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The tetrapeptide *N*-acetyl-Pro-Pro-Tyr-Leu in skin care formulations—Physicochemical and release studies

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ABSTRACT

Recently there has been a growth of interest in the novel skin care formulations containing active ingredients such as low molecular weight peptides. In this paper we present new skincare formulations such as hydrogels, oil-in-water emulsions and water-in-oil emulsion containing a tetrapeptide (*N*-acetyl-Pro-Pro-Tyr-Leu). These formulations were characterized in terms of physicochemical parameters (pH, viscosity), stability and particle size distribution. Additionally, the diffusion parameters of the peptide in the obtained formulations were calculated based on the Einstein–Smoluchowski equation. Furthermore, in order to determine the penetration of the tetrapeptide through membranes its release kinetics were investigated. On the basis of release curves, the release rate constants were determined. The results proved that the properties of the formulations strongly determined the release rate of the tetrapeptide. The higher viscosity of the semisolid, the slower was the permeation through the membrane.

1. Introduction

In vitro release tests are applied to quantify the amount and extent of the active compound released from solid and semisolids dosage forms. These studies are useful in the design and development of novel formulations as well as in quality control. The release results are indicative of the formulation performance and can be used for screening of a product prior to *in vivo* testing. The release profile of an active ingredient is determined by various factors such as the size of the dispersed particles, the interfacial tension between water and the oil phases and the rheological properties of the semisolid dosage form (Olejnik et al., 2012; Yang et al., 2008; Bonacucina et al., 2006). Nowadays very often, the common active components of the semisolid formulations are low molecular weight peptides. These compounds have recently revolutionized the skincare and pharmaceutical industries and have become one of the major bioactive ingredients in anti-aging cosmetic compositions (Lupo and Cole, 2007). Peptides are formed from amino acids that mimic fragments of endogenous peptides that exhibit biological activity (Buraczewska et al., 2007). Peptides used in cosmetics products are generally divided into four categories such as carrier peptides, neuro-transmitters, enzyme modulators and signal peptides (Draelos, 2011). The great demand for these components is connected with their activity because peptides are involved in various natural processes with relevance to skincare, such as cell migration, modulation of cell proliferation, melanogenesis, angiogenesis and protein synthesis and their regulation (Fields et al., 2009; Zhang and Falla, 2009). Some researchers state that there is no significant evidence that peptides work better than moisturizers although other investigators claim that they represent a group of compounds with future potential (Bowler, 2009). In fact peptides can be found in a large number of cosmetics and cosmeceuticals (Zhang and Falla, 2009) including anti-aging, anti-wrinkle and skin moisturizing products, therefore it is important to analyze the formulations that contain these compounds. In order for the action of a dermal semisolid formulation to occur, the active compound must first be released from the vehicle in order to penetrate through the skin layers (Goebel et al., 2013). The release of active components is to a large extent dependent on the properties of the semisolid dosage form.

Therefore, the aim of this study was to characterize the formulations containing the tetrapeptide *N*-acetyl-Pro-Pro-Tyr-Leu (AcPPYL) and to evaluate the release rate of this tetrapeptide from various semisolid formulations. This tetrapeptide (Fig. 1) is used in cosmeceutical compositions as an anti-aging active ingredient (Rodrigues et al., 2008). Pauly et al. (2008) revealed in *in vitro* studies that AcPPYL commercially known as acetyl-tetrapeptide-11 stimulates keratinocyte cell growth and syndecan-1 synthesis. *In vivo* studies showed a significant increase in the biomechanical parameters of the superficial layers of epidermis.

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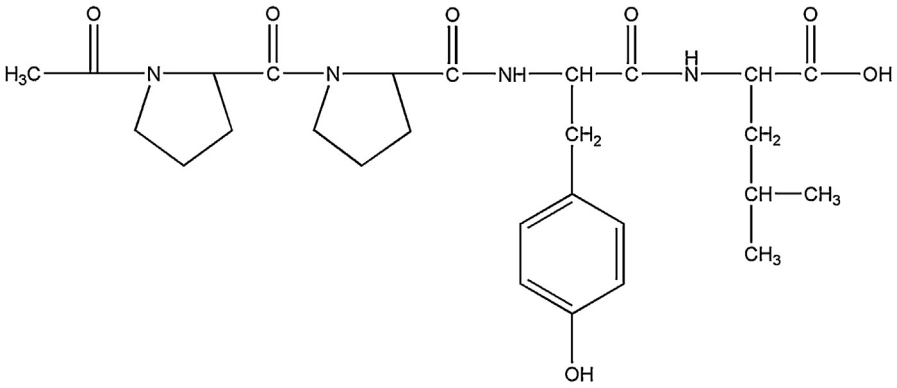

Fig. 1. Chemical structure of the tetrapeptide AcPPYL.

Creams with acetyl-tetrapeptide-11 demonstrated 5–10% better effects than placebos (Pauly et al., 2008; Gorouhi and Maibach, 2010).

2. Materials and methods

2.1. Materials

The tetrapeptide AcPPYL was synthesized by Lipopharm (Poland). Potassium phosphate buffer was purchased from J.T. Baker® (USA). Cuprophan (regenerated cellulose) was obtained from Agilent Technologies (USA).

2.2. Semisolids preparation

2.2.1. Preparation of emulsion A in cold process (Olejnik et al., 2013)

Cragel EZ7 and Alphaflow 20 were mixed in a beaker until the phase becomes homogenous. Then the water phase was added slowly, while mixing gently at room temperature, until the proper viscosity was fully developed. Next the tetrapeptide was added.

2.2.2. Preparation of emulsion B

The ingredients of the oil phase (Tego Care 450, Tego Alkanol 1618,Tegin 4100 Pellets Cetiol 868, Masocare SQV) were heated to 70 °C. At the same time water in another flask was also heated to 70 °C. When all of the oil phase ingredients were melted, water was added. The obtained mixture was stirred and cooled to 50 °C. Afterwards the emulsion was homogenized by a homogenizer (yellow line DI25 basic, IKA-Werke GmbH & Co.KG, Germany) and cooled to 30 °C. Then glycerin, citric acid and tetrapeptide were added and stirred until cool.

2.2.3. Preparation of emulsion C

The ingredients of the oil phase (Tego Alkanol 1618, parafin oil, isopropylpamitate and vaseline) were heated to 70 °C. At the same time water was heated to the same temperature. When all of the oil phase ingredients were melted, water was added and both phases were mixed until a homogeneous emulsion was obtained. After cooling down to 30 °C citric acid and tetrapeptide were added.

2.2.4. Preparation of hydrogel A

Tego Carbomer 340 was dispersed for 2 h in water by a magnetic stirrer. Then isopropanol with tetrapeptide was introduced, and next sodium hydroxide was added until a pH of 6.5 was reached.

2.2.5. Preparation of hydrogel B

Water was mixed with glycerin and heated to 80 °C. Afterwards hydroxyethylcellulose, citric acid and tetrapeptide were added and stirred until the hydrogel formulation was obtained.

2.3. Characterization of obtained formulations

2.3.1. Centrifugation test

Centrifugation tests were performed (by MPW-360R Centrifuge, MPW MED Instruments, Poland) for emulsions immediately after preparation, after 24 h and after 60 days of preparation. Between the measurements the samples were stored at room temperature. Centrifugation working conditions were 4000 rpm.

2.3.2. Stability test by multiple light scattering

The stability measurements were conducted directly after preparation of the emulsions and at different times for 30 days by using Turbiscan Lab Expert (Formulaction, France). Multiple light scattering was used to measure the stability of the emulsions at 25 °C. All of the emulsions were compared using Turbiscan Stability Index (TSI) that provides information regarding the general behavior of the samples. TSI is calculated as the sum of all of the destabilization processes occurring in the sample cell (Zhao et al., 2014; Carbone et al., 2015).

2.3.3. Particle size distribution analysis by laser diffraction

The particle size distributions of formulations containing tetrapeptide were analyzed by using Mastersizer 2000 (Malvern, UK) equipped with a hydro dispersion unit. The pump speed was settled at 2000 rpm. The measurements were carried out at room temperature in distilled water. The mean droplet diameter was presented as $d_{3,2}$ known as the Sauter diameter (Pérez-Mosqueda et al., 2015). Measurements were performed in triplicate. Then the average value was determined.

2.3.4. Determination of pH

The pH values of samples were determined by a pH-meter (Testo, Australia). The measurements were performed at room temperature in triplicate, and then the average value was determined.

2.3.5. Viscosity

All viscosity measurements were done at room temperature by using a rotational viscometer equipped with a temperature sensor (RC02 Viscometer, Rheotec, Germany). All of the samples were previously equilibrated at room temperature.

2.4. Release studies

In vitro release studies were performed with a USP Apparatus 2 (Vankel 7010, Varian, USA) connected with a UV–vis Cary 50 Bio (Varian, USA). Each sample containing peptides was placed into the enhancer cell and covered with a cellulose-based membrane (Cuprophan). The analysis was performed in a potassium