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Synthesis and characterization of Gefitinib and Paclitaxel dual drug loaded Cockle shell (*Anadara granosa*) derived Calcium carbonate nanoparticles

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Outline

Introduction

Nanomedicine – future of medicine

Nanoparticles – effective drug delivery system as they possess higher surface area to volume ratio (Din et al., 2017)

Cockle shells- (*Anadara granosa*)- source of Calcium carbonate (Aragonite polymorph)

Nano vectors – Targeted drug loaded nano materials

Dual-drug loaded system

Distribution of blood cockle shells

(FAO, n.d.)

Sustainable source of $CaCO_3$

Aragonite polymorph-biocompatible, safe, pure (Kamba *et al.*, 2013; Hammadi *et al.*, 2017; Danmaigoro *et al.*, 2017)

pH dependent drug release (Hammadi *et al.*, 2017; Danmaigoro *et al.*, 2017)

Cockle shell derived aragonite calcium carbonate nanoparticles ($CSCaCO_3NP$)

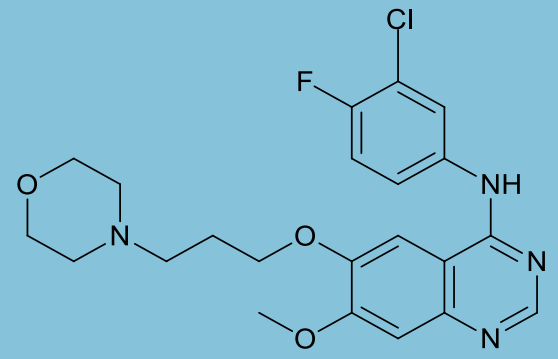
For delivering hormonal agents (Jaji *et al.*, 2017)

For delivering chemotherapeutic agents (Danmaigoro *et al.*, 2017; Ibiyeye et al., 2020)

To deliver anti-bacterial agents (Saidykhan *et al.*, 2016; Idris et al., 2019)

Iressa (Gefitinib, ZD 1839)

$C_{22}H_{24}ClFN_4O_3$

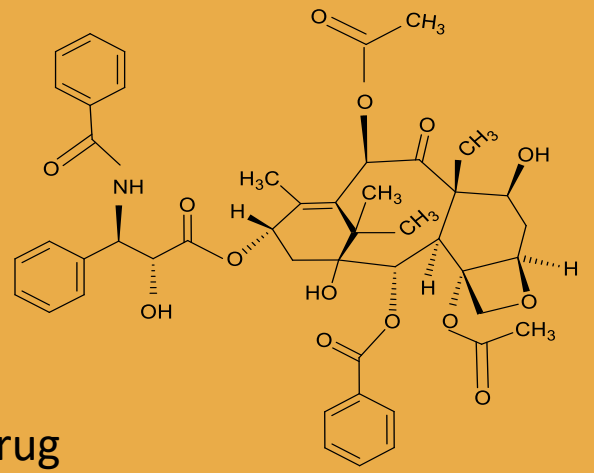


Small molecule EGFR-TKI
Reversible competitive inhibitor of EGFR tyrosine kinase (Ward *et al.*, 1994)
447 kD
FDA approved
-NSCL

NO approved drugs

Paclitaxel

$C_{47}H_{51}N\,O_{14}$

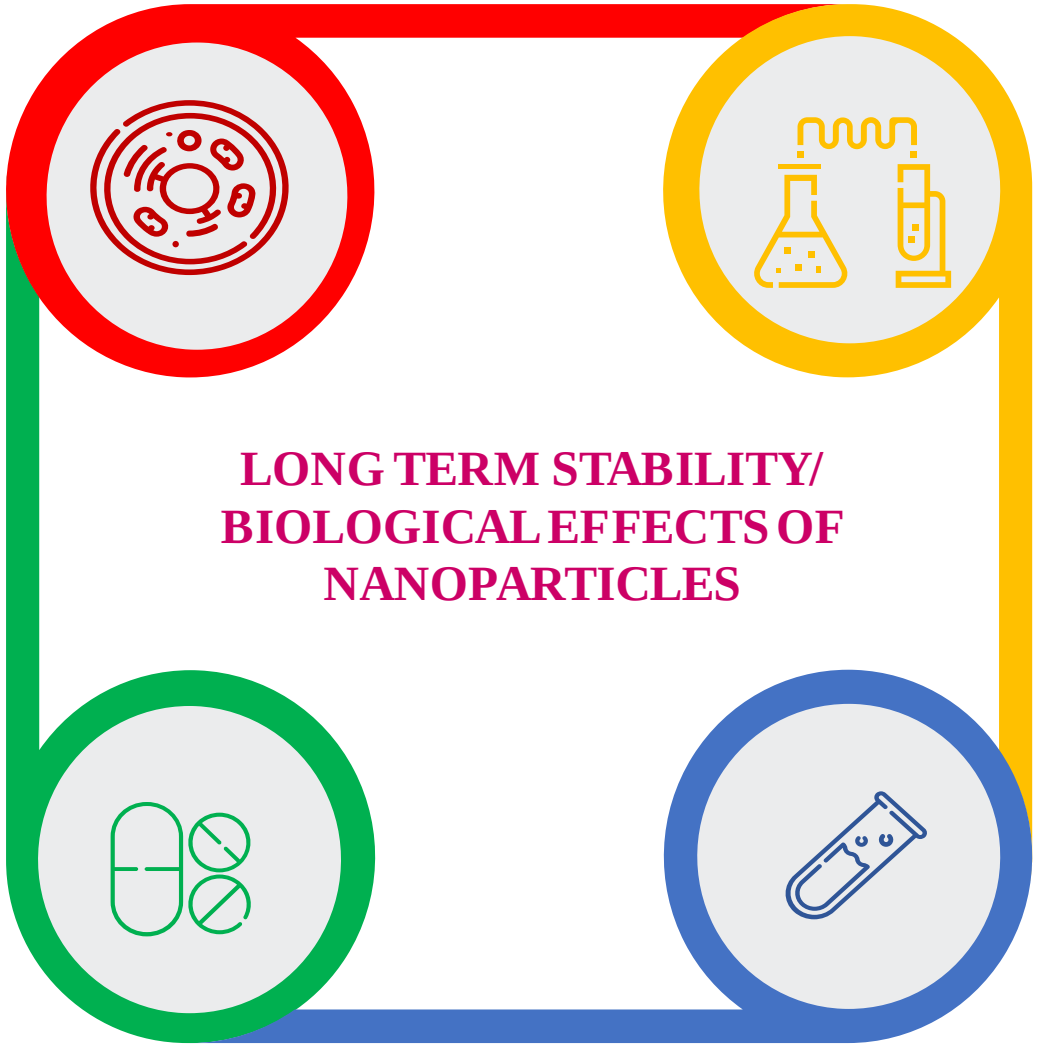


Cytotoxic drug
Promotor of tubulin polymerization and stabilizes microtubules to depolymerization (Nikolic *et al.*, 2011)
853.89kD
FDA approved -* Node positive breast cancer + adjuvant therapy, Metastatic BC
-Kaposi sarcoma
-NSCL
-Ovarian cancer

Abraxane, 2005

Size, PDI, shape, Surface chemistry,

Drug loading and encapsulation efficiency



Chemical composition, bonding and in vitro drug release kinetics

Impurities present

(Hosokawa et al., 2007)

Materials

- GEF and PTXL (Gold Biotechnology, St. Louis, MO)
- DMSO (Fisher Scientific U.K)
- Tween 80 (R & M marketing, U K)
- Cockle shells (local market in Serdang, Malaysia)
- Deionized water Milli-Q integral Water Purification System(Millipore Sigma, USA)
- Double beam UV-VIS spectrophotometer (Shimadzu 1650PC)
- High speed centrifuge (Optima XPN, Beckman Coulter instruments Inc., CA, USA)
- Magnetic stirrer (Dhaihan WiseStir® Systematic Multi-Hotplate Stirrer, South Korea)
- Hot air oven (Memmert UM500, GmbH Co, Germany)
- Programmable ball miller (BML-6'', Diahan scientific®, Korea)
- Transmission Electron Microscope(HRTEM, JOEL JEM-2100F, Japan)
- Field emission scanning electron microscope (Nova Nanosem 230, Japan)
- Carbon-coated copper grid (Sigma- Aldrich, St. Louis, MO, USA)
- Zetasizer Nano ZS (Ver.7.2; Malvern Instruments Ltd., Malvern, UK)
- XRD (Shimadzu XRD- 6000 powder diffractometer)
- FT-IR (Model spectrum 100; Perkin Elmer, USA)
- Micromeritics (Tristar II Plus, USA).

Methods

A. Synthesis of $CSCaCO_3$ NP and GEF-PTXL- $CSCaCO_3$ NP

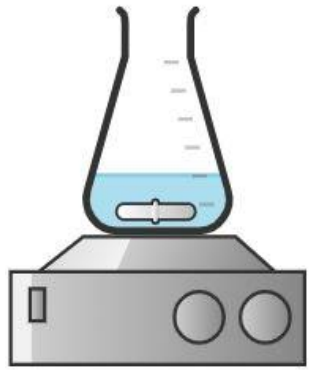
1. Top down synthesis of $CSCaCO_3$ nanoparticles from cockle shells (*Anadara granosa*)

Washed & dried

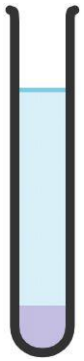
Powdered

Sieved-75µ mesh

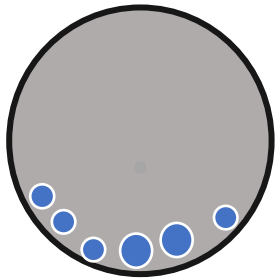
Micron-sized cockle shell powder (MAC)



Deionized water: Tween 80 (1:20), MAC



Washed & centrifuged 16,000 RPM



Ball milling 120hrs @ 120 RPM

Cockle shell derived $CaCO_3$ nanoparticles ($CSCaCO_3$ NP)

2. Synthesis of GEF-CSCaCO$_3$, PTXL-CSCaCO$_3$ and GEF-PTXL-CSCaCO$_3$ nanoparticles

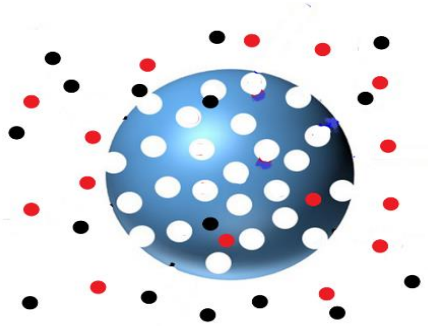
CSCaCO$_3$NP

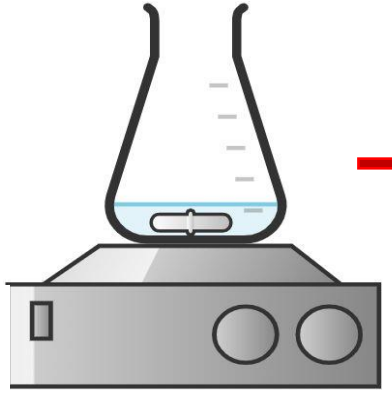
GEF

DMSO

PTXL

0.05%Tween 80



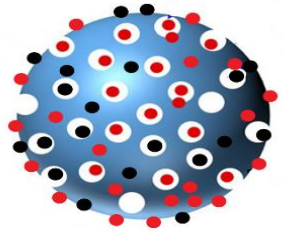


Stirred 12 hours

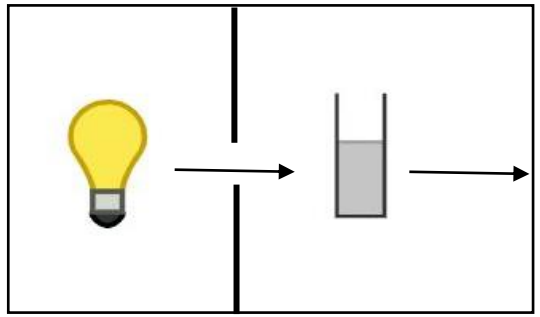
supernatant

drying

sediment



GEF-PTXL-CSCaCO$_3$NP



UV-Vis Spectrophotometer

3. Loading content and encapsulation efficiency of GEF-PTXL-CS$CaCO_3$ nanoparticles

The encapsulation efficiency (EE%) and loading content (LC%) was determined as the average measurement of 3 independent measurements (Fu *et al.*, 2017).

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Wt} - \text{Wf}}{\text{Wt}} \times 100 \quad \text{--- (1)}$$

Where,

Wt is the total weight of drug fed

Wf is the weight of non-encapsulated free drug.

$$\text{Loading content (\%)} = \frac{\text{Wt} - \text{Wf}}{\text{Wnp}} \times 100 \quad \text{--- (2)}$$

Where,

Wt is the total weight of drug fed

Wf is the weight of non-encapsulated free drug

Wnp is the weight of the nanoparticles

B. Physicochemical characterization of $CSCaCO_3$ NP and GEF-PTXL- $CSCaCO_3$ NP

1. Transmission Electron Microscopy (TEM) and Field emission Scanning Electron Microscopy (FESEM) of $CSCaCO_3$NP and GEF-PTXL- $CSCaCO_3$NP

2. Electro-kinetic zeta potential, hydrodynamic diameter, and Poly-dispersity Index (PDI) of $CSCaCO_3$NP and GEF-PTXL- $CSCaCO_3$NP

3. Powder X- ray powder Diffraction (PXRD) of $CSCaCO_3$NP and GEF-PTXL- $CSCaCO_3$NP

4. Fourier-transform infrared spectroscopy (FT-IR) of $CSCaCO_3$NP and GEF-PTXL- $CSCaCO_3$NP

5. Specific Surface area and Pore Size of $CSCaCO_3$NP and GEF-PTXL- $CSCaCO_3$NP

Results & Discussion

1. Loading content and encapsulation efficiency of GEF-PTXL-$CSCaCO_3$ nanoparticles

Table 1: Loading content (%) and Encapsulation efficiency (%) of various groups of GEF-PTXL- $CSCaCO_3$NP

Groups	Drugs	$CSCaCO_3$NP (µg)	Loading content (%)	Encapsulation efficiency (%)
GEF1-PTXL	GEF (400 µg)	10,000	1.98 ± 0.11	50.01± 2.18
	PTXL (200 µg)		0.92 ± 0.01	45.60 ± 0.32
GEF2-PTXL	GEF (400 µg)	15,000	1.14 ± 0.23	42.95 ± 8.98
	PTXL (200 µg)		0.50 ± 0.08	37.45 ± 5.73
GEF3-PTXL	GEF (400 µg)	20,000	1.12 ± 0.19	45.03±10.37
	PTXL (200 µg)		0.44 ± 0.08	43.93 ± 7.25

The loading efficiency of drugs into the nanoparticles is also governed by the surface area availability on the $CSCaCO_3$ nanoparticles and water solubility of the drugs employed (Govender *et al.*, 2000).
The lower loading content of less than 10% is usually observed for inorganic carrier based nanoparticles. Similar result is reflected in the loading content obtained in the current experiment (Shen *et al.*, 2017).

B. Physicochemical Characterization

1. TEM and FESEM of $CSCaCO_3NP$ and GEF-PTXL- $CSCaCO_3NP$

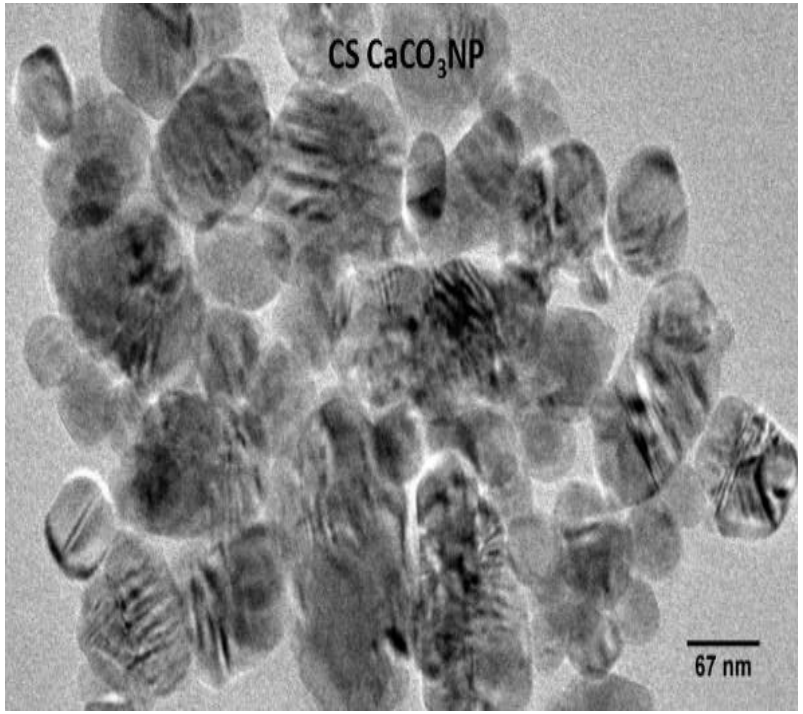


Figure 1: Transmission Electron micrograph of $CSCaCO_3NP$ @50nm

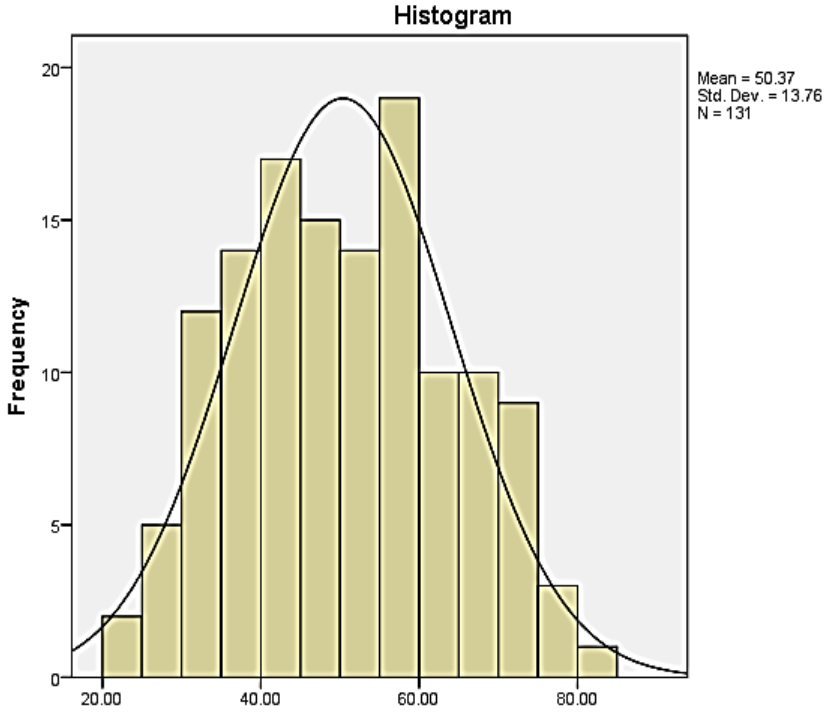


Figure 2: Size distribution chart of $CSCaCO_3NP$

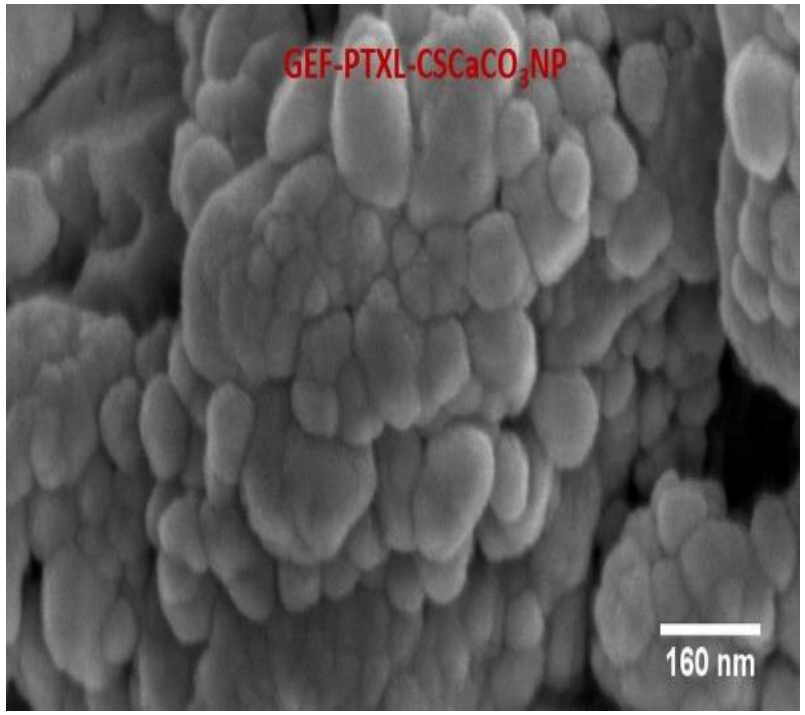


Figure 3: Scanning Electron micrograph of GEF-PTXL-$CSCaCO_3NP$ @ 87nm

Similar results were obtained by Ibiyeye *et al.*, where $CSCaCO_3NP$ had similar average diameters of 53.65 ± 10.29 nm and $CSCaCO_3NP$ loaded with Thymoquinone /Doxorubicin, had an average diameter of 60.49 ± 11.36 nm (Ibiyeye *et al.*, 2020).

2. Electro-kinetic zeta potential, hydrodynamic diameter, and Poly-dispersity Index (PDI)

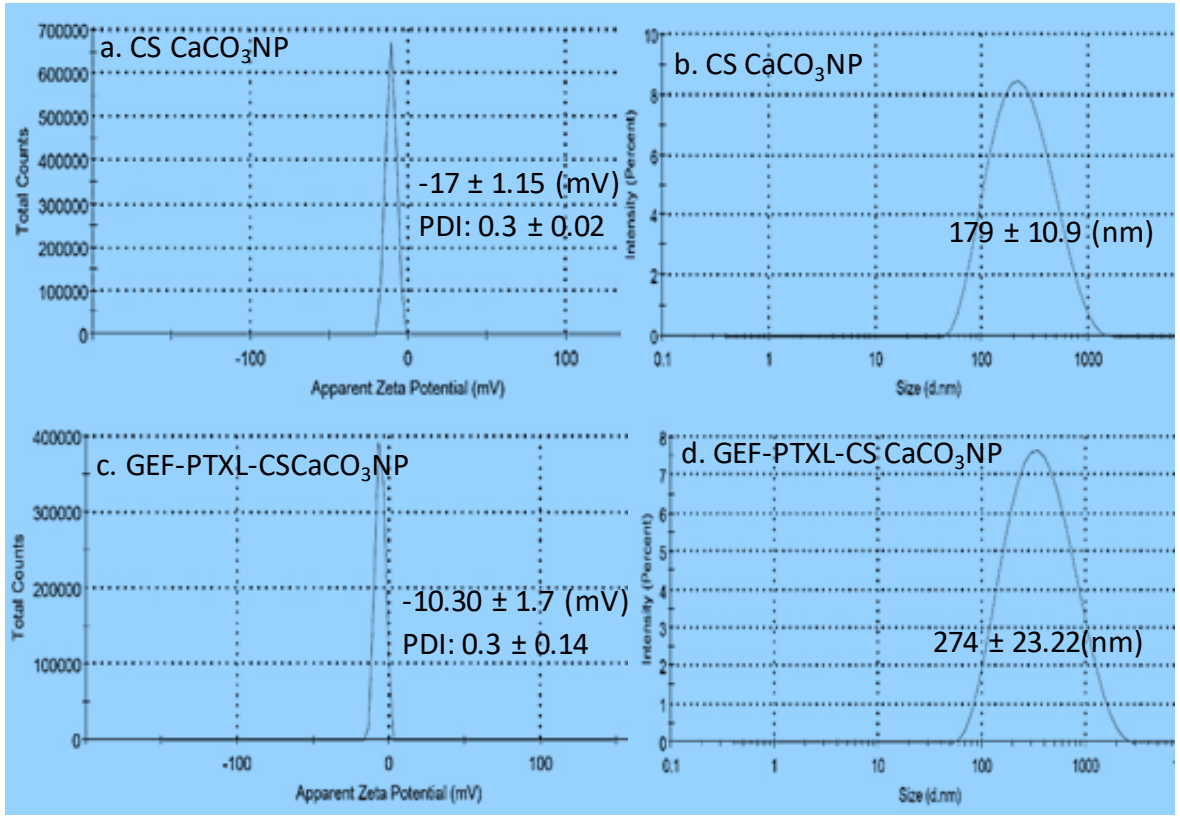


Figure 4: DLS results showing the apparent Zeta Potential and the Particle size distribution in deionized water and PBS with 0.2%Tween 80 for CS$CaCO_3$NP (a & b) and GEF-PTXL- CS$CaCO_3$NP (c & d), respectively.

The negative Zeta potential is in concurrence with the results from other researchers (Danmaigoro *et al.*, 2017; Idris *et al.*, 2019; Ibiyeye *et al.*, 2020).

The hydrodynamic diameter of both of the nanoparticles was larger than the Doxorubicin loaded CS$CaCO_3$NP obtained by other researchers (Danmaigoro *et al.*,2017 and Hamidu *et al.*, 2019).

3. Powder X- ray Diffraction (PXRD)

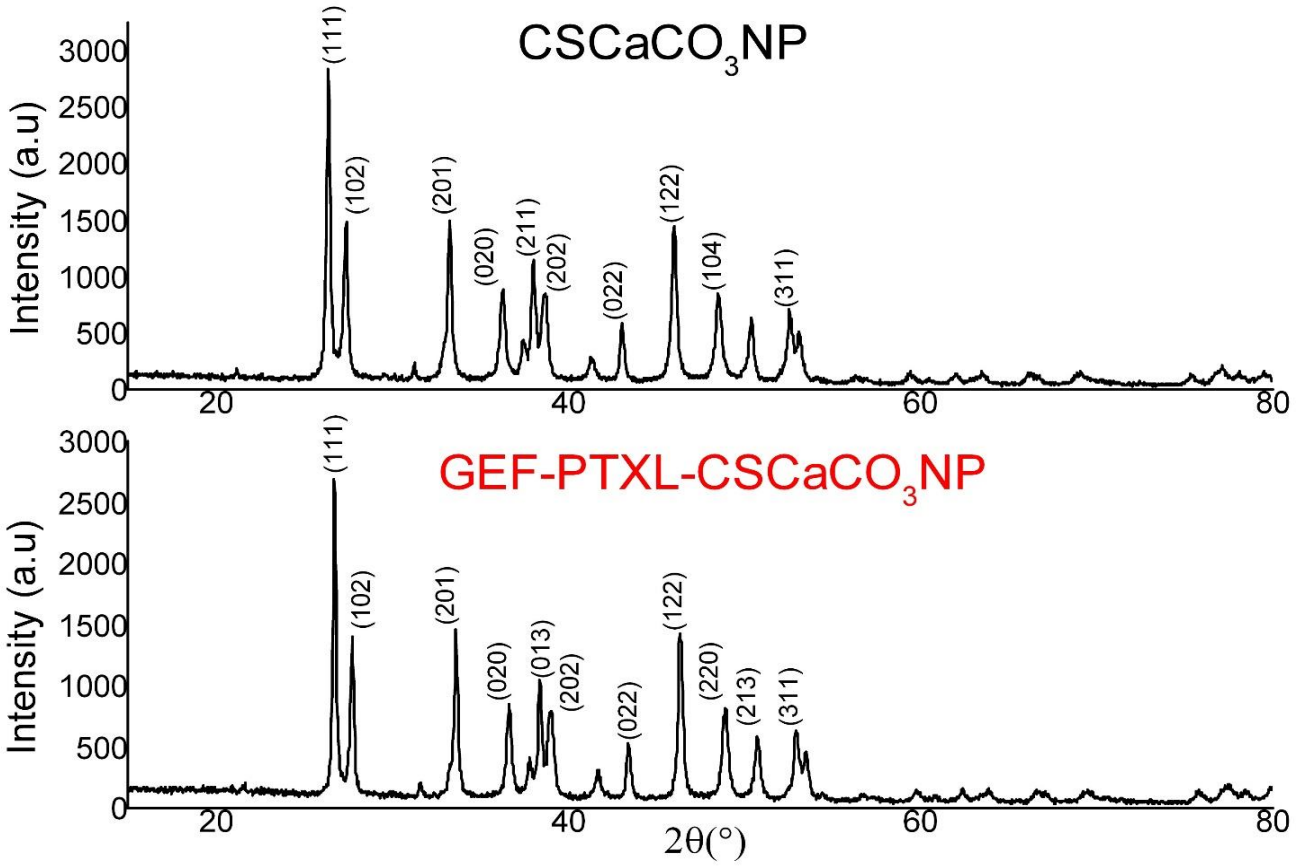


Figure5: PXRD patterns demonstrates aragonite crystalline phase in both the nanoparticles and labelled are the miller indices planes of the synthesized crystals

Raw data of the PXRD, when analyzed in X'Pert High score Plus software, showed the highest score for aragonite phase of $CaCO_3$.

This result is in agreement with the results obtained by other researchers where various other drugs like Vancomycin (Saidykhan *et al.*,2017), Doxorubicin (Danmaigoro *et al.*,2017), Thymoquinone, and Doxorubicin (Ibiyeye *et al.*, 2020) that have been loaded onto the $CSCaCO_3NP$.

4. Fourier-transform infrared spectroscopy (FT-IR)

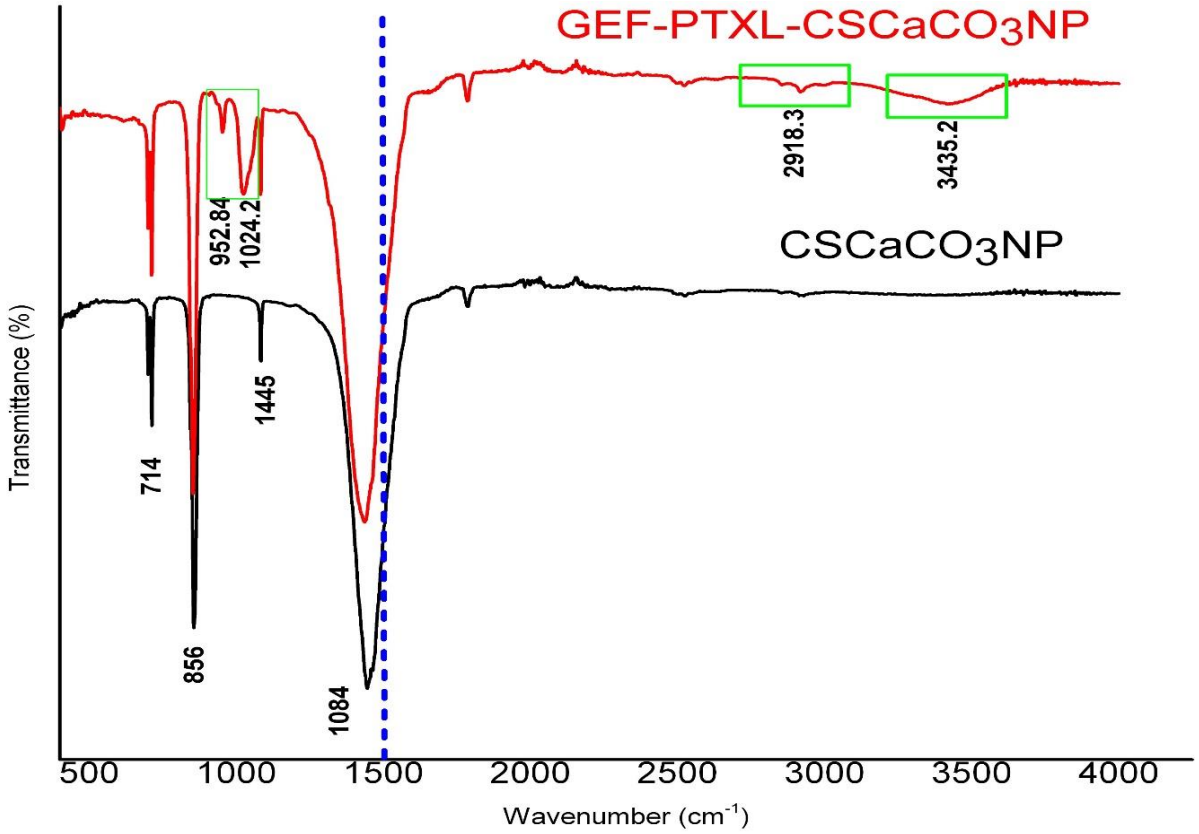


Figure 6:FT-IR pattern of $CSCaCO_3NP$ and formation of new peaks (green box) in the spectra of GEF-PTXL-$CSCaCO_3NP$

$CSCaCO_3NP$:

1445, 1084, 856, and 714 cm^{-1}.

The largest and strongest band-1445 cm^{-1} C-O stretching band.

The other peaks at 1084 and 856 cm^{-1} are attributed to CO_3^{2-} in the molecular structure of the calcium carbonate.

The derived spectra are similar to the spectra obtained by other researchers for cockle shell derived $CaCO_3NP$ (Hammadi *et al.*, 2017; Danmaigoro *et al.*,2017 and Fu *et al.*, 2017).

GEF-PTXL- $CSCaCO_3NP$;

New vibrational band assignments at 952.84 (cyclohexane), 1024.20 (C-F stretch), 2918.30 (C-H stretching) and 3435.22 (aromatic amine and OH^- stretch) cm^{-1} (Renuga Devi and Gayathri, 2010 and Talari *et al.*, 2017)

5. Specific Surface area and Pore Size

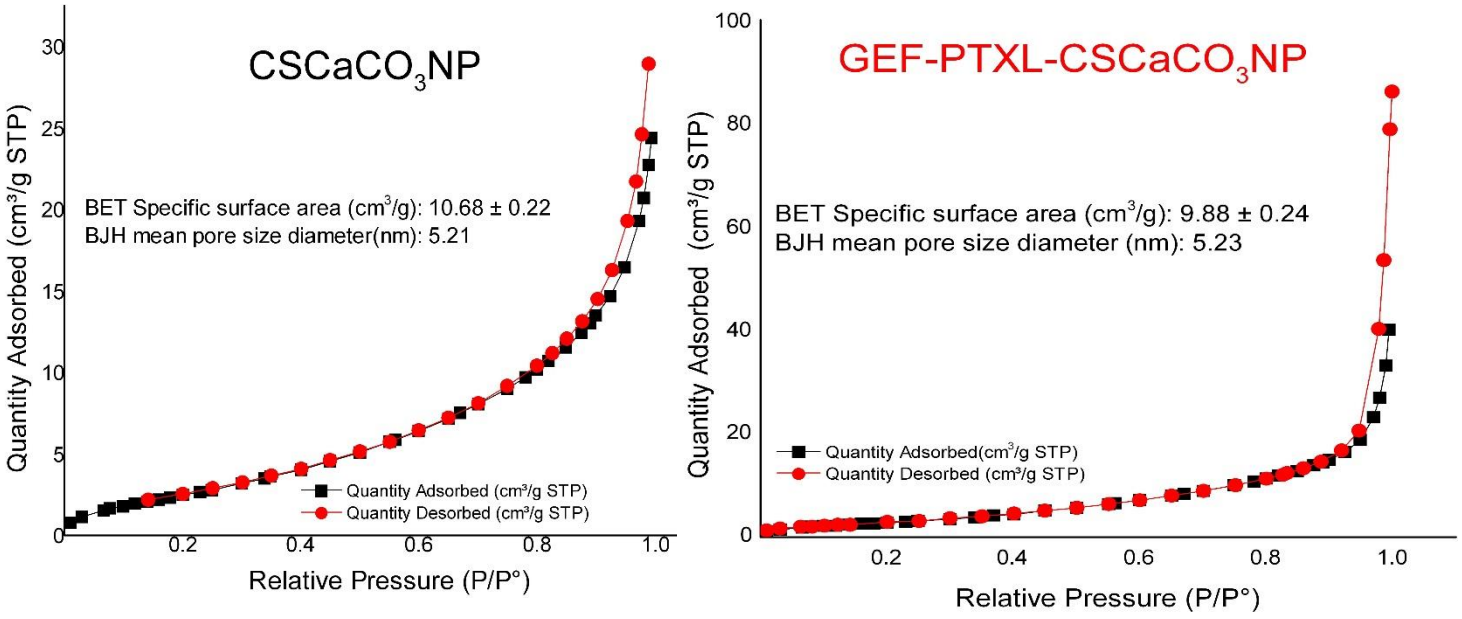
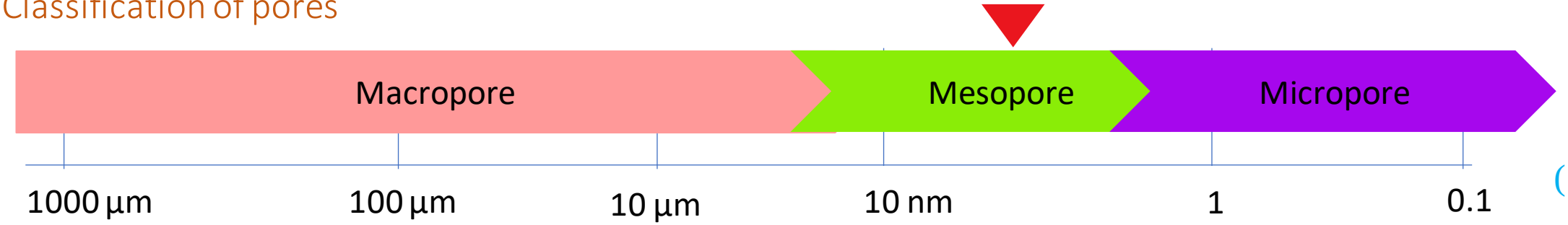


Figure 7:BET nitrogen adsorption isotherms revealing the characteristic isotherms of plain $CSCaCO_3NP$ and GEF-PTXL- $CSCaCO_3NP$

BET Brunauer-Emmett-Teller
Type IV isotherm + "hysteresis loop H1"
initial loop- mono-multi layer adsorption,
2nd loop- desorption of gases
(Sing, 1982; Thommes *et al.*, 2015)

According to Hammadi *et al.*, there is increase in surface area have been observed for the drug loaded Cockle shell derived $CaCO_3NP$.

Classification of pores



(Sing, 1982)

Conclusions

$CSCaCO_3NP$

- The top down method of synthesis of Cockle shell derived ($CSCaCO_3NP$) resulted in nanoparticles of average size of 52.36 ± 15.82 nm and spherical shaped nanoparticles.
- -17 ± 1.15 (mV) of zeta potential, with PDI of 0.3 is indicating stability
- XRD data revealed that the $CSCaCO_3NP$ is purely aragonite crystals
- FTIR analysis shows that the synthesized $CSCaCO_3NP$ possessed the characteristic spectra of calcium carbonate compound.
- The BET pore size of 5.21 nm, with good surface area of 10.68 cm^3/g makes it a good candidate as a drug carrier.

GEF-PTXL-$CSCaCO_3NP$

- The Loading content (%) and encapsulation efficiency (%) for GEF and PTXL in dual drug-loaded NP (GEF-PTXL-$CSCaCO_3NP$) was 1.98 ± 0.11, 50.01 ± 2.18 and 0.92 ± 0.01, 45.60 ± 0.32.
- The synthesized GEF-PTXL-$CSCaCO_3NP$ had an average size of 87.20 ± 26.66 nm.
- -10.30 ± 1.7 (mV) of zeta potential and PDI of 0.3 is indicating stability
- XRD data revealed that the GEF-PTXL-$CSCaCO_3NP$ belong to the aragonite signature even after loading of the drugs
- FTIR analysis shows that the certain functional groups of the drugs are found in the loaded GEF-PTXL-$CSCaCO_3NP$.
- The BET pore size of 5.23 nm, with surface area of 9.88 cm^3/g, reduction in surface area could be due to the loaded drugs on the surface and pores of the $CSCaCO_3NP$

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Thank you.