Research Article

Design of an Inflammation-Sensitive Polyelectrolyte-Based Topical Drug Delivery System for Arthritis

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Abstract. The most successful treatment strategy for arthritis is intra-articular injections that are costly and have reduced patient compliance. The purpose of the current study was to develop an inflammation-sensitive system for topical drug administration. Multi-macromolecular alginate-hyaluronic acid-chitosan (A-H-C) polyelectrolyte complex nanoparticles, loaded with indomethacin were developed employing pre-gel and post-gel techniques in the presence of dodecyl-L-pyroglytamate (DLP). In addition to *in vitro* studies, *in silico* simulations were performed to affirm and associate the molecular interactions inherent to the formulation of core all-natural multi-component biopolymeric architectures composed of an anionic (alginate), a cationic (chitosan), and an amphi-ionic polyelectrolytic (hyaluronic acid) macromolecule. The results demonstrated that DLP significantly influenced the size of the synthesized nanoparticles. Drug-content analysis revealed higher encapsulation efficiency (77.3%) in the presence of DLP, irrespective of the techniques used. Moreover, *in vitro* drug release studies showed that indomethacin release from the nanosystem was significantly improved (98%) in Fenton's reagent. Drug permeation across a cellulose membrane using a Franz diffusion cell system showed an initial surge flux (0.125 mg/cm²/h), followed by sustained release of indomethacin for the post-gel nanoparticles revealing its effective skin permeation efficiency. In conclusion, the study presents novel nanoparticles which could effectively encapsulate and deliver hydrophobic drugs to the target site, particularly for arthritis.

KEY WORDS: alginate; chitosan; dodecyl-L-pyroglytamate; drug delivery; hyaluronan; inflammation; topical.

INTRODUCTION

Arthritis is a degenerative joint disease which causes chronic pain and reduced quality of life. Arthritis responds to the oral administration of NSAIDS for relieving pain and inflammation. However, it is associated with gastrointestinal adverse effects. The US Food and Drug Administration (FDA) has approved the use of intra-articular hyaluronic acid formulations for osteoarthritis of the knee, thereby minimizing the side effects. However, this preparation often requires frequent injections which carry a high financial burden and reduces patient compliance (1). Therefore, there is a need for an alternative drug administration route which will increase patient compliance.

Topical drug delivery is one of the best non-invasive routes of administration of drugs due to its increased patient compliance. Skin provides a widely used alternative route for drug delivery, allowing greater comfort for the patient and

avoiding the side effects associated with oral and parenteral administration (2). Topical drug delivery has many advantages, several of which include long application times, large available surface areas, and low enzymatic degradation (3). Hair follicles were previously seen as being insignificant as a possible route of drug delivery, but their potential has been recently reviewed (2). The hair follicles extend deep into the skin where there is a rich capillary blood supply available for transport of solutes diffusing out of the follicle (3). Studies on follicular penetration of solid particles have been conducted, and results suggest that the movement of hair from massaging of the skin aided the penetration of the particles into the follicle and ultimately served as a reservoir for nanoparticle-based drug delivery (2).

The principal pharmaceutical interest of nanoparticles stems from their potential as carriers for drugs and other active entities (4). Biodegradable nanoparticles are often used to improve the therapeutic value of drugs since they are better encapsulated and allow improved bioavailability, solubility, and permeability (5). In addition, nanoparticulate drug delivery systems improve patient compliance by minimizing drug dosage frequency as well as providing novel and innovative treatment, facilitating targeted drug delivery and controlled release (5). There are several reports suggesting the efficiency

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of nanoformulation for topical drug delivery applications (6–8). Various nanoparticles were reported to be effective for the administration of anti-inflammatory drugs for the treatment of dermatitis as well as rheumatoid arthritis (9–12).

In arthritic disorders, a vast amount of circumstantial evidence implicates that oxygen-derived free radicals, especially superoxide and hydroxyl radicals, are the mediators of inflammation (13,14). According to Kennett *et al.*, there is an enhanced capillary permeability in the first stages of inflammation which results in an accumulation of polymorphonuclear leukocytes and other phagocytic cells at the inflammation site (15). Hydroxyl radicals that serve as a bactericidal agent are produced by the phagocytic cells and other inflammation-generating compounds. Evidently, hydroxyl radicals are present at the site of inflammation in arthritis, and this mediator could possibly be a target for anti-inflammatory therapy. Therefore, an inflammation-sensitive drug delivery system would provide more benefits to the arthritic patients by having the propensity to penetrate the skin and release the incorporated anti-inflammatory drug at the site/s of inflammation. These systems could lengthen drug retention times, decrease the clearance of drug into joint cavities, enhance the therapeutic effect of pharmaceutical agents, and increase patient compliance (1).

Hence, the objective of the current study is to design an inflammation-sensitive nanosystem having the potential to be topically applied for the treatment of inflammation in arthritic patients by employing inflammation-sensitive polymers with the ability to degrade in the presence of inflammation and release the anti-inflammatory drug. Based on the prerequisite for inflammation-sensitive drug delivery, we developed a novel, multi-macromolecular, inflammation-sensitive nanosystem. The premise of the design was to encapsulate the drug in an alginate carrier system, shielded by the inflammation-sensitive polymer-hyaluronic acid, bridged by chitosan. The mechanism by which hyaluronic acid degrades in acute inflammatory processes in rheumatoid arthritis is due the presence of hydroxyl radicals (14). This proposed mechanism will be manipulated to produce an inflammation-sensitive biodegradable drug delivery system.

MATERIALS AND METHODS

Materials

Chemicals such as low molecular weight chitosan, sodium alginate, hyaluronic acid, calcium chloride, sodium lauryl sulphate, oleic acid, dodecyl-L-pyroglytamate (DLP), indomethacin, and other reagents such as hydrochloric acid, potassium dihydrogen phosphate, sodium hydroxide, ferrous sulphate, and hydrogen peroxide were all purchased from Sigma-Aldrich Corp.

Preparation of Inflammation-Sensitive Nanoparticles

The inflammation-sensitive nanoparticles were prepared using an ionotropic pre-gelation (16) and post-gelation method. In the pre-gel method, drug molecules were incorporated before polymeric gelation, whereas the post-gel nanoparticles were synthesized by adding drug

after polymeric gelation. Briefly, a 1% chitosan solution was prepared by dissolving chitosan in 0.2 N HCl with continuous stirring. A solution of 0.75% of sodium alginate and 0.3% hyaluronic acid were separately prepared in deionized water. In the pre-gelation method, 0.1% indomethacin containing 0.55% calcium chloride solution was added drop-wise into the sodium alginate solution using a 2 ml syringe attached to a 0.8×40 mm sterile hypodermic needle with continuous stirring. Thereafter, 0.3% HA solution and 1% chitosan solution was added in small volumes while stirring. Once a homogenous solution was formed, 0.2 g dodecyl-L-pyroglytamate was incorporated. Thereafter, the solution was subjected to ultrasonication (Ultrasonic Cleaner SB5200D, Zhejiang, China) at an amplitude of 80% to enable (alginate-hyaluronic acid-chitosan) A-H-C nanoparticle formation. The ionotropic post-gelation method differs from the pre-gelation method primarily because of the difference in the time of incorporation of indomethacin during the nanoparticle synthesis. In this method, indomethacin was added after gelation, *i.e.*, after the addition of calcium chloride to the sodium alginate solution. The nanoparticles were further modified by adding 2 ml oleic acid at the beginning of sonication to form A-H-C/OA pre-gel and post-gel NPs. The resultant NP solutions were then stored in a freezer (Sanyo Ultralow Temperature freezer, MDF-U73V, Sanyo Electric company, Japan) for 24 h at −80°C to solidify the product in a mold. Thereafter, the product was lyophilized (Labconco Freeze-Dry Systems, Labconco Corp., Kansas City, MO, USA) for 48 h, resulting in a freeze-dried powder. Table I describes the six different nanoparticle formulations and their composition in detail.

Determination of the Particle Size and Zeta Potential

The mean diameter of the NPs were determined by photon correlation spectroscopy (DTS (nano), Malvern instruments Ltd, Worcestershire, UK). Each sample was diluted in deionized water and analyzed with a detector at a 90° angle. As a measure of the particle size distribution, the polydispersity index (PI) was used. For each batch, the mean diameter and size distribution were the mean of three measures. For each formulation, the mean diameter and PI were calculated as the mean of three different batches. The zeta potential (ζ) of the NPs surface was measured in deionized water by means of a Zetasizer Nano Z (Malvern, UK). An average of three measurements was taken as the data point.

Scanning Electron Microscopic Analysis

The surface morphology of the pre- and post-gel nanoparticles was analyzed by scanning electron microscopy (FEI Nova Nanolab 600 FIB/SEM, USA). After re-dispersing the lyophilised samples in deionized water, samples were allowed to dehydrate on aluminium stubs and prepared for visual analyses by sputter-coating with a thin layer of carbon. The shape, homogeneity, and the surface morphology were ascertained and photomicrographs were captured at different magnifications to assess the aforementioned characteristics.

Table I. Representing the Composition of Different Nanoformulations

Sample	Composition
Pre-gel	
F1	Chitosan, sodium alginate, hyaluronic acid, and indomethacin
S1	Chitosan, sodium alginate, hyaluronic acid, indomethacin, and oleic acid
D1	Chitosan, sodium alginate, hyaluronic acid indomethacin, oleic acid, and DLP
Post-gel	
F2	Chitosan, sodium alginate, hyaluronic acid, and indomethacin
S2	Chitosan, sodium alginate, hyaluronic acid, indomethacin, and oleic acid
D2	Chitosan, sodium alginate, hyaluronic acid indomethacin, oleic acid, and DLP

Fourier Transform Infrared Spectroscopy

The structures of the native polymers, namely chitosan, alginate, and hyaluronic acid, as well as the drug indomethacin were assessed by FTIR using a Spectrum 2000 spectrometer with a MIRTGS detector (PerkinElmer Spectrum 100, Wales, and UK). Furthermore, the pre- and post-gel indomethacin-loaded cross-linked lyophilized samples were evaluated to assess any variations in the vibrational frequencies as a result of crosslinking during gel formation. Samples were placed on a diamond crystal and processed by a universal ATR polarization accessory for the FTIR spectrum series at a resolution of 4 cm⁻¹. Samples were analyzed at wavenumbers ranging from 600 to 4000 cm⁻¹.

Simulated Inflammatory Solution (Fenton's Reagent)

A solution simulating an inflammation-like environment was required to determine the degradation of the nanoparticles and the release of drug in the presence of inflammation. A combination of H₂O₂ and FeSO₄ was employed to generate hydroxyl radicals (14). The reaction is as follows (14):

0.566 g ferrous sulphate (FeSO₄) was dissolved in 20 ml distilled water. Then, 20 ml of a 0.1 M hydrogen peroxide solution was added to 40 ml phosphate buffer at pH 7.4. The solution was filtered through a 125-mm filter paper to produce a clear solution.

Drug Entrapment Studies

Drug encapsulation efficiency was determined by UV-Vis spectrophotometer (SPECORD 400, Win Aspect). One hundred milligrams of freeze-dried nanoparticles were incubated in phosphate-buffered saline (PBS) (pH 7.4) at room temperature and centrifuged at 12,000 rpm for 30 min. The concentration of drug in the supernatant was quantified using the absorbance maxima of the drug indomethacin (λ_{max} indomethacin = 318 nm). A calibration curve of indomethacin in PBS (pH 7.4) was also prepared. The calibration curves were linear in the ranges

of standard concentrations measured. Equations for drug encapsulation efficiency (DEE) are as follows:

$$\% \text{DEE} = \frac{\text{Weight of the drug (mg)} \times 100}{\text{Total weight (nanoparticles + drug)}} = \frac{\text{Actual yield} \times 100}{\text{Theoretical yield}}$$

Drug Release Studies

Drug release rates of the nanoparticles were performed in two different conditions as follows: 100 mg of lyophilized nanoparticles were dispersed in 50 ml of PBS of pH 7.4 and Fenton's reagent and placed in three separate dialysis bags (MWCO: 12,400 Da), which were immersed in separate beakers and contained 50 ml of the respective buffers. Samples were placed into an Orbital Shaker Incubator that was maintained at a temperature of 37°C and a speed of 25 rpm. Samples were incubated and at suitable time intervals, 2 ml of each of the samples were removed and filtered through a 0.22-μm Millipore filter into a cuvette for analysis by UV spectroscopy. The 2 ml that was removed for analysis was replaced in order to maintain sink conditions.

Skin Permeation Studies

The skin penetration efficiency of the drug was analyzed by a Franz diffusion (Perme Gear, Amie Systems, USA) method to determine the amount of drug crossing a semi-permeable cellulose dialysis membrane. The membrane with a molecular weight of 12,400 MWCO was employed to serve as the proposed skin for topical administration. Fifty milligrams of indomethacin-loaded nanoparticles was placed into the donor compartment with 2 ml of phosphate buffer solution of pH 7.4, and 8 ml of Fenton's reagent was placed in the receptor compartment. The surface area of the membrane exposed to the solution was 1.767 cm². The diffusion cells were immersed in a water bath maintained at 37°C by a temperature bath heating system (17). Samples of 1 ml were withdrawn from the receptor compartment hourly for 4 h and replaced with 1 ml of fresh Fenton's reagent after each removal to maintain sink conditions. All of the experiments were conducted in triplicate.

Molecular Modeling Simulations

The molecular modeling simulations were performed using HyperChem™ 8.0.8 Molecular Modeling System (Hypercube Inc., Gainesville, Florida, USA) wherein the structure of polysaccharides (four monosaccharide units) were built employing standard bond lengths and angles on the default Sugar Builder Module on HyperChem 8.0.8. The models were sequentially optimized using the MM+ Force Field algorithm followed by Assisted Model Building and Energy Refinements (AMBER 3) Force Field algorithm. A complex of one polymer molecule with another was assembled by parallel disposition, and the energy minimization was repeated to generate the final models: ALG-CHT, CHT-HYA, HYA-ALG, and ALG-HYA-CHT. Full geometrical optimization was conducted in vacuum employing the Polak–Ribiere Conjugate Gradient method until an RMS gradient of 0.001 kcal/mol was reached (18).

Molecular Mechanics Assisted Model Building and Energy Refinements

A molecular mechanics conformational searching procedure was employed to acquire the data employed in the statistical mechanics analysis, and to obtain differential binding energies of a Polak–Ribiere algorithm and to potentially permit application to polymer composite assemblies. Molecular mechanics energy relationship (MMER), a method for analytico-mathematical representation of potential energy surfaces, was used to provide information about the contributions of valence terms, noncovalent Coulombic terms, and noncovalent van der Waals interactions for polymer/polymer interactions. The MMER model for potential energy factor in various molecular complexes can be written as follows:

$$E_{\text{molecule/complex}} = V_{\Sigma} = V_b + V_{\theta} + V_{\varphi} + V_{ij} + V_{hb} + V_{el} \quad (1)$$

where V_{Σ} is related to total steric energy for an optimized structure, V_b corresponds to bond stretching contributions (reference values were assigned to all of a structure's bond lengths), V_{θ} denotes bond angle contributions (reference values were assigned to all of a structure's bond angles), V_{φ} represents torsional contribution arising from deviations from optimum dihedral angles, V_{ij} incorporates van der Waals interactions due to non-bonded interatomic distances, V_{hb} symbolizes hydrogen-bond energy function, and V_{el} stands for electrostatic energy.

In addition, the total potential energy deviation, ΔE_{total} , was calculated as the difference between the total potential energy of the complex system and the sum of the potential energies of isolated individual molecules, as follows:

$$\Delta E_{\text{Total(A/B)}} = E_{\text{Total(A/B)}} - (E_{\text{Total(A)}} + E_{\text{Total(B)}}) \quad (2)$$

The molecular stability can then be estimated by comparing the total potential energies of the isolated and complexed systems. If the total potential energy of complex is smaller than the sum of the potential energies of isolated individual

molecules in the same conformation, the complexed form is more stable and its formation is favored (19).

Statistical Analysis

Student's *t* test was used to evaluate the statistical significance for drug encapsulation efficiency. $p < 0.05$ was considered statistically significant.

RESULT AND DISCUSSION

Physicochemical Characterization of Nanoparticles

Zeta sizing, polydispersity index, and zeta potential of all six formulations were analyzed. Zeta sizing of both pre-gelation and post-gelation nanoparticles, F1 and F2, shows in the range of 300–500 nm. However, a significant reduction in the size of nanoparticles was observed after modification with oleic acid, a permeation enhancer and followed by the ultrasonication. Both pre-gel and post-gel nanoparticles modified with oleic acid, S1 and S2 revealed NP sizes of 177 and 165 nm, respectively. However, the addition of DLP further decreased the particles sizes to 48 and 51 nm for pre-gel and post-gel NP (D1 and D2), respectively, without significant difference between the particles. This decrease in particle size upon the addition of DLP was attributed to its non-ionic surfactant nature (20). Typically, non-ionic surfactants consist of polyoxyethylene moieties with a hydrophilic head and a hydrophobic tail (20–22). During ultrasonication, interparticulate collisions occur which causes the agglomeration of particles. However, the hydrophilic head of DLP attaches to the particles surface leaving the hydrophobic tail away from the particles surface (20). This preferential adsorption of DLP onto the particles surface restricts the interconnection of particles and prevents agglomeration, thus reducing the particle size (20–22). The polydispersity index of all the formulations was in the range of 0.2 to 0.8, revealing its monodispersity nature for all the synthesized nanoparticles (Table II). The zeta potential analysis of the formulations revealed highly negative zeta values in the range of –20 mV to –63 mV (Table II). In practice, zeta potential values more than 30 V and less than –30 V were attributed to the high stability and shelf life of the synthesized formulations (23). The results were further confirmed with scanning electron microscopic images (Fig. 1a, b).

Determination of the Molecular Transitions of the Nanoparticle Formulations

FTIR was performed in order to investigate the structural composition of the developed nanoparticles. Typical FTIR spectra of post-gel nanoparticles incorporated with indomethacin as well as those of native materials such as chitosan, sodium alginate, and hyaluronic acid are displayed in Fig. 2. In the spectra of chitosan (Fig. 2a), the peaks at 3298 cm^{-1} corresponds to the amine and hydroxyl group, the –OH stretching causes a peak at 2872 cm^{-1} . The binding vibrations of the N-H bond were seen at 1585 cm^{-1} , and the peak at 1374 corresponds to the N-H stretching. In hyaluronic acid, the broad spectrum at 3284 cm^{-1} can be assigned to hydrogen-bonded O-H

Table II. Showing the Zeta Size, Polydispersity Index, and Zeta Potential of Different Formulations

Sample	Zeta size (nm)	Polydispersity index	Zeta potential (mV)
Pre-gel			
F1	259.2±5.05	0.312	0.312
S1	177.7±8.61	0.353	-22.4±0.34
D1	48.0±4.02	0.352	-19.9±0.62
Post-gel			
F2	499.0±5.63	0.851	-22.7±0.98
S2	165.9±8.28	0.271	-61.8±1.67
D2	51.6±5.42	0.420	-63.1±0.56

and N-H stretching vibrations. A small peak observed at 2916 cm^{-1} is due to the C-H stretching vibrations. The bands at 1607 and 1400 cm^{-1} can be attributed to the asymmetric (C=O) and symmetric (C-O) stretching modes of the planar carboxyl groups in hyaluronate (Fig. 2b). The bands around 1030 cm^{-1} (C-O-C) presenting in the IR spectrum of sodium alginate were attributed to its saccharide structure. Moreover, the peaks at 1585 and 1374 cm^{-1} are assigned to the asymmetric and symmetric stretching of carboxylate salt groups (Fig. 2c).

The broad band spectrum in the post-gel nanoparticle was shifted to 3321 cm^{-1} . Characteristic peaks of chitosan and sodium alginate (1585 cm^{-1}) and hyaluronic acid (1607 cm^{-1}) were shifted to 1661 and 1711 cm^{-1} , respectively, showing the interaction of these polymers during the nanoparticle formation. Functional groups that were present in all of the individual polymer spectrums include peaks at 1662 – 1711 cm^{-1} assigned to C=C alkenyl stretching and a peak at 1244 cm^{-1} indicating a C-C stretch as well

as a peak at 1080 cm^{-1} indicating skeletal vibrations. The peak at 1244 cm^{-1} showed a decrease intensity that may be attributed to the incorporation of the indomethacin. All these peaks indicate interactions between the various polymers (24). Characteristic peaks between 2955 and 2917 cm^{-1} indicate the formation of new bonds that were not present in either of the polymers, and were associated with a methylene C-H symmetrical/asymmetrical stretch. This new bond indicates that crosslinking occurred between the hyaluronic acid-chitosan and sodium alginate polymers.

Molecular Interpretation of the Formation/Formulation of the Tripolymeric Architectures

The molecular interactions affecting the formation and formulation of the final tripolymeric nanoarchetype were studied through molecular mechanics simulations in vacuum phase (Tables III and IV). The energetic stability of the *in*

Fig. 1. **a, b** Showing representative SEM images of post-gel nanoparticles. **a** Lower and **b** higher magnifications. **c** Representative graphs for zeta sizing and **d** zeta potential of nanoparticle formulation

Fig. 2. FTIR spectra of *a* Chitosan, *b* alginate, *c* hyaluronic acid, and *d* indomethacin-loaded nanoformulation

silico generated bimolecular [alginate-chitosan (ALG-CHT), chitosan-hyaluronic acid (CHT-HYA), and hyaluronic acid-alginate (HYA-ALG)] and trimolecular [alginate-chitosan-hyaluronic acid (ALG-HYA-CHT)] complexes was considered as the benchmark for the determination of preferential orientation of the polymers to form the final coherent polymeric matrix geometric conformations as discussed below:

1. Bipolymeric alginate-chitosan (ALG-CHT) molecular assembly: The geometrically optimized structure of ALG-CHT bipolymeric complex revealed the involvement of bonding torsional strain and non-bonding H-bonding, electrostatic interactions, and van der Waals forces toward the final energy minimization of -41.465 kcal/mol (Tables I and II). Figure 3A(a) clearly indicates the H-bonding involvement of $-\text{COOH}/-\text{NH}$ and $-\text{OH}/-\text{OH}$ functional groups with the former molecular interaction confirming the formation of a polyelectrolyte complex.
2. Bipolymeric chitosan-hyaluronic acid (CHT-HYA) molecular assembly: Theoretically, chitosan containing cationic amino-functionality and hyaluronic acid with anionic carboxylic-functionality appear to form a $-\text{NH}/-\text{COOH}$ polyelectrolyte complex. However, notably hyaluronic acid itself is partially charge balanced due to the presence of amido-groups and hence CHT-HYA may only form a partial-polyelectrolyte complex as shown in Fig. 3A(b). With an interaction energy of -40.136 kcal/mol, CHT-HYA displayed an extensive intra-molecular functional interaction profile with -

$\text{NH}/-\text{OH}$, $-\text{OCO}/-\text{OH}$, $-\text{OH}/-\text{OH}$, $-\text{NH}/-\text{COOH}$, and $-\text{OH}/-\text{COOH}$ H-bonds. Similar to ALG-CHT; the CHT-HYA molecular complex was mainly stabilized by van der Waals forces and electrostatic interactions.

3. Bipolymeric hyaluronic acid-alginate (HYA-ALG) molecular assembly: HYA-ALG demonstrated best compatibility with interaction energy of -49.146 kcal/mol among the three bipolymeric entities tested in this *in silico* study. As opposed to ALG-CHT and CHT-HYA, HYA-ALG displayed both intra- and inter-molecular associations with no evident polyelectrolyte complex formation, no van der Waals space infiltration, and hence higher compatibility ($-\text{COOH}/-\text{COOH}$ and $-\text{OH}/-\text{OH}$) and geometric stabilization (Fig. 3A(c)). The high torsional strain and destabilized dihedral contribution in HYA-ALG (ΔE (V_{ϕ}) = $+5.703$ kcal/mol) can be attributed to the inter-molecular repulsion experienced by HYA and ALG owing to the presence of bulky carboxylic functionalities in both the molecules.
4. Tripolymeric alginate-hyaluronic acid-chitosan (ALG-HYA-CHT) molecular assembly: The final ALG-HYA-CHT polymeric complex consisted of a very unique approach wherein an anionic polymer with primarily carboxylic acid moieties (alginate) and a cationic polymer with primarily amino moieties (chitosan) were reacted in the presence of an amphi-ionic polymer with pendent carboxylic acid moieties and weak amide groups (hyaluronic acid) forming an ionic quadrilateral (25). In contrast to bipolymeric complexes, ALG-HYA-

Table III. Inherent Energy Components Representing the Molecular Assemblies Modeled Using Static Lattice Atomistic Simulations in Vacuum

Molecule	V_{Σ}^a	V_b^b	V_{θ}^c	V_{φ}^d	V_{ij}^e	V_{hb}^h	V_{el}^g
ALG	20.719	2.689	21.034	25.652	5.393	-0.462	-33.588
CHT	15.222	1.288	7.393	10.105	6.727	0.000	-10.292
HYA	8.242	1.778	17.688	15.493	8.628	0.000	-35.347
ALG-CHT	-5.524	4.320	32.810	33.524	1.820	-1.858	-76.143
CHT-HYA	-16.672	3.340	28.322	25.065	0.163	0.000	-73.238
HYA-ALG	-20.185	4.563	38.606	46.848	4.281	-0.099	-114.386
ALG-CHT-HYA	-49.426	6.410	50.722	60.378	-12.32	-1.862	-151.854

^a Total steric energy for an optimized structure^b Bond stretching contributions^c Bond angle contributions^d Torsional contribution arising from deviations from optimum dihedral angles^e van der Waals interactions^f Hydrogen-bond energy function^g Electrostatic energy

CHT evidenced a complete destabilization of bonding interactions and substantial stabilization of non-bonding interactions leading to the formation of a “well-connected” energetically and geometrically optimized 3D-polymer architecture (Fig. 3b). Furthermore, ALG-HYA-CHT [$\Delta E(V_{\Sigma}) = -93.609$ kcal/mol] depicted higher bonding destabilization and better non-bonding stabilization for all-three respective energy components confirming the formation of a perfectly energy balanced structure wherein the bond stretching [$\Delta E(V_{\Sigma}) = +0.655$ kcal/mol], angle [$\Delta E(V_b) = +4.607$ kcal/mol], and torsional [$\Delta E(V_{\theta}) = +9.128$ kcal/mol] contributions sterically adjusted to accommodate the inherent overlap of van der Waals spaces [$\Delta E(V_{ij}) = -33.068$ kcal/mol], inter- and intra-molecular H-bonding [$\Delta E(V_{hb}) = -1.400$ kcal/mol], and the electrostatic interactions [$\Delta E(V_{el}) = -72.627$ kcal/mol]. Under different binding modes and positions, ALG-HYA-CHT formation was accompanied by -COOH/-COOH, -OH/-OH, -NH/-OH, -OCO/-OH, -COOH/-OH, and -COOH/-NH H-

bonds with bond lengths ranging between 1.729 and 3.0978. In conclusion, the tripolymeric complex proved to be energetically, geometrically, and functionally more preferable and rational than individual bipolymeric complexes.

Drug Encapsulation Efficiency

The drug encapsulation efficiency of indomethacin to the pre-gel and post-gel nanoparticles was analyzed using UV-Vis spectrophotometry. The loading efficiency of indomethacin on A-H-C nanoparticle was significantly increased by the presence of DLP both in pre-gel as well as post-gel nanoparticles. In the case of D1 and D2 nanoparticles, the drug loading efficiency was found to be 72 and 73.04%, respectively, which were approximately five- to sixfold higher than the loading efficiencies of F1, F2, S1, and S2 (Fig. 4a). It was already established that in addition to the drug permeation-enhancing property, DLP improves drug diffusibility and solubility through micelle formation. This makes indomethacin more compatible with hydrophilic polymeric systems (26,27). In the case of F1, F2, S1, and S2 formulations, interaction of

Table IV. Resultant Stabilized Energy Attributes Representing the Molecular Assemblies Modeled Using Static Lattice Atomistic Simulations in Vacuum

Molecule	$\Delta E(V_{\Sigma})^a$	$\Delta E(V_b)^c$	$\Delta E(V_{\theta})^d$	$\Delta E(V_{\varphi})^e$	$\Delta E(V_{ij})^f$	$\Delta E(V_{hb})^g$	$\Delta E(V_{el})^h$
ALG-CHT	-41.465	0.343	4.383	-2.233	-10.3	-1.396	-32.263
CHT-HYA	-40.136	0.274	3.241	-0.533	-15.192	0.000	-27.599
HYA-ALG	-49.146	0.096	-0.116	5.703	-9.740	0.363	-45.451
ALG-CHT-HYA	-93.609	0.655	4.607	9.128	-33.068	-1.400	-72.627

^a $\Delta E_{(A/B)} = E_{(A/B)} - [E_{(A)} + E_{(B)}]$ ^b Total steric energy for an optimized structure^c Bond stretching contributions^d Bond angle contributions^e Torsional contribution arising from deviations from optimum dihedral angles^f van der Waals interactions^g Hydrogen-bond energy function^h Electrostatic energy

Fig. 3. **a** Energy minimized geometrically conformation displaying the bipolymeric complex of (a) alginate (*tubes*) and chitosan (*ball-and-tube*); (b) chitosan (*ball-and-tube*) and hyaluronic acid (*stick*); and (c) hyaluronic acid (*stick*) and alginate (*tubes*) after molecular mechanics simulations. Color codes for elements: carbon (*cyan*), hydrogen (*white*), nitrogen (*blue*), and oxygen (*red*). **b** Energy minimized geometrically conformation displaying the tripolymeric complex of alginate (*tubes*), chitosan (*ball-and-tube*), and hyaluronic acid (*stick*) after molecular mechanics simulations. Color codes for elements in (a): carbon (*cyan*), hydrogen (*white*), nitrogen (*blue*), and oxygen (*red*). Color codes for structures in (b): alginate (*red*), chitosan (*blue*), and hyaluronic acid (*yellow*)

indomethacin with the hydrophilic polymers were limited, allowing low encapsulation. Hence, the higher loading efficiency observed in D1 and D2 formulation is primarily attributed to the involvement of DLP. The same parameters that decrease the mean size of the particles could also increase the encapsulation efficiency (20,22). The chain length and size of the hydrophilic head group of non-ionic surfactants affect the entrapment efficiency as is evident with DLP which has a long chain length (C12) and correspondingly higher entrapment efficiency (22,28).

***In Vitro* Drug Release Study**

By considering the application of the present study, further experiments were conducted using two nanoparticle formulations, *viz.* nanoparticle without DLP (S1 and S2) and with DLP (D1 and D2) because of their smaller particle size. The *in vitro* release profile of the developed nanoparticles was studied in PBS (pH 7.4) as well as simulated inflammation-like solution, *viz.* Fenton's reagent. Fenton's reagent provided an environment similar to that of an *in vivo* inflammatory system, while the phosphate buffer solution of pH 7.4 represented the blood compartment (29). Figure 4 shows that after 8 h of incubation, the percentage of drug release in PBS was 3.8 and 7.2% for S1 and S2 NP respectively (Fig. 4b). However, a significantly higher drug release, *viz.* 44.8 and 59.2%, was observed in Fenton's reagent for S1 and S2 respectively

(Fig. 4c). Similarly, D1 and D2 showed 82.1 and 98.2% indomethacin release respectively in Fenton's reagent (Fig. 4b) and which was significantly higher than their release profile (44.1 and 58.1%) in PBS (Fig. 4c).

This result confirmed the sensitivity of our nanoparticle system to the inflammatory environment which responds more to the hydroxyl radicals in Fenton's reagent than sodium hydroxide in the buffer solution. Hyaluronic acid was employed to impart inflammation-sensitive characteristics (14). *In vivo* HA degradation is either by the hyaluronidase enzyme or via hydroxyl radical as a source of active oxygen. During inflammation, an increase in capillary permeability results in the accumulation of polymorphonuclear leukocytes and other phagocytic cells at the inflammation site. These phagocytic cells can produce hydroxyl radicals which in turn cause the depolymerisation of hyaluronic acid which results in the enhanced drug release profile. The drug released from pre-gel nanoparticles S1 and S2 was lower than post-gel nanoparticles D1 and D2 both in PBS and Fenton's reagent. This may be attributed to the comparatively lower drug loading efficiency of S1 and S2. Interestingly, the presence of DLP in the post-gel nanoparticle synthesis was found to have an influence in the higher loading efficiency as well as drug release from the nanoparticle systems (28). This could be attributed to the lipophilic nature of DLP, which could enhance entrapment of the poorly water-soluble indomethacin (20). Furthermore, this emanates in enhanced solubilization of the drug in the

Fig. 4. **a** Graph showing percentage of drug encapsulation efficiency (DEE) of indomethacin encapsulated nanoformulations, **b** percentage of indomethacin release in phosphate buffer saline (pH 7.4), and **c** percentage of indomethacin release in simulated inflammatory solution (Fenton's reagent). Panel **d** shows indomethacin drug flux through cellulose membrane in Franz diffusion cell

nanoparticulate system, with a greater amount of indomethacin in the amorphous form. This enhances the bioavailability of the low-solubility drug and would provide higher dissolved-drug concentrations than if the drug were purely in the crystalline form resulting in the enhanced dissolution of indomethacin from D1 and D2. Drug release was lower in the phosphate buffer solution, showing the efficacy of the hyaluronic acid; however, it does degrade in the phosphate buffer solution but to a limited extent. The drug release kinetics depicted by the nanoparticle D2 from 46.8 to 98.2% within 8 h shows an ideal concept, as rapid and sustained indomethacin release ensuring its effect in the targeted inflammatory environment.

Analysis of Drug Permeation through Skin

Drug penetration studies were carried out on pre- and post-gel samples with DLP (D1 and D2) because of their inflammation-sensitive drug release profile. Samples extracted were analyzed by measuring the absorbance using a UV Spectrophotometer (SPECORD 400, Win Aspect) at a $\lambda_{\max}$ of 318 nm. Indomethacin drug penetration through the simulated skin membrane was determined using Eq. 3 as follows:

$$J_s = \frac{Q_r}{A} * T \quad (3)$$

Where J_s is the flux of indomethacin, Q_r is the quantity (mg) of drug which permeated through the cellulose dialysis membranes, A is the cross-sectional area (cm²) of the dialysis

membrane which formed the surface area for drug flux, and T is the time of exposure of the drug to the dialysis flat membrane.

The skin permeation strategy can be enhanced by the use of chemicals that can reversibly compromise the skin barrier's function and thereby allow entry of nanoparticles through the skin. The most widely investigated permeation enhancer is oleic acid, an FDA approved, monostructured fatty acid and membrane fluidizing agent. Several studies have suggested that oleic acid can stimulate the lipid bilayer of stratum corneum of skin, hence, the modification of nanoparticles with oleic acid can open the channels in the stratum corneum and thereby enhance the permeation of macromolecules into the deeper layer of the skin (30,31). In the current study, we have used two permeation enhancers, viz. oleic acid and dodecyl-L-pyroglytamate, for the synthesis of indomethacin-loaded nanoparticles. There are various reports suggesting the role of oleic acid and dodecyl-L-glutamate for improved skin permeation (32,33). The permeation-enhancing effects of DLP can be explained by its similarity in structure to other permeation enhancers such as laurocapram which has a hydrophilic head group and a 12-carbon hydrophobic tail which was established as the optimum chain length for enhanced transdermal absorption (34). In addition, DLP has been shown to disrupt the stratum corneum due to its amphiphilic nature which allows it to penetrate the lipid bilayer of the skin (35). Several earlier studies show that topically applied nanoparticle formulations were unable to permeate the skin layer, releasing drug on the skin surface and very few successful studies were done so far showing the nanoparticle permeation through hair follicles (36). Nanoparticles larger than

700 nm have been found to aggregate superficially in follicular openings (37).

Zeta sizing analysis shows the size of D1 and D2 nanoparticles in the range of 48–51 nm, which is an acceptable size to cross through pores of the skin as demonstrated by employing the dialysis flat membrane (MWCO 12400). The cellulose dialysis membrane with a molecular weight of 12,400 Da correlates with other investigations on skin permeation (38). The tubing will retain most particles of molecular weight 12,400 or greater. Moreover, optimal drug encapsulation for D1 and D2 nanoparticles (75%) indicates that an adequate amount of drug was encapsulated which could penetrate through the skin. Drug flux for sample D2 showed a surge in release in the first hour, thereafter declining slowly and reaching zero at 8 h (Fig. 4d), whereas sample D1 showed very slow permeation efficiency till 4 h then declined rapidly to reach a stable concentration. The rapid nanoparticle permeation for D2 is primarily due to the method of incorporation of drug molecule, *i.e.*, after initial gelation. Here, the drug molecules can easily release from the system compared to D1, where the drug is completely entombed and shielded by alginate molecules. This phenomenon was confirmed in the previous section that the drug release was significantly lower for D1 than D2 which now evidently apparent in the drug flux data during the permeation study. Though the nanoparticles permeated the membrane to the receptor compartment, the release assumed to be lower for D1 compared to D2.

CONCLUSIONS

Nanoparticulate drug delivery systems are rapidly replacing conventional methods and are becoming a more viable alternative to delivering drugs into the body. The current study designed to develop an inflammation-sensitive nano-drug delivery system for topical administration of anti-inflammatory drugs for arthritis. The developed chitosan/alginate/hyaluronic acid post-gel nanoparticle system showed stable, optimum nanosize with uniform distribution for topical drug delivery application. The higher drug encapsulation efficiency, drug release kinetics, and drug permeation capability of the dodecyl-L-pyroglytamate and oleic acid incorporated post-gel nanoparticle (D2) in simulated inflammatory environment highlighted the novel application of this formulation. Hence, our study presents a novel, multi-macromolecular inflammation-sensitive nanosystem particularly for topical drug delivery application.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors confirm that there are no conflicts of interest.

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