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Research Article

Development of Controlled Drug Delivery System for a Traditional Medicine and Study of Its Antimicrobial Property

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ABSTRACT

Development of a novel drug delivery system to provide sustainable and controlled release of a traditional Ayurvedic medicine, Makaradhwaja (enhances the power of immunity and prevents inflammatory and degenerative changes) has been attempted by using the process of encapsulation into nano porous silica gel matrix. Following entrapment of Makaradhwaja, the synthesized gels have been subjected to characterization by FTIR, HRTEM and UV-visible spectroscopic analysis. FTIR and HRTEM studies had been done to authenticate the presence of drug molecule into the gel matrix. In FTIR spectrum the broad peaks at 3474 $cm^{-1}$and 1637 cm^{-1} are detected due to the presence of Makaradhwaja into silica gel. The entrapment has also been proved by the HRTEM image. Release kinetics in a Simulated Body Fluid (SBF) has been subsequently observed and the UV-visible spectroscopy reveals up to 50% release of drug molecule in the Simulated Body Fluid (SBF) in 240 hours. The significant antimicrobial activity of the formulation had been observed against three pathogenic bacterial strains i.e. *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* by using disc diffusion method Acute oral toxicity study on mice had been done and throughout the two weeks of the treatment no significant changes had been observed in behavioral pattern, clinical sign and body weight of the animals. All data indicates that the formulation could be used as bioactive controlled drug delivery systems.

Key words: Makaradhwaja, traditional medicine, disc diffusion, controlled drug delivery.

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Introduction

To increase drug bioavailability and the accumulation of drug molecules in required zone, to minimize drug degradation or loss and to reduce the harmful side-effects different types of drug delivery systems are currently developed.[1] While newer and more powerful drugs continue to be developed, increasing attention is being given to the methods by which these active substances are administered. For last few years, controlled drug delivery systems have become an important area for the researcher. Controlled drug delivery would be the optimal way for drug administration if the drug could be precisely matched with physiological needs.[4] Traditional Systems of Medicine dealt with the medicines made by using drug materials from plant, mineral and animal origin. The healing properties of these medicines are documented in traditional and scientific literatures. In the present study Makaradhwaja i.e. the compound of purified mercury, sulphur and gold (8:16:1ratio) has been used.[3, 7] In Makaradhwaja preparation initially gold and mercury (purified by using particular methods)[17] have been triturated in stone mortar pestle. Then sulphur was added to the mixture and triturated well to prepare a homogenous mixture. This way a black mixture has been produced and then it was subjected to sublimation in sand bath.[2, 17] The yield product (Makaradhwaja) has been triturated again to get an odorless, tasteless, fine powder with bright red colour. The pH of the drug is 5.2 and it is insoluble in water or alcohol. The drug is soluble in Aqua regia. The XRD (Figure. 1) of powder Makaradhwaja (by using Rigaku Ultima III with a Bragg – Brentano geometry and a Cu-kα radiation λ= 0.154) has been done for standardization and Scanning Electron Microscopy (SEM) analysis (Figure. 2a and 2b) was done by using JEOL, JSM (Model no: 6360). All the drug samples had shown the presence of Hg, S and Au in the X-Ray Diffraction analysis. No free molecule of Hg or S was present in the sample and they are all in the form of HgS. Side by side the presence of Gold molecule in the sample has also been detected.

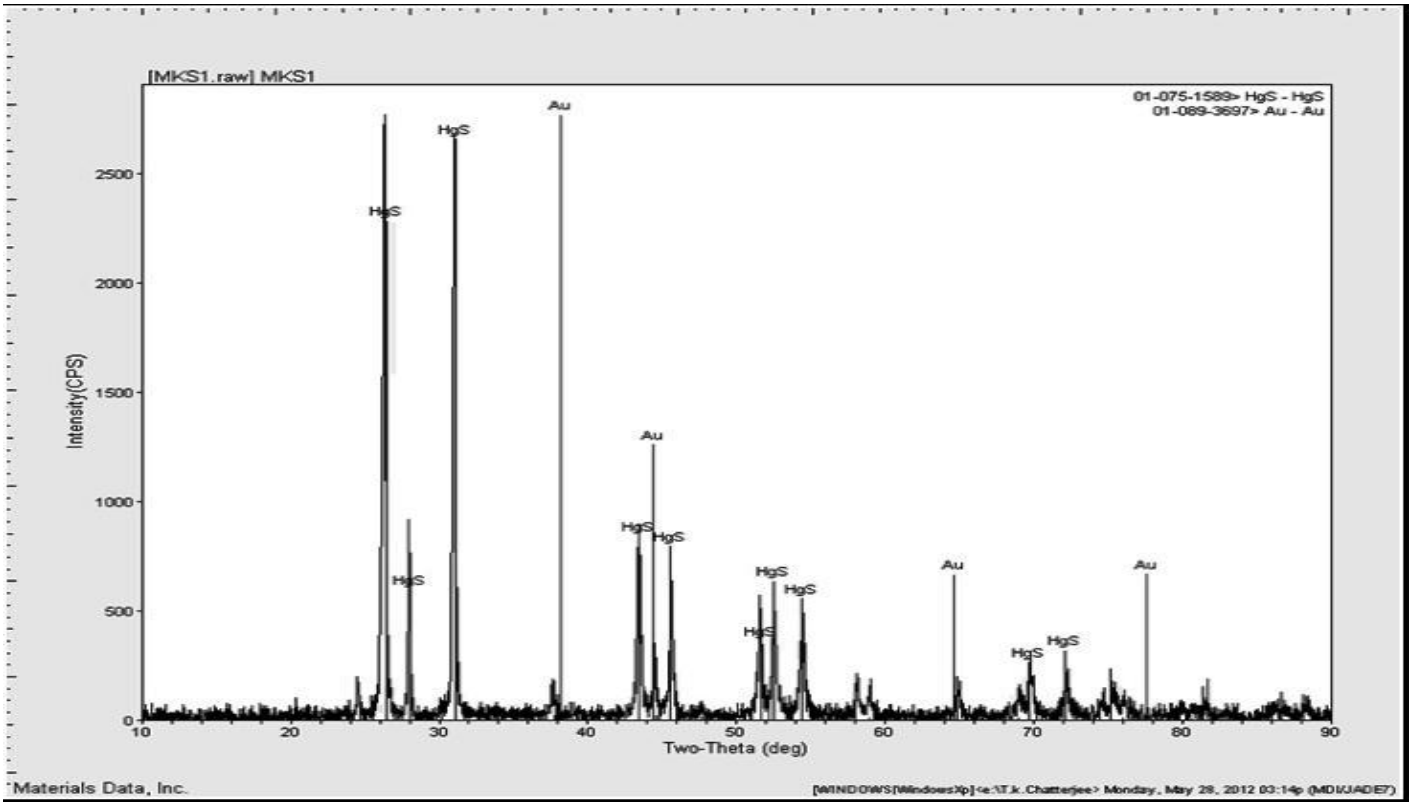


Figure.1: The XRD graph of powder Makaradhwaja

The crystallite size of the powder drug was determined from X-ray line broadening using Scherrer's equation

$$D = 0.94 \lambda / (\beta \cos \theta)$$

Where D= the average crystallite domain size perpendicular to the reflecting planes; λ =X-ray wavelength; β =Full width at half maximum (FWHM); θ=the diffraction angle. The average mean size powder of Makaradhwaja is ranging from 28.62 – 29.08nm.[24]The image of the drug in Scanning Electron Microscopy (SEM) reveals that the molecules are spherical shaped and scattered over an uneven surface. According to traditional medicine Makaradhwaja is very much effective in degenerative diseases, tuberculosis, diabetes, cardiac and respiratory diseases, acute fever, common cough and cold, diarrhoea, inflammation of the G.I. tract, insomnia, mental anxiety etc. It has the property of immunomodulation and stress adaptation. It is also used for rejuvenation to combat the aging process.[2, 3, 20]The present study has been designed to prepare the silica gel entrapped nanoparticle of powder Makaradhwaja to get maximum effect by administration of

minimum quantity of drug along with sustained release action. The gel has been synthesized in room temperature by using easy method of silica gel preparation. The molecule of interest has been added in required quantities in the liquid media and distributed uniformly by continuous mixing. The silica gel prepared in this process is very porous in nature and rigid framework of sol gel polymers provide stabilization of entrapped drug molecule and prevent their escape from the gel matrix.[15] After encapsulation characterization have been done with the help of different analytical methods such as FTIR, HRTEM, and UV-visible spectroscopy.[5, 9] The objective of adopting analytical methods was to authenticate the method of encapsulation. A comparative study between the crude drug and gel entrapped drug molecules has also been performed to evaluate the antimicrobial activity of this new formulation against different bacterial strains (i.e. *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus*). Acute oral toxicity study (in mice) has been carried out to observe the changes in behavioral pattern, clinical sign and body weight of the animals after treatment .[21, 22, 23]

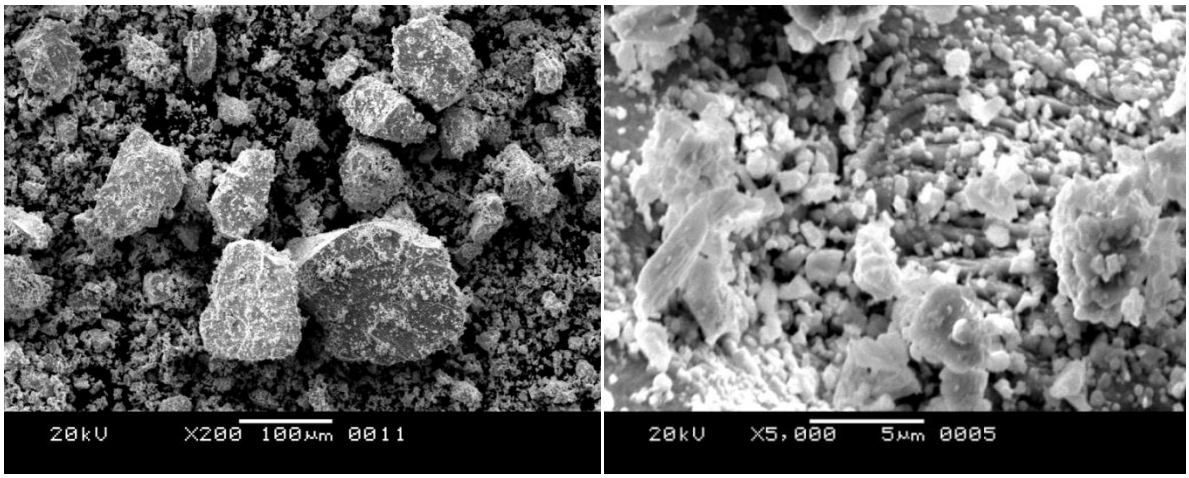


Figure.2a *Figure.2b*

Figure.2a and 2b: Scanning electron microscopy (SEM) analysis of powder Makaradhwaja

Materials and methods

Source of the Drug

The powder Makaradhwaja used in this study has been kindly supplied by the Dr. P. K. Prajapati of Department of Rasashastra & Bhaishajya Kalpana, Gujrat Ayurved University, Jamnagar. After procurement tests for standardization have been performed through various physical, analytical and instrumental methods.

Synthesis of silica gel

The hydrolysis and condensation reactions are the two main chemical reactions that take place during the synthesis of silica gel. The hydrolysis reaction (that can be either acid or base catalyzed) replaces oxide groups with hydroxyl groups. Siloxane bonds (Si-O-Si) are formed during condensation reaction. Alcohol and water are the by-products of the condensation reaction and both evaporate during drying.

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

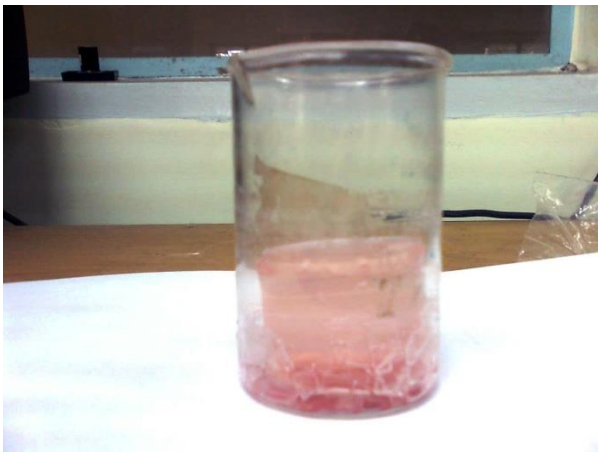
$$[Si(OH)_4]_n \rightarrow [Si\text{-}O\text{-}Si\text{-}O\text{-}Si]_n$$

Theoretically the overall reactions is as follows,

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

Completion of the reaction and chemical composition of the resulting product depend on certain factors such as the ratio of silica precursor, ethyl alcohol, 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. Tetra ethyl orthosilicate (TEOS purchased from Sigma Aldrich) has been used as precursor of silica. Addition of aqueous solution of Makaradhwaja during the formation of oxide backbone at room temperature facilitates their encapsulation within the oxide matrix leading to the production of composite gel with the active ingredient homogenously distributed throughout the gel. The prepared gel was subjected to drying until the weight (of the gel) became constant. The processes of gel formation, condensation and drying, produce a glassy solid gel matrix. These gel matrixes are resorbable, highly porous and nano structured (pore size from 1 to 5 nm). Their structural properties can be controlled by altering sol-gel processing parameters.[5] In this way Makaradhwaja were entrapped into silica gels.[5, 6, 7, 11] Addition of Poly Ethylene Glycol, 400 (purchased from Merck) has

been incorporated to provide better release of drug particles from the matrix. Figure 3a and 3b showing images of the synthesized gel.



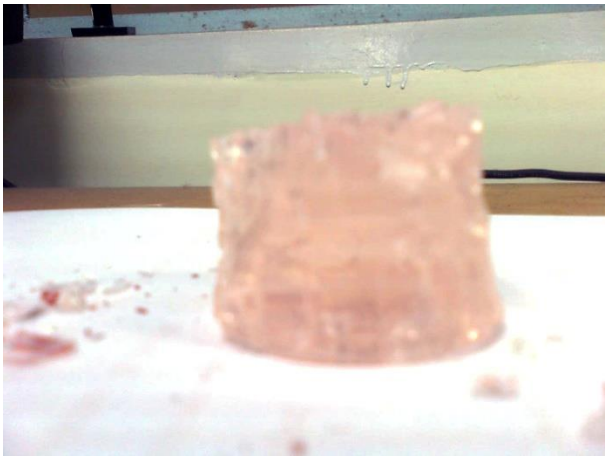


Figure.3a *Figure.3b*

Figure 3a and 3b: Different images of the synthesized gel.

Characterization of gel encapsulated drug molecule

After preparation of silica gel entrapped Makaradhwaja, different analytical tests have been performed for characterization of the formulation. The Transmission Electron Microscopy (TEM) analysis of the synthesized gel has been done by using HRTEM JEOL-TEM 2100 to obtain the image of drug entrapped silica gel matrix. The optical absorption spectra of the different samples of gel entrapped drug molecule over 300nm – 800 nm were taken using Perkin Elmer, Lambda 35 UV-Visible spectrophotometer. The percentage of drug release was studied and plotted against time.[11] FTIR spectrum of the bare silica gel and gel entrapped Makaradhwaja were taken by using Schimadzu (Model: prestige 22) FTIR spectrophotometer.[24]

Microbial analysis by incorporating disc diffusion method

The antibacterial activity of powder Makaradhwaja and silica gel entrapped Makaradhwaja has been observed against three different strains of bacteria i.e. *Escherichia* coli (gram-negative, non capsulated bacillus causing dysentery); *Klebsiella pneumoniae* (Gram-negative bacillus causing upper respiratory tract infection) and *Staphylococcus aureus* (gram-positive diplococcic bacteria causing skin, soft tissue and respiratory tract infections). For the present study three strains of bacteria i.e. *Staphylococcus aureus* ATCC25923, *Escherichia Coli* wild type and *Klebsiella pneumonia* MDR were taken. [10] The gel entrapped drug molecule was made into fine powder. Sterile antibiotic discs (Himedia) were used for the present investigation.[16, 18]The aqueous solution of powder Makaradhwaja, silica gel entrapped Makaradhwaja and silica gel entrapped Makaradhwaja with PEG were incorporated into the sterile disc individually by using micropipette. Precautions were taken to prevent the flow of the solvent extract from the disc outer surface. The discs were placed on the Muller-Hinton agar plates on which the bacteria were inoculated and spread uniformly and incubated at 37°C for 24 hour. The diameter of inhibition zone was measured in mm. [10, 24]

Acute oral toxicity study on mice

The experiment was conducted on 24 healthy Swiss albino mice of either sex weighing from 25 gram to 35 gram and aged approximately 10 weeks. The mice were distributed into four groups. The experimental procedures related to the animals were authorized by the institutional animal Ethical committee (Proposal No. IAEC/2012/08). One week acclimatization to the laboratory conditions was performed before starting the experiment. Drinking water and food were provided *ad libitum* throughout the experiment, except for the short fasting period where the drinking water was still in free access but the food supply was discontinued 12 hour prior to treatment. [21] Acute oral toxicity study was conducted to avoid the undesirable effects on consumers. The animals were treated with single dose and observed for a period of 14 days. Human therapeutic dose of Makaradhwaja is 2 mg/ kg body weight per day.[19]The suitable dose for mice had been calculated for the toxicological studies. The animals of group.1 has been treated with the formulation containing ten times of active ingredient (Makaradhwaja) than human therapeutic dose, converted to the animal dose ; the animals of group.2 has been treated with half dose of group.1; the animals of group.3 had received half dose of group.2. The animals of group.4 have been taken as control group. Food was provided to the animal one hour after treatment. The mice were observed scrupulously for any indications of toxicity effect within the first six hours after the treatment period, and daily further for

a period of 14 days. Animals were weighed and visual observations for mortality, behavioral pattern, changes in physical appearance and signs of illness were conducted daily during this period.[22, 23]

Result and discussion

HRTEM analysis

Transmission Electron Microscopy of the synthesized gel was performed to visualize the evolution of the meso structure after the loading of drug molecule. Figure.4a and 4b represent the HRTEM image of the porous structure of drug entrapped silica gel matrix. The TEM images of the sample evidenced the formation of a silica matrix with network-like porous structures. Pores of the silica gel act as the host of the drug molecule.[11] Framework rigidity of gel matrix stabilize of entrapped drug molecule prevent their leakage.

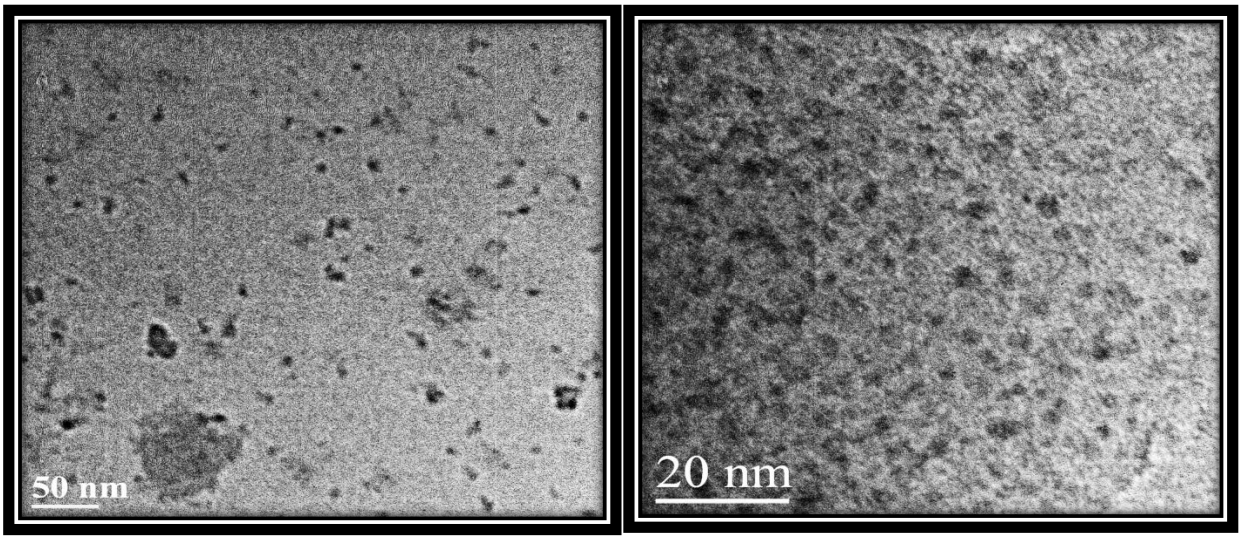


Figure.4b *Figure.4a*

Figure.4a and 4b: TEM images of the Gel entrapped Makaradhwaja

The amount of release percentage study

Figure.5a shows the percentage of release of Makaradhwaja from silica gel matrix with time. The amount of drug released in Simulated Body Fluid (SBF) buffer with on concentrations nearly equal to those of human blood plasma from the silica matrix has been measured. Then the absorbance at 180 nm has been measured and there by plotted against time. By this way the percentage of release has been studied from the curves.[6, 13] From the literature review the absorption maxima was found to be at 290 nm OD.[8] But in the present study it is seen to be reduced to 180nm which may be due to the encapsulation or due to the selection of solvent. The release kinetics was initially very rapid and followed first order reaction up to around 100 hours. Then the release concentration attains a steady state or followed the zero order reaction. The release of drug particle has been thoroughly observed up to 240hours.[6, 13]UV-visible spectroscopic analysis shows 50% release of the total amount in a period of 240hrs. Later on another type of gel has been synthesized by addition of Poly Ethylene Glycol (PEG 400 by MERCK) in the solution. A comparative study between the gels with PEG (mk3) and without PEG (mk1) reveals that the amount of release percentage is higher in the former one. In Figure.5b the curves mk3 and mk1 represent the percentages of drug release of the gel with PEG and gel without PEG respectively.

Fourier Transform Infrared Spectroscopy (FTIR analysis)

The synthesized gels have been characterized by FT-IR spectrometry. Figure 6a represents the FTIR spectra of bare silica gel and Figure 6b represents the FTIR spectra of silica gel entrapped Makaradhwaja within the range of 4000- 400 cm^{-1}. In the sample of bare silica gel peaks at 459 cm^{-1} and 804cm^{-1} and in the sample of doped silica gel peaks at 457 cm^{-1} and 792cm^{-1} were found due to the Si-O-Si bending vibration. In the sample of bare silica gel peaks at 1086 cm^{-1} and at 960 cm^{-1} were formed due to the Si-O stretching and Si-OH stretching vibration respectively.[14] Similarly peaks at 1088 cm^{-1} and 955 cm^{-1} were formed due to the Si-O stretching and Si-OH stretching vibration respectively in the sample of doped silica gel. [6,7,12] From the literature review it has been found that broad peaks at 3400 cm^{-1} and 1600 cm^{-1} arise in the FTIR spectra of Makaradhwaja due to characteristic OH stretching (γ-OH) H OH bending (δ OH) vibrational band due to adsorbed water in the sample.[8] In FTIR spectrum of the gel entrapped Makaradhwaja (Figure 6b) the broad peaks at 3474 $cm^{-1}$and 1637 cm^{-1} were formed due to the same reasons. In the loaded silica gel broad FTIR peaks at 3474cm^{-1} indicates the presence of phenolic OH group and at 1637cm^{-1} indicates the presence keto group which is probably due to the entrapment of Makaradhwaja. [9, 11]

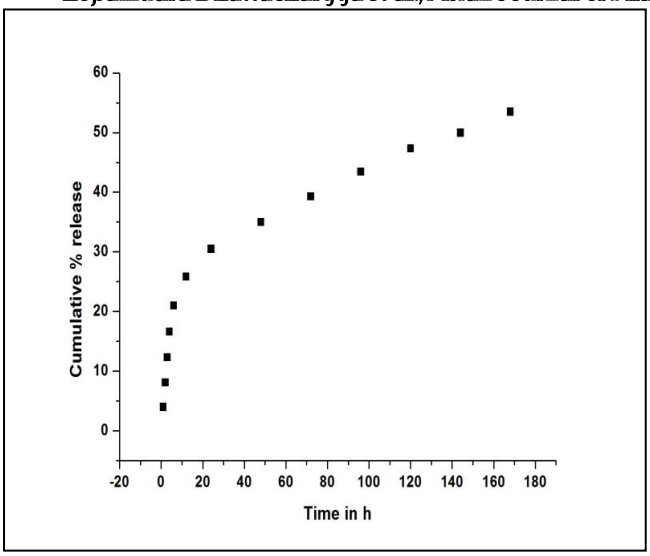


Figure.5a: The percentage of release of Makaradhwaja from silica gel matrix with time

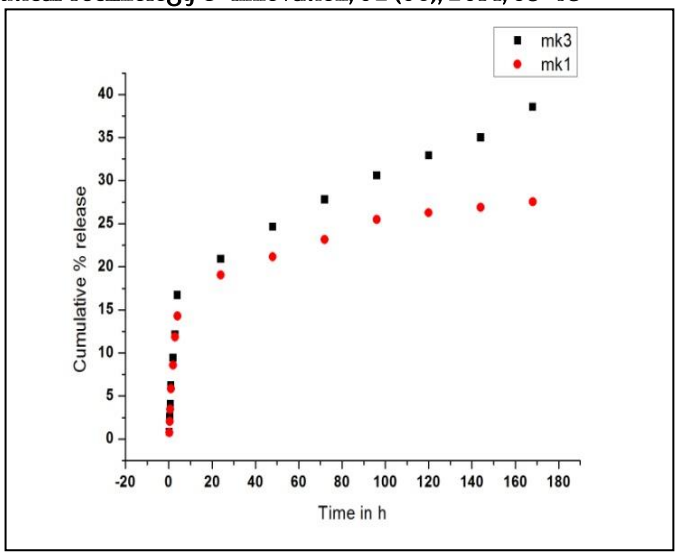


Figure.5b: The percentage of release of mk3 and mk1 from silica gel matrix with time mk3 = the gel with PEG and mk1 = the gel without PEG

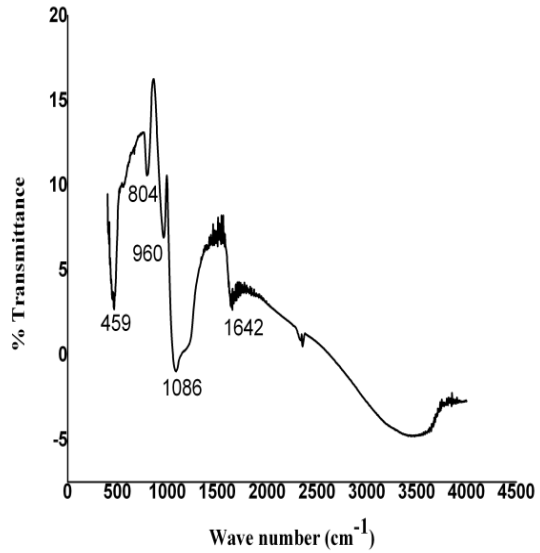


Figure.6a FTIR spectra of bare silica gel

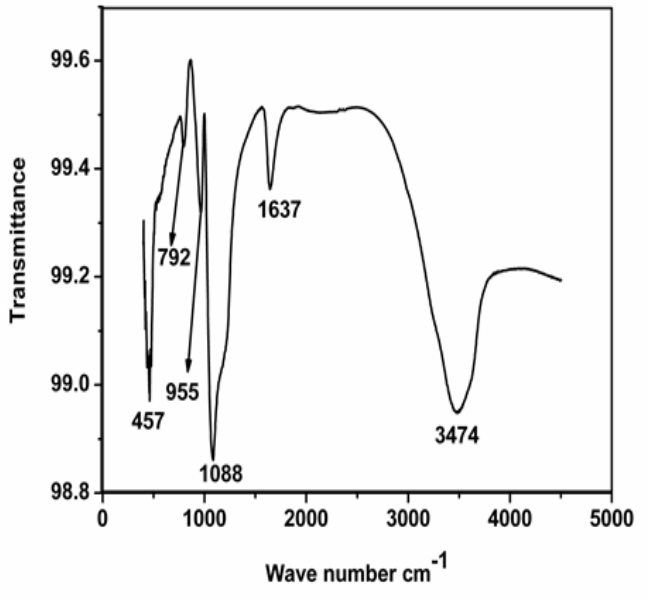


Figure.6b FTIR spectrum of the silica gel entrapped Makaradhwaja

Microbial analysis study by disc diffusion method

The comparative study between the silica gel entrapped Makaradhwaja and silica gel entrapped Makaradhwaja with PEG regarding the effect on pathogenic bacterial strains by Disc Diffusion method was performed. The powder Makaradhwaja did not show sensitivity to any of the pathogenic bacterial strains. The inhibitory effect of silica gel entrapped Makaradhwaja with PEG against *Escherichia* coli and *Klebsiella pneumonia* is found to be more than the silica gel entrapped Makaradhwaja. But in case of *Staphylococcus aureus* only the silica gel entrapped Makaradhwaja with PEG had produced zone of inhibition. The silica gel entrapped Makaradhwaja did not show any significant result against *Staphylococcus aureus*. Figure.7 represents the comparison between the mean diameters of zone of inhibitions against pathogens produced due to the antimicrobial activity of silica gel entrapped Makaradhwaja with PEG and silica gel entrapped Makaradhwaja.[10, 24] The mean diameter of zone of inhibition in case of *Escherichia* coli is 25.9 mm and 20.98 mm due to the effect of silica gel entrapped Makaradhwaja with PEG and silica gel entrapped Makaradhwaja respectively. The silica gel entrapped Makaradhwaja with PEG and the silica gel entrapped Makaradhwaja have produced zone of inhibition with mean diameter of 22.93 mm and 19.05 mm respectively in case of *Klebsiella pneumonia*. The silica gel entrapped

Makaradhwaja with PEG had produced the zone of inhibition measuring mean diameter of 24.8 mm against *Staphylococcus aureus.*

Study of Acute oral toxicity on mice

The animals were treated with single dose and observed for a period of 14 days in respect to their behavioral pattern, physical sign and body weight. The gain in body weight of the treatment and control mice were studied. There were gradual increases in body weight of treatment and control mice. The percentage increase in body weight of treatment mice were more as compared to the control mice.[22]The percentage increase in body weight of treatment mice in Group.1 on Day 7 and Day 14 were found 4.92% and 11.48%, respectively. The percentage increase in body weight of treatment mice in Group.2 on Day 7 and Day 14were found 4.42% and 7.72%, respectively. The percentage increase in body weight of treatment mice in Group.3 on Day 7 and Day 14 were found 2.56% and 6.27%, respectively. The percentage increase in body weight of mice in Group.4 (control) on Day 7 and Day 14 were found 1.45% and 4.03%, respectively. The results signify that the rate of weight gain is directly proportionate to the amount of drug consumed by the animals (Figure 8). The physical appearance and behavioral observation record in animals of Group.1, Group.2, Group.3 & Group.4 are represented by Table.1. The test drug did not produce any mortality up to the ten times of its therapeutic dose (2 mg/ kg body weight, orally) even after encapsulation in porous gel matrix. The test drug did not produce any observable toxic effect except mild lethargy for the animals in Group.1. Based on the observation made and recorded in Table.1 it can be concluded that the test drug is without any toxic potential at ten times of its therapeutic dose even after encapsulation. [23]

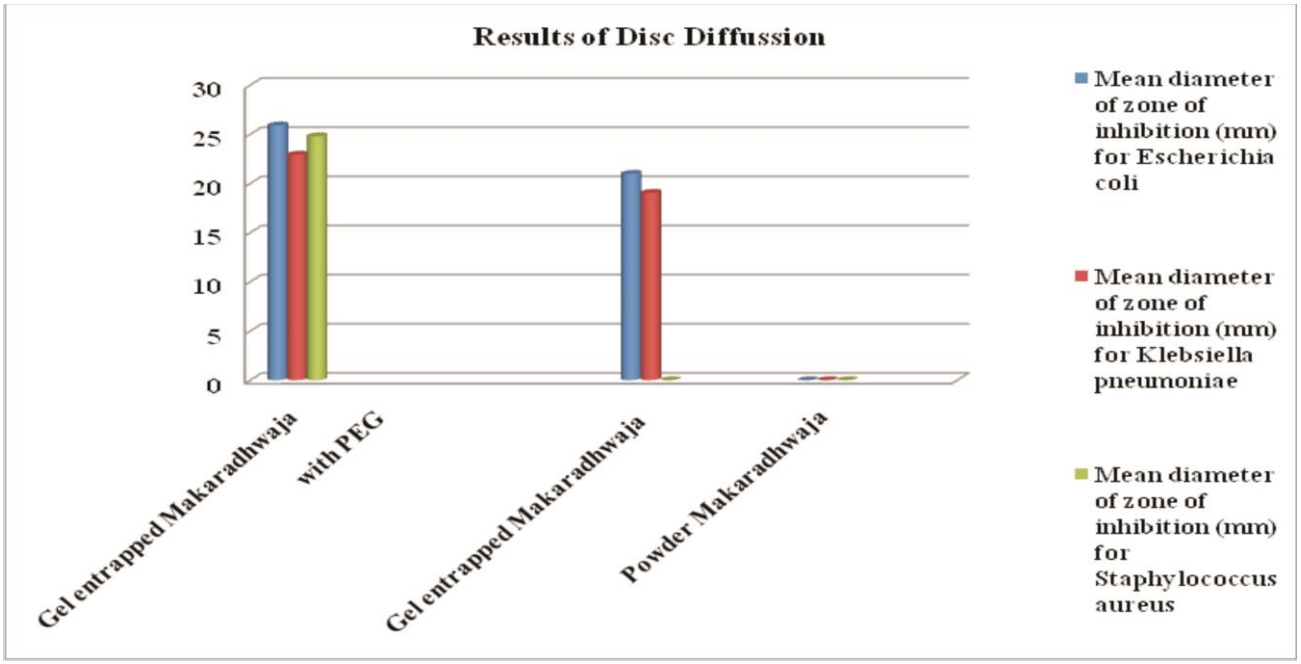


Figure 7 Comparative study between the powder Makaradhwaja, the gel entrapped Makaradhwaja with PEG and the gel entrapped Makaradhwaja in Disc diffusion method

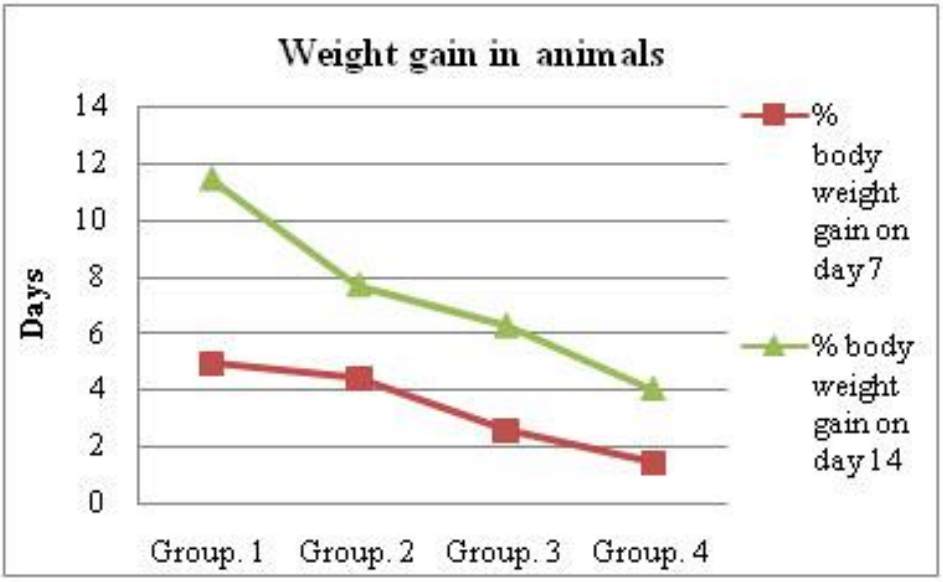


Figure.8 *Percentage of body weight gain in animals of four groups in acute oral toxicity study*

Observation	$^{1}/_{2}$hr	1hr	6hrs	12hrs	24hrs	48hrs
Skin and fur	normal	normal	normal	normal	normal	normal
Eyes	normal	normal	normal	normal	normal	normal
Mucous membrane	normal	normal	normal	normal	normal	normal
Motor activity (increased/decreased)	normal	normal	normal	normal	normal	normal
Convulsion	-	-	-	-	-	-
Straubs reaction	-	-	-	-	-	-
Muscle spasm	-	-	-	-	-	-
Catatonia	-	-	-	-	-	-
Opisthotonus	-	-	-	-	-	-
Hyperasthesia	-	-	-	-	-	-
Muscle relaxation	-	-	-	-	-	-
Anaesthesia	-	-	-	-	-	-
Arching & rolling	-	-	-	-	-	-
Lacrimation	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-
Respiration	normal	normal	normal	normal	normal	normal
Salivation	normal	normal	normal	normal	normal	normal
Writhing	normal	normal	normal	normal	normal	normal
Grip strength(Kg) mean	normal	normal	normal	normal	normal	normal
Visual placing response	normal	normal	normal	normal	normal	normal
T ail pinch response	normal	normal	normal	normal	normal	normal
Auditory response	normal	normal	normal	normal	normal	normal

Table.1

Table.1: Physical appearance and behavioral observation record in animals of Group. 1, 2, 3& 4
**normal = No significant change in animals of Group. 1, 2, 3& 4*
"-"= Not found in animals of Group. 1, 2, 3& 4

Conclusion

A sustainable and controlled release drug delivery system had been developed by encapsulation of Makaradhwaja (a traditional Ayurvedic medicine) into silica gel matrix. The proper formation of gel has been evidenced by the FTIR spectrometry and supported by the HRTEM micrograph. The drug encapsulated nano porous silica gel provides sustainable and controlled release (50%) for more than 240 hours (which has been proved by UV-visible spectroscopic analysis). So a simple method of development of resorbable and biocompatible formulation for controlled release has been established. The significant antimicrobial activity of gel encapsulated drug molecule was found against three bacterial strains. But the crude drug has not shown sensitivity towards the pathogens. So it can be said that the Makaradhwaja entrapped into the gel matrix is biologically more active in comparison to the crude drug. No significant physical and behavioral change has been observed (except mild lethargy in higher dose) in the treated animals in acute oral toxicity study. All the animals belonging to the treatment and control group were survived throughout the study.

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