Hindawi
International Journal of Polymer Science
Volume 2018, Article ID 6387826, 10 pages
https://doi.org/10.1155/2018/6387826

Review Article

Analytical Methods to Characterize and Purify Polymeric Nanoparticles

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Received 30 June 2018; Accepted 10 July 2018; Published 5 August 2018

Academic Editor: Zhonghua Peng

Advances in polymeric nanoparticles as novel nanomedicines have opened a new class of diagnostic and therapeutic tools for many diseases. However, although the benchtop research studies in the nanoworld are numerous, their translation to currently marketed products is still limited. This lack of transference can be attributed, among other factors, to problems with nanomedicine characterization. Characterization techniques at the nanoscale could be divided in three categories: characterization of physicochemical properties (e.g., size and surface charge), characterization of nanomaterials interactions with biological components (e.g., proteins from the blood), and analytical characterization and purification methods. Currently available literature of this last group only describes methodologies applied for a specific type of nanomaterial or even methods used for bulk materials, which are not completely applicable to nanomaterials. For this reason, the current review aims to become a scholastic guide for those scientists starting in the nanoworld, giving them a description of analytical characterization techniques aimed to analyze polymers forming nanoparticles and possible forms to purify them before being used in preclinical and clinical applications.

1. Introduction

The field of nanotechnology and, more specifically, nanomedicine emerged about 20 years ago and since then, it experienced an exponential progress both in the fundamental study of nanosystems and in their multiple applications. Specifically, studies on polymeric nanoparticles have gained attention due to the multiple advantages that are attributed to this kind of nanosystems in terms of safety, versatility, and robustness [1–3]. Although the number of benchtop research studies developing novel nanosystems intended for biomedical applications is enormous, their use as clinically effective products is still limited. One of the main concerns of pharmaceutical industries for the production of novel formulations based on polymeric nanoparticles is the complexity of a deep characterization, which would enable their safe production and use in humans [4]. Therefore, a wide characterization of nanomedicines is a must before their testing in preclinical and clinical stages. However, the current technology is challenging in the sense that many characterization techniques have been applied directly from those methods used for bulk materials (not at the nanoscale) or using conditions that do not simulate biological environment [1, 2]. Therefore, many efforts must be devoted to the enhancement of the performance of current techniques. Nanomedicine characterization can be divided in three steps: first, an analytical characterization, useful for characterizing the materials they are composed of as well as to find out the impurities present and develop purification processes; second, a physicochemical characterization of the main parameters that will define the performance of nanomaterials *in vivo*, such as size, surface charge, and stability in biological conditions; and third, the study of their interaction with biological components (Figure 1). Although many reviews

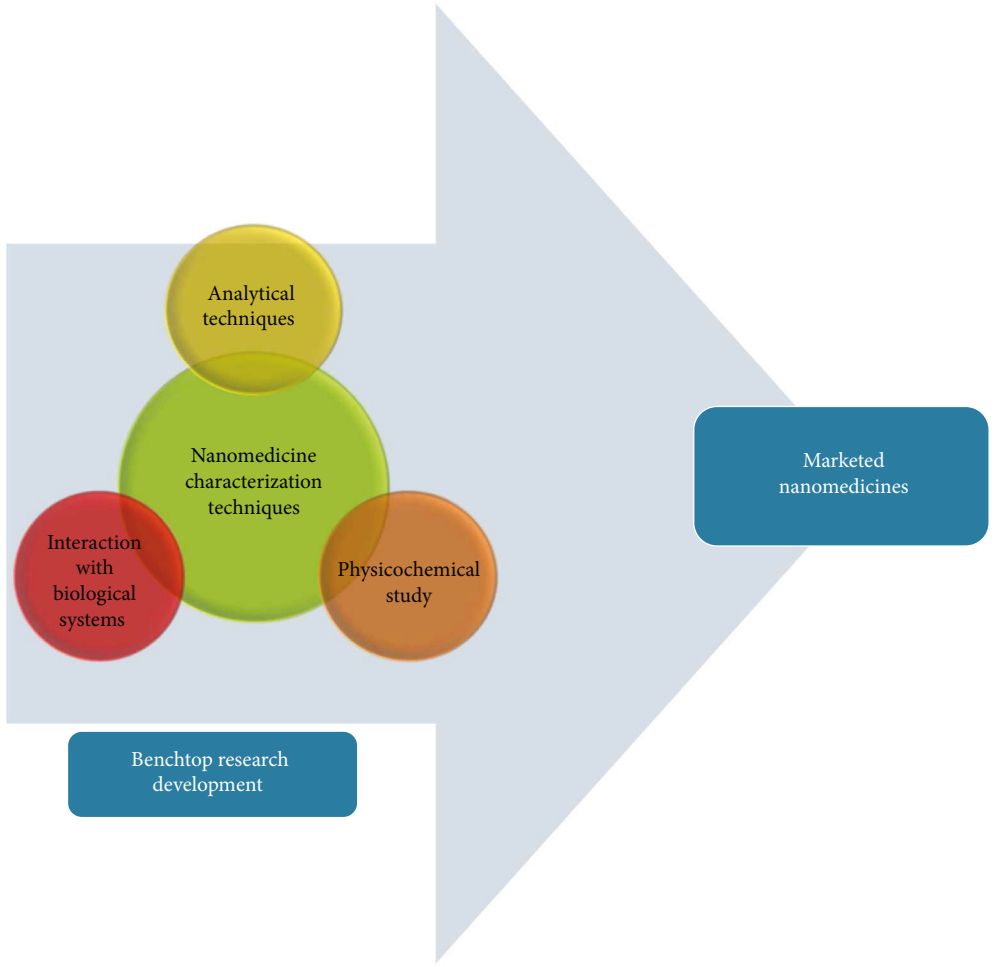

Figure 1: Schematic representation of the process of nanomedicine characterization before translating to pharmaceutical production.

for the characterization of polymeric nanoparticles designed as nanomedicines exist, most of them give a particular point of view, signaling only some techniques [2, 5, 6]. Therefore, scientists working on the development of novel nanoformulations find themselves lost in the huge but dispersed existent bibliography. This is the reason that motivated the authors to write a series of three reviews with a scholastic character, to enable those scientists starting in the nanoworld to have guidelines for the correct characterization of polymeric nanoparticles. The first review was devoted to the characterization of nanomaterial interactions with biological systems [2]; the second one describes the physicochemical characterization techniques at the nanoscale, to assess size, surface charge, and stability of polymeric nanoparticles [7]; and the present one (third one) devoted to describe analytical characterization and purification techniques useful for nanomedicine study of polymeric nanoparticles (see a schematic representation of these techniques in the SI). Therefore, the purpose of the present review is to be a first practical guideline for those scientists initiating their studies in the nanoworld. It should be noted that it was not the objective of the authors to get deep into description of each individual technique but rather describe briefly each methodology to help the readers to select the most appropriate technique for their study and look for more specific information in the numerous references given for each technique.

These methods have been classified not as a function of what is characterized but as a function of the technique: chromatographic, spectroscopic, calorimetric, and purification techniques. Authors will guide the reader through them with the objective to help in the selection of one or other technique depending on the parameter to study. Physicochemical techniques, mainly used to characterize size (e.g., light scattering or microscopy), are out of the scope of the present review [7].

2. Chromatographic Techniques

Chromatographic techniques, in general, are a group of techniques devoted to the separation of various compounds [8] as a step prior to their characterization. Their advantages are related with their high power of separation of substances, and the easy and simple manipulation, although they have as drawback the nonspecific interactions and the difficulty in method optimization [8, 9]. In this work, various types of chromatographic techniques are briefly described (Table 1). All of them could be also classified as purification techniques (see Section 5), since they are also able to separate compounds.

Table 1: Analytical chromatographic techniques.

Technique	Characteristics that analyses	Advantages	Disadvantages	References
Gel permeation chromatography (GPC)	Molecular weight	Rapid and simple High resolution	Interaction sample with column filling	Williams [10] Neverova and Van Eyk [8] Cho et al. [1]
High-performance liquid chromatography (HPLC)	Quantification of actives	High resolution Rapid and easy performance	Interaction sample with column filling	Neverova and Van Eyk [8]
	Purification	Low cost/sample Small sample volumes		Sapsford et al. [9]
Size exclusion chromatography (SEC)	Molecular weight	High resolution	Interaction sample with column filling	Kostanski et al. [25]
		Rapid and simple	Need of a labelling tag	Sapsford et al. [9] Rebolj et al. [26]

2.1. Gel Permeation Chromatography (GPC). The gel permeation chromatography (GPC) is a widely used technique to determine the molecular weight of materials dissolved in organic solvents as well as the physical stability of assembled nanomaterials [1, 10]. The nanomaterials are eluted as a function of their molecular weight: the bigger the nanomaterials, the faster the elution. The quantification of the eluted samples is performed by means of UV-Vis absorption or changes in the refractive index.

This technique could be useful, for example, to study the stability of a polymer dissolved in an organic solvent. After various periods of time, the dissolved polymer should be analyzed through the GPC and its molecular weight assessed by using a calibration curve. Other examples of the use of GPC in the nanoscale could be the assessment of the polymer molecular weight, the degree of polymerization of a synthesized polymer, or even the number of monomer subunits that a polymer contains [11–14]. In some cases, GPC has also been used for the purification of quantum dots or carbon nanotubes, for example [15, 16].

GPC is advantageous in terms of short-time experiments. However, an important drawback of this technique is the possible interaction between the nanomaterials and the column filling, which could interfere the size assessment [1, 8].

2.2. High-Performance Liquid Chromatography (HPLC). High-performance liquid chromatography (HPLC) is the most used type of chromatography not only for colloidal nanosystem studies but also for other type of materials (e.g., proteins). In the vast majority of studies, it is used for the fine quantification and separation (purification) of actives, such as drugs [5, 8, 9]. Briefly, it consists in the injection of the liquid sample using a pump that introduces it to a flow (mobile phase) that passes through a separative column (stationary phase), which entraps the molecules depending on their nature. The more interactions the molecules have with the column filling, the later they will be eluted. Further, molecules are eluted in a characteristic pattern for each compound. It results a chromatogram with the peaks of each compound (Figure 2).

The quantification of the actives is required in any study of the encapsulation efficiency of drugs in the nanosystems or their release kinetics, as well as the percentage of conjugation to some nanosystems [9]. Examples of studies using HPLC for drug quantification exist are numerous [17–19]. For example, Fornaguera et al. [19] studied the encapsulation and release kinetics of dexamethasone (an anti-inflammatory drug) from polymeric nanoparticles. They were able to determine very low concentrations of the drug in a release study receptor solution, due to the high sensibility that offers the HPLC technique.

The resolution of the HPLC depends on the filling of the column (on the stationary phase properties), which is commonly composed of silica with attached alkyl chains, being the reversed phase C_{18}-type columns the most widely used, since it enables a differential retentionship depending on the polarity of the compounds [8, 9].

The advantages of HPLC are the high resolution, the low volumes required, and an easy, rapid, and economic manipulation [8, 9]. However, some drawbacks derived from the interaction of the samples with the stationary phase (column filing) could take place [9].

In 2004, it appeared the ultra-HPLC (UHPLC) technique, with many advantages among the traditional HPLC. It uses a column filling of particles of sub-2 micron size, while conventional HPLC uses particles between 2.5 and 5 microns. This reduction on the filling particle size enables a finer separation of similar compounds. In addition, the working pressure of UHPLC equipment is markedly higher than that supported by conventional HPLC, which enables more rapid flow rates, resulting in shorter elution times and decrease on the solvent amount used [20, 21].

2.3. Size Exclusion Chromatography (SEC). Size exclusion chromatography (SEC), together with ion-exchange and affinity chromatography are classified as low-pressure liquid chromatography [9]. It is useful to characterize and separate (purify) nanomaterials with different properties, dispersed in an aqueous solution. For example, it has been useful to separate antibody-conjugated nanoparticles from the free

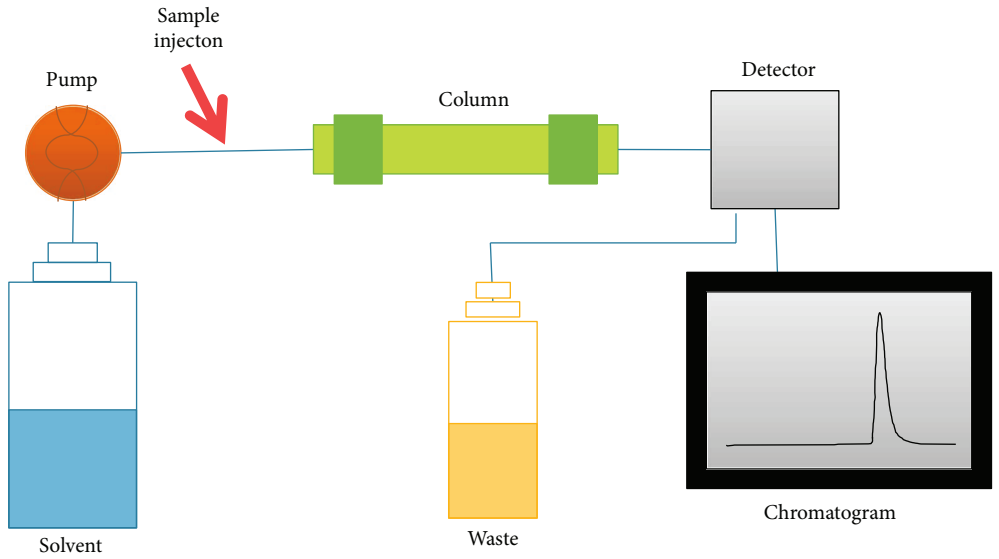

Figure 2: Schematic representation of a HPLC system.

antibody and nanoparticles [22, 23]. Another example of its use is the characterization of the molecular weight of nanoobjects, such as proteins or polymers [24]. The separation of the compounds depends not only on their molecular weight but also on their 3D dimensions, due to differences in pore permeation [25, 26]. It has the same advantages than other chromatographic techniques.

3. Spectroscopic Techniques

Spectroscopic techniques are those that give information on the interaction of an electromagnetic radiation with a sample, thus resulting in an absorption that depends on the excitation wavelength. Therefore, a wavelength spectrum with absorption/emission peaks that depend on the material is produced [9].

These techniques, summarized in Table 2, in general, are useful to characterize materials, which includes also nanomaterials, since spectra are characteristic of each material. A specific use of them, for example, could be the determination of the formation of bioconjugates between nanomaterials and conjugated moieties [9].

3.1. Infrared Spectroscopy. Fourier transformed infrared spectroscopy (FTIR) is a spectroscopic technique based on the measurement of vibrational transitions between different excitation states of molecules [27]. IR radiation is absorbed by molecules with dipoles that oscillate with the same frequency of the incident IR light. The IR absorption is an energy transfer of molecules and, if a change on their vibration occurs (by stretching, bending, or twisting of the bonds), the IR absorption changes; and this change can be characterized (e.g., covalent attachment of a carboxylic group to an amine group that results in an amide group) [5, 9].

It is a widely used technique, not only in the nanomedicine field but also in a variety of scientific fields. It has many advantages (Table 2), such as the fast and inexpensive performance and the obtaining of characteristic IR fingerprints of each compound, since they are a combination of the vibrational state of each atom [5]. In addition, it can be a quantitative tool in some specific conditions [27]. However, as drawbacks, there is the sample preparation (dried samples required, although some apparatus are prepared for liquid samples, but in this last case, a high concentration of the compounds to analyze is required), a requirement of technique optimization in most cases and interference with water molecules, which strongly absorb and need of a background signal [9]. Concerning nanomedicines experimentation, Sapsford et al. [9] and Lin et al., [5] for example, used FTIR to confirm the attachment of biomolecules onto nanomaterials surface, since FTIR results in a band pattern as a function of the chemical groups.

3.2. UV-Visible Spectroscopy. UV-Visible spectroscopy is a spectroscopy type that emits radiation of wavelength between 190 and 800 nm, widely used for the quantification of compounds concentration, and, in some cases, even size and shape, since each material absorbs at a determined wavelength and changes in the spectra could be related with changes in the aggregation of nanomaterials [5, 9]. It has been used, for example, to determine the conjugation and ratio of conjugation of biomolecules to nanomedicines [2, 5, 6, 8, 9].

It is a simple, fast, and cost-effective technique that can be applied to a variety of nanomaterials (Table 2). However, since most of the materials produce absorption at some wavelength, interferences between absorption of different compounds have to be taken into account [9].

3.3. Fluorimetry. Fluorimetry is a very sensitive spectroscopic technique that consists in the quantitative measurement of fluorescent signals, usually produced by aromatic molecules,

Table 2: Summary of the main advantages and disadvantages of spectroscopic techniques.

Technique	Use	Advantages	Disadvantages	References
Fourier transformed infrared (FTIR)	Chemical composition	Fast and inexpensive Characteristic of each material Possible quantification	Complicated sample preparation Interference of water Relatively low sensitivity Requires dried samples Destructive	Alben and Fiamingo [27] Pretsch 2003 Sapsford et al. [9] Lin et al. [5]
UV-Vis	Quantification of concentration Size and shape determination	Cost effective Simple and fast Useful for a variety of compounds	Interference between materials	Sapsford et al. [9] Lin et al. 2014 [5]
Fluorimetry	Quantitative fluorescence determination	High sensitivity Compound specificity	Limited to fluorescent compounds Limited fluorescence lifetime	Lakowicz [28]

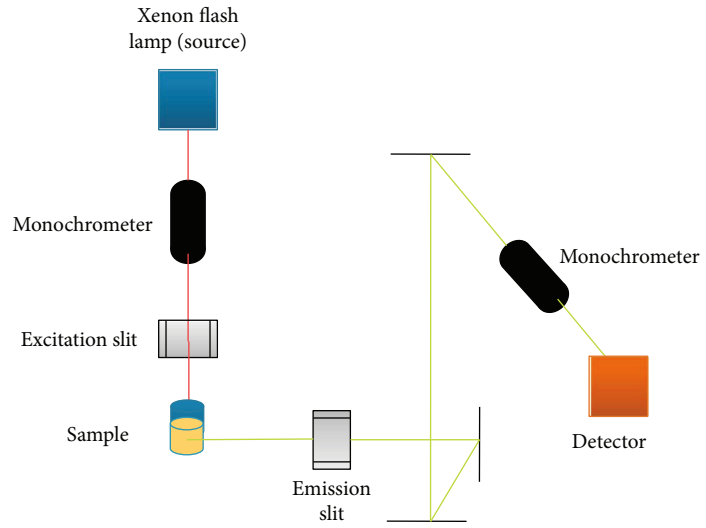

Figure 3: Schematic representation of a fluorescence spectrophotometer model.

for the detection and characterization of organic and inorganic compounds thanks to the application of a fluorescent laser to a sample (Figure 3) [28]. The fluorescent signal is composed of

(i) Excitation: it is produced by the absorption of the electromagnetic radiation by the sample to study.

(ii) Losses of vibrational energy: they are produced after the absorption of energy and before the emission of fluorescence, due to the internal collision between the molecules of the sample.

(iii) Emission: it consists of the energy produced by a molecule of the sample when it drops to a lower vibrational level (corresponding to lower energy and longer wavelengths), thus emitting energy in the form of fluorescence. The fluorescence is obtained as a spectrum and not a single band because not all the molecules of the sample drop to the same vibrational level.

This technique has been used for various applications, all of them taking advantage of the capacity of the studied materials to be excited under a fluorescent laser and emit fluorescence of another wavelength. For example, Lin et al. [5] used fluorescence to study conformational changes of biomaterials and their conjugation with nanomaterials. Fornaguera et al. [29], in contrast, took advantage of the fluorescent properties of galantamine drug to quantify its encapsulation efficiency in polymeric nanoparticles and its release. Chen et al. [30] used fluorimetry to confirm covalent bond formation onto polymeric nanoparticle surface. In addition, since the excitation/emission are wavelength

Table 3: Summary of the main advantages and disadvantages of calorimetric techniques.

Technique	Use	Advantages	Disadvantages	References
Differential scanning calorimetry (DSC)	Glass transition Melting temperature	Low amounts of sample High precision and sensitivity	Requires sample preparation Requires an appropriate reference	Hunt [36] Haq [35] Lu and Li [37] Sapsford et al. [9]
Thermogravimetry (TGA)	Weight loss	Low amounts of sample High precision and sensitivity	Requires sample preparation Requires an appropriate reference	Berger et al. [38] Sapsford et al. [9] Lin et al. [5]

specific, it can be also used to quantify two colocalized fluorescent dyes, due to the simultaneous detection of the different wavelengths.

Although the varied uses of fluorescence, it has two main drawbacks (Table 2): only some materials have fluorescence and the fluorescence lifetime is limited, with a wavelength dependent on the specific compound [28].

Recently, advanced techniques combining the advantages of fluorescent signal have appeared. Of special importance is the Förster resonance energy transfer or FRET technology. It consists in the combination of two fluorescent probes labeling pairs of two compounds, the first one called the donor and the second one called the acceptor. Fluorescence of excitation wavelength of the donor is directed to the compound, which, under the specific fluorescence will emit fluorescence in another wavelength. The acceptor is excited specifically by the emission wavelength of the donor (energy transference) and emits fluorescence in another wavelength. Therefore, if both compounds are very close (<10 nm), when exciting the donor, only fluorescence signal of the acceptor emission wavelength will be detected. In contrast, if both molecules are not close enough, when exciting the donor, fluorescence emission of this donor wavelength will be detected. Therefore, this technique is very useful to study two compound aggregation, and it is starting to play an important role in nanosystem studies [31–33]. Lai et al. [31], for example, studied the drug release from porous silica nanoparticles using this technique. Liu et al. [32], in contrast, used FRET technology as a nanodiagnostic system to detect the presence of chrome in urine, which produces the dissociation of FRET pairs, specifically designed for this purpose.

4. Calorimetric Techniques

Calorimetric techniques are a type of techniques that apply a temperature change to the samples to study physical phenomena, such as the crystalline transition, fusion, vaporization, sublimation, absorption, adsorption, and desorption and chemical phenomena, such as chemisorptions, desolvation, decomposition, oxidative degradation, solid-state, and solid-gas reactions [34]. Two main calorimetric techniques will be described in this review (Table 3).

4.1. Differential Scanning Calorimetry (DSC). Differential scanning calorimetry (DSC) is a technique that continuously measures the apparent specific heat of a system as a function of the temperature [35]. It is useful for the measurement of the glass transition and melting temperatures of materials including nanosystems. The glass transition temperature (Tg) is the temperature at which a material in a solid state changes its conformation. The melting temperature is always higher than the Tg [36, 37]. Therefore, in some cases, the determination of the melting temperature is not performed because it requires too high temperatures.

It is a useful technique to determine the structure and stability of nanomaterials, as well as their conformation, since material transitions will change depending on the nanomaterial composition [5, 35].

4.2. Thermogravimetric Assays (TGA). Thermogravimetric assays (TGA) are another type of calorimetric techniques which measure the weight loss of the samples [34, 38].

It is a useful technique to determine the amount of nanoconjugation, since the change on the nanomaterial composition produces changes in the temperature weight loss [5, 9]. For example, Fornaguera et al. [39] used TGA analysis to confirm the covalent attachment of a dendritic carbosilane wedge to polymeric nanoparticles.

Since both calorimetric techniques have a similar performance, their advantages and disadvantages have been summarized together. Calorimetric techniques are advantageous in terms of small amount of sample required, high precision and sensitivity. However, an appropriate reference as well as the preparation of the sample is required [9, 35, 38].

5. Purification Techniques

The composition of colloidal nanomaterials is a key point, since it affects not only its transport, delivery, and biodistribution *in vivo*, but, most importantly, it can contribute to toxicity-related problems [4–6, 9]. For this reason, to ensure a safe formulation, free of contaminants, a purification step is strongly recommended, followed by a physicochemical characterization, before starting preclinical and clinical analysis.

Although various methods exist for the colloidal nanomaterial purification, such as the magnetic separation for magnetic nanoparticles, for example [40, 41], in most cases, when nanosystems do not possess any specific inherent property that facilitates purification, this step may represent a difficult and tedious process, being sometimes impossible to confirm the presence of a purified compound [9]. In the following, common purification techniques, useful for a variety of nanosystems, are summarized (Table 4). Specific

Table 4: Summary of the main advantages and disadvantages of the described purification techniques.

Technique	Use	Advantages	Disadvantages	References
Filtration	Purification Sterilization	Useful for thermolabile compounds	Time consuming	Scopes [44]
	Concentration	Rapid and simple	Bigger sizes determined by the cut-off size	Sapsford et al. [9]
	Dispersant changement	Commercially available devices	Single-use devices	Roy et al. [43]
	Reduce size polydispersity	Cost effective		Dobrovolskaia and McNeil [42]
Centrifugation	Purification	High efficiency	Special equipment required for large volumes	Scopes [44]
	Concentration Dispersant changement	Rapid, facile, and economic Low amounts of sample Appropriate for different kinds of nanomaterials	Difficult to resuspend soft matter	Sapsford et al. [9]
Dialysis	Purification	Rapid, facile, and economic	Limited to the membranes MWCO	Vauthier et al. [46]
	Concentration Dispersant changement	Commercially available devices No sample pretreatment	High receptor solution volumes	Sapsford et al. [9]
Electrophoresis	Purification	Simple and economic High resolution and sensitivity	Postelectrophoresis purification steps Need of charged compounds	Sapsford et al. [9] Jason [51] Scopes [45]

techniques, useful only for a determined material have not been described in this review.

It is worth remarking the contamination by endotoxins. Although methodology to purify from endotoxins is out of the scope of the present review, authors would like to remark the importance of producing nanomedicines clean from this type of contamination, which could be produced from many lipopolysaccharides, of the omnipresent Gram-negative bacteria. It is important to clean all material used before starting the production (e.g., by cleaning with sodium hydroxide or heating treatments) of nanomedicines and to confirm that resulting nanomedicines are clean from endotoxins [6].

5.1. Filtration. Filtration is a purification method, specifically used to sterilize nanomaterial colloidal dispersions. This method is advantageous as a sterilization technique for thermolabile compounds [42]. In addition, it represents a rapid, commercially available, cost-effective, and simple technique. Although it can be performed under atmospheric pressure, usually, it is performed taking advantage of special devices with filters, which are centrifuged, thus increasing the speed and efficiency of the process [9, 43]. An aspect that could be considered as a drawback is that filtration of large volumes could produce clogging of the filters, reason for which filters are single-use devices [44].

In addition, it can also be useful for other purposes, such as for the concentration of colloid nanomedicines or the reduction of their polydispersity. For example, Roy et al. [43] produced Au and CdS nanoparticles and passed them through a multiwall carbon nanotube, which was used as a filter to control the maximum nanoparticle size, thus eliminating the bigger ones and reducing nanoparticle size polydispersity [43, 45–50].

5.2. Centrifugation. Centrifugation is another technique useful for the purification of nanomaterials. It consists of the application of a centrifugal force to enhance the precipitation of nanomaterials due to the increased gravitational field [44]. Different kinds of centrifugation exist, such as the conventional centrifugation, ultracentrifugation, and gradient centrifugation, whose use depends on the objective of the study and on the nanomaterials type, specifically on their size [9].

Centrifugation is more efficient than filtration. It is a rapid, facile, and economic technique, able to be used for different kinds of nanomedicines. In addition, low amounts of sample are required. However, the centrifugation of large volumes requires special equipment and in some cases, difficulties on resuspending sediment nanomaterials appear, specifically when working with soft matter, which are not always possible to recover their dispersion liquid state [9, 44].

Apart from the use of centrifugation to purify nanomedicines, it has been also used to concentrate nanomedicines, to change their dispersant, and to separate conjugated nanomaterials from those nonconjugated [9]. For example, Fornaguera et al. [19] centrifuged PLGA nanoparticles to purify them from the surfactant traces and to concentrate them.

5.3. Dialysis. Dialysis consist on changing the nanomaterial dispersant by means of submerging a semipermeable dialysis bag (or a dialysis dispositive) filled with the nanomaterial dispersion, in a receptor solution. Therefore, it is not only useful for the nanomaterial purification but also for nanomedicine concentration and to change the dispersant to achieve the desired properties (e.g., dialysis with PBS to achieve the physiological pH and osmolality). The liquid diffuses through the membrane from the more concentrated solution (sample or receptor solution) to the less concentrated one, to

achieve an osmotic equilibrium. Therefore, osmotic conditions have to be completely controlled to avoid volume changes; except in the case of nanosystem concentration, where a decrease of the sample volume is required and it can be achieved using a hypotonic receptor solution [9]. It is worth noting that not only the liquid but also molecules with smaller molecular weight than the molecular weight cut-off of the membrane can also diffuse to achieve the osmotic equilibrium. For example, Vauthier et al. [46] used and demonstrated inverse dialysis as an appropriate methodology to concentrate polymeric nanoparticles using a receptor solution with a high osmotic pressure.

This technique is advantageous in terms of minimal sample manipulation, without the need of any pretreatment, but it is limited to the existent dialysis membranes. If the sample to dialyse is expected to interact with the membrane or if the molecular weight cut-off is not appropriate, this technique cannot be used. In addition, high volumes of the receptor solution are usually required [9, 46].

5.4. Electrophoresis. Electrophoretic techniques consist of the application of an electric field to a polymeric gel (composed mainly of agarose or polyacrylamide) submerged in a liquid buffer; through which charged molecules run depending on their charge and/or on their molecular weight. Although electrophoresis is used with many objective, one of the uses of this technique is the separation and purification of determined nanomaterials, which has been widely reported [9]. General examples of the purification use of electrophoresis are the following. Meyer et al. [47] reported the use of PAGE (polyacrylamide gel) electrophoresis for the purification of RNAs extracted from enzymatic synthesis or from cells, for example. They also demonstrated both native and denaturing electrophoretic conditions for the selection of RNAs of the required size.

The main advantages of this technique are its economic and simple performance together with the high resolution and sensitivity. However, only charged compounds can be purified through electrophoresis, and once the compound is separated in the gel, further purification steps are required to extract it from the gel [9, 45, 51].

Although it is not specifically a technique for the purification of nanosystems, it was considered appropriate to remark an electrophoretic type widely used to characterize the formation of nanocomplexes by the electrostatic interactions between their components. This technique is called electrophoretic mobility shift assay (EMSA). EMSA consists in a native (nondenaturing) polyacrylamide electrophoresis where samples migrate depending on their charge, under an electric field [48]. It is widely used in the nanomedicine field for the determination of the complexation ratio between two compounds, for example, polymeric nanoparticles and antisense oligonucleotides (Figure 4) [39]. The complexation is detected due to a retardation of the oligonucleotide band migration when complexes are formed, since complexes migrate slower than free nucleic acids [48].

Its rapid performance, simplicity, robustness, and high sensitivity make this methodology the choice for the study of electrostatic complexes formation of a wide range of compounds. Although EMSA is usually applied as a qualitative technique, under the appropriate conditions, it can be also useful to quantify the stoichiometry of complexation. It is also remarkable that multiple EMSA variants exist for various purposes, such as the time-course EMSA to measure the dissociation kinetics or the circular permutation to measure the DNA bending [48–50]. However, EMSA has also some disadvantages, such as the performance of the assay in non-chemical equilibrium [48].

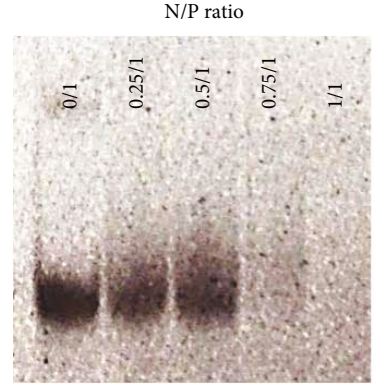

Figure 4: Example of an EMSA of polymeric nanoparticles conjugated with antisense oligonucleotides at different nanoparticle/oligonucleotide (N/P) charge ratios. When the complexation is achieved (zero surface charge), the band is diffused (at 0.75/1 in this case).

6. Conclusions

A complete characterization of nanomaterials intended for biomedical purposes (diagnostic, treatment, or theragnostic) is a must before the translation to preclinical and clinical studies. Classical chemistry analytical techniques can be applied for the characterization of some aspects of nanomaterials, since nanomaterial properties usually change from those of their components. Chromatographic techniques can be useful for the determination of the molecular weight of nanosystems, as well as for the purification of the produced nanoobject from raw materials and impurities. Spectroscopic techniques, in parallel, can be very useful for the confirmation of the formation of the nanosystems, assessing the materials of which it is composed as well as its aggregation/attachment state. Calorimetric techniques can be useful for the study of the nanomaterial behavior when submitting them to temperature changes. Finally, it is always required a purification step to ensure the obtaining of a safe nanosystem, free of impurities and raw materials. Therefore, as a general conclusion of this review, it is strongly recommended to take a look on the variety of existent techniques to look for all the aspects that must be known before the translation of a novel nanomaterial to a human diagnostic or therapeutic formulation.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was funded by Spanish Ministry of Economy and Competitivity, MINECO (grant CTQ2014-52687) and Generalitat de Catalunya (grant 2014-SGR-1655). CIBER-BBN is an initiative funded by the VI National R&D&I Plan 2008–2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions and financed by the Instituto de Salud Carlos III with assistance from the European Regional Development Fund. Cristina Fornaguera is grateful to MINECO for their PTQ2015 grant.

Supplementary Materials

Schematic representation of analytical techniques to characterize a nanoparticle dispersion, as a summary of the whole review. *(Supplementary Materials)*

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