AFRICAN UNIVERSITY OF SCIENCE & TECHNOLOGY [AUST]

MATERIAL SCIENCE DEPARTMENT

BIOSYNTHESIZED GOLD NANOPARTICLES AND MICROPARTICLES FOR CANCER DETECTION AND TREATMENT

BY

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AT THE

US/AFRICA WORKSHOP ON CANCER RESEARCH

HOLDING AT

PRINCETON UNIVERSITY

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OUTLINE

- Introduction/background
- Biosynthesis
- Experimentals

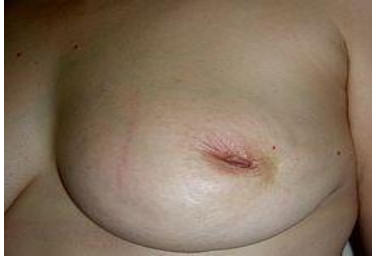
(a) Synthesis with plant materials

(b) Synthesis with bacteria

(c) Functionalization of the gold nanoparticles

- Encapsulation of prodigiosin in micro-particles
- Synergy between nanoparticles and microparticles
- Summary/Conclusion

BACKGROUND/MOTIVATION



- **Cancer is an emerging public health problem in Africa!!!**

- **In a 2008 survey by GLOBOCAN, 7.6 million deaths occurred out of 12 million** cases of cancer, by 2030 **26.4million** with **17 million deaths** (American cancer society, 2010).

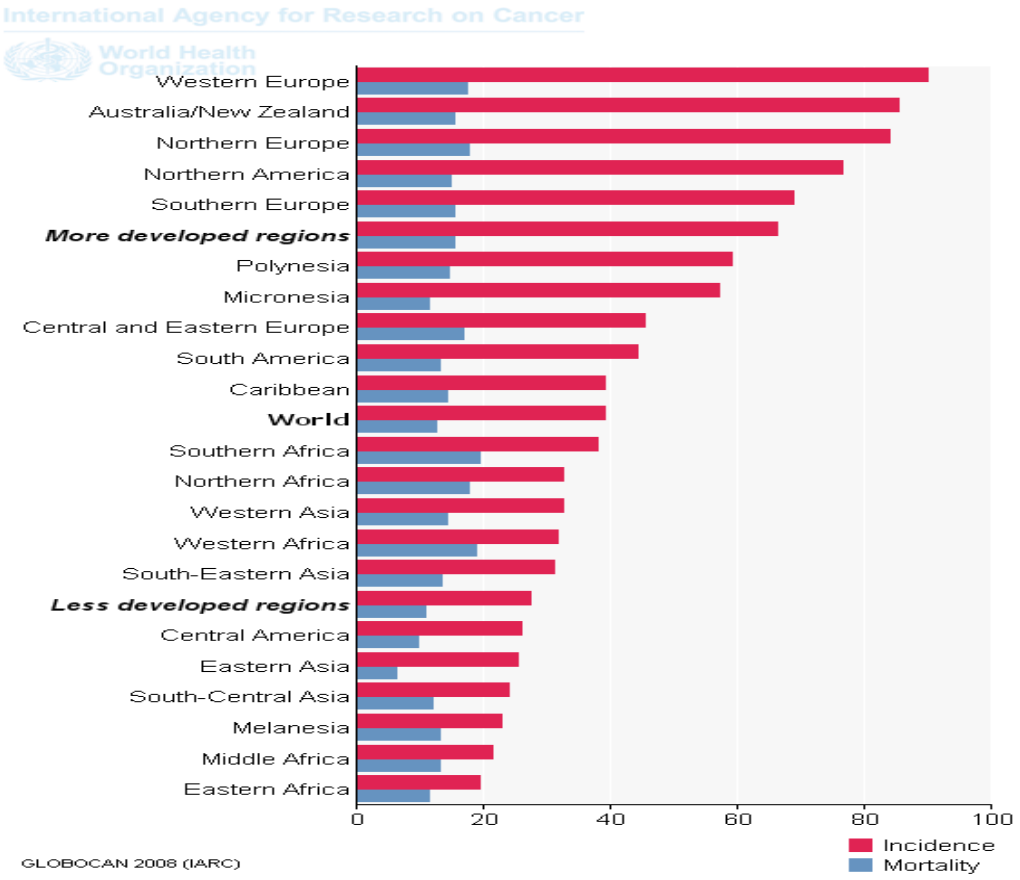
- Most of these new cases of cancer are expected to occur in the developing world, particularly, India, China and **Africa.**

- Mostly due to the adoption of behaviors and lifestyles, such as smoking, unhealthy diet, and physical inactivity (WHO,2008)

- **Breast cancer is the most common type of cancer in Nigerian women (**Adebamowo and Ajayi, 2000 **).**

- **LATE DETECTION, a major challenge** , as **most women present in advanced stages (**Lagos State Ministry of Health, 2009 **).**

BREAST CANCER INCIDENCE AND MOTALITY IN AFRICA



REASONS FOR HIGH MORTALITY RATE

- **Low income**
- **Lack of awareness/knowledge**
- **Lack of access to treatment**
- **Cultural stigmas**

- **Lack of Trained personnel. In 2004, Uganda had just 1 cancer specialist in a nation of 34 million people** (Uganda Cancer Institute/Hutchinson Center Cancer Alliance, 2013).

- **Environmental exposures e.g. toxic substances**

•LATE DETECTION !

The prevalent type of breast cancer in Africa

- **An estimated 1 million cases of breast cancer are diagnosed annually worldwide** (Anders and, Carey, 2009)

- **Of these, approximately 170,000 are of the triple-negative (ER–/PR–/HER2–) phenotype** (Anders and, Carey, 2009)

- **TNBC is the most prevalent type of breast cancer in Africa** (Foulkes *et al.*, 2010)

- **About 47% of women in Africa are diagnosed with Triple negative breast cancer (TNBC)**

Issues with TNBC

- TNBC affects **mostly young women (between age 25 - 40,** as against age 40 - 60 or older, as obtainable with other types of breast cancer).

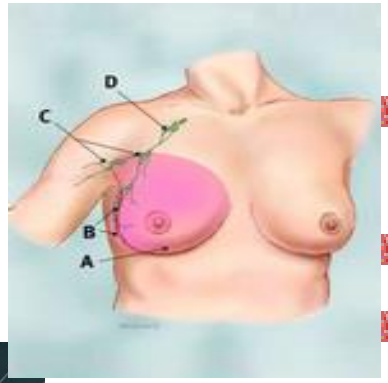
- This type of breast cancer shows
  - **Early recurrence**
  - **Poor prognosis**
  - **Prone to visceral metastasis and the central nervous system**

(Schneider *et al.*, 2008; Dent *et al.*, 2007; De Laurentiis *et al.*, 2010; Chacon and Costanzo , 2010; Lin *et al.*, 2008).

Standard Treatments and Problems

Treatment of breast cancer usually fall into one of the following categories:

- **surgery,**
- **radiation,**
- **chemotherapy,**
- **immunotherapy,**
- **hormone therap**
- **gene therapy**



Radiation Therapy and Mastectomy for Breast Cancer Treatment

PROBLEMS

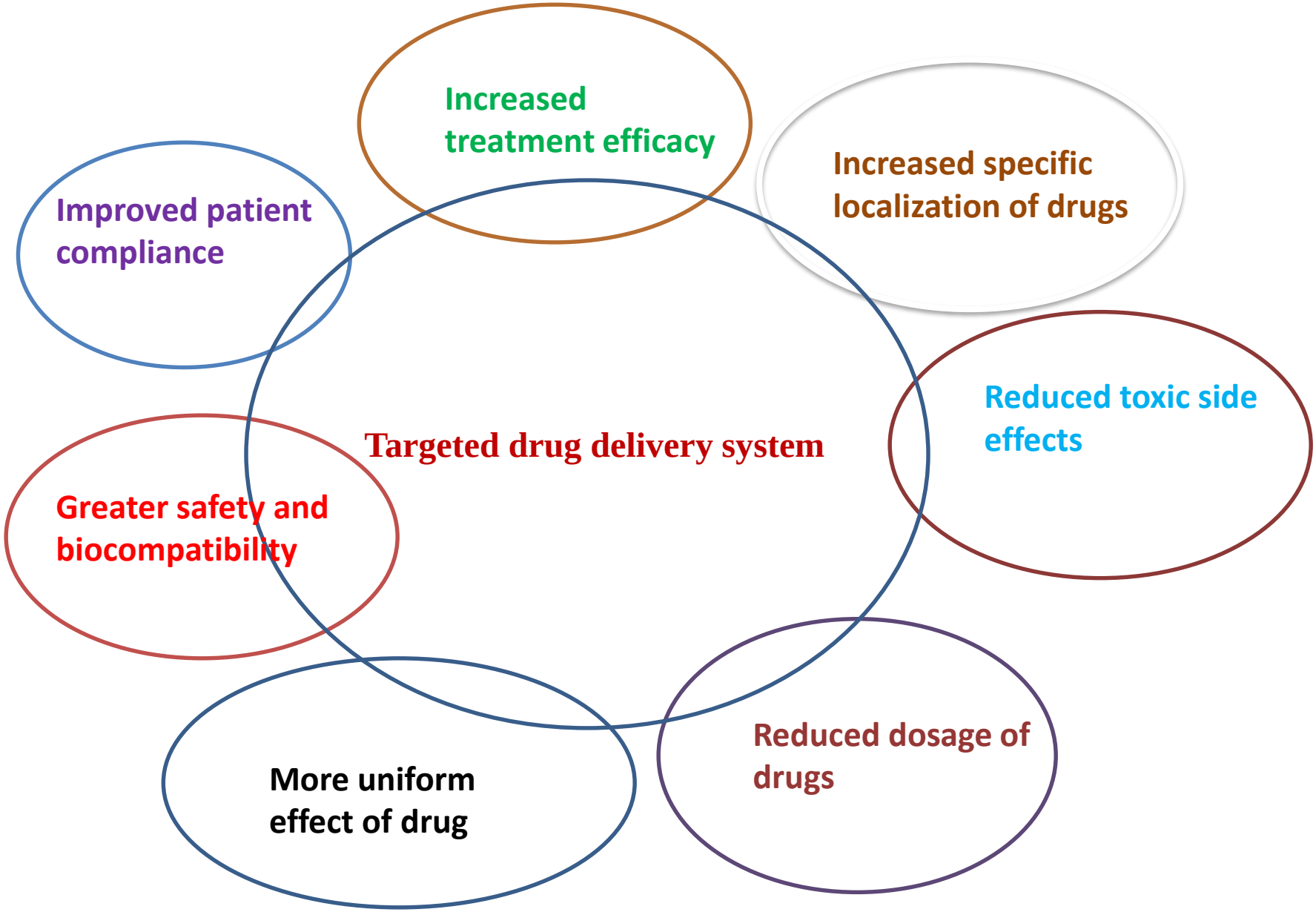
- Not site-specific to tumor
- High toxicity
- Devastating short- and long-term side effects
- Low efficacy: <1% of drug makes it to tumor cells

GOLD NANOPARTICLES (AuNPs)

- **Nanoparticles (NPs) are particles of dimensions within the range of 1-100 nm**
- **Gold nanoparticles play very important roles in biomedical field.**
- **They have been variously applied in targeted drug delivery**
- **They are non-toxic**
- **Biocompatible with human cells**
- **Exhibit optical and catalytic properties**

- **The optical property is being utilized in hyperthermia treatment of cancer.**

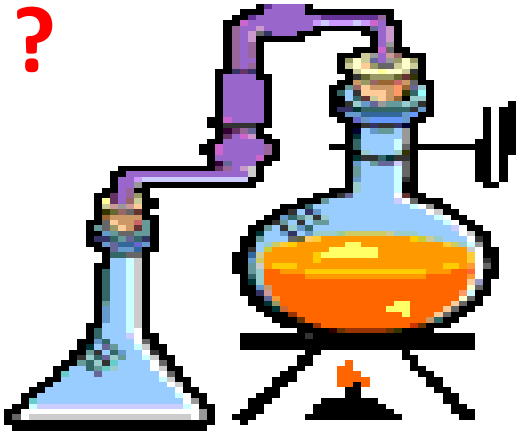
TARGETED DRUG DELIVERY



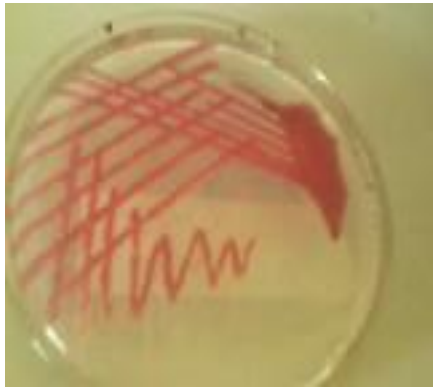
BIOSYNTHESIS OF GOLD NANOPARTICLES (AuNPs)

WHY BIOSYNTHESIS ?

- **Chemical synthesis:**
  - **Toxic chemicals (** sodium citrate, tetraoctylammonium bromide, tolune, Sodium borohydride, hydroquinone)
  - **High temperatures**
  - **High pressures**
  - **Issues with stability in aqueous solution, toxicity and control over their size/shape**
- **Biosynthesis of AuNPs**
  - **Non-toxic,**
  - **Ecofriendly**
  - **Cost effective**
  - **Use bacteria, fungi, and plants**
  - **Faster rate of production**
  - **Control over size and shapes**



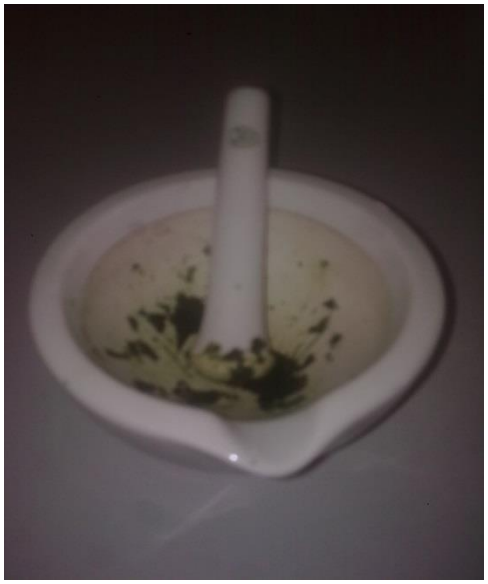
CHEMICAL SYNTHESIS
(TOXIC)



BACTERIA MEDIATED
(NATURAL/NON-TOXIC)

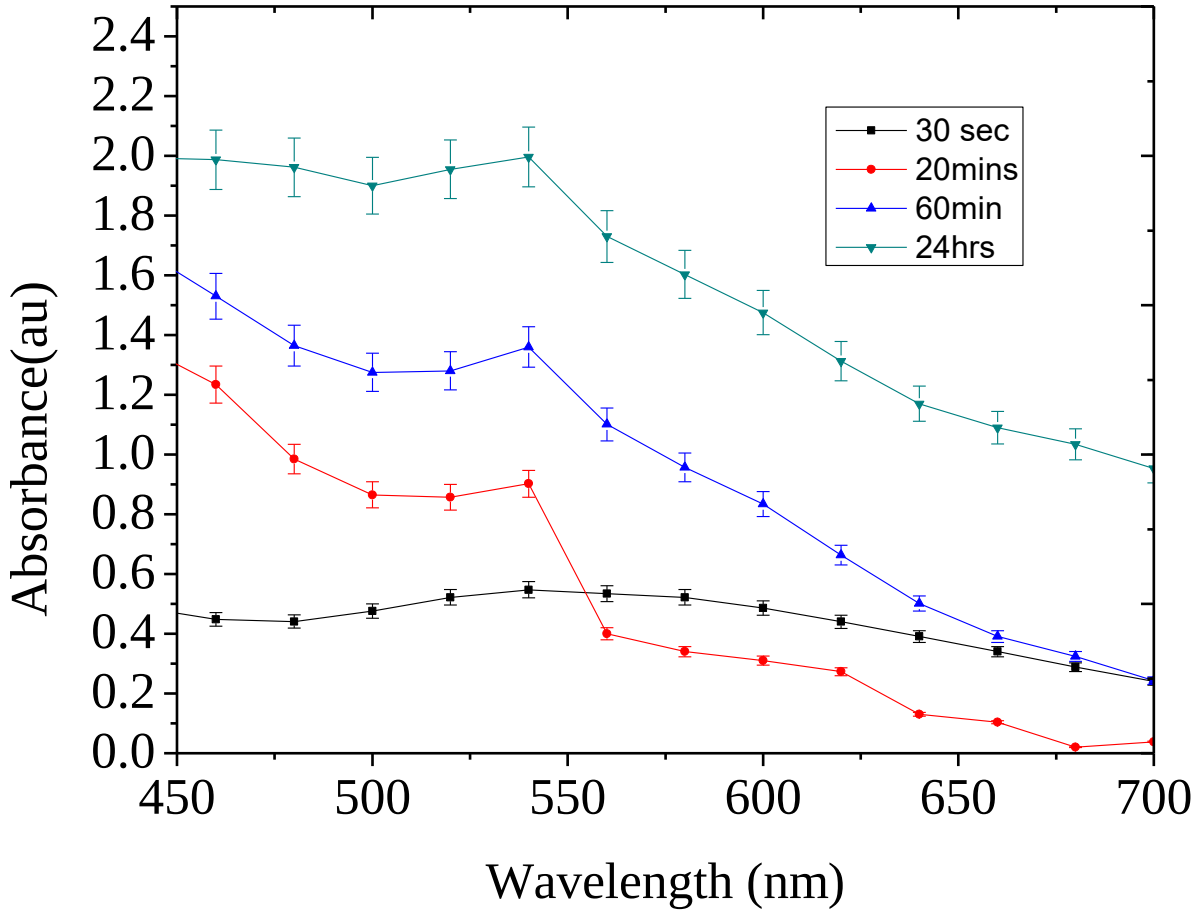
PLANT SYNTHESIS
(NATURAL/NON-TOXIC)

Processing of *Nauclea latifolia*



CHARACTERIZATION

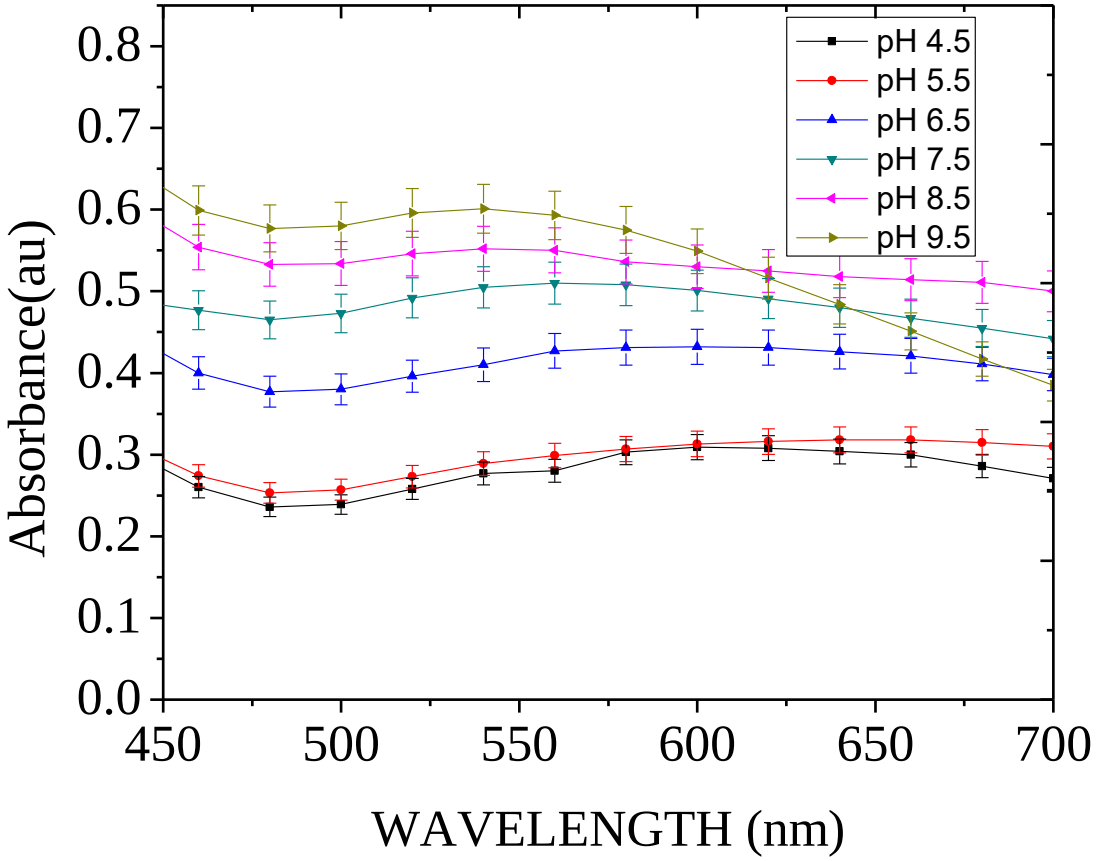
- ❑ **AuNPs were synthesized within 30 sec.**
- ❑ **The naoparticles remained stable even after 24 hours**



UV/Vis Spectra Showing the Effect of Time on the Formation of Gold Nanoparticles with *Nauclea latifolia* leaves

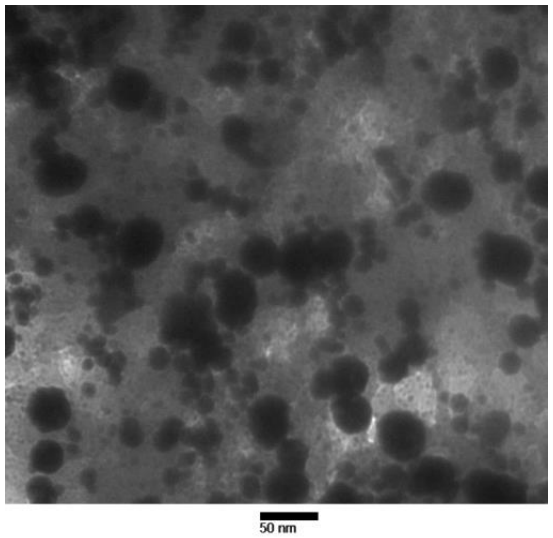
CHARACTERIZATION (cont.)

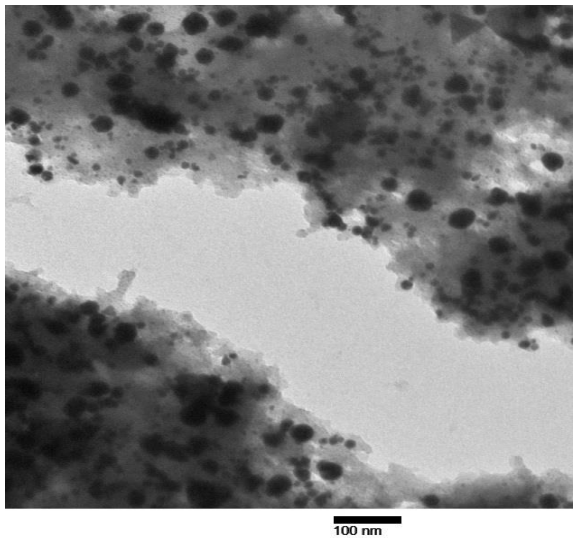
- **Alkaline pH favours synthesis of AuNPs**
- **No synthesis at pH 4.5 and 5.5**

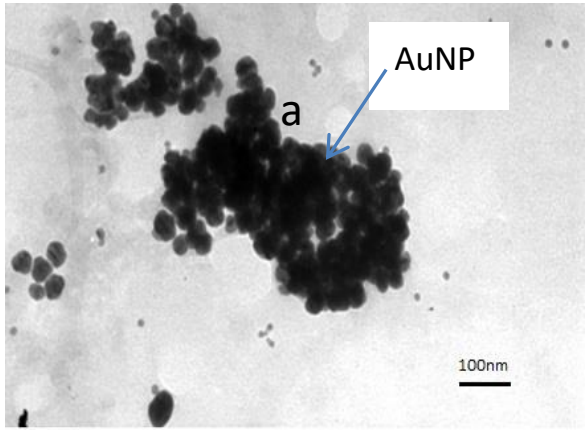


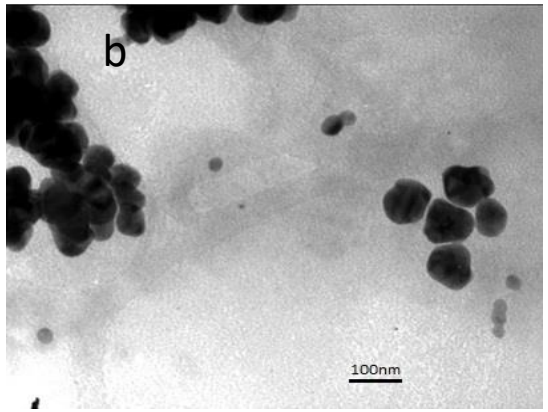
UV/Vis Spectra obtained For AuNPs produced From *Nauclea latifolia* leaves Showing Effect of pH

CHARACTERIZATION (cont.)









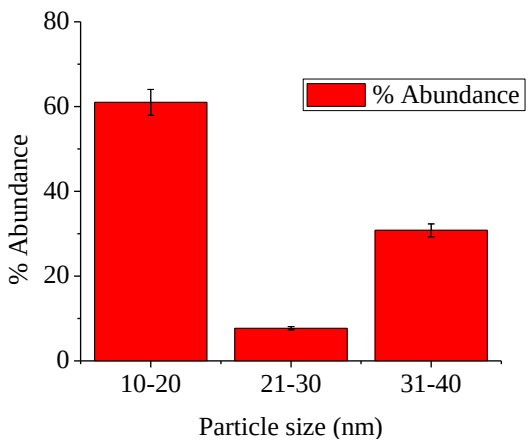
(e)

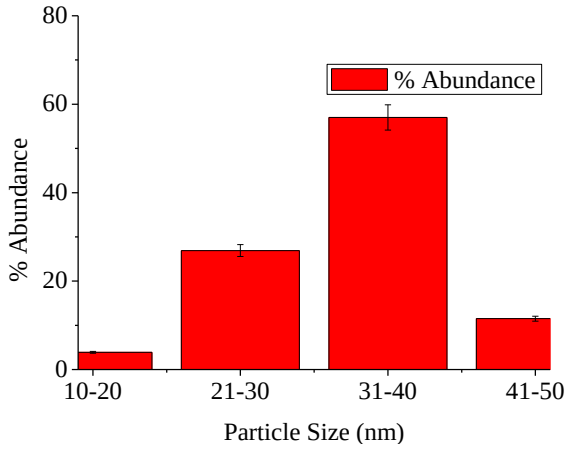
TEM images of AuNPs from Nauclea latifolia: (a) neat (dH2O); (b) at pH 6.5; (c) at pH 7.5 ; (d) at pH 9.5

d

c

Cont.





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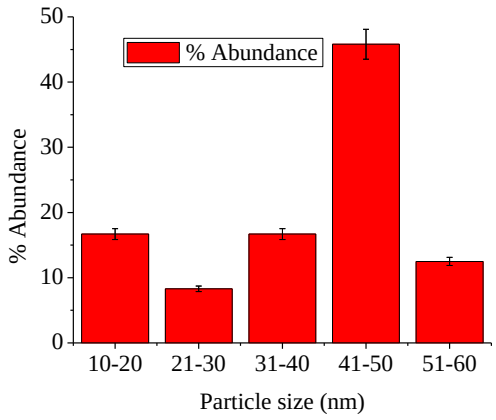

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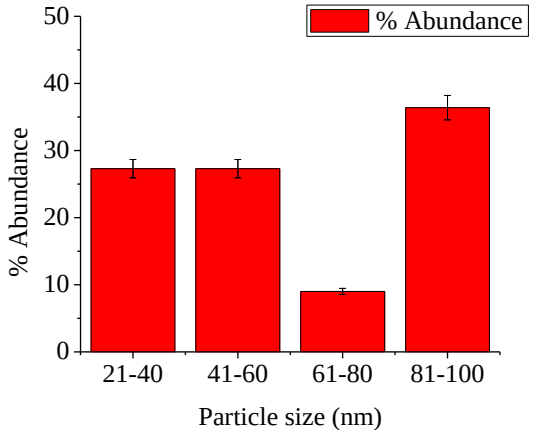
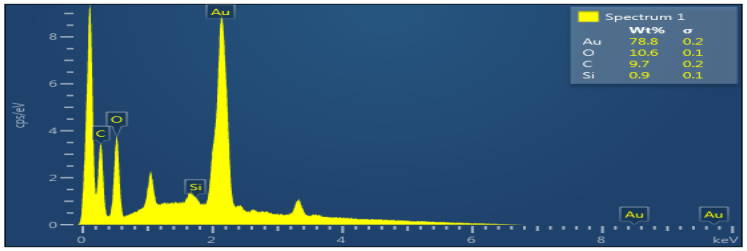
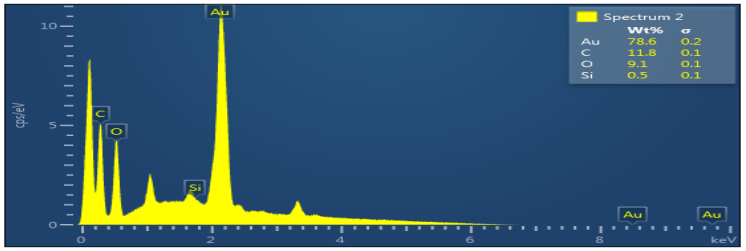


Histogram distributions that show the particle size of AuNPs from *Nauclea latifolia* (a) Neat; (b) at pH 6.5; (C) at pH 7.5 and (d) at pH 9.5

Characterization cont.





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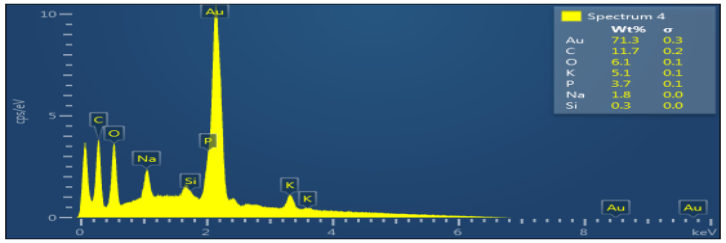

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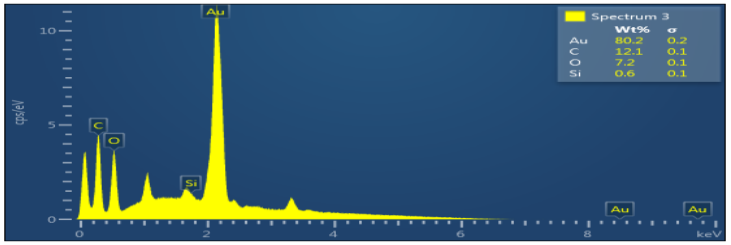

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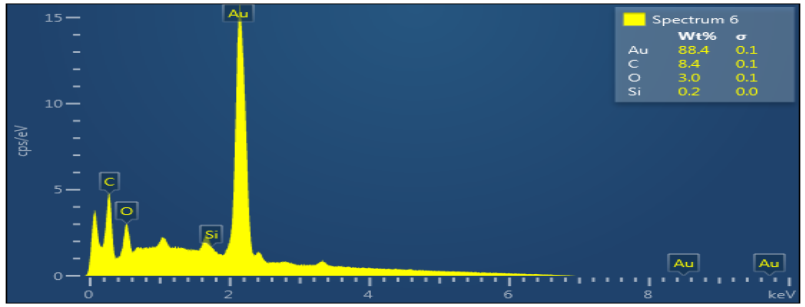
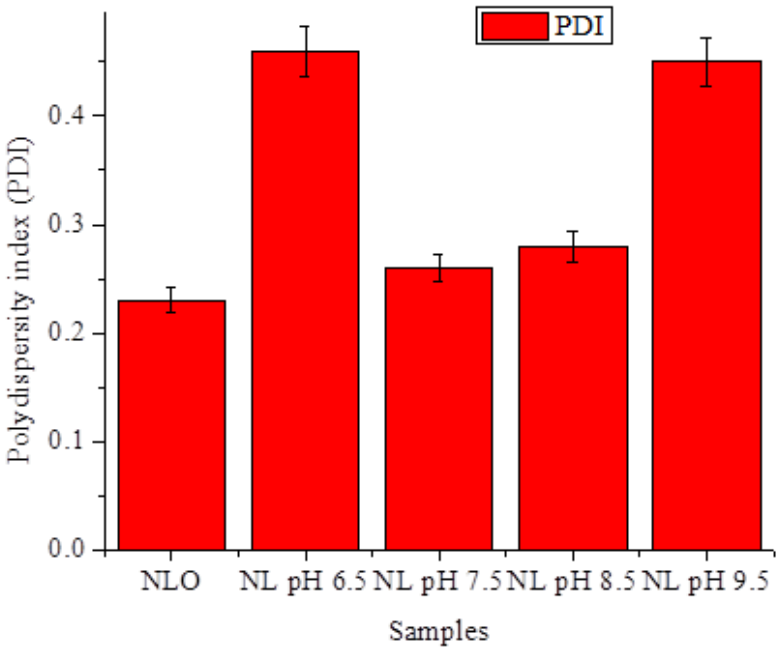
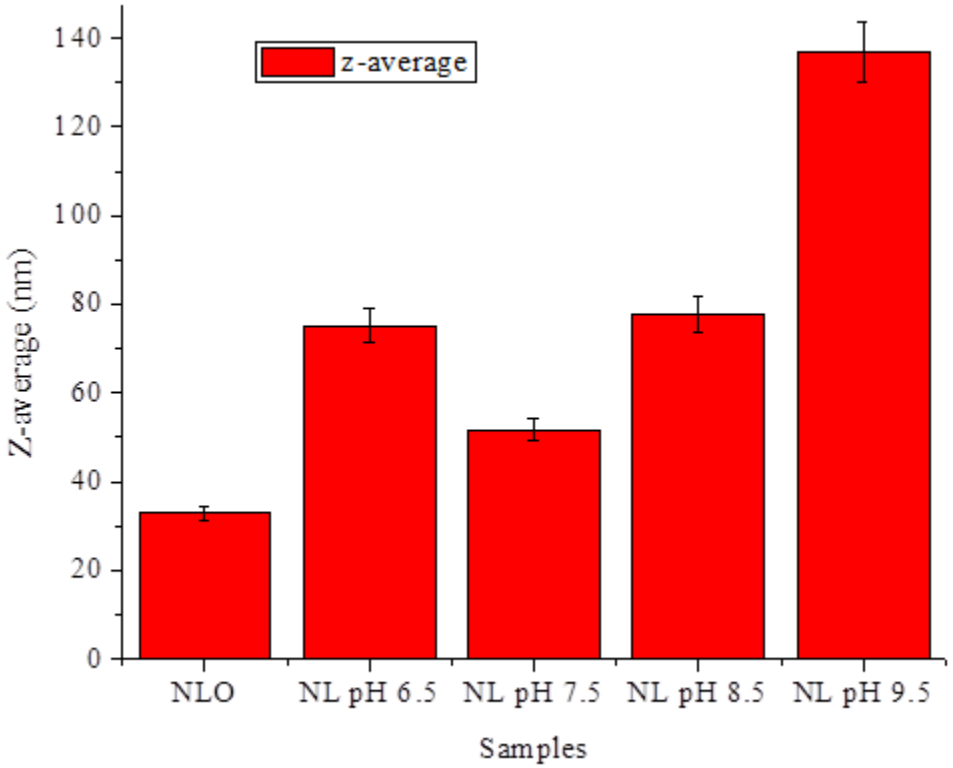


EDX showing elemental composition of AuNPs from *Nauclea latifolia* (a) Neat (dH2O); (b) at pH 6.5; (c) at pH 7.5; (d) at pH 8.5, and (e) at pH 9.5

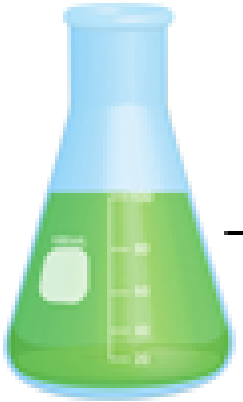
Cont.





Polydispersity index (PDI) and Z-average results of the NL synthesized gold nanoparticles at varying pH

AuNPs SYNTHESIS WITH BACTERIA



S. marcescens
cultured in (PGB)
48 hours at 30°C

Centrifuged/Washed Cells

Reactions:
+ 2.5 mM $HAuCl_4$
at pH 4 , ; 5.5,
6.5, 7.5 and 8.5

24 hours

Characterization
UV-Visible Spectroscopy;
SEM, EDS, TEM

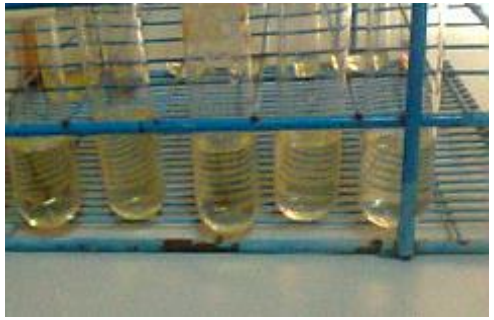
Filtered to Remove Cells
Cell free extract

*Controls:
Cells in water, 2.5mM $HAuCl_4$, Cell Free, ENB + 2.5mM $HAuCl_4$
at pH 4.0; 5.5, 6.5, 7.5 and 8.5

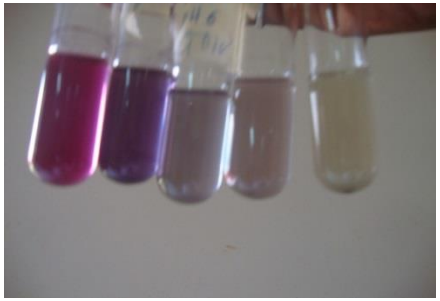
The Reaction

- ***Nauclea latifolia and Serratia marcescens*** have the potential to reduce Au^{3+} to form gold nanoparticles (Au^0) according to reaction;

- $HAuCl_4 + 3e^- \longrightarrow Au + 4Cl^- + H^+$

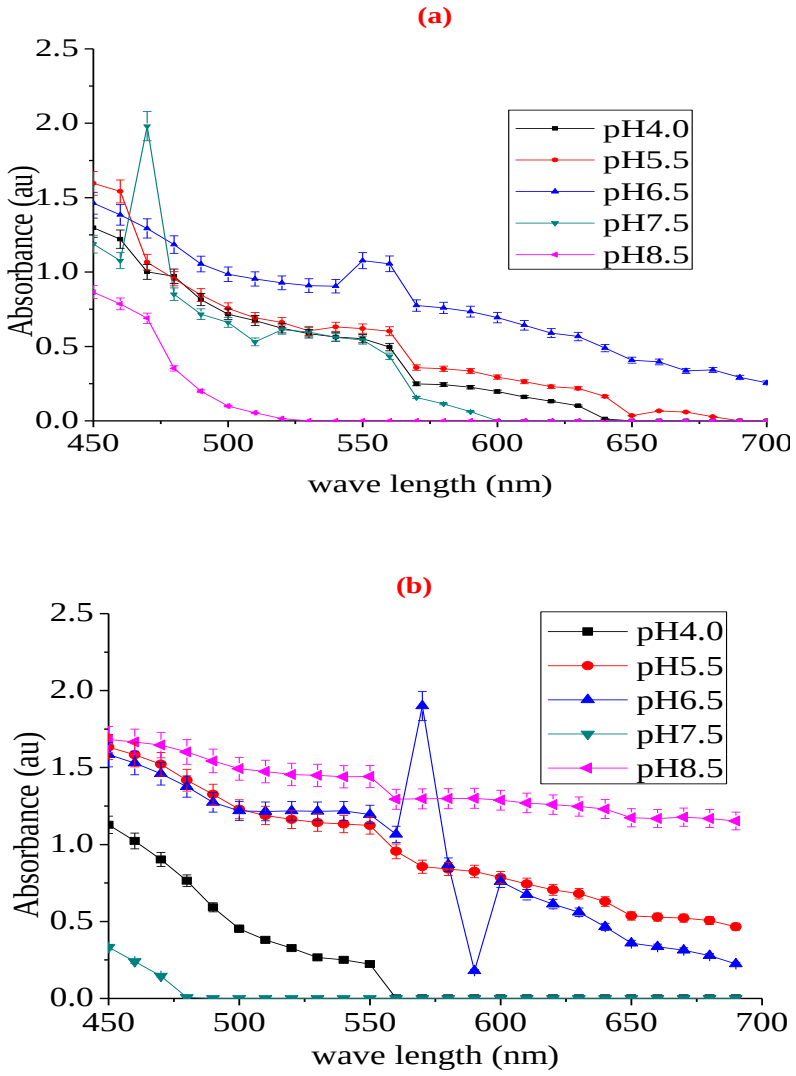


at zero hour



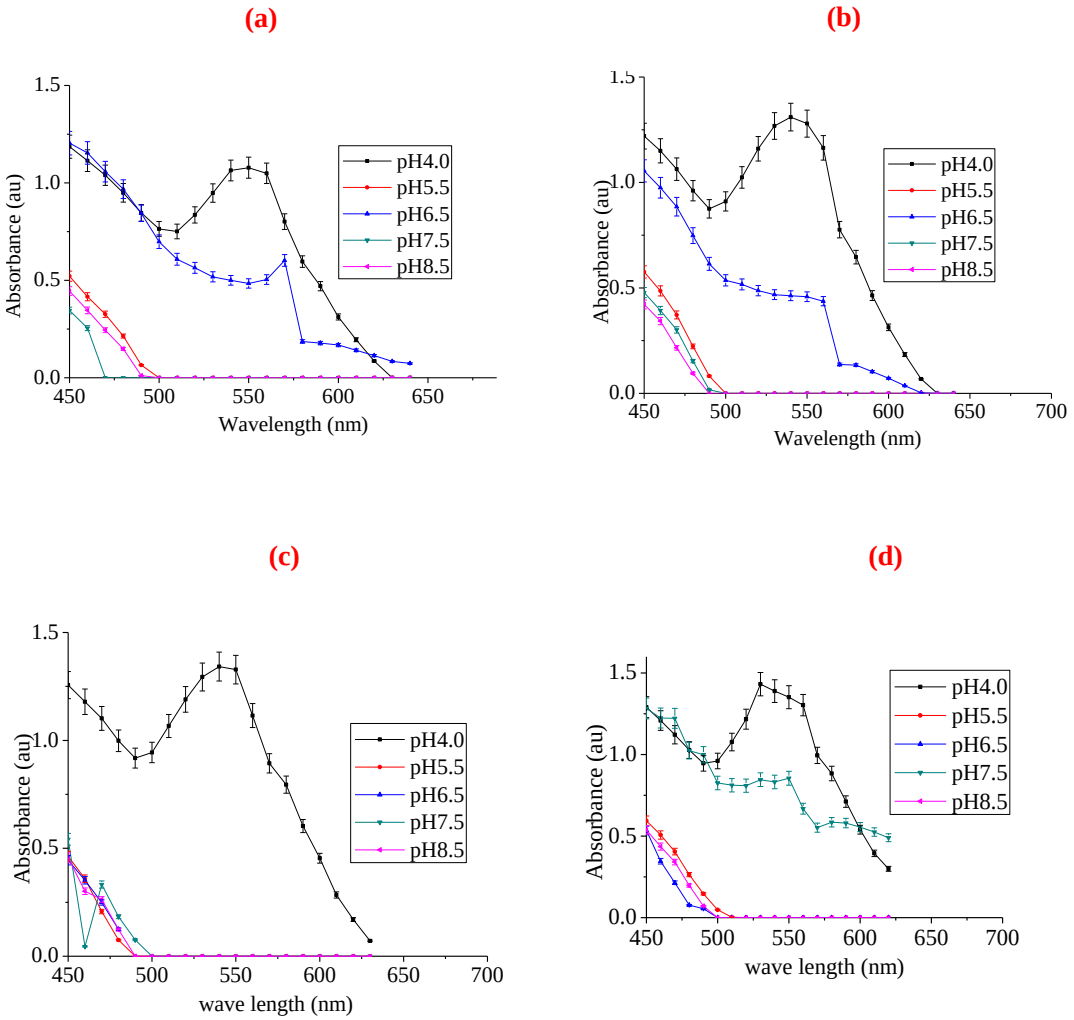
After 24hours

CHARACTERIZATION



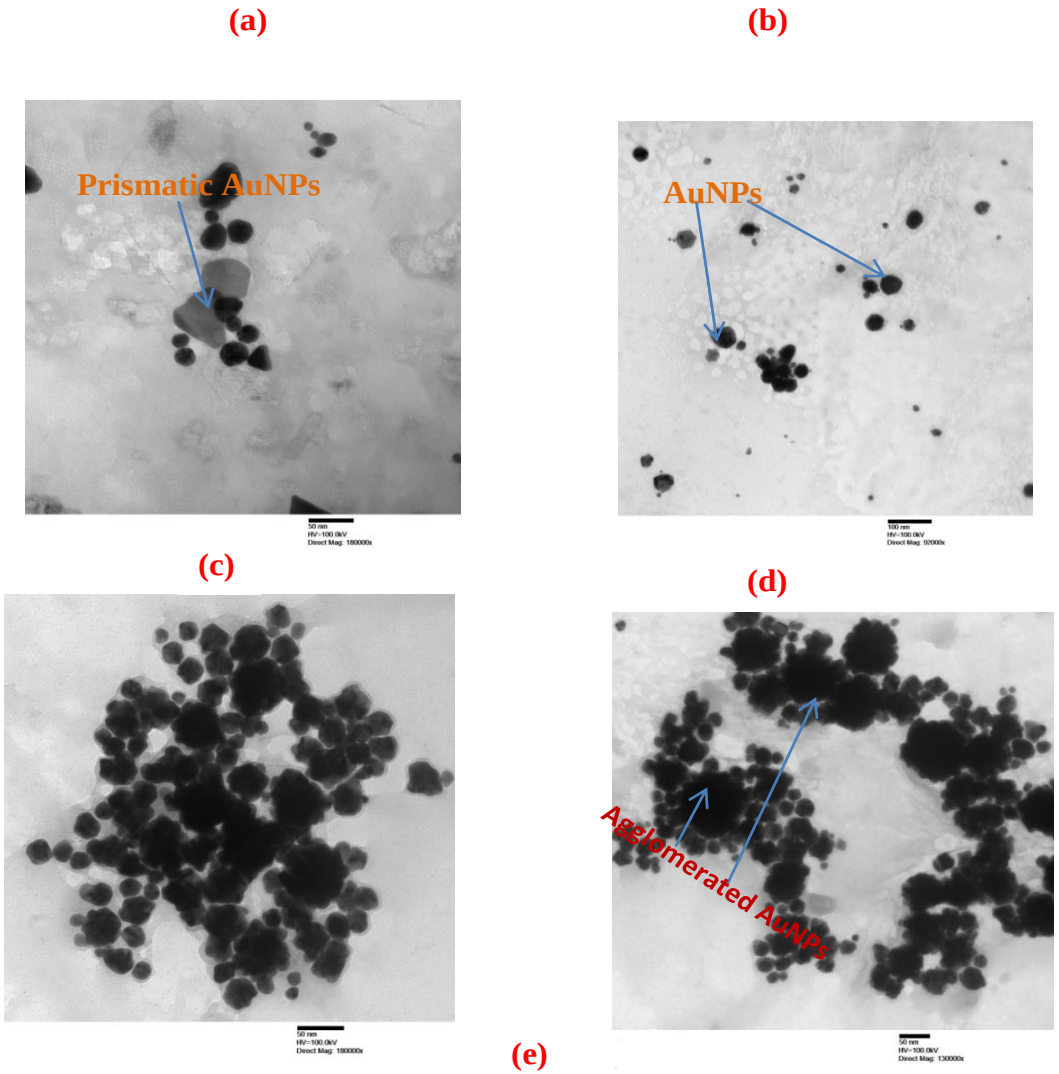
UV/Vis spectra of gold nanoparticles synthesis with the biomass at (a) 6 days, and (b) 10 days.

CHARACTERIZATION (cont.)



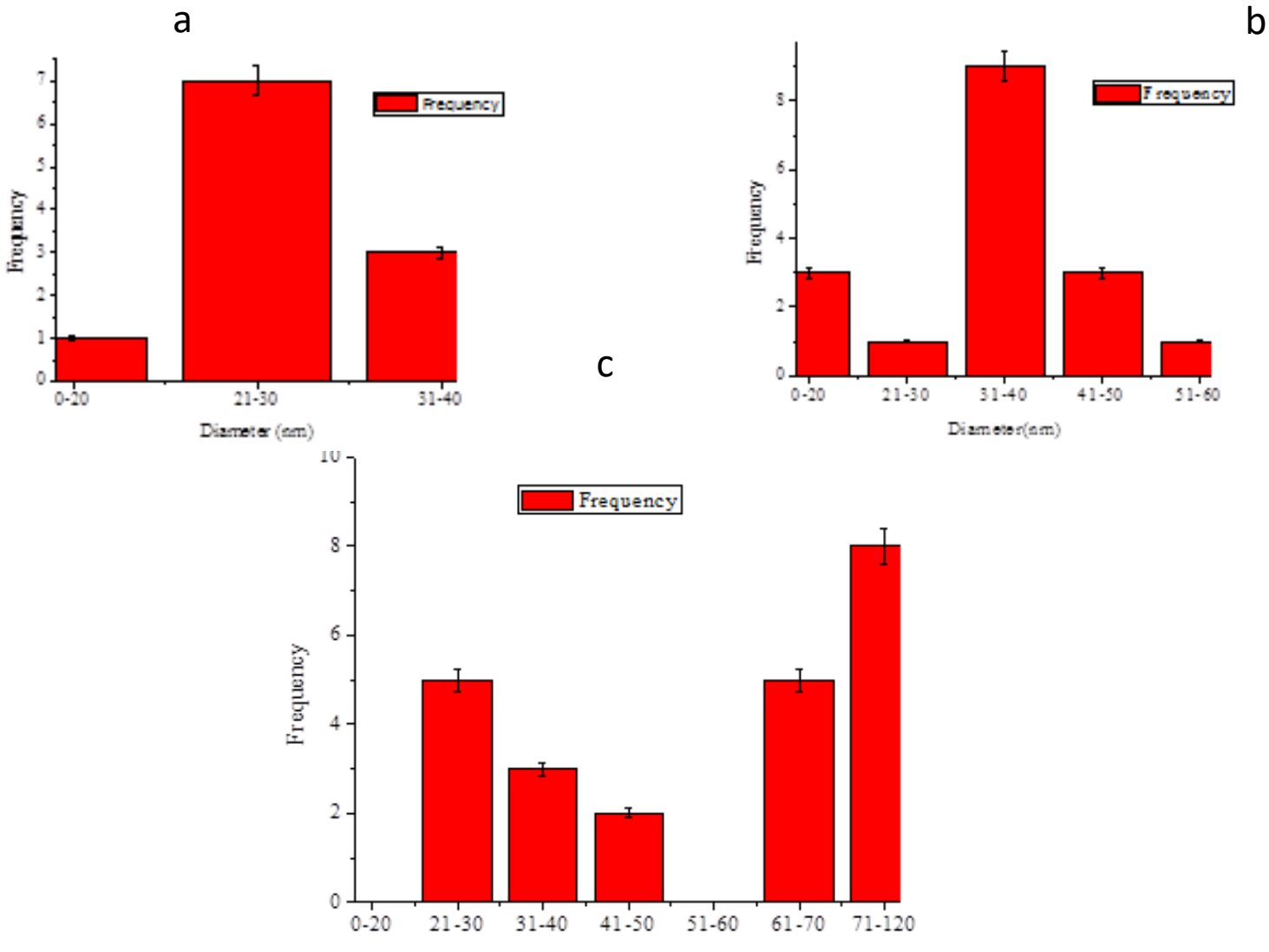
Effects of pH on UV/Vis spectra of gold nanoparticles synthesis with the cell-free extract of *Serratia marcescens* at; (a) 24hrs; (b) 48hrs (c) 72hrs, and (d) 96hrs

CHARACTERIZATION (cont.)



TEM images of the biosynthesized gold nanoparticles obtained from cell-free extract of Serratia marcescens at: (a) pH 4 on day 1; (b) pH 6.5 on day 1. From biomass at: (c) pH 5.5 after 24 hours; (d) pH 6.5 on day 6

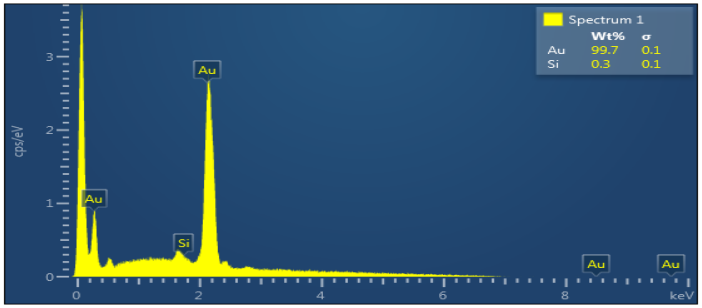
CHARACTERIZATION (Cont)



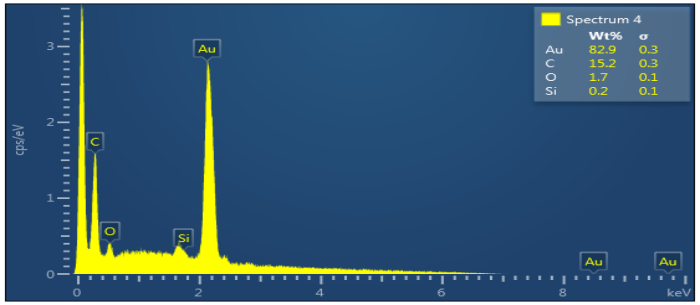
Histograms showing the size distribution of the gold nanoparticles synthesized (a) from cell-free extract of *Serratia marcescens* at pH4 ; (b) at pH6.5 ; (c) from biomass of *Serratia marcescens* at pH

CHARACTERIZATION (Cont)

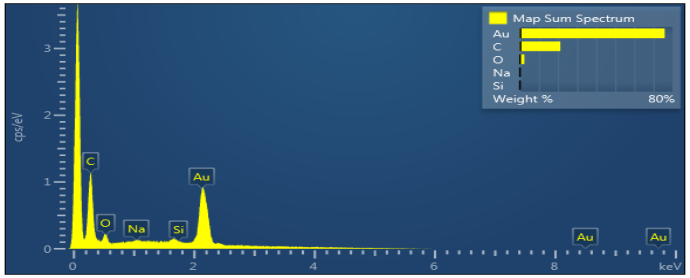
(a)



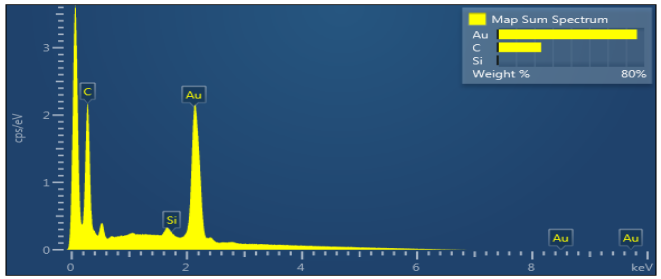
(b)



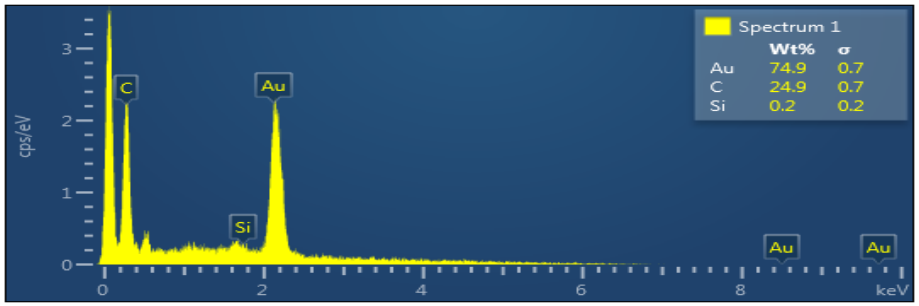
(c)



(d)

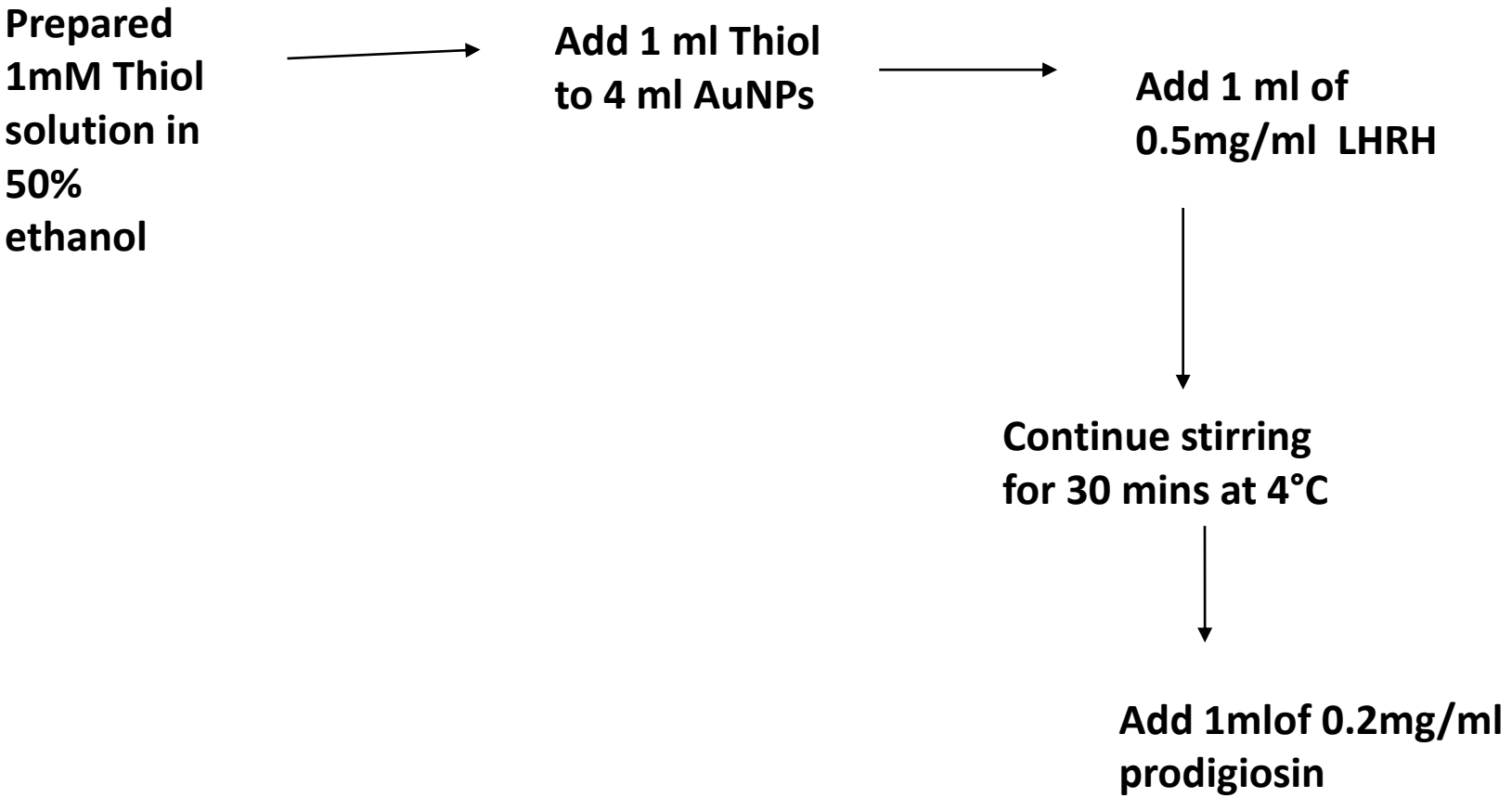


(e)

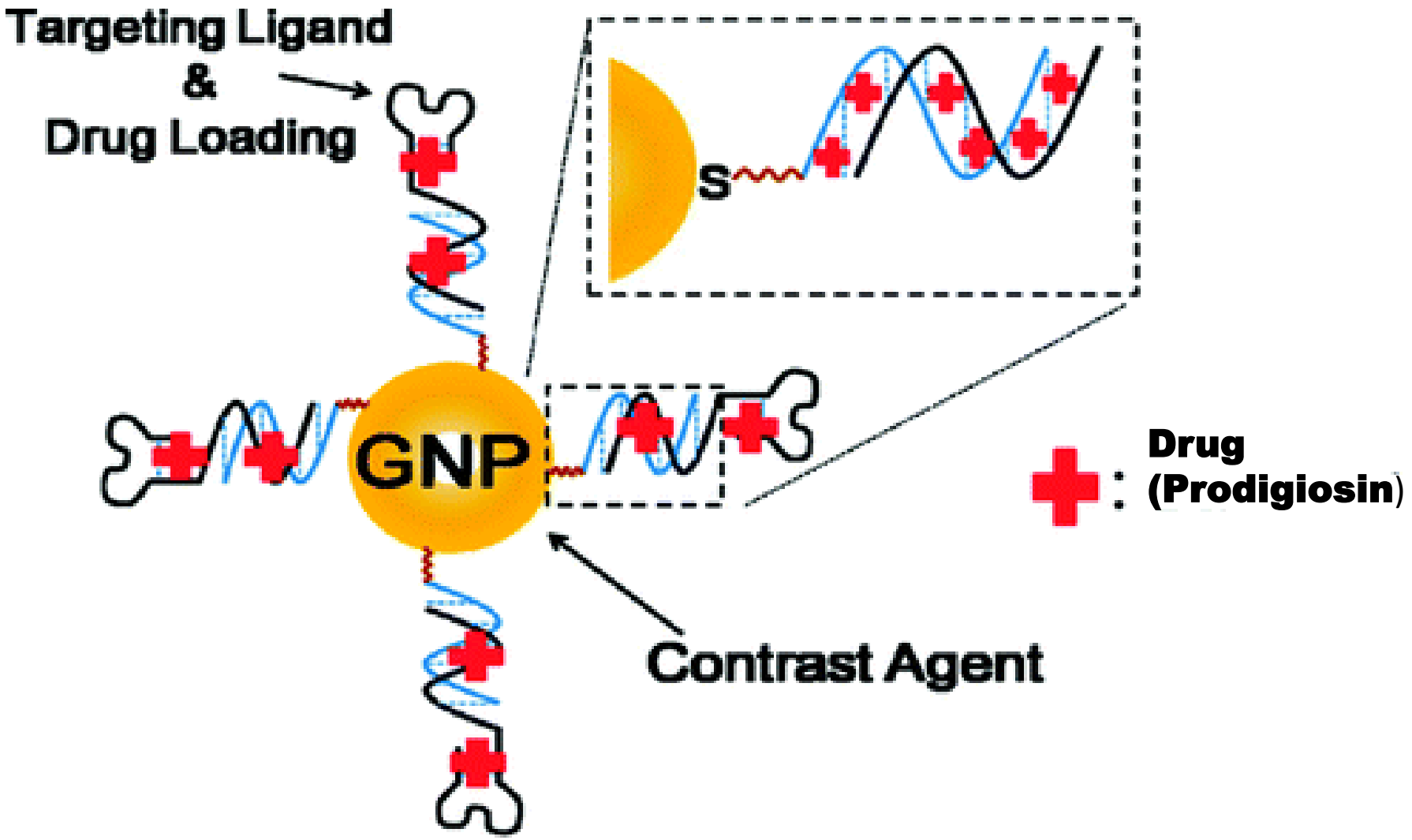


EDS analysis of the synthesized AuNPs indicating the presence of elemental gold at varying pH

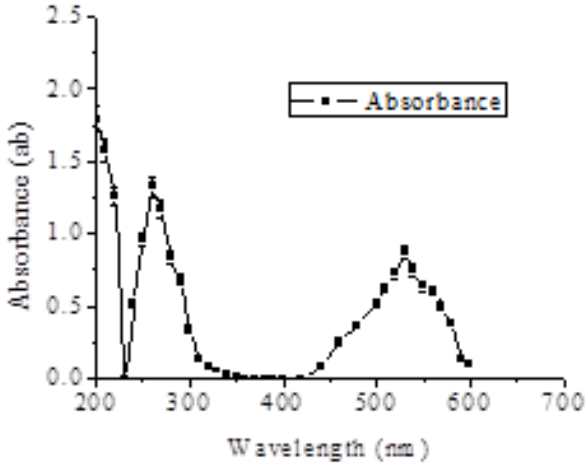
CONJUGATION OF AuNPs WITH LHRH

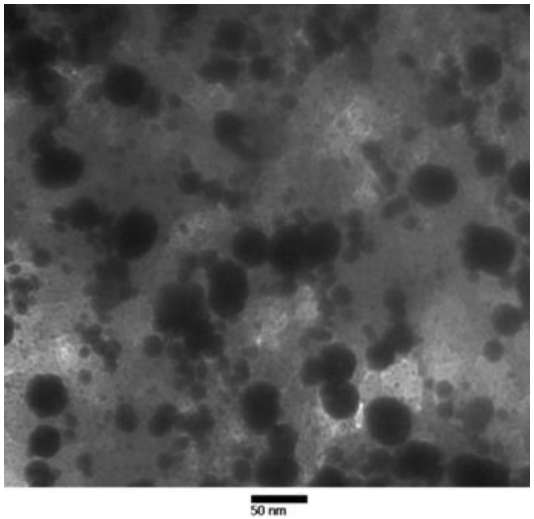


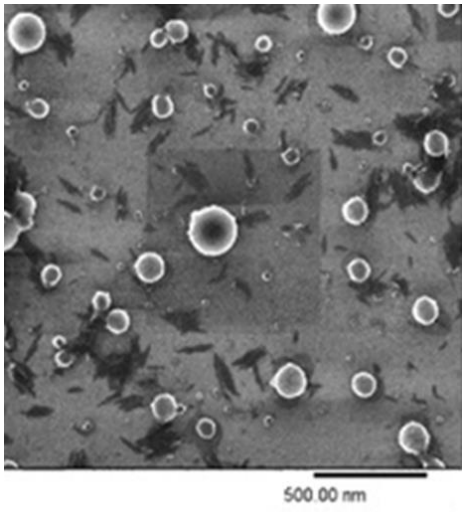
Conjugation of AuNPs with LHRH and Prodigiosin

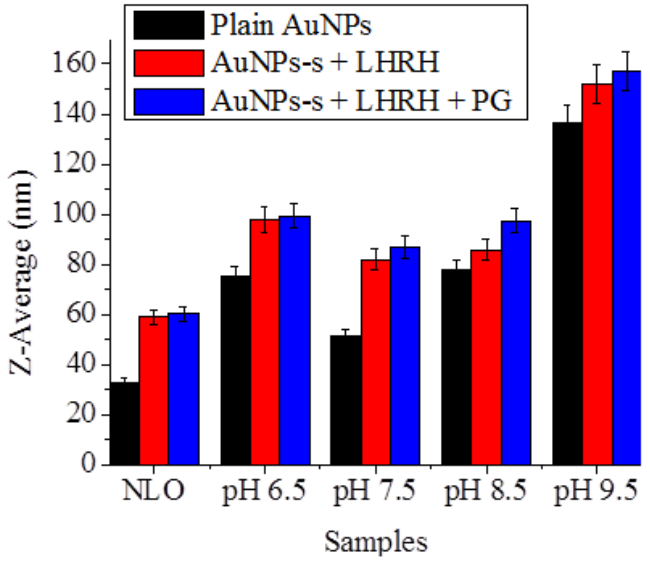


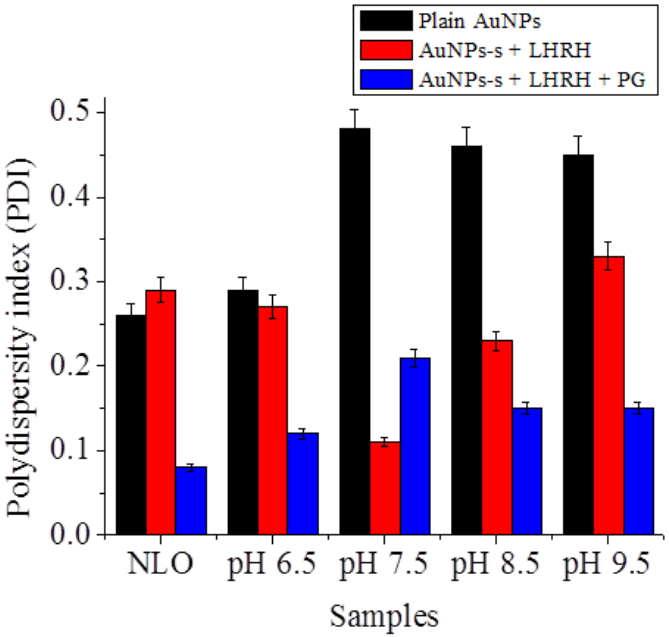
CHARACTERIZATION AFTER CONJUGATION





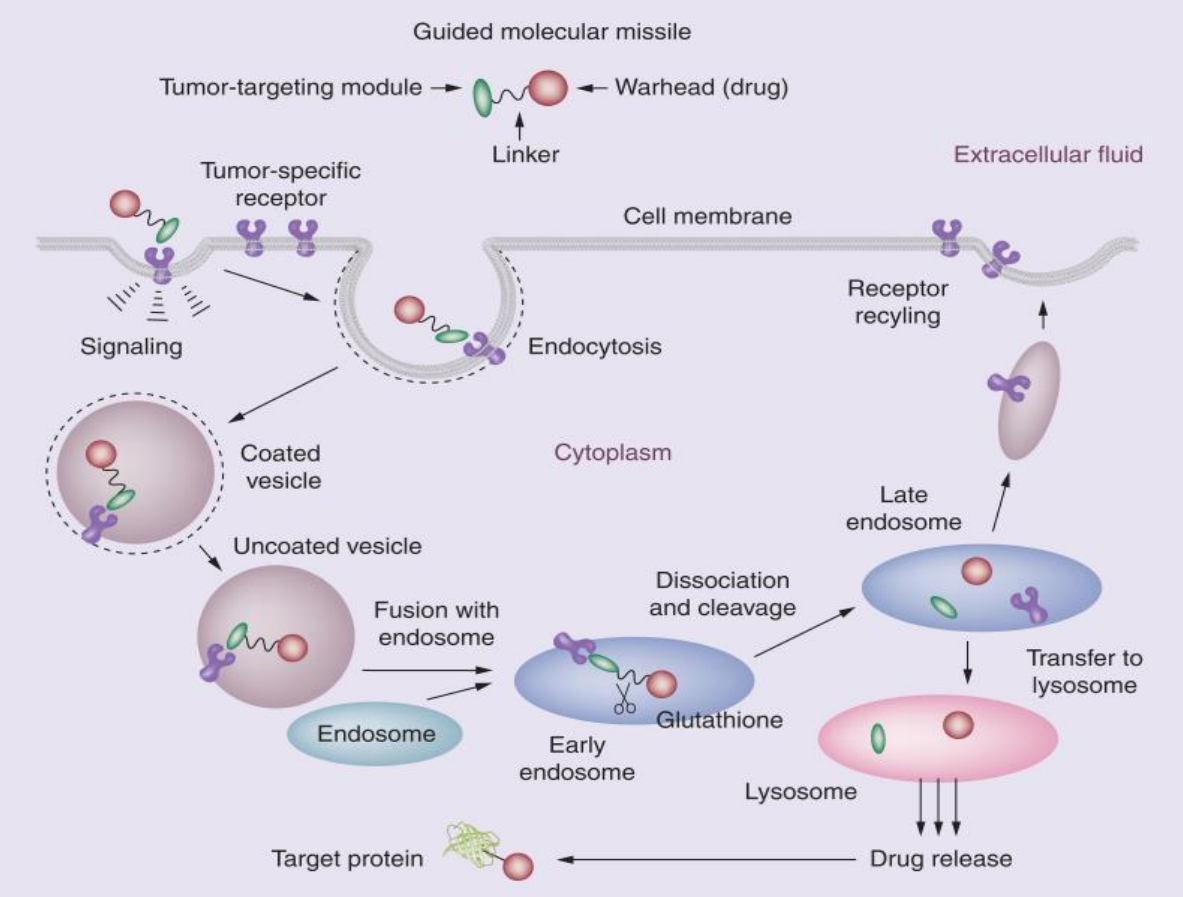






MODE OF TARGETING: ACTIVE TARGETING

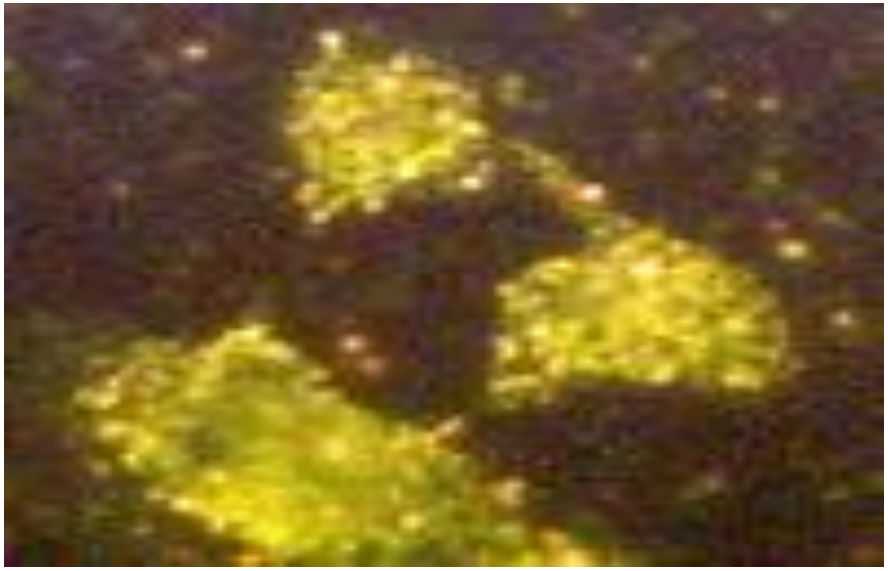
- **LHRH-AuNPs - target is taken into the cell by binding to the LHRH receptor at the cell surface.**
- **On reaching early endosomes, the LHRH-AuNPs dissociates from the receptor, and the receptor can be recycled to the cell surface.**
- **The LHRH-AuNPs remains in the endosome and is delivered to lysosomes for processing.**
- **LHRH-AuNPs - target dissociates because of the slightly acidified environment of the early endosome, generated by a vacuolar membrane proton pump V-ATPase**

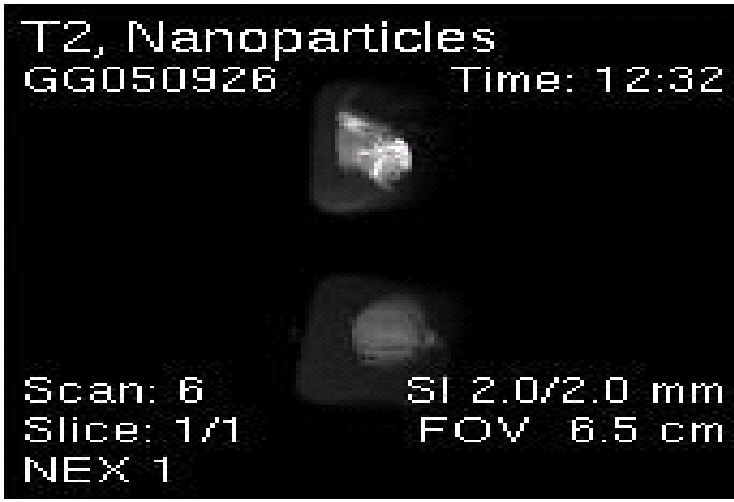


USE OF AuNPs IN EARLY DETECTION

Diagnosis can be done either by

- **Biopsy**
- **MRI,**
- **Mammogram**
- **Ductogram**

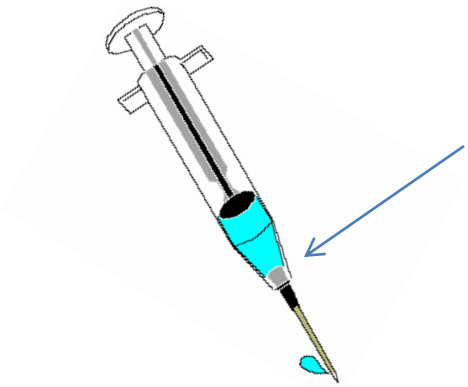




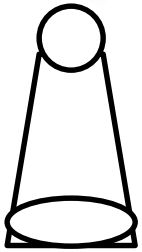
Light scattering images of malignant cells after incubation with anti-EGFR antibody conjugated gold nanoparticles.

LOCALIZED DRUG DELIVERY BY THE USE OF MICROPARTICLES

ENCAPSULATION OF PRODIGIOSIN IN CHITOSAN MICROSPHERES



0.25% chitosan
+ 2% (v/v) acetic acid
+ prodigiosin (5% of polymer)



250ml Paraffin
+ 0.5% Tween 20

Stirred at 2000rpm for 20min at 2-8°c

Add gluteraldehyde for cross-linking, stir for 2½hr

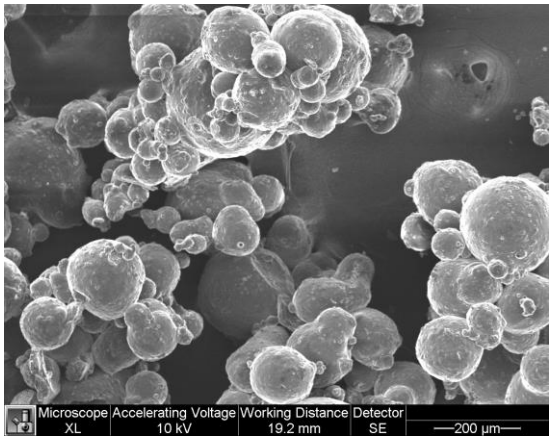
Centrifuge at 5000rpm

Wash with n-hexane/ DH_2O/ acetone

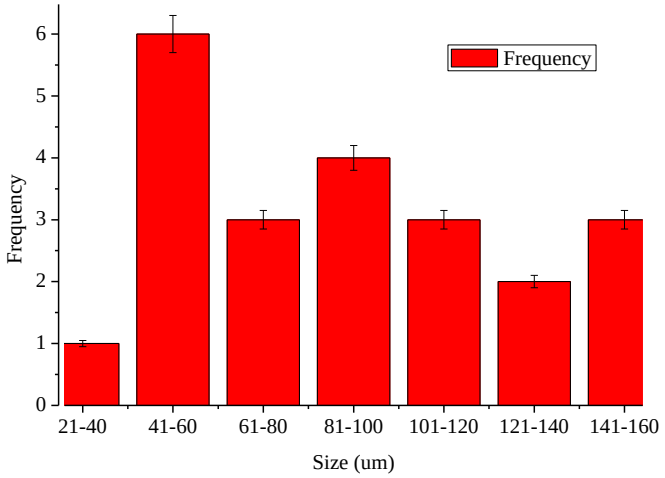
Dry microspheres at room temperature

Characterization

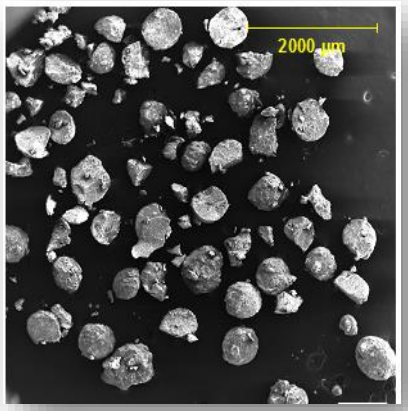
a



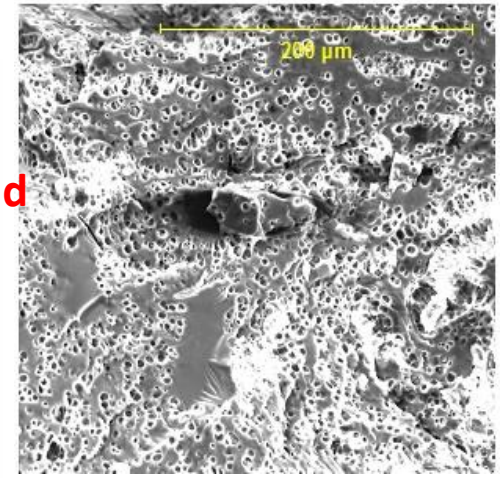
b



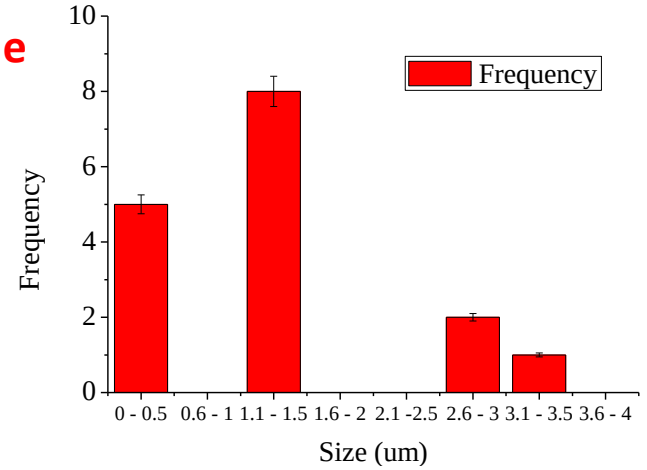
c



d



e



SEM images of (a) spherical shaped chitosan microspheres (b) size distribution of chitosan microspheres (c) microspheres cut open (d) enlarged micropores within the microspheres (e) histogram of size distribution for the micro-pores.

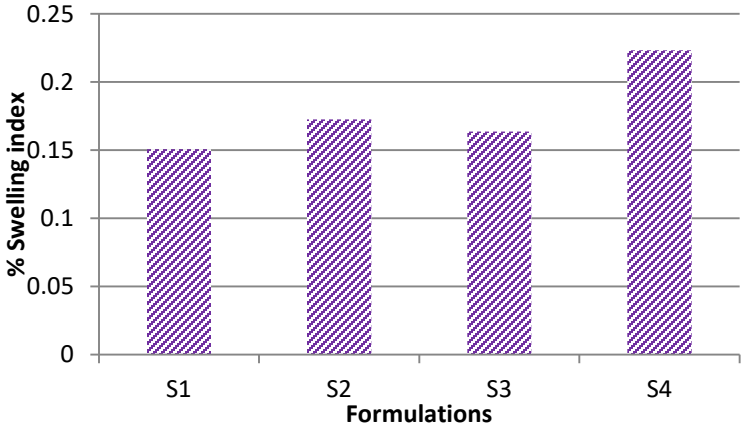
Table showing the encapsulation efficiency, drug loading capacity and % yield of the prodigiosin encapsulated chitosan microspheres

Samples (mg/ml)	S_1 (1mg/ml)	S_2 (1.5mg/ml)	S_3 (2mg/ml)	S_4 (5mg/ml)
% Encapsulation efficiency	66.7±3.3	80±4	76.7±3.8	90± 4
% Drug loading capacity	6.7±0.3	12±0.6	15.3±0.8	45± 2.3
% Yield	55.5±2.8	52.4±2.6	50±2.5	42±2.1

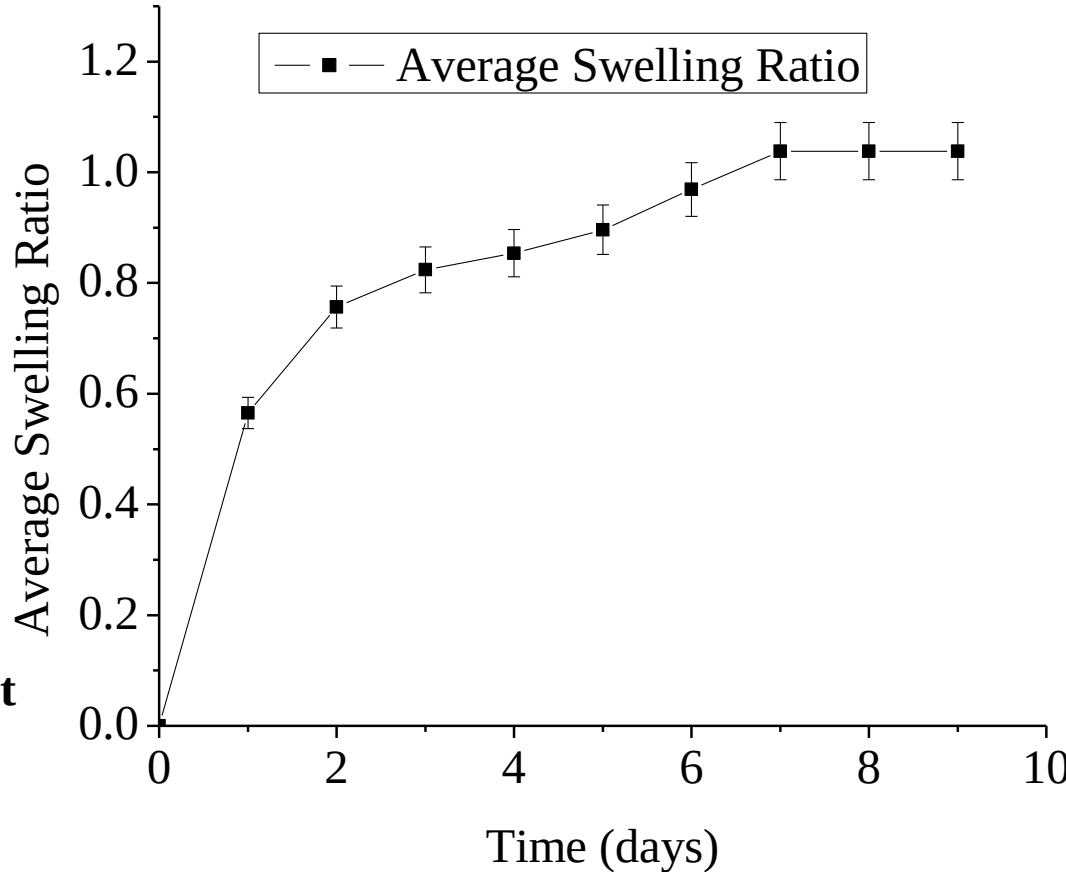
Swelling index (S.I) = $\frac{W_t - W_o}{W_o}$

Where W_t = Final weight of microspheres

W_o = Initial weight of microspheres



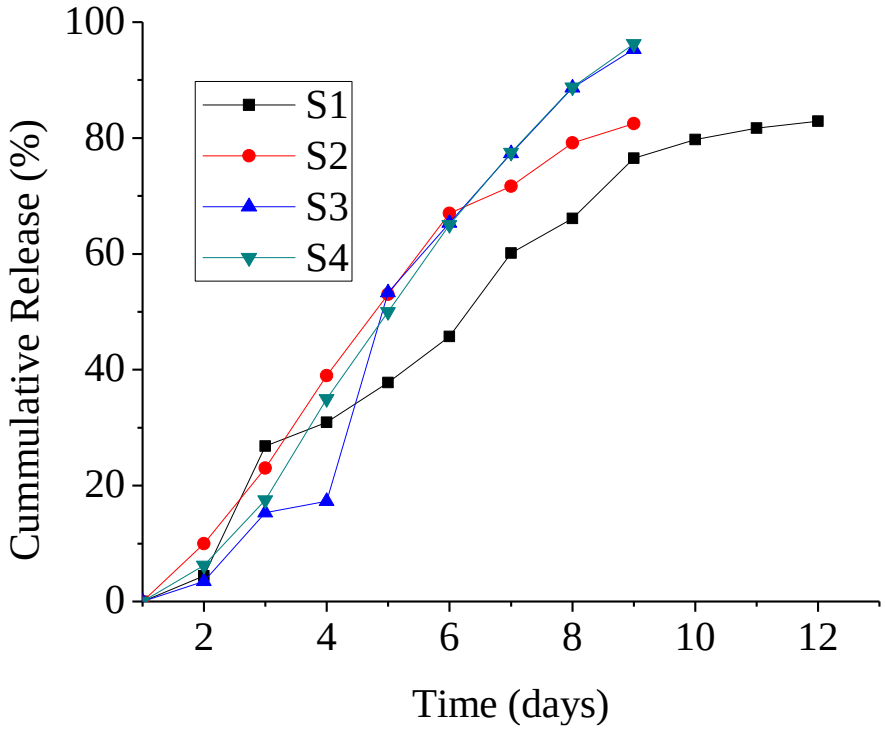
Comparison of swelling index of different formulation of microspheres



Average swelling ratio of the microparticles

DRUG RELEASE STUDIES

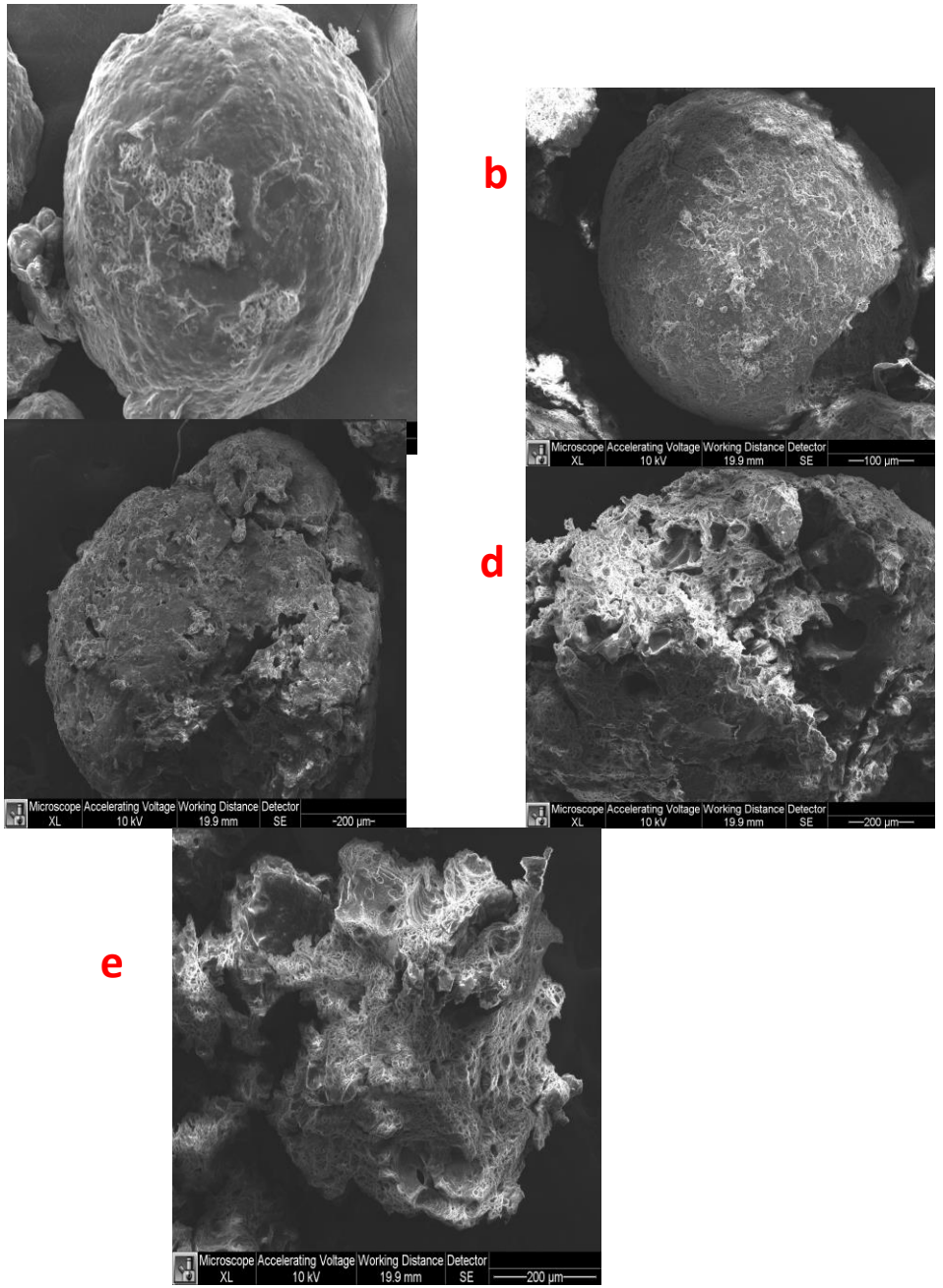
Samples (mg/ml)	S_1 (1mg/ml)	S_2 (1.5mg/ml)	S_3 (2mg/ml)	S_4 (5mg/ml)
%cumulative Release	82.92	82.5	96.25	96.67



Release pattern of prodigiosin from the microspheres

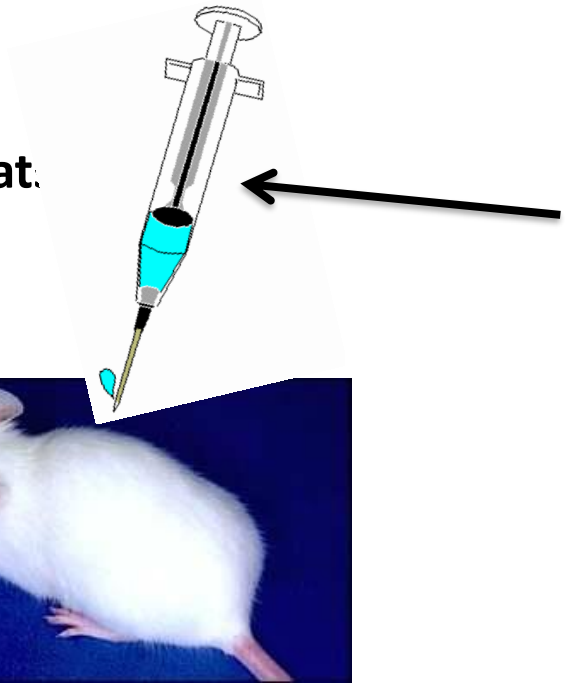
SEM Images showing the degradation process of the microspheres

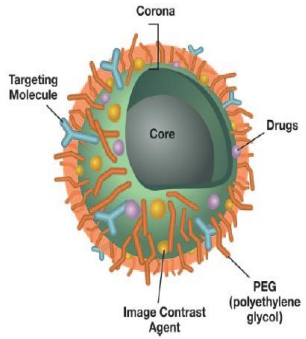
- (a) microsphere before immersing in the buffer
- (b) microsphere after 2 days, crack being initiated
- (c) increase in number of cracks after 5 days
- (d) at day 9 degradation has set in
- (e) after 12 days.

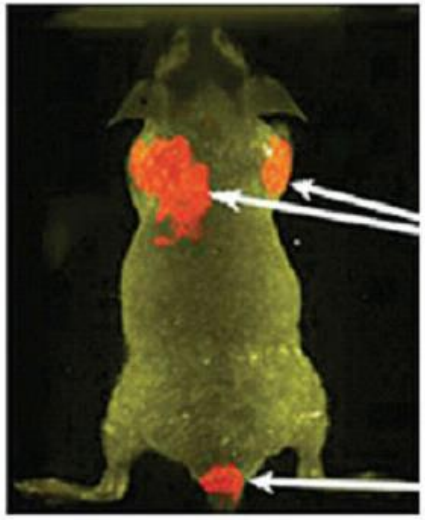


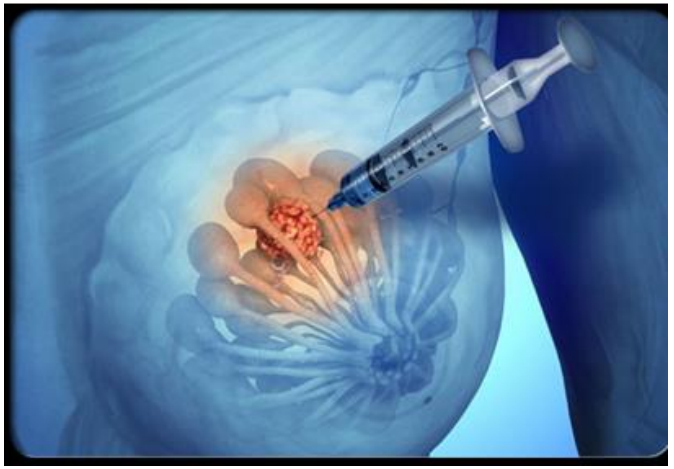
Synergy between Nanoparticles and Microparticles

- Acquire laboratory animals (female rat
- Infect with breast cancer
- Inject with the drug design
- Carry out clonogenic assay









SUMMARY/CONCLUSION

- **Breast cancer is on the increase and requires urgent attention**
- **Biosynthesis is a cheap and faster means for producing gold nanoparticles**
- **Prodigiosin, an alternate cancer drug, cheap and readily available**
- **Gold nanoparticles enhance early detection**
- **The use of microspheres-based therapy allows drug to be released at a controlled rate at the specific site of interest**

SUMMARY/CONCLUSION

- **Drug released over a period of 12 days thereby helping to reduce the frequency of taking drugs.**

- **The initial drug released is due to diffusion and burst process, as the drug can travel through the pores formed during the hardening phase, followed by a later degradation controlled release for a small fraction of the drug**

- **A combination of targeted drug delivery and localized drug delivery will ultimately give a better result.**

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