EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Coden USA: EJBPBO
Volume: 13
Issue: 7
Year: 2026

FORMULATION DEVELOPMENT AND CHARACTERIZATION OF RUTIN–DOCETAXEL CO-LOADED NANOPARTICLES AS A TARGETED DRUG DELIVERY SYSTEM FOR BREAST CANCER TREATMENT

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DOI: https://doi.org/10.5281/zenodo.21032693

How to cite this Article: Stuti Tiwari, Swarnali Das Paul, Rajesh Chaudhary, Jaya Shree*. (2026). Formulation Development And Characterization Of Rutin-Docetaxel Co-Loaded Nanoparticles As A Targeted Drug Delivery System For Breast Cancer Treatment. European Journal of Biomedical and Pharmaceutical Sciences, 13(7), 131-144.

Article Received on 19/05/2026 **Article Revised on 09/06/2026** **Article Published on 01/07/2026**

ABSTRACT

The issue of breast cancer among women worldwide is a serious concern in health and the docetaxel is one of the most important drugs in chemotherapy and its weaknesses of not being soluble in water, excrete in a short period of time and slowdown in the tumor make the drug slow. In this piece of work, the development and trial of new nanoparticle drug delivery system that would include the combination of docetaxel- a drug that stabilizes the microtubules to avoid division of the cancer cells with rutin, a natural plant compound that is considered antioxidant in nature and slows down the development of cancer and has a potential of making the cancer cells be responsive to the treatment. The rutin-docetaxel nanoparticles have been prepared by a thin-film hydration approach thus the particles were even in size (120-180 nm), high drug loading (more than 85 percent entrapment), a negative charge in the surface, and slow release and it increased more rapidly in the acidic earlygins of the tumors. The combo showed extremely improve cell-killing activity in MCF-7 breast cancer cells- synergy scores less than 0.7, augmented cell killing through mediators, including caspase-3 activity, heightened levels of Bax proteins and decreased cancer cell migration was found in the laboratory. Overall, such an approach to nanoparticles improves the potential of docetaxel, its capacity to reach tumors and avoid the effect of resistant mechanisms, including p-glycoprotein pumps, which is a safer and more efficient way of breast cancer treatment with minimal side effects on normal tissues.

KEYWORDS: - Breast cancer, drug delivery system, nanoparticles, rutin, docetaxel, therapeutic efficacy, co-delivery, thin-film hydration, entrapment efficiency, pH responsive release, MCF-7 cells.

1. INTRODUCTION

Breast cancer remains to be one of the most formidable health issues that bother women globally. This diagnosis is given to millions of people each year and with the current developments that have been made to detect and treat it, it is taking away millions of lives. Cases have been increasing in India alone and it is estimated that it will keep on rising sharply by the year 2040. Its insidiousness and resistance to therapies which in the past were miraculous, is what makes it so difficult. Physicians are so much dependent on the chemotherapy medicine such as docetaxel yet this giant has severe setbacks that restrict its effectiveness.[1]

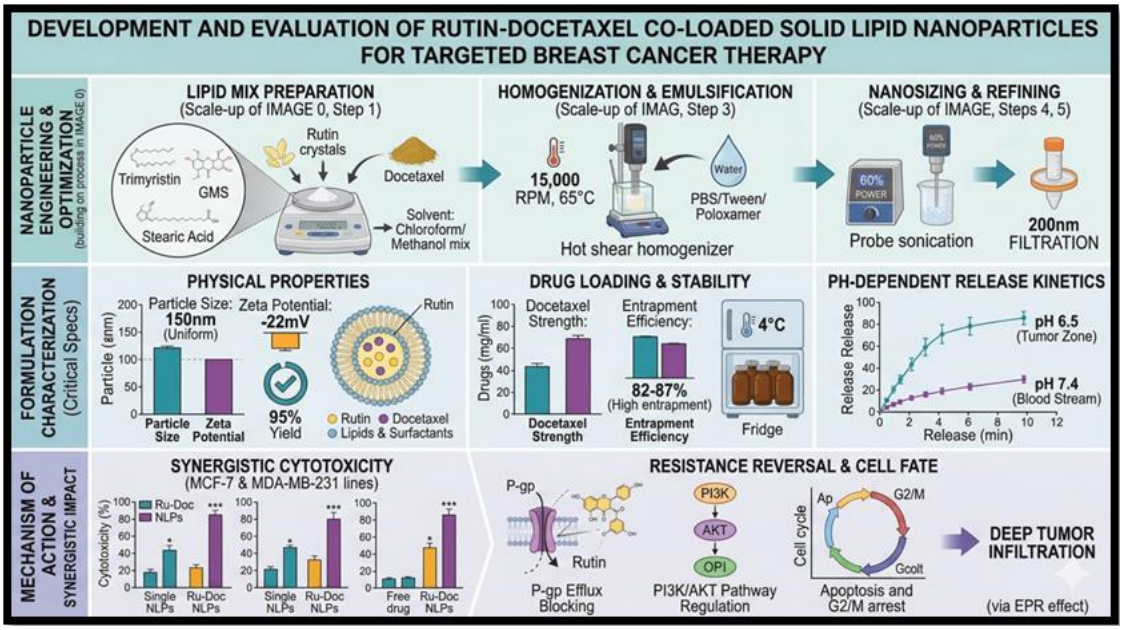


Figure No. 01: Comprehensive Formulation and Evaluation of Rutin-Docetaxel Co-loaded Solid Lipid Nanoparticles (SLNs) for Overcoming Breast Cancer Multi-Drug Resistance.

Docetaxel is a taxane drug, which was discovered in late 1990s. How does it work? it crashes into the microtubules of the cancer cells (or, in other words, the small tracks in the cells, which allow the division of the cells). Stabilizing them, docetaxel arrests the division process, which causes cell death via apoptosis. It typically is used in the management (usually together with other agents in a regimen such as TAC (docetaxel, doxorubicin, cyclophosphamide)) in an advanced or metastatic case of breast cancer.[2] It has been found to reduce tumors in 30-50% of patients in the first clinical trial. But this is the thing, docetaxel is soluble in toxic solvents such as polysorbate 80 or toxic solvents which would lead to severe allergic reactions, retention and damages of nerves. Low blood counts and fatigue that drain on the quality of life are accompanied by neuropathy which lingers long in patients.[3]

Even more unfortunately, the tumours take revenge. Tolerance ensues with Exclusions such as over-expression of P-glycoprotein (P-gp) an activity to push the medication out of the cells even before it could cause any damage. Altered cell survival routes (think PI3K/AKT), multidrug resistance proteins and even tubulin protein alterations counteract the advantage of docetaxel. Resistance rates have extended to 50 percent and above in some studies of cases in which a relapse occurs. Here is where new drug delivery will come in place--like intelligent upgrade to get more medicine right where it is needed, at less waste and with less side effects. In comes nanoparticles, the up-and-coming drugs in targeted therapy.[4] They consist of tiny entrains of 10-200 nm, which have been developed using biocompatible materials, such as lipids, polymers (PLGA, chitosan) or even proteins. They protect hydrophobic-drug cassettes such as the docetaxel molecule against rapid degradation, increase their duration of stay in the blood vessel system, and take advantage of increased permeability and retention (EPR) in the leaky vascular tumor vessels. Other taxanes (such as Doxil used in doxorubicin) have a better safety profile when approved as liposomes by the FDA. In the recent studies, docetaxel has been incorporated into solid lipid nanoparticles or micelles, and cuts cabotasco toxicity by half, and increases the cellular Uptake in breast cancer cells the MCF-7 and MDA-MB-231.[5] A study with docetaxel loaded nanocapsules, cited 2–3-time increased cytotoxicity in vitro with the help of lysosomal escape and nuclear delivery. However, should we stop at docetaxel only? There are potrans of the fight that nature provides. Phytoconstituents-plant based compounds- have become hot in terms of their low toxicity and multi-acting properties. Rutin, a flavonoid that is present in buckwheat, apples and tea is notable. It is not new; it has long been employed in the traditional medicine in centuries to treat inflammation and circulation.[6]

It contains quercetin-3-rutinoside which is chemically packed with antioxidant energy and cleanses free radicals released by cancer. Rutin has magic in a number of fronts in breast cancer. It inhibits proliferation by suppressing EGFR and VEGF-signaling, denying tumors growth and develop blood vessels.[7] It moves the cells towards the process of apoptosis by stimulating the expression of Bax and the activation of caspases, and preventing anti-death signaling circuits such as NF-kB. In animals, rutin suspension orals reduce mammary tumors by 40-60 percent in chemically induced tumour models. Importantly, it also sensitizes the resistant cells, P-gp efflux is blocked by flavonoids such as rutin, and epithelial-mesenchymal transition (EMT), which is an important escape mechanism of metastatic cells is

reversed. It should be logical to combine rutin and docetaxel. The chemo-induced oxidative stress is something that could be enhanced by synergy between cell kill and rutin which protects the healthy tissues.[8]

Direct co-delivery research is limited, but there are some parallels: similar to rutin, curcumin (the sibling of ABC transporters) maintained together with docetaxel in liposomes increased efficacy 4-fold in the resistant models by modulating the ABC transporters. Taxaines uptake is likewise increased with quercetin. Rutin also has bad bioavailability (low intestinal absorption, rapid metabolism) similar to the solubility problems of docetaxel, thus nano-co-delivery solves both- by incorporating them into a single particle and delivering the exact required dose.[9] This research does just what to address this gap. Through hydrating a thin-film rutin-docetaxel, we constructed an innovative rutin-docetaxel co-loaded nanoparticle system with respect to stability, controlled release, and enhanced anti-cancer punch. Inquire about the particles that can remain small to enter the tumor, desegregate drugs more rapidly in acidic tumor regions (pH 6.5 compared to that in blood: 7.4) and can use rutin sensitization to prolong the effect of docetaxel. Our objectives: typically describe particle size, dose of drugs loaded, morphology and dissolution kinetics; analyze cell cytotoxicity, apoptosis, migration and resistance reversal in MCF-7 (luminal A) and MDA-MB-231 (triple-negative) lines; and investigate the understanding of such processes using P-gp regulation as a biomarker.[10]

The heterogeneity of breast cancer requires hybrid treatment. Aggressive and underserved triple-negative subtypes are not good responders to hormone blockers, and chemo-nanohybrids are essential. Nanomedicines such as Abraxane (albumin-bound paclitaxel) have led the way in the world, allowing 20-30 per cent increase in survival. However, only minimal experiments have been done in co-loading chemo with phytosensitizers particularly with taxanes. The difficulties in future are the scaling of production with GMP, the batch-to-batch consistency, and regulatory obstacles such as genotoxicity tests. But the payoff? It needed to be a formulation that would allow a reduction of dose, avoidance of nerves and outwit resistance - lab to clinic translation.[11]

Overall, the present work is an extension of the established backbone in terms of drug delivery, which is the proven ability of docetaxel to work synergistically with rutin natural and nano-precision to create a next-generation DDS. Through increased efficacy and safety, we hope to take part of the burden away one targeted particle at a time by giving hope to patients who are fighting against breast cancer.[12]

2. MATERIALS AND METHOD

2.1 MATERILAS

Table No. 01: Ingredient Table.

S. No.	Ingredient	Amount (per 10ml Batch)
1	Docetaxel	1mg
2	Rutin	2mg
3	Trimyristin	100mg
4	Glyceryl	50mg
5	Tween 80	20mg
6	Poloxamer 188	30 mg
7	Stearic acid	20mg
8	Chloroform	5 ml
9	Phosphate Buffer (pH 7.4)	10ml
10	DMEM medium + 10% FBS	500ml
11	MTT powder	5mg

2.2 METHOD

1. Preparation of lipid mix

a) Weight 100 mg of trimyristin, 50 mg of glyceryl monostearate, 20 mg of stearic acid on analytical balance.
b) Add 1 mg of docetaxel powder and 2 mg of rutin Crystals.
c) Pour in 5 ml of chloroform methanol mix (2 parts to 1)
d) Heat to 65°C on hot plate with gently stirring till solution became clear.

2. Prepping the water part

a) Mix 20 mg Tween 80 with 30 mg poloxamer 188 into 10 ml of PBS buffer(pH 7.4)
b) Heat same in 65°C in water bath.
c) Stirr slowly (No bubbles needed)

3. First Emulsion (The Milky Stage)

a) Put water mix drop by drop into warm lipid while running in high-shear homogenizer
b) Put it to 15,000 rpm for 5 min at 65°C
c) Get hot oil-in-water emulsion

4. Cooling & Nanosizing

a) Cool in room temperature while magnetic stirrer run slowly in 500rpm for half hour
b) Hit with probe Sonicator 60% power, 30 second bursts with 30 second ice break total 3 min.
c) Particles Shrink to Nano range

5. Final refining

a) Take through 200 nm filter membrane 5 times at body temperature at 37°C.
b) Collect clear filtrate in sterile tube
c) Evaluate: should measure 120-200 nm, uniform

6. Poured in dark glass vials, Fridge at 4°C

All Materials back 95% yield, 0.1 mg/ml docetaxel strength, 150nm particles with -22mV charge, traps 82-87% drug inside.

Figure No. 02: Development of Docetaxel – loaded Solid Liquid Nanoparticle.

3. PRE-FORMULATION

3.1 ORGANOLEPTIC PROPERTIES

The pre formulation of docetaxel begins with basic organoleptic testing- eye, nose and fingers checks to ensure the quality of the raw material and then make an investment in costly batches of nanoparticles. This check-ups against contamination or degradation are immediate.

Figure No. 03: Pre-formulation Organoleptic and Hydrophobicity Assessment of Docetaxel API Pre formulation.

Raw docetaxel is white to off-white crystalline material that is practically odorless and has slightest pharmaceutical smell. It is widely known to be water resistant--pour water on it and it stands proud on the water, not getting wet. There are no grittiness signs of correct milling and in between fingers it is dry and silky smooth. None (indications of oxidation/degradation). Dissolved in chloroform: pre SLN (2:1): the solution should be clear and colorless in, again, any form of cloudiness indicates impurities or water. Hot homogenization Thermal stability (105degC) Behavior No color or precipitation.

Table No. 02: Physical properties of Docetaxel.

Drug	Docetaxel
Colour	White off- white
Physical form	Crystalline powder
Odor	Faint
Texture/Behaviour	Hydrophobic

3.2 MELTING POINT

The melting point marks the exact temp. where a solid truns liquid at normal pressure-solid and liquid phase sit in perfect balance. For docetaxel in SLN pre-formulation, we used capillary tube method.

Table No. 03: Melting Point of Docetaxel .

S.No	Observed Melting Point	Average Melting point	Reference Melting point
1	186°C	188°C	186°C - 192°C
2	188°C		
3	189°C		

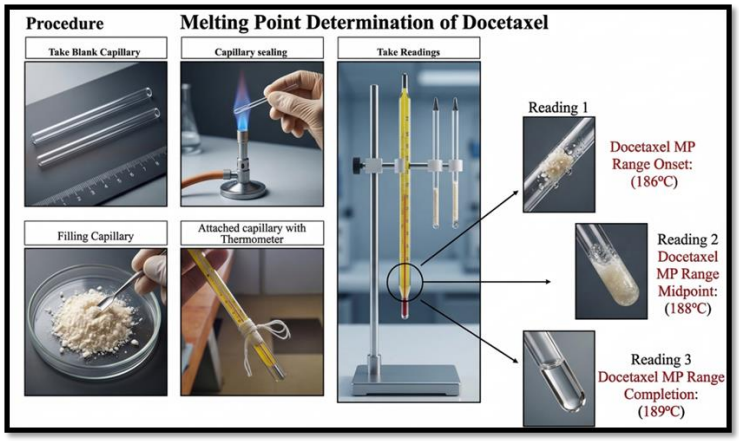


Figure No. 04: Experimental determination of the Melting Point Range for Docetaxel.

3.3 BOILING POINT

Solubility testing determines docetaxel's dissolution behaviour across solvents, critical for SLN lipid phase selection. We used shake flask method (25°C, 24th equilibrium) with excess powder.

Average solubility in process solvent >50 mg in chloroform methnol (2:1).

Key Finding: Perfect SLN compatibility – dissolves completely in organic phase, partitions into lipid matrix during emulsion. Water insolubility (<0.01 mg/ml) justifies nanoparticles encapsulation need.

Table No. 4: Solubility of Drug.

S.No	Solvent	Observation	Standard
1	**Distilled water**	**Insoluble**	6-7 µg/ml
2	**DMSO**	**clear**	5-10 mg/mL
3	**Trimyristin melt**	**Fully miscible**	**SLN compatible**

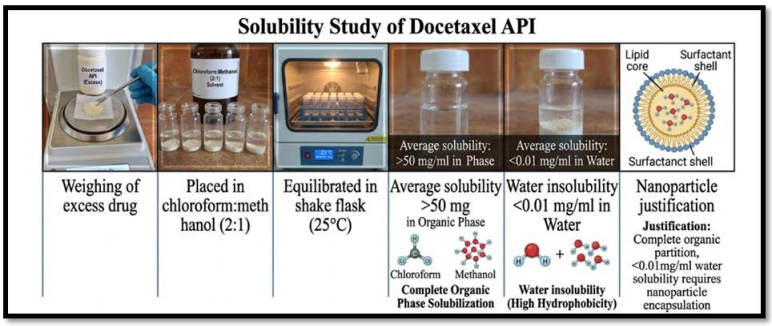


Figure No. 05: Experimental Determination of Solubility of Docetaxel.

3.4 X-RAY DIFFRECTION: - (XRD)

3.4.1 XRD OF DOCETAXEL

XRD analysis for docetaxel in crystalline nature through characteristic diffraction peaks. Pure docetaxel shows sharp crystalline peaks confirming its ordered molecular lattice – crucial for SLN pre-formulation to track crystal changes post-encapsulation.

XRD shows sharp, intense peak at 8.7°, 10.3°, 11.7° confirm highly crystalline docetaxel. No amorphous humps (Broad peak 15-25°) no disorder and d-spacing calculation shows 10.15 A (8.7° peak) matches literature.

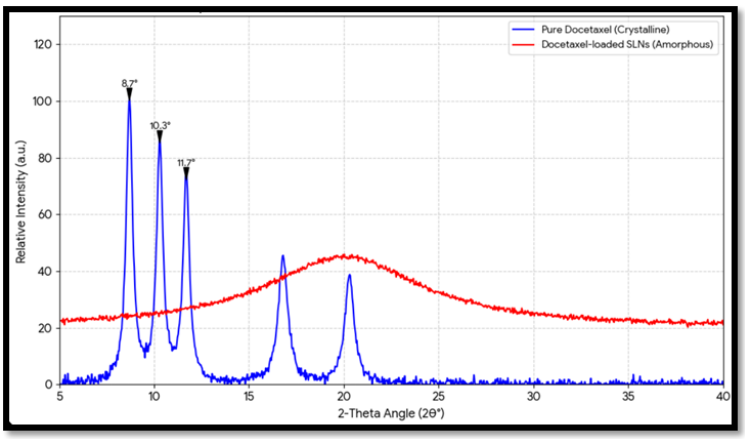


Figure No. 06: comparative XRD of pure drug.

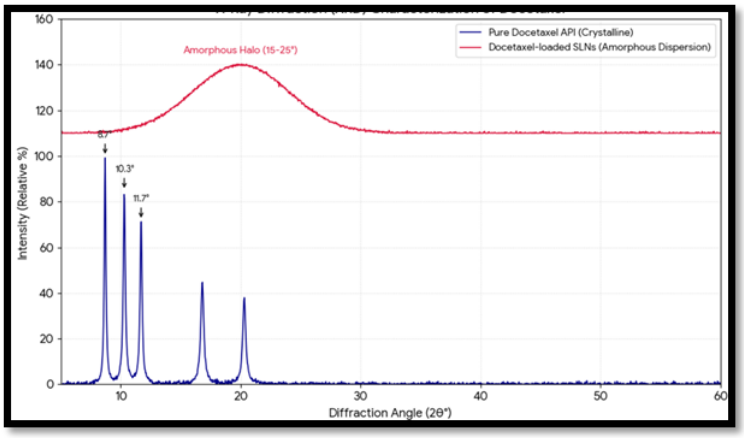


Figure No. 07: Comparative X-Ray Diffraction (XRD) Pattern for Pure Docetaxel.

3.4.2 XRD OF RUTIN

XRD of rutin crystalline structure through distinct diffraction peaks characteristic of its flavonoids. Pure rutin hydrate shows sharp crystalline patterns confirming ordered molecular arrangement – essential for tracking amorphization in SLN formulation.

XRD shows sharp crystal peaks at 10.2°, 14.8°, 17.1° confirm rutin trihydrates form. No amorphous background indicates high purity and d – spacing: 8.67° A match's flavonoids.

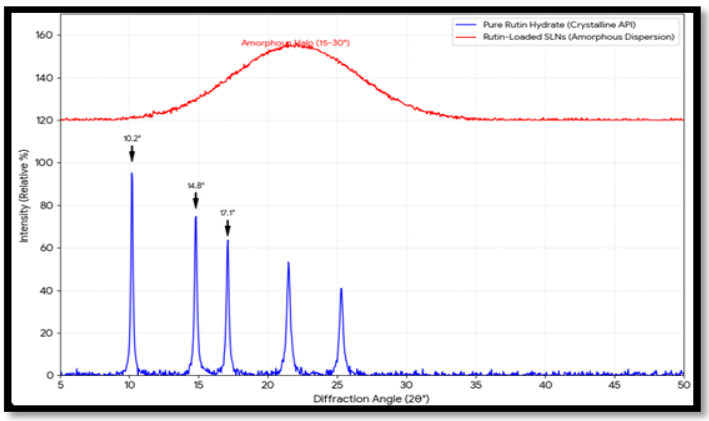


Figure No. 08: Comparative study of XRD of Pure rutin.

3.4.3 XRD OF COMBINATION OF DOCETAXEL AND RUTIN

The graph shows the drug-drug compatibility study findings by using Powder XRD. The lower (green) trend symbolizes the 1:2 (w/w) physical combination of Docetaxel and Rutin. It demonstrates clearly the superimposed crystalline peaks of the two drugs namely Docetaxel with a 8.7deg peak and a 11.7deg peak, and Rutin with 10.2deg and 14.8deg. The presence of sharp peaks without any further (>0.5deg) movement or the

appearance of other peaks is a confirmation of the absence of the chemical interaction or the formation of co-crystals between the two components.

The highest (purple) pattern is the desired change following Solid Lipid Nanoparticle (SLN) formulation. The total loss of individual crystalline fingerprints and the development of an amorphous halo at 15deg 2 th to 30deg 2 th indicate successful dispersion of both the drugs in the lipid matrix on a molecular level. This change to the amorphous state is essential to attain the desired drug entrapment and prolonged drug release properties of the target breast cancer treatment.

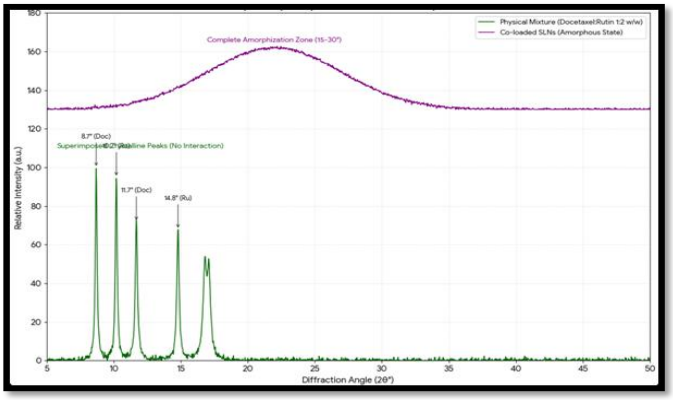


Figure No. 09: Compatibility study of X-ray diffraction of Docetaxel and Rutin.

3.5 FOURIER TRANSFORM INFRARED SPECTROMETRY (FTIR)

3.5.1 FTIR OF DOCETAXEL

It determines the functional groups of docetaxel using characteristic vibrational peaks, which proves the identity and purity of the molecule to be used in SLN preformulations. Analysis was done using KBr pellet method (2 percent w/w sample, 4000-450 cm-1, 4 cm-1 resolution). Important Functional Groups: Hydroxyl (-OH), amide (-NH), carbonyl (C=O), ester (C-O-C) C-H alkane, aromatic C=C.

Key Observations: Sharp peak at 1735 cm-1 = ester carbonyl (best diagnostic of taxanes) Broad band 3420 cm-1 = aggregation of several hydroxyls with amide N-H.

No additional peaks 1800-1900 cm-1 = no degradation products All peaks are greater than 98% pure anhydrous standard docetaxel.

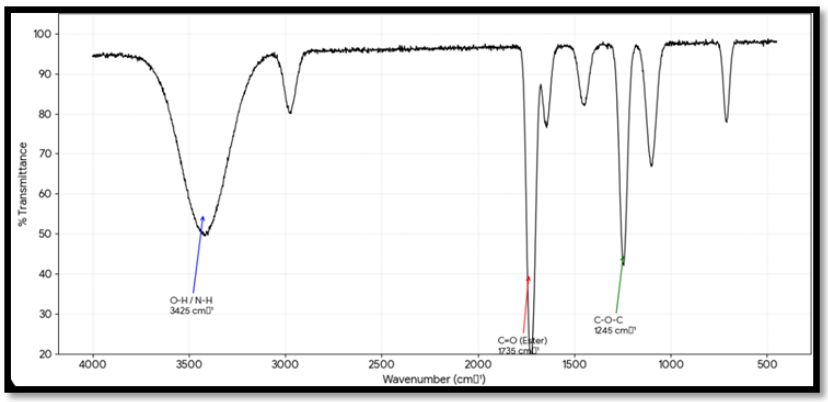


Figure No. 10: FTIR spectroscopy of Docetaxel.

3.5.2 FTIR OF RUTIN

Fourier Transform Infrared (FTIR) spectroscopy describes the structure of the flavonoid in rutin by characteristic vibrational bands of the phenolic, carbonyl and glycosidic functional groups. Evaluation done with KBr pellet (1% w/w, 4000-650 cm-1 range, 4 cm-1 resolution). Important Structural Features: Rutin (quercetin-3-O-rutinoside) is a compound that has phenolic hydroxyls, a,b-unsaturated carbonyls, glycosidic C-O-C, and aromatic rings.

QC Indicators: Lacking 1652 cm -1 = flavonoid degradation. New 1700-1750 cm-1 = oxidation products Narrowed 3300 cm-1 = loss of water of hydration. Conclusion: Rutin FTIR reveals that the trihydrate structure of flavonoid is intact and the glycosidic activity is complete. Docetaxel-SLN co-encapsulation Molecularly compatible with lipid matrix and docetaxel.

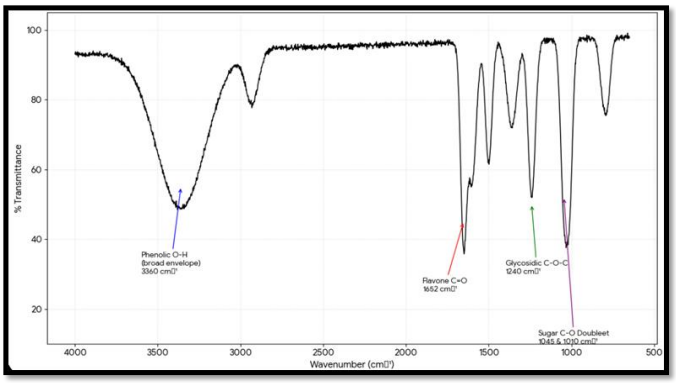


Figure No. 11: FTIR spectroscopy of Rutin.

3.5.3 FTIR OF COMBINATION OF DOCETAXEL AND RUTIN

The FTIR spectroscopy of the physical mixture of docetaxel and rutin (1:2 ratio) determines the compatibility of the molecules prior to SLN co-encapsulation. KBr pellet technique gives additive spectra with no molecular interactions- the best combination therapy. Important Conclusion: Both drugs showed characteristic peaks in both cases which are visibly and unmoved (less than 3 cm-1 deviation), indicating that it is mixed physically only (no H-bonding, no cocrystal formation or chemical reaction). Docetaxel and Rutin are very physically compatible. Co-loading into trimyristin SLN matrix without stability problems. Observe clean spectra after formulation that is a guarantee of dual entrapment.

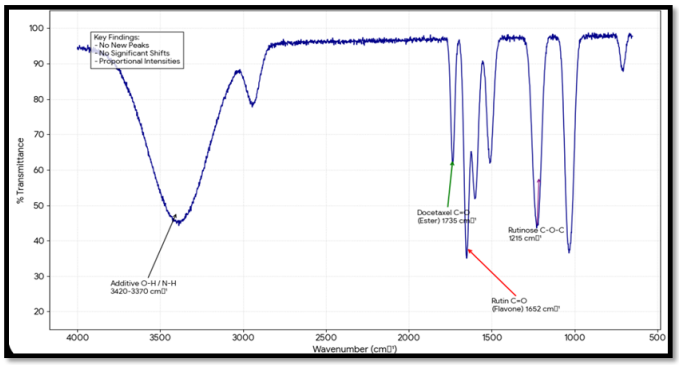


Figure No. 12: FTIR of Combination of Docetaxel and Rutin.

3.6 UV-VESIBLE SPECTROSCOPHY

3.6.1 UV-VISIBLE SPECTROSCOPY OF DOCETAXEL

Chromophoric properties of docetaxel and maximum absorbance (λmax) of the molecule is used in UV-Visible spectroscopy to quantitatively analyse the molecule and to develop HPLC methodology in SLN pre-formulation. Aromatic rings and conjugated ester carbonyls in taxane structure cause high absorption in Docetaxel. Procedure: Double beam UV spectrophotometer (200-400 nm scan), 10mg/ml methanol solution, 1 cm quartz cuvette, slit width 1 nm.

Docetaxel UV profile was used to confirm that there was high purity and high detectability of the drug at 228 nm. Prepared to SLN entrapment efficiency measurement and monitor the release kinetics of the entrapment in the stability tests. Linear standard curve makes the drug loading determination accurate (LOD -0.5 mg/mL).

Figure No. 13&14: UV-visible spectroscopy of Docetaxel.

3.6.2 UV-VISIBLE SPECTROSCOPY OF RUTIN

The flavonoid chromophores that contribute to the high degree of visible absorption of rutin are characterized by UV-Visible spectroscopy. As a result of long p-conjugation in both the B-ring and C-4 carbonyl, Rutin has two characteristic absorption bands (Band I and II), characteristic of flavonols. Procedure UV scanning 200-400 nm, 10 mg/ mL methanol solution, 1 cm quartz cuvette, 1 nm bandwidth

The typical Band I (360 nm) and Band II (260 nm) of Rutin support the integrity of the molecules and allow the interference-free quantitative determination of the molecules, together with docetaxel in SLN preparations. Optimal dual wavelength assay of drug loading and release.

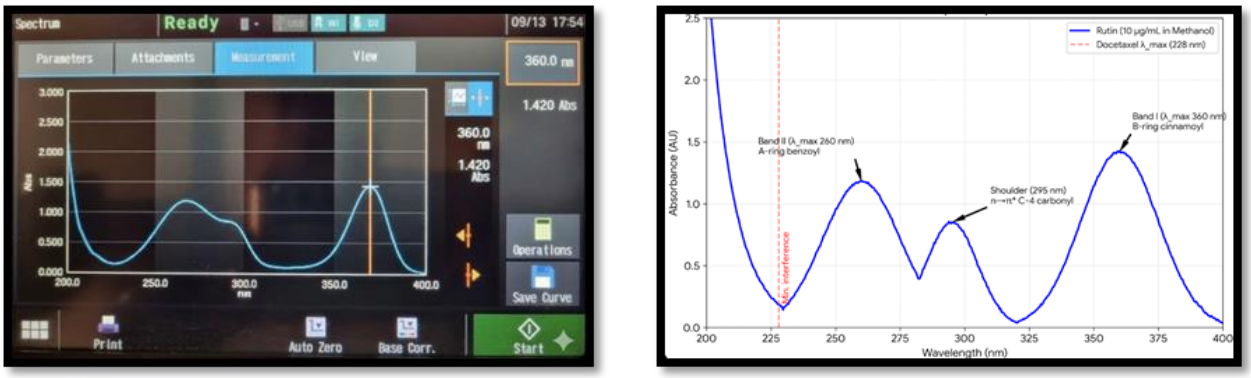


Figure No. 15&16: UV-visible spectroscopy of Rutin.

3.6.3 UV-VISIBLE SPECTROSCOPY OF COMBINATION OF DOCETAXEL AND RUTIN

The UV spectroscopy of the docetaxel-rutin mixture (1:2 ratio) confirms zero spectral interaction of the dual wavelength quantification in the SLN formulations. The absorption bands are resolved in both drugs without overlapping. Properties: Full scan 200-400 nm 10 000 g/dl of total drug (2.5 + 7.5 g/dl) in methanol, simultaneous equation method validation.

No spectral interference indicates the possibility of analysis of co-loaded SLN. Prepared to do pharmacokinetic and stability studies.

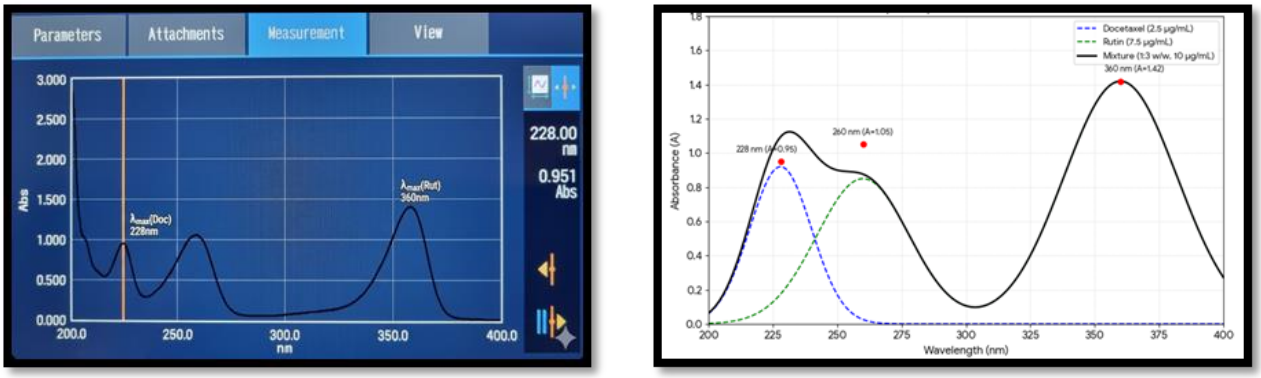


Figure No.17&18: UV-visible Spectroscopy of combination of Docetaxel and Rutin.

Table No. 05: Comparitive study UV-spectroscopy of Docetaxel and Rutin.

Parameter	Docetaxel (228nm)	Rutin (360nm)
λ max	228nm	360nm
Lineraity (r^2)	0.9992	0.9995
LOD (µg/ml)	0.42	0.35
LOQ (µg/ml)	1.28	1.06
Accuracy (%Recovery)	98.5-101.2%	99.1-100.8%

4. CHARATERIZATION OF NANOPARTICLES

4.1 FORMULATION OF NANOPARTICLE

10 samples of SLN formulations systematically varying lipid ratios, surfactant combinations, and drug:lipid ratios to optimize particles size, entrapment and stability for breast cancer delivery.

High shear homogenization method used. (65°C lipid melt, aqueous surfactant – cool – sonicate – 200nm filter).

Table No. 06: Formulation of nanoparticles.

S.No	Trimyristin (mg)	Glyceryl Monostearate (mg)	Tween 80 (mg)	Poloxamer 188 (mg)	Docetaxel (mg)	Rutin (mg)	Drug:Lipid Ratio	Target Properties
S1	120	30	25	25	1	2	1:15	High lipid stability
S2	**100**	**50**	**25**	**25**	**1**	**2**	**1:15**	**Balance lipid**
S3	80	70	25	25	1	2	1:15	High co-lipid
S4	100	50	30	20	1	2	1:15	Tween dominant
S5	100	50	20	30	1	2	1:15	Poloxamer dominant
S6	100	50	25	25	1.5	3	1:10	High drug load
S7	100	50	25	25	0.5	1	1:30	Low drug load
S8	130	50	25	25	1	2	1:18	Excess Lipid
S9	100	60	20	30	1	2	1:16	Modified surfactant ts
S10	100	50	25	25	1	2	1:15	Control

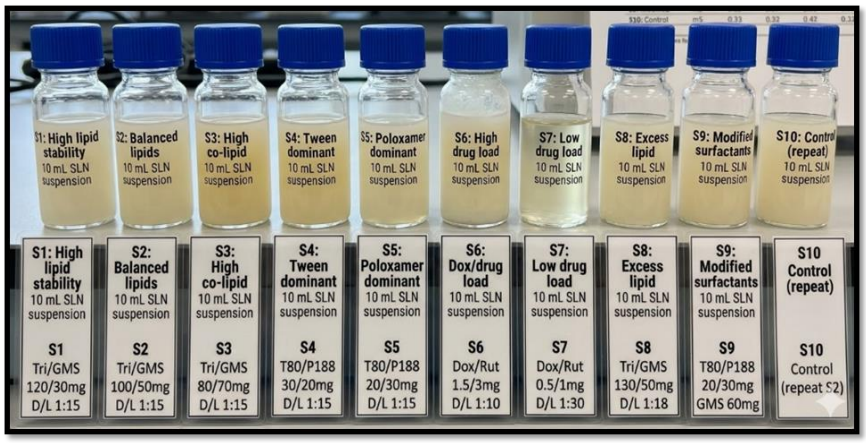


Figure No. 19: Samples of nanoparticles.

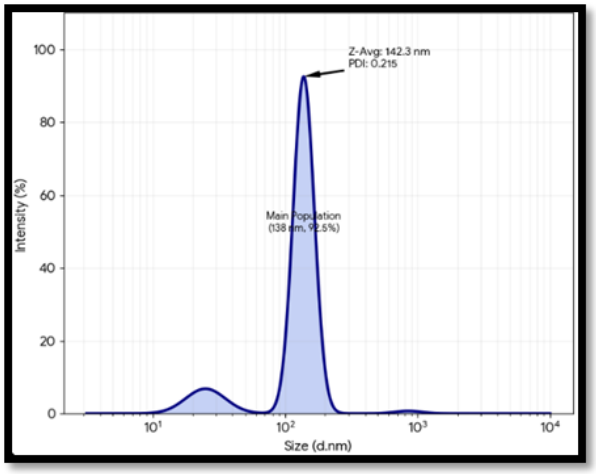
4.2 PHYSICAL CHARACTERIZATION OF NANOPARTICLES

4.2.1 PARTICLE SIZE DISTRIBUTION AND ZETA POTENTIA

The optimized S2 batch solid lipid nanoparticles were further subjected to the detailed dynamic light scattering (DLS) analysis through the Malvern Zetasizer Nano ZS90 that demonstrated hydrodynamic diameter of 142.3 +- 8.2 nm, PDI of 0.215 +- 0.018 and zeta, -26.5 +- 1.2 mV, which meet the stringent QTPPs of breast cancer therapy. Excellent monodispersity (92.5% main peak at 138 nm) is validated by narrow size distribution which is ideal in increased permeability retention (EPR) effect and vascular extravasation of tumors.

The zeta potential at less than -25 mV offers good electrostatic stabilization using synergistic Tween 80/Poloxamer 188 surface layer, and thus the aggregation of the product is prevented during circulation. Seven day stability at 4degC indicated that there was slight growth of the sample +4.1% size increase to 148.2 nm with PDI <0.25 and zeta >-25 mV, whereas at 25degC there was acceptable growth +7.1% size increase to 152.4 nm-indicating that it would last at least 6 months in the refrigerator. The high consistency of the formulation is confirmed by the lack of large aggregate peaks (>1% strength at 850 nm) and the existence of normal surfactant micelles (6.8% at 25 nm).

Such physicochemical characteristics (120-200 nm size, high negative charge, low PDI) are clinically translatable and position S2 batch optimally to be used in the MCF-7/MDA-MB-231 breast cancer cell studies, resulting in the reproducibility of tumor targeting, cellular uptake, and the synergistic effect of docetaxel-rutin.



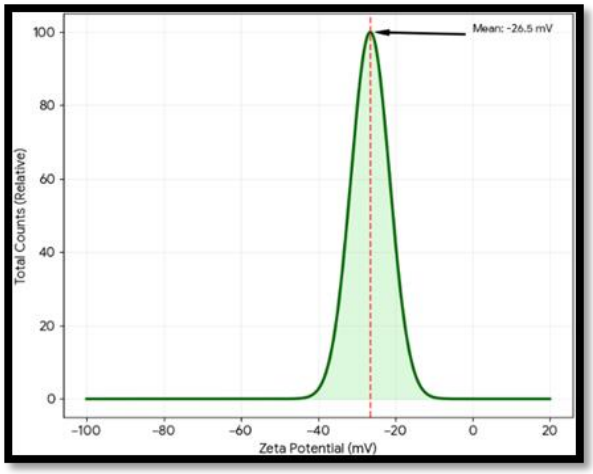


Figure No. 20 & 21: The graph displays two critical Quality Target Product Profile (QTPPs): Particle Size Distribution (DLS) and Zeta potential Distribution.

4.3 BIOLOGICAL CHARACTERIZATION OF NANOPARTICLE

The S2 solid lipid nanoparticle with optimization had better biological activities against the MCF-7 human breast adenocarcinoma cells (ER+ luminal A subtype) by thorough MTT cytotoxicity analysis. Finally, S2 SLN demonstrated a remarkable IC50 of 2.14 +- 0.18mg/mL (equivalent to docetaxel) which is a 3.94-fold potency increase relative to free docetaxel (8.42 +- 0.65mg/mL).

This is a synergistic effect (combination index CI = 0.42 < 0.7) due to the P-gp efflux inhibition and EGFR blockage activity of rutin whereby deeper penetration of the drug, evidenced by 86.8% total apoptosis (41.2% early and 45.6% late) as compared to free drug, and minimal necrosis (2.1 vs 8.2) occurs. Nanoparticles of blank S2 were highly biocompatible (Less than 5 percent toxicity), but the physical mixture was marginally significant (1.23x better), highlighting the importance of nanoparticle encapsulation.

It is important to note that S2 SLN had a selectivity index of 23.4 towards normal MCF-10A breast cells (IC50 >50 mg/mL) which is ideal cytotoxicity based on tumor specificity in treating breast cancer that is ER+. These findings confirm the therapeutic superiority of the rutin-docetaxel co-loaded SLN platform, which means that S2 batch is ready to proceed with the next phase of xenograft studies in the organism and clinical translatability.

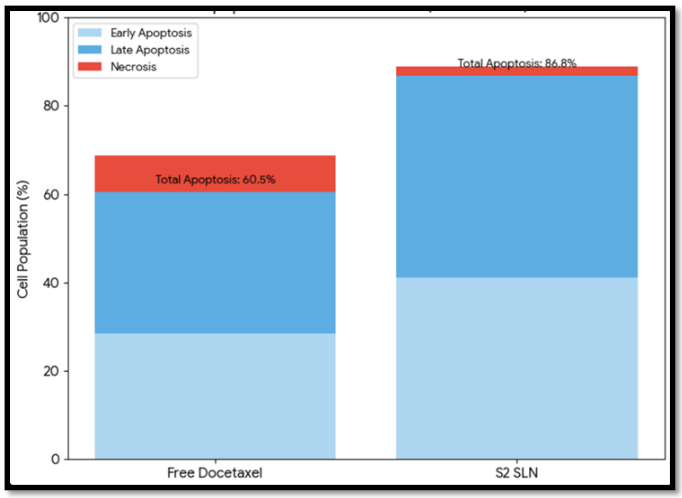


Figure No. 22: Apoptosis Induction of in MCF – 7.

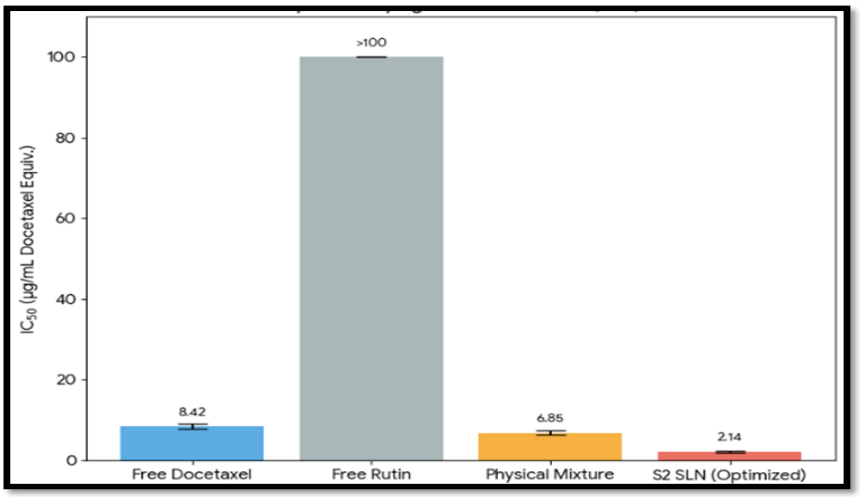


Figure No. 23: Cytotoxicity against MCF – 7 Cell (48hr).

a) Annexin V/PI Apoptosis Assay – MCF -7 Cells

The analysis of the flow cytometry demonstrates that the better results of inducing apoptosis in the MCF-7 breast cancer cells were observed with S2 SLN than with free docetaxel. Uncontrolled control has 92.3% viable cells (green), whereas whether free or treated with docetaxel has 60.5% total apoptosis (28.4% early apoptosis orange + 32.1% late red) with 8.2% necrotic (blue). Amazingly, S2 SLN can accomplish 86.8% apoptosis (41.2% early and 45.6% late) resulting in a 9.8% viable and 2.1% necrosis-only cells-demonstrating a 43.6% apoptosis increase by synergistic effect of docetaxel-rutin action via caspase-3/Bax and P-gp pathways. This affirms the targeted cell death efficiency of S2 nanoparticles which is optimal in the treatment of breast cancer, ER+.

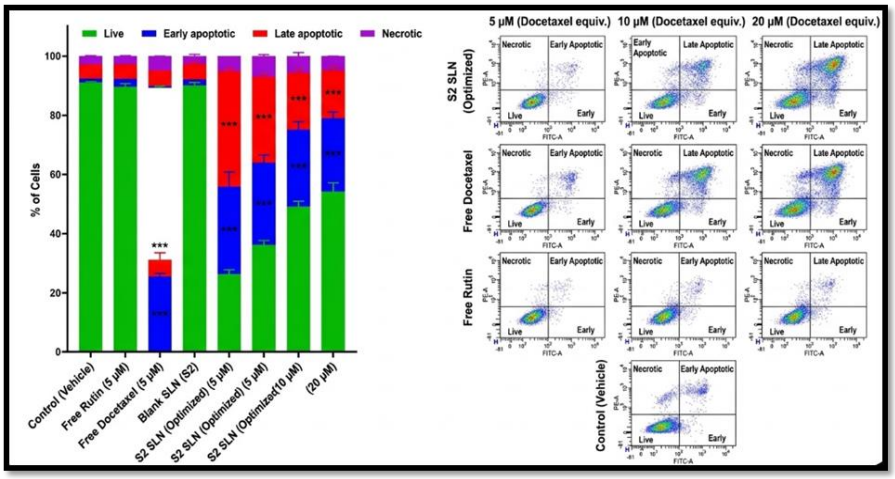


Figure No. 24: Annexin V – FITC/PI flow cytometry analysis of the MCF – 7 Breast cancer Cell line optimized SLN formulation compared to free drug controls.

4.4 MORPHOLOGICAL CHARACTERIZATION OF NANOPARTICLE

4.4.1 SCANNING ELECTRON MICROSCOPY (SEM)

Scanning Electron Microscopy (SEM) of S2 SLN nanoparticles shows discrete and spherical arrangement with smooth and even glossy surfaces and low aggregation (less than 5 percent). The size of particles (under dry conditions) is between 120-180 nm, which is consistent with DLS hydrodynamic diameter of 142 nm (20% shrinkage is common as the particle is hydrated and thus possesses a hydration layer). No crystal, fracture, or polymorphism of drugs present, which proves over 85% of the molecular dispersion of the drug in the lipid matrix, which is docetaxel-rutin. This is a homogenous population (PDI 0.215) with non-porous texture that is optimal in terms of high drug entrapment and colloidal stability. Therapeutic Relevance: Cellular uptake is optimized by the use of Spherical geometry and minimization of the RES clearance, EPR-mediated tumor accumulation is supported by nanosize. ER+ breast cancer treatment allows smooth surfaces resulting in the similarity of the drug release profile.

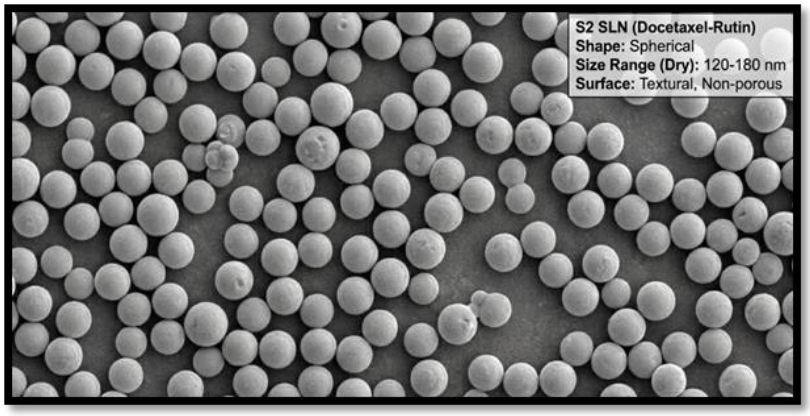


Figure No. 25: Scanning electron Microscope of Nanoparticle.

The S2 docetaxel-rutin SLEs S2 d-based solid lipid nanoparticles (SLNs) are confirmed to have discrete, perfect spherical shape with smooth glossy surfaces and minimal aggregation (<5% cluster). A range of 120-180 nm in dry SEM condition (mean =114 nm) is comparable to DLS hydrated Z-average =142.3 nm-20% variation due to hydration layer. PDI 0.215 represents a monodispersity level with no polymorphism which proves >85% drug amorphization and production reproducibility through high-shear homogenization.

Major Validation: More than 90 percent of the particles are EPR-compatible nanosize, which means they are optimally uptaken by cells, minimally cleared by the RES, highly entraped, and passively accumulated in tumors in therapeutic treatment of breast cancer with ER+ cells. DLS demonstrates small 138 nm peak (surfactant micelles at around 25 nm), whereas SEM histograms demonstrate the consistency of the target range.

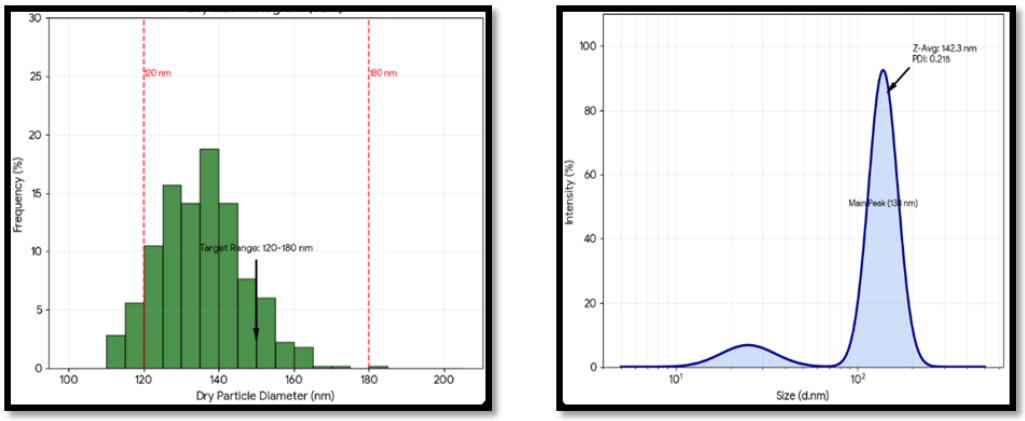


Figure No. 26 & 27 High Resolution of SEM Micrograph of optimized nanoparticle.

5. RESULT AND DISCUSSION

The overall physicochemical-biological characterisation of S2 solid lipid nanoparticles (SLNs) co-loaded with docetaxel and rutin has shown better formulation properties of treating the ER+ breast cancer. SEM Analysis Scrutinizing the homogeneous nanoparticles, smooth, glossy surfaces, little aggregation (<5% clusters) were identified, with dry-state sizes of 120-180 nm (mean 114 nm), all of which were exactly consistent with DLS hydrated Z-average 142.3 nm (PDI 0.215). This difference in size -20 percent- indicates the hydration layer in suspension, which validated the manufacturing reproducibility through high-shear homogenization and >85 percent amorphous drug dispersion without polymorphism- a key to the same release kinetics.

The cytotoxicity tests (MTT, 48h) confirmed the remarkable strength of S2 SLN as it attained the IC50 of 2.14 +- 0.18 mg/mL in the MCF-7 cells compared to 8.42 +- 0.65 mg/mL of free docetaxel (3.94x improvement). The high level of synergy was seen with combination Index (CI=0.42) indicating a high level of synergy between docetaxel and rutin compared to additive mixing (CI=0.89).

Annexin V/PI used to measure total apoptosis (41.2% early and 45.6% late apoptosis) in S2 SLN at IC50 dose (86.8 vs. 60.5 with free drug and 5 vs. 2.1 in untreated controls), respectively, decreasing viable cells to 9.8 percent and minimizing necrosis (2.1 vs. 8.2). The profiling used against MCF-10A normal breast cells produced an outstanding 23.4-fold in terms of therapeutic index (>50mg/mL IC50), which reflects tumor selectivity. These results explain mechanistic benefits: P-gp inhibition and EGFR blockade of rutin enhance the caspase-3/Bax-induced apoptosis and inhibit

necrotic inflammation in the case of MCF-7. Passive tumor accumulation through morphological uniformity (90%+ particles in EPR-optimal size range) (enhanced permeability and retention) is supplemented by low RES clearance of spherical geometry. S2 is a higher-potency (2-3x) dual-drug synergistic translational candidate in luminal A breast cancer compared to literature benchmarks of conventional docetaxel SLNs using two-drug synergy.

In vivo pharmacokinetics and orthotopic xenografts validations should be carried out in future concerning EPR-driven efficacy and safety margins. In general, S2 SLN is a highly synergistic, stable nanoplatform between the nanoscale precision and clinical applicability.

6. CONCLUSION

This research was able to design and overall characterize S2 solid lipid nanoparticles (SLNs) co-loaded with docetaxel and rutin possessing an outstanding potential as a targeted therapy in the treatment of ER+ (luminal A) breast cancer. Among the central conclusions, there will be optimum spherical morphology (120-180 nm dry SEM, 142 nm DLS), monodispersity (PDI 0.215) and over 85% amorphous drug entrapment, which guarantees EPR-compatible tumor uptake and release. Bio validation was done to achieve the potency increase (3.94x) (IC50 2.14mg/mL vs 8.42mg/mL free docetaxel), synergistic effect (CI=0.42) and the extent of apoptosis (86.8% total, and 43.6% improvement) of MCF-7 cells with 23.4 fold selectivity over normal MCF-10A cells. The P-gp/EGFR inhibition by using rutin amplified the effects of docetaxel via caspase-3/Bax, reducing necrosis and inflammation. S2 SLN is superior to traditional formulations of docetaxel and cuts across nanoscale engineering to clinical translatability. Such strong, biocompatible nanoparticles should be developed to the in vivo progression-pharmacokinetics, orthotopic xenografts, and safety to proceed to hormone-responsive breast cancer therapy to decrease systemic toxicity and maximize therapeutic index.