International Journal of Research in Advent Technology, Vol.6, No.11, November 2018
E-ISSN: 2321-9637
Available online at www.ijrat.org

Characterization and Comparison of Drug Release from Modified and Unmodified Chitosan Microspheres

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Abstract- Chitosan is a renewable, linear polysaccharide with reactive $–NH_2$ (at C_2) and –OH (at C_3 and C_6) group. It is preferred over other polymers due to its properties such as biodegradability, biocompatibility, non-toxicity, availability and ease of chemical modification. Its solubility is limited to aqueous acidic solution and it also has higher swelling index in aqueous environment. Various efforts have been made to overcome these limitations. Most efficient and versatile tool for modifying chitosan chemically is graft copolymerization which includes substitution of a variety of functional group at C_2,C_3 or C_6 positions. Grafting does not change the basic structure of chitosan but results in a variety of derived products with improved properties. In the present study, graft copolymerization of 2-hydroxyethyl methacrylate onto chitosan was carried out. Ceric ammonium nitrate had been used as free radical initiator. Evidence of grafting was confirmed by various analytical techniques such as fourier transform infrared spectroscopy, scanning electron microscopy, X-ray diffraction, differential scanning calorimetry and nuclear magnetic resonance. Microspheres of modified and unmodified chitosan were then prepared by emulsion cross-linking method using glutaraldehyde as cross-linking agent. Optimized microspheres were evaluated for their percentage yield, particle size, % drug entrapment efficiency and surface morphology. *In-vitro* drug release from chitosan microspheres and modified chitosan microspheres was studied in 0.1N HCl for two hours and in pH 6.8 phosphate buffer for next seven hours. An increase in % cumulative drug release might be due to enhancement in solubility of chitosan after modification with monomer. An increase in water uptake capacity of modified chitosan might be another reason for increased % CDR.

Keywords: Chitosan; Graft copolymerization; 2-Hydroxyethyl methacrylate (2-HEMA); Ceric ammonium nitrate (CAN); Glutaraldehyde; Microspheres.

1. INTRODUCTION

N-deacetylation of chitin using strong alkali results in formation of chitosan (1). Chitosan is a linear, semi crystalline, polycationic polysaccharide with two glucosamine units. (2). Due to its various advantageous biological properties, chitosan is preferred over other polymers for pharmaceutical and biomedical applications. One of the most important advantages of this polymer is its ability to be used in different drug delivery systems such as microspheres, nanoparticles, hydrogels, films, enteric coatings and many others (3). It possess reactive hydroxyl (at C_3 and C_6 positions) and amine (at C_2 position) groups which are responsible for its less crystalline nature (4) and possibility of stable covalent bond formation with other molecules (2). These groups can be modified to obtain chitosan derivatives with new properties (5).

Among numerous techniques of chemical modification, graft copolymerization is the most efficient one (6). The chemical reaction between natural and synthetic polymers by means of grafting is widely used nowadays for modifying the physical and chemical properties of a polymer (7,8). New properties of polymer depend on selection of side chains. Grafting of acrylates onto chitosan provides product with new properties including enhanced aqueous solubility, thermal stability, optimal swelling capacity and improved mechanical property. (6).

Grafting can be initiated mainly by three methods namely by using chemicals, radiation or enzymes (9). Generally chemical initiators have been used to initiate grafting process onto chitosan. Mostly used among them are Cerric Ammonium Nitrate (CAN), Ammonium Per Sulphate (APS), Potassium Per Sulphate (KPS), AIBN and AMPN (6). Among these initiator, CAN is the most efficient and widely used and the reaction must proceed in acidic medium (4).

Microspheres constitute an important part of particulate drug delivery system due to their small size and efficient carrier capacity (10). These are carrier linked drug delivery system in which particle size ranges from 1-1000 µm in diameter (11). These consist a core of drug and entirely outer layers of protein or synthetic polymers, which are biodegradable in nature (12, 13). Microspheres are formulated so as to provide constant and prolonged

therapeutic effect thereby improving patient compliance and reducing dosing frequency. Microsphere allows a controllable variability in degradation and drug release. Other reasons for microencapsulation may be- masking of taste and odour of many drugs, conversion of liquid drugs in to a free flowing powder, prevention of incompatibility among drugs and alteration of site of absorption (14, 15).

Several techniques of microsphere preparation namely single emulsion method, double emulsion method, coacervation method, polymerization method, cross-linking method, spray drying and spray congealing method, melt dispersion method, solvent extraction method and emulsion solvent evaporation method (12, 16).

The present work was aimed to modify chitosan using 2-HEMA by graft copolymerization technique. Initiallychemical modification of chitosan was carried out by reacting with 2-Hydroxyethyl methacrylate (2-HEMA). The grafting was confirmed by various analytical methods such as FTIR, SEM, XRD and DSC. The optimized modified chitosan fyrther was used to prepare microspheres using emulsion cross-linking method.The *in-vitro* drug release was then compared with the microspheres prepared from modified and unmodified chitosan.

2. EXPERIMENTAL PART

2.1 *Materials*

Chitosan (from shrimp shells, degree of deacetylation >75%) was purchased from Himedia Laboratories Pvt. Ltd., Nashik. Ceric ammonium nitrate was obtained from Qualigens fine chemicals (A division of Glaxo India Pvt. Ltd., Mumbai). 2-Hydroxyethyl methacrylate was supplied by Merck Schuchardt, OHG, Germany. Other chemicals and reagents used are of analytical grade.

2.2*Synthesis of chitosan graft copolymer*

A 1% (w/v) chitosan stock solution was prepared in 1% (v/v) acetic acid aqueous solution. A known volume of above solution was taken and known amount of initiator (CAN) was added to it and stirred for 10 minutes. A known amount of monomer (2-HEMA) was added to it and stirred until complete homogenization. Resulting solution was transferred to a petridish and allowed to form a film. Film formed was removed using1% NaOH aqueous solution and filtered. Resulting solution was extracted in Soxhlet apparatus. Final product was dried at room temperature.

2.3 *Characterization of graft copolymers*

2.3.1Determination of functional groups by fourier transform infrared spectroscopy

IR spectra of samples were recorded on KBr pellet and with nujol solution whichever appropriate. About 2 mg of solid samples were grounded with KBr and pellets were prepared using a hydraulic press under a pressure of 600 kg/cm^2. Liquid samples were mixed with nujol solution and analyzed further. Samples were scanned for the spectral range of 400-4000 cm^{-1} using Perkin Elmer spectrum-2-89258.

2.3.2Study of surface morphology using scanning electron microscope

Surface morphology of grafted copolymer was observed with scanning electron microscopy. SEM of graft copolymer was obtained by ZEISS EVO 40 EP at 20 kV. Powdered sample was taken and fixed on aluminium stubs and coated with gold using QUORUM, Q150RES and viewed through the microscope.

2.3.3Determination of glass transition temperature by differential scanning calorimeter

DSC was carried out using DSC Q20. The sample was placed into aluminium pan and sealed. As reference, an empty aluminium pan of approximately same weight was taken. The samples were heated from 0-400^{0}C at a heating rate of 1^{0}C/min in a nitrogen atmosphere.

2.3.4Determination of crystalline nature by X-ray diffractometer

X-ray diffraction studies were performed using PAN analytical diffractometer (Model X'Pert PRO) with copper as a target material in an X-ray tube. To perform XRD studies samples were mounted in a sample holder and XRD scans were recorded under operational conditions 45kV, 40mA, and wavelength between 1.39225A to 1.54443A in angle range 0-70^0.

2.3.5Determination of chemical structure by nuclear magnetic resonance

Proton nuclear magnetic resonance (H_1-NMR) spectra were recorded with a VNMRS-500, "Agilent NMR" spectrophotometer operating at 8012.8 Hz and solvent used was methanol with TFA.

2.4. Preparation of microspheres

Microspheres were prepared by emulsion cross-linking method using glutaraldehyde as a cross-linking agent. Method involved mixing of model drug (metronidazole) in modified and unmodified chitosan solution. This mixture was added drop wise into an oily phase containing paraffin oil and an emulsifier, span 80 with continuous stirring. Glutaraldehyde was added twice to the mixture, once after 1hour and then 2hours with

continuousand constant stirring. The prepared microspheres were then isolated by vacuum filtration and washed with petroleum ether and dried at room temperature.

2.5 Evaluation of optimized microspheres

Optimized modified and unmodified microspheres wereevaluated in terms of percentage yield, particle size, drug entrapment efficiency and surface morphology.

2.5.1 Particle size analysis

The particle size of theoptimized modified and unmodified chitosan microspheres were analyzed by using optical microscope (CH20i). The samples were dispersed in liquid paraffin and individual microsphere's diameter was measured using micrometer. The mean particlediameter of microspheres was then calculated from the data.

2.5.2 Drug entrapment efficiency

A weighed amount of drug loaded modified and unmodified chitosan were taken and crushed in a glass mortar and pestle. The powdered microspheres were suspended in 10ml of 0.1N HCl. The solution was then shaken for 24 hours and was filtered. The solution was then suitably diluted and was analyzed for drug content using UV-Visible spectrophotometer at desired λ_{max}. The experiment was done in triplicate and mean % drug entrapment efficiency was calculated using the following formula:

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

2.5.3 Surface morphological study

The external morphology of modified and unmodified chitosan microspheres was observed by scanning electron microscopy. The powdered samples were fixed on aluminium stubs with double sided tape and coated with gold using QUORUM, Q150RES. Samples were then viewed with the scanning electron microscope, ZEISS EVO 40 EP at 20 kV.

2.6 In-vitro drug release study

In-vitro drug release study was carried out in USP type II apparatus. Microspheres, equivalent to 100 mg of drug was accurately weighed and placed in cellophane dialysis bag and then inserted into dissolution flask containing 200 ml of dissolution media. The temperature was maintained at 37±0.5°C The study was carried out in 0.1N HCl for first two hours and then media was transferred with pH 6.8 phosphate buffer. An aliquot of samples were withdrawn at regular intervals and after suitable dilution, the concentration of drug was determined.The release study wasperformed upto 9 hours.

2.7 Comparison of dissolution profileof microspheres

Comparison of dissolution profileof microspheres prepared by unmodified and modified chitosan was done by using similarity factor (f_2). The $f_2$value assures the similarity in dissolution profiles. The f_2 value between 50-100 indicates similarity between two dissolution profiles. It can be calculated using following formula:

$$f_2 = 50 \times \log\{1 + {}^{1}/_{n}\left[\sum (R_t - T_t)^2\right]^{-0.5} \times 100$$

Where,

R_t = dissolution value of reference (chitosan) batch at time t

T_t= dissolution value of test (modified chitosan) batch at time t

n = number of time points.

3. RESULTS AND DISCUSSION

3.1 Characterization of graft copolymers

Free radical polymerization technique was used for grafting of 2-HEMA onto chitosan. Graft copolymer was optimized by varying reaction parameters such as monomer and initiator concentration, reaction time and temperature. The optimized copolymer was characterized by various analytical techniques.

3.1.1 Determination of functional groups by FTIR spectroscopy

The FTIR spectra of chitosan, 2-HEMA, physical mixture of chitosan and 2-HEMA, chitosan grafted 2-HEMA is given in Fig. 3.1**.**Peak at 1100cm^{-1} is characteristic peak of polysaccharide bone whereas in grafting double bond absorption was absent. FTIR spectra of chitosan showed a peak at 3415.23cm^{-1} which is attributed to -OH stretching vibration. Peaks around 1078.93cm^{-1}, 1638.12cm^{-1} and 1375.29cm^{-1} are due to C-O-C bond, amide C=O stretching and symmetric stretching of carboxylate C=O. In IR spectra of 2-HEMA, the absorption at 1723.66 cm^{-1} and 1405.2cm^{-1} is attributed to C=O asymmetric stretching of the ester group and presence of vinylic double bond. In the IR spectra of chitosan grafted with 2-HEMA, there is absence of peak near 1405.2cm^{-1} whereas this peak was found in physical mixture of chitosan and 2-HEMA which is characteristic for the absorption peak of C=O of monomer. The absence of such peak indicates the absence of monomer as residuals. Presence of a new peak at 1276.86 cm^{-1} due to -CN group along with broadening of

-OH stretching peak near 3416.24 cm^{-1} confirmed grafting of 2-HEMA onto chitosan.

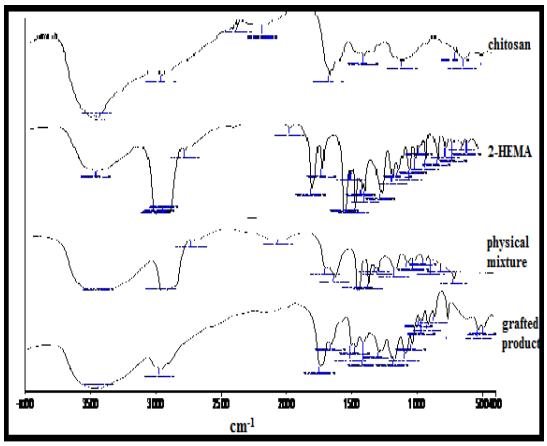
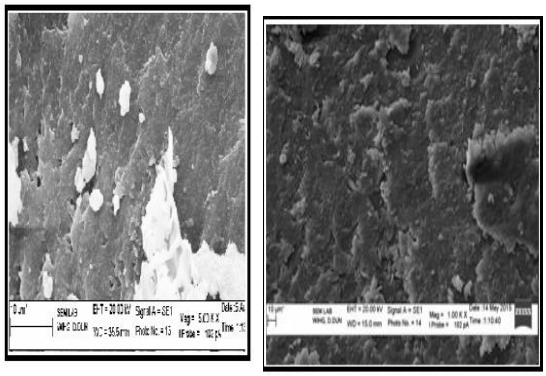


Fig. 3.1IR spectra of chitosan, 2-HEMA, physical mixture of chitosan and 2-HEMA, chitosan grafted 2-HEMA.

3.1.2 Determination of surface morphology using Scanning Electron Microscope

A SEM image of chitosan and chitosan-g-HEMA is shown in Fig.3.2 (a) and (b) respectively.The copolymerization of 2-HEMA modified the surface morphology of original chitosan. SEM images of modified chitosan showed dense and rough surface morphology as compared to chitosan which may be due to attachment of monomer on chitosan backbone. The results are in agreement with the findings of K.Wang et al. (2005).



(a) (b)

Fig. 3.2 SEM image of (a) chitosan, (b) chitosan-g-HEMA

3.1.3 Determination of crystalline behaviour by X-ray Diffraction

Fig. 3.3 illustrates the XRD patterns of chitosan and grafted chitosan. As shown in diffractogram of chitosan, crystalline band at 2θ value of 19.9437° confirms the crystalline nature of chitosan. Grafting copolymerization of 2-HEMA on chitosan changed the crystalline nature of chitosan into amorphous nature. Absence of peaks in diffractogram of modified chitosan confirmed it. The disarrangement in ordered structure of crystal lattice of chitosan may be due to random attachment of monomer molecules on chitosan backbone. Grafting had changed the crystalline nature of chitosan. The alteration in inter and intra molecular H-bonding of chitosan was the reason behind this change.

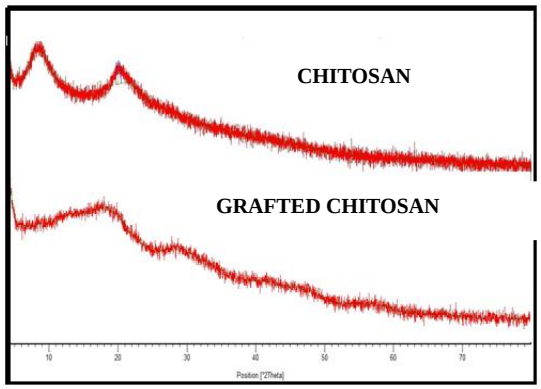
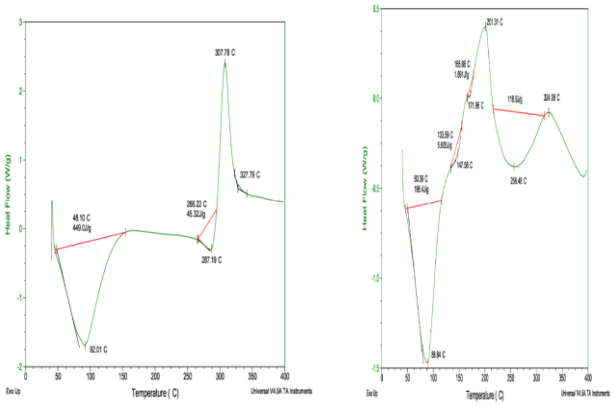


Fig. 3.3 XRD patterns of chitosan and grafted chitosan

3.1.4 Determination of glass transition temperature by Differential Scanning Calorimetry

Fig. 3.4 shows the DSC of (a) chitosan and (b)grafted chitosan. The glass transition temperature (T_g) of chitosan appeared at92.01°C, whereas for modified chitosan T_g had been shifted towards lower temperature of 88.84°C. This lowering in endothermic peak value of chitosan might be due to grafting of 2-HEMA onto chitosan. The characteristic exothermic transition peak at 307.76°C might correspond to decomposition of chitosan during heating. The modified chitosan had exothermic peak at 324.08°C which indicates that grafting had improved the thermal stability of chitosan.



(a) (b)

Fig. 3.4 DSC of (a) chitosan and (b) grafted chitosan

3.1.5 Determination of chemical structure by nuclear magnetic resonance

Fig. 3.5 shows the ^{1}H-NMR of grafted chitosan. Multiplets at 3-3.5 ppm corresponds to the ring methine protons. The copolymer also shows multiplet peaks at 1-2 ppm due to methylene protons. Peaks at 4-5 ppm indicates presence of hydroxyl group in modified chitosan. The peak at 1.76 ppm was due to the acetyl protons of the N-acetyl glucosamine units of chitosan.

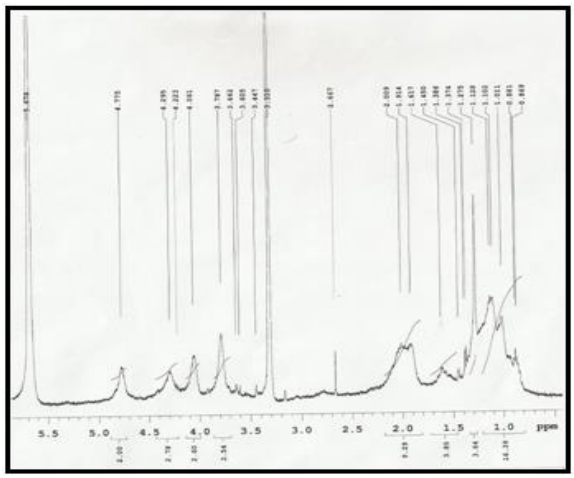
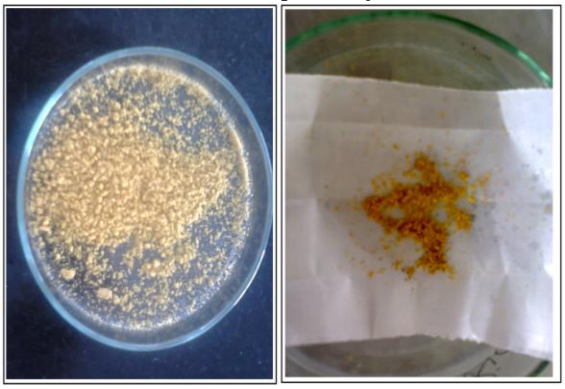


Fig. 3.5 NMR spectra of grafted chitosan

3.2Preparation and optimization of microspheres

3.2.1 Preparation of microspheres

Microspheres were prepared by emulsion cross-linking method. For both microspheres drug: polymer ratio taken was 1:1, 2.5ml of glutaraldehyde and 25mg of magnesium stearate. Optimized stirring rate for chitosan and modified chitosan microspheres were 500 rpm and 800 rpm respectively and stirring was carried out for 2 hours and 3 hours respectively.



(a) (b)

Fig. 3.6 (a) Chitosan microspheres (b) modified chitosan microspheres

3.3Evaluation of optimized microspheres

3.3.1 Particle size analysis

The particle size analysis was performed on the microspheres using optical microscope. The mean particle size of chitosan and modified chitosan microspheres was found to be 62.5µm and 102µm.

3.3.2 Drug entrapment efficiency

The drug entrapment efficiency of chitosan and modified chitosan microspheres was found to be 76.24% and 82.36% respectively. The modification in chitosan had resulted in enhancement of drug entrapment efficiency of microspheres.

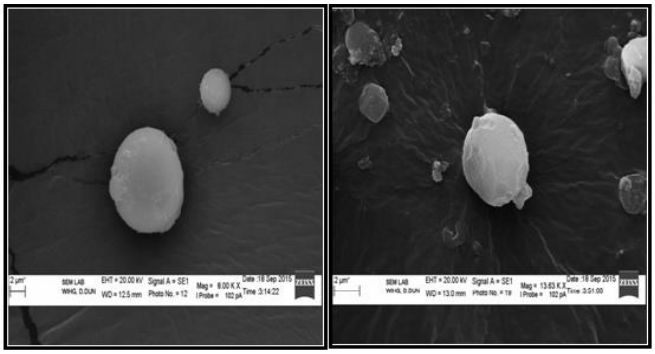
Table 3.1 Various parameters of chitosan and modified chitosan microspheres

Parameters	**Chitosan microspheres**	**Modified chitosan microspheres**
Particle size (µm)	62.5	102
Drug entrapment efficiency (%)	76.24	82.36

3.3.3 Surface morphological study

SEM images of chitosan and modified chitosan are given in Fig. 3.7 (a) and (b) respectively. Chitosan microspheres exhibited a smooth surface morphology. These spheres were found to have regular spherical geometry. The SEM of modified chitosan

microspheres showed a rough surface morphology that might be due to the polymer grafted onto chitosan. The lack of uniformity in geometry of these spheres is evident in SEM images. The insufficient cross-linking might be the reason for the uneven formation of microspheres.



(a) (b)

Fig. 3.7 SEM images of microspheres of (a) chitosan and (b) modified chitosan

2.6 In-vitro drug release study

In-vitro drug release from chitosan microspheres and modified chitosan microspheres was studied in 0.1N HCl for two hours and in pH 6.8 phosphate buffer for next seven hours.

In first two hours, the drug released from chitosan microspheres and modified chitosan microspheres was found to be 20.17% and 24.85%. In next seven hours, % cumulative drug release from chitosan and modified chitosan microspheres was 67.52 and 92.05, respectively. The reason for increase in % cumulative drug release might be due to enhancement in solubility of chitosan after modification with monomer. An increase in water uptake capacity of modified chitosan might be another reason for increased % CDR.

K.G. Subramanian et al., (2012) synthesized and evaluated chitosan-g-poly (2-HEMA *co* itaconic acid) as a drug carrier for controlled release (17). The drug release rate of tablet was greater for pure chitosan than that of grafted chitosan in both SGF and SIF which is contradictory with our result. The reason for greater release was attributed to the burst release of drug from tablet while this phenomenon was absent with multiple unit dosage form such as microspheres. Hence, microspheres might have advantage over tablets for controlled release of drug.

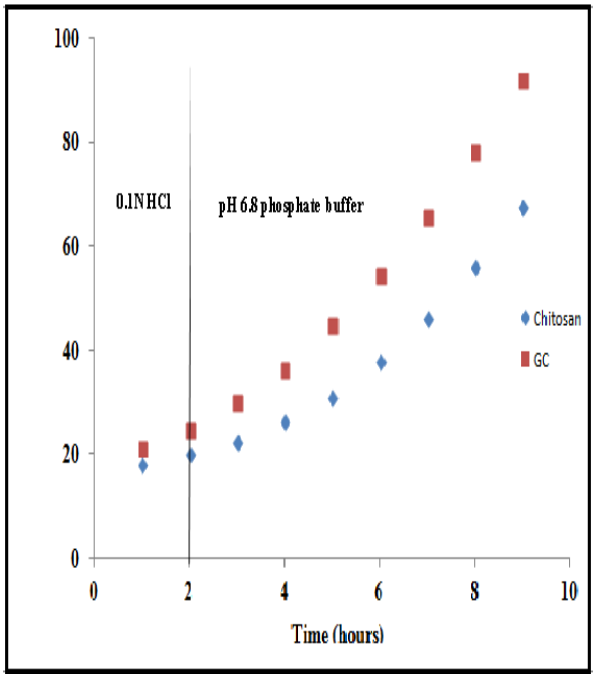


Fig 3.8*In-vitro* drug release studies of chitosan and modified chitosan microspheres

2.7 Comparison of dissolution profile of microspheres

The dissolution profile of chitosan and modified chitosan microspheres were compared by similarity factor (f_2).Using the formula for similarity factor, f2 value was found to be 40.58. As it is known that f2 value equals to or greater than 50 indicate similarity in two dissolution profiles. Hence, result confirmed that there is a significant difference in dissolution profile of chitosan and modified chitosan microspheres. Microspheres prepared from modified chitosan showed faster % CDR as compared to chitosan microspheres.
The f2 value in acidic pH was 68.95 which showed that there is no significant difference between the profiles when the release was performed in gastric media. Modification of chitosan does not alter its solubility in gastric media. The f2 value of 38 in intestinal media indicates a significant difference in dissolution profile.

4. CONCLUSION

The grafting of water soluble 2-hydroxyethyl methacrylate onto chitosan has been done successfully. The copolymer was characterized by various analytical techniques. Further the microspheres were prepared using modified chitosan and comparison of drug release from modifiedand unmodified chitosan microspheres was done. Modified chitosan microspheres exhibited faster

dissolution release as compared to chitosan microspheres.

DIRECTIONS FOR FUTURE RESEARCH

These modified chitosan microspheres can be used for enhancing the drug release by providing the controlled release of drugs thereby improving its bioavailability. The future research of these microspheres can be extended for its site specific targeting. The present research on modified chitosan microspheres strongly supports its potential as a versatile and effective drug delivery system for *in-vivo* studies which can be further thoroughly studied.

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