Naringin as Sustained Delivery Nanoparticles Ameliorates the Anti-inflammatory Activity in a Freund’s Complete Adjuvant-Induced Arthritis Model

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ABSTRACT: Naringin (NAR), a naturally occurring essential flavonoid, present in grapefruit and Chinese herbal medicines, creates great interest in researchers due to its diverse biological and pharmacological activities. However, further development of NAR is hindered due to its poor water solubility and dissolution rates in GIT. To address these limitations, in this study, we report polymeric nanoparticles (NPs) of NAR (NAR-PLGA-NPs) for enhancing the oral NAR efficiency, with a biodegradable polymer (PLGA) to improve its absorption and bioavailability. NAR-PLGA-NPs were fabricated by a modified solvent emulsification—evaporation technique. Physicochemical properties were evaluated by SEM, particle size distribution, entrapment efficiency, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and differential scanning calorimetry (DSC). In vitro drug release and ex vivo permeation studies were carried out in phosphate buffer (pH 6.8) for 24 h. Furthermore, in vivo anti-arthritic studies were performed on a mouse model, and the results were compared with free NAR. The modulation of inflammatory mediators was also evidently supported by docking studies. Optimized nanoformulation FN4 (NAR-PLGA-NPs) prepared with acetone−ethanol (2:1) as a solvent system in a combination of stabilizers, i.e., poloxamer-188 and sodium deoxylate (1:1), along with 2% PVA solution, was prepared. From size characterization studies, it was observed that nanoformulations possessed a low particle size (179.7 ± 2.05 nm), a low polydispersity index (0.206 ± 0.001), and a negative zeta potential (−9.18 ± 0.78 mV) with a maximum entrapment efficiency (74 ± 3.61%). The drug release followed a Korsmeyer−Peppas release kinetic model (anomalous non-Fickian diffusion), providing greater NAR release after lyophilization (82.11 ± 3.65%) drug release in pH 6.8 phosphate buffer for 24 h. Ex vivo permeation analysis through an isolated goat intestinal membrane revealed 80.02 ± 3.69% drug release in 24 h. Encapsulation of a drug into PLGA is well described by the results of FTIR, DSC, and XRD. Finally, the therapeutic efficacy of optimized FN4 (NAR-PLGA-NPs) and its possible application on RA were further confirmed in a Freund’s complete adjuvant-induced rat arthritic model as against free NAR at a dose of 20 mg/kg body wt. Our findings demonstrate that sustained action of NAR from optimized FN4 NPs with a rate-controlling polymeric carrier system exhibited prolonged circulation time and reduced arthritic inflammation, hence indicating the possibility as a novel strategy to secure the unpropitious biological interactions of hydrophobic NAR in a gastric environment.

1. INTRODUCTION

Naringin (NAR) is a promising nutraceutical with diverse pharmacological effects such as anticancer, anti-inflammation, anti-oxidant, and blood lipid-lowering activities.1,2 Grapefruits are a source of this phytochemical where it is extracted as a glycosylated flavanone, formed by naringenin and the disaccharide neohesperidose.3,4 NAR formulations are widely used in Traditional Chinese Medicine to treat various ailments as long-term administration has proved it to be a safe and efficient drug.3 From a recent study, it was reported that arthritis is the UK’s second-most burden disease where there is a need for discovery and development of novel anti-arthritic agents. In this context, natural compounds such as curcumin, berberine, and quercetin are helpful for their potent therapeutic effect and fewer side/toxic effects.

Apart from these, NAR has also shown promising results in the treatment of osteoarthritis (OA), rheumatoid arthritis (RA), and ankylosis spondylitis, restoring the inflamed cartilage tissues that severely affect a patient’s life. Recently, NAR’s potential was well noticed in averting cartilage ruination of the monosodium-iodoacetate-induced OA model in rats (5 and 10 mg/kg, oral route, pure drug). These studies revealed the improvement of OA rats in weight-bearing ability, with the inhibition of NF-kB in
affected rats, which is considered to be the key mediators of OA progression.\textsuperscript{6,7} In parallel, NAR exhibited therapeutic efficacy in the disuse-induced osteoporosis animal model due to mechanical unloading and was mostly observed in astronauts or any bedridden patients. Wei et al. showed improved levels of calcium and phosphorus, and the osteoporotic condition when NAR was administered in the retinoic acid-induced OA model of rats at different doses of 20, 40, and 100 mg/kg.\textsuperscript{8} Ramasamy et al. demonstrated Notch activity of endothelial cells that plays a vital role in osteogenesis promotion and bone angiogenesis, suggesting NAR’s osteoprotective effect.\textsuperscript{9} Ahmed et al. investigated NAR treatment in autoimmune arthritis that was able to significantly regulate expressions of IL-4 and T regulatory cells, which mitigated cartilage proteoglycan exhaustion induced by pro-inflammatory cytokines.\textsuperscript{10} However, in the CIA rat model, oral treatment of NAR significantly ameliorated the damage of knee joints (intercondylar), infiltration of the synovium, and pannus development.\textsuperscript{11} Recent investigations on the anti-arthritis activity of NAR also well correlated with various cell mediators and inflammatory proteins that are responsible for facilitating cellular infiltration.\textsuperscript{12} From the available reports, it is conclusive that NAR has potent chondroprotective effects in osteoarticular degenerative diseases like RA.

Despite NAR’s diverse curative potentials in many pathological conditions, particularly in autoimmune degenerative illness, it is yet to be approved for clinical translation. A few known reasons are as follows: NAR displays instability in harsh pH (acidic) and is enzymatically cleaved by β-glycosidase in the stomach. Also, NAR degrades in the blood circulation when administered intravenously, with a half-life of 2.6 h.\textsuperscript{13,15} However, its susceptibility to oxidation, poor water solubility, and dissolution rates hinders its bioavailability (about 8.8%), resulting in slow irregular absorption (BCS-class II). To overcome these limitations, alternative formulations of NAR are required for improving its bioavailability and absorption so as to protect the drug against degradation in GIT by delivering them to the intestine with a sustained release effect.

In recent years, the nanotechnology-based drug delivery system has proved to be successful in enhancing the effectiveness of both pharmaceuticals as well as nutraceuticals. Among these, polymeric NPs serve as an attractive tool to address problems of poor water solubility. It facilitates improvement in solubility, bioavailability, and pharmacokinetics of the entrapped drug and prevents degradation. To minimize the unwanted side effects, control of particle size and particle size distribution (polydispersity) is of great significance. For example, nano-formulations of berberine employing PLGA have shown higher efficacy against free berberine in Freund’s complete adjuvant (FCA)-induced arthritic rats.\textsuperscript{16}

To date, there are different approaches that are being adopted to enhance the bioavailability and poor water solubility problem associated with NAR. Mohamed et al. developed NAR polymeric micelles based on PF68 and reported the cytoprotective activity in the ethanol-induced ulcer rat model and antitumor activity against carcinoma (Ehrlich ascites) in mice.\textsuperscript{17} In another study, naringenin liposomes (elastic) were prepared for topical medication including Tween-80 and cholesterol (in different proportions), suggesting that nanoparticles are promising tissue-specific targeting structures with increased drug loading and sustained release characteristics.\textsuperscript{18} Kandhare et al. showed the capability of NAR ointment formulation in wound healing in experimental rats through the regulation of apoptotic and growth mediators.\textsuperscript{19}

Poly-co-glycolic acid (PLGA) is an FDA-authorized amiphathic copolymer that can be developed into NPs easily due to its excellent biocompatibility and biodegradability nature.\textsuperscript{20,21} Furthermore, these PLGA-NPs manifest remarkable enhancement in the solubility, pharmacokinetics, and stability of the encapsulated drug. However, hydrolytic degradation of a polymer in aqueous suspension is a major drawback in these PLGA-NPs where lyophilization is considered a viable solution. In this context, we used mannnitol as a cryoprotectant, bulking agent, and stabilizer for a stable NAR-PLGA lyophilisate preparation in the current work.

Poloxamer-188, commonly known as Pluronic F68, is a triblock copolymer that serves as a non-emulsifier in the formulation development, results in small and narrow size distribution particles, with improved solubility and bioavailability of many hydrophobic drugs.\textsuperscript{22} Furthermore, adsorption of this non-ionic P188 surfactant into PLGA through a poly(ethylene oxide) center block results in minimization of zeta potential and augmentation of the drug release in NAR-PLGA-NPs. The adsorption is responsible for changing the smooth surface of PLGA into a rough one due to extended chains of PEO in solution.\textsuperscript{23} In addition, mixtures of two water-immiscible organic solvents (acetone and ethanol) were employed instead of DCM + acetone in preparing NAR-PLGA-NPs for improved yield, superior powder property, and prevention of aggregates as suggested by the literature.\textsuperscript{24} Sodium deoxycholate is an amphiphilic molecule that helps in sustained release activity. This copolymer did not influence its inherent sol–gel transition behavior but increased the sol–gel behavior.\textsuperscript{25}

RA is a disease with inconclusive pathophysiology, which leads to disability and destruction of joints. Apart from different biological pathways, the inflammatory cytokines also play a pivotal role in pathogenesis of RA, inducing signal transduction and intracellular signaling. Since flavonoids have an immense potential in blocking targeted cytokines, in the current study, we are exploring molecular docking studies to check the effect of NAR on the chosen RA-associated cytokine target proteins (IL-6, TNF-α, IL-10, and INF-γ).

To our knowledge, there is no previous report exploring systemic optimization and therapeutic benefits of NAR-loaded PLGA-NPs using poloxamer-188 (Pluronic F68) and sodium deoxycholate as stabilizers in RA. In this context, the objective of present investigation is to formulate polymeric NPs of NAR (hydrophobic) with PLGA as the polymer to evaluate their efficacy as sustained release formulations, employing in vivo, in vitro, ex vivo, and in silico docking methodologies. The prepared NPs may open new avenues toward targeting of anti-inflammatory drugs to the desired pharmacological sites.

2. RESULTS AND DISCUSSION

2.1. Effect of Formulation Variables during Formulation Development. In the current study, 10 biodegradable polymeric NPs (FN1–FN10) were prepared using the modified solvent emulsification–evaporation technique. For the solubilization of hydrophobic drug NAR with a log P value of −0.44 (Pubchem), a molar mass of 80.54 g/mol, and a water solubility of 1.9 μg/mL (Drug Bank) in NAR-PLGA-NPs, different solvent systems were implemented such as dichloromethane (DCM) + acetone, dimethyl sulfoxide (DMSO) + acetone, and ethanol + acetone (in ratios of 1:1, 2:1, and 3:1,
respectively). The solvent systems were selected to avoid any agglomeration.26

A formulation (FN1) containing organic solvent systems, water-immiscible DCM with water-soluble acetone (i.e., DCM + acetone), and a formulation (FN2) containing organic water-miscible solvents (DMSO + acetone) were executed as the yield was poor. Further, developments were with the solvent system ethanol + acetone in ratios of 1:1, 2:1, and 3:1 and were executed for the present study. An ideal solvent displacement method with poloxamer-188 (surfactant) was used as a cryoprotectant and bulking agent. Table 1 summarizes 10 different formulations (FN1–FN10) of NAR-PLGA-NPs using different excipients.

Out of the 10 different prepared NAR-PLGA-NPs, the smallest NPs (FN4) were obtained with an optimized solvent system (ethanol + acetone) (2:1) on the basis of the absence of agglomeration, along with a combination of stabilizers (viz., P188 + sodium deoxycholate) and PVA (2%), showing a low polydispersity index of 0.206 ± 0.001 and a better entrapment efficiency (74 ± 3.61%).

2.2. Characterization of NAR-PLGA-NPs. PLGA is one of the most safe, widely recommended, FDA-approved, semi-synthetic polymers, which gets metabolized easily by the Krebs cycle in our body due to its biodegradable and biocompatible features with the least systemic toxicity.27,28 To stabilize NPs, PVA is a great choice as the presence of hydroxyl groups can easily hydrate the NPs, resulting in its better adsorption onto the NP surface.29 The size of NPs also plays a vital role in drug delivery as nanosized particles effectively reach the target areas of the synovium in the RA condition. In addition, lower polydispersity index (PDI) values (0.2 or less) are usually acceptable for polymer-based nanoparticles.30 Furthermore, zeta potential (ZP) values play a major role in detecting prolonged pharmacokinetics of entrapped hydrophobic drugs. It further predicts the dispersion stability. A higher ZP value suggests higher charge (electric) on the NP surface, facilitating the absence of aggregation.31 Meanwhile, poloxamer-188 (non-ionic surfactant) used in this study exerts extra stearic stabilization with adsorption of P188 into PLGA, avoiding agglomeration of particles, which is as per the literature.28 Prepared PLGA-NPs exhibited a low particle size (179.7 ± 11.03 nm), along with a polydispersity index range from 0.206 to 0.581, and high values of zeta potential (−9.11 to −11.6 mV) and revealed colloidal stability and finer homogenization of NPs because of their negative surface charge (Table 2 and Figure 1A,B). Revealed PGLA-NPs provide physicochemical stability to NAR against gastric degradation, thereby expanding blood retention time, which allows better therapeutic efficacy.

2.3. Surface Morphological Analysis. Figure 1C represents SEM micrographs of pure drug and prepared nanoformulations. It could be observed that pure drug NAR was an irregular crystal, but after formulation development, using the optimized methodology, the prepared NPs showed small, smooth, globular particles without drug crystals, suggesting that they can more easily penetrate into cells as compared to the free drug. Lindwre et al. previously suggested that PVA in the concentration range of 2–4% (w/v) may have interrupted the agglomeration of NPs.53 This aspect may be responsible for our studies also, although the effect of PVA concentration was not determined in our work.

### Table 1. Formulation Table of NAR-PLGA-NPs

| formulation code | FN1  | FN2  | FN3  | FN4  | FN5  | FN6  | FN7  | FN8  | FN9  | FN10 |
|------------------|------|------|------|------|------|------|------|------|------|------|
| NAR (mg)         | 50   | 50   | 50   | 50   | 50   | 50   | 50   | 100  | 100  | 100  |
| PLGA (mg)        | 500  | 500  | 500  | 500  | 500  | 1000 | 1000 | 1000 | 1000 | 1000 |
| sodium deoxycholate (mg) | 50   | 50   | 50   | 50   | 50   | 50   | 50   | 100  | 50   | 100  |
| n-mannitol (mg)  | 500  | 500  | 500  | 500  | 500  | 500  | 500  | 1000 | 1000 | 1000 |
| dichloromethane (mL) | 1    |      |      |      |      |      |      |      |      |      |
| PVA (mg)         |      |      |      |      |      |      |      |      |      |      |
| DMSO (mL)        |      |      |      |      |      |      |      |      |      |      |
| acetone (mL)     | 1    | 1    | 1    | 2    | 1    | 1    | 3    | 2    | 2    |      |
| P188             | 50   | 50   | 50   | 100  | 100  | 100  | 100  |      |      |      |
| ethanol (mL)     | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |      |

### Table 2. Composition and Physicochemical Characteristics of NAR-PLGA-NPs

| formulation particle size (nm) | PDI | zeta potential (mV) | EE (%) | drug dissolution (%) |
|-------------------------------|-----|---------------------|--------|----------------------|
| FN1  | 492.8 ± 2.71 | 0.512 ± 0.001 | −10.14 ± 0.12 | 32 ± 4.19 | 56.50 ± 4.62 |
| FN2  | 504.8 ± 1.10 | 0.029 ± 0.003 | −10.26 ± 0.07 | 34 ± 4.38 | 56.80 ± 4.62 |
| FN3  | 301.5 ± 0.96 | 0.028 ± 0.003 | −10.7 ± 0.45 | 40 ± 3.57 | 61.62 ± 2.82 |
| FN4  | 179.7 ± 2.50 | 0.206 ± 0.001 | −9.18 ± 0.78 | 74 ± 3.61 | 82.11 ± 3.65 |
| FN5  | 248.4 ± 3.40 | 0.322 ± 0.002 | −11.6 ± 1.65 | 64 ± 4.20 | 69.07 ± 4.20 |
| FN6  | 292.3 ± 1.40 | 0.287 ± 0.004 | −11.4 ± 1.65 | 42 ± 3.59 | 56.47 ± 2.38 |
| FN7  | 265.4 ± 1.91 | 0.427 ± 0.003 | −11.03 ± 0.48 | 55 ± 4.60 | 54.11 ± 3.72 |
| FN8  | 323 ± 2.96  | 0.581 ± 0.004 | −11.0 ± 1.63 | 40 ± 3.57 | 56.10 ± 4.62 |
| FN9  | 283.9 ± 0.80 | 0.011 ± 0.001 | −10.99 ± 0.49 | 40 ± 3.57 | 53.07 ± 3.46 |
| FN10 | 229.7 ± 2.51 | 0.350 ± 0.005 | −9.11 ± 0.78 | 70 ± 3.58 | 80.35 ± 3.69 |
2.4. **In Vitro and Ex Vivo Drug Release Studies with Mathematical Kinetic Modeling.** The dissolution profiles of all the formulations FN1–FN10 are reported in Figure 1D.

When compared to other formulations, prepared NAR-encapsulated PLGA-NPs (FN4) have shown the highest % drug release of 82.11 ± 3.65% in pH 6.8 phosphate buffer with a

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**Figure 1.** (A) Particle size distribution. (B) Zeta potential curve. (C) SEM studies: (a) naringin, (b) FN4, (c) FN10, (d) FN5, (e) FN7, and (f) FN6. (D) *In vitro* dissolution. (E) *Ex vivo* dissolution.
sustained effect, which may be due to slow decline/degradation of PLGA in the media used. A lower polymer/naringin ratio, having both stabilizers (P188 and sodium deoxydate) in a ratio of 1:1 and a solvent system of acetone + ethanol in a ratio of 2:1, met the USP requirements on SR formulations.

The drug release from NAR-PLGA-NPs was analyzed by four kinetic models, viz., zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetic models. To predict the kinetics and drug release mechanism, the indicator that was chosen for model fitting is $R^2$. When the value of $R^2$ is close to 1, it suggests

Table 3. In Vitro Release Modeling for NAR-PLGA-NPs

| formulation | zero order ($C = K_0t$) | first order ($\ln C = \ln C_0 + K_1t$) | Higuchi order ($Q = K_2t^{1/2}$) | Korsmeyer–Peppas ($M_t/M_\infty = K_3t^{n}$) | $N$ |
|-------------|--------------------------|--------------------------------|---------------------------------|---------------------------------|----|
| FN1         | 0.547                    | 0.263                        | 0.845                          | 0.961                           | 0.617|
| FN2         | 0.551                    | 0.263                        | 0.848                          | 0.961                           | 0.617|
| FN3         | 0.673                    | 0.311                        | 0.922                          | 0.978                           | 0.613|
| FN4         | 0.672                    | 0.284                        | 0.925                          | 0.971                           | 0.633|
| FN5         | 0.637                    | 0.285                        | 0.885                          | 0.967                           | 0.627|
| FN6         | 0.634                    | 0.323                        | 0.889                          | 0.979                           | 0.615|
| FN7         | 0.478                    | 0.265                        | 0.781                          | 0.952                           | 0.643|
| FN8         | 0.477                    | 0.220                        | 0.796                          | 0.931                           | 0.614|
| FN9         | 0.552                    | 0.315                        | 0.822                          | 0.958                           | 0.640|
| FN10        | 0.720                    | 0.321                        | 0.942                          | 0.984                           | 0.645|

Figure 2. (A) FTIR of NAR-PLGA-NPs: (a) pure drug, (b) FN4, (c) FN10, (d) FN5, (e) FN7, and (f) FN6. (B) DSC of NAR-PLGA-NPs: (a) pure drug, (b) FN4, (c) FN10, (d) FN5, (e) FN7, and (f) FN6. (C) XRD of NAR-PLGA-NPs: (a) pure drug, (b) FN4, (c) FN10, (d) FN5, and (e) FN7.
the best fit kinetic model for the delivery system. The obtained results as mentioned in Table 3 confirmed that NAR-PLGA-NPs followed the Korsmeyer–Peppas kinetic model with good linearity (0.931–0.984). Furthermore, diffusion exponent values (n) as calculated by the Korsmeyer–Peppas model revealed that the NAR release occurred by more than one mechanism. Both diffusion and the swelling mechanism are responsible, suggesting anomalous non-Fickian diffusion, which is in accordance to the study of Jardim et al.32 Figure 1E shows ex vivo permeation analysis, which revealed 80.02 ± 3.69% drug releases in 24 h. The in vitro release details were found to be in congruence with ex vivo permeation results through the isolated goat intestinal membrane.

2.5. Stability Studies. Optimized FN4 NAR-PLGA-NPs were freeze-dried using mannitol as a cryoprotectant and were stored under three different conditions: refrigerated (2–8 °C), ambient (room temp.), and accelerated conditions (40 ± 2 °C and 75 ± 5% RH). No significant changes were noted even after 6 month storage in size, PDI, and %EE. Also, developed formulations did not show any precipitate formation or phase separation during the storage period.

2.6. FTIR Analysis. FTIR was conducted for the measurement of the IR spectrum of different formulations along with pure drug substances. The study was conducted to determine any alteration in both physical and chemical properties of different formulations. From the results, it was observed that there was no interference in the functional group, as the principle peaks of drug substances were found to be unchanged in the drug polymer physical mixture. NAR exhibits polyhydroxy groups; hence, it showed the characteristic bands at 3447.91 cm⁻¹, 1551.80 cm⁻¹ (C=O), 1639.56 cm⁻¹ (C−O), 2982.08 cm⁻¹ (C−H aliphatic stretching bands), and 1309.72 cm⁻¹ (phenolic −OH) (Figure 2A). These peaks are in accordance with a reported study.33 From the literature, PLGA peaks were observed at 3450–3500 cm⁻¹ (O−H), 2885–3010 cm⁻¹ (C−H), 1762.6 cm⁻¹ (C=O), 1186–1089 cm⁻¹ (C−O), and 1450 cm⁻¹ (C−H); sodium deoxycholate peaks were at 2938.1 cm⁻¹, 2864.3 cm⁻¹, and 1561.2 cm⁻¹, and peaks of P188 were at 2883 cm⁻¹.

Figure 3. Result of NPs on (A) arthritic score, (B) PAW volume, (C) dorsal flexion test, (D) RF, and (E) CRP in FCA-induced arthritic rats. Values are revealed in mean ± SEM. Group I, normal rats; group II, FCA-induced rats; group III, standard drug indomethacin (10 mg/kg); group IV, naringin (20 mg/kg); group V, blank PLGA-NPs; group VI, NAR-PLGA-NPs (20 mg/kg).
cm\(^{-1}\) (C\(\cdot\)H), 1113 cm\(^{-1}\) (C\(\cdot\)O), 1341 cm\(^{-1}\) (O\(\cdot\)H), and 1099 cm\(^{-1}\) (C\(\cdot\)O).

FTIR spectra of formulations showed a few characteristic peaks of drugs but with slight shifts. The shift of drug peaks in the spectra of prepared NAR-PLGA-NPs from 3447.91 to 3377.50 cm\(^{-1}\) and 1639.56 to 1644.39 cm\(^{-1}\) in FN4 indicates probable drug encapsulation (Figure 2A).

2.7. DSC Analysis. Analysis of DSC thermograms showed a sharp endothermic peak of pure drug NAR within a range of 150 to 170 °C (\(T_{\text{onset}} = 157.48 \pm 2.1^\circ C, T_{\text{peak}} = 162.92 \pm 3.2^\circ C, \text{ and } T_{\text{endset}} = 167.92 \pm 4.5^\circ C\) (Figure 2B). Existing literature reveals that PLGA exhibits an endothermic peak at 60 °C and the sodium deoxycholate peak was at 149.15 °C, indicating a larger disarray in crystallinity of sodium deoxycholate.34 However, no new endothermic peak was observed in prepared formulations other than characteristic peaks of NAR, PLGA, and sodium deoxycholate. Reduced crystalline peaks of the drug (NAR) and polymer (PLGA and sodium deoxycholate) were seen evidently in DSC thermograms of FN4, confirming successful NAR incorporation (Figure 2B).

2.8. X-ray Diffractometry (XRD). Generally, this study is conducted to identify the phase identification of crystalline properties and measure the purity of different samples. An XRD study was conducted using an X-ray diffractometer by taking a 2\(\theta\) value from 0° to 70°. Different peaks are obtained, which determined the crystalline properties of different samples. Diffraction peaks of NAR were predominant at 2\(\theta\) = 19.38° and 23.52°. From the literature, PLGA broad peaks were evident in the range of 10°–40°.

In the prepared nanoparticles, principle peaks of NAR and the polymer exist, but sharpness peaks of NAR, which were noticed in pure drug thermograms, disappeared for NAR-PLGA-NPs, which suggested that NAR was successfully encapsulated in an amorphous form35 (Figure 2C).

From the above results, it was concluded that out of all the prepared formulations of NAR-PLGA-NPs, nanof ormulation FN4 (NAR-PLGA-NPs) containing the solvent system acetone–ethanol (2:1) with a combination of stabilizers, i.e., poloxamer-188 and sodium deoxy (1:1), along with 2% PVA solution, was optimized with a minimum particle size (179.7 ± 2.05 nm), low polydispersity index (0.206 ± 0.001), negative zeta potential (−9.18 ± 0.78 mV), and maximum encapsulation efficiency (74 ± 3.61%). The drug release kinetics resulted in the Korsmeyer–Peppas model contributing higher release of NAR after lyophilization, i.e., 82.11 ± 3.65% drug release in 6.8 phosphate buffer for 24 h. Ex vivo permeation analysis through the isolated goat intestinal membrane revealed 80.02 ± 3.69% drug release in 24 h. Hence, formulation FN4 was found to be the best as it does not evidence any phase separation/precipitate formation. Therefore, further experiments related to animal studies were carried out using this optimized FN4 formulation (NAR-PLGA-NPs).

2.9. In Vivo Potency Estimation in the Chronic Arthritic Rat Model. Paw swelling and arthritic score are two important predictions in the RA condition as it manifests the diseased condition. Our results demonstrated that NAR-loaded PLGA-NPs had a significant reducing effect on the arthritic score in treated rats (from 3.88 ± 0.32 to 1.25 ± 0.12). This decreased level in conjunction with the reduced paw volume (from 2.34 ± 0.28 to 1.84 ± 0.08) was well marked in the treated rats after 21 days (Figure 3B), which indicates the efficiency of the NAR-PLGA encapsulation method in improving the arthritic condition. Treatment of FCA rats with NAR alone also significantly downturn the severity of RA but to a lesser extent (2.06 ± 0.18) (Figure 3A). The optimized FN4 (NAR-PLGA-

![Figure 4. Effect of NPs on pro-inflammatory and anti-inflammatory cytokines on rats: (A) IL-6, (B) TNF-\(\alpha\), (C) INF-\(\gamma\), and (D) IL-10.](https://doi.org/10.1021/acsomega.1c03066)
NPs)-treated group showed a greater reduction in arthritis severity in FCA than those treated only with NAR. As a consequence, it can be concluded that the entrapment of NAR in PLGA-NPs increases the NAR efficacy. However, blank PLGA-NPs do not show any effect significantly.

The arthritic rats exhibited an approximately 8-fold increase in serum rheumatoid factor (RF-factor) levels coupled with a 14-fold increase in C-reactive protein (CRP) levels, indicating severe inflammation in the RA synovium. It may be due to pannus formation, infiltration, and synovial hyperplasia. Both CRP and RF are considered as the valuable markers in rheumatic inflammation, which gets released with interleukin IL-6, T-cells, and B-cells, respectively. Efficacy differences were observed clearly between the NAR-PLGA-NPs and pure drug at a dose of 20 mg/kg (Figure 3D,E). A considerable decrease in the serum rheumatoid factor and C-reactive protein to about normal ranges [RF (0–20 IU/mL) and CRP (0–6 mg/dL)] was noticed with oral administration of PLGA-NPs for 21 days as contrasted to the FCA-treated group ($p < 0.05$).36 Here also, blank PLGA-NPs failed to show any significant effect.

Figure 3C depicts the effect of NAR treatment on motility and dorsal flexion pain in FCA-induced arthritic rats. Dorsal flexion pain was noticed throughout the experiment in the toxic control group (FCA-induced), which gets attenuated with time from 10 to 3.5 in treated rats (NAR-PLGA-NPs) as against the median score of 6 in the NAR-treated group alone. This data gives a clear picture regarding effect of NAR treatment on the pain threshold, as the rheumatic patients feel severe pain in the joints. The motility test done for 5 min showed that the negative control rats (group II) were stagnant in their particular position and were unable to climb the stairs starting from the 6th day onward, which further gets deteriorated with each passing day. The situation seems to have worsened by the end of this experiment (i.e., on the 21st day). However, treatment with drug (NAR) alone, NPs, and standard drug indomethacin (10 mg/kg) enhanced the motility capacity with significant differences being noticed. Blank NPs failed to restore the motility capacity. Restoration of this motility capacity was well marked in NAR-PLGA-NPs at a dose of 20 mg/kg.

### 2.10. Evaluation of Serum Inflammatory Cytokines (IL-10, TNF-α, IL-6, and INF-γ).

It is obvious that a large number of cytokines IL-10, TNF-α, IL-6, and INF-γ cause increased cell infiltration, pannus formation, and high grades of synovial inflammation in joints. Different immune cells, which are evident in the RA synovium, include B-cells, T-cells, mast cells, macrophages, and a number of mediators like TNF-α, IL-17A, and IL-β that have a connection with the RA disease progression. Our sandwich ELISA study revealed a better effect with nanoparticulate formulations of NAR, which significantly suppresses INF-γ, IL-6, and TNF-α (pro-inflammatory cytokines) with the increment in IL-10 (anti-inflammatory cytokine) at the same time, as compared to free drug NAR and blank NPs (Figure 4A–D).

### 2.11. In Silico Molecular Docking Studies.

Molecular docking studies indicate the interaction and binding muddled between polymers and proteins with NAR. The in silico docking pose of polymers PLGA, P188, sodium deoxycholate, and NAR (Figure S5A) has shown that there is slight interaction between the drug (NAR) and polymers with a docking score ranging between −1.54 and −2.24 kcal/mol, whereas the in silico pose of protein PDB, namely, IL-6, TNF-α, IL-10, INF-γ, and NAR (Figure S5B), has shown very good promising interactions with a docking score ranging between −7.89 to −9.04 kcal/mol (Table 4). From the docking results of NAR with the RA-associated cytokine target proteins, it was confirmed that the compound NAR exerted strong inhibitory effects against TNF-α (PDB ID: 2AZS), human interleukin-6 (PDB ID: 1ALU), human interferon-γ (PDB ID: 1EKU), and IL-10 (PDB ID: 2H24), implying their therapeutic potential for use in RA, and was more stable with the involvement of a higher number of H-atoms. The drug under investigation was analyzed in respect of its pharmacologic potency. The same can be indicated by the data as obtained by the docking score (Table 4 and Figure S5A,B). A lower docking score of the drug and polymers suggests that the NAR is supposed to encapsulate into NPs effectively.

![Figure 5. Cavity prediction of NAR from in silico studies: (A) drug + polymer binding and (B) drug + protein binding.](image-url)
Furthermore, the interacting amino acids and interaction distance for protein PBD were successfully analyzed, viz., the amino acid for TNF-α (PDB ID: 2AZS) is Asp45 (NH, 2.507 Å), those for IL-6 (PDB ID: 1ALU) are Glu93 (OH, 1.928 Å) and Glu172 (2.038 Å), that for INF-γ (PDB ID: 1EKU) is Lys194 (1.949 Å), and that for IL-10 (PDB ID: 2H24) is Tyr72 (1.42 Å). Results obtained from in silico studies performed for NAR and a polymeric carrier system can be well correlated with the in vivo and in vitro experiments.

3. CONCLUSIONS
A significant decline in arthritic progression via decreased pro-inflammatory levels, increased anti-inflammatory levels ($p < 0.05$), and attenuation of RF and CRP levels was well noticed upon administration of NAR-PLGA-NPs in comparison to blank NPs and their free drug counterparts at a dose of 20 mg/kg, suggesting the protective potential of NAR-PLGA-NPs in mitigating FCA-induced arthritis in rats.

In conclusion, this polymeric NP approach seems suitable for delivering the flavonoid naringin to the intestine, avoiding the gastric degradation of NAR and improving their bioavailability from oral solid dosage forms.

4. MATERIALS AND METHODS
4.1. Materials. Polymer PLGA (75:25) (RG 752 H) was a generous gift from Evonik Germany. Naringin (molecular weight, 580.5 g/mol), sodium deoxycholate, and poloxamer-188 were procured from Sigma-Aldrich Co. (USA). Analytical grade chemicals and solvents were used in this work.

4.2. Formulation of NAR-PLGA-NPs. The original PLGA-NPs were fabricated with a double emulsion method followed by the solvent evaporation technique as described previously with slight modifications.43 PLGA was dissolved in acetone + ethanol or DCM + ethanol solution, to which NAR was added and mixed at 800 rpm using a magnetic stirrer. To a previously prepared PVA and sodium deoxycholate solution, the drug polymer emulsion was added dropwise and further emulsified with vigorous stirring at 800 rpm for 30 min. The resultant emulsion was centrifuged at 20,000 rpm for 25 min at 40 °C. The resultant NPs were washed out with double-distilled water to remove the solvents. Further, they were diluted with 10 mL of distilled water and lyophilized at −80 °C for 48 h.

4.3. Physicochemical Characterization of NAR-PLGA-NPs. 4.3.1. Determination of the Entrapment Efficiency of NAR. To measure the amount of NAR quantification, briefly, 100 μL of lyophilized NAR-PLGA-NPs was mixed with methanol and water (450 μL each) for 10× dilution so as to solubilize the polymeric particles. Then, the sample was analyzed spectrophotometrically at 282 nm, multiplied 10× for estimating the concentration of naringin present, using a UV spectrophotometer (Thermo Fisher Evolution 201 model). Quantification of free drugs was also carried out after centrifugation of 1 mL of sample at 10,000 rpm for 15 min at 4 °C. All the tests were done in triplicate. The percentage entrapment efficiency (EE%) of NAR in PLGA-NPs was estimated using the equation:

$$EE\% = \frac{\text{weight of drug in NPs}}{\text{weight of initially added drug}} \times 100\%$$

4.3.2. Size Distribution and Zeta Potential Estimation of NAR in NAR-PLGA-NPs. Size distribution and zeta potential of prepared NAR-NPs were measured using DLS Instruments (dynamic light scattering, Malvern Zetasizer, UK). Dried powder of NAR-PLGA-NPs was made in purified water and was transferred to a transparent sizing cuvette for polydispersity index (PDI) and particle size measurements. All the measurements were done in a set of three, and results were depicted as mean ± SD (standard deviation).

4.3.3. Morphological Estimation. The selected NAR-PLGA-NPs morphology was accomplished using a scanning electron microscope (Tokyo, Japan). The shape morphologies of prepared NPs were investigated by SEM.

4.3.4. Fourier Transform Infrared Spectrometry (FTIR) Analysis. The drug polymer interaction study was carried out using an FTIR spectrophotometer (Thermo Fisher Scientific). Potassium bromide with a disc of individual samples was scanned over a wavenumber of 500−4000 cm$^{-1}$.

4.3.5. X-ray Diffraclometry (XRD) Analysis. This study was conducted using an X-ray diffractometer (Diano Woburn, USA) by taking the 2θ value from 0° to 70° so as to obtain the phase identification of crystalline properties and measure the purity of different samples.

4.3.6. Differential Scanning Calorimetric (DSC) Analysis. DSC was conducted to determine the thermal characteristics of different formulations and pure drug using DSC-4, PerkinElmer Inc., USA. The measurement was carried out at a temperature range of 50−350 °C at 10 °C/min, with heating of samples under (N2) nitrogen gas flow.

4.3.7. Drug Release Studies (In Vitro). The in vitro release rate of NAR containing different formulations was determined using USP dissolution testing apparatus-2 (paddle method). The dissolution was performed using phosphate medium (pH 6.8) for 24 h to mimic the in vivo environment. Nine hundred milliliters of medium was used for the study. Ten milliliters of samples was withdrawn by using a 0.45 μm Millipore in a 10 mL syringe and simultaneously replaced with the fresh dissolution medium at different time intervals to retain the conditions of sink. The absorbances of different samples were estimated at a fixed wavelength of 282 nm, and graphs were plotted between cumulative % drug release vs time. Figure 1D summarizes the in vitro drug release profile for all the prepared NPs formed up to 24 h.

4.3.7.1. Mathematical Modeling Release Kinetics. The in vitro release kinetic study of the drug can be calculated by fitting the cumulative DR (drug release) into various mathematical models, viz., zero-order, first-order, Higuchi, and Korsmeyer−Peppas kinetic models. Obtained correlation coefficients ($R^2$) demonstrate the various drug release kinetics.

4.3.8. Ex Vivo Permeation Study. Ex vivo permeation analysis was performed through the isolated goat intestinal membrane. The small intestine of a goat was obtained from the local slaughter house. Also, without storing it for longer time, it was kept in Krebs-Ringer Solution (buffer fluid), cleaned properly to separate the mesentery, and finally cut into different sections. Each section was fixed on its location via a thread and was inverted on a Teflon rod. With slight modifications as suggested by Meriani et al., the experiments were conducted. Briefly, a cylindrical glass vessel (intestine-holder) was interconnected with a U-glass tube where the intestine was placed. The intestine-holder represents the receiver unit, which was filled with buffer fluid. To keep the intestine cells alive throughout experimentation, both the donor and receiver phases were continuously aerated. Fresh buffer was replaced at regular time intervals, and the drug concentration was analyzed using a UV spectrophotometer.
4.3.9. Stability Study. The stability studies were performed for the optimized NAR-PLGA-NPs as per ICH guidelines and were analyzed for any change in particle size and percentage drug content. Briefly, 100 μL of lyophilized NAR-PLGA-NPs was mixed with methanol and water (450 μL each) for 10× dilution so as to solubilize the polymeric particles. Then, to ascertain the EE%, the sample was analyzed spectrophotometrically at 282 nm, multiplied 10× for estimating the concentration of naringin present using a UV spectrophotometer (Thermo Fisher Evolution 201 model). Quantification of free drugs was done via centrifugation of 1 mL of sample at 10,000 rpm for 15 min at 4 °C. All the tests were carried out in triplicate. The percentage entrapment efficiency (EE%) of NAR in PLGA-NPs was estimated using the equation:

\[
EE\% = \frac{\text{weight of drug in NPs}}{\text{weight of initially added drug}} \times 100\%
\]

4.4. Animal Experiments (In Vivo Studies). Protocols of all the animal experiments were approved by the CPCSEA committee and was in accordance with the institutional guidelines (approval no. IAEC/SPS/SOA/15/2018). Adult male Wistar rats (body wt., 150–250 g) were kept for 21 days in the SPS animal house under controlled conditions of 25 °C, 60–80% RH, and 12 h light/dark cycles and nourished with proper palate diet and sufficient water. The experimental animals in the FCA-induced arthritic model were distributed equally into six groups with six animals per group. Animals in group I (normal control) received saline water. Group II (toxic control) received free drugs equally into six groups with six animals per group. Animals in groups III, IV, V, and VI were treated with standard drug indomethacin (10 mg/kg), pure drug NAR (20 mg/kg), blank PLGA-NPs, and optimized FN4 NAR-PLGA-NPs (20 mg/kg), respectively. Arthritis was induced in rats of all the groups except group I. On day 0, 0.1 mL of FCA was injected intradermally for immunizing the rats through the subplantar region of the right hind paw. After this day, the pure drug NAR, blank formulation, and optimized NAR-PLGA-NPs (formulation FN4) (dosed at 20 mg/kg p.o.), along with the reference standard drug indomethacin (10 mg/kg p.o.), were administered for 21 days. The animals were anesthetized to collect the blood from the heart for biochemical estimation studies and were sacrificed. Observation of C-reactive protein (CRP) and rheumatoid factor (RF) levels was carried out.

4.4.1. Isolation of Rat PBMCs and Analysis of Serum Inflammatory Cytokines (IL-6, IL-10, TNF-α, and INF-γ). Blood obtained from rats through cardiac puncture was allowed to clot at room temperature for about an hour. The obtained serum was centrifuged at 10,000 rpm for 15 min at 4 °C until evaluation. The serum obtained from rats through cardiac puncture was allowed to clot at room temperature for about an hour. The obtained serum was centrifuged at 10,000 rpm for 15 min at 4 °C until evaluation. The serum obtained from rats through cardiac puncture was allowed to clot at room temperature for about an hour. The obtained serum was centrifuged at 10,000 rpm for 15 min at 4 °C until evaluation. The serum obtained from rats through cardiac puncture was allowed to clot at room temperature for about an hour. The obtained serum was centrifuged at 10,000 rpm for 15 min at 4 °C until evaluation. 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V.B.K. and S.M. designed the work, conducted the formulation studies, and were involved in the characterization. A.P. and S.M. performed the pharmacological studies. T.S. carried out the in silico studies. S.M. and A.P. analyzed the data. S.C.S. contributed the reagents. S.M. provided the draft of the paper. V.B.K. provided corrections to the draft.

Notes
The authors declare no competing financial interest.

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References
(1) Wang, D.-M.; Yang, Y.-J.; Zhang, L.; Zhang, X.; Guan, F.-F.; Zhang, L.-F. Naringin enhances CaM KKII activity and improves long-term memory in a mouse model of Alzheimer’s disease. Int. J. Mol. Sci. 2013, 14, 5576–5586.
(2) Yin, L.; Cheng, W.; Qin, Z.; Yu, H.; Yu, Z.; Zhong, M.; Sun, K.; Zhang, W. Effects of naringin on proliferation and osteogenic differentiation of human periodontal ligament stem cells in vitro and In Vivo. Stem Cells Int. 2015, 2015, 1.
(3) Zhang, J.; Gao, W.; Liu, Z.; Zhang, Z.; Liu, C. Systematic analysis of main constituents in rat biological samples after oral administration of the methanol extract of Fructus aurantii by HPLC-ESI-MS/MS. Iran. J. Pharm. Res. 2014, 13, 493–503.
(4) Choutrou, Y.; Gargouri, B.; Kebiche, M.; Fetoui, H. Naringin abrogates cisplatin-induced cognitive deficits and cholinergic dysfunction through the down-regulation of AChE expression and iNOS signaling pathways in hippocampus of aged rats. J. Mol. Neurosci. 2015, 56, 349–362.
(5) Kim, Y. H.; Tabata, Y. Dual-controlled release system of drugs for bone regeneration. Adv. Drug Deliv. Rev. 2015, 94, 28–40.
(6) Xu, Q.; Zhang, Z.; Sun, W. Effect of Naringin on Monosodium Iodoacetate-Induced Osteoarthritis Pain in Rats. Med. Sci. Monit. 2017, 23, 3746–3751.
(7) Zhao, Y.; Li, Z.; Wang, W.; Zhang, H.; Chen, J.; Su, P.; Li, W. Naringin Protects Against Cartilage Destruction in Osteoarthritis Through Repression of NRX-RF Signaling Pathway. Inflammation 2016, 39, 385–392.
(8) Wei, M.; Yang, Z.; Li, P.; Zhang, Y.; See, W. C. Anti-Osteoporosis Activity of Naringin in the Retinoic Acid-Induced Osteoporosis Model. Am. J. Clin. Med. 2007, 35, 663–667.
(9) Ramasamy, S. K.; Kusumbe, A. P.; Wang, L.; Adams, R. H. Endothelial Notch activity promotes angiogenesis and osteogenesis in bone. Nature 2014, 507, 376–380.
(10) Ahmad, S. F.; Zohair, K. M. A.; Abdel-Hamied, H. E.; Ashour, A. E.; Bakheet, S. A.; Attia, S. M.; Abd-Allah, A. R. A. Amelioration of autoimmune arthritis by naringin through modulation of T regulatory cells and Th1/Th2 cytokines. Cell. Immunol. 2014, 287, 112–120.
(11) Chen, R.; Qi, Q. L.; Wang, M. T.; Li, Q. Y. Therapeutic potential of naringin: an overview. Pharm. Biol. 2016, 54, 3203–3210.
(12) Zhu, L.; Wang, J.; Wei, T.; Gao, J.; He, H.; Chang, X.; Yan, T. Effects of Naringenin on inflammation in complete Freund’s adjuvant-induced arthritis by regulating Bax /Bcl-2 balance. Inflammation 2015, 38, 245–251.
(13) Lauro, M. R.; De Simone, F.; Sansone, F.; Iannelli, P.; Aquino, R. P. Preparations and release characteristics of naringin and naringenin gastro-resistant microparticles by spray-drying. J. Drug Delivery Sci. Technol. 2007, 17, 119–124.
(14) Mohanty, S.; Sahoo, A. K.; Konkimalla, V. B.; Pal, A.; Si, S. C. Naringin in Combination with Isosorbide Mononitrate as Liposomal Formulations Potentiates the Anti-inflammatory Activity in Different Acute and Chronic Animal Models of Rheumatoid Arthritis. ACS Omega 2020, 5, 28319.
(15) Bai, Y.; Peng, W.; Yang, C.; Zou, W.; Liu, M.; Wu, H.; Fan, L.; Li, P.; Zeng, X.; Su, W. Pharmacokinetics and Metabolism of Naringin and Naringenin Active Metabolite Naringenin in Rats, Dogs, Humans, and the Differences Between Species. Front. Pharmacol. 2020, 11, 364.
(16) Fan, X.-x.; Xu, M.-z.; Leung, E. L.-H.; Jun, C.; Yuan, Z.; Liu, L. ROS-Responsive Berberine Polymeric Micelles Effectively Suppressed the Inflammation of Rheumatoid Arthritis by Targeting Mitochondria. Nano-Micro Lett. 2020, 12, 76.
(17) Mohamed, E.; Abu Hashim, I.; Yusríf, R.; Shaaban, A.; El-Shekh, A.; Hamed, M.; Badria, F. Polymeric micelles for potentiated antioxidant and anticancer activities of naringin. Int. J. Nanomed. 2018, 13, 1009–1027.
(18) Pluegueuzuelos-Villa, M.; Mir-Palomo, S.; Díez-Sales, O.; Buso, M. A. O. V.; Sauri, A. R.; Nácher, A. A novel ultraforceformable liposomes of Naringin for anti-inflammatory therapy. Colloids Surf., B 2018, 162, 265–270.
(19) Kandhare, A. D.; Alam, J.; Patil, M. V. K.; Sinha, A.; Bodhankar, S. L. Wound healing potential of naringin ointment formulation via regulation the expression of inflammatory, apoptotic and growth mediators in experimental rats. Pharm. Biol. 2016, 54, 419–432.
(20) Sharma, S.; Parmar, A.; Kori, S.; Sandhir, R. PLGA-based Nanoparticles: A new paradigm in biomedical applications. TrAC, Trends Anal. Chem. 2016, 80, 30–40.
(21) Elnowafy, E. M.; Tiboni, M.; Soliman, M. E. Bio-compatibility, biodegradation and biomedical applications of poly (lactic acid)/ poly (lactic-co-glycolic acid) micro and nanoparticles. J. Pharm. Invest. 2019, 49, 347–380.
(22) Pankaj, S.; Singh, O.; Betlem, K.; Aswal, V.; Peeters, M.; Mahajan, R. ACS Omega 2019, 4, 11251–11262.
(23) Yan, F.; Zhang, C.; Zheng, Y.; Mei, L.; Tang, L.; Song, C.; Sun, H.; Huang, L. The effect of poloxamer 188 on nanoparticle morphology, size, cancer cell uptake, and cytotoxicity. Nanomedicine 2010, 6, 170–178.
(24) Jain, D.; Athawale, R.; Bajaj, A.; Shrikhande, S.; Goel, P. N.; Gude, R. P. Studies on stabilization mechanism and stealth effect of poloxamer 188 onto PLGA nanoparticles. Colloids Surf., B 2013, 109, 59–67.
(25) Samstein, R. M.; Perica, K.; Balderrama, F.; Look, M.; Fahmy, T. M. The use of deoxycholic acid to enhance the oral bioavailability of biodegradable nanoparticles. Biomaterials 2008, 29, 703–708.
(26) Song, K. C.; Lee, H. S.; Choung, I. Y.; Cho, K. I.; Ahn, Y.; Choi, E. J. The effect of type of organic phase solvents on the particle size of poly (d, l-lactide-co-glycolide) Nanoparticles. Colloids Surf., A 2006, 276, 162–167.
(27) Danhier, F.; Ansoarena, E.; Silva, J. M.; Coco, R.; Le Breton, A.; O. V.; Sauri, A.; Nácher, A. A novel ultradeformable liposomes of Naringin for anti-inflammatory therapy. Colloids Surf., B 2018, 162, 265–270.
(28) Kandhare, A. D.; Alam, J.; Patil, M. V. K.; Sinha, A.; Bodhankar, S. L. Wound healing potential of naringin ointment formulation via regulation the expression of inflammatory, apoptotic and growth mediators in experimental rats. Pharm. Biol. 2016, 54, 419–432.
polymerization of poly (vinyl alcohol) on the preparation and properties of poly (dl-lactide-co-glycolide) nanoparticle. *Int. J. Pharm.* 1997, 149, 43–49.

(30) Jang, J.-H.; Jeong, S.-H.; Lee, Y.-B. Preparation and In Vitro/In Vivo Characterization of Polymeric Nanoparticles Containing Methotrexate to Improve Lymphatic Delivery. *Int. J. Mol. Sci.* 2019, 20, 3312.

(31) Honary, S.; Zahir, F. Effect of zeta potential on the properties of nano-drug delivery systems—a review (Part 2). *Trop. J. Pharm. Res.* 2013, 12, 265–273.

(32) Jardim, K. V.; Joanitti, G. A.; Azevedo, R. B.; Parize, A. L. Physico-chemical characterization and cytotoxicity evaluation of curcumin loaded in chitosan /chondroitin sulfate nanoparticles. *Mater. Sci. Eng., C* 2015, 36, 294–304.

(33) Cordonevisi, L. M.; Sponchiado, R. M.; Campanharo, S. C.; Cassia, G. V.; Raffin, P. R.; Schapoval, S. E. E. Study of flavonoids present in Pomelo (Citrus máxima) by DSC, UV-VIS, IR, 1H AND 13C NMR AND MS. Drug Anal. Res. 2017, 1, 31–37.

(34) Palmeiro-Roldán, R.; Fonseca-Berzal, C.; Gómez-Barrio, A.; Ará, V. J.; Escario, J. A.; Torrado-Durán, S.; Torrado-Santiago, S. Development of novel benzimidazole formulations: Physicochemical characterization and in vivo evaluation on parasitemia reduction in Chagas disease. *Int. J. Pharm.* 2014, 472, 110–117.

(35) Malathy, S.; Iyer, R. P. Naringin Loaded Chitosan Nanoparticle for Bone Renervation: A Preliminary in vitro study. *J. Nanomed. Nanotechnol.* 2018, 9, 4172.

(36) Guha, P.; Subbhashis, P.; Das, A.; Halder, B.; Bhattarcharjee, S.; Chaudhuri, T. K. Analyses of Human and Rat Clinical Parameters in Pomelo (Citrus máximum) and obtain the complete Adjuvant induced monoarthritis. *Pharmacol. Toxicol. Methods* 2020, 6, 298–301.

(37) Geetha, P.; Sivaram, A. J.; Jayakumar, R.; Gopi Mohan, C. Integration of in silico modeling, prediction by binding energy and experimental approach to study the amorphous chitin nanocarriers for cancer drug delivery. *Carbohydr. Polym.* 2016, 142, 240–249.

(38) Anthony, T.; Fong, P.; Goyal, A.; Saltzman, W. M.; Moss, R. L.; Breuer, C. Development of a parathyroid hormone-controlled release system as a potential surgical treatment for hypoparathyroidism. *J. Pediatr. Surg.* 2005, 40, 81–85.

(39) Harilahar, S.; Bhardwaj, V.; Bala, I.; Sitterberg, J.; Bakowsky, U.; Ravi Kumar, M. N. V. Design of estradiol loaded PLGA nanoparticulate formulations: a potential oral delivery system for hormone therapy. *Pharm. Res.* 2006, 23, 184–195.

(40) Thamake, S. I.; Raut, S. L.; Ranjan, A. P.; Gryczynski, Z.; Vishwanatha, J. K. Surface functionalization of PLGA nanoparticles by non-covalent insertion of a homo-bifunctional spacer for active targeting in cancer therapy. *Nanotechnology* 2011 Jan 21, 22, No. 035101.

(41) Seju, U.; Kumar, A.; Sawant, K. K. Development and evaluation of olanzapine-loaded PLGA nanoparticles for nose-to-brain delivery: in vitro and in vivo studies. *Acta Biomater.* 2011, 7, 4169–4176.

(42) Parhizkar, M.; Reardon, P. J. T.; Knowles, J. C.; Browning, R. J.; Stride, E.; Barbara, P. R.; Harker, A. H.; Edirisinghe, M. Electro-hydrodynamic encapsulation of cisplatin in poly (lactic-co-glycolic acid) nanoparticles for controlled drug delivery. *Nanomedicine* 2016, 12, 1919–1929.

(43) Ali, H.; Weigmann, B.; Collnot, E. M.; Khan, S. A.; Windbergs, M.; Lehr, C. M. Budesonide Loaded PLGA Nanoparticles for Targeting the Inflamed Intestinal Mucosa- Pharmaceutical Characterization and Fluorescence Imaging. *Pharm. Res.* 2016, 33, 1085–1092.

(44) Bansal, S.; Aggarwal, G.; Chandel, P.; Harikumar, S. L. Design and development of cefdinir niosomes for oral delivery. *J. Pharm. Bioall. Sci.* 2013, 5, 318–325.

(45) Meriani, F.; Cocceani, N.; Sirotti, C.; Voinovich, D.; Grassi, M. In vitro Nimesulide absorption from different formulations. *J. Pharm. Sci.* 2004, 93, 540–552.

(46) Jain, S.; Mittal, A.; Jain, A. K.; Mahajan, R. R.; Singh, D. Cyclosporin A Loaded PLGA Nanoparticle: Preparation, Optimization, In-Vitro Characterization and Stability Studies. *Curr. Nanosci.* 2010, 6, 422–431.