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DEVELOPMENT AND EVALUATION OF INULIN-LOADED ETHYL CELLULOSE NANOPARTICLES AS ORAL PREBIOTIC SUPPLEMENT FOR SELECTIVE EUBIOSIS.

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ABSTRACT

In recent days, ethyl cellulose polymer is being used in various biomedical applications due to its unique biocompatibility, non-toxicity and cost effectiveness. The aim of the present investigation was to prepare, characterize and evaluate the inulin-loaded ethyl cellulose nanoparticles. Inulin, a prebiotic, was encapsulated by the two-step desolvation process using ethyl cellulose. Characterization of the nanoparticles was performed using scanning electron microscopy (SEM), dynamic light scattering (DLS) and fourier transform infrared spectroscopy (FTIR). The nanoformulations were uniform in size, smooth surface, globular in shape and homogenous in nature. The mean average diameter of the nanoparticles was ranged between 174.2 and 217.9 nm and zeta potential was found to be -16.0±1.48 mV. From FTIR, it was clear that there was physisorption between the drug inulin and ethyl cellulose polymer. The drug loading capacity and entrapment efficiency of nanoparticles were found at 2.6% and 40%. The *in vitro* drug release profile exhibited a biphasic phenomenon indicating controlled drug release. The nanoformulations were found to be safe as evidenced by hemolysis assay. Based on the data available inulin-loaded ethyl cellulose nanoformulation would be a promising carrier system for the production of synbiotic system for oral delivery of probiotics.

KEYWORDS: **Prebiotic inulin, Ethyl cellulose, Desolvation, Controlled release, Hemolysis.**

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INTRODUCTION

The polymer ethyl cellulose (EC) is a non-ionic, non-toxic, stable, compressible, inert, pH independent and hydrophobic cellulose ether but soluble in many polar organic solvents. Considering the fact that the high molecular weight, hydrophobicity and the lack the digestive enzyme necessary to metabolize ethyl cellulose, it will be excreted in stools essentially unchanged through the gastrointestinal tract following oral ingestion. We could not find published literature regarding absorption of ethyl cellulose. It has been widely used to prepare pharmaceutical dosage forms like a non-swellable component in coating systems, used to coat active ingredients of a formulation to prevent them from reacting with one other, to prevent discoloration of easily oxidizable substances, to prepare sustained release film coatings.[1-4] Ethyl cellulose is often chosen in dosage processing because of water sensitivity of the active ingredient. Several researchers like Mura *et al.,*[5]Soskolne *et al.,*[6]Friedman *et al.,*[7] have successfully demonstrated the sustained release of drugs using ethyl cellulose. As per Code of Federal Regulations, ethyl cellulose is a food additive which is permitted for direct addition to food for human consumption, as long as the quantity of the ethyl cellulose is of appropriate food grade and handled as a food ingredient simultaneously does not exceed the amount reasonably required to accomplish its intended effect in food.[8]Ethyl cellulose is permitted in feed and drinking water of animals.[9] However, research articles pertaining to usage of inexpensive polymer ethyl cellulose in the form of nano-carrier is very less.[10-12]Due to these factors in this study we have chosen ethyl cellulose as an encapsulating agent which could deliver drug cost-effectively to colonic region. Prebiotics are touted as growth promoters for the good growth of bacterial community such as *Bifidobacteria*, in the gut region.[13-16]Prebiotics like inulin are basically non-digestible carbohydrates richly present in plants like chicory chemically composed of β (2-1) linked fructose units to form oligomers of 5000 Daltons. Reddy *et al.,* have demonstrated that dietary oligo-fructans reduce the number of aberrant crypt foci[17], purported pre-neoplastic lesions in the colon of rats.[18] Short-chain fructo-oligosaccharides have also been reported to reduce colon tumours in Min mice, resembling a genetic model mimicking human colon cancer.Prebiotic inulin is known to promote a favorable environment for the growth of native flora, and selectively fermented by bacteria living in the intestinal ecosystem.[19-20]Although several researchers have prepared synbiotic preparations, no attempt has been made to prepare prebiotic nanoformulations for probiotics delivery. In the present investigation, we have attempted to fill the gap in a way of providing prebiotic-inulin for the probiotic organisms of interest for oral delivery.

MATERIALS AND METHODS

Ethyl cellulose [E0854, Viscosity 18 - 24 cP, Ethoxy content 48-49%] was purchased from Rolex, Tween-80 and Inulin A.R. [RM103-25G] was purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai), Ethyl alcohol [99.99%] from MERCK.Water used for all experiments was triple distilled water or Milli-Q water. All other chemicals were of highest purity available and were used without further purification. The study was approved by Research Centre for Nanoscience & Technology of DAVANGERE UNIVERSITY with the approval no :DU/BMS/2014-15/05.

Preparation of nanoparticles

Inulin-loaded ethyl cellulose (IEC) nanoparticles were prepared by a two-step desolvation method (Fig 1), previously described by Weber *et al.,*[21]In brief: 2mg inulin was dissolved in 2ml distilled water under constant stirring. At the same time 24 mg of ethyl cellulose is dissolved in 8 ml of 100% ethanol. To this solution the prepared inulin solution is added drop wise to form nanoparticles. At the end 1% tween-80 is added drop wise as stabilizer under sonication (Sonics Ultrasonic processor, model no VCX750, USA) for 4 min at 20 amplitude. The resultant nanoparticles solution was centrifuged (Hettich, Universal 320R) at 3000rpm for 15min. The supernatant was collected in watch glass and the remaining organic solvent was evaporated by keeping it on hot plate stirrer at 40ºC (until the solution evaporates). The pellets (which contain free drug/polymer) were lyophilized and stored at 2-8°C for further experiments. Drug free ethyl cellulose (Blank EC) nanoparticles were prepared according to the same procedure by omitting the drug.

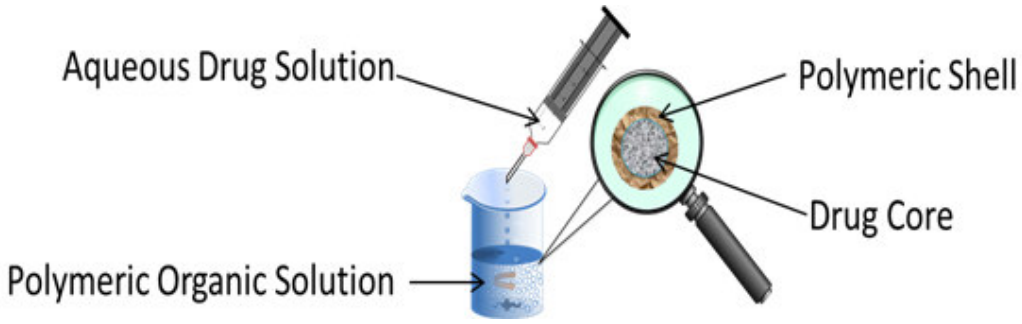

Figure 1
Desolvation method.

In order to determine the exact solid content of the nanoparticles in suspension the accurate number of nanoparticles in the dried powder was assessed by weighing the vials after drying. The nanoparticles recovery which is also referred to as nanoparticles yield in the literature was calculated using following equation.

$$Nanoparticles\ recovery\ (\%) = \frac{Mass\ of\ Ethyl\ cellulose\ nanoparticles\ recovered}{Mass\ of\ polymer, drug\ and\ stabilizer\ used\ in\ formulation} X\ 100$$

Physicochemical characterizations

Scanning electron microscopy analysis

The inulin-loaded ethyl cellulose nanoparticles were redispersed in Milli-Q water under bath sonication for 60 seconds at 40 amplitude and then air-dried on aluminum sheet mount. The sample was observed with a FEI ESEM Quanta 200 scanning electron microscope to obtain their morphologies. SEM photographs were taken from a FEI ESEM Quanta 200 instrument operated at an accelerating voltage of 20 kV in high vacuum mode using Everhart-Thornley detector.

Particle size and sizedistribution

The average particle size and zeta potential of the nanoparticles were measured using a Zetasizer NanoZS (Malvern Instruments, Malvern, UK). Particle size and polydispersity index were determined using non-invasive back scatter (NIBS) technology, which allows sample measurement in the range of 0.6nm-6µm. Freshly prepared particles suspension (20µl of sample added to 980µl of double distilled water) was placed in a clear disposable zeta cell (Clear disposable zeta cell, DTS 1060C). The measurement was carried out using a 4mW He-Ne laser (633nm) as light source at a fixed angle 173°. The following parameters were used for experiments; material refractive index 1.60, dispersant refractive index 1.330, medium viscosity 0.8872 cP, a dielectric constant of 78.54, temperature 25°C. Each size measurement was performed at least 10 runs; however, the determination of number of run was left to instrument. All measurements were carried out in triplicate directly after nanoparticle preparation, and the results were expressed as mean size ± S.D.

Zeta potential measurements

After the size measurement, zeta potential was measured with the Malvern patented laser interferometric technique M3-Phase Analysis Light Scattering. A Smoluchowski constant *F* (K_a) of 1.5 was used to achieve zeta potential values from electrophoretic mobility. Each zeta potential measurement was performed automatically at 25°C. All measurements were carried out in triplicate directly after nanoparticle preparation, and the results were expressed as mean size ± S.D.

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (Perkin Elmer Spectrum One FT-IR spectrometer, USA) of ethyl cellulose and inulin alone, inulin-loaded ethyl cellulose and inulin free ethyl cellulose nanoparticles dried by a hot plate were recorded in potassium bromide pellets. All the samples in powder form was mixed with dried and ground potassium bromide (Merck, FT-IR grade) pelletized (2mg sample in 200mg KBr) and the spectrum was recorded between 4000 cm^{-1} and 400cm^{-1} using a high energy ceramic source and DLaTGS (Deuterated Lanthanum α Alanine doped TriGlycine Sulphate) detectors.

Determination of drug entrapment efficiencies

Drug loading for inulin-loaded ethyl cellulose (IEC) nanoparticles was determined using resorcinol method-an indirect estimation method.[22] The inulin loading or content (%) was calculated using the following formula

$$Drug(Inulin)\ content\ \left(\%\frac{W}{W}\right) = \frac{Mass\ of\ Inulin\ in\ Ethyl\ cellulose\ \ nanoparticles}{Mass\ of\ Ethyl\ cellulose\ nanoparticles\ recovered}\ X\ 100$$

$$Entrapment\ Efficiency\left(\%\frac{W}{W}\right) = \frac{\text{Mass of Inulin in Ethyl cellulose nanoparticles}}{\text{Starting Mass of Inulin}}\ \text{X 100}$$

In vitro drug release assay

About 100mg of prepared nanoformulation was redispersed in PBS (pH 7.4) containing 0.1% Tween-80 and then placed in the dialysis bag (Mw cut-off 12,000, HiMedia, Mumbai, India). Hermetically sealed bag was immersed in 100ml phosphate buffer (pH 7.4) solution under sink conditions. The entire system was kept at 37°C with continuous magnetic stirring at 200 rpm. At predetermined time points, 1ml aliquot of the sample was drawn and immediately another 1ml of fresh medium added to maintain the volume. From the aliquots, the amount of inulin leeched from the nanoformulations was evaluated by using resorcinol method-an indirect estimation method[22]and the experiments were carried out in triplicate. A standard curve prepared was used for data analysis with necessary corrections for the dilution factors.

Hemolysis assay of nanoformulations

Fresh blood (5ml) from a healthy volunteer was taken with prior consenting and standard protocol approved by departmental doctoral review committee and added with EDTA (1.8mg/ml). The blood sample was then centrifuged in a cold centrifuge (Hettich, universal 320R) at 1000 rpm for 15 min after mixing thoroughly. Buffy coat was removed and the packed cells were washed thrice with normal saline (0.9 % NaCl). After final wash, normal saline was added to the cells to obtain 50% hematocrit. Hemolysis experiments were in accordance with a method with slight modifications.[10] Upon adding 100µl cell suspension to normal saline (3ml), it was observed for hemolysis. An appropriate negative control in normotonic condition and a positive control with 100µl of RBC suspension in double-distilled deionized water (3ml) were used to compare RBC's lysis. Drug-loaded nanoparticle suspension, was added as 100µl of nanoparticle solution in different concentration of ethyl cellulose nanoparticles per milliliter of RBC suspension. The blood samples were then centrifuged at 2000 rpm

for 10 min and the absorbance of supernatant was measured at 520nm in UV Spectrophotometer (Shimadzu, Model: UV1800). Percent hemolysis was calculated using formula:

$$Hemolysis(\%) = \frac{\text{Absorbance of sample}}{\text{Absorbance of Positive control}} \text{ X } 100$$

RESULTS

Scanning electron microscopy analysis

The SEM images of inulin-loaded ethyl cellulose nanoformulation revealed the spherical nature. The SEM showed that nanoformulations were globular in shape with smooth surface and found to be homogenous (Fig 2). This was an important observation in order to further our investigation *in vivo* as homogeneous spherical forms will not show much interference during their journey in the systemic circulation.

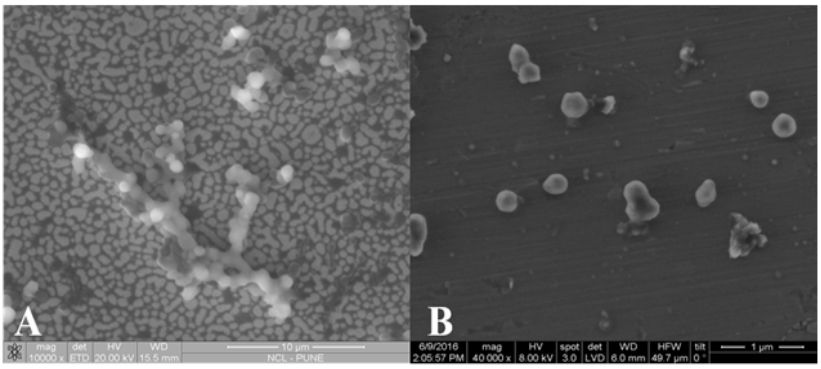

Figure 2
Scanning electron micrograph analysis of (A) Drug free Ethyl cellulose nanoparticles and (B) Inulin-loaded Ethyl cellulose nanoparticles.

Particle size and size distribution

Nanoparticles were characterized for their mean particle diameter and size distribution. The average diameters of drug free ethyl cellulose nanoparticles and inulin-loaded ethyl cellulose nanoparticles are tabulated below (Table 1). The average diameters of drug free ethyl cellulose nanoparticles (Fig 3A) and inulin-loaded ethyl cellulose nanoparticles (Fig 3B) from the preparations ranged between 174.2±0.896 and 217.9±1.747nm respectively. The polydispersity indices obtained from the measurements were around 0.2 or lower indicating narrow deviations in size.

Table 1
Size distribution of drug free ethyl cellulose nanoparticles and inulin-loaded ethyl cellulose nanoparticles.

Nanoparticles	Mean Diameter (nm)	Polydispersity index
Drug free ethyl cellulose nanoparticle	174.2±0.896	0.155±0.052
Inulin-loaded ethyl cellulose nanoparticles	217.9±1.747	0.195±0.094

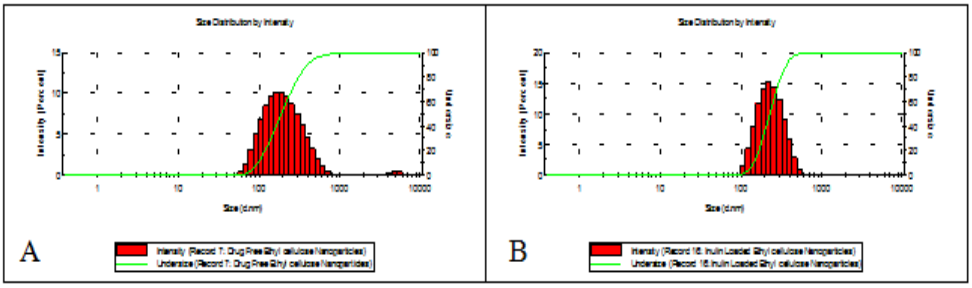

Figure 3
Size distribution of (A) drug free ethyl cellulose nanoparticles and (B) inulin-loaded ethyl cellulose nanoparticles

Zeta potential measurements

High potentials values should be achieved in order to achieved to ensure a high-energy barrier[23] and favor a good stability. Friberg[24] considers that a zeta potential of about -25mV allows an ideal stabilization of nanoparticles because the repulsive forces prevent aggregation upon ageing. Accordingly, -8.64±0.77mV which was the value obtained with inulin-free ethyl cellulose nanoparticles (Fig 4A) and -16.0±1.48mV which was the value obtained with inulin-loaded ethyl

cellulose nanoparticles (Fig 4B) shows to favor the best stability among the designed nanoparticles. In this investigation, the zeta potential of the nanoparticles was negative. The negative zeta potential can be mainly attributed to the steric repulsion caused by use of nonionic surfactant Tween-80. The Zeta potential measurements indicated that the interface is negatively charged. This charge will enhance the stability of the emulsion by causing double layer repulsion between droplets.

Table 2
Zeta potential of drug free ethyl cellulose nanoparticles and inulin-loaded ethyl cellulose nanoparticles.

Nanoparticles	Zeta potential (mV)
Drug free ethyl cellulose nanoparticles	-8.64±0.77
Inulin-loaded ethyl cellulose nanoparticles	-16.0±1.48

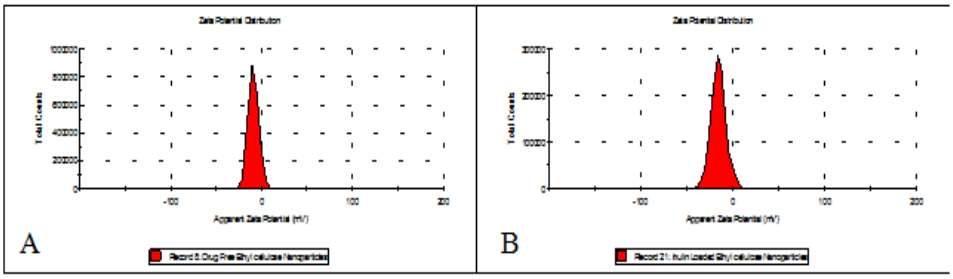

Figure 4
Zeta potential distribution of (A) drug free ethyl cellulose nanoparticles and (B) inulin-loaded ethyl cellulose nanoparticles.

Fourier transform infrared spectroscopy

FTIR studies were performed on the drug-loaded particles in order to obtain information regarding possible chemical interaction between drug and polymer, and surface chemistry of the particles prepared. Empty polymer particles were chosen as a reference and compared to drug loaded particles. FTIR spectra were measured by PerkinElmer Spectrum One FT-IR spectrometer. FTIR spectra of inulin (free drug), ethyl cellulose, inulin-loaded ethyl cellulose and drug-free ethyl cellulose nanoparticles were obtained.

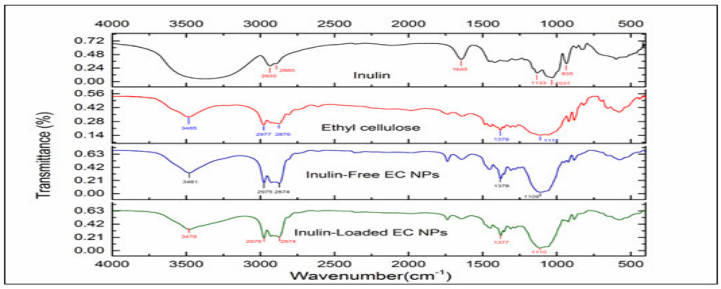

Figure 5
FT-IR Spectra of Inulin, Ethylcellulose, Inulin-Free Ethylcellulose NPs and Inulin-loaded Ethylcellulose NPs.

From the FTIR studies, it was quite difficult to observe any chemisorption between the drug inulin and ethyl cellulose polymer (Fig 5). As compared to standard spectra of inulin, we observed that similar IR spectra with the most intensive broad band at ~1031cm^{-1}along 2876 cm^{-1} due to C-H stretching vibration peak. The -OH stretching vibration was observed at 3485cm^{-1}. The other important peaks at 1111 and 1379cm^{-1} corresponded to C-O-C stretching and C-H bending, respectively.From the graph, the peaks of Inulin from 2930cm^{-1} and 2870cm^{-1}was found to be masked by the ethyl cellulose peaks from 2977cm^{-1} and 2876cm^{-1}. This may be due to the fact that the polymer is used in higher quantity when compared with drug. In inulin-loaded ethyl cellulose nanoformulations, the -OH stretching vibration peak at 3483cm^{-1} was found be to displaced to slightly with two shoulders at ~940cm^{-1}and 1130cm^{-1}. Similarly, a broad band with low intensity is at ~1640cm^{-1}and two overlapped bands at ~2930cm^{-1} and ~2870cm^{-1}are indicative of carbohydrates.IR spectra of ethylcellulose showed similar characteristic peaks at 2977 cm^{-1} and lower wavenumber and the peak was slightly broadened due to the presence of inulin. These observations would help us to confirm that the drug and polymer are physisorbed rather than chemisorption and intact form of drug was preserved in the nanoformulation.

Drug entrapment efficiency

A number of studies have reported quantification of drug entrapment using various method of estimation based on determination of free drug after entrapment

compared to total amount of drug used. Initial studies carried out focused on this method of determination for drug loading and entrapment efficiency. Based on the result obtained from indirect estimation, around 40% of drug was entrapped within the particles. Free drug was analyzed spectrophotometrically. Standard solution of known concentration of inulin was analyzed at 520nm on a Spectrophotometer and a calibration curve was prepared (Fig 6). Samples were analyzed for free drug and determine the percentage of drug entrapment.

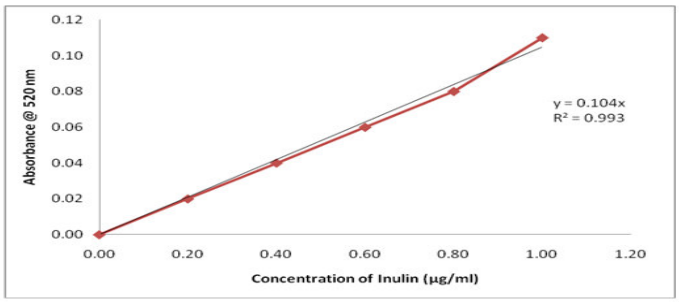

Figure 6
Calibration curve for inulin solution using spectrophotometer.

$$Drug(Inulin)\ content\ \left(\%\frac{W}{W}\right) = \frac{\text{Mass of Inulin in Ethyl cellulose nanoparticles}}{\text{Mass of Ethycellulose nanoparticles recovered}}\ \text{X } 100$$

= (0.8mg/27mg) *100 = 2.926 %

$$Entrapment\ Efficiency\left(\%\frac{W}{W}\right) = \frac{\text{Mass of Inulin in Ethyl cellulose nanoparticles}}{\text{Starting Mass of Inulin}}\ \text{X } 100$$

= (0.8mg/2mg) *100= 40%

The calibration curve for inulin was used to calculate the amount of inulin in the sample. The result obtained from the indirect estimation (Table 3), around 40% of drug was entrapped within the particles.

Table 3
Estimation of inulin in inulin-loaded ethyl cellulose nanoparticles

Sl. No	Sample Name	Conc. of sample(ml)	OD 520 (nm)
1	IEC-1	1	0.12
2	IEC-2	1	0.12
3	IEC-3	1	0.11

In vitro drug release assay

Understanding the profile of drug release *in vitro* would help to assess the possible biological effect of inulin. Membrane diffusion techniques are the most widely used experimental methods for the study of the *in vitro* release profiles of inulin incorporated in nanoparticles.

The *in vitro* release profile of inulin from ethyl cellulose nanoparticles in PBS (pH 7.4) containing 0.1% Tween-80 is presented in Fig 7, which shows a rapid diffusion of inulin; 30 to 40% of inulin is released after 3h and ~94% after 24h. This release profile shows a biphasic phenomenon.

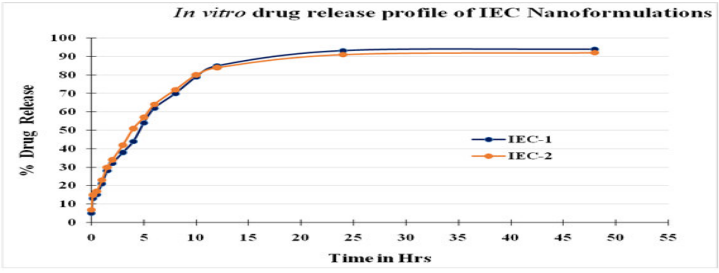

Figure 7
***In vitro* release assay of nanoformulations.**

Hemolysis assay of nanoformulations

In vitro hemolysis assays of nanoformulations were carried out to ascertain the hemocompatibility in the systemic circulation (Table 4). In this observation, drug and ethyl cellulose is having the toxicity maximum of 4% at maximum drug concentration of about 1.75mg/ml. This suggests that drug and ethyl cellulose had negligible amount of toxicity to RBC's. Hemolysis (%) assay with distilled water as control shows that inulin released from inulin loaded ethyl cellulose nanoparticles would cause hemolysis in a concentration-dependent manner (Fig 8). It was evident from the experiment that

prevention of hemolysis was almost 100% at the end of 1hr. This would reveal the information that drug-loaded nanoparticles are pharmacologically active and sustained releases of inulin from the carrier polymer nanoparticles prevent hemolysis

Table 4
Hemolysis assay of nanoformulations

Sl. No	Sample concentration	%Hemolysis		
	mg/ml	Drug Free EC NPs	IEC-1 NPs	IEC-2
1	0.25	0.821	0.836	0.83
2	0.75	2.345	2.412	2.397
3	1.25	3.187	3.226	3.216
4	1.75	4.123	4.127	4.125

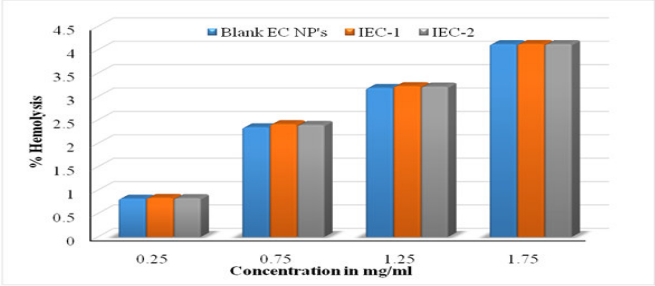

Figure 8
Hemolysis assay of nanoformulations

DISCUSSION

Various formulation variables like interaction between dissolution media, polymer and drug; influence drug release rate[25-26], to greater or lesser extent loading.[27-28]Drug/polymer ratio[29] and drug particle size have been shown to affect drug release from ethyl cellulose matrices. Through different characterizations, the prepared nanoformulations were found to be smooth surfaced, globular in shape having narrow deviations in size with a negative zeta potential. As a general rule, decrease in the nanoparticles diameter causes increase in the ratio of surface to volume.[30] Spreading of charge in larger particle surface may have resulted in lower zeta potential. At present study results (Table 2) were on the contrary regarding the small nanoparticles. Although it was quite difficult to observe any interaction between the drug and polymer from the FTIR studies, the encapsulation efficiency and biphasic *in vitro* release profile showed successful entrapment of drug inside the polymer matrix. Ethyl cellulose retained its non-toxicity feature even after formation of nanoparticles. After the release of drug from its core ethyl cellulose may act as laxative and naturally excreted via stool.

CONCLUSION

Inulin-loaded ethyl cellulose nanoformulations which were successfully prepared with repetitive results by two-step desolvation method using tween-80 as stabilizer. The formulations were characterized in detail and found to have moderately better entrapment efficiency. The repetitive formulations exhibited slow and sustained release of inulin in 48-hour study duration. The delivery of prebiotic-inulin through the inulin-loaded ethyl cellulose nanoformulations were proven safe with negligible hemolytic activity, suggesting that the nanoformulations would be a better candidate for oral drug delivery applications.

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CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Alpar HO, Walters V. The prolongation of the in vitro dissolution of a soluble drug (phenethicillin potassium) by microencapsulation with ethyl cellulose. Journal of Pharmacy and Pharmacology. 1981;33(1):419-22.
2. Al-Omran MF, Al-Suwayeh SA, El-Helw AM, Saleh SI. Taste masking of diclofenac sodium using microencapsulation. Journal of Microencapsulation. 2002;19(1):45-52.
3. Ouwehand AC, Salminen S, Isolauri E. Probiotics: an overview of beneficial effects. Lactic Acid Bacteria: Genetics, Metabolism and Applications: Springer Science + Business Media; 2002. p. 279-89.
4. Heller J, Helwing R, Baker R, Tutte M. Controlled release of water-soluble macromolecules from bioerodible hydrogels. Biomaterials. 1983;4(4):262-6.
5. Mura P, Faucci MT, Manderioli A, Bramanti G, Parrini P. Thermal Behavior and Dissolution

Properties of Naproxen From Binary and Ternary Solid Dispersions. Drug Development and Industrial Pharmacy. 1999;25(3):257-64.
6. Soskolne A, Golomb G, Friedman M, Sela MN. New sustained release dosage form of chlorhexidine for dental use. Journal of Periodontal Research. 1983;18(3):330-6.
7. Friedman M, Golomb G. New sustained release dosage form of chlorhexidine for dental use. Journal of Periodontal Research. 1982;17(3):323-8.
8. Food and Drugs : Food additives permitted for direct addition to food for human consumption. (as on 05/08/2016).Retrieved from Code of Federal Regulations database (172.868). US Government Publishing Office, USA.
9. Food and Drugs : Food additives permitted in feed and drinking water of animals. (as on 05/08/2016). Retrieved from Code of Federal Regulations database (573.420). US Government Publishing Office, USA.
10. Ravikumara NR, Madhusudhan B, Nagaraj TS, Hiremat SR, Raina G. Preparation and Evaluation of Nimesulide-loaded Ethylcellulose and Methylcellulose Nanoparticles and Microparticles for Oral Delivery. Journal of Biomaterials Applications. 2009;24(1):47-64.
11. Suwannateep N, Banlunara W, Wanichwecharungruang SP, Chiablaem K, Lirdprapamongkol K, Svasti J. Mucoadhesive curcumin nanospheres: Biological activity, adhesion to stomach mucosa and release of curcumin into the circulation. Journal of Controlled Release. 2011;151(2):176-82.
12. Pan-In P, Wanichwecharungruang S, Hanes J, Kim AJ. Cellular trafficking and anticancer activity of Garcinia mangostana extract-encapsulated polymeric nanoparticles. International journal of nanomedicine. 2014;9:3677-86.
13. Sanders ME. Lactic Acid Bacteria as Promoters of Human Health. Functional Foods: Springer Science + Business Media; 1994. p. 294-322.
14. Roberfroid MB. Defining functional foods. Functional Foods: Elsevier BV; 2000. p. 9-27.
15. Borriello SP, Hammes WP, Holzapfel W, Marteau P, Schrezenmeir J, Vaara M, et al. Safety of Probiotics That Contain Lactobacilli or Bifidobacteria. Clinical Infectious Diseases. 2003;36(6):775-80.
16. Konishi M, Tabata Y, Kariya M, Hosseinkhani H, Suzuki A, Fukuhara K, et al. In vivo anti-tumor effect of dual release of cisplatin and adriamycin from biodegradable gelatin hydrogel. Journal of Controlled Release. 2005;103(1):7-19.
17. Reddy B. Effect of dietary oligofructose and inulin on colonic preneoplastic aberrant crypt foci inhibition. Carcinogenesis. 1997;18(7):1371-4.
18. Rao BS, Murthy KVR. Studies on rifampicin release from ethylcellulose coated nonpareil beads. International Journal of Pharmaceutics. 2002;231(1):97-106.
19. Gibson GR, Probert HM, Loo JV, Rastall RA, Roberfroid MB. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutrition Research Reviews. 2004;17(02):259.
20. Khutoryanskiy V. Hydrogen-bonded interpolymer complexes as materials for pharmaceutical applications. International Journal of Pharmaceutics. 2007;334(1-2):15-26.
21. Weber C, Coester C, Kreuter J, Langer K. Desolvation process and surface characterisation of protein nanoparticles. International Journal of Pharmaceutics. 2000;194(1):91-102.
22. Ashwell G. [12] Colorimetric analysis of sugars. Methods in Enzymology: Elsevier BV; 1957. p. 73-105.
23. Benita S, Levy MY. Submicron emulsions as colloidal drug carriers for intravenous administration: Comprehensive physicochemical characterization. Journal of Pharmaceutical Sciences. 1993;82(11):1069-79.
24. Friberg SE. A review of: " COLLOID CARRIERS FOR CONTROLLED DRUG DELIVERY AND TARGETING: " Modification, Characterization and In Vivo Distribution, R.H.Müller, ed., CRC Press, Boca Raton, FL, 1991. Journal of Dispersion Science and Technology. 1995;16(5):395-.
25. Wichterle O, LÍM D. Hydrophilic Gels for Biological Use. Nature. 1960;185(4706):117-8.
26. Gander B, Johansen P, Nam-Trân H, Merkle HP. Thermodynamic approach to protein microencapsulation into poly(D,L-lactide) by spray drying. International Journal of Pharmaceutics. 1996;129(1-2):51-61.
27. Ei-Arini SK, Leuenberger H. Modelling of drug release from polymer matrices: Effect of drug loading. International Journal of Pharmaceutics. 1995;121(2):141-8.
28. Neau SH, Howard MA, Claudius JS, Howard DR. The effect of the aqueous solubility of xanthine derivatives on the release mechanism from ethylcellulose matrix tablets. International Journal of Pharmaceutics. 1999;179(1):97-105.
29. Billon A, Petit M, Doko MB, Bataille B, Jacob M. Effects of Cellulose Derivatives and Additives in the Spray-Drying Preparation of Acetaminophen Delivery Systems. Drug Development and Industrial Pharmacy. 1999;25(11):1149-56.
30. 30. Bischoff KB, Denn MM, Seinfeld JH, Stephanopoulos G, Chakraborty A, Peppas N, et al. Nanostructured Materials: Elsevier Science; 2001.