IJBPAS, November, 2022, 11(11): 5414-5426

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

pH/TEMPERATURE CHANGE INDUCED GELATION OF INJECTABLE CHITOSAN/N-ISOPROPYL ACRYLAMIDE LIQUID SYSTEM FOR TARGETED DELIVERY OF CISPLATIN FOR ANTI-TUMOR ACTIVITY: A PILOT STUDY

AHUJA S, PATEL A, PATEL M, TAILOR K AND HARSHITA

Department of Chemistry, Parul Institute of Applied Sciences, Parul University

Limda-391760 Waghodiya, Vadodara, Gujarat, India

***Corresponding Author: Dr. Sonam Ahuja: E Mail: sonam.ahuja82106@paruluniversity.ac.in**

Received 28th Dec. 2021; Revised 26th March 2022; Accepted 16th May 2022; Available online 1st Nov. 2022

https://doi.org/10.31032/IJBPAS/2022/11.11.6599

ABSTRACT

In this work, we have used a combination of stimuli responsive polymers, namely Chitosan (Ch) and poly-N-isopropyl Acrylamide (poly(NIPAAm)),as a injectable liquid mixture to deliver the entrapped anti-cancer drug Cis-Platin(CP) at the simulating tumor environment(STE), with minimum drug release in the blood environment. A solution, containing Ch, poly (NIPAAm) and CP was exposed to simulating blood environment (SBE) and then to the simulating tumor environment (STE) at 37^0C. It was found that injectable liquid mixture was co-precipitated with minimum release in SBE while a 14 times higher release was observed in STE, thus indicating that liquid mixture was successful to deliver most of the drug at the tumor site with minimum release in the blood stream. The combination of chitosan and poly (NIPAAm) appears to be a injectable system that could undergo sol-gel transition on exposure to pH and/or temperature variations.

Keywords: Cancer, Tumor, injectable hydrogels

1. INTRODUCTION

Recently, there have been great efforts to fabricate injectable hydrogels, that could deliver the anti-cancer drug at the tumor site. These biocompatible and biodegradable injectable hydrogels are usually prepared by either *in situ* polymerization or the sol–gel phase transition **[1]**. Apart from targeted deliver, they have also been used as cell carriers and as scaffolds for tissue regeneration **[2]**. These drug-loaded injectable hydrogels are, in fact, liquid mixture with good flowing property. When they are injected into blood stream, they undergo sol-gel transition due to changing simulating conditions of temperature and/or pressure, thus releasing the entrapped biologically active ingredient at the targeted site **[3]**.These injectable hydrogels are better options as compared to the surgery of tumors. After injecting them in the blood stream, they undergo gelation via various approaches like thermal gelation, ionic complex, self-assembly, chemical cross linking and photo polymerization **[4]**. In recent past, there have been tremendous work do on the preparation and function of these injectable hydrogel systems **[5]**. Due to biodegradable and biocompatible nature, bio-polymers have been the first choice for fabrication of injectable hydrogels **[6]**. Like cellulose and starch, chitosan is also reported to be the one of the most abundant biopolymers. It is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) **[7]**. However, chitosan is only soluble in the presence of an acid, which restricts its application. The specific property of chitosan to dissolve only in acidic pH makes it susceptible to phase transition on exposure to change in pH from acidic to alkaline one **[8]**. Hydrogels, undergoing volume phase transition with change in physical parameters like pH, and temperature, find tremendous applications in biomedical fields **[9]**. Indeed, pH and temperature are the most significant parameters related with the physiology of human being **[10]**. Poly (N-isopropylacrylamide) (PNIPAm) is one of the most well-known temperature sensitive polymers with a lower critical solution temperature (LCST) at 32°C around body temperature **[11]**. The combination of chitosan and poly(NIPAAm) appears to be a smart hydrogel system that could undergo sol-gel transition on exposure to pH and/or temperature variations. The aim of this work is to develop cyto-compatible pH/thermo-responsive injectable hydrogels based on

Chitosan and poly (N-isopropyl acrylamide) for delivery of Cisplatin to target tumor site. In this novel work, a liquid mixture of Ch, poly(NIPAAm) and drug CP has been used as tumor targeted delivery system . The work is a hypothetical approach to investigate the fact that Ch/poly(NIPAAm)/CP liquid mixture release the minimum drug in the blood environment and maximum release could be obtained at Tumor environment.

2. Experimental

2.1 MATERIALS AND METHOD

Chitosan (CS, molecular weight 120000) with a degree of de-acetylation of 93% and N-isopropylacrylamide (PNIPAm) were obtained from Sigma, U.S.A. The initiator potassium persulfate (KPS), and other chemicals used to prepare physiological fluid were obtained from E. Merck, Mumbai, India. The double distilled water was used throughout the investigations. The anticancer drug Cisplatin (CP) (Cisplatin injection BP 10mg/10mL, CSI1414AC) was obtained from a local medical shop.

2.2. Preparation of un-crosslinked poly-(N-isopropyl acrylamide)(poly(NIPAAm)

The poly(NIPAAm) was prepared by free radical polymerization of monomer N-isopropyl acrylamide (NIPAAm). In brief, definite amounts of monomer NIPAAm and initiator KPS were dissolved in distilled water and one drop of TEMID was added to accelerate the reaction. Now, the reaction mixture was placed in electric oven (Tempstar) at 60^0C for a period of 2 h to ensure complete polymerization. The product poly (NIPAAm) was filtered and kept in a dust free chamber for further use.

2.3. Preparation of chitosan/ poly(NIPAAm) solution

A definite amount of chitosan was dissolved in 0.5 % acetic acid solution, followed by addition of a pre-calculated quantity of poly(NIPAAm) under normal stirring so that both the polymers dissolved completely to give a transparent solution. We shall call this solution asCh/poly(NIPAAm) solution .In order to prepare drug loaded solution, a pre-calculated quantity of drug Cisplatin was also dissolved in the above solution.

2.4. Preparation of Injectable Hydrogel

10mL of Ch solution was mixed with 10mL 2 % Poly(NIPAAm) solution and then 10mg/mL Cp was added to this solution at 27^OC(probable pH 5).

2.5. Investigation of thermal and pH induced precipitation

This investigation was carried out in following 6 steps.

(1) 0.1 ml of Chitosan solution was poured into 25 ml of physiological buffer of pH 7.4.

The pH-induced precipitation of chitosan took place. The turbidity of the precipitated colloidal solution was measured.

(2) 0.1 ml of poly(NIPAAm) solution was poured into 25 ml of buffer pre-maintained at 37^0C. The thermal-induced precipitation occurred which was measured by recording turbidity.

(3) 0.1 mL of each chitosan and poly(NIPAAm) solutions were mixed in to 25 mL of pH 7.4 solution at 37^0C (mimicking human blood environment) and the turbidity was recorded.

(4) 0.1 mL of each chitosan and poly(NIPAAm) solutions were mixed in to 25 mL of pH 4.0 solution at 37^0C (mimicking tumor environment) and the turbidity was recorded.

(5) 0.2 mL of hydrogel were mixed in to 25 mL of pH 7.4 solution at 37^0C (mimicking human blood environment) and the turbidity and drug release was recorded.

(6)0.2 mL of hydrogel were mixed in to 25 mL of pH 4.0 solution at 37^0C (mimicking tumor environment) and the turbidity and drug release was recorded.

2.6. Characterization of (Ch/poly(NIPAAm) composite particles

The size of the Ch/poly(NIPAAm) composite particles was determined using Transmission Electron Microscopy(TEM) at Bombay IIT, Mumbai. The FTIR spectrum of composite particles was carried out using Shimadzu spectrophotometer (8400, Japan) by KBr pelleting method.

2.7. Measurement of drug release

The amount of drug CP released in blood and tumor environments were measured by Uv-Vis spectrophotometer (Thermo Spectronic) at 778 nm. The transformation of absorbance into concentration was done using Lambert-Beer law obtained for a series of solutions of known concentrations **Figure 1**.

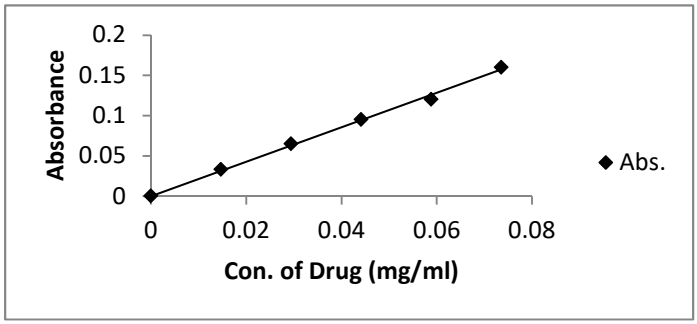

Figure 1: Lambert-Beer law obtained for a series of solutions of known concentrations

3. RESULTS AND DISCUSSION

3.1. Mechanism of Cisplatin

In an aqueous environment, the chloride ligands of cisplatin are replaced by water molecules generating a positively charged electrophile. This electrophile reacts with nucleophilic sites on intracellular macromolecules to form DNA, RNA, and protein adducts **[12]**. Following mechanisms showing in **Figure 2**.

3.2. Preparation of poly (N-isopropyl acrylamide)

The free radical initiated polymerization of poly(N-isopropyl acrylamide) can be described as shown below(**Figure 3**).

Furthermore, poly (N-isopropyl acrylamide) is fairly soluble at room temperature but gets precipitated at 32^0 C, which is its Critical Solution Temperature (CST) **[13]**. Macromolecular chains of uncross linked pol(NIPAAm) are shown at room temperature and above 32^0C respectively. It is clear that at room temperature polymeric segments are in the fully relaxed state thus showing complete dissolution. However above the temperature of 32^0C the hydrophobic interactions become predominant and the polymer is in precipitated state, as shown in the following **Figure 5**.

It has been reported that production of lactic acid under anaerobic conditions and the hydrolysis of ATP in an energy-deficient environment contribute to the acidic microenvironment which has been found in many types of tumor **[14]**. Considering that environment of tumor is acidic with pH around 4, we made a strategy to make use of these two polymers to prepare injectable drug carrier with sol-gel property. This sol-gel injectable drug carrier could be targeted to deliver most of the entrapped drug at tumor site, with least effecting healthy cells.

3.3. Prediction for the pilot study

We predicted that if an aqueous solution of mixture of chitosan (Ch), poly(NIPAAm) and anticancer drug Cisplatin (CP) is injected in to blood stream, the Ch and poly(NIPAAm) shall be co-precipitated along with drug, entreated within the precipitated mass since temperature of blood is 37^0 C (thermal gelation of poly(NIPAAm) takes place) and its pH is 7.4 (at which chitosan gets precipitated).The poly(NIPAAm) and Chitosan shall remain as drug loaded composite particulates. When these particulates travel along the blood stream and reach the tumor site, the acidic pH of site causes chitosan to dissolve. As a result drug Cisplatin is released appreciably. In this way the

Ch/poly(NIPAAm)/ Cp) system can be used to deliver drug around tumor environment with minimum release in the blood stream , thus protecting healthy cells. The following scheme-I illustrates the overall strategy of the proposed drug delivery system **Figure 6**.

Figure 7(a) shows a clear image of Ch solution in 0.5% glacial acetic acid at room temperature along with turbidity, when 0.1 ml of this solution is mixed into 25 mL of pH 7.4 buffer, there is precipitation of Ch due to pH-induced phase change and the images also show of precipitated Ch solutions, along with respective turbidity.

Similarly, **Figure 7(b)** shows clear solution of poly (NIPAAm) at room temperature. Addition of 0.1mL of this solution into pH 7.4 buffer at 37^0 C results in precipitation. Their respective turbidities are also shown along with the image. Finally the mixture of Ch/PNIPAAm is shown in **Figure 7(b)** at room temperature and at 37^0C.

3.4. Preparation of injectable hydrogel

10mL of Ch solution was mixed with 10mL 2 % Poly(NIPAAm) solution and then 10mg/mL Cp was added to this solution at 27^OC (probable pH 5), this is shown in **Figure 8**.When 0.2 mL of Ch/Poly(NIPAAm)/Cp solution is mixed into 25 mL of pH 7.4 at 37^0 C, both of the polymers namely Ch and Poly(NIPAAm) and drug get precipitated **Figure 9(a)**.

This co precipitated solution centrifuged at high speed and then 0.2 mL of this centrifuged solution was added in to 25 mL of pH 4 buffer at 37^0C, (Tumor environment), the poly(NIPAAm) gets precipitated but Ch still remains in the dissolved state **(Figure 9(b))**.

3.5. Interpretation of data

The turbidity data given in above experiments support our hypothesis that mixture of Ch/Poly(NIPAAm)/Cp give low turbidity in pH 4.0 at 37^0 C (i.e. Simulating Tumor Environment) and show maximum turbidity in pH 7.4 at 37^0 C (Simulating Blood Environment). Therefore, it may be concluded from precipitation experiment that there is possibility that drug loaded composite particles shall release minimum drug in blood stream and they shall deliver the active ingredient in tumor environment. In this way the targeted release of cis-platin at tumor site can be achieved without affecting the healthy cells.

3.6. Characterization of composite Ch/poly(NIPAAm) particles

3.6.1. FTIR Analysis

The FTIR spectrum of poly (N-isopropyl acrylamide) /chitosan composite particles shows N-H stretching of secondary

amide of chitosan at 3585.79 cm^{-1}. In addition, asymmetric and anti-symmetric stretching of C-H bond of poly (N-isopropyl acrylamide) and C-H stretching of $–CH_2$ groups of chitosan are indicated at 2816.16 and 2731.29 cm^{-1}. Similarly, peaks around 1666.55 and 1552.75 cm^{-1} indicate presence of amide I and amide II group of chitosan and N-H stretching of amide II bond of poly (N-isopropyl acrylamide) .1386.86 and 1356.00 cm^{-1} peaks indicate the C-H bending of chitosan and anti-symmetric deformation of Isopropyl group of poly (N-isopropyl acrylamide).Further, 1085 cm^{-1} peak indicates –C-O- stretching of chitosan **Figure 10**.

3.6.2. TEM analysis

Figure 11 shows images of Ch/poly(NIPAAm) composite particles .It can be seen that co-precipitated particles do not have uniform size, but exhibit a size range of 40 to 90 nm. Such a smaller size range is attributable to the fact that in the medium of pH 7.4 and temperature of 37^0C, the co-precipitated particles are in shrunken state due to thermoreversibility of one component poly(NIPAAm). This particle size is quite satisfactory to have obstacle-free flow in the blood stream.

3.7. Cisplatin Release study **Figure 12** shows amount of drug CP released in the pH 7.4 (blood environment) and pH 4.0 (tumor environment) respectively. It can be seen that 0.46μg of drug is released when solution of mixture of Ch/poly(NIPPAm)/drug is placed in pH 7.4 (i.e. blood environment). This is because of the fact that under the pH and temperature conditions of 7.4 and 37^0C, the Ch and poly(NIPAAm) are co-precipitated in the presence of drug. In this way most of the drug remains entrapped in the Ch/poly(NIPAAm) composite particles. The precipitated Ch and poly poly(NIPAAm) composite particles keep the entrapped drug almost protected within them. However, in the pH 4.0 (simulating tumor environment), the chitosan is dissolved from the composite particles. As a result, 6.0μg of drug comes out or released in the acidic solution of pH 4 (tumor environment). It is clear that almost fourteen times less drug is released in pH7.4 as compare to the pH 4. Therefore it may be concluded that if Ch/poly(NIPAAm)/CP solution if injected into blood, it shall release minimum amount of drug due to co-precipitation of Ch/poly(NIPAAm) particles. This shall prevent the healthy cells from damage. However, when these composite particles reach the tumor (where environmental pH is 4) they shall release a fairly high quantity of drug due to dissolution of chitosan. The drug released shall be able

to kill the cancerous cells, with least adverse effects on the healthy cells. The **Figure 11** shows various quantities ofdrug CP released in pH 7.4 and 4.0 respectively.

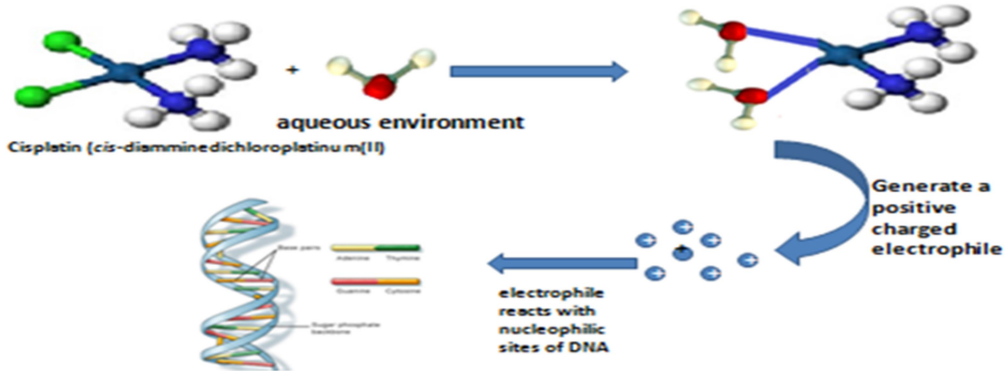

Figure 2: Mechanism of Cisplatin

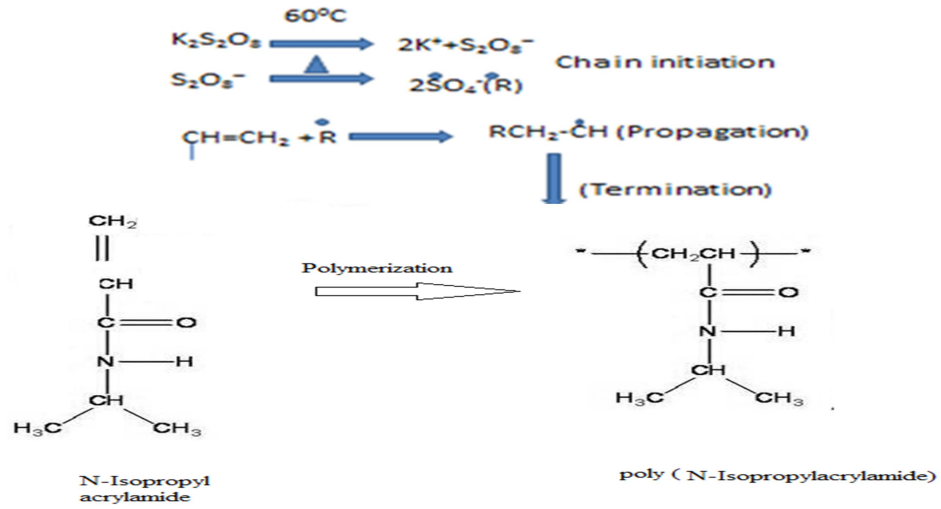

Figure 3: Polymerization reaction of N-Isopropyl acrylamide

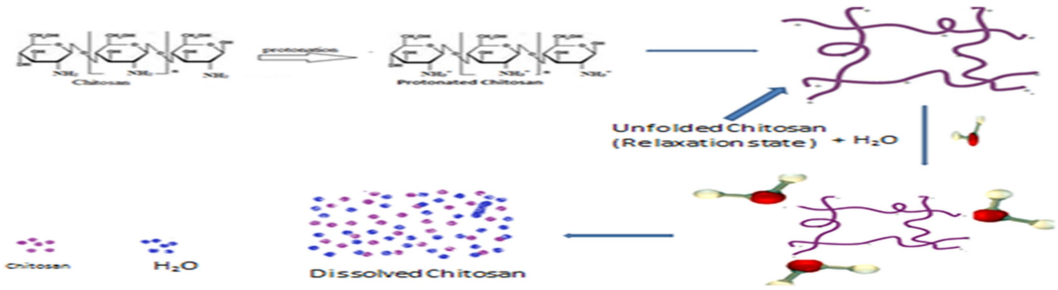

Figure 4: Protonation of chitosan

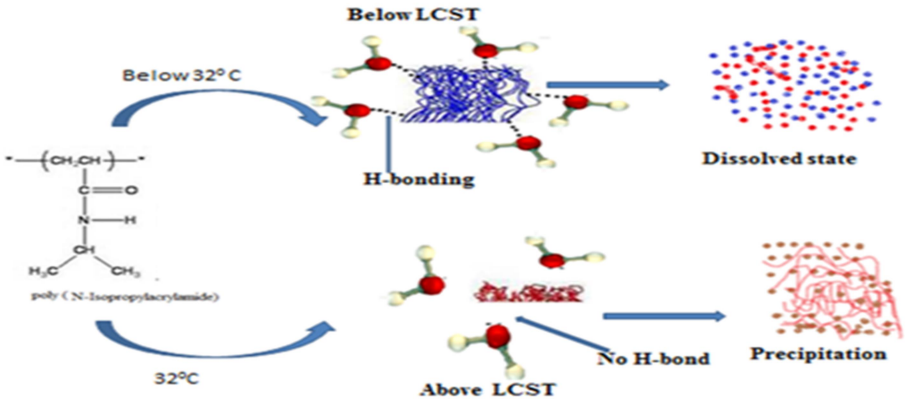

Figure 5: Hydrophobic and Hydrophilic nature of poly(N-Isopropylacrylamide)

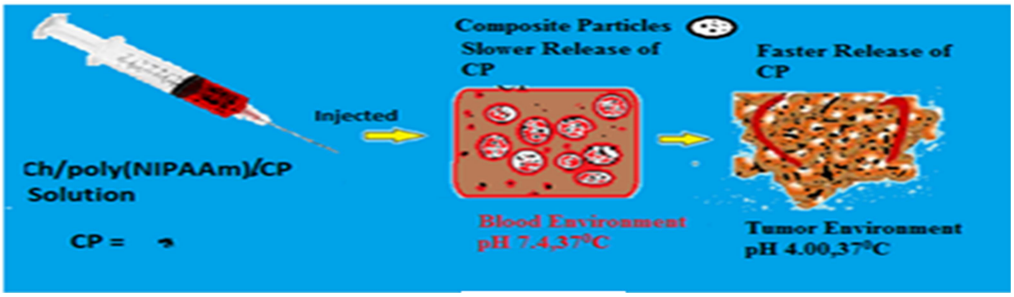

Figure 6: Proposed drug delivery system

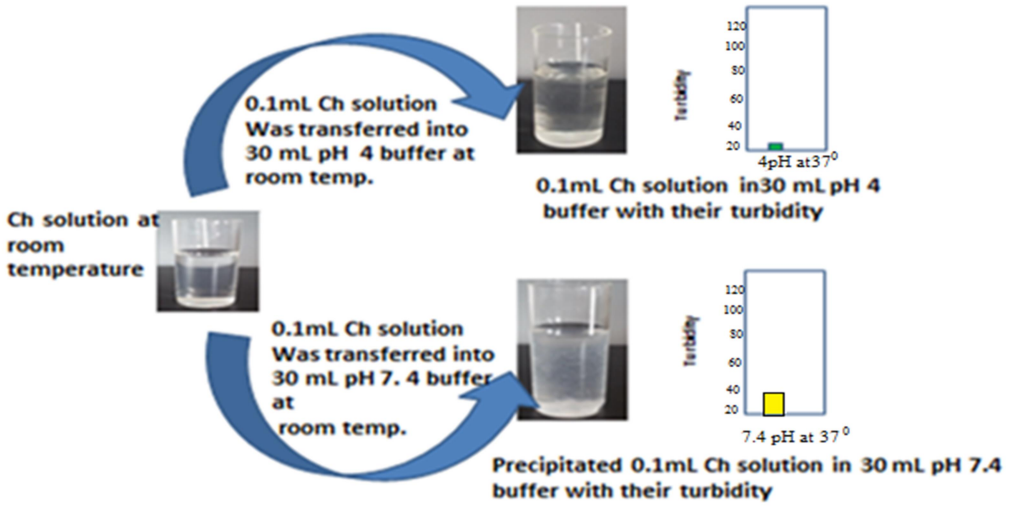

Figure 7(a): A clear image of Ch solution in 0.5% glacial acetic acid at room temperature along with turbidity

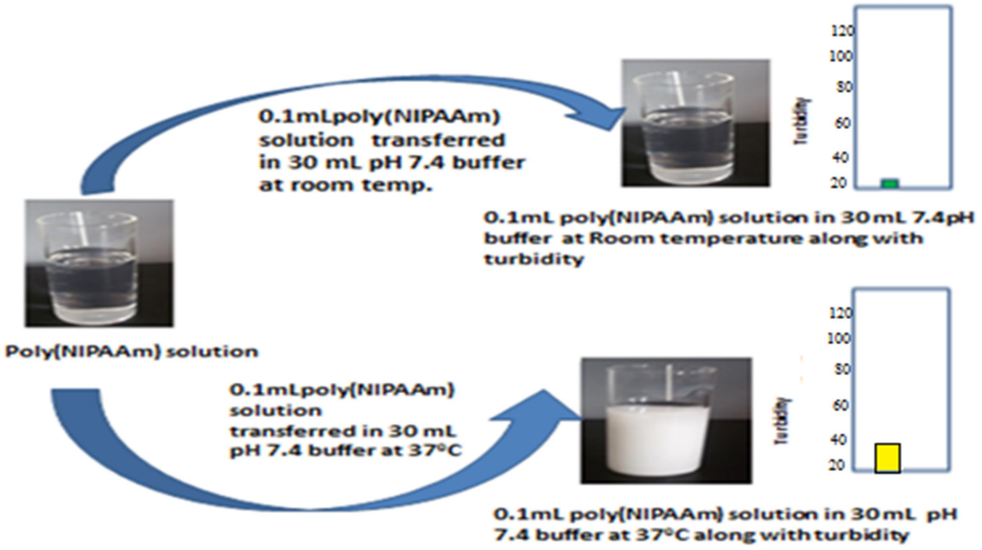

Figure 7(b): A clear solution of poly (NIPAAm) at room temperature

Figure 8: Injectable hydrogel

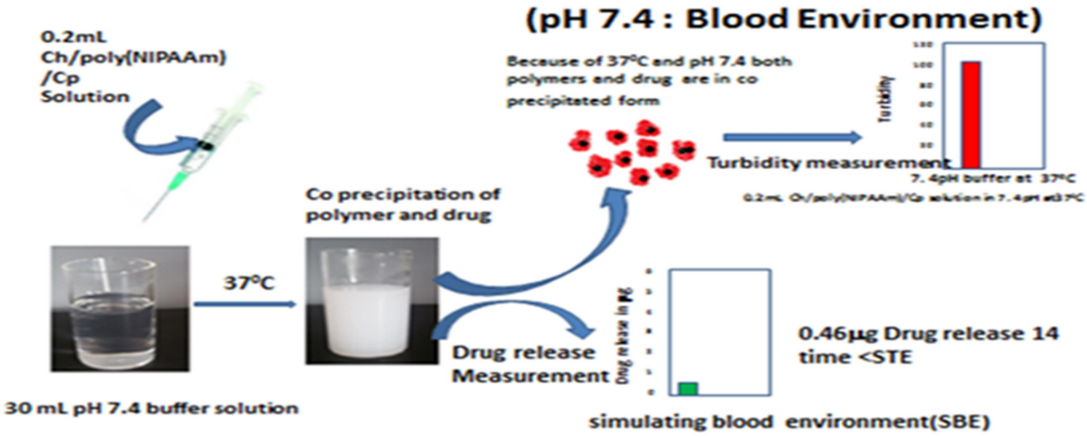

Figure 9(a): 0.2 mL of Ch/Poly(NIPAAm)/Cp solution is mixed into 25 mL of pH 7.4 (Blood environment)at 37^0 C

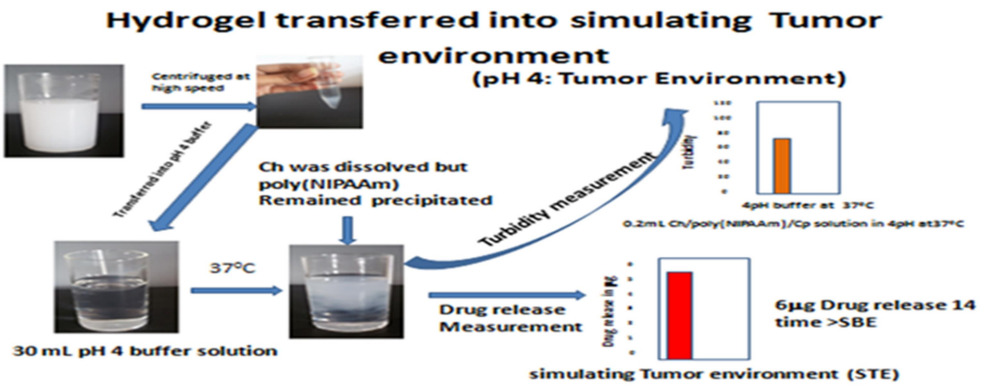

Figure 9(b): 0.2 mL of co- precipitated solution is mixed into 25 mL of pH 4 buffer at 37^{0}C, (Tumor environment)

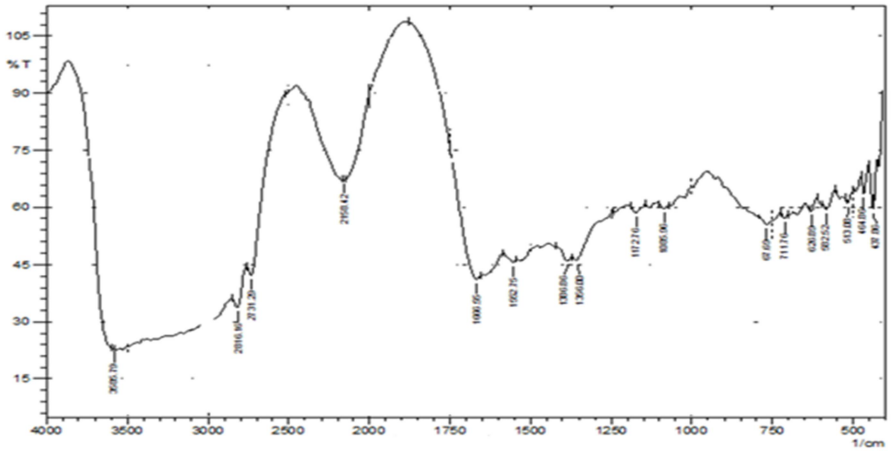

Figure 10: FTIR spectrum of poly(N-isopropyl acrylamide) /chitosan composite particles

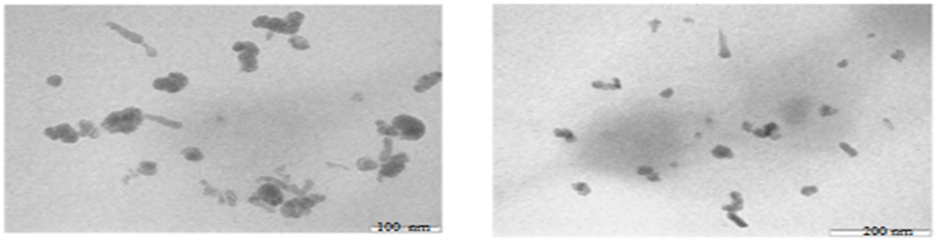

Figure 11: Tem an alysis of Ch/poly(NIPAAm) composite particles

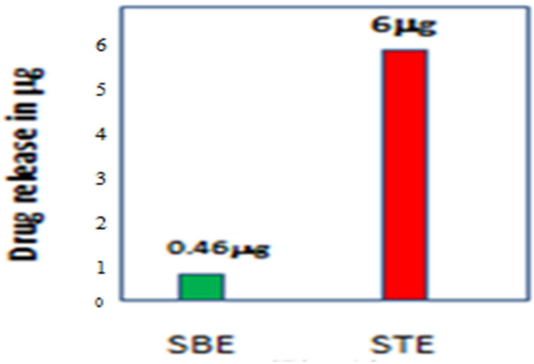

Figure 12: Drug release profile in the pH 7.4 (blood environment) and pH 4.0 (tumor environment)

CONCLUSION

It may be concluded from the above study that a solution mixture of Ch, poly(NIPAAm) and drug CP may be used as a tumor – targeted drug delivery system. The injectable hydrogel system is capable of delivering the most of the entrapped drug in the medium of pH 4.0 (which mimics the tumor environment), while minimum drug is released in the physiological medium of pH 7.4 (simulating blood environment). Although this is just a pilot study with no in-vivo or in-vivo investigations, but the study surely puts forward a step towards achieving the goal of targeted drug delivery for tumors.

Acknowledgment

The authors are Thankful to IIT, Mumbai .India for providing facilities for Transmission Electron Microscopy (TEM) analysis.

REFERENCES

[1] Jun, C., Xiaojian, Li., Liqian, G., Yi Hu Wen, Z., Malcolm, M.Q. X. A Facile strategy for in controlled delivery of doxorubicin with a pH-Sensitive Injectable hydrogel 4(3), 2014, 286-295.

[2] Janani, R., Uma, M. K., Swaminathan, S. Hydrogel based injrctable scaffolds fpr cardiac tissue regeneration Biotechnology Advances, 2014, 32(2): 449–4612

[3] Na, L., Chang, Y. G., Zhi, Y.Q., Feng, L., Cheng, W., He, L. W., Yu, Q. W. Therapeutic application of injectable thermosensitive hydrogel in preventing local breast cancer recurrence wound healing in a mouse model nanoscale 4(18), 2012, 5686-5693.

[4] Jung-Kyo, C., Ji, M. H., Taesu, H., Han-Kwang, Y., Soo-Chang, S. Injecatable and biodegradable poly (organophophazene) hydrogel as a delivery system of docetaxel for cancer treatment Journal of Drug Targeting, 2013, 21(6): 564-573.

[5] Rong jin, LS Moreira texixeira, Pieter J Dijkstra, M Karperien, CA Van blitterswijik, ZY Zhong, Jan Feijen : injectable chitosan-based hydrogels for cartilage tissue engineering . Biomaterials 30(13), 2544-2551, 2009

[6] Ki H. B., Li-Shan, W., Motoichi, K. Injcectable biodegradable hydrogels: progess and challenges, J. Mater. Chem, 2013, B 1(40), 5371-5388.

[7] Ana Elizabeth, C. F., Thayza, C.M. S., Thatiana, M. S., Petrus, D., Amorim S., Marta, C., Freitas, D. S., Galba, M., Campos-Takaki , and Tânia, L.M. S. physio-chemical characteristics and functional properties by mucoe circinelloides using yam bean as substrate Molecules, 2011, 16(8): 7143-7154.

[8] Liu, H., Du, Y., Yang, J., Zhu, H. Structural characterization and antimicrobial activity of chitosan/betaine derivative complex Carbohydr. Polym. 55(3), 2004, 291-297.

[9] Ajazuddin, Alexander A., Khan J., Giri T. K., Tripathi D. K., Saraf S. Advancement in stimuli triggered in situ gelling delivery for local and systemic route, Expert Opinion on Drug Delivery, 2012, 9(12): 1573–1592.

[10] Carreira, A.S., Goncalves, F.A.M.M., Mendonca, P.V., Gil, M.H., Coelho, J.F.J. Temperature and pH responsive polymer based on chitosan:application and new graft copolymerization strategies based on living radical polymerization Carbohydrate Polymers, 2010 80(3),: 618-630.

[11] Wei, H., Cheng, S. X., Zhang, X. Z., Zhuo, R. X. thermo-senstive polymer micelles based on poly(N-isopropylacrylamide) as drug carriers Progress in Polymer Science, 2009, 34(9): 893–910.

[12] Pascoe, J.M., Roberts, J.J. interaction between mammalian cell DNA inorganic platinum compound-2: interstrand cross-linking of isolated and cellular DNA by platinum 4 compounds Biochem.Pharmacol, 1974, 23(9): 1359–1365.

[13] Sun, B., Lin Y., Wu, P. Structure analysis of poly(N-isopropylaacrylamide) using near infrafred spectroscopy and generalized two-dimensional correlation infrared spectroscopy Applied Spectroscopy,2007, 61(7): 765-771.

[14] Ian, F., Tannock, Daniela, H. I.Acid pH in tumors and its potential for therapeutic exploitation Cancer Research, 1989, 49(16): 4373-4384.