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HPLC Method development and validation for Nano drug delivery system

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ABSTRACT
Advances in polymeric nanoparticles as novel nanomedicines have opened a replacement class of diagnostic and therapeutic tools for several diseases. However, although the benchtop research studies within the nanoworld are numerous, their translation to currently marketed products remains limited. This lack of transference are often 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 selected sort of nanomaterial or maybe methods used for bulk materials, which aren't completely applicable to nanomaterials. For this reason, the present review aims to become a scholastic guide for those scientists starting within the nanoworld, giving them an outline of analytical characterization techniques aimed to analyze polymers forming nanoparticles and possible forms to purify them before getting used in preclinical and clinical applications.

Keywords: - Nanoparticles, Analytical characterization and purification, preclinical and clinical.

I. 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 nano systems 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 nano systems in terms of safety, versatility, and robustness [1–3]. Although the number of benchtop research studies developing novel nano systems 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 com plexity of a deep characterization, which would enable their safe production and use in humans [4]. Therefore, a good characterization of nanomedicines may be a must before their testing in preclinical and clinical stages. However, , the present technology is challenging within the sense that a lot of characterization techniques are applied directly from those methods used for bulk materials (not at the nanoscale) or using conditions that don't simulate biological environment [1, 2]. Therefore, many efforts must be devoted to the enhancement of the performance of current techniques. Nanomedicine characterization are often divided in three steps: first, an analytical characterization, useful for characterizing the materials they're composed of also on determine 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 componentsfor the characterization of polymeric nanoparticles designed as nanomedicines exist, most of them give a particular point of view, signaling just some techniques [2, 5, 6]. Therefore, , scientists working on the development of novel nano formulations find themselves lost in the huge but dispersed existent bibliography. This is the

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rationale that motivated the authors to write down a series of three reviews with a scholastic character, to enable those scientists starting within the nano22world to possess 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 character inaction 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 those techniques within the SI). Therefore, the purpose of the present review is to be a first practical guide line for those scientists initiating their studies in the nano world.

It should be noted that it had been not the target of the authors to urge deep into description of every 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 are classified not as a function of what's characterized but as a function of the technique: chromatographic, spectroscopic, calorimetric, and purification techniques. Authors will guide the reader through them with the target to assist within the selection of 1 or other technique counting on the parameter to review. Physicochemical techniques, mainly used to characterize size (e.g., light scattering or microscopy), are out of the scope of the present review [7].

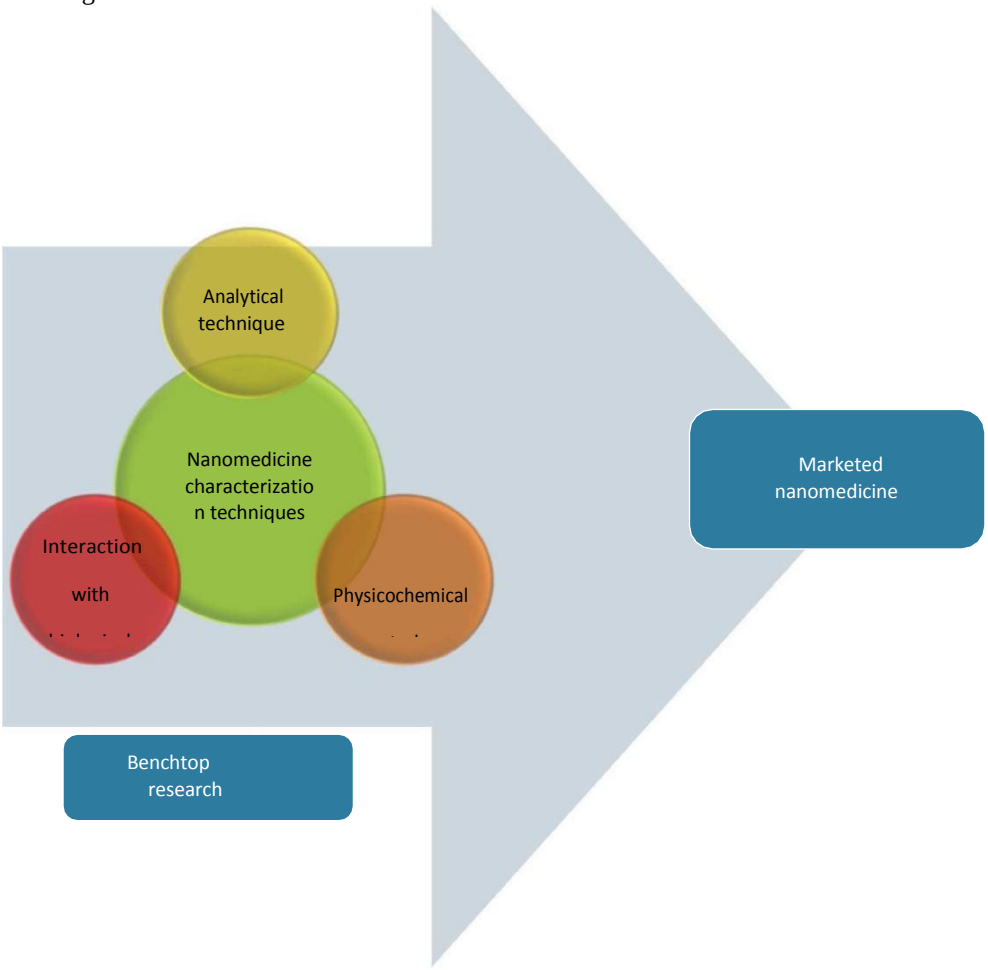

Figure 1

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HPLC

High-performance liquid chromatography, formerly referred to as high-pressure liquid chromatography, is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture.

The separation principle of HPLC is based on the distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (packing material of the column). Depending on the chemical structure of the analyte, the molecules are retarded while passing the stationary phase.

High-Performance Liquid Chromatography (HPLC).

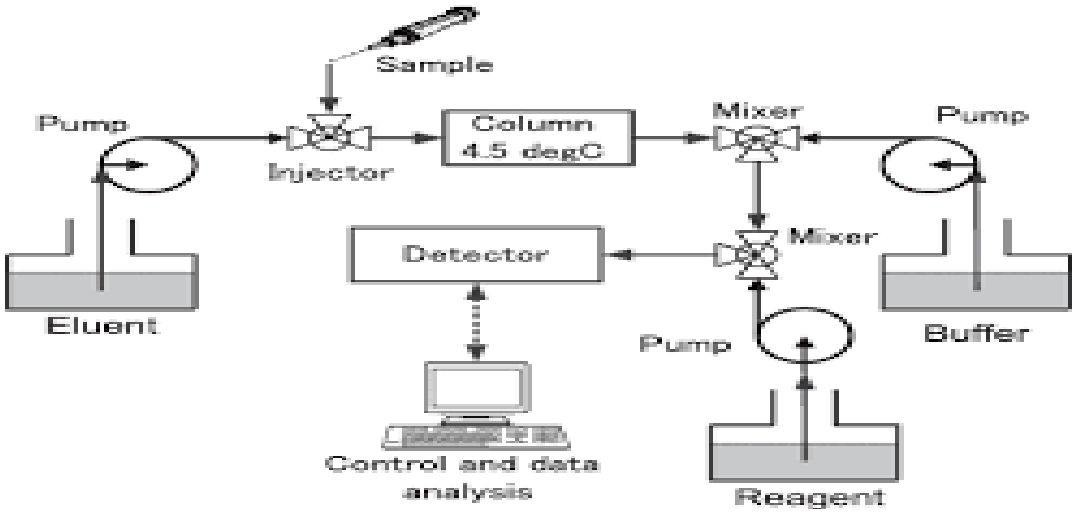
High-performance liquid chromatography (HPLC) is the most used type of chromatography not only for colloidal nanosystem studies but also for other type of materials(e.g., proteins).Inthevastmajorityofstudies,itisusedforthefine quantification and separation (purification) of actives, such asdrugs[5,8,9].Briefly,itconsistsintheinjectionofthel iquid 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 com- pound. It results a chromatogram with the peaks of each compound (Figure2).

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 com-monly composed of silica with attached alkyl chains, being the reversed phase C18-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 manip- ulation[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].



HPLC Diagram Figure no 2

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Development and validation of an HPLC method for the determination for Different Nano Drug Delivery system

1) fluorouracil in polymeric nanoparticles

Theobjectiveofthisworkwastodevelopandvalidatearapidhighperformanceliquidchromatography (HPLC) method for the quantitative analysis of fluorouracil (5-FU) in polymeric nanoparticles. Chromatographic analyses were performed on an RP C18 column with a mobile phase consisting of acetonitrile and water (10:90, v/v) at a flow rate of 1 mL/min. The 5-FU was detected and quantitated usingaphotodiodearraydetectoratawavelengthof265 nm.Themethodwasshowntobespecificand linearintherangeof0.1-10µg/mL(r=0.9997).Theprecision(intra-andinter-day)wasdemonstrated because the maximum relative standard deviation was 3.51%. The method is robust relative to changes in flow rate, column and temperature. The limits of detection and quantitation were 10.86and 32.78 ng/mL, respectively. The method fulfilled the requirements for reliability and feasibility for application to the quantitative analysis of 5-FU in polymeric nanoparticles.(12)

2) Development and validation of a stability-indicating RP-HPLC method to separate low levels of dexamethasone

Betamethasone (BM) is an active pharmaceutical ingredient (API) or an intermediate which is used to manufacture various finished pharmaceutical products. Betamethasone is also used as a starting material to manufacture other APIs that are related to this steroid family. It is quite a challenging task to separate dexamethasone (DM) peak (the alpha epimer) and other structurally related compounds from BM. A stability-indicating reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed which can separate and accurately quantitate low levels of DM and other related compounds from BM and also from each other. This method was successfully validated for the purpose of conducting stability studies of betamethsone in quality control (QC) laboratories. The stability-indicating capability of this method was demonstrated by adequate separation of DM and all the degradation product peaks from BM peak and also from each other in aged stability samples of betamethasone. A gradient mobile phase system consisting of (A) water: acetonitrile (90:10, v/v) and (B) acetonitrile: isopropanol (80:20, v/v) was used with an ACE Phenyl column (10 cm × 4.6 mm, 3 m particles, 100 Å pore size) and an ultraviolet (UV) detection at 240 nm. (22).

3) Development and Validation of the HPLC Method for Simultaneous Estimation of Paclitaxel and Topotecan

There are no results for a simple, rapid, accurate and precise high performance liquid chromatography (HPLC) method for simultaneous analysis of Paclitaxel and Topotecan was developed. Different analytical parameters, such as linearity, accuracy, precision, specificity with intentional degradation, limit of detection and limit of quantification (LOQ), were determined according to the ICH guidelines. Acetonitrile–water (70:30, 0.1% trifluoroacetic acid) was run on a Phenomenex Luna C-18(2) column in isocratic mode at a flow rate of 1.2 mL/min for simultaneous analysis of the two drugs using a UV detector set at 227 nm. The proposed method showed a retention time (RT) of 14.56 min for Topotecan and 23.81 min for Paclitaxel with a continuous run up to 30 min. The linearity of the calibration curves foreach analyte in the desired concentration range was found to be good (r 2 > 0.9995). The recovery ranged from 97.9 to 101% for each drug with a relative standard deviation (%RSD) of <2%. Peaks corresponding to each of the drugs exhibited positive values for the minimum peak purity index over the entire range of integrated chromatographic peak indicating high purity of the peaks. Stability analysis revealed that the drugs remained stable for sufficient time. Thus, the developed method was found to be robust, and it can be employed to quantify Paclitaxel and Topotecan in commercial sample and rat blood/serum (23).

II. APPLICATION

1. It is applicable in Food Analysis, Drug interaction studies, Preparative Analysis, Forensic Sciences, and in natural product analysis.
2. HPLC is much more useful for the bioanalytical studies .it is used to determine the drug in biological matrices such as blood, plasma, urine, serum and faeces. Thus it is useful in pharmacokinetics and bioequivalence studies.
3. HPLC has got a prime importance is the pharmaceutical industry. It is used in the department such as R&D, quality control and F&D. it is used for the analysis of samples from initial step that is raw material analysis till the final product analysis.
4. The validity of the suggested method was

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assessed by their application to the determination of the cited drugs in spiked human plasma and in their separate tablets dosage forms. A statistical comparison of the obtained results by the suggested method and the reported method [44].

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