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Nanoformulations of Poorly-Water Soluble Molecules

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Abstract Around forty percent of new chemical entities (NCEs) are reported to be poorly soluble in water. This translates to poor bioavailability and, thereby, limits the development of these molecules as drug candidates. It is suggested that nanoformulations of poorly-water soluble NCEs/Drugs are viable routes to increase the bioavailability of these molecules. The role of particle size in molecular dissolution is discussed. Different nanostructures that can incorporate poorly-water soluble NCEs/Drugs and which have been previously reported in the literature such as nanoemulsions, nanosuspensions and nanoscale NCE/Drug-Polymer composites are presented. Methods for the preparation of these nanostructures are discussed. Characterization techniques such as Powder X-Ray Diffraction (PXRD), Dynamic Light Scattering (DLS), Zeta Potential Measurement, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM) and Differential Scanning Calorimetry (DSC) are discussed. Finally, an example of an NCE-Polymer Nanocomposite is presented.

Keywords Pharmaceutical Nanotechnology, Nanoformulations, Poorly-Water Soluble Drugs, Nanoemulsions, Nanosuspensions, NCE/Drug-Polymer Nanocomposites

1. Introduction

The classic book, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems provides a definition of a drug [1]. It is as follows: 'A drug is defined as an agent intended for use in the diagnosis, mitigation, treatment, cure or prevention of disease in humans or in other animals.' Blockbuster, patented drugs record annual sales in the billions of dollars in regulated markets. For example, it is reported that, in 2016, AbbVie's's drug Humira® (adalimumab) recorded a sales figure of US$ 16.078 billion and Gilead Sciences' drug Harvoni® (ledipasvir 90 mg/ sofosbuvir 400 mg) recorded a sales figure of US$ 9.081 billion[2]. This clearly shows that the discovery of drugs, which can be protected by patents in therapeutic areas where they can fetch large revenues, has huge commercial implications.

The process of drug discovery is long, arduous and expensive. It can take anywhere between ten to fifteen years to bring a patented molecule to market as a drug. The cost involved in doing this is estimated to lie around US$ 2.6 billion [3]. Therefore, anything that reduces the research and development burden is most welcome. From a statistical perspective it is clear that the greater the number of new chemical entities (NCEs) which have the potential of becoming drugs, the greater is the chance that a drug will be discovered from that collection of NCEs. One of the factors that limit the development of NCEs into drugs is the poor aqueous solubility of the NCEs. According to Prof. Robinson of Wisconsin, around 40% of NCEs are sparingly soluble in water even though they may be otherwise promising as drug candidates [4]. This is a large number and clearly represents a great deal of the synthetic effort and research expenses involved.

The question then is whether something can be done about this so as to enable these molecules to progress further in the drug discovery pipeline.

It turns out that one of the answers to this burning question lies in nanotechnology. To understand this let us examine some basic aspects of size-effects in molecular dissolution.

2. Size-effects in Molecular Dissolution

The role of size-effects in molecular dissolution has been discussed previously in the literature [5, 6]. We build upon these considerations. There are two factors that contribute to the increase in the rate of dissolution of a substance when the particle size of a given mass of a substance is reduced. In order to understand these factors let us first examine the Noyes-Whitney equation [7], (1), which provides an expression for the rate of dissolution of a solid in a liquid medium. This equation is as follows:

$$\frac{dC_x}{dt} = \frac{DA}{h}(C_s - C_x) \quad (1)$$

In this equation,

$\frac{dC_x}{dt}$ is the rate of dissolution

D is the diffusion coefficient

A is the surface area of the particle

h is the thickness of the diffusion layer

C_s is the concentration of the molecule in a thin saturated liquid film (boundary layer) adjacent to the solid surface, and C_x is the concentration in the surrounding bulk liquid medium.

The two factors that lead to the observed size-effects in molecular dissolution are as follows:

(1) The total surface to volume ratio of a given mass of matter, and

(2) The concentration gradient, $\frac{C_s - C_x}{h}$

We shall take a closer look at each one of these factors.

2.1. Role of Surface to Volume Ratio of a Given Mass of Solid Matter: The Noyes-Whitney equation (1) clearly indicates that the rate of dissolution of a molecular solid in a liquid medium is directly proportional to the surface area of the solid particle that is exposed to the liquid medium. For a given volume of solid mass, the smaller the size of the particles comprising the solid mass the greater is the total surface area of the solid mass exposed to the liquid medium. Let us consider the following example to get a feeling for the magnitude of the enhancement in the surface area and correspondingly for the magnitude of enhancement in the rate of dissolution that is possible by size reduction of solid particles to the nanometer scale.

Example: Consider a spherical particle that has a diameter of 10 μm. Its surface area is 3.142×10^{-10} m^2. Let us break this particle into spherical fragments where each particle has a diameter of 10 nm. We get 1×10^9 particles. The total surface area becomes 3.142×10^{-7} m^2 leading to an increase in the surface to volume ratio by three orders of magnitude causing in turn an increase in the dissolution rate of the given mass of solid by three orders of magnitude.

2.2. Role of the Concentration Gradient in Molecular Dissolution: It has been shown by Mueller and Boehm [5] that, for spherical particles, the quantity C_s increases as the size of the particle decreases. Additionally, they have mentioned that the quantity *h* decreases as the size of the particle decreases. This leads to the situation described by equation (2).

$$\frac{C_{sN} - C_x}{h_N} \gg \frac{C_{sM} - C_x}{h_M} \quad (2)$$

Where C_{sN} represents the quantity C_s for a nanometer-scale particle, which may be of diameter in the range from 10 nm to 100 nm. C_{sM} represents the quantity C_s for a micrometer-scale particle, which may be of diameter in the range from 40 μm to 1000 μm (which is the size range of normal pharmaceutical powder [1]). The quantity h_N is the

thickness of the diffusion layer for a nanoparticle of the dimensions indicated above and h_M is the thickness of the diffusion layer for a microparticle of the dimensions indicated above.

Since both the increase in surface area as well as the increase in the concentration gradient contribute to the increase in the rate of dissolution of molecules from a given mass of molecular solid, it is clear that reducing the particle size (to the nanometer range) of powders comprising a given mass of NCE/drug will increase the rate of dissolution (or dissolution velocity to use a synonym) by several orders of magnitude.

3. Nanostructures that can Incorporate NCEs/Drugs

We have looked at factors that indicate that the reduction of the particle size of a substance to the nanometer-scale can enhance the rate of aqueous solubility of the substance considerably. In fact, it has been shown in the case of many poorly-water soluble molecules that incorporating them in suitable nanostructures can increase their rates of dissolution to extents that have saved these molecules from oblivion [8].

The following nanostructures may be used to deliver poorly-water soluble molecules:

(1) Nanoemulsions,
(2) Nanosuspensions, and
(3) Nanoscale NCE/Drug-Polymer Composites.

In this section, we shall examine some of the characteristics of materials (1) and (2). We shall discuss material (3) in a subsequent section of this report when we consider an example.

3.1. Nanoemulsions: An oil/water (o/w) emulsion is a dispersion of droplets of oil in water with which it is immiscible. When the size of the oil droplets is in the nanometer range the emulsion is referred to as a nanoemulsion. An appropriate surfactant or an appropriate combination of surfactants is added to the system in order to provide stability to the o/w emulsion. Figure 1 shows a schematic of an o/w emulsion particle. The poorly-water soluble NCE/drug is accommodated in the oil phase. For this technique to work, the poorly-water soluble NCE/drug has to be solubilized in the oil phase. O/w nanoemulsions are advantageous from both formulation and business perspectives.

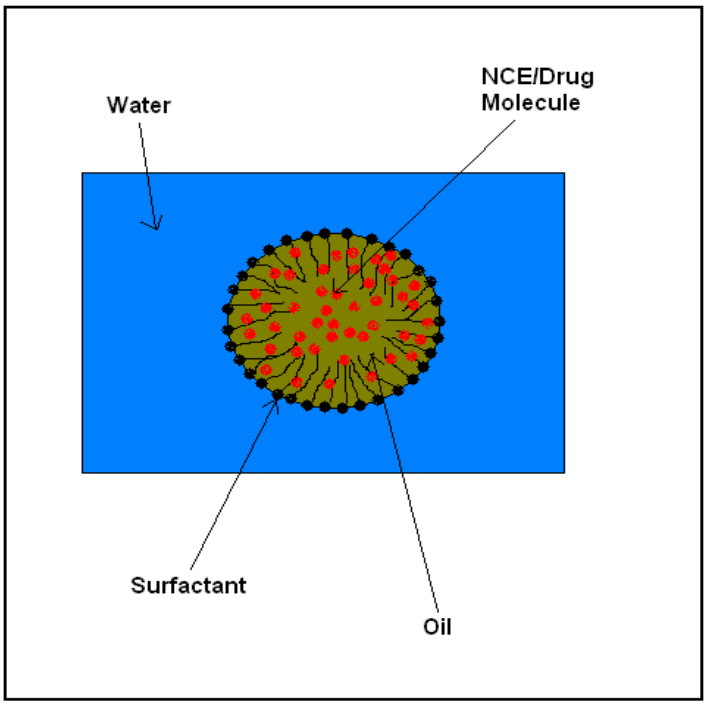

Figure 1: A schematic diagram of an NCE/drug containing o/w nanoemulsion particle. A surfactant shell encloses an oil droplet in which the NCE/drug has been solubilized

From a formulation perspective they are advantageous in that they are biodegradable, biocompatible and relatively easy to produce on a large scale [9]. According to Fresenius Kabi Clayton L. P. [10], from a business perspective, the advantage that they provide are the following:

'Business advantages for using emulsions for using emulsions for the drug delivery of poorly aqueous soluble compounds in contrast to nanoparticulate or cyclodextrin encapsulation technologies is that emulsion technology is non-proprietary.'

Examples of nanoemulsions taken from ref. 9 include the following:

(1) Diazemuls (diazepam emulsion)
(2) Diprivan (propofol submicron emulsion), Zeneca, UK
(3) Fluosol
(4) Etomidat Lipuro (etomidate emulsion)
(5) Liple (alprostadil emulsion)
(6) Limethason (dexamethasone palmitate emulsion)

3.2. Nanosuspensions: Nanosuspensions are made up of solid NCE/drug particles dispersed in a liquid medium (which in pharmaceutical applications is usually an aqueous medium). The surfaces of these particles are suitably derivatized in order to prevent aggregation. Figure 2 shows a schematic diagram of a dispersed-phase particle in a nanosuspension.

Following are the advantages of using a nanosuspension formulation:

(1) Since the dispersed-phase consists of the solid drug and not the drug incorporated in a matrix material, greater drug loading is possible,
(2) The avoidance of a polymeric or lipidic matrix material reduces the type and amount of excipients in the formulation. Any adverse affects that may arise from the use of such excipients can be avoided, and
(3) A suitably stable crystalline form of the drug can be developed which would lead to predictable pharmacokinetic behavior.

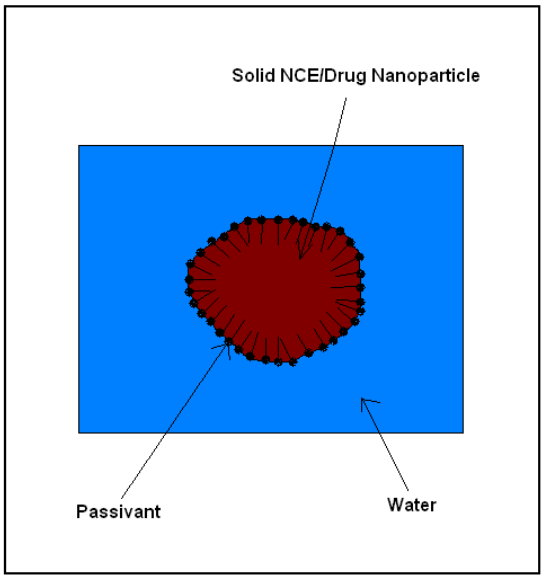

Figure 2: Schematic diagram of the dispersoid-phase (solid NCE/drug nanoscale particle whose surface has been suitably derivatized) of an NCE/drug nanosuspension.

Examples of nanosuspension based formulations in the market or that are in various stages of development taken from ref. 8 are as follows:

(1) Rapamune (Elan Nanosystems/Wyeth)
(2) Emend (Elan Nanosystems/Merck)

(3) Silver (NUCRYST)
(4) Paclitaxel (American BioScience/American Pharmaceutical Partners)
(5) Busulfan (SkyePharma/Supergen)
(6) Budesonide (Elan Nanosystems/Sheffield Pharmaceuticals)

4. Method for the Preparation of NCE/Drug-Containing Nanoemulsions and Nano-suspensions

There are several different methods to prepare drug-loaded nanoemulsions. In what follows, I discuss one such method which may be modified and/or extended to prepare nanosuspensions and solid lipid nanoparticles. This method works when the drug dissolves sufficiently in a suitable oil-phase.

4.1. Preparation of NCE/Drug-Containing Nanoemulsions: One possible scheme for the preparation of NCE/drug-containing nanoemulsions is described. In this process, the first step is to prepare an o/w emulsion containing micron-sized droplets of the drug-loaded oil phase stabilized by suitable surfactant or combination of surfactants. All chemicals must be pharmaceutically acceptable for the development of pharmaceutical emulsions. This can be achieved from a composition containing the drug-loaded oil, the surfactant or combination of surfactants, and water by treating it with a high-speed homogenizer. Once this has been achieved the next step is to pass the emulsion through a high-pressure homogenizer in which the droplet size of the oil phase can be reduced to the nanometer range in one or more passes.

4.2. Preparation of Nanosuspensions from Nanoemulsions: In this technique the poorly-water soluble NCE/drug is loaded into a suitable oil phase which is then dispersed in the form of nanometer-scale droplets in an aqueous phase using the method described above. The oil phase is then evaporated from the liquid leading to precipitation of the drug within a nanometer-scale space enclosed by the surfactant or combination of surfactants. Since the starting emulsion contained oil droplets dispersed in water, when the chemical constitutents are appropriately chosen, the precipitated drug nanosolids passivated by the surfactant or combination of surfactants also remain dispersed in water yielding a nanosuspension.

An important factor to note here is that the surfactants used must adhere strongly to the precipitated nanosolids to give stability to the nanosuspension. This depends upon the particular NCE/drug and surfactant combination. Is there a general solution to this problem? It turns out that one of the methods may be to dissolve the NCE in a molten lipid material (that is a solid at room temperature) and to disperse it in an aqueous surfactant solution. Then an emulsion is formed by high-speed stirring which is passed through a high speed homogenizer to form an o/w nanoemulsion. The hot o/w nanoemulsion is then cooled down to room temperature to cause recrystallization of the lipid material. The dispersed nanosolid thus formed is covered with either surfactant or the combination of surfactants used and contains the NCE/drug molecule. The solid material may be one in which the drug is molecularly dispersed or dispersed as nanometer-scale particles within a matrix formed by the chosen lipid. In the selection of the lipid material it is ensured beforehand that the lipid material and the surfactant (or combination of surfactants) have the requisite bonding characteristics. The advantage here is that the same lipid matrix may support a variety of drugs, and when the formulation is developed a previously optimized lipid matrix-surfactant combination can be chosen. In fact, such a system is known as a Solid Lipid Nanoparticle (SLN) and has been extensively discussed in the literature [6].

5. Characterization of the Drug Containing Nanostructures

We are interested in obtaining the particle size distributions of drug containing nanostructures, a measure of the stability of nanoemulsions and nanosuspensions, as well as in obtaining a picture of the form of the drug-containing nanostructures.

In order to achieve the desired outcomes, as described above, a number of characterization techniques are employed. I shall list them and briefly examine each technique in the context of the study of nanostructured drugs. The techniques are as follows:

(1) Powder X-Ray Diffraction (PXRD)
(2) Dynamic Light Scattering
(3) Zeta Potential Measurement
(4) Scanning Electron Microscopy
(5) Transmission Electron Microscopy
(6) Atomic Force Microscopy, and
(7) Differential Scanning Calorimetry.

5.1. PXRD: Powder x-ray diffraction [11] is a useful technique that has been applied for studying the characteristics of both NCE/drug solids as well as that of the solid matrix material. Firstly, using this technique it can be ascertained whether these materials are crystalline or amorphous. Secondly, in the case of nanostructures that comprise a matrix containing the NCE/drug, it can be used to ascertain whether the NCE/drug is present in the matrix as solid precipitates or whether it is molecularly dispersed. Thirdly, when the particle size of the crystalline component of the drug-containing nanostructures is in the range from 10 nm to 100 nm, an estimate of the particle size can be obtained from the line widths of the x-ray diffraction peaks of the material in question by using the Scherrer equation [11].

5.2. Dynamic Light Scattering: Dynamic Light Scattering [12] is a useful technique for obtaining the size of particles in nanoemulsions and nanosuspensions. Since the technique may not be familiar to everybody, I shall briefly touch upon how it operates.
Using a laser as a source of light, light is made to scatter from a given volume of the sample, which may be either a nanoemulsion or a nanosuspension. Due to the Brownian motion of the particles in the nanoemulsion or nanosuspension, the scattering intensity fluctuates with time. This signal is processed with a digital autocorrelator to yield the autocorrelation function in real time. The decay constant in the normalized autocorrelation function is a function of the particle's Brownian diffusion coefficient. From this, the particle size is calculated using the Stokes-Einstein equation. There are many manufacturers who supply instruments that can measure particle size distributions in the range from 1 nm to 6 μm.

5.3. Zeta Potential Measurement: Zeta Potential, ζ, is defined as the potential difference between the surface of the tightly bound layer (shear plane) and the electroneutral region of the solution [13]. As discussed by Martin, ζ determines the degree of repulsion between adjacent, similarly charged dispersed particles. The value of this quantity provides a measure of the stability of colloidal systems such as nanoemulsions and nanosuspension formulations. Klang and Benita [9] have pointed out that, in most cases, in order to formulate stable emulsions, high values of the zeta potential (values above 30 mV) must be achieved. As may be discernable, this is to ensure that there is a sufficiently high-energy barrier that prevents the flocculation of droplets.

5.4. Scanning Electron Microscopy: Scanning Electron Microscopy [14] can, in principle, be used to image solid NCE/drug-containing nanostructures that are of a size greater than the instrumental resolution. In modern instruments the instrumental resolution is as good as a few nanometers (say between 2 to 5 nm). Under favourable conditions, it should be possible to obtain information about the morphology, the spatial and size distribution of the particles.

5.5. Transmission Electron Microscopy [15]: Nanometer-scale objects usually permit the electron beam in a transmission electron microscope to pass through the sample. In addition, those samples that scatter part of the beam sufficiently provide the desired level of contrast when compared to the background and can, therefore, be imaged. Because of the differences in the scattering of the electron beam by the drug solid and the matrix material, imaging of the constituents can be done and information about the particle morphology, size and spatial distributions of the

particles can be obtained. Additionally, electron diffraction can be performed in a transmission electron microscope and this information can be used to determine the crystalline or amorphous nature of the components of the solid drug-containing nanostructures.

5.6. Atomic Force Microscopy [16]: Developed just after Scanning Tunneling Microscopy had been invented, Atomic Force Microscopy (AFM) is a suitable technique for imaging the surface of insulating materials that may be soft as well. When drug-loaded nanostructures are immobilized on a suitably flat surface, AFM may be used to obtain images of individual or aggregates of drug loaded nanostructures. This information may be useful to obtain the particle size distribution of drug-containing nanostructures. When the convolution of the tip with the sample does not obscure the image, images where morphologies (of drug-containing nanostructures) are visible can also be obtained. In the case where the shape of the particles may be obscured by the convolution of the tip with the sample, suitable deconvolution procedures may be used.

5.7. Differential Scanning Calorimetry [17]: Differential Scanning Calorimetry (DSC) is a tool that can be used to study the melting and recrystallization behavior of solid materials [6]. For a particular crystalline material the decrease in the onset and the maximum temperature of melting can be attributed to the small size effects (the fact that the particle size of the material is in the nanometer range). Further, the presence of impurities can be examined from the DSC thermograms. Let us take the example of a solid lipid nanoparticle here. DSC can provide evidence to distinguish between the two cases of the drug being dispersed molecularly and as particles. In addition, the melting enthalpy of the material can be used to provide support for the degree of disorder present within the material [6].

6. Example: NCE-Polymer Nanocomposites: By spray drying a given NCE and water-soluble polymeric material we were able to prepare a powdered material that contained NCE particles. Evidence in support of this is provided by PXRD as shown in Figure 3.

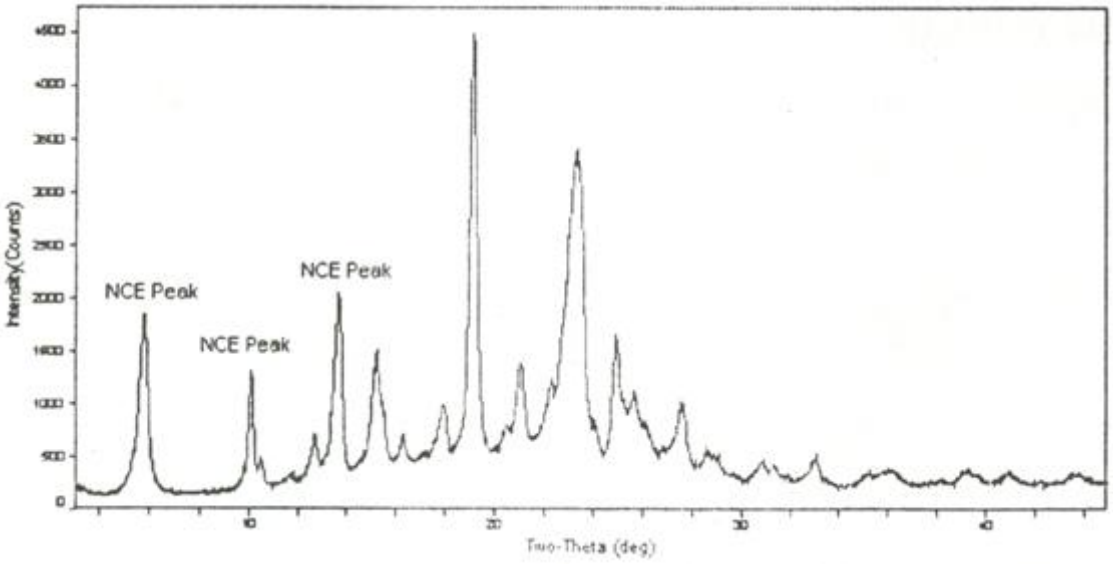

Figure 3: X-ray diffractogram of the powdered material containing the NCE and the water-soluble polymer. Some of the peaks due to diffraction from nanocrystalline NCE particles have been identified in the figure.

We used the Scherrer equation to estimate the particle size to lie in the range from 14nm to 50 nm. In addition to PXRD experiments we performed dissolution experiments of the powdered material in aqueous media. We found that this powdered material disintegrates in aqueous media but under the time-scales of our experiment it does not release the NCE into aqueous media in any appreciable amounts. However, upon the use of a suitable chemical agent we find that we are able to solubilize the NCE in aqueous media. Based on our experiments, the following

three observations can be made which support the contention that the prepared powders are composites containing nanometer-scale crystalline dispersoids of the NCE in a water-soluble polymer matrix:

(1) The size of the NCE particles being in the nanometer range as indicated by PXRD studies.
(2) The fact that the prepared powder disintegrates in aqueous media but does not release the NCE into water in an unaided manner.
(3) The fact that the NCE can be solubilized in aqueous media by the utilization of a suitable chemical agent.

Clearly, observation (1) indicates that the NCE is in the nanometer size range. Observations (2) and (3) support the contention that the NCE particles are trapped in a polymeric matrix. The three observations put together support the contention that we have prepared a composite containing nanocrystalline dispersoids of the NCE in a polymeric matrix. A schematic diagram of this material is shown in Figure 4.

The fact that the NCE is crystalline and not amorphous is advantageous from stability perspectives where reproducible pharmacokinetic behavior can be expected under conditions of long-term storage. If the NCE were amorphous then under storage it may become crystalline, thereby, resulting in different pharmacokinetic behavior that the one based upon which the formulation was optimized.

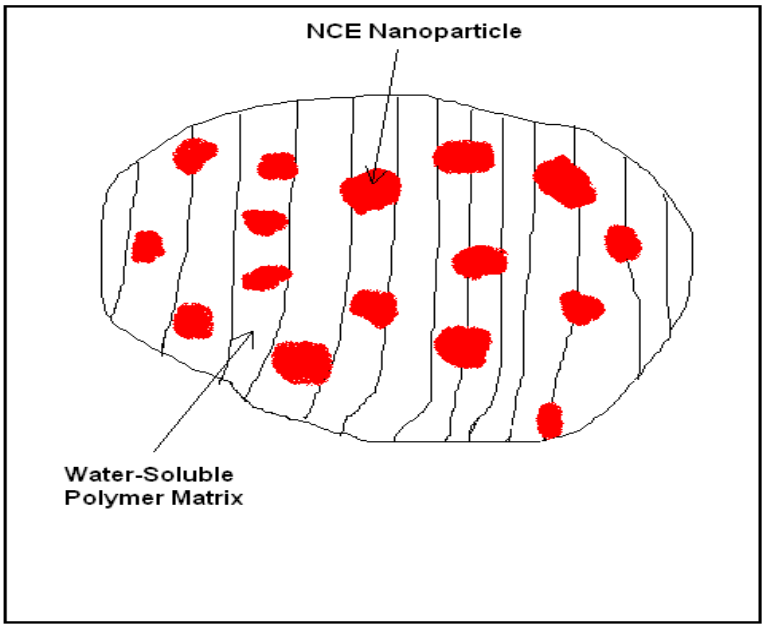

Figure 4: Schematic diagram of a composite particle containing nanocrystals of an NCE in a water-soluble polymer matrix.

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