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Preparation and characterization of chitosan encapsulated beta-lactam antibiotic and Baicalein nanoparticles

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Abstract
Antibiotic resistance is an emerging problem faced by the dairy industry. To improve the targeted drug delivery and bioavailibilty, nanotechnology is such an alternative and fascinating developing field of science. In this study Baicalein, traditional Chinese medicine due to its the intrinsic poor solubility and low bioavailability limits its biomedical applications. Chitosan encapsulation was performed with resistant beta-lactam antibiotic, amoxicillin against Methicillin resistant Staphylococcus aureus. This study envisages the preparation of Chitosan encapsulated baicalein and amoxicillin for increasing bioavailability and enhancing targeted delivery. The yield of the Chitosan encapsulated baicalein and amoxicillin particles was 83.4±3.04959% and the corresponding encapsulation efficiency was 83.8 ±2.58%. The size distribution of the chitosan encapsulated amoxicillin and baicalein particles were of size 251nm. The *in vitro* release study revealed a 28.3 percent burst release in the initial 6 hours, followed by a sustained slow and steady release of baicalein from the chitosan encapsules. This is important to maintain the minimum drug concentration at the site of infection. and improve the therapeutic amoxicillin release profile showed significant release of 32% in the first 5 hours, followed by extended release. These findings imply that CH encapsulated BC with beta-lactam could be a promising mastitis treatment.

Keywords: Baicalein, chitosan, encapsulation, methicillin resistant *staphylococcus aureus*, amoxicillin

1. Introduction
The methicillin-resistant *S. aureus* (MRSA) is one of the most prominent pathogens that cause mastitis and incidence of MRSA mastitis is rising in dairy cows worldwide (Tchamba *et al.*, 2021) [13]. Although the antibiotic is the cornerstone of mastitis treatment, but it often fails in treatment of MRSA mastitis because of its resistant to methicillin and other β-lactam antibiotics. There is active interest in searching for new therapeutic approaches to curb the antimicrobial resistance or reduce its dose and duration for antibiotic therapy. Drug combinations offer a promising strategy to overcome bacterial resistance mechanisms (Worthington and Melander, 2013) [15].
The *S. aureus* mastitis model is frequently used as it appears to be very suited for studying ruminant mastitis due to similarities between mice and cows (Notebaert and Meyer, 2006) [8].
Baicalein, a major flavonoid constituent, is found in the *Scutellaria baicalensis Georgi*, a kind of traditional Chinese medicine, have been used to treat bacterial infections (Shang *et al.*, 2010; He *et al.*, 2015) [12, 4]. It exhibits antibacterial action through inhibiting the quorum sensing, biofilm formation, and the expression of virulence genes of bacteria (Peng *et al.*, 2019) [10]. Aside from the antibacterial properties, baicalin also shows anti-inflammatory activity by inhibiting the MAPK pathway and lowering the expression of pro-inflammatory cytokines including MMPs (Fujita *et al.*, 2015) [3]. In spite of enormous therapeutic potential, the intrinsic poor solubility and low bioavailability limit its biomedical applications. The baicalein is reported to exhibit *in vitro* synergistic antimicrobial effect on MRSA when used in combination with β-lactam antibiotics (Liu *et al.*, 2000) [6].
Hence, a coating that is both bioactive and antimicrobial properties may address such shortcomings and improve the performance of the molecule (Palierse *et al.*, 2021) [9]. Utilization of nanoparticles for the intracellular delivery of drug molecules is valid approach to

restore the effectiveness of antimicrobials.

In the current study, baicalein and resistant β-lactam antibiotic, Amoxicillin was encapsulated in chitosan nanoparticles and its characterization, determination of percent yield, encapsulation efficiency and drug release profile was performed

2. Materials and methods

Baicalein (purity 98%), chitosan and amoxicillin were purchased from Sigma Chemical Co (St. Louis, MO, USA).

2.1 Preparation of blank chitosan nanoparticles

Chitosan (CH) nanoparticles were prepared by ionotropic gelation with tripolyphosphate (TPP, Sigma Chemical Co., USA) (Koukaras *et al.*, 2012) [5]. Chitosan blank particles were dissolved in distilled water containing 0.2% acetic acid (12mL) and stirred at 900 rpm for 12h and pH was adjusted to 5.0 with 1N sodium hydroxide (NaOH). After that 10mL of 0.5% sodium TPP was added drop by drop to 4mL (8mg) CH solution and solution kept for stirring at 900 rpm for one hour. Blank CH nanoparticles obtained by ultracentrifugation at 18000 rpm at 4ºC for one hour. The sediment obtained was washed several times with deionised water and freeze dried (Zaki and Hafez, 2012) [16].

2.2 Preparation of Chitosan encapsulated Beta-lactam antibiotic and Baicalein

Chitosan (CH) blank particles were dissolved in distilled water containing 0.2% acetic acid and stirred at 9000 rpm for 12 hrs. adjusting pH 5.0 with 1N NaOH. After that 0.5% TPP was added drop by drop to 4mL (8mg) CH solution and solution was kept for stirring at 900 rpm for one hour. Baicalein solution (6mg) was gradually added drop-wise to CH solution on magnetic stirrer maintained at 37ºC and cured for 1h at 900rpm. Shortly, after amoxicillin (5mg) was added and stirred for one hour on magnetic stirrer. The CH encapsulated baicalein and amoxicillin were obtained after ultracentrifugation at 18000 rpm at 4ºC for one hour. The sediment was washed several times with deionised water and freeze dried.

2.3 Determination of percent yield and encapsulation efficiency

Chitosan encapsulated amoxicillin and baicalein nanoparticles was dispersed into 6 mL of phosphate buffer solution (PBS) and centrifuged at 12,000 rpm for 30 min. The supernatant was collected to measure the ultraviolet absorption at 272nm. The loading efficiency and encapsulation efficiency of particles were calculated as per method described earlier (Li-Weber, 2009) [7].

Loading efficiency $=W_0/W\times100\%$

Encapsulation efficiency$=W_0/W_1\times100\%$

Where

W_0 is the weight of baicalein enveloped in the Chitosan encapsulated Amoxicillin and Baicalein,

W is the weight of CH nanoparticles, and W_1 is the amount of baicalein added in the system.

2.4 Characterization of Chitosan encapsulated Amoxicillin and Baicalein

The size, zeta (ζ) potential and polydispersity index (PdI) of the nanoparticles were determined by particle size analyzer (MicrotracNanotrac Wave II, USA) for preliminary screening. The shape and size of the nanoparticles was determined by scanning electron microscope (SEM) (JSM-6610 LV, JOEL, USA). Nanoparticles were spreaded on a carbon tape of an aluminium stab and coated with gold sputter coating using JFC-1600 Auto Fine Coater and observed under SEM. The digital images were captured for analyzing the surface morphology and size of the nanoparticle.

2.5 *In vitro* release kinetics

For *in vitro* release kinetics study, the lyophilized Chitosan encapsulated Amoxicillin and Baicalein nanoparticles (10 mg) was dissolved in 10 mL PBS (pH 7.3±0.1). The solution was taken into the microcentrifuge tubes (500µl). The tubes were kept in an orbital shaker stirring at 50 rpm and maintained at 37°C. At the predetermined intervals, each tube was centrifuged at 3000 rpm for 10 min to sediment the baicalein released from the particles. The sediment was re-dissolved in 1 mL of ethanol and the absorbance was measured in spectrophotometer at 272 nm. The concentration of the baicalein released was calculated using a standard curve of baicalein in ethanol from 0 hr to 168 hrs (Babu and Kannan, 2012) [2].Similarly, the concentration of the amoxicillin released was calculated using a standard curve of amoxicillin in Phosphate buffer saline from 0 hr to 168 hrs.

3. Results

3.1 Preparation and characterization of chitosan encapsulated baicalein and amoxicillin particles

The yield of the Chitosan encapsulated baicalein and amoxicillin particles was 83.4±3.04959% and the corresponding encapsulation efficiency of tricomplex was 83.8 ±2.58% (Table 1). Particle size analysis by Nanotrac Wave II (MICROTRAC) revealed narrow size distribution for the optimized batch of chitosan particles. The size distribution for the optimized batch of chitosan encapsulated amoxicillin and baicalein nanoparticles ranged from 238nm to 271nm, with a mean particle size of 251nm (Table 2). Zeta potential of nanoparticles was measured by Nanotrac Wave II. Zeta potential of nanoparticles was 6.7 mv (Fig 1). The SEM images of Chitosan encapsulated Amoxicillin and Baicalein revealed that nanoparticles had a more or less spherical shape with a narrow size distribution. (Fig 2) The release profile of baicalein from the chitosan nanoparticles in PBS at 37ºC showed initial burst release of 28.3% in first 6 hours and followed by a slow and steady release of baicalein from the chitosan encapsulated particles (Fig. 3). Similarly, the release profile of Amoxicillin from the chitosan encapsulated nanoparticles in PBS at 37ºC showed initial burst release of 32% in first 5 hours and followed by a continuous release of baicalein from the chitosan encapsulated particles (Fig 4).

Table. 1: Yield (%) and encapsulation efficiency (%) of chitosan encapsulated amoxicillin and baicalein

Samples	Yield (%)	Encapsulation efficiency (%)
1	82	80
2	79	87
3	85	83
4	87	85
5	84	84
Mean±SD	83.4±3.04959	83.8 ±2.58

Table. 2: Size distribution of chitosan encapsulated amoxicillin and baicalein

Samples	Particle size (nm)
1	250.62
2	238.51
3	251.71
4	271.71
5	243.42
Mean ± SE	251.2±24.66

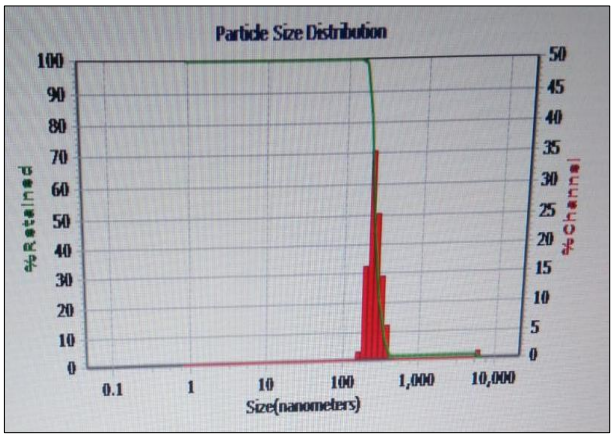

Fig 1: Particle size analyzer findings

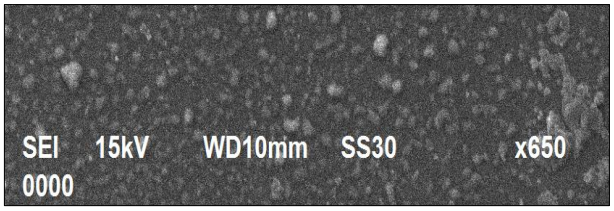

Fig 2: Surface Morphology of chitosan encapsulated amoxicillin and baicalein

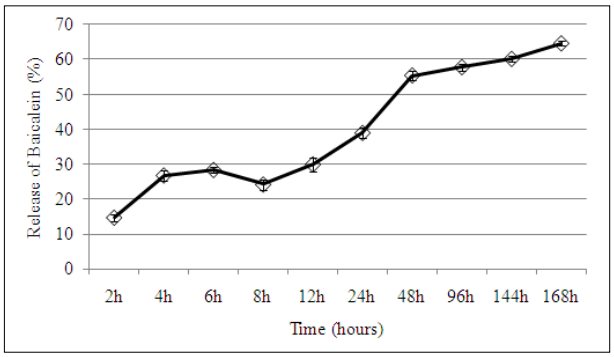

Fig 3: *In vitro* release graph for Baicalein from the chitosan encapsulated particles

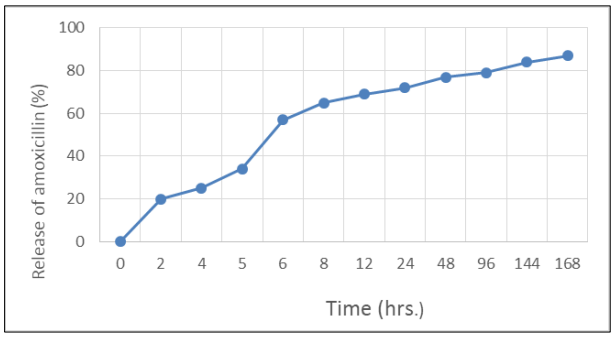

Fig 4: *In vitro* release graph for Amoxicillin from the chitosan encapsulated particles

4. Discussion

In the current study, the yield and corresponding encapsulation efficiency of BC were 83.4±3.04959and 83.8 ±2.58%, respectively and the findings are accordance with earlier study (Wang *et al.*, 2012) [14]. The *in vitro* release study showed an initial burst release of 28.3% in the first 6h, followed by continuous slow and steady release of baicalein from the chitosan encapsules, which could be beneficial in maintaining the minimum drug concentration at the site of infection and improving the therapeutic potential (Safhi *et al.*, 2016; Ahmed *et al.*, 2020) [11, 1]. The ζ potential and PdI of tricomplex were 6.7mV and 0.53, respectively, indicating the fineness and non-aggregating nature of the particles and thus their stability during storage (Ahmed *et al.*, 2020) [1].

5. Conclusion

The encapsulation of bacialin and amoxicillin by chitosan in the current study may have additionally aided baicalin internalization and intracellular persistence for a long time in oral use. Very recently, it was demonstrated that the encapsulation of baicalin in lactobionic acid modified chitosan increases the local bioavailability of therapeutic agents upon oral administration. These findings suggest that CH encapsulated BC with β-lactam may be a potential therapeutic agent for mastitis. However, more research on target animals are warranted before its application in clinical practice.

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7. References

1. Ahmed IS, Rashed HM, Fayez H, Farouk F, Shamma RN. Nanoparticle-mediated dual targeting: An approach for enhanced baicalin delivery to the liver. Pharmaceutics. 2020;12:107.
2. Babu VN, Kannan S. Enhanced delivery of baicalein using cinnamaldehyde cross-linked chitosan nanoparticle inducing apoptosis. Int. J Biol. Macromol. 2012;51:1103-1108.
3. Fujita M, Shiota S, Kuroda T, Hatano T, Yoshida T, Mizushima T, Tsuchiya T. Remarkable synergies between baicalein and tetracycline, and baicalein and β-lactams against methicillin-resistant *Staphylococcus aureus*. Microbiol. Immunol. 2015;49:391-396.
4. He X, Wei Z, Zhou E, Chen L, Kou J, Wang J, *et al.*

Baicalein attenuates inflammatory responses by suppressing TLR4 mediated NF-κB and MAPK signaling pathways in LPS-induced mastitis in mice. Int. Immunopharmacol. 2015;28:470-476.
5. Koukaras EN, Papadimitriou SA, Bikiaris DN, Froudakis GE. Insight on the formation of chitosan nanoparticles through ionotropic gelation with tripolyphosphate. Mol. Pharma. 2012;9:2856-2862.
6. Liu IX, Durham DG, Richards RME. Baicalin synergy with β-lactam antibiotics against methicillin-resistant *Staphylococcus aureus* and other β-lactam resistant strains of *S. aureus*. J Pharm. Pharmacol. 2000;52:361-366.
7. Li-Weber M. New therapeutic aspects of flavones: The anticancer properties of *Scutellaria* and its main active constituents Wogonin, Baicalein and Baicalin. Cancer Treat. Rev. 2009;35:57-68.
8. Notebaert S, Meyer E. Mouse models to study the pathogenesis and control of bovine mastitis. A review. Veterinary quarterly. 2006;28(1):2-13.
9. Palierse E, Hélary C, Krafft JM, Génois I, Masse S, Laurent G, *et al*. Baicalein-modified hydroxyapatite nanoparticles and coatings with antibacterial and antioxidant properties. Mater. Sci. Eng. C. Mater. Biol. Appl. 2000;118:111-537.
10. Peng LY, Yuan M, Wu ZM, Song K, Zhang CL, An Q *et al*. Anti-bacterial activity of baicalin against APEC through inhibition of quorum sensing and inflammatory responses. Sci. Rep. 2019;9:1-11.
11. Safhi MM, Sivakumar SM, Jabeen A, Zakir F, Islam F, Bagul US, *et al*. Therapeutic potential of chitosan nanoparticles as antibiotic delivery system: Challenges to treat multiple drug resistance. Asian J Pharm. 2016;10: S61-S66.
12. Shang X, He X, He X, Li M, Zhang R, Fan P, *et al*. The genus Scutellariaan ethno pharmacological and phytochemical review. J Ethno Pharmacol. 2010;3128:279-313.
13. Tchamba CN, Duprez, JN, Lucas P, Blanchard Y, Boyen F, Haesebrouck F, *et al*. Comparison of the Staphylococcal Chromosome Cassette (SCC) mec in Methicillin-Resistant Staphylococcus aureus (MRSA) and Non-aureus Staphylococci (MRNAS) from Animals and Humans. Antibiotics. 2021;10(3):256.
14. Wang W, Xi M, Duan X, Wang, Y, Kong F. Delivery of baicalein and paclitaxel using self-assembled nanoparticles: synergistic antitumor effect *in vitro* and *in vivo*. Int. J. Nanomed. 2015;10:3737.
15. Worthington RJ, Melander C. Combination approaches to combat multidrug-resistant bacteria. Trend Biotechnol. 2013;31:177-184.
16. Zaki NM, Hafez MM. Enhanced antibacterial effect of ceftriaxone sodium-loaded chitosan nanoparticles against intracellular Salmonella typhimurium. Aaps Pharm SciTech. 2012;13(2):411-421.