CONTROLLED RELEASE STUDIES OF HYDROXYCHLOROQUINE SULPHATE (HCQ) DRUG-USING BIODEGRADABLE POLYMERIC SODIUM ALGINATE AND LIGNOSULPHONIC ACID BLENDS

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ABSTRACT

HCQ an antimalarial drug is one of the most in-demand medications globally since COVID-19 pandemics. To study the controlled release of HCQ in physiological environments to address the side effects, biodegradable polymeric blends of Sodium alginate (NaAlg) and Lignosulphonic acid (LS) blends are loaded with HCQ as a model drug. Drug-loaded NaAlg/LS blends of the ratio 80:20 is crosslinked using calcium chloride solutions at different time intervals of time to study HCQ release in different pH conditions of 1, 4, 7, and 9.2 using a UV-Visible spectrophotometer. The HCQ drug release is studied for its release in variable pH conditions to mimic the gastrointestinal conditions in the stomach and intestine. The HCQ drug-loaded NaAlg/LS (80:20) blends are also studied for chemical interactions using FT-IR. The results showed that NaAlg/LS (80:20) blends are more suitable carriers and promising strategy for HCQ control release and FTIR results confirm, that HCQ is very much compatible with alginate blend matrix.

Keywords: Hydroxychloroquine Sulphate, Biodegradable Polymers, Sodium Alginate, Controlled Release, Hydrogels.

RASAYAN J. Chem., Vol. 14, No.4, 2021

INTRODUCTION

The world at its a difficult situation where people are suffering from infections such as Novel Coronavirus disease (COVID-2019) which was described as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The scientists are on their toes struggling to cure suffering globally of infections. Several medicines such as Lopinavir and Ritonavir, associate with nursing HIV drug combinations that are tried on Covid-19 treatment have emerged out with side effects include liver injury, nausea, and diarrhea.¹ These medicines possess a half-life of ~4–6 hr,² which means that concentrations can vary by the aspect of eight between peak and trough. By minimizing the drug concentration, developing a controlled-release formulation with zero-order release kinetics to retain the least effective drug concentration may reduce these side effects. Although achieving zero-order *in-vivo* drug release for an oral or injectable delivery mechanism remains impossible, even formulations with reasonably achievable first-order release kinetics could help minimize toxicity.³⁻⁴ This strategy is not likely to support all drugs under consideration for COVID-19. However, in the midst of 2020, Hydroxychloroquine sulphate (HCQ) emerged as a prescription drug for COVID-19 infections with a systemic drug concentration that is unlikely to vary dramatically between daily doses due to a biological half-life of approximately 50 days, maximum average drug absorption concentrations are unlikely to differ significantly among daily doses.⁴⁻⁸ Clinical trials have reported that in later stages that it induces side effects.⁹⁻¹⁰ Many research articles are reported on how to prevent side effects, in particular biodegradable polymers, which are advanced materials used to mitigate side effects by embedding the drug into its matrix and delivering the drug in a therapeutic range at a controlled dosage.¹¹⁻¹⁵

Sodium alginate a seaweed product and Lignosulphonic acid is a plant by-product are the biodegradable polymers available plenty in nature. Sodium alginate is a gel-forming polymer and it has two polymers in

its chain, they are α -L-Guluronic acid and β -D-Mannuronic acid extracted from seaweed product brown algae.¹⁶⁻¹⁷ NaAlg is extensively used because of its gel-forming property and non-toxicity in controlling drug delivery applications, sustainable drug delivery, wound healing, scaffolds, biodegradable sutures, interpenetrating networks, food toppings, etc.¹⁸⁻²¹ Lignosulphonic acid is a biodegradable polymer that is commonly used in cement, pesticides, water treatments, dye dispersants, etc.²²⁻²⁴ To obtain stable NaAlg beads, LS is blended with NaAlg in various proportions of 80/20, 60/40 and 50/50 and these blends are investigated for various properties such as swelling, morphological, crystalline, and drug delivery are reported.²⁵⁻²⁶

Hydroxychloroquine sulphate is typically prescribed for the treatment of malaria, rheumatoid arthritis, and lupus erythematosus.^{4,27-28} The controlled release studies are a significant tool for the design of effective and reliable pharmaceutical products.

An in-depth literature review indicates that no prior studies are available or HCQ control release formulations are reported. In this article, an attempt has been made to report the control release formulations of HCQ loaded in NaAlg/LS blends.

The present study describes the HCQ release *in-vitro* in the variable pH medium to imitate the fluids present in the stomach and GI tract. Further HCQ loaded NaAlg /LS (80:20) blends are characterized for drug-polymer interactions and drug compatibility by Fourier Transform Infra-Red (FT-IR) analysis.

EXPERIMENTAL

Materials

Biodegradable Sodium alginate (Mw10,000-6,00,000) a seaweed product and Lignosulphonic acid (Mw 52000) a plant by-product purchased from Sigma Aldrich, Bengaluru. Calcium chloride, Hydrochloric acid, Buffer tablets of 4, 7, 9.2 are obtained from Nice chemicals. Hydroxychloroquine sulphate (HCQ) was obtained from IPCA Laboratories Ltd, India. All these chemicals are used as obtained. All experiments are care carried using double distilled water.

Synthesis of Drug Loaded NaAlg/LS (80:20) Blends

Pre-soaked 4% Sodium alginate (NaAlg) in 50ml of double-distilled water, as well as Lignosulphonic acid (LS), are taken in the proportion of (80:20). To this mixture, 20ml of HCQ solution (1mg HCQ/1ml) is added and constantly stirred for 30 minutes using a magnetic stirrer and sonicated for 30minutes for proper agitation.

Crosslinking of NaAlg /LS Blends

The HCQ loaded NaAlg/LS viscous mixture in a 5ml syringe was added dropwise in 25ml of 2% CaCl_2 solution. The beads are allowed to crosslink for various time intervals of 10, 20, and 30, and 60 mins respectively to form stable beads. After crosslinking the beads are removed from the aqueous solution and dried at 60°C for 24hr.

Characterization

In-Vitro HCQ Drug Release Studies

The NaAlg /LS (80:20) beads are evaluated for release kinetics of HCQ drug at different pH solutions. The drug release was studied in 4 typical conditions; pH1 (0.1N HCl) & pH4 (mixture of Acetic acid-Sodium acetate), acts as simulated gastric juice (SGF), and pH 7 & pH 9.2 buffer solutions (mixture of NaH_2PO_4 – Na_2HPO_4) acts as simulated intestinal fluid (SIF) were chosen to study the drug release. The drug-loaded beads are taken in 25ml buffer solutions of pH1, 4, 7, and 9.2, and a physiological temperature of $37 \pm 1^\circ\text{C}$ is maintained as shown in Fig.-1. At every 1hr the release of HCQ drug from beads was recorded using Shimadzu 2600 UV-Visible Spectrophotometer at 342nm which is a typical peak of HCQ spectrum seen in Figure. 2. HCQ drug release tests were performed in triplicate for each formulation evaluated.

Fourier Transform Infra-Red Spectroscopy Studies

FT-IR spectra of NaAlg/LS (80:20) blends, HCQ drug, and HCQ loaded NaAlg /LS (80:20) blends are investigated for chemical interaction using Shimadzu Fourier Transform Infra-Red Spectrophotometer, IR Spirit- L, Model no.206-31000-58. The blend composite pellets are made using KBR containing 2-4% by

weight of the sample. The samples were scanned within the absorption range of $400\text{--}4000\text{cm}^{-1}$. The interferogram is generated by the signal process of 45 scans at a resolution rate of 4cm^{-1} with LAB SOLUTION software.

Fig.-1: HCQ Drug release from Crosslinked NaAlg/LS (80/20) Beads in a pH Medium of 1, 4, 9.2 & 7.

RESULTS AND DISCUSSION

Control Release Kinetics of HCQ Drug from NaAg/LS Blends

The HCQ drug-loaded NaAg/LS (80:20) beads are crosslinked for various time intervals (10, 20, 30 mins) are investigated for release of the drug, in different pH mediums (1, 4, 7, and 9.2) for the reason that, when the drug-loaded beads are taken *in-vivo*, it passes through the different medium of fluids in stomach, intestine, and colon. To investigate the drug release kinetics and to mitigate different pH mediums in the human digestive system, the beads are subjected to drug release in different pH solutions at a physiological temperature of $37\pm 1^\circ\text{C}$. The calibration curve (Fig.-2) is plotted for different HCQ concentrations in 25ml double distill water for evaluating its release from NaAlg/LS blends in different pH mediums.

Fig.-2: (a) UV Spectra of HCQ Drug, (b) Calibration Curve of HCQ Drug

Release Studies of HCQ Drug in pH 1 Medium

In an aqueous medium of pH 1, the HCQ drug release profiles of NaAlg/LS (80:20) blends of varying crosslinking timings were investigated for 48hrs. It was observed from Fig.-3a, that 10mins crosslinked beads releases most of the HCQ drug within 7hrs. The plots demonstrate that drug release gradually decreases in 20min crosslinked NaAlg/LS(80/20) beads and releases a maximum of $70\pm 6\%$ HCQ drug in 8hrs, thereafter drug releases constantly up to 24hr and beyond. 30min crosslinked NaAlg/LS beads, on the other hand, release only 50% of the HCQ drug for the first 7 hrs and then release at a very low pace for the next 48 hours. This is because the uronic acid present in NaAlg gels is stabilized with intermolecular hydrogen bonding²⁹ and shrinks in alginate structure at low pH, resulting in very low bead swelling.

Release Studies of HCQ Drug in pH 4 Medium

Similar trends were observed for 30min crosslinking drug-loaded NaAlg/LS (80/20) beads as depicted in pH4 medium (Fig.-3b). Whereas 10- and 20-mins crosslinked blends show more release of HCQ drug compared to pH 1 medium.

Release Studies of HCQ Drug in pH 7 Medium

The HCQ drug release was investigated in pH 7 medium, which is considered to be SIF in the GI tract.³⁰ The HCQ drug release was very fast in 10min crosslinked NaAlg/LS beads and releases the complete drug within 3-4hrs. It was noted that in 10mins crosslinked NaAlg/LS (80/20) beads develop some cracks, which could be one of the main reasons for the faster release of HCQ drug. The moderate release was observed in 20min crosslinked beads as shown in Fig.-3c, whereas controlled drug release was observed in 30min crosslinked beads up to 10hrs. It is reported in our previous publication²⁶ that, the rapid release in 10min crosslinked beads is because of the exchange of Ca^{2+} ions present in crosslinked beads with Na^+ ions present in pH 7 (Na_3PO_4 buffer solution). It is noticed and confirmed from results obtained, that time of exposure of NaAlg to crosslinking medium influences the strength of the beads. Since 10min crosslinking beads are exposed for a minimum period may lose their structures in pH 7 medium and promote uptake of more water causing swelling of beads. These swelled beads could not retain the alginate matrix structure and gradually beads start disintegrating (Figure.4), thereby releases most of the HCQ drug.³¹

Release Studies of HCQ Drug in pH 9.2 Medium

For HCQ drugs, further to elucidate how higher pH affects the drug release the HCQ loaded NaAlg/LS beads are subjected for swelling and release studies. As shown in Fig.-3d the HCQ drug release was very minimal in pH9.2 because the drug release is swelling dependent and the swelling of beads are very poor in alkaline solutions.³² It was observed that only 25-30% HCQ drug was released up to 48hrs in 20 and 30min crosslinked beads (Fig.-4c).

Fig.-3: HCQ Release Profiles of HCQ Drug-loaded NaAlg/LS (80:20) Beads Crosslinked for time intervals of 10, 20 and 30 mins: (a) pH1 (b) pH4 (c) pH7 and (d) pH9.2.

Fig.-4: (a) Dry Beads of HCQ Loaded NaAlg/LS (80:20); (b) Swelled NaAlg/LS (80:20) Beads; (c) Stable Beads releasing HCQ Drug after 48hr.

HCQ Drug Release in Physiological Medium

Attempts are made to investigate control release of HCQ drug in a variable pH solution of 1 and 7 because as drugs are taken orally, it passes through the human digestive system (SGF) for 3hrs where pH ranges between 1 to 4 and then passes through intestinal fluids and pH is between 7 to 9.2. To mimic these digestive systems, the HCQ drug-loaded NaAlg/LS (80:20) beads are allowed to release the drug in a variable pH medium. The HCQ drug release was very minimum in pH 1 up to 3hrs as seen in Fig.-5; this is because protonation of sodium alginates in beads doesn't swell much, thereby affecting the drug release. Further, the same NaAlg/LS beads are subjected to study the controlled release in pH 7 medium, which is assumed to be SIF³⁰, the beads release 75±5% drug up to 12hrs due to appreciable amount of hydrolysis of alginate beads.

Fig.-5: HCQ Drug Release in the Physiological Medium of pH1 (up to 3hrs) and pH7 (after 3hrs)

FT-IR Studies of HCQ, NaAlg/LS (80:20) and HCQ loaded NaAlg/LS (80:20) Blends

The FT-IR analysis of HCQ was carried out, the FT-IR absorption spectrum from Fig.-6 reveals the following bands; 3447 cm^{-1} was due to the OH group, and the absorption band at 2977 cm^{-1} is due to NH deformations. The bands at 3100–2500 cm^{-1} is indicative of secondary and tertiary amines, with intermolecular interactions within hydrogen bonds overlapping the stretching of the C–H group. The axial deformation of aromatic compounds and alkenes by C=C and C=N bonds shows the absorbance peak at 1622 cm^{-1} . In the bands between 1177 and 1373 cm^{-1} , symmetric and asymmetric deformations of the S=O bonds can be seen. Finally, the peaks observed between 550 and 850 cm^{-1} are due to the C–Cl bond.³³

Fig.-6: FT-IR Spectrum of (a) Hydroxychloroquine Sulphate, (b) NaAlg/LS (80:20) Blend, (c) HCQ Drug-loaded NaAlg/LS (80:20)

The FT-IR spectrum of NaAlg/LS blends (Fig.-6) shows absorption peaks at 1609 and 1414 cm^{-1} accounted for the asymmetric and symmetric stretching vibration of the COO^- group present in NaAlg³⁴.

Whereas, absorption peaks at 3442 and 1047 cm^{-1} are of stretching vibration of the O-H group and C-C stretching of aromatic rings.³⁴ On the other hand, the absorption bands due to LS in NaAlg/LS blend, which is observed at 1511 and 1046 cm^{-1} , are attributed to C-C stretching in aromatic rings, whereas 654 cm^{-1} is due to =C-H bending vibration.²⁹

An overlap of bands was observed for mixture shows the axial deformation of -OH, -NH, and -CH present in aromatic compounds present in HCQ drug in the FTIR spectra of HCQ drug-loaded NaAlg/LS (80:20) beads (Fig.-6). The HCQ drug present in the NaAlg/LS matrix is confirmed by the flattening range observed in HCQ loaded NaAlg/LS blends between 990-1183 cm^{-1} , which shows the potential interactions.

CONCLUSION

Biodegradable blends of NaAlg and LS are used to load HCQ drug and its control release in the physiological medium is reported. HCQ drug is known for its antimalarial dosage prescribed during COVID-19 pandemic situations. Control release profiles of HCQ from the NaAlg/LS matrix are reported to avoid the side effects caused during HCQ treatment. Crosslinking HCQ loaded NaAlg/LS (80/20) beads using calcium chloride are studied, in which crosslinking timings have shown a major role in controlling the release of the drug. It was observed that HCQ drug was released up to 48hrs and beyond in 30min crosslinking beads in the gastrointestinal medium. The FT-IR analysis shows that the HCQ drug is compatible and drug concentration is unaffected in NaAlg/LS (80/20) blends. The present investigations describe the control release profiles of HCQ release from NaAlg/LS (80/20) blend and nontoxicity of biodegradable blends which could overcome the side effects. These formulations promote intestinal absorption and demonstrate controlled release to the prolonged periods of therapeutic drug doses. The NaAlg/LS (80/20) blends are likely to make a very important contribution to be a suitable HCQ drug carrier for the possible effective treatment for COVID-19.

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[RJC-6508/2021]