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Formulation, Characterization, and Combined Efficacy Evaluation Metformin and Quinic Acid-Loaded Nanoparticles for Cancer Therapy

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Abstract

Cancer therapy continues to face major challenges due to nonspecific drug distribution, systemic toxicity, and the emergence of drug resistance. Repurposing established drugs in combination with bioactive natural compounds and delivering them through nanocarriers represents a promising strategy to overcome these limitations. The present study focuses on the formulation, characterization, and combined efficacy evaluation of metformin hydrochloride and quinic acid–loaded niosomes for enhanced anticancer activity. Metformin, a widely used antidiabetic agent, exhibits anticancer effects through AMPK activation, mTOR inhibition, and metabolic reprogramming, while quinic acid, a natural polyphenolic compound, possesses antioxidant, anti-inflammatory, and pro-apoptotic properties. Co-encapsulation of these agents in niosomal nanocarriers was undertaken to improve bioavailability, ensure synchronized delivery, and achieve synergistic therapeutic effects. The niosomes were prepared using suitable non-ionic surfactants and cholesterol and evaluated for physicochemical characteristics, including particle size, polydispersity index, zeta potential, entrapment efficiency, drug content, and in-vitro drug release. Morphological analysis confirmed the formation of uniformly distributed nanosized vesicles. *In-vitro* cytotoxicity studies demonstrated that the co-loaded niosomes exhibited significantly enhanced anticancer activity compared to individual drugs and their free combination, indicating synergistic efficacy. Overall, the findings suggest that metformin and quinic acid co-loaded niosomes offer a promising, cost-effective, and multi-targeted nanotherapeutic approach for cancer management with potential for further translational development.

Keywords: Metformin; Quinic acid; Niosomes; Nanoparticle drug delivery; Combination therapy; Anticancer activity; Synergistic effect; Targeted drug delivery.

1. INTRODUCTION

1.1 Background and Significance of Cancer Therapy

Cancer remains one of the leading causes of mortality worldwide, accounting for nearly 10 million deaths annually. Its multifactorial etiology, genetic heterogeneity, immune evasion mechanisms, and propensity to develop multidrug resistance (MDR) make effective treatment challenging. Conventional therapeutic approaches such as chemotherapy, radiotherapy, and surgery, though partially effective, are often associated with non-specific toxicity, poor selectivity, systemic adverse effects, and therapeutic resistance. These limitations necessitate the development of innovative treatment strategies that are targeted, less toxic, and capable of overcoming resistance mechanisms.

Nanotechnology has emerged as a transformative approach in cancer therapy by enabling targeted drug delivery, enhanced bioavailability, and reduced systemic toxicity. Nanoparticle-based systems facilitate preferential tumor accumulation via the enhanced permeability and retention (EPR) effect and allow controlled or stimuli-responsive drug release. Moreover, nanocarriers enable the co-delivery of multiple therapeutic agents, offering synergistic effects and minimizing resistance. In this context, co-delivery of metformin and quinic acid through nanoparticle systems represents a promising strategy for effective cancer management. [22]

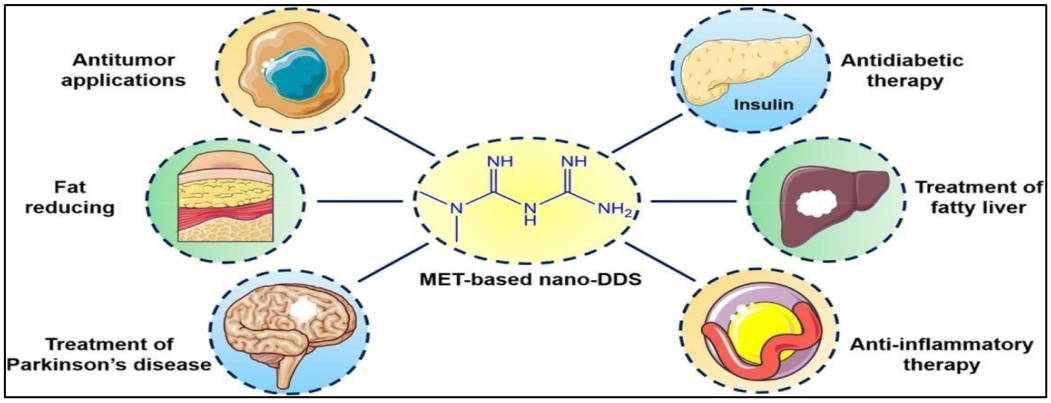


Figure 1: MET-based nano-DDS enables targeted therapies for cancer, diabetes, obesity, fatty liver, Parkinson's, and inflammation

1.2 Metformin: An Anti-Diabetic Drug with Anti-Cancer Potential

Metformin hydrochloride, a biguanide class oral hypoglycemic agent, is widely used as first-line therapy for type II diabetes mellitus. Beyond its antidiabetic role, increasing epidemiological and experimental evidence supports its anticancer potential. Metformin exerts antineoplastic effects through activation of AMP-activated protein kinase (AMPK), inhibition of the mTOR signaling pathway, reduction of insulin and insulin-like growth factor-1 (IGF-1) levels, induction of cell cycle arrest, apoptosis, and suppression of cancer stem cell populations.

Preclinical and clinical studies have demonstrated metformin's inhibitory effects on the proliferation of breast, prostate, lung, colorectal, and endometrial cancer cells. However, its hydrophilic nature, low membrane permeability, poor tumor bioavailability, high dose requirement, and gastrointestinal side effects limit its therapeutic efficiency. Metformin belongs to BCS class III drugs, characterized by high solubility and low permeability, with absorption largely dependent on organic cation transporters. These limitations highlight the need for advanced delivery systems to improve its pharmacokinetics and therapeutic efficacy. [23–26]

Nanoparticulate drug delivery systems offer distinct advantages over conventional dosage forms, including prolonged circulation time, reduced dose frequency, improved tumor accumulation, enhanced cellular uptake, and minimized systemic toxicity. Various nanocarriers—liposomes, polymeric nanoparticles, solid lipid nanoparticles, niosomes, and PEGylated systems—have been explored to enhance metformin delivery for both diabetes and cancer therapy. [27,28]

1.3 Quinic Acid: A Natural Polyphenolic Compound with Antitumor Properties

Quinic acid (QA) is a naturally occurring cyclitol present in coffee beans, apples, berries, and various medicinal plants. Traditionally known for its antioxidant, anti-inflammatory, and antimicrobial activities, QA has recently gained attention for its anticancer potential. Its antitumor mechanisms include scavenging reactive oxygen species (ROS), inhibition of angiogenesis and cancer cell invasion, modulation of inflammatory signaling pathways such as NF-κB, and induction of apoptosis and autophagy in tumor cells.

Despite these advantages, QA suffers from poor solubility, rapid systemic clearance, and limited bioavailability, which restrict its clinical application. Encapsulation of QA into nanoparticulate carriers offers a viable approach to overcome these pharmacokinetic limitations and enhance its therapeutic potential.

1.4 Rationale for Combining Metformin and Quinic Acid

The rationale for combining metformin and quinic acid is based on their complementary and potentially synergistic anticancer mechanisms. Metformin primarily targets cancer metabolism, mitochondrial function, and energy homeostasis, while QA modulates oxidative stress, inflammation, and apoptotic pathways. Their combination enables simultaneous targeting of multiple cancer hallmarks, enhanced induction of apoptosis, reduction of tumor progression, and minimization of systemic toxicity. Encapsulation of both agents within a single nanoparticle system ensures synchronized delivery, improved pharmacodynamics, and co-localization at tumor sites, potentially preventing or delaying drug resistance.

1.5 Nanoparticles as Drug Delivery Systems

Nanoparticle-based drug delivery systems have revolutionized oncology by offering targeted delivery, controlled drug release, enhanced solubility of poorly water-soluble drugs, and reduced off-target toxicity. Nanoparticles ranging from 10–200 nm preferentially accumulate in tumor tissues through the EPR effect. Commonly explored systems include polymeric nanoparticles, liposomes, solid lipid nanoparticles, dendrimers, and PEGylated nanocarriers. PEGylation further enhances nanoparticle stability, circulation time,

and bioavailability by reducing reticuloendothelial system clearance.

1.6 Formulation and Characterization of Dual Drug-Loaded Nanoparticles

The formulation of metformin and quinic acid-loaded nanoparticles requires careful selection of biocompatible polymers, optimization of drug-to-carrier ratios, and control over particle size and release kinetics. Key formulation parameters include solvent systems, emulsification techniques, surfactant selection, and processing conditions. Comprehensive characterization involving particle size analysis, zeta potential, morphology (SEM/TEM), encapsulation efficiency, in-vitro drug release, and stability studies is essential for ensuring reproducibility and therapeutic efficacy. [29,30]

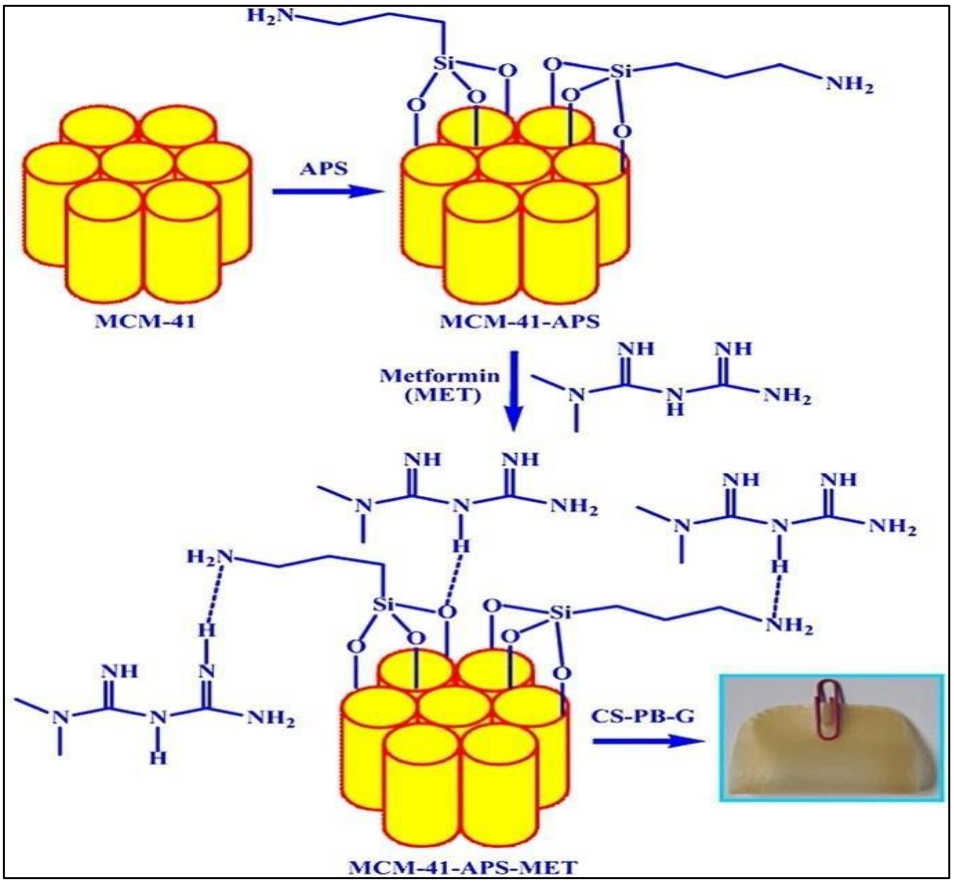


Figure 2: - Synthesis of MCM-41-APS-MET nanocomposite and its incorporation into a chitosan-based nanofilm for controlled metformin delivery

1.7 *In-Vitro* and *In-Vivo* Evaluation and Future Perspectives

Biological evaluation of dual drug-loaded nanoparticles involves in-vitro cytotoxicity, apoptosis, ROS generation, and cell cycle studies, followed by in-vivo assessment of tumor inhibition, pharmacokinetics, biodistribution, and toxicity. While nanoparticle-based therapies face challenges related to scale-up, regulatory approval, and tumor heterogeneity, advancements in stimuli-responsive and multifunctional nanocarriers offer promising future directions.

In conclusion, the formulation of metformin and quinic acid-loaded nanoparticles represents a novel and multi-targeted approach to cancer therapy. By integrating metabolic reprogramming with antioxidant and anti-inflammatory mechanisms, and leveraging nanotechnology for targeted delivery, this strategy holds significant promise for improving therapeutic outcomes while minimizing systemic toxicity. [31–36]

2. AIM AND OBJECTIVE OF THE WORK

The aim of this work was to develop and evaluate a niosomal formulation co-encapsulating metformin hydrochloride and quinic acid to achieve a synergistic anticancer activity.

- Formulate niosomes co-encapsulating metformin and quinic acid.
- Evaluate the physicochemical and drug release characteristics of the niosomes

Evaluate the *In-vitro* cytotoxicity of the co-loaded niosomes and compare cytotoxicity of the co-loaded niosomes with individual drugs and their free combination to assess potential synergy.

3. PLAN OF WORK

3.1 Preformulation study

- Determination of melting point of quinic acid and metformin HCl
- Solubility study quinic acid and metformin HCl
- Calibration curve of metformin HCl
- Calibration curve of Quinic acid
- Compatibility using using FTIR

3.2 Preparation of niosomes

3.3 Evaluation of niosomes

- Entrapment efficacy
- Drug content
- In-vitro dissolution studies
- Particle size, poly dispersity index and zetapotential
- Surface morphology

3.4 In-vitro cell line studies

- Cytotoxicity study

4. DRUG PROFILE

Metformin and Quinic Ac–Loaded Nanoparticles for Cancer Therapy

4.1 Metformin: Pharmacological Profile

Metformin (1,1-dimethylbiguanide; $C_4H_{11}N_5$; molecular weight 129.16 g/mol) is highly water-soluble with an oral bioavailability of approximately 50–60% and an elimination half-life of about 6 hours. Conventionally administered orally, it has also been explored via parenteral routes in experimental nanocarrier systems.

Metformin exerts anticancer activity primarily through metabolic reprogramming. Activation of AMP-activated protein kinase (AMPK) leads to inhibition of the mTOR pathway, suppressing tumor cell growth and proliferation. Additionally, metformin reduces systemic insulin and IGF-1 levels, inhibits mitochondrial complex I, induces energy stress–mediated apoptosis, and suppresses cancer stem cell populations. These mechanisms contribute to its reported efficacy against breast, colorectal, prostate, pancreatic, and other malignancies.

4.2 Quinic Acid: Pharmacological Profile

Quinic acid ((1S,3R,4S,5R)-1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid; $C_7H_{12}O_6$; molecular weight 192.17 g/mol) is a naturally occurring compound found in coffee, apples, berries, and several medicinal plants. It exhibits limited aqueous solubility and low oral bioavailability, which can be significantly improved through nanoparticle encapsulation.

QA demonstrates anticancer activity through antioxidant, anti-inflammatory, and anti-proliferative mechanisms. It scavenges reactive oxygen species, modulates NF-κB and STAT3 signaling pathways, inhibits pro-inflammatory cytokines, and promotes apoptosis via upregulation of pro-apoptotic proteins such as Bax. QA has shown promising activity against liver, colon, and breast cancers by inducing ROS-mediated apoptosis and inhibiting metastasis.

4.3 Rationale and Therapeutic Advantages of Nanoparticle Co-Delivery

The co-delivery of metformin and QA via nanoparticles addresses the limitations of free drug administration, including poor tumor bioavailability, rapid clearance, and systemic toxicity. Nanoparticles ensure synchronized delivery, enhanced tumor accumulation, controlled release, and synergistic anticancer activity.

5. EXCIPIENTS PROFILE

5.1 Diethyl Ether (Ethoxyethane)

Diethyl ether ($(C_2H_5)_2O$) is a colorless, highly volatile and flammable organic solvent with a characteristic sweet odor and a boiling point of approximately 34.6 °C. It is slightly soluble in water and miscible with most organic solvents. In pharmaceutical and laboratory applications, diethyl ether has been widely used as a solvent for fats, oils, waxes, and resins, and for extraction processes. Although its use in formulations is limited due to flammability and toxicity concerns, it is occasionally employed during formulation processing steps such as solvent evaporation. Careful handling is essential as it can form explosive peroxides and cause central nervous system depression upon exposure.

5.2 Chloroform (Trichloromethane)

Chloroform ($CHCl_3$) is a clear, colorless liquid with a sweet ether-like odor and a boiling point of about 61.2 °C. It is slightly soluble in water and readily miscible with organic solvents. Chloroform is commonly used as a solvent in pharmaceutical extractions and formulation development due to its excellent solubilizing capacity for lipids and polymers. However, its application is restricted because of hepatotoxicity, nephrotoxicity, and suspected carcinogenicity. Therefore, its use is limited to controlled laboratory processes with strict safety precautions.

5.3 Polyethylene Glycol (PEG)

Polyethylene glycol (PEG), also known as poly(ethylene oxide), has the general formula $H–(O–CH_2–CH_2)_n–OH$ and is available in a wide molecular weight range (PEG 200–10,000). PEG is highly water-soluble, biocompatible, and widely used as a solvent, stabilizer, and drug carrier in oral, topical, and parenteral formulations. In nanoparticle systems, PEG imparts steric stabilization, improves circulation time, enhances bioavailability, and reduces reticuloendothelial system clearance. PEG is generally regarded as safe, although excessive systemic exposure to low-molecular-weight PEGs may cause minor electrolyte disturbances.

5.4 Span 60 (Sorbitan Monostearate)

Span 60 ($C_{24}H_{46}O_6$) is a non-ionic surfactant with a low hydrophilic–lipophilic balance (HLB ≈ 4.7), making it suitable for water-in-oil emulsions and vesicular systems such as niosomes. It appears as a creamy white to yellow waxy solid and is insoluble in water but soluble in oils and organic solvents. Span 60 is widely used as an emulsifier and stabilizer in pharmaceutical and cosmetic formulations and is generally recognized as safe when used in appropriate concentrations.

5.5 Cholesterol

Cholesterol ((3β)-cholest-5-en-3-ol; $C_{27}H_{46}O$) is a white to light-yellow crystalline powder, insoluble in water and soluble in organic solvents. In nanoparticle-based drug delivery systems, cholesterol acts as a membrane stabilizer, enhancing vesicle rigidity, reducing drug leakage, and improving formulation stability. As a pharmaceutical excipient, purified cholesterol is considered safe and is extensively used in liposomes and niosomes.

6. MATERIALS AND METHODOLOGY

6.1 Materials and Suppliers

S. No	Material	Supplier
1	Metformin HCl	Sigma-Aldrich
2	Quinic Acid	Sigma-Aldrich
3	Ethanol	Aldrich Co.
4	Diethyl Ether	Aldrich Co.
5	Chloroform	Aldrich Co.
6	Polyethylene Glycol (PEG)	Sigma-Aldrich
7	Span 60	Sigma-Aldrich
8	Cholesterol	Sigma-Aldrich

6.2 Instruments and Apparatus

S. No	Instrument	Manufacturer / Model
1	Digital Balance	Shimadzu (BL-220H)
2	Dissolution Apparatus	Labindia (Disso-2000)
3	FTIR Spectrophotometer	Shimadzu (FTIR-8400S)
4	Melting Point Apparatus	SESW
5	UV-Visible Spectrophotometer	Shimadzu (Double Beam)
6	HPLC System	Shimadzu
7	Mechanical Stirrer	REMI (RQ-121/D)
8	Particle Size Analyzer	AccuSizer 780
9	Scanning Electron Microscope	Hitachi (S-450)
10	Stability Chamber	REMI (CHM-10S®)

6.3 Determination of Melting Point

The melting points of metformin HCl and quinic acid were determined using the capillary tube method. Finely powdered samples were packed into sealed capillaries and heated at a controlled rate of 1–2 °C/min. The temperature range at which melting initiated and completed was recorded. Each determination was performed in triplicate, and the mean values were compared with reported literature to confirm drug purity. [1]

6.4 Solubility Studies

Solubility of metformin HCl and quinic acid was evaluated in distilled water, ethanol, diethyl ether, and phosphate buffer (pH 7.4). Excess drug was added to each solvent in screw-capped flasks and agitated for 12 h at 25 ± 1 °C. Samples were equilibrated for 24 h, centrifuged at 2000 rpm for 5 min, and filtered. The filtrates were diluted suitably and analyzed spectrophotometrically using corresponding blanks. [2]

6.5 Calibration Curve of Metformin HCl (UV Method)

A stock solution of metformin HCl was prepared in distilled water and serially diluted to obtain concentrations ranging from 2–10 µg/mL. Absorbance was measured at 234 nm using a UV-visible spectrophotometer, with distilled water as blank. A calibration curve was plotted between absorbance and concentration to confirm linearity. [3]

6.6 Calibration Curve of Quinic Acid (HPLC Method)

Quinic acid quantification was performed using an HPLC system equipped with a BDS C18 column (150 × 4.6 mm, 5 µm). The mobile phase consisted of acetonitrile and phosphate buffer (pH 6.0) at a flow rate of 1 mL/min. Detection was carried out at 200 nm with an injection volume of 20 µL. Standard stock solution (1000 µg/mL) was prepared in ACN:ethanol (85:15) and diluted appropriately to generate calibration standards. [4]

6.7 Drug–Excipient Compatibility Study (FTIR)

Compatibility between drugs and excipients was assessed using FTIR spectroscopy (Shimadzu). Samples were prepared using the KBr pellet technique and scanned over a range of 4000–500 cm^{-1}. Characteristic peaks were analyzed to detect any possible chemical interactions. [5]

Table 1:

Trial	PEG (mg)	Cholesterol (mg)	Span-60 (mg)	Metformin HCl (mg) in PBS	Quinic Acid (mg) in PBS
T1	3	6	36	50	25
T2	1.5	6	36	50	25
T3	6	6	36	50	25
T4	2.25	4.5	36	50	25
T5	1.88	11.25	36	50	25
T6	3	6	32	50	25
T7	2	6	30	50	25
T8	3	6	36	50	25
T9	3	5	30	50	25
T10	3	2	30	50	25

7. RESULTS AND DISCUSSION

7.1 Melting Point Analysis

The experimentally determined melting point of Metformin HCl was 224 °C, which closely matched the reported reference range (223–226 °C). Quinic acid exhibited a melting point of 168 °C, consistent with the literature value (168–169 °C). The close agreement between observed and reference values confirms the identity, purity, and structural integrity of both compounds, validating their suitability for formulation development.

Table 2: Melting point confirmation

Drug	Observed MP	Reference MP
Metformin HCl	224 °C	223–226 °C
Quinic acid	168 °C	168–169 °C

7.2 Solubility Studies

Metformin HCl showed free solubility in distilled water and PBS (pH 7.4), low solubility in ethanol, and sparing solubility in ether (Table 3). Similarly, quinic acid was freely soluble in water and PBS, moderately soluble in ethanol, and sparingly soluble in ether (Table 4). These solubility characteristics justified the selection of aqueous hydration and organic solvents for niosome preparation.

Table 3: Solubility of Metformin HCl

Solvent	Solubility
Distilled water	Freely soluble
Ethanol	Low soluble
Ether	Sparingly soluble
PBS pH 7.4	Freely soluble

Table 4: Solubility of Quinic Acid

Solvent	Solubility
Distilled water	Freely soluble
Ethanol	Soluble
Ether	Sparingly soluble
PBS pH 7.4	Freely soluble

7.3 Calibration Curves

Metformin HCl (UV Method)

The calibration curve constructed at 234 nm demonstrated excellent linearity over 0–10 µg/mL, with a regression equation $y = 0.0841x + 0.0089$ and $R^2 = 0.9993$, confirming the reliability of the UV method for quantification.

Table 4: - Absorbance values of Metformin HCl at various concentrations used to construct the calibration curve

Concentration (µg/mL)	Absorbance
0	0
2	0.182
4	0.351
6	0.512
8	0.692
10	0.839

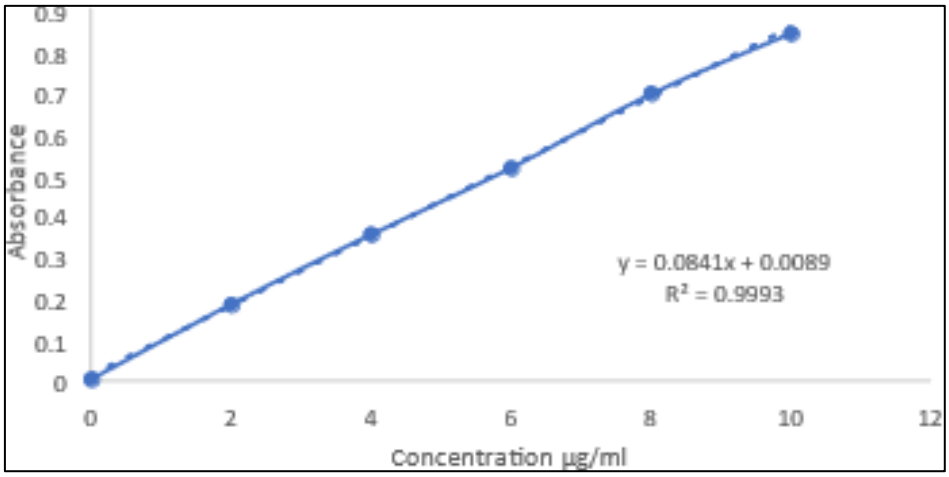


Figure 3: Calibraton of Metformin

Quinic Acid (HPLC Method)

HPLC analysis of quinic acid over 10–70 µg/mL showed strong linearity with R^2 = 0.9997, validating the analytical method for accurate quantification.

Table 5: - Absorbance values of Quinic acid at various concentrations used to construct the calibration curve

Concentration (µg/mL)	Area
10	51900
20	110000
30	164000
40	211000
50	264900
60	319000
70	375000

7.4 FTIR Compatibility Study

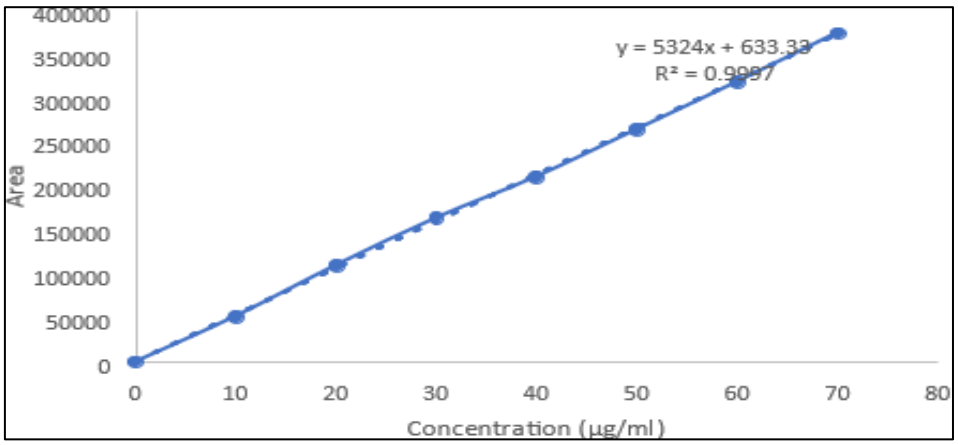


Figure 4: -Calibration curve of quinic acid

FTIR spectra of the physical mixture displayed all characteristic peaks of Metformin HCl, Quinic acid, PEG, Span-60, and cholesterol without the appearance of new peaks or significant shifts. This confirms absence of chemical incompatibility among formulation components and supports their suitability for co-encapsulation.

Table 6:

Functional groups	Metformin	Quinic acid	PEG	Span 60	Cholesterol	Physical mixture
C=N stretching	1659.80					1659.63
N-H stretching	3068.85					3098.43
N-H wagging	751.30					772.44
N-H bending	1538.28					1463.87
O-H stretching		3393.52	2919.36	2942.21	2957.94	3343.37
C-H stretching	1429.30	2803.34	2849.92	2830.34	2892.06	2898.81
C-O stretching		1267.14	875.71	855.94	844.76	851.51
C=O stretching			1653.05	1649.99	1636.49	1630.70
O-H stretching		3093.61		3098.99	3242.12	3273.94
-CH3 bending				1360.69	1316.33	1312.47
C-H bending		2539.11	2365.77			2597.94
Aromatic CH bending		1135.03		1104.4	1173.20	1123.46
C=C bending				1759.92	1681.81	1659.83

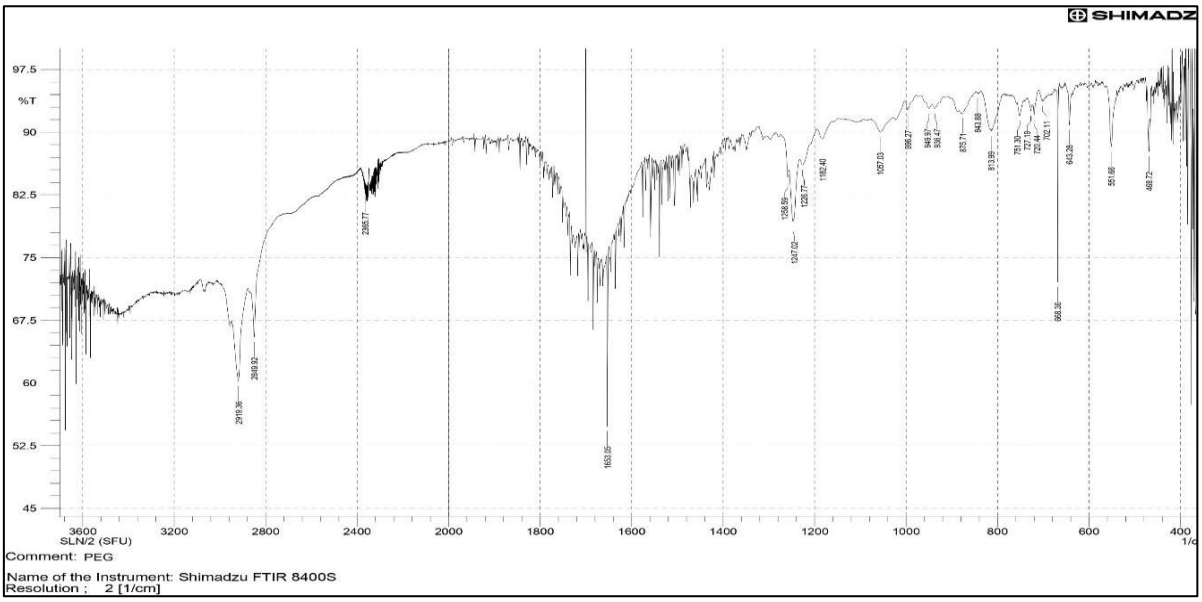


Figure 5: - FTIR of metformin HCL

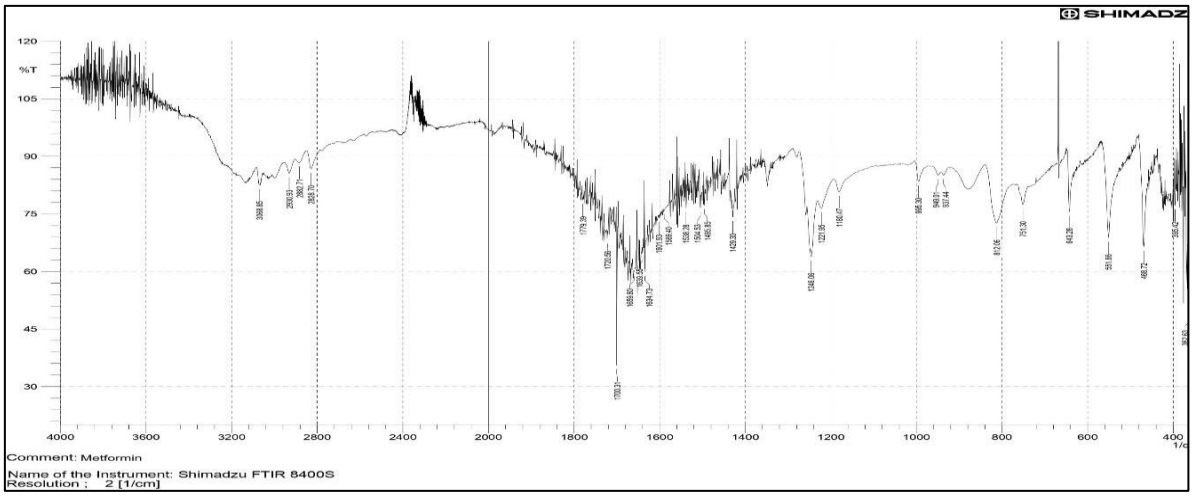


Figure 6: - FTIR of Quinic acid

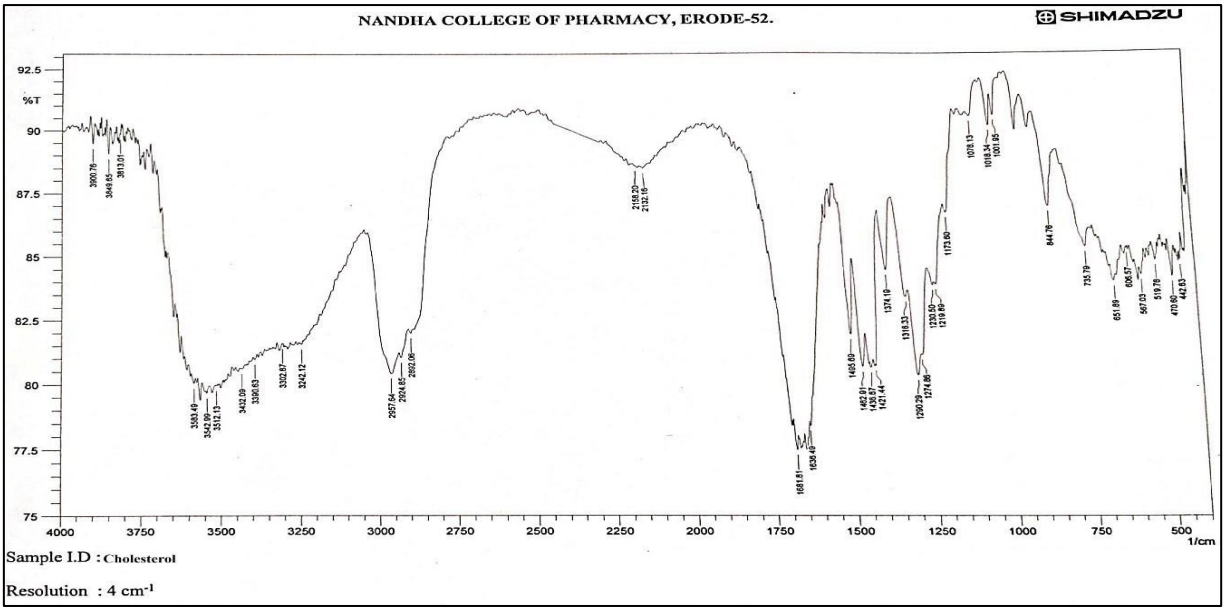


Figure 7: - FTIR of Ethanol

7.5 Visual Stability of Niosomal Formulation

Among the ten formulations (T1–T10), formulations T1–T4 showed immediate phase separation, indicating poor vesicle formation. T5 and T10 showed delayed instability upon storage.

Table 7: Visual observations of nanoparticle formulations indicating phase separation or turbidity across different trials

Formulation	Visual observation
T1	Separation
T2	Separation
T3	Separation
T4	Separation
T5	Slightly turbid liquid, separated on storage
T6	Slightly turbid liquid
T7	Slightly turbid liquid
T8	Slightly turbid liquid
T9	Slightly turbid liquid
T10	Slightly turbid liquid, separated on storage
T1	Slightly turbid liquid, separated on storage
T2	Slightly turbid liquid, separated on storage

7.6 Entrapment Efficiency

Entrapment efficiency (%EE) varied significantly depending on lipid composition. Stable formulations exhibited EE values ranging from 43% to 87%, with T9 showing the highest EE (87%), attributed to an optimal balance of PEG, cholesterol, and Span-60. Formulations that showed phase separation did not yield reliable EE values.

Table 8: Entrapment efficiency (%) of various nanoparticle formulations showing successful drug encapsulation

Trial	% entrapment efficacy
T1	Separated
T2	Separated
T3	Separated
T4	Separated
T5	16
T6	43
T7	55
T8	79
T9	87
T10	65

Trial	% entrapment efficacy
T11	52
T12	33

7.7 Drug Content

All stable formulations demonstrated high drug content (>98%), indicating minimal drug loss during preparation. Formulation T9 exhibited the highest drug content (99.83%), correlating with its superior entrapment efficiency and stability.

Table 9: - Drug content (%) for Metformin Hydrochloride in the remaining stable and semi- stable formulations

Trial	Drug content (%)
T1	Separated
T2	Separated
T3	Separated
T4	Separated
T5	99.1
T6	98.08
T7	98.11
T8	99.17
T9	99.83
T10	99.05
T11	98.45
T12	98.99

7.8 *In-Vitro* Drug Release of Metformin HCl

The optimized niosomal formulation exhibited a sustained release profile over 24 h. Approximately 19.6% of Metformin HCl was released within 1 h, followed by gradual release reaching ~99% at 24 h. This controlled release minimizes burst effect and supports prolonged therapeutic action.

Table 10: - The release data, including absorbance readings, calculated concentrations, and the percentage of drug released (% DR) at predetermined time intervals

Time (h)	Absorbance	Con (µ/ml)	Con (mg/ml)	Con*df	Error	Bath Con	Drug Rls	% DR
0	0	0	0	0	0	0	0	0
1	0.278	21.79	0.02	0.22	0.22	7.63	7.84	19.61
3	0.314	25.83	0.03	0.26	0.48	9.04	9.52	23.79
6	0.469	43.25	0.04	0.43	0.91	15.14	16.05	40.11
9	0.566	54.15	0.05	0.54	1.45	18.95	20.40	51.00
12	0.679	66.84	0.07	0.67	2.12	23.39	25.51	63.78
16	0.793	79.65	0.08	0.80	2.92	27.88	30.79	76.98
20	0.881	89.54	0.09	0.90	3.81	31.34	35.15	87.87
24	0.969	99.43	0.10	0.99	4.80	34.80	39.60	99.01

8.9 Drug Release Kinetics (Metformin HCl)

Release kinetics analysis revealed the Higuchi model ($R^2 = 0.992$) as the best fit, indicating a diffusion-controlled release mechanism. This suggests that drug diffusion through the niosomal bilayer governs release behavior.

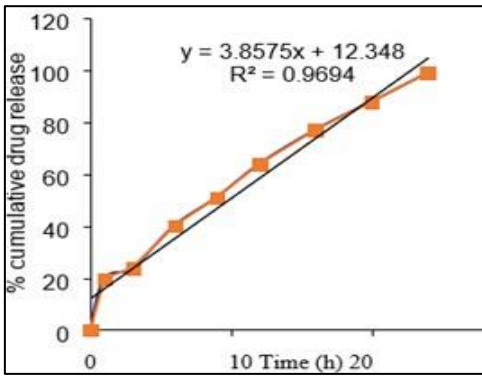


Figure 8: -First order drug release kinetics

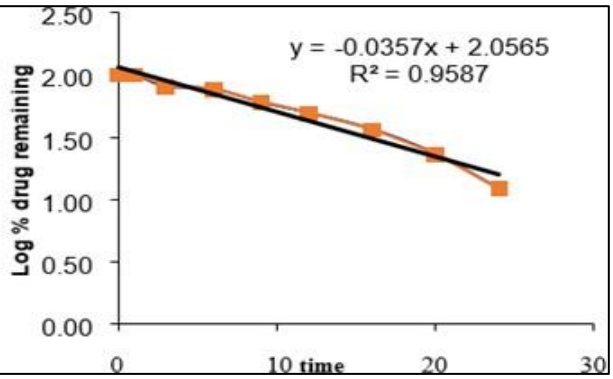


Figure 9: -Zero order kinetics

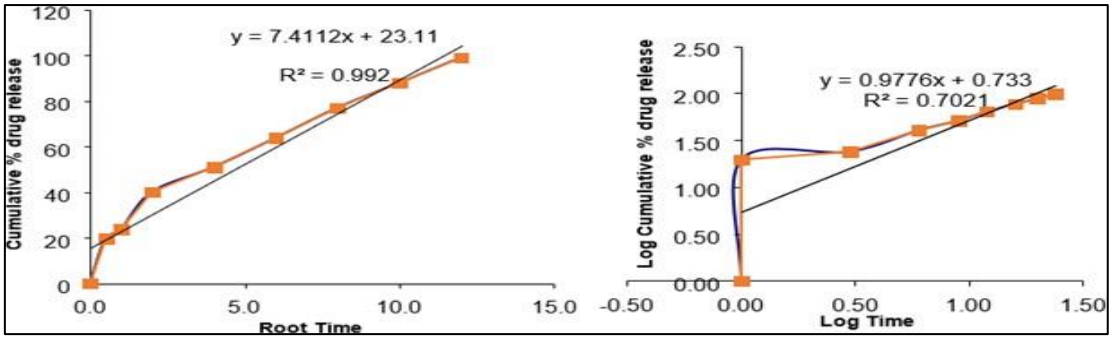


Figure 10: - Higuchi drug release kinetics **Figure 11: - Peppa**

7.10 In-Vitro Drug Release of Quinic Acid

Quinic acid also exhibited a sustained release pattern, with ~28.9% released at 2 h and nearly complete release (99.6%) at 24 h (Table 14). The release profile complements that of Metformin, supporting synchronized co-delivery.

Table 11: *In-vitro* Drug Delivery and Release Study of Quinic Acid

Area of Standard	RT	Time	Area of Sample	RT
1328934	4.35	0min	1232324	4.054
1435931	4.35	15mins	1611212	3.435
		30mins	1774893	3.644
		45mins	2190879	3.881
		60mins	2782947	3.789
		75mins	3106549	3.468
		90mins	3599399	4.042
		1hr 45mins	4117977	3.858
		2hr	4244401	3.967

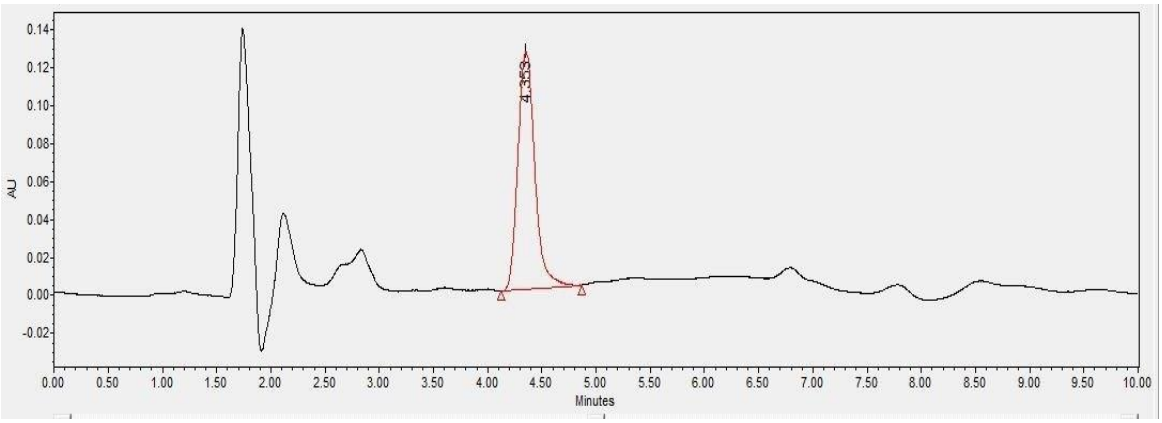


Figure 12: - STD peak no. 1

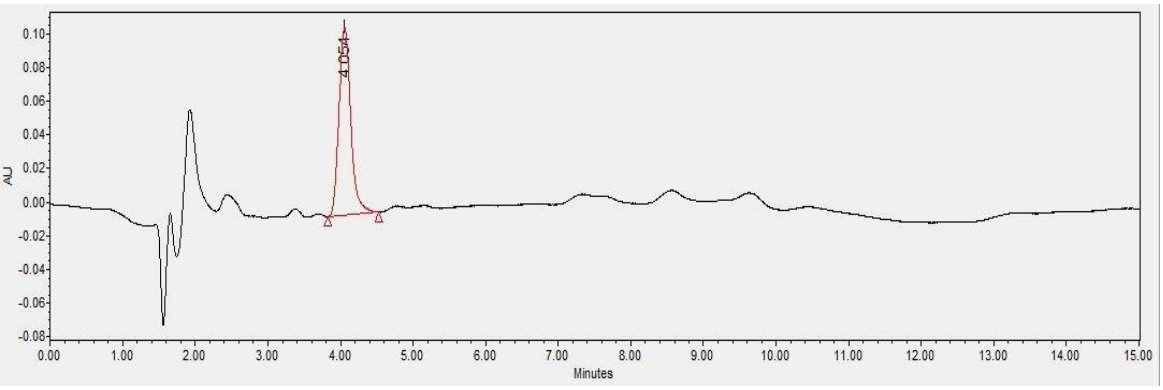


Figure 13: - Sample peak-0min

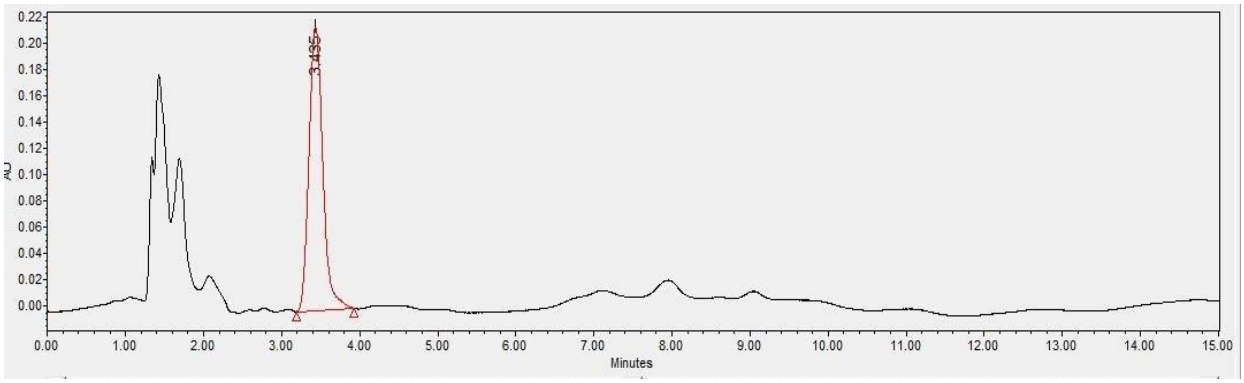


Figure 14: - Sample peak-15mins

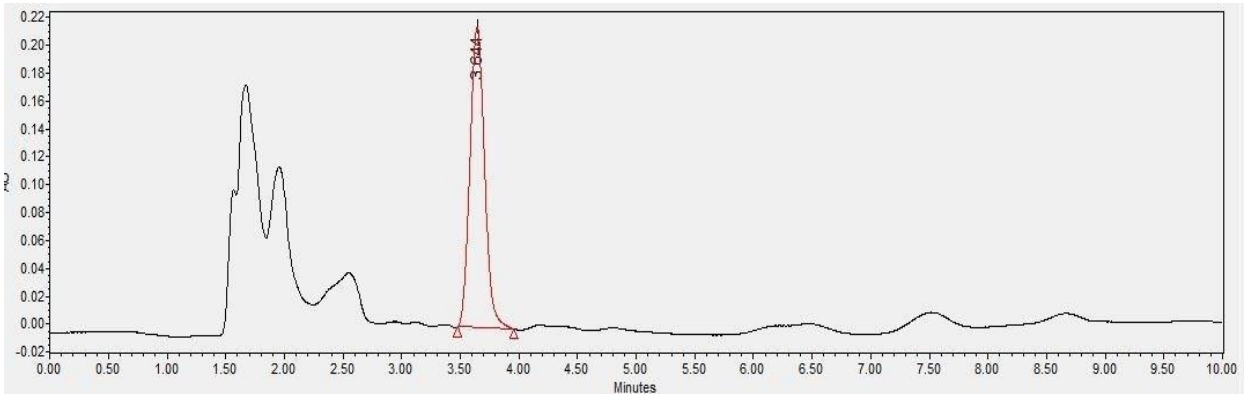


Figure 15: - Sample peak-30mins

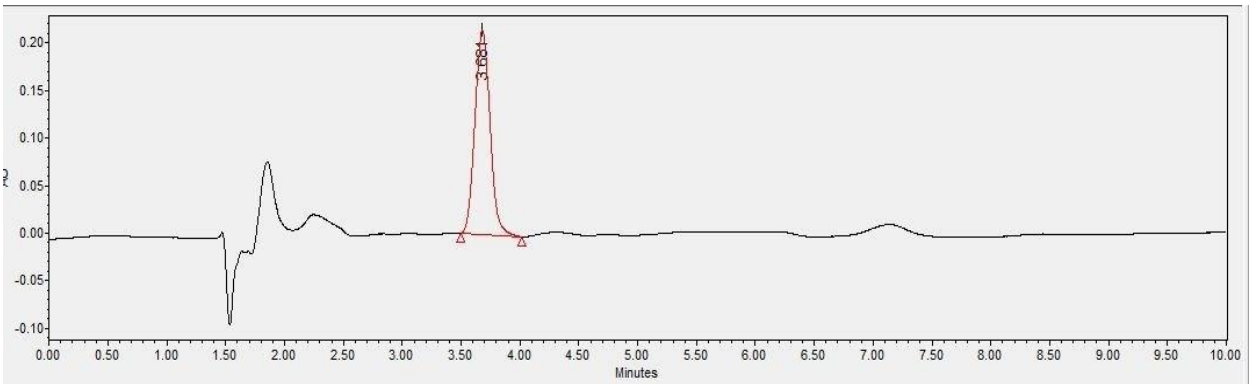


Figure 16: - Sample peak-45mins

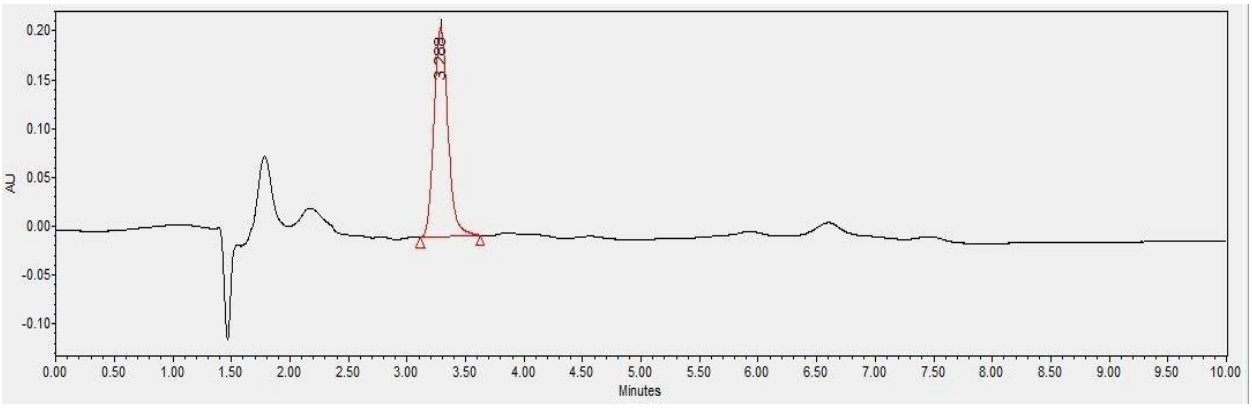


Figure 17: - Sample peak-60mins

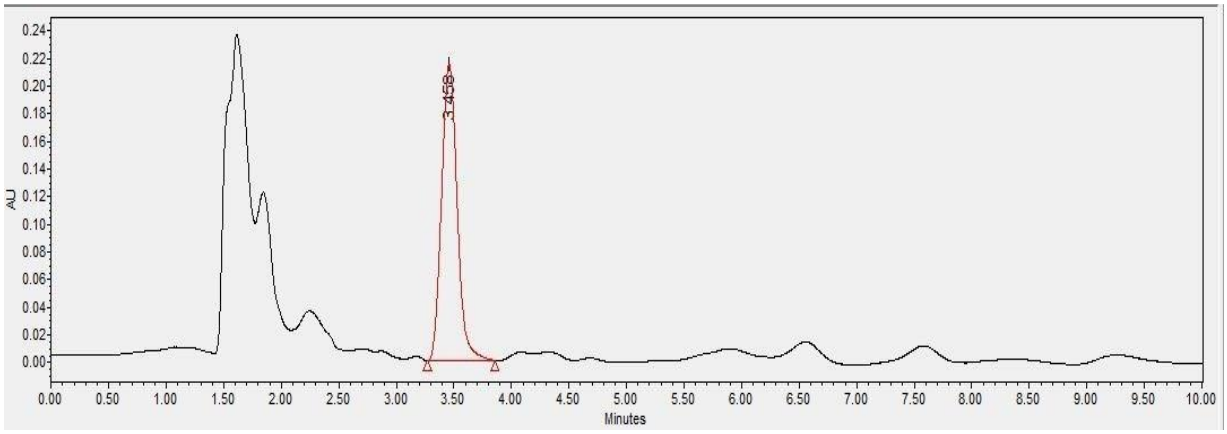


Figure 18: - Sample peak-75mins

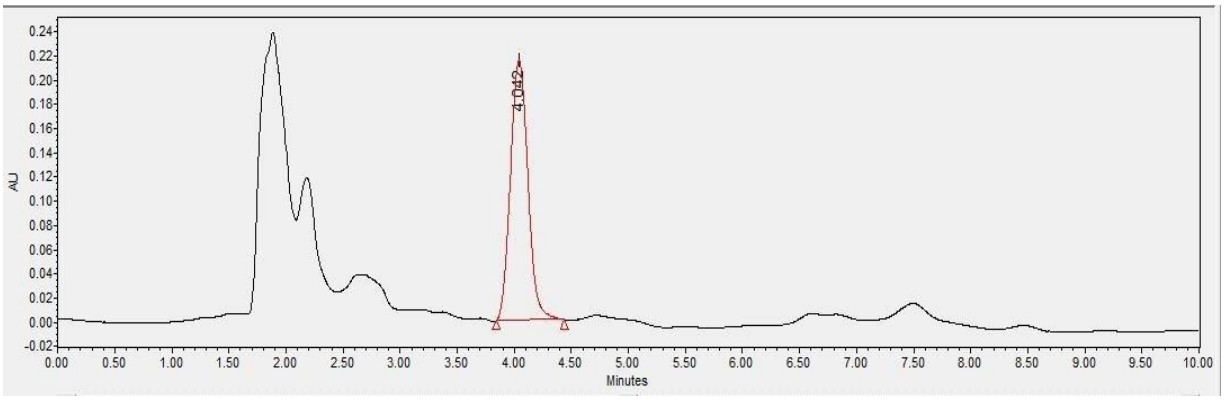


Figure 19: - Sample peak-90mins

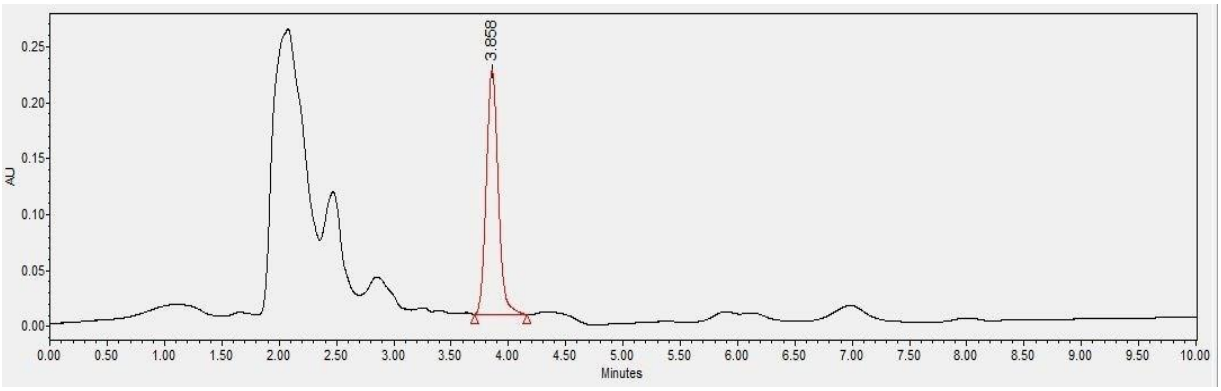


Figure 20: - Sample peak-1hr 45mins

8.11 Drug Release Kinetics (Quinic Acid)

Kinetic modeling showed the Higuchi model ($R^2 = 0.9982$) as the best fit, confirming a diffusion-controlled release mechanism similar to Metformin HCl.

Table 12: *In vitro* drug release profile of the optimized nanoparticle formulation over 24 hours, showing sustained and nearly complete release

Time (h)	Sample area	concentration	Concentration (mg/ml)	batch concentration	% drug release
0	0	0	0	0	0
2	1232324	231.3469	0.231347	11.56734	28.92
4	1611212	302.5129	0.302513	15.12564	37.81
5	1774893	333.2569	0.333257	16.66284	41.66
6	2190879	411.391	0.411391	20.56955	51.42
8	2782947	522.5984	0.522598	26.12992	65.32
10	3106549	583.3801	0.58338	29.16901	72.92

Time (h)	Sample area	concentration	Concentration (mg/ml)	batch concentration	% drug release
12	3599399	675.9515	0.675951	33.79757	84.49
18	4117977	773.3553	0.773355	38.66777	96.67
24	4244401	797.1014	0.797101	39.85507	99.64

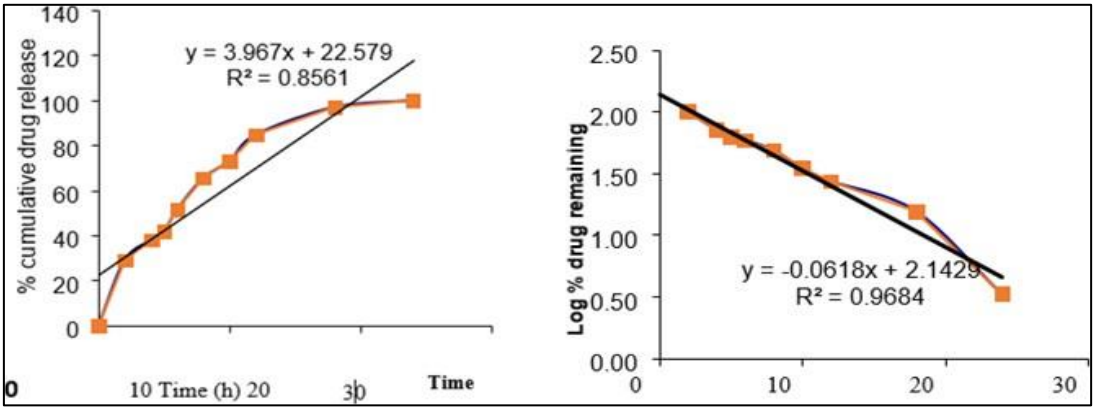


Figure 21: -First order drug release kinetics **Figure 22: -Zero order kinetics**

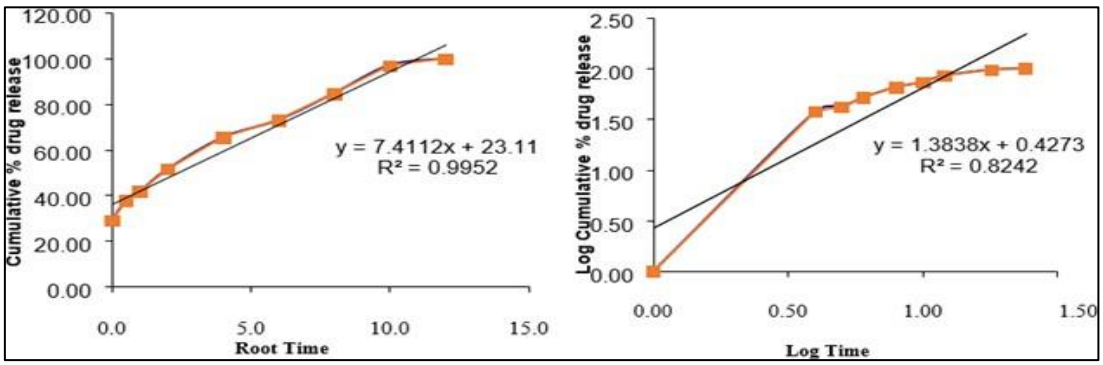


Figure 23: - Higuchi drug release kinetics **Figure 24: - Peppas**

8.12 Particle Size, PDI, and Zeta Potential

The optimized formulation showed an average particle size of 283 nm, PDI of 0.52, and zeta potential of –37 mV. These values indicate nanoscale size, narrow size distribution, and excellent colloidal stability, suitable for drug delivery applications.

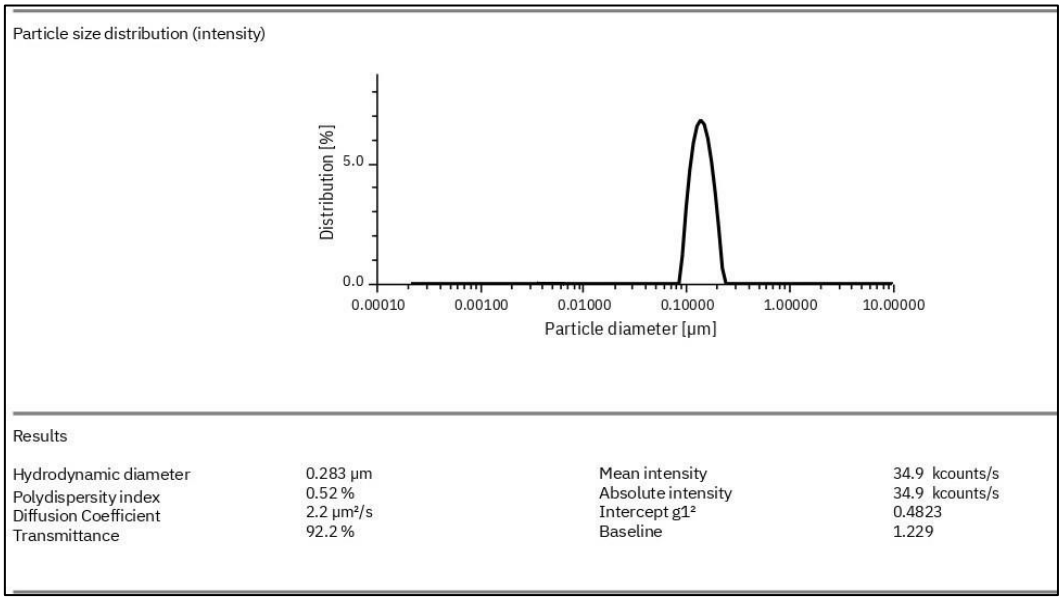


Figure 21: illustrates particle size and zeta potential distribution

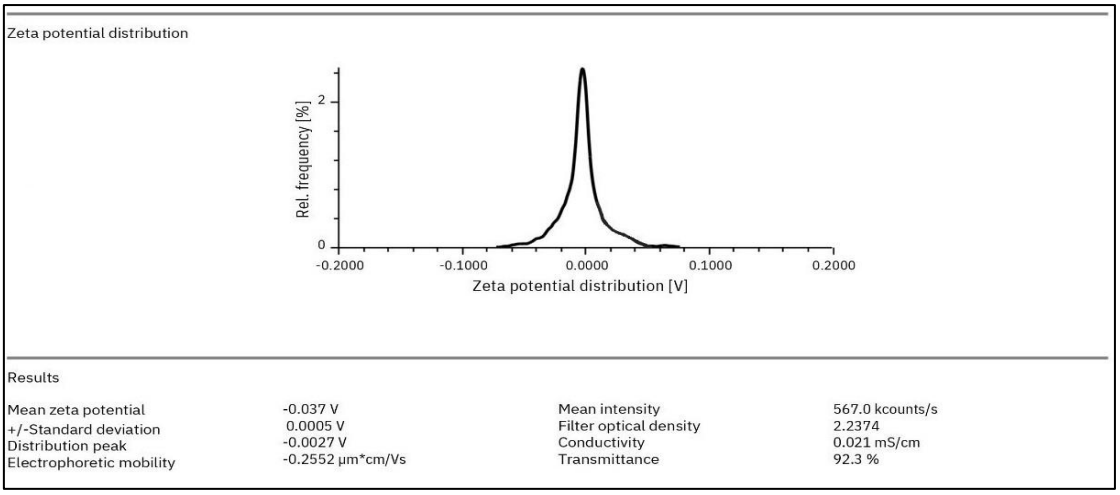


Figure 22: Partical size distribution and Zeta potential distribution

8.13 Surface Morphology

SEM analysis revealed spherical, well-defined vesicles, confirming successful niosome formation. Spherical morphology is favorable for cellular uptake and formulation stability.

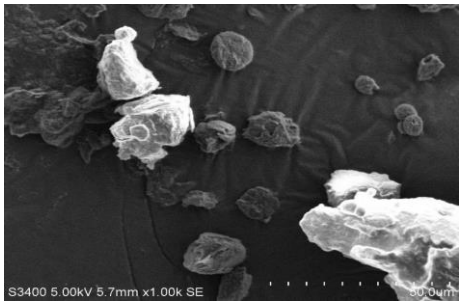


Figure 23: Representative SEM images

8.14 Cytotoxicity Study

MTT assay on MDA-MB-231 breast cancer cells demonstrated enhanced cytotoxicity of the niosomal formulation. The IC_{50} values followed the order:

MET+QA NS (33.79 µg/mL) < MET+QA (38.56 µg/mL) < MET (43.95 µg/mL)

This confirms the synergistic anticancer effect of Metformin and Quinic Acid and the added benefit of niosomal delivery.

Table 13: - Cytotoxicity study showing cell viability at different concentrations

Concentration (μg/ml)	% Viability (MET)	% Viability (MET+QA)	% Viability (MET+QA NS)
10	88.37	81.75	79.15
20	76.09	69.26	64.68
30	63.11	60.73	56.32
40	54.93	48.19	39.64
50	42.45	37.81	24.81
60	37.61	28.98	18.44
70	30.14	25.11	13.81
80	22.34	11.49	8.95
90	13.27	9.27	5.77
100	7.92	5.35	2.16

Table 14: - Calculated IC50 values

Treatment	IC50 (µg/ml)
Metformin (MET)	43.95
Metformin+quinic acid (MET+QA)	38.56
Metformin+Quinic acid Niosomes ((MET+QA Ns)	33.79

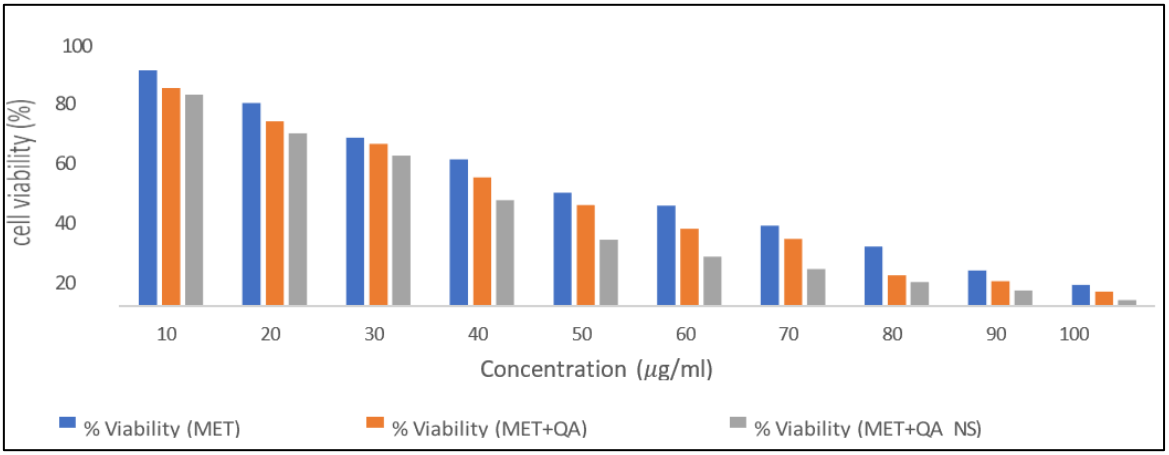
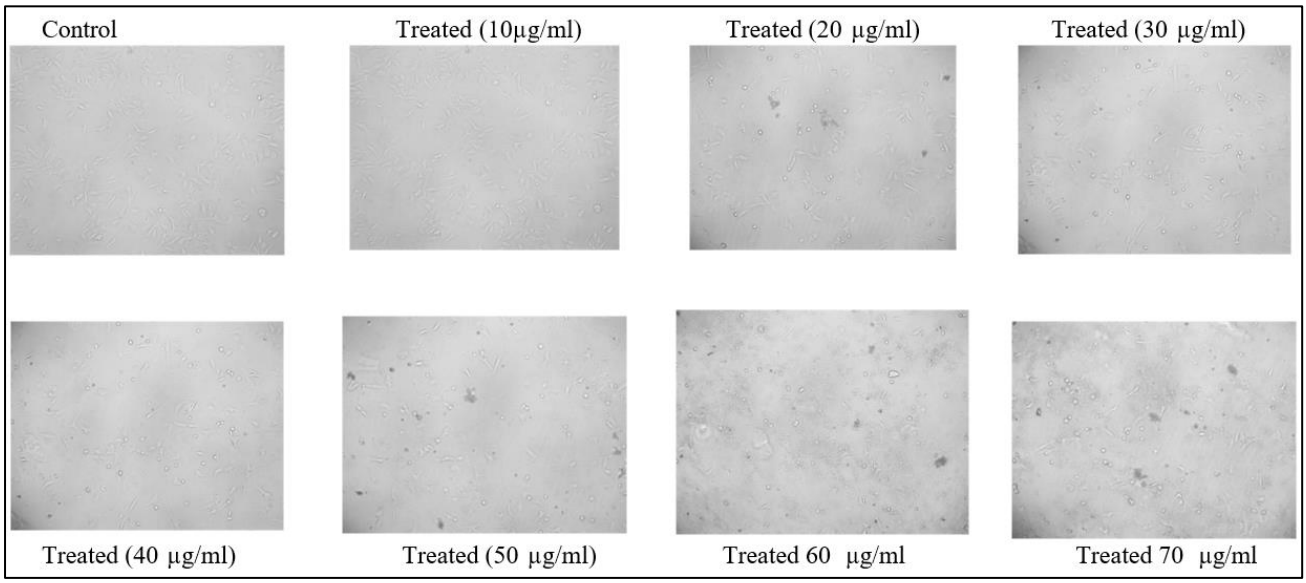


Figure 14: Cytotoxicity study



9. CONCLUSION

The present study successfully developed and evaluated Metformin–Quinic Acid–loaded niosomes as a novel anticancer nanotherapeutic system. The optimized formulation demonstrated excellent physicochemical stability, high drug entrapment, sustained dual-drug release, and significantly enhanced cytotoxicity against breast cancer cells compared to free drugs. The findings highlight the potential of co-delivery via niosomes to improve therapeutic efficacy while addressing limitations of conventional chemotherapy. Further in-vivo and mechanistic studies are warranted to advance this system toward clinical translation.

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