

INCORPORATION OF NEW BENZIMIDAZOLE COMPOUNDS INTO LIPID NANOSTRUCTURES IN ORDER TO OBTAIN PHOTOPROTECTIVE FORMULATIONS

IRINEL GRAȚIELA MĂNESCU¹, GABRIELA BADEA^{2*}, LUCIAN ISCRULESCU¹, MIRCEA IOVU¹, TEODORA BALACI¹

¹"Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, Bucharest, Romania

²Polytechnic University of Bucharest, Faculty of Applied Chemistry and Materials Science, Bucharest, Romania

*corresponding author: gabriela.badea8@gmail.com

Manuscript received: December 2014

Abstract

The exposure to UVA and UVB radiation is responsible for damaging the skin's natural defence, having mechanism various adverse effects, from sunburns to skin cancer. The skin application of cosmetic products that possess photo protective activity raises some concerns associated with the toxicity of the synthetic organic UV filters used on market today. Thus, it is necessary to develop new photo protective products based on safe to use UV filters. The objective of study was to encapsulate several non-toxic benzimidazole derivatives, which absorb in the UVA and UVB domains, into lipid nanostructures in order to improve their photo protective action. The developed lipid nanostructures were embedded into topical cosmetic emulsions and the obtained preparations were evaluated *in vitro* for their sun protection factor (SPF).

Rezumat

Expunerea la radiațiile UVB și UVA este responsabilă pentru afectarea mecanismului natural de apărare al pielii, determinând efecte adverse variate, de la eritem solar până la cancer de piele. Aplicarea pe piele a produselor cosmetice cu acțiune fotoprotectoare pe domeniile UVA și UVB ridică însă probleme datorită conținutului de substanțe active sintetice periculoase utilizate în prezent pe piață. Astfel, este necesară dezvoltarea de noi produse cosmetice pe bază de filtre solare sigure în utilizare. Obiectivul studiului a constat în introducerea unor derivați netoxici ai benzimidazolului, care absorb în domeniile UVA și UVB, în nanostructuri lipidice pentru a le îmbunătăți acțiunea fotoprotectoare. Nanostructurile lipidice dezvoltate au fost încorporate în emulsii dermato-cosmetice cărora li s-a determinat *in vitro* factorul de protecție solară (SPF).

Keywords: lipid nanostructures, sun protection factor (SPF), benzimidazole derivatives

Introduction

The radiations emitted by the sun are light radiations, infrared and ultraviolet radiations (200-400 nm). These ultraviolet radiations are divided into three groups: UVC (200-280 nm), which are absorbed into the atmosphere, UVB (280-320 nm) and UVA (320-400 nm). Recent studies have shown the detrimental effect of ultraviolet radiation (UV) in the occurrence of different forms of skin cancer [4] and also in premature skin aging [7]. UVA and UVB have a role in pathogenic and photosensitive diseases but also in photo-allergic and photo-toxic reactions to drugs [21]. Protection against UV radiation is performed with *inorganic screens* and *organic filters*. The *inorganic* filters used in industry for the preparation of sunscreens are zinc oxide and titanium dioxide, and organic filters are salicylic acid derivatives, benzoic acid derivatives, camphor derivatives [1] and cinnamates [8, 18]. Currently, organic UV filters are the most used ingredients for the production of sunscreens [3].

The organic UV filters are not beneficial to the human body because they present estrogenic activity and can damage the cellular DNA at the skin level [9, 10]. Due to their lipophilic character they are bio accumulated in the body and contaminate the environment in significant concentrations. Thus, it is necessary to develop safe organic UV filters that absorb on both domains, UVB and UVA. In recent studies, in order to reduce the toxicity and the amount of UV filters used in cosmetic formulations, UV filters were incorporated into nanostructured lipid carriers (NLCs). These lipid nanostructures are new colloidal systems developed from oil/ water emulsions type, where the oil phase was replaced by a mixture of solid and liquid lipids. NLCs are submicron particles with spherical shape and average diameters ranging of 40-500 nm that are generally used for the delivery of lipophilic compounds. The lipid matrix is formed by physiological lipids that are not toxic and the production process is generally cheap, fast and effective.

Several studies used NLCs in order to develop photo protective nano-formulations by encapsulating organic UV filters such as benzophenone-3 [12], oxybenzone [19], avobenzone [16, 17], octocrylene [11], and diethylamino hydroxybenzoyl hexyl benzoate [2].

Benzimidazoles are a large range of biological properties [22] but in the present research, derivatives of benzimidazole with photo protective activity were synthesized. The compounds absorb UVB or UVA radiations, having extended conjugation throughout the molecule. These compounds do not present toxicity [14]. To improve their photo protective action benzimidazole compounds were incorporated in NLCs developed based on rice bran oil. The feasibility of benzimidazole derivatives in developing NLCs were evaluated in terms of mean particle size and zeta potential. They were further formulated into a cosmetic emulsion with photo protective effect.

The photo-protective properties of the developed formulations were evaluated *in vitro* by using TransporeTM tape, a plastic material which has a non-uniform surface topography similar to that of human skin *stratum corneum* [15].

Materials and Methods

Materials

The synthesis of benzimidazole derivatives was performed using commercial reagents (Fluka, Merck and Aldrich) phenylenediamine, benzaldehyde,

Amberlite IR -120, ethanol-water, methoxybenzoic acid, acetic acid, formaldehyde, aminobenzoic acid. Polyoxyethylenesorbitan monolaurate (Tween 20) was purchased from Merck (Germany). Synperonic PE/F68 (Poloxamer 188) and L- α -Phosphatidylcholine were obtained from Sigma Aldrich Chemie GmbH (Munich, Germany). The solid lipids used were: *n*-Hexadecyl Palmitate (CP) obtained from Acros Organics (USA) and Glyceryl Stearate (GS) from Cognis GmbH. The rice bran oil was purchased from Elemental SRL (Romania). All the substances that were used to obtain the dermato-cosmetic emulsion were of pharmaceutical purity.

Methods

Synthesis of benzimidazole compounds. The benzimidazole derivatives used were: 1-bezyl-2-phenyl-1H-1,3-benzimidazole (**C1**), 1-(4-methylbenzyl)-2-(4-methylphenyl)-H-1,3benzimidazole (**C2**), 1-(4-carboxy)-phenyl-2-(4-methoxyphenyl)-H-1,3benzimidazole (**C3**), 1-(4-dimethylamino)benzyl-2 -(4-dimethylaminophenyl)-H-1,3 benzimidazole (**C4**). Compounds C1 and C2 were obtained by condensation of phenylenediamine with benzaldehyde and, respective by with toluene aldehyde. For the synthesis of 1,2-disubstituted benzimidazoles, two-stages operating mode was applied: the synthesis of 2-substituted benzimidazoles, followed by the application of Mannich base to the nitrogen atom at position 1. The progress of the synthesis reaction was controlled by HPLC.

Table I
Benzimidazole derivatives synthesized

Symbol	Molecular Formula	Molecular Mass	Yield %	Melting Point (°C)	Wavelength λ_{max} (nm)	Molecular Structure
C1	C ₂₀ H ₁₆ N ₂	284	93	130	286	
C2	C ₂₂ H ₂₀ N ₂	312	90	129	291,6	
C3	C ₂₂ H ₁₈ N ₂ O ₃	358	92	167	289	
C4	C ₂₄ H ₂₆ N ₄	370	88	135	324	

Synthesis of benzimidazole-NLC compounds. The nanostructured lipid carriers were composed of a

lipid phase, which consisted in rice bran oil, cetyl palmitate, glyceryl monostearate and one of the

compounds C1÷4 in ratio of 3:3.5:3.5:1, and by an aqueous phase containing in 3.5% surfactant mixture (Tween 20:Poloxamer:Phosphatidylcholine) in a ratio 4.66:1:1. The preparation method of NLCs was the melt emulsification method coupled with high shear homogenization method [13]. The two phases were separately heated at 85°C while stirring. Then, the lipid phase was gradually added over the aqueous phase. The emulsion was heated under stirring at 85°C for 1 h, and then homogenized by applying 25000 rpm for 10 min (High-shear homogenizer PRO250, Germany). To remove the water, the dispersions were frozen for 24 h and then lyophilized using a Christ Alpha 1-2 LD Freeze Dryer (Germany) for 72 h at -55°C.

NLCs characterization

Particle size analysis. The particles size was measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS system (Malvern Instruments, Malvern, United Kingdom). The average particle size of nanoparticles in dispersion and the polydispersity index (PDI) were measured at 90° scattering angle and at a temperature of 25°C. Before measurements, the dispersion was diluted with deionized water to get an adequate scattering intensity. The average diameters and the PDI were presented as the average of three measurements.

Zeta potential analysis. The Zeta potential (ZP) of nanostructured lipid carriers was determined by measuring the electrophoretic mobility of the nanoparticles in an electric field using a Zetasizer Nano ZS system (Malvern Instruments, Malvern, United Kingdom). The electrophoretic mobility was converted into zeta potential using Helmholtz-Smoluchowski equation. Before measurements, the nanoparticles in dispersion were diluted with a solution of NaCl (0.9%, w/v) to adjust the conductivity to 50 µS/cm. All measurements were made in triplicate at 25°C and the average value was reported.

Formulation and obtaining the cosmetic preparations

For an appropriate formulation of the sunscreen preparations a number of issues that might affect their efficiency were considered: the amount in which the UV filter was incorporated, previously processed in NLCs, depending on the absorption potential of the UV radiation, and the selection of appropriate cosmetic ingredients, taking into account the physicochemical characteristics of the UV filter agent and the desired cosmetic properties of the finished product.

Considering the physicochemical properties of the selected photo protective compounds, including the NLCs, and the general methods for preparing the ointments – emulsions, the ingredients were processed in the form of a cosmetic cream, of which the formulation is shown in Table II.

Calendula extract was used for its antibacterial and antioxidant properties [6].

Table II

Qualitative and quantitative composition of the photo protective preparations

Composition	Quantity (g)
Lyophilized nanostructures 1-4	10
Titanium dioxide	1
Zinc oxide	1
Allantoin	0.2
<i>Calendula</i> extract	1
Collagen	0.5
Vitamin A	150.000 UI
Shea butter	5
Olive oil	35
lanolin	7
Beeswax	5
Cholesterol	1
Cetaceum	3
Cetyl alcohol	3
Sodiumhydrogencarbonate	0.2
Butylhydroxyanisole	0.05
Phenoxyethanol	0.1
Perfume	q.s.
Distilled water	q.s.

The lipophilic phase consists of Shea butter, lanolin, beeswax, cholesterol, cetaceum, cetyl alcohol and olive oil. Beeswax, lanolin and cholesterol are natural products which act as emulsifying agents. Lanolin presents a remarkable emollient capacity and ideal emulsifying properties. Sodium hydrogen carbonate causes the formation of alkali soaps which serve to stabilize the emulsion. Butylatedhydroxyanisole is an antioxidant and phenoxyethanol is an antimicrobial agent.

Cosmetic qualities, moisturizing, nourishing and healing have been improved by adding allantoin, vitamin A, *calendula* extract and collagen.

Fatty components and zinc oxide were melted together on a water bath at a temperature of about 60-70°C. The aqueous phase in which titanium dioxide was dispersed, was heated separately at a temperature slightly higher than that of the lipid phase, and then they were gradually brought together while stirring. The obtained emulsion was cooled to a temperature of 30°C, temperature that allowed the incorporation of NLCs, collagen, vegetal extract, vitamin and perfume. Throughout the preparation, an important role was played by the stirring, which ensured a stable and homogeneous formulation. The cosmetic preparations were stored in appropriate conditions, in tightly closed boxes and in a cool place, away from light.

Quality control of the photo protective preparations

The quality control consisted in the following determinations:

- the organoleptic control: appearance, colour, smell, finesse, adhesion - was

performed according to FRX guidelines and literature data;

- evaluation of sensory characteristics. The use of inorganic UV filters in formulating photoprotective products causes undesirable sensorial characteristics: visible white film on the skin surface, unpleasant sensation upon application, skin drying. Therefore, it is necessary to evaluate the characteristics of the obtained cosmetics.

Pharmacotechnical control

The pH determination of the samples was carried out in an aqueous phase, which was obtained by shaking the cream into adequate water content.

Determination of the display capability using the extensimetric method

The determination was performed to check the spread-ability of the preparation on the skin or mucosa. This analysis determines the deformation ability of the preparation when several forces are acting on it, by using Ojeda Arbussa method.

The evaluation of the viscosity was performed using a RC 1 rheometer (Rheotec, Germania), by determining the shear stress. Rheological measurements were performed in accordance with the instructions set out in the technical specification of the apparatus. Shear speed was increased progressively (10 speed steps). The variation of the viscosity was measured and recorded, and the shear stress with increasing shear speed, respectively.

Determining the degree of hydration by corneometer method

The measuring principle of the Corneometer CM 825 is based on the capacitance measurement of a dielectric medium. Any change of the dielectric constant due to variation of the skin surface hydration alters the capacitance of a condenser. The reproducibility of the measurements is very high and the measurement time is very short (one second). Practically, the technique is used to measure the difference in the hydration of the stratum corneum before and after the application of a cosmetic treatment on the skin. The test was performed on three areas of the skin, which were different by treated (zone 1 –blank - without any cosmetic product, zone 2 – base emulsion and zone 3 – the obtained emulsion with active ingredients) at room temperature. Before measurements, there was an adaption time to room conditions without covering the areas with clothes. All the measured values were expressed as mean average of 10 records. The measurements were made exactly in the same places. There is a change of hydration status depending on location. The measuring place was at the middle of the forearm. The corneometer was used and calibrated at the value of the baseline for each part of the skin portion before applying the

preparations. In the first 30 minutes after application, the water content is usually higher than normal. Measuring water content at this time can lead to errors. The following readings were performed at 60 minutes, 120 minutes and 240 minutes.

Evaluation of the photo protective properties

In vitro determination of SPF (sun protection factor)

Studies have shown that the results of the photo protective properties determinate *in vitro* were similar with those measured *in vivo*. In this study, the SPF was determined *in vitro* according to the methodology of Diffey-Robson [5], using an UV-VIS Spectrometer V670 apparatus (Jasco) equipped with integrating sphere and adequate software. The method is based on measuring the transmittance of radiation transmitted by the sample deposited on the substrate (2 mg/cm^2), by recording the photocurrent from 5 to 5 nm on 290-400 nm (UVB, UVA). The sample under analysis consists in the prepared cosmetic emulsion in which the lyophilized NLCs loaded with UV filters were added. The cosmetic preparations were applied to a synthetic skin (Transpore tape) and subjected to spectrophotometric analysis in six different points. The average recorded values were used to calculate the SPF, according to the equation [19]:

$$\text{SPF} = \sum E_{\lambda} B_{\lambda} / \sum (E_{\lambda} B_{\lambda}) / \text{MPF}_{\lambda}$$

where E_{λ} is the spectral irradiance of terrestrial sun light under defined conditions; B_{λ} is the erythema effectiveness; MPF_{λ} is the monochromatic protection factor for each wavelength increment (the ratio of the detector signal intensity without formulation applied to the substrate to that with applied formulation to the substrate).

In vitro determination of UVA-PF (UVA-protection factor)

In vitro determination of the UVA-PF of the cosmetic product was made starting from the method used to measure the SPF [19]. UVA-PF represents the efficiency of the formulation to absorb the UVA radiation and is calculated according to the equation [20]:

$$\text{UVA-PF} = \sum E_{\lambda} B_{\lambda} / \sum (E_{\lambda} B_{\lambda}) / \text{MPF}_{\lambda}$$

Results and Discussion

Characterization of lipid nanostructures

Nanostructured lipid carriers loaded with benzimidazole derivatives having average sizes of 207-240 nm and polydispersity index (PdI) of 0.235-0.371 were obtained (Figure 1). The best results were found for NLC 1 (207 nm), which present relatively homogeneous populations of nanoparticles reflected by a PdI of 0.25. This fact is attributed to the lower molecular weight of the

compound C1 in relation to the other three synthesized compounds. In the case of NLC 2 and NLC 3, they present bigger sizes and Pdl values higher than 0.36, which reflects polydisperse populations of nanoparticles that in time can cause a decrease of the stability of the colloidal system formed due to phase separation phenomena.

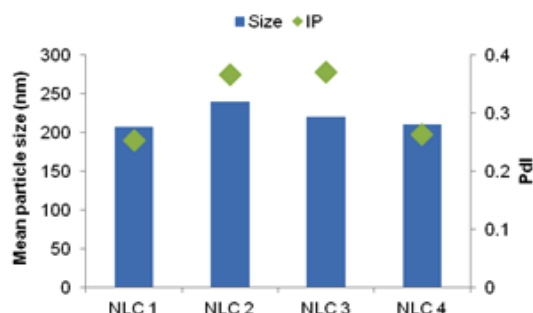


Figure 1.

Mean particle size (nm) and Pdl of benzimidazole-lipid nanostructures in dispersion

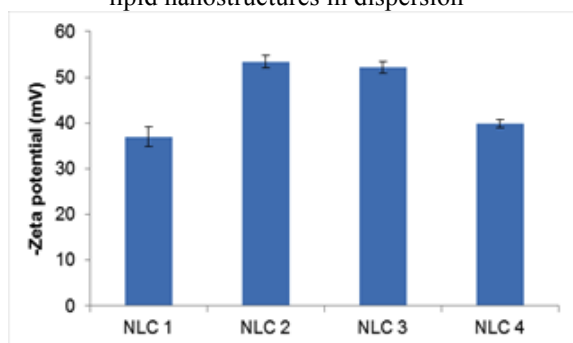


Figure 2.

Benzimidazole-lipid nanostructures stability evaluated by zeta potential values

The physical stability of benzimidazole-NLCs was evaluated by determining the zeta potential (ZP), which reflects the electric charge on the particle surface (Figure 2). Particles with ZP values more negative than -30 mV are considered to possess a moderate stability, while ZP values more electronegative than -40 mV in generally represent a sufficient electrostatic repulsion that provides a

good physical stability. NLC 1 and NLC4 have a moderate stability with values bigger than -37 mV (Figure 2). NLC 2 and NLC 3 show ZP values more electronegative than -50 mV, thus, this electric charge can assure a good physical stability over time.

In vitro determination of the photo protective properties

The UV absorption properties of the non-encapsulated benzimidazole derivatives formulated into an emulsion which formulation is shown in Table II with a concentration of 6.9% C1-4 compounds as UV absorber (Figure 5a) were compared with the UV absorption properties of the encapsulated benzimidazole derivatives into lyophilized lipid nanostructures, having the same concentration of C1-4 compounds (Figure 5b). Figure 6a and 6b illustrates their corresponding SPFs and UVA-PFs, the formulations containing non-encapsulated (free) and encapsulated benzimidazole derivatives into lipid nanostructures, respectively. It can be observed that C1, C2 and C3 compounds have a low absorption in the UVB domain (290 - 320 nm) and among these compounds; C3 presents the highest absorption in this domain. Only the benzimidazole derivative C4 shows a slight absorbance in the UVA domain (320 - 400 nm). These compounds formulated into an emulsion do not present significant photo protective properties having SPFs ranging between 1.5 and 2.1, and UVA-PFs of about 1. By encapsulation into lipid nanostructures, the resulted formulations based on benzimidazole compounds present an increase in the ultraviolet absorption in the UVB domain for NLC 1 - 3 and in the UVB and UVA domain for NLC 4. Thus, the photo protective properties of the benzimidazole derivatives encapsulated into lyophilized NLCs are improved having higher SPF values (from 2.7 to 5.8) and UVA-PFs (around 2). Even though, this increase does not lead to the obtaining of efficient sunscreen products, their UV absorption properties being insufficient for assuring a good skin photo protection.

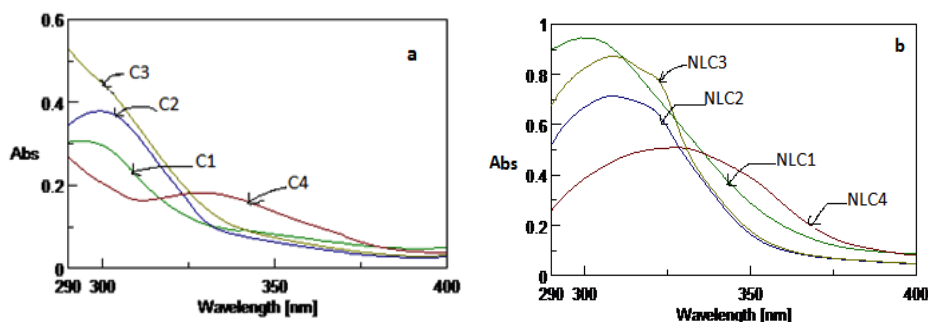


Figure 3.

Superposed absorption spectra of a) emulsions containing benzimidazole compounds C1-4 and b) lyophilized lipid nanostructures (NLCs) loaded with C1-4

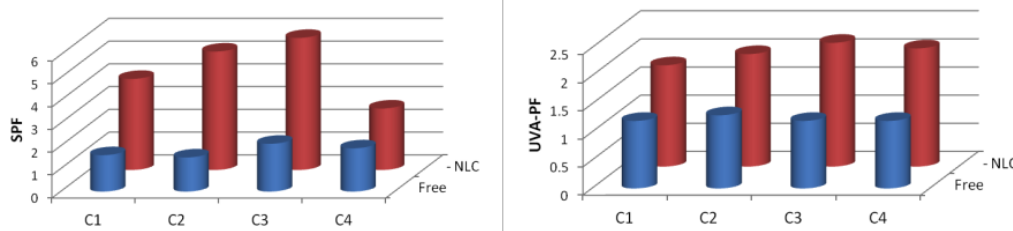


Figure 4.

UV protection factors of non-encapsulated (free) and encapsulated benzimidazole compounds into lipid nanostructures: a) SPF values and b) UVA-PF values

In order to offer high SPF with values over 15, the commercially available sunscreen products contain more than one organic UVB and/or UVA filter coupled with an inorganic filter. In this sense, the benzimidazole – lipid nanostructures were further incorporated into a cosmetic preparation containing zinc oxide and titanium dioxide as inorganic UV

filters. The composition of the cosmetic preparations is presented in Table II. The UV absorption spectra and the UV protection factors of the preparations containing lipid nanostructures are shown in Figure 4. It can be observed that all four formulations offer a high and broad photoprotection on both UVA and UVB domains with SPF values over 60 and UVA-PF over 45.

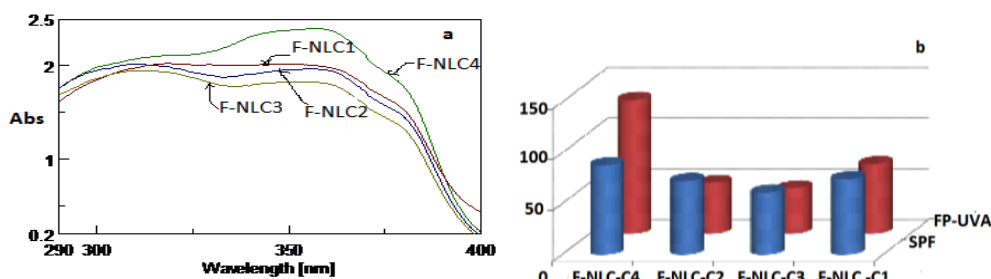


Figure 5.

a) Superposed absorption spectra and b) UV protection factors of the cosmetic formulations containing benzimidazole-lipid nanostructures

Properties evaluation of the cosmetic formulation based on benzimidazole-NLC

The obtained cosmetic formulations showed a homogeneous appearance, with white to yellowish colour and a pleasant smell. All these properties are making the emulsion attractive and pleasant for skin application. These characteristics remained unchanged during storage. The formulations presented a pH of 6, immediately after preparation, and 6.2 after 30 days. Thus, the pH falls within the limits of FRX. These values are compatible with the skin pH and do not causes skin irritation at application. The spreadability control results are shown in Figure 6. It is found that in all cases the ability to display increases with increasing the added weights and by increasing the area of application a better display ability of the formulations is obtained. It was also observed that the encapsulation of the photo protective compounds inside the lipid nanoparticles had a slightly influence on the consistency of the cream by comparing with the blank preparation.

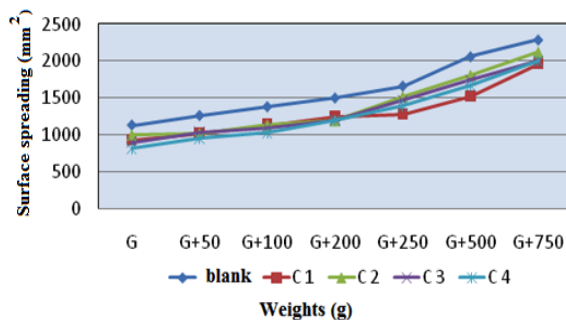


Figure 6.

Surface spreading of cosmetic formulations containing benzimidazole-lipid nanostructures

The viscosity control results are shown in Figure 7. By analysing the obtained rheograms, it was found that the benzimidazole derivatives incorporated into NLCs did not significantly influence the viscosity of the final formulations. The blank cream presented lower values of the viscosity when compared to those of the creams containing the photo protective compounds. By increasing the shear speed, the viscosity of the cosmetic formulations has decreased (pseudo plastic flowing).

Regarding the hydration degree, it was obtained that all the five formulations (one blank and four NLCs-based creams) exhibited a hydration effect from 55% (control skin, untreated) to 74-89 %. There were no significant differences between the NLCs-based creams.

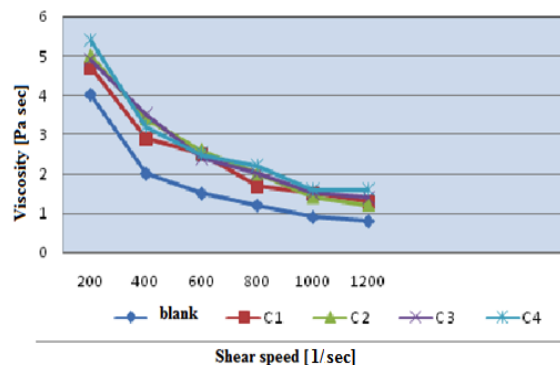


Figure 7.

Variation of viscosity cosmetic formulations [Pa sec] (cosmetic formulation containing photo protective compounds and blank cosmetic formulation) depending on shear speed [1/sec]

Conclusions

The encapsulation procedure into lipid nanostructures has led to an improvement of the UV absorption properties of the benzimidazole compounds. Starting from the lyophilized benzimidazole-lipid nanostructures, efficient cosmetic formulations with broad photo protective properties were obtained by using the new benzimidazole compounds as organic UV filters, metal oxides as inorganic filters, and vegetable extracts, oils and other ingredients with emollient, sedative and moisturizing effect. The obtained cosmetic formulations have proved qualities for skin application possessing suitable physicochemical characteristics (pH, viscosity) and spreadability.

References

- Agar N.S., Halliday G.M., Barnetson R.S., Ananthaswamy H.N., Wheeler M. Jones A.M., The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: a role for UVA in human skin carcinogenesis. *Proc. Natl. Acad. Sci. USA*, 2004; 101: 4954-4959.
- Rău G., Mogoșanu G.D., Pisoschi C.G., Stănculescu C.E., Synthesis, physico-chemical characterisation and mesomorphic properties of some novel azoderivatives. *Farmacia*, 2014; 62(3): 494-503.
- Chaudhuri R.K., Lascu Z., Puccetti G., Deshpande A.A., Paknikar S.K., Design of photostabilizer having butyl in antioxidant functionality and its utility in obtaining broad-spectrum sunscreen formulation. *Photochem. Photobiol.*, 2006, 82823-82828.
- Ciurlea S., Dehelean C., Pinzaru Cîntă S., Fălămaș A., Mureșan A., Loghin F., Raman spectroscopy investigations of the CD1 Nu/Nu mouse skin pre-carcinoma damages. *Farmacia*, 2012; 60(3): 448-456.
- Diffey B.L., Robson J.J., A new substrate to measure sunscreen protection factors throughout the ultraviolet spectrum. *J. Soc. Cosmet. Chem.*, 1989; 40(3): 127-133.
- Hîrjău V., Lupuleasa D., Balaci T., Budura E., Rădulescu F., Hîrjău M., The formulation and evaluation of photoprotection preparations containing a filter agent. *Practica Farmaceutică*, 2011; 4(3-4): 161-166.
- Grether-Beck S., Marini A., Jaenicke T., Krutmann J., Photoprotection of human skin beyond ultraviolet radiation. *Photodermatology Photoimmunology Photomedicine*, 2014; 30: 167-174.
- Fierascu I., Bunghez I.R., Fierascu R.C., Ion R.M., Dinu-Pîrvu C.E., Nuță D., Characterization and antioxidant activity of phytosynthesised silver nanoparticles using *Calendula officinalis* extract. *Farmacia*, 2014; 62(1): 129-136.
- Kunz P.Y., Fent K., Estrogenic activity of UV filter mixtures. *Toxicol. Appl. Pharmacol.*, 2006; 217(1): 86-99.
- Kunz P.Y., Fent K., Estrogenic activity of ternary UV filter mixtures in fish (*Pimephales promelas*) – An analysis with nonlinear isobolograms. *Toxicol. Appl. Pharm.*, 2009; 234(1): 77-88.
- Lacerda S.P., Cerize N.N.P., Ré M.I., Preparation and characterization of carnauba wax nanostructured lipid carriers containing benzophenone-3. *Int. J. Cosmetic Sci.*, 2011; 33(4): 312-321.
- Lacatusu I., Niculae (Badea) G., Badea N., Stan R., Popa O., Oprea O., Meghea A., Design of soft lipid nanocarriers based on bioactive vegetable oils with multiple health benefits. *Chemical Engineering Journal*, 2014; 246: 311-321.
- Lacatusu I., Badea N., Murariu A., Meghea A., The encapsulation effect of UV molecular absorbers into biocompatible lipid nanoparticles. *Nanoscale Research Letters*, 2011; 6(1), 73: 1-9.
- Mănescu I.G., Negreș S., Iscrulescu L., Ștefănescu E., Dinu M., Olaru O.T., Iovu M., Synthesis and pharmacological researches of 2- and 1,2 disubstituted benzimidazoles. *Revista de Chimie*, 2015; in print.
- McKinlay A.F., Diffey B.L., A reference action spectrum for ultraviolet induced erythema in human skin. *CIE Journal*, 1987; 6: 17-22.
- Niculae G., Lacatusu I., Bors A., Stan R., Photostability enhancement by encapsulation of α -tocopherol into lipid-based nanoparticles loaded with a UV filter. *Comptes Rendus Chimie*, 2014; 17: 1028-1033.
- Puglia C., Damiani E., Offerta A., Rizza L., Triendi G.G., Tarico M.S., Curreri S., Bonina F., Perrotta R.E., Evaluation of nanostructured lipid carriers (NLC) and nanoemulsions as carriers for UV-filters: characterization, *in vitro* penetration and photostability studies. *Eur. Journal Pharm. Sci.*, 2014; 51: 211-217.
- Saewan N., Jimtaisong A., Photoprotection of natural flavonoids. *Journal of Applied Pharmaceutical Science*, 2013; 3: 129-141..

-
19. Sanad R.A., Abdel Malak N.S., El-Bayoomy T.S., Formulation of a Novel Oxybenzone-Loaded Nanostructured Lipid Carriers (NLCs). *AAPS Pharm. Sci. Tech.*, 2010; 11(4): 1684-1694.
 20. Sanad R.A., Abdel Malak N.S., El-Bayoomy T.S., Preparation and characterization of oxybenzone-loaded solid lipid nanoparticles (SLNs) with enhanced safety and sunscreens efficacy: SPF and UVA-PF. *Drug Discov. Ther.*, 2010; 4: 472-483.
 21. Vilela F.M.P., Fonseca Y.M., Jabor J.R., Vicentini F.T.M.C., Fonseca M.J.V., Effect of ultraviolet filters on skin superoxide dismutase activity in hairless mice after a single dose of ultraviolet radiation. *Eur. J. Pharm. Biopharm.*, 2012; 80: 387-392.
 22. Zamani L., Fatemeh Mirjalili B., Zomorodian K., Namazian M., Khabnadideh S., Mirzaei E.F., Synthesis of benzimidazoles in the presence of nano-TiCl₄SiO₂ as antifungal study of some products. *Farmacia*, 2014, 62(3): 467-482.