Toxicology and Applied Pharmacology 342 (2018) 14–21

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Safety assessment of starch-based personal care products: Nanocapsules and pickering emulsions

J. Marto[a], P. Pinto[a,b], M. Fitas[b], L.M. Gonçalves[a], A.J. Almeida[a], H.M. Ribeiro[a,*]

[a] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal
[b] PhD Trials, Rua das Murtas, n°1B, 1° - 1700-309, Lisboa, Portugal

ARTICLE INFO

Key-words:
Safety assessment
Biological effects
Starch vehicles
Cosmetics
Excipients
Topical application

ABSTRACT

The safety profile of the ingredients used in topical dosage forms and its evaluation is an issue of utmost importance. A suitable equilibrium between safety and efficacy is crucial before promoting a dermatological product. The aim of this work was to assess the safety and biological effects of starch-based vehicles (St-BV) used in such products. The hazard, exposure and dose-response assessment were used to characterize the risk of each ingredient. The EpiSkin™ assay and human repeat insult patch tests were performed to compare the theoretical safety assessment to *in vitro* and *in vivo* data. The efficacy of the St-BV was studied using biophysical measurements in human volunteers during 28 days, showing that all ingredients and their combinations were safe for the consumer. Tissue viability determined using the EpiSkin™ testing reached values between 84.0 ± 5.0% and 98.0 ± 8.6% after application of St-BV, which were considered as non-irritant to the skin. These observations were confirmed by the *in vivo* studies where the St-BV did not induce any sensitization on the volunteers, being safe for human use. Moreover, St-BV increased skin hydration and microcirculation, emerging as an attractive alternative to chemical raw materials.

1. Introduction

Emulsions and, more recently, micro- and nanoparticulate systems have been widely used in pharmacy and cosmetics because they could be a support for therapies and, as vehicles to deliver drugs and cosmetic agents to the skin. These pharmaceutical dosage forms must fulfill a number of regulatory requirements, *e.g.* acceptable physical and chemical stability, satisfactory safety and efficacy profile, while presenting optimal sensory attributes. In addition, they must be non-irritant to the skin and easily applied. In order to meet all these attributes, several excipients have been investigated often resulting in complex, multicomponent formulations containing several emulsifiers, co-emulsifiers, polymers, emollients and preservatives (Raposo et al., 2013b, 2014). Thus, it is crucial to evaluate the safety profile of the ingredients used in such vehicles, particularly those that may present safety concerns (*e.g.* preservatives, solubilizers and surfactants). Although human external contact with these ingredients barely results in significant systemic exposure (skin care products mainly produce local exposure), it cannot be completely excluded and, in specific circumstances, may cause skin reactions (Nohynek et al., 2010). This is why cosmetic products must undergo an extensive safety assessment before marketing, including skin acceptability or compatibility studies carefully conducted on volunteers to confirm their good tolerance (SCCS, 2015).

Human studies may also be performed to measure skin benefits and document product claims, or to collect consumer's feedback before the product is launched in the market. In all these studies, any undesirable health effect is taken into account to complement and strengthen the overall safety assessment on the product (Pauwels and Rogiers, 2010).

Cosmetic products fall under the general requirements of the EC Cosmetics Regulation 1223/2009 (EC, 2009), whereby the toxicological profile of all used ingredients and detailed knowledge of the product-specific exposure are required as fundamental for the safety assessment. As imposed by the European legislation, cosmetics are considered to be safe for the consumer. Although this appears to be self-evident, there is a whole scientific exercise preceding this “obvious” conclusion (Pauwels and Rogiers, 2010). The safety of a cosmetic product is determined based on the safety assessment of its ingredients, which is performed using literature data, *in vitro* tests and human tests since in the European Union (EU) finished cosmetic products are no longer tested in animals.

The key factors in the management of topical diseases are not only related to the use of effective topical agents but also in providing skin hydration and barrier repair. The selected ingredients to such vehicles are extremely important and should present a suitable equilibrium

* Corresponding author at: Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Professor Gama Pinto, 1649-003 Lisboa, Portugal.
E-mail address: hribeiro@campus.ul.pt (H.M. Ribeiro).

https://doi.org/10.1016/j.taap.2018.01.018
Received 17 October 2017; Received in revised form 8 January 2018; Accepted 25 January 2018
Available online 04 February 2018
0041-008X/

Table 1
Qualitative and quantitative composition of the St-BV.

Ingredients	Quantitative composition (%, w/w)			Main functions/additional functions	Main characteristics
	ASt-emulsion with LP	ASt-emulsion with CT	StNC		
Phase A (external)					w/o emulsions Stabilized by starch particles
Liquid paraffin	69	–	–	Emollient	
Caprylic/capric acid triglyceride	–	69	–	Emollient	
Phase B (solid particles)					
Aluminum starch octenylsuccinate	6	6	–	Absorbent and viscosity controlling	
Phase C (internal)					
Purified water	25	25	–	Solvent	
Phase A (ethanolic phase)					Particle size distribution:
Ethanol	–	–	32.53	Solvent and preservative	d(10) – 171 nm
Cetrimonium bromide	–	–	0.25	Emulsifying	d(50) – 293 nm
Capric/caprylic triglycerides	–	–	0.55	Emollient	d(90) – 429 nm
Phase B (aqueous phase)					Span – 0.884
Purified water	–	–	65.47	Solvent	Zeta potential = 34,4 mV
Polysorbate 80	–	–	1.13	Emulsifying	
Pregelatinized modified corn starch	–	–	0.07	Polymer	

between safety and efficacy (Serup, 2001). Emollients or moisturizers are often used in the treatment of topical diseases with the aim of improving skin hydration and barrier repair (Carneiro et al., 2011).

In recent years, there has been an increased interest in developing delivery systems and, also, exploring new ways of using approved excipients, such as starch. Using safe approved excipients is a smart strategy to obtain improved cosmetics products. Due to its unique properties, starch has been extensively used in various topical pharmaceutical applications, *i.e.* as a sensorial enhancer, a stabilizer and polymer. The modification of the native starch generates improved starches with different and enhanced physicochemical properties that allowed its use as a more efficient pharmaceutical excipient (Marto et al., 2015). These modified starches can act as gelling agents, originating gels with good spreadability and stability. Also, as they can be effective as an emulsion stabilizer they can avoid the use of hazardous surfactants. Starch is also a good candidate for nanoparticulate carriers. As starch nanoparticles have excellent mechanical and functional properties, they can be seen as highly valuable for their potential as drug carrier material in pharmaceutical industry (Marto et al., 2016b). Their small size promotes skin permeation and their large surface area ensures drug release and absorption. While starch is a multifunctional excipient with endless advantages, many developments are expected in this area, which might represent a renewed vision for the pharmaceutical industry.

The base-concept of this study was to develop and characterize novel starch-based formulations for dermatological application. We developed two types of novel starch-based vehicles, specifically, emulsions stabilized by starch particles (Octenyl succinic anhydride starch) and nanocapsules produced by pregelatinazed modified corn starch. Therefore, the major aim of this research study was to evaluate the safety profile and biological effects of starch-based vehicles (St-BV), using literature data and a systematic approach for safety assessment, comparing it with *in vitro* and *in vivo* data obtained using skin bioengineering methodologies and tests performed on human volunteers, such as trans-epidermal water loss (TEWL), epidermal capacitance, skin surface lipids and microcirculation. It should be mentioned that St-BV can be marketed as cosmetic products, being of great importance to study their safety profile and biological effects according to the relevant European regulatory framework.

2. Materials and methods

2.1. Materials

Aluminum starch octenylsuccinate (ASt) (DryFlo® Plus) was purchased from AkzoNobel (Amsterdam, Netherlands). The oils used were liquid paraffin (LP) purchased from Mosselman (Ghlin, Belgium) and caprylic/capric acid triglyceride (Tegosoft® CT) (CT), a kind gift from Evonik Industries AG (Essen, Germany). Cetrimonium bromide (Cetrimide) was a gift from DS Produtos Químicos (São Domingos de Rana, Portugal). Polysorbate 80 (Tween® 80) was obtained from Merck (Kenilworth, USA) and ethanol was from Carlo Erba Reagents (Cornaredo, Italy). Pregelatinized modified starch (Instant Pure-Cote® B793) was a gift from Grain Processing Corporation (Washington, USA). Purified water was obtained by reverse osmosis, electro deionization (Millipore, Elix 3) followed by filtration (pore 0.22 μm).

2.2. Methods

2.2.1. Preparation of starch-based vehicles (St-BV)

2.2.1.1. Starch-stabilized emulsions (ASt-emulsion). The w/o emulsion stabilized by ASt granules was developed using purified water as disperse phase and CT or LP as continuous phase. The ASt particles were first dispersed in the oil phase using a vortex mixer until total dispersion. The oil and aqueous phases were then mixed together with an UltraTurrax® T25 homogenizer (IKA®-Werke GmbH & Co. KG, Germany) (Table 1).

2.2.1.2. Starch nanocapsules (StNC). The StNC were emulsion-solvent evaporation method, using prepared by capric/caprylic triglycerides (CT) as the lipid component, Tween®80 and cetrimide as surfactants, pregelatinized modified corn starch as a polymer and ethanol. Briefly, capric/caprylic triglycerides were dissolved in an ethanol solution containing the cationic surfactant and then added to the aqueous phase containing Tween®80 and the hydrated polymer, under constant magnetic stirring. The nanoparticle dispersion was then kept under stirring at 25 ± 2 °C (Table 1). Particle size distribution and the surface charge (zeta potential) was performed according a previous published work (Marto et al., 2016b).

2.2.2. Safety assessment of St-BV

The safety assessment of St-BV was accomplished according to the Scientific Committee on Consumer Safety's (SCCS) Notes of Guidance for Testing of Cosmetic Ingredients and their Safety Evaluation (SCCS, 2015). The information for each ingredient was obtained from the respective supplier.

2.2.2.1. Hazard identification. The physical, chemical and toxicological properties of each ingredient, as well as the results of the *in vitro* and *in vivo* testing, and clinical studies, were taken into account to infer if the