Development of thiamine and pyridoxine loaded ferulic acid-grafted chitosan microspheres for dietary supplementation

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Abstract Therapeutic potential of water soluble vitamins has been known for long and in recent times they are being widely supplemented in processed food. Phenolic acid-grafted chitosan derivatives can serve as excellent biofunctional encapsulating materials for these vitamins. As a proof of concept, thiamine and pyridoxine loaded ferulic acid-grafted chitosan microspheres were developed. Ferulic acid was successfully grafted on chitosan by a free radical mediated reaction and the structure was confirmed by FTIR and NMR analysis. When compared to FTIR spectra of chitosan, intensity of amide I (at around 1644 cm^{-1}) and amide II (at around 1549 cm^{-1}) bands in spectra of ferulic acid-grafted chitosan were found increased, indicating formation of new amide linkage. Strong signals at $\delta=6.3\text{--}7.9\text{ ppm}$ corresponding to methine protons of ferulic acid were observed in NMR spectra of ferulic acid-grafted chitosan, suggesting the successful grafting of ferulic acid onto chitosan. Grafting ratio of the derivative was 263 mg ferulic acid equivalent/g polymer. Positively charged particles

(zeta potential 31 mv) of mean diameter 4.5 and 4.8 μ , corresponding to number distribution and area distribution respectively were observed. Compact microspheres with smooth surfaces and no apparent cracks or pores were observed under scanning electron microscope. Efficient microencapsulation was further proved by X-ray diffraction patterns and thermal analysis. Preliminary anti-inflammatory activity of the vitamin-loaded microspheres was demonstrated.

Keywords Vitamin · Microencapsulation · Antioxidant · Anti-inflammatory · Phenolic acid

Highlights

- Microencapsulation of thiamine and pyridoxine in ferulic acid-grafted chitosan
- Bioactive wall material for microencapsulation
- Anti-inflammatory activity of vitamin loaded microspheres
- Controlled release of thiamine and pyridoxine

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Introduction

Biofunctional chitosan derivatives, resulting from graft copolymerization of phenolic acids with chitosan are of recent interest to chitosan chemistry (Cho et al. 2011; Aytekin et al. 2011). These biomaterials have been promoted as multifunctional packaging materials (Schreiber et al. 2013) and novel antioxidants (Pasanphan et al. 2010). In recent literature, phenolic acids have been proposed as a potential treatment for many disorders including Alzheimer's disease, cancer, cardiovascular diseases, diabetes mellitus and skin disease (Mancuso and Santangelo 2014; Roy and Prince 2013). Grafting phenolic acids on chitosan may provide these compounds with the much needed stability and also aid their slow release. In consonance to this, recently, anti-diabetic potential of chitosan gallic acid conjugate has been documented (Liu et al. 2013). Our research group has previously demonstrated synthesis of four phenolic acid-grafted chitosan derivatives with improved grafting ratio, antioxidant activity and broad spectrum antimicrobial activity against food spoilage bacteria (Chatterjee et al. 2015). Further we observed that phenolic acid grafted chitosan derivatives may find potential

application in functional food development. We hypothesized that phenolic acid-grafted chitosan derivatives may serve as novel encapsulating wall materials for microencapsulation of nutraceuticals.

Microencapsulation is a novel technology that can improve the retention time of the nutrient in food and allow controlled release at specific times, during food consumption or in the intestinal gut. Edible micro-particles as release systems of nutraceuticals are finding many potential applications in food (Matalanis et al. 2011). Phenolic acid-grafted chitosan derivatives can be novel edible bioactive wall materials for microencapsulation of nutrients and nutraceuticals in contrast to the typically used inert wall materials. Chitosan is an approved food additive in Japan and Korea (The Japan Food Chemical Research Foundation 2015; Korea Food Additives 2004). European Food Safety Authority has also approved chitosan as a supplement which regulates blood lipid (EFSA 2011). Commonly used chemicals in food processing like ascorbic acid and hydrogen peroxide were used as the redox pair reagent for grafting phenolic acid onto chitosan. Hence, the resultant micro-particles may have potential application as dietary supplement formulation.

Health benefits of nutraceuticals and their role in preventive and alternative medicine have been substantiated in recent literature (Braithwaite et al. 2014). Supplementation of key nutrients is a new treatment approach where these substances exert a protective effect on the body by preventing the onset of adverse health conditions (Gupta et al. 2010). Among the water soluble vitamins, anti-nociceptive and anti-inflammatory effects of thiamine are well known (Moallem et al. 2008). Recent studies have demonstrated a significant association between Parkinson's disease, Wernicke encephalopathy and low levels of thiamine in serum, which suggests that elevated thiamine levels might provide protection against these diseases (Lu'o'ng and Nguyen 2012; Klooster et al. 2013). Similarly, pyridoxine plays a key role in human metabolism, immune function, and cardio vascular wellbeing (Bowling 2011; Ji et al. 2006). However these vitamins are known to be thermally unstable (Uherová et al. 1993), hence microencapsulation may improve thermal stability and open up new possibilities of dietary supplementation through functional food.

Advantages of microencapsulation with chitosan by spray drying for food and other industry applications have been documented in recent literature (Estevinho et al. 2013). However to our knowledge, so far phenolic acid grafted-chitosan derivatives were not explored as a bioactive wall material for microencapsulation of nutrients/ nutraceuticals. Hence as a proof of concept, the authors report development and characterization of thiamine and pyridoxine loaded ferulic acid grafted-chitosan microspheres. Simultaneous encapsulation of both the vitamins in ferulic acid grafted chitosan was demonstrated to emphasize the possibilities of developing

tailor made functional food ingredients employing this approach. Loading capacity and in vitro release of thiamine and pyridoxine were studied. The microspheres were preliminarily evaluated for anti-inflammatory activity in carrageenan induced paw edema of albino rats.

Materials and methods

Materials

Chitosan from shrimp shells (MW=100 kDa, 90 % degree of deacetylation) was prepared at the pilot plant facility of Central Institute of Fisheries Technology, Cochin (Weska et al. 2007). Ferulic acid (Fe), thiamine hydrochloride, pyridoxine hydrochloride, Folin-ciocalteu reagent and ferrous chloride (FeCl_2) were obtained from Sigma-Aldrich (USA). Acetic acid, hydrogen peroxide (H_2O_2), ascorbic acid, sulfuric acid (96 % w/w), trisodium phosphate, ammonium molybdate, ammonium thiocyanate and ninhydrin were obtained from Merck Millipore (Germany).

Synthesis and characterization of ferulic acid grafted chitosan

The synthesis of ferulic acid-grafted chitosan was performed employing free radical mediated grafting method as reported by Curcio et al. (2009) with some modifications. Briefly, 10 g of chitosan was dissolved in 2 L of 1 % acetic acid solution (v/v) in a 5 L three necked round bottom flask. Then 20 mL of 1 M H_2O_2 containing 1.08 g of ascorbic acid was added dropwise to the chitosan solution. The reaction vessel was then flushed with nitrogen for 30 min with continuous stirring, followed by addition of 10 g ferulic acid dissolved in 100 mL ethanol. The reaction vessel was stoppered and sealed with Parafilm® to minimize further entry of oxygen. The reaction was carried out for 24 h at 25 °C with constant stirring over a magnetic stirrer. The reaction mixture was later dialyzed against distilled water (with 12 changes of water) with a 14,000 Da molecular weight cutoff membrane for 72 h to remove unreacted phenolic acids. The dialyzate was spray dried (inlet temperature 140 °C, outlet temperature 77 °C) in a pilot plant spray drier (SM Scientech, Kolkata, India) to afford water soluble ferulic acid-grafted chitosan derivative.

Absorbance maxima of ferulic acid-grafted chitosan was determined by recording UV–Vis absorption spectra using a Hitachi U-2910 spectrophotometer (Tokyo, Japan). FTIR spectra was recorded using a Thermo Nicolet, Avatar 370 spectrometer (Waltham, USA) as KBr pellets over a spectral range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} . ^1H NMR spectra of ferulic acid-grafted chitosan was recorded in 400 MHz NMR (Bruker, Massachusetts, USA) using D_2O as solvent. Grafting ratio of ferulic acid on chitosan was

estimated using Folin-Ciocalteu reagent procedure (Curcio et al. 2009).

Microencapsulation of thiamine and pyridoxine

Thiamine and pyridoxine were encapsulated following spray drying technique using ferulic acid- grafted chitosan as wall material. The wall material was synthesized following optimized protocol, starting with 20 g of chitosan and 20 g of ferulic acid. The dialysate (3 L) was mixed with thiamine and pyridoxine (2 g each) in deionized water (400 mL) followed by homogenization at 16,000 rpm for 15 min. The mixture was spray dried (inlet temperature 140 °C, outlet temperature 77 °C, feed pump flow 10 mL/min) to afford microparticles of encapsulated thiamine and pyridoxine.

Characterization of microparticles

Particle size analysis of the thiamine and pyridoxine loaded micro-particles was performed in a dynamic light scattering (DLS, Microtrac, Montgomeryville, USA) particle size analyzer. For particle size measurement, 50 mg of the microparticles were dispersed in 10 mL of deionised water by vortex. Surface morphology was observed under scanning electron microscope (SEM, JEOL Model JSM - 6390LV, Tokyo, Japan) using instrumentation facility of Sophisticated Test and Instrumentation (STIC) Centre of Cochin University of Science and Technology, Cochin, Kerala, India. X-ray diffraction (XRD, Bruker Kappa Apex II, Massachusetts, USA) studies (over a 2θ range from 3 to 40°, using a scan rate of $0.04^\circ \text{ min}^{-1}$) and thermal analysis (DTG, Perkin Elmer, Diamond TG/DTA, Waltham, USA) (heated from 30 to 600 °C under nitrogen atmosphere at a heating rate of $10^\circ \text{ C min}^{-1}$) of ferulic acid-grafted chitosan and vitamin loaded microparticles were also performed using instrumentation facility of STIC. Loading capacity was determined by estimating thiamine and pyridoxine in pre-weighed amount of the microencapsulated particles and expressed as mg of thiamine and pyridoxine per gram of sample.

LC-MS/MS analysis of thiamine and pyridoxine

A highly sensitive method for estimation of thiamine and pyridoxine was developed using AB Sciex API4000 triple quadrupole mass spectrometer (Framingham, USA) in multiple reaction monitoring (MRM) mode. Method parameters are presented in Appendix A.

In vitro release study

In vitro release behavior of thiamine and pyridoxine from the encapsulated particles was studied. Briefly, 1 g of the sample was placed inside an empty tea bag and was incubated in

10 mL of sodium acetate buffer of pH 4 containing Tween 80 (0.5 % wt) at 37 °C with gentle shaking (100 rpm). At specific intervals, the entire release medium was removed and replaced by pre-warmed fresh medium. The release medium was analyzed by LC-MS/MS to quantify released thiamine and pyridoxine. All results were the mean of three samples, and all data were expressed as the mean $\pm$ SD.

Preliminary evaluation of anti-inflammatory activity in rat paw edema model

Anti-inflammatory activity was evaluated by the carrageenan-induced edema method as per literature (Akindele and Adeyemi 2007) with slight modification. Male albino rats (200–250 g), maintained under standard environmental conditions and diet at the Institute animal laboratory facility were used. The animals were housed individually in polypropylene cages under hygienic environmental conditions ($28\pm 2^\circ \text{ C}$, humidity 60–70 %, 12 h light/dark cycle). The animals were provided a standard diet with metabolizable energy of 3600 Kcal (M/s Sai Feeds, Bangalore, India) and water ad libitum. The entire experiment was carried out strictly as per the guidelines prescribed by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. The experiment was approved by the Institute Animal Ethics Committee (Clearance No. CIFT/B&N/IAEC/13(2)/2015). The animals were divided into three groups of six rats each and housed in individual cages. Animals in group I were administered with distilled water (10 mL/kg body weight) and considered as control. Group II was treated orally with vitamin loaded ferulic acid grafted chitosan microspheres (250 mg/kg body weight), dispersed in water for 7 days. Group III animals were treated similarly as control animals, however on 8th day, they were treated orally with 10 mg/kg of ibuprofen used as reference. After 1 h, acute inflammation was induced in all the animals by injection of λ -carrageenan (0.2 mL, 1 % w/v in saline) into the subplantar tissue of the right hind paw. Thickness of the footpad was measured with a digital vernier caliper, immediately before and after induction of inflammation at intervals of 1 h for a period of 3 h.

Results and discussion

Synthesis and characterization of ferulic acid grafted chitosan

Ferulic acid-grafted chitosan was successfully synthesized by free radical mediated reaction of ferulic acid with chitosan (1:1 w/w) for 24 h under inert environment at 25 °C. The free radical mediated grafting was achieved by using a “Hydrogen peroxide-Ascorbic acid” redox initiator system.

Conventional initiator systems (i.e., azo compounds and peroxides) require relatively high reaction temperature to ensure their rapid decomposition. Interestingly, redox initiators show several advantages over conventional initiator systems. First of all, this kind of system does not generate toxic reaction products; moreover, it is possible to perform the reaction processes at lower temperatures, reducing the risks of antioxidant degradation. Again, as compared to carbodiimide (EDC) and N-hydroxysuccinimide (NHS) mediated and laccase catalyzed polymerization, free radical mediated grafting of phenolic acid has been considered as rapid and ecofriendly (Curcio et al. 2009). Yield of the chitosan-phenolic acid conjugates varied between 58 and 65 %. Grafting of phenolic acids on chitosan was confirmed by TLC, FTIR spectroscopy and UV–Vis spectrophotometry. No spots corresponding to the individual phenolic acids were observed on the developed TLC plates for chitosan phenolic acid conjugates confirming absence of free phenolic acid and successful grafting of the phenolic acids on chitosan (Appendix B). FTIR spectroscopy of native chitosan and ferulic acid grafted chitosan (Fig. 1) confirmed formation of new covalent bonds. As compared to chitosan, intensity of amide I (at around 1644 cm^{-1}) and amide II (at around 1549 cm^{-1}) bands in ferulic acid-grafted chitosan increased, indicating formation of new amide linkages. It was also observed that integral ratio of the C H stretching band (at around 2933 cm^{-1} , belonging to chitosan and gallic acid) to the pyranose band (at around 896 cm^{-1} , belonging to chitosan), i.e., $12933/1896$ was higher in ferulic acid grafted chitosan as compared to chitosan (Woranuch and Yoksan 2013), implying successful grafting of ferulic acid onto chitosan. Compared to chitosan, ferulic acid-grafted chitosan showed new band at around 1515 cm^{-1} (C=C aromatic ring). The derivative showed primary UV–Vis absorption peak at 323 nm which signifies the grafting of ferulic acids onto chitosan (Appendix C). Similar observation was made by Woranuch and Yoksan (2013). The chemical structure of ferulic acid grafted chitosan was further confirmed by ^1H NMR technique (Fig. 2). The proton signals of chitosan appeared at $\delta=2$ ppm (NHCOCH_3), $\delta=2.6$ ppm (H-2), $\delta=3.2\text{--}3.9$ ppm (H-3-H-6) and $\delta=4\text{--}5$ ppm (H-1, D_2O). Strong signals at $\delta=6.3\text{--}7.9$ ppm corresponding to methine protons of ferulic acid was observed, suggesting the successful grafting of ferulic acid onto chitosan. Similar observation was made in other reports of synthesis of ferulic acid-grafted chitosan (Woranuch and Yoksan 2013; Liu et al. 2014).

Grafting ratio of the derivative was 263 ± 5.72 mg ferulic acid equivalent/g polymer. Elsewhere, it has been reported that the grafting ratio of ferulic acid-grafted chitosan by free radical mediated method was 66.7 mg ferulic acid equivalent/g polymer (Liu et al. 2014). Higher grafting ratio in the present study may be due to longer reaction time and inert reaction environment. However, we had reported earlier that with further increase of reaction time, the grafting ratio of phenolic

acid on chitosan did not improve significantly (Chatterjee et al. 2015).

It is important to note that spray drying conditions did not affect the stability of the ferulic acid-grafted chitosan as ascertained by the higher grafting ratio of ferulic acid on chitosan achieved in the present study. Spray drying has been noted in literature as one of the best drying methods to convert, in a single step, fluid materials into solid or semi-solid particles. The rapid evaporation of the fluid feed material in contact with hot air, keeps the droplets' temperature relatively low. Hence, the product quality is not significantly or negatively affected (Murugesan and Orsat 2012).

Thiamine and pyridoxine loaded microspheres

Yield (actual yield as percentage of theoretical yield) of vitamin loaded microspheres through spray drying varied between 63.58 and 65.12 % for different batches prepared. The theoretical yield was calculated as 25.26 g considering initial amount of 20 g chitosan and 5.26 g of grafted ferulic acid (calculated from grafting ratio). The product yield can be considered satisfactory as similar yields for microencapsulation through spray drying were reported in recent literature (Klein et al. 2015; Bastos et al. 2012). Total encapsulation efficiency (TEE) was determined as ratio of actual content and theoretical content (2 g thiamine/pyridoxine in 25.26 g microspheres) of thiamine and pyridoxine per gram of the microspheres. TEE was found to be 91 ± 2.31 % in case of thiamine and for pyridoxine the encapsulation efficiency was 83 ± 3.17 %. Relatively lower encapsulation efficiency for pyridoxine may be due to high inlet and outlet temperature during spray drying. It is known that pyridoxine is thermally more unstable than thiamine and stability of thiamine improves in acidic solution (Uherová et al. 1993). We could not compare the attained encapsulation efficiency, since there are no previous reports for microencapsulation of thiamine and pyridoxine. However Desai et al. (2006) reported encapsulation of ascorbic acid in chitosan microspheres by spray drying. The size, yield and encapsulation efficiency of ascorbic acid loaded chitosan microspheres ranged from $3.9\text{--}7.3\text{ }\mu\text{m}$, 54.5–67.5 % and 45.72–68.7 % respectively.

Particle size and surface morphology analysis of the microspheres

Particle size analysis of the microspheres (Fig. 3) showed positively charged particles (zeta potential $+37\text{--}44\text{ mV}$) of mean diameter 4.5 and $4.8\text{ }\mu\text{m}$, corresponding to number distribution (MN) and area distribution (MA) respectively. Particle size varied from 3.2 to $6.5\text{ }\mu\text{m}$. It is important to note that the particle size was less than $10\text{ }\mu\text{m}$ and particles were homogeneous in dimension. This is important in food application since these factors are directly related to controlled release of

Fig. 1 FTIR spectra of native chitosan and ferulic acid grafted chitosan

the loaded bioactive compound and ease of uptake by cells (Bastos et al. 2012). Zeta potential is an indicator of the surface charge property of microspheres. It signifies the electric

potential of particles as influenced by the wall material of the microspheres and the medium in which it is dispersed. It was observed that microspheres with a zeta potential above $\pm$

Fig. 2 ^1H NMR spectra and tentative structure of ferulic acid grafted chitosan

30 mV form stable suspensions, as the surface charge prevents aggregation of the particles (Selvaraj et al. 2012). In our study, all the batches of vitamin loaded microspheres were having zeta potential more than +35 mV, confirming good dispersibility in water. Compact microspheres with smooth surface and no apparent cracks or pores were observed in SEM images (Fig. 4). SEM micrograph also confirmed less than 10 μ particle size and homogeneous dimension of the particles. This suggests an efficient microencapsulation.

XRD analysis of the vitamin loaded microspheres

XRD spectra of ferulic acid-grafted chitosan showed characteristic broader peak at about 2θ 20° and less crystallinity as compared to chitosan. This observation is consistent with earlier reports (Woranuch and Yoksan 2013) and might be

attributed to the destruction of inter and intra molecular hydrogen bonds of chitosan by bulky ferulic acid. Thiamine and pyridoxine loaded microspheres showed further flat peak at around 2θ 20° indicating reduction in crystallinity (Fig. 5). This can be attributed to further folding of the polymer chains to produce micro particles. However no characteristic peaks for thiamine and pyridoxine were observed. This indicates that the vitamins are dispersed at the molecular level in the polymer matrix and signifies successful microencapsulation. Similar observations were made by Agnihotri and Aminabhavi (2004) for clozapine encapsulation in chitosan.

Fig. 3 Particle size analysis of thiamine and pyridoxine loaded ferulic acid grafted chitosan microspheres

Fig. 4 SEM images showing surface morphology of vitamin loaded ferulic acid grafted chitosan

Fig. 5 XRD spectra of ferulic acid grafted chitosan (a) and vitamin loaded ferulic acid grafted chitosan microspheres (b)

Thermo gravimetric analysis of the vitamin loaded microspheres

The derivative of weight loss (DTG) thermograms of ferulic acid-grafted chitosan (FCS) and vitamin loaded microspheres (VMS) are presented in Fig. 6. FCS showed three distinct stages of weight loss. The first weight loss with a peak at 78.93 °C can be attributed to loss of water from the polymer.

The second and third stages of weight loss with peaks at 174.71 and 294.88 °C were due to decomposition of grafted ferulic acid (Melting point 168–172 °C) and chitosan respectively. This decreased decomposition temperature of chitosan is due to reduced crystallinity after grafting of ferulic acid. These observations are consistent with earlier reports (Liu et al. 2014). In case of VMS, decomposition temperature (peak at 163.23 and 257.56 °C) further decreased due to

Fig. 6 Derivative of weight loss (DTG) thermograms of ferulic acid grafted chitosan (FCS) and vitamins loaded microspheres (VMS)

reduced crystallinity of the microspheres as observed by XRD analysis. Reduced thermal stability of modified chitosan as compared to native chitosan was also observed by Stulzer et al. (2008) while studying malonyl chitosan microspheres for controlled release of acyclovir. However, the beginning of melting of the microspheres occurred at higher temperatures than melting point of pyridoxine (159 °C) and thiamine (248 °C). Rate of weight loss (Fig. 6) was also much lower for VMS (0.548 mg/min and 0.320 mg/min) as compared to FCS (1.244 mg/min and 1.037 mg/min). A small weight loss peak after 300 °C in case of VMS (Fig. 6) may indicate the decomposition of vitamins in the core. Rosa et al. (2013) explained the same behavior for microencapsulated gallic acid in chitosan. Reduced rate of weight loss due to microencapsulation was also observed for protein antigen loaded chitosan microspheres (Walke et al. 2015). This signifies proper microencapsulation and thermal stability of the microencapsulated thiamine and pyridoxine.

In vitro controlled release study

Cumulative release of thiamine and pyridoxine in aqueous medium of acidic pH, as percentage released against time was observed. Sustained release of thiamine and pyridoxine was observed over a period of 100 h. After 2 h, 24±2.45 % of thiamine and pyridoxine was released. At 100 h, a maximum

of 87±3.14 % of thiamine and pyridoxine was found to be released from the microspheres. The initial rapid increase was typical of microspheres prepared with chitosan and chitosan derivatives. Cho et al. (2012) made similar observations while studying the controlled release of Vitamin C from N-acyl chitosan nano particles. The release of Vitamin C had reached a plateau after 21 h and after 168 h around 90 % of the loaded Vitamin C was released. Hence the slow release of thiamine and pyridoxine in acidic condition over a period of 100 h shows the effectiveness of encapsulation methodology.

In vivo anti-inflammatory activity

As shown in Table 1., thiamine and pyridoxine loaded microspheres (250 mg/kg body weight) showed significant ($p<0.05$) inhibition (52.3–56.9 %) of carrageenan induced paw edema in albino rats. Whereas, for Ibuprofen 34.7–40.3 % inhibition of paw edema was observed. Literature reports suggest that treatment of thiamine/pyridoxine (100 mg/kg body weight intraperitoneal for 7 days) partially reduce formaldehyde-induced hindpaw edema in experimental rats (Franca et al. 2001). The good anti-inflammatory activity observed in our study, at much lower effective dose of thiamine/ pyridoxine can be explained as cumulative bioactivity of ferulic acid and the vitamins. Therapeutic potential of ferulic acid as a function of its anti-inflammatory and

Table 1 Effect of thiamine and pyridoxine loaded ferulic acid grafted chitosan microspheres on carrageenan induced paw edema in albino rats

Groups	Paw edema (cm) at time T (h)		
	1 h	2 h	3 h
Control	1.70±0.14	1.78±0.07	1.81±0.16
Ibuprofen (10 mg/ kg body weight)	1.11±0.07 (34.7 %)	1.11±0.09 (37.6 %)	1.08±0.05 (40.3 %)
Microspheres (250 mg/ kg body weight)	0.81±0.10* (52.3 %)	0.81±0.08* (54.4 %)	0.78±0.06* (56.9 %)

Values are mean±SD, values in parentheses suggest % inhibition

* $P<0.05$ vs. control, Student's *t*-test ($N=6$)

antioxidant action is well known (Srinivasan et al. 2007). Interestingly, synergistic effect of thiamine and pyridoxine for inhibition of thermal hyperalgesia in rats has also been reported (Wang et al. 2005). Hence as a proof of concept, the present study emphasizes the preventive and therapeutic potential of thiamine and pyridoxine loaded ferulic acid grafted chitosan microspheres.

Conclusions

Ferulic acid grafted chitosan can serve as a functional wall material for microencapsulation of vitamins and micronutrients. As a proof of concept, the authors successfully demonstrated microencapsulation of thiamine and pyridoxine with ferulic acid-grafted chitosan. The vitamin loaded microspheres showed potential anti-inflammatory activity and may find application as novel dietary supplement or ingredient of functional food. Controlled release kinetics of the vitamins from microspheres, in vivo pharmacokinetics and detailed bioactivity evaluation will form part of our future studies.

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