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REVIEW ARTICLE

Nanosuspension Technology: Recent Patents on Drug Delivery and their Characterizations

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Abstract: ***Background*:** Nanosuspension has arisen as a remunerative, lucrative as well as a potent approach to improve the solubility and bioavailability of poorly aqueous soluble drug entities. Several challenges are still present in this approach which need more research. The prime aim of this review is to identify such challenges that can be rectified in the future.

***Methods*:** Through this review, we enlighten the recent patents and advancement in nanosuspension technology that utilize the different drug moieties, instruments and characterization parameters.

***Results*:** Nanosuspension has been found to possess great potential to rectify the several issues related to poor bioavailability, site-specific drug delivery, dosing frequency, *etc*. In the past decade, nanosuspension approach has been complementarily utilized to solve the developed grievances, arisen from poorly soluble drugs. But this field still needs more attention to new discoveries.

***Conclusion*:** Nanosuspension contributes a crucial role in administering the different drug entities through a variety of routes involving oral, transdermal, ocular, parenteral, pulmonary, *etc*. with solving the different issues. This review also confirms the significance of nanosuspension in safety, efficacy, and communal as well as the economic expense associated with healthcare.

Keywords: Nanosuspension, drug moieties, bioavailability, solubility, dosing frequency, nanosuspension technology.

1. INTRODUCTION

Solubility, as well as bioavailability, have found major critical hindrance that affects the fabrication of new pharmaceutical product especially if the drug belongs to BCS class II [1]. In order to achieve a product having sufficient bioavailability, the pharmaceutical researchers are persistently involved in finding new ways and around more than 1/3rd drugs have been reported to exhibit poor aqueous solubility. A copious number of newly developed drugs demonstrate bioavailability problems because of their diminished water solubility. This has arisen as a major trouble for the developers. Conventional solubility enhancement approaches possess limited applicability, especially if a drug is poorly soluble in both aqueous and non-aqueous solvent. Thus, nanosuspension technology has proven a unique and lucrative approach to improve the bioavailability of poorly soluble drugs. Nanosuspension is dispersion of very fine colloidal solid drug particles which are biphasic in nature, in an aqueous vehicle and stabilized through surfactants. These are simple to prepare and more advantageous than other techniques. Nowadays, nanosuspension has become an integral part of nano-carriers by a dint of their several advantages such as reduced toxicity, improved bioavailability, targeted drug delivery to a specific site, reduced dosing frequency, sustained and controlled release effects, high patient compliance, better stability, ease of administration through different routes, *etc.* [2]. The acceptable particle size range of pharmaceutical nanosuspension is less than 1 µm with an average range between 200-600 nm [3]. This can be achieved through a number of approaches such as bottom up or top down methods, *etc.* The top-down methodology includes the conversion of large particles into fine particles [4-6] by using different techniques like high-pressure homogenization, media milling [7-9]. However, this approach is quite expensive and has the probability of heavy metal contamination [10, 11]. The bottom-up approach involves the dissolution of the drug into the solvent system after which drug precipitation is done through the introduction of anti-solvent [12, 13]. Different techniques may be used to fabricate nanosuspension either alone or in combination such as media milling, nanoedge or high-pressure homogenization, nanopure or precipitation method and several other combinational methodologies which may solve the problems arising from the hydrophobic drugs.

1.1. Advantages and Need of Nanosuspension

Nanosuspension has several special features that can be used as a potential approach to deliver the drugs, and are enlisted below [14, 15].

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- Reduced particle size, enhance dissolution rate, enhanced rate, and extent of absorption.
- Long-term physical stability.
- Drugs having a high log P value can be formulated as nanosuspensions in order to enhance their bio-availability.
- Nanosuspension can be formulated with compounds insoluble in water but exhibit solubility in oil.
- The pharmaceutical nanosuspension can be delivered through various routes such as oral, topical, parentral, ocular, pulmonary, *etc.* In fact, nanosuspensions can be incorporated in tablet, pellet, hydrogel, and suppositories.
- Drug nanosuspension can provide passive targeting of the drug.
- It can Improve the *in vivo* performance due to high dissolution rate and saturation solubility of the drug,
- Ease of manufacturing and scale-up for large scale production.
- The possibility of surface-modification for site-specific drug delivery.
- The nanosuspension technology can increase the amorphous fraction in the particles that may lead to a potential change in the crystalline structure and solubility.

1.2. Limitations of Nanosuspension

Besides the several advantages, nanosuspension may be subjected to physical instability due to an elevation in the sedimentation rate of dispersed nanoparticles during storage. This may arise as a major problem with nanosuspension, although this crunch can be rectified by the use of suitable polymers. Pharmaceutical nanosuspension may also be subjected to wear and tears during handling or transportation due to their bulkiness. Hence, they require sufficient care during handling & transportation.

2. RECENT ADVANCEMENT IN NANOSUSPENSION WITH UTILIZED DRUGS

A copious number of nanosuspensions have been developed successfully with different drug candidates by using a variety of approaches. As a large number of drugs exhibit poor aqueous solubility, the main motto to design and develop these nanosuspensions is to improve the solubility as well as the bioavailability of the Active Pharmaceutical Ingredient (API). This review has focused on various recent advances in nanosuspension technology, summarized briefly in Table **1**.

Table 1. Nanosuspension formulations with utilized drug moieties.

Drug	Type of Studies	Particulars	Refs.
Valsartan	*In vitro* studies	The prepared valsartan nanosuspension indicated high drug solubility in hypromellose. The FTIR data reflected no interaction between the drug and polymer.	[16]
Aceclofenac	*In vitro* studies	Prepared nanosuspension of aceclofenac showed quick release behaviour. The formulation demonstrated better solubility and fast dissolution rate in comparison of marketed preparations for effective treatment of pain.	[17]
Diacerein	*In vitro* studies	Diacerein nanosuspension reflected high dissolution rate and solubility and around 400 times increased saturation solubility of the bulk drug in comparison to free drug in order to effectively treat osteoarthritis.	[18]
Metformin	*In vitro* studies	The formulation utilized a high ratio of stabilizer & polymer that induced nanosuspension with fine particle size, better release rate, and encapsulation efficiency. The mean particle size was estimated at 399 nm.	[19]
Ezetimibe	*In vitro* studies	In this research, the optimized formulation exhibit the 99% of drug release in 25 minutes for efficaciously treatment of hyperlipidemia.	[20]
Celecoxib	*In vivo* and *in vitro* studies	Fabricated nanosuspension of celecoxib possessed enhanced solubility and *in vitro* dissolution rate. The *in vivo* pharmacokinetic data also revealed enhanced oral bioavailability of celecoxib in rats.	[21]
Zerumbone	*In vitro* studies	*In vitro* data of zerumbone nanosuspension, which was prepared with hypromellose (HPMC), demonstrated increased solubility as well as the rate of drug dissolution.	[22]
Bifonazole Nitrate	*In vitro* studies	The topical formulation of nanosuspension reflected enhanced saturation solubility with controlled release rate. The developed formulation also reduces the side effects of Bifonazole like burning and itching.	[23]
Tinidazole	*In vivo* and *in vitro* studies	The prepared tinidazole nanosuspension showed better stability, palatability with increased dissolution rate and oral bioavailability.	[24]

Table (1) contd….

Drug	Type of Studies	Particulars	Refs.
Azoxystrobin	*In vitro* studies	Azoxystrobin nanosuspension exhibited better anti-fungal activity, high cell wall permeability, reduced toxicity up to 1.7 times than other marketed preparations.	[25]
Naringenin	*In vitro* studies	Developed nanosuspension reflected the increased *in vitro* dissolution rate and oral bioavailability of naringenin with diminished adverse effects.	[26]
Cefdinir	*In vitro* studies	The fabricated cefdinir nanosuspension displays enhanced rate of release of a drug that results in the improvement of antibacterial efficacy of cefdinir.	[27]
Silymarin	*In vitro* studies	The optimized formulation confirmed improved dissolution and *in vitro* release parameters of silymarin.	[28]
Nifedipine	*In vivo* and *in vitro* studies	Nifedipine dispersed nanosuspension reflected the enhanced *in vitro* drug release with greater bioavailability that was found up to 2 times as compared to conventional formations.	[29]
Clopidogrel	*In vitro* studies	The optimized formulation of Pluronic F-127 entrapped clopidogrel nanosuspension demonstrated increased dissolution rate up to 2 times in 0.1 N HCl and 10 times in pH 6.8 phosphate buffer in comparison to conventional suspensions.	[30]
Fisetin	*In vitro* studies	Polyvinyl alcohol containing fisetin nanosuspension showed better stability and dissolution rate with a mean particle size of 406 nm.	[31]
Rosiglitazone	*In vitro* studies	The formulated nanosuspension demonstrated the improvement in solubility and site specificity of the rosiglitazone.	[32]
Lafutidine	*In vitro* studies	The developed lafutidine nanosuspension appeared as an effective one that indicated enhanced dissolution rate and oral bioavailability of drug to treat ulcers.	[33]
Meloxicam	*In vivo* and *in vitro* studies	Bovine serum albumin facilitated meloxicam nanosuspension reported the enhanced half-life, better bioavailability of meloxicam than the free drug.	[34]

3. RECENT PATENTS ON NANOSUSPENSION TECHNOLOGY

In the past decades, nanosuspensions have been investigated for their potent applications in drug delivery systems and it has been observed that nanosuspensions possess the capability to enhance the bioavailability and effectiveness of drug candidates. Various patents have been granted over nanosuspension technology. Table **2** demonstrates an overview of these patents.

The patent WO2016135753 is related to the method used to develop a topical nanosuspension. Such nanosuspension contains the water-soluble active ingredient or its salt, with a wetting agent and a non-aqueous solvent system. This non-aqueous nanosuspension was further converted into a topical nanosuspension [35].

The patent WO2016081593 describes a pharmaceutical nanosuspension that comprises the therapeutically active moiety such moiety may be an active nutraceutical ingredient that has poor solubility. The patents indicate that at least one alginate moiety was selected *i.e.* either sodium alginate or potassium alginate for developing the nanosuspension [36]. Another US patent 20160317534 demonstrates the nanosuspension of a freeze-dried (lyophilized) drug. This freeze-dried drug nanosuspension was found to possess sufficient stability for long term storage [37].

US patent 20160206577 describes a method used for the fabrication of nanosuspension of an antibacterial moiety. This patent indicated that the developed formulation has better stability and lower toxicity [38].

US patent 20150238446 is linked with the stable nanosuspension that possesses a chitin synthesis inhibitor particularly hexaflumuron, which is used as an injectable in order to control sea lice [39].

Chinese patent 105708844 provides information about a developed method of ophthalmic nanosuspension. In this method, tobramycin and dexamethasone were used as active medicament. The developed process of such nanosuspension was found to be reproducible, effective, stable and convenient for use [40].

Another Chinese patent 105315249 is related to the development of the nanosuspension method using the simvastatin drug candidate. The researchers reported that the method enhanced efficiency of drug delivery system [41]. As one more Chinese patent 105534947 describes the manufacturing method that is suitable for developing the nanosuspension capsule of celecoxib, which was converted into the solidified powder through freeze-drying [42].

Chinese patent 104814926 demonstrates the nanosuspension of lurasidone that was fabricated through the combination method of nano-precipitation and high-pressure homogenization. This method was found suitable for enhancing bioavailability as well as stability of the drug [43].

US patent 9023886 is related to the development of nanosuspension using the poorly soluble drug. In this patent, the researchers used the microfludization technique for the development of nanosuspension. The prepared nanosuspension was found to be suitable to use with better bioavailability [44].

Table 2. Recent patents on nanosuspensions.

Patent/ Application Number	Publication/ Application Date & Year	Patent Description	Refs.
WO2016135753Al	Sept. 1, 2016	This patented work involves the development of a methodology for topically utilized nano-suspension through the process of milling.	[35]
WO2016081593Al	May 26, 2016	The patented invention describes the nanosuspension fabricated with a therapeutically active moiety. Such moiety is an active nutraceutical having poor solubility profile.	[36]
US20160317534A1	Nov. 3, 2016	This patent gives information about a nanosuspension prepared with the lyophilized drug. Such nanosuspension possessed sufficient stability during long-term storage.	[37]
US20160206577	Jul. 21, 2016	This patented study reflects the method of fabrication of nanosuspension of an antibacterial moiety that improves the stability and reduced toxicity of the drug.	[38]
US20150238446A1	Aug. 27, 2015	The researchers reported the development of stable hexaflumuron nanosuspension that can be injected into fishes for controlling sea lice.	[39]
CN105708844A	June 29, 2016	This patented work describes the development method of ophthalmic nanosuspension of tobramycin & dexamethasone. The process was found to be reproducible, effective, stable and convenient.	[40]
CN105315249A	Feb. 2, 2016	This patent is related to the development method of simvastatin nanosuspension to enhance the efficiency of drug delivery systems.	[41]
CN105534947A	Feb. 16, 2016	The patented work involves a method of developing a celecoxib nanosuspension capsule which can be converted into solidified powder through freeze drying.	[42]
CN104814926	Aug. 5, 2015	This invention stated that the nanosuspension of lurasidone was fabricated through the combination of nano-precipitation and high-pressure homogenization method.	[43]
US9023886B2	May 5, 2015	The patented invention demonstrates the formation of nanosuspension of poor water soluble drug through microfludization technique.	[44]

4. CHARACTERIZATION APPROACH/ TECHNOLOGIES FOR NANOSUSPENSION

Evaluation of nano-materials including nanosuspension is quite significant for knowing their unique characteristics as well as applications. These techniques are helpful for an efficient comparison among formulations and for the development of an optimized product. As each technique exhibits its own strengths and limitations, the choice of appropriate characterization approaches is a matter of perplexity for researchers and technologists. To overcome these challenges, some of the reliable techniques are required with sufficient reproducibility. Various evaluative techniques that can be efficiently utilized for fabricating the optimum quality products are described in this review article.

4.1. Energy Dispersive X-Ray Spectroscopy (EDX)

Energy dispersive X-ray spectroscopic approach is generally used for chemical or elemental analysis of the sample. EDX is based on the principle that each material exhibits a different atomic structure that allows the emission of X-rays from it. This spectroscopy involves the interaction between electromagnetic radiations and material so that X-rays emitted from the material after charged particles collide [45, 46]. The highly focused beam of electrons/ protons is radiated to the sample which excites the electrons of lower energy level so that they migrate to higher energy shells. This migration of electrons creates the electron holes at the place where the electrons exactly were, which was filled by the electrons of higher energy shells. This difference in energy between the higher and lower energy shell shall be released in the form of X-ray. The extent of X-ray emitted from the sample would be measured through the dispersive spectrometer. By determining the energies of X-rays emitted from a specific area excited by the electron beam, the elements present in the sample can be estimated.

There are some limitations in EDX by which such technology cannot be used extensively to characterize the samples. The elements that do not have sufficient electrons to produce characteristics X-rays, cannot be analyzed using this technology such as; the first three elements of the periodic table; hydrogen, helium and lithium do not possess sufficient electrons to produce characteristics X-rays and hence they are not detectable by EDX. Additionally, many elements show overlapped peaks in EDX spectra due to the low spectral resolution.

4.2. Particle Size Analyzer

Particle size is a crucial parameter for the evaluation of nanoparticles of nanosuspension. Particle size analyzer is an ideally favourable technique utilized for the determination of particle size in nanometer (nm) range. For this purpose, the series of Malvern zeta analyzer is exclusively used that exhibits high sensitivity to estimate the minute particle size of the sample even in nano-range that possess low concentra-

tion [46]. In fact, backscatter optics help in determining the particles with a high concentration in comparison to other conventional equipment. Dynamic Light Scattering (DLS) approach is a widely employed technique for estimating the dispersed nanoparticles of nanosuspension.

It is difficult to accurately quantify the amount of any aggregates present in the sample. So, the fractions of size cannot be correlated with a specific composition. This is the main limitation associated with DLS instrument. This technique also requires the refractive index of the solvent. The sample has a mixture of nanoparticles, and analysis is weighted towards the more refractive particles. Additionally, the average particle size is intensity-based on the large or contaminant particles within a sample.

4.3. Scanning Electron Microscopy (SEM)

Scanning electron microscopy is extensively employed as a characterization technique for nanosuspensions especially for morphology and surface characterization of particles. The nanoparticles of nanosuspension are turned into a dry form for SEM characterization *i.e.* mounted on a sample holder coated with a metal like gold for high conductivity. SEM provides images of the high resolution of a sample surface, can produce the details about particles having a size in less than 5 nm. In SEM, an electron gun produces a finely focused beam of electrons passing through the electromagnetic lens and rapidly moves over the surface of the specimen that causes the release of secondary electrons from the surface of the specimen. The surface characteristics can be examined through these secondary electrons emitted from the surface. The intensity of these secondary electrons depends on the shape and chemical composition of the object. This equipment works very fast, often complete Secondary Electron Detector (SED), and X-Ray Detector (EDS) analysis in less than five minutes. In addition, modern SEM allows the generation of data in digital form.

However, this instrument is quite expensive and needs complementary information regarding the distribution of size [47]. Its maintenance requires a steady voltage, current to electromagnetic coils as well as circulation of cool water. In fact, special training is essential for operating SEM and preparing the samples. Additionally, SEM is limited to solid inorganic samples that should be small enough to fit inside the vacuum chamber that can handle sufficient vacuum pressure. This equipment possesses a small risk of radiation exposure.

4.4. Transmission Electron Microscope (TEM)

Transmission electron microscopy is the utmost powerful characterization technique with high magnification utilized for predicting the morphological characters and surface examination of the samples. It uses a different working principle than SEM but provides similar data. Bright field mode and dark field modes are used for imaging materials to the nanometer scale. The working principle behind the TEM is that when the finest beam of electron passes through the thin section of the specimen, electrons start to scatter and are transmitted through the object. The beam passes through the object lens, which magnifies images and makes diffraction in the back focal plane and the image of the sample in the image plane. Some intermediate projecting lenses re-magnify the image and project it on fluorescence screen. TEM provides high-quality images with detailed information. The instrument is able to yield information about surface, shape, size and structure.

The main limitation of this equipment is the size of equipment, expensiveness and sample preparation takes much time as the ultra-thin section of the specimen is favourable. TEM does not provide colourful images as it provides black and white images. Like SEM, it also requires maintaining voltage, current to electromagnetic coils and circulation of cooling water. Additionally, the handling of the instrument needs special training. The samples which are electron transparent, able to tolerate vacuum chamber and sufficiently small to fit in the chamber can only be analyzed by transmission electron microscopy.

4.5. X-Ray Diffraction (XRD)

X-ray diffraction is a suitable analytical approach to get valuable insight regarding crystalline structures, the lattice structure of a crystalline material, nature of phase and crystallographic structures of both natural and synthesized substances. XRD is based on the dual nature of X-Rays *i.e.* wave or particle. It relies upon characteristics secondary rays emitted from substances which when excited by a high X-ray energy source are primarily utilized to predict specific elements in compounds. In X-ray diffraction, the electromagnetic radiations are produced from the cathode ray tube, which is then filtered to generate monochromatic rays. The interaction of these incident-derived rays with specimen causes constructive interference when the condition satisfies Bragg's law *i.e.* $n\lambda = 2d\,Sin\theta$. Where λ is the wavelength of incident X-Rays at a certain angle of diffraction (θ) and the distance between atomic layers in a crystal (d) and n is an integer [48].

XRD has a size range limitations as it can more accurately analyze the large crystalline structures in comparison to small ones. The small structures that are available only in trace quantity would not be detected by the equipment, which can result in skewed results. Besides this, the equipment is expensive.

4.6. Thermogravimetric Analysis (TGA)

The thermo-gravimetric analysis is a thermal analytical approach that determines the variation in the mass of substance and composition of stabilizers in a controlled environment. This technology involves the heating of nanosample, degradation, and vaporization of components at different degradation temperatures. After that, the temperature and mutation in mass are recorded by the TGA instrument [49]. It is commonly utilized in research and testing to predict the characteristics of substances such as polymers to deliberating their degradation temperatures, absorbed moisture content of compounds, the extent of inorganic and organic components in materials, decomposition points of explosives, and solvent residues.

TGA require several scans in order to optimize run conditions, which act as the main disadvantage of the instru-

ment. The equipment needs a longer duration to analyze the sample. In fact, an inappropriate choice of parameters may produce artefacts. The equipment is also expensive and large.

4.7. Ultraviolet (UV) Spectroscopy

Ultraviolet spectroscopy or Ultraviolet-visible spectroscopy is widely used to evaluate nano-range substances. It is based upon the Lambert-beer's law which states that when a monochromatic light ray passes through a transparent medium, the rate of intensity of light decreases with the thickness of medium which is directly proportional to the intensity of light. UV spectroscopy utilized as a facile and potential characterizing tool especially for those nanoparticles that exhibit optical characteristics sensitive to agglomeration, size, shape, concentration and refractive index near the surface of nanoparticles. It works through comparing the intensity of radiations reflected from the sample as well as the intensity of radiations pass through reference substance [49].

The main disadvantage of UV spectroscopy is the broad spectrum that can be impossible to use as a chemical analysis tool to figure out content in the unknown sample. In order to use the equipment for concentration analysis, one needs to be in the low concentration region. Secondly, the equipment needs a lot of time to initiate the analysis.

4.8. Zeta Potential

Zeta potential is used to estimate the nature and intensity of the surface charge of the dispersed nanoparticles of nanosuspension. Zeta potential helps in ascertaining their interaction with the biological environment and their electrostatic interaction with bioactive compounds. Zeta potential is the potential difference existing between the surface of solid particles immersed in a conducting liquid and bulk of the liquid. It is estimated in volts (V) or milli-volts. Colloidal or storage stability of dispersed nanoparticles can be predicted through zeta potential. It may also provide information about the nature of material encapsulated or coated on the surface of the particle [50].

4.9. Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS) technique is widely employed an evaluative technique for estimating the particle size of dispersed nanoparticles of nanosuspension. It is well known as photon correlation spectroscopy. The earliest light scattering experiments were introduced by John Tyndall, that evaluated light scattering from colloidal suspensions, where the particles were larger than the wavelength of incident light (λ) [51]. It is based on the principle that when a monochromatic light ray is incident on solution possessing dispersed particles, light scatters in different directions due to varying shape and size of dispersed particles. The intensity of scattered light is recorded, which provides the knowledge on molecular mass and size of the dispersed particles. However, if there are fluctuations in light intensity, the diffusion coefficient (Dt) may be obtained which is related to the hydrodynamic size of particles [52].

The main limitation associated with DLS instrument is that the quantification of aggregates present in any sample. So, the fractions of size cannot be correlated with a specific composition. This technique also requires the refractive index of the solvent. For the sample that possesses a mixture of nanoparticles, the analysis is weighed towards the more refractive particles. Additionally, the average particle size is intensity based on the large or contaminant particles within a sample.

4.10. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry is a thermal analytical technique which involves the measurement of the difference to the extent of heat necessary to increase the temperature of sample and reference as a function of temperature. Both the reference and sample are kept at an identical temperature during the experiment. In DSC, the temperature of the sample holder increases linearly. Lesser amount of material is needed to perform the analysis. DSC is based on the principle that when a sample comes under a physical transformation like a phase transition, high or low amount of heat will be required by the sample in comparison to reference in order to maintain both at the similar temperature and this process depends upon the type of process *i.e.* exothermic or endothermic [53]. This technique is extensively utilized by a dint of its speed, simplicity, availability [54] and quantitative analysis [55].

Besides the various appreciable advantages, some demerits are present in DSC that include the equipment cannot control the rate of experiment, the analysis depends on many parameters, the equipment is very sensitive to any changes, results depend upon the operator and the procedure of standard parameters evaluation is not described precise and has lack of thermodynamic background.

4.11. Atomic Force Microscopy (AFM)

Atomic force microscopy gives utmost high resolution in terms of particle size determination. It involves the utility of probe tip for the physical scanning of a sample of submicron size range [56]. This approach provides the topographical information of the sample depending on the force between the tip and sample surface. On the basis of characteristics of compounds, samples can be analyzed both in contact or non- contact mode. The surface properties are determined by tapping the probe on the surface across the sample in contact mode. While in non-contact mode, the probe levitates upon the conducting surface. The non-conducting samples like polymeric nano-materials, micro-crystals, *etc.* can be easily characterized by this approach that is the main advantage of this technique [57].

The AFM cannot scan images for analysis as fast as an SEM and requires several minutes for a typical scan, which is the major limitation of AFM instrument. There is also a possibility of image artefacts and the images can be affected with nonlinearity, hysteresis as well as the creep of piezoelectric material. Additionally, the AFM image does not display the topography of true sample but rather represents the interaction of the probe with the sample surface.

4.12. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy is another remarkable approach which is broadly employed to analyze the small particles and molecules. This technique provides significant information about the three-dimensional data of structures derived from the X-ray diffraction. In FTIR, the vibration frequencies of a given compound are expected in a particular region which is based on the type of atoms as well as the type of chemical bonds present in it [58]. This technique is extensively used as a potential tool for the characterization of biomolecules [59]. FTIR can be used for evaluating microbial samples because of the availability of economic minicomputers and potent different algorithms for statistical analysis and pattern determination methodologies [60]. It has been realized that infrared spectra of bacteria can be utilized for identification [61] and differentiation [59, 62]. In the past decade, Fourier transforms infrared spectroscopy has arisen as a remunerative and potent approach for analyzing the biological macro-molecules [63] and the complicated biological systems like cells and tissues [64-66].

However, FTIR does not measure spectra; it measures interferograms which are difficult to interpret without first performing FTIR to produce a spectrum. This is a major limitation associated with the instrument. The equipment bears the high initial cost and has adequate maintenance issues. Additionally, the latter arises as the quality of an FTIR spectrum degrades with equipment maladjustment and faster than a grating spectrum.

5. SOME IMPORTANT PATENTS OVER THE CHARACTERIZATION TECHNIQUES

Nano-materials can be characterized on the basis of their morphology, shape, size, surface area, surface charge, *etc.* The characterization can be performed by using several instruments such as Scanning the electron microscope, X-ray diffraction, Zeta size analyzer, Zeta potential, Differential scanning calorimetry, Fourier transform infrared spectroscopy, Transmission electron microscope, Energy dispersive X-ray diffraction, Thermo-gravimetric analyzer, *etc.* Several patents have been taken over such devices by the inventors. Some of these patents on the characterization technologies are summarized in Table **3**.

US patent 7294834 describes a patent that was granted over the Scanning Electron Microscope (SEM) and used for the morphological studies of the sample, efficiently. In developed SEM, the primary electron beam diverts to an angle of 45 degrees just prior to incidence on a specimen and then provides sample images with high magnification [67]. Similarly, US patent 8232523 is related to the method of sample analysis using scanning electron microscopy. The working method of SEM involves focusing of a fine beam of electrons over the surface of the specimen. This provides high magnifying images of specimens and helps in studying their surface morphology [68]. Another US patent 2010/0230590 germane to the compact Scanning Electron Microscope (SEM) that generates high-quality images of the sample for their characterization. The researchers claimed that this compact form of SEM is easy to operate, robust and does not require special utilities. The equipment provides the images of the sample rapidly, after its insertion [69].

US patent 7796726 is associated with the equipment of X-Ray Diffraction (XRD) and X-ray fluorescence. The patented work also described the analysis methods of X-ray diffraction without the preparation of the samples. The X-ray source and the photon counting X-ray spectrometer are arranged in a reflection geometry which is related to the predetermined coordinate [70]. Another US patent 5491738 deals with the X-ray diffraction apparatus based on a Charge-Coupled Array (CCD) which is used to predict the X-ray diffraction pattern of the sample. The equipment uses a source of the beam for generating a collimated X-ray beam, a sample holder and a charged coupled device for detection [71]. One more US patent 7564947 was based on a topographic energy dispersive X-ray diffraction instrument which consists of a radiation Source, and energy dispersive detectors that possess collimators. According to inventors, this equipment can be utilized as a better imaging tool than conventional devices [72].

US patent 8431897 demonstrates the Transmission Electron Microscope (TEM) that utilizes optical fibres as optical guiding media. The equipment obtains high-resolution images for better characterization of the sample. It was also found that the microscope provides high angle scattering pictures by radiating at the surface of the specimen [73]. Another US patent 2012/0120226 related to the Transmission Electron Microscopy (TEM) was employed to produce images of living cells in their native liquid surrounding using a microfluidic chamber with electron transparent windows [74].

US patent 5059909 reflects a method used for estimating the particle size and zeta potential of dispersed particles in a bulk liquid. According to this patent, the particle size and surface charge can be estimated using the equipment by measuring the amplitude of particle viscosity as well as the phase lag between an applied electric field and particle viscosity [75].

The patent WO2014/039376 is associated with the operating technique for a quasi-adiabatic differential scanning calorimeter to diminish the leakage of heat flow rate. The equipment is based on the principle that the temperature of equipment is independent of the temperature of a measuring system which causes the difference of temperature between the sample and reference container and hence diminishes the leakage of heat flow rate [76]. Another US patent 6561692 demonstrates a modulated differential scanning calorimeter and its method of utilization that provides more accurate information about the heat flow with better resolution in less time interval [77].

US patent 2004/0141541 is associated with the thermogravimetric analysis instrument and its method, which relies on the micro-electromechanical system in order to detect the changes occuring in the mass of the sample [78]. Another US patent 6146013 is associated with the combined form of differential thermal analysis and dynamic mechanical analysis equipment and its method of analysis as well. The researchers claimed that this equipment can effectively predict the

Table 3. Patents on characterization techniques.

Patent Number	Publication/ Application Date	Patent Description	Refs.
US7294834B2	Nov. 13, 2007	This patent is associated with a Scanning Electron Microscope (SEM) in which primary electron beam diverts to an angle of 45 degrees just prior to incidence on a specimen and provides the sample images with the high magnification.	[67]
US8232523B2	Jul. 31, 2012	This patent tells about the method of analyzing a sample *via* scanning electron microscopy by focusing the fine beam of electrons on the surface of the specimen.	[68]
US2010/0230590A1	Sep. 16, 2010	This patented innovation germane to compact scanning electron microscope that generates high-quality images of the sample for morphological characterization.	[69]
US7796726B1	Sep. 14, 2010	This patented work is related to the equipment of X-ray diffraction and X-ray fluorescence and their methods of analysis without the fabrication of the sample.	[70]
US5491738	Feb. 13,1996	The patent describes the X-ray diffraction apparatus based on a charge-coupled array which is used to predict the X-ray diffraction pattern of the sample.	[71]
US7564947B2	July 21, 2009	This patent is based on a tomographic energy dispersive X-ray diffraction instrument which consists of a radiation Source, energy dispersive detectors that possess collimators. This equipment was utilized as a better imaging tool than conventional devices.	[72]
US8431897B2	Apr. 30, 2013	The invention demonstrates the transmission electron microscope that utilizes optical fibres in order to obtain high-resolution images by radiating the surface of the specimen.	[73]
US2012/0120226A1	May 17, 2012	The invention is associated with transmission electron microscopy that is employed to produce images of living cells in their native liquid surrounding by using a microfluidic chamber with electron transparent windows.	[74]
US5059909	Oct. 22, 1991	This patented study reflects a method used for estimating the particle size and zeta potential of dispersed particles in a bulk liquid.	[75]
WO2014/039376A3	Mar. 13, 2014	This present invention describes the operating technique for a quasi-adiabatic differential scanning calorimeter in order to diminish the leakage of heat flow rate.	[76]
US6561692B2	May 13, 2003	The patent demonstrates the modulated differential scanning calorimeter and its method of utilization that provides more accurate information about heat flow with better resolution in less time interval.	[77]
US2004/0141541A1	July 22, 2004	The patented innovation is associated with the thermo-gravimetric analysis instrument and its method which relies on the micro-electromechanical system in order to detect the changes occurs in the mass of the sample.	[78]
US6146013A	Nov. 14, 2000	The invention reflects the combined form of differential thermal analysis and dynamic mechanical analysis equipment and its method of analysis as well, in order to predict physical properties of the sample in a more precise manner.	[79]
US5321719A	June 14, 1994	The patented work is based on the thermo-gravimetric device that possesses a ceramic balance beam and its approaches for determining the composition, phase, structure of the sample.	[80]
US7111504B2	Sep. 26, 2006	This patent is associated with the atomic force microscope and its operating method to get surface images of an object with high magnification.	[81]
US6818891B1	Nov. 16, 2004	This patented work describes atomic force microscopy and its operating method in order to reduce contact forces between a probe tip as well as biological Specimen.	[82]
US8656510B1	Feb. 18, 2014	This patent describes the instrument of atomic force microscope, method of operating and method for simultaneously measuring atomic force microscopy and fluorescence for a single molecule.	[83]
US8739309B2	May 27, 2014	The patented invention is related to the scanning probe microscopes that specifically involve atomic force microscopes and its operating method which give better control at high speed with high resolution.	[84]

Table (3) contd….

Patent Number	Publication/ Application Date	Patent Description	Refs.
US6031233	Feb. 29, 2000	This patented work is germane to the portable infrared spectrometer instrument which is utilized for the analysis of a substance on the basis of its optical reflectance.	[85]
US7034944B2	Apr. 25, 2006	This patent is associated with an infrared spectrometer that possesses a single element detector for the determination of compounds.	[86]
US2003/0103209A1	June 5, 2003	This patent is linked with an imaging infrared spectrometer that contains an optical light source coupled with an interferometer which divides the light ray produced by the source into two partial light rays.	[87]
US2010/0282958A1	Nov. 11, 2010	The patented invention is related to the operating methodology of Fourier transform infrared spectrometer and spectrometer themselves.	[88]
WO2010/096081Al	Aug. 26, 2010	The patent is related to Fourier transform infrared spectrometer which is based on micro-electromechanical systems and can be utilized for the monitoring of the environment, security of the homeland, safety of food products and fingerprints of the unknown materials.	[89]
US8102518B2	Jan. 24, 2012	This patent portrays a photon spectrometer, utilized for persistently monitoring physical and chemical parameters of water and other fluids.	[90]
US8573404B2	Nov. 5, 2013	This patented study demonstrates the field flow fractionation which is combined with electrophoresis for determining the surface charge and other characteristics of Suspended particles.	[91]
US9217810B2	Dec. 22, 2015	This patented research displays an infrared gas detection system for identifying and estimating the quantities and types of gases flowing from a wellbore.	[92]
WO2015/116876Al	Aug. 6, 2015	The patented study is linked with the portable Ultraviolet disinfection apparatus, used for the disinfecting different places like hospitals, rooms, food products, *etc.*	[93]
US6641300B1	Nov. 4, 2003	This patent portrays the differential scanning calorimeter and its utility for measuring thermal resistance and capacitance and hence estimates the extent of heat flows to the sample.	[94]
US2011/0170095A1	Jul. 14, 2011	This patented study is related to an analytical approach which is a combination of differential scanning calorimetry and Raman spectroscopy which is utilized to analyze the single sample.	[95]

physical properties of the sample in a more precise manner [79]. Similarly, US patent 5321719 deals with the thermogravimetric device that possesses a ceramic balance beam. This balance beam has hot as well as the cold end. The ceramic platform is attached rigidly with the hot end of the ceramic balance beam. An inert metallic liner which consists of platinum or its alloys is placed on the ceramic plate. A thermocouple is attached directly to the metallic liner so that it could perform functions effectively [80].

US patent 7111504 is related to the atomic force microscope and its operating method. The apparatus provides the surface images of an object with high magnification [81]. Another US patent 6818891 describes the atomic force microscopy and its operating method to reduce contact forces between a probe tip as well as biological specimen. This instrument provides better images of the biological samples for their surface characterization [82]. Similarly, another US patent 8656510 reflects the instrument of atomic force microscope, method of operating and method for simultaneously measuring atomic force microscopy and fluorescence for a single molecule [83]. One more US patent 8739309 discloses the information about the scanning probe microscopes that specifically involve atomic force microscopes and their operating method that give better control even at high speed and high-resolution graphics [84].

US patent 6031233 is related to the portable infrared spectrometer instrument, which is utilized for the analysis of a substance on the basis of its optical reflectance [85]. US patent 7034944 is associated with the Fourier Transformed Infrared Spectrometer (FTIR) that possesses a single element detector for the determination of compounds. The detector analyzes a light beam emitted by the sample, with a unit to digitize the voltage [86]. Another US patent 2003/0103209 is linked with an Imaging Fourier Transformed Infrared Spectrometer (FTIR) that contains an optical light Source coupled with an interferometer, which divides the light ray produced by the source into two partial light rays. The light beam is first passed through the interferometer and then focused on the sample that creates an image on the surface of detector array [87]. Similarly, US patent 2010/0282958 also relates to the operating methodology of Fourier transform infrared spectrometer and spectrometer themselves in which the validation or calibration of the spectrometer can be done in the cyclically recurring durations [88]. The patent WO2010/096081 is related to the Fourier Transform Infrared spectrometer (FTIR) which is based on micro-electromechanical systems. According to inventors, this equipment can be utilized for monitoring of the environment, the security of the homeland, the safety of food products and fingerprints of the unknown materials [89].

US patent 8102518 relates to a photon spectrometer and its method which is utilized for monitoring the optical parameters of water and other fluids. The optical sensor of the instrument detects the abnormalities that change the optical characteristics of water or fluids [90]. Another US patent 8573404 demonstrates the field flow fractionation which is combined with a micro-electrophoresis system for determining the surface charge and other characteristics of Suspended particles in the solvent system [91].

US patent 9217810 reveals the information about an infrared gas detection system for identifying and estimating the quantities, and types of gases flowing from a wellbore. The gas detection system consists of a control module, infrared sensor, as well as first and second portable type pumps [92]. Another patent WO2015/116876 is linked with the portable Ultraviolet disinfection apparatus, used for disinfecting different places like hospitals, rooms, food products, *etc.* [93]

US patent 6641300 reveals the Differential Scanning Calorimeter (DSC) and its utility for measuring thermal resistance, capacitance and hence estimate the extent of heat flows to the sample [94]. Similarly, US patent 2011/0170095 is related to an analytical approach, which is a combination of differential scanning calorimetry and Raman spectroscopy. This is utilized to analyze a single sample in the experiment. The equipment provides information about the thermal properties of the sample by measuring the thermal conductivity, specific heat or chemical reactions [95].

6. MAIN PROBLEMS IN MANUFACTURING AND SCALABILITY OF NANOSUSPENSION

Besides the several advantages of nanosuspension, there are some drawbacks associated with nanosuspension such as manufacturing complications, stability issues and nanotoxicity. Stability is a crucial facet that ensures the safety and efficacy of the product. The agglomeration of particles may be a serious problem arising in pulmonary delivery of the drug as it affects the drug efficacy. Similarly, if a pharmaceutical nanosuspension has particle size more than 5 μm, it can obstruct the blood flow due to capillary blockage [96]. Therefore, during storage conditions particle size should be monitored carefully. Sometimes, stability issues arise during the manufacturing of nanosuspensions since high pressure and temperature may produce a change in crystalline structures of drug particles [97, 98]. The various common stability grievances associated with nanosuspension are sedimentation of particles, crystal formation, and change in the crystalline state, caking, and agglomeration [96].

In the sedimentation phenomena, the drug particles present in nanosuspension may settle down depending upon their density. Both flocculated and deflocculated suspension displays phenomena of sedimentation. However, their rate of sedimentation and nature of sediment differs from each other. The sedimentation rate of suspension may be described by Stoke's law [96]. The law states that a particle attains a constant sedimentation rate when moving through the viscous fluid, depending upon the viscosity of liquid and diameter of moving particle. In order to overcome the issue of sedimentation, the nanosuspensions are converted into the form of dry powder through spray drying or freeze drying approach. In case of oily nanosuspensions, freeze drying is less utilized since the oily medium cannot be frozen. However some other methods such as particle engineering etc. may be used to rectify the stability issue.

One of the important stability issues that may arise during or after the manufacturing of nanosuspension is crystal growth. In fact, it is a phenomenon in which small particles (present in solution) get dissolved and start to deposit on larger particles to achieve a more stable thermodynamic state. It is a most common issue that occurs in nanosuspension and is responsible for the alteration in particle size and their distribution. This spontaneous thermodynamic driven process occurs because the large particles are more stable than small particles. This phenomenon of crystal growth is also called Ostwald ripening. This process occurs due to the non-identical solubility profile of particles, and depends upon their size. Since small particles possess high surface energy than large particles, which give rise to high saturation solubility. Due to this difference in saturation solubility, a concentration gradient develops between the small and large particles. This results in the diffusion of molecules from higher concentration to lower concentration. This causes the development of supersaturated solution around large particles that leads to drug crystallization onto drug particles. Therefore, small particles exhibit a low energy level after their conversion into large particles. The difference in saturation solubility and concentration gradient can be minimized by enhancing the presence of uniform size into the solution medium. This will be significantly helpful in inhibiting the Ostwald ripening.

Another problem associated with nanosuspension is a change in the crystalline state of Active Pharmaceutical Ingredient (API). This problem affects the drug solubility, stability, and efficacy of the drug. This problem is concerned with amorphous drug formation or conversion of high shear-induced APIs. The various Top-down manufacturing methods of nanosuspension involve media milling *etc.* applying high shear to a formulation while some bottom-up methods may also convert the drug particles into an amorphous state. Various technologies may be used to determine the state of APIs such as solid-state nuclear magnetic resonance, Infrared Spectrometer (IR), X-ray diffraction, differential scanning calorimetry [96]. These problems affect the manufacturing and scalability of nanosuspension either directly or indirectly. These issues can be controlled by controlling the different factors that affect the formulation development and their scalability like process factors and formulational factors.

7. MARKETED PRODUCTS APPROVED BY FDA

NanoCrystal® is a patented technology discovered by the ELAN Corporation; Ireland [99]. It is a significant technology used for evaluating the new chemical entities that show poor aqueous solubility and work as a potent tool for optimizing the performance of current drugs. NanoCrystal can be applied to both oral and parenteral formulations. This nano-sized formulation displays an enhancement in surface area, which utterly increases the solubility and is stabilized by using suitable stabilizers [100]. With the help of this technology, various nano-medicines were developed and mar-

Table 4. The various marketed formulations prepared by using nanosuspension technology and approved by the Food and Drug Administration (FDA).

Company	Drug	Brand name	Formulation	Application	References
First Horizon Pharmaceutical	Fenofibrate	Triglide®	NanoCrystal®	Lipid disorders	[101,102]
Novartis	Methylphenidate HCL	Ritalin® LA	NanoCrystal®	Attention deficit disorders	[102]
Novartis	Dexmethylphenidate HCL	Focalin® XR	NanoCrystal®	Attention deficit disorders	[102]
PAR Pharmaceutical	Megestrol Acetate	Megace ES®	NanoCrystal®	Act as Anti-anorexic	[101-103]
Merck	Aprepitant	Emend®	NanoCrystal®	Nausea occurs in chemotherapy	[101-103]
King Pharmaceutical	Morphine sulfate	Avinza®	NanoCrystal®	Brain stimulant	[102]
Abbott	Fenofibrate	TriCor®	NanoCrystal®	hypercholesteremia	[101-103]
Wyeth	Sirolimus	Rapamune®	NanoCrystal®	Immunosuppressant	[101-103]
Acorda	Tizanidine HCL	Zanaflex®	NanoCrystal®	Muscle relaxant	[102]

keted. These include TRICOR® (fenofibrate), FOCALIN® XR (dexmethylphenidate HCl), RAPAMUNE® (Sirolimus), EMEND® (Aprepitant), ZANAFLEX® (Tizanidine HCl) and MEGACE® ES (megestrol acetate), *etc.* Table **4** provides a sufficient glimpse of these marketed products approved by the FDA.

8. REGULATORY ASPECTS OF NANOSUSPENSION

In recent years, nanosuspension technology has arisen as a lucrative approach to rectify the problems associated with the poor aqueous solubility. Several pharmaceutical industries are utilizing this technology to enhance the solubility of these drug moieties. All nanosuspensions available in the market have been approved by the Food and Drug Administration (FDA) according to their already existing legislation without any unique testing. Therefore, the approval of these nano-pharmaceuticals has challenged the regulatory guidelines of the FDA. With reference to FDA legislative guidelines, all products that are submitted to the FDA for their approval are evaluated on the basis of their category. The several categories of drug products such as biological products, drug products as well as medical devices, etc. are assigned for evaluations to the Centre for Biologics Evaluation and Research (CBER), Centre for Drug Evaluation and Research (CDER), and Centre for Devices and Radiological Health (CDRH) [104]. In 2011, the FDA issued guidelines for industries with title "Consideration whether FDA regulated products involved the application of Nanotechnology" to demonstrate the current perspective on nanotechnology and to seek public views [105]. The approval of combinational drug products produces several complications as these products contain two or more components. The FDA has set up an office with the name 'office of Combinational products' to sort out these complications. Now, FDA also utilizes the formula of Primary Mode Of Action (PMOA) in order to assign a combinational nano-product to the suitable category.

The FDA has found that nanosuspension and other aspects of nanotechnology are an emerging approach that can be potentially used for FDA-regulated products such as drug moieties, biological drug products, *etc.* Thus, FDA took various steps for the sufficient growth and development of nanopharmaceuticals products. To fulfill this need, the FDA organizes the nanotechnology regulatory science program periodically for filling the gap in knowledge, methods required as well as tools essential for the assessment of the products based on nanotechnology [104].

CONCLUSION

The development of pharmaceutical formulations that comprise poorly soluble drugs is the major trouble for formulators. By the dint of several appreciable advantages, nanosuspension is now a promising approach for delivering several kinds of therapeutically active ingredients. This technology may solve the problems that arise due to hydrophobic drugs like poor solubility and bioavailability. Nanosuspension contributes a significant role in administering different drug entities through a variety of routes involves oral, transdermal, ocular, parenteral, pulmonary, *etc.* The nanosuspension technology can be a fruitful approach for the betterment of humans due to its simplicity, lucrativeness, improved solubility, and dissolution. From this review study, it can be concluded that nanosuspension may enhance the quality of life of patients with better efficacy, less adverse drug effects, diminished communal as well as the economic expense associated with healthcare.

9. CURRENT & FUTURE DEVELOPMENTS

In recent years, nanosuspension approach has been complementarily utilized to solve the grievances developed due to poorly soluble drugs. The nanosuspensions having better solubility or redispersibility in the aqueous medium have attracted the attention of formulators due to their unique properties. Nanosuspension of poorly soluble drugs can be fabricated through a variety of methodologies including media milling, ultra-sonication, high-pressure homogenization,

precipitation, *etc.* as these techniques are observed to be quite fruitful and productive for obtaining nanosuspension. However, the nanosuspension obtained through such techniques may be subjected to some kinds of stability problems like crystals growth, Ostwald ripening, *etc.* hence suitable stabilizers/polymers are imparted into nanosuspension to make them stable. The selection of stabilizers is very complicated and challenging as it takes too much time and efforts. In fact, in the field of pharmacy, the well-stabilized nanosuspensions that do not possess any stabilizers are in the clamour. As such, nanosuspensions are more compatible with the human body and also reduce the complications of imparting stabilizers. For this purpose, some scientists are working on the self-stabilization concept. However, this field is quite challenging with several complications, therefore this field requires utmost research work for developing nanosuspensions without the stabilizers. By making emphasis on nanosuspension technology, human society shall be benefitted due to its simplicity, lucrativeness, improved solubility, and dissolution.

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