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REVIEW ARTICLE

Nanocrystal Technology For Oral Delivery of Poorly Water-Soluble Drugs

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Summary

Nanotechnology has been improving and has a variety of application fields. One of the major application areas of nanotechnology in pharmacy is nanoparticular drug delivery systems. Preparation of drug nanocrystals to improve the solubility of poorly water-soluble drugs for oral delivery is also one of the important applications. Nanocrystal dispersions comprise water, active drug substance and a stabilizer. They are physically stable due to the presence of stabilizers that prevent reagragregation of the active drug substance. Different techniques can be used to prepare nanocrystal formulations of a drug powder such as homogenization, co-precipitation, spray drying and milling. There are several advantages of nanocrystal formulations such as, enhanced oral bioavailability, improved dose proportionality, reduced food effects, suitability for administration by all routes and possibility of sterile filtration due to decreased particle size range. The first nanocrystal drug product, Rapamune® (Sirolimus), was approved by the FDA in 2000. In the following years, several other nanocrystal drug products came out on the pharmaceutical market. Nanocrystal technology is cheap and easy to apply; thus, it appears that this technology will be substantially useful for the manufacture of poorly water-soluble drug products for oral delivery.

Key Words: *Nanotechnology, nanocrystal, dissolution rate, particle size, bioavailability*

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Çözünürlüğü zayıf olan ilaçların oral uygulaması için nanokristal teknolojisi

Özet

Nanoteknoloji son yıllarda çok farklı uygulama alanlarında hızlı bir şekilde gelişmektedir. Eczacılık alanında nanoteknolojinin uygulama alanı ise, nanopartiküler ilaç taşıyıcı sistemlerdir. Oral uygulama için kullanılan suda çözünürlüğü zayıf olan ilaç etkin maddelerinin çözünürlüğünü artırma yöntemlerinden biri ilaç nanokristallerinin hazırlanmasıdır. Nanokristal dispersiyonları su, etkin madde ve stabilizan içermektedirler. Nanokristaller, partiküllerin aglomerizasyonunu engellemek için formülasyonlara ilave edilen stabilizanların varlığında fiziksel olarak dayanıklı sistemlerdir. Nanokristal formülasyonlarının hazırlanmasında homojenizasyon, birlikte çöktürme, püskürterek kurutma ve öğütme gibi çeşitli yöntemler vardır. Nanokristal formülasyonlarının oral biyoyararlanımının artması, doz orantısallığının iyileştirilmesi, yiyecek etkisini azaltılması, tüm dozaj şekillerine uygulanabilir olması ve partikül büyüklüğü çok küçük olduğu için filtrasyonla sterilizasyona uygun olması gibi birçok üstünlüğü vardır. Rapamune® (Sirolimus) FDA tarafından 2000 yılında kabul edilen ilk nanokristal ilaç ürünüdür. Sonraki yıllarda pek çok farklı nanokristal ilaç ürünü, ilaç pazarına çıkmıştır. Nanokristal teknolojisi ucuz olup, uygulaması kolaydır; bu nedenle, bu teknoloji ilerleyen yıllarda da suda çözünürlüğü zayıf olan ilaçların oral uygulama için üretimlerinde yararlı olmaya devam edecektir.

Anahtar Kelimeler: *Nanoteknoloji, nanokristal, çözünme hızı, partikül büyüklüğü, biyoyararlanım*

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INTRODUCTION

Nanotechnology is derived from the Latin word "nano", which means dwarf. It is used in the production of materials at submicron or molecular level in engineering, electronics, physics and material science. Nanosized materials may be a device, a system of supramolecular chemistry, complexes or compounds. In 1959, the term of nanotechnology was first used by the physicist Richard Feynman (1). He indicated that by producing materials and devices at molecular level, nanostructures can be measured and nanotechnology can be used for many new purposes. The major application areas of nanotechnology are materials and manufacturing sector, nano electronics and computer technology (fiber optic communications networks), aviation and space research, environment and energy, agriculture, chemical engineering, defense industry, biology, biotechnology, medicine and pharmaceutics.

In medicine and pharmaceutics, nanotechnology is used to improve human health at a molecular level. The novel and potential applications of nanotechnology in pharmaceutics are; development of diagnostic tools, formulation of drug carrier systems and gene therapy (2). The advantages of nanotech drugs compared to conventional counterparts lie on the basis of particle size. Drugs/drug products with nano dimension can be used at a lower concentration and can lead to early onset of bioactivity (3). Nano drug delivery systems (nanopharmaceutics) are, but not limited to, nanocapsules, nanospheres, nanosponges, nanoemulsions, solid lipid nanoparticles, nanovesicular systems (liposomes, niosomes), molecular systems (inclusion complexes) and nanocrystals.

The dissolution rate of drugs in the GI tract affects absorption rate and degree of drugs. Absorption of a drug is defined as the transition of a drug from the applied place to the blood and/or lymphatic circulation. The amount of absorbed active drug substance depends on physicochemical properties of the active drug substance, pharmaceutical dosage form and physiological characteristics. For complete absorption of the active drug substance from the gastrointestinal tract (GI), it must be completely dissolved (Figure 1). However, most of the new chemical compounds developed as a drug have poor solubility in water. Water solubility of 40% of the active drug substances that are currently in use and 60% of the active drug substances that are at the investigation stage is very low (4,5). Low water solubility limits absorption and bioavailability of these drugs. For oral administration, conventional formulations of poorly water-soluble drugs are associated with erratic absorption in the GI tract and low/variable bioavailability (6).

Thus, bioavailability of poorly water-soluble drugs will be affected positively when their dissolution rate is increased. These drugs show serious adverse clinical effects like non-steady absorption due to variability among patients and individual patient dosing. Drugs which have high permeability but low solubility (Class II according to Biopharmaceutics Classification System) are not easily dissolved (7) so they may not be absorbed from the GI tract sufficiently (8,9). Moreover, such drugs incorporated into conventional dosage forms are usually affected by the fasted or fed state of the patient. This situation eventually causes inappropriate dosing and low bioavailability (10-12).

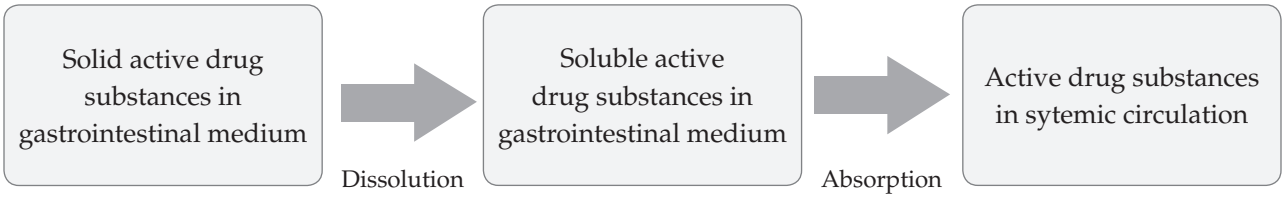

Figure 1. Schematic representation of events that an active drug substance faces in the GI medium.

There are several classical pharmaceutical approaches for improving solubility of drugs in order to enhance oral bioavailability. Solubilization of poorly water-soluble drugs increases dissolution rate and absorption leading to a significant improvement of drug bioavailability. Approaches to improve the solubility or to increase the available surface area for dissolution are classified as physical and chemical modifications. Physical modifications are decreasing particle size (micronization, nanosuspensions), formation of polymorphs/pseudopolymorphs (including solvates), complexation/solubilization (use of surfactants or cyclodextrines, addition of cosolvents) and preparation of drug dispersions in carriers (eutectic mixtures, non-molecular solid dispersions, solid solutions). The chemical modifications are synthesis of soluble prodrugs and salts (13).

Approaches such as adding a surfactant/cosolvent, complexation with cyclodextrins or/and preparing oil–in-water emulsions for intravenous applications have been developed for poorly water-soluble drugs, but these approaches have limited application since the active drug substance must have specific physicochemical properties (for example, cyclodextrins must have suitable molecular weight for optimal conjugation of the conical structure with the drug for such applications to be successful) (4). Solid dispersions are theoretically one of the appropriate methods for increasing dissolution rate, but molecules in the amorphous state are not thermodynamically stable; they can convert to the crystal form during storage (14-16). This transformation is undesirable because the dissolution rate can significantly change due to transition to the crystal structure from the amorphous structure. The use of surfactants or cosolvents sometimes leads to increased side effects and toxic reactions in the body. Potential disadvantages of salt forms include, high reactivity with atmospheric carbon dioxide and water resulting in precipitation of the poorly water-soluble drug. Polymorphs are different crystalline forms of a drug that may have different physicochemical properties and biological activities. The polymorphs differ from each other with respect to morphology, density, melting point, hardness, compression, solubility and bioavaliability. Therefore, the preparation of actual drug polymorphs is crucial during preformulation studies. An alternative drug delivery approach called micronization, has been developed to overcome poor solubility in water. Micronisation of poorly soluble drugs, increases the dissolution rate of the drug due to the increase in surface area, but does not change the saturation solubility. In order to increase solubility and oral bioavailability, going down to the micron level may sometimes not be sufficient, so the next step, going down to the nano level, may be necessary.

Dissolution Rate vs. Particle Size

Intrinsic solubility can be explained as the number of moles of a substance per liter which dissolves in a particular solvent. Thus, dissolution is the process by which a solid substance dissolves. There are many factors that effect solubility; such as pH, co-solvent, surfactants, temperature and particle size. By changing these factors, solubility of active drug substances can be modified.

Dissolution rate has been first described by Noyes and Whitney in 1897. In 1904, Nernst and Brunner explored the dissolution rate constant and difussion coefficient of solutes. Factors that affect dissolution rate are mixing, temperature, viscosity, surface active substances, crystal shape, pH and particle size (17, 18). According to the Nernst Brunner equation, dissolution rate is proportional to the surface area of the active drug substance in contact with the dissolution medium.

$$\frac{dw}{dt} = \frac{D}{h} \times S\left(Cs - Ct\right) \qquad \text{Exuation 1}$$

dw/dt: Dissolution rate (mg/s)
S: Effective surface area of the solid drug (cm^2)
Cs-Ct: Concentration gradient (mg/mL)
Cs: Saturated concentration(mg/mL)
Ct: Concentration of the solute at time t (mg/mL)
h: Effective diffusion layer thickness (cm)
D: Diffusion coefficient (cm/s)

It can be deduced from the formula that changing particle size of the active drug substance, it is possible to change the specific surface area and the

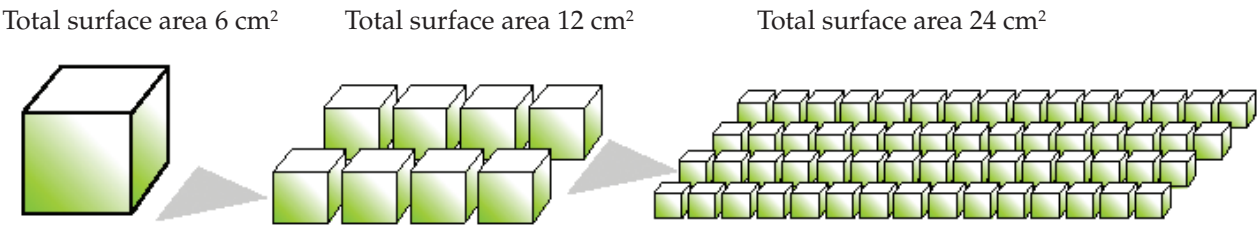

Figure 2. Surface area enlargament by particle size reduction (18).

dissolution rate of the active drug substance in body fluids (Figure 2).

With this basic information in hand, it is clear that the dissolution rate and bioavailability of poorly water-soluble drugs can be increased by decreasing the particle size of active drug substances. Additionally, particle size reduction results in a decrease in the diffusion layer thickness surrounding the drug particles and an increased concentration gradient between the surface of the drug particles (19).

Nanocrystal Technology

Preparation of drug nanocrystals is basically a nano-sizing method, which is utilized to enhance the oral bioavailability of poorly water-soluble drugs. Drug nanocrystals are nanoscopic crystals of the drug with dimensions less than 2000 nm as defined in the first patents in this field (21-23). Nanocrystal dispersions contain dispersion media (water, aqueous solutions or nonaqueous media), active drug substances and surface active agents or polymers required for stabilization (24). If necessary, other substances such as buffers, salts and sugars can be added.

There are many advantages of nanocrystal formulations designed for oral administration. They are as follows:

- Increased rate of absorption,
- Increased oral bioavailability,
- Rapid effect,
- Improved dose proportionality,
- Reduction in required dose,
- Applicability to all routes of administration in any dosage form. Contrary to micronized drugs, nanocrystals can be administered via several routes. Oral administration is possible in the form of tablets, capsules, sachets or powder; preferably in the form of a tablet. Nanosuspensions can also be administered via the intravenous route due to very small particle size, and in this way, bioavailability can reach 100 %.
- Reduction in fed/fasted variability,
- Rapid, simple and cheap formulation development (19, 25, 26).
- Possibility of high amounts (30-40 %) of drug loading,
- Increased reliability. Usually side effects are proportional to drug concentration, so decreasing the concentration of active drug substances leads to an increased reliability for patients (27, 28).
- Sustained crystal structure. Nanocrystal technology leads to an increase in dissolution rate depending on the increase in surface area obtained by reduction of the particle size of the active drug substance down to the nano size range preserving the crystal morphology of the drug (29).
- Improved stability. They are stable systems because of the use of a stabilizer that prevents reaggregation of active drug substances during preparation (19). Suspension of drug nanocrystals in liquid can be stabilized by adding surface active substances or polymers.
- Applicability to all poorly soluble drugs because all these drugs could be directly disintegrated into nanometer-sized particles.

Nanocrystal Preparation Methods

Several preparation methods for drug nanocrystals have been investigated. These are schematically depicted in Figure 3.

Today, implemented preparation methods of nanocrystal formulations can be classified as "bottom up", "top-down", "top down and bottom up" and

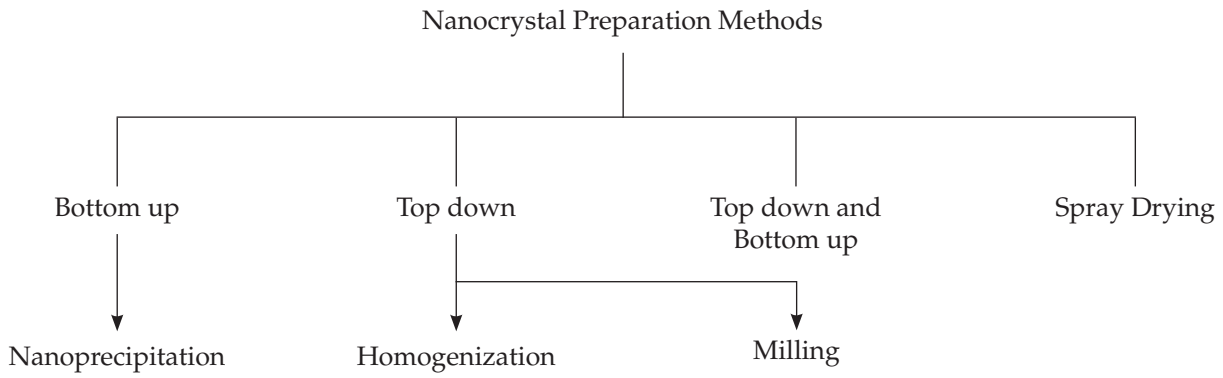

Figure 3. Preparation methods of drug nanocrystals

"spray drying". "Bottom up" technology begins with the molecule; active drug substance is dissolved by adding an organic solvent, and then, solvent is removed by precipitation. "Top-down" technology applies dispersing methods by using different types of milling and homogenization techniques. "Top-down" technology is more popular than "Bottom up" technology; it is known as "nanosizing". In other words, it is a process which breaks down large crystalline particles into small pieces. In "top down and bottom up" technology, both methods are utilized together (30). Spray drying is also a method for preparing drug nanocrystals, which is faster and more practical compared to the other methods.

Bottom up technology

"Bottom up" technology relies on precipitation (31, 32). The principle of this method is based on the dissolution of the active drug substance in an organic solvent which is then added into a nonsolvent (miscible with the organic solvent). In the presence of stabilizers, thereafter, the nanocrystals are precipitated. Basic advantage of the precipitation technique is that it is simple and has a low cost. Also, scale up is simple in this method. Examples of products manufactured by the precipitation method are, Hydrosols and Nanomorph™, which are developed by Sucker and Soliqs/Abbott respectively (4, 33). It should be kept in mind that several parameters; such as stirring rate, temperature, solvent/nonsolvent rate, drug concentration, viscosity, type of solvent and stabilizer should be controlled in order to obtain homogenous nanocrystals by this technique (34, 35).

Top down technology

As it is shown in the Figure 3, "top down" technology can be applied by either homogenization or milling. In the milling method; pearl, bead or ball mills can be utilized to prepare a nanocrystal formulation. In this method, the active drug substance and the stabilizer are dispersed in the dispersion medium, and this mixture is then put into a grinder chamber (36). Balls are rotated at a very high speed and particle size of the drug gets smaller until nanocrystals are obtained. Physicochemical characteristics of the nanocrystals depend on the number of milling balls, the amount of drug and stabilizer, milling time and speed, type of grinding chamber and temperature. In contrast with high pressure homogenization, it is a low energy technique (24). Grinder chambers are made from stainless steel, porcelain or hard material, and the balls are made from porcelain, glass, zirconium oxide, stainless steel, chromium, agate, or special polymer materials. The type of material that the balls are made of is very important since an interaction could take place between the material and the drug substance. In this regard, agate balls are frequently used in the pharmaceutical field as the possibility of such an interaction is minimized. Another important factor is the size of balls. When the balls with small diameter are used, grinding time is extended but smaller particles are obtained. The mechanism of ball milling is that while grinding chamber is rotated, balls are rotated too, and at the end of this procedure, the particle size of the active drug substance is reduced by mechanical energy. Thus, particle size of active drug substances can be adjusted by changing the diameter and number

of balls. Rotational speed of the grinding chamber is also very important. If the rotational speed is too low, balls can not rotate effectively and grinding can not be done efficiently. If rotational speed is very high, balls will remain at the edge of the grinding chamber due to centrifugal forces and grinding can not be done effectively. Moreover, for efficient grinding the volume of the balls should be 30 % to 50 % of the grinding chamber (18). The milling time depends on many factors such as the surfactant content, hardness of the drug, viscosity, temperature and energy input. The milling time can change from 30 minutes to hours or several days (10). This simple method was developed by G. Liversidge and coworkers and has been used by the Elan company (36).

The main problem in this method is the contamination of the product as a result of erosion of balls or grinding chamber. Therefore, grinding time and the material of balls/grinding chamber should be selected very carefully. This interaction can be reduced by using surface active agents or polymers (22). Nanocrystals of drugs; such as N-[[4-(5-Bromo-2-pyrimidinyloxy)-3-chlorophenyl]amino] carbonyl]-2–nitrobenzamide, naproxen, cilostazol, paclitaxel, 3,9-bis(N,N dimethylcarbamoyloxy)-5H-benzofuro[3,2-c] quinoline-6-one have been prepared by this method and highly improved bioavailability values were obtained (9, 10, 33, 37, 38).

The other "top down" method is homogenization. One of the preparation methods of nanocrystals is homogenization by ultasonification. Ultrasonic probes are used to decrease the particle size in liquid or solid dispersed phase. Ultrasonic homogenization is quite effective for reducing the size of hard and soft particles. Homogenization by ultasonification is based on high frequency mechanical vibrations. Liquids are exposed to intense sound waves transmitted with ultrasonification. Ultrasonic probe provides controlled and reproducible ultrasonification. This is important for the quality of manufactured products and scale up studies (39).

Another homogenization method is high pressure homogenization in which two types of homogenizers, namely, microfluidizers and piston gap homogenizers are used. Here, the main goal is to reduce particle size with high pressure homogenization. Microfluidizer technology (Insoluble Drug Delivery – Particles, IDD-P™ technology) is utilized to achieve production of submicron particles of poorly soluble drugs. Piston gap homogenization is performed in water (DissoCubes®), water mixtures or nonaqueous media (Nanopure®) (4, 40).

Top down and bottom up technology

In "top down and bottom up" technology, both methods are used together. NanoEdge® is a product obtained by such a combination technology. As can be inferred, precipitation is followed by high pressure homogenization in this technology (4).

Spray Drying

One of the preparation methods of nanocrystals is spray drying. This method is usually used for drying of solutions and suspensions. In a conical or cylindrical cyclone, solution droplets are sprayed from top to bottom, dried in the same direction by hot air and spherical particles are obtained. Spraying is made with an atomizer which rapidly rotates and provides scattering of the solution due to centrifugal effect. The solution, at a certain flow rate, is sent to the inner tube with a peristaltic pump, nitrogen or air at a constant pressure is sent to the outer tube. Spraying is provided by a nozzle. Droplets of solution become very small due to spraying; therefore, surface area of the drying matter increases leading to fast drying. Concentration, viscosity, temperature and spray rate of the solution can be adjusted and particle size, fluidity and drying speed can be optimized. The dissolution rate and bioavailability of several drugs, including hydrocortisone, COX-2 Inhibitor (BMS-347070) were improved utilizing this method (41, 42).

Selection of stabilizers for nanocrystal preparation

Selection of stabilizers is very important in nanocrystal formulations (31) because the type and concentration of stabilizer affect the final size of the particles, and also, stabilizers prevent nanocrystals from aggregating. Polymers or surface active agents exert their effect by covering the surface of drug nanocrystals and providing stabilization by creating

a steric barrier. At the nano size, forces between particles due to dispersion or van der Waals forces come into play. Nanosized particles with a high surface area have high surface free energy (ΔG). Thus, particles tend to agglomerate in order to decrease the surface free energy leading to an increase in particle size and reduction in the surface area. Therefore, a stabilizer leads to a decrease in ΔG by decreasing the interfacial tension $\gamma_{s/1}$ (Equation 2) (43, 44).

$$\Delta G = \Delta A \bullet \gamma_{s/1} \qquad \text{Equation 2}$$

$\gamma_{s/1}$: interfacial tension between the surface of solid and the surrounding liquid phase (joule/m^2)
ΔG: change in surface free energy (joule)
ΔA: change in the surface area (m^2)

Jonghwii Lee and coworkers have reduced the particle size of seven different active drug substances by preparing nanocrystals. They investigated two different stabilizers (polyvinylpyrollidone and hydroxypropyl cellulose), their interaction with the active drug substances, and effects on surface energy and particle size. They found that interactions between stabilizers and active drug substances were complex and depended on many variables, such as presence of functional groups and surface energy (45). The concentration of the stabilizer is an important factor that affects the physical stability of the final product (4, 10).

Hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC), povidone (PVP K30), and pluronics (F68 and F127) are polymers suitable for use as stabilizers. The chains should be long enough to provide a steric layer, but not too big to slow down dissolution. Polysorbate 80 (nonionic), sodium laurylsulfate (SLS) and docusate sodium (DOSS) (both anionic) are some examples of suitable surfactant stabilizers for physical stability. Also, surfactants often help in the wetting, electrostatic stabilization and dispersion of the drug particles, which are usually very hydrophobic. HPMC E3, Povidone, DOSS, and SLS are some of the stabilizers that have been used in the nanocrystal formulations of drugs that are on the market today (30).

Current status of nanocrystal technology in pharmaceutics

Since the first patended product came up, studies related to nanocrystal technology have tremendously increased. In a study made by R. Mauludin et al., nanocrystals were prepared by lyophilization and physicochemical properties; such as re-dispersability, particle size, morphology and dissolution behavior were investigated (46). Nanocrystal-loaded tablets were produced using direct compression. As seen in figure 4, % amount of drug dissolved from all

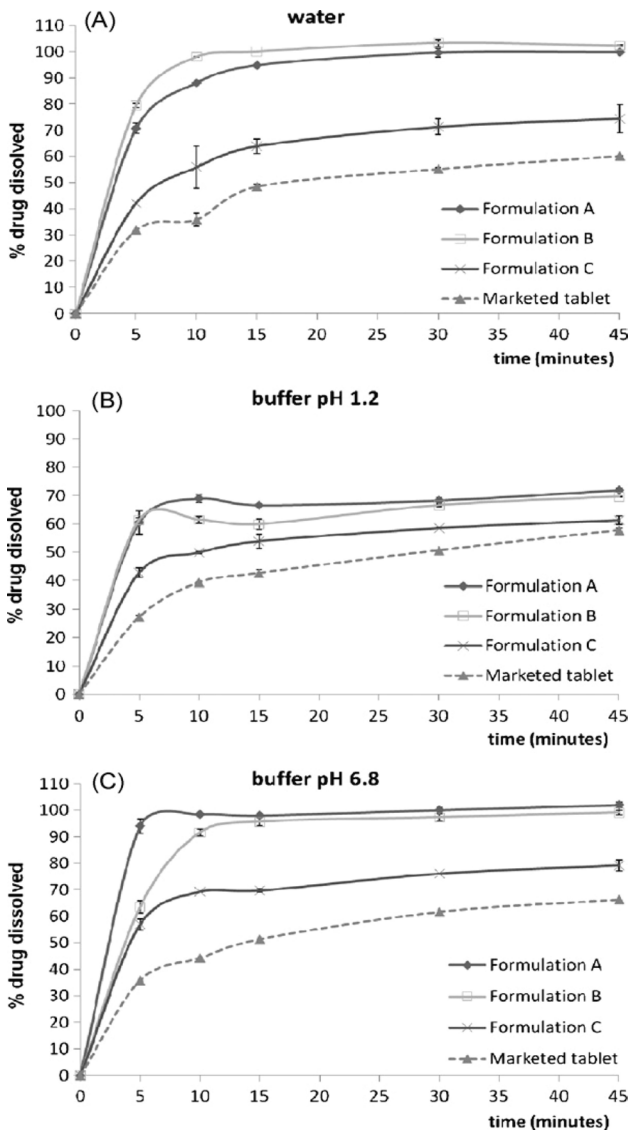

Figure 4. Percentage of dissolved drug from nanocrystal-loaded tablets (formulation A, B) and from microcrystal-loaded tablets (formulation C and marketed tablet) in water (A), pH 1.2 buffer (B) and pH 6.8 buffer (C) (46).

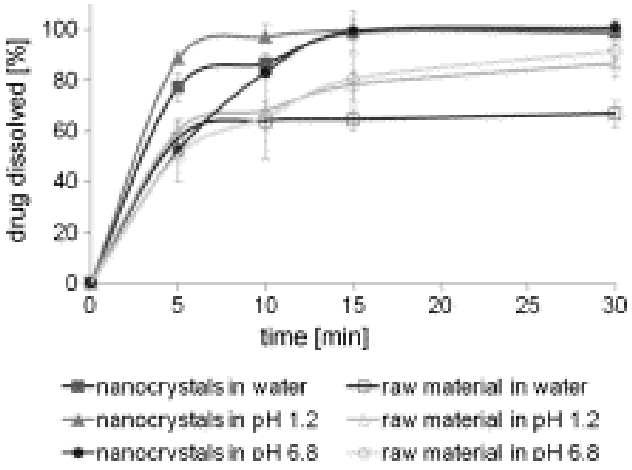

Figure 5. Dissolution profile of the nanocrystals and the raw material in water, pH 1.2 buffer and pH 6.8 buffer (47).

formulations was evaluated in three different dissolution media; of the formulations tested, formulation A and B were prepared by nanocrystal technology. The dissolution rate of the nanocrystal-loaded tablet was faster compared to the microcrystal-loaded tablet.

In another study, nanosuspensions were prepared by high pressure homogenization followed by lyophilization and spray drying. Nanocrystals were obtained and particle size analyses, crystallinity, kinetic solubility and dissolution studies were conducted. Figure 5 demonstrates the improved dissolution rate that can be achieved by reducing the particle size with nanocrystal technology. Dissolution rates of the nanocrystals were observed to be distinctively superior compared to the raw material (47).

In one of the recent studies, T. Gülsün et al. have prepared ezetimibe nanocrystals utilizing ball milling and homogenization. Ezetimibe is a poorly water-soluble drug, which is a lipid-lowering compound that selectively inhibits the absorption of cholesterol and related phytosterols from the intestine. Pluronic F127 was chosen as a surface modifier to stabilize the nanocrystal formulations. Tablets were prepared containing ezetimibe nanocrystals formed by high speed homogenization (ultrasonic) and ball milling according to the results of particle size measurements and in vitro dissolution rates of the nanocrystal formulations. As a result of these experiments, it was found that the dissolution rate of the nanocrystal formulations increased and although tablet formulations, which

Table 1. FDA approved nanocrystal products on the pharmaceutical market

Product	Drug	Indication	Technology by/ licenced to	Year of FDA approval
Rapamune®	Sirolimus	Immunosuppressant	Elan/Wyeth	2000
Emend®	Aprepitant	Antiemetic	Elan/Merck	2003
Tricor®	Fenofibrate	Treatment of high cholesterol and high triglyceride levels	Elan/Abbott	2004
Megace ES®	Megestrol Acetate	Palliative treatment of some breast and uterine cancers	Elan/Par Pharm	2005
Triglide®	Fenofibrate	Treatment of high cholesterol and high triglyceride levels	SkyePharma/First Horizon Pharmaceuticals	2005
Invega Sustenna®	Paliperidone palmitate	Treatment of schizophrenia	Elan/ Johnson and Johnson	2009

did not contain any solubilizing agent like sodium lauryl sulfate, the dissolution profile of these formulations were found similar to the commercial product (f2>50) (48-50).

The first nanocrystal product on the market was Rapamune® , introduced by Elan/Wyeth in 2000. Commercial products, which are prepared by nanocrystal technology, are listed in Table 1.

CONCLUSION

According to literature, about 60 % of all synthesized drug candidates are poorly water-soluble. Thus, it appears that nanocrystal technology will continue to thrive as a useful tool in pharmaceutics for the improvement of drug solubility, oral absorption, and hence, bioavailability. The fact that this technology has many advantages; such easy production and scale up, and low cost, make this approach a very attractive means for solving a very serious problem of drugs, poor water-solubility in conjunction with low oral absorption and bioavailability.

REFERENCES

1. Feynman, R. P. (1959). There's plenty of room at the bottom,Caltech. Retrieved September 1, 2002, from http://www.its.caltech.edu/~feynman/plenty.html
2. Zhang L, Webster TJ. Nanotechnology and Nanomaterials: Promises for Improved Tissue Regeneration. *Nano Today* 4: 66-80, 2009.
3. Chan VSW. Nanomedicine: An Unresolved Regulatory Issue. *Regul Toxicol Pharmacol* 46: 218-224, 2006.
4. Keck CM, Müller RH. Drug Nanocrystals of Poorly Soluble Drugs Produced by High Pressure Homogenisation. *Eur J Pharm and Biopharm* 62: 3-16, 2006.
5. Müller RH, Runge S, Ravelli V, Mehnert W, Thünemann AF, Souto EB. Oral Bioavailability of Cyclosporine: Solid Lipid Nanoparticles (SLN®) Versus Drug Nanocrystals. *Int J Pharm* 317: 82-89, 2006.
6. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 46(1-3):3-26, 2001.
7. Amidon GL, Lennernäs H, Shah VP, Crison JR. A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of In Vitro Drug Product Dissolution and In Vivo Bioavailability. *Pharm Res* 12: 413-420, 1995.
8. Crisp MT, Tucker CJ, Rogers TL, Williams RO, Johnston KP. Turbidimetric Measurement and Prediction of Dissolution Rates of Poorly Soluble Drug Nanocrystals. *J Control Release* 117: 351- 359, 2007.
9. Jinno J, Kamada N, Miyake M, Yamada K, Mukai T, Odomi M. 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, 2006.
10. Merisko-Liversidge E, Liversidge GG, Cooper ER. Nanosizing: A Formulation Approach for Poorly-Water-Soluble Compounds. *Eur J Pharm Sci* 18: 113-120, 2003.
11. Venkatesh, S, Lipper RA. Role of the Development Scientist in Compound Lead Selection and Optimization. *J Pharm Sci* 89: 145-154, 2000.
12. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular Properties That Influence the Oral Bioavailability of Drug Candidates. *J Med Chem* 45: 2615-2623, 2002.
13. Leuner C, Dressman J. Improving drug solubility for oral delivery using solid dispersions. *Eur J Pharm and Biopharm* 50: 47-60, 2000.
14. Law D, Wang W, Schmitt AE, Qiu Y, Krill SL, Fort JJ. Properties of Rapidly Dissolving Eutectic Mixtures of Poly(ethylene glycol) and Fenofibrate: The Eutectic Microstructure. *J Pharm Sci* 92: 505-515, 2003.
15. Serajuddin ATM. Solid Dispersion of Poorly Water-Soluble Drugs: Early Promises, Subsequent Problems, and Recent Breakthroughs. *J Pharm Sci* 88: 1058-1066, 1999.
16. Pokharkar VB, Mandpe LP, Padamwar MN, Ambike AA, Mahadik KR, Paradkar A. Development, Characterization and Stabilization

of Amorphous Form of a Low Tg drug. *Powder Tech* 167: 20–25, 2006.

17. Çapan Y. et al. Farmasötik Teknoloji Temel Konular ve Dozaj Şekilleri. In: Gürsoy AZ, editor. Çapan, Y. Çözünürlük ve çözünme hızına etki eden faktörler. 1st ed. İstanbul: Kontrollü Salım Sistemleri Derneği; Piksel Bilişim ve Matbaacılık Reklamcılık ve Filmcilik hizmetleri Ltd. Şti.Press; 2004. p. 85-96.
18. Acartürk, F., Ağabeyoğlu, İ., Çelebi, N., Değim, T. Modern Farmasötik Teknoloji. Değim, Z. Öğütme. 1st ed. Ankara: Türk Eczacıları Birliği Eczacılık Akademisi Yayını, Fersa Matbaacılık Ltd. Şti.Press; 2006. p.1-7.
19. Technology Focus Helping you bring products to market. Serial online: 23 March 2007 Availablefrom: URL: http://www.elan.com/Images/Elan_Technology_07v1_tcm3-17145.pdf
20. Galli C. Experimental determination of the diffusion boundary layer width of micron and submicron particles. *Int J Pharm* 313: 114–122, 2006.
21. Hovey D, Pruiit J, Ryde T, inventors; Elan Pharma International Limited, assignee. Nanoparticulate megestrol formulations. US patent 7,101,576. 2006 September 5.
22. Liversidge G, Jenkins S, inventors; Elan Pharma International Limited, assignee. Nanoparticulate candesartan formulations. WO 074218. 2006 July 13.
23. Ruddy SB, Ryde NP, inventors; Elan Pharma International Limited, assignee. Solid dose nanoparticulate compositions. WO 666,539. 2002 March 28.
24. Junghanns JUAH, Müller AH. Nanocrystal technology, drug delivery and clinical applications. *Int J Nanomedicine* 3(3):295–309, 2008.
25. Müller RH, Keck CM. Challenges and Solutions for the Delivery of Biotech Drugs: a Review of Drug Nanocrystal Technology and Lipid Nanoparticles. *J Biotech* 113: 151-170, 2004.
26. Möschwitzera JM, Achleitner G, Pomper H, Müller RH. Development of an Intravenously Injectable Chemically Stable Aqueous Omeprazole Formulation Using Nanosuspension Technology. *Eur J Pharm and Biopharm* 58: 615–619, 2004.
27. Coppola D. Nanocrystal Technology Targets Poorly Water-soluble Drugs. *Pharm Tech* 27: 20, 2003.
28. Riehemann K, Schneider SW, Luger TA, Godin B, Ferrari MF, Fuchs H. Nanomedicine – challenge and perspectives. *Angew Chem Int Ed* 48: 872–897, 2009.
29. Waard H, Hinrichs WLJ, Frijlink HW. A Novel Bottom–Up Process to Produce Drug Nanocrystals: Controlled Crystallization During Freeze-Drying. *J Control Release* 128: 179-183, 2008.
30. Kesisoglou F, Panmai S, Wu Y. Nanosizing – Oral formulation development and biopharmaceutical evaluation. *Adv Drug Deliv Rev* 59: 631–644, 2007.
31. Rabinow BE. Nanosuspensions in drug delivery. *Nat Rev Drug Discov* 3(9):785-796, 2004.
32. Liversidge EM, Liversidge GG, Cooper ER. Nanosizing: a formulation approach for poorly-water-soluble compounds. *Eur J Pharm Sci* 18(2):113-120, 2003.
33. Liversidge GG, Conzentino P. Drug Particle Size Reduction for Decreasing Gastric Irritancy and Enhancing Absorption of Naproxen in Rats. *Int J Pharm* 125: 309-313, 1995.
34. Chen H, Khemtong C, Yang X, Chang X, Gao J. Nanonization strategies for poorly water-soluble drugs. *DDT* 00: 00, 2010.
35. Gao L, Zhang D, Chen M. Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system. *J Nanopart Res* 10:845–862, 2008.
36. Suman K, V S R Chandrasekhar P, Balaji S. Drug Nanocrystals: A Novel Formulation Approach for Poorly Soluble Drugs. *Int J PharmTech Res* 1(3):682-694, 2009.
37. Yamada T, Saito N, Imai T, Otagiri M. Effect of Grinding with Hydroxypropyl Cellulose on the Dissolution and Particle Size of a Poorly Water-Soluble Drug. *Chem Pharm Bull* 47: 1311- 1313, 1999.
38. Kondo N, Iwao T, Masuda H, Yamanoouchi K, Ishihara Y, Yamada N, Haga T, Ogawa Y, Yokoyama K. Improved Oral Absorption of a Poorly Water-Soluble Drug, HO-221, by Wet-Bead Milling Producing Particles in Submicron Region. *Chem Pharm Bull* 41: 737-740, 1993.

39. Ultrasonic Devices for Liquid Processing. Available from: URL: http://www.hielscher.com/ultrasonics/products.htm
40. Li P, Zhao L. Developing early formulations: Practice and perspective. *Int J Pharm* 341: 1–19, 2007.
41. Corrigan OI, Crean AM. Comparative Physicochemical Properties of Hydrocortisone / PVP Composites Prepared Using Supercritical Carbon Dioxide by the GAS Anti-Solvent Recrystallization Process, by Coprecipitation and by Spray Drying. *Int J Pharm* 245: 75-82, 2002.
42. Yin SX, Franchini M, Chen J, Hsieh A, Jen S, Lee T, Hussain M, Smith R. Bioavailability Enhancement of a COX-2 Inhibitor, BMS-347070, from a Nanocrystalline Dispersion Prepared by Spray-Drying. *J Pharm Sci* 94: 1598–1607, 2005.
43. A. Martin, P. Bustamante, A.H.C. Chun, Interfacial phenomenon, Physical Pharmacy, Lippincott Williams & Wilkins, Maryland, 1993, pp. 362–392.
44. Sharma P, Garg S. Pure drug and polymer based nanotechnologies for the improved solubility, stability, bioavailability and targeting of anti-HIV drugs. *Adv Drug Deliv Rev* 62:491–502, 2010.
45. Choi JY, Yoo JY, Kwak HS, Uk Nam B, Lee J. Role of Polymeric Stabilizers for Drug Nanocrystal Dispersions. *Curr Appl Phys* 5: 472-474, 2005.
46. Mauludin R, Müller RH, Keck CM. Development of an oral rutin nanocrystal formulation. *Int J Pharm* 370: 202–209, 2009.
47. Mauludin R, Müller RH, Keck CM. Kinetic solubility and dissolution velocity of rutin nanocrystals. *Eur J Pharm and Biopharm* 36 (4-5): 502-510, 2009.
48. Gülsün T, Gürsoy RN, Öner L. Characterization of nanocrystal formulations which contain poorly water soluble drug, ezetimibe. 7th Central European Symposium on Pharmaceutical Technology & Biodelivery Systems, Ljubljana-Slovenia (2008).
49. Gülsün T, Gürsoy RN, Öner L. Application of Nanotechnology for the Improvement of Dissolution Properties of a Poorly Water-Soluble Drug, Ezetimibe, 6th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Barcelona- Spain (2008).
50. Gülsün T, Gürsoy RN, Öner L, Improving Dissolution Profile of Ezetimibe, When Variability Becomes an Issue in Drug Development: How to Understand, Predict and Manage?, Verona- İtalia (2008).