Synthesis and characterization of imidazolium ILs based chitosan–tripolyphosphate microparticles

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Abstract. In this work, two imidazolium based ILs, 1–butyl–3– methylimidazolium hydrogen sulfate [bmim][HSO_4] and 1–butyl–3– methylimidazolium acetate [bmim][Ac] were blended with Chitosan (CS) to synthesize the CS–IL–TPP microparticles. The prepared microparticles were characterized by Zetasizer, FTIR, TGA, SEM and elemental analysis (EDX). The addition of these ILs resulted in controlled sized microparticles which possessed good thermal stability. The elemental analysis indicated that the better crosslinking of tripolyphosphate (TPP) with CS was achieved in the presence of IL, [bmim][Ac] as compared with [bmim][HSO_4] and CS–TPP formulation alone. The better crosslinking might result in the formation of stable and compact microparticles which could have a better control over the drug release applications. Therefore, this kind of IL might be suitable for improving the characteristics of chitosan as a drug carrier which could ultimately provide controlled release of unstable drugs.

Keywords—Chitosan, Ionic Liquid, microparticles, Drug delivery

1. Introduction

Chitosan (CS) is water–immiscible polysaccharide which can be obtained by the deacetylation of Chitin at 100–160 ˚C by using 40 to 50 % aqueous alkaline solution. This polysaccharide undergoes protonation when dissolved in weak acids. Acetic acid is one of the weak acids which is mostly used for its solubilization and it possess positive charge which is considered as one of its important properties. The presence of free amino groups in CS plays an important role in cross–linking with other anions enabling it suitable for various applications such as food and textile industry, wastewater treatment and pharmaceutical applications. Chitosan (CS) has been used with a number of polymers/materials to improve the characteristics of drugs [1, 2]. There are several factors which can affect the release profiles of drugs such as nature of polymer/drug, the method of preparation, particle size and stability of particles etc are the important factors which affect the release profiles of drugs [3–5]. Despite the great potential of CS, it is less stable in acidic pH as well as high temperature medium. This deficiency of CS creates a barrier for its commercialization in drug formulation [6]. Therefore, investigations are required which may improve the characteristics of CS. In this regard, one of the ways is to optimize and improve the synthesis process of CS microparticles (MPs). Finding the best conditions in terms of Molecular weight (MW) Concentration of CS, initial pH of CS solution, speed

and time of mixing, binder type/concentration, the mass ratio of CS–binder may result in enhanced stability of particles. The second way is to find and investigate the new materials which may enhance the stability of these particles.

Ionic liquids have emerged as alternative solvents for various applications. Their unique properties can tailor the shortcomings of various drugs. Though the purity, toxicity and unknown biodegradability of several ILs is a big issue for their use in pharmaceutical applications, on the other side, the toxicity of ILs does not obstruct their use for pharmaceutical applications. Fortunately, some reports have indicated the possibility of synthesizing non–toxic ILs. There are several FDA approved counterions such as anionic salts (bromide, chloride, phosphate, mesylate, citrate, titrate and acetate and cationic salts (sodium, ammonium, N–methylglucamine, diethylamine, ethylenediamine) [7]. The toxicity study of 1–butyl–3–methylimidazolium chloride [bmim][Cl] showed that this IL did not possess the acute toxic effects (EC50≈13μM) on marine algae and enzymes such as Acetylcholinesterase. Some other studies also supported some of the imidazolium ILs in terms of low/negligible toxicity towards Caco–2 cells. The specified test on rats (LD50) also indicated the low toxicity (LD50=1300 mg/kg) of imidazolium ILs [8].

Dorota et al (2013) prepared the oil–water emulsion by using hydrophilic IL, 1–hexyl–3–methylimidazolium chloride [hmim][Cl] and water–oil emulsion by using hydrophobic IL, 1–butyl–3–methylimidazolium hexafluorophosphate [bmim][PF_6]. The use of these ILs resulted in a stable emulsion with low cytotoxicity and good microbial activity showing the potential of these ILs to be used as preservatives [9]. The use of these ILs resulted in a stable emulsion with low cytotoxicity and good microbial activity showing the potential of these ILs to be used as preservatives. Moniruzzaman et al (2010) investigated the imidazolium based ILs for micro–emulsion formation in drug delivery and reported that IL–oil micro–emulsion increased the topical and transdermal drug delivery six times higher than normal formulation. Extension to this work resulted in the formation of stable IL–micelle formation with stronger shelf life as compared with typical drug formulation [10–12]. Sometimes these solvents can be a good alternative to enhance the solubility, absorption and bioavailability of several drugs through oral route as compared with volatile, flammable and toxic polar solvents such as pyridine, N, N–Dimethylformamide (DMF) and Dimethyl sulfoxide (DMSO) [8]. Therefore, ILs in combination with CS could be a good approach to alter the release characteristics of various drugs. In the present work imidazolium based CS–IL–TPP MPs have been synthesized and their characterization is conducted.

2. Methodology

2.1. Materials

Chitosan (CS) with high purity and HMW (kDa) (CS) was purchased from Sigma Aldrich, Sodium tripolyphosphate (TPP) with purity ≥ 94.00 % from SRL Chemicals, Hydrochloric acid (HCL) with purity ≥ 37 % and Sodium hydroxide (NaOH) with purity ≥ 98.00 % were purchased from the Merk. Acidic acid was purchased from R and M Chemicals. The Ionic Liquids were purchased from the Merk. The stock solutions of TPP, HCL and NaOH were prepared by using distilled water, while the CS stock solution was prepared in 1.0 % acetic acid.

2.2. Synthesis of CS– IL–TPP Microparticles

The CS–IL microparticles (MPs) were prepared by using ionic gelation method by using TPP as a crosslinker [13]. In detail, the CS stock solution was prepared by dissolving CS in acetic acid (1 % w/v). Further, this solution was diluted with distilled water to make a concentration of (0.05 % w/v). The ILs, [bmim][HSO_4] (0.05 % w/v) and [bmim][Ac] (0.05 % w/v) were added to this solution and stirred for 4 hours to obtain the homogeneous mixtures. The pH of both CS–[bmim][HSO_4] and CS–[bmim][Ac] solutions were maintained at pH=4.80 by using 1.0 M NaOH solution. Afterward, the TPP (0.05 % w/v) solution was prepared in distilled water. The concentration of CS (0.05 % w/v), initial pH of CS (pH=4.80), concentration of TPP (0.05 % w/v), initial pH of TPP (pH=9.30), CS–TPP mass ratio (5:1) and operating parameters such as rpm (700) and stirring time (60 min) were kept constants.

The CS–IL–TPP MPs were prepared by adding the CS–IL solutions into the TPP solution at room temperature. After preparation, these MPs were collected by centrifugation at rpm 4000 and freeze dried.

2.3. Characterization of CS–IL–TPP Microparticles

The properties such as particle size, zeta potential and surface morphology have significance importance in controlling the release profile of various drugs therefore, it is necessary to characterize the modified MPs. This could provide a better understanding of modified MPs before they could use for drug release application. The freeze–dried MPs were dispersed in the deionized water, sonicated for 30 minutes at room temperature and particle size and zeta potential was estimated by using the Malvern Zetasizer Nano ZS.

FTIR analysis was conducted to study the structure of synthesized CS–IL–TPP MPs. This analysis was carried out by using SHIMADZU FTIR–8400S in the range of 4000–400 cm^{-1} for synthesized CS–IL–TPP MPs as well as for pure CS and TPP. Thermal degradation of the prepared MPs was studied by using a software based and autosampler Thermogravimetric analyzer (Perkin Elmer TGA 4000). For this purpose, a temperature range of 20–40010 ˚C was selected and % mass loss was recorded versus heat rate (10 ˚C/min). Scanning Electron Microscopy (SEM ZEISS) was used to study the morphology of the prepared MPs. Further, elemental analysis (EDX) was carried out to find the presence of elements after modification.

3. Results and Discussion

The particle size and zeta potential of CS–[bmim][HSO_4]–TPP MPs were found in the range of 800 ± 90 nm and 15.0 ± 0.50 mV respectively. On the other hand, the particle size and zeta potential of CS–[bmim][Ac]–TPP MPs were found in the range of 900 ±105 nm and 4.98 ± 0.70 mV. In both cases, the NPs were also produced but the average sizes of particles are reported. The particle size of later MPs is bigger than the former, while the zeta potential value is less. In comparison to zeta potential of CS (0.05 % w/v), the zeta potential of prepared MPs is shifted to low positive values giving a confirmation of the modification of these particles.

FTIR analysis for the pure CS, TPP, [bmim][HSO_4] and [bmim][Ac] based MPs was carried out in the range of 4000–400 cm^{-1}. In case of pure CS, the significant peaks were found in the regions 3600–3000 cm^{-1}, 1700–1500 cm^{-1} and 1190–850 cm^{-1}. The broader peak at wavelength 3370 cm^{-1} was due to the O–H stretching caused by the hydroxyl group. The small peak at wavelength 2871 cm^{-1} is due to the result of C–H vibration, whereas the peaks at the 1019 cm^{-1} indicate the C–O stretching. Similarly, another small peak was found at 1665 cm^{-1}. The IR spectra of the TPP showed two significant peaks, one at 890 cm^{-1} and second at 1140 cm^{-1} representing the P–O–P and P–O stretching respectively. The IR spectra of CS solution (0.05 % w/v) was taken as a reference to see the effects of ILs on the structural changes of MPs. The main peaks in this spectrum were found at 3440cm^{-1}, 1640 cm^{-1} and 1084 cm^{-1}. In case of CS–[bmim][HSO_4]–TPP and CS–[bmim][Ac]–TPP MPs, the major peaks were almost at the same wavelength as that of CS solution. However, some shifting was found in the fingerprint regions which can be seen in Figure 1. In case of CS–[bmim][HSO_4]–TPP MPs, the peaks in fingerprint region shifted from reference CS solution (1084 cm^{-1}) to 1087 cm^{-1} as well as from 893 cm^{-1} to 888 cm^{-1} respectively. In case of [bmim][Ac]–TPP MPs, these peaks from were shifted 1084 cm^{-1} to 1077 cm^{-1} and from 893 cm^{-1} to 897 cm^{-1} respectively.

The morphology of the CS–TPP MPs, CS–[bmim][HSO_4]–TPP MPs and CS–[bmim][Ac]–TPP MPs were studied by the SEM analysis. The images of the prepared MPs are given in Figure 2. Freeze drying is usually used for the long–term stability of particles, but it affects the shape of particles. Similar effects were seen in present work as prepared MPs somehow possessed uneven shapes after freeze drying.

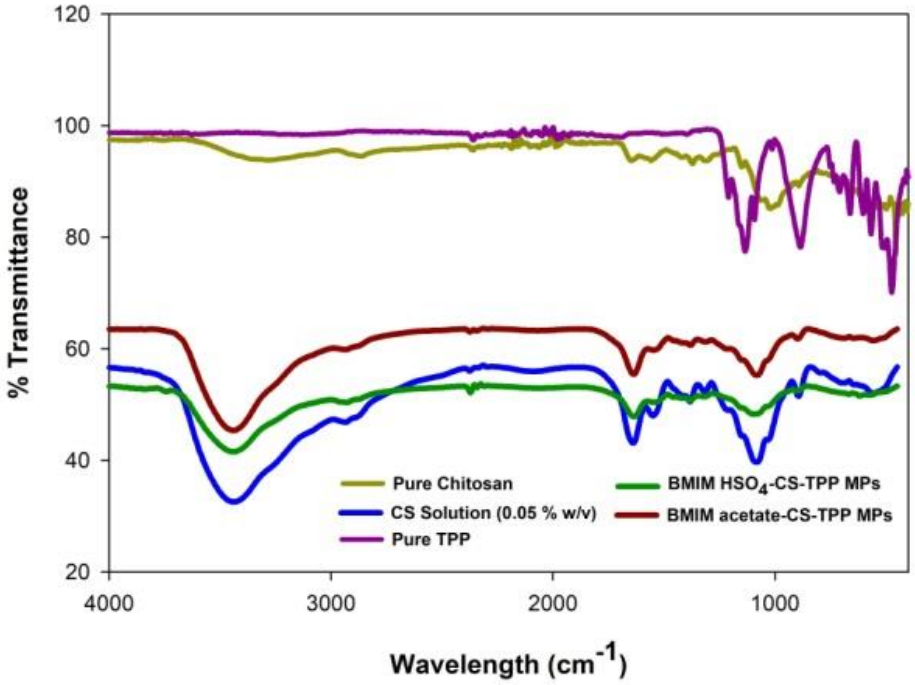

Figure 1. FTIR spectra of pure TPP, CS, aqueous CS and IL–CSS–TPP MPs

These results can be supported by the findings of Leoner et al (2008) [14], who investigated the effects of freeze drying on particle shape. Overall, the CS–TPP MPs, CS–[bmim][HSO_4]–TPP MPs and CS–[bmim][Ac]–TPP MPs had a similar structure with a fact that CS–[bmim][HSO_4]–TPP MPs possessed more agglomeration than other combinations. The reason could be low positive zeta potential which resulted in less stable particles. Overall, SEM analysis somehow showed the bigger sizes as compared with Zetasizer. As mentioned earlier, the reason could be the agglomeration of particles which formed clusters beside each other and at such magnification (100 X) appeared as bigger particles. While the analysis by using the Zetasizer showed reasonable particle size, the possible reason in this case could be the sonication technique which broke down the agglomerated particles into their actual sizes.

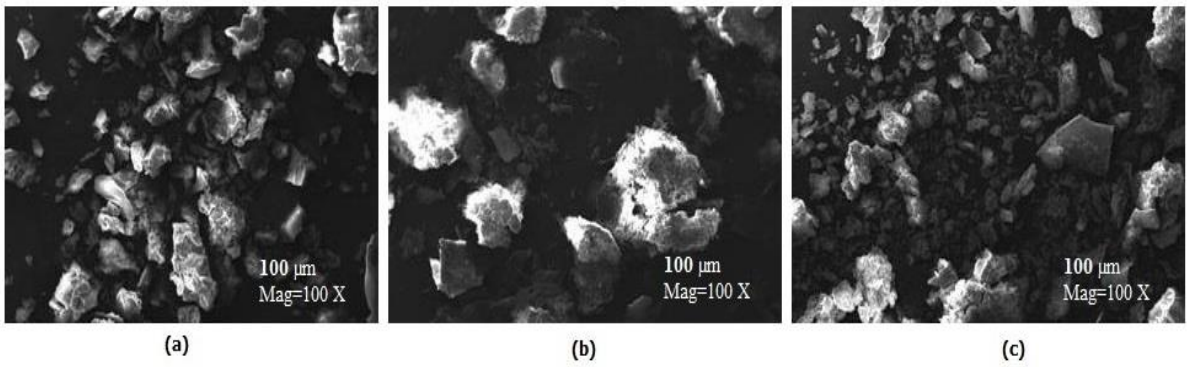

Figure 2. SEM Images for: (a), CS–TPP MPs; (b), CS–[bmim][HSO_4]–TPP MPs and (c), CS–[bmim][Ac]–TPP MPs

The cloud images and elemental analysis (EDX) are presented in Figure 3. The percentage of elements Carbon (C), Oxygen (O), Nitrogen (N) and Phosphorous (P) was found in the order of CS–

[bmim][Ac]–TPP MPs > CS–[bmim][HSO_4]–TPP MPs > CS–TPP MPs, CS–[bmim][HSO_4]–TPP MPs > CS–TPP MPs > CS–[bmim][Ac]–TPP MPs, CS–TPP MPs=CS–[bmim][HSO_4]–TPP MPs > CS–[bmim][Ac]–TPP MPs and CS–[bmim][Ac]–TPP MPs > CS–TPP MPs > CS–[bmim][HSO_4]–TPP MPs respectively.

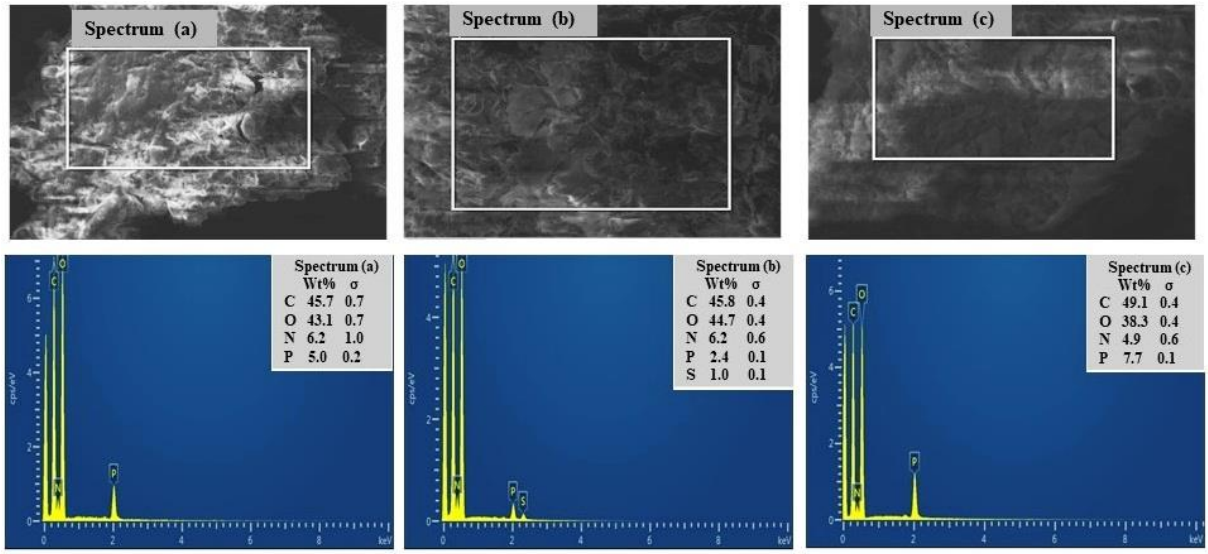

Figure 3. Electron Images and elemental analysis for: (a), CS–TPP MPs; (b), CS–[bmim][HSO_4]–TPP MPs and (c), CS–[bmim][Ac]–TPP MPs

The considerable sulfur (S) contents are only found in CS–[bmim][HSO_4]–TPP MPs. From the results it was found that the phosphorous contents are higher in the microparticles prepared by the [bmim][Ac] as compared with other studied combinations. This means that better crosslinking of Chitosan and TPP was achieved by using this IL, ultimately resulting in compact particles. The compact and mechanically stable particles are essential to control the release mechanism of drugs.

The TGA results (Figure 4) indicated that the CS–TPP MPs are more stable than pure CS, CS–[bmim][HSO_4]–TPP MPs and CS–[bmim][Ac]–TPP MPs. The % mass loss of these MPs within temperature range 40–215 ˚C was found in the order of CS–[bmim][HSO_4]–TPP MPs > CS–[bmim][Ac]–TPP MPs > pure CS > CS–TPP MPs. For the temperature range 216–260 ˚C, % mass loss was found in order of CS–[bmim][Ac]–TPP MPs > CS–[bmim][HSO_4]–TPP MPs > pure CS > CS–TPP MPs. Finally, for the temperature range 261–400 ˚C, the % mass loss was found in the order of CS–[bmim][HSO_4]–TPP MPs > CS–[bmim][Ac]–TPP MPs > pure CS > CS–TPP MPs. Overall, all the microparticles exhibited good thermal stability which supports their use as carrier materials for in vitro drug release applications.

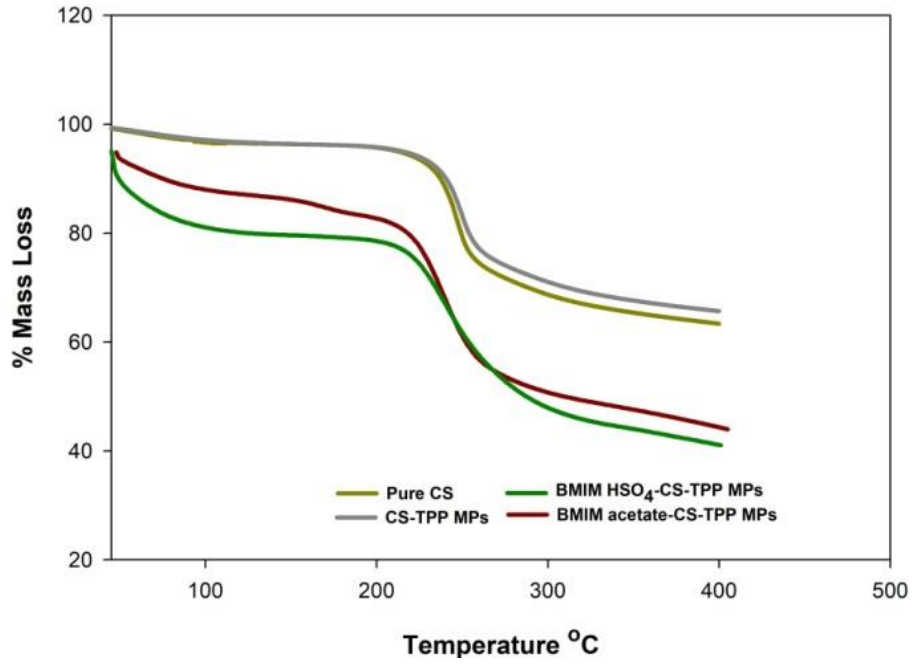

Figure 4. TGA of pure CS, CS–TPP MPs, CS–[bmim][HSO_4]–TPP MPs and CS–[bmim][Ac]–TPP MPs

4. Conclusion

In this study two imidazolium based ILs were used for the preparation of IL–CS–TPP microparticles. The prepared MPs were characterized in terms of size, zeta potential, morphology and thermal stability. The results concluded that the prepared MPs possess good thermal stability. Use of IL, [bmim][Ac] resulted in more stable and compact CS–TPP MPs which could be useful in altering the shortcomings of drugs and better release profiles can be obtained. Further research in this area can bring improvement in drug formulations.

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