Radiation cross-linked carboxymethyl sago pulp hydrogels loaded with ciprofloxacin: Influence of irradiation on gel fraction, entrapped drug and in vitro release

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HIGHLIGHTS
- Carboxymethyl sago pulp (CMSP) with ciprofloxacin is irradiated to form hydrogels.
- 20% CMSP at 25 kGy has produced stable hydrogels with the highest gel fraction.
- Crystalline ciprofloxacin converted to amorphous during hydrogel formation.
- Hydrogel in disc form sustained the drug release drug up to 36 h.
- Irradiation cross-linked polymeric chain of CMSP resulted in controlled swelling.

ABSTRACT
Carboxymethyl sago pulp (CMSP) with 0.4 DS, viscosity 184 dl/g and molecular weight 76,000 g/mol was synthesized from sago waste. 10 and 20% w/v solutions of CMSP were irradiated at 10–30 kGy to form hydrogels and were characterized by % gel fraction (GF). Irradiation of 20% CMSP using 25 kGy has produced stable hydrogels with the highest % GF and hence loaded with ciprofloxacin HCl. Drug-loaded hydrogels were produced by irradiating the mixture of drug and 20% CMSP solution at 25 kGy. After irradiation, the hydrogels were cut into circular discs with a diameter of 6 ± 1 mm and evaluated for physicochemical properties as well as drug release kinetics. The ciprofloxacin loading in the disc was 14.7 ± 1 w/w with an entrapment efficiency of 73.5% w/w. The low standard deviation of drug-loaded discs indicated uniform thickness (1.5 ± 0.3 mm). The unloaded discs were thinner (1 ± 0.4 mm) and more brittle than the drug-loaded discs. FESEM, FT-IR, XRD, DSC and TGA analysis revealed the absence of polymer–drug interaction and transformation of crystalline to amorphous form of ciprofloxacin in the discs. The disc sustained the drug release in phosphate buffer pH 7.4 over 36 h in a first-order manner. The mechanism of the drug release was found to be swelling controlled diffusion and matrix erosion. The anti-bacterial effect of ciprofloxacin was retained after irradiation and CMSP disc could be a promising device for ocular drug delivery.

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1. Introduction

Hydrogels comprise networks of hydrophilic polymers chains, which can be formed from a range of both synthetic and natural polymers. Over the past decade, hydrogels fabricated from natural polymers have become the biomaterials of tremendous interest in biomedical applications, such as drug-delivery systems. Sago waste, which is a by-product of the sago palm (Metroxylon sagu), is one of the cheapest, biodegradable and most readily available renewable natural polymers existing in Malaysia. In Malaysia, the state of Sarawak is recognized as the largest sago-growing area, which is presently the world’s greatest exporter of the sago. The synthesis and characterization of carboxymethyl sago pulp (CMSP) from sago waste was reported by Pushpamalar et al. (2006).
The unique anatomy, biochemistry and physiology of the eye offer many challenges in developing effective ophthalmic drug-delivery systems. Topical delivery into cul-de-sac is by far the most common route of ocular drug delivery. CMSP hydrogels swell to the maximum at and above pH 7 (Pushpamalar et al., 2013a), indicating their potential for promoting a sustained drug release at the ophthalmic pH. Carboxymethyl cellulose has been reported in the literature for ophthalmic drug-delivery systems (Jain et al., 2010; Kim, 2006; Sasaki et al., 1999), however, the production of CMSP discs made from sago waste is still relatively a new area.

Ciprofloxacin HCl (1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid hydrochloride) is a broad-spectrum fluoroquinolone antibiotic which was used as a model drug. Ciprofloxacin HCl is active against aerobic Gram positive and negative bacteria (Charoo et al., 2003; Egger et al., 2001). Resistance to this drug develops slowly, and a minimal elimination half-life (Padhy et al., 2013). Multiple administrations are required for the maintenance of the pharmacological action for conventional liquid ophthalmic drops or ointment formulation. Thus, a controlled-release dosage form of ciprofloxacin HCl could increase pre-corneal residence time, the possibility of releasing the drugs at a slow and constant rate, enhance ocular bioavailability, and reduce the frequency of administration.

In the present investigation, we have incorporated ciprofloxacin HCl in CMSP discs that were formulated by cross-linking with irradiation. Drug-loaded CMSP discs were then characterized for drug loading, entrapment efficiency, size and weight uniformity, field emission scanning electron microscopy (FE-SEM), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction, differential scanning calorimetric (DSC) studies, thermo gravimetric analysis (TGA), in vitro release studies, swelling dynamics and antimicrobial efficacy studies to evaluate their potential as drug delivery devices.

2. Materials and methods

2.1. Materials

Sago waste was obtained from Ng Kia Heng Kilang Sagu Industrial Sdn. Bhd., Malaysia. Ciprofloxacin HCl was purchased from Afine Chemicals Limited, China. Methanol and ethanol (denatured 95%) were obtained from HmbG chemicals and Bumi-Pharma Sdn. Bhd., respectively. Sodium chloride (80% technical grade) was obtained from Sigma-Aldrich and sodium monochloroacetate from Fluka. Sodium hydroxide pellets were obtained from Friedemann Schmidt, Malaysia. Glacial acetic acid, isopropanol and potassium dihydrogen phosphate were obtained from R&M chemicals. All chemicals used in the study were of analytical grade.

2.2. Methods

2.2.1. Preparation of carboxymethyl sago pulp

Carboxymethyl sago pulp (CMSP) with 0.4 DS, viscosity 184 dl/g and molecular weight 76,000 g/mol was synthesized from sago waste as described by Pushpamalar et al. (2006). Briefly, 20 g of the sago waste was transferred into a 1000 ml Erlenmeyer flask and suspended in 640 ml of hot distilled water. Four ml of glacial acetic acid and 6 g of sodium chloride were subsequently added. The mixture was later heated at 70 °C for 3 h, then filtered and washed with cold distilled water. The resultant white residue (sago pulp) was dried in the oven to its constant weight.

Five grams of the sago pulp were added into 100 ml of isopropanol and 10 ml of 30% sodium hydroxide. The mixture was stirred for an hour at 160 rpm in a thermostated water bath with a horizontal shaker (Model SW22, Julabo, Germany). The carboxymethylation reaction was started by adding 3 g of sodium monochloroacetate to the reaction mixture. Then, the stirring (160 rpm) was continued at 45 °C for 3 h. The mixture was filtered and suspended in 300 ml of methanol overnight and neutralized with glacial acetic acid. The mixture was filtered again and washed thoroughly with 150 ml of ethanol to remove undesirable by-products and dried in an oven at 60 °C. The degree of substitution was determined by a titration method as reported by Pushpamalar et al. (2006). The molecular weight was determined from the intrinsic viscosity measured in 0.1 M NaCl at 25 °C with an AVS 440 Ubbelohde viscometer (Schott, Germany), using the following relationship given by the Mark–Houwink empirical equation as below

\[ \eta = kM_a^a \]

where \( \eta \) is the intrinsic viscosity, \( k \) and \( a \) are the parameters that depend on the solvent/polymer pair and \( M_i \) is the viscosity-average molecular weight of the polymer.

2.2.2. Determination of irradiation dose for cross-linking

A 10 and 20% (w/v) of CMSP solution was prepared in distilled water. The samples were then poured separately into the lids of sterile petri dishes (clear polystyrene, 6 mm thickness and 90 cm diameter, BioAn, Malaysia). The lids were covered with a plastic sheet (clear polypropylene, 0.03 mm thickness, OCS Plas Corporation, Malaysia) and then irradiated with the doses of 10, 20, 25 and 30 kGy using an electron beam (EB) accelerator (EPS–3000, 2 MeV energy, 10 mA current and 20 kW power), at room temperature, in air using a dose rate of 5 kGy/pass (Malaysian Nuclear Agency, Malaysia). The irradiation doses were measured using the cellulose triacetate film dosimeter (CTA-FTR-125, Fuji film, Japan), with the size of 8 mm width and 0.125 mm thickness. The uncertainty of the given doses determined using the CTA dosimeter was less than 5%. Petri dish lids were used as moulds to ensure uniform distribution of the irradiation dose. The volume/thickness for the CMSP samples in the lids was kept constant. The same surface area and penetration depth in each sample being irradiated ensure uniform irradiation dose. Moreover, a thin plastic sheet was placed on the surface of the lid to minimize the occurrence of air bubbles that may affect the EB penetration.

All the irradiated samples were transferred into individual tea bags and suspended in beakers containing large amounts of deionised water overnight to obtain the formed hydrogels. The hydrogel residues were then transferred to individual plastic bags and dried in an oven at 70 °C for 2 days. The percentages of sol fractions (% sol) and gel fractions (% GF) were then calculated as per the equations below, where \( w_0 \) and \( w_1 \) are the weights of the initial wet hydrogel and the dried hydrogel, respectively

\[ \text{Sol fraction} (\%) = \frac{w_0 - w_1}{w_0} \times 100\% \]

\[ \text{Gel fraction (GF)} (\%) = 100 - \text{sol fraction} \]

2.2.3. Preparation of ciprofloxacin HCl loaded with CMSP discs by radiation cross-linking

20% (w/v) of CMSP solution was prepared by dissolving synthesized CMSP in 100 ml of distilled water, and the pH was adjusted to 7 using acetic acid. To this 20% (w/w) of ciprofloxacin HCl based on CMSP weight was added and stirred at 500 rpm for 60 min using an automated stirrer. The solution was covered with aluminium foil to minimize light exposure for the entire duration. The samples were then poured separately into the petri dish lid, covered with a plastic sheet and subjected to the irradiation dose...
of 25 kGy using electron-beam accelerator as explained in the previous section. The samples were then left covered overnight on an even surface, with no light exposure and dried at 60 °C until a constant weight was achieved. Small circular discs were cut out using a 6 mm single hole puncher and then kept in light-resistant containers, which were stored in the desiccator. The unloaded discs were also prepared in the same manner but without the addition of the drug.

2.2.4. Determination of drug loading and entrapment efficiency

Pre-weighted quantities of drug-loaded discs (≈ 250 mg) were placed in a volumetric flask containing 100 ml of 2 N NaOH and stirred for 24 h at 40 °C with a thermostated water bath with a horizontal shaker to extract the drug. The suspensions were subsequently filtered, and the ciprofloxacin HCl was measured at 271 nm using a UVmini-1240 UV–vis spectrophotometer (Shimadzu, USA). Absorbance of standard ciprofloxacin HCl solution exposed to identical condition was used as reference to determine the unknown quantity of the drug in the disc. The drug entrapment efficiency (DEE) was then calculated as follows:

\[
\text{Drug entrapment efficiency (DEE)} \, \% = \left( \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \right) \times 100\%
\]

\[
\text{Theoretical drug loading (\%)} = \left( \frac{\text{Weight of CMSP}}{\text{Weight of drug added}} \right) \times 100\%
\]

2.2.5. Size and weight uniformity of discs

The diameter, thickness and weight variation of randomly chosen unloaded and ciprofloxacin-loaded discs were measured using a dial calliper (Series 505, Mitutoyo, Japan) and an electronic balance (AX224, Sartorius, Germany). Readings were taken in triplicate.

2.2.6. Field emission scanning electron microscopy (FE-SEM) studies

A field emission scanning electron microscope (SU8010, Hitachi, Japan) was used to study the surface morphology of the discs. Samples were initially coated with a thin layer of platinum using a Q150R S rotary-pumped sputter coating system (Quorum Technologies, UK) before being observed at 100 and 1800x magnifications.

2.2.7. Infrared spectroscopy studies

Approximately 0.2 mg of unloaded and ciprofloxacin HCl-loaded discs were separately ground using a mortar and pestle, and their infrared spectra measured between 600 and 4000 cm\(^{-1}\) by a 640-IR FTIR spectrophotometer using attenuated total reflection (ATR) accessory (Varian, USA).

2.2.8. X-ray diffraction

An X-ray diffraction apparatus (InXitu BTXII, Olympus, USA) was used to obtain the X-ray diffraction patterns of the unloaded and drug-loaded discs. Each sample was ground to produce an even surface, with no light exposure and dried at 60 °C until a constant weight was achieved. Small circular discs were cut out using a 6 mm single hole puncher and then kept in light-resistant containers, which were stored in the desiccator. The unloaded discs were also prepared in the same manner but without the addition of the drug.

2.2.9. Differential scanning calorimetry

Differential scanning calorimetry (DSC) analyses were carried out in the temperature range from 25 to 350 °C on DSC 4000 (Perkin Elmer, USA). Approximately 5 mg of each respective sample were weighed and sealed into the aluminium pans. The heating rate was 15 °C min\(^{-1}\) while the nitrogen flow rate was 20 °C min\(^{-1}\).

2.2.10. Thermo gravimetric analysis

The thermo gravimetric analyses (TGA) were performed on a Q50 TGA (TA Instruments, USA). About 10 mg of samples were analysed over the temperature range from 25 to 500 °C at the heating rate of 10 °C min\(^{-1}\) under the flow of nitrogen at the rate of 50 ml min\(^{-1}\).

2.2.11. In vitro release studies

The in vitro release studies were carried out in an EDC-07 Franz diffusion cell chamber (Electrolab, India) using 5 ml of phosphate buffer (pH 7.4) as the release medium (Gupta et al., 2007). Drug-loaded discs (n=3) were placed in individual chambers, and the dissolution rates were measured at 37.0 ± 0.5 °C and 50 rpm. One ml of samples was withdrawn at regular intervals, and the same volume of the release medium was replaced. After filtration, the amount of the drug present in the sample was estimated at 271 nm using a UV–vis spectrophotometer (Shimadzu, USA).

2.2.12. Swelling kinetics

The swelling behaviour of the unloaded CMSP discs (n=3) was studied. The unloaded discs were weighed and added individually into Schott bottles containing 50 ml of pH 7.4 phosphate buffer. The swelling rates were measured at 37.0 ± 0.5 °C. The discs were removed over a 30 h period using a plastic sieve and blotted carefully with minimum pressure to remove any excess surface liquid. The swollen discs were then weighed using an AX224 electronic balance (Sartorius, Germany). The swelling ratio of the respective discs was determined using the following equation:

\[
\text{Swelling ratio(SR)} = \frac{w_t}{w_0}
\]

where \(w_0\) is the initial weight of the dry disc and \(w_t\) is the weight of the swollen disc at time \(t\). The equilibrium swelling ratio (ESR) of the respective discs was also determined, where ESR refers to the swelling ratio (SR) at equilibrium of the discs.

2.2.13. Antimicrobial efficacy studies

Microbiological studies were carried out to ascertain the biological activity of the loaded discs in comparison with standard ciprofloxacin discs (5 µg/disc) (Oxoid, UK) against microorganisms (Staphylococcus aureus strain ATCC 33591 and Pseudomonas aeruginosa strain ATCC 10145). The discs were placed on the agar surface using sterile forceps. The plates were kept at room temperature for 15 min and then incubated at 37 °C for 24 h. The diameter of the zone of inhibition (ZOI) thus obtained was measured by a ruler. Readings were taken in triplicate.

3. Results and discussion

3.1. Preparation of ciprofloxacin HCl loaded CMSP discs

The CMSP hydrogel solution of 20% (w/v) was formed intact and uniform discs after irradiation at 10, 20, 25 and 30 kGy due to the presence of more gel fractions as shown in Fig. 1a. Hydrogel prepared with 10% (w/v) CMSPs at a similar irradiation dose range were thinner and fragile as they were easily cracked during handling due to the presence of lower gel fractions (Fig. 1b). Gel fraction in 20% CMSP increased with increasing radiation (Fig. 1) dose from 10 to 25 kGy, indicating cross-linking predominates over scission and thus gel fraction is proportional to the applied radiation dose (Vijayabaskar et al., 2004; Thenapakiam et al., 2013). The reduction in % GF beyond 25 kGy is possibly due to more scission and oxidative degradation taking place as compared to cross-linking (Pushpamalar et al., 2013b; Thenapakiam et al., 2013). Similarly, gel fraction of 10% CMSP solution increased until
20 kGy and after that started declining at higher radiation doses. Irradiation of 20% (w/v) CMSP solution using 25 kGy resulted in the highest % GF and hence was used to cross-link the CMSP-ciprofloxacin mixture. Moreover, 25 kGy is the radiation dose for sterilization, which is an ideal and legal requirement for products applied in the eye.

Ciprofloxacin HCl is a zwitterionic drug and has dissociation constants $pK_a1$ and $pK_a2$ values at 6.1 and 8.7, respectively (Gu and Karthikeyan, 2005). The cationic form of molecules occurs when the pH is lower than 6.1 attributing to the protonation of the amine group in the piperazine moiety. In contrast, the anionic form dominates the above pH 8.7 due to the ionization of the carboxylic groups. When the pH is between 6.1 and 8.7, it is in the zwitterionic form. Besides that, ciprofloxacin stability in the aqueous mediums is also dependent on the pH, with maximum instability of the drug obtained at pH 12 (Adam et al., 2012). Hence, the CMSP solution was maintained at pH 7 before the incorporation of ciprofloxacin to ensure that it maintains neutral charge (zwitterion) and low solubility. This deters any possible drug–polymer interaction from occurring while also minimizing drug degradation.

### 3.2. Drug loading and entrapment efficiency

The amount of ciprofloxacin in the CMSP discs was evaluated by dissolving the discs in 2 N NaOH, which would break down the discs and completely retrieve the drug from the interior of the hydrogel matrix. The % of ciprofloxacin present in the disc was 14.7±1 w/w with an entrapment efficiency of 73.5% w/w. The high loading could be contributed by the high gel fraction produced after irradiation. The dense cross-linking within the CMSP matrix likely resulted in the immobilization of the drug particles within the polymeric network after irradiation.

### 3.3. Size and weight uniformity

The diameter of unloaded and loaded discs was 6 ± 1 mm and found to be uniform discs-shaped as shown in Fig. 2a and b, respectively. The thicknesses of the unloaded and loaded discs were 1 ± 0.4 mm (Fig. 2c) and 1.5 ± 0.3 mm (Fig. 2d), respectively. The low standard deviation of the values indicated uniform thickness. The unloaded discs were thinner and more brittle than the drug-loaded discs. This could be due to relatively higher cross-linking in the unloaded disc. The weights of the unloaded and drug-loaded discs were found to be uniform and were in the range of 29.1 ± 4 mg to 38.4 ± 5 mg, respectively, as they are affected by the increase or decrease of the film thickness proportionally. Based on average weight and drug loading, the amount of the drug present in each disc was calculated as about 5.64 mg. The thinner unloaded discs could be due to the relatively higher cross-linking within the polymer network in the absence of the drug. Increased cross-linking will shrink hydrogel, which is a known concept, thus resulting in relatively thinner and brittle disc. In the presence of the drug, the degree of cross-linking might be less and hence result in a relatively little increase in the thickness. In addition, the presence of the drug in the CMSP solution caused a slight increase in the viscosity and density of the drug–polymer mixture. The passage of radiation is also influenced by these parameters and thus may result in lower cross-linking.

Examples of ophthalmic inserts available for commercial usages (Macoul and Pavan-Langston, 1975; Lamberts et al., 1978) are the elliptical (Ocusert) and rod (Lacrisert)-shaped systems. The design and shape of these systems necessitate the need for alignment of the geometrical axis of the device with eyelid margin. The symmetrical circular design of the CMSP discs eliminates this need, supporting its suitability for eye application as less time and manual dexterity are required for insertion. The minidisc inserts, first described by Bawa et al. (1988), are designed like a miniature contact lens with 4 mm diameter. The thickness of the CMSP discs is slightly larger than the currently researched ocular inserts (Aburahma and Mahmoud, 2011; Deshpande et al., 2010). However, the thickness and diameter of the CMSP discs could be modified using a smaller hole punch or lower casting volume of the drug-loaded CMSP hydrogel in the petri dish moulds.

### 3.4. Field emission scanning electron microscopy (FE-SEM)

As seen in the FE-SEM photograph, the unloaded discs have a smooth surface (Fig. 2e) as the CMSP hydrogels have extensive cross-linking between the polymer chains. Whereas a great crosslinking and high polymer-to-drug ratio resulted in a smoother surface (Fig. 2f) for the drug-loaded discs, no visible drug particles were observed on the surface of the loaded discs (Fig. 2g), which was attributed to the inclusion of the drug inside the CMSP polymeric matrix.

The cross-sectional view of the discs was observed to evaluate in situ distribution of the drug inside the CMSP hydrogel networks. As shown in the FE-SEM image, the cross-section of the unloaded discs (Fig. 2g) was smooth and free from particles. In contrast, the cross-section of the loaded discs (Fig. 2h) reveals the entrapment of drugs within the cross-link network of the CMSP polymer matrix. The drug particles form a homogeneous distribution of the drug throughout the CMSP hydrogel networks in a highly dispersed state. Crystalline form of the ciprofloxacin HCl is shown in Fig. 2i, which was converted to fine dispersion as shown in Fig. 2h and both pictures were taken in the same magnification for comparison.

### 3.5. Infrared spectroscopy studies

The FT-IR spectrum of ciprofloxacin HCl is shown in Fig. 3a. The prominent characteristic peak at 3350–3500 cm$^{-1}$ confirms −OH stretching vibration due to intermolecular hydrogen bonding in ciprofloxacin. The characteristic absorption bands at 1623 and 1271 cm$^{-1}$ correspond to the quinolones, and the vibration of the phenyl framework conjugated to −COOH, respectively. The stretching vibration at 1705 cm$^{-1}$ represented the carbonyl C=O stretching. A peak at 2926 cm$^{-1}$ was observed for vibration of C–H from the phenyl framework. The peaks of ciprofloxacin HCl are well in agreement with those reported in literature (Tom et al., 2004; Sahoo et al., 2011).

The IR spectrum of CMSP (Fig. 3b) shows a broad peak around 3350 cm$^{-1}$ due to the stretching vibration of the −OH group. The peak at 2986 cm$^{-1}$ indicates the C–H stretching vibration. The presence of a strong absorption band at 1599 cm$^{-1}$ confirms the presence of COO– functional group and acts as evidence of
carboxymethylation of the sago pulp (Biswal and Singh, 2004). The bands at 1415 and 1324 cm\(^{-1}\) are assigned to \(-\text{CH}_2\) scissors and \(-\text{OH}\) bending vibration, respectively. The broad bands around 1026 cm\(^{-1}\) which can be ascribed to sugar ring absorption (Barbucci et al., 2000; Wang et al., 2007).

The characteristic ciprofloxacin HCl peaks mentioned earlier also appeared in the spectrum of the drug-loaded discs (Fig. 3c), inferring the stable nature of the drug in the formulated discs even after irradiation. The intensity of the drug peaks was lower in the disc due to the low concentration of the drug present on it. Some
of the drug peaks in the loaded disc were slightly shifted or merged with CMSP peaks and this could be due to the physico-chemical changes occurring upon the formation of the CMSP discs. However, the vital bands observed in the CMSP hydrogels also appeared in the drug-loaded discs.

3.6. X-ray diffraction

X-ray diffraction studies were performed to investigate the physical state of the drug in the discs, as it can influence the release characteristics of the drug. The crystalline state of ciprofloxacin HCl was evidenced in the XRD diffractogram (Fig. 4a); the profile exhibited sharp, intense and fewer diffused peaks that represent the highly crystalline structure (Sahoo et al., 2011) of the drug. No prominent peaks were observed in the unloaded CMSP discs (Fig. 4b), indicating the amorphous nature of CMSP in the formulated discs (Kamarudin and Isa, 2013). The X-ray diffraction pattern of ciprofloxacin-loaded disc demonstrated (Fig. 4c) the loss of drug crystallinity in the disc. The X-ray diffraction pattern of the drug-loaded discs was almost similar to that of the unloaded discs. The sharp drug diffraction peaks disappeared in the drug-loaded disc, which indicated the conversion of crystalline drug into the amorphous form during the preparation process. This observation also supports the FFE-SEM image (Fig. 2h) that shows the remarkable reduction in the crystalline nature of the drug in the radiation cross-linked CMSP matrix. The reduction in crystallinity in the formulation is a desirable property as the dissolution rate of poorly soluble drugs can be enhanced (Takeuchi et al., 2005; Meziani et al., 2009). As crystalline ciprofloxacin is less soluble at pH 7.4, the amorphous form could enhance dissolution, and hence bioavailability.

3.7. Differential scanning calorimetry

Fig. 5a shows the DSC thermograms of the ciprofloxacin HCl, unloaded CMSP discs, and drug-loaded CMSP discs. The thermal profile of ciprofloxacin HCl exhibited two endothermic peaks at 156 and 322 °C (Fig. 5ai). The small peak at 156 °C corresponds to the complete loss of water molecules as the drug possessed hygroscopic property (Yong et al., 2005). The dominant peak at 322 °C corresponds to the melting point of the crystalline ciprofloxacin HCl (Turel and Bukovec, 1996).

Unloaded CMSP discs showed two endothermic melting peaks at 162 and 182 °C (Fig. 5aii). The first peak is caused by dehydration since CMSP also possesses hygroscopic property (Yong et al., 2005), while the second peak is associated with the structural transitions of the polymer chains (Lojewskia et al., 2005). During the heating process, the residual non-substituted O6H6 groups in the CMC chains are oxidized, producing the peak at 182 °C (Li et al., 2009; Watanabe et al., 2006). The DSC thermogram of the physical mixture (1:1) of the drug and CMSP (Fig. 5aiii) showed one broad and two sharp peaks. The broad de-hydrated peak is due to the fusion of the dehydration stages of both the drug and CMSP. The two later sharp peaks were similar to those of unloaded CMSP and crystalline drug thermograms, corroborating the absence of any drug and CMSP chemical interaction.

For the DSC thermogram of drug-loaded discs (Fig. 5aiv), an almost identical thermal behaviour with a slight change in the peaks (163.25 °C and 188.40 °C) as those of unloaded CMSP discs was observed. No characteristic endothermic peaks corresponding to the drug appeared in the DSC thermogram of the drug-loaded discs, indicating the absence of the crystalline state of the drug. This evidence was supported by XRD studies, showing that
The commercially available ciprofloxacin eye drops (Ciloxan) strength is 0.3% and the first day recommended dose (MIMS, 2011) for the treatment of corneal ulcer is about 0.1 ml every 15 min for the first 6 h and 0.1 ml every 30 min for the remaining hours. The cumulative dose crosses 12 mg. On subsequent days, the dose is gradually reduced. The formulated disc has a relatively low amount (∼5 mg) of drug for 24 h sustained effect. This would expect to give better antimicrobial effect than conventional drops as the disc is meant for single administration.

3.10. Swelling behaviour

To support the drug release mechanism, swelling studies of unloaded CMSP were performed in phosphate buffer pH 7.4. CMSP discs begin to swell above pH 5–6 due to the development of osmotic swelling forces (Pushpamalar et al., 2013a). This phenomenon arises as a result of their unprotonated COO− groups, which are completely ionized at this pH. The swelling profile (Fig. 6b) for the unloaded CMSP discs showed a comparatively high extent of swelling, which could be due to the hydrophilic nature of CMSP (Boppana et al., 2010). The high CMSP cross-link density was induced by irradiation, which has produced a more compact hydrogel matrix due to the tightening of the polymer chains. This radiation cross-linked hydrogel is capable of swelling up to 36 h, giving rise to a higher equilibrium swelling ratio. The swelling profile of CMSP correlates to the drug release profile. As the drug-loaded disc starts to swell, the drug diffuses through the porous CMSP matrix to the release medium in a sustained manner. The lag time in swelling confirms the reason for the very slow release of the drug in the first 5 h.

3.11. Release kinetics

Data obtained from in vitro release studies were fitted to zero- and first-order models (Saravanan et al., 2004). The subsequent plots were made: $Q_t$ vs. $t$ (zero-order kinetic model), and log ($Q_t - Q_0$) vs. $t$ (first-order kinetic model), where $Q_t$ is the quantity of drugs released at time $t$ and $Q_0$ is the initial quantity of the drug present in the drug-loaded discs. Additionally, to verify the mechanism of drug release, first 60% drug release was fitted in Korsmeyer–Peppas model where $M_t/M_\infty$ is the fraction of drug released at time $t$, $k$ is the rate constant and $n$ is the release exponent. The $n$ value is used to illustrate different release mechanisms (Peppas, 1985).

Zero-order models are commonly used to describe the hydrophobic drug release from delivery systems such as matrix tablets and osmotic systems, where the drug release would be directly proportional to time (Varelas et al., 1995). In contrast, first-order models can describe the release from the delivery systems containing hydrophilic drugs dispersed in porous matrices, where drugs would be released at the rates proportional to the amounts of drug remaining in the interior of the delivery system (Gibaldi...
and Feldman, 1967; Mulye and Turco, 1995). The 20% (w/w) loaded discs have followed the first-order release, showing higher correlation ($r^2 = 0.99$) than zero order ($r^2 = 0.77$). This could be attributed to the tightly bounded drug within the CMSP polymeric matrix and the steady swelling rate (Fig. 5b) which contributed to the slow sustained release which increases over time, and thus showed a first-order release kinetic (Wagner, 1969).

The release profile also showed a high correlation ($r^2 = 0.97$) with the Korsmeyer–Peppas equation. The value of the exponent $n$ was 0.82, depicting non–Fickian or anomalous release mechanism (Korsmeyer et al., 1983; Ritter and Peppas, 1987). This might indicate that the drug loaded in the CMSP discs is released by the cooperative mechanism of diffusion and matrix erosion. Drug release from CMSP hydrogel mainly occurred by the rates proportional to the amounts of drug remaining in the system as commonly observed in the first-order kinetics. The drug release rate is important because in an ophthalmic application, the tear film is drained and replenished at a regular rate. For the eye application, the delivery system must be able to maintain effective concentrations of the drug throughout the prescribed wearing period, as unabsorbed drug is eliminated from the ocular surface. Slower release rates enable higher drug concentration in the eye (Lesher and Gunderson, 1993) and therefore, the CMSP disc shows promising scope in the treatment of eye infections.

3.12. Antimicrobial efficacy studies

*S. aureus* and *P. aeruginosa* are among the leading causes of bacterial conjunctivitis (Schein et al., 1989; Wahl et al., 1991). These microorganisms can cause severe ocular infection, keratitis that can progress rapidly, resulting in intense inflammation, irreversible stromal scarring, and probable loss of vision. Presently, infective conjunctivitis is treated with topical application of fluoroquinolones. Ciprofloxacin, a second-generation fluoroquinolone, is considered as a potent drug and has virtually replaced the standard therapy regimen (fortified tobramycin and cefazolin) for bacterial corneal ulcers treatment (Hynduiq et al., 1996; Gangopadhyay et al., 2000). Therefore, the antimicrobial efficacy of the formulated drug-loaded discs was tested against these two microorganisms.

The results of antimicrobial effect of the standard ciprofloxacin discs against *S. aureus* and *P. aeruginosa* are shown in Fig. 7a and b, respectively. The drug-loaded CMSP discs showed slightly lesser activity against *Staphylococcus aureus* than *Pseudomonas aeruginosa*. This study indicated that ciprofloxacin retained its antimicrobial efficacy when incorporated in the CMSP discs even after radiation exposure. However, the ZOI of CMSP disc (5.6 mg/disc) was comparatively smaller than the standard commercial disc (5 mg/disc) in terms of drug content probably due to the diffusion limitation of the drug from CMSP discs to the agar surface. As evidenced in Fig. 6a and b, some level of swelling is required for drug diffusion and hence low ZOI was observed in the CMSP disc.

4. Conclusion

The present investigation clearly indicated the prospect of developing an ocular drug delivery system using CMSP. The amorphous nature of the drug in formulated discs was shown by XRD, DSC, and TGA studies. FTIR and DSC revealed no significant drug–polymer interaction in the formulation. The drug release was sustained with kinetic obeying first-order’s model. The CMSP discs show promising scope in the ophthalmic delivery of ciprofloxacin as they can improve ocular bioavailability by increasing the duration of the contact of the drug with corneal tissue and eradicating the frequency of administration that is essential for

![Fig. 7. Zone of inhibition (ZOI) ($n=3 \pm sd$) of standard ciprofloxacin HCl against *Staphylococcus aureus* (a) and *Pseudomonas aeruginosa* (b). The figure also shows ZOI ($n=3 \pm sd$) of ciprofloxacin-loaded CMSP disc against *Staphylococcus aureus* (c) and *Pseudomonas aeruginosa* (d).](image-url)
conventional eye drops. Moreover, applied radiation dose fulfills the requirement of sterilization, which is one of the requirements of ocular drug delivery. However, further in vivo studies are required to confirm its efficacy.

Conflict of interest

The authors declare that they have no conflicts of interest.

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