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Anti-inflammatory drug nanocrystals: state of art and regulatory perspective

Luiza de O. Macedo [a], Eduardo J. Barbosa [a], Raimar Löbenberg [b,1], Nadia A. Bou-Chacra [a,*]

[a] University of Sao Paulo, Faculty of Pharmaceutical Sciences, 580, Prof. Lineu Prestes Avenue, – Bloco 15, Caixa-Postal 6608, CEP 05508-900, São Paulo – SP, Brazil
[b] University of Alberta, Faculty of Pharmacy and Pharmaceutical Sciences, Edmonton – AB, Canada T6G 2N8

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ABSTRACT

Anti-inflammatory drugs have been prescribed extensively for a wide range of diseases. Combined with over-the-counter use, approximately 30 billion doses of non-steroidal inflammatory drugs (NSAIDs) are consumed annually in the USA. The global market of glucocorticoids (GCs) is forecast to reach US$ 8.6 billion by 2025. Severe adverse effects have been reported for NSAIDs, GCs, and COX-2 selective NSAIDs (COXIBs). Furthermore, the overwhelming majority of these drug substances are BCS class II, which limits their bioavailability due to poor water solubility. Drug nanocrystals, a carrier-free nanosystem, can increase saturation solubility, dissolution rate, and the mucoadhesiveness of these drugs. The enhancement of these properties was highlighted in our findings. These features improve the efficacy and safety of anti-inflammatory drugs. In this review, we show that drug nanocrystals are an attractive strategy that contributes to an important shift in the development of innovative products for different routes of administration. The possibility of targeting can minimize the adverse effects and improve the efficacy in the management of inflammatory conditions. We comprehensively review the critical quality attributes (CQAs) in the anti-inflammatory drug nanocrystals preparation, which are fundamental to developing a successful marketable product. Despite the advantages, maintaining properties such as average particle size, surface properties, and physicochemical stability of these preparations during shelf life poses challenges to be overcome.

1. Introduction

Inflammation is a natural response of the organism, involving events responsible for releasing chemical mediators and migrant cells to return the homeostasis (Netea et al., 2017). For being a complex response, recruiting immunological and molecular components and physiological process, this response may be more damaging than the initial harmful stimulation (Hall, 2017), requiring pharmacological intervention to relieve the general and nonspecific symptoms such as pain, redness, heat, swelling, and loss of tissue function (Takeuchi and Akira, 2010).

Abbreviations: non-steroidal anti-inflammatory drugs (NSAID), glucocorticoid (GC), COX- selective NSAID (COXIB), phospholipase A (PLA_2), unstirred water layer (UWL), mononuclear phagocytic system (MPS), enhanced permeability and retention (EPR), wet bead milling (WBM), high pressure homogenization (HPH), polydispersivity index (PDI), drug:stabilizer ratio (D/S), x-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy (FTIR), attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR), dynamic light scattering (DLS), laser diffraction (LD), stability studies (SS), liquid chromatography coupled with mass spectrometry (LC-MS), high performance liquid chromatography (HPLC), ultraviolet (UV), high performance liquid chromatography coupled with ultraviolet (HPLC-UV), scanning electron microscopy (SEM), environmental scanning electron microscopy (ESEM), field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), light microscopy (LM), contact angle measurement (CAM), surface plasmon resonance (SPR), thermogravimetric analysis (TGA), Raman spectroscopy (Raman), freeze-drying (FD), polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), hydroxypropyl methylcellulose (HPMC), d-alfa tocopheryl polyethylene glycol 1000 succinate (TPGS), poly(ethylene oxide) (PEO), orally disintegrating tablets (ODT), new drug application (NDA), abbreviated new drug application (ANDA), local accumulation efficiency (LAC), electric double layer (EDL), poly (ethylene oxide) (PEO).

* Corresponding author: Nadia Araci Bou-Chacra, 580, Prof. Lineu Prestes Avenue, Bloco 15, Caixa-Postal 6608, CEP 05508-900, São Paulo – SP, Brazil. Tel. +55 11 3091-3628

E-mail addresses: luiza-macedo@hotmail.com (L.O. Macedo), edujbarbosa@hotmail.com (E.J. Barbosa), raimar@ualberta.ca (R. Löbenberg), chacra@usp.br (N.A. Bou-Chacra).

[1] Tel.: +1 780 492 1255; fax. +1 780 492 1217

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Anti-inflammatory drugs are listed on the World Health Organization (WHO) Model List of Essential Medicines (WHO, 2019), and more than 70 million prescriptions are written each year for them (Medscape, 2017).

The pharmacological intervention in the inflammation response is mediated by its action in the arachidonic acid pathway. The release of prostanoids involved in inflammation is due to the activity of the cyclooxygenase enzyme (COX). COX has two isoforms: isoform 1 (COX-1) is constitutive of various tissues, and isoform 2 (COX-2) is induced in the presence of inflammatory mediators, and it is responsible for the most of inflammation symptoms (Su and O'Connor, 2013). Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit non-selectively both COX isoforms. Within this group, there are the COX-2 selective NSAIDs (COXIBs), aiming to act only on the inflammatory response. This mechanism allows avoiding the adverse effects by inhibiting COX-1, such as decreasing the gastric cytoprotective effect, and increased susceptibility to acute renal failure, hepatic and cardiovascular disorders (Rang et al., 2016; Scheiman, 2016). In addition to the anti-inflammatory activity, NSAIDs have been investigated as an anti-cancer agent (Gurpinar et al., 2014; Kumar et al., 2016). Estimates show that NSAIDs, including COXIBs, are regularly taken by 15% of the USA population and, through occasional use, more than 30 billion doses are taken each year (Harvard Health Publishing, 2018).

Another group used to fight inflammation is glucocorticoids (GCs), which comprise potent anti-inflammatory and immunosuppressive activity drugs with extensive pharmacological usage in treating other distinct diseases. In inflammation, GCs have an inhibitory effect on the phospholipase A_2 enzyme (PLA_2), responsible for the arachidonic acid formation, suppressing the entire inflammatory cascade. Genomic mechanisms also have a role in the anti-inflammatory effect of GCs (Ingawale et al., 2015), and its extensive applicability for the treatment of various diseases and conditions has been reflected in GCs global market size. According to the 'Global Steroids Industrial Chain Market Insights, Growth Trends and Competitive Analysis 2025', it is estimated that GCs segment can achieve US$ 8.6 billion by 2025 end, expanding the compound annual growth rate in 1.8 % on this period evaluated (QY Research, 2018)

The lack of selectivity of conventional NSAIDs between both COX isoforms results in a high number of adverse effects, especially in COX-1 inhibition, as we mentioned before. Although COXIBs minimize gastrointestinal complications, the selective inhibition of COX-2 involves decreasing vasodilatory and anti-aggregation prostaglandin production, leading to an increase of cardiovascular risk as myocardial infarction, thrombosis, and ischemic stroke (Mendes et al., 2012). Furthermore, GCs is also responsible for a wide range of adverse effects, such as osteoporosis, muscle atrophy, hyperglycemia, fat redistribution,

Table 1
Physicochemical and pharmacological considerations of conventional BCS class II anti-inflammatory drugs

Drug	Molecular weight (g/mol)	pKa (pH 1.2–7.5)	logD (pH 7.4)	Calculated solubility (mg/mL)	Group	Conventional dosage (mg)	Main indication	Adverse effects
Aceclofenac	354.19	3.44 (acidic)	0.49	0.01	NSAID	100–200	Joint diseases and pain relief	Nausea, vomiting and other gastrointestinal effects
Betamethasone	392.46	Neutral	1.68	0.01	GC	0.5–4	Topical inflammation and allergic reactions	May have systemic effects when applied topically, skin fragility
Budesonide	430.53	Neutral	2.73	0.01	GC	0.4–9	Topical inflammation, respiratory conditions	Systemic absorption in nasal use, hepatic impairment, dysphonia
Celecoxib	318.37	Neutral	4.01	0.01	COXIB	100–400	Rheumatic diseases, pain relief	Severe skin and cardiovascular reactions
Diclofenac	296.15	4.00 (acidic)	1.10	0.1	NSAID	75–200	Musculoskeletal disorders, sprain, and strain	Gastrointestinal discomfort, hepatotoxicity, thrombotic events
Etodolac	287.35	4.73 (acidic)	0.83	0.01	NSAID	600–1000	Joint diseases, acute pain	Nausea, agranulocytosis, abdominal pain
Hydrocortisone acetate	362.46	Neutral	1.72	0.01	GC	30–100	Topical inflammation, respiratory conditions	Skin fragility, increase in intraocular pressure
Ibuprofen	206.28	4.85 (acidic)	1.34	0.01	NSAID	600–2400	Headache, dysmenorrhea, reduce fever	Gastrointestinal reactions, nausea, vomiting
Indomethacin	357.8	3.79 (acidic)	0.26	0.01	NSAID	75–200	Rheumatic diseases, pain associated with orthopedic procedures	Gastrointestinal and hematological effects, vertigo, headache, kidney toxicity
Ketoprofen	254.3	3.88 (acidic)	0.39	0.01	NSAID	100–300	Joint diseases, reduce fever	Careful use in hepatic and renal patients, skin reactions in topical use
Mefenamic acid	241.3	3.89 (acidic)	2.18	0.01	NSAID	500–1500	Headache, musculoskeletal pain	Cutaneous rash, diarrhea, drowsiness
Meloxicam	351.41	4.47 (acidic)	-1.10	0.01	NSAID	7.5–15	Rheumatic and joint diseases	Gastrointestinal effects, pruritus, headache, dizziness
Nabumetone	228.29	Neutral	3.22	0.01	NSAID	500–1000	Rheumatic and joint diseases	Gastrointestinal, nausea, vomiting
Naproxen	230.26	4.19 (acidic)	-0.05	0.01	NSAID	500–1500	Joint diseases, headache	Gastrointestinal, slightly increase in blood pressure
Nimesulide	308.31	6.70 (acidic)	1.20	0.01	NSAID	100–200	Acute pain, reduce fever	Hepatotoxicity
Piroxicam	331.35	3.79 (basic) and 4.76 (acidic)	-1.52	0.01	NSAID	10-30	Joint diseases, acute pain	Gastrointestinal, slightly increase of blood pressure, skin reactions
Triamcinolone*	394.4	Neutral	0.24	0.01	GC	4–48	Topical inflammation	Skin fragility, risk of systemic absorption

Molecular weight, conventional dosage, main indication and adverse effects data were obtained from Brayfield (2017) and Drugbank (2020) database. Solubility values obtained from Drug Delivery Foundation (2020) database; pKa and logD values obtained from ChEMBL (2020) database. NSAID: non-steroidal anti-inflammatory drug; GC: Glucocorticoid; COXIB: COX-2 selective NSAID

* BCS class IV

skin fragility, and adrenal insufficiency (Vandewalle et al., 2018). The resistance of GCs by several molecular mechanisms is also a concern in chronic inflammation therapies (Ingawale et al., 2015).

Table 1 shows some representative of each group and the primary indication, such as pain, fever, rheumatic and joint diseases, headache, and others. In addition to the adverse effects caused by the mechanism of action itself, 94% of our findings show that these drugs belong to the biopharmaceutical classification system (BCS) class II, with low water solubility and high permeability. This feature reduces the dissolution rate, and the drug could not achieve therapeutic levels, resulting in low bioavailability and requiring high doses (Mohammad et al., 2019). The use of nanotechnologies is one of the most prevalent methods for bioavailability enhancement (Malamatari et al., 2018). Among them, drug nanocrystal is an approach to overcome poor absorption of low water-soluble drugs, which comprise 40% of new chemical entities in the pharmaceutical industry (Savjani et al., 2012).

During drug nanocrystal preparation, problems regarding the quality of final drug product can be predicted by systematic control of important variables involved in the process. Critical quality attributes (CQAs) are physical, chemical, and biological properties present at any step of the process, which should be within a specific range to guarantee that the final drug product has the desired quality (ICH Q8(R2), 2009). Average drug particle size and distribution, chemical composition, general shape, and stability are among the critical properties related to drug nanocrystals (FDA, 2017).

Thus, this review aims to explore recent findings and a regulatory perspective of anti-inflammatory drug nanocrystals. This article includes insights about direct targeting, the selection of stabilizers and preparation techniques, and particular attention to CQAs.

2. Drug nanocrystals: a tool for bioavailability improvement of anti-inflammatory drugs

Nanocrystals are undoubtedly an important strategy for improving the physicochemical properties of BCS class II drugs, which has gained attention in the nanotechnology field. According to the 'Nanotechnology in Drug Delivery - Global Market Trajectory & Analytics', the nanotechnology drug delivery global market is estimated at US$ 124.7 billion in 2027, where drug nanocrystal has forecast to reach US$ 83.1 billion in the end of the analysis period, even considering the coronavirus pandemic crisis and consequent economic recession (Research and Markets, 2020).

By definition, drug nanocrystals are nanoparticles with crystalline structure, particle size below 1000 nm, and composed of drug and stabilizer system, without matrix material (Junghanns and Müller, 2008). When the drug nanocrystals are dispersed in an aqueous media, they are referred to as nanosuspensions. The change of the intrinsic properties by decreasing drug particle size improves the bioavailability of BCS class II drugs, and it may benefit BCS class IV, with poor solubility and poor permeation, since higher drug water solubility increases the concentration gradient and it enhances drug permeability (Peltonen and Hirvonen, 2018). This increased permeability by drug nanocrystal was already described by the diffusion of nanoparticles through the unstirred water layer (UWL) (sometimes referred to as the aqueous boundary layer). UWL is a region of fluid adjacent to membrane cells in which the solvent movement is slower than in the bulk medium (Wood et al., 2018). Erythrocytes and intestine cells were already used as cellular models to study this phenomenon (Barlow et al., 2017). UWL offers resistance to drug diffusion before the permeation through membrane cells. Hence, the drug gradient concentration between bulk and UWL may affect the overall permeability (di Cagno and Stein, 2019; Korjamo et al., 2009). Nanocrystals could overcome UWL resistance due to their higher diffusivity than larger particles. Along with the higher dissolution rate, this would provide a higher amount of dissolved molecules available to permeation (Imono et al., 2020; Roos et al., 2018). Sometimes the mucus layer is considered a biological barrier to drug absorption, considering the overall composition, including mucin glycoproteins, lipids, inorganic salt, and water. The mechanism proposed includes fast diffusion or higher penetration for nanoparticles as well (Guo et al., 2019b; Liu et al., 2020).

The following four main advantages of drug nanocrystal formulation are related to size reduction: increase of dissolution rate, enhance of saturation solubility, improved mucoadhesiveness, and minimization of fed/fasted state variation. When drug particle size is decreased to nanoscale, it increases the surface-area-to-volume ratio, making the dissolution faster, and it can be represented by the Noyes-Whitney equation (Eq 1) (Noyes and Whitney, 1897):

$$\frac{dc}{dt} = \frac{D \times A \times (Cs - Cx)}{h} \quad (1)$$

Where dc/dt is the dissolution velocity (change of concentration by time), D is the diffusion coefficient, *A* is the surface area, *h* is the diffusional distance, *Cs* is the saturation concentration, and *Cx* is the bulk concentration. The dissolution rate is proportional to the concentration gradient, and this *in vitro* assay may be consistent with *in vivo* results. In addition to improving aceclofenac release by 2.10-fold, as shown in Table 2, when orally administered in rats, it showed almost 2-fold increase in C_{max} and 1.55-fold increase in AUC, both compared with the raw drug (Narayan et al., 2017). The higher dissolution velocity can enhance the rate and extent of the absorption, reducing the conventional dose of aceclofenac. Additionally, the bioavailability enhancement provided by nanocrystal technology causes a rapid onset of action, bringing quality of life in patients with anti-inflammatory drug treatment for pain relief.

Similarly, particle size affects saturation solubility. Factors such as dissolution medium, temperature, and the crystalline drug structure may also influence (Chogale et al., 2016). Saturation solubility is associated with the Kelvin equation, which was first proposed to describe the relationship between the curvature of the liquid drop's surface and the vapor pressure in liquid-vapor systems (Wang et al., 2020; Xia et al., 2020). In this model, molecules passing from the drop to the gas phase constitute the vapor pressure, which is enhanced when the drop surface curvature increases (Yarom and Marmur, 2015). Hence, this phenomenon is analogous to the transference of molecules from a solid nanoparticle towards the liquid phase. The reduction in the nanoparticle's curvature increases the dissolution pressure, contributing to enhancing saturation solubility (Junyaprasert and Morakul, 2015). The Ostwald-Freundlich equation (2) directly describes the relationship between saturation solubility and particle size (Fontana et al., 2018):

$$\log \frac{Cs}{C\alpha} = \frac{2\sigma V}{2.303RT\rho r} \quad (2)$$

Where Cs is the saturation solubility, Cα is the solubility of large particles, σ is the interfacial tension of the drug substance, V is the molar volume of the particle material, R is the gas constant, T is the absolute temperature, ρ is the density of the solid, and r is the radius. This enhancement is more relevant for particles in the nanoscale range. The fundamental difference from this model to the Noyes-Whitney equation (1) is the variable time, since in this latter the dissolution kinetics is the parameter associated with surface area and, consequently, particle size.

Evaluating the saturation solubility, flurbiprofen nanosuspensions in dermal delivery demonstrated an increase of saturation solubility of 5.3-fold compared to a coarse powder, as mentioned in Table 3 (Oktay et al., 2018). The same study showed the nanosuspension has a permeated drug amount 2.2-fold higher than the suspension in rat's skin during *ex vivo* evaluation. The improved permeation using flurbiprofen nanosuspensions is correlated with an increase of gradient concentration between formulation and skin due to the enhancement surface-area-to-volume ratio, saturation solubility, and dissolution rate when reducing the drug particle size. Additionally, these features improve adhesiveness to surfaces due to an increase in the contact area

Table 2
Anti-inflammatory drug nanocrystals prepared using wet bead milling (WBM) approach

Drug (conc.)	Route of administration	Stabilizer (conc.)	Milling speed (time)	Final particle size (nm) (PDI)	Solubility	Dissolution	Characterization	Ref.
Indomethacin (100 mg/mL)	Oral	Poloxamer 407 (50 mg/mL)	400 rpm (24 h)	260-270 (0.11-0.21)	-	-	DLS, TEM, NMR, Raman, HPLC, SS	Kuroiwa et al. (2018)
Meloxicam (0.15 g)	Oral	Tween 80 (0.5% w/w)	1200 rpm (4 h)	88.0	-	80-100% release after 10 min in pH 7.4	XRPD, DLS, LD, SEM	Tao Liu et al. (2018)
Budesonide (1 g)	Pulmonary	Poloxamer 188 (0.3 g)	500 rpm (120 min)	259.0 ± 2.0 (0.18 ± 0.08)	-	Faster dissolution before encapsulation in the first 4 h	XRPD, DSC, DLS, FESEM, HPLC	Tingting Liu et al. (2018)
Ibuprofen (0.5 g)	Pulmonary	HPMC (10% w/w of ibuprofen)	200 rpm (180 min)	533.0 ± 28	-	Complete dissolution after 5 min in pH 6-7	XRPD, DSC, TGA, DLS, LD, SEM	Malamatari et al. (2017)
Aceclofenac (200 mg)	Oral	PVA(0.25%)	400 rpm (4 h)	484.7 ± 54.12 (0.108 ± 0.009)	Increase of 1.99-fold in water and 1.65-fold in HCl	2.19-fold higher in 2h	XRPD, DSC, DLS, UV, HPLC, SEM, SS	Narayan et al. (2017)
Dexamethasone (0.05% w/v)	Dermal	Poloxamer 407 (0.49% w/v)	800 rpm (3 h)	221.0 ± 4.0 (0.08 ± 0.03)	-	Complete dissolution after 5 h	DLS, SEM, UV, LC-MS	Döge et al. (2016)
Meloxicam (10%)	Intranasal	PVA (0.5 g)	400 rpm (50 min)	135.0 ± 0.002	~3.08 higher in pH 5.5 as nasal spray	-	DLS, LD, SEM	Bartos et al. (2015)
Indomethacin (1 g)	Oral	Poloxamine 908 (0.4 g)	1100 rpm	286.0 ± 15.0 (0.22 ± 0.01)	-	~100% was dissolved after 1 min at pH 5.0	DLS, SEM, SS, CAM, SPR	Liu et al. (2015)
Sodium diclofenac acid (2:1 w/w)	Dermal	Poloxamer 188 (2:1 w/w)	3000 rpm (60 min)	279.0 ± 8 (0.17 ± 0.01)	3-fold higher than coarse crystals	-	XRPD, DSC, DLS, SEM, HPLC, SS	Pireddu et al. (2015)
Naproxen (1% w/v)	Oral	Tween 80 (0.2% w/v)	3200 rpm (4 h)	~ 270.0	-	-	XRPD, DSC, ATR-FTIR, DLS, HPLC-UV, LC-MS	Kumar and Burgess (2014)
Meloxicam (20 mg)	Oral	PVP k30 (20 mg/mL)	2000, 400 rpm (2 min, 1 min, respect.)	119.0	-	23.07-fold higher in pH 1.2 and 2.08-fold higher in neutral media	XRPD, DLS, LD, SS, HPLC-UV	Ochi et al. (2014)
Nimesulide (1% w/v)	Oral	Poloxamer 407 (0.5 w/v)	175 rpm (15 min)	702.6 ± 10.1 (0.324 ± 0.04)	59-fold higher in pH 1.2 than raw powder	-	XRPD, DSC, FTIR, DLS	Gülsün et al. (2013)
Indomethacin (1 g)	Oral	Poloxamer 188 (0.6 g)	1100, 100 and 850 rpm (30 min each)	340.0 ± 4.0 (0.24 ± 0.06)	Significantly high in pH 5	Significantly high in pH 5	DLS, HPLC	Liu et al. (2013)

Conc.: concentration; Ref.: references; PDI: Polydispersity index; XRPD: X-ray powder diffraction; DSC: differential scanning calorimetry; DLS: dynamic light scattering; LD: laser diffraction; SS: stability studies; UV: ultraviolet; SEM: scanning electron microscopy; FESEM: field emission scanning electron microscopy FTIR: Fourier-transform infrared spectroscopy; ATR-FTIR: attenuated total reflectance Fourier-transform infrared spectroscopy; TGA: Thermogravimetric analysis; LC-MS: liquid chromatography coupled with mass spectrometry; HPLC: high-performance liquid chromatography; NMR: Nuclear magnetic resonance; HPLC-UV: high-performance liquid chromatography coupled with ultraviolet; CAM: contact angle measurement; SPR: surface plasmon resonance; Raman: Raman spectroscopy; TEM: transmission electron microscopy.

of small particles (Saini and Kumar, 2018). Celecoxib nanosuspensions have improved adhesion in mucus and intestinal villus in rats, which contributes to the drug delivery across the four segments of the intestine, mainly in the duodenum (He et al., 2017). The absorption enhancement by drug nanocrystal may benefit high-dose anti-inflammatory drugs, requiring less to produce the effect and facilitating patient compliance

The anti-inflammatory drugs with multiple-dose regimen could have its administration frequency decreased by drug particle size reduction in nanoscale. Pharmacokinetic studies using animal model demonstrate that drug nanocrystal presents a rapid increase in plasma concentration. However, the elimination is equivalent compared to the conventional drug/microsuspension, mainly evaluated as half-life ($t_{1/2}$). For instance, aceclofenac nanocrystal did not significantly differ the $t_{1/2}$ and elimination rate constant comparing to the conventional drug (Narayan et al., 2017). Similar results were observed in fluticasone and etodolac nanosuspensions (Afifi et al., 2015; Fu et al., 2019). In healthy humans, budesonide nanocrystal for inhalation delivery presented a C_{max} increase and T_{max} reduction compared to Pulmicort Respules (budesonide as a dry-powder inhaler, AstraZeneca, Wilmington, DE), indicating rapid drug delivery and absorption (Kraft et al., 2004). Simultaneously, the $t_{1/2}$ was corresponding to the marketed drug product. For other pharmacological classes, similar results have been extensively found in the animal model (Imono et al., 2020; Ma et al., 2017; Paredes et al., 2020; Sattar et al., 2017; Sharma and Mehta, 2019; Zhang et al., 2020). Therefore, the increase in drug nanocrystal absorption and an equivalent decrease in its $t_{1/2}$ and other pharmacokinetics parameters may support the reduction of the conventional dose or keep it, and reduce the administration throughout the day. However, studies comparing the effects of a single dose of drug nanocrystal with multiple-dose of the conventional drug is paramount to test this hypothesis and assure its safety.

The increased saturation solubility of drug nanocrystals may also eliminate the food effect in oral administration. Poorly water-soluble drugs are mainly ingested with food to improve bioavailability since the gastrointestinal system facilities the dissolution rate by increased gastric fluid, delayed gastric emptying, and increase of splanchnic flow

Table 3
Anti-inflammatory drug nanocrystals prepared using high-pressure homogenization (HPH) approach

Drug (conc.)	Route of administration	Stabilizer (conc.)	Milling pressure (cycle or time)	Final particle size (nm) (PDI)	Solubility	Dissolution	Characterization	Ref.
Flurbiprofen (4%)	Dermal	Plantacare ® 2000 (2.5:1 D/S)	30,000 psi (25 cycles) (microfluidization)	654.3 ± 9.7 (0.30 ± 0.02)	5.3-fold than coarse powder	-	XRPD, DSC, LD, DLS, SEM, HPLC, SS	Oktay et al. (2018)
Meloxicam (0.8 g)	Oral	Tween 80 (0.5% w/w)	1300 bar (20 cycles)	± 400.0	-	80-100% dissolved in the first 10 min	XRPD, DLS, LD, SEM	Tao Liu et al. (2018)
Ibuprofen (0.25% w/v)	Oral	Tween 80 + PVP k30 (0.2% and 1.2% w/v, respect.)	1000 bar (20 min) (80°C)	79.0 (0.126)	-	-	DLS, SS	Fernandes et al. (2017)
Celecoxib (4:1 D/S)	Oral	TPGS (4:1 D/S)	200 and 800 bar (5 and 20 cycles)	232.5 ± 3.0 (0.20 ± 0.02)	~4-fold greater than coarse celecoxib	More than 80% released within 10 min	XRPD, DSC, FTIR, DLS, LD, SEM, TEM, SS	He et al. (2017)
Meloxicam (0.75% w/v)	Oral (ODT)	Poloxamer 188 (1% w/v)	500 and 1000 bar (2 and 20 cycles)	463.5 ± 9.71 (0.312 ± 0.014)	-	90% was dissolved after 6 min (influenced by freeze-drying) in pH 7.4	XRPD, DLS, SEM	Iurian et al. (2017)
Piroxicam (2.5% w/w)	Oral (ODT)	Poloxamer 188 (1.5% w/w)	500 and 1500 bar (3 and 30 cycles)	414.3 ± 21.1 (0.40 ± 0.02)	-	All formulations with high dissolution rate than coarse ODT	XRPD, DSC, FTIR, DLS, SEM, UV	Lai et al. (2014)
Hydrocortisone acetate (10% w/w)	Oral	Poloxamer 188 (1% w/w)	150, 500 and 1500 bar (2, 2, and 30 cycles)	677.0 ± 55.0	-	-	DLS, LD, ESEM	Möschwitzer and Müller (2013)
Indomethacin (0.5% w/v)	Oral	HPMC (0.5% w/v)	18,000 psi (70 min) (microfluidization)	428.0 ± 13.0	-	-	XRPD, DLS, SEM, LM, SS	Verma et al. (2011)

Conc.: concentration; Ref.: References; PDI: polydispersity index; D/S: drug:stabilizer ratio; ODT: orally disintegrating tablets; XRPD: X-ray powder diffraction; DSC: differential scanning calorimetry; FTIR: Fourier-transform infrared spectroscopy; DLS: dynamic light scattering; LD: laser diffraction; SS: stability studies; HPLC: high-performance liquid chromatography; UV: ultraviolet; SEM: scanning electron microscopy; ESEM: environmental scanning electron microscopy; TEM: transmission electron microscopy; LM: light microscopy.

in food presence (Jinno et al., 2006; Junyaprasert and Morakul, 2015). The fed/fasted state may vary the bioavailability, leading to a sub-optimal therapeutic level and poor performance (Merisko-Liversidge et al., 2003). Drug nanocrystals uniformly formulated can minimize this variation because the dissolution rate is fast enough, even under the fasted state (Junyaprasert and Morakul, 2015). A good example is aprepitant nanocrystals, an antiemetic agent marketed as Emend® by Merck. The conventional formulation of aprepitant requires food presence to achieve therapeutic levels, where is an unavailable approach for patients with nausea. In addition to the bioavailability improvement, the fed/fasted state variation was eliminated during *in vivo* evaluation (Merisko-Liversidge, 2015). For anti-inflammatory drugs, this type of assessment requires further studies. Despite being more effective in the postprandial state to minimize their gastrointestinal side effects, the variation of absorption of anti-inflammatory drugs can be decreased when using nanocrystal formulation.

The most prominent adverse effect of NSAIDs is gastrointestinal irritation, impairing gastric compartment defense by local and systemic mechanisms. As for the local effect, NSAIDs cause damage to epithelial cells, and decrease of mucus and bicarbonate secretion (Scheiman, 2016). Hence, the mucosa becomes less resistant to the stomach acidic environment. Regarding the systemic effect, COX-1 inhibition reduces prostaglandin E_2 production, which has an essential role in protecting gastric mucosa. The systemic effect is observed regardless of the administration route (Takeuchi, 2012). Additionally, poorly water-soluble drug substances promote a high and prolonged drug concentration in the gastric compartment, leveraging the NSAIDs toxicity.

The anti-inflammatory drug nanocrystals can reduce the gastrointestinal adverse effect by a systemic and local effect. The systemic effect is attributed to increased drug bioavailability, decreasing drug dosage, and attenuating the direct stimulation in the gastrointestinal compartment (Nagai et al., 2020). Meloxicam nanocrystals presented minimal gastrointestinal lesions compared with the conventional drug dispersion after repetitive administration in rats for a month. The nanocrystal formulation used a dose 4-fold reduced, which was the same dose that presented an increase of bioavailability, and an equivalent decrease in paw edema (Nagai et al., 2020). Despite the mucoadhesive property, drug nanocrystals will quickly disperse and dissolve comparing with larger coarse API particles, although further specific studies are necessary to address this issue mechanistically.

Liversidge and Conzentino (Liversidge and Conzentino, 1995) prepared naproxen nanocrystals, where the effect in minimizing the gastric irritation followed by oral administration was evaluated in rats. The naproxen nanocrystals were obtained by the top-down approach using poloxamer 188 as a stabilizer, with no particles above 400 nm. Additionally, they presented 4-fold increase in naproxen absorption, and the gastric irritancy was significantly lower than coarse naproxen. It shows that anti-inflammatory drug nanocrystal is a safe and efficient alternative to minimize the adverse effects of the conventional anti-inflammatory drugs.

The benefits of anti-inflammatory drug nanocrystals approach for different routes of administration have been reported recently, and some of those benefits are shown in Table 2–4. In addition to the 26-fold enhancement in dissolution rate as we show in Table 4, dexibuprofen nanocrystals presented almost equivalent analgesic potency than the conventional drug with dose 4-fold reduced after oral administration in BALB/c mice (Ullah et al., 2018). Diclofenac acid nanocrystals in dermal delivery improved skin deposition and permeation compared to the commercial topical formulation in *in vitro* transdermal evaluation (Pireddu et al., 2015), which may require fewer applications to produce the desirable anti-inflammatory and analgesic effect. Fluticasone nanosuspensions exhibited significant anti-inflammatory activity in the lungs compared to microsuspensions after pulmonary delivery in mice, even in

Table 4
Anti-inflammatory drug nanocrystals prepared using a bottom-up technology

Drug (conc.)	Route of administration	Bottom-up method (solvents)	Stabilizer (conc.)	Final particle size (nm) (PDI)	Solubility	Dissolution	Characterization	Ref.
Beclomethasone dipropionate (10 mg/mL)	Dermal	Antisolvent (ethanol + aqueous solution)	Poloxamer 407 (1% w/v)	622.0 ± 21.0 (0.30 ± 0.01)	745.5 higher than raw powder	-	XRPD, DSC, DLS, TEM	Assem et al. (2019)
Meloxicam (5:1 D/S)	Sublingual	Acid-basic neutralization (NaOH + HCl)	Poloxamer 407 + Tween 80 (50% w/w of drug, 5:5 each)	178.7 ± 4.5 (0.197 ± 0.015)	4.81-fold increase in pH 6.8	Almost complete dissolution within 10 min as fast dissolving sublingual films	XRPD, DLS, SEM, SS	Song et al. (2018)
Dexibuprofen (30 mg/mL)	Oral	Antisolvent (ethanol + aqueous solution)	HPMC and PVP (1% w/v)	85.0 ± 2.5 (0.17 ± 0.01)	~ 5-fold increase in water	26-fold increase compared with marketed tablets	XRPD, DSC, DLS, SEM, TEM, FTIR, HPLC-UV, SS	Ullah et al. (2018)
Meloxicam (0.5% w/v)	Transdermal	Acid-basic neutralization (NaOH + HCl)	Poloxamer 407 + Tween 80 (0.1% w/v, 80:20 each)	175.0 ± 4.0 (0.167 ± 0.030)	-	-	XRPD, DSC, DLS, SEM, HPLC-UV, SS	Yu et al. (2018)
Aceclofenac (20 mg)	-	Antisolvent (acetone + aqueous solution)	Tween 80 (20 mg)	716.0 ± 65.0	3.45x higher in water and 4.03x higher in pH 6.8	Slightly decrease than raw drug	DSC, FESEM, HPLC	Park et al. (2016)

Conc.: concentration; Ref.: References; PDI: polydispersity index; D/S: drug: stabilizer ratio; XRPD: X-ray powder diffraction; DSC: differential scanning calorimetry; DLS: dynamic light scattering; SS: stability studies; HPLC: high-performance liquid chromatography; HPLC-UV: high-performance liquid chromatography coupled with ultraviolet; SEM: scanning electron microscopy; FTIR: Fourier transformed infrared; FESEM: field emission scanning electron microscopy; TEM: transmission electron microscopy.

6 h post-administration, indicating that drug nanocrystal can prolong the fluticasone retention in the lungs (Fu et al., 2019). This can be attributed to the drug penetration enhancement, where fluticasone nanocrystals penetrate the periciliary liquid layer, escaping from mucociliary clearance. Also, nanosized particles are essential for this route of administration since depositing GCs back of the mouth and the throat could lead to localized immune suppression and favor local opportunistic infections (Gao et al., 2012). For intravenous administration (IV), drug nanocrystal is preferred because particles larger than 5 μm can lead to capillary blockade and embolism (Gao et al., 2012). For ophthalmic delivery, drug nanocrystal has a rapid onset of action compared to other techniques since this approach does not use matrix material (Peters et al., 2020), and it is less prone to cause ocular irritation and blurry vision compared to other techniques to overcome the poor water-soluble drugs problem (Sharma et al., 2016). Thus, it is a promising technique to improvement of poorly water-soluble anti-inflammatory drugs efficacy, feasible for different administration routes.

Drug nanocrystals were first introduced at the beginning of the 1990s (Liversidge et al., 1992). Rapamune® (sirolimus) was one of the first products on the market in 2000 by Wyeth Pharmaceuticals (Malamatari et al., 2018). In contrast, Celebrex® (celecoxib) had already been approved in 1998 as a new drug application (NDA) of oral anti-inflammatory drug nanocrystals by Pfizer (Chen et al., 2017). In 2006, Naprelan® was approved as tablets of naproxen nanocrystals by Wyeth (Jarvis et al., 2019). Ilevro® containing nepafenac nanocrystals for ophthalmic delivery by Alcon also represents anti-inflammatory drug nanocrystals on the market, launched in 2013 (Chen et al., 2017).

Furthermore, submissions to the Food and Drug Administration (FDA) for nanocrystal drug products from 1973 to 2015 comprised approximately 30% of all applications containing nanomaterials, in which 32% were abbreviated new drug applications (ANDA) (Chen et al., 2017). Regarding therapeutic areas, 11% had anti-inflammatory indication. The high percentage of ANDA nanocrystals can be explained because nanocrystals are readily translatable technology in contrast to other nanomaterials.

3. Targeting anti-inflammatory drug nanocrystals

Anti-inflammatory drug nanocrystals can be targeted towards inflammation sites to improve the *in vivo* behavior and minimize adverse effects. The targeting process can be divided into active targeting and passive targeting, as we illustrated in Figure 1. In active targeting is used ligands such as proteins, antibodies, and small molecules to enhance delivery to specific organs. In passive targeting, the drug particles can be internalized by cells and delivered through the cell route. The targeting process depends on the drug particle size, dissolution rate, route of administration, and surface modification. Under 1000 nm, drug nanocrystals can be uptake by the mononuclear phagocytic system (MPS) cells and, below 100 nm, they can be endocytosed by other cells (Müller et al., 2011). Despite this phenomenon, long-acting intramuscular prodrug injection in the submicron range in rats revealed the API uptake by MPS cells. The pharmacokinetics and histopathological findings indicated that prodrug uptake by macrophages promoted a slower second phase dissolution and conversion to the active drug. It shows that long-acting injection drugs, even in the submicron range, can have its pharmacokinetics behavior influenced by the MPS uptake (Darville et al., 2016, 2014).

The MPS cells comprise a phagocytic cells system, predominantly macrophages, present in the liver, spleen and lungs (Gustafson et al., 2015; Müller et al., 2011). For instance, Kupffer cells, the most important MPS cells of the liver, is responsible for the major drug nanoparticle accumulation in this organ (Samuelsson et al., 2017). They represent 80-90% of the total body macrophage population (Bertrand and Leroux, 2012). Furthermore, the liver presents fenestrations in the endothelial cells allowing the nanoparticles to be trapped in them, contributing to additional accumulation in this organ (Blanco et al., 2015; Van Haute and Berlin, 2017).

For oral delivery, the recognition by phagocytic cells is minimal once the dissolution is fast enough until the absorption process occurs (Lu et al., 2017; Müller et al., 2011). For intravenous delivery, the MPS cells have a significant role in the drug nanocrystal uptake (Fuhrmann et al., 2014). The nanoparticles recognized as an exogenous material will be opsonized, and further uptake by MPS cells in the bloodstream, which will carry the engulfed material to its organ of residence, leading drug nanoparticle accumulation (Gustafson et al., 2015; Jokerst et al., 2011). The persistent presence of naturally toxic drugs in these organs, such as chemotherapeutic agents, can compromise the safety promised by the nanocrystal preparation (Zhou et al., 2016).

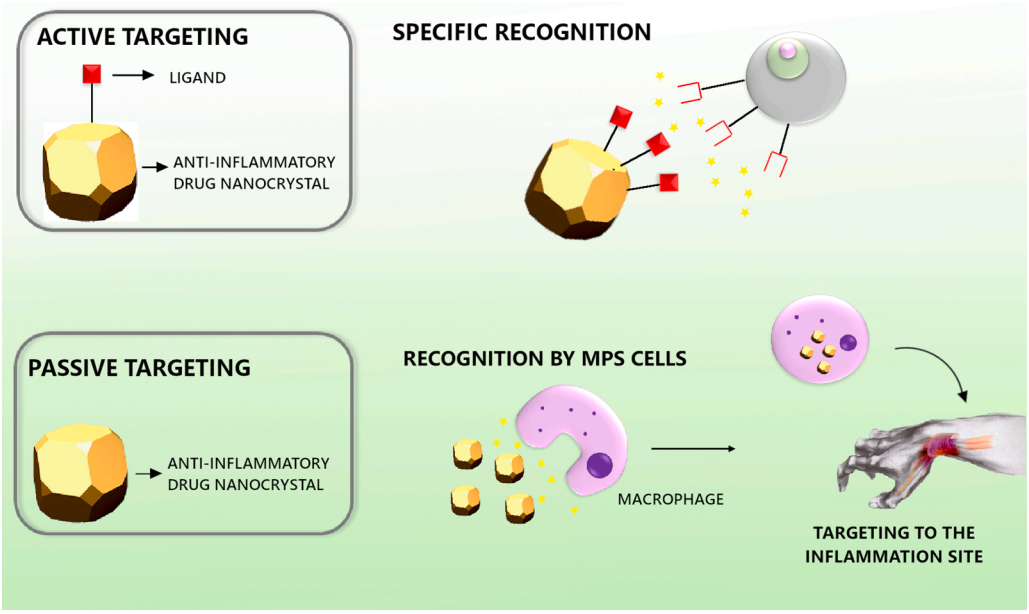

Figure 1. Anti-inflammatory drug nanocrystal recognition by active and passive targeting. While in the active targeting the nanocrystal is specifically recognized due surface decoration by ligands such as proteins, antibodies and its fragments, peptides, and specific molecules, in the passive targeting anti-inflammatory drug nanocrystals could be recognized by macrophages and penetrate in the inflammation site.
MPS: Mononuclear phagocytic system

The nanocrystal clearance by MPS cells may not occur instantly. Ritonavir nanocrystals remained 68% intact into monocyte-derived macrophages after 24 h post-uptake by passive targeting (Kadiu et al., 2011). The internalization of drug nanocrystal will act as a depot, dissolving the drug slowly until diffusing out of the cells, whose mechanism has been explored to specific targeting in different therapies, such as HIV and cancer (Fuhrmann et al., 2014; Lu et al., 2017, 2015; Zhou et al., 2018). In active targeting, the drug surface is decorated with specific ligands to avoid the phagocytic cells' clearance and increase the target specificity. For instance, folic acid is a ligand of folate receptor-β expressed on the surface of the activated macrophage, and it is currently used to improve drug nanocrystal targeting in HIV since this phagocytic cell acts as a viral reservoir (Liu et al., 2010; Wong et al., 2019; Zhou et al., 2018).

As for anti-inflammatory drug nanocrystal, targeting macrophages is also an interesting hypothesis for therapy improvement. This approach has already been investigated by matrix nanosystems, such as lipid-based, polymeric, and chitosan nanoparticles, whether by active or passive targeting (Chuang et al., 2018; Dolati et al., 2016). For nanocrystals, this strategy remains unexplored. The enhanced permeability and retention (EPR) frequently present in inflammation enables the drug nanoparticles with the appropriate size (approximately 200-700 nm) to permeate through vascular endothelium gaps. They will then be slowly released in the site of inflammation (Chuang et al., 2018). Based on this principle, anti-inflammatory drug nanocrystals could permeate through inflamed leaky vessels due to the EPR effect and achieve organism sites with limited drug delivery, such as arthritic joints, and then will be taken up by macrophages. In some inflammatory diseases, macrophages act as central players, such as rheumatoid arthritis (Udalova et al., 2016). The hypothesis is based on the internalization of anti-inflammatory drug nanocrystals by macrophages, which would be directly targeted into the inflammatory site by combining EPR effect and passive targeting, improving the efficacy and safety of these drugs. Nevertheless, it is necessary to consider the observations related to the particle size, dissolution rate, and administration route presented in this chapter. The necessity to explore this insight in further studies to evaluate the behavior of anti-inflammatory drug nanocrystal at the intracellular level is also paramount.

4. Preparation techniques of anti-inflammatory drug nanocrystals

4.1. Bottom-up technology

The drug nanocrystal preparation involves the bottom-up and top-down technologies, as well as combinations of both of them. The bottom-up consists of the growth of small particles from individual molecules. This process is also called nanoprecipitation because drug particles are precipitated from a supersaturated solution (Sinha et al., 2013). The supersaturation is the driving force for the growth of a crystal, achieved by decreasing the temperature or addition of an anti-solvent (De Waard et al., 2011). Bottom-up approaches have the following main advantages in preparing drug nanocrystals: minimal or no use of mechanical energy in the process, fewer expenses compared to other techniques, and suitable for thermolabile drugs (Miao et al., 2018).

Although a few authors have used bottom-up to prepare anti-inflammatory nanocrystals, as shown in Table 4, our findings related that this technique is promising, with particle size varying from 85.0 to 716 nm. In addition to preparing nanocrystals using solvent-antisolvent precipitation with saturation solubility approximately 700-fold higher than raw powder, beclomethasone dipropionate nanocrystals presented amount retention on the skin in an *ex vivo* study 2.49-fold higher than the marketed product (Assem et al., 2019). Furthermore, local accumulation efficiency (LAC) of nanocrystals was approximately 25-times higher than brand formulae. Thus, bottom-up approaches in this report not only enhanced the drug deposition on the skin but also decreased the amount permeating into the systemic circulation, providing a safer product.

Acid-basic neutralization is another type of bottom-up strategy that also offers anti-inflammatory drug nanocrystals pH-dependent solubility with excellent properties (Mou et al., 2011). A report of meloxicam, a weak acid drug with pH-dependent solubility as dissolving sublingual film nanocrystal, was performed in acid-basic neutralization. It showed a mean particle size below 200 nm and a great polydispersity index (PDI) (Table 4), even after redispersion on sublingual films (Song et al., 2018). Moreover, the films containing these nanocrystals showed an increased AUC of 4.33-fold, and C_{max} 5.42-fold higher of raw meloxicam in rats. It indicates that drug nanocrystals prepared by acid-basic neutralization can be a promising delivery system to improve the

bioavailability of poorly water-soluble anti-inflammatory drugs that are pH-dependent.

Despite advantages, bottom-up approaches have difficulty in controlling the particle size growth, and may produce amorphous materials (Peltonen and Hirvonen, 2018). Thorough removal of residual organic solvents is also important to prevent problems in physical and chemical stability (Mahesh et al., 2014), and toxicity for the patients. Environmental contamination is another concern when using organic solvents, showing that the process needs to be very well controlled to prepare successful anti-inflammatory drug nanocrystals using bottom-up technologies.

4.2. Top-down approach

The top-down process is the most important industrial technology in particle size reduction (Salazar et al., 2012). This process differs from bottom-up in not requiring organic solvents, better control of particle-shape and size, and ease of scale-up (Müller et al., 2001). It is a high-energy process, where micron-sized drugs are suspended into an aqueous or non-aqueous dispersion medium containing stabilizers and, under mechanical attrition (wet bead milling (WBM)) or high pressure (high-pressure homogenization (HPH)), it reduces drug particles to the nanoscale (Tuomela et al., 2016b; Van Eerdenbrugh et al., 2008). WBM is the most common method to produce drug nanocrystal in the industry (Kumar and Burgess, 2014) and, as can be seen in Table 2, it is also the most reported anti-inflammatory drug nanocrystal method in our findings (50%).

WBM is performed dispersing the drug and stabilizer in aqueous media, then it is placed in a jar with ball milling media while moving the beads at high speed (Gülsün et al., 2009). Particle size reduction is obtained by impact, friction, and shear forces produced from the movement of milling media. Usually, the grinder chamber is made of stainless steel, porcelain, or hard material, and the beads can be made of porcelain, yttrium-stabilized zirconium, glass, agate, or special polymer materials. Zirconia or yttrium zirconia beads (stabilized version) are frequently used as milling agent. The size can vary from < 0.1 mm to 20 mm, and smaller milling beads produce finer nanoparticles due to the increase of the collision frequency between drug and the beads, drug and drug, and drug and the vessel wall (Malamatari et al., 2018). Indomethacin nanosuspension, with smallest particle size (340.0 ± 4.0 nm), was obtained using zirconium oxide beads of 1 mm, in contrast to 5 and 10 mm ones (Liu et al., 2013). On the other hand, very small beads ($<$ 0.03 mm) may not generate sufficient energy to reduce the drug particle size (Malamatari et al., 2018). This literature review found that anti-inflammatory drug nanocrystals prepared by WBM mostly used beads of 0.5 mm (data not shown). However, an optimal bead size to obtain smaller drug particle size will depend on different parameters, including drug hardness, an appropriate stabilizer selection, milling speed, and time.

The drug particle reduction is proportional to the increase of rotation speed and time. However, our findings revealed that increasing speed and grinding time does not necessarily result in an additional reduction of nanocrystal particle size. On the contrary, extended milling time may produce nanocrystals with larger particle size due to aggregation (Kumar and Burgess, 2014; Tao Liu et al., 2018; Pireddu et al., 2015). Kumar and Burgess (Kumar and Burgess, 2014) prepared naproxen nanocrystals by WBM with Tween 80 or HPMC E-15 as stabilizers. While naproxen nanocrystals stabilized with Tween 80 presented no aggregation in all stabilizer concentration and milling intensities, providing particle size below 300 nm as mentioned in Table 2, the opposite was observed when stabilized with HPMC E-15. The particle size of naproxen nanocrystals increased independently of HPMC-E15 concentration in the higher milling intensity (3400 rpm), showing that increasing the stabilizer concentration or milling intensity does not obtain smaller drug particle size. Naproxen nanocrystals stabilized with HPMC-E15 showed aggregation and chemical instabilities, where degradation in a decarboxylated product of naproxen took place in the WBM process. It shows that other parameters must also be considered during grinding to obtain smaller sizes of anti-inflammatory drug nanocrystals. This includes the surfactant selected, hardness of the drug, viscosity, and temperature (Junghanns and Müller, 2008).

HPH is the second most important technique to produce drug nanocrystals, considering a highly productive process with low batch-to-batch variation (Junyaprasert and Morakul, 2015; Möschwitzer, 2013). The drug particle size reduction can be divided into microfluidization technology (IDD-P™), where the collision of two jet streams under high pressure can reduce drug particle size by shear and cavitation forces (Junyaprasert and Morakul, 2015). Piston gap in water (Dissocubes®) is another HPH method, where the dispersion (containing the drug, stabilizer, and aqueous media) is forced to pass through narrow channels with the help of a piston under high pressure. Drug nanocrystals is obtained by cavitation, high shear forces and turbulent flow (Joseph and Singhvi, 2019). The difference between the Dissocubes® and Nanopure® is the dispersion media of the latter, composed of polyethylene glycol (PEG) or fatty acids, and the absence of cavitation forces, in which the particle size reduction occurs by shear forces, particle collisions, and turbulence (Junyaprasert and Morakul, 2015).

Table 3 shows anti-inflammatory drug nanocrystals with particle size from 79 to 677.0 nm. It can be directly influenced by the homogenization pressure, where a higher pressure will provide high energy to reduce drug particle size. Additionally, it is necessary enough homogenization cycles to maintain sufficient energy to break down the particles and obtain uniform drug nanocrystals, and both homogenization pressure and cycles will also depend on the drug hardness (Junyaprasert and Morakul, 2015). Despite the benefits, HPH demands high equipment costs, and the process energy increases the temperature of dispersion even at lower pressures (approximately 10 °C at 500 bar), which can compromise thermolabile drugs and the stabilizer efficiency (Junyaprasert and Morakul, 2015; Shrimal et al., 2020). WBM and HPH can render the process more susceptible to contamination due to extended milling time and homogenization cycles. Additionally, process parameters have to be adequate to avoid porous and fragile nanoparticles (Tao Liu et al., 2018).

It is possible combining the bottom-up and top-down methods as an option to improve the effectiveness of drug nanocrystal preparation. The patented Nanoedge™ by Baxter was the first combination of technologies to obtain drug nanocrystal (Chang et al., 2015). This technique combines the precipitation method and HPH, a great strategy to overcome crystal growth and promote the long-term stability of bottom-up technology (Mirza et al., 2017). Additionally, smartCrystal® is a patented technique by PharmaSol GmbH, later acquired by Abbot. It comprises different combination processes, not limited to the bottom-up and top-down association. HPH is the main treatment, and each pre-treatment differs in smartCrystal®, which can be: spray drying (called H 42), precipitation (H 69), and lyophilization (H 96) (Bansal et al., 2012). These techniques are advantageous since they can produce drug nanocrystal with particle size below 100 nm, which is very difficult to obtain using HPH alone (Shegokar and Müller, 2010). Pre-treatment steps such as spray drying and lyophilization lead drug powder easier to particle size reduction by HPH.

Furthermore, smartCrystal® includes combinative technology (CT), where it is possible to combine WBM and HPH. Fluticasone nanosuspension was prepared combining these top-down techniques (Fu et al., 2019). The drug particle size was reduced below 250 nm, and the dissolution rate was improved 1-33-fold compared to the microsuspensions, even after the internalization of the drug particles by Calu-3 cells. The nanosuspensions maintained stable for three months. These results indicate that combining the benefits of WBW and HPH techniques is a feasible approach to anti-inflammatory drug nanocrystal preparation.

5. Stabilization of anti-inflammatory drug nanocrystals

An essential common feature of all methods to prepare drug nanocrystals discussed in Section 4 is that they all use stabilizers. Its use is vital to prepare nanocrystals with excellent physical and chemical characteristics. The particle size reduction changes Gibbs free energy, and the nanosuspension becomes thermodynamically unstable, resulting in agglomeration and formation of large particle size, decreasing the surface-area-to-volume ratio (Ghosh et al., 2011). Over time, stability issues may also emerge due to Ostwald ripening. This phenomenon occurs due to the difference in dissolution rate and saturation solubility among particles with different sizes, which allows the increasing of larger particles from the dissolution of the smaller ones (Skrdla and Yang, 2019).

An adequate stabilizer should provide a barrier to agglomeration (Van Eerdenbrugh et al., 2008), and its selection is as fundamental as other process parameters. There is still no rationale in the selection of stabilizers, with this process driven by trial and error. It is also necessary to be safe, non-toxic, and adequate for the intended route of administration. Stabilization can be divided into electrostatic, comprising cationic and anionic surfactants (as sodium dodecyl sulfate, benzalkonium chloride) and steric, such as polymers and nonionic surfactants (povidones, cellulosederivates, poloxamers) (Van Eerdenbrugh et al., 2008). The cationic and anionic stabilizers prevent physical instabilities by electrostatic repulsion, while nonionic and polymers by forming a steric barrier (Tuomela et al., 2016a). To obtain these advantages, it is possible to combine both electrostatic and steric stabilizers.

Although a standard mechanism in the stabilizer selection is unclear, there are some methods to support it following a physicochemical coherence. Ochi and colleagues (Ochi et al., 2014) prepared meloxicam nanocrystals and screened hydrophilic polymers based on the anti-precipitant by the solvent shift method. In this approach, polymers should promote a crystal growth inhibition in a supersaturated drug solution, which was previously correlated with stability by acting as a precipitation inhibitor (Chauhan et al., 2013). The viscosity and pH of the polymer solution presented no correlation with the precipitation of meloxicam, and the efficiency of crystal growth inhibition was not influenced by the polymer's polymerization degree. It indicates that the effect of stabilizers on the meloxicam precipitation is strongly correlated with drug-stabilizer interactions, such as hydrogen bonds and van der Waals forces. In this report, PVP-k30 presented the highest supersaturation ratio (given by dissolved concentration/equilibrium solubility), which was directly correlated with the efficiency of crystal growth inhibition. Furthermore, meloxicam nanocrystal stabilized with PVP-k30 showed a great particle size reduction and a increase of dissolution rate, as mentioned in Table 2, along with excellent pharmacokinetic results. The efficiency of crystal growth inhibition by PVP-k30 also reflected in meloxicam nanocrystal stability as expected, where presented minimal aggregation during 21 days of storage in comparison of other polymers evaluated. Thus, the precipitation by solvent shift is a promising approach to select an adequate polymer during drug nanocrystal preparation, providing mechanistic relationships about the key factors that are influencing the stabilization efficiency. However, this technique cannot be applied in electric stabilizers screening because the aggregation inhibition could overestimate the drug solubility (Ochi et al., 2014).

In addition to the traditional role of stabilizing drugs and improving process efficiency, the stabilizers can promote other benefits in preparing drug nanocrystals. Dexamethasone acetate nanocrystals associated with polymyxin B for ophthalmic delivery were produced using both cationic stabilizers, cetylpyridinium chloride and benzalkonium chloride (Romero et al., 2016). The particle size was 200 to 250 nm and was maintained after 6 months of storage at 5 °C, room temperature and 40 °C. The mucoadhesion itself promoted by the decrease of drug particle size was intensified with the use of a cationic system due to electrostatic interactions between stabilizer and mucosa. It shows that a drug nanocrystal formulation with an excellent stabilization system can be an alternative for ocular delivery, with increased performance than standard eye drop formulations.

As shown in Tables 2–4, in most of the studies we reviewed of anti-inflammatory drug nanocrystals, the authors used polymers or nonionic surfactants as stabilizers, followed by associated polymers + nonionic surfactants. The performance of nanocrystals prepared was variable, also depending on other factors, as we discussed in the previous section. The amount of drug and stabilizer can vary, mostly ranging from 0.1% to 1% w/w, but still does not follow a pattern. Although some studies have investigated several stabilizers to select the best scheme among them, only 38% have performed stability studies. This assay is essential to ensure that anti-inflammatory drug nanocrystals can maintain their physical and chemical properties, especially of drug nanocrystals intended for commercial marketing.

6. Physical and chemical properties and regulatory perspective

It is essential to understand CQAs to guarantee the benefits that drug nanocrystals can offer are present. By determining the physical and chemical properties, it is possible to ensure a successful preparation. Intended for commercialization, a full description of these characteristics such as identity and quality must be provided as any drug submitted to FDA, along with CQAs that are specific to nanomaterials, such as particle size distribution and physical stability, and attributes that potentially affect the efficacy, safety, or quality of the final drug product (FDA, 2017). The essential parameters are (i) chemical composition, (ii) average particle size, (iii) size distribution, (iv) shape and morphology, (v) stability, (vi) dissolution rate and saturation solubility (FDA, 2017; Müller et al., 2001). Ideally, it is recommended to perform at least two types of techniques for each parameter to validate the results obtained by more than one method. Few authors in the studies we reviewed performed all of these assays in anti-inflammatory drug nanocrystal preparation, as can be seen in Tables 2–4. The absence of these assays can be attributed to the high cost of performing these techniques.

The properties that anti-inflammatory drug nanocrystals offer are attributed to their dimensions (FDA, 2017), consequently drug particle size measurement is vital to guarantee if the size reduction has been successful. Average particle size (Tables 2–4) was determined by dynamic light scattering (DLS). Briefly, this technique provides the hydrodynamic diameter, which is determined based on the fluctuation in the intensity of scattered light (Bhattacharjee, 2016). As recommended by the FDA, some studies also performed a second method such as laser diffraction (LD). In this case, the particle size is measured from the scattered light and reported as the volume of an equivalent sphere (Xu, 2015). The volumetric median diameter is obtained based on the contribution of each particle in the volume distribution, which is often presented as a D-value (D10, D50, D90).

In addition, our findings revealed that scanning electron microscopy (SEM) was the most used technique for morphological evaluation of drug nanocrystal, followed by transmission electron microscopy (TEM) (Tables 2–4). Whereas SEM was designed to examine on surface morphology, TEM provides information on the internal structure of the nanocrystals (Inkson, 2016), and both techniques provide particle information in the submicron range (0.0012-5 μm), where drug particles are analyzed individually in high resolution (Peltonen and Strachan, 2015; Sangolkar et al., 2012).

Regarding solid-state material, thermal analysis is commonly used for drug nanocrystal characterization. Among the techniques, thermogravimetric (TG) and differential scanning calorimetry (DSC) are the most used. In TG analysis, the percentage of mass loss of the sample upon heating is related to temperature or time. This relationship allows obtaining information such as water loss and degradation kinetics (Coty et al., 2020). Besides, it is possible to identify when the events occur (onset temperature) and the degradation products. In this case, the analysis is coupled with equipment based on gas analysis, such as a mass spectrometer (Mansfield, 2015). In DSC, the sample's heat flow upon

heating is compared with a reference material, being related to the temperature's function. Hence, thermogram profile and thermodynamic parameters such as heat capacity and enthalpy provide information about the sample, such as amorphous/crystalline state and phase transition (Mansfield, 2015). Besides, it allows studying the compatibility of the drug with the excipients (Thakkar and Misra, 2020).

Another commonly used technique to characterize drug nanocrystals is X-ray powder diffraction (XRPD). It is based on the organization pattern between crystalline and amorphous materials. In the first, the molecules present long-range order at the atomic level, lacking the latter (Peltonen and Strachan, 2020). Hence, Bragg peaks emerge from crystalline materials when incited with X-ray radiation, allowing differentiating crystalline structures from amorphous materials (Guo et al., 2019a; Rahman et al., 2019). It is worth noting that the physicochemical characterization of nanocrystals may require previous special sample preparation. For instance, as drug nanocrystals are commonly prepared in the aqueous medium, drying techniques such as spray drying or lyophilization may be required to obtain the necessary amount of solid sample (Guo et al., 2019a; Koradia et al., 2018; Mansfield, 2015).

The crystalline structure is thermodynamically stable and prevents solid-state modification after storage (Surwase et al., 2013). The amorphous drug exhibits short-range molecular order against a three-dimensional long-range in crystalline compounds (Colombo et al., 2018). The crystal-packing energy is the necessary energy to disrupt the crystal lattice and remove the molecules from the structure during the solubilization (Jermain et al., 2018). It is the driving force of drug solubility, and an amorphous state minimizes this energy disrupting crystalline drug structure (Jermain et al., 2018). The amorphous drug has improved thermodynamic properties, resulting in the highest saturation solubility, and consequently, bioavailability than drug nanoparticles in the crystalline state (Junghanns and Müller, 2008; Sun et al., 2012).

Despite the benefits, amorphous form represents the most energetic solid state, which is stored like a compressing spring that will release its energy, and therefore, the compound into a supersaturated state (Jermain et al., 2018). The amorphous drug must resist its thermodynamic tendency to nucleation and crystal growth in the supersaturated state, critical during its storage (Gajera et al., 2019). Thus, the amorphous state is sometimes undesirable due to this issue on formulation stability. Solid-state materials rarely have a pure crystalline or amorphous nature. It means that drug nanocrystals may present some degree of long-range molecular disorganization, whereas amorphous materials may present short-range order at the atomic or molecular level. Since nanoparticles tend to aggregate easily, additional stability issues such as hygroscopicity and crystallization tendency should be considered for amorphous nanoparticles (Peltonen and Strachan, 2020).

Thus, Colombo and colleagues (Colombo et al., 2018) performed amorphous indomethacin nanosuspensions intending to obtain the aforementioned benefits associated with the nanotechnology to provide a synergistic effect in the saturation solubility. The amorphous state was promoted by quench cooling and showed no diffraction peaks by XRPD, indicating amorphization. After WBM, the XRPD diffractograms and further confirmed by FTIR showed that poloxamer 407 does not maintained the amorphous state, working only as a stabilizer. The amorphous indomethacin nanosuspension promoted saturation solubility 422% higher than amorphous powder, and this result was even 103% higher than indomethacin nanocrystals. Hence, the most significant disadvantage of an amorphous drug state was observed in the same report: the nanoparticles were stable for only 10 days, which means that the shelf life of the product remains a challenge to be overcome and improved.

Dissolution is another test required by FDA for new submissions containing drug nanoparticles. It is is not a specific CQA for nanomaterials, but it is necessary to validate the quality and chemical performance courses with the lifecycle of the drug (FDA, 2017). This assay depends on agitation or rotation speed, apparatus, the media, concentration of drug and stabilizer, and others. Additionally, according to the FDA (FDA, 2017), the drug release should achieve a plateau and at least 85% release in the labeled amount for the immediate release dosage form. Approximately 58% of our findings in anti-inflammatory drug nanocrystals performed a dissolution test with excellent results as shows Tables 2–4, while some studies have reached almost complete dissolution in less than 10 minutes (He et al., 2017; Iurian et al., 2017; Liu et al., 2015; Tao Liu et al., 2018; Malamatari et al., 2017; Song et al., 2018). Furthermore, most anti-inflammatory drugs from Table 1 presents an acidic pKa in physiological pH, which makes their dissolution profile strongly pH-dependent such as meloxicam (Ochi et al., 2014) and indomethacin (Liu et al., 2013) nanocrystals. Considering acid pKa values (Table 1), the aqueous solubility of drug substances tends to be higher in the intestine than in the stomach (Pobudkowska and Domańska, 2014). Besides, their lipophilicity at pH 7.4 tends to be lower than the neutral compounds. Both features are due to the ionization of these compounds as pH increases.

The FDA also warns in its guide for nanomaterials that drugs can interact with some compartments of the dissolution test. Also, to evaluate the pH influence, sample ratio, and paddle agitation speed, Liu and colleagues (Liu et al., 2013) showed that indomethacin nanocrystals could interact with the filter material during the dissolution study. Dissolution tests have a full application in quality control studies of anti-inflammatory drug nanocrystals, but these parameters must be well controlled so that dissolution profiles are successfully defined.

There are some points to consider relative to the improvement of dissolution rate and saturation solubility. A very rapid drug dissolution can result in high plasma peak and very early T_{max} (Junghanns and Müller, 2008). In this case, a modify drug delivery system has to be considered to overcome this problem. Budesonide nanocrystals were prepared by WBM and loaded into hyaluronic acid to enhance the mucoadhesivity in pulmonary delivery (Tingting Liu et al., 2018). During the *in vitro* evaluation, the loaded nanocrystal prolonged the retention time on the porcine tracheal mucosa than budesonide nanocrystal alone. In the pharmacokinetic evaluation, the budesonide nanocrystal absorption was so fast that it could not be possible to be observed in the experimental conditions, with C_{max} similar to the nanocrystal intravenous administered. On the one hand, it shows that the nanocrystal was capable of increasing the dissolution rate and the budesonide absorption, which is practically insoluble in water, as shown in Table 1. However, the rapid absorption decreases drug retention in the lungs, as was predicted in *in vitro* evaluation, reducing the therapeutic performance. Loaded budesonide nanocrystals into hyaluronic acid showed a gradual increase of absorption, with drug concentration declining slowly until 24 h. Thus, modify the drug delivery system is an alternative to avoid too fast absorption, which can be undesirable in some treatments using anti-inflammatory drugs.

Besides, it is crucial to evaluate the role of an increase in drug permeability during topical administration in the nanocrystal approach. For NSAIDs, such as diclofenac, ketoprofen, and indomethacin, the permeability enhancement is beneficial as it allows the drug easily reach deeper structures, such as muscles, joints, and bones, improving therapies of rheumatic diseases, sprain, and musculoskeletal pain. Also, the dermal delivery of anti-inflammatory drugs is an alternative to minimize gastrointestinal damages. However, the permeability increase can be a problem for GCs. Drug nanocrystals can reach systemic absorption and potentiate the several adverse effects of these drugs, as mentioned in Section 1, which is already possible to be seen in the conventional topical formulations of GCs (Dhar et al., 2014). Regarding drug nanocrystal penetration, similar results were reported in a comparative study between ethylcellulose nanocarriers and nanocrystals approaches for dexamethasone intended to dermal delivery (Döge et al., 2016). In this case, the nanocrystal formulation presented increased penetration instead of permeability. Likewise, a fast penetration might not maintain the retention required to nanocrystals produce the effect, resulting in higher application frequency and increasing the risk of systemic absorption of a GC. In this report, ethylcellulose nanocarriers of dexamethasone could prolong release kinetic, being a better option for

topical dexamethasone delivery in this case. Despite that, the use of mucoadhesivity enhancers for dexamethasone nanocrystals, such as hyaluronic acid and derivates, as we mentioned above, is an option to overcome this challenge, improving the safety of nanocrystal formulation.

Before the stability studies, it is possible to predict drug nanocrystals behavior during storage by PDI determination. It describes the uniformity degree, where a uniform nanoparticle distribution is when PDI is closer to 0.0 (Bera, 2015). A high PDI (> 0.7) indicates that the sample has multiple particle size distributions (Danaei et al., 2018), with the potential to increasing particle size due to crystal growth (Ostwald ripening). Hence, a narrow particle size could diminish the difference in saturation solubility between small and larger particles, which could inhibit crystal growth during storage (Wang et al., 2013; Wu et al., 2011). Stabilizer concentration and temperature may also influence crystal growth. If stabilizer concentration is high enough to increase drug solubility, it could potentialize Ostwald ripening; regarding temperature, it was already described that higher temperatures promoted crystal growth during stability study (Malamatari et al., 2018; Wu et al., 2011).

Similarly to PDI, zeta potential (ZP) is another parameter that affects stability. When charged drug nanoparticles are dispersed, an electric double layer (EDL) is created on its surface, and ZP is the interface between the EDL of moving particles and the dispersant layer around it during the application of an electric field (Bhattacharjee, 2016). This interface is called slipping potential, and then ZP is the slipping potential of a drug particle moving under an electric field (Wang et al., 2013). It affects stability since ZP indicates the degree of repulsion between the dispersion charges, where high values – positively or negatively charged – prevent aggregation due to the strong repellent forces among the nanocrystals (Das et al., 2012). This value depends on the type and amount of stabilizer to provide sufficient surface coverage repulsion among the particles. The electrostatic and steric forces may be simultaneously present in the nanosuspension, where the ZP absolute value of 20 mV is adequate to stabilize the formulation (Zhang et al., 2020). Our findings revealed a preference for non-ionic or polymeric stabilizers to develop anti-inflammatory drug nanocrystal, resulting in ZP values from -0.0725 to -35.8 mV (data not shown), which may indicate sterically stabilized preparations.

Indomethacin nanosuspension was prepared to evaluate the stabilization efficiency of poloxamers and poloxamines (Liu et al., 2015). The different values among the non-ionic (poloxamer 188, -24 mV) and the protonable stabilizers (poloxamine 908, -14 mV and poloxamine T1107, -16 mV) is attributed to the dense hydrated poly(ethylene oxide) (PEO) layer of the protonable ones that efficiently covers the surface of indomethacin nanosuspension. This characteristic can neutralize the negative drug charges, providing lower ZP compared to the non-ionic stabilizer. Moreover, these three stabilizers proved to be efficient in maintaining the physical stability of indomethacin nanosuspension, where drug particle size presented a minimal variation of 30 nm after storage of 1.5 years — independent of the evaluated temperature: 4 °C, 25 °C or 40 °C —. It shows that ZP is a useful method to predict the stability of drug nanocrystal, even though stability studies have paramount importance and it is irreplaceable to date.

Our findings showed that the stability tests were conducted on a short-time evaluation to obtain preliminary results about the quality of the drug product prepared on a laboratory scale, usually a few weeks to months at 25 °C (data not shown). The temperature values are generally based on the ICH Q1A (R2) guideline, which is established considering climatic conditions of the European Union, Japan, and the USA (temperate, subtropical, and Mediterranean climates – climatic zones I-II) (ICH Q1A (R2), 2003). However, drug nanosuspension intended to be commercialized in countries such as Brazil, India, or Egypt (hot dry or humid climate – climatic zones III-IV), when based in ICH Q1A (R2), may have its stability studies non-consistent due to their different value (30 °C) established for long-term testing conditions (WHO, 2018).

The nanosuspension accelerated stability studies (40 °C and 75% RH) are challenging. At high temperatures, the micellization will take place in surface adsorption instead of the stabilizer (Wang et al., 2013). Thus, the increase of kinetic energy promoted by elevated temperatures will turn the electrostatic repulsion among the particles easier to overcome, leading to aggregation (Wang et al., 2013). It shows that the stabilizer is strongly affected by the temperature increase. The indomethacin nanosuspension stability was evaluated under stress conditions (45 °C and thermal cycling) (Verma et al., 2011). Depending on the stabilizers type and their concentration, the drug presented an increase of particle size after three months of storage, with whose change accompanies the temperature increase (15 °C, 25 °C, 35 °C, and 45 °C). When using HPMC in a 1:1 drug:stabilizer ratio, an excellent stabilization was observed, possibly due to the excess of the stabilizer used to prepare this formulation. This ratio (1:1) was sufficient to surface coverage even at 45 °C and provided less growth of the average particle size (108%) than HPMC 10:1 (210%).

7. Final considerations

The nanocrystal approach is an excellent alternative to overcome the poor water-soluble problem present in many anti-inflammatory drugs. It is a feasible, safe, and easy scale-up technology, where it has a potential for bioavailability improvement, with the possibility of dose reduction and requiring fewer administrations throughout the day. In addition to other benefits, the increase of the dissolution rate of anti-inflammatory drug nanocrystal avoids a higher and prolonged concentration in the gastric compartment, reducing the local gastrointestinal damages. For other administration routes such as pulmonary and dermal delivery, anti-inflammatory drug nanocrystals can increase drug retention, promoting a higher local drug delivery, and avoiding systemic absorption. Furthermore, the nanocrystal formulation can be target towards inflammation sites, minimizing adverse effects, and also contributing to dose reduction, although it requires further studies to understand the mechanisms involved comprehensively. Understanding quality attributes are also vital while seeking FDA approval of a nanomaterial product, selecting an adequate stabilizing complex to maintain the supersaturated state, preparing anti-inflammatory drug nanocrystals with consistent particle size, morphology, and solid-state results, confirming its properties through dissolution and solubility tests, and evaluating all this development chain during stability tests. Hence, understanding the general mechanism involved in its preparation is possible to obtain anti-inflammatory drug nanocrystal with excellent permeation, absorption, and bioavailability, minimizing the adverse effects attributed to its mechanism of action.

Declaration of Competing Interest

None.

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References

Afifi, S.A., Hassan, M.A., Abdelhameed, A.S., Elkhodairy, K.A., 2015. Nanosuspension: An emerging trend for bioavailability enhancement of etodolac. Int. J. Polym. Sci 2015. https://doi.org/10.1155/2015/938594 https://doi.org/.

Assem, M., Khowessah, O.M., Ghorab, D., 2019. Nano-crystallization as a tool for the enhancement of beclomethasone dipropionate dermal deposition: Formulation, in vitro characterization and ex vivo study. J. Drug Deliv. Sci. Technol. 54, 101318 https://doi.org/10.1016/j.jddst.2019.101318 https://doi.org/.

Bansal, S., Bansal, M., Kumria, R., 2012. Nanocrystals: current strategies and trends. Int. J. Res. Pharm. Biomed. Sci. 3, 406–419.

Barlow, N.E., Bolognesi, G., Haylock, S., Flemming, A.J., Brooks, N.J., Barter, L.M.C., Ces, O., 2017. Rheological droplet interface bilayers (rheo-dibs): probing the unstirred water layer effect on membrane permeability via spinning disk induced shear stress. Sci. Rep 7. https://doi.org/10.1038/s41598-017-17883-0 https://doi.org/.

Bartos, C., Ambrus, R., Sipos, P., Budai-Szucs, M., Csányi, E., Gáspár, R., Márki, Á., Seres, A.B., Sztojkov-Ivanov, A., Horváth, T., Szabó-Révész, P., 2015. Study of sodium hyaluronate-based intranasal formulations containing micro- or nanosized meloxicam particles. Int. J. Pharm. 491, 198–207. https://doi.org/10.1016/j.ijpharm.2015.06.046 https://doi.org/.

Bera, B., 2015. Nanoporous silicon prepared by vapour phase strain etch and sacrificial technique. Int. J. Comput. Appl. Micro 42–45.

Bertrand, N., Leroux, J.C., 2012. The journey of a drug-carrier in the body: An anatomo-physiological perspective. J. Control. Release 161, 152–163. https://doi.org/10.1016/j.jconrel.2011.09.098 https://doi.org/.

Bhattacharjee, S., 2016. DLS and zeta potential – What they are and what they are not? J. Control. Release 235, 337–351. https://doi.org/10.1016/j.jconrel.2016.06.017 https://doi.org/.

Blanco, E., Shen, H., Ferrari, M., 2015. Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nat. Biotechnol. 33, 941–951. https://doi.org/10.1038/nbt.3330 https://doi.org/.

Brayfield, A., 2017. Martindale: The Complete Drug Reference, thirty nin. ed. Pharmaceutical Press, London.

Chang, T., Zhan, H., Liang, D., Liang, J.F., 2015. Nanocrystal technology for drug formulation and delivery. Chem. Sci. Eng. 9, 1–14. https://doi.org/10.1007/s11705-015-1509-3 https://doi.org/.

Chauhan, H., Hui-Gu, C., Atef, E., 2013. Correlating the behavior of polymers in solution as precipitation inhibitor to its amorphous stabilization ability in solid dispersions. J. Pharm. Sci. 102, 1924–1935. https://doi.org/10.1002/jps.23539 https://doi.org/.

ChEMBL, 2020. ChEMBL. https://www.ebi.ac.uk/chembl/ (accessed 12 November 2020).

Chen, M.L., John, M., Lee, S.L., Tyner, K.M., 2017. Development considerations for nanocrystal drug products. AAPS J 19, 642–651. https://doi.org/10.1208/s12248-017-0064-x https://doi.org/.

Chogale, M.M., Ghodake, V.N., Patravale, V.B., 2016. Performance parameters and characterizations of nanocrystals: A brief review. Pharmaceutics 8, 1–18. https://doi.org/10.3390/pharmaceutics8030026 https://doi.org/.

Chuang, S., Lin, C., Huang, T., Fang, J., 2018. Lipid-based nanoparticles as a potential delivery approach in the treatment of rheumatoid arthritis. Nanomaterials 8, 1–16. https://doi.org/10.3390/nano8010042 https://doi.org/.

Colombo, M., Minussi, C., Orthmann, S., Staufenbiel, S., Bodmeier, R., 2018. Preparation of amorphous indomethacin nanoparticles by aqueous wet bead milling and in situ measurement of their increased saturation solubility. Eur. J. Pharm. Biopharm. 125, 159–168. https://doi.org/10.1016/j.ejpb.2018.01.013 https://doi.org/.

Coty, J.B., Martin, C., Telò, I., Spitzer, D., 2020. Use of Spray Flash Evaporation (SFE) technology to improve dissolution of poorly soluble drugs: Case study on furosemide nanocrystals. Int. J. Pharm. 589, 119827 https://doi.org/10.1016/j.ijpharm.2020.119827 https://doi.org/.

Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S., Mozafari, M.R., 2018. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics 10, 1–17. https://doi.org/10.3390/pharmaceutics10020057 https://doi.org/.

Darville, N., Van Heerden, M., Mariën, D., De Meulder, M., Rossenu, S., Vermeulen, A., Vynckier, A., De Jonghe, S., Sterkens, P., Annaert, P., Van Den Mooter, G., 2016. The effect of macrophage and angiogenesis inhibition on the drug release and absorption from an intramuscular sustained-release paliperidone palmitate suspension. J. Control. Release 230, 95–108. https://doi.org/10.1016/j.jconrel.2016.03.041 https://doi.org/.

Darville, N., Van Heerden, M., Vynckier, A., De Meulder, M., Sterkens, P., Annaert, P., Van Den Mooter, G., 2014. Intramuscular administration of paliperidone palmitate extended-release injectable microsuspension induces a subclinical inflammatory reaction modulating the pharmacokinetics in rats. J. Pharm. Sci. 103, 2072–2087. https://doi.org/10.1002/jps.24014 https://doi.org/.

Das, S., Kiong, W., Tan, R.B.H., 2012. Are nanostructured lipid carriers (NLCs) better than solid lipid nanoparticles (SLNs): Development, characterizations and comparative evaluations of clotrimazole-loaded SLNs and NLCs? Eur. J. Pharm. Sci 47, 139–151. https://doi.org/10.1016/j.ejps.2012.05.010 https://doi.org/.

De Waard, H., Frijlink, H.W., Hinrichs, W.L.J., 2011. Bottom-up preparation techniques for nanocrystals of lipophilic drugs. Pharm. Res. 28, 1220–1223. https://doi.org/10.1007/s11095-010-0323-3 https://doi.org/.

Dhar, S., Seth, J., Parikh, D., 2014. Systemic side-effects of topical corticosteroids. Indian J. Dermatol. 59, 460–464. https://doi.org/10.4103/0019-5154.139874 https://doi.org/.

di Cagno, M.P., Stein, P.C., 2019. Studying the effect of solubilizing agents on drug diffusion through the unstirred water layer (UWL) by localized spectroscopy. Eur. J. Pharm. Biopharm. 139, 205–212. https://doi.org/10.1016/j.ejpb.2019.04.005 https://doi.org/.

Döge, N., Hönzke, S., Schumacher, F., Balzus, B., Colombo, M., Hadam, S., Rancan, F., Blume-Peytavi, U., Schäfer-Korting, M., Schindler, A., Rühl, E., Skov, P.S., Church, M.K., Hedtrich, S., Kleuser, B., Bodmeier, R., Vogt, A., 2016. Ethyl cellulose nanocarriers and nanocrystals differentially deliver dexamethasone into intact, tape-stripped or sodium lauryl sulfate-exposed ex vivo human skin - assessment by intradermal microdialysis and extraction from the different skin layers. J. Control. Release 242, 25–34. https://doi.org/10.1016/j.jconrel.2016.07.009 https://doi.org/.

Dolati, S., Sadreddini, S., Rostamzadeh, D., Ahmadi, M., Jadidi-Niaragh, F., Yousefi, M., 2016. Utilization of nanoparticle technology in rheumatoid arthritis treatment. Biomed. Pharmacother. 80, 30–41. https://doi.org/10.1016/j.biopha.2016.03.004 https://doi.org/.

Drug Delivery Foundation, 2020. Drug Delivery Foundation: Provisional BCS Classification. http://www.ddfint.net/search.cfm (accessed 27 March 2020).

Drugbank, 2020. Drugbank. https://www.drugbank.ca/ (accessed 15 December 2019).

Fernandes, A.R., Ferreira, N.R., Fangueiro, J.F., Santos, A.C., Veiga, F.J., Cabral, C., Silva, A.M., Souto, E.B., 2017. Ibuprofen nanocrystals developed by 2^2 factorial design experiment: A new approach for poorly water-soluble drugs. Saudi Pharm. J. 25, 1117–1124. https://doi.org/10.1016/j.jsps.2017.07.004 https://doi.org/.

Fontana, F., Figueiredo, P., Zhang, P., Hirvonen, J.T., Liu, D., Santos, H.A., 2018. Production of pure drug nanocrystals and nano co-crystals by confinement methods. Adv. Drug Deliv. Rev. 131, 3–21. https://doi.org/10.1016/j.addr.2018.05.002 https://doi.org/.

Food and Drug Administration, 2017. Drug Products, Including Biological Products, that Contain Nanomaterials Guidance for Industry. https://www.fda.gov/files/drugs/published/Drug-Products–Including-Biological-Products–that-Contain-Nanomaterials-Guidance-for-Industry.pdf (accessed 05 January 2020).

Fu, T.T., Cong, Z.Q., Zhao, Y., Chen, W.Y., Liu, C.Y., Zheng, Y., Yang, F.F., Liao, Y.H., 2019. Fluticasone propionate nanosuspensions for sustained nebulization delivery: An in vitro and in vivo evaluation. Int. J. Pharm. 572, 118839 https://doi.org/10.1016/j.ijpharm.2019.118839 https://doi.org/.

Fuhrmann, K., Gauthier, M.A., Leroux, J.C., 2014. Targeting of injectable drug nanocrystals. Mol. Pharm. 11, 1762–1771. https://doi.org/10.1021/mp5001247 https://doi.org/.

Gajera, B.Y., Shah, D.A., Dave, R.H., 2019. Development of an amorphous nanosuspension by sonoprecipitation-formulation and process optimization using design of experiment methodology. Int. J. Pharm. 559, 348–359. https://doi.org/10.1016/j.ijpharm.2019.01.054 https://doi.org/.

Gao, L., Liu, G., Ma, J., Wang, X., Zhou, L., Li, X., 2012. Drug nanocrystals: In vivo performances. J. Control. Release 160, 418–430. https://doi.org/10.1016/j.jconrel.2012.03.013 https://doi.org/.

Ghosh, I., Bose, S., Vippagunta, R., Harmon, F., 2011. Nanosuspension for improving the bioavailability of a poorly soluble drug and screening of stabilizing agents to inhibit crystal growth. Int. J. Pharm. 409, 260–268. https://doi.org/10.1016/j.ijpharm.2011.02.051 https://doi.org/.

Gülsün, T., Budak, C., Vural, I., Sahin, S., Öner, L., 2013. Preparation and characterization of nimesulide containing nanocrystal formulations. Pharm. Dev. Technol. 18, 653–659. https://doi.org/10.3109/10837450.2012.663390 https://doi.org/.

Gülsün, T., Gürsoy, R.N., Öner, L., 2009. Nanocrystal technology for oral delivery of poorly water-soluble drugs. Fabad J. Pharm. Sci. 34, 55–65.

Guo, M., Dong, Y., Wang, Y., Ma, M., He, Z., Fu, Q., 2019a. Fabrication, characterization, stability and in vitro evaluation of nitrendipine nanocrystals by media milling. Powder Technol 358, 20–28. https://doi.org/10.1016/j.powtec.2018.08.018 https://doi.org/.

Guo, Mengran, Wei, M., Li, W., Guo, Meichen, Guo, C., Ma, M., Wang, Y., Yang, Z., Li, M., Fu, Q., Yang, L., He, Z., 2019b. Impacts of particle shapes on the oral delivery of drug nanocrystals: Mucus permeation, transepithelial transport and bioavailability. J. Control. Release 307, 64–75. https://doi.org/10.1016/j.jconrel.2019.06.015 https://doi.org/.

Gurpinar, E., Grizzle, W.E., Piazza, G.A., 2014. NSAIDs inhibit tumorigenesis, but how? Clin. Cancer Res 20, 1104–1113. https://doi.org/10.1158/1078-0432.CCR-13-1573 https://doi.org/.

Gustafson, H.H., Holt-Casper, D., Grainger, D.W., Ghandehari, H., 2015. Nanoparticle uptake: The phagocyte problem. Nano Today 10, 487–510. https://doi.org/10.1016/j.nantod.2015.06.006 https://doi.org/.

Hall, J.E., 2017. Guyton and Hall Textbook of Medical Physiology, thirteenth. ed. Elsevier Inc., Philadelphia.

Harvard Health Publishing, 2018. Are you taking too much anti-inflammatory medication? Harvard Med. Sch. https://www.health.harvard.edu/blog/are-you-taking-too-much-anti-inflammatory-medication-2018040213540 (accessed 25 March 2020).

He, J., Han, Y., Xu, G., Yin, L., Ngandeu Neubi, M., Zhou, J., Ding, Y., 2017. Preparation and evaluation of celecoxib nanosuspensions for bioavailability enhancement. RSC Adv 7, 13053–13064. https://doi.org/10.1039/c6ra28676c https://doi.org/.

ICH Q1A (R2), 2003. Q1A(R2) Stability Testing of New Drug Substances and Products https://www.fda.gov/media/71707/download (accessed 29 October 2020).

ICH Q8(R2), 2009. ICH Harmonised Tripartite Guideline, Pharmaceutical Development Q8(R2) https://database.ich.org/sites/default/files/Q8_R2_Guideline.pdf (accessed 05 March 2020).

Imono, M., Uchiyama, H., Yoshida, S., Miyazaki, S., Tamura, N., Tsutsumimoto, H., Kadota, K., Tozuka, Y., 2020. The elucidation of key factors for oral absorption enhancement of nanocrystal formulations: In vitro - in vivo correlation of nanocrystals. Eur. J. Pharm. Biopharm. 146, 84–92. https://doi.org/10.1016/j.ejpb.2019.12.002 https://doi.org/.

Ingawale, D.K., Mandlik, S.K., Patel, S.S., 2015. An emphasis on molecular mechanisms of anti-inflammatory effects and glucocorticoid resistance. J. Complement. Integr. Med. 12, 1–13. https://doi.org/10.1515/jcim-2014-0051 https://doi.org/.

Inkson, B.J., 2016. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) for materials characterization. In: Hübschen, G., Altpeter, I., Tschuncky, R., Herrmann, H.-G. (Eds.), Materials Characterization Using Nondestructive Evaluation (NDE) Methods. Woodhead Publishing, pp. 17–43. https://doi.org/10.1016/B978-0-08-100040-3.00002-X https://doi.org/.

Iurian, S., Bogdan, C., Tomuță, I., Szabó-Révész, P., Chvatal, A., Leucuța, S.E., Moldovan, M., Ambrus, R., 2017. Development of oral lyophilisates containing meloxicam nanocrystals using QbD approach. Eur. J. Pharm. Sci. 104, 356–365. https://doi.org/10.1016/j.ejps.2017.04.011 https://doi.org/.

Jarvis, M., Krishnan, V., Mitragotri, S., 2019. Nanocrystals: A perspective on translational research and clinical studies. Bioeng. Transl. Med. 4, 5–16. https://doi.org/10.1002/btm2.10122 https://doi.org/.

Jermain, S.V., Brough, C., Williams, R.O., 2018. Amorphous solid dispersions and nanocrystal technologies for poorly water-soluble drug delivery – An update. Int. J. Pharm. 535, 379–392. https://doi.org/10.1016/j.ijpharm.2017.10.051 https://doi.org/.

Jinno, J.I., Kamada, N., Miyake, M., Yamada, K., Mukai, T., Odomi, M., Toguchi, H., Liversidge, G.G., Higaki, K., Kimura, T., 2006. Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs. J. Control. Release 111, 56–64. https://doi.org/10.1016/j.jconrel.2005.11.013 https://doi.org/.

Jokerst, J.V, Lobovkina, T., Zare, R.N., Gambhir, S.S., 2011. Nanoparticle PEGylation for imaging and therapy. Nanomedicine 6, 715–728. https://doi.org/10.2217/nnm.11.19 https://doi.org/.

Joseph, E., Singhvi, G., 2019. Multifunctional nanocrystals for cancer therapy: a potential nanocarrier. In: Grumezescu, A.M. (Ed.), Nanomaterials for Drug Delivery and Therapy. Elsevier Inc, pp. 91–116. https://doi.org/10.1016/b978-0-12-816505-8.00007-2 https://doi.org/.

Junghanns, J.U.A.H., Müller, R.H., 2008. Nanocrystal technology, drug delivery and clinical applications. Int. J. Nanomedicine 3, 295–309. https://doi.org/10.2147/ijn.s595 https://doi.org/.

Junyaprasert, V.B., Morakul, B., 2015. Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs. Asian J. Pharm. Sci. 10, 13–23. https://doi.org/10.1016/j.ajps.2014.08.005 https://doi.org/.

Kadiu, I., Nowacek, A., Mcmillan, J., Gendelman, H.E., 2011. Macrophage endocytic trafficking of antiretroviral nanoparticles. Nanomedicine 6, 975–994. https://doi.org/10.2217/nnm.11.27 https://doi.org/.

Koradia, K.D., Sheth, N.R., Koradia, H.D., Dabhi, M.R., 2018. Ziprasidone nanocrystals by wet media milling followed by spray drying and lyophilization: Formulation and process parameter optimization. J. Drug Deliv. Sci. Technol. 43, 73–84. https://doi.org/10.1016/j.jddst.2017.09.011 https://doi.org/.

Korjamo, T., Heikkinen, A.T., Mönkkönen, J., 2009. Analysis of unstirred water layer in in vitro permeability experiments. J. Pharm. Sci. 98, 4469–4479. https://doi.org/10.1002/jps.21762 https://doi.org/.

Kraft, W.K., Steiger, B., Beussink, D., Quiring, J., Fitzgerald, N., Greenberg, H.E., Waldman, S.A., 2004. The pharmacokinetics of nebulized nanocrystal budesonide suspension in healthy volunteers. J. Clin. Pharmacol. 44, 67–72. https://doi.org/10.1177/0091270003261490 https://doi.org/.

Kumar, R., Siril, P.F., Javid, F., 2016. Unusual anti-leukemia activity of nanoformulated naproxen and other non-steroidal anti-inflammatory drugs. Mater. Sci. Eng. C 69, 1335–1344. https://doi.org/10.1016/j.msec.2016.08.024 https://doi.org/.

Kumar, S., Burgess, D.J., 2014. Wet milling induced physical and chemical instabilities of naproxen nano-crystalline suspensions. Int. J. Pharm. 466, 223–232. https://doi.org/10.1016/j.ijpharm.2014.03.021 https://doi.org/.

Kuroiwa, Y., Higashi, K., Ueda, K., Yamamoto, K., Moribe, K., 2018. Nano-scale and molecular-level understanding of wet-milled indomethacin/poloxamer 407 nanosuspension with TEM, suspended-state NMR, and Raman measurements. Int. J. Pharm. 537, 30–39. https://doi.org/10.1016/j.ijpharm.2017.12.028 https://doi.org/.

Lai, F., Pini, E., Corrias, F., Perricci, J., Manconi, M., Fadda, A.M., Sinico, C., 2014. Formulation strategy and evaluation of nanocrystal piroxicam orally disintegrating tablets manufacturing by freeze-drying. Int. J. Pharm. 467, 27–33. https://doi.org/10.1016/j.ijpharm.2014.03.047 https://doi.org/.

Liu, F., Park, J., Zhang, Y., Conwell, C., Liu, Y., 2010. Targeted cancer therapy with novel high drug-loading nanocrystals. J. Pharm. Sci. 99, 3542–3551. https://doi.org/10.1002/jps.22112 https://doi.org/.

Liu, J., Tu, L., Cheng, M., Feng, J., Jin, Y., 2020. Mechanisms for oral absorption enhancement of drugs by nanocrystals. J. Drug Deliv. Sci. Technol. 56 https://doi.org/10.1016/j.jddst.2020.101607 https://doi.org/.

Liu, P., De Wulf, O., Laru, J., Heikkilä, T., Van Veen, B., Kiesvaara, J., Hirvonen, J., Peltonen, L., Laaksonen, T., 2013. Dissolution studies of poorly soluble drug nanosuspensions in non-sink conditions. AAPS PharmSciTech 14, 748–756. https://doi.org/10.1208/s12249-013-9960-2 https://doi.org/.

Liu, P., Viitala, T., Kartal-Hodzic, A., Liang, H., Laaksonen, T., Hirvonen, J., Peltonen, L., 2015. Interaction studies between indomethacin nanocrystals and PEO/PPO copolymer stabilizers. Pharm. Res 32, 628–639. https://doi.org/10.1007/s11095-014-1491-3 https://doi.org/.

Liu, Tingting, Han, M., Tian, F., Cun, D., Rantanen, J., Yang, M., 2018. Budesonide nanocrystal-loaded hyaluronic acid microparticles for inhalation: In vitro and in vivo evaluation. Carbohydr. Polym. 181, 1143–1152. https://doi.org/10.1016/j.carbpol.2017.11.018 https://doi.org/.

Liu, Tao, Yao, G., Zhang, X., Zuo, X., Wang, L., Yin, H., Möschwitzer, J.P., 2018. Systematical investigation of different drug nanocrystal technologies to produce fast dissolving meloxicam tablets. AAPS PharmSciTech 19, 783–791. https://doi.org/10.1208/s12249-017-0889-8 https://doi.org/.

Liversidge, G.G., Conzentino, P., 1995. Drug particle size reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats. Int. J. Pharm. 125, 309–313. https://doi.org/10.1016/0378-5173 https://doi.org/.

Liversidge, G.G., Cundy, K.C., Bishop, J.F., Czekai, D.A., 1992. Surface modified drug nanoparticles. 5, 145,684.

Lu, Y., Chen, Y., Gemeinhart, R.A., Wu, W., Li, T., 2015. Developing nanocrystals for cancer treatment. Nanomedicine 10, 2537–2552. https://doi.org/10.2217/nnm.15.73 https://doi.org/.

Lu, Y., Qi, J., Dong, X., Zhao, W., Wu, W., 2017. The in vivo fate of nanocrystals. Drug Discov. Today 22, 744–750. https://doi.org/10.1016/j.drudis.2017.01.003 https://doi.org/.

Ma, J., Yang, Y., Sun, Y., Sun, J., 2017. Optimization, characterization and in vitro/vivo evaluation of azilsartan nanocrystals. Asian J. Pharm. Sci. 12, 344–352. https://doi.org/10.1016/j.ajps.2016.09.008 https://doi.org/.

Mahesh, K.V., Singh, S.K., Gulati, M., 2014. A comparative study of top-down and bottom-up approaches for the preparation of nanosuspensions of glipizide. Powder Technol 256, 436–449. https://doi.org/10.1016/j.powtec.2014.02.011 https://doi.org/.

Malamatari, M., Somavarapu, S., Kachrimanis, K., Buckton, G., Taylor, K.M.G., 2017. Preparation of respirable nanoparticle agglomerates of the low melting and ductile drug ibuprofen: Impact of formulation parameters. Powder Technol 308, 123–134. https://doi.org/10.1016/j.powtec.2016.12.007 https://doi.org/.

Malamatari, M., Taylor, K.M.G., Malamataris, S., Douroumis, D., Kachrimanis, K., 2018. Pharmaceutical nanocrystals: production by wet milling and applications. Drug Discov. Today 23, 534–547. https://doi.org/10.1016/j.drudis.2018.01.016 https://doi.org/.

Mansfield, E., 2015. Recent advances in thermal analysis of nanoparticles: methods, models and kinetics. In: Tewary, V., Zhang, Y. (Eds.), Modeling, Characterization and Production of Nanomaterials. Woodhead Publishing, Cambridge, pp. 167–178.

Medscape, 2017. Nonsteroidal Anti-inflammatory Drug (NSAID) Toxicity. https://emedicine.medscape.com/article/816117-overview (accessed 22 March 2020).

Mendes, R.T., Stanczyk, C.P., Sordi, R., Otuki, M.F., dos Santos, F.A., Fernandes, D., 2012. Selective inhibition of cyclooxygenase-2: risks and benefits. Rev. Bras. Reumatol. 52, 767–782. https://doi.org/10.1590/S0482-50042012000500011 https://doi.org/.

Merisko-Liversidge, E., 2015. Nanosizing : “ end-to-end ” formulation strategy for poorly water-soluble molecules. In: Templeton, A., Byrn, S., Haskell, R. (Eds.), Discovery and Developing Molecules with Optimal Drug-Like Properties. Springer, pp. 437–467. https://doi.org/10.1007/978-1-4939-1399-2 https://doi.org/.

Merisko-Liversidge, E., Liversidge, G.G., Cooper, E.R., 2003. Nanosizing: A formulation approach for poorly-water-soluble compounds. Eur. J. Pharm. Sci. 18, 113–120. https://doi.org/10.1016/S0928-0987(02)00251-8 https://doi.org/.

Miao, X., Yang, W., Feng, T., Lin, J., Huang, P., 2018. Drug nanocrystals for cancer therapy. Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology 10, 1–22. https://doi.org/10.1002/wnan.1499 https://doi.org/.

Mirza, R.M., Ahirrao, S.P., Kshirsagar, S.J., 2017. A nanocrystal technology: to enhance solubility of poorly water soluble drugs. J. Appl. Pharm. Res. 5, 1–13.

Mohammad, I.S., Hu, H., Yin, L., He, W., 2019. Drug nanocrystals: Fabrication methods and promising therapeutic applications. Int. J. Pharm. 562, 187–202. https://doi.org/10.1016/j.ijpharm.2019.02.045 https://doi.org/.

Möschwitzer, J.P., 2013. Drug nanocrystals in the commercial pharmaceutical development process. Int. J. Pharm. 453, 142–156. https://doi.org/10.1016/j.ijpharm.2012.09.034 https://doi.org/.

Möschwitzer, J.P., Müller, R.H., 2013. Factors influencing the release kinetics of drug nanocrystal-loaded pellet formulations. Drug Dev. Ind. Pharm. 39, 762–769. https://doi.org/10.3109/03639045.2012.702347 https://doi.org/.

Mou, D., Chen, H., Wan, J., Xu, H., Yang, X., 2011. Potent dried drug nanosuspensions for oral bioavailability enhancement of poorly soluble drugs with pH-dependent solubility. Int. J. Pharm. 413, 237–244. https://doi.org/10.1016/j.ijpharm.2011.04.034 https://doi.org/.

Müller, R.H., Gohla, S., Keck, C.M., 2011. State of the art of nanocrystals - Special features, production, nanotoxicology aspects and intracellular delivery. Eur. J. Pharm. Biopharm. 78, 1–9. https://doi.org/10.1016/j.ejpb.2011.01.007 https://doi.org/.

Müller, R.H., Jacobs, C., Kayser, O., 2001. Nanosuspensions as particulate drug formulations in therapy: Rationale for development and what we can expect for the future. Adv. Drug Deliv. Rev. 47, 3–19. https://doi.org/10.1016/S0169-409X(00)00118-6 https://doi.org/.

Nagai, N., Ogata, F., Otake, H., Kawasaki, N., 2020. Oral administration system based on meloxicam nanocrystals: decreased dose due to high bioavailability attenuates risk of gastrointestinal side effects. Pharmaceutics 12, 1–15. https://doi.org/10.3390/pharmaceutics12040313 https://doi.org/.

Narayan, R., Pednekar, A., Bhuyan, D., Gowda, C., Koteshwara, K.B., Nayak, U.Y., 2017. A top-down technique to improve the solubility and bioavailability of aceclofenac: In vitro and in vivo studies. Int. J. Nanomedicine 12, 4921–4935. https://doi.org/10.2147/IJN.S141504 https://doi.org/.

Netea, M.G., Balkwill, F., Chonchol, M., Cominelli, F., Donath, M.Y., Giamarellos-Bourboulis, E.J., Golenbock, D., Gresnigt, M.S., Heneka, M.T., Hoffman, H.M., Hotchkiss, R., Joosten, L.A.B., Kastner, D.L., Korte, M., Latz, E., Libby, P., Mandrup-Poulsen, T., Mantovani, A., Mills, K.H.G., Nowak, K.L., O'Neill, L.A., Pickkers, P., Van Der Poll, T., Ridker, P.M., Schalkwijk, J., Schwartz, D.A., Siegmund, B., Steer, C. J., Tilg, H., Van Der Meer, J.W.M., Van De Veerdonk, F.L., Dinarello, C.A., 2017. A guiding map for inflammation. Nat. Immunol. 18, 826–831. https://doi.org/10.1038/ni.3790 https://doi.org/.

Noyes, A.A., Whitney, W.R., 1897. The rate of solution of solid substances in their own solutions. J. Am. Chem. Soc. 19, 930–934. https://doi.org/10.1021/ja02086a003 https://doi.org/.

Ochi, M., Kawachi, T., Toita, E., Hashimoto, I., Yuminoki, K., Onoue, S., Hashimoto, N., 2014. Development of nanocrystal formulation of meloxicam with improved dissolution and pharmacokinetic behaviors. Int. J. Pharm. 474, 151–156. https://doi.org/10.1016/j.ijpharm.2014.08.022 https://doi.org/.

Oktay, A.N., Karakucuk, A., Ilbasmis-Tamer, S., Celebi, N., 2018. Dermal flurbiprofen nanosuspensions: Optimization with design of experiment approach and in vitro evaluation. Eur. J. Pharm. Sci. 122, 254–263. https://doi.org/10.1016/j.ejps.2018.07.009 https://doi.org/.

Paredes, A.J., Camacho, N.M., Schofs, L., Dib, A., Zarazaga, P., Litterio, N., Allemandi, D. A., Sánchez, S., Lanusse, C., Palma, S.D., 2020. Ricobendazole nanocrystals obtained by media milling and spray drying: Pharmacokinetic comparison with the micronized form of the drug. Int. J. Pharm. 585, 119501 https://doi.org/10.1016/j.ijpharm.2020.119501 https://doi.org/.

Park, J.J., Meghani, N., Choi, J.S., Lee, B.J., 2016. Development and evaluation of decorated aceclofenac nanocrystals. Colloids Surfaces B Biointerfaces 143, 206–212. https://doi.org/10.1016/j.colsurfb.2016.03.022 https://doi.org/.

Peltonen, L., Hirvonen, J., 2018. Drug nanocrystals – Versatile option for formulation of poorly soluble materials. Int. J. Pharm. 537, 73–83. https://doi.org/10.1016/j.ijpharm.2017.12.005 https://doi.org/.

Peltonen, L., Strachan, C., 2015. Understanding critical quality attributes for nanocrystals from preparation to delivery. Molecules 20, 22286–22300. https://doi.org/10.3390/molecules201219851.

Peltonen, L., Strachan, C.J., 2020. Degrees of order: A comparison of nanocrystal and amorphous solids for poorly soluble drugs. Int. J. Pharm. 586, 119492 https://doi.org/10.1016/j.ijpharm.2020.119492 https://doi.org/.

Peters, M.C.C., Santos Neto, E., Monteiro, L.M., Yukuyama, M.N., Machado, M.G.M., de Oliveira, I.F., Zanin, M.H.A., Löbenberg, R., Bou-Chacra, N., 2020. Advances in ophthalmic preparation: the role of drug nanocrystals and lipid-based nanosystems. J. Drug Target. 28, 259–270. https://doi.org/10.1080/1061186X.2019.1663858 https://doi.org/.

Pireddu, R., Sinico, C., Ennas, G., Marongiu, F., Muzzalupo, R., Lai, F., Fadda, A.M., 2015. Novel nanosized formulations of two diclofenac acid polymorphs to improve topical bioavailability. Eur. J. Pharm. Sci. 77, 208–215. https://doi.org/10.1016/j.ejps.2015.06.006 https://doi.org/.

Pobudkowska, A., Domańska, U., 2014. Study of pH-dependent drugs solubility in water. Chem. Ind. Chem. Eng. Q. 20, 115–126. https://doi.org/10.2298/CICEQ120531116P https://doi.org/.

QY Research, 2018. Global Steroids Market: Corticosteroids Segment to Reach Value of US$ 8.6 Bn by 2025 end. https://www.prnewswire.com/news-releases/global-steroids-market-corticosteroids-segment-to-reach-value-of-us-8-6-bn-by-2025-end-qy-research-inc–853616656.html (accessed 19 August 2020).

Rahman, M., Arevalo, F., Coelho, A., Bilgili, E., 2019. Hybrid nanocrystal–amorphous solid dispersions (HyNASDs) as alternative to ASDs for enhanced release of BCS Class II drugs. Eur. J. Pharm. Biopharm. 145, 12–26. https://doi.org/10.1016/j.ejpb.2019.10.002 https://doi.org/.

Rang, H.P., Ritter, J.M., Flower, R.J., Henderson, G., 2016. Rang & Dale's Pharmacology, eighth. ed. Churchill Linvingstone, London.

Research and Markets, 2020. Nanotechnology in Drug Delivery - Global Market Trajectory & Analytics. http://www.researchandmarkets.com/reports/2769238/nanotechnology_in_drug_delivery_global_market (accessed 19 August 2020).

Romero, G.B., Keck, C.M., Müller, R.H., Bou-Chacra, N.A., 2016. Development of cationic nanocrystals for ocular delivery. Eur. J. Pharm. Biopharm. 107, 215–222. https://doi.org/10.1016/j.ejpb.2016.07.005 https://doi.org/.

Roos, C., Dahlgren, D., Sjögren, E., Sjöblom, M., Hedeland, M., Lennernäs, H., 2018. Jejunal absorption of aprepitant from nanosuspensions: role of particle size, prandial state and mucus layer. Eur. J. Pharm. Biopharm. 132, 222–230. https://doi.org/10.1016/j.ejpb.2018.09.022 https://doi.org/.

Saini, J.K., Kumar, S., 2018. Development of nanocrystal formulation with improved dissolution. J. Drug Deliv. Ther. 8, 118–129. https://doi.org/10.22270/jddt.v8i5.1946 https://doi.org/.

Salazar, J., Ghanem, A., Müller, R.H., Möschwitzer, J.P., 2012. Nanocrystals: Comparison of the size reduction effectiveness of a novel combinative method with conventional top-down approaches. Eur. J. Pharm. Biopharm. 81, 82–90. https://doi.org/10.1016/j.ejpb.2011.12.015 https://doi.org/.

Samuelsson, E., Shen, H., Blanco, E., Ferrari, M., Wolfram, J., 2017. Contribution of Kupffer cells to liposome accumulation in the liver. Colloids Surfaces B Biointerfaces 158, 356–362. https://doi.org/10.1016/j.colsurfb.2017.07.014 https://doi.org/.

Sangolkar S., S., Adhao S., V., Mundhe G., D., Sawarkar S., H., 2012. Particle size determination of nasal drug delivery system: a review. Int. J. Pharm. Sci. Rev. Res. 17, 66–73.

Sattar, A., Chen, D., Jiang, L., Pan, Y., Tao, Y., Huang, L., Liu, Z., Xie, S., Yuan, Z., 2017. Preparation, characterization and pharmacokinetics of cyadox nanosuspension. Sci. Rep. 7, 1–9. https://doi.org/10.1038/s41598-017-02523-4 https://doi.org/.

Savjani, K.T., Gajjar, A.K., Savjani, J.K., 2012. Drug solubility: importance and enhancement techniques. ISRN Pharm. 2012, 1–10. https://doi.org/10.5402/2012/195727 https://doi.org/.

Scheiman, J.M., 2016. NSAID-induced gastrointestinal injury. J. Clin. Gastroenterol 50, 5–10. https://doi.org/10.1097/mcg.0000000000000432 https://doi.org/.

Sharma, M., Mehta, I., 2019. Surface stabilized atorvastatin nanocrystals with improved bioavailability, safety and antihyperlipidemic potential. Sci. Rep. 9, 1–11. https://doi.org/10.1038/s41598-019-52645-0 https://doi.org/.

Sharma, O.P., Patel, V., Mehta, T., 2016. Nanocrystal for ocular drug delivery: hope or hype. Drug Deliv. Transl. Res. 6, 399–413. https://doi.org/10.1007/s13346-016-0292-0 https://doi.org/.

Shegokar, R., Müller, R.H., 2010. Nanocrystals: Industrially feasible multifunctional formulation technology for poorly soluble actives. Int. J. Pharm. 399, 129–139. https://doi.org/10.1016/j.ijpharm.2010.07.044 https://doi.org/.

Shrimal, P., Jadeja, G., Patel, S., 2020. Chemical engineering research and design a review on novel methodologies for drug nanoparticle preparation: microfluidic approach. Chem. Eng. Res. Des. 153, 728–756. https://doi.org/10.1016/j.cherd.2019.11.031 https://doi.org/.

Sinha, B., Müller, R.H., Möschwitzer, J.P., 2013. Bottom-up approaches for preparing drug nanocrystals: Formulations and factors affecting particle size. Int. J. Pharm. 453, 126–141. https://doi.org/10.1016/j.ijpharm.2013.01.019 https://doi.org/.

Skrdla, P.J., Yang, H., 2019. On the stability of nano-formulations prepared by direct synthesis: simulated Ostwald Ripening of a typical nanocrystal distribution post-nucleation. AAPS PharmSciTech 20, 1–6. https://doi.org/10.1208/s12249-018-1257-z https://doi.org/.

Song, Q., Shen, C., Shen, B., Lian, W., Liu, X., Dai, B., Yuan, H., 2018. Development of a fast dissolving sublingual film containing meloxicam nanocrystals for enhanced dissolution and earlier absorption. J. Drug Deliv. Sci. Technol. 43, 243–252. https://doi.org/10.1016/j.jddst.2017.10.020 https://doi.org/.

Su, B., O'Connor, J.P., 2013. NSAID therapy effects on healing of bone, tendon, and the enthesis. J. Appl. Physiol. 115, 892–899. https://doi.org/10.1152/japplphysiol.00053.2013 https://doi.org/.

Sun, Y., Zhu, L., Wu, T., Cai, T., Gunn, E.M., Yu, L., 2012. Stability of amorphous pharmaceutical solids: Crystal growth mechanisms and effect of polymer additives. AAPS J 14, 380–388. https://doi.org/10.1208/s12248-012-9345-6 https://doi.org/.

Surwase, S.A., Boetker, J.P., Saville, D., Boyd, B.J., Gordon, K.C., Peltonen, L., Strachan, C.J., 2013. Indomethacin: new polymorphs of an old drug. Mol. Pharm. 10, 4472–4480. https://doi.org/10.1021/mp400299a https://doi.org/.

Takeuchi, K., 2012. Pathogenesis of NSAID-induced gastric damage: Importance of cyclooxygenase inhibition and gastric hypermotility. World J. Gastroenterol. 18, 2147–2160. https://doi.org/10.3748/wjg.v18.i18.2147 https://doi.org/.

Takeuchi, O., Akira, S., 2010. Pattern recognition receptors and inflammation. Cell 140, 805–820. https://doi.org/10.1016/j.cell.2010.01.022 https://doi.org/.

Thakkar, S., Misra, M., 2020. Electrospray drying of docetaxel nanosuspension: A study on particle formation and evaluation of nanocrystals thereof. J. Drug Deliv. Sci. Technol. 60, 102009 https://doi.org/10.1016/j.jddst.2020.102009 https://doi.org/.

Tuomela, A., Hirvonen, J., Peltonen, L., 2016a. Stabilizing agents for drug nanocrystals: effect on bioavailability. Pharmaceutics 8, 1–18. https://doi.org/10.3390/pharmaceutics8020016 https://doi.org/.

Tuomela, A., Saarinen, J., Strachan, C.J., Hirvonen, J., Peltonen, L., 2016b. Production, applications and in vivo fate of drug nanocrystals. J. Drug Deliv. Sci. Technol. 34, 21–31. https://doi.org/10.1016/j.jddst.2016.02.006 https://doi.org/.

Udalova, I.A., Mantovani, A., Feldmann, M., 2016. Macrophage heterogeneity in the context of rheumatoid arthritis. Nat. Rev. Rheumatol. 12, 472–485. https://doi.org/10.1038/nrrheum.2016.91 https://doi.org/.

Ullah, N., Khan, S., Ahmed, S., Govender, T., Faidah, H.S., De Matas, M., Shahid, M., Minhas, M.U., Sohail, M., Khurram, M., 2018. Dexibuprofen nanocrystals with improved therapeutic performance: fabrication, characterization, in silico modeling, and in vivo evaluation. Int. J. Nanomedicine 13, 1677–1692. https://doi.org/10.2147/IJN.S151597 https://doi.org/.

Van Eerdenbrugh, B., Van den Mooter, G., Augustijns, P., 2008. Top-down production of drug nanocrystals: Nanosuspension stabilization, miniaturization and transformation into solid products. Int. J. Pharm. 364, 64–75. https://doi.org/10.1016/j.ijpharm.2008.07.023 https://doi.org/.

Van Haute, D., Berlin, J.H., 2017. Challenges in realizing selectivity for nanoparticle biodistribution and clearance: lessons from gold nanoparticles. Ther. Deliv. 8, 763–774. https://doi.org/10.4155/tde-2017-0057 https://doi.org/.

Vandewalle, J., Luypaert, A., De Bosscher, K., Libert, C., 2018. Therapeutic mechanisms of glucocorticoids. Trends Endocrinol. Metab. 29, 42–54. https://doi.org/10.1016/j.tem.2017.10.010 https://doi.org/.

Verma, S., Kumar, S., Gokhale, R., Burgess, D.J., 2011. Physical stability of nanosuspensions: Investigation of the role of stabilizers on Ostwald ripening. Int. J. Pharm. 406, 145–152. https://doi.org/10.1016/j.ijpharm.2010.12.027 https://doi.org/.

Wang, Y., Shardt, N., Lu, C., Li, H., Elliott, J.A.W., Jin, Z., 2020. Validity of the Kelvin equation and the equation-of-state-with-capillary-pressure model for the phase behavior of a pure component under nanoconfinement. Chem. Eng. Sci. 226, 115839 https://doi.org/10.1016/j.ces.2020.115839 https://doi.org/.

Wang, Y., Zheng, Y., Zhang, L., Wang, Q., Zhang, D., 2013. Stability of nanosuspensions in drug delivery. J. Control. Release 172, 1126–1141. https://doi.org/10.1016/j.jconrel.2013.08.006 https://doi.org/.

WHO, 2018. Annex 2 to WHO Technical Report Series, No. 953: Stability testing of active pharmaceutical ingredients and finished pharmaceutical products. https://www.who.int/medicines/areas/quality_safety/quality_assurance/StabilityConditionsTable2updatedAugust2018.pdf?ua=1 (accessed 30 October 2020).

Wong, M.E., Jaworowski, A., Hearps, A.C., 2019. The HIV reservoir in monocytes and macrophages. Front. Immunol. 10, 1–16. https://doi.org/10.3389/fimmu.2019.01435 https://doi.org/.

Wood, F.L., Houston, B., Hallifaz, D., 2018. Importance of the unstirred water layer and hepatocyte membrane integrity in vitro for quantification of intrinsic metabolic clearance. Drug Metab. https://doi.org/10.1124/dmd.117.078949 https://doi.org/.

World Health Organization, 2019. World Health Organization model list of essential medicines https://apps.who.int/iris/bitstream/handle/10665/325771/WHO-MVP-EMP-IAU-2019.06-eng.pdf (accessed 28 January 2020).

Wu, L., Zhang, J., Watanabe, W., 2011. Physical and chemical stability of drug nanoparticles. Adv. Drug Deliv. Rev. 63, 456–469. https://doi.org/10.1016/j.addr.2011.02.001 https://doi.org/.

Xia, G., Wang, Jiahao, Zhou, W., Ma, D., Wang, Jun, 2020. Orientation effects on liquid-vapor phase change heat transfer on nanoporous membranes. Int. Commun. Heat Mass Transf 119. https://doi.org/10.1016/j.icheatmasstransfer.2020.104934 https://doi.org/.

Xu, R., 2015. Light scattering: A review of particle characterization applications. Particuology 18, 11–21. https://doi.org/10.1016/j.partic.2014.05.002 https://doi.org/.

Yarom, M., Marmur, A., 2015. Vapor-liquid nucleation: the solid touch. Adv. Colloid Interface Sci. 222, 743–754. https://doi.org/10.1016/j.cis.2014.07.011 https://doi.org/.

Yu, Q., Wu, X., Zhu, Q., Wu, W., Chen, Z., Li, Y., Lu, Y., 2018. Enhanced transdermal delivery of meloxicam by nanocrystals: Preparation, in vitro and in vivo evaluation. Asian J. Pharm. Sci. 13, 518–526. https://doi.org/10.1016/j.ajps.2017.10.004 https://doi.org/.

Zhang, X., Li, Z., Gao, J., Wang, Z., Gao, X., Liu, N., Li, M., Zhang, H., Zhang, A., 2020. Preparation of nanocrystals for insoluble drugs by top-down nanotechnology with improved solubility and bioavailability. Molecules 25, 1–21. https://doi.org/10.3390/molecules25051080 https://doi.org/.

Zhou, T., Lin, Z., Puligujja, P., Palandri, Di., Hilaire, J., Araínga, M., Smith, N., Gautam, N., McMillan, J., Alnouti, Y., Liu, X., Edagwa, B., Gendelman, H.E., 2018. Optimizing the preparation and stability of decorated antiretroviral drug nanocrystals. Nanomedicine 13, 871–885. https://doi.org/10.2217/nnm-2017-0381 https://doi.org/.

Zhou, Y., Du, J., Wang, L., Wang, Y., 2016. State of the art of nanocrystals technology for delivery of poorly soluble drugs. J. Nanoparticle Res 18. https://doi.org/10.1007/s11051-016-3575-y https://doi.org/.