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Dermal anti-oxidant, anti-inflammatory and anti-aging effects of Compritol ATO-based Resveratrol colloidal carriers prepared using mixed surfactants

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ABSTRACT

In this study, Compritol ATO-based Resveratrol colloidal carriers (CCCs) were prepared and subjected to characterization and evaluation. In most formulae, the use of a binary-mixture of surfactants improved the physicochemical properties. CCC6 (containing P407/P188 as bi-surfactants) attained the highest drug loading, release efficiency during 24 h and occlusive effect for 48 h; in addition, it showed a uniform particle size distribution within the desired range. *In-vivo* studies were done based on the analysis of anti-oxidant markers [catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD)], anti-inflammatory markers [interleukin 6 (IL-6), interleukin 8 (IL-8) and rat Nuclear factor-kappa B (NF-κB)] and anti-wrinkling markers [matrix metalloproteinase (MMP-1) and Granulocyte-macrophage colony-stimulating factor (GM-CSF)], after UVB-irradiation. Results were significantly different when comparing the positive control and the negative control groups ($p < 0.05$). Rats pre-treated with CCC6 showed a great amelioration, and the level of the biochemical markers was significantly different compared to those of the positive control group and those pre-treated with the drug suspension ($p < 0.05$). Also, the high skin protective effect of CCC6 was proved by visual and histopathological examination of the rats' skin. Therefore, the current study proves the beneficial effects of the designed dermal Resveratrol-loaded colloidal system.

1. Introduction

Recently, lipid nanoparticles have attracted great attention as a colloidal drug carrier for topical use.

The advantages of this carrier comprise safety, negligible skin irritation, protective properties for the active substances, and controlled drug release (Mei et al., 2003; Muller et al., 2002). Also, its occlusive effect favors the drug penetration into the skin (Wissing and Muller, 2002). The nanometric size leads to a high surface area of the particles allowing better contact of the encapsulated drug with the stratum corneum and the superficial junctions of corneocyte clusters and furrows between corneocyte islands, this can result in the accumulation for several hours leading to sustained drug release (Cevc, 2004), therefore the lipid nanoparticles have a skin targeting capability (Liu et al., 2007).

Nonionic surfactants improve the solubility of poorly soluble drugs, they are superior compared to other surfactants due to compatibility, stability, less toxicity, and less cellular irritating effect (Jiao, 2008). A previous study has proved that the use of the non-ionic surfactants, Poloxamer or Tween-80, as emulsifiers can improve the stability of the nanosystem by increasing the electrostatic repulsion potential energy among the nanoparticles, and hence, the steric stabilization (Han et al., 2008). It is well established that the use of a combination of emulsifiers (co-emulsifiers) can be more effective than the use of a single emulsifier. In this context, Kamel et al. have reported that the use of a binary mixture of non-ionic surfactants improved the delivery of the hydrophobic drug Rutin (Kamel et al., 2013).

Aging is a biological process threatening living organisms and predisposing to different diseases and disorders (Smith and Forbes, 1996). Skin, being the largest and most exposed human body organ, is highly susceptible to aging/photo-aging which results in skin damage, cancer and many others deleterious harmful effects; the main toxic effects caused by the long exposure to ultraviolet radiation are due to the oxidative stress created by reactive oxygen species (ROS) leading to photo-aging. ROS play a critical role in many pathological conditions (Gilchrest and Yaar, 1992). The innovation of delivery systems providing continuous skin protection is necessary. Some previous studies have proved the photoprotective effect of lipidic nanostructured preparations containing natural components (Kamel et al., 2017; Kamel and Mostafa, 2015).

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Antioxidants are generally unstable and are susceptible to photo-degradation (Kaur et al., 2007). The incorporation of such molecules within lipid nanosystems can provide stable and efficient dermal products. Resveratrol (Res) (*trans*-3, 4, 5-trihydroxystilbene), a phytoalexin found in grapes, nuts, fruits, and red wine, has different potent beneficial biological effects which can combat aging, these include: anti-oxidant, anti-inflammatory anti-angiogenic, and anti-proliferative properties (Afaq et al., 2003; Kasiotis et al., 2013). However, its delivery is accompanied by some limitations due to its poor aqueous solubility and its instability (Shi et al., 2008), therefore the creation of a suitable carrier overcoming these shortcomings is necessary. In some previous studies, Resveratrol was loaded in nanocarriers (Montenegro et al., 2017; Caddeo et al., 2013), however, the use of a binary mixture of surfactants was not previously investigated and none of these literatures was oriented towards the study of the dermal localized biological effects related to the photo-protective potential of the suggested formulae. The current study is focused on the preparation and evaluation of topical Compritol ATO-based Resveratrol colloidal carriers (CCCs), and the effect of the surfactant type was studied. Compritol® ATO 888 (US/NF: glyceryl behenate, is a mixture of monoglycerides, diglycerides, and triglycerides of behenic acid) was used as the lipid material. The non-ionic surfactants used, alone or as a binary mixture, in the preparation of the CCCs are: Tween 80 (PEG Sorbitan monooleate), Poloxamer 407 and Poloxamer 188 (triblock copolymer of polyoxyethylene and polyoxypropylene). The selected formula was subjected to *in-vivo* experiments and biochemical assays to study its localized anti-oxidant, anti-inflammatory and anti-aging potential.

2. Materials and methods

2.1. Materials

Resveratrol (trans, 98% content) was purchased from Behr GmbH., Stuttgar, Germany. Poloxamer 188 (P188) and Poloxamer 407 (P407) were obtained from Sigma-Aldrich Chemie GmbH, Germany; Compritol® ATO 888 (C 888) was a free sample from Gattefossè, France. Tween 80 (T80) was purchased from Adwic, El-Nasr Pharmaceutical Chemicals Co., Egypt. Cellulose membrane (molecular weight cut-off 12,000 g/mole) was purchased from Sigma Chemical Company, USA. All other chemicals used in the study were of analytical grade and were obtained from the El-Nasr Company for Pharmaceutical Chemicals (Cairo, Egypt). Catalase (CAT) and rat Nuclear factor-kappa B(NF-κB) ELISA Kit was purchased from Hubei, China. Rat Superoxide Dismutase (SOD), Interleukin 8 (IL-8) and Rat Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) ELISA Kit was purchased from MyBioSource, San Diego, US. Matrix metalloproteinase (MMP-1) ELISA kits was purchased from Lifespan Bioscience, North America, Interleukin 6 (IL-6) was purchased from CUSABIO, Inc., Wuhan, Hubei, China and Glutathione peroxidase (GSH) was purchased from ShangHai BlueGene Biotech CO., China.

2.2. Methods

2.2.1. Preparation of Compritol ATO-based Resveratrol colloidal carriers (CCC)

The Resveratrol-loaded CCC was prepared by high pressure homogenization technique (Hot Homogenization) (Abdelbary and Fahmy, 2009). The weighed amount of the lipid Compritol®(C888) and the drug (Res) were heated together at temperature 75 °C till they melt forming the oil phase (melting point of C888 is between 69 °C and 74 °C (Faham et al., 2000). The aqueous phase was prepared by dissolving the emulsifying agent (P188, P407 or T80) or a binary mixture of them in a ratio of 1:1 (according to Table 1) in distilled water, and then heated to the same temperature of the oil phase. The hot aqueous phase was added to the melted lipid phase and directly homogenized using Heidolph homogenizer (Germany). Res-loaded CCCs were finally obtained by allowing the hot nanoemulsions to cool to room temperature. The formulae were kept at room temperature under dry conditions till further investigations.

Table 1
Composition of the surfactants used to prepare the CCCs and, particle size analysis: particle size (PS), size distribution (PDI) and zeta potential (Z) values of the CCCs.

Formula	Surfactant used	PS (nm)	PDI	Z (mV)
CCC1	T80	841.20 ± 28.99	0.59 ± 0.20	−35.1 ± 7.20
CCC2	P188	270.40 ± 7.07	0.13 ± 0.04	−6.3 ± 0.28
CCC3	P407	230.40 ± 7.78	0.13 ± 0.06	−16.6 ± 5.23
CCC4	T80/P188	521.00 ± 50.61	0.22 ± 0.07	−20.00 ± 5.20
CCC5	T80/P407	435.80 ± 38.42	0.22 ± 0.00	−27.20 ± 5.30
CCC6	P188/P407	248.10 ± 2.12	0.07 ± 0.02	−28.23 ± 6.15

T80: Tween 80.
P188: Poloxamer 188.
P407: Poloxamer 407.
Values = mean ± SD.

2.2.2. Particle size analysis and Zeta potential

The Particle size analysis of Res-loaded CCCs was performed using Particle size analyzer (Zeta Sizer Nano ZEN 3600). The measurements were executed in triplicate for each sample and the average values were taken.

The CCCs surface charge was determined by the measurement of the zeta potential using Zeta Sizer Nano ZEN 3600.

2.2.3. Determination of the drug content and encapsulation efficiency

Accurately weighed amounts (100 mg) of Res-loaded CCCs were dissolved in 50 ml ethanol under sonication for 60 min. to ensure complete extraction of the drug. After filtration using 0.45 μ Millipore filter, the samples were assayed for drug content by UV-Spectrophotometry (Shimadzu UV Spectrophotometer) at 303 nm. Blank experiments were done simultaneously (Kamel and Mostafa, 2015). The amount of drug loaded in 100 mg preparation (drug loading) was validated and the % encapsulation efficiency (EE) was calculated as follows:

$$EE = (\text{Calculated drug amount}/\text{Theoretical drug amount}) \times 100$$

2.2.4. In vitro release studies

In-vitro release studies were performed by the dialysis bag diffusion technique using 50 ml phosphate buffer pH 5.5; and shacked in a water bath shaker at 100 rpm and 32 ± 2 °C (Memmert GmbH, Germany). An amount of the CCCs equivalent to 10 mg drug was placed in a cellulose acetate dialysis bag (Sigma) which was sealed at both ends. At predetermined time intervals, 2 ml samples of the receiver medium were withdrawn and replaced by equivalent volume of fresh medium to maintain constant volume and sink conditions. The samples were analyzed for drug content spectrophotometrically using Shimadzu UV Spectrophotometer at 306 nm.

2.2.5. Occlusive properties study

Twenty-five ml beakers were filled with 15 ml distilled water and covered with Whatman® filter paper No 41 with pore size of 8 μm (England) and sealed with silicon. The CCCs were applied on the filter papers, in 10 mg/cm^2, and evenly spread with a spatula. The beakers were weighed and stored in an incubator (Shellab model 1545, USA) at 32 °C and 50% RH for 48 h. The beakers were weighed at zero, 6, 24 and 48 h and the occlusion factor (F) was calculated according to the equation (Shiva et al., 2012):

$$F = 100 * (A - B)/A$$

where A = weight difference (water loss) in case of control (without any application) and B = weight difference in case of tested formulae.