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Dual Drug Loaded Nanoparticles For Enhanced Pain Relief And Anti-Inflammatory Actions

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) and steroidal anti-inflammatory drugs (corticosteroids) are curative management regimen for alleviating pain and anti-inflammatory effects. The aim of study was to create a novel dual-drug of NSAID-corticosteroid encapsulated into chitosan (CS) nanoparticles to increase their solubility, attain a sustained release system and reduce systemic adverse effects. The ionic gelation method, which provides a mild and effective way for drug encapsulation, was utilized to create naproxen-dexamethasone (nap-dex) loaded chitosan nanoparticles (CNPs). Nap-dex loaded CNPs were defined by UV-Visible spectrophotometry, zeta size, scanning electron microscope (SEM) and fourier transform infrared spectroscopy (FTIR). Zeta size analysis identified nanoparticles with an adequate size and stability for drug delivery, while the UV and FTIR spectra confirmed successful drug loading and interaction with the chitosan matrix. SEM analysis revealed a spherical shape with smooth surface properties. *In vitro* drug release study was done by using dialysis membrane method revealing a sustained and controlled release profile of the dual drug. T-test confirmed that all pairwise comparisons between time intervals were statistically significant ($p < 0.05$). The prepared nano dual drug is a novel therapy that showed a

sustained release profile and enhanced bioavailability with an excellent anti-inflammatory activity

Introduction

Pain is a serious medical condition which affects not just those who experience it but also the larger society and economy (Li *et al.,* 2024; Aqsa Naseem *et al.,* 2026). Neuropathy, arthritis, and musculoskeletal problems are among the most prevalent and significant causes of disease, pain, and disability (Hua and Cabot, 2013). Opioids and corticosteroids are used for pain management but there are numerous drawbacks to this one-drug approach to treatment (Yong *et al.,* 2022; Fiza Nasir *et al.,* 2026). Currently, opioids, adjuvant analgesics (anticonvulsants and antidepressants), and non-opioid painkillers (steroids, local anesthetics, and NSAIDs) are utilized to alleviate pain (Paul *et al.,* 2021).

The reaction of immune system to hazardous signals like infections, injured cells, radiation, or toxins, aimed at removing these threats and starting the body's repair processes (Öztürk *et al.,* 2020; Salma Sabir *et al.,* 2026). The production of prostaglandins is highly influenced by the cyclooxygenase enzymes (COX-1 and COX-2) (Laine *et al.,* 2008; Chen *et al.,* 2017). Prostaglandins are essential in various biological functions, such as immune regulation, kidney formation, reproductive processes, and maintaining gastrointestinal health (Nguyen *et al.,* 2009).Corticosteroids and NSAIDs are two often administered kinds of anti-inflammatory medications (Tahreem Fatima *et al*., 2026; Muhammad Usman Arif *et al*., 2025).

Aspirin and other NSAIDs block cyclo-oxygenase irreversibly and reversibly, respectively (Rabiya Javaid *et al.,* 2025). However, their use has been correlated with gastrointestinal problems like stomach ulcers and bleeding, which can sometimes turn into deadly illnesses (Muhammad Nabeel Sharif *et al.,* 2025). An estimated 15–30% of chronic NSAID users develop stomach ulcers and bleeding (Mizushima, 2010). One synthetic glucocorticosteroid with little mineralocorticoid action is dexamethasone. It is regarded as a strong anti-inflammatory medication (Soumya and Joe, 2021). Among the several kinds of drugs used to treat pain, non-opioid drugs like corticosteroids, local anesthetics, and non-steroidal anti-inflammatory drugs are advised for minor to moderate discomforts (Maestrelli *et al.,* 2016).

Combination therapy has been extensively studied in labs and clinical settings with the goal of demonstrating non-overlapping toxicities and synergistic pharmacological mechanisms (Mokhtari *et al.,* 2017). In recent years, several sophisticated delivery methods have been created to improve the safety, drug effectiveness and bioavailability of known pain-relievers in order to get around the drawbacks and restrictions of traditional formulations (Muhammad Aleem *et al*., 2026). A promising treatment for inflammatory diseases is to utilize nanomedicines (nanoparticles encapsulated with a therapeutic active principle) to treat inflammation by identifying molecules overproduced on the endothelial cells surface or active macrophages, increasing the permeability of the vasculature, or even biomimicry (Mishra *et al.,* 2024). The powerful treatment of all human diseases depends on the potential to deliver extremely powerful therapeutic chemicals precisely to diseased locations (Ren *et al.,* 202). Dual drug-loaded nanoparticles have emerged as a viable strategy for improving pain relief and anti-inflammatory properties (Assali *et al*., 2018). Arbitrary arrangement of β-(1,4)-linked glucosamine and N-acetylglucosamine residues to form chitosan, an aminoglucopyran made from chitin that is present naturally (Hembram *et al.,* 2016). Chitosan finds multiple applications because it is non-toxic, biodegradable and has antimicrobial characteristics (Divya and Jisha, 2017). Dual drug loaded chitosan nanoparticles were formulated by ionic gelation method (Peer *et al.,* 2007; Fariha *et al.,* 2025).

METHODOLOGY

Ionic gelation method for Nap-Dex loaded chitosan nanoparticles (N-DLCNPs)
Dual drug loaded chitosan nanoparticles were synthesized by ionic gelation method. 1% acetic acid solution (1 mL acetic acid in 99 mL of deionized water) was prepared. pH of solution was adjusted from 4-5. Sodium tripolyphosphate solution was prepared by adding 50 mg of TPP in 8 mL of deionized water. Ultrasonic bath was also used for 2-3 minutes to ensure mixing of drugs and to form a homogenized solution. To synthesize Nap-Dex loaded chitosan nanoparticles, 20 mL of prepared (1%) chitosan solution was added in a beaker and put on magnetic stirrer. Each drop of TPP interacts with the chitosan, neutralizing its positive charge, causing it to form a complex and put it on magnetic stirrer for almost 8 hours for the suspension creation of chitosan nanoparticles. The whole suspension was transferred in plastic falcon tubes and allow it to centrifuge in ultracentrifugation machine at 15,000 rpm for 30 minutes at -4℃. After centrifugation pellet was visible.It was dried in Hot air oven for 3-4 hours. After that fine powder of nanoparticles was obtained for further analysis.

Physiochemical properties of formed nanoparticles
Ultraviolet–visible Spectroscopy (UV-Vis)-
To confirm the creation of nanoparticles the solution went through scanning in the range from 200-800 nm in a spectrophotometer using a quartz cuvette with deionized water as the reference.

Scanning Electron Microscopy (SEM)-
Dried chitosan nanoparticles' size and shape were investigated in the Hitachi S-238ON (Central Hi-Tech lab, UAF). A tiny quantity of produced chitosan nanoparticle samples placed on a SEM stub using two-sided adhesive tape and heated to 50 mA for six minutes using a sputter. The sample-containing stub was then put into the scanning electron microscopy (SEM) chamber. 20 KV of acceleration voltage was utilized to take the photomicrograph.

Dynamic Light Scattering (DLS)-
Particle size distribution of drug loaded chitosan nanoparticles was examined through DLS with Litesizer 500 (Hi-tech lab, UAF). The analysis was carried out at a Back scatter (automatic) measuring angle, at 23°C temperature by using dispersed nanoparticles in deionized water (pellet was dissolved in 1mL of deionized water and dispersed by vortex). The nanoparticles size dispersion expressed as polydispersity index (PDI), mean hydrodynamic diameter (MHD) and standard deviation (SD).

Fourier transform-infrared (FT-IR) spectroscopy-
To identify degree of deacetylation and nanochitosan structure were executed by FTIR spectroscope (Cary 630, Agilent Technologies, USA) functioned in the 4000-650 cm^{-1} wavelength range. FTIR is necessary to guarantee the creation of the nanoparticle solution through the assessment of the functional groups of chitosan and STPP in the chitosan nanoparticle solution. By correlating its functional group and structure, FTIR offers details about the functional characteristics of nanochitosan.

***In-vitro* drug release profile**
Membrane diffusion method was utilized for *in-vitro* drug release profile of naproxen and dexamethasone from nap-dex loaded CNPs. Phosphate buffered saline (PBS) solution was prepared with pH of 7.4 at 37℃. Nap-dex loaded CNPs were first centrifuged to obtain a pellet, which was dispersed in 4 mL of medium separately, placed into pre-soaked dialyzing membrane and sealed safely. The dialysis bag was submerged in 50 mL of PBS buffer. The solution was put on a magnetic stirrer at 200rpm and 37℃. A 3 mL aliquot of the dissolving fluid was taken out at various time

intervals and replaced with the same amount of new media each time. The collected samples were withdrawn at different time intervals and UV-visible spectrophotometer was used for analysis at specific wavelength according to (Paswan and Saini, 2021) and the % cumulative release was accessed.

Modeling drug release

The release of drug from nanoparticles is crucial in determining its pharmacological effects.. *In vitro* techniques for nano-formulations are increasingly being used to predict in vivo drug release. Estimating the processes of drug release, aiding in creation of formulation, and creating methods of controlled drug release are just a few of the many benefits of mathematical models. Both the dose forms of NPs and the evaluation of drug release are intricate. The models that are used to study drug release are Korsmeyer-Peppas model, Higuchi Model, First order Release Kinetics Model, Zero-order Release Kinetics Model.

RESULTS AND DISCUSSION

Preparation of (Nap-Dex) loaded chitosan nanoparticles

Naproxen-Dexamethasone loaded chitosan nanoparticles were synthesized by Ionic gelation method. The non-toxic, organic solvent-free, easy, and controlled nature of the ionic gelation technique has drawn a lot of attention (Fan *et al.*, 2012).

The ionic gelation approach relies on the ionic interactions between the negatively charged groups of polyanion, like sodium tripolyphosphate (TPP), which is the commonly utilized ion cross-linking agent because of its versatile and non-toxic qualities, and the positively charged primary amino groups of chitosan (Shu and Zhu, 2002).

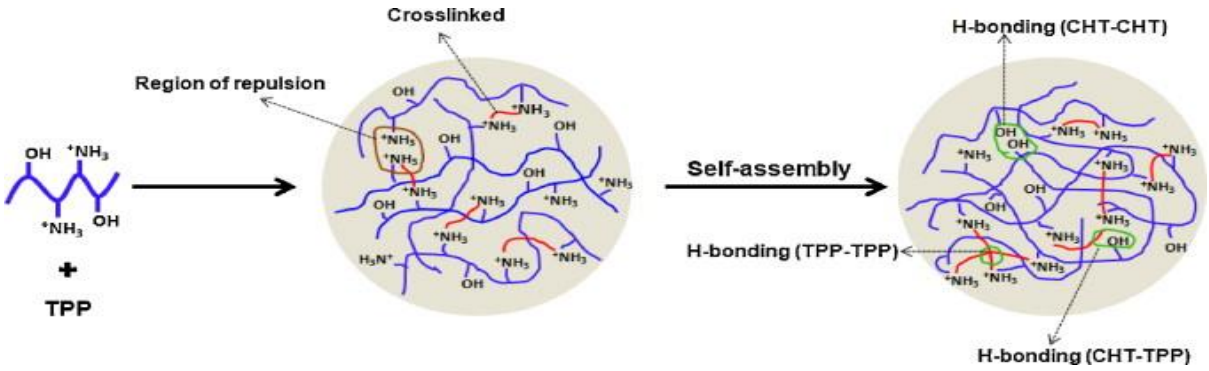


Fig. 1- Schematic demonstration of ionic crosslinking reaction between chitosan and TPP

Characterization of nanoparticles

UV-Visible spectroscopy

There is a prominent peak in absorbance occurring roughly between 280nm and 300nm with the maximum absorbance value reaching slightly above 0.2. It provides evidence for effective loading of therapeutic agents in chitosan nanoparticle system. In a study by Agarwal *et al.*, a peak at 226 nm was observed in the U.V. spectrophotometer used to characterize the prepared CSNPs; this could be because chitosan contains an amido group (Agarwal *et al.*, 2018). In 2021, Liu *et al.*, observed a peak of chitosan at 201 nm (Liu *et al.*, 2021).

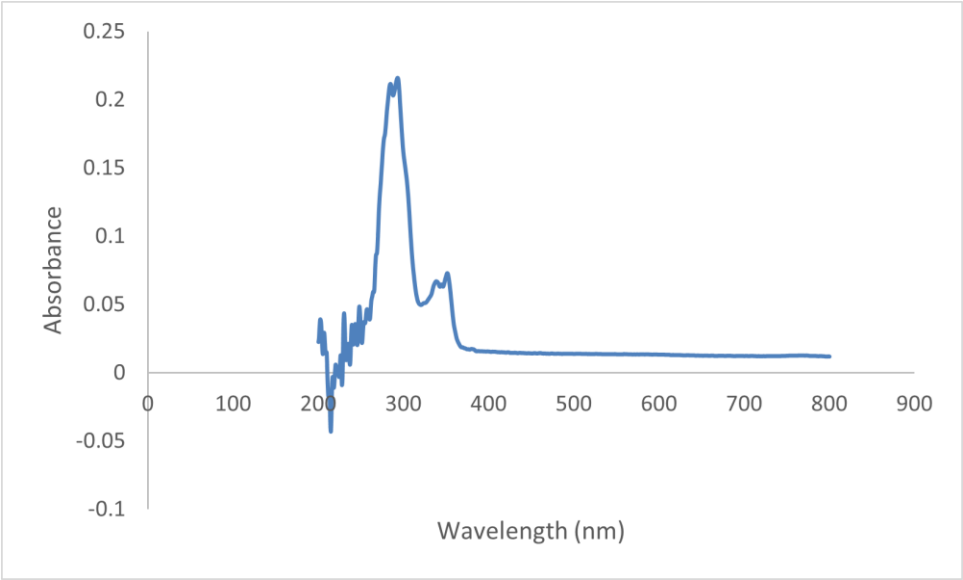


Fig. 2- UV-Visible Spectroscopy

Dynamic Light Scattering (DLS) Analysis-

Using the Dynamic Light Scattering (DLS) approach, which is based on the diffusion of Brownian motion, one can analyze the size and homogeneity of nanoparticles (Rosyada *et al.,* 2019. Hydrodynamic diameter was measured in the nanoscale range using DLS. Nanoparticles are solid particles with a size ranging from 10-1000 nm (Mohanraj and Chen, 2006) and the findings of this research fall within these ranges. In a previous study done by Zegre *et al.,* the particle size of Minocycline-Voriconazole NPs obtained from DLS was 466.8 ± 13.9 nm (Zegre *et al.,* 2025). According to Avadi *et al*., the polydispersity index (PDI) ranges from 0 to 1, with a value near 0 indicating a homogeneous nanoparticle solution and a value above 0.5 indicating a heterogeneous nanoparticle solution (Avadi *et al.,* 2010).

In this study, a 0.1% chitosan concentration with an 85% deacetylation degree was employed. The nanoparticles will get bigger as the concentration rises. Mechanical treatment is another element that affects how chitosan nanoparticles develop. Below 1000 nm, size reduction with a magnetic stirrer can result in more stable particles of a more uniform size.

SEM Analysis

The morphology of CNPs was examined and the results were obtained. CNPs revealed a very homogenous morphology and they are spherical in shape. In a published study, ionic gelation was used to create chitosan nanoparticles cross-linked with TPP that were loaded with gentamicin and salicylic acid.

FTIR Analysis

Using an FTIR spectrophotometer, the structure and degree of deacetylation of the nanochitosan were determined. By assessing the existence of chitosan and TPP functional groups in the chitosan nanoparticle solution, this is necessary for ensuring the creation of the nanoparticle solution.

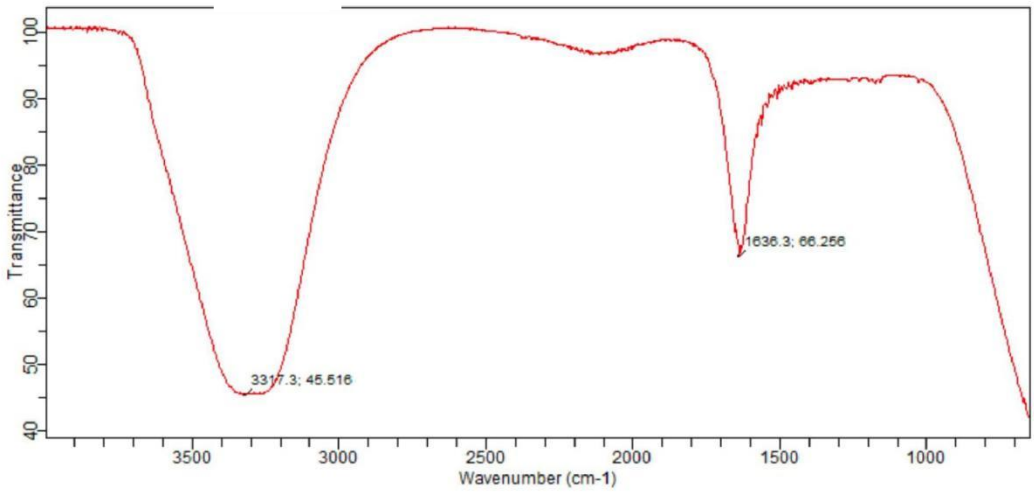


Fig. 3- FTIR analysis

A wide peak between 3500-3000 cm^{-1} areas was attributed to hydrogen-bonded (O-H) stretching vibration also overlapping of N-H stretching from primary amine groups in chitosan. The peak formed at 1636.3 cm^{-1}indicated amide I band (C=O stretching) from chitosan's residual N-acetyl groups and overlap with C=O stretching of naproxen (carboxylate group) and dexamethasone (ketone).

The use of tripolyphosphate (TPP) and surfactants (Tween 80) may improve the ionic crosslinking between chitosan molecules and improve the mechanical qualities of chitosan, which are inherently fragile. When compared to micro-scale materials, this technique has been shown to enhance the mechanical, thermal, barrier, and physicochemical properties of the nanoparticles. In fruit cell tissue, the chitosan's adsorption capability can be increased, and it became more stable.

Table 1- Characterization of prepared nap-dex loaded CNPs

Sr. no	Methods	Results
1	UV Spectroscopy	Peak observed at 295 approximately
2	DLS	Size 339.2 ± 106.4nm
3	SEM	Homogenous and spherical in shape
4	FTIR	Peak of OH group of chitosan becomes wider and was obtained at 3317.3 cm^{-1}

***In-vitro* drug release profile**

In-vitro release profile of nap-dex-CNPs was observed over 24 hour period by UV-Visible spectrophotometer. The release study demonstrated a sustained and controlled release behavior for the co-encapsulated drugs, naproxen sodium and dexamethasone.

Table 2- Absorbance peaks (330nm) for each time point

Time	Absorbance (Mean ± SD)	Concentration (µg/mL ± SD)	% Cumulative release	Progress
1 hour	0.742 ± 0.001	27.77 ± 0.045	58.5 ± 0.09	Initial burst release
4 hr	0.746 ± 0.001	27.92 ± 0.045	58.8 ± 0.09	Slight modification
20 hr	0.897 ± 0.001	33.97 ± 0.045	71.1 ± 0.09	Sustained release phase
24 hr	1.254 ± 0.001	47.46 ± 0.045	100 ± 0.0	Maximum release (plateau)

The cumulative percentage of drug release increased gradually from 58.5% at 1 hour to 100% at 24 hours, indicating a consistent and prolonged release pattern. The initial phase (1–4 hours) showed a minor increase from 58.5% to 58.8%, suggesting a limited burst release, likely due to surface-associated drug molecules. From 4 to 20 hours, the release rise to 71.1%, followed by a significant increase to complete release (100%) at 24 hours. The increased maximum cumulative release of minocycline in Min-Vor-NPs points to variations in drug packing within the polymer matrix when voriconazole is present, which increases the overall release of minocycline.

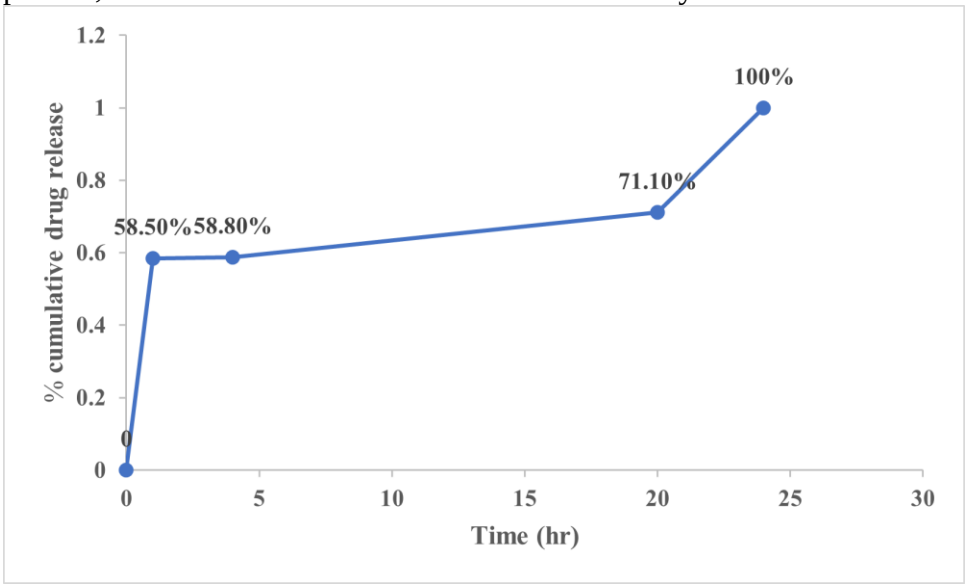


Fig. 4- % Cumulative release profile vs Time

The ionic gelation method used for nanoparticle synthesis is also known to create tight, stable matrices, contributing to sustained release.

Drug release kinetics

To determine drug release mechanism from nap-dex loaded CNPs, Korsmeyer-Peppas model, Higuchi Model, First order Release Kinetics Model, Zero-order Release Kinetics Model were used.

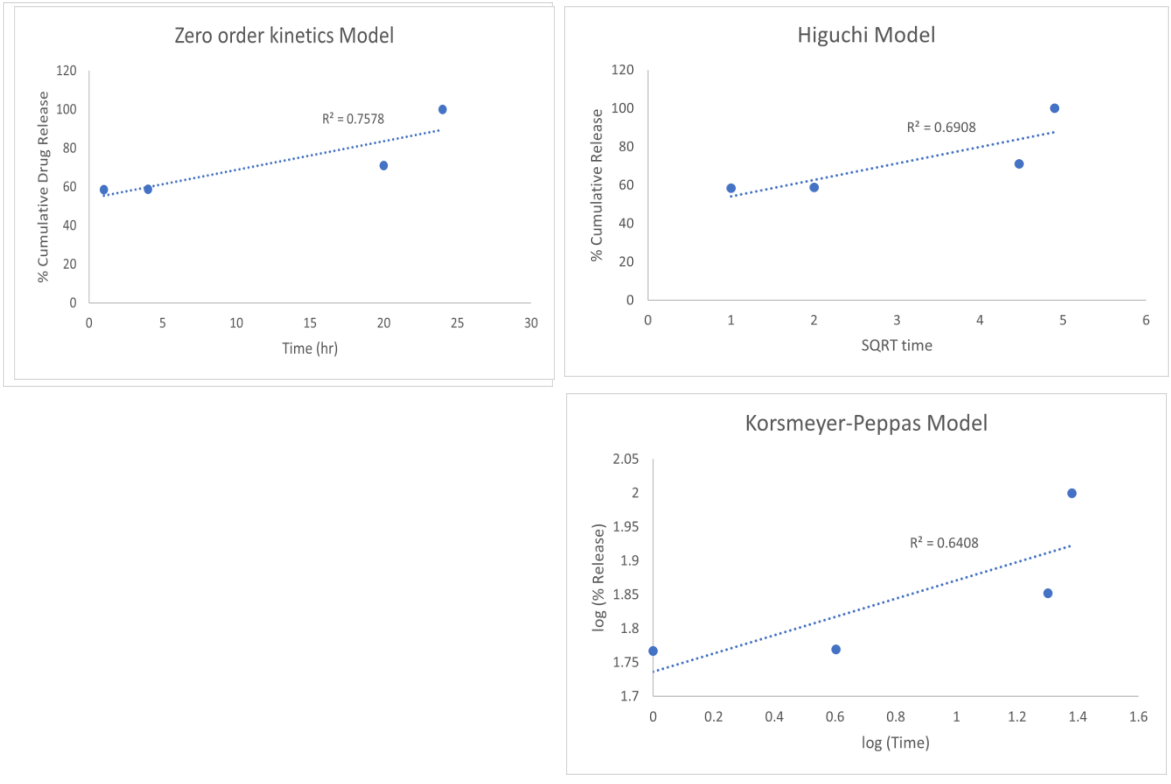


Fig. 5- Graphs showing different Order kinetics

Among all models, first order kinetics model displaying the highest correlation coefficient (R^2 = 0.9832), indicating that this model best describes the drug release kinetics. It suggested that release rate is proportional to the remaining drug concentration in the nanoparticles, supporting a concentration dependent release mechanism. This model is compatible with controlled release systems in which the predominant mechanism is diffusion through the polymer matrix.
The release exponent from Korsmeyer–Peppas model n = 0.192 (less than 0.45), points to a Fickian diffusion mechanism. This model does not, however, accurately reflect the complete release profile, as evidenced by the lower R^2 value and divergence from linearity brought on by the initial burst release and the 100% release at 24 hours.

Table 3- Drug Release Kinetics - Model comparison

Model	**R^2 value**	**Mechanism Indicated**	
Zero Order	0.758	Constant release rate	Moderate fit
First Order	**0.983**	Concentration-dependent release	Best fit; explains controlled release
Higuchi	0.691	Fickian diffusion	Fair fit; lack accuracy after 4hr
Korsmeyer-Peppas	0.640	Fickian diffusion (n=0.192)	Poor fit, due to burst and plateau

Based on the kinetic modeling, the first-order model best describes the release behavior of the dual drug-loaded chitosan nanoparticles, indicating a concentration-dependent, controlled release mechanism suitable for long-term therapeutic action.

Statistical Analysis

According to the statistical analysis of *in vitro* drug release data, drug release from the dual drug-loaded chitosan nanoparticles varied significantly over time. The release of the co-encapsulated medicines was not constant but rather increased over time, as evidenced by the extremely significant variations in mean absorbance values across all time periods ($p < 0.0001$) found by one-way ANOVA. To further identify where these differences occurred, Tukey's (HSD) post hoc test was applied. The results confirmed that all pairwise comparisons between time points were statistically significant ($p < 0.05$), thereby validating that each time interval represented a distinct stage in the drug release process.

CONCLUSION

dual drug-loaded chitosan nanoparticles encapsulating naproxen sodium and dexamethasone were successfully formulated via ionic gelation method as a novel nanotechnology-based combination therapy. The optimized formulation exhibited nanometric size (339.2 nm), spherical morphology, and efficient dual-drug entrapment with sustained biphasic release following first-order kinetics. This system offers synergistic anti-inflammatory and analgesic effects while overcoming the limitations of conventional monotherapies such as high dosage, systemic side effects, and poor bioavailability. By providing controlled, concentration-dependent release, it minimizes dosing frequency and improves patient compliance. Thus, Nap-Dex loaded CNPs represent a promising, biocompatible, and clinically translatable platform for effective pain and inflammation management, warranting further in-vivo and stability studies.

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