoemulsion is restricted. Due to this reason, the statistical method such as response surface methodology (RSM) is preferable. A literature survey reveals many papers related to the formulation of herbal in TFS, but no reported paper was found on specifically using KMO in a TFS.

1.3 Objectives of the study

The main objectives of this research was to develop a nanoemulsion system containing kojic monooleate (KMO) in thin film system (TFS). Therefore, the specific objectives of this study were:

1. To investigate the effect of different plasticiser and solvent as well as different composition on KMO nanoemulsion.
2. To optimise the size of droplet for polymeric TFS from KMO nanoemulsion using Response Surface Methodology (RSM).
3. To determine the physicochemical properties of polymeric TFS using transmission electron microscopy (TEM), viscosity, and stability study.
4. To evaluate both *in vitro* (antimicrobial, penetration study and DPPH assay) and *in vivo* studies (cytotoxicity, TEWL and hydration) of polymeric TFS.

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