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# Controlled release of antimicrobial Cephalexin drug from silica microparticles

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ABSTRACT

Release of antimicrobial drugs in a controlled fashion for extended duration of time has been investigated for long. Such controlled-drug-releasing materials show promising applications in medicinal bandages. Along with antimicrobial agents, one could also incorporate other therapeutic drugs, to make such bandages more versatile. In this context, silica micro particles were synthesized using direct reduction method, in which the synthesis was done in the presence of Cephalexin. Cephalexin was chosen as an antimicrobial candidate. The morphological characterization shows formation of monodispersed, silica microparticles of ~200 nm in size. The FTIR spectroscopy shows weak interaction of the drug molecule at its hydroxide (OH) site with oxygen ions on the silica surface. Upon conjugation, the UV–vis spectroscopy shows persistence of the Cephalexin signature, especially its R group, confirming its antimicrobial activity even after conjugation. Loading studies reveal 12% Cephalexin loading on silica. The antimicrobial studies were done on three micro-organisms, namely, *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli*. Using zone-of-inhibition studies, it was found that *E. coli*, did not respond to the delivery of Cephalexin either directly or via microparticles. However, for both *S. aureus* and *B. subtilis*, the particles showed controlled release of Cephalexin for the duration of 48 h and continued maintenance and even increase in the zone of inhibition. This work demonstrates an effective protocol to prepare antimicrobial patches for controlled drug delivery.



## 1. Introduction

Advances in science and technology have seen unprecedented growth of pharmaceutical industry, especially due to the evolution of submicron science. The medical diagnostic tools and formulations have become miniaturized. Less dosage of drugs is now required with better efficacy. Additionally, a capability of on-site-drug-delivery has given rise to lesser side-effects. Sustained drug delivery/release is one such area, which is benefitted from such advancement of nano and submicron science. Many attempts are done by polymer chemists and organic chemists to develop bio-compatible polymers which can be used for drug storage. These drugs can be then either released in-vivo or used in-vitro for sustained release, depending upon the applications envisaged. Researchers have tried inorganic porous particles which could do similar function, like ZnO, $TiO_2$ and silica. In materials like silica [1–5], the porosity is largely dependent upon the particle–particle spacing, the surface area and the pore volumes. The smaller the particles, the larger are the surface areas, which result in better drug loading capacity. Furthermore, if some chemical functionality is introduced into these inorganic moieties, then drug can be released in a selective fashion. These attempts are much underway.

Other than silica, various polymeric systems have also been well studied in the context of drug carriers [6–13], such as PLGA nanoparticles [6], polymer hydrogels [7], polyketals [8], polyvinyl alcohol and starch polymers [9], to name a few. Such polymers and silica nanoparticles have also been used to carry the drug pay-load, which is especially poorly water-soluble. Though drugs have high therapeutic values, many of them have limited bio-availability, especially because they are poorly water-soluble. Zhao et al. [10] have shown a drug, Honokiol, which has poor water solubility, and has been loaded onto polyethylene glycol–polylactic acid polymer system to improve drug delivery. On similar lines, similar polymeric micellar combinations have been used by Shin et al. [11] to load and deliver multiple anti-cancer drugs, simultaneously. Scholsky et al. [12] have studied polyacrylate ester latex particles in the diameter range of 40–400 nm synthesized with the drug moieties attached by ester linkages, to form pendant drug-like structures. They have further studied and reported sustained release of these active materials by either acid- or base-catalyzed hydrolysis of the pendant linkages. One of the challenging areas of research which is being explored in recent times [14] is the stimuli-responsive organic and inorganic systems, which could be used in developing environmentally sensitive macromolecules

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which could be crafted into new smart materials; commonly being referred as "smart delivery systems".

In this context, we report on the synthesis of silica microparticles and loading Cephalexin drug over these microparticles. The particles have been studied for their sustained release using 3 different micro-organisms, namely, *Staphylococcus aureus, Bacillus subtilis and Escherichia coli*. Zone-of-inhibition studies have been performed to evaluate the efficacy and efficiency of release.

## 2. Materials and methods

Tetraethyl orthosilicate $Si(OC_2H_5)_4$ (TEOS 99%, Sigma Aldrich), ammonium hydroxide solution ($NH_4OH$ 25%, Thomas Baker), ethanol (99.9%, Changshu Yangyuan Chemical) and Cephalexin (Sigma Aldrich) were used for synthesis of Cephalexin conjugated silica microparticles. Test cultures used in this study were *E. coli* (ATCC 8739) *S. aureus* (ATCC 29737) and *B. subtilis* (ATCC 6633). These were grown for 16 h in sterile Luria Bertani broth (procured from HiMedia Laboratories, India). All the cultures were routinely maintained by sub culturing on Luria Bertani (LB) agar. The growth medium components were purchased from HiMedia Laboratories, India.

### 2.1. Synthesis of silica microparticles

Out of many synthesis procedures already documented by many [15–17], standard Stober method [15] was used. In this method the starting solution was prepared from 15 ml ethanol (99.9%), 4 ml D I water, 0.75 ml ammonia (25%), in the molar ratio of $C_2H_5OH::H_2O:NH_3$ is 17.12 M:55.5 M:42.86 M. After 30 minute stirring, 1.2 ml of TEOS was added (4.55 M) to the solution. The whole solution was stirred for 3 h, finally a whitish precipitate was obtained which was centrifuge at 3000 rpm, washed and dried at 60 °C.

### 2.2. Synthesis of conjugated Silica Cephalexin (Si:Cephalexin) microparticles

On similar lines as mentioned in Section 2.1 above, silica microparticles were prepared by hydrolysis and condensation of TEOS in ethanol (15 ml) and in presence of ammonium hydroxide (0.75 ml). Starting solution was prepared from 15 ml ethanol, 4 ml D I water, and 0.75 ml ammonia. Cephalexin (4 mg/ml) was additionally added simultaneously along with TEOS (1.2 ml). The whole solution was stirred for 3 h which gave rise to whitish precipitate, which was further washed and centrifuged at 3000 rpm and dried at 60 °C. The samples are further referred to as Si:Cephalexin.

### 2.3. Construction of bandages/samples for antimicrobial release studies

Chemical spray pyrolysis was used and well adherent films were deposited. The distance between nebulizer to substrate was 50 cm and the temperature of the substrate was maintained at 80 °C. The spray nozzle, with the help of the argon carrier gases, accomplished the atomization of the chemical solution into aerosols. In this process, first the solution of 100 mg Si:Cephalexin in 10 ml of D I water was pulverized (sprayed) by means of argon carrier blow and deposited on the glass substrate. The deposition time was 10 min. Control samples were also deposited using the same technique, namely: only Cephalexin (12 mg/10 ml) and only silica (88 mg/10 ml). The concentrations were decided using the drug loading studies as mentioned in coming paragraphs. Similar samples were deposited on thin bandage cloth materials (procured from George and George Hygiene Products Pvt Ltd., India) as well.

### 2.4. Antimicrobial study (zone of inhibition) assay-methodology

The test organisms (*S. aureus*, *B. subtilis* and *E. coli*) were grown for 16 h in sterile Luria Bertani broth. 100 μl of this broth culture was added to 10 ml of sterile soft agar solution and mixed well. The soft agar was then overlaid onto basal LA (Luria Bertani agar). The thin film of Si:Cephalexin coated on the surface of a cover slip was then gently placed with the help of a forcep on top of the agar plate containing the test organism. The plate was then incubated at 30 °C till 72 h to observe for increase in the zone of inhibition as the test compound is progressively released from the thin film surface. These zones were measured and were reported in the units of diametric lengths.

### 2.5. Release studies

To evaluate the release studies, the following procedure was employed. The conjugated particles of Si:Cephalexin were taken at ~10 mg in 20 ml water. Such 4 samples were made and kept in individual test-tubes. These tubes were kept on a simple shaker and after every 12 h, one test tube was removed from the shaker and subjected to 3000 rpm of centrifugation for 2 min. This was done to collect the released Cephalexin drug in water from the silica surface after definite time intervals (because silica micro particles settle at 3000 rpm, leaving only the released Cephalexin in the water medium at supernatant level). The supernatant was then removed with a quantity of 5 ml. The sample was then evaluated using UV–vis spectrometer to observe the signature of Cephalexin. Handling individual sample each time after 12 h ensured that the sample does not get concentrated with time, and the readings are not ambiguous. The data was taken for duration of 48 h. The whole procedure was repeated four times to get the final values. This was done to ensure repeatability and correctness of the data.

Fourier transform infrared (FTIR, Shimadzu Systems) spectroscopy, thermogravimetric analysis (TGA, PerkinElmer Inc.), scanning electron microscopy (SEM, JEOL made), atomic force microscopy (AFM, Asylum Research Labs), surface area analysis (BET, Metrohm Systems) and UV–vis spectroscopy (JASCO (model V-570)) have been used for characterizing the synthesized samples. The following formula was used for calculation of percentage of Cephalexin loading:

$$\%\text{Loading Drug} = \frac{W_1 - W_2}{W_1} \times 100$$

$W_1$ initial weight of the Silica:Cephalexin sample
$W_2$ weight of the sample at which Cephalexin decomposed completely

## 3. Results and discussion

### 3.1. Structural and loading characterizations

Fig. 1 shows the FTIR spectrum of silica (a), Cephalexin (b) and Si:Cephalexin (c). The typical signatures of silica were observed in (a) with bands at 798, 950 and 1050–1100 $cm^{-1}$. The bands at 950, 798 and 1070 $cm^{-1}$ were due to Si – OH stretching, Si – O – Si symmetric and asymmetric stretching vibrations respectively [18]. The bands at 798 $cm^{-1}$ were attributed to the ring structure of the $SiO_4$ tetrahedral. The highest frequency band near 1094 $cm^{-1}$ was associated with the Si – O – Si stretching vibration. The 950 and 571 $cm^{-1}$ bands were due to Si – O – groups. The band at 440–480 $cm^{-1}$ was of Si – O – Si bending vibration mode. The broadening and slight shifting of Si – OH (3200–3700 $cm^{-1}$) signatures in Si:Cephalexin system (as shown in Fig. 1(c)) as compared to both silica (Fig. 1(a)) and Cephalexin (Fig. 1(b)) hinted that it was due to adsorption of Cephalexin. The other characteristic FTIR spectrum of Cephalexin with bands at 1770 $cm^{-1}$ (C═O group) of four membered ring lactams (1418, 1395 and 1357 $cm^{-1}$) and COOH functional group, R-group bands at 1697 $cm^{-1}$ (C═O group), 1605 $cm^{-1}$ (primary amine), 1505 $cm^{-1}$ (C – C ring stretch) and