International Journal of Pharmaceutics 521 (2017) 156–166

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

In situ determination of the saturation solubility of nanocrystals of poorly soluble drugs for dermal application

Miriam Colombo[a], Sven Staufenbiel[a], Eckart Rühl[b], Roland Bodmeier[a,*]

[a] College of Pharmacy, Freie Universität Berlin, Kelchstr. 31, 12169 Berlin, Germany
[b] Physical Chemistry, Institute for Chemistry and Biochemistry, Freie Universität Berlin, Takustr. 3, 14195 Berlin, Germany

ARTICLE INFO

Article history:
Received 20 January 2017
Received in revised form 9 February 2017
Accepted 10 February 2017
Available online 20 February 2017

Keywords:
Dermal nanocarrier
Dissolution rate
In situ methods
Nanocrystals
Saturation solubility
Wet bead milling

ABSTRACT

The aim of this study was to determine, *in situ*, the saturation solubility and dissolution rate of nanocrystals of three poorly water-soluble drugs for dermal application. The nanocrystals were prepared by wet bead milling. Their size could be controlled by various process parameters. The saturation solubility was measured in water or in the presence of surfactant at 32 °C with a Sirius® inForm based on *in situ* UV–vis spectroscopy. The saturation solubility of nanocrystals with sizes of ~300 nm increased for each drug in comparison to non-milled drug powders, with factors of increase in the range 1.3–2.8. The tacrolimus solubility was further analyzed with excess nanocrystal amounts four and ten times higher than the drug powder solubility. The corresponding solubility increases were 2.8 and 6.6 and thus dependent on the amount of excess nanocrystals. The higher increase was due to the presence of a larger fraction of small size particles, and only crystals far below 1 μm showed supersaturation. The solubility increase for nanocrystals determined *in situ* was remarkably lower than the one previously reported with the use of non *in situ* methods. Nanomilling increased the drug dissolution rates: the highest increase was obtained with ibuprofen (rate increase ~30).

1. Introduction

Most of the new drug candidates are characterized by a poor water solubility and consequently often by a low bioavailability. Solid dispersions (Van Den Mooter, 2012; Vasconcelos et al., 2007; Xie and Taylor, 2016) and the use of solubilizers (Balakrishnan et al., 2004; Yueksel et al., 2003) are strategies to overcome this problem. However, the use of solid dispersions is limited because of their recrystallization tendency (Wegiel et al., 2013), and solubilizers increase the colloidal solution concentration but often lower the solute thermodynamic activity, thereby not increasing the diffusive flux, which depends on activity and not on concentration gradients (Raina et al., 2015).

Another approach is based on nanosuspensions (nanocrystals) (Keck and Müller, 2006; Rabinow, 2004; Shegokar and Müller, 2010). Products for oral administration based on nanocrystal technology are already on the market for the therapy of hypercholesterolemia, hypertriglyceridemia, and immunosuppression after renal transplants, *e.g.* Tricor® and Rapamune® (Junghanns and Müller, 2008; Kesisoglou and Mitra, 2012). Nanocrystals are promising not only for the oral route but also for an improved skin delivery of poorly soluble drugs (Zhai et al., 2014). Their advantages regarding dermal application (Müller and Keck, 2004; Zhai et al., 2014) consist of (a) a higher surface coverage/enhanced adhesiveness, (b) hair follicle targeting potential, (c) an increased dissolution rate and (d) an increased flux caused by an increased saturation solubility. However, the question whether the solubility of a drug is only dependent on its chemical structure and its solid state modification/degree of crystallinity or also on its particle size is an ongoing debate.

The increased solubility of nanocrystals is explained by different theories. It is hypothesized that the behavior described by the Kelvin equation (Skinner and Sambles, 1972; Thomson, 1871) is transferable to the solid–liquid interface of particles with sizes below 1000 nm. The higher dissolution pressure would shift the equilibrium solubility to an increased kinetic solubility. Furthermore, the solubility depends on the particle size (*cf.* Ostwald–Freundlich equation (Von Helmholtz, 1886)) and size reduction to the nanometer range results in a higher solubility. The surface tension between nanoparticles and medium is, however, difficult to measure and this value is needed for the calculation of the solubility with the Ostwald–Freundlich equation. Indeed, the

* Corresponding author.
E-mail address: roland.bodmeier@fu-berlin.de (R. Bodmeier).

http://dx.doi.org/10.1016/j.ijpharm.2017.02.030
0378-5173/

surface tension depends on the particle size in case of colloidal systems (Stauff, 1960).

Two types of methods are generally known for the determination of the solubility: (a) non *in situ* methods and (b) *in situ* methods. The first ones are based on centrifugation/filtration techniques and are not suitable for the analysis of the solubility of nanocrystals (Anhalt et al., 2012). After centrifugation, a fraction of very small particles is partially still in the supernatant, even if the latter one is clear (Sinha, 2013) and would thus be incorrectly added to the solubility value. In case of ultra-centrifugation, small drug particles could immediately diffuse in the supernatant at the end of the process. The challenges in case of filtrations are: (a) a filter pore size which cannot exclude particles in the lower nanometer range and the rapid occlusion of small pores and (b) the absorption of drug on the filter or on the membrane and (c) an insufficient resolution in time. The latter is crucial for the determination of the kinetic solubility because the recrystallization of drugs from the supersaturated solution can occur during the dissolution measurement. The insufficient resolution in time is a problem of all non *in situ* methods. A factor of increase in solubility of 20 has been reported with non *in situ* methods (Mishra et al., 2013). The increase is very high, especially when compared to the factor of increase of ~1.2 reported with an *in situ* method (Anhalt et al., 2012). *In situ* methods are preferable and different methodological approaches are reported. One is based on calorimetric measurements (Kayaert et al., 2010) but differentiating calorimetrically dissolution occurrences from other molecular interactions is challenging. A method based on light scattering is described in the literature (Anhalt et al., 2012). It uses photon correlation spectroscopy (PCS) for the solubility determination of nanoparticles. A disadvantage of this method is the pronounced particle size dependency, indeed PCS is used for the determination of the size of nanoparticles.

In this study, an *in situ* method based on UV–vis spectroscopy was used to determine solubility profiles. The light absorbing potential of particles was controlled by an adequate Tyndall–Rayleigh correction of the obtained spectra. The equipment used was a Sirius® inForm. It is an automated platform which provides support during the formulation development and quality control. This technique can also be used for acquiring dissolution profiles and dissolution rates, which could also be obtained under biorelevant conditions and with pH-titration.

The topically used drugs investigated were dexamethasone, tacrolimus, and ibuprofen. Most topical formulations of poorly water-soluble drugs contain solvents which have the capability to penetrate through the skin faster than the drug, leading to recrystallization of the drug on the skin and resulting in a low dermal bioavailability (Berardesca et al., 2013). It is hypothesized that the efficiency of topicals could be improved by formulating these drugs as nanocrystals. An improved *in vitro* skin penetration has been reported in the literature (Romero et al., 2015). Moreover, nanocrystals with a proper size could target the hair follicle pathway as a route to obtain a higher penetration of drug into the skin (Patzelt and Lademann, 2013).

To evaluate if nanocrystals have an increased saturation solubility, which could lead to the mentioned advantages, the aim of this study was to determine, *in situ*, the kinetic water solubility at 32 °C (average skin temperature (Burton, 1935)). The drugs were chosen considering their relevance for dermal application and in order to cover a broad range of solubility values. The preparation of the nanocrystals was investigated in terms of bead size, milling speed, and time in order to obtain nanosuspensions of various particle sizes.

2. Materials and methods

2.1. Materials

Dexamethasone (Fagron GmbH, Barsbüttel, Germany), ibuprofen 70 (BASF SE, Ludwigshafen, Germany), tacrolimus (LC Laboratories, Woburn, USA), poloxamer 407 (Kolliphor® P407, BASF SE, Ludwigshafen, Germany), poloxamer 188 (Pluronic® F68, BASF SE, Ludwigshafen, Germany), decyl glucoside (Plantacare® 2000 UP, BASF SE, Ludwigshafen, Germany), Lecithin (Carl Roth GmbH, Karlsruhe, Germany), D-α-Tocopherol polyethylene glycol 1000 succinate (Speziol® TPGS Pharma, Cognis GmbH, Monheim am Rhein, Germany), ultrapurified water purified by a Milli-Q-apparatus (Millipore GmbH, Darmstadt, Germany), 0.1 mm zirkonium beads (Hosokawa Alpine AG, Augsburg, Germany), 0.3 mm zirkonium beads (YTZ® Grinding Media, Tosoh, Tokyo, Japan).

2.2. Preparation of nano- and microsuspensions

Dexamethasone, ibuprofen, and tacrolimus nanocrystals were prepared by wet bead milling with a batch size of 35 mL. Dexamethasone and ibuprofen powders were dispersed in aqueous solutions of 1% (w/v) poloxamer 407 (stabilizer) in order to obtain 1% and 0.1% (w/w) nanosuspensions. Tacrolimus 0.05% (w/w) was dispersed in aqueous solutions of 0.1% or in 0.05% (w/v) poloxamer 407. The drug dispersions were first homogenized with an Ultra Turrax T-25 (IKA®-Werke GmbH & Co. KG, Staufen, Germany). The zirkonium beads and a magnetic stirrer were added to a 100 mL Erlenmeyer flask and the dispersions were poured into the flask. The suspensions were stirred on a magnetic stirring plate (IKA®-Werke GmbH & Co. KG, Staufen, Germany). Nanomilling was investigated with regard to type and concentration of surfactant and as a function of stirring time, speed and bead size (Tables 1 and 2, Fig. 1). For comparison, regular (micro-)

Table 1
Characterization of dexamethasone nanocrystal suspensions with different surfactants.

Surfactant	Concentration, % w/v	Size, nm	PDI	Stability, weeks
Poloxamer 407	0.01–0.20	178–256	0.06	2–3
	0.50	225	0.08	>4
	1.00	231	0.11	>4
Poloxamer 188	0.02	208	0.12	2
	0.20	196	0.05	1
Vitamin E-TPGS	0.02	188	0.09	1
	0.20	206	0.15	2–3
Lecithin	0.02	165	0.10	1
	0.20	181	0.22	–
Poloxamer 188 + Lecithin	0.02	184	0.08	1
	0.20	209	0.20	–
Plantacare 2000 UP	0.02	183	0.06	1
	0.20	197	0.10	2