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Original Article

MICROWAVE ASSISTED NANOPARTICLES FOR DRUG DELIVERY SYSTEMS

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ABSTRACT

Objective: The aim of this study was to evaluate the prospective of nanoparticles as carriers for an anticancer drug ampicillin in an attempt to increase the drug bioavailability and prolonging the therapeutic effect.

Methods: Chitosan molecule is quite a large molecule with pendent –OH and –NH2 groups. Graft copolymer based on chitosan has been synthesized by grafting acryl amide and poly (ethylene glycol) onto polysaccharide molecule in aqueous medium using microwave irradiation. The formulations were evaluated for swelling properties and *in-vitro* release profile. Furthermore, the nanoparticles were investigated by FT IR, XRD, AFM studies.

Results: In order to achieve efficient polymerization, the concentration of the monomers added should be more than 30%, less than it leads to jelly like material. Precipitation of the polymers occurs only when the pH of the reaction mixture is increased above 6.5. Efficient precipitation occurs when pH reaches to 8. The swelling degree of hydrogel samples was determined at 37° C as function of time, in buffer solutions at pH 4.0 and 9.2, as well as in dilute solutions of calcium chloride, and in distilled water (pH 7.0).It can be seen that the hydrogel swelled the most in acidic pH as compared with basic pH. The drug release profile of ampicillin from the chitosan- acrylamide- PEG hydrogel in potassium di hydrogen phosphate pH of 6.8 at 37° C. The drug release was calculated in the intervals of 1hr and it was found to be 19%, 31%, 39%, 43%, 49%, 50% and 51% respectively.

Conclusion: This shows that 51% of drug was released at the end of 7 hrs it confirms the sustained drug release which is necessary to increase the drug bioavailability and prolonging the therapeutic effect

Keywords: Nanohydrogel, polymerization, Microwave radiation, Ampicillin, Drug Delivery

INTRODUCTION

Chitin, the second most abundant natural polysaccharide, is synthesized by a number of living organisms [1]. Chitin occurs in nature as ordered micro fibrils, and is the major structural component in the exoskeleton of arthropods and cell walls of fungi [2]. Chitosan is an excellent excipient because it is non-toxic, stable, biodegradable, and can be sterilized. These properties also make chitosan a very versatile material with extensive application in the biomedical and biotechnological fields [3]. Chitin and chitosan are biocompatible, biodegradable, and non-toxic, and are anti-microbial and hydrating agents. Chitin and chitosan are easily processed into gels [4][1].Recently, numerous studies have utilized cationic hydrogel systems for gene and antisense therapies and development of viral and non viral vectors of DNA and oligonucleotide delivery [5]. For successful drug delivery application of nanohydrogels, three important aspects should be taken into consideration: (a) protection of the active substance during both storage and use, (b) control of the amount of active substance to be released, and (c) targeting of specific tissues[1, 5, 6].

Chitosan hydrogel have been prepared with a variety of different shapes, geometries, and formulations that include liquid gels, powders, beads, films, tablets, capsules, microspheres, micro particles, sponges, nano fibrils, textile fibers, and inorganic composites [7]. Chitosan has shown to facilitate wound healing, reduce serum cholesterol levels and to stimulate the immune system. Drugs can be easily encapsulated into the nanohydrogels, which is stable enough to circulate in the blood stream over long time periods without allowing much leaching [8].

However, the nanoparticles easily degrade once inside target cells hydrolyzed by enzymes in the cell cytoplasm, releasing the drug being carried. Last, but by no means least, the fragments of the degraded nanohydrogels can be eliminated by the body and show no toxic effects on cells [9]. Nanohydrogels have been developed and studied with regard to their application in several biomedical fields, e.g. separation techniques, soft-actuators and controlled drug delivery systems [10].

The scope of this paper is to explore the use of double emulsions in enhancing the first aspect of nanohydrogels applications, that is, the protection during storage and use. The carrier was synthesized by block polymerization of Acryl amide monomer and poly (ethylene glycol) onto Chitosan using glutaraldehyde as a cross linker. Grafting method initiated by Microwave radiations in aqueous solution. The polymer structures were characterized by FTIR, AFM and XRD techniques. The swelling behaviors were investigated in buffer solutions of varying pH and in salt solutions in terms of time of swelling. The drug ampicillin was loaded and the release behavior of nanohydrogels was studied.

MATERIALS AND METHODS

Chemicals

Chitin (CAS NO: 1398-61-4) [Himedia laboratories private limited, India], Acryl amide [Sisco laboratories private limited, India], poly (ethylene glycol)-4000 [Qualigens fine chemicals private limited, India], Glutaraldehyde 25% LR [Sd fine chemicals limited].

Method

Conventional synthesis of Chitosan

In a typical procedure, an aqueous solution of chitosan (2 g) was prepared by dissolving chitosan by dissolved in 100 ml of 40, 50 and 60% w/w Sodium hydroxide (NaOH) solution at 120 °C in a heating mantle for 7 hours. Solid crystals obtained from each concentration were washed twice with distilled water and finally with acetone, using vacuum distillation pump[11].

Microwave assisted synthesis of Chitosan

Microwave technology is emerging as an alternative energy source powerful enough to accomplish chemical transformations in

minutes, instead of hours or even days. About 2 g of chitin was dissolved in100 ml of 40, 50 and 60% of sodium hydroxide solution and the polymerization reaction was performed in a beaker placed in a domestic microwave oven at a power 480 W. During this process the sample is heated for 2 minutes; after that, the microwave is turned off, leaving the sample for 60s. The cycle is repeated up to 50 minutes. After cooling, the polymers were purified by precipitation with methanol-acetone and washed with distilled water using vacuum distillation pump. The washed samples obtained were placed in the vacuum desiccators for complete removal of the moisture [12, 13].

Characterization of nanoparticles

Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR Analysis was carried out as per the methods of Arunachalam et al, 2013[14, 15]. FTIR was used to characterize the presence of specific chemical groups in the chitosan hydrogel and hybrid networks, reflecting the effectiveness of the developed procedure for producing different nanostructure materials. Fourier transform infrared (FTIR) spectra were recorded between 400 and 4000 cm^{-1} with a 4 cm^{-1} resolution from KBr pellets on a Perkin Elmer Spectrum BX FTIR system. Hybrids were milled and mixed with dried KBr powder. Samples were placed in a sampling cup and 32 scans were acquired at 2 cm−1 resolution with the subtraction of KBr background [16].

X-Ray Diffraction (XRD) Analysis

To characterize the purified silver & gold nanoparticles XRD measurements were conducted using XRD-6000 X-ray diffractometer (Shimadzu, Japan) operated at a voltage of 40kV and 30mA with Cu Kα radiation in θ–2θ configurations. The crystallite domain size was calculated from the width of the XRD peaks by assuming that they were free from non-uniform strains using the following Scherer formula [17].

$$D = \frac{0.94\,\lambda}{\beta cos\theta}$$

where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM) and θ is the diffraction angle**[**18,19,20**].** To expel the added instrumental broadening, the FWHM was corrected using the FWHM from a large-grained Si sample.

$$\beta\ corrected = \left(FWHM^2_{sample} - FWHM^2_{si}\right)_{1/2}$$

This modified formula is valid only when the crystallite size is smaller than 100 nm [21].

Microscopic Evaluation

Visualization by Atomic force microscope

The atomic force microscope (AFM) is ideally suited for characterizing nanoparticles. It works in a similar way like phonometer or profilometer only difference is that AFM use very smaller scale for detection. In it a small tip is dragged across a sample surface and the change in vertical position (denoted by 'z' axis) reflects the topographical image of the surface. By collecting the data for a succession of lines it is possible to form a three dimensional visualization of the surface features and the size of the sample is measured by receiving laser light deflection as it touches the sample [16, 22, 23].

The Nanoparticle sample was first mixed with acetone and then sonicated until the particles get uniformly distributed in the solution. After that a drop of the sample was taken and slide was prepared by spreading it uniformly over the glass slide. The slide was then allowed to dry.AFM image was obtained by PicoScan5100 PicoSPM AFM at room temperature.

Swelling measurement

The swelling measurement was carried out as per the modified methods of Ghasem Rezanejade Bardajee et al., 2011[24].The degree of swelling was determined by routine gravimetric method. A 100 mesh nylon screen containing an accurately dried weighed powdered sample (0.1 ± 0.01 g) with average particle sizes between 40–60 mesh (250–400 μm) was immersed entirely in distilled water (400 ml) and allowed to soak for 60 min at room temperature. The equilibrium swelling (ES) capacity was measured twice at room temperature using the following formula:

$$Es = \frac{W2 - W1}{W1}$$

Where, W1 and W2 are the weights of dried and swollen gel, respectively.

For swelling kinetics, at consecutive time intervals, the water absorbency of the hydrogel was measured according to the mentioned method and for swelling measurements.

***In-Vitro* Drug Release**

0.05 g of powdered nanoparticle was added in 60 ml of tetracycline hydrochloride (TH) solution (0.1mol/l) and put in the dark place for 24 h to complete drug loading. The release rate experiments were performed in a glass apparatus at 25 °C under unstirred conditions in solutions with desired pH. The hydrogel (0.05 g) containing a known amount of TH was added to the release medium (100 ml). At a given time interval, 1ml samples were withdrawn and assayed for the amount of released TH as a function of time. The amount of released TH was determined by UV spectroscopy at 275 nm using a calibration curve constructed from a series of TH solutions with standard concentrations. The results are expressed as released amount (mg/g gel) and cumulative release ratio (amount of released TH/all amount of loaded TH).

RESULTS AND DISCUSSION

FT IR spectroscopy of formulations

For the conventional method, the IR spectrum of the chitosan has strong peak at 3436.9 $cm^{-1}$and for the graft copolymer the peak at 3436.63 cm^{-1}. Amide-I and amide-II bands are observed at 1673.97 and 1613.07 cm^{-1} respectively shown in (figure1), however, these peaks are seen masked with sharp peak present in the chitosan around 1611.70 cm^{-1} in (figure 2).Peak around 1400 cm^{-1} due to C–N stretching in graft copolymer further supports grafting is shown in (figure 4).

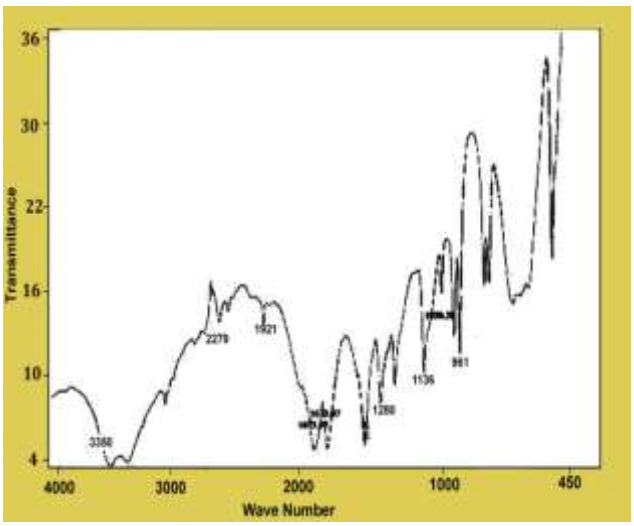

Fig.1: FT- IR spectrum of Acrylamide

X Ray Diffraction spectrometry

The grafting was also supported by XRD analysis. The X-ray diffraction spectra of the grafted chitosan with acrylamide is illustrated in Figure (6), show many crystalline areas between 2θ

22-59° and 65-78° (due to polyacrylamide grafts at the chitosan backbone), while no such peaks are visible in the XRD of the chitosan itself in figure (5) and the grafting of chitosan with acryl amide and PEG show many crystalline areas between 2θ 23- 31° and 74- 81° is shown in figure (7).

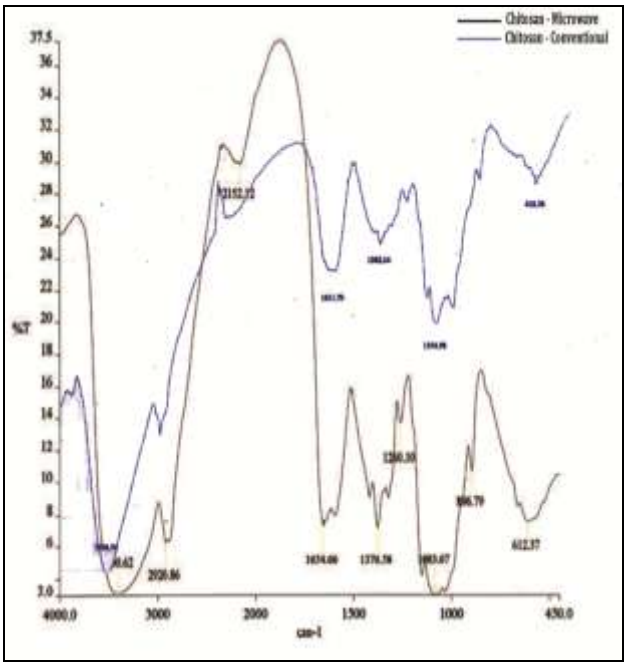

Fig. 2: FT-IR spectrum of Chitosan by conventional and Microwave Method

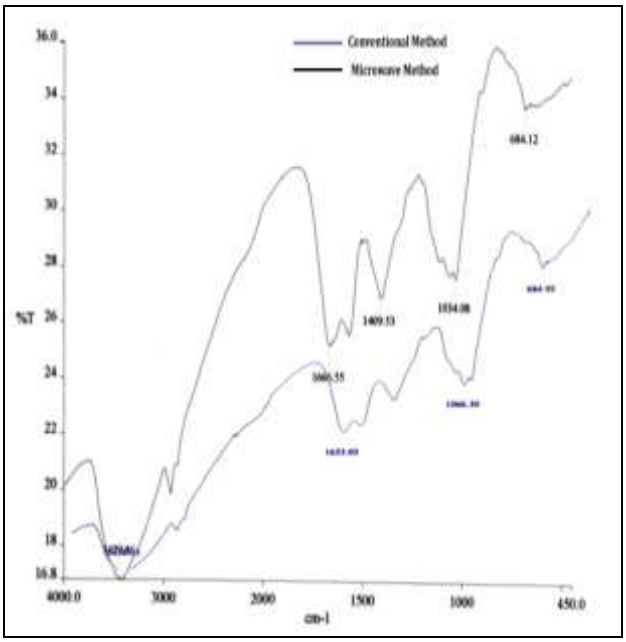

Fig. 3: FT-IR spectrum of Chitosan- g- Acrylamide by Conventional and Microwave method

Visulaisation of Atomic Force Microscopy

The morphology and size of the nano particles can be studied by using the AFM results. The normal and three dimensional structure of the tri block can be shown in figure (8). The size of the particles can be calculated by using line graphs is illustrated in figure (9) and figure (10).

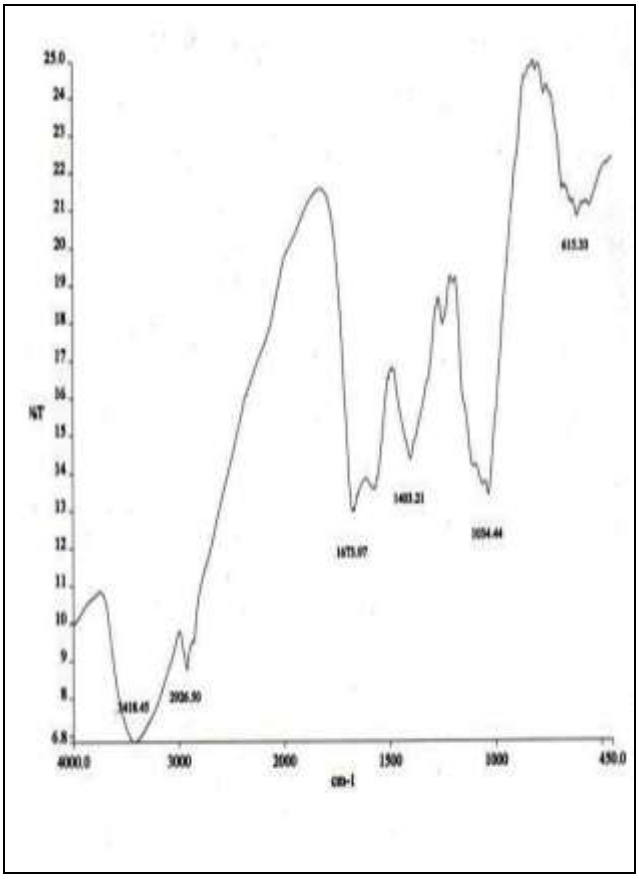

Fig. 4: FT-IR spectrum of Chitosan-g- acrylamide-g-polyethylene glycol

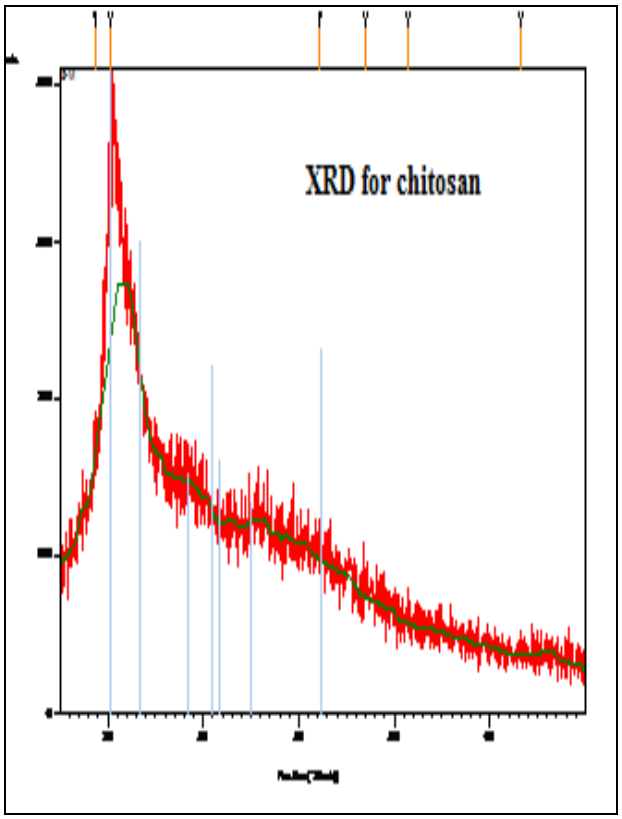

Fig. 5: XRD plot for chitosan (microwave method)

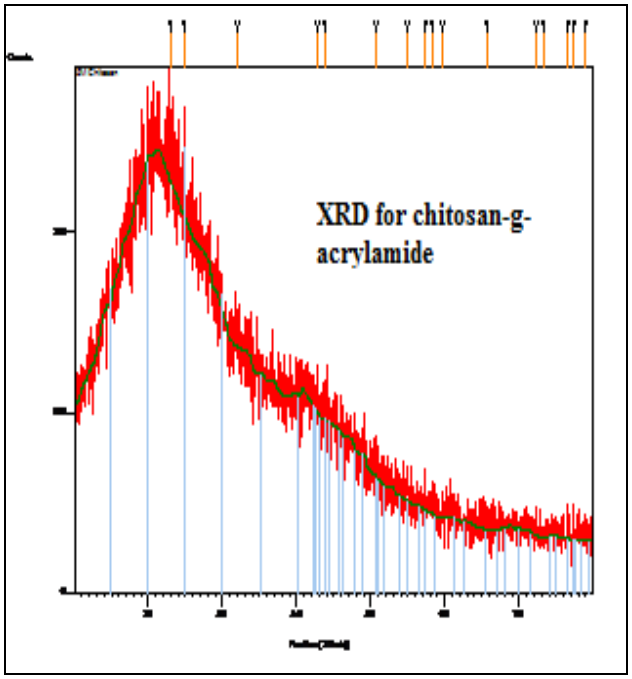

Fig.6: XRD plot for chitosan-g- acrylamide

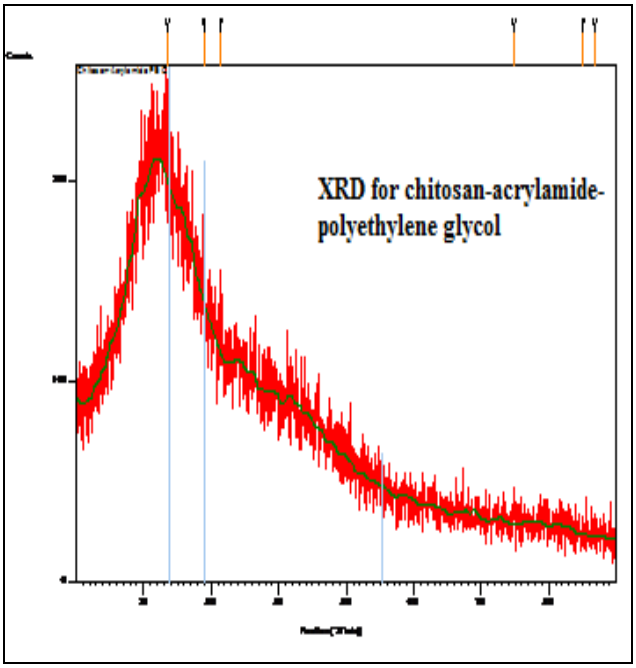

Fig.7: XRD plot for chitosan-g-acrylamide-g-polyethyleneglycol

Swelling profile

The swelling kinetics for four different degrees of grafting was shown in figure (11). For %G = 267 shows a very fast swelling at acidic pH, reaching its maximum in few minutes and decreases with longer time. The quick response of the hydrogel can be attributed to a fast protonation of the amine groups of chitosan. The interaction of NH3 + groups with amido groups on poly acrylamide grafted chains leads to a closed structure allow greater amount of acid solution, at longer times and diffuse to the exterior until reaching the equilibrium. However, it can be noted that the hydrogel network swells quickly and strongly at basic pH and in calcium chloride solutions.

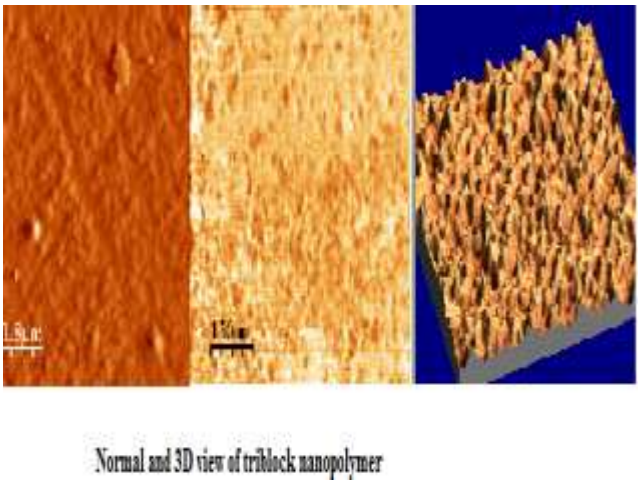

Fig.8: Atomic force microscopy micrographs of chitosan-g-acrylamide-g-polyethyleneglycol

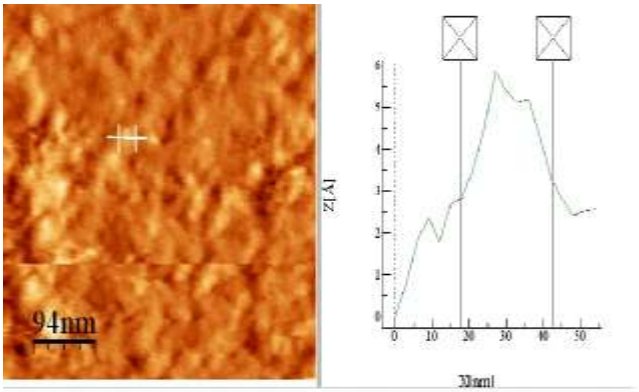

Fig. 9: Atomic Force Microscopy photograph of tri block polymer of 24nm

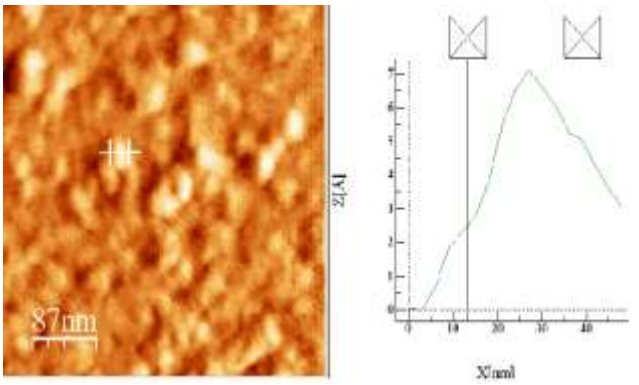

Fig.10: Atomic Force Microscopy photograph of Tri block polymer of 27nm

Drug Release Studies

Figure (12) shows the drug release profile of ampicillin from the chitosan – acrylamide - PEG hydrogel in potassiumdihydrogen phosphate pH of 6.8 at 37° C. The drug release was calculated in the intervals of 1hr and it was found to be 19%, 31%, 39%, 43%, 49%, 50% and 51% respectively. This shows that 51% of drug was released at the end of 7 hrs it confirms the sustained drug release which is necessary to increase the drug bioavailability and prolonging the therapeutic effect.

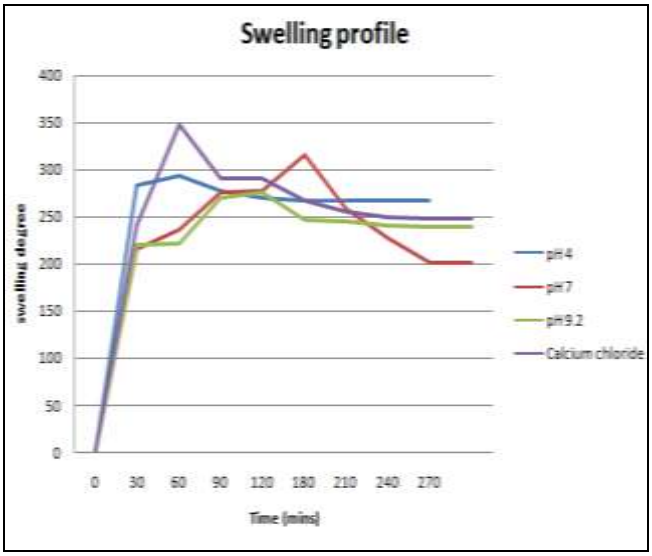

Fig.11: Swelling profile of triblock polymer

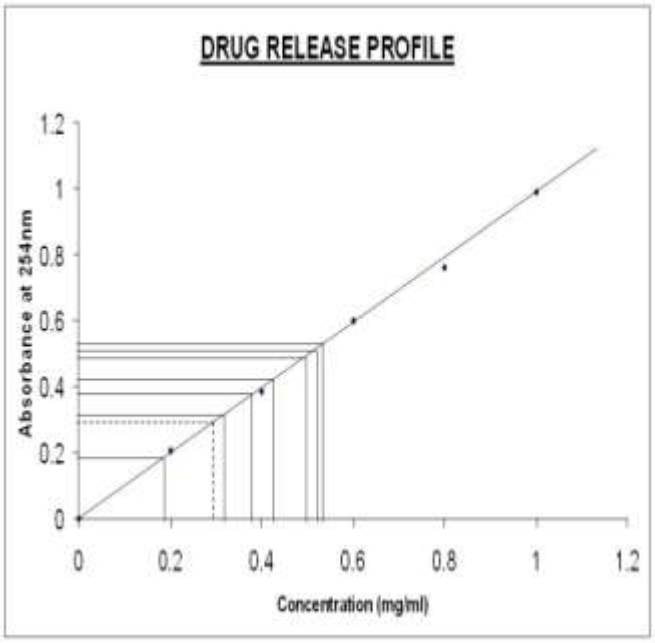

Fig. 12: Ampicillin release profile

CONCLUSION

This work focuses on the design, preparation and *in vitro* characterization of polymeric nanoparticles. Radiation polymerization was selected for their fabrication by means of cross-linking of chitosan, acrylamide and poly (ethylene glycol).This polymerization technique allows for control over size, is flexible in respect to initiation and composition, and proceeds to full double-bond conversion in relatively short times. Incorporation of monomers in the polymeric networks offers the formation of hydrogel. Characterization studies were performed using FT IR, AFM and XRD techniques. Swelling nature was studied in various buffer solutions and salt solution. The particles are visualized as nano scale, three dimensional polymeric networks which allow the accommodation of drugs through hydrophobic interactions was experimentally studied using ampicillin drug. Release studies of the drug were performed using potassium dihydrogen phosphate as solvent. *in vitro* studies shows that drug released at a level of about 51 % at the 7th hour. In conclusion, the nanoparticles presented here are well suited for drug delivery applications, because of the sustained release of the drug.

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DISCLOSURE

The authors declare no conflicts of interest in this work.

REFERENCES

1. Jayakumar R, Prabaharan M, Nair S V, Tamura H, *Novel chitin and chitosan nanofibers in biomedical applications*.Biotechnology Advances, 2010. 28 (1): p.142–50.
2. Ravi Kumar, M.N., *A review of chitin and chitosan applications*. Reactive and Functional Polymers,2000. 46: p.1–27.
3. Bhattarai, N., Gunn, J., Zhang, M., *Chitosan-based hydrogels for controlled, localized drug delivery*, Advanced Drug Delivery Reviews, 2010. 62(1): p.83–99.
4. Gao, D., Xu, H., Philbert, M., Kopelman, R., *Bioeliminable nanohydrogels for drug delivery*, Nano Letters, 2008. 8(10): p.3320–4.
5. Rojas, E.C., Sahiner, N., Lawson, L.B., John, V.T., Papadopoulos, K.D., *Controlled release from a nanocarrier entrapped within a microcarrier*, Journal of Colloid Interface Science,2006.301: p.617–23.
6. Moya-Ortega, M.D., et al*., Cyclodextrin-based nanogels for pharmaceutical and biomedical applications*.International journal of Pharmceutics, 2012. 428: p.152–63.
7. Denkbas, E,B., *Perspectives on Chitosan Drug Delivery Systems Based on their Geometries.* Journal of Bioactive and Compatible Polymers, 2006. 21: p.351–368.
8. Zhang J., et al., *The targeted behavior of thermally responsive nanohydrogel evaluated by NIR system in mouse model.* Journal of controlled release, 2008. 131 (1): p.34–40.
9. Gao, D., et al., *Bioeliminable nanohydrogels for drug delivery.*Nano Letters, 2008. 8: p.3320–3324.
10. 10. Liu, K.H., et al., *Drug release behavior of chitosan-montmorillonite nanocomposite hydrogels following electrostimulation*.Acta Biomaterialia, 2008. 4(4): p.1038–45.
11. Ryu, J.H., et al., *Chitosan-g-hematin: Enzyme-mimicking polymeric catalyst for adhesive hydrogels.* Acta Biomaterialia, 2014.10(1): p.224–33.
12. Tharun, J., *Microwave assisted preparation of quaternized chitosan catalyst for the cycloaddition of CO2 and epoxides*. Catalysis Communications,2013, 31: p.62–65.
13. Singh, V., *Microwave synthesized chitosan-graft-poly(methylmethacrylate): An efficient Zn2+ ion binder.* Carbohydrate Polymers, 2006, 65: p.35–41.
14. Arunachalam, K.D., Annamalai, S.K, Hari S, *One-step green synthesis and characterization of leaf extract-mediated biocompatible silver and gold nanoparticles from Memecylon umbellatum.* Internatinal Journal of Nanomedicine, 2013, 8:p.1307–1315.
15. Arunachalam, K.D., Annamalai, S.K., *Chrysopogon zizanioides aqueous extract mediated synthesis of crystalline silver & Gold Nanoparticles for Biomedical Applications.* International journal of Nanomedicine, 2013, 8: p.2375 – 2384.
16. Mansur, H.S., Oréfice, R.L., Mansur, A., *Characterization of poly(vinyl alcohol)/poly(ethylene glycol) hydrogels and PVA-derived hybrids by small-angle X-ray scattering and FTIR spectroscopy*. Polymer (Guildf),2004, 45: p.7193–7202.
17. Haix, C., Jurado, E.L.D., *Domain size distribution of y-tzp nano-particles using xrd and hrtem*. Image Anaysisand Stereology,2001;*20157-161* 2001: p.157–161.
18. Shameli, K., et al., *Green biosynthesis of silver nanoparticles using Curcuma longa tuber powder.* International Journal of Nanomedicine, 2012, **7**: p.5603–10.
19. Narayanan, K.B., Sakthivel, N., *Green synthesis of biogenic metal nanoparticles by terrestrial and aquatic phototrophic and*

heterotrophic eukaryotes and biocompatible agents. Advances in Colloid and Interface Science, 2011, 169: p.59–79.

20. Shameli, K., et al., *Synthesis of silver nanoparticles in montmorillonite and their antibacterial behavior*. International Journal of Nanomedicine, 2011, 6: p.581–90.
21. Dipankar, C., Murugan, S., *The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from Iresine herbstii leaf aqueous extracts.*Colloids and Surfaces B: Biointerfaces, 2012, 98: p.112–9.
22. Vasnev, V, A., *Synthesis and properties of acylated chitin and chitosan derivatives.* Carbohydrate Polymers, 2006, 64: p.184–189.
23. Mi, Y., Zhao, J., Feng, S.S., *Targeted co-delivery of docetaxel, cisplatin and herceptin by Vitamin E TPGS-cisplatin prodrug nanoparticles for multimodality treatment of cancer.*Journal of Controlled Release,2013.
24. Bardajee, G.R., Pourjavadi, A., Soleyman, R., *Novel nano-porous hydrogel as a carrier matrix for oral delivery of tetracycline hydrochloride*. Colloids Surfaces A: Physicochemical Engineering Aspects, 2011, 392: p.16–24.