The *in vitro* release features of 5-Fluorouracil from tablets with chitosan, soy protein extract and chitosan-soyprotein extract blend as carriers and their thermal degradation characteristics

Subramanian K.*, Harivignesh G. and Jeevitha A.
Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam, Erode, Dt, Tamil Nadu, INDIA
*drksubramanian@rediffmail.com

Abstract

Natural polymers are finding widespread applications in drug delivery, scaffold fabrication, bio plastic production, food packaging, wound dressing etc. due to their availability, biodegradability, biocompatibility, renewable nature, ease of modification to achieve the desirable properties etc. In the present study, the commercially available soy protein extract (SPE), chitosan(CSN) and their physical blend(CSN-SPE) have been chosen as drug carrier matrices to campare their in vitro drug release features in simulated intestinal fluid for controlled release applications taking 5-fluorouracil (5-FU) as a typical drug. The percentages of 5-FU released from the SPE, CSN and CSN-SPE tablets as a function of time were quantified by reverse phase High Performance Liquid Chromatography using KH_2PO_4 solution (pH 6.8) as the mobile phase and C-18 column as the stationery phase.

The observed drug release features were found to be different for these polymer carriers. The percentages of drug released during the initial periods upto 60 min were less in the CSN-SPE tablet than those observed for CSN and SPE tablets. This implied that the drug release rate can be modified by the proper choice of natural polymers in the blends as carriers. The thermal degradation characteristics of CSN, SPE and CSN-SPE blend and their 5-FU tablets were also analysed by simultaneous Thermogravimetry (TG) and Differential Thermal Analysis (DTA).

Comparative analysis of the TG and DTA traces of these polymers and their 5-FU tablets implied that there may be a drug-matrix interaction. This along with different degrees of swellability and degradability of these polymers might account for the differences in the drug release features. The structures of CSN, SPE and CSN-SPE blend were characterized by FT-IR spectroscopy.

Keywords: Soy protein extract, Chitosan, 5- Fluorouracil, *in vitro* drug delivery, Reverse Phase Chromatography, Thermogravimetry, FT-IR spectra.

Introduction

In the last five decades, significant research efforts were made to understand the interactions between biomaterials and targets that resulted in the creation of advanced products with different biomedical uses. In recent years, a paradigm shift from biostable biomaterials to hydrolytically and enzymatically biodegradable biomaterials for medical and allied applications has been observed. Many of the permanent prosthetic devices used for temporary therapeutic applications may be replaced by biodegradable devices that could support the body to repair and regenerate the damaged tissues. Biopolymers are the suitable choice materials for these applications. Biopolymers are carbon neutral and are always renewable because they are made from plant materials which can be grown indefinitely.

Hence the use of biopolymers would create a sustainable biomaterial industry. Polymeric blends from combination of different types of biopolymers have been used for the development of biosensors, body implants, scaffolds in tissue engineering, wound dressing and drug carriers. Biopolymers are increasingly used as carriers for targeted delivery of drugs, genes and vaccines with controlled rate and time. Excipient development had become a core area of research in pharmaceutical drug delivery because it influences the formulation development and drug delivery process in various ways.

Since, the material properties of biopolymers are suitable for various industrial and medical applications with plethora of advantages over conventional synthetic polymers, they had garnered a great deal of interest both in academic and industrial research. The use of biodegradable biopolymers for biomedical applications is continually increasing and evolving.[8].

Among the biopolymers, the versatile polysaccharides and proteins are finding tremendous applications as carriers in drug, protein, gene etc., delivery systems with improved pharmacokinetic features, scaffold in tissue engineering, hydrogels in wound dressings, packaging material and edible films in food industry, bioplastics due to their biodegradability, biocompatibility, low cost, renewable and ecofriendly nature, high adsorption capabilities etc. [1,5,21] The biopolymers can be processed into various forms like films, beads, fibers, nanoparticles, foams etc. The main advantage of biodegradable polymers was that their degradation products were non-toxic and could be completely eliminated

from the body by natural metabolic pathways with minimal or negligible side effects.[7,10,11,18,11,26,28]

The applications of food-grade biopolymer for the encapsulation, protection and controlled release of bioactive food ingredients were also gaining increasing interest in the research fields of functional foods and pharmaceutics. Plant proteins (mainly soy proteins, zein and wheat gliadins), which are widely available and environmentally economic compared to animal derived proteins, can be made into various delivery platforms such as micro and nanoparticles, fibers, films and hydrogels[24,29].

Protein nanoparticles had promising properties like biodegradability, non-antigenicity, metabolizability, greater *in vivo* and storage stability, relative ease of preparation and particle size monitoring etc.[3,25] These polymers due to their biochemical similarity with human extracellular matrix components were readily recognized and accepted by the body. Natural bio polymers inherit numerous advantages including natural abundance, relative ease of isolation and room for chemical modification through the multiple functional groups to meet the various technological and specific needs.

Many polysaccharides had been chemically modified to achieve consistent physicochemical properties including mechanical stability, degradability and bioactivity and were processed into microparticles, hydrogels and 3D porous structures for tissue regeneration applications[14,16,22]. Proteins and polysaccharides had been widely used for a variety of biotechnological and pharmaceutical applications such as medical scaffolding and implants, carriers in drug delivery, inert diluent for drug and wound dressing because of their high biocompatibility and biodegradability[14,16,22].

Modified biopolymers were widely studied as a potential carrier material for site specific drug delivery applications. Chitosan was one of the most valuable biopolymers for biomedical and pharmaceutical applications due to biodegradability, biocompatibility, antimicrobial nature, non-toxicity and anti-tumor properties[4,19]. Conjugated chitosan by thiolation, glycosylation and folation with improved biocompatibility was also used as drug carrier. But due to limited thermal stability, biopolymers undergo significant degradation during prolonged industrial thermal processing. Hence, understanding the thermal characteristics of these polymers and their blends need to be investigated for broadening their applications. Thermal analysis was widely used to investigate the thermal and thermo-oxidative degradation features of biopolymers.[13,17,27]

The present study involves the thermal degradation behavior of the chitosan (CSN), soyprotein extract (SPE), CSN-SPE blend (50:50, w/w) and *in vitro* release characteristics of 5-fluorourcil (5-FU) anticancer drug with these polymers as drug carriers. CSN is a random biocopolymer of glucosamine and N-acetyl-glucosamine and is made by deacetylation of chitin, a natural polymer from the exoskeletons of crustaceans. It is extensively used in tissue engineering, wound healing and drug delivery applications due to its inherent biocompatibility and biodegradability. It also had unique characteristics such as pH dependent molecular conformation and solubility in the environment in which it is found, mucoadhesivity and the ease of overcoming the barriers in epithelial cell junction[6].

Soy protein, the major component of soybean, is biodegradable, non toxic, environmentally friendly and readily available from the abundant renewable plant resource. It had been considered as an interesting starting material for biotechnological, biomedical and biodevice applications. But the soy protein films are highly brittle and hygroscopic. To surmount this problem, soy protein had been blended with other proteins or polysaccharides. This had improved the mechanical and water vapour barrier properties of the protein films. Soy protein is a complete protein as it meets all the essential amino acid requirements to support normal growth and development of infants and children. Soy protein isolates consist of 90% protein and its major components were glycinin (11S, 34%) and P-conglycinin, (7S, 27%). The rest of the protein consisted of whey proteins (y-conglycinin), the basic 7S globulin, lipoxygenase, agglutinins, P-amylases and trypsin inhibitors (in 2S fraction)[20].

Material and Methods

Chitosan (CSN,High molecular weight) was purchased from Marine Hydrocolloids, Cochin. Soy Protein Extract (SPE) was prepared from natural soyabean. 5-Fluorouracil (5-FU, >99%, anticancer, Himedia) was used as purchased. Pancreatin, sodium carbonate, NaOH and monobasic potassium phosphate (KH_2PO_4) (Himedia, Mumbai) and conc. hydrochloric acid were used as received. Simulated intestinal fluid (SIF, pH 7.4) was prepared as per United States Pharmacopeia. The structures of 5-FU and CSN are shown in figure 1.

Soy Protein Extract (SPE): Commercial soya bean was purchased from the local market and soaked in water for about 2 hrs to remove the hull. It was then blanched in water (1 g in 4 ml water) and ground into a paste. To the paste, sodium carbonate solution was added, stirred and the pH was adjusted to 8.5. The alkali treated paste was then centrifuged to remove the debris. The supernatant was treated with the HCl and its pH was adjusted to its isoelectric pH (4 to 5). The precipitated protein was allowed to settle down and then filtered out. It was vacuum dried and then lyophilized to get the dry powder form.

Preparation of chitosan- soyprotein extract (CSN-SPE) blend: A physical blend of CSN and SPE in the ratio 1:1 (w/w) was made by grinding both the polymers into fine powders individually and then grinding them together using a mortar and pestle.

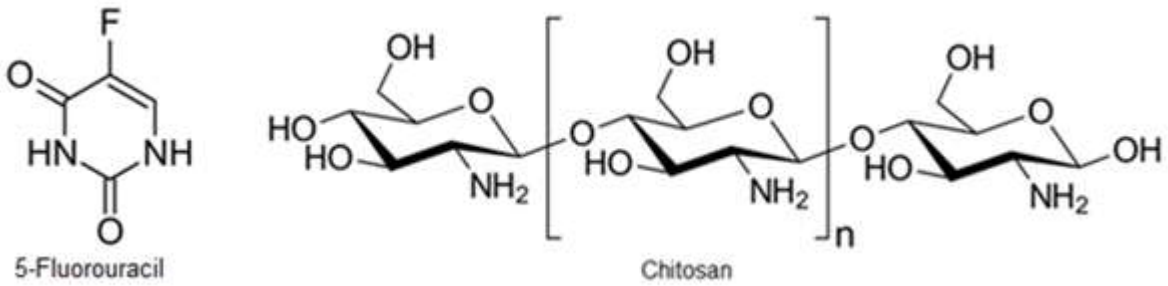

Figure 1: Chemical structures of 5-fluorouracil and chitosan

Preparation of SIF: 0.5 g of pancreatin dissolved in 150 ml of water was mixed with 38.5 ml of 0.2N NaOH and 3.4 g of KH_2PO_4 in 250 ml of Millipore water. The pH was adjusted to 7.4 and then the volume was made up to 500 ml. The SIF thus prepared was used for drug dissolution studies of the tablets CSN-5FU, SPE-5FU and CSN-SPE-5FU.

Fourier Transform-Infrared Spectroscopy (FT-IR): FT-IR spectra of the polymer samples under ATR mode were recorded on the powder samples for the spectral range 400-4000 cm^{-1} using Shimadzu FT-IR spectrophotometer at a resolution of 2 cm^{-1} with 32 scans.

Thermal studies: Thermal degradation studies on CSN, SPE and their 1:1(w/w) blend and their tablets with 5-FU were performed via simultaneous Thermogravimetry(TG) and Differential Thermal Analysis (DTA) on NETZSCH STA-2500 Regulus both under nitrogen and air atmospheres at a heating rate of 10^oC per minute. The sample size was 3-6 mg.

HPLC analysis: HPLC analysis of 5-FU samples was carried out on Binary Gradient Agilent 1220 HPLC infinity series fitted with C18 column (5 mm diameter, Agilent technology) using 0.5M monobasic potassium phosphate as mobile phase at a flow rate of 1ml/min under isocratic elution. Injection volume was 20 µL and run time was 12 min. Variable wave length UV detector at 265 nm was used.

Preparation of Tablets: The polymers samples CSN, SPE and CSN-SPE (1:1 w/w) blend were finely powdered using mortar and pestle. In a typical tablet (2.5 mm thickness and 13 mm diameter) formulation, exactly 200 mg of dried polymer matrix was loaded with 25mg of 5- FU by mixing and grinding in a mortar and pestle to ensure homogeneity and pelletized as tablet using KBr press at 1.5 psi pressure.

***In vitro* drug dissolution study:** *In vitro* drug dissolution studies were performed in SIF at 37^OC by embarking the compressed tablet in stainless steel cylindrical mesh immersed in 150 ml of SIF taken in a thermostated 250 ml beaker. Aliquots of samples (2 ml each) were withdrawn at 10, 20, 60, 120 and 180th minutes of the dissolution experiments for estimating the quantity of drug released using HPLC (Binary Gradient HPLC Agilent 1220 infinity) with UV (265 nm) detector and C18 column. After each withdrawal, a volume equivalent (2 ml) of aliquot sample was incubated fresh SIF solution was added into experimental solution to maintain the volume of the solution constant.

Quantification of 5-FU released: The percentages of drug released at different known intervals of time was estimated by HPLC. 20 microlitre of the solution from the 2 ml of aliquot sample withdrawn from the drug release experiment was syringed out using a 50 µL syringe and injected into the column in each experiment. Chromatograms of pure 5-FU of known concentrations were also recorded under the identical experimental conditions used for the 5-FU released from the tablets in drug release experiment.

Results and Discussion

FT-IR spectra: The FT-IR spectra of CSN, SPE and CSN-SPE (1:1 w/w) blend shown in figure 2 dispalyed several characteristics absorption peaks in the range 3400 - 550 cm^{-1}. The absorption peaks assignments[2,6,9,12,15] were furnished in table 1.

Analysis of TG/DTA thermograms: The recorded TG/DTG and DTA thermograms for the CSN, SPE and CSN-SPE polymer carriers and their 5-FU tablets were shown in figures 3 to 6 respectively. The TG/DTA thermogram of pure 5-FU was shown in figure 7. The onset degradation temperatures for polymers CSN, SPE and CSN-SPE were greater than 150^oC. Hence these polymer carriers were sufficiently thermally stable for their use as drug carriers in drug delivery applications.

Comparison of TG thermograms of CSN, SPE and CSN-SPE blend (Figure 3) indicated that their thermal stability decreased in the order CSN>SPE>CSN-SPE (1:1 w/w blend) under nitrogen atmosphere. The initial weight loss was attributed to the loss of moisture and other volatile impurities. The residual masses at 500^oC for CSN, SPE and CSN-SPE were 40.1, 32.8 and 28.55% respectively. The multistep degradation in SPE was attributed to the presence of its different constituent proteins such as glycinins, conglycinins, agglutinin etc. In the DTG thermogram of the blend CSN-SPE, the DTG peak corresponding to the major degradation step of CSN component had broadened compared to that in pure CSN.

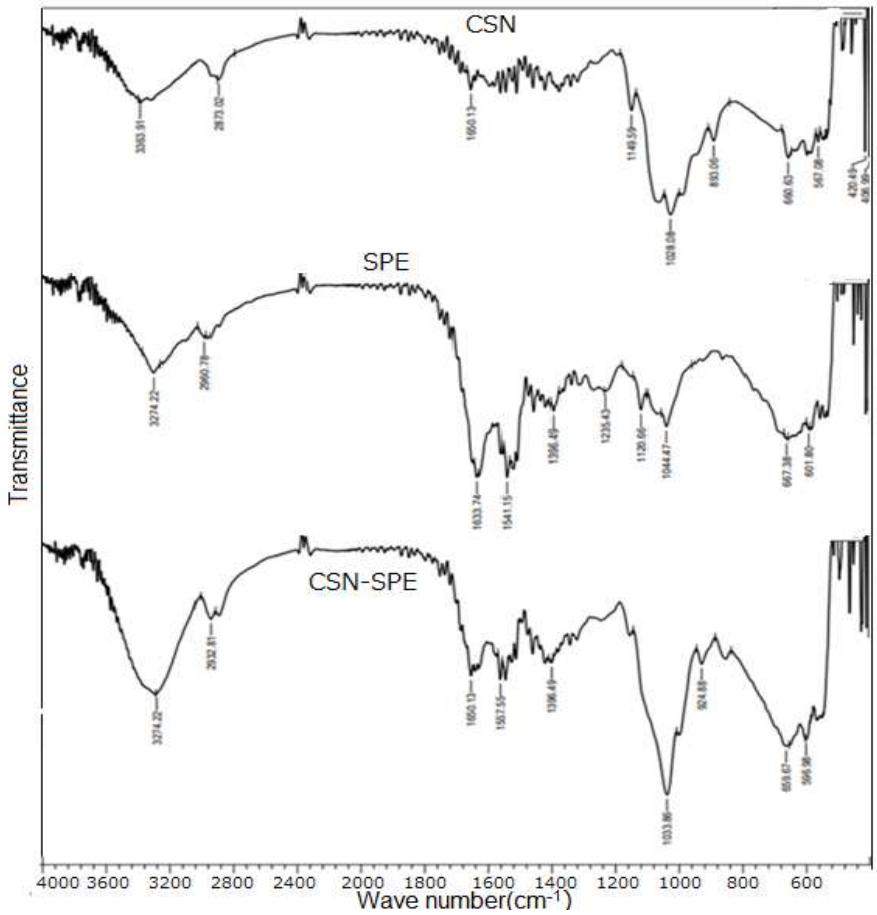

Figure 2: FT-IR spectra of chitosan(CSN), soyaprotein extract(SPE) and CSN-SPE blend

Table 1
Assignment of absorption peaks in FT-IR spectra of chitosan(CSN), soya protein extract (SPE) and CSN-SPE blend

Sample	Frequency of absorbance peak (cm^{-1})	Peak assignment
Chitosan[6,9,12,15]	3200-3363	O-H & N-H stretching
	1650.1	C=O stretching of amide (from residual N-acetyl group)
	1109	Asymmetric stretching of C-O-C bridge
	1028	C-O stretching vibration
	1260	OH bending vibration
	833	CH bending out of the plane of the ring monosaccharide
Soy protein extract[2]	1633	Amide I (C=O stretch)
	3274	N-H & O-H stretching
	1541	Amide II (N-H bending & C-N stretching)
	1044	C-O stretching
	1120	Asymmetric stretching of C-O-C bridge
	2960	CH_3 asymmetric stretching
Chitosan- soy protein extract blend	3274.2	O-H and N-H stretching
	2932	CH_3 asymmetric stretching
	1650	C=O stretching of amide
	1033	C-O stretching

It appeared that in the presence of SPE, the degradation of CSN might be enhanced. But there was no significant difference in the DTG peaks of CSN degradation in CSN-SPE-5-FU and CSN-5-FU tablets (Figure 4). This difference might be due to polymer-polymer and drug-polymer interactions in the tablet. This might be also one reason for the observed differences in the drug release features of the tablets.

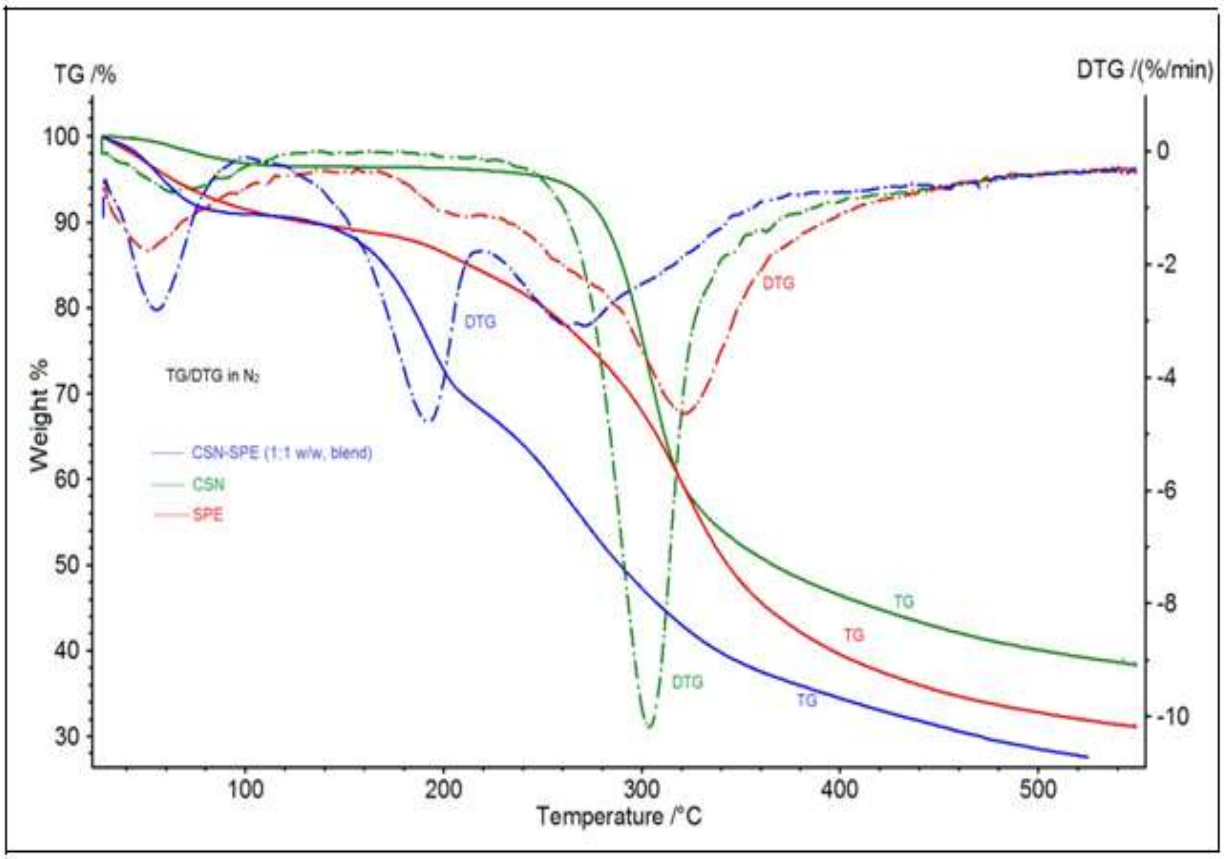

Figure 3: TG/DTG thermograms of chitosan(CSN), soy protein extract(SPE) and 1:1 (w/w) blend of CSN and SPE under nitrogen

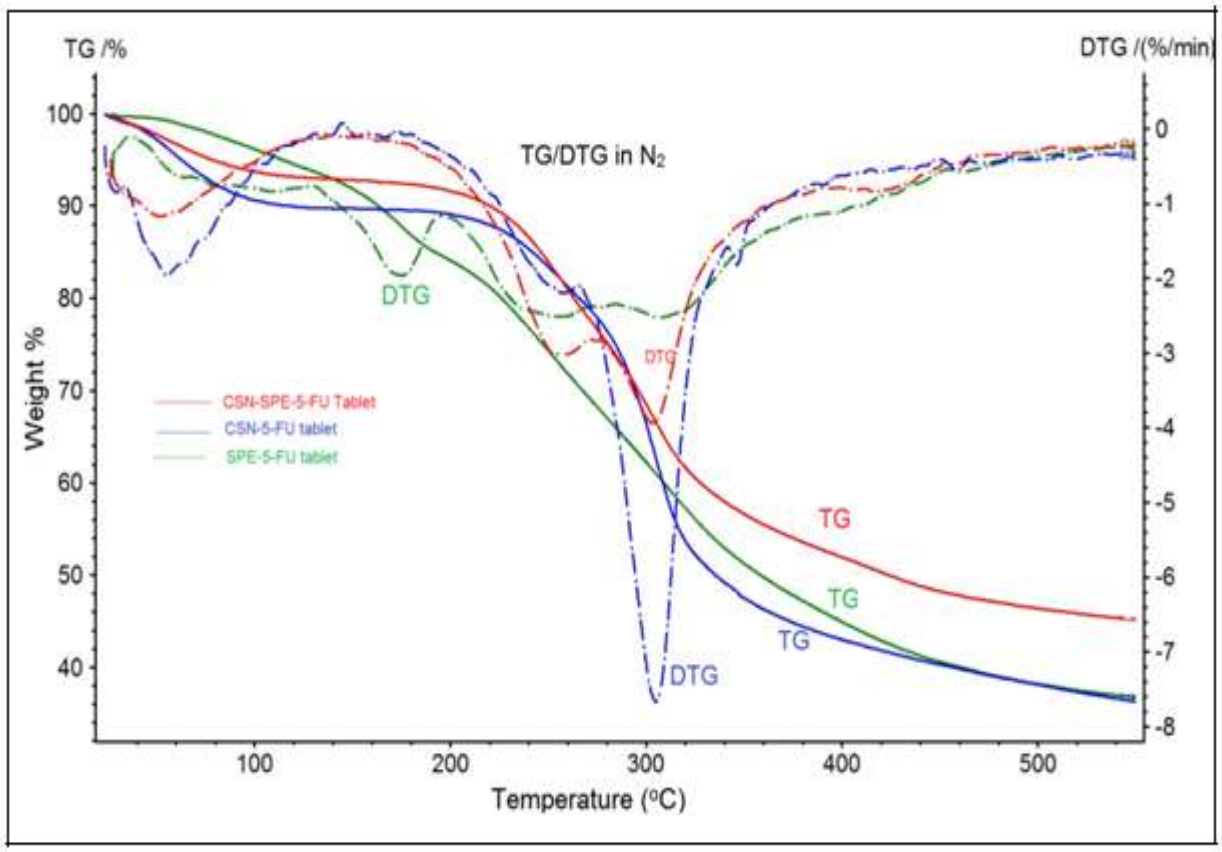

Figure 4: TG/DTG thermograms of CSN-5-FU, SPE-5-FU and CSN-SPE-5-FU tablets under nitrogen

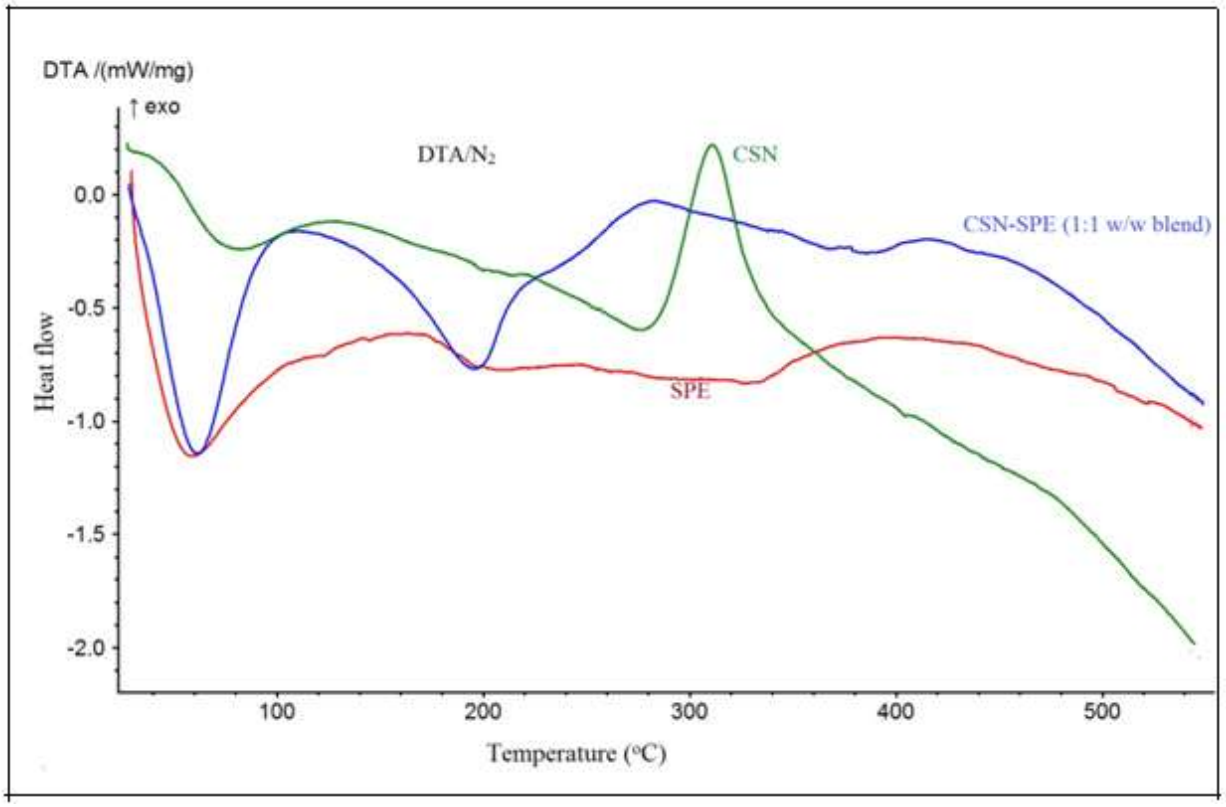

Figure 5: DTA traces of CSN, SPE and CSN-SPE (1:1 w/w blend) recorded in N_2

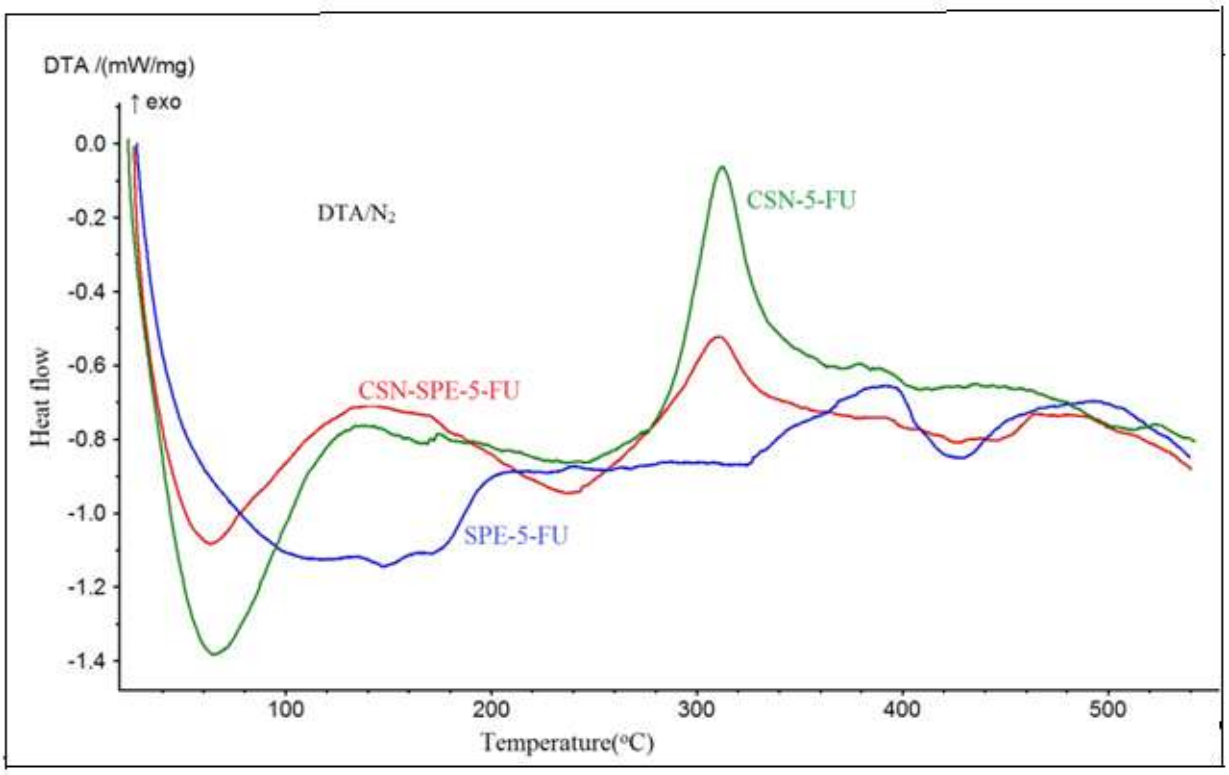

Figure 6: DTA traces of CSN-5-FU, SPE-5-FU and CSN-SPE (1:1 w/w)-5-FU (1:1 w/w blend) recorded in N_2

The thermal degradation patterns for the polymer components namely CSN, SPE and CSN-SPE blend in their corresponding 5-FU tablets (Figure 6) were slightly different from those of pure polymers and the blend (Figure 5). This might be due to drug- matrix interaction[23]. In the presence of drug the residual weight at 500°C displayed in the TG trace (Figure 6) was more for the CSN-SPE-5-FU tablet than those for CSN-5-FU and SPE-5-FU tablets. The onset weight loss temperature for pure 5-FU was observed at 294° C (Figure 7).

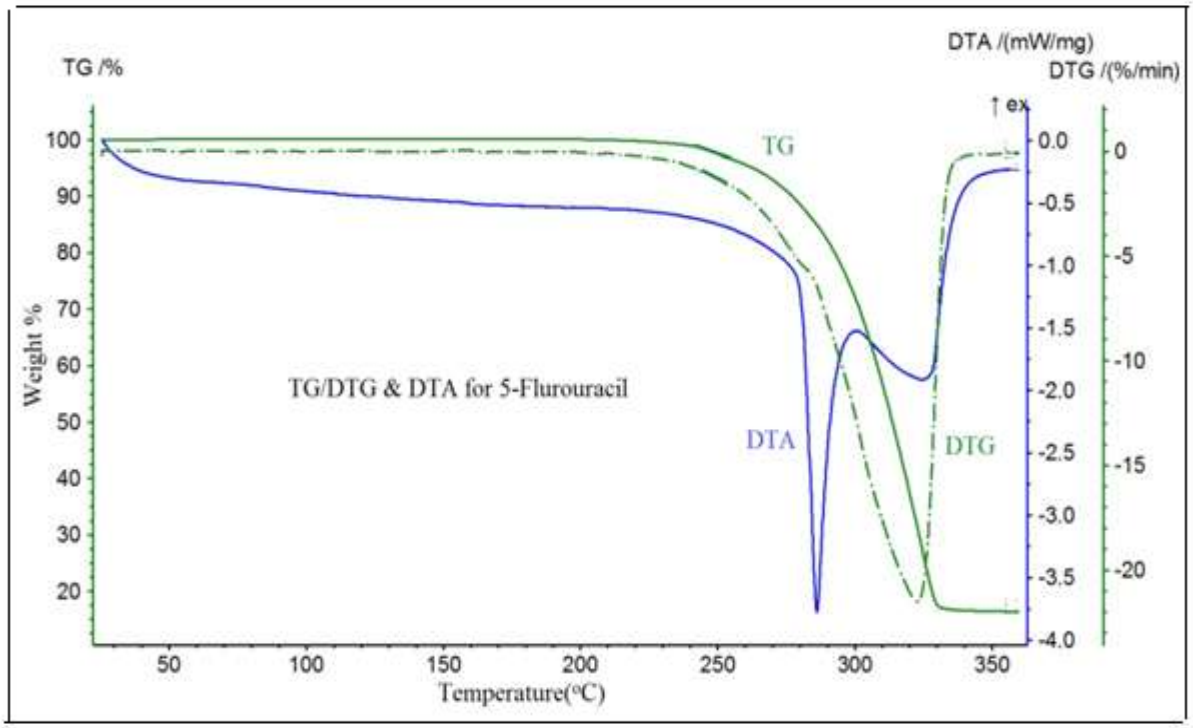

Figure 7: TG/DTG and DTA thermograms of 5-FU recorded under nitrogen

Analysis of DTA thermograms shown in figure 5 indicated that the CSN degrades exothermically around 310-315° C and SPE degraded with broad endotherm around 145°C with multistep degradation. The endotherms below 80°C (Figures 5 and 6) in CSN, SPE and CSN-SPE and their 5-FU tablets were attributed to the loss of moisture and other volatile impurities if any. The exothermic peak due to CSN degradation in the CSN-SPE blend was shifted to slightly lower temperature and broader compared to that observed in pure CSN.

A similar trend was noticed for the corresponding degradation temperature observed in the DTG trace (Figure 3). This may be due to the plasticizing effect of the constituent proteins present in SPE.[20] But unlike the broad exotherm of CSN in CSN-SPE, the exotherm of CSN in CSN-SPE-5-FU tablet (Figure 6) was narrow and only slightly shifted to lower temperature.

Chromatographic estimation of 5-FU: Chromatograms of 5-FU solutions of various known concentrations (5-30 µg/ml) prepared in SIF were recorded using Agilent HPLC as per the experimental parameters mentioned earlier to find the retention time of the sample peak and the concentration dependent peak areas. The retention time was found to be 6.2 min for pure 5-FU. A typical chromatogram of a standard solution of 5-FU (25 µg/ml) in SIF was shown in figure 8(A).

A representative chromatogram of the 5-FU released experimental solution from SPE-5-FU tablet dissolution study in SIF at 60 minutes was furnished in figure 8(B). The standard plot of chromatographic peak area (at retention time= 6.2-6.3 min) vs 5-FU concentration for the standard 5-FU solutions was shown in figure 9. The peak area varied linearly with 5-FU concentration for the chosen experimental conditions.

The amounts of drug released in the drug dissolution study for the different intervals of time were calculated using the calibration curve (Figure 9) and the 5-FU peak areas (retention time 6.2-6.3 min) of the recorded chromatograms of the experimental solutions at different time intervals of dissolution study. The 5-FU release curves namely percentage of 5-FU released vs time (min) in SIF for the CSN-5-FU, SPE-5-FU and CSN-SPE-5-FU tablets at 37°C were shown in figure 10.

Comparison of the release profiles (Figure 10) implied that the percentage of drug release was more for CSN-5-FU tablet in the initial 60 min time compared to those for the other two tablets. After 60 min of tablet dissolution, the 5-FU release rate decreased in CSN-5-FU tablet and only 15% drug release was observed at 180 min. In SPE-5-FU and CSN-SPE-5-FU tablets, the percentage of drug release increased nearly linearly with time and a net drug release of 40 and 20 % respectively were observed after 180 minutes. Initially the percentage release of 5-FU followed the order SPE-5-FU > CSN-5-FU> CSN-SPE-5-FU. But at 180 minutes, the percentage of 5-FU released from the tablets followed the order SPE-5-FU> CSN-SPE-5-FU> CSN-5-FU.

Conclusion

SPE prepared from soya bean,CSN and CSN-SPE (1:1 w/w) blend were used as drug carriers for *invitro* controlled release of 5-FU in simulated intestinal fluid(SIF).

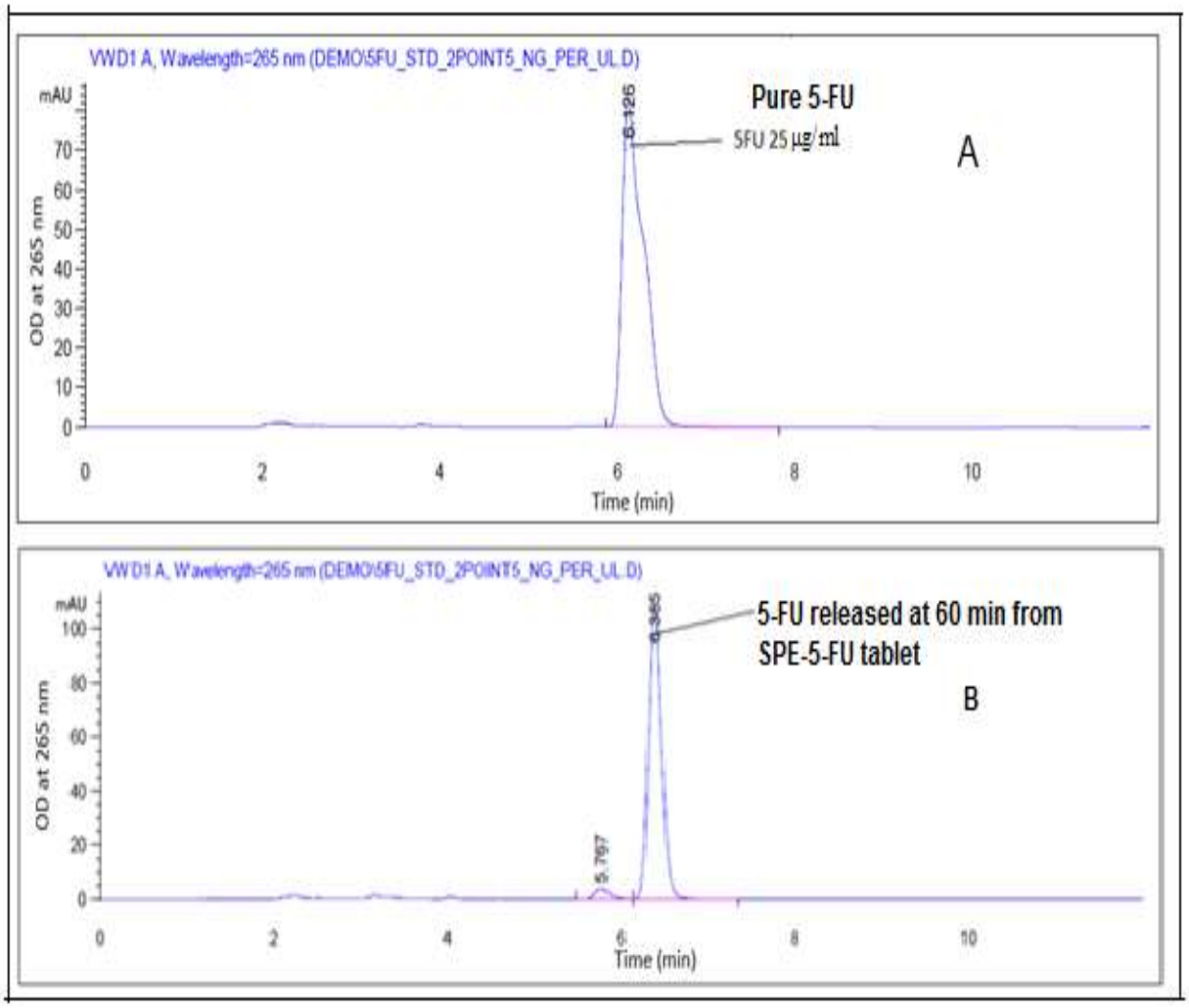

Figure 8: Chromatograms of (A) a standard 5-FU solution (25 µg/ml in SIF) and (B) 5-FU released at 60 min from SPE-5-FU tablet with 0.5 M monobasic potassium phosphate buffer as mobile phase

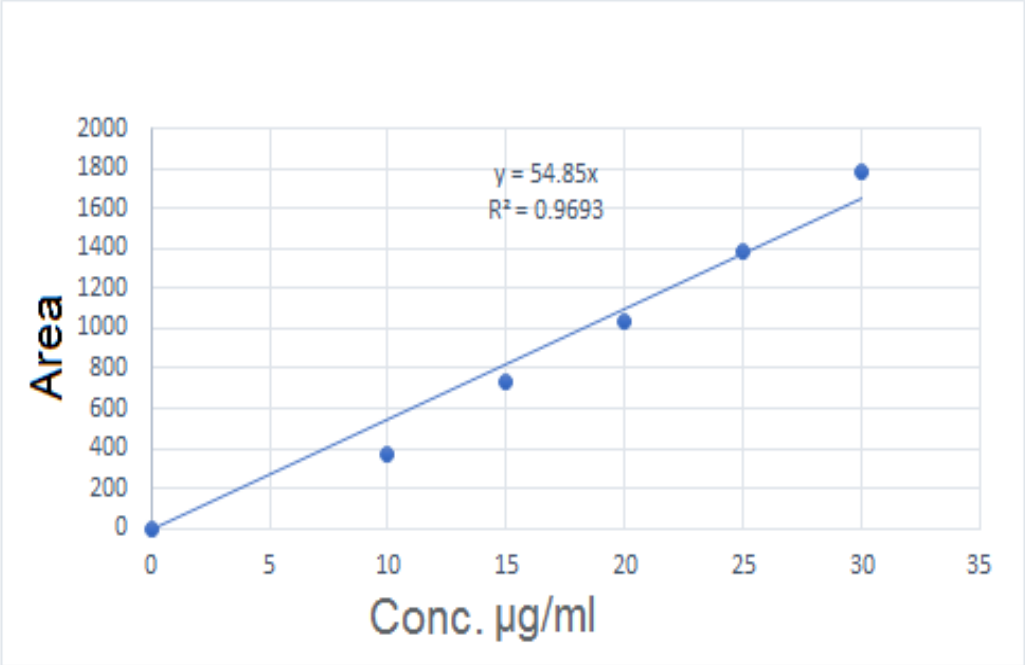

Figure 9: HPLC Calibration curve for the standard 5-FU drug

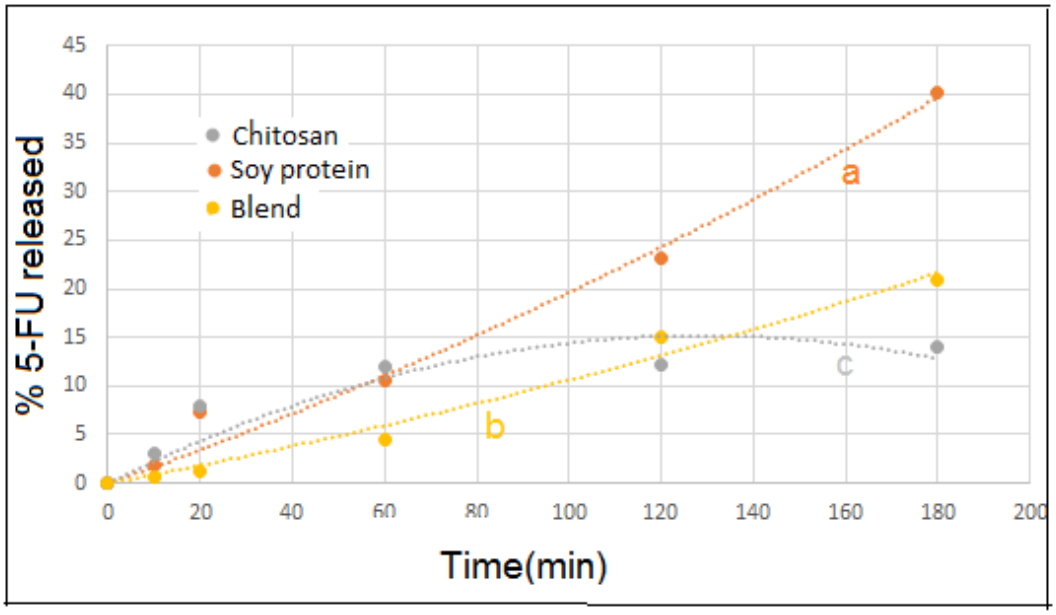

Figure 10: 5-FU release profiles in SIF at 37ºC from (c) CSN-5-FU, (a) SPE-5-FU and (b) CSN-SPE-5-FU tablets

The drug release profiles constructed by estimating the percentage of 5-FU released using reverse phase HPLC with C18 column and variable wavelength UV detector (265 nm) were quite different for the three carriers. The percentage of drug released from CSN-SPE-5-FU tablet was much lower than those observed for the CSN-5-FU and SPE-5-FU tablets. This might be attributed to the differences in drug-polymer interaction and swellability of tablets in SIF.

The polymers and tablets were also characterized for their thermal degradation and structure by simultaneous TGA/DTA and FT-IR spectroscopic studies respectively. The onset degradation temperatures for CSN, SPE and CSN-SPE blend were greater than 150°C. Hence the polymers were thermally stable enough for drug delivery use as carriers. Analysis of TG and DTA traces of the polymers and their 5-FU tablets indicated the more likely presence of polymer-drug interactions. The study revealed that the CSN-SPE blend may be a promising carrier for slow release of 5-FU and other similar drugs. The polymer blend may also serve as a promising scaffold material for tissue engineering applications.

Acknowledgement

The authors are thankful to DST-FIST, New Delhi, Government of India facility at Bannari Amman Institute of Technology(BIT), India and AICTE-MODROBS, New Delhi India for extending the thermal analysis and HPLC facilities to carry out this research work and to the management of BIT for encouraging and supporting this research work.

References

1. Adikwu M.U. and Esimone C.O., eds., Biopolymers in drug delivery: Recent advances and challenges, Bentham Science, DOI: 10.2174/97816080507891090101 **(2009)**

2. Chen X., Ru Y., Chen F., Wang X., Zhao X. and Ao Q., FT-IR spectroscopic characterization of soy proteins obtained through AOT reverse micelles, *Food Hydrocoll.*, **31(2),** 435 **(2013)**

3. DeFrates K., Markiewicz T., Gallo P., Rack A., Weyhmiller A., Jarmusik B. and Hu X., Protein polymer based nanoparticles: Fabrication and medical applications, *Int. Mol. Sci.*, **19(6),** 1717 **(2018)**

4. Dattagupta D.S., Jadhav V.M. and Kadam V.J., Chitosan: A Propitious Biopolymer for Drug Delivery, *Curr. Drug. Deliv.*, **12(4),** 369 **(2015)**

5. Dubey K.A., Chaudhari C.V., Bhardwaj Y.K. and Varshney L., Polymers, blends and nanocomposites for implants, scaffolds and controlled drug release applications, *Adv. Struct. Mater*., **66**, 1 **(2017)**

6. Fernandes Queiroz M., Melo K., Sabry D., Sassaki G. and Rocha H., Does the Use of Chitosan Contribute to Oxalate Kidney Stone Formation?, *Mar. Drugs*, **13(1)**, 141 **(2014)**

7. Foox M. and Zilberman M., Drug delivery from gelatin-based systems, *Expert Opin. Drug Del.*, **12(9),** 1547 **(2015)**

8. Gopi S., Amalraj A., Sukumaran N.P., Haponiuk J.T. and Thomas S., Biopolymers and Their Composites for Drug Delivery: A Brief Review, *Macromolecular Symposia*, **380(1)**, 1800114 **(2018)**

9. Hayes M. Chitin, Chitosan and their Derivatives from Marine Rest Raw Materials: Potential Food and Pharmaceutical Applications, In Hayes M., Editor, Marine Bioactive Compounds, Springer, New York, 115 **(2012)**

10. Huang G., Liu Y. and Chen L., Chitosan and its derivatives as vehicles for drug delivery, *Drug Deliv.*, **24(2),** 108 **(2017)**

11. Jain A., Gupta Y. and Jain S.K., Perspectives of biodegradable natural polysaccharides for site- specific drug delivery to the colon, *J. Pharm. Pharm. Sci.*, **10(1)**, 86 **(2007)**

12. Jayakumar R., Menon D., Manzoor K., Nair S.V. and Tamura H., Biomedical applications of chitin and chitosan based nanomaterials- A short review, *Carbohydr. Polym.*, **82**, 227 **(2010)**

13. Kumar S.P., Chaudhary V.A., Tiwari P. and Katiyar V., Thermal Degradation Kinetics of Biopolymers and their composites: Estimation of appropriate kinetic parameters, *J. Energy Environ. Sustain.*, **3,** 10 **(2017)**

14. Kumbar S.G., Toti U.S., Deng M., James R., Laurencin C.T., Aravamudhan A., Harmon M. and Ramos D.M., Novel mechanically competent polysaccharide scaffolds for bone tissue engineering, *Biomed. Mater.*, **6(6)**, 174 **(2011)**

15. Laurienzo P., Marine Polysaccharides in Pharmaceutical Applications: An Overview, *Mar Drugs*, **8,** 2435 **(2010)**

16. Malafaya P.B., Silva G.A. and Reis R.I., Natural–origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications, *Adv. Drug Deliv. Rev*., **59(4–5)**, 207 **(2007)**

17. Moussout H., Ahlafi H., Aazza M. and Bourakhouadar M., Kinetics and mechanism of the thermal degradation of biopolymers chitin and chitosan using thermogravimetric analysis, *Polym. Degrad. Stab*., **130**, 1 **(2016)**

18. Nair L.S. and Laurencin C.T., Polymers as biomaterials for tissue engineering and controlled drug delivery, *Adv. Biochem. Eng. Biotechnol.*, **102,** 47 **(2006)**

19. Orienti I., The Role of Chitosan in Drug Delivery: Current and Potential Applications, *Amer. J. Drug Deliv.*, **1(1),** 43 **(2003)**

20. Petruccelli S. and Anon M.C., Soy Protein Isolate Components and Their Interactions, *J. Agr. Food Chem.*, **43(7**), 1762 **(1995)**

21. Ramakrishna S., Mayer J., Wintermantel E. and Leong K.W., Biomedical applications of polymer-composite materials: a review, *Compos. Sci. Technol.*, **61(9),**1189 **(2001)**

22. Shelke N.B., James R., Laurencin C.T. and Kumbar S.G., Polysaccharide biomaterials for drug delivery and regenerative engineering, *Polym. Adv. Technol.*, **25(5),** 448 **(2014)**

23. Subramanian K. and Vijayakumar V., Synthesis and evaluation of chitosan-graft-poly(2-hydroxy ethyl methacrylate-co-itaconic acid) as a drug carrier for controlled release of tramadol hydrochloride, *Saudi Pharm. J.*, **20,** 263 **(2012)**

24. Teng Z., Luo Y. and Wang Q., Carboxymethyl chitosan-soy protein complex nanoparticles for the encapsulation and controlled release of vitamin D3, *Food Chem.*, **141**, 524 **(2013)**

25. Teng Z., Luo Y. and Wang Q., Nanoparticles Synthesized from Soy Protein: Preparation, Characterization and Application for Nutraceutical Encapsulation, *J. Agric. Food Chem.*, **60**, 2712 **(2012)**

26. Vaz C.M., van Doeveren P.F., Reis R.L. and Cunha A.M., Soy matrix drug delivery systems obtained by melt-processing techniques, *Biomacromolecules*, **4(6),** 1520 **(2003)**

27. Villetti M.A., Crespo J.S., Soldi M.S., Pires A.T.N., Borsali R. and Soldi V., Thermal Degradation of natural polymers, *J. Therm. Anal. Calorim*., **67(2)**, 295 **(2002)**

28. Wan Z.L., Guo J. and Yang X.Q., Plant protein-based delivery systems for bioactive ingredients in foods, *Food Funct.*, **6**, 2876 **(2015)**

29. Zhang J., Field C.J., Vine D. and Chen L., Intestinal uptake and transport of vitamin B12-loaded soy protein nanoparticles, *Pharm. Res.*, **32,** 1288 **(2015)**.

(Received 26th June 2020, accepted 27th August 2020)