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Metallic and non metallic nanoparticles incorporated meropenem and ceftazidime synthesis for the improved antibacterial activity against human pathogenic bacteria

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ABSTRACT

In the present study improved antibacterial activity of meropenem and ceftazidime incorporated with free and chitosan coated egg albumin nanoparticles, silver nanoparticles against Escherichia coli and Salmonella paratyphi has been carried out. Egg albumin nanoparticles incorporated drugs were synthesized by coacervation method and chitosan coated nnao drug conjugate by ionic gelation method. Synthesized nano drug conjugates were characterized by scanning electron microscopy (SEM),energy dispersive x ray spectroscopy (EDX) and Fourier Transform Infrared spectroscopy (FTIR).All the nanoformulations showed distinct antibacterial activity against both the tested bacteria and further studies will be helpful to formulate in bulk volume and commercialization possible through proper clinical trials

Key words; meropenem, Ceftazidime, nanoformulation,*Escherichia coli*,*Salmonella paratyphi*

INTRODUCTION

Nowadays, development of new colloidal drug delivery system for controlled and targeting release of drugs has become more interest. One of the most important aspects of ideal drug delivery system is transportation of the associated drug to its desired site of action and release drug at an optimum rate to improve the therapeutic index [1,2]. Therefore, expectations from suitable drug carriers and effective drug delivery systems are: a) improving drug bioavailability through enhancing aqueous solubility b) increasing the residence time in the body (increasing the half-life for clearance/increasing specificity for its cognate receptor) c)targeting the drug to a specific location in the body with a concomitant reduction in the quantity of drug required and dosage toxicity enabling the safe delivery of toxic therapeutic drugs and protection of non-target tissues and cells from severe side effects d) degradation of carriers in vivo in order to not accumulate indefinitely in the tissues, e) non-toxicity of carriers[3,4]

Drug delivery systems based on nanoparticles, can be designed to improve the pharmacological and therapeutic properties of drugs. The strength of drug delivery systems is their ability to alter the pharmacokinetics and bio distribution of the drug [5,6].When designed to avoid the body's defense mechanisms, nanoparticles have beneficial properties that can be used to improve drug delivery. Where larger particles would have been cleared from the body, cells take up these nanoparticles because of their size. Complex drug delivery mechanisms are being developed, including the ability to get drugs through cell membranes and into cell cytoplasm. Efficiency is important because many diseases depend upon processes within the cell and can only be impeded by drugs that make their way into the cell. Triggered response is one way for drug molecules to be used more efficiently [7,8,9]. Drugs are placed in the body and only activate on encountering a particular signal. For example, a drug with poor solubility will be replaced by a drug delivery system where both hydrophilic and hydrophobic environments exist, improving the solubility. Also, a drug may cause tissue damage, but with drug delivery, regulated drug release can eliminate the problem. If a

drug is cleared too quickly from the body, this could force a patient to use high doses, but with drug delivery systems clearance can be reduced by altering the pharmacokinetics of the drug [10,11]. Poor biodistribution is a problem that can affect normal tissues through widespread distribution, but the particulates from drug delivery systems lower the volume of distribution and reduce the effect on non-target tissue. Potential nanodrugs will work by very specific and well-understood mechanisms; one of the major impacts of nanotechnology and nanoscience will be in leading development of completely new drugs with more useful behavior and less side effects [12,13].

Meropenem belongs to carbapenem group of β-lactams antibiotics with a broad spectrum of activity against gram-negative, gram-positive and anaerobic microorganisms. Meropenem has clinical and bacteriological efficacy in treatment of various serious infections in adults and children. Meropenem exerts its bactericidal action by interfering with vital bacterial cell wall synthesis. The ease with which it penetrates bacterial cell walls, its high level of stability to all serine b-lactamases and its marked activity for the penicillin binding proteins (PBPs) explain the potent bactericidal action of meropenem against a broad spectrum of aerobic and anaerobic bacteria [14,15].The activity profile of meropenem has been well established in *in vitro* studies and more recently in large surveillance studies. *Invitro* antibacterial spectrum of meropenem includes the majority of clinically significant Gram-positive and Gram-negative, aerobic and anaerobic strains of bacteria including methicillin-sensitive *Staphylococcus aureus, Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Enterococcus faecalis*, *Escherichia coli*,*Salmonella Paratyphi*,*Klebsiellapneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Burkholderiacepacia*, Acinetobacter spp., *Hemophilusinfluenzae*, and *Bacteroidesfragilis*. As the spectrum of activity of meropenem extends to over 200 clinically significant bacterial species [16]. Ceftazidime belongs to third generation cephalosporin group of β-lactams antibiotics with a broad spectrum of activity against gram-negative andgram positive microorganisms causing urinary tract,lower respiratory,bone and joint,abdominal,central nervous ststem infections and septicaemia [17,18]. In the present study, free silver, coated with chitosan,egg albumin nanoparticles incorporated meropenem was synthesized and the formulated meropenem was evaluated against pathogenic bacteria.

MATERIALS AND METHODS

Preparation of Egg Albumin nanoparticles incorporated Meropenem and Ceftazidime Nano drug conjugate (EA NPs-Mer,EANps-CF)

EA Np-Mer and EANps-CF nano drug conjugate was prepared by coacervation method [19]. 30mg of Egg Albumin was added in 50ml deionized water and ethanol was added drop wise until the suspension turns turbid 0.001mg of respective antibiotic was added into the suspension and stirred continuously using magnetic stirrer for 12hrs. After 12hrs, 50µl of 25% Glutaraldehyde was added as a cross linking agent and kept stirring for 1-2hrs. The homogeneous slurry was centrifuged at 4000rpm for 25min. The pellet was then lyophilized to form fine powder.

Preparation of Silver nanoparticles incorporated Nano drug conjugate (AgNps-Mer,AgNps-CF)

Silver nanoparticles incorporated nano drug conjugate was prepared by chemical reduction of metal salt precursor (0.01M AgNO 3) with reducer (0.01M NaBH4) followed by the addition of 0.001mg of respective antibiotic,the reaction mixture was kept under stirring for 2 hours [20].The homogeneous slurry was centrifuged at 4000rpm for 25min. The pellet was then lyophilized and used for further studies.

Preparation of Chitosan coated Egg Albumin nano drug conjugate (CS-EANps-Mer,CS-EANps-CF)

Chitosan coated egg albumin nanoparticles incorporated drug conjugate was prepared by ionotropic gelation method [21]. Respective egg albumin nanoparticles incorporated drug conjugate were mixed with 100mg chitosan suspended in 1% acetic acid and sodium tripolyphosphate solution was added drop wise into the reaction mixture, and kept stirring for 2hours. The slurry thus obtained was lyophilized and used for further studies.

Preparation of Chitosan coated Silver- Nano drug conjugate

Chitosan coated silver nano drug conjugate was prepared by ionotropic gelation method. Respective silver nano drug conjugate was mixed with 100mg chitosan, 1% acetic acid and sodium tripolyphosphate solution was added drop wise and kept stirring for 2 hours. The slurry thus obtained was lyophilized and used for further studies.

ANTIBACTERIAL ACTIVITY OF ANTIBIOTIC LOADED NANOPARTICLES

Antibacterial activity ofEA-Mer NPs, EA-Ctz NPs, Ag-Mer NPs, Ag-Ctz NPs, CsEA-Mer NPs, CsEA-Ctz NPs, CsAg-Mer NPs and CsAg-Ctz NPs were analyzed on clinically isolated pathogenic strains of *E. coli* and *S. paratyphi*. The respective bacterial organisms were uniformly spread onto the sterile MH agar plates with sterile cotton swabs and antibiotic loaded nanoparticles of 10, 25, 50, 75,100µlwas loaded onto the wells that were made on culture seeded MH agar plates and incubated for 12-24hrs at 37^{o}C. The zone of inhibition after incubation period was observed and recorded.

RESULTS AND DISCUSSION

Nanoparticles have been emerged as essential strategy for drug delivery. Nanoparticles have many unique properties that make them suitable as effective drug carriers. Advantages of using these particles are including:(I) Easy manipulation of particle size and surface characteristics of nanoparticles to can be prepared passive and active drug targeting after parenteral administration. (II) In order to enhancement of drug therapeutic efficiency, nanoparticles able to control, modify and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug. Also they capable to reduce unwanted effects by controlled release.(III) Selection of matrix constituents gives this possibility to modulate Controlled release and particle degradation characteristics. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction, this property is important for preserving the drug activity [22,23]. In the present study,meropenem and ceftazidime formulated with metallic and non metallic nanoparticles nano drug conjugate was prepared and the synthesized nano drug conjugate was evaluated for the improved anti bacterial activity.

Table 1. Zone of inhibition of meropenem and ceftazidime against E. coli and S. Paratyphi

S.No	Concentration (ug/ml)	Zone of inhibition			
		Meropenem		Ceftazidime	
		E. C.	*S. P.*	*E. C.*	*S. P.*
1	10	14	12	10	13
2	25	15	14	13	14
3	50	16	16	15	16
4	75	20	19	18	19
5	100	22	20	20	21

Table 2. Zone of inhibition of meropenem and ceftazidime loaded Egg albumin nanoparticles against *E. coli and S. paratyphi*

S.No	Concentration (ug/ml)	Zone of inhibition			
		EA-Mer NPs		EA-Ctz NPs	
		E. C.	*S. P.*	*E. C.*	*S. P.*
1	10	25	24	24	24
2	25	26	25	25	25
3	50	26	26	26	26
4	100	28	28	27	28

Table 3. Zone of inhibition of meropenem and ceftazidime loaded silver nanoparticles against *E. coli and S. paratyphi*

S.No	Concentration (ug/ml)	Zone of inhibition			
		Ag-Mer NPs		Ag-Ctz NPs	
		E. C.	*S. P.*	*E. C.*	*S. P.*
1	10	18	16	13	14
2	25	19	17	16	15
3	50	20	19	18	19
4	100	21	21	19	20

Table 4: Zone of inhibition of meropenem and ceftazidime loaded chitosan coated Egg albumin nanoparticles against *E. coli and S. paratyphi*

S.No	Concentration (ug/ml)	Zone of inhibition			
		Ag-Mer NPs		Ag-Ctz NPs	
		E. C.	*S. P.*	*E. C.*	*S. P.*
1	10	17	16	15	15
2	25	18	17	16	17
3	50	20	18	17	19
4	75	22	19	18	20
5	100	23	21	19	22

Table.5: Zone of inhibition of meropenem and ceftazidime loaded chitosan coated silver nanoparticles against *E. coli and S. paratyphi*

S.No	Concentration (ug/ml)	Zone of inhibition			
		Ag-Mer NPs		Ag-Ctz NPs	
		E. C.	*S. P.*	*E. C.*	*S. P.*
1	10	15	13	14	15
2	25	16	14	16	17
3	50	17	16	17	19
4	75	19	18	18	20
5	100	21	19	19	22

Figure 1.Scanning electron microscopy image of EA-Mer NPs

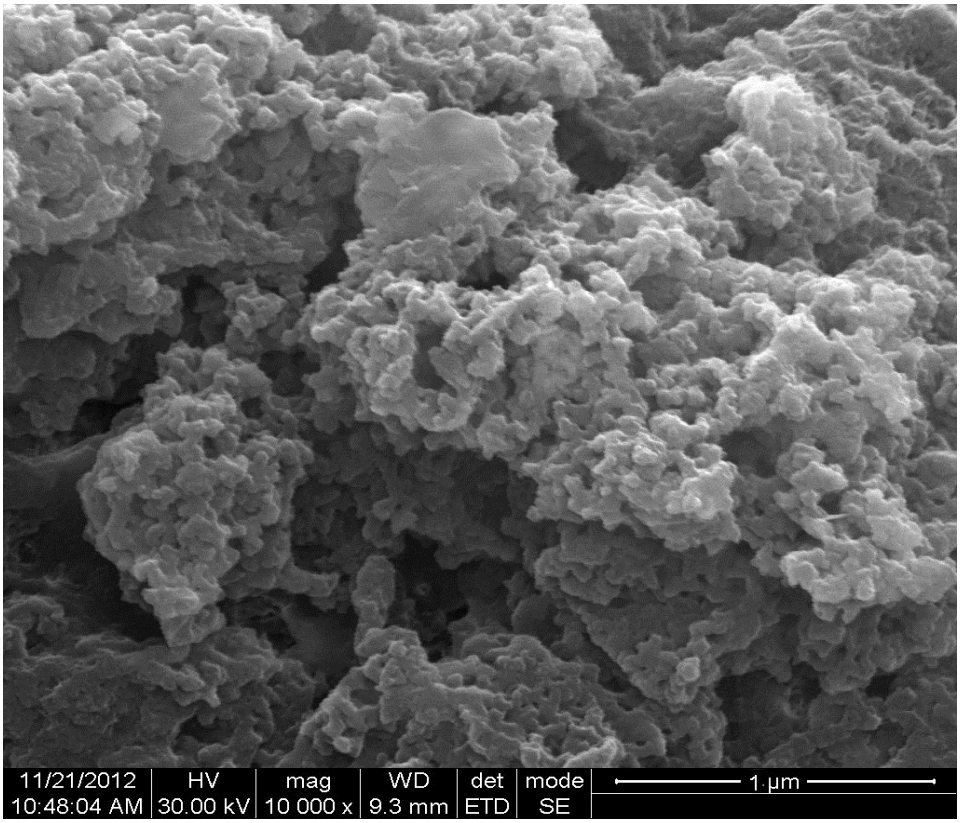


Figure 2.Scanning electron microscopy image of EA Nps-CF

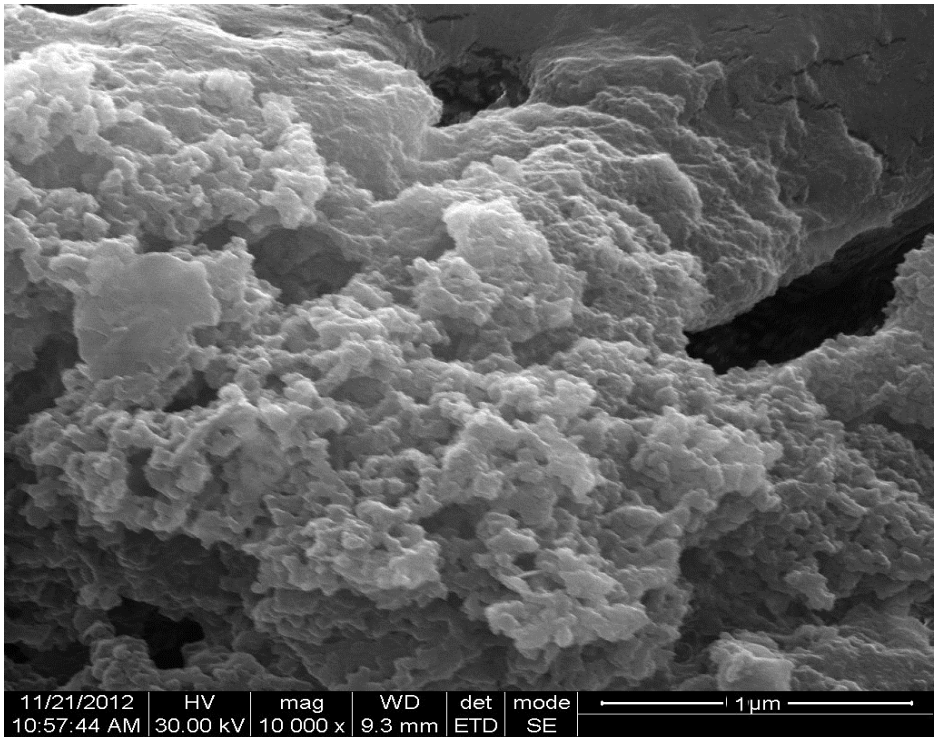


Figure 3. FTIR of EA-Mer NPs

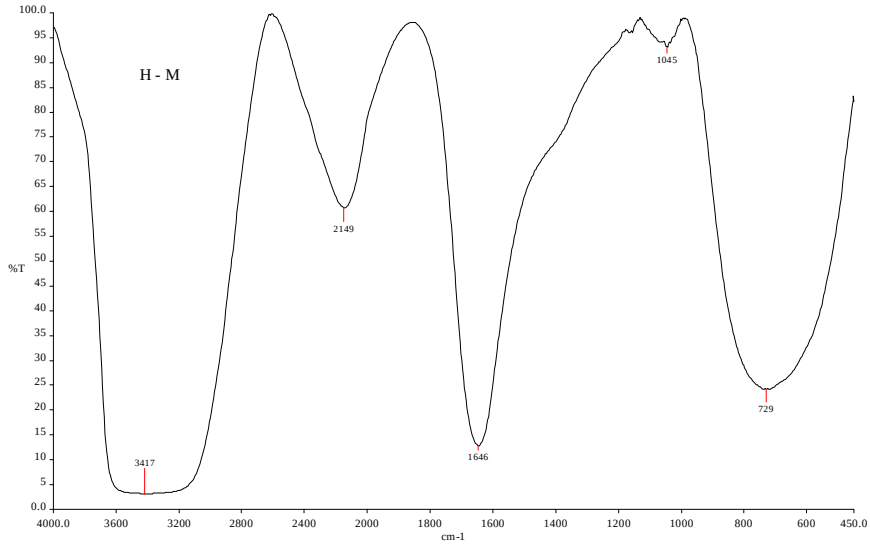
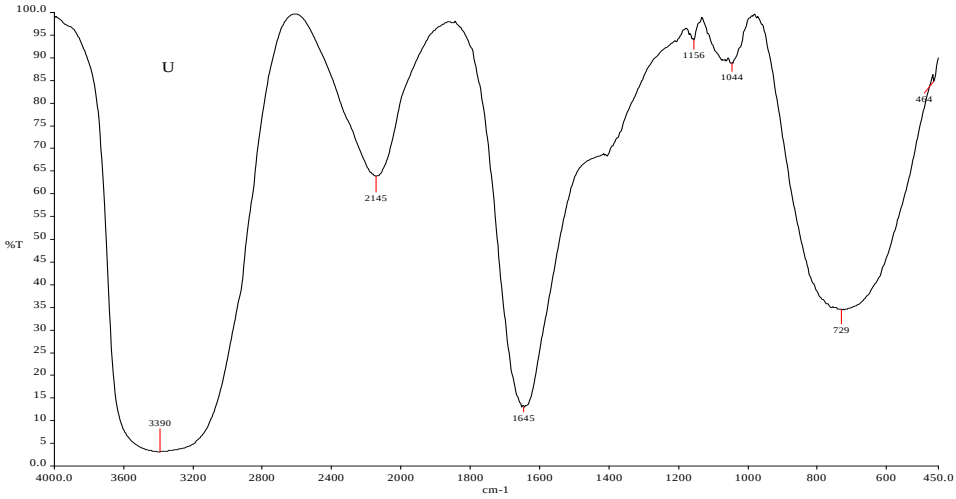


Figure 4. : FTIR of EA-Ctz NPs



EA Np-Mer and EANps-CF nano drug conjugate was prepared by coacervation method and the morphology of the nano drug conjugate was studied by SEM.Scanning Electron Microscopy (SEM) which revealed nanospheres with electron dense core shell particles in the size range of 100 to 140nm (EA Np-Mer) and 100 to 140nm (EANps-CF) (Figure 1,2).Further characterization was carried out by FTIR which shows distinct pattern of peaks (Figure 3,4).Silver nanoparticles incorporated drug nano conjugate was also prepared .Characterization of nano drug conjugate was studied by SEM(Figure 5,6) which revealed spherical particles with the size range of 25-52nm (AgNp-Mer) and 58.64nm (AgNp-CF).Chitosan coated egg albumin and silver nano drug conjugate was prepared by ionic grlation method.CS-EANp-Mer and CS-EANps-CF showed spherical shape morphology with the size range of 110 to 154nm and 120-150nm respectively.

Antibacterial activity reveals that both the free antibiotics were found to be active against *E.coli* and *S. paratyphi* as dose dependent manner. (Table 1,2).But, all the prepared nano drug conjugate showed improved antibacterial activity against both the tested bacteria (Table 3-5). In the case of EANps-Mer, the zone of inhibition against E.coli and S.paratyphi was increased in all the tested concentration.Similar Improved activity has also been observed in all the tested nano drug conjugate .Enhanced anti bacterial activity of BSA Np-ofloxacin drug nano conjugate against clinical isolate of *Pseudomonas aeruginosa* [24]. The present study would suggests the possible utilization of nano drug conjugate as the anti bacterial agent against human pathogenic bacteria.

Figure 5. SEM image of Silver-Meropenem nanoparticles

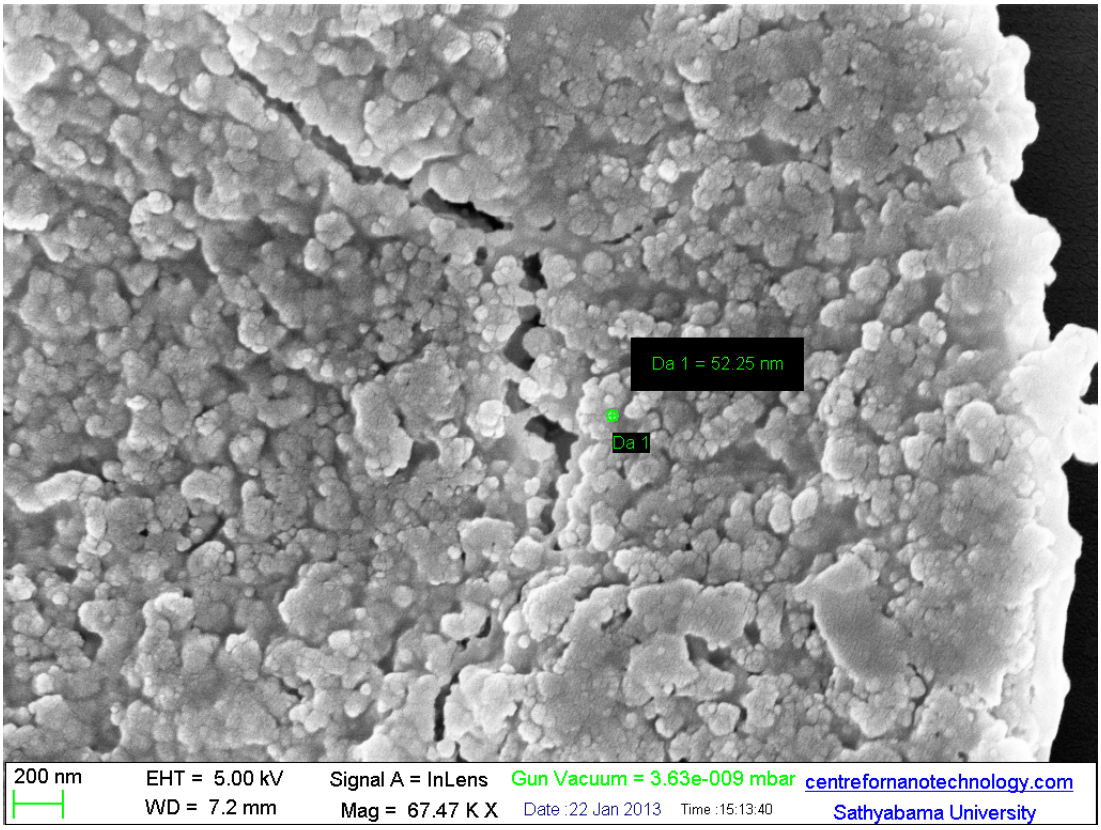
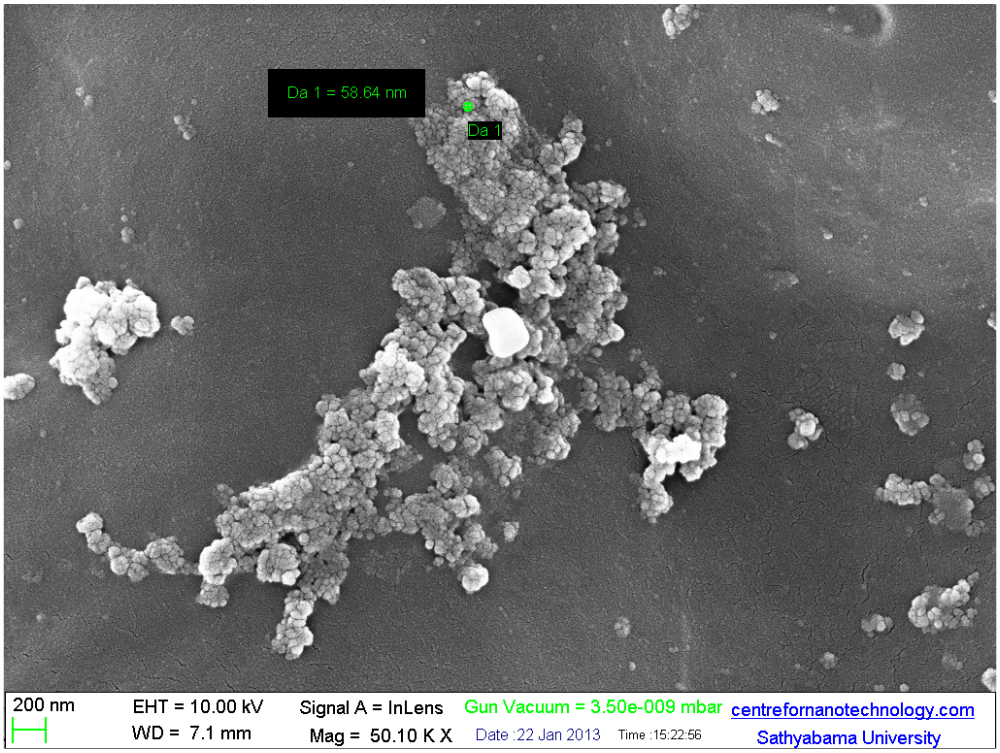


Figure 6. SEM image of silver-Ceftazidime nanoparticles



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