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

Presented by
Chemmalar S, M.V.Sc
PhD Scholar in Nanomedicine,
Institute of Bioscience,
University Putra Malaysia, Malaysia
Authors

Chemmalar S, Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; gs52461@student.upm.edu.my

Intan Shameha Abdul Razak, Department of Veterinary Pre-Clinical Science, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; intanshameha@upm.edu.my

Che Abdullah Che Azurahanim, Institute of Bioscience and Biophysics Laboratory, Department of Physics, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; azurahanim@upm.edu.my

Nor Asma Abdul Razak, Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; norasmarazak@upm.edu.my

Loqman Haji Mohamad Yusof, Department of Companion Animal Medicine and Surgery, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; loqman@upm.edu.my and

Md Zuki bin Abu Bakar, Laboratory of Molecular Biomedicine, Institute of Bioscience, and Department of Veterinary Pre-Clinical Science, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; zuki@upm.edu.my
Outline

Introduction
Materials and methods
Results and discussion
Conclusions
Acknowledgement
References
Introduction
Nano vectors – Targeted drug loaded nano materials

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)

Dual-drug loaded system
Distribution of blood cockle shells

(FAO, n.d.)
Cockle shell derived aragonite calcium carbonate nanoparticles (CSCaCO\textsubscript{3} NP)

- Sustainable source of CaCO\textsubscript{3} (Kamba et al., 2013; Hammadi et al., 2017; Danmaigoro et al., 2017; Jaji et al., 2017)
- 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)
- 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
$C_{47}H_{51}N_1O_{14}$

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

Abraxane, 2005
Drug loading and encapsulation efficiency

Impurities present

Size, PDI, shape, Surface chemistry, Chemical composition, bonding and in vitro drug release kinetics

LONG TERM STABILITY/ BIOLOGICAL EFFECTS OF NANOPARTICLES

(Hosokawa et al., 2007)
Materials
• GEF and PTXL (Gold Biotechnology, St. Louis, MO)
• DMSO (Fisher Scientific U.K)
• Tween 80 (R & M marketing, UK)
• 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 Nanoem 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 cockle shells
   - Powdered cockle shell powder (MAC)
   - Sieved-75µ mesh
   - Ball milling 120hrs @ 120 RPM
   - Washed & centrifuged 16,000 RPM
   - Deionized water: Tween 80 (1:20), MAC

   Cockle shell derived CaCO$_3$ nanoparticles (CSCaCO$_3$ NP)
2. Synthesis of GEF-CSCaCO$_3$, PTXL-CSCaCO$_3$ and GEF-PTXL-CSCaCO$_3$ nanoparticles

- Stirred 12 hours
- DMSO
- 0.05% Tween 80
- GEF
- PTXL

Supernatant

Drying

Sediment

UV-Vis Spectrophotometer

GEF-PTXL-CSCaCO$_3$NP

CSCaCO$_3$NP
3. Loading content and encapsulation efficiency of GEF-PTXL-CSCaCO₃ nanoparticles

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

Encapsulation efficiency (%) = \frac{W_t - W_f}{W_t} \times 100 \quad (1)

Where,

- \( W_t \) is the total weight of drug fed
- \( W_f \) is the weight of non-encapsulated free drug.

Loading content (%) = \frac{W_t - W_f}{W_{np}} \times 100 \quad (2)

Where,

- \( W_t \) is the total weight of drug fed
- \( W_f \) is the weight of non-encapsulated free drug
- \( W_{np} \) 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drugs</th>
<th>CSCaCO$_3$NP (µg)</th>
<th>Loading content (%)</th>
<th>Encapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEF1-PTXL</td>
<td>GEF (400 µg)</td>
<td>10,000</td>
<td>1.98 ± 0.11</td>
<td>50.01 ± 2.18</td>
</tr>
<tr>
<td></td>
<td>PTXL (200 µg)</td>
<td></td>
<td>0.92 ± 0.01</td>
<td>45.60 ± 0.32</td>
</tr>
<tr>
<td>GEF2-PTXL</td>
<td>GEF (400 µg)</td>
<td>15,000</td>
<td>1.14 ± 0.23</td>
<td>42.95 ± 8.98</td>
</tr>
<tr>
<td></td>
<td>PTXL (200 µg)</td>
<td></td>
<td>0.50 ± 0.08</td>
<td>37.45 ± 5.73</td>
</tr>
<tr>
<td>GEF3-PTXL</td>
<td>GEF (400 µg)</td>
<td>20,000</td>
<td>1.12 ± 0.19</td>
<td>45.03 ± 10.37</td>
</tr>
<tr>
<td></td>
<td>PTXL (200 µg)</td>
<td></td>
<td>0.44 ± 0.08</td>
<td>43.93 ± 7.25</td>
</tr>
</tbody>
</table>

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 CSCaCO₃NP and GEF-PTXL- CSCaCO₃NP

Figure 1: Transmission Electron micrograph of CSCaCO₃NP @50nm

Figure 2: Size distribution chart of CSCaCO₃NP

Figure 3: Scanning Electron micrograph of GEF-PTXL-CSCaCO₃NP @ 87nm

Similar results were obtained by Ibiyeye et al., where CSCaCO₃NP had similar average diameters of 53.65 ± 10.29 nm and CSCaCO₃NP 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)

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 CSCaCO$_3$NP obtained by other researchers (Danmaigoro et al., 2017 and Hamidu et al., 2019).

**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 CSCaCO$_3$NP (a & b) and GEF-PTXL-CSCaCO$_3$NP (c & d), respectively.
3. Powder X-ray Diffraction (PXRD)

Figure 5: PXRD patterns demonstrate 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$_3$NP.
4. Fourier-transform infrared spectroscopy (FT-IR)

**CSCaCO$_3$NP:**

- 1445, 1084, 856, and 714 cm$^{-1}$.
- The largest and strongest band at 1445 cm$^{-1}$ is the 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$_3$NP (Hammadi et al., 2017; Danmaigoro et al., 2017 and Fu et al., 2017).

**GEF-PTXL-CSCaCO$_3$NP:**

- 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).

---

**Figure 6:** FT-IR pattern of CSCaCO$_3$NP and formation of new peaks (green box) in the spectra of GEF-PTXL-CSCaCO$_3$NP
5. Specific Surface area and Pore Size

Figure 7: BET nitrogen adsorption isotherms revealing the characteristic isotherms of plain CSCaCO$_3$NP and GEF-PTXL-CSCaCO$_3$NP

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$_3$NP.

Classification of pores

- Macropore
- Mesopore
- Micropore

(Sing, 1982)
Conclusions
The top down method of synthesis of Cockle shell derived (CSCaCO$_3$NP) 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$_3$NP is purely aragonite crystals

FTIR analysis shows that the synthesized CSCaCO$_3$NP 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.

The Loading content (%) and encapsulation efficiency (%) for GEF and PTXL in dual drug-loaded NP (GEF-PTXL-CSCaCO$_3$NP) was 1.98 ± 0.11, 50.01 ± 2.18 and 0.92 ± 0.01, 45.60 ± 0.32.

The synthesized GEF-PTXL-CSCaCO$_3$NP 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$_3$NP 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$_3$NP.

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$_3$NP.
References


Acknowledgement
This research was funded by MINISTRY OF HIGHER EDUCATION MALAYSIA, under the Fundamental Research Grant Scheme (FRGS/1/2019/SKK15/UPM/02/4), Project grant code: 04-01-19-2097FR
Thank you.