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Chitosan-Based Nanoemulgel for Enhanced Co-Delivery and Sustained Release of Lipophilic Cynaropicrin and Hydrophilic Salicin: Formulation, Physicochemical Characterization, and Stability

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Abstract: The co-delivery of therapeutic agents with differing physicochemical properties presents significant formulation challenges. This study aimed to develop and characterize a chitosan-based nanoemulgel for the simultaneous encapsulation and sustained delivery of the lipophilic anticancer agent cynaropicrin and the hydrophilic anti-inflammatory/anticancer agent salicin. Nanoemulsions were prepared by integrating an oil phase containing cynaropicrin (Medium-Chain Triglyceride oil) with an aqueous phase of chitosan and salicin, followed by high-shear homogenization and sonication, and subsequently incorporated into a Carbopol gel. Five distinct formulations (F1-F5) were systematically developed and characterized for pH, viscosity, spreadability, particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency (EE%), and morphology via TEM. Optimized nanoemulgels exhibited desirable particle sizes ranging from 81.63 ± 0.47 nm to 106.13 ± 0.75 nm, low PDI values (0.249 ± 0.002 to 0.337 ± 0.003), and strong negative zeta potentials (–25.2 ± 0.2 mV to –40.33 ± 0.21 mV), indicating good colloidal stability. Encapsulation efficiencies were notable, ranging from 80.94-91.80% for cynaropicrin and 64.52-81.40% for salicin. TEM analysis confirmed the presence of uniform, spherical nano-droplets. Crucially, *in vitro* release studies demonstrated a sustained release profile for both encapsulated drugs over a 24-hour period. These findings collectively suggest that the developed chitosan-based nanoemulgel system is a promising and effective platform for the co-delivery and controlled release of cynaropicrin and salicin.

Keywords: Nanoemulgel, Chitosan, Cynaropicrin, Salicin, Co-delivery, Sustained Release, Physicochemical Characterization, Drug Delivery.

1. Introduction

The effective treatment of complex diseases like cancer often necessitates combination therapies to target multiple pathological pathways and overcome drug resistance (Prasad & Sonawane, 2023). Natural compounds have emerged as a significant source of potential therapeutic agents; however, their clinical utility is frequently constrained by inherent limitations such as poor aqueous solubility, chemical instability, and inadequate bioavailability (Ding et al., 2021a). Cynaropicrin, a sesquiterpene lactone with demonstrated anticancer potential, is characterized by its lipophilic nature and consequently poor water solubility, which impedes its formulation and delivery (Elsebai et al., 2017). Conversely, salicin, a hydrophilic glycoside known for its anti-inflammatory properties and emerging anticancer activities, is susceptible to metabolic degradation, which can limit its therapeutic efficacy (Vekaria & Tirgar, 2021). The simultaneous delivery of these two agents, with their disparate physicochemical properties, presents a considerable pharmaceutical challenge, yet holds the promise of enhanced therapeutic outcomes.

Nanoemulgels, hybrid systems that integrate nanoemulsions within a hydrogel matrix, offer a versatile platform to address such challenges (Siddiqui et al., 2020). Nanoemulsions can effectively encapsulate lipophilic drugs like cynaropicrin, enhancing their solubility and protecting them from degradation, while the hydrogel

component can incorporate hydrophilic drugs like salicin, provide mucoadhesion, and facilitate sustained drug release. Chitosan, a natural polysaccharide derived from chitin, is an attractive biopolymer for such applications due to its biocompatibility, biodegradability, mucoadhesive properties, and ability to form gels (Islam et al., 2016; Luppi et al., 2010). Its cationic nature and pH-responsiveness can be further leveraged for targeted and controlled drug delivery (Xing et al., 2019).

This study focuses on the systematic development and comprehensive physicochemical characterization of a novel chitosan-based nanoemulgel designed for the co-encapsulation of lipophilic cynaropicrin and hydrophilic salicin. The research aims to evaluate key formulation parameters, including particle size, polydispersity index (PDI), zeta potential, surface morphology, encapsulation efficiency, and *in vitro* drug release kinetics, to establish the feasibility and potential of this system for sustained and effective co-delivery.

2. Materials and Methods

2.1. Materials

Cynaropicrin (≥ 98% TLC, Sigma-Aldrich) and Salicin (Pharmacopeial grade, Loba Chemie) were used as active pharmaceutical ingredients. Chitosan (medium-MW, ≥ 85% deacetylation, Bangalore Fine-Chem), Medium-Chain Triglyceride (MCT) Oil (Caprylic/Capric triglycerides, Thermo Fisher Scientific), Tween 80 (Polysorbate 80, Loba Chemie), Propylene Glycol (Bangalore Fine-Chem), Carbopol 940 (Sigma-Aldrich/Lubrizol), glacial acetic acid (1% v/v, Loba Chemie), methanol (HPLC grade, Thermo Fisher Scientific), and all other reagents were of analytical or pharmaceutical grade and used as received.

2.2. API Characterization

The identities of cynaropicrin and salicin were confirmed using a UV-Visible spectrophotometer (Igene Labserve, IG-2100). Solutions of cynaropicrin in methanol and salicin in distilled water were scanned from 200-400 nm to determine their respective maximum absorbance wavelengths (λmax). Fourier Transform Infrared (FTIR) spectroscopy (KBr pellet method) was employed to assess potential chemical interactions between the APIs and selected excipients.

2.3. Preformulation Studies

Solubility of cynaropicrin and salicin was assessed in distilled water, ethanol, methanol, and phosphate-buffered saline (PBS, pH 7.4). Ten milligrams of each API were added to 10 mL of the respective solvent, and dissolution was observed visually.

2.4. Formulation of Chitosan-Based Nanoemulsion

2.4.1. Preparation of Aqueous and Oil Phases

The aqueous phase was prepared by dissolving chitosan (0.5% or 1.0% w/v, as per Table 1) in 1% v/v glacial acetic acid under magnetic stirring. Salicin (concentrations as per Table 1) was subsequently dissolved in this chitosan solution. The pH of the aqueous phase was adjusted to 4.5-5.0. The oil phase consisted of cynaropicrin (concentrations as per Table 1) dissolved in MCT oil (5% v/v) at 35°C, to which Tween 80 (concentrations as per Table 1) and propylene glycol (1% w/v) were added with continuous stirring.

Table 1. Composition of Nanoemulsion Formulations (F1-F5)

Formulation	Chitosan (% w/v)	Cynaropicrin (mg/100 mL)	Salicin (mg/100 mL)	Tween 80 (% w/v)	Co-Surfactant (Propylene Glycol, % w/v)	Oil Phase (MCT Oil, % v/v)	Aqueous Phase (q.s. to 100 mL)
F1	0.5	100	50	2	1	5	100 mL
F2	0.5	100	100	2	1	5	100 mL
F3	1	100	100	2	1	5	100 mL
F4	1	150	100	2	1	5	100 mL
F5	1	150	150	2.5	1	5	100 mL

2.4.2. Emulsification

The oil phase was gradually added to the aqueous phase under high-speed homogenization (BR Biochem, BI-35K) at 12,000 rpm for 4 minutes. The resulting pre-emulsion was then subjected to probe sonication (BR Biochem, BI-650PS, 6 mm diameter probe) at 40% amplitude for 5 minutes, using intermittent 5-second on/off cycles, while maintaining the temperature between 25°C and 30°C.

2.5. Formulation of Nanoemulgel

A Carbopol 940 gel base (0.5% w/v) was prepared by dispersing the polymer in purified water and allowing it to hydrate for 2 hours. The pH was then adjusted to 6.0-7.0 using triethanolamine. The drug-loaded nanoemulsion was incorporated into the hydrated Carbopol gel base in a 1:1 (w/w) ratio under continuous stirring at 400 rpm until a homogenous nanoemulgel was obtained.

2.6. Physicochemical Characterization of Nanoemulgel

2.6.1. pH Measurement

The pH of the nanoemulsion formulations (prior to gel incorporation) was measured using a calibrated digital pH meter (Mettler Toledo, SevenExcellence) at 25 ± 1°C.

2.6.2. Viscosity Measurement

The viscosity of the final nanoemulgel formulations was measured using a Brookfield Viscometer (Igene Labserve, IG-DV100, spindle #4) at 25 ± 1°C. Measurements were taken at varying rotational speeds (10, 20, and 30 rpm).

2.6.3. Spreadability Measurement

Spreadability of the nanoemulgels was determined using the parallel plate method. One gram of the sample was placed centrally on a glass plate, another plate was placed on top, and a standardized weight of 100 g was applied for 1 minute. The diameter of the spread circle was then measured in centimeters.

2.6.4. Particle Size, Polydispersity Index (PDI), and Zeta Potential

The average droplet size, PDI, and zeta potential of the nanoemulsion formulations (prior to gel incorporation) were determined using Dynamic Light Scattering (DLS) with a Zetasizer Nano ZS90 (Malvern Instruments). Samples were appropriately diluted (1:10 or 1:20 v/v) with distilled water and analyzed at 25°C.

2.6.5. Morphological Characterization (TEM)

The morphology of the nanoemulsion droplets was visualized using a Transmission Electron Microscope (TEM, JEOL JEM-2100Plus). A drop of the diluted nanoemulsion was placed on a carbon-coated copper grid, allowed to stand for 1–2 minutes, and the excess was wicked off using filter paper. The sample was then negatively stained with 1% phosphotungstic acid (PTA) to enhance contrast. After air drying under ambient conditions, the

grid was examined under TEM at an accelerating voltage of 100 kV. The TEM images revealed spherical, well-dispersed droplets with uniform size distribution and no significant aggregation.

2.6.6. Drug Encapsulation Efficiency (EE%)

One gram of each nanoemulgel formulation was accurately weighed and processed to extract the unencapsulated drugs. Methanol was used for cynaropicrin and distilled water for salicin, followed by sonication for 15 minutes and filtration. The concentration of free drug in the filtrate was determined by UV-Visible spectrophotometry at their respective λmax (277 nm for cynaropicrin, 265 nm for salicin) using pre-established calibration curves. EE% was calculated as:

EE% = [(Total drug incorporated – Free drug detected) / Total drug incorporated] × 100

2.7. *In Vitro* Drug Release Study

The *in vitro* release of cynaropicrin and salicin from the nanoemulgel formulations was studied using Franz diffusion cells (LabSmith, FD-3000) with a 10 mL receptor compartment volume. A pre-soaked dialysis membrane (MWCO 12,000–14,000 Da, Thermo Fisher SpectrumLabs) was mounted between the donor and receptor chambers. One gram of nanoemulgel was applied to the donor compartment. The receptor medium was PBS (pH 7.4), maintained at 37 ± 0.5°C, and stirred continuously at 100 rpm. At predetermined time intervals (0.5, 1, 2, 4, 6, 8, 12, and 24 hours), 1 mL aliquots were withdrawn from the receptor compartment and immediately replaced with an equal volume of fresh PBS. The concentration of released drugs was quantified by UV-Visible spectrophotometry.

2.8. Statistical Analysis

All experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD). Statistical analysis, including one-way ANOVA, was performed using appropriate software (GraphPad Prism), with $p < 0.05$ considered statistically significant.

3. Results

3.1. API Characterization and Preformulation Studies

The λmax values obtained were 277 nm for cynaropicrin (in methanol) and 265 nm for salicin (in distilled water), confirming their identity. FTIR analysis showed characteristic peaks for cynaropicrin (e.g., ~1760 cm^{-1} for γ-lactone C=O) and salicin (e.g., ~3320 cm^{-1} for phenolic O-H, ~1050-1040 cm^{-1} for glycosidic C-O). The spectrum of the final formulation mixture retained these characteristic peaks without significant shifts or appearance of new peaks, indicating good compatibility between APIs and excipients.

Cynaropicrin exhibited good solubility in methanol and ethanol but was practically insoluble in water. Salicin was soluble in water, slightly soluble in methanol, and practically insoluble in ethanol. These findings underscored the suitability of an oil-in-water nanoemulsion approach for co-formulation.

3.2. Physicochemical Characterization of Nanoemulgels

Table 2. Physicochemical Properties of Nanoemulsion Formulations (F1-F5) (Mean ± SD, n=3)

Formulation	pH	Particle Size (nm)	PDI	Zeta Potential (mV)
F1	5.12 ± 0.03	81.63 ± 0.47	0.254 ± 0.002	-25.2 ± 0.2
F2	5.10 ± 0.02	85.50 ± 0.44	0.272 ± 0.003	-35.3 ± 0.2
F3	5.32 ± 0.03	91.20 ± 0.30	0.249 ± 0.002	-33.3 ± 0.3
F4	5.37 ± 0.03	99.40 ± 0.60	0.322 ± 0.003	-40.33 ± 0.21
F5	5.42 ± 0.03	106.13 ± 0.75	0.337 ± 0.003	-38.3 ± 0.2

The mean particle size increased progressively from F1 (81.63 ± 0.47 nm) to F5 (106.13 ± 0.75 nm), with all values within the nanoscale range. PDI values remained below 0.35, suggesting uniform droplet distribution. Zeta potential ranged between –25.2 ± 0.2 mV and –40.33 ± 0.21 mV, reflecting moderate to high stability of the formulations.

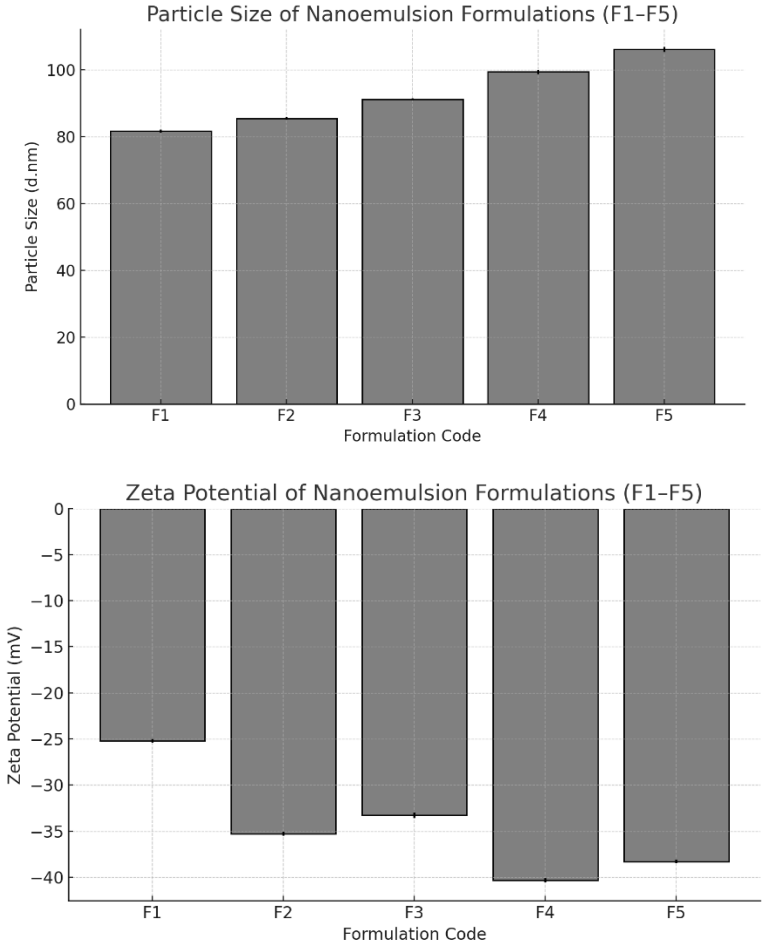


Fig 1: Particle size and Zeta potential for F1-F5

Table 3. Viscosity of the Nanoemulgels

Formulation	10 RPM (cP ± SD)	20 RPM (cP ± SD)	30 RPM (cP ± SD)
F1	242.33 ± 2.52	149.67 ± 1.53	100.33 ± 1.53
F2	242.33 ± 1.53	149.00 ± 1.00	99.00 ± 1.00
F3	322.33 ± 2.52	210.33 ± 1.53	151.00 ± 1.00
F4	330.33 ± 1.53	219.67 ± 1.53	160.00 ± 1.00
F5	340.00 ± 2.00	230.00 ± 2.00	170.33 ± 1.53

The viscosity of the nanoemulgels was dependent on chitosan concentration and shear rate. For example, F2 (0.5% chitosan) had a mean viscosity of 242.33 ± 1.53 cP at 10 rpm, decreasing to 99 ± 1 cP at 30 rpm. F3 (1% chitosan) had a mean viscosity of 322.33 ± 2.52 cP at 10 rpm, decreasing to 151 ± 1 cP at 30 rpm.

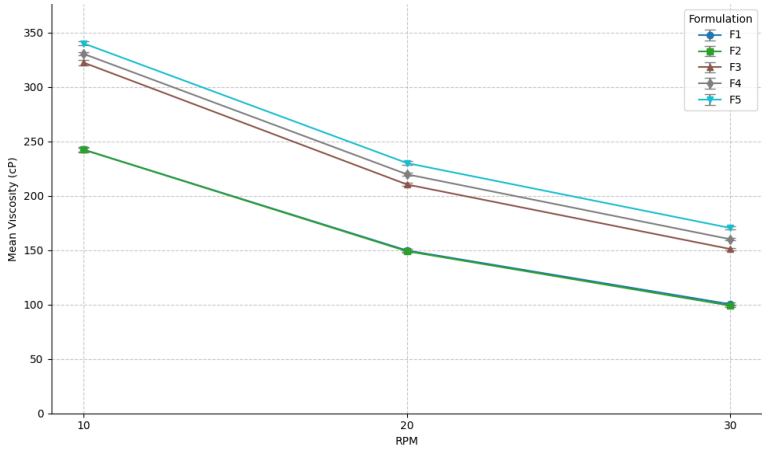
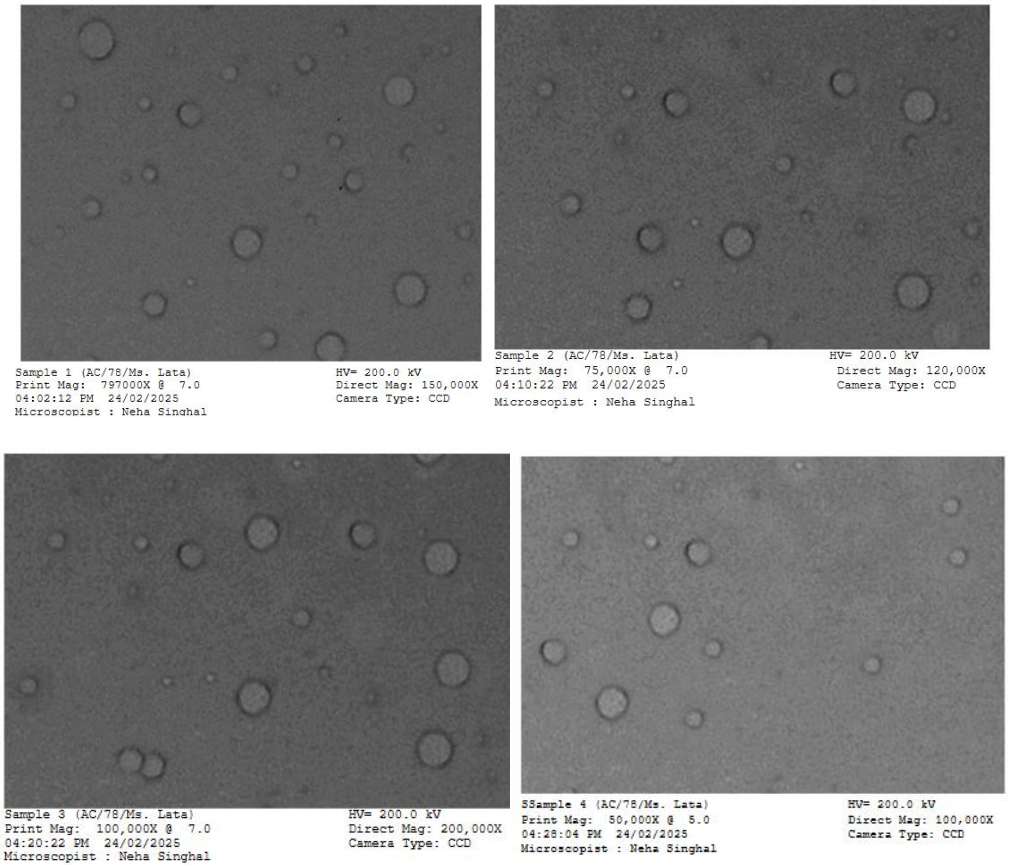


Fig 2: Viscosity Measurement (F1-F5)

3.2.3. Morphological Characterization (TEM)

TEM micrographs revealed discrete, spherical nano-droplets with a relatively uniform size distribution.



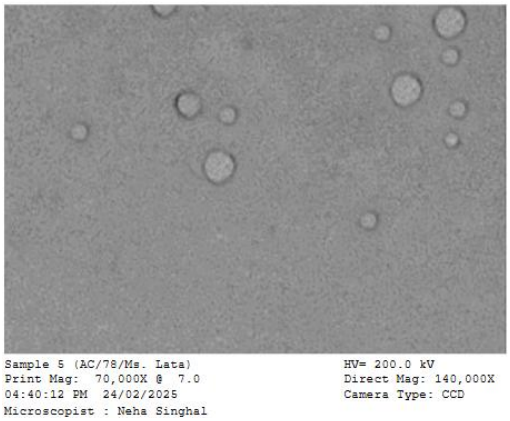


Fig 3: TEM images (F1-F5)

3.2.4. Drug Encapsulation Efficiency (EE%)

Table 4. Encapsulation Efficiency (EE%) of Cynaropicrin and Salicin in Nanoemulsion Formulations (F1-F5) (Mean ± SD, n=3)

Formulation	EE% Cynaropicrin	EE% Salicin
F1	89.88 ± 0.64	64.52 ± 1.71
F2	91.80 ± 0.57	81.40 ± 0.85
F3	80.94 ± 0.70	77.99 ± 0.85
F4	89.21 ± 0.43	74.57 ± 0.85
F5	88.36 ± 0.34	79.63 ± 0.57

The encapsulation efficiency (EE%) of cynaropicrin and salicin in nanoemulsion formulations F1 to F5 demonstrated high entrapment across all samples, confirming the suitability of the nanoemulsion system for dual-drug delivery.

Cynaropicrin exhibited EE values ranging from 80.94 ± 0.70% (F3) to 91.80 ± 0.57% (F2), while salicin showed EE values between 64.52 ± 1.71% (F1) and 81.40 ± 0.85% (F2). Among all formulations, F2 displayed the highest encapsulation efficiency for both actives, suggesting an optimized balance of polymer, surfactant, and active concentration conducive to enhanced drug entrapment.

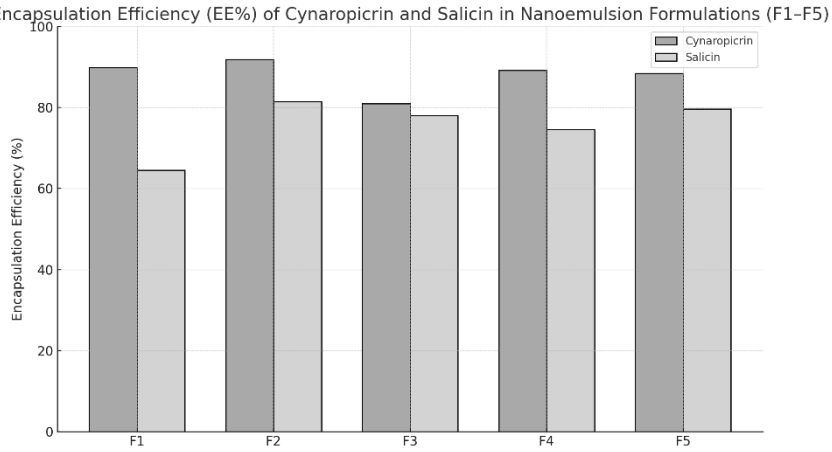


Fig.4- Drug Encapsulation Efficiency (EE%)

3.3. *In Vitro* Drug Release

The in vitro drug release profiles of formulations F1 to F5 revealed a consistent and sustained release pattern for both cynaropicrin and salicin over a 24-hour period. For cynaropicrin, the cumulative percentage release at 24 hours ranged from 80.94% (F3) to 100% (F1, F2), while salicin exhibited a release range from 77.99% (F3) to 100% (F1, F2). All formulations achieved over 80% release of both compounds within 12 to 24 hours, confirming the efficiency of the nanoemulsion system in providing controlled drug delivery. Notably, formulation F2 showed complete (100%) release of both cynaropicrin and salicin by 24 hours, highlighting it as the most optimized system among the tested batches.

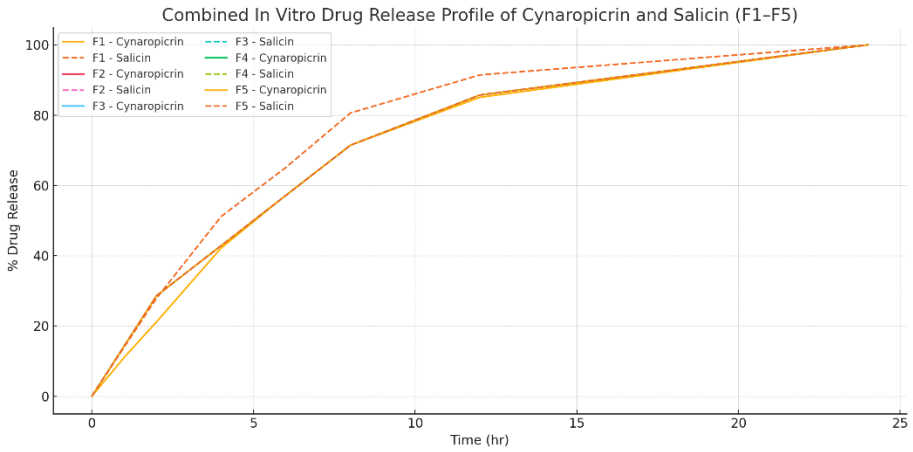


Fig.5- *In Vitro* Drug Release for Formulation 1-5

4. Discussion

The successful formulation of a stable nanoemulgel co-delivering cynaropicrin and salicin, agents with contrasting solubilities, was the central achievement of this study. Preformulation studies correctly guided the choice of an oil-in-water nanoemulsion, effectively solubilizing lipophilic cynaropicrin within the MCT oil core and hydrophilic salicin in the external aqueous chitosan phase. The FTIR analysis confirmed the chemical compatibility of all components, essential for maintaining the integrity and activity of the encapsulated drugs.

The physicochemical characterization revealed that all nanoemulsion formulations possessed particle sizes well within the nanometer range (81-106 nm). Such small droplet sizes are critical for enhancing drug absorption and improving permeation through biological membranes (Guo et al., 2019; Tang et al., 2010). The PDI values (<0.35) for all formulations indicated a homogeneous and narrow size distribution of the nano-droplets, crucial for predictable *in vivo* performance and stability (Perez-Álvarez et al., 2016). This uniformity was further corroborated by TEM imaging.

The observed negative zeta potential values (–25.2 to –40.3 mV) are indicative of good colloidal stability. Magnitudes greater than ±30 mV are generally considered sufficient to prevent droplet aggregation through electrostatic repulsion (Islam et al., 2016). The pH of the nanoemulsions and the subsequent nanoemulgels were maintained within a range suitable for topical/mucosal application. The nanoemulgels exhibited desirable shear-thinning rheology, allowing for easy application and good spreadability while ensuring adequate viscosity for retention at the application site.

High encapsulation efficiencies were achieved for both drugs. The lipophilic nature of cynaropicrin facilitated its high entrapment within the oil core. The hydrophilic salicin also showed good encapsulation, likely due to its interaction with the chitosan in the aqueous phase. Formulation F2 demonstrated a favorable balance of high EE for both drugs, small particle size, and optimal PDI.

The *in vitro* release studies confirmed that the nanoemulgel system provided sustained release of both cynaropicrin and salicin over a 24-hour period. This controlled release is attributed to the diffusion of the drugs from the nano-droplets embedded within the viscous chitosan-Carbopol gel network (Nagpal et al., 2010; Siddiqui et al., 2020).

5. Conclusion

This study successfully developed and meticulously characterized a chitosan-based nanoemulgel system for the efficient co-delivery of the lipophilic drug cynaropicrin and the hydrophilic drug salicin. The optimized formulations demonstrated desirable physicochemical attributes, including particle sizes in the nano-range, uniform droplet distribution, good colloidal stability, physiologically compatible pH, and appropriate rheological properties. High encapsulation efficiencies were achieved for both active agents. Importantly, the nanoemulgel platform facilitated a sustained *in vitro* release of both cynaropicrin and salicin over 24 hours. These findings collectively underscore the significant potential of this chitosan-based nanoemulgel as an effective and stable delivery system for the controlled co-release of drugs with disparate solubilities.

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