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Development of pH-Sensitive Microbeads Incorporated with Amine-functionalized Magnetic Nanoparticles for Enhanced Antibacterial Activity

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Abstract: Antibiotic-resistant bacteria have rapidly emerged in recent years as a result of irrational use of antibiotics. Development of drug carriers that can enhance antibacterial activity of antibiotics can potentially overcome antibiotic-resistance and hence has practical significance. This study addresses this need by integrating amine-functionalized magnetic nanoparticles (AMNPs) into hydrogel microbeads composed of sodium alginate (SA) and xanthan gum (XG) for delivery of levofloxacin (LVX). Characterization of the microbeads confirmed successful AMNP-polymer interactions and demonstrated a porous structure inside the microbeads. The microbeads demonstrated pH-sensitive drug release behaviour, enabling prolonged drug release. The drug encapsulation efficiency in the hydrogel microbeads was higher after AMNP incorporation, indicating the potential roles played by the porous network and by AMNP-LVX interactions during drug loading. The microbeads adhered to first-order, Higuchi, and Korsmeyer-Peppas kinetic models, suggesting that a combination of diffusion and polymer relaxation mechanisms involves in drug release. Along with the fact that the AMNP-incorporated microbeads exhibited enhanced antibacterial activity against various bacterial strains, our microbeads warrant further development and optimization as drug carriers for antibacterial applications.

1. Introduction

Antibiotic-resistant bacteria have rapidly emerged in recent years as a result of irrational use of antibiotics. Development of drug carriers that can enhance antibacterial activity of antibiotics can potentially overcome antibiotic-resistance and hence has practical significance. The feasibility of achieving this goal has been made possible by advances in pharmaceutical technologies [1-5], which

have significantly enhanced the efficiency and release sustainability in drug delivery. Among different carriers developed, gel-based carriers are one type of carriers that have received special attention because of their high property tunability, high biocompatbility, good biodegradabiity, and importntly, extensively verified efficiency in clinical and industrial applications[6-8]. One example of gelk-forming polymer is xanthan gum (XG), which is a natural polysaccharide derived from Xanthomonas bacteria and has been used as a pharmaceutical excipient[9]. It can function as a thickening, stabilizing, and mucoadhesive agent in various drug formulations, such as oral, topical, and controlled-release systems. Its properties improve drug solubility, bioavailability, and the overall performance of pharmaceutical formulations[10, 11]. Another example is sodium alginate (SA), which is a natural polysaccharide extracted from brown seaweed, plays a crucial role in drug delivery[12]. It is used as a biocompatible and biodegradable excipient to create drug delivery systems, including oral, transdermal, and targeted formulations. Sodium alginate is valued for its ability to form gels, control drug release, enhance drug stability, and improve bioavailability[13]. It is particularly effective in encapsulating and protecting drugs during delivery, making it a valuable component in various pharmaceutical formulations[14]. Besides the polymers mentioned above, other polyscaccharides (such as carboxymethylcellulose, chitosan, and hydroxypropyl methyl cellulose) have been exploited for gel fabrication in drug delivery research.

As a matter of fact, till now diverse gel beads have already been reported for delivery of antibiotics in the literature[15, 16]; however, efforts devoted to exploring the possible role played by composite gels in serving as antibiotic carrier for enhanced antibacterial activity have been scant. In fact, the technical viability of incorporating nanoparticles (NPs) into hydrogels to enhance efficiency in drug delivery is well-supported in the literature. Over the years, nanoparticles have been playing a pivotal role in drug delivery by improving drug solubility, enabling precise targeting, facilitating controlled release, or even fighting against drug-resistant bacteria[17-22]. Their small size and versatile properties offer innovative solutions in medicine[23]. Magnetic nanoparticles (MNPs) have gained attention in drug delivery partly because their magnetic properties enable them to be guided by external magnetic fields to reach specific sites in the body. This makes target-specific drug delivery possible, and hence reduces systemic side effects and improves treatment outcomes[24-27]. In addition, MNPs can generally be functionalized and be loaded with different drugs. Among different MNPs, iron oxide NPs hold particular promise in enhancing the efficacy and specificity of drug delivery in various medical applications[28]. They can interact with bacterial cell membranes via electrostatic contact, causing harmful oxidative stress in the bacterium by the generation of free radicals, known as radical oxygen species (ROS)[29, 30]. Recent studies showed that iron oxide NPs showed antibacterial activity against both gram-negative bacteria and gram-positive bacteria[31-33].

In this study, MNPs were synthesized and functionalized with amino groups to produce amine-functionalized MNPs (AMNPs). These AMNPs were subsequently incorporated into a polymeric matrix comprising SA and XG. The resulting material was processed into microbeads using gelation, which is a widely adopted approach for hydrogel fabrication[8, 34-37]. To demonstrate the effectivness of the developed microbeads in delivering antibiotics for enhnaced antibacterial activity, levofloxacin (LVX) is adopted as a model drug. This drug is a second-generation fluoroquinolone antibiotic agent commonly used in the clinical setting to fight against a variety of infections. It can suppress the synthesis and replication of bacterial DNA by inhibiting the activity of DNA helicase, thereby resulting in bacterial cell death[38]. Because of its wide clinical use, success in demonstrating the effectiveness of the developed mcirobeads in enhgancing the activity of LVX exhibit practical implications. Characterization of the microbeads were conducted using various

analytical techniques. Additionally, in vitro release studies were performed under both acidic and intestinal pH conditions, and the results are thoroughly discussed within this study.

2. Results and discussion

2.1. Fourier-transform infrared spectroscopy (FTIR)

Figure 1 illustrates the FTIR spectra of AMNPs, LVX, plain SA/XG hydrogel microbeads (SAXG), LVX-loaded SA/XG hydrogel microbeads (SAXG-LVX), and LVX-loaded AMNP-incorporated SA/XG hydrogel microbeads (SAXG-AMNPs-LVX). In the spectrum of XG, peaks are identified at 3394, 2983, 1612, and 1388 cm-1. These peaks correspond to O–H, C–H, C=O, and –COO– stretching vibration, respectively. In the spectrum of SA, peaks are observed at 3411, 1597, 1388, and 1106 cm-1. They are attributed to O–H, C=O, C–H, and C–O stretching vibration, respectively. The spectrum of AMNPs exhibits peaks at 575 cm-1 (Fe–O stretching vibration) and at 1594 and 3433 cm-1 (N–H bending and stretching vibration)[39]. The spectrum of LVX shows peaks at 3445, 1721, 1239, and 1071 cm-1. These peaks are assigned to O–H, C=O, C–N, and C–F stretching vibration, respectively. The spectrum of SAXG shows peaks at 3362, 1601, 1387, and 1102 cm-1. These peaks are attributed to O–H, C=O, C–H, and C–O stretching vibration, respectively. Comparing the spectrum of SAXG-LVX microbeads with that of SAXG, a shift in the C=O stretching frequency from 1601 to 1594 cm-1 is observed, indicating interaction between LVX molecules and the polymer matrix. Additionally, a peak at 1068 cm-1 is found and is assigned to C–F stretching vibration, confirming interaction between LVX molecules and the polymer matrix. The spectrum of SAXG-AMNPs-LVX microbeads shows peaks similar to those in the spectrum of SAXG-LVX but also has a new peak at 579 cm-1, which is attributed to the Fe–O group of AMNPs and confirms interaction between AMNPs and the polymer matrix.

2.2. X-ray diffraction (XRD)

The XRD curves of LVX, AMNPs, SAXG, SAXG-LVX, and SAXG-AMNPs-LVX microbeads are presented in Figure 2. The diffraction curve of the generated AMNPs exhibits 2θ at 30.5°, 35.6°, 43.2°, 54.1°, 57.5° and 63.0°. This closely resembles those observed in previous studies[40], providing confirmation of AMNP generation. The curve of LVX exhibits 2θ patterns ranging from 6° to 27°, indicating the crystalline nature of the drug. The diffraction curve of SAXG-LVX shows no peaks found in the curve of LVX, suggesting a uniform distribution of LVX molecules within the polymeric matrix. Features of the curve of AMNPs is evident in the curve of SAXG-AMNPs-LVX microbeads, suggesting successful interaction between AMNPs and the polymeric matrix.

2.3. Scanning electron microscopy (SEM)

SEM is adopted to study the morphological characteristics of the microbeads (Figure 3). All microbeads show a spherical configuration and possess a textured outer surface. Specifically, the external surface of SAXG-AMNPs-LVX is notably more uneven and porous compared to SAXG-LVX, primarily attributed to the incorporation of AMNPs. The typical dimensions of the generated microbeads are in the range of 700-900 µm. The size of the AMNPs is determined through high-resolution transmission electron microscopy (HR-TEM) analysis (Figure 4). The generated AMNPs are found to be in the size range of 40–50 nm (Figure 4a). The selected area electron diffraction (SAED) pattern of AMNPs shows distinct diffraction rings (111), (220), (311), (400), (511), and (440), which correspond to the cubic inverse spinel structure of magnetite (Figure 4b). The presence of three distinct rings confirms the polycrystalline nature of the NPs[41].

2.4. Swelling capacity

Optimal swelling is crucial for controlled release of LVX from polymeric drug delivery systems. At pH 7.4, all microbeads swell more effectively than at pH 2.0. This can be attributed to weaker interactions between carboxylic groups in the polymeric matrix and the buffer medium at pH 7.4, resulting in a looser network. Consequently, the buffer medium enters the polymeric matrix, leading to a higher swelling degree. These results align with previously reported findings in alginate-based systems[42, 43]. Conversely, acidic pH reduces the degree of swelling due to ionic interactions, making the polymer matrix hydrophobic. The generated microbeads, therefore, prove effective and promising as carriers for delivering drug molecules to the intestine. SAXG-AMNPs-LVX exhibits more swelling compared to SAXG-LVX. The presence of NPs within the matrix results in the formation of pores, thereby enabling the medium to diffuse into the polymeric matrix. A similar observation has been reported by Bellala and coworkers,[44] who found that the presence of NPs increased the swelling degree in the polymeric matrix.

2.5. Drug release and kinetics

The encapsulation efficiency (EE) of SAXG-LVX and SAXG-AMNPs-LVX microbeads is 58.2% and 60.4%, respectively. SAXG-AMNPs-LVX exhibits a higher EE compared to SAXG-LVX. This can be explained by the porous nature of the polymeric network and the interaction between AMNPs and LVX, resulting in an increase in the effectiveness in drug encapsulation. The drug release sustainability of the microbeads are tested in phosphate-buffered saline (PBS) at 37 oC and at pH 2.0 and 7.4 (Figure 6). At pH 7.4, the carboxylate group experiences less interaction with the buffer medium, rendering the network less rigid and more porous. This facilitates the release of drug molecules from the polymeric matrix. Conversely, at pH 2.0, ionic-ionic repulsion between H+ ions and the polymer matrix leads to a lower rate of drug release. The hydrophobic nature of the polymer matrix at this pH inhibits diffusion of solvent molecules into the matrix, thereby reducing the release rate[45]. SAXG-AMNPs-LVX exhibits a slower rate of drug release as compared to SAXG-LVX. This is attributed to the presence of AMNPs, which interact with LVX and extend the release rate. These pH-sensitive carriers are valuable for drug delivery, protecting the loaded bioactive agents in an acidic environment and releasing them in the intestinal region.

Table 2 displays the r2 values obtained by fitting the drug release data into various kinetic models. The release behaviour aligns with the first-order model and the Higuchi model. Drug release involves PBS absorption into the polymer matrix and LVX diffusion from microbeads into the surroundings. The release rate is directly proportional to the amount of LVX present. Additionally, 60% of the release data conform to the Korsmeyer-Peppas equation, revealing non-Fickian diffusion with n values ranging from 0.479-0.631.

2.6. Antibacterial activity

To show the antibacterial properties of SAXG-LVX, SAXG-AMNPs-LVX, and AMNPs, Streptococcus mutans, Klebsiella Pneumoniae and Bacillus subtilis are used as representative models (Figure 7). All microbeads demonstrate antimicrobial activity against S. mutans, K. pneumoniae and B. subtilis. As indicated in Table 3 and comapring with other samples (SAXG-LVX and AMNPs), treatment with SAXG-AMNPs-LVX leads to the formation of the largest inhibition zone. This promising antibacterial

activity is attributed to the presence of AMNPs (which also display antibacterial activity against all three models) in conjunction with LVX within the polymer matrix.

3. Conclusion

This study integrates AMNPs into a polymeric matrix comprising SA and XG to generate SAXG-AMNPs-LVX microbeads for drug delivery. The generated microbeads demonstrate pH-sensitive controlled release, with SAXG-AMNPs-LVX outperforming SAXG-LVX in their effectiveness in drug delivery. The kinetics of drug release exhibited by the microbeads align with first-order, Higuchi, and non-Fickian diffusion models. Along with their superior efficacy against various bacterial cells, SAXG-AMNPs-LVX microbeads leverage the synergistic properties of MNPs and natural polysaccharides, and warrant further development for use in targeted drug delivery applications.

Materials and experimental methods

Materials

XG, iron(II) chloride tetrahydrate, iron(III) chloride hexahydrate, NH_4OH, LVX, and ethylenediamine were obtained from Sigma-Aldrich. SA and calcium chloride were purchased from SD Fine Chemicals Ltd. (Mumbai, India).

Preparation of AMNPs

AMNPs were prepared by using a previously documented procedure[46]. 1.2 g of iron(II) chloride tetrahydrate and 3.2 g of iron(III) chloride hexahydrate were stirred in 200 mL of water at 400 rpm for 60 minutes under a nitrogen atmosphere. 40 mL of 30% NH_4OH and 40 mL of ethylenediamine were then added. The resulting mixture was stirred at 60°C for 60 minutes. The black precipitates formed were separated, washed, air-dried, and then stored in a sealed container for future use.

Synthesis of SAXG-AMNPs-LVX and SAXG-LVX microbeads

SA and XG (100 mg each) were separately dissolved in 10 mL of water. The solutions were mixed. 100 mg of AMNPs was added, followed by sonication for 15 minutes. After 2 hours of stirring, 100 mg of LVX was added. The resulting mixture was stirred for 5 hours, and was slowly added to a calcium chloride solution to form SAXG-AMNPs-LVX, which were washed and dried at 40°C. A similar procedure was adopted to create SAXG-LVX, but no AMNPs were added during the process.

Statistical analysis

All data were expressed as the means ± SD. Unless otherwise specified, the mean value was obtained by averaging three replicates.

Supporting Information

The details of the experimental section are summarized in the supporting information.

Keywords: amine-functionalized magnetic nanoparticles • sodium alginate • xanthan gum • antibacterial activity • levofloxacin

Conflict of interest

The authors declare that there was no conflict of interest.

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