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Research Article

Sodium Tri Poly Phosphate Mediated Synthesis of Curcumin Loaded Chitosan-Carboxymethyl Cellulose Microparticles for Drug Delivery

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ABSTRACT

In this study, curcumin (CUR) was encapsulated into chitosan (CS) and carboxymethyl cellulose (CMC) microparticles using sodium tripolyphosphate (TPP) as chelator. Here, different concentrations (0.1%, 0.3% and 0.5%) of sodium tripolyphosphate (TPP) were utilised to synthesise microparticles. Microparticles were characterized by Fourier Transform Infra-Red Microscopy (FTIR) and Scanning Electron Microscope (SEM). All the CUR encapsulated microparticles were analysed for their drug encapsulation efficiency and the drug release kinetics. Microparticles were studied for the *invitro* controlled drug release against *Pseudomonas aeruginosa*.

Keywords: Chitosan (CS); carboxy methyl cellulose (CMC); sodium tripolyphosphate (TPP)

INTRODUCTION

The main objective of any nanoparticles/ microparticles based drug carriers is to entrap drug into a biodegradable polymer and to deliver the drug slow and steady[1-4]. Microparticles with size between 1 and 1000µm have acquired significant interest and can be used in drug delivery. These microparticles are expected to be biocompatible and biodegradable. This property is solely dependent on the nature of the polymers and methods followed[5-8]. There are several natural biopolymers like chitosan (CS), polyhydroxyalkanoates (PHA), carboxymethyl cellulose (CMC) etc can be used for drug delivery[9-11] as they are biodegradable and biocompatible[12,13]. CS, a versatile and second most abundant natural polysaccharide, found to be used in pharmaceutical excipients as it is absolutely biocompatible and biodegradable[14,15]. It is obtained from partially deacetylated chitin with β- (1-4)-linked 2-amino-2-deoxy-b-D-glucopyranose[16,17]. CS can be used for preparing microparticles by crosslinking the matrix with chemical crosslinking agents such as glutaraldehyde[18], NaOH[19], tripolyphosphate[20,21] etc. Amongst all these, TPP is found to be nontoxic and it interacts with amino groups of CS to form a gel[21]. In acidic environment, amino groups of CS react with an anionic group of other polymers, such as CMC[22,23]. Thus a biocompatible and biodegradable CMC[24] can be incorporated into CS. CS and CMC microparticles have been well studied for drug delivery[20,25-28]. Thus, this study is intended to load curcumin (CUR), a curcuminoid with various bioactivities[29,30] isolated from rhizome of *Curcuma longa* L.[31] into TPP chelated CS-CMC microparticles and studied for its controlled drug release *invitro* against *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Materials

The reagents and chemicals used were of analytical grade. Chitosan ($C_6H_{11}NO_4$), curcumin and Agar Powder were the make of Sisco Research Laboratories Pvt. Ltd. Acetic acid, Sodium Tripolyphosphate, Carboxymethyl Cellulose and Ethyl Alcohol AR were obtained from Qualigens Fine Chemicals, LOBA Chemie, Micro Fine Chemicals, (India) and Changshu Yangyuan Chemicals respectively. All the chemical preparations were done with Milli-Q water.

Preparation of microcarriers using TPP

Microparticle production was done by adding oppositely charged crosslinking agents[9,32]. 100ml of 0.1N acetic acid was added with 0.4% CS. Meanwhile, 0.1%, 0.3% and 0.5% TPP were prepared separately in 50 ml Milli-Q water each. The prepared TPP was taken in a burette and added dropwise to CS solution with continuous stirring at room temperature. 50 ml Milli-Q water was added with 0.15g CMC (0.3%) was taken in a burette and added dropwise to CS chelated with TPP solution and left undisturbed for 2 hours, after that, it was centrifuged at 5000 rpm for 15 min. Supernatant was discarded and the pellet was lyophilised. The lyophilised microparticles synthesised with 0.1%, 0.3% and 0.5% TPP were labelled as CS-CMC-TPP1, CS-CMC-TPP2 and CS-CMC-TPP3 respectively.

Preparation of CUR encapsulated microparticles

Here, 0.05g CUR was dissolved in 50ml ethanol and was added to 100ml of 0.1N acetic acid with 0.4% CS and it

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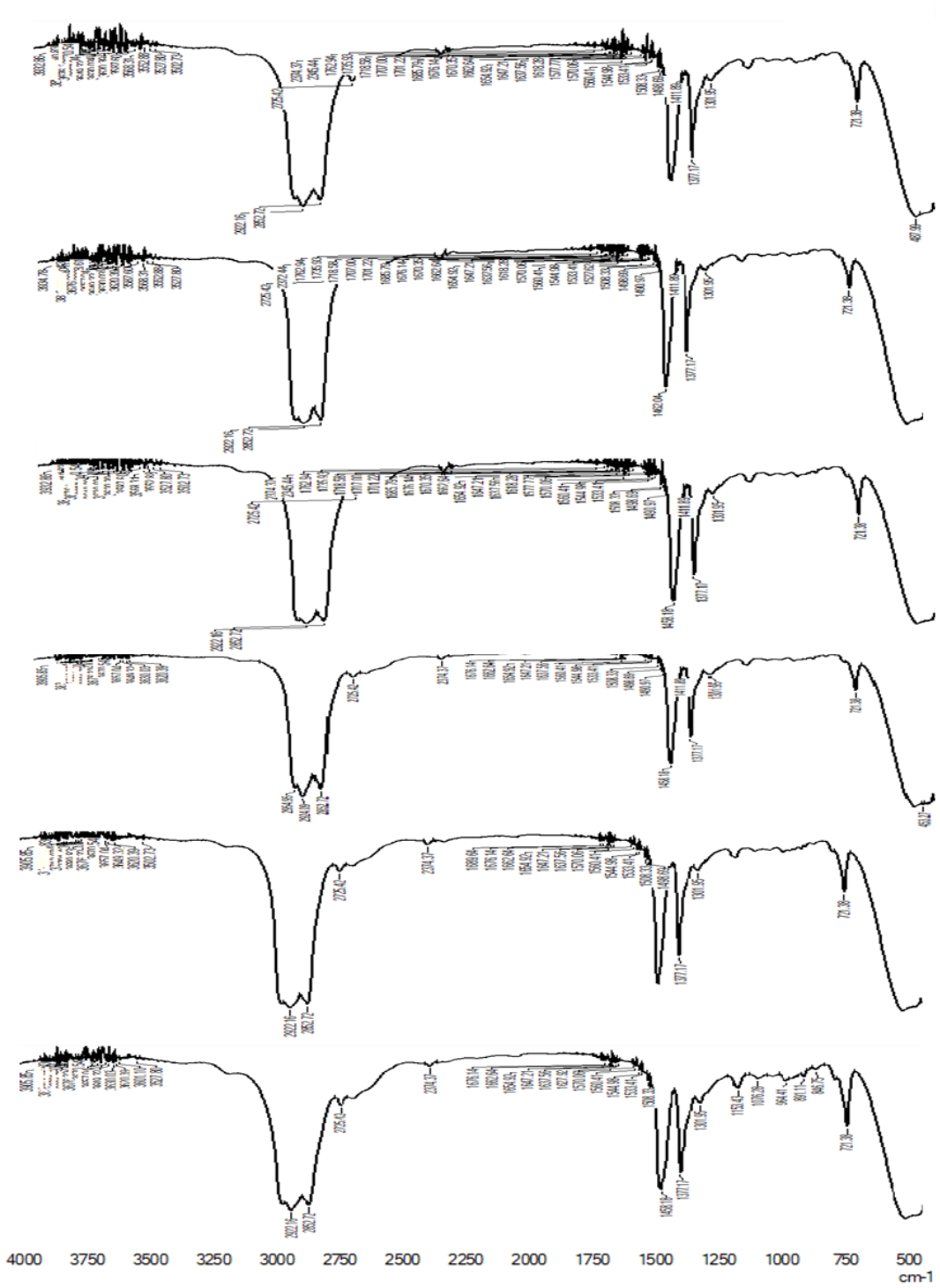

Figure 1: FTIR analysis of CS microparticles chelated with TPP a) chelated with 0.1% TPP and without drug (CS-CMC-TPP1), b) chelated with 0.1% TPP and loaded with drug (CS-CUR -CMC -TPP1), c) chelated with 0.3% TPP and without drug(CS-CMC-TPP2), d) chelated with 0.3% TPP and loaded with drug (CS- CUR-CMC- TPP2), e) chelated with 0.5% TPP and without drug (CS-CMC-TPP3), f) chelated with 0.5% TPP and loaded with drug (CS-CUR-CMC- TPP3). (LABEL a-f THE FTIR IMAGES).

was chelated with different concentrations of TPP solutions (0.1%, 0.3%, 0.5%) followed with addition of CMC as described above. The curcumin loaded microparticles prepared with 0.1%, 0.3% and 0.5% TPP were labelled as CS- CUR-CMC-TPP1, CS- CUR-CMC-TPP2 and CS- CUR-CMC-TPP3 respectively.

Characterization of microparticles

Fourier Transfer Infra-Red Spectroscopy (FTIR) Analysis

All the drug loaded and unloaded microparticles were analysed with transmission mode scan in spectral region of 4000-400 cm^{-1} in IR Affinity-1s (Shimadzu, Japan) instrument.

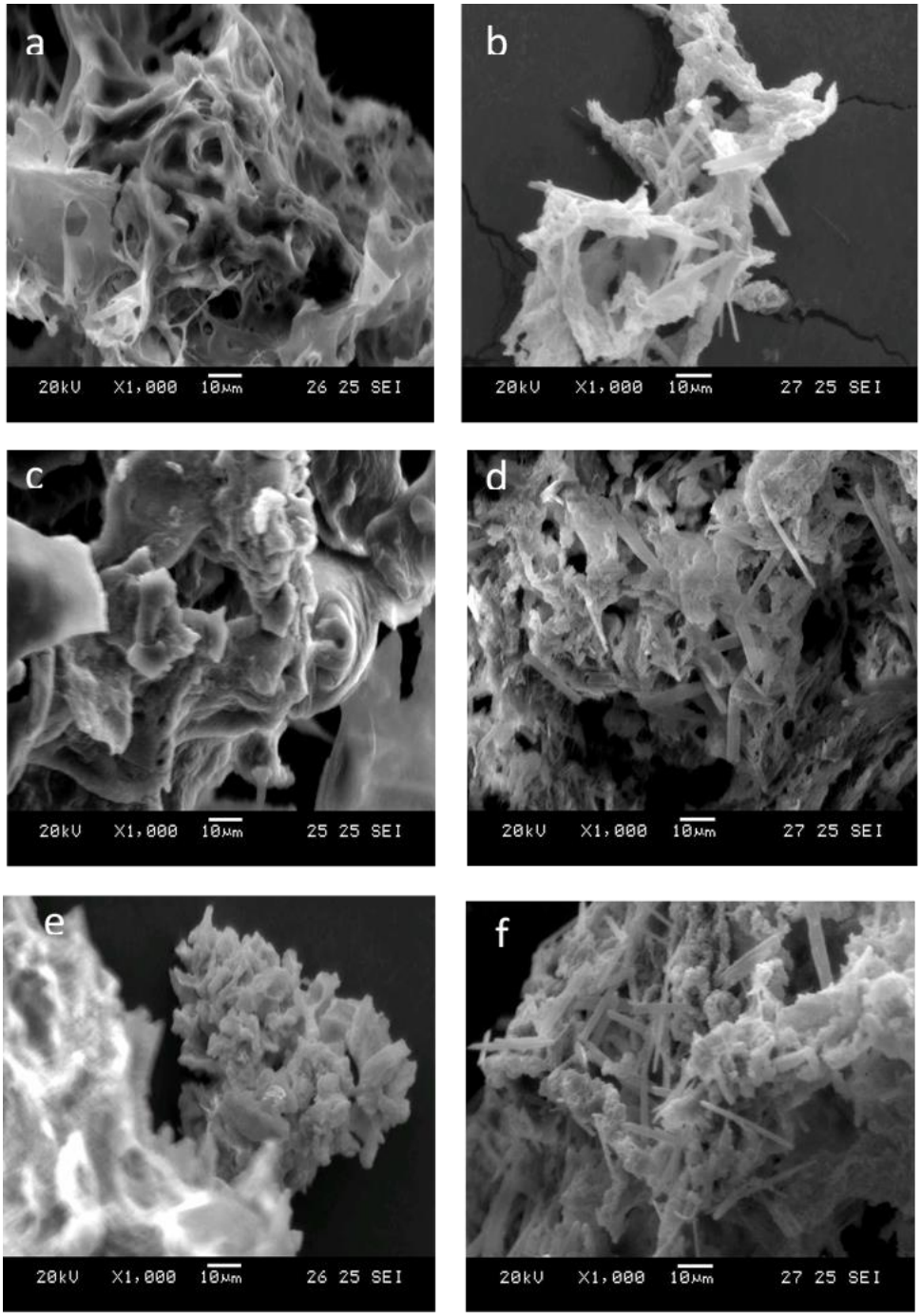

Figure 2: SEM Analysis of CS microparticles chelated with TPP a) chelated with 0.1% TPP and without drug (CS-CMC-TPP1), b) chelated with 0.1% TPP and loaded with drug (CS- CUR-CMC- TPP1), c) chelated with 0.3% TPP and without drug(CS-CMC-TPP2), d) chelated with 0.3% TPP and loaded with drug (CS- CUR-CMC-TPP2), e) chelated with 0.5% TPP and without drug (CS-CMC-TPP3), f) chelated with 0.5% TPP and loaded with drug (CS-CUR-CMC-TPP3).

Scanning Electron Microscope (SEM) Analysis

All the microparticles (both the CUR loaded and unloaded) were sprayed and sputter coated with gold for the examination under SEM (JEOL JSM-5610LV).

Evaluation of encapsulation efficiency

Drug encapsulation efficiency (EE) of the CUR loaded microparticles were evaluated by measuring the absorption at 425 nm (λ_{max} of curcumin) at various time intervals of the supernatant liquid (after centrifugation at 5000 rpm for 15 min) using UV Spectrophotometer (Systronics)[33-35]. At each time interval three samples were taken. Mean value of the samples were calculated and standard error was determined. Graphs were plotted having time interval in x axis and drug encapsulation absorbance in y axis.

InVitro drug release kinetics

Dialysis membrane technique was used to study drug release kinetics of CUR loaded microparticles[36]. 10 mg of CUR loaded microparticles (i.e. CS- CUR-CMC-TPP1,

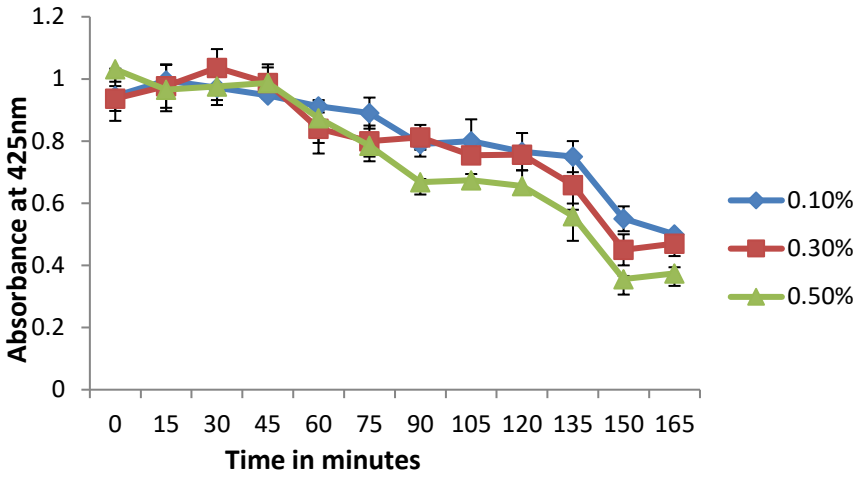

Figure 3: Encapsulation efficiency of CUR microparticles chelated with different concentrations of TPP.

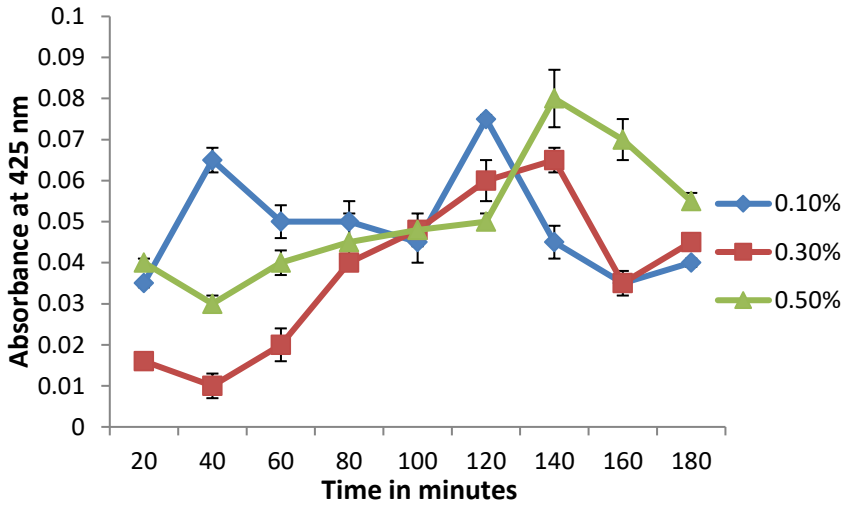

Figure 4: *Invitro* drug release kinetics of CUR microparticles chelated with different concentrations of TPP.

CS- CUR-CMC-TPP2 and CS- CUR-CMC-TPP3) were separately tied in three dialysis membranes (AV flat width-32.34mm, AV diameter-21.5mm, Capacity approx.-3.63m/cm). They were immersed separately in 50 ml of phosphate buffer solution (PBS) (pH 6.8) and left undisturbed at room temperature. 1 ml (three samples were taken at every time interval) of the released solution was collected at regular time intervals. Absorbance was read at 425 nm in UV visible Spectrophotometer (Systronics) (λ_{max} of CUR). Mean value of the samples were calculated and standard error was determined. Graphs were plotted having time interval in x axis and drug release absorbance in y axis.

InVitro controlled drug release studies

Anti-Bacterial activity

To release the CS-CUR-CMC encapsulated drug different solvents are required to break the CS-CMC complex, thus this study was done with four different solvents such as water, ethanol, PBS and acetic acid. *InVitro* controlled drug release studies and bactericidal activity of CUR loaded microparticles was studied by agar well diffusion method[37, 38]. *Pseudomonas aeruginosa* was swabbed over Mueller hinton agar plates, wells were bored using a sterile punching kit. CS-CUR-CMC microparticles were dissolved in different solvents [water, ethanol, PBS (pH 7) and acetic acid (1mg/ml)] and were used to analyse the drug release study. Different concentrations of microparticles (10µg, 20µg, 30µg and 40µg) were poured onto the wells. After 48h the zone of clearance was measured and recorded.

RESULTS AND DISCUSSION

Fourier Transfer Infra-Red Spectroscopy (FTIR) Analysis

The FTIR spectrum of CUR unloaded (Figure 1 a,c,e) and ~~un~~loaded microparticles (Figure 1b,d,f) are shown in Figure 1. The characteristic peaks for amide bonds of CS-CMC-TPP1, CS-CMC-TPP2 and CS-CMC-TPP3 were observed at 1637.56 cm^{-1} which shows the presence of N-acetylglucosamine[33]. The C-N stretching vibration peaks for CS and CMC were observed at 1377.17 for all carrier samples CS-CMC-TPP1, CS-CMC-TPP2 and CS-CMC-TPP3. TPP characteristic bands at 1206-1215 (P—O stretching), 1135-1157 (PO_2 groups), 1090-1115 (PO_3 groups) and 880 - 895 cm^{-1} (P—O—P asymmetric

Table 1: Antibacterial activity of CUR against *P. aeruginosa* using water as solvent.

Type of microparticles	Positive control	Negative control	Zone of inhibition at various Concentrations (in cm) 10µg	20 µg	30 µg	40 µg
CS- CUR-CMC-TPP1	-ve	-ve	-ve	-ve	-ve	-ve
CS- CUR-CMC-TPP2	-ve	-ve	-ve	-ve	-ve	-ve
CS- CUR-CMC-TPP3	-ve	-ve	-ve	-ve	-ve	-ve

-ve – negative

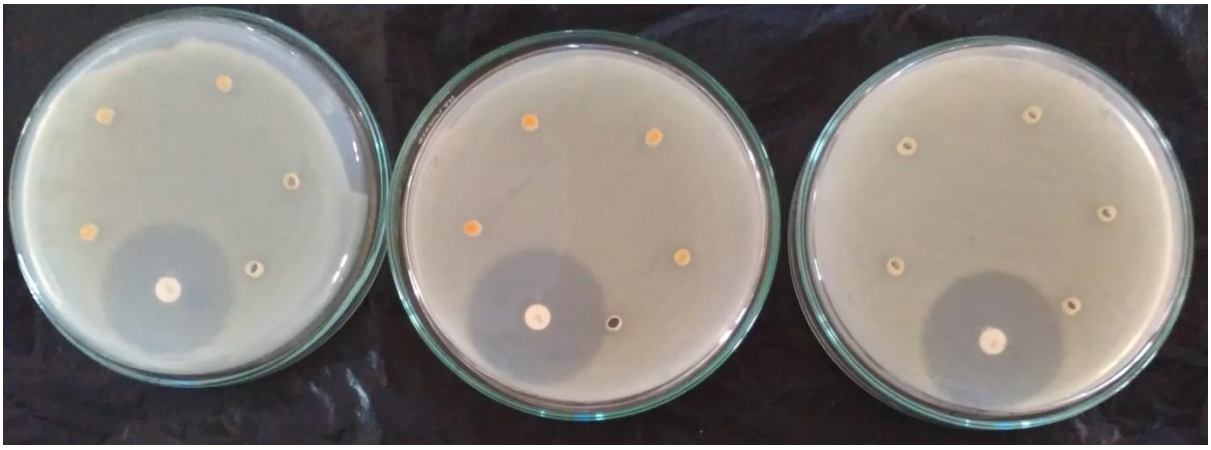

Figure 5: Antibacterial activity of CUR against *P. aeruginosa* using water as solvent. a) chelated with 0.1% TPP and loaded with drug (CS- CUR-CMC- TPP1), b) chelated with 0.3% TPP and loaded with drug (CS- CUR-CMC- TPP2), c) chelated with 0.5% TPP and loaded with drug (CS- CUR-CMC- TPP3).

Table 2: Antibacterial activity of CUR against *P.aeruginosa* using PBS as solvent.

Type of microparticles	Positive control	Negative control	Zone of inhibition at various Concentrations (in cm) 10µg	20 µg	30 µg	40 µg
CS- CUR-CMC-TPP1	-ve	-ve	-ve	-ve	-ve	-ve
CS- CUR-CMC-TPP2	-ve	-ve	-ve	-ve	-ve	-ve
CS- CUR-CMC-TPP3	-ve	-ve	-ve	-ve	-ve	-ve

-ve – negative

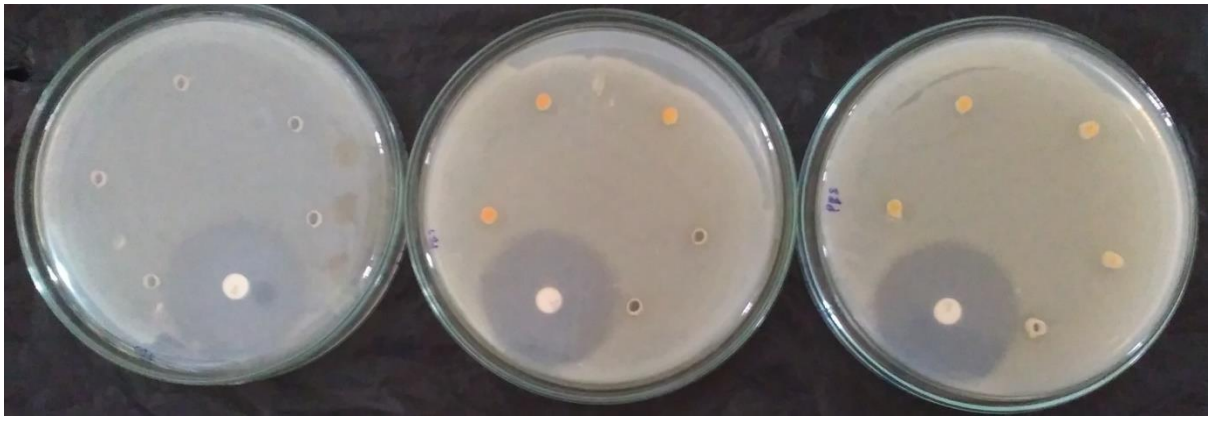

Figure 6: Antibacterial activity of CUR against *P. aeruginosa* using PBS as solvent a) chelated with 0.1% TPP and loaded with drug (CS- CUR-CMC-TPP1), b) chelated with 0.3% TPP and loaded with drug (CS- CUR-CMC-TPP2), c) chelated with 0.5% TPP and loaded with drug (CS- CUR-CMC- TPP3).

stretching)[39,40] (Figure 1a,c&e) were seen. The C=O frequency were observed next to amide bond stretching which ranges from 1647-1654 cm^{-1}. A broad trough was observed at 3502, 3527, 3502,3520,3520 and 3527 cm^{-1} was observed in CS-CMC-TPP1, CS- CUR-CMC- TPP1, CS-CMC- TPP2, CS- CUR-CMC- TPP2 CS-CMC- TPP3, and CS- CUR-CMC- TPP3 respectively which shows the presence of either carbonyl or hydroxyl groups. The C-H

Table 3: Antibacterial activity of CUR against *P.aeruginosa* using ethanol as solvent.

Type of microparticles	Positive control	Negative control	Zone of inhibition at various Concentrations (in cm) 10µg	20 µg	30 µg	40 µg
CS- CUR-CMC- TPP1	-ve	-ve	-ve	-ve	-ve	-ve
CS- CUR-CMC- TPP2	-ve	-ve	-ve	-ve	-ve	-ve
CS- CUR-CMC- TPP3	-ve	-ve	-ve	-ve	-ve	-ve

-ve – negative

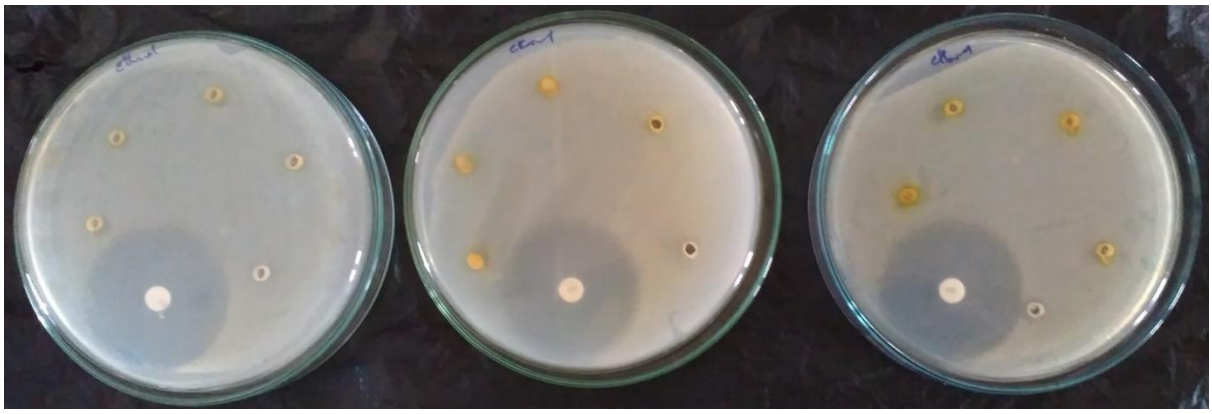

Figure 7: Antibacterial activity of CUR against *P.aeruginosa* using ethanol as solvent. a) chelated with 0.1% TPP and loaded with drug (CS- CUR-CMC- TPP1), b) chelated with 0.3% TPP and loaded with drug (CS- CUR-CMC- TPP2), c) chelated with 0.5% TPP and loaded with drug (CS- CUR-CMC- TPP3).

Table 4: Antibacterial activity of CUR against *P.aeruginosa* using acetic acid as solvent.

Type of microparticles	Positive control	Negative control	Zone of inhibition at various Concentrations (in mm) 10µg	20 µg	30 µg	40 µg
CS- CUR-CMC-TPP1	-ve	-ve	-ve	-ve	-ve	-ve
CS- CUR-CMC-TPP2	-ve	-ve	-ve	2	4	6
CS- CUR-CMC-TPP3	-ve	-ve	-ve	4	5	7

-ve – negative

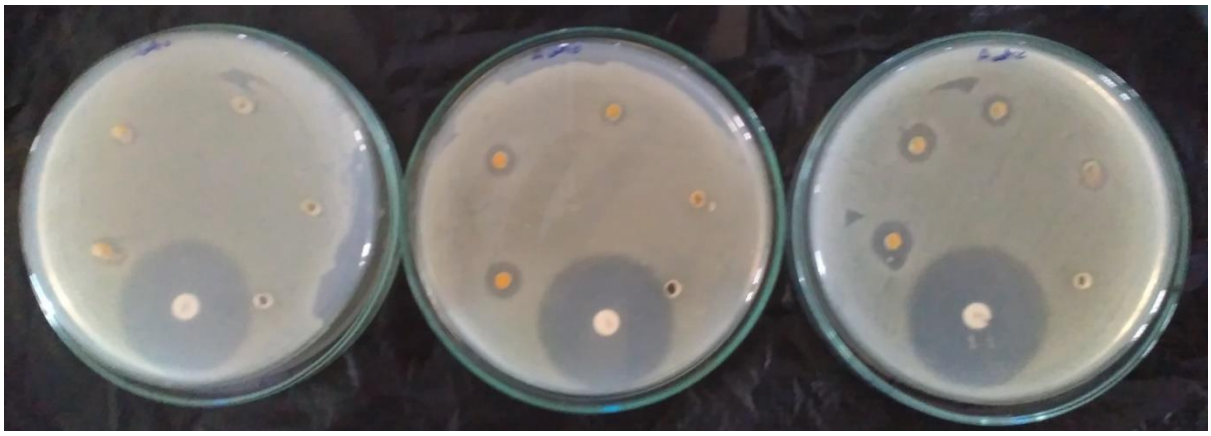

Figure 8: Antibacterial activity of CUR against *P.aeruginosa* using acetic acid as solvent. a) chelated with 0.1% TPP and loaded with drug (CS- CUR-CMC- TPP1), b) chelated with 0.3% TPP and loaded with drug (CS- CUR-CMC- TPP2), c) chelated with 0.5% TPP and loaded with drug (CS- CUR-CMC- TPP3).

peak of CS was absorbed in all the drug loaded and unloaded microparticles at 2922.16, 2922.16, 2922.16, 2924.09, 2922.16 and 2922.16 for CS-CMC-TPP1, CS-CUR-CMC- TPP1, CS-CMC- TPP2, CS- CUR-CMC-TPP2, CS-CMC- TPP3, and CS- CUR-CMC- TPP3 respectively (Figure 1 a-f). C=O stretching was observed at 1654 for CUR loaded microparticles. The characteristic peaks of OCH_3 for the presence of CUR were observed at 1153 cm^{-1} for all the CUR loaded microparticles. C-N stretching was observed at 1377.17 for all microparticles

(Figure 1a-f). The absorption peaks appeared between 1560 and 1570 cm^{-1} were due to secondary amide group (N-H bending)[41].

Scanning Electron Microscope (SEM) Analysis

SEM analysis of CS-CMC-TPP1, CS-CMC-TPP2 and CS-CMC-TPP3 were fluffy in appearance and smooth irregular in shape (Figure 2 a,c,e,), whereas the SEM analysis of CS-CUR-CMC-TPP1, CS-CUR-CMC-TPP2 and CS-CUR-CMC-TPP3 were irregular and rod in appearance (Figure 2 b,d,f,). Our previous work of CS-CMC microparticle production with 0.2%, 0.4% and 0.6% concentrations of TPP was found to produce a fluffy to irregular shape only[20]. Liuyun et al[42] showed the scaffold made of hydroxyl apatite CS-CMC to have irregular porous structure together with good interconnections. Barakat and Almurshedi[43] produced CS microparticles ranged between 675–887µm in diameter. They also found that the viscosity and concentration of CS plays important role in the formation of microparticles. CS/TPP molar ratio and its pH also influence the microparticle formation[20].

Encapsulation Efficiency

Encapsulation of CUR in all the microparticles was time dependent (Figure 3). CS-CMC-TPP3 was showing a faster encapsulation than others. Increased TPP concentration was reported to affect the encapsulation efficiency of the nanoparticles[44], but in this study is on par with our earlier study[20] where it was found to have good encapsulation at higher concentrations of TPP.

InVitro drug release kinetics

Drug release kinetics of CUR loaded microparticles was studied at pH 6.8 in phosphate buffer by dialysis membrane technique. The plots of CS-CUR-CMC-TPP1, CS-CUR-CMC-TPP2 and CS-CUR-CMC-TPP3, showed similar kind of drug release kinetics. Higher amount of drug is released in first 40 min and lowered down in next 40min, it maintained a steady state upto 180 min and that of CS-CUR-CMC -TPP3 shows steady release in drug till 120 min and there was a sharp peak at 140min (Figure 4). Overall drug release kinetic study reveals that CS-CUR CMC-TPP3 holds drug for more time and gradually releases it. Microparticles were getting hydrated slowly in buffer (pH 6.8) and then were diffusing slowly. This is the characteristic of positively charged amines present in CS which allows the acidic media to enter slowly and then release its contents[45].

InVitro controlled release studies

No antibacterial activity was observed when water, PBS and ethanol were used as solvent in microparticles chelated with TPP (Table 1, 2, 3). This is due to the inability of the solvent to dissolve the CS microparticles. Zone of inhibition was observed when acetic acid was used as solvent to dissolve microparticles (Table 4), it was found that it dissolves CS and releases the loaded CUR better[20].

CONCLUSION

In this study, different concentrations of TPP (0.1%, 0.3% and 0.5%) were used to synthesise CS-CMC microparticles. Irregular to rod shaped CS-CMC microparticles were produced. Microparticles were able to encapsulate 0.5% of TPP cross-linked CS-CUR-CMC -TPP3 microparticles which showed faster encapsulation efficiency and slower drug release. Acetic acid alone was found to release the encapsulated CUR to the medium which was evidenced by its antimicrobial activity against *Pseudomonas aeruginosa*. Thus, an acidic environment favours the drug release.

CONFLICT OF INTEREST

Authors have no conflict of interest

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REFERENCES

1. Xiao, K., Luo, J., Fowler, W.L., Li, Y., Lee, J.S., Xing, L., Cheng, R.H., Wang, L., Lam, K.S. A self-assembling nanoparticle for paclitaxel delivery in ovarian cancer, *Biomaterials*. 2009, 30, 6006-6016.
2. Shiny, J., Ramchander, T., Goverdhan, P., Habibuddin, M., Aukunuru, J.V. Development and evaluation of a novel biodegradable sustained release microsphere formulation of paclitaxel intended to treat breast cancer. *Int. J. Pharma. Investig*. 2013. 3, 119-125.
3. Ruan, G., Feng, S.S. Preparation and characterization of poly (lactic acid)-poly(ethylene glycol)- poly(lactic acid) (PLA-PEG-PLA) microspheres for controlled release of paclitaxel. *Biomaterials*. 2003.24, 5037-5044.
4. Boudou, T., Kharkar, P., Jing, J., Guillot, R., Pignot-Paintrand, I., Auzely-Velty, R., Picart, C. Polyelectrolyte multilayer nanoshells with hydrophobic nanodomains for delivery of Paclitaxel. *Journal of Control Release*. 2012. 159, 403-412.
5. Edwards, D.A., Caponetti, J.G., Mintzes, J., Hrkach, J., Lotan, N., Langer, R. Large porous particles for pulmonary drug delivery. *Science*. 1997. 276 (5320), 1868-1871.
6. Andrianov, A.K., Payne, L.G. Polymeric carriers for oral uptake of microparticles. *Adv. Drug. Deliv. Rev.* 1998. 34, 155–70.
7. Zimmer, A., Kreuter, J. Microspheres and nanoparticles used in ocular delivery systems. *Adv. Drug. Deliv. Rev.* 1995. 16,61–73.
8. Kurita, K. Chemical modification of chitin and Chitosan. In: Muzzarelli RA, Jeuniaux C, Gooday GW, editors. Chitin in Nature and Technology. *New York: Plenum*. 1986. 287–93.
9. Zhao, L.M., Shi, L.E., Zhang, Z.L., Chen, J.M., Shi, D.D., Yang, J., Tang, Z.X. Preparation and application of chitosan nanoparticles and nanofibers. *Brazilian Journal of Chemical Engineering*. 2011. 28(3), 353 - 362.
10. Wang, Q., Jamal, A., Detamore, M.S., Berkland, S. PLGA-chitosan/PLGA-alginate Nanoparticle Blends as Biodegradable Colloidal Gels for Seeding Human Umbilical Cord Mesenchymal Stem Cells. J.*Biomed.Mater. Res.A*. 2011. 96(3), 520–527.
11. Yue, Z., You, Z., Yang, Q., Lv, P., Yue, H., Wang, B., Ni, D., Su, Z., Wei, A., Ma, G. Molecular structure matters: PEG-b-PLA nanoparticles with hydrophilicity

and deformability demonstrate their advantages for high-performance delivery of anti-cancer drugs. *J.Mater.Chem.B.* 2013. 1,3239-3247

12. Steinbuchel, A. Non-biodegradable biopolymers from renewable resources: perspectives and impacts. *Curr.Opin.Biotechnol*. 2005. 16, 607–613.
13. Akar, A., Akkaya, E.U., Yesiladali, S.K., Celikyilmaz, G.,. Cokgor, E.U, Tamerler, C. Accumulation of polyhydroxyalkanoates by *Microlunatus phosphovorus* under various growth conditions, *J.Ind.Microbiol.Biotechnol*. 2006. 33,215–220.
14. Struszczyk, S.M.H. Chitin and chitosan - Part II. Applications of chitosan. *Polimery*. 2002. 47(6), 396-403.
15. Puvvada, Y.S., Vankayalapati, S., Sukhavasi, S. Extraction of chitin from chitosan from exoskeleton of shrimp for application in the pharmaceutical industry. *Inter.Curr. Pharm. J.* 2012. 1(9), 258-263.
16. Tomihata, K., Ikada, Y. *In vitro* and *In vivo* degradation of films of chitin and its deacetylated derivatives. *Biomaterials.* 1997. 18(7), 567-575.
17. Roberts, G.A.F. Preparation of chitin and chitosan. *The Macmillan UK: London Press* 1992.
18. Jameela, S.R., Jayakrishnan, A. Glutaraldehyde crosslinked chitosan microspheres as a long acting biodegradable drug delivery vehicle: studies on the *In vitro* release of mitoxantrone and *In vivo* degradation of microspheres in rat muscle. *Biomaterials.* 1995. 16, 769-775.
19. Vasudev, S.C., Chandy, T., Sharma, C.P. Development of chitosan/polyethylene vinyl acetate co-matrix: controlled release of aspirin/heparin for preventing cardiovascular thrombosis. *Biomaterials*. 1997. 18, 375-381.
20. Samrot, A.V., Akanksha, Jahnavi, T., Padmanaban, S., Philip, S.A., Burman, U. A., Rabel, M. Chelators influenced synthesis of chitosan-carboxymethyl cellulose microparticles for controlled drug delivery. *Advanced nanoscience*. 2016. 6 (8), 1219-1231.
21. Shu, X.Z., Zhu, K.J. A novel approach to prepare tripolyphosphate/chitosan complex beads for controlled drug delivery. *Int. J.Pharm.* 2000. 201, 51-58.
22. Chandy T., Sharma, C.P. Chitosan—as a biomaterial. *Biomater. Artif.Cells Artif. Organs.* 1990. 18, 1–24.
23. Takishima, J., Onishi, H., Machida, Y. Prolonged intestinal absorption of cephradine with chitosan-coated ethylcellulose microparticles in rats. *Biol.Pharm.Bull.* 2002. 25, 1498– 1502.
24. Smart, J.D., Kellaway, I.D., Worthington, H.E.C. An *In vitro* investigation of mucosa-adhesive materials for use in controlled drug delivery. *J Pharmacol.* 1994. 36(5), 295-299.
25. Cerchiara, T., Abruzzo, A., Parolin, C., Vitali, B., Bigucci, F., Gallucci, M.C., Nicoletta, F.P., Luppi, B. Microparticles based on chitosan/carboxymethylcellulose polyelectrolyte complexes for colon delivery of vancomycin. *Carbohydrate Polymers.* 2016. 143, 124–130.
26. Bigucci, F., Abruzzo, A., Vitali, B., Saladini, B., Cerchiara, T., Gallucci, M.C., et al. Vaginal inserts based on chitosan and carboxymethylcellulose complexes for local delivery of chlorhexidine: Preparation, characterization and antimicrobial activity. *Inter. J. Pharm*. 2015. 478, 456–463.
27. Garcia, A., Leonardi, D., Piccirilli, N.G., Mamprin, M.E., Olivieri, A.C., Lamas, M.C. Spray drying formulation of albendazole microspheres by experimental design. *In vitro-in vivo* studies. *Drug Development and Industrial Pharmacy*. 2015. 41,244–252.
28. Lu, S., Liu, M., Ni, B. An injectable oxidized carboxymethylcellulose/N-succinyl-chitosan hydrogel system for protein delivery. *Chemical Engineering Journal*. 2010. 160, 779–787.
29. Wright, L.E., Frye, J.B., Gorti, B., Timmermann, B.N., Funk, J.L. Bioactivity of Turmeric-Derived Curcuminoids and Related Metabolites in Breast Cancer. *Curr. Pharm. Des*. 2013. 19(34), 6218–6225.
30. Niamsa, N., Sittiwet, C. Antimicrobial activity of Curcuma longa aqueous extract. *Journal of Pharmacology and Toxicology*. 2009. 4(4), 173–177.
31. Ammon, H.P.T., Wahl, M.A. Pharmacology of *Curcuma longa*. *Planta Med*. 1991. 57(1), 1-7.
32. Calvo, P., Remunan-Lopez, C., Vila-Jato, J.L., Alonso, M.J. Novel Hydrophilic Chitosan–Polyethylene Oxide Nanoparticles as Protein Carriers. *Journal of Applied Polymer Science*. 1997. 63, 125–132.
33. Parize, A.L., Stulzer, H.K., Laranjeira, M.C.M., Brighente, M.D.C., de-Souza, T.C.R. Evaluation of chitosan microparticles containing curcumin and cross linked with sodium tripolyphosphate produced by spray drying. *Quím Nova*. 2012. 35 (6).
34. Mukerjee, A., Vishwanatha, J.K. Formulation, Characterization and Evaluation of Curcumin-loaded PLGA Nanospheres for Cancer Therapy. *Anticancer Research*. 2009. 29(10), 3867-3875.
35. Benetton, S.A., Kedor-Hackmann, E.R.M., Santoro, M.I.R.M., Borges, V.M. Visible spectrophotometric and first-derivative UV spectrophotometric determination of rifampicin and isoniazid in pharmaceutical preparations. *Talanta*. 1998. 47(3), 639–643.
36. Hua, S. Comparison of *In vitro* dialysis release methods of loperamide-encapsulated liposomal gel for topical drug delivery. *Int.J.Nanomedicine.* 2014. 9, 735–744.
37. Dima, C., Cotarlet, M., Alexe, P., Dima, S. Microencapsulation of essential oil of pimento [Pimenta dioica (L) Merr.] by chitosan/k-carrageenan complex coacervation method. *Innovative Food Science and Emerging Technologies*. 2014. 20, 203-211.
38. Buzia, O.D., Dima, C., Dima, S. Preparation and characterization of chitosan microspheres for vancomycin delivery. *Farmacia*. 2015. 63(6).
39. Mi, F.L., Shyu, S.S., Lee, S.T., Wong, T.B. Kinetic study of chitosan-tripolyphosphate complex reaction and acid-resistive properties of the chitosan-tripolyphosphate gel beads prepared by in-liquid curing

method. *Journal of Polymer Science B*. 1999. 37, 1551–1564,

40. Martins, A.F., de Oliveira, D.M., Pereira, A.G.B., Rubira, A.F., Muniz, E.C. Chitosan/TPP microparticles obtained by microemulsion method applied in controlled release of heparin. *Inter. J.Biol. Macromol.* 2012. 51 (5), 1127–1133.
41. Banik, N., Iman, M., Hussain, A., Ramteke, A., Boruah, R., Maji, T.K. Soy flour nanoparticles for controlled drug delivery: effect of crosslinker and montmorillonite (MMT). *New.J.Chem.* 2013. 37, 3981-3990
42. Liuyun, J., Yubao, L., Chengdong, X. Preparation and biological properties of a novel composite scaffold of nano-hydroxyapatite/chitosan/carboxymethyl cellulose for bone tissue engineering. *J.Biomed. Sci.* 2009. 16, 65.
43. Barakat, N.S., Almurshedi, A.S. Design and development of gliclazide-loaded chitosan microparticles for oral sustained drug delivery: in-vitro/in-vivo evaluation. *J.Pharm.Pharmacol.* 2011. 63(2),169-78.
44. Zhang, H., Wu, A., Tao, Y., Zang, L., Su, Z. Preparation and Characterization of Water-Soluble Chitosan Nanoparticles as Protein Delivery System. *J.Nanomater.* 2009. 2010.
45. Mukhopadhyay, P., Sarkar, K., Bhattacharya, S., Mishra, R., Kundu, P.P. Efficient oral insulin delivery by dendronized chitosan: *In vitro* and *In vivo* studies. *RSC Advances*. 2014. 4 (83), 43890-43902.