



# Ibuprofen nanocrystals: Production, lyophilization and release profile

P. Severino<sup>1,2,3\*</sup>; T. Almeida<sup>1,2</sup>; T.C. Barbosa<sup>1,2</sup>; C. Rodrigues<sup>4</sup>; MA. Costa<sup>4</sup>; JN. Ferreira<sup>4</sup>; J. Dias-Ferreira<sup>4</sup>; AR. Fernandes<sup>4</sup>; EB. Souto<sup>4</sup>

\*Corresponding author: e-mail address: [pattseverino@gmail.com](mailto:pattseverino@gmail.com)

**Abstract:** Ibuprofen (IBU) is a poorly water-soluble non-steroidal anti-inflammatory drug with proven effectiveness for treating inflammatory, musculoskeletal, and rheumatic disorders. Nanocrystals (NCs) have been proposed as drug delivery systems to improve the solubility and bioavailability of poorly water-soluble compounds. Ibuprofen NCs (IBU-NCs) have been produced by the melt-emulsification method using a combination of Tween<sup>®</sup>80 (1.0%, w/v)/Span<sup>®</sup>80 (0.5%, w/v) as surfactant as these molecules are generally recognized as safe (GRAS) as non-toxic, non-irritating and are of low cost. The obtained main particle size (z-Ave) and polydispersity index (Pdl) were  $159.4 \pm 3.265$  nm and  $0.24 \pm 0.007$ , respectively. Lyophilization slightly increased the mean particle size and Pdl compared to the non-freeze-dried IBU-NCs. The obtained IBU-NCs powders were of white and fine texture. The type and concentration of cryoprotector (trehalose, glucose, sucrose) influenced both the size and the in vitro release profile tested in Franz diffusion cells. Due to the smaller z-Ave, NCs:Trehalose (2:1) of  $170.6 \pm 3.880$  nm ( $0.417 \pm 0.050$ ), NCs:Glucose (3:1) of  $275.3 \pm 8.351$  nm ( $0.144 \pm 0.021$ ) and NCs:Sucrose (4:1) of  $223.3 \pm 10.35$  nm ( $0.402 \pm 0.016$ ) were selected for the in vitro drug release tests. Within the first 6 hours, resuspended lyophilized nanocrystals released between 50–70% of the drug.

**Keywords:** Ibuprofen. Nanocrystals. Melt-Emulsification Methodology. Cryoprotectants. Lyophilization.

## Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for their analgesic and antipyretic actions<sup>(1)</sup>. Their mechanism of action concerns non-selective inhibition of the type 1 cyclooxygenase (COX-1) and type 2 cyclooxygenase (COX-2), which is listed as its principal adverse effects by oral ingestion<sup>(2)</sup>.

Among NSAIDs, ibuprofen has been recommended to treat inflammatory, musculoskeletal and rheumatic disorders<sup>(3-5)</sup>. However, the challenge in pharmaceutical development of such drug is due to its high plasma protein binding (90–99%), short plasma half-life (2.2 hours) with rapid urine removal, reaching the peak plasma concentration in just 1–2 hours<sup>(6)</sup>. Also, IBF shows lipophilicity, a high partition coefficient and low bioavailability through the skin<sup>(7)</sup>.

Many approaches have been exploited to overcome the skin barrier for IBF drug delivery. Nanocrystals (NCs) have significant advantages in improving the bioavailability of poorly water-soluble drugs, due to their high solubilization capacity and the possibility to control the delivery rate<sup>(6,8,9)</sup>. NCs are homogeneous, optically transparent, of low viscosity, thermodynamically stable and can be produced without the need of specialized equipment other than to use a few adjuvants<sup>(9)</sup>.

Nanocrystals have emerged as an interesting drug delivery system for clinical usage. It is defined as a nanotechnology-based formulations with a particles size below 1000 nanometers and have a stable performance by adding an appropriate stabilizer such as polymeric steric stabilizers or surfactant in a aqueous medium – nanosuspension<sup>(10,11)</sup>.

These stabilizers have an important role in nanosuspension,

preventing aggregation among nanocrystals due to their high surface energy which contributes to aggregation between them. They are largely used to overcome the bioavailability and dissolution difficulties of using poor water-soluble drugs in traditional pharmaceutical formulations, which is one of the chief challenges that scientists face in drugs formulations<sup>(10,11)</sup>.

The nanoparticle size allows to increase the surface area and, consequently, enhance the dissolution velocity according with the Noyes-Whitney equation, improving kinetic drug performance. Another important characteristic which made nanocrystals a special formulation is each nanoparticle contain 100% of the drug inside and a polymeric or lipid matrix enclose them, thus, considerable drug concentration is transporting to the target cells, enhancing the bioavailability and, consequently, achieving an appropriate drug concentration for the pharmaceutical effect<sup>(9)</sup>.

The aim of this work has been to develop an ibuprofen nanocrystal formulation by melt-emulsification technique, and evaluate the effect of lyophilization on its physical stability and release profile using Franz diffusion cells.

## MATERIALS AND METHODS

### Materials

Ibuprofen has been donated by Medifar (Amadora, Portugal). Polysorbate 80 (Tween<sup>®</sup>80) was obtained from Uniqema (Everberg, Belgium). Trehalose, sucrose, and glucose were purchased from Merck S.A. (Lisboa, Portugal). Phosphate buffered saline (pH 7.4), and sorbitan

<sup>1</sup>University of Tiradentes (Unit), Biotechnological Postgraduate Program. Av. Murilo Dantas, 300, 49010–390 Aracaju, Brazil.

<sup>2</sup>Institute of Technology and Research (ITP), Nanomedicine and Nanotechnology Laboratory (LNMed), Av. Murilo Dantas, 300, 49010–390 Aracaju, Brazil.

<sup>3</sup>Tiradentes Institute, 150 Mt Vernon St, Dorchester, MA 02125, USA.

<sup>4</sup>Faculty of Pharmacy, University of Coimbra (FFUC), Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000–548 Coimbra, Portugal –Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra (FFUC), Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000–548 Coimbra, Portugal.

<sup>5</sup>Department of Biology and Environment, University of Trás-os-Montes e Alto Douro, UTAD, Quinta de Prados, P-5001–801 Vila Real, Portugal.

<sup>6</sup>CEB – Centre of Biological Engineering, University of Minho, Campus de Gualtar 4710–057 Braga, Portugal.

Received 05 June 2020 Accepted 21 August 2020; Available online 25 September 2020.

DOI: <https://doi.org/10.52466/ijamb.v3i2.37>

monooleate (Span®80) were purchased from Sigma–Aldrich (Steinheim, Germany). Cellulose membrane with an average pore size of 0.22 µm was obtained from Millipore® HA. Ultra–purified water was obtained from the Milli®Q Plus system.

### Nanocrystal production by melt emulsification

The melt–emulsification methodology was used to produce nanocrystals as previously described<sup>(3,4)</sup>. Ibuprofen 0.25% (w/v) was added to the aqueous surfactant solution composed of Tween® 80 (1.0%, w/v) and Span® 80 (0.5%, w/v) heated at 80°C. The suspension was homogenized by high shear homogenization in Ultra Turrax® (T25, IKA) for 10 minutes to obtain a coarse emulsion. The obtained emulsion was poured into a high–pressure homogenizer (EmulsiFlex®–C3, Avastin) and homogenized at 1000 bar for 20 minutes in the continuous mode under heating at 80°C. Finally, the hot formulation was then cooled down using an ice bath for approximately 20 minutes.

### Lyophilization

Nanocrystals were freeze–dried using three different cryoprotectants, trehalose (T), glucose (G) and sucrose (S). Nanocrystal dispersions were diluted with aqueous cryoprotectant solutions at 4:1, 3:1 and 2:1 (v/v) prior to freeze–drying, and frozen to –80°C in a freezer immediately before freeze–drying in a Gamma 2–20 freeze dryer (Christ, Osterode a.H., Germany) operating at –25°/0.025 mBar for a period of 24 h (for the main freezing) and over 6 h drying<sup>(12)</sup>. The lyophilized samples were then dispersed in double distilled water and filtered by a 0.22 µm pore membrane and reanalyzed for their mean particle size and PI<sup>(13)</sup>.

### Particle size analysis

The mean particle size (z–Ave) and polydispersity index (PI) were measured by dynamic light scattering (DLS), in freshly prepared nanocrystals, using a particle size analyzer (DelsaNano C Submicron, Beckman Coulter Delsa, Krefeld, Germany). The measurements were run in triplicate, recording 10 readings per run<sup>(14)</sup>.

### In vitro release profile

The release profile of ibuprofen from the obtained nanocrystals (before and after freeze–drying) was studied using vertical Franz diffusion cells (n = 3 cells/sample). In each cell, a cellulose Millipore HA membrane with an average pore size of 0.22 µm was used previously soaked for at least 1 hour with receptor fluid (phosphate–buffered saline (PBS) at pH 7.4). At pre–determined time intervals, 200 µL of the samples were collected using a syringe, and the same volume was replaced with PBS. The samples were analyzed by ultraviolet (UV) assay at 264 nm, in a Synergy™ HT Multi–Mode Microplate Reader. PBS at pH 7.4 was used as receptor medium and maintained at 37°C during the tests (3).

### Results and discussion

Ibuprofen (IBU), a name derived from isobutyl propanoic phenolic acid, is non–steroidal anti–inflammatory drug with proven effectiveness for treating pain, fever, and inflammation. IBU is one of the most commonly used non–steroidal anti–inflammatory drugs (NSAIDs). It is the weakest of the NSAIDs but reversely has a prominent analgesic and antipyretic actions. Its mechanism of action concerns a non–selective inhibition of cyclooxygenase type 1 (COX–1) and cyclooxygenase type 2 (COX–2). Its side effects are mainly due to the oral intake of this drug which produces an inhibitory action on cyclooxygenases, which are related with prostaglandins' synthesis. Prostaglandins are associated with the production of pain, inflammation and fever.

Extensive research has been put forward with the aim to improve the use of IBU in topical application to overcome its common side effects and low bioavailability<sup>(15,16)</sup>. Our group has described nanocrystal suspensions of IBU stabilized with a combination of surfactants and melt–emulsification processes<sup>(3,4)</sup> using different types of surfactants. In this work, we highlight the combination of Tween® 80/Span® 80 to stabilize IBU nanocrystals in aqueous dispersion. Both surfactants are widely used in pharmaceutical formulation. These surfactants are generally recognized as safe (GRAS) as non–toxic, non–irritating and of low cost. The method production was based on homogenization because it is easily be performed on a small and large scale concerning industrial pharmaceutical.

The produced IBU–NCs were analyzed by particle size and Pdl. The fresh sample showed a particle size and Pdl of 159.4 ± 3.265 nm and 0.24 ± 0.007, respectively. The size of NCs formulation is dependent on the composition/concentration of formulation and method of production. Generally, the high homogenization produces small droplets due to high pressure and fast cooling. Particle size is important for skin permeation, and the ideal size for penetration of the skin is under 700 nm<sup>(17)</sup>. Also, Lademann et al.<sup>(18)</sup>, Adib et al.<sup>(19)</sup> suggested that a particle size between 300–600 nm promote drug penetration significantly more in–depth into the skin layer. The Pdl is a parameter for defining the particle size distribution. Samples with a wide particle size distribution usually have a polydispersion index > 0.7<sup>(20)</sup>. Pdl of 0.4 shows a heterogeneous particle size distribution, and the presence of agglomerates in the dispersion<sup>(21)</sup>. In this study, the obtained Pdl is attributed to the

rearrangements of polysorbate and sorbitan monooleate chains on the surface of nanocrystals. The obtained Pdl value was considered low, and a homogeneous sample was produced.

The choice of cryoprotectant and its concentration is essential for obtaining a stable and reproducible product. The absence of cryoprotecting agents in the drying stage can occasionally produce NCs with sticky and thick components, and if reconstituted, there is the production of aggregates. Thus, the correct choice will allow easy redispersion of the freeze-dried formulation<sup>(22)</sup>. As shown in Table I, the lyophilization slightly increased the mean particle size of IBU-NCs measured after their reconstitution with double distilled water, however remaining within the nanometer range. A low Pdl could be seen in particular for the NCs:Glucose (3:1) combination. All IBU-NCs powders were of white, fine and uniform texture. The NCs:Trehalose (2:1), NCs:Glucose (3:1) and NCs:Sucrose (4:1) for further studies.

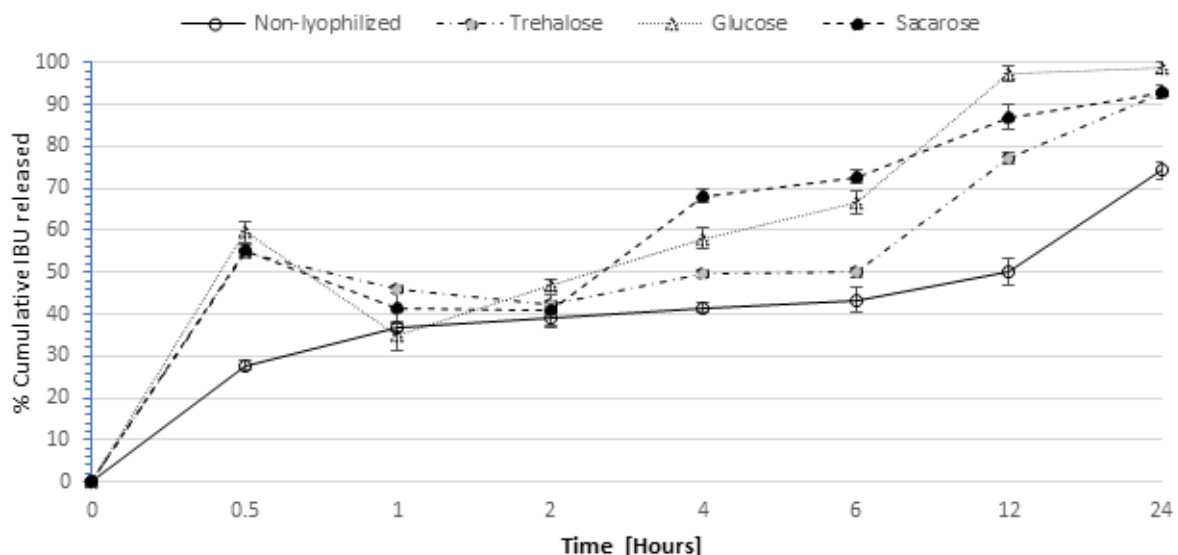
In vitro release profile of ibuprofen from non-lyophilized nanocrystals has been compared with the profiles of the resuspended nanocrystals freeze-dried with different cryoprotectant, over a period of 24 hours (Figure 1). Selected cryoprotectant was based on the lowest mean particle size and polydispersity index, namely NCs: Trehalose (2:1) of  $170.6 \pm 3.880$  nm (PI  $0.417 \pm 0.050$ ), NCs:Glucose (3:1) of  $275.3 \pm 8.351$  nm (PI  $0.144 \pm 0.021$ ), and NCs:Sucrose (4:1) of  $223.3 \pm 10.35$  nm (PI  $0.402 \pm 0.016$ ), covered by a combination of Tween<sup>®</sup> 80 and Span<sup>®</sup> 80.

As shown in Figure 1, the release profile allowed the identification of a burst release occurring within the first 10 min, during which approximately 50% of the drug was immediately released in all lyophilized samples. Within the first 6 h, NCs:Trehalose (2:1), NCs:Glucose (3:1) and NCs:Sucrose (4:1) released about 70%, 65% and 50% of the drug, respectively. The slower drug release observed among the samples can be attributed to the interaction between the surfactants, cryoprotection and the drug. Within 24 h, all resuspended freeze-dried samples released almost 100% of the drug. A delayed release was observed for the non-lyophilized IBU nanocrystals; within the first 30 mins only 30% of the drug has been quantified in the receptor medium in contrast to ca. 55% of the lyophilized formulations. The higher hydration capacity of these latter justifies the higher cumulative IBU released over the course of the experiment.

According Patel et al., 2018<sup>(23)</sup> the release profile observed in this study is typical of nanocrystal formulations. Nanocrystal interact with skin overcoming the barrier and reaching cellular level and increase the saturation solubility and concentration gradient easing passive penetration through the skin. Also, this technology is widely used in cosmetics and ointments and emulsions to improve improve formulation stability, tolerance and obtain more product esthetically. Similar results were obtained by rutin<sup>(24)</sup>, flavonoids<sup>(25)</sup> and quercetin<sup>(26)</sup>.

**Table I.** Mean particle size (z-AVE) and polydispersity index (Pdl) of ibuprofen nanocrystals before (non-lyophilized) and after lyophilization. Nanocrystals were re-suspended in ultra-purified water prior to measurements.

<b>Samples</b>	<b>z-AVE (nm)</b>	<b>Pdl</b>
<b>NCs (non-lyophilized)</b>	<b><math>159.4 \pm 3.265</math></b>	<b><math>0.24 \pm 0.007</math></b>
NCs:Trehalose (4:1)	$188.2 \pm 3.696$	$0.409 \pm 0.01$
NCs:Trehalose (3:1)	$234.2 \pm 5.217$	$0.440 \pm 0.024$
<b>NCs:Trehalose (2:1)</b>	<b><math>170.6 \pm 3.880</math></b>	<b><math>0.417 \pm 0.050</math></b>
NCs:Glucose (4:1)	$306.8 \pm 5.488$	$0.751 \pm 0.012$
<b>NCs:Glucose (3:1)</b>	<b><math>275.3 \pm 8.351</math></b>	<b><math>0.144 \pm 0.021</math></b>
NCs:Glucose (2:1)	$376.4 \pm 3.972$	$0.552 \pm 0.026$
<b>NCs:Sucrose (4:1)</b>	<b><math>223.3 \pm 10.35</math></b>	<b><math>0.402 \pm 0.016</math></b>
NCs:Sucrose (3:1)	$223.9 \pm 13.33$	$0.489 \pm 0.030$
NCs:Sucrose (2:1)	$347.6 \pm 5.147$	$0.665 \pm 0.040$



**Figure 1.** *In vitro* IBU release profiles of nanocrystals dispersions before freeze-drying (non-lyophilized) and after freeze-drying with trehalose (2:1), Glucose (4:1) and Sucrose (3:1). Data were recorded at pre-defined time points for a period of 24 h.

## Conclusions

The advantages of IBU nanocrystals as delivery system is attributed to the possibility to enhance the drug solubility and stability. Nanocrystals can be lyophilized to enhance their stability retaining their capacity to modify the release profile. The type and concentration of the cryoprotectant were found to influence the mean particle size, polydispersity index and release profile.

## Acknowledgments

This work was financed through the projects M-ERA-NET/0004/2015 and UIDB/04469/2020, from the Portuguese Science and Technology Foundation, Ministry of Science and Education (FCT/MEC) from national funds, and co-financed by FEDER, under the Partnership Agreement PT2020. Also, this research was funded by Coordenação Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de Sergipe (FAPITEC), and Conselho Nacional de Desenvolvimento Científico e Tecnológico.

## REFERENCES

- [1]. Jahnvi K, Reddy PP, Vasudha B, Narender B. Non-steroidal anti-inflammatory drugs: an overview. *J Drug Deliv* 2019;9(1-s):442-8.
- [2]. Moilanen E, Vuolteenaho K. Nonsteroidal Anti-inflammatory Drugs. *Nijkamp and Parnham's Principles of Immunopharmacology*: Springer; 2019. p. 689-707.
- [3]. Fernandes A, Dias-Ferreira J, Cabral C, Garcia M, Souto E. Release kinetics and cell viability of ibuprofen nanocrystals produced by melt-emulsification. *Colloids and Surfaces B: Biointerfaces* 1. 2018;166:24-8.
- [4]. Fernandes A, Ferreira N, Fangueiro J, Santos A, Veiga F, Cabral C, et al. Ibuprofen nanocrystals developed by 22 factorial design experiment: a new approach for poorly water-soluble drugs. *Saudi Pharm J* 2017;25(8):1117-24.
- [5]. Fernandes AR, Dias-Ferreira J, Ferreira-da-Silva C, Severino P, Martins-Gomes C, Silva AM, et al. Drug nanocrystals: Present, past and future. *Applications of Nanocomposite Materials in Drug Delivery*: Elsevier; 2018. p. 239-53.
- [6]. Hofmann M, Thieringer F, Nguyen MA, Månsson W, Galle PR, Langguth P. A novel technique for intraduodenal administration of drug suspensions/solutions with concurrent pH monitoring applied to ibuprofen formulations. *Eur J Pharm Biopharm.* 2019;136:192-202.

- [7]. Alves TFR, Barros CT, Baldo D, Amaral VA, Sever M, Santos C, et al. Preparation, Characterization and ex vivo Intestinal Permeability Studies of Ibuprofen Solid Dispersion. *J Dispers Sci Technol*. 2019;40(4):546–54.
- [8]. Fangueiro JF, Marques IR, Severino P, Santana MHA, Souto EB. Desenvolvimento, produção e caracterização de nanocristais de fármacos pouco solúveis. *Química Nova*. 2012.
- [9]. Fangueiro JF, Marques IR, Severino P, Santana MHA, Souto EB. Development, production and characterization of nanocrystals of poorly soluble drugs. *Química Nova*. 2012;35(9):1848–53.
- [10]. Teeranachaideekul V, Junyaprasert VB, Souto EB, Muller RH. Development of ascorbyl palmitate nanocrystals applying the nanosuspension technology. *Int J Pharm*. 2008;354(1–2):227–34.
- [11]. Muller RH, Runge S, Ravelli V, Mehnert W, Thunemann AF, Souto EB. Oral bioavailability of cyclosporine: solid lipid nanoparticles (SLN) versus drug nanocrystals. *Int J Pharm*. 2006;317(1):82–9.
- [12]. Doktorovova S, Shegokar R, Fernandes L, Martins–Lopes P, Silva AM, Muller RH, et al. Trehalose is not a universal solution for solid lipid nanoparticles freeze–drying. *Pharmaceutical development and technology*. 2014;19(8):922–9.
- [13]. Gol D, Thakkar S, Misra M. Nanocrystal–based drug delivery system of risperidone: lyophilization and characterization. *Drug Dev Ind Pharm*. 2018;44(9):1458–66.
- [14]. Cavendish M, Nalone L, Barbosa T, Barbosa R, Costa S, Nunes R, et al. Study of pre–formulation and development of solid lipid nanoparticles containing perillyl alcohol. *JTAC*. 2019:1–8.
- [15]. Salim N, García–Celma MJ, Escribano E, Nolla J, Llinàs M, Basri M, et al. Formation of Nanoemulsion Containing Ibuprofen by PIC Method for Topical Delivery. *Mater Today Proc*. 2018;5:S172–S9.
- [16]. Bagde A, Patel K, Kutlehria S, Chowdhury N, Singh M. Formulation of topical ibuprofen solid lipid nanoparticle (SLN) gel using hot melt extrusion technique (HME) and determining its anti–inflammatory strength. *Drug Deliv Transl Res*. 2019:1–12.
- [17]. Alvarez–Román R, Naik A, Kalia Y, Guy RH, Fessi H. Skin penetration and distribution of polymeric nanoparticles. *J Control Release*. 2004;99(1):53–62.
- [18]. Lademann J, Richter H, Meinke MC, Lange–Asschenfeldt B, Antoniou C, Mak WC, et al. Drug delivery with topically applied nanoparticles: science fiction or reality. *Skin Pharmacol Physiol*. 2013;26(4–6):227–33.
- [19]. Adib ZM, Ghanbarzadeh S, Kouhsoltani M, Khosroshahi AY, Hamishehkar H. The effect of particle size on the deposition of solid lipid nanoparticles in different skin layers: A histological study. *Adv Pharm Bull*. 2016;6(1):31.
- [20]. Nidhin M, Indumathy R, Sreeram K, Nair BU. Synthesis of iron oxide nanoparticles of narrow size distribution on polysaccharide templates. *Bull Environ Contam Toxicol* 2008;31(1):93–6.
- [21]. Krause B, Mende M, Pötschke P, Petzold G. Dispersability and particle size distribution of CNTs in an aqueous surfactant dispersion as a function of ultrasonic treatment time. *J Carbon*. 2010;48(10):2746–54.
- [22]. Sharma M, Mehta I. Surface stabilized atorvastatin nanocrystals with improved bioavailability, safety and antihyperlipidemic potential. *Sci Rep*. 2019;9(1):1–11.
- [23]. Patel V, Sharma OP, Mehta T. Nanocrystal: A novel approach to overcome skin barriers for improved topical drug delivery. *Expert opinion on drug delivery*. 2018;15(4):351–68.
- [24]. Pyo SM, Meinke M, Keck CM, Müller RH. Rutin—increased antioxidant activity and skin penetration by nanocrystal technology (smartCrystals). *Cosmetics*. 2016;3(1):9.
- [25]. Gujar K, Wairkar S. Nanocrystal technology for improving therapeutic efficacy of flavonoids. *Phytomedicine*. 2020:153240.
- [26]. Manca ML, Lai F, Pireddu R, Valenti D, Schlich M, Pini E, et al. Impact of nanosizing on dermal delivery and antioxidant activity of quercetin nanocrystals. *Journal of Drug Delivery Science and Technology*. 2020;55:101482.