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Synthesis and Characterization of Chitosan based Interpenetrating Network Microgels for Controlled Release of Olmesartan

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Abstract: The aim of this present study was to develop a pH sensitive interpenetrating polymeric network (IPN) hydrogels (HG) based on chitosan-acrylamide-*grafted*-poly ethylene glycol followed by hydrolysis that are cross-linked with glutaradehyde Olmesartan is an antihypertensive drug was successfully loaded in these IPNHG's. The HGs formed were characterized by Fourier transform infrared spectroscopy (FTIR) to confirm the grafting and cross linking response, X-ray diffraction (XRD) to monitor drug encapsulation in the polymer matrix. HG's gels were evaluated for swelling index, drug content, *in–vitro* release performed in both acidic and basic conditions. The IPN matrix in this study was able to extend the release of Olmesartan more than 12 hours.
Keywords: Chitosan, poly ethylene glycol, micro gels, cross-linking, controlled release, Olmesartan.

Introduction

Hypertension is a chronic medical which affects approximately one billion people worldwide. The renin– Angiotensin aldosterone system (RAAS) modulates blood pressure[1] and it is the major system associated with hypertension. Many classes of antihypertensive medication are developed to act on RAAS. Angiotensin's II receptor blockers (ARBs) have been designed to inhibit the binding of angiotensin II (AII) onto the G-protein coupled AT1 receptor, and consequently decrease blood pressure. Apart from complications such as stroke, ischemic heart disease, vascular remodeling and diabetic nephropathy, AII is associated also with inflammation, oxidative stress and cell growth. Olmesartan medoxomil belongs to the antihypertensive class of ARBs. This drug is an ester prod rug of the active metabolite[2], which is DE esterified in the gastrointestinal tract. The IUPAC name of this active metabolite Olmesartan is 5-(2-hydroxypropan-2-yl)-2- propyl-3-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] imidazole- 4-carboxylic acid. Olmesartan medoxomil is commonly prescribed with a thiazide diuretic in general, hydrochlorothiazide (HCT)) and/or a calcium channel blocker to ameliorate its effect. The Food and Drug Administration (FDA) approved Olmesartan medoxomil on April2002[3], which was the seventh drug in the class of ARBs. It has been marketed as an antihypertensive drug in United States, Japan and European countries. The most recent approval by FDA was announced on July 2010 for Tribenzor(Olmesartan medoxomil, amlodipine, hydrochlorothiazide), a new three-in-one combination product

for the treatment of hypertension, which contains an ARB, Calcium channel blocker and a diuretic. Although Olmesartan belongs to ARB class, it is proposed that its pharmacological profile is distinct from the others. Generally, not only pharmacological similarities but also differences are observed among different ARBs. The blends of hydrophilic polymers are sensitive to the condition of the surrounding environment and this property has been exploited in many fields such as medicine, pharmacy, biotechnology etc. These blends are obtained from the macromolecular networking either by physical (complexation or by aggregation which resulted entangled networks) or by chemical (covalently) links. These covalent links are developed by the addition of cross-linkers such as glutaraldehyde (GA), formaldehyde, genipen, N,N–methylenebis acrylamide or by ionizing radiations The cross-linkers that were used need a purification step during manufacturing to remove the unreacted cross-linker which is sometimes very difficult[4].Interpenetrating polymeric network (IPN) hydrogels were prepared by varying the concentration of cross-linking agent (glutaraldehyde)[5]. The amount of chitosan (CS), poly acrylic acid (PAA)[6], poly vinyl pyrrolidone (PVP) and N, N-methylenebisacrylamide were kept constant in all the formulations of Clarithromycin in different formulation of IPN hydrogels. Spherical, semi-interpenetrating polymer network beads of chitosan and glycine, cross-linked with different concentrations of glutaraldehyde[7] were prepared for controlled release of Chlorphenramine maleate drugs. An IPN composed of polyvinyl alcohol (PVA) and PAA was used to release Theophylline in controlled manner using polymeric material comprising an IPN network of a polyol (allyl carbonate)[8]The aim of the present study was to i) formulate Olmesartan microspheres based on cross-linked chitosan and acryl amide-grafted-poly ethylene glycol, ii) characterize by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscopy(SEM),*in- vitro* study to evaluate pH-sensitive controlled release of Olmesartan from microspheres at different conditions.

Experimental

Method and Materials

Apparatus:

The FTIR spectra were recorded on an FTIR system (JASCO-4100, Japan) using KBr disc in the scanning range of 4000 - 400 cm^{-1}, electronic weighing balance (model AUY220, Shimadzu, Japan),X-ray diffraction (XRD) (Bruker D8 Advance diffractometer, Germany, CuKά radiation, Nickel filter) were performed to determine crystalline patterns of the drug in the cross-linked polymer network. Differential scanning calorimeter (DSC Q1000V9.4 Build 287) was used to find out drug loading in the polymeric system. The particles size of the microsphere was measured by Raman spectrometer (Lesser 536 Aglitron USA) equipped with camera-microscope system. The surface and cross sectional morphologies of the microspheres were examined using high resolution scanning electron microscopy (FEI Quanta FEG 200).

In-vitro drug release studies were performed using USP paddle single stage digital apparatus with 900 ml of phosphate buffer (pH 7.4) and HCl/KCl buffer (pH 2), absorbance measured by using spectrophotometry (model-V-670PC, JascoUV-visible-NIR spectrophotometer). Statistical analysis was performed using Origin 7.0 software.

Reagents:

OlmesartanUSP was obtained as a gift from Kosher Pharmaceuticals, India. High molecular weight chitosan poly (D-glucosamine) was purchased from Sigma–Aldrich, Belgium; acryl amide was purchased from Qualigens Mumbai, India; while polyethylene glycol 4000, hydrochloric acid, glutaraldehyde, acetic acid and sodium hydroxide were purchased from Sd Fine Chemicals, India. All other chemicals used in this work were of analytical reagent grade.

Synthesis of polyethylene glycol grafted –acrylamide

Polyethylene glycol 4000 was dissolved in water at 60-65^{o}C and treated with acryl amide under nitrogen gas atmosphere[9] followed by addition of trace quantity of potassium persulfate under continuous stirring at a temperature of 65^{o}C for 5 h.The resulting product was washed with water: methanol (1:1 v/v), filtered and kept in vacuum for drying at 60^{o}C. About 2 wt % (100ml) solution of PEG-grafted acryl amide was hydrolyzed using 50 ml of equimolar concentration of sodium hydroxide solution at 60^{o}C for 5 h. The final

product was filtered and dried under vacuum at 60^{o}C.

Drug loading

Approximately 2.0 g of the polymer blend obtained above was dissolved in 2 % acetic acid (v/v) containing a required amount of Olmesartan[10] (100 mg) and was dispersed with equal quantities of light liquid paraffin stirred at high speed and the resulting water-oil emulsion was stabilized by addition of 1 % Tween 80 solution. Thereafter, the aqueous phase of the emulsion was hardened to form microgels. Glutaradehyde (5ml) was added drop-wise to the emulsion and stirred for 30 min at room temperature to stabilize the prepared microsphere. The mixture was then left to cool at 10-15^{o}C for 30 min. The microspheres were collected by filtration using Whatman filter paper 41 of pore size 25µm. Finally, the microspheres were washed with 30ml of hexane and water to remove paraffin oil, acid, water and excess glutaraldehyde after cross-linking with glutaraldehyde. In order to determine the drug release characteristics of the micro gels, different formulations were prepared by varying amount of drug and blend ratio of chitosan with PEG, PEG-grafted copolymer as well as hydrolyzed PEG-grafted copolymer matrices. Drug concentration with different polymeric blends and their formulation codes are given in Table 1. Formulations containing varying Olmesartan and polymeric blend concentrations were prepared and studied.

Fourier transforms infrared spectroscopy (FTIR)

The FTIR spectra of Olmesartan and drug-loaded microsphere were recorded on an FTIR system (JASCO-4100, Japan) using KBr disc in the scanning range of 4000 - 400 cm^{-1} in order to assess structural changes that could have occurred in the drug due to drug-polymer interaction following microsphere formation.

Evaluation of water content and swelling ratio of microspheres

The microsphere formulations (beads) (50 mg) were each placed in 50 mL of distilled water and at 30 min intervals, beads were taken out, excess water removed from them using filter paper and weighed immediately on an electronic weighing balance (Model AUY220,Shimadzu, Japan). Swelling ratio was expressed as % weight gain.

X-ray diffraction studies

X-ray diffraction (XRD) studies (Bruker D8 Advance diffractometer, Germany, CuKά radiation, Nickel filter) were performed to determine crystalline patterns of the drug in the cross-linked polymer network. The XRD patterns of pure Olmesartan drug-loaded chitosan microspheres and blank microspheres were recorded and compared.

Scanning electron microscopy studies (SEM)

The surface and cross sectional morphologies of the microspheres were examined using high resolution scanning electron microscopy (FEI Quanta FEG 200).

***In-vitro* drug releasestudies**

In-vitro drug release studies were performed using USP paddle single stage digital apparatus with 900 ml of phosphate buffer (pH 7.4) and HCl/KCl buffer (pH 2) respectively. It is the mixture of hydrochloric acid and potassium chloride solution (50 mL 0.2M KCl+13mL of 0.2M HCl) and is made up to 200ml with distilled water as dissolution medium. At predetermined time intervals, samples (5ml each) were withdrawn from the medium and the medium was replenished immediately with the same volume of fresh dissolution medium to maintain sink conditions. The amount of dissolved drug in the sample taken was measured (after filtration through Whatman filter paper 4 of pore size 25µm spectrophotometric ally atλ_{max} of 288 nm. Data obtained from *in vitro* release studies were fitted to Higuchi model to find out the mechanism of drug release from the chitosan microsphere. The tests were carried out in triplicate.Aliquots were withdrawn, and assayed for Olmesartan content spectrophotometrically.

Determination of drug content

A sample of the formulation (approx. 100 mg) was weighed accurately, transferred quantatively into

a 100 mL volumetric flask and diluted up to mark using distilled water. The mixture was stirred overnight to allow total release of the drug from the microspheres. After filtration through Whatman filter paper 41 of pore size 25µm, the filtrate was assayed spectrophotometrically at λ_{max} 264 nm.

Statistical analysis

Statistical analysis was performed using Origin 7.0 software. All the tests were run in triplicate and the data were analyzed by one-way ANOVA for drug release. Statistical significance was set at $p< 0.05$.

Results

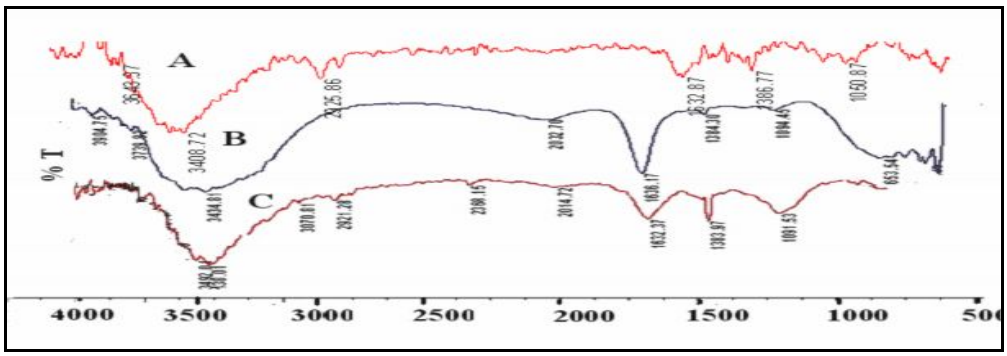

Figure 1 FTIR spectra of (1a) hydrolyzed PEG-g–copolymer (1a A), PEG-g–copolymer (1a B) and plain PEG (1a C)

The FTIR spectra of plain PEG, PEG-grafted copolymer and hydrolyzed PEG-grafted copolymer are shown in Figure 1.The broad band appearing at 3234.09 cm^{-1}corresponds to the associated –OH stretching vibrations of the hydroxyl group of the grafted copolymer (Fig. 5.1a-A). A new peak appeared at 3019.24 cm^{-1} and the related peak at 1522.35cm^{-1} corresponds to –NH bending vibrations of the primary amides of acryl amide. In the spectra of hydrolyzed PEG-grafted copolymer, the shoulder peak (826 cm^{-1}) disappeared but two new peaks appeared around 1265 cm^{-1}and1061cm^{-1}, respectively, which are due to the anti-symmetric vibrations of –COOH groups.

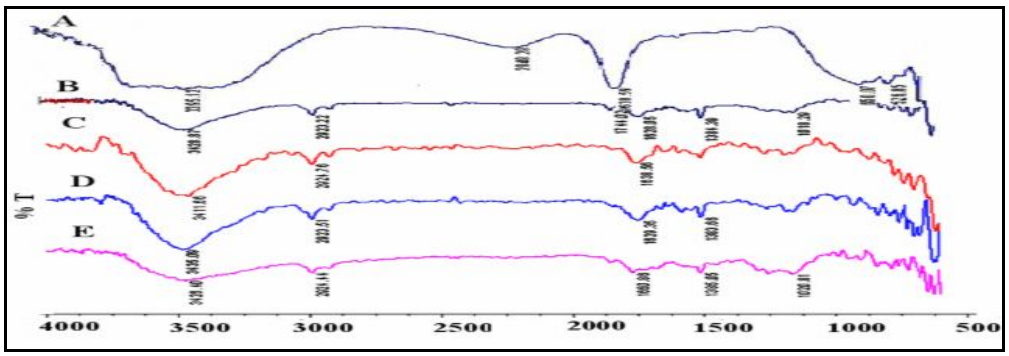

Figure 2: FTIR spectra of A) Chitosan blend with hydrolyzed poly ethylene-g-acryl amide. B) Cross linked Chitosan, C) Chitosan blend with poly ethylene, D) Chitosan blend with poly ethylene-g-acryl amide, E) pure Chitosan

The FTIR spectrum of plain Chitosan showed two peaks around 910cm^{-1} and 1218cm^{-1} corresponding to saccharine structure (Figure 2). The observed sharp peaks at 1356 cm^{-1} and 1508cm^{-1} are assigned to -CH_3 group. A broad band appearing around 1105cm^{-1} indicates the C-O stretching vibration of Chitosan. Another band appearing around at 3486cm^{-1} is due to amine N-H symmetric stretching vibration. A new peak appeared at 1604cm^{-1} due to imine bonds (-C-N) as a result of cross linking reaction between amino

groups in Chitosan and aldehydic group in the glutaraldehyde (Figure 2). In spectra (c), blends of Chitosan with poly ethylene glycol, ether linkage observed at 1150cm^{-1}, In spectra (d), two distinguishing peaks at 1420cm^{-1} and 1575cm^{-1} In spectra (e), Chitosan hydrolyzed complex observed around 1575-1590cm^{-1} due to -NH_2

X-ray diffraction studies

XRD aids in assessing changes in the crystallinity of a drug after formulation or processing. Olmesartan showed characteristic intense peaks at 10and 30^{o} due to its crystalline nature However, these peaks were not seen in the drug-loaded matrix complex, placebo microgels and drug-loaded microgels which indicated that the encapsulated drug became amorphous.

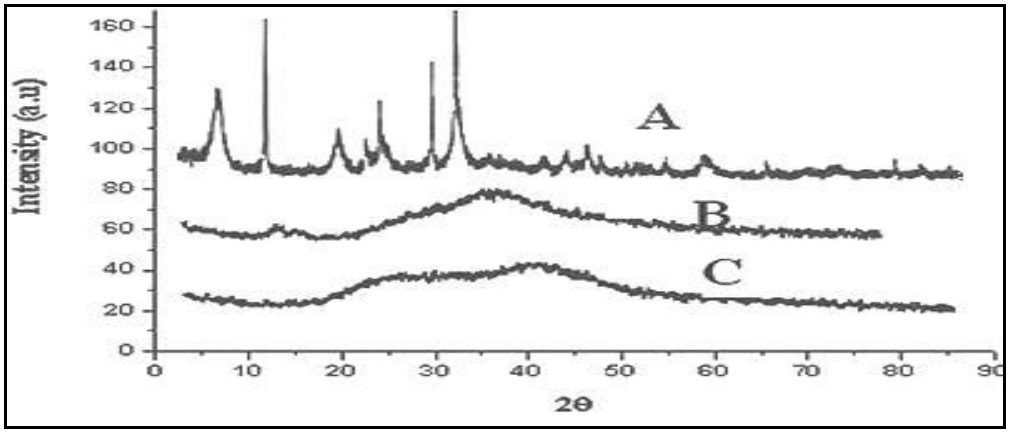

Figure 3 XRD diffractograms of A) Pure drug, B) Drug-loaded chitosan microspheres and C) Blank microspheres

Swelling of microgels

As shown in Table, the equilibrium swelling (%) of plain chitosan microspheres in pH 2 media is higher than that in pH 7.4. Swelling increased from 190% to193% in pH 7.4 media, where in pH 2 media it increased from 181% to 206%. The equilibrium swelling (%) for blend microsphere of chitosan and PEG is higher than that of plain chitosan microspheres. % equilibrium swelling increased from 239% to 240% for the formulation of C-PEG-50 to C-PEG-100 in pH 7.4 media and 216% to 209% in pH 2 media whereas in C-grafted copolymer-50 to C-grafted copolymer-100, equilibrium swelling(%) increased from 278% to 288% in pH 7.4 media and 266% to 277% in pH 2 media. Equilibrium swelling (%) increased from 279% to 288% in pH 7.4 and 266% to 277% in pH 2 for the formulation containing C-grafted copolymer-50 (hydrolyzed) to C-grafted copolymer-100 (hydrolyzed), whereas equilibrium swelling (%) decreased from 294% to 291% in pH 7.4 and 261% to 248% in pH 2.

Table1: Encapsulation efficiency and equilibrium swelling of Olmesartanformulation

Formulation code	Drug Loading (%)	Encapsulation Efficiency (%)*	Particle size(µm)	pH 2 (%swelling)	pH 7.4 (%swelling)
C-50	50	72.42±0.2	59 ± 0.2	181	190
C-100	100	81.02±0.8	73.3±0.5	206	193
C-PEG-50	50	82.96±0.4	92.9±0.9	239	259
C-PEG-100	100	89.19±0.1	107±0.4	240	268
C-grafted copolymer 50	50	89.03±0.3	109±0.2	251	269
C-grafted copolymer 100	100	90.28±0.5	118±0.2	262	273
C-grafted copolymer 50 (hydrolyzed)	50	93.04±0.2	120±0.5	266	279
C-grafted copolymer-100 (hydrolyzed)	100	95.01±0.9	129±0.1	277	288
C-grafted copolymer-100 (hydrolyzed)-(2.5GA)	100	91.4±0.5	130±0.1	261	294
C-grafted copolymer-100 (hydrolyzed)-(5.0GA)	100	84.9±0.8	117±0.1	249	298
C-grafted copolymer-100 (hydrolyzed)-(7.5GA)	100	79.9±0.2	110±0.11	248	291

SD = standard deviation; *Mean ± SD (n = 3); GA = glutaraldehydecross-linked; C = chitosan.

SEM Studies

Surface morphology of polymeric complex was studied under a scanning electron microscope (SEM). The micro gels are spherical and polymeric materials are seen around the micro gels. Blending of micro gels with polymeric materials has no effect on surface properties as shown in Figure 4.

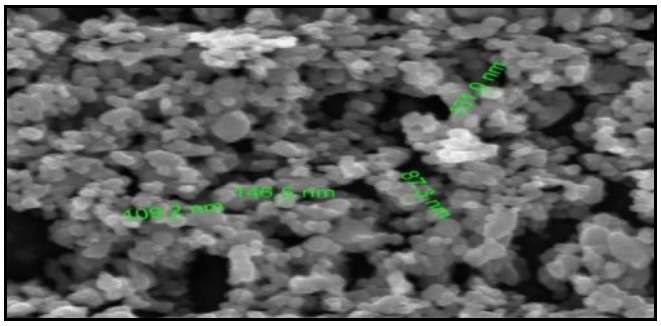

Figure 4 SEM micrograph of micro sphere

***In- vitro* drug release**

In order to study the usefulness of using the modified PEG blends with chitosan as a hydrophilic drug carrier Olmesartan drug-loaded microsphere were prepared by w/o emulsion method. As shown in the figure compares the release profiles of chitosan with all other blends. In both pH media drug release was found to be much faster in the case of plain chitosan than the polymeric matrix in pH 2. The release of drug is almost complete within 10 h in pH 2 whereas in pH 7.4 release of drug is about 43% within the same time period. Thismay be due to blending of different polymer with chitosan and release rate of blended micro gels get much delayed in comparison with plain chitosan. Thus drug release depends upon the nature of the polymer matrix as well pH media.

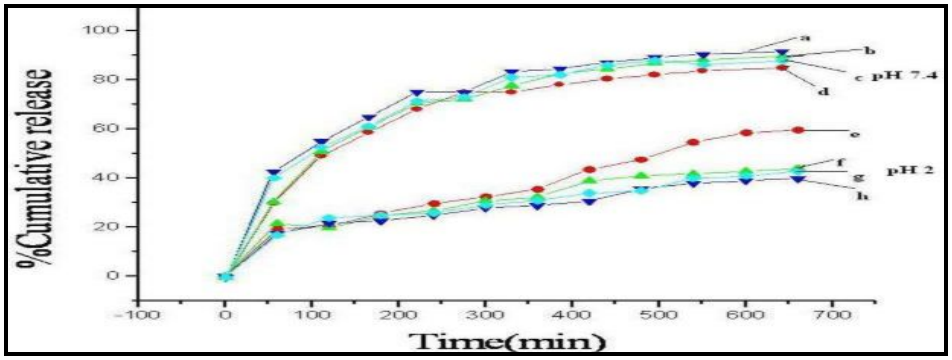

Figure 5 Cumulative release of drug (%) vs. time for chitosan-grafted PEG (hydrolyzed) encapsulated with different amount of drug at pH 7.4.and pH 2

Conclusion

The hydrophilic nature of polyacrylamide modified poly ethylene glycol was used to synthesize microgels blended with chitosan. The blend microgels showed favorable controlled releases *i.e.* release rate was more than 10 h. These blended microgels could be used for controlled release of Olmesartan. Further research works warrant its practical applications *in vivo*.

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