GRAPHENE NANOPLATELETLOADED DRUG DELIVERY SYSTEM FOR BREAST CANCER TREATMENT

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Keywords

Breast Cancer, Graphene Nanoplatelets

Introduction

Among cancers encountered in women, breast cancer is the most prevalent, with the highest mortality rate. It is a type of cancer that originates in the cells of the breast, most commonly in the milk-producing ducts or the glandular tissue known as lobules. Metastasis, the spread of cancer cells from the primary tumor to distant sites, plays a pivotal role in the progression and lethality of breast cancer. Breast cancer's progression can indirectly affect the lungs through metastasis, where cancer cells migrate from the breast to the lungs, forming secondary tumors. Additionally, aggressive forms of breast cancer may trigger systemic inflammation and immune system dysfunction, creating a microenvironment conducive to the development of lung cancer.Early diagnosis, the use of high performing screening methods and of selective and adequate treatments have increased survival rate. In the past years, breast cancer therapy has substantially progressed and new therapies are emerging. (Sevastre et al., 2021) The main types of breast cancer treatment are: surgery, RT (radiation therapy), CT (chemotherapy), ET (endocrine therapy), and targeted therapy. Compared to conventional dosage forms, nanomedicines have particular characteristics that allow them to provide augmented safety, bioavailability and specificity.

Graphene nanoplatelets (GNPs) are regarded as a new type of nanomaterial, which are made from Graphite and structurally similar to nanocarbons, but have excellent performance characteristics compared to other nanomaterials. Graphene nanoplatelets possess ‘platelet’ morphology, meaning that they are very thin, can also be used for drug delivery. Graphene nanoplatelets, with an average thickness of the 5–10nm, are offered in varying sizes of up to 50µm. Their potential lies in providing a platform for controlled release due to their layered structure, allowing for the encapsulation of drugs between the layers (Zamiri *et al.*, 2016) .

Hence the main objective of the study was to design a novel drug delivery system for the management of breast cancer.

Objectives

The extended objectives are as follows

1. To perform the docking studies for the selected targets and ligands (drug)
2. To carry out the pre-formulation studies
3. To formulate and evaluate graphene nanoplatelet particulate drug delivery system using the selected drug
4. To perform the *In-vitro* cell assay using suitable cell line
5. To perform the stability studies as per ICH guidelines

Methodology

Materials:

Active Ingredient (Indomethacin) purchased from Himedia

Graphene Nanoplatelet: Ramaiah Institute of technology Laboratory

Methods:

Objective 1: To perform the docking studies for the selected targets and ligands (drugs)

- Ligands were downloaded from PUBCHEM
- Targets were downloaded from RCSB PDB Database

Ligands:

1. Naproxen
2. Aspirin
3. Indomethacin
4. Diclofenac Sodium
5. Ibuprofen

Targets with PDB Ids

1. HER2 (5MY6)
2. ER-alpha (6CHW)
3. CDK4 (7SJ3)
4. VEGFA (5HHC)
5. mTOR (5GPG)
6. TfR1 (6OKD)

Table 1: Binding Scores of Ligands with Targets

TARGETS	BINDING SCORE OF DRUGS	BEST DRUG WITH BEST BINDING SCORE
CDK4	2244(Aspirin) = -6.6 3033(Diclofenac Na) = -7.9 3672(Ibuprofen) = -7.5 3715(Indomethacin)= -9.2 156391(naproxen)= -7.9	3715(Indomethacin)= -9.2
ER alpha	2244(Aspirin) = -6.1 3033(Diclofenac Na) = -6.9 3672(Ibuprofen) = -7.2 3715(Indomethacin)= -7.1 156391(naproxen)= -7.7	156391(naproxen)= -7.7
HER2	2244(Aspirin) = -5.8 3033(Diclofenac Na) = -7 3672(Ibuprofen) = -5.8 3715(Indomethacin)= -7.2 156391(naproxen)= -6.3	3715(Indomethacin)= -7.2
mTOR	2244(Aspirin) = -5.9 3033(Diclofenac Na) = -6.9 3672(Ibuprofen) = -6.9 3715(Indomethacin)= -7.9 156391(naproxen)=-7.4	3715(Indomethacin)= -7.9
TfR1	2244(Aspirin) = -5.6 3033(Diclofenac Na) = -7 3672(Ibuprofen) = -5.7 3715(Indomethacin)= -7.1 156391(naproxen)= -6.5	3715(Indomethacin)= -7.1

VEGFA	2244(Aspirin) = -5.3 3033(Diclofenac Na) = -5.7 3672(Ibuprofen) = -6.1 3715(Indomethacin)= -6.1 156391(naproxen)= -6.9	156391(naproxen)= -6.9

Standard: Binding Score of Paclitaxel= -9.62

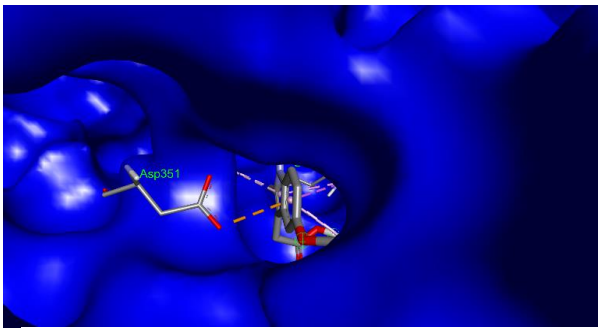


Fig. 1:
3D Diagram (Binding interaction)
- Target: CDK-4 (7SJ3)
- Drug: Indomethacin

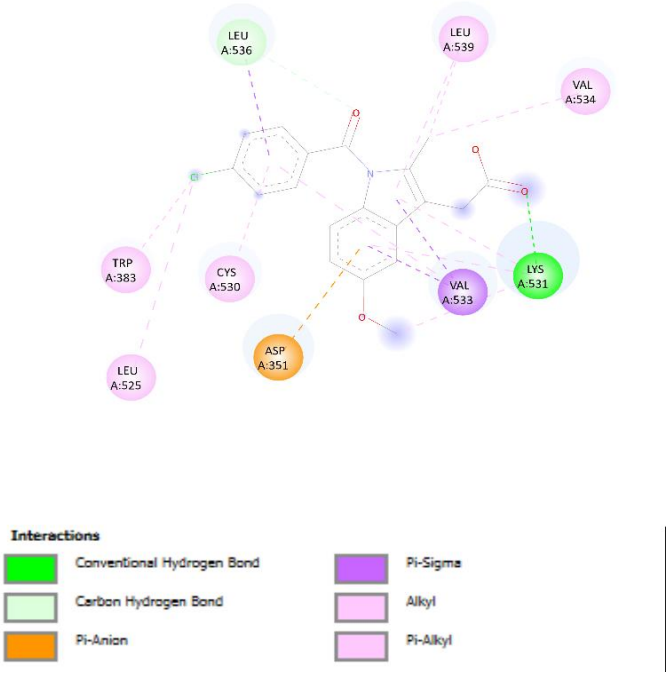


Fig. 2:
2D Diagram (Binding interaction)
- Target: CDK-4 (7SJ3)
- Drug: Indomethacin

Objective 2: To carry out the pre-formulation studies

Standard Calibration Curve of Indomethacin: at 281nm using Phosphate buffer 7.4

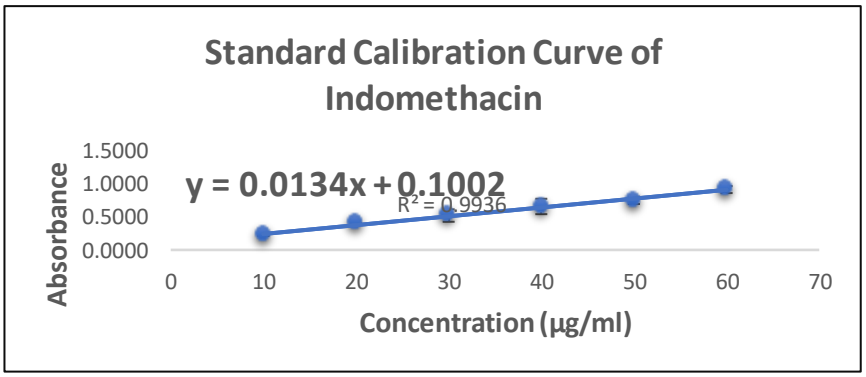


Fig. 3: Standard Calibration Curve of Indomethacin

Infrared (IR) Spectrophotometry:

Fig. 4: Indomethacin:

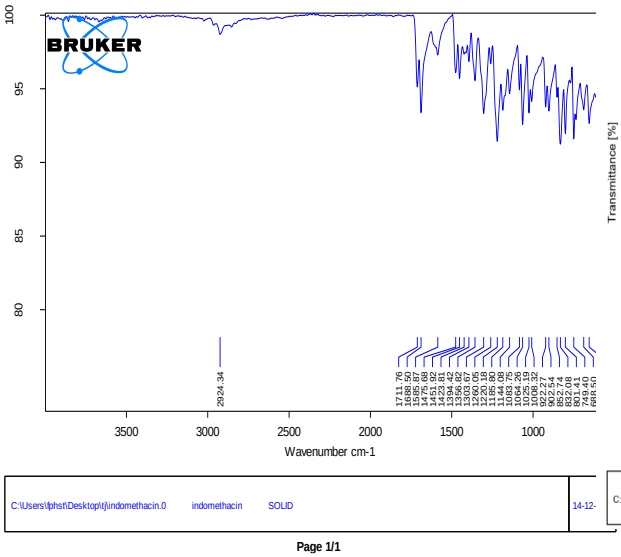


Fig. 5: Graphene Nanoplatelet (GNP)

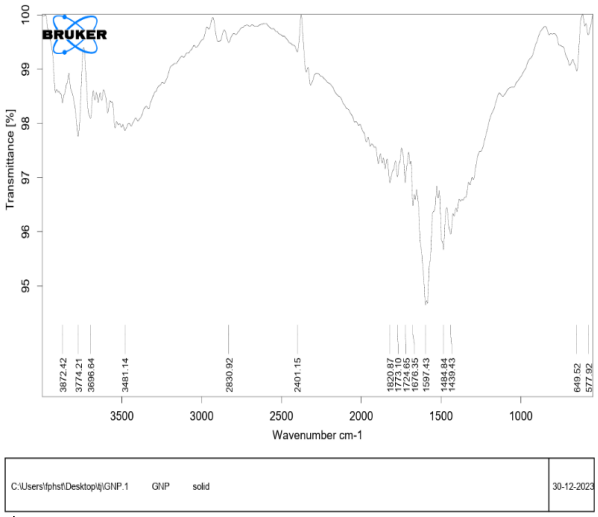
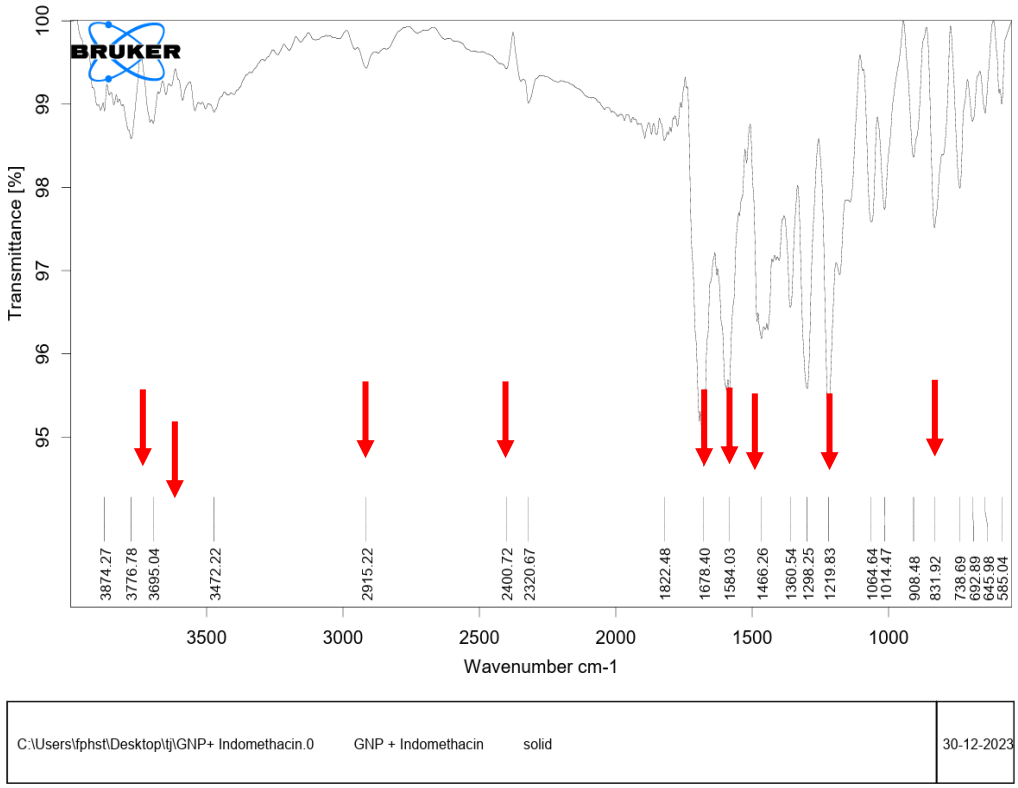


Fig. 6: Physical Mixture of GNP and Indomethacin



Objective 3: To formulate and evaluate graphene nanoplatelet particulate drug delivery system using the selected drug

- Activation of GNP: with 0.1 N NaOH (3h)

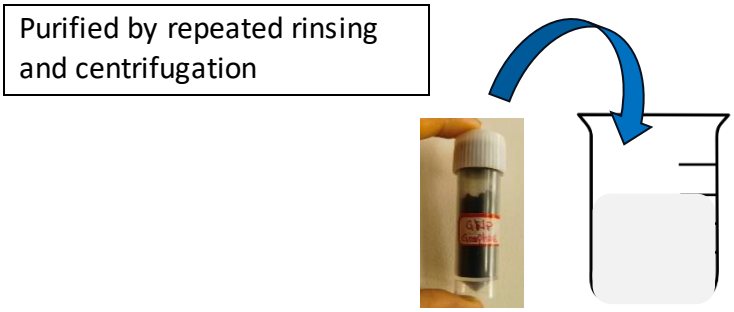


Fig.7 : Activation of GNP

- Drug was dissolved in DMSO and added to the activated graphene nanoplatelets with constant stirring for 8 hours

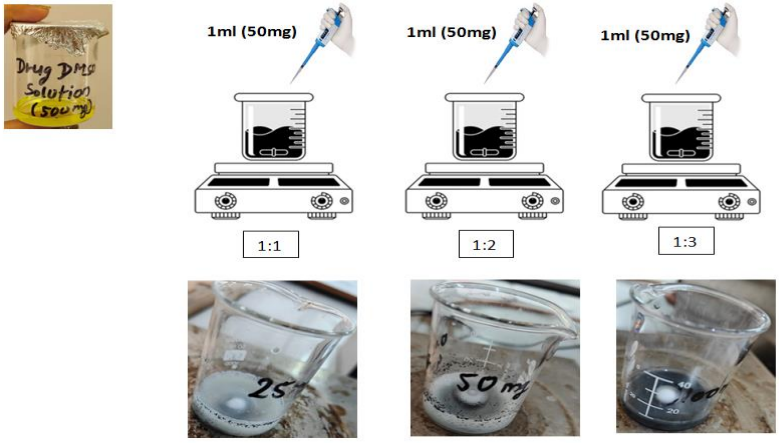


Fig. 8: Drug Loading

- **Entrapment Efficiency (EE):**

  Centrifugation was carried out at 8000 rpm for 1.5 hours and supernatant was collected to check the EE.

Trials	Concentration (Drug: Polymer)	Absorbance	Amount of Drug present in Supernatant(mg)*	Entrapment Efficiency (%)*
Trial 1	1:1	0.3646	1.23	97.5 %
	1:2	0.4329	1.51	96.9 %
	1:3	0.3865	1.33	97.3 %
Trial 2	1:1	0.3927	11.13	77.7 %
	1:2	0.2720	6.41	87.18 %
	1:3	0.3100	7.82	84.3 %

Average of Two Trials = 90.40 %

- Determination of particle size, zeta potential and PDI

Formulation	Zeta Potential (mV) *	Particle Size (nm) *	PDI *
1:1	+21	240	0.213
1:2	+24	240	0.610
1:3	+34	254	0.44
Average	+ 26.3	244.6	0.42

Differential Scanning Calorimetry:

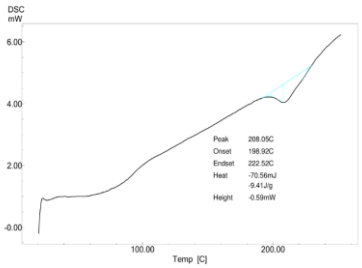


Fig.9: GNP DSC Curve

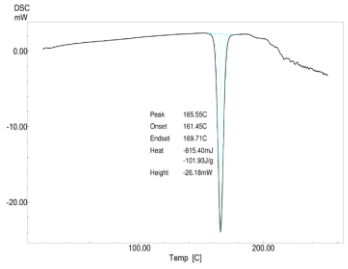


Fig.10: GNP + Indomethacin DSC

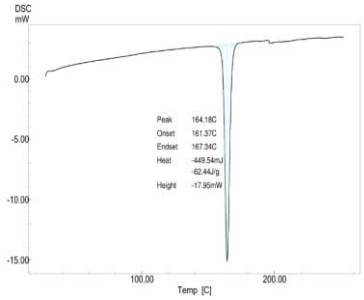


Fig.11: Indomethacin DSC

Objective 4: To perform the *In-vitro* cell assay using suitable cell line

The MDA-MB-231 cell line (isolated at M D Anderson from a pleural effusion of a patient with invasive ductal carcinoma) is commonly used to model late-stage breast cancer. This cell line is ER, PR, and E-cadherin negative and expresses mutated p53.

The cytotoxicity studies and cell line studies are under process.

Objective 5: To perform the stability studies as per ICH guidelines (Yet to commence)

Accelerated Stability Studies:

To provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity & light and enables recommended storage conditions, re-test periods & shelf lives to be established.

Accelerated Stability Study at 40 °C ± 2 °C/75% RH ± 5% RH

Study	0th Day	7th Day	14th Day	30th Day
Colour	Greyish	No change	No change	No change
pH	6.7	6.7	6.5	6.5
Entrapment Efficiency	95.6%	87.18%	97.3%	84.2%

Conclusion:

Preliminary Screening Studies was carried out for the shortlisted repurposed drugs (Aspirin, Naproxen, Indomethacin, Ibuprofen and Diclofenac Sodium) towards breast cancer targets (HER2, ER alpha, CDK-4, VEGFA, m TOR and TfR1) by Molecular Docking studies. Indomethacin was found to have highest binding affinity (-9.2) amongst all other selected NSAIDs.

Drug Interaction studies was carried out by IR and DSC. The IR spectra of Indomethacin, GNP and Physical Mixture (Indomethacin + GNP) was recorded. The characteristic peaks of indomethacin were recorded at 1711 cm^{-1}, 2924 cm^{-1}, 1688 cm^{-1}, 1475 cm^{-1}, 1585 cm^{-1}, 1200 cm^{-1},1451 cm^{-1},749 cm^{-1} as shown in Fig.4 and

GNP at 2830, 3696, 1484, 1439, 1712, 2401 respectively as shown in Fig. 5. Physical mixture of GNP and Indomethacin as shown in Fig. 6, where all the major peaks of drug and GNP were retained. This shows that the polymer did not show any interaction with drug.

DSC results shows the drug peak at 164.8 ^{0}C which is retained in the DSC of physical mixture of Drug + GNP. This shows that the drug in the physical mixture is intact.

The formulation involved activation of GNP followed by drug loading. The drug loading was performed at 3 different ratios of Drug: Polymer (1:1, 1:2, 1:3).The entrapment efficiencies were estimated for Drug: Polymer ratios (1:1,1:2, 1:3). Trials were done in duplicate. The entrapment efficiency was found to be greater than 77 % and the maximum entrapment was found to be 97% at varying polymer concentrations. This desirable drug loading efficiency was largely due to the larger surface area of GNP, boosting the capacity of the nanoparticles for entrapping more drug molecules. The average particle size and average zeta potential was found to be 244.6 nm and +26.3 respectively.

The cytotoxicity studies and cell line studies are under process.

Innovation in the project

The project focusses on the use of repurposed drugs (FDA approved drugs) in the treatment of cancer.

Further, graphene nanoplatelets has not been widely explored as carrier for drug delivery. The large surface area allows for high drug loading capacity making them efficient drug delivery carriers. We would like to explore this unique property of GNP's and study its suitability in the treatment of breast cancer.

Hence, in this study we intend to formulate graphene nanoplatelet loaded with repurposed drug which could be used for the management of breast cancer.

Future Scope:

To perform pre-clinical and clinical studies for drug-loaded GNP formulation to prove its safety and efficacy.