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Research Article

SUSTAINED RELEASE OF SILICA GEL ENTRAPPED HERBAL VALUES AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Sol-gel derived silica gel was synthesized in room temperature and two important herb extracts (*Ocimum sanctum*, *Terminalia chebula*) were entrapped into the silica gel. FTIR study revealed the proper absorption of the herbal extracts into the nano porous silica gel. Almost 28% release of herbal values from entrapped condition was attained at 216 hours. The silica gel entrapped herb extracts remain functionally active and shows its antibacterial effect. Minimal inhibitory concentrations (MIC) of the gel entrapped extracts were also determined.

Keywords: Silica gel, FTIR, MIC, Herb Extract, Sustained release, Ocimum sanctum, Terminalia chebula.

INTRODUCTION

Sol gel processed materials represent a controlled release of biologically active agents. Room temperature processed sol gel derived silica has been explored for various biomedical applications. Acid catalyzed or base catalyzed silica gel have been used for the encapsulation of enzyme, proteins, DNA, RNA, cells and living tissue in their viable state [1]. This room temperature process provides easily reproducible silica gel properties. Large quantities of biological agents can be added and uniformly distributed in the liquid sol. These materials are resorbable, highly porous and nano structured (pore size from1-5 nm). The silica gel materials are biodegradable and are known to cause no adverse effects in the surrounding tissue or various organs. They are bioactive, they form bond to bone and have osteoconductive properties. These can be used in biomedical and dental applications [2].

Our aim is to study this silica gel material as a sustained delivery system for herbs like *Ocimum sanctum* and *Terminalia chebula* and also determine the MIC value of gel entrapped herbal extract. The *Terminalia chebula* fruit extract has been reported to have strong antioxidant capacity and high amount of phenolic compounds including gallic acid, ellagic acid and corilagin are present in it. These compounds have been shown to have anti-cancer, antimicrobial, and anti-inflammatory activities [3].The extract of *Ocimum sanctum* leaves is hypoglycemic immunomodulatoy, anti-stress, anti-inflammatory, anti-ulcerogenic, anti-hypertensive, radio-protective, anti-tumour and antibacterial. Eugenol is considered the active constituent of *Ocimum sanctum* and is largely responsible for the therapeutic activity of *Ocimum sanctum* [4].

Data regarding determination of sustained release of herbal extract as well as MIC in gel entrapped condition is not available in any literature reviewed.

MATERIALS AND METHODS

a) Preparation of herbs extract

Terminalia chebula and Ocimum *sanctum* were subjected to aqueous extraction by using soxhlet apparatus. The aqueous extract of *Terminalia chebula* (5 gm) and the Ocimum *sanctum* (5gm) were used for further experiment.

b) Synthesis of silica gel

The chemical reactions that take place during the synthesis of silica gel are hydrolysis and condensation reactions. The hydrolysis reaction which can be either acid or base catalyzed, replaces alkoxide groups with hydroxyl groups. Siloxane bonds (Si-O-Si) are formed during subsequent condensation.

Alcohol and water are the byproducts of the condensation reaction which evaporate during drying.

$$Si(OEt)_4 + H_2O \xrightarrow{H^+} Si(OH)_4 + EtOH$$

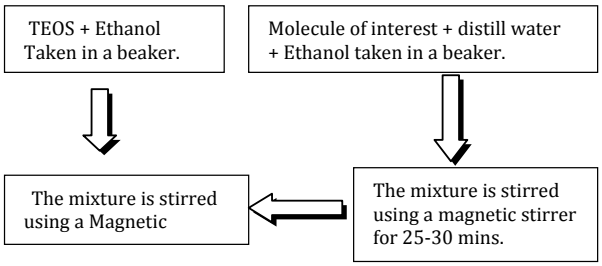
$$[Si(OH)_4]_n \rightarrow [\mathbf{Si\text{-}O\text{-}Si\text{-}O\text{-}Si}]_n$$

Theoretically the overall reactions is as follows,

$$n\,Si(OR)_4 + 2n\,H_2O \xrightarrow{H^+} n\,SiO_2 + 4n\,ROH$$

Completion of the reaction and chemical composition of the resulting product depend on the ratio of silica precursor, ethanol, water and also the concentration of the solvents , pH of the sol, temperature, aging and drying schedules. All silica gels are synthesized by a room temperature process using silica precursor as tetra ethyl ortho silicate (TEOS).

In general the procedure is as follows



After mixing, the sols were cast into cylindrical beaker and the beakers were sealed with porous plastic packs. Subsequently the beaker was allowed to dry until the gel's weight became constant. In this way *Ocimum sanctum* and *Terminalia chebula* were entrapped into silica gels[2, 5,6] .

Characterizations

The optical absorption spectra of the samples over 300nm – 800 nm were taken using (Perkin Elmer, Lambda 35 UV-VIS spectroscope). FTIR spectrum of the silica gel and gel entrapped *Ocimum sanctum* and *Terminalia chebula* extract were taken by using Schimadzu (Model: prestige 22) FTIR spectroscope. SEM analysis was done by using JEOL, JSM (Model no: 6360).

The MIC was carried out in Micronaut system (Merlin, Germany) with the help of Multiskan EX (Thermo, Finland) spectrophotometer system.

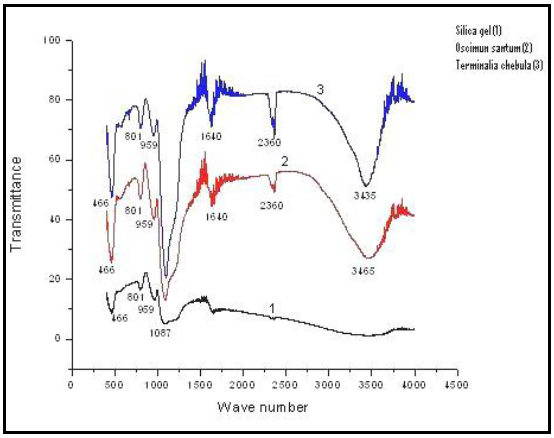


Fig. 1: FTIR Spectrum of bare Silica Gel along with *Ocimum sanctum* and *Terminalia chebula* extract entrapped in silica gel.

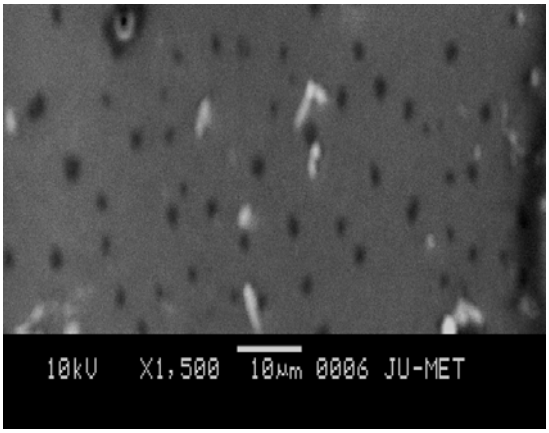


Fig. 2: SEM micrograph of porous silica gel.

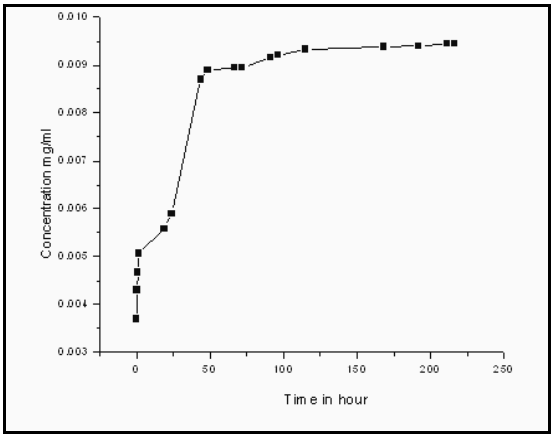


Fig. 3: Cumulative release of *Terminalia chebula* for 240 hours.

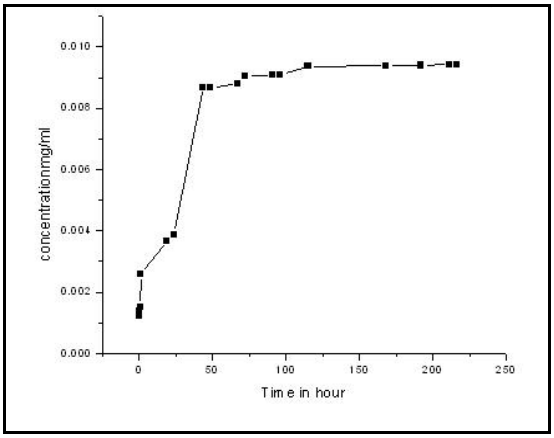


Fig. 4: Cumulative release of *Ocimum sanctum* for 240 hours.

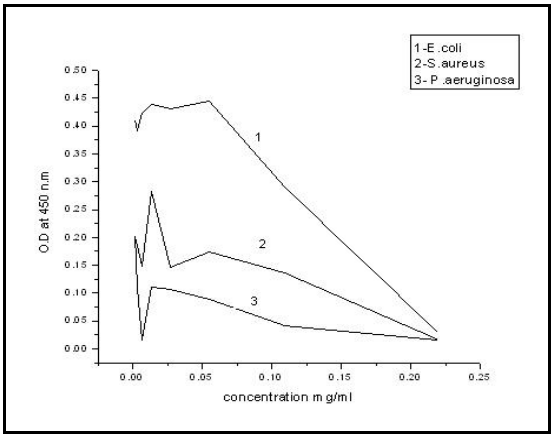


Fig. 5: Minimum inhibitory concentration determination graph of *Ocimum sanctum* extract entrap silica gel against different bacterial strain.

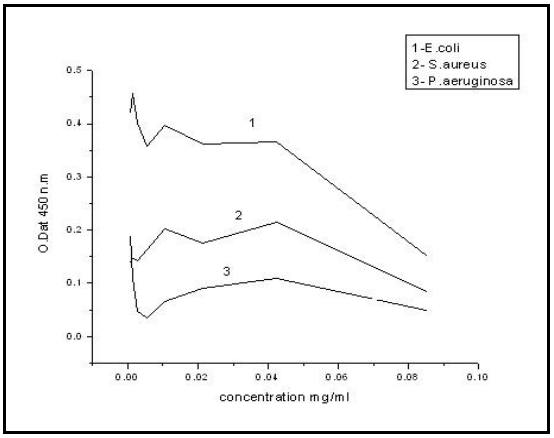


Fig. 6: Minimum inhibitory concentration determination graph of *Terminalia chebula* extract entrap silica gel against different bacterial strain.

Table 1: It has been found that total percentage of *Terminalia chebula* and *Ocimum sanctum* extract released after 216 hours are 27.82%and 27.76% respectively.

Time (Hour)	Cumulative concentration (mg/ml) (*Terminalia chebula*)	Cumulative concentration (mg/ml) (*Ocimum sanctum*)
0.0833	0.0037	0.00122
0.25	0.0043	0.00139
1	0.00467	0.00152
1.25	0.0051	0.00257
19	0.0053	0.00366
24	0.0059	0.00387
43.5	0.0087	0.00869
48.5	0.00889	0.00869
67	0.00894	0.0088
72	0.00895	0.00904
91	0.00916	0.00908
96	0.0092	0.00908
115	0.00933	0.00937
168	0.00938	0.00939
192	0.00940	0.00940
211	0.00945	0.00943
216	0.00945	0.00943

RESULT AND DISCUSSION

a) FT Infra Red analysis

Fig 1 represent the FTIR spectra of bare silica gel 1, *Ocimum sanctum* extract 2 and *Terminalia chebula* extract 3 loaded silica gels respectively, within the range of 4000-400 cm^{-1}. In all the samples peaks at 470 and 810cm^{-1} were found for the Si-O-Si bending vibration, peak at 1100 cm^{-1} and at 960 cm^{-1} are due to the Si-O stretching and Si-OH stretching vibration respectively . Peak arises at 1087cm^{-1} is for Si-OR stretching vibration[7,8].While in graphs b & c [encapsulated form] two distinct peaks at 3466cm^{-1} and at 1640cm^{-1} were found for phenolic OH and conjugated keto group respectively along with the peaks of silica. Various compounds containing these groups are present in the extracts of *Ocimum sanctum* and *Terminalia chebula* [9,10]. So it can be concluded the herbal extract had been successfully entrapped in the silica gel matrix.

b) SEM analysis

Fig 2 represents the SEM image of the porous structure of silica gel. Dark pores of nanometric dimension acts as the host for the herbal extract whose incorporation is confirmed by FTIR studies.

c) Amount of release percentage study

Fig 3 and Fig 4 show the cumulative release of *Terminalia chebula* and *Ocimum sanctum* from silica xerogel matrix with time. We have measured the released amount of extracts within PBS buffer from the absorbance at 276 nm for *Terminalia chebula* and 278 nm for *Ocimum sanctum*, with respect to time and calculated the concentration from standard curves [11]. In both cases the release kinetics were initially very rapid and follows first order reaction upto around 50 hours. Then the release concentration attains a steady state or follows zero order reaction. We have thoroughly observed the released extract upto 216 hours[2, 12].

The total calculation of concentration with respect to time is given in table [1] for *Ocimum sanctum* and *Terminalia chebula* respectively.

d) Microbial analysis

Three strains (*Staphylococcus aureus* ATCC 25923*, Escherichia Coli* ATCC25922 *Pseudomonas aeruginosa* ATCC27853*)* were taken. Gel entrapped herbal values were powered in a mortar pestle and was added to 1000µl of Mueller Hinton broth and centrifuged at 1200 rpm for 5 minutes. Then 25 µl of supernatant was collected and mixed with 75 µl of fresh Mueller Hinton broth and serially double diluted upon eight microwells. Then 10 µl of bacterial culture broth were added in each wells of the 96 well microtitre plates. The O.D at 450 nanometer had been taken at zero hour and after four hours of incubation.

MIC study analysis

Graph 5 shows the inhibitory action of *Ocimum sanctum* on different bacteria. MIC was approximately 0.05 mg/ml and complete inhibition of growth was at >0.212mg/ml. Graph 6 shows the inhibitory action of *Terminalia chebula* on different bacteria. MIC was observed near about 0.04 mg/ml and complete inhibition is at > 0.08 mg/ml.

DISCUSSION

Based on the in vitro and in vivo analysis, Room temperature synthesized silica gel can be characterized as resorbable and biocompatible material for the controlled release of herbal extracts. FTIR analysis revealed that herbal values of *Ocimum sanctum* and *Terminalia chebula* were adsorbed into the nano pore of silica gel matrix. Porous nature of silica gel was characterized by SEM analysis. The extracts showed a broad spectrum of antibacterial activity against gram-positive and gram-negative bacteria. *Ocimum sanctum* showed greater antibacterial efficacy against the gram-negative bacteria due to the presence of eugenol. Hydrophobicity of eugenol enables it to penetrate the lipopolysaccharide layer of the gram-negative bacterial cell membrane, and disrupts the cell structures leading to leakage of cell content [4, 13]. Silica gel entrapped *Terminalia chebula* showed significant antimicrobial activity against gram-negative bacteria where as sole extraction of this herb shows antimicrobial efficacy mainly against gram-positive bacteria [14,15].

CONCLUSIONS

FTIR study revealed the proper adsorption of herbal value into the nanoporous gel. SEM analysis proved that the gel is porous in nature U.V visible spectroscopy showed 49% release of gel entrapped extract from the pore after 240 hr. Thus Silica gel is considered as control release system. MIC value of *Terminalia chebula* was 0.04 mg/ml and that of *Ocimum sanctum* was 0.05 mg/ml against *Staphylococcus aureus, Escherichia Coli* and *Pseudomonas aeruginosa.* The compounds present in herbal extract are functionally active in gel entrapped condition.

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