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Comprehensive review on nano drug delivery systems bioanalysis

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Abstract

Nanoparticulate drug delivery systems (NDDSs) offer a revolutionary strategy to fighting ailments. The NDDSs are uniquely and deliberately constructed as vessels for the transport of active pharmaceutical components (APIs), which leverages their distinct physicochemical traits, such as lengthened circulation period, enhanced targeting capability and evasion of drug resistance. Although the last three decades have seen considerable progress, our comprehension of how NDDSs' pharmacokinetics in vivo can affect their safety profiles is still insufficient. Examining NDDSs is way more complex than analyzing small molecules since their structure and composition are completely different, almost making regular analytical techniques inadequate for measuring correctly its behavior within living systems. The following advanced bioanalysis techniques are summarized to trace the in vivo fate of NDDSs: liquid chromatography tandem mass spectrometry (LC-MS/MS), enzyme-linked immunosorbent assay (ELISA), magnetic resonance imaging (MRI), radiolabeling, fluorescence spectroscopy, laser ablation inductively coupled plasma MS (LA-ICP–MS,) and size exclusion chromatography. These tools provide an invaluable way of examining a drug's journey through the body. By leveraging the most modern technologies, we are able to conduct a thorough survey of NDDSs and their various changes in structure, composition and form. This review can be beneficial for accurately assessing pharmacokinetics as well as gauging efficacy and safety profiles of new drug delivery systems. We hope this comprehensive overview will serve as an effective basis for application methodology when analyzing NDDSs.

Keywords: Nanoparticles; Mass Spectrometry; Bioanalysis; Polymer; Nanoparticulate drug delivery systems and Pharmacokinetics

1. Introduction

Over the past three decades, nanotechnology has been a blossoming field with its applications ranging from medical diagnosis to pharmaceutical discovery and tissue engineering. Nanomedicine delivery systems (NDDS), in particular, have recently become increasingly popular due to their various advantages. The first NDDS approved by the FDA was Doxil®, liposomal doxorubicin (DOX) for treatment of AIDS-related Kaposi's and ovarian cancer, which decreased side effects associated with traditional treatments while ensuring passive tumor targeting efficacy. Over the last several years, numerous NDDSs have been researched and tested in preclinical and clinical studies, including liposomes, micelles, polymer-based nanoparticles (NPs), nanoemulsions, nanogels, inorganic NPs as well as core/shell combinations of both organic and inorganic substances[1–5].

In nanodrug delivery systems (NDDSs), small molecular drugs or biotherapeutics are housed in nanoparticles either chemically bound or trapped. These NDDSs, unlike traditional pharmaceuticals, provide exceptional properties related to drug delivering post-administration. Nanoparticle Drug Delivery Systems (NDDS) have been scientifically proven to promote the performance of parent drugs by increasing their circulation duration, potency and targeting capacity, reducing toxicities and immunogenicity as well as combating drug resistance. This has generated an immense surge in global investment into nanomedicine research with 623 million US dollars poured into it from 2011-2019 alone

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according to NIH figures. Yet despite this profound progress there are still only a mere 51 approved nanomedical products by FDA till now. The incomplete understanding of NDDSs' pharmacokinetic properties caused the low clinical transition ratio. It is still hard to comprehend the biological fate of nanocarriers and payloads at once with conventional methods such as fluorescence labeling, making it difficult to track them in vivo[5–8].

In this paper, we examine the recent developments of bioanalytical techniques for pharmacokinetic study on non-conventional delivery systems (NDDSs) for the first time. With details about how to measure and results from released drug within a polymer carrier of NDDSs as well as their bio distribution, along with any obstacles or perspectives that may arise when using these technologies, readers will gain in-depth knowledge into the use of NDDSs.

2. Classical NDDS

A range of nanoscale drug delivery systems (NDDSs), including liposomes, micelles, polymer-based NPs, nanoemulsions, nanogels, inorganic NPs and core/shell inorganic/organic NPs have been developed to transport APIs precisely to their intended site. As awareness grows about the advantages NDDSs offer compared with traditional methods of administering drugs, we are now seeing a rapid increase in the number that progress from preclinical through to clinical phases [9–11]

2.1. Liposomes

Liposomes are microscopic vesicles, measuring only around 400 nanometers in size that form a concentric bilayer from either natural or synthetic phospholipids. The molecular structure of these lipids consists of a phosphate group with two hydrophobic fatty acid chains which assemble and fuse to create the closed-off bilayer when immersed in water. Boasting size and hydrophobic properties, as well as an exterior electrostatic charge, liposomes are uniquely suited to storing both water-soluble APIs in the cavity or oil soluble ones within the lipid bilayer (Fig. 1A). The phosphate groups that line its external and internal surfaces enable recognition by mononuclear phagocyte system (MPS), allowing a rapid clearance response. With this functionality, liposomes can be confidently used for transportation of active pharmaceutical ingredients. To overcome this obstacle, PEGylation has become a prevalent technique to modify the surface of liposomes with amphiphilic polymers like poly (ethylene glycol). This coating provides "stealth properties" that shield the liposome from enzymatic and immunologic clearance. Consequently, it reduces opsonization while also lengthening its circulation time. In 1995, the approval of Doxil® brought about a revolutionary surface-bound methoxy polyethylene glycol liposomal formulation (STEALTH®), which allowed for an impressive half-life of approximately 55 h in humans[12,13].

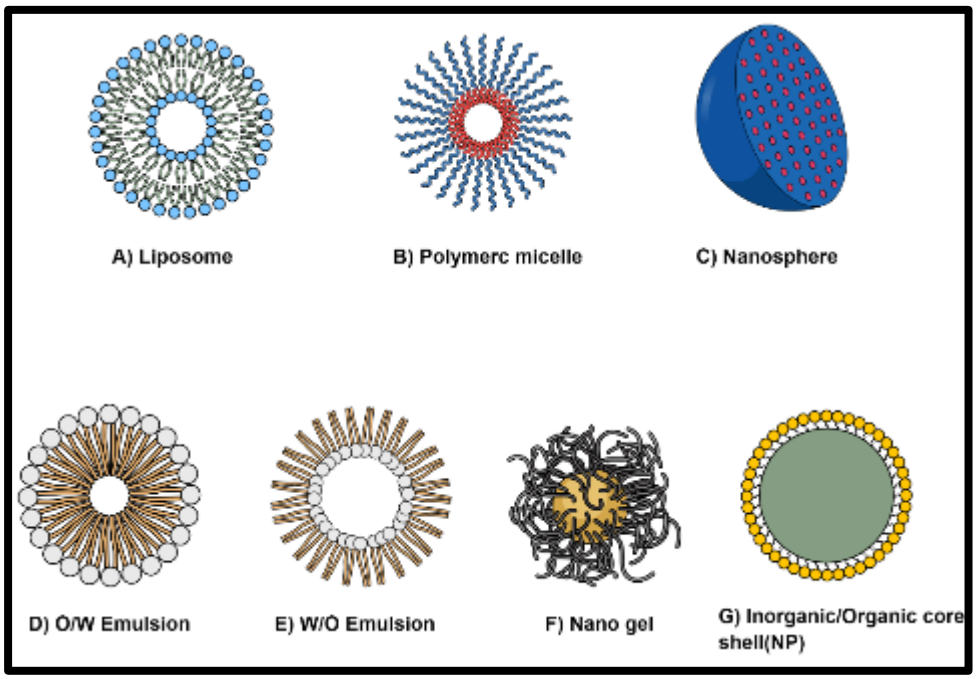

Figure 1 Nano drug delivery system (NDDSs) used in drug delivery

2.2. Polymeric micelles

Polymeric micelles, which are composed of amphiphilic copolymers containing hydrophobic and hydrophilic blocks, magically form solid spherical aggregates when the concentration of this special polymer surpasses its critical micelle concentration (CMC). These aggregates can range in size between 10 nm to 100 nm with a unique construction: they have an internal hydrophobic core accompanied by a protective outer layer known as the "corona" that is made out of water-attracting compounds. Through their ability to increase lipophilic drug concentrations in an aqueous medium, polymeric micelles had been used extensively for medical applications such as drug delivery (Fig 1B). After being administered via injection, polymeric micelles can be diluted beneath their Critical Micellar Concentration (CMC) in the bloodstream. This may cause them to disassemble into unimers. The CMC is heavily influenced by various aspects such as the polymer's structural composition and molecular weight of each polymeric block. Nanoxel-PM® offers docetaxel in an mPEG-PDLLA micelle formulation, while Genexol®-PM contains PEG-PLA micelles filled with paclitaxel[14–17].

2.3. Polymeric NPs

Polymeric particles ranging from 40 to 400 nm can transport APIs either embedded within their polymeric matrix or absorbed on the surface. These NPs are divided into nanospheres and nanocapsules, depending on the type of polymer used, where the API is localized, and how it's produced. Nanospheres have a solid structure with an outer shell that contains both dissolved as well as externally loaded APIs (Fig. 1C). On the contrary, nanocapsules are storage systems composed of a polymeric shell and APIs either stored in an inner liquid core or adsorbed on its surface. These polymeric nanoparticles may be synthesized from natural materials such as chitosan or dextran as well as synthetic ones including PLA (poly (lactide)), PLGA (Poly (lactide-co-glycolide)), Poly (alkylcyanoacrylates), and Poly (epsilon-caprolactone)). The FDA has granted its approval to synthetic polymers such as PEG, PLA and PLGA for human use over the span of decades. The 2018-approved lipid nanoparticle drug Onpattro® consists of two lipids: heptatriaconta-6, 9, 28, 31-tetraen-19-yl -4-(dimethylamino) butanoate (DLin MC3 DMA) and a-(3'-[1 & 2 di (myristyloxy)-proponoxy] carbonylaminopropyl)-u methoxypoly oxyethylene (PEG 2000 CDMG)[18–22].

2.4. Nanoemulsions

Nanoemulsions are a stable, effective dispersion of two immiscible liquids; one is dispersed as droplets in the other and stabilized by an amphiphilic surfactant (Fig. 1 D and E). These nano-sized droplets typically range from 10 to 600 nm in diameter making them ideal for delivering both hydrophobic and hydrophilic drugs with improved solubility and protection against hydrolysis or degradation due to enzymes. The release of drugs from nanoemulsions involves solute distribution from the droplets into their surfactant layers, before diffusing out to a wider environment. Unfortunately, nanoemulsions that are produced using low-energy emulsifying methods usually require an extensive amount of stabilizing surfactants - which can pose other problems such as cell membrane fluidization if too concentrated. Another issue with these particle suspensions is a limited stability and poor adhesivity.In 2002, the FDA gave its approval of Restasis®, an oil-in-water nanoemulsion consisting of cyclosporine A in castor oil droplets and with polysorbate 80 as the emulsifying agent, for treating keratoconjunctivitis sicca[23].

2.5. Nanogels

Nanogels, ranging from 100nm to 200 nm in size and composed of a cross-linked hydrophilic polymer network, are revolutionary drug carriers capable of absorbing up to one thousand times their dry weight. By forming a huge 3D framework when swollen, nanogels can be used for the containment of drugs, polymers and liquid dispersions (Fig. 1G). Not only have that but due to its versatility with both hydrophobic and hydrophilic compounds, nanogels become indispensable tools in modern pharmaceutical delivery systems. Due to their unique property of swelling and shrinking, flexibility in shape, high water content, and large surface area; nanogels are able to produce a controlled release effect. With multiple formulations currently available on the market ranging from cosmetic remedies to toothpaste formulation and personal skin care products , there is something for everyone[24,25]

2.6. Inorganic NPs and inorganic/organic (core/shell) nanoparticles

Inorganic nanopharmaceuticals (NPs) have been around for many years, such as the use of platinum compounds in cancer therapy and silver to fight bacterial infections. These NPs include metals and metal oxides like gold (Au), silver (Ag), platinum (Pt), iron oxide (Fe_3O_4), titanium oxide (TiO_2), copper oxide (CuO) and zinc oxide (ZnO). In addition, these inorganic pharmaceuticals can gain an upper hand with nanomedicines by enhancing drug loading, targeting capabilities, immune system evasion while improving their pharmacokinetic performance. Inorganic/organic (core/shell) nanoparticles are complexation of inorganic NPs with an organic polymer shell (Fig. 1H). The polymeric protective shell can promote purely steric repulsions so as to reduce the range and strength of electrostatic and van der Waals interactions between the colloids. Ferumoxytol (Feraheme ®), which have been officially accepted by the FDA

for treating iron deficiency in adults with chronic kidney disease since 2009, are hybrid aggregates that normally measure around 100 nm and demonstrate exceptional colloidal stability despite ionic strength variations[26,27].

3. Quantification of the *in vivo* trafficking of NDDSs

To realize the desired biological effect, the active pharmaceutical ingredients (APIs) must be released from nanoparticles (NPs) and reach their target site. Although nanodelivery drug systems have been applied as carriers for over two decades now, pharmacokinetics studies usually focus on total drug concentrations while overlooking polymers involved. Moreover, characterizing a systematic release profile of drugs loaded in particles remains an incomplete task. As opposed to the conventional pharmacokinetic studies of APIs, NDDSs (Novel Drug Delivery Systems) also necessitate analyses of encapsulated drugs and carrier polymers. The primary technical complication hindering bioanalysis on these systems is the quantitation and discrimination between released and encapsulated drug molecules. Mercifully, thanks to modern advancements in technology, numerous methods have been developed for identifying liberated vs. entrapped drugs in vivo - such as LC-MS/MS; ELISA etc.

3.1. Quantitation methods for the released and encapsulated drug in vivo

The NDDS system of encapsulating drugs not only preserves the payloads, but it also extends their circulation time and solubilization capabilities. This is typically referred to as a circulating “reservoir” for drug delivery. Countless studies have been conducted in an attempt to uncover the release profiling of these NDDS systems.

3.1.1. LC-MS/MS

As LC-MS/MS is now considered the preferred method for examining drug release from NDDSs in vivo, researchers must identify a way to differentiate between released and encapsulated drugs. Smits et al., with their novel approach of utilizing LC-MS/MS to quantify prednisolone phosphate loaded liposomes in whole blood and liver tissue, were the first individuals to successfully do so. Therefore, this method provides an invaluable asset when it comes to accurately assessing these types of medications. To examine the released drug, prednisolone phosphate was applied as a surrogate analyte since it is immediately dephosphorylated by phosphatases in vivo. An analogous study surrounds the determination of release from polymeric micelles and has been documented in Braal et al.'s research. CriPec® is an approximately 65 nm micelle that's stably covalently conjugated to docetaxel at pH 5.0 temperature conditions. To quantify released docetaxel, we can do so without interference from the conjugated drug. Meanwhile, to ascertain the amount of attached docetaxel, it must first be detached from micelles at a temperature of 37° degrees Celsius and pH 7.4 over three days. Unfortunately, our LC-MS/MS technique cannot differentiate between newly disjoined meds and earlier released molecules; thus we need to separate drug-laden micelle particles first before further analysis can be done.

Unlike protein precipitation method[28,29], the solid Phase Extraction (SPE) is a valuable tool in separating encapsulated and released drug. The standard SPE procedure involves three steps: 1) loading the sample; 2) water washing, which ensures that liposomes with hydrophilic surfaces are not retained on the column; and 3) eluting adsorbed drugs with a hydrophobic solvent. Deshpande et al., Su et al., Xie et al. have all utilized this process to understand the pharmacokinetic behavior of amphotericin B and DOX liposomes when given to living organisms. Wang et al. followed a comparable methodology to profile the DOX liposome release and uptake process in healthy tissues and tumors. This particular SPE separation technique has also been conditionally used with polymeric NPs; Song et al. were trailblazers as they applied this same approach for determining gedatolisib released from PLA-PEG NPs. By subtracting the released gedatolisib values from their total values, we were able to ascertain the concentration of encapsulated gedatolisib. Taking into consideration the payload drug's features, an MCX SPE column was chosen instead of an HLB SPE column. To assess the efficacy of ultrafiltration as a liposomal separation technique, Xie et al. [46] conducted an experiment with DOX liposomes as their sample. Unfortunately, they discovered that the recovery rate of DOX by way of this method was only 10%. It's speculated that adsorption to device and plasma proteins might have been to blame for such low success rates. Fortunately, Chen et al. introduced a novel class of separation techniques which could provide more promising results in future experiments. To separate biotin-DTX-liposomes from plasma, streptavidin-Fe^3O_4@PDA was used since its particular binding with biotin and streptavidin is so efficient. Through the presence of a magnetic field in this process, an incredible 75% recovery efficiency has been achieved[30–36].

3.1.2. ELISA

The ELISA, or Enzyme-Linked Immunosorbent Assay, is an immunological technique used to detect various biological molecules such as proteins, antibodies, hormones and cytokines. It has also been applied to analyze the release profile of bio macromolecule-loaded nanoparticles. The most common form of ELISA utilizes a “sandwich” format where two

different types of antibodies bind with the targeted antigen between them; these dual antibody forms are known as capture and secondary for their roles in capturing and confirming analytical antigens respectively. The T antigens captured can be identified and tracked with the help of an enzyme-linked specific antibody, referred to as a detecting antibody. The attached enzyme serves its purpose by amplifying the detected antigen's signal optically while also allowing for quantification. Wang et al. employed a sandwich ELISA method in their research that measured both stromal cell-derived factor 1 (SDF-1) and bone morphogenetic protein 2 (BMP2) release payloads to determine encapsulation efficiency and kinetics of chitosan oligosaccharide/heparin NPs in vitro. Azie et al. utilized latent transforming growth-factor beta (TGF-b) and conjugated it to super paramagnetic iron oxide nanoparticles (SPIONs). Afterwards, the active TGF-b release profile from these SPIONs was monitored through ELISA. Although this ELISA has a great sensitivity advantage, there certainly exist certain drawbacks like limited varieties of commercial kits available, narrow linear ranges of results as well as potential cross reactivity issues[37–39].

3.1.3. Quantitation methods for polymer

Following ingestion, the polymeric material of NDDSs may be subject to disassembly, distribution, metabolism and excretion. In comparison to the actual drug payload, our understanding concerning its in vivo fate is uncertain. Although pharmaceutical polymer excipients are generally believed to act as inert ingredients for NDDSs, ADR reports due to these components such as hypersensitivity reactions, cell vacuolation or splenomegaly continue rising alarmingly. The potential toxicity that can come from the accumulation of polymers has been a topic of concern for regulatory authorities. To analyze these materials, multiple analytical techniques have been developed such as colorimetry methods, nuclear magnetic resonance (NMR), high-performance liquid chromatography (HPLC), and size exclusion chromatography (SEC). However, many of these technologies lack sufficient sensitivity; thus currently LC-MS/MS and ELISA are being used to accurately quantify the polymer in vivo with much greater precision.

3.1.4. LC-MS/MS

LC-MS/MS has revolutionized small molecular drug analysis, making a tremendous impact on the quantitative study of polymers. However, one major challenge arises from this technique: due to their heterogenous structure that consists of multiple homologues with differing degrees of polymerization and MWs, accurate quantification is often difficult. In order to tackle this problem, a variety of mass spectroscopy data gathering techniques such as selected ion monitoring (SIM), multiple reaction monitoring (MRM), in-source collision induced dissociation (CID) and MSALL were employed for the analysis of polymers found in biological matrices. This review will discuss bioanalytical strategies for detecting substances like PEG, PLA, hyaluronan (HA), chitosan and cyclodextrin (CD).

PEG

PEG is one of the most fundamental synthetic polymers in the pharmaceutical industry. It's commercially produced from ethylene oxide monomers and can be seen illustrated in Fig. 6A. PEG has a variety of uses, acting as solubilizer, stabilizer, and release-modifier or conjugated with drug molecules (PEGylation) and utilized to create drug delivery vehicles such as liposomes, micelle, and nanoparticles. Moreover, its ability to lengthen drugs' half-lives while improving bioavailability and reducing immunogenicity was approved by FDA for human use over ten years ago, making it an indispensable part of modern medicine today[40–43].

In 2004, Zhang and collaborators developed a Flow Injection Mass Spectrometry (FIMS) method for quantifying PEG300 in commercial drug formulations. This was done using the Selected Ion Monitoring (SIM) scanning mode - whereby only precursor ions are passed through both Q1 and Q3 quadrupole mass analyzers; with CE of Q2 set at a level which does not cause any noticeable fragmentation. Comparatively, this method of analysis yielded better results in terms of its lower limit of quantitation (LLOQ) at 136 ng/mL - a far cry from Gas Chromatography-Mass Spectrometry (GC-MS)'s 1 mg/mL, Semi-Preparative HPLC's 0.73 mg/ mL, HPLC's 50mg / ml[68] and SEC's 1.15 mg /ml. Although SIM methods are highly effective for analyzing biological samples, they're often neglected due to their inferior selectivity and background noise levels. Ashiru et al. pioneered the first LC-MS/MS methodology to quantify PEG400 concentration in biological samples, but this method was hindered by limited selectivity of SIM; as a result, its LLOQ (2.5 mg/mL) exceeded that of the preceding flow injection MS technique[44–48].

MRM is a particularly delicate and discerning scan mode, which is frequently employed in the LC-MS/MS bioanalysis of small-molecular drugs. This method entails selecting the precursor ions of your respective analyte by way of Q1, followed by effective fragmentation in Q2. Afterward, using the second mass spectrometer -Q3-, a uniquely specific product ion can be identified from among multiple product ions for detection purposes; this bolsters selectivity and significantly enhances signal-noise ratio. Bhaskar et al. implemented an MRM analysis to investigate the bioanalytical assay for PEG400 using plasma. In this study, nine of the most frequent oligomers and their shared product ion (at m/z

89) were monitored in Q1 and Q3 respectively. Further, each analyte peak was combined together to identify precisely how much PEG400 was found in the plasma with a LLOQ of 1.01 mg/mL- which is suitable for low MW PEGs. This approach fails when used on high MW PEGs due to its wide range of homologues and multicharged ions; thus making it difficult to monitor them all through scrupulous MRM analysis -which is mandatory for quantitation purposes. Warrack et al. proposed a two-step approach for determining high molecular weight PEGs (1.4 to 40 kDa) found in biological samples, which begins by subjecting them to in-source CID declustering potential ionization that leads to the production of fragment ions. These fragments are then subjected to MRM scans as surrogate precursor ions; however, this method still holds some limitations due its limited ability at fragmentation under DP and concomitant decline in sensitivity during the MRM scan stage. For PEG, the lower limit of quantification (LLOQ) with in-source CID is 300 ng/mL. To heighten fragmentation efficacy, Zhou et al. developed a MSALL based technique for liquid chromatography triple-quadruple and time-of flight mass spectrometry analysis (LC-QTOF MS). QTOF MS brings together an array of elements - including Q1, Q2 and high resolution TOF mass analyzer – to offer incredible precision. The exclusive scan mode allows all precursor ions to pass through each element before being fragmented while passing through Q2 via the MSALL method. Subsequently, all the product ions were scrutinized with a high-resolution TOF analyzer (Fig. 7D). In comparison to earlier strategies, MSALL is an effective technique for measuring PEGs in biological samples. Consequently, this approach can also be used as a quantitative analysis tool for both PEG and its derivatives like PEGylated DOX, paclitaxel and gemcitabine[49–53].

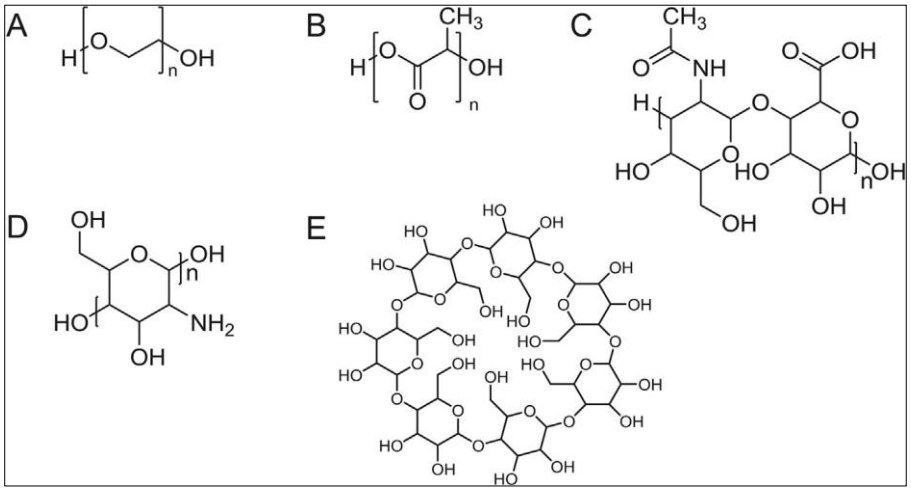

Figure 1 Structures of (A) poly (ethylene glycol) (PEG), (B) poly(lactide) (PLA), (C) hyaluronan (HA), (D) Chitosan, and (E) b-cyclodextrin (β-CD).

PLA

is a widely implemented biodegradable polymer due to its low toxicity and biocompatibility (Fig.6B). The different types of PLA available in the market are poly-L-lactic acid (PLLA), poly-D-lactic acid (PDLA), and poly-DL lactic acid (PDLLA). It's usually copolymerized with PEG to form an amphiphilic copolymer which can assemble into micelles for drug encapsulation. Shi et al. reported that it was possible to quantify PEG -PLA in plasma using CID technique. The PLA-specific fragment ions were generated in the source, which then further fragmented into product ions of m/z 505.0 / 217.0 in Q2 detected with higher sensitivity for quantification of PEGPLA. MRM transition monitoring was also utilized to track the PEG-specific fragmentations and it proved successful when applied to a pharmacokinetic study on mPEG2000-PDLLA2500-COOH conducted on rats[54–56].

HA

Hyaluronic acid (HA) is an incredibly advantageous linear polysaccharide comprised of D-glucuronic acid and N-acetyl-D-glucosamine, commonly found in vertebrate soft tissues including connective tissue and extracellular matrixes (Fig.6C). Its hydrophilic properties make it particularly attractive for use due to its biocompatibility, biodegradability, non-toxicity and lack of immunogenic qualities. However HA powerful interaction with proteins is combined with a low ionization efficiency results in difficulty when preparing biological samples for quantitative LC-MS/MS analysis. Simek et al. developed a complex LC-MS/MS method for determining the levels of DOX and oleyl hyaluronan (HAC18: 1) in plasma and tissue homogenates. This involved two enzymatic work-up procedures, one by protease to free up any HA from proteins, and another through hyaluronate lyase digestion for further break down. Through this process, Simek et al were able to accurately measure the quantities of these substances present in samples taken from biological sources.

Hyaluronate lyase was used to reduce the length of HA chains in order for greater ionization efficiency. This approach was applied when studying the pharmacokinetics of DOX and HA-C18:1 after intravenous administration of DOD loaded into a HA-C18:1 polymeric micelle. A variation between the profiles of DOX and HA-C18:1, hinting at premature disruption within these micelles during vivo conditions[57].

Chitosan

Chitosan, a linear polysaccharide formed from β-1, 4 bonds between D-glucosamine and N-acetyl-D-glucosamine is obtained by deacetylation of chitin through alkaline or enzymatic processes (Fig. 6D). However its importance lies not only in its production but also the fact that high molecular weight Chitosan has lower solubility than low MW Chitosan; additionally with an MW less than 3.9 kDa it gains the common name of chitooligosaccahride (COS). Research suggests this variant has reduced degradation rates and higher toxicity as compared to other forms. In modern studies, LC-MS/MS is predominantly used to examine chitosan's properties. Li et al. published a Multiple Reaction Monitoring (MRM) method for distinguishing COS oligomers in chitosan samples ranging from monomeric D-glucosamine all the way up to heptamers. Since ESI sources could fragmentize chitin easily, an In-source Collision Induced Dissociation (CID) process was developed without any Q2 fragments so as to accurately detect and quantify the 139.7 ± 6 kDa of it; with this technique we achieved excellent linearity between 20-10000 ng/mL ranges at r > 0.99 values[58–60].

CD

Cyclic oligosaccharides or Cycle Dextrins (CDs) are composed of six (αCD), seven (βCD) or eight (γCD) a-1, 4-linked glucose units that have been shown to have the capacity to load drugs (Fig. 6C). The creation of CD-drug complexes increases solubility and enhances the physicochemical properties of medications in question. In particular, HP-β-cyclodextrin – short for 2 hydroxypropyl β cyclodextrin – is regularly utilized as an excipient due to its abundance; this homologue encompasses over two million possible combinations which makes it highly versatile. Jiang et al. created a two-dimensional liquid chromatography integrated with fluorescence detection and mass spectrometry (2D-LC-IF-MS/MS) method as well as a reversed phase ultra-performance liquid chromatography integrating with mass spectrometry technique (RP-UPLC-MS/MS) for the diagnosis of HPβCD in human plasma and cerebrospinal fluid respectively. The formation of sodium adducts can impede the fragmentation efficiency, which is why ammonium salt was added to the mobile medium. This allowed us to develop UPLC MS/MRM transistion at m / z 1326.5 / 383 for quantifying HPβCD and set a lower limit of quantification (LLOQ) as 50 ng/mL in human plasmic samples and 5 μg/mL LLOQ for CSF analysis. In addition, when employing 2D-LC-MS/MS method, we were able to detect using in-source CID and MRM with an LLOQ in human plasma and CSF of 10 ng/mL respectively 100 ng/mL - rendering this technique highly successful. The HPβCD underwent an in-source CID and formed a 2-hydroxypropyl substituted dihydro pyrylium fragment at m/z 203. Subsequent fragmentation transition to the product ion of 4-hydroxypyrylium was monitored by MRM at m/z 97, providing characteristics of its detection in plasma samples. Initially, separation was attempted on a HILIC column, yet it revealed that glycerophosphocholine species suppressed the detecting potential for HPβCD. To negate matrix interference, both 1D and 2D separations were carried out using a C_{18} guard column followed by a HILIC column. The more sensitive 2D-LC-MS/MS method was complemented with the UPLC-MS/MS method that had shorter runtime to improve throughput. Both these methods have been successfully applied to evaluate HPβCD pharmacokinetics in humans[61–63].

3.1.5. ELISA

By developing antibodies that bind to PEG conjugates, the ELISA method can be used for detecting PEGylated drugs. Richter and Akerblom pioneered the generation of anti-PEG antibodies from immunized rabbits; these polyclonal antibodies offer an LLOQ of 1mg/ml or lower when applied to PEGylated drugs. The binding affinity of such anti-PEG antibodies is significantly weaker than their affinity for free PEGs. To refine accuracy, mouse monoclonal antibodies were generated to differentiate and bind PEG-conjugates exclusively. By utilizing a sandwich ELISA approach with these antibodies, it was possible to measure the concentrations of PEG-conjugated in complex biological samples down to 1.2 ng/mL. Danika et al. further advanced upon this by creating a sensitive LC-MS/MS method (LLOQ 0.125 mg/mL) for determining PCK3145 levels in mouse plasma - providing unprecedented insight into previously inaccessible research data. The LC-MS/MS has long been used to quantify peptides, but still struggles with PEGylated peptides due to their high polydispersity of PEG, huge mass and poor ionization efficiency. As a result, the team created an indirect ELISA method for detecting PCK3145 that utilized Enzo Life Sciences' PEGylated protein ELISA kit. This anti-PEG sandwich ELISA achieved unprecedented sensitivity (with LLOQ at 0.132 ng/mL) and can be applied in a variety of circumstances concerning PEGconjugates. The accuracy of this technology has yet to be confirmed. Furthermore, ELISA is not suitable for the evaluation of free PEGs, which serves as a constraint when monitoring the biological impact of PEG-based NDDSs[64–69].

3.2. Quantitation methods for the NPs

Despite the unknown activity and uptake mechanisms of NDDSs, numerous in vitro and in vivo studies have verified an elevated cell intake of nanostructures. This increased cell uptake has become a critical subject within NDDS research due to its possible effect on drug payload distribution. At present, MRI imaging, radiolabeling methodologies, fluorescence spectroscopy and LAICP-MS are being used to measure NPs quantitation.

3.2.1. Radiolabeling

By labeling nanoparticles with radionuclides, their bio distribution, targeting and clearance can be tracked in vivo. Classical radiochemistry techniques commonly entail complexifying the particle surface, core or coating by attaching a radio-tag. However, this process might modify the original pharmacokinetic and toxicity profiles of NPs due to large lipophilic prosthetic tags or metal ion chelate-tags being introduced into the system.To maintain the surface properties of NPs, researchers have been exploring a multitude of alternative radiolabeling methods including radiochemical doping, physisorption, direct chemisorption and isotope exchange. Additionally, particle beam or reactor activation and cavity encapsulation can also be utilized to achieve this goal. To gain information on their in vivo bio distribution through modern imaging techniques such as positron emission tomography (PET), NDDSs need to be effectively labeled with radioactivity. Engudar et al.'s study revealed that when 124I liposomes were remotely loaded, PET/CT imaging in vivo could be used to evaluate them and exhibit a prolonged blood circulation half-life of 19.5 hours Additionally, lower accumulation of the radiolabeled liposomes was detected in spleen, liver, kidney and tumors compared with traditionally long-circulating ones[70–73].

3.2.2. Fluorescence spectroscopy

Recently, nanostructures known as quantum dots (QDs) have revolutionized fluorescence spectroscopy. This technologies has been a tremendous asset when it comes to studying the bio distribution of NPs in cells and tissues. Compared to traditional organic dyes, QDs possess optical transitions in the near-infrared region where tissue absorption is low or nonexistent. As such, this renders them ideal for use within biomedical fields that require precise measurements. Kenesei et al. used spectral imaging fluorescence microscopy to observe the dispersal of fluorescent polystyrene nanoparticles altered with PEG or carboxylic acid groups in male and pregnant female mice. The combination of this advanced imaging technology along with subsequent spectrum analysis enabled for effective visualization of these particles in many different tissues, thus circumventing any obstacles posed by the high auto fluorescence existing within native tissue structures[74–76].

3.2.3. LA-ICP-MS

ICP-MS is a well-known tool used to measure minuscule amounts of metals and metalloids. LA-ICP-MS, an extension of ICP-MS with the addition of a laser ablation system for vaporizing samples, allows us to map out the distribution of metal nanoparticles (NPs) within tissues using high resolution imagery. By scanning a tissue sample on its surface with lasers, we're able to achieve spatial mapping that provides unparalleled insight into NP absorption in various organs. Elci et al. created an LA-ICP-MS method to accurately map the bio distributions of PEGylated AuNPs for future biomedical uses. This imaging process will generate invaluable data regarding tissue/organ distribution, which will undoubtedly improve nanomaterial design and investigation[77–79].

3.2.4. Metabolite analysis techniques for Novel-Drug Delivery Systems (NDDSs)

When it comes to NDDSs, the metabolism and elimination of polymer materials are extremely essential. Unexpected accumulation of polymers or their metabolites in organs like liver, spleen, and kidney may cause iatrogenic illness. Fortunately for us, certain polymers such as PLA and HA are biodegradable which can be degraded into low molecular weight monomers/oligomers that will rapidly pass out from our body's system with ease. High molecular weight and shape are essential elements to consider when dealing with non-biodegradable polymers such as PEG, since they can greatly influence glomerular filtration and the rate at which something is eliminated. Combining biodegradable and non-biodegradable polymer blocks in a copolymer provides an effective solution for achieving balance between performance and excretion. For example, incorporating low MW PEG into PLA (polylactic acid) yields a block copolymer that can be degraded inside the body into smaller, renal excretory segments of non-degradable PEG.It is therefore of utmost importance to monitor synthetic polymers according to the ADME concept in order to identify and prevent iatrogenic illnesses due to progressive accumulation. One major component of a polymer balance study includes determining the excretion routes, as well as identifying metabolites found in urine, bile or feces. To do so, solid-phase or liquid extraction techniques can be used for recovering and enriching important polymer components based on their molecular weight (MW) and quantity. To investigate the molecular weight of cleared polymers, SEC or LC-MS methods are used. Additionally, fluorescence and radiometric techniques can be utilized to measure the amount of polymers

present in excreta samples. As both fluorescent and radio labeling strategies are surrogate measurements, they are usually combined with SEC for quantifying polymers. Radio labeling, fluorescence, and refractive index (RI) were used to analyze the degradation of poloxamer 188, HA, chitosan and PVA. Polymers don't absorb UV light very well; therefore RI was a viable option for quantifying higher concentrations at greater purity. Subsequently, SEC or HPLC combined with RI allowed us to measure the breakdown of PLGA, PLA, Chitosan and HA[5,80].

4. Conclusion

To ensure optimal results, NDDSs should include APIs with characteristics such as sustained release, extended circulation time, improved stability and solubility. Each year we gain a greater understanding of the pharmacokinetic properties of drug-loaded NDDSs; however there are still limited numbers of approved nanotechnology-based products due to their low drug pass through rate. We must continue investing in research so that these invaluable drugs can be fully realized and utilized for their many benefits. In this review, we explore the latest advancements in analyzing NDDSs. This includes exploring both the released and encapsulated drugs as well as examining their pharmacokinetic activities. We find that LC-MS/MS is by far the most comprehensive method for either tracking a drug's pharmacokinetics in clinical trials or quantifying its polymer material within living organisms. With the large discrepancy between released (in-) active ingredients and NPs in their pharmacokinetics, it is essential to comprehend the behavior of NDDSs within a living organism. To further support this idea, we must develop efficient bioanalytical methods that can assess these drugs accurately. We anticipate our review will be beneficial to those who are evaluating NDDSs for clinical applications by providing critical implications on its *in vivo* fate.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare that they don’t have any conflict of interest.

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