Review Article

Analytical strategies for the detection and quantification of nano-formulated antibiotics: Updates and perspectives

Abstract: The rapid development of drug resistant micro-organisms is a challenge to the mankind. Nano formulated compounds have proved to be effective strategy to combat bacterial drug resistance. Currently nanoparticulate systems such as nanoantibiotics are getting major attention due to their low inherent toxicity, biodegradability, bioincompatibility and tuneable mechanical characteristics. Nano formulated antibiotics are generally obtained by emulsification and gelification techniques. The effective uses of polymers in encapsulation of antibiotics show enhancement of the efficacy of antibiotics. Combined with techniques like diffraction laser spectroscopy (DLS), electron microscopy (EM) and atomic force microscopy (AFM), morphological research of nanoformulated antibiotics are conducted. The detailed study of the polymers used in the preparation of antibiotics nanoparticles as well as their impact on interactions is done by bio-analytical techniques. Antibiotics attached to nanoparticles can avoid the action of enzymes produced by drug resistant bacteria. Nano antibiotics show higher efficacy and bioavailability so a lot of new formulations using nano methods can be developed with the help of bioanalytical techniques. The development as well as the estimation of antibiotics prepared as nano-formulations as per the recent advanced techniques is illustrated in this review.
Keywords: Nano-antibiotics, surface properties, efficacy, resistance, bio-analytical techniques.

1. Introduction

Increased use of several antibiotics has boosted the level of danger to public health. Despite conventional antibiotics have yet to be seen to be effective, it has been generally assumed that most widely used antibiotics have lost their efficacy[1]. Specialized therapies for these infectious diseases are completely necessary, particularly in view of the increasingly evolving technologies for producing them [2-4].

Results of many nanotechnology studies have highlighted the strategic applications of living systems. The development of medicinal products is possible by streamlined nanometric technology, which offers molecular features and therapeutic effects. Researchers have recently discovered a way of growing the therapeutically effective biomolecular carriers known as nanotechnology, which are widely associated with antiviral, antifungal, anticancer, and antibiotic molecules [5-8].

The chemical compounds used to cure disease, which are known as antibiotics, are administered to inhibit the growth of unhealthy bacteria or to kill pathogens [9-10]. Antibiotics form a subgroup of endogenous anti-infective agents derived from bacteria or moulds poisonous to other bacteria. The word antibiotic, however, is often used broadly to describe anti-infectious substances derived from synthetic and semisynthetic compounds [11]. Efficacy of antibiotics can be assessed on several factors, such as administration route, site of infection, presence of intervention agents, drug concentration in the bloodstream, and pathogen presence [12]. Antibiotics being solid, shows precise calculation of the intensity is critical in pharmacology in order to optimize the effectiveness of these drugs [13].

Many methods of manufacturing nanostructures used for prescription drugs, which involve different forms of interactions among the antibiotic molecules and polymers are depicted in table 1.

The provided study has its emphasis on antibiotic growth and development. For the most part, recent work has concentrated on developing the antibiotics. The purpose of the current review is to present recent developments in antibiotic nano formulations.

Table 1. The antimicrobial behavior, immune response activation and toxic effects of nanostructured drugs.

Chemical class	Drug	Polymer	Method/structure	Results	Reference
Fluoroquinolone antibiotic	Levofloxacin	PLGA (Poly lactic-coglycolic acid)	Standard methods/Single emulsification and Solvent-evaporation (ESE) / Double-emulsification solvent-evaporation (DESE)	MIC = 2µg.ml^{-1} Dose used in study: 7±0.3µgml^{-1} killing 99.9% of *P. aeruginosa*	[35]
β -lactam antibiotic	Amoxicillin	PECA (Poly ethyl cyanoacrylate)	Emulsion polymerization of ethylcyanoacrylate	MIC did not show but Eradicated *Helicobacter pylori*	[41]
β -lactam antibiotic	Penicilin	Polyacrylate	Free radical emulsion polymerization in water	MIC = 16 µg.ml^{-1} against methicillin-resistant *Staphylococcus aureus*	[33]
Fluoroquinolone antibiotic	Levofloxacin	PCL (Poly caprolactone)	Emulsification/ solvent evaporation	MIC of nanoparticles against *E. coli* biofilm cells at 0.15 µg.ml^{-1}. 99.9% effective against *E. coli*	[32]
Aminoglycoside antibiotics	Gentamicin	PLGA	Water-oil-water / solvent evaporation	Prolongated the in vivo activity of gentamicin at MIC 1.5 mg.kg^{-1}	[40]
Immunomodulatory peptide	P10 peptide	PLGA	Water-oil-water / solvent evaporation	Killed *Paracoccidioides brasiliensis*	[29]

2. Advanced methods for development of nanoformulated antibiotic

Study of morphemes and encapsulation

In the beginning, to learn about the relationship between encapsulated drugs and nanostructures was the first step in designing nanostructured antibiotics. Next, further investigation is performed on nanostructures, mainly on what their functionalities are, as well as the modes of action, the pharmacodynamic and pharmacokinetic properties. Morphological analysis is performed using DLS, EM, AFM, or a combination of these approaches.

They are designed to gather information about their different traits, such as size and shape, as well as processing with nanostructures on the exterior. The studies in this article are intended to monitor the functions of nanoparticles with the use of the physical and chemical properties of nanostructures [14-16].

Additionally, antimicrobial peptides reflect structural forms of nanocarrier growth; dendritic polymers, solid-core nanoparticles, liposomes, or carbon nanotubes are all common types of nano formulating agents, used in a number of ways, which is that there are different ways of nanofabrication [17].

This method, together with scanning electron microscopy and scanning transmission electron microscopy, provides vital knowledge on the modes of action of nanoparticles, including that of nanoparticle-membrane interactions. Another advantage of DLS, also known as Systemic Synergistic Research, is that it allows accurate measurements of nanostructures and nanocomposites with respect to polydispersity, the consequences of drug-polymer interactions, and drug-controlled release.[18-19] Other important characteristics to remember when using DLS include the Zeta potential. Electrophoresis is the tool used to perform this form of research [20].

Many different experiments have employed these techniques to assess nanostructure stability. Zeta potential must be -70 mV to +70 mV. Structure voltage reaches + 70 mV and structures are more robust because they have a higher frequency of operation. However, nanostructures designed for medicinal use are unlikely to be as durable as they are when being published. Accordingly, researchers proposed that nanostructures should have -30 mV to + 30 mV values [21-22].

Other essential facts of the production of nano-particles include control release and toxicity reduction. The peptide-polymer polymer-drug link helps in better regulation of drug release, as a result resulting in a lower amount of host system absorption [23].

The main criteria is to analyze the clinical value and bio-security of the nano-structural drug delivery system. Nanostructured drugs as illustrated in the Fig 1 are tested for their antimicrobial, immune system activation, and toxic effects.

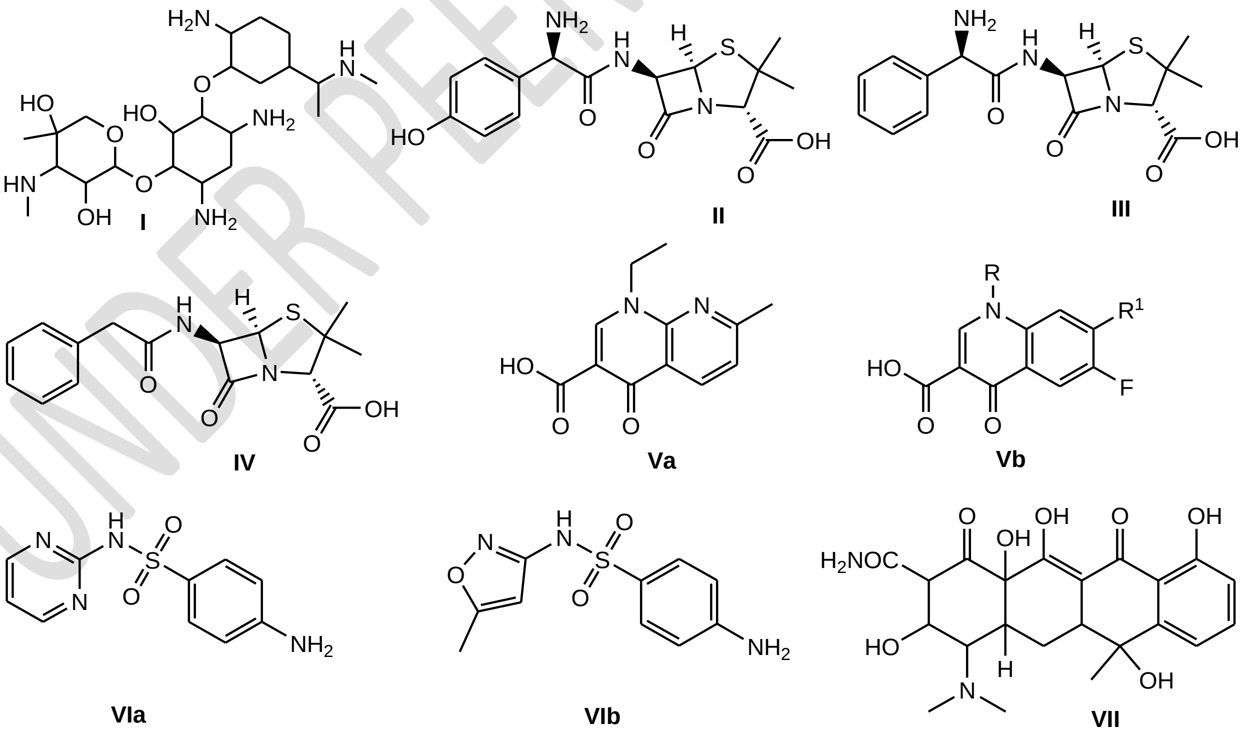

Fig 1. Chemical structures of gentamicin **(I)**, amoxicillin **(II)**, ampicillin **(III)**, penicillin G **(IV)**, nalidixic acid **V(a)**, Fluoroquinolone **V(b)**, sulfadiazine**(VIa)**, sulfamethoxazole **(VIb)** and tetracycline **(VII)**.

3. Nanoparticles comprising antibiotic complexes

Preformed polymers or monomers are used in the production of drug carriers in order to meet nanometric structures. The importance of the polymer-biomolecule relationship in bio nanotechnology cannot be overstated [23]. To quote one more example, Pinto Reis and coworkers update the requirement for polymer structure pharmacokinetic characteristics and is attentive to the methodology used. It has been proposed that characteristics such as the correct technique and safer nanoparticle-drug relations could add nanostructures with less toxicity and improved encapsulation effectiveness as soon as they emerge. Shemetov and colleagues debated the association among nanoparticles, peptides, and proteins in this context. Although nanoparticle properties including charge energy, morphology, and polarity are taken into account, the authors note that the biochemical and biological effects of nanoparticle-biomolecule interactions should be dependent on nanoparticle properties such as charge energy, morphology, and polarity [23].

Nanoparticles may adhere to the host systems when the outer surface can come into contact with the environment [23]. As well, both of the polymeric and peptide-constructed structures could decide nanoparticle physicochemical characteristics. Since the last interconnected nanostructures will show diverse properties of isolated polymers, this instance can contain them [24].

The idea is also interesting for drug carriers which plan to build a polymer-based drug delivery system that violates the hosts' immune system. The combined effects of cautious polymer-environment connections and improved target specificity could further boost the goal specificity and mitigate damage [24]. As can be seen in the illustration above, different materials can have various functional characteristics (e.g., structural stability, biodegradability, rate of release, morphology, etc.).

Other than charges and chemical structures, electrical charges and the composition of molecules can also have an effect on the way the tissues are categorised. Solubility is additionally helpful when encapsulated medications are given, since this improves the potency of the pharmacokinetics, resulting in a simple improvement in the pharmacokinetics [24].

Another category of polymers used for creating nano-antibiotics are polysaccharides, vinyl polymers, poly (amino acids), poly (ethylene glycol) and proteins. There are various block structures used in a radical polymerization; they are all based on the incorporation of free radicals. Nanostructures may have different types of structures that differ in their ability to activate the immune system, release molecules, improve solubility, stability, and biological activity.In the other hand, if it is not, natural polymers, such as chitosan, agarose, alginate, and chitin derivative, will also be worthwhile candidates for advancing nanodevices. Complex polymers' inherent chemical assemblies can give a superior combination of favourable characteristics and stability in a simple lined chain, so long as the polymers' distinctive biodegradability and biocompatibility when used as a drug carrier are maintained [25].

To generate nano antibiotics, three methods are used: interfacial polycondensation, interfacial polymerization, and emulsion polymerization [25]. Various methodologies, such as emulsification/evaporation of solvents, displacement of solvents, and interfacial deposition, emulsification/diffusion of solvents, in addition, salting can be used to manufacture nanoparticles from polymers that have been pre-designed. Nanoparticles, such as chitosan, agarose, and alginate, may be produced by thermal, gelification, or chemical treatment methods for the manufacturing of natural supermolecules, such as chitosan, agarose, and alginate [26].

Among several methods of encapsulation, the effective implementation of PLGA nanoparticles for azithromycin and rifampicin encapsulation has been obtained with respect to increased internal build up and intracellular battle, this encapsulation process significantly increased the efficacy of antibiotics (which ranged from 5 ng/ml for rifampicin to 40 ng/ml for azithromycin). 25 22% rifampin and 25% azithromycin PLGA nanostructures developed a release of 12% in 3 days with a width of ~260 nm. In this case, there was 1 mg of all antibiotics, in equal parts, added to 1 mg of polymer. Even so, the final antibiotic concentrations were 32 mg of rifampin and azithromycin, which was higher than the concentrations in the sample [25]. Due to the biocompatibility and biodegradability, the use of PLGA has a range of distinct benefits relative to other non-degradable polymers as soon as degradation materials can be immediately ingested by the body [27]. There is so much chance of manipulate the rate of deprivation of nanoparticle PLGA within the body, from days to months by merging its components [28]. In addition, to maximise the immunogenicity of the delivery molecules, PLGA nanoparticles is used that communicates an advantageous validity to immunogenic distribution molecules that function as a minor immunological retort through triggering protection cells[29-30].

Bacteria bound with antibiotics could be taken straight to cells, enhancing subsequent treatment and preventing the-lactamase resistance against methicillin-resistant *Staphylococcus aureus* (MRSA). Covalent connection among amoxicillin and polyacrylate was developed with the intention of discovering something new. The architecture of nanoparticles maintains the activity of a drug on the host system while preventing lactamase activity, which occurs in hydrophobic nanoparticle structures consisting of PLGA chains.[31]

Additionally, the nanoparticle's synthesis used an emulsifying process and was then preformed when a water-soluble radical initiator is added. In their article, the authors noted that the MIC (Minimum Inhibitory Concentration) of conjugated penicillin was 16 μg/ml. The average free penicillin is 260 μg/ml in *S. aureus* [31]. A supplementary paper appeared in the same year, suggesting a successful improvement in antimicrobial activity attributable to the nanoparticle preparation and control methods [32]. The preparation of the penicillin-containing polyacrylate nanoparticles for use in detecting MRSA beta-lactamase involved preparation the nanoparticles using free radical emulsion polymerization in water [33].

A biodegradable polymer with direct antimicrobial action has the possibility of being used in the production of modern pharmaceuticals. Polycarbonate nanoparticles with antimicrobial activity were described in this review [31-33]. An attractive selectivity was developed for the microbial membrane, and anionic microbial nanoparticles were formulated with functional cyclic carbonate and the organocatalytic ring-opening polymerization of cyclic carbonate that is free of metal precursors. Gram-positive bacteria membranes have the unusual property of producing amphiphilic and cationic nanoparticles [34].

Biocompatibility, biodegradability, low inherent toxicity, and tunable mechanical properties make polycarbonate nanoparticles ideal for medical use. Because of these features, microbial and human cells can be handled differently; decreasing the toxicity of mammalian cells. There is also a toxicity problem in mammalian cells that needs to be studied. Toxicity and minimum inhibitory concentration measurements of cells are completely important for all samples examined by all these authors [35].

4. Antibiotics and analytical methodologies

This term, "antibiotic" can be used to describe both microorganisms and their distantly developed components, as can be found in this article. It is important to track antimicrobial proteins in tissues for compliance with the legal structure, to ensure product consistency, and we have analysed the antimicrobial properties, therapeutic utility, and analyte recognition methods used in antimicrobial classes under the specifications accepted in the following subsections. The methodologies that are most important for each antibiotic class are stressed, since the purification and extraction steps are critical for the effectiveness of these treatments.

4.1 Aminoglycosides (AG)

AGs are potent class of bactericidal antibiotics which are active against all aerobic, gram-negative bacteria and gram-positive microorganisms. The most widely used amino-glycoside is gentamicin (Fig. 1). Based on MRLs of aminoglycosides, it can not be used as growth promoter in food [36].

4.1.1Extraction and clean-up procedures

Amino glycosides are hydrophilic in nature and solid phase extraction (SPE) technique is adopted for maximum extraction of AG followed by purification with HPLC. Amongst other processes, trichloroacetic acid extraction was utilised to ensure complete extraction of the analytes from the matrix [37-38]. An anion exchanger is used to neutralise the acid in the matrix and a cation exchanger is coupled with SPE catridge for complete elution of AG [39].

4.1.2 Methodologies for determination

AG may be quantified by using spectrophotometric, liquid chromatography, immunochemical, or microbiological techniques [40]. AGs are derivatised for fluorescence detection. Mass spectrometry is used for specific and unambiguous identification and validation of AGs [41]. Another approach is using hydrophilic contact chromatography (HILIC). However, it involves solid ionic buffer solutions and highly advanced chromatography columns. But derivatization of AG's with phenyl isocyanate produced derivates can be separated using a typical reversed-phase column that elimininate the use of HILIC liquid chromatography or ion-pair reagents.

4.2 β-lactam antibiotics

β-lactam antibiotics possess four membered cyclic amines. The major class of drugs such as penicillins, cephalosporins, monobactams, carbapenems, and β-lactamase inhibitors are included in this section (Fig. 1). Commonly, these antibiotics are used in both human and animal bacterial infections [42].

4.2.1Extraction and clean-up procedures

This class of drugs are highly unstable and thermolabile. Also, it get degraded on exposure to heat, alcohol and lose its therapeutic activity on alteration in pH [43].

4.2.2 Methods for determination

LC-MS/MS analysis is adopted to analyse, identification, validation of β-lactam antibiotics residues in food items. EU imposes higher limits on drug residues such as amoxicillin, ampicillin, cloxacillin, dicloxacillin, oxacillin, and benzylpenicillin in edible animal tissue for controlled medications [44].

4.3 Quinolones

Quinolones are a synthetic group of antibiotics used in both human and veterinary applications. Fluorinated quinolones have been applied to the medicinal arsenal to help treat septicaemia [45-46].

4.3.1. Extraction and clean-up procedures

In general, the quinolone antibiotics are extracted by solvent extraction processes. Also, some special extraction techniques such as QuEChERS extraction, solid phase exxtraction, dispersive solid phase extraction (DSPE) are used to optimise the extraction process for better resoultion of detection [47].

4.3.2 Methods for determination

HPLC, UV and fluorescence detection methods are used to study quinolone antibiotics [48]. Besides this, LC-MS/MS and LC-FLD analysis are widely used for routine quality control of quinolones [49].

4.4 Sulphonamides

Sulphonamides are p-aminobenzene sulphonamide derivate classs of drugs used in animals and humans. It includes sulphadiazine, sulphamethizole, sulphamethoxazole, sulphasalazine, and sulphisoxazole class of drugs (Fig. 1) [50].

4.4.1Extraction and clean-up procedures

Solid-phase dispersion and liquid-phase extraction methods are used for sulphonamide extraction. Sample size reduction method is followed to minimize matrix interferences, reduce solvent consumption and avoid SPE catridges [51].

4.4.2 Methods for determination

The conventional approach of determining sulphonamide by use of GC-MS method is found to be inappropiate, So, an advanced method of matrix solid-phase dispersion technique with hot water extraction followed by LC-MS is adopted for it. Also, HPLC-PDA method is used for analysis but false positive errors may arise due to matrix interferences. So, this problem can be overcomed by use of UHPLC-MS/MS technique [52].

4.5 Tetracyclines

Tetracycline antibiotics (TC) are used widely in the agriculture industry to help with growth in animals, and in human medicine to treat and avoid bacterial infections. TC resistance among bacterial species has become widespread as a result of wide use [53].

4.5.1Extraction and clean-up procedures

TCs are soluble in polar-organic solvents, bases, and acids. So, it is difficult to these compounds from tissues. Aqueous-based extraction by using EDTA treated C18 SPE cartridge is accepted for extraction of TCs [54].

4.5.2 Methods for determination

TCs are identified by using multiple techniques such as immunoassays, capillary electrophoresis, liquid chromatography. However, LC-MS/MS method is widely accepted method for its improved sensitivity and accuracy in determination of TC as compared to UV and fluorescence methods [55].

5. Conclusion

In the past, antibiotics were intended to modify the particular biochemical pathways of the target species, but the application of trace amounts into the environment is now correlated with a possibility of accidentally altering other, distinct and unknown biochemical pathways also in nontarget organisms, and a possible encouragement of other, distinct and unknown results at even lower concentrations. In order to control the impact on antibiotic residue in the food chain, new environmental matrices are still required.

Competing interests disclaimer

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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