IJPPR (2016), Vol. 7, Issue 2 *Research Article*

International Journal of Pharma Professional's Research

Received on 20 March, 2016; received in revised form, 01 April, 2016; accepted, 15 April, 2016; published 30 April, 2016

DEVELOPMENT AND CHARACTERISATION OF ZOLMITRIPTAN THIOLATED CHITOSAN NANOPARTICLES

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Key words:
Thiolation, Nanoparticles, Intranasal drug delivery, Migraine

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ABSTRACT:

The present study was aimed to develop thiolated chitosan nanoparticles of Zolmitriptan for brain targeting in migraine via intranasal drug delivery. The thiolated chitosan was prepared using thiogycolic acid and chitosan in presence of catalyst and characterized using FTIR and DSC studies. Drug loaded nanoparticles were prepared by ionic gelation method using Sodium tripolyphosphate. The prepared NPs were characterized by FTIR spectroscopy and DSC techniques to confirm the drug loading without any chemical interactions between the drug and excipients. The morphology and size of the nanoparticles was affirmed using TEM analysis..

INTRODUCTION:

Zolmitriptan is a second generation triptan derivative act as a serotonin 5 HT_1 receptor agonist and primarily used in acute migrain in adults (Belvis R et al, 2009). It directly and selectively constricts intracranial, extracerebial blood vessels and inhibits the release of the vasoactive neuropeptides from perivascular nerves to prevent neurogenic vasodilation and extravasation in the dura matter. Centrally, triptans inhibit excitability of cells in the trigeminal nucleus caudalis via 5-HT_1 D agonism (Girotra P et al, 2014; Pascual J, 1998).

Due to hydrophilic nature it is well absorbed by oral and intranasal administration with bioavailability approximately 40-42%, reaches peak plasma levels in 2–4 hours. The appearance of zolmitriptan's active metabolite (N-desmethyl zolmitriptan) in plasma following intranasal administration occurs at about 30 minutes after nasal administration. Thus first-pass metabolism in the liver is avoided with the nasal formulation, this active metabolite of zolmitriptan (ZM) may contribute to the increase in therapeutic efficacy of the drug as compared to oral formulations (De Hoon JNJM et al, 2000). Intranasal administration transported drug directly to brain via olfactory nerve pathways. Intranasal route has been investigated to bypass blood brain barrier and to avoid systemic side effects for many drugs acting on central nervous system (Dhuria SV et al, 2010). Thus intranasal administration offers a

practical, noninvasive, and an alternative route of administration for rapid drug delivery to brain in migraine.

Chitosan is a mucoadhesive biodegradable polymer and widely studied for the preparation of polymeric nanoparticles for the nose to brain drug delivery. It increases the permeability of the epithelial membrane by opening the tight junctions and facitilate paracellular tranport across the nasal epithelial membrane. It also increases the residence time of the formulation over the olfactory epithelia by mucoadhesive forces. Thiolation of chitosan further improves the mucoadhesive potential. Thiolated chitosan tightly adhere to mucosal epithilia through covalent bonding with mucin gylcoprotiens via thiol-disulfide linkage (Margit D H et al, 2003)

In the present study thiolated chitosan nanoparticles of Zomitriptan (ZM-TCH-NPs) was synthesized from chitosan by using thioglycolic acid. The TCS was prepared by thiogycolic acid by using (1-ethyl-3-(3-dimethyl amino-propyl) carbodimide hydrochloride and catalyst characterized by FTIR and DSC studies. Zolmitriptan loaded thiolated chitosan nanoparticles were formulated using ionic gelation method and characterized for particle size, zeta potential, drug loading of nanoparticles are critical parameter to be studied for drug delivery.

Materials and methods

Drug and chemicals

Zolmitriptan was gifted by Alembic PharmaceuticalsLtd., (Baroda), India. Chitosan was obtained as gift sample from CIFT, Kochi, India. Sodium tripoly phosphate (TPP), 1-ethyl-3-(3-dimethyl amino-propyl) carbodimide hydrochloride (EDAC) and Thioglcolic acid (TGA) was purchased from sigma. Dialysis tubing (capacity approx. - 3.63 ml/cm) was purchased from Himedia and all other chemicals used are of analytical grade.

Synthesis Of Thiolated Chitosan

Briefly 500 mg of chitosan was dissolved in 50 ml of 1% acetic acid. EDAC dissolved in 1ml deionized water was added to a final concentration of 125mM. Thereafter both of the above solutions were properly mixed and to this 500 mg TGA was added. Then pH of the medium was adjusted to 5 and the reaction mixture was incubated for 4h in dark at room temperature under constant stirring. For the isolation of unreacted TCS from the reaction mixture, the polymer solution was dialyzed in tubings of cellulose membrane (molecular weight cut -off 12-14 KDa) for 3 days in dark against 5mM HCl , then twice against the same medium containing 1% NaCl to reduce the ionic interactions between the cationic polymer and the anionic sulfhydryl groups. Dialysis process was carried out in dark at 4^{o}C for avoiding the oxidation of sulfhydryl groups. After dialysis, the polymer sample was lyophilized and the freeze dried polymer was used for futher studies.

Characterization of Thiolated Chitosan

FTIR spectra of chitosan and TCH were carried on perkin Elmer spectrum Fourier transforms infrared spectrophotometer using KBr method. The new amide bond formation and thiol group substitution in thiolated chitosan can be confirmed by the presence of characteristic peaks in FTIR spectra.

Nanoparticles Preparation

ZM-TCH-NPs were obtained by ionic gelation method . In this method chitosan was crosslinked with TPP. Nanoparticles were obtained as result of the dropwise addition of TPP solution to the aqueous solution of polymer by continous stirring, TCS to TPP weight ratio used is 3:1. As a result of ionic cross linking, a turbid solution was obtained, which was kept for half an hour stirring. The resultant nanoparticles were separated by centrifugation at 13,000 rpm for 1h at 4^{o}C. The pellets was redispersed in water and lyophilized by using cryoprotectant. The sample was further used for characterization.

Characterization of nanoparticles

Measurement of particle size and zeta potential

Average particle size (z-average), polydispersity index (PDI) and zeta potential of the prepared

nanoparticles were determined by dynamic light scattering analysis using Malvern Zetasizer. All the measurements were carried out by dispersing the nanoparticles in appropriate volume of HPLC grade water at 25 °C.

Percent Drug Entrapment Efficiency (%Dde)

The supernatant of formulations after centrifugation were collected and filtered through 0.45 µm filter. The amount of drug present was determined by UV spectrophotometer. The Percentage drug entrapment efficiency (DDE) was calculated using formula:

$$\%\ DDE = \frac{The\ amount\ of\ drug\ (W) - Free\ drug\ in\ supernatant(w)}{Total\ amount\ of\ drug\ (W)} \times 100$$

Transmission Electron Microscopy (Tem)

The size and morphological characterstics of the prepared ZM-TCH-NPs were further confirmed by TEM. For TEM NPs suspension was diluted in HPLC grade water and sonicated for 5 min to produce disaggregation of the particles. One drop of the sample was stained with 2% phosphotungstic acid, deposited on a 300 mesh formvar coated grid and then examined under HRTEM equipment (TECHNAIS TWIN) operated at 200 kV.

Fourier Transform Infrared (Ftir) Spectroscopy

In order to study the chemical interaction between the drug and polymer, FTIR spectra was taken by Foureir transform infrared spectrophotometer (Perkin Elmer, USA) using KBr method. The Lyophillised TCS NPs were mixed with KBr pressed in to pellet and scanned in the frequency range of 4000 to 400 cm^{-2}.

Differential Scanning Calorimetry (Dsc)

DSC thermograms of lyophilized TCS, chitosan, Drug loaded NPs and pure drug (ZM) were obtained on a simultaneous TGA/DSC equipment (TA Instruments DSC SDTQ600), in the temperature range of 10◦–600◦C, at a heating rate of 10◦C/min, with continuous purging of nitrogen gas (flow rate100 ml/min) for examining the morphological changes, degradation steps as well as physical interaction of drug with the carriers in the formulation. The data was analyzed using the software Universal Analysis 2000 (SDT Instruments).

Results and discussion

Particle size, zeta potential and drug incapsulation efficiency

The average particle size of the ZM-TCH-NPs was found to be 127nm (Figure:1) having zeta potential value +37.2 mV. Drug encapsulation efficiency of nanoparticles was found to be 89.5%.

Transmission Electron Microscopy (Tem)

ZM-TCH-NPs observed under HRTEM, revealed the particles to be spherical in shape. The particles size observed under TEM image are in range 50 to 70 nm (Figure: 2). The difference in size of particles measured from Zetasizer explained due to removal hydrodynamic layers covering the particles, during the sample preparation for TEM analysis, so leads to decrease in size.

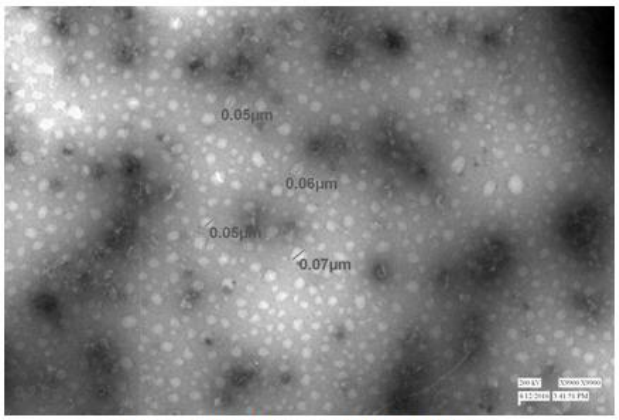

Figure 1. TEM image of ZM-TCH-NPs

FTIR SPECTROSCOPY

FTIR spectra of Zolmitriptan, TCH and ZM-TCH-NPs were shown in In the spectrum of chitosan, the characterstic peaks are at 3431cm-1 (υo-H and υN-H), 2925 cm-1 (υC-H), 1645 cm-1, 1517 cm-1 (δo-H), 1078 cm-1 (υC-N), 617 cm-1 (δNH), 1376 cm-1 (δC-H), 1248 cm-1 (δo-H), 1154 cm-1 (δC-O-C). TCH Shows all the peaks of chitosan and additional peaks due to new amide bond formation occurs between amino groups and carboxyl groups, so characteristic peak of amide bonds are 1527 cm-1 (amide band), 1638 cm-1 (amide band). The peak at 1248 cm^{-1}

due to thiol substitution. The FTIR spectrum of ZM-TCH-NPs represented all the characteristic peaks of the drug as well as excipients, thereby confirming the absence of any chemical interaction between them.

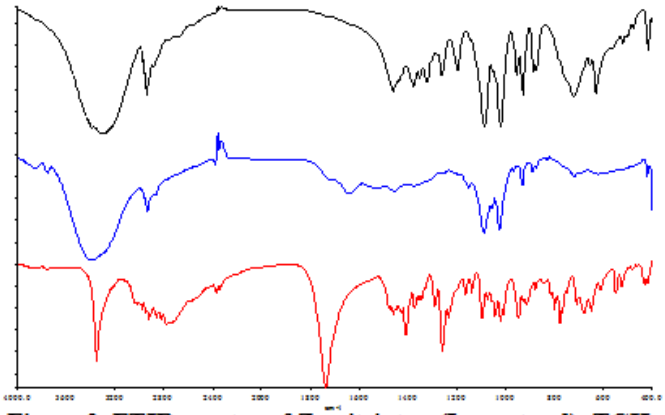

Figure 2. FTIR spectra of Zomitriptan (Lowest red), TCH (Middle Blue) and ZM-TCH-NPs (Highest Black).

DSC-TGA THERMOGRAMS

Chitosan started to degrade near about 300 and has broad degradation temperature range with a high char at 550 (Figure 5). Degradation involves dehydration, deacetylation and chain scission (Kast CE., & Bernkop-Schnürch, A 2001) The degradation first started with loss of water. TCS degradation start at lower temperature (150) compared to chitosan due presence of weaker amide bond (-NH-CO-CH_2-SH) formed in thiolation (Figure 6). ZM-TCH-NPs degradation is more difficult than TCS due to TPP cross linking (Figure 5).

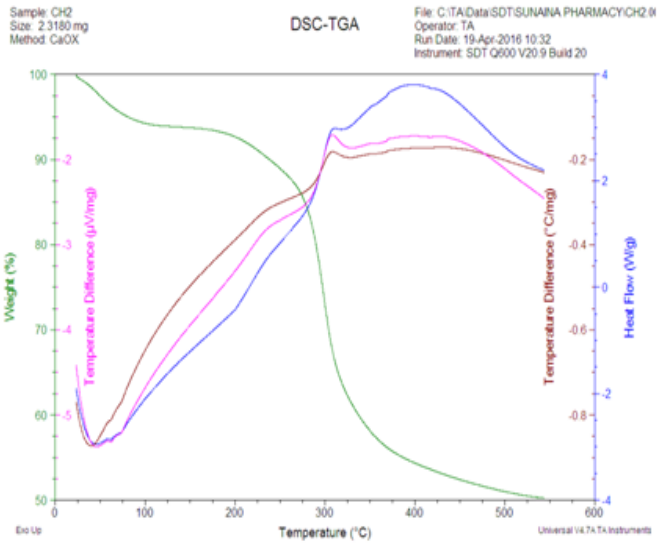

Figure 3. DSC-TGA thermogram of chitosan

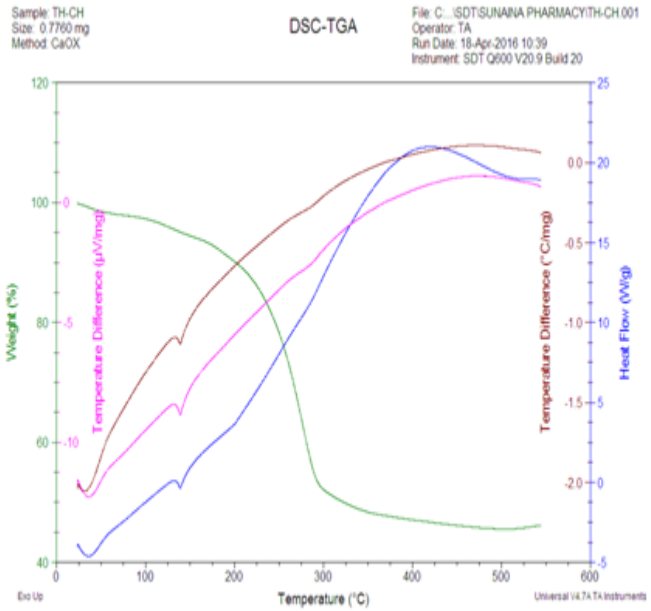

Figure 4. DSC-TGA thermogram of TCH

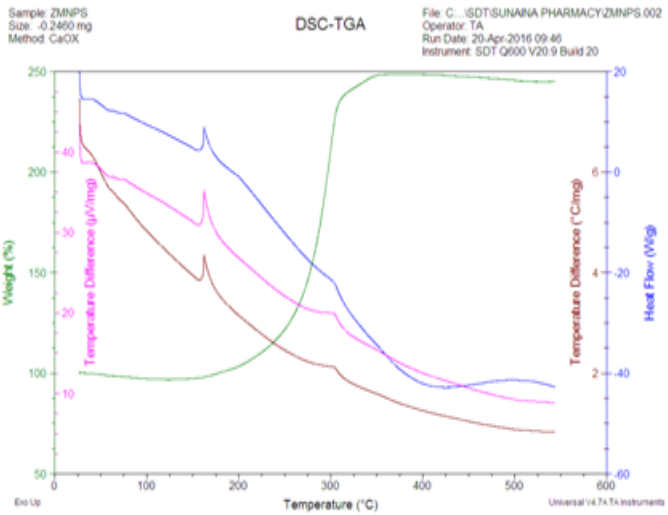

Figure 5. DSC-TGA thermogram of ZM-TCH-NPs

CONCLUSION

The mucoadhesive polymer, TCS was synthesized and characterized by FTIR and DSC studies. The prepared NPs were characterized using FTIR, TEM and DSC studies. The prepared NPs possess a size range 50-70 nm and spherical in shape. By Zeta potential measurement the TCS NPs was found to be stable and having positive surface charge. Thus Thiolated chitosan based Zolmitriptan nanoparticles have potential drug drug delivery tool for migraine treatment and further confirmed by *in vivo* studies.

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