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RESEARCH PAPER

Controlled Electrostatic Self-Assembly of Ibuprofen-Cationic Dextran Nanoconjugates Prepared by low Energy Green Process – a Novel Delivery Tool for Poorly Soluble Drugs

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ABSTRACT

Purpose The direct effect of electrostatic interaction between ibuprofen and cationic dextran on the system-specific physicochemical parameters and intrinsic dissolution characteristics of ibuprofen was evaluated in order to develop drug-polymer nanoconjugate as a delivery strategy for poorly soluble drugs.

Methods Amorphous ibuprofen-DEAE dextran (Ddex) nanoconjugate was prepared using a low energy, controlled amphiphile-polyelectrolyte electrostatic self-assembly technique optimized by ibuprofen critical solubility and Ddex charge screening. Physicochemical characteristics of the nanoconjugates were evaluated using FTIR, DSC, TGA, NMR and SEM relative to pure ibuprofen. The *in vitro* release profiles and mechanism of ibuprofen release were determined using mathematical models including zero and first order kinetics; Higuchi; Hixson-Crowell and Korsmeyer-Peppas.

Results Electrostatic interaction between ibuprofen and Ddex was confirmed with FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy. The broad and diffused DSC peaks of the nanoconjugate as well as the disappearance of ibuprofen melting peak provided evidence for their highly amorphous state. Low concentrations of Ddex up to 1.0×10^{-6} g/dm^3 enhanced dissolution of ibuprofen to a maximum of 81.32% beyond which retardation occurred steadily. Multiple release mechanisms including diffusion; discrete drug dissolution; anomalous transport and super case II transport were noted.

Conclusions Controlled assembly of ibuprofen and Ddex produced a novel formulation with potential extended drug release dictated by Ddex concentration.

KEY WORDS amorphous ibuprofen-Ddex nanoconjugate · controlled electrostatic self-assembly · low energy green process · multiple release mechanisms · particle surface modification

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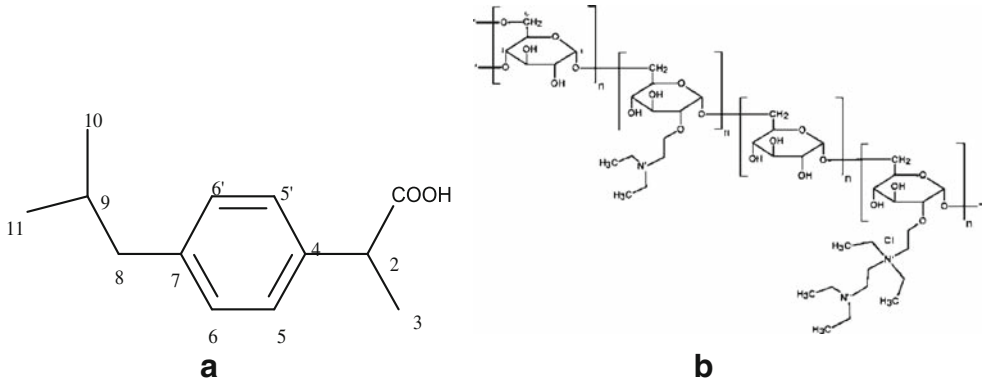
ABBREVIATIONS

ANOVA	Analysis of variance
CAPESA	Controlled amphiphile-polyelectrolyte electrostatic assembly
CE	Conjugation efficiency
Ddex	Diethyaminoethyl Dextran
DE	Dissolution efficiency
DSC	Differential scanning calorimetry
FT-IF	Fourier Transform Infra-Red spectroscopy
Ibu	Ibuprofen
MANOVA	Multivariate analysis of variance
MDR	Mean dissolution rate
MDT	Mean dissolution time
NMR	Nuclear Magnetic Resonance
SEM	Scanning Electron Microscopy
TGA	Thermal Gravimetric Analysis

INTRODUCTION

Ibuprofen [(±) -2-[4 – (2 – methylpropyl) phenyl] propanoic acid] is one of the most popular and safest non-steroidal anti-inflammatory drug (NSAID) for the management of pain, feverish and inflammatory conditions including symptoms of rheumatoid arthritis and osteoarthritis (1). It is a colourless needle-like (acicular) crystalline material (2) and practically insoluble in water (49 μg/ml at 25°C) with a pKa of 4.5 (3). Ibuprofen (Scheme 1a) exhibits hydrophobic, viscoelastic and high cohesive characteristics as well as poor flow, compression and dissolution properties (4–6). These properties have been reported to be responsible for most of the formulation difficulties encountered during manufacturing of ibuprofen such as caking of suspensions (7), capping and lamination of tablets etc. (8). Variation in crystal lattice arrangement of a drug has a huge impact on its physicochemical properties such as flow properties, solubility, compression characteristics, dissolution

Scheme I Structural formula of (**a**) Ibuprofen and (**b**) Ddex



profile etc. (4). However consistent research efforts to date have not been able to establish the desired improvement in the physicochemical and biopharmaceutical properties of ibuprofen (8). Therefore the problems of poor solubility, compression and dissolution characteristics remain unresolved and research interest in this area is continuous.

Ibuprofen has a short biological half-life (2 h) and is rapidly absorbed from the GIT exhibiting peak plasma concentration and complete excretion at 2 and 24 h respectively (9). It has high permeability but low solubility (Biopharmaceutics Classification System (BCS) class II) hence dissolution is the rate limiting step for absorption after oral administration (10, 11). The low solubility phenomenon and short plasma half-life translate to limited systemic bioavailability and rapid clearance which may necessitate high and multiple dosing in order to maintain steady state plasma concentration for therapeutic action. The usual consequence includes wasted dosing and potentially serious side effects (6). However research attempts to enhance the solubility of BCS class II drugs have been shown to correlate well with increased bioavailability (12). Solubility of ibuprofen increases as a function of the surrounding pH, for instance it exhibits a solubility of 0.049, 0.053 and 6.02 mg/ml in water, pH 1.2 and pH 7.4 respectively (3, 13).

In the recent past, biodegradable polyelectrolyte nanocarriers such as nanoparticles, nanocapsules, micellar systems, and nanoconjugates have been a focus of intensive research for the delivery of poorly soluble drugs because they provide great opportunities in the area of site specific drug delivery due to their submicron size. The intention was to modify the physicochemical characteristics and system specific parameters of the active drug; improve the release profile of poorly soluble drugs; improve therapeutic benefits and minimise side effects (14).

Polyelectrolytes are polymers whose repeating units contain ionisable groups attached to their structure enabling them to dissociate in aqueous solutions and release low molecular weight counterions to the solution giving the polymer chain a defined charge (15). A mixture of the charged polymer solution and oppositely charged drug solutions would produce drug-polyelectrolyte self-assembly (complex) by electrostatic attraction. This technique which is a green and low energy process is relatively new and is attracting a lot of research interest as a formulation strategy to improve physicochemical properties and therapeutic quality of poorly soluble drugs as well as optimizing their delivery efficiency (5, 6). However there has been a slow progress in transforming polymer-drug conjugates into clinically useful medicines for regulatory approval because of unspecific drug release profile (8). Therefore research interest in this area is continuous.

Diethylaminoethyl-Dextran (Ddex) is the cationic polyelectrolyte (Scheme 1b) used in this study because of its ability to form nanoconjugates with many drug molecules as well as its good stability, biocompatible and biodegradable characteristics (16). Jiang *et al.*, 2005a (17) demonstrated the formulation of ibuprofen-loaded Ddex nanoparticles using co-precipitation technique. They concluded that the technique produced a core-shell nano-structure at pH 6.0 and ibuprofen-Ddex weight ratio of 1:5. However macroscopic needle-like crystal precipitate of ibuprofen was detected in the milieu especially at lower pH than 6.0. In a separate study Jiang *et al.*, 2005b (18) prepared ibuprofen-loaded nanoparticles with an average of 200 nm size and 73–74% loading efficiency using Ddex and polylactide polymers crosslinked with glutaraldehyde.

Chen *et al.*, 2013 (19) prepared ibuprofen-loaded poly-(methylvinyl ether-co-maleic anhydride (PVM/MA) nanoparticles using solution-enhanced dispersion by supercritical CO_2 (SEDS) process. The nanoparticles prevented the release of ibuprofen at low pH while ~90% release within 48 h was reported at higher pH (8.5) suggesting a reduced adverse effects caused by ibuprofen in the stomach and colon targeted release respectively. However 20 ppm of organic solvent residue was detected by GC analysis. The authors have previously utilized particles from gas-saturated solutions (PGSS^{TM})-based technique to prepare PEG6000-ibuprofen composite nanoparticles (20–500 nm). In order to avoid degradation of nanoparticles by gastric lipase, Dian *et al.*, 2013 (20) utilized non-digestible lipid phytantriol to produce ibuprofen loaded cubic nanoparticles with mean particle size of 238.1±2.8 nm using homogenization (*high energy)* technique.

Pathak *et al.*, 2005 (21) used a modified traditional rapid expansion supercritical solution (RESOLV) method for the production of nanoscale (less than 100 nm) particles. However post-production stabilization scheme was required to protect the drug nanoparticles from agglomeration. Ye and Squillante (22) also produced ibuprofen-loaded poly (lactide-co-glycolide) (PLGA) nanoparticles using modified spontaneous emulsion solvent diffusion (SESD) method. They concluded that this method provided narrow size distribution, high encapsulation efficiency, and high batch-to-batch reproducibility.

Previously, we have reported the crystal habit, micromeritic properties, solubility, dose distribution, pre-compression characteristics and dissolution profile of ibuprofen-cationic dextran conjugate crystanules prepared by melt-*in situ* granulation-crystallization technique. The technique demonstrated the formulation of a novel stable amorphous ibuprofen-Ddex conjugate crystanule which exhibited improved dose distribution, enhanced pre-compression characteristics, controlled release, extended and complete release profiles (5, 6). However the process involved the utilization of high energy. To our knowledge, the controlled amphiphile-polyelectrolyte electrostatic self-assembly of ibuprofen-cationic dextran nanoconjugates, a *low energy and green* process has not been reported in literature. In the same vein, quantification of the direct effect of drug-polymer interaction on the physicochemical and *in vitro* release characteristics of ibuprofen is also lacking in literature. Therefore in this study the electrostatic interaction between ionized ibuprofen species (weak organic acid amphiphile) and cationic dextran was envisioned to prepare a stable amorphous ibuprofen-Ddex nanoconjugate and the direct effect of the polymer-drug association on the physicochemical and *in vitro* release characteristics of ibuprofen was evaluated. By understanding the impact of this interaction, it may be possible in the future to design specifically tailored drug-polymer formulations with more desirable physicochemical and drug release profiles than those currently available.

MATERIALS AND METHODS

Materials

Ibuprofen was purchased from Fargon UK (Newcastle upon Tyne, UK), diethylaminoethyl dextran (Ddex) with molecular weight of 500,000 g/mol was purchased from Sigma-Aldrich (Gillingham, Dorset, UK). Both materials were used in this study without any further purification. Other chemical reagents were of analytical grade.

Methods

Preparation of Ibuprofen-Ddex Nanoconjugates

A novel *controlled amphiphile-polyelectrolyte electrostatic self-assembly (CAPESA)* was employed in this study to prepare the ibuprofen-Ddex nanoconjugates. Briefly, double strength molar concentrations of Diethylaminoethyl Dextran (Ddex) ranging from 5.0×10^{-4} to 1.6×10^{-2} mM was prepared in 25 ml de-ionized water at room temperature (25°C) under continuous magnetic stirring (1000 rpm) in a jacketed vessel on a water bath (B Braun Certomat WR Shaker water bath, Germany). Double strength molar concentrations of ibuprofen (2.42, 4.85, 9.70 and 19.40 mM) were dissolved in 3–5 ml of sodium hydroxide (0.1 M) and made up to 25 ml with distilled water. Ibuprofen solution was added drop wise to the Ddex solution with continuous stirring for 24 h. The total volume of the conjugate colloidal dispersion (50 ml) contains 1.21, 2.42, 4.85 and 9.70 mM of ibuprofen and 2.5×10^{-4} to 8.0×10^{-3} mM of Ddex respectively. The colloidal dispersion of each drug polymer molar ratio was transferred quantitatively into separate dialysis tube (MWCO 14000 Da) and placed in 900 ml deionised water in a USP XXI six stage paddle dissolution apparatus at 25°C and 50 rpm paddle rotation. The deionised water was changed three times after each dialysis cycle time of 3 h. At the end of the three cycles (9 h), all the water washings were pooled together and evaluated spectroscopically to determine the amount of un-conjugated ibuprofen at 264 nm.

The colloidal dispersions of the conjugates were transferred into small 10 ml vials and freeze dried for a total of 68 h. Primary drying cycle included freezing samples to −35°C within the first hour and maintained for a further 8 h. Shelf temperature was decreased to −30°C under the vacuum (0.1mBar) for 1 h. Samples were kept under these conditions for 48 h. During the secondary drying cycle the temperature ramp occurred up to 20°C for 6 h and it was maintained for further 4 h. The freeze dried samples were kept in the refrigerator (4–8°C) until ready for analysis.

Zeta Potential of Ibuprofen-Dex Nanoconjugates

ZetaPlus Zeta Potential Analyser (Brookhaven Instruments Corporation) was used to determine the zeta potential of the nanoconjugates. Samples were diluted appropriately with distilled water and injected into the sample cell at 25°C and the electric field strength of 14.95 V/cm. The electrophoretic mobility of the dispersed nanoconjugate was measured in a charged field. All measurements of individual samples were a mean of 10 runs.

Ibuprofen-Ddex Conjugation Efficiency

The amount of ibuprofen that forms the ibuprofen-Ddex conjugate was calculated as the difference between the amounts of

ibuprofen added and the amount of non-conjugated ibuprofen in the dialysis washings after 9 h of dialysis process (Eq. 1). 5 ml of the dialysis washings was passed through a 0.45 μm disposable Millipore membrane filter (Sartorius, Germany) and diluted to a suitable concentration with de-ionized water. The non-conjugated ibuprofen concentration was determined using UV – visible spectrophotometer (ThermoFischer Evolution 60 UV Spectrophotometer, UK) at 264 nm. All measurements were an average of six determinations.

$$Conjungation\ efficiency = \frac{M_i - M_n}{M_i} X\ 100\% \quad (1)$$

Where M_i is the initial amount of ibuprofen added and M_n is the amount of non-conjugated ibuprofen in the dialysis washings after 9 h.

Morphological Characteristics of Ibuprofen-Ddex Nanoconjugates Using Scanning Electron Microscope (SEM)

The shape and surface topography of pure ibuprofen crystals, Ddex and the nanoconjugates were determined by using Carl Zeiss SEM EVO High Definition 15 Scanning Electron Microscope (Carl Zeiss, Germany) operating at 15 kV. The samples were mounted on a metal stub with double-sided adhesive tape and gold-coated under vacuum in an argon atmosphere prior to observation. Particle size was determined using *SmatTiff* software and average of a minimum of twenty nanoconjugates was determined within each microscopic view.

Fourier Transforms Infrared Spectroscopy (FT-IR)

The method described previously (5) was adapted to investigate the structural changes in ibuprofen-Ddex nanoconjugates compared with the pure ibuprofen. Briefly, about 10 mg of each sample was placed on the diamond surface plate of the Perkin-Elmer Precisely Spectrum One FTIR Spectrometer with Universal ATR Sampling Accessory (Perkin Elmer, USA). Sufficient pressure (100–120 units) was applied for close contact compression. The spectrum for each sample was recorded within the wave number range of 4000–650 cm^{-1} at an average of 16 scans and resolution of 4 cm^{-1}. All measurements were taken in replicate of six determinations.

Differential Scanning Calorimetry (DSC)

As described previously (5), Perkin Elmer Precisely Jade DSC machine with a Perkin Elmer Intracooler SP cooling Accessory and Pyris Software (PerkinElmer Ltd., Beaconsfield, UK) was utilized to evaluate the thermal behaviour of the ibuprofen-Ddex nanoconjugates. The temperature and heat flow of the instrument were calibrated using an indium and zinc standards. The sample sizes in the range of 5–8 mg were heated in hermetically sealed aluminium pans under nitrogen flow (40 ml/min) using a scanning rate of 20°C/min from −50 to 300°C. Empty aluminium pan was used as a reference. All measurements were an average of four determinations and expressed as mean ± S.D.

Thermal Gravimetry Analysis (TGA)

Perkin Elmer Pyris 1 Thermogravimetric Analyser (PerkinElmer Ltd., Beaconsfield, UK) was used to evaluate the rate and extent of weight change of the ibuprofen-Ddex nanoconjugates with temperature. Standard references (Alumel and nickel) were used to calibrate the weight profile. Samples of known weight (3–5 mg) were analysed in crimped alminium pans placed in crucible baskets at a scanning rate of 10°C/min between 25 and 500°C. All measurements were an average of four determinations and expressed as mean ± S.D.

NMR Spectroscopy

As described previously (5), Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectra of the nanoconjugates were generated using the Bruker AV-400 (Ultrashield, Germany) spectrometer equipped with a pulsed field gradient accessory (490 mT m^{-1} z-field gradient) at 25°C (298 K) and a probe tuned at 5.13 MHz. The samples were prepared in DMSO and equilibrated in the probe for about 30 min at 25°C before the NMR experiments. ^{1}H-NMR spectra of the pure ibuprofen and the ibuprofen-Ddex nanoconjugates were studied in 5 mm QNP (1H/15) tubes and recorded at room temperature; frequency 399.94 MHz; 45° pulse; relaxation delay of 1 s; acquisition time of 7.9168 s and spectra width of 8.278 kHz. One hundred and twenty eight scans were recorded for each sample. ^{13}C-NMR analysis was also performed on the same set of samples at frequency of 399.94 MHz; acquisition time 1.3665 s and spectra width of 23.980 kHz. All chemical shifts were assigned relative to Tetramethylsilane (TMS) as external reference. One thousand and twenty four scans were recorded for each sample.

In Vitro Release Kinetics

Previously described method (5) was adapted. The USP XXI six stage dissolution apparatus II (paddle) method was used on Pharma Test Dissolution tester DT70 with 900 ml of phosphate buffer (pH 7.4) as the dissolution medium. The temperature of the medium was maintained at 37 ± 0.5°C throughout the study. The samples of pure ibuprofen and ibuprofen-Ddex nanoconjugate containing 100 mg of ibuprofen or its equivalent were placed in the dissolution medium and stirred at 50 rpm using a rotating paddle. 5 ml aliquot samples were withdrawn at pre-determined time intervals (5,10,15,20,25,30, 40,50,60,70,80,90,100,110,120 min; every hour up to 8 h and

every 24 h for 2 days). 5 ml of fresh dissolution medium was replaced after each sampling to maintain a constant volume. Each sample was filtered through a 0.22 μm disposable Millipore membrane filter (Sartorius, Germany) and diluted appropriately with the dissolution medium. The absorbance of the diluted solutions were measured at 264 nm using UV – Visible Spectrophotometer (Evolution 60S, Thermo Scientific, China) against the dissolution medium as the blank. Each measurement was an average of six determinations. Percentage drug release was calculated using the equation obtained from the calibration curve of ibuprofen secondary standard prepared under the same experimental conditions.

Mechanism of Ibuprofen Release from Ibuprofen-Ddex Nanoconjugate

The rate and extent of ibuprofen release from the ibuprofen-Ddex nanoconjugates were fitted into mathematical models including zero order kinetics; first order kinetics; Higuchi; Hixson-Crowell and Korsmeyer-Peppas (23). Dissolution profiles of up to 60% were selected for the model fitness analysis. The degree of fitness into the mathematical models was used to evaluate the mechanism of drug release

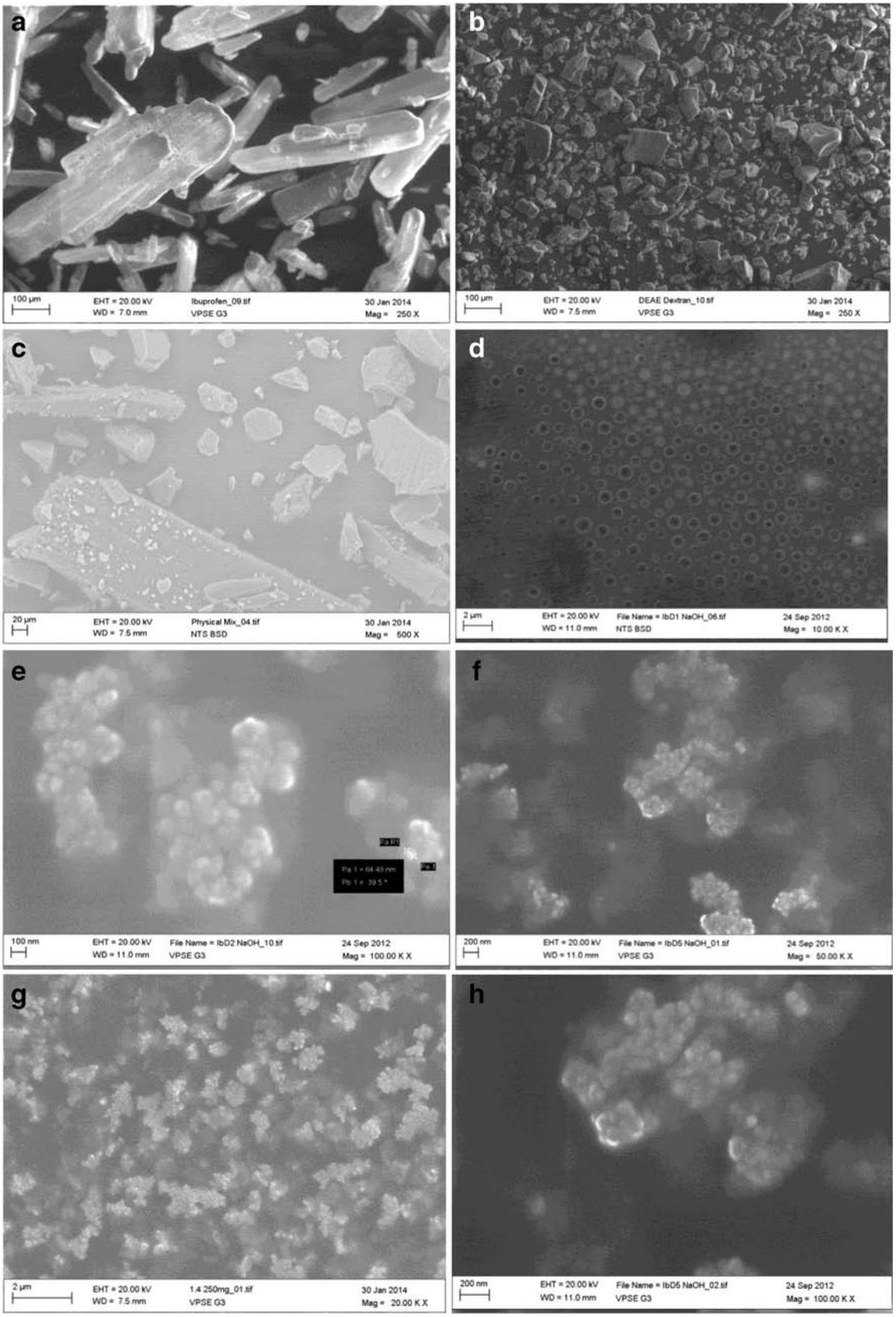
Statistical Analysis

Quantitative data were analyzed using one way non-paired analysis of variance (ANOVA) to test differences in the percent of ibuprofen dissolved at each time point separately while multiple variate (MANOVA) was used to compare the dissolution profiles of nanoconjugates with different concentrations of Ddex and at each time point. Pairwise comparison of the nanoconjugate against reference pure ibuprofen was also performed. SPSS 8.0 for Windows (SPSS, Chicago, IL) was employed for the ANOVA based technique while Student *t*-test was used to determine any significant differences between test samples and the control. Differences were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Formulation and Optimization of Ibuprofen-Ddex Self-Assembled Nanoconjugates

The process of ibuprofen-Ddex electrostatic self-assembly was controlled by optimizing the formulation variables such as pH, amount of ibuprofen, drug-polymer ratio, mixing time, mixing speed, temperature and order of drug-polymer addition (results not presented). Ibuprofen was ionized at high pH in 0.1 M NaOH to produce a highly soluble carboxylate species (24). In contrast, although Ddex is soluble and stable aqueous medium with pH between 4 and 10, it is usually uncharged at high pH however as pH decreases the amino group would be protonated to give a net positive charge. It was noted that the ibuprofen nanoparticles was enclosed *via* electrostatic self-assembly as concentrations of Ddex increased (Fig. 1d). In a similar study, Jiang *et al.* (17) prepared ibuprofen-loaded nanoparticles by co-precipitation technique. They reported that interaction between hydrophilic polymer and oppositely charged drug molecule retards further accumulation through hydrostatic and charge repulsion forces leading to the formation of nanoparticles with a core-shell structure. They demonstrated that ibuprofen became supersaturated at pH below its pKa value (4.5) and precipitation occurred however ibuprofen and Ddex could not be perfectly coacervated to form compact particles at pH 9 and above as a very loose and fluffy particle structure was formed. Therefore in this study the pH and consequently the solubility of ibuprofen were controlled to ensure that the concentration of ibuprofen was lower than its critical solubility so that the nanoconjugate was formed by simple self-assembly in order to prevent co-precipitation. The nanoconjugates were further consolidated by increasing concentration of the Ddex and excess Ddex (positive charge) was screened using three cycles of 3 h dialysis process. Physicochemical properties of ibuprofen, Ddex and their binary nanoconjugates, such as surface tension, conductivity, turbidity and pH were evaluated to determine the critical association concentration and polymer saturation point which in turn was used to determine the appropriate ibuprofen concentration and drug-polymer ratio for the formulation of the nanoconjugates. It was noted that 2.42×10^{-3} g/dm^3 of ibuprofen exhibited the smallest particle size (85.26–157.10 nm) across the varying concentrations of Ddex used in this study (Table I). It also showed consistent and reproducible physicochemical characteristics. It was hypothesized that this particle size range will exhibit complete dissolution by diffusion and burst release profiles. Therefore the direct effect of ibuprofen-Ddex interaction and the varying concentration of Ddex on the physicochemical characteristics and dissolution profile of ibuprofen-Ddex nanoconjugates have been investigated. In a similar study, Reddy and Gudsookar prepared gliclazide-Polyethylene glycol conjugate using the solid dispersion technique in organic solvent (25). The authors reported particle size reduction resulting in increased surface area and gliclazide wettability. However the use of organic solvent to dissolve the drug may impact the safety of the final product. Therefore in this study, the binary (Ibuprofen-Ddex) nanoconjugates were prepared by a low energy, controlled amphiphile-polyelectrolyte electrostatic self-assembly (CAPESA) technique which is a safe, green and environment-friendly process. It precludes the use of high energy and toxic organic solvents. Ibuprofen was ionized in order to enhance the propensity and extent of its interaction with the cationic polymer and ensure formation of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◀ **Fig. 1** Representative Scanning Electron Micrographs of Ibuprofen-Ddex nanoconjugates showing (**a**) pure ibuprofen crystals; (**b**) pure Ddex; (**c**) physical mixture of ibuprofen and Ddex; (**d**)–(**h**) ibuprofen-Ddex nanoconjugates at weight ratios 1:0.5; 1:1; 1:2; 1:4 (×20,000 magnification) and 1:4 (×100,000 magnification) respectively.

nanoconjugates with predictable formulation characteristics and potentials to enhance the delivery of poorly soluble drugs.

Surface Morphology and Particle Size Analysis of Ibuprofen-Ddex Nanoconjugates

Figure 1 shows the morphologic characteristics of pure ibuprofen, Ddex and the nanoconjugates under Scanning Electron Microscope (SEM). Pure ibuprofen (Fig. 1a) exhibits distinct rod-like shape with smooth regular surface and average aspect ratio (length / width) of 5.16±1.15 (n=20) which was within the reported literature range of 4–6 (26). The physical mixture of ibuprofen and Ddex exhibited the features of both components (Fig. 1c) while the nanoconjugates exhibited spherical structures with varying sizes within nanometre range. Conversion of rod-like ibuprofen crystals (453.88±29.8469× 97.12±5.4267 μm) into nanoconjugates (85.2±4.4461 to 157.10±10.0214 nm) by the simple green process is of great value in the delivery of poorly soluble drugs. The size of the individual nanoconjugates decreased steadily with increasing amount of Ddex from 157.10±10.0214 nm at 2.5×10^{-7} g/dm^3 Ddex to 85.26±4.4461 nm at 4.0×10^{-6} g/dm^3 Ddex. It was evident that this technique effectively produced nanoconjugates however they associated to form loose aggregates whose size increased from 323.30±11.7144 to 1009.12± 28.7991 nm with increasing concentration of Ddex (Fig. 2). It was noted that the individual nanoparticle in the aggregate maintained their spherical identity suggesting a reversible physical aggregation rather than particle growth. In theory, if the kinetic energy or velocity of the particles is sufficiently high they will collide, coalesce and grow in size (26). The values of Zeta potential of the nanoconjugates are positive increasing from 0.96 ±0.2400 to 9.08±0.8122 mV with increasing concentration of Ddex (Table I) indicating surface modification of the nanoconjugates by cationic dextran. These values are quite low relative to the minimum Zeta potential (±20 mV) required for electrostatic stability of nanosuspensions to minimize particle aggregation (27). As an amphiphilic molecule, ibuprofen can interact with biocompatible polymers to form micelle-like association above critical aggregation concentration (*cac*) (28). Therefore the loss of crystalline nature of ibuprofen may be explained by the molecular interaction between ionized ibuprofen and the cationic dextran which engenders the formation of micelle-like nanoconjugates by disrupting the crystal lattice of ibuprofen. This spontaneous electrostatic self-assembly of the oppositely charged molecules may have prevented ibuprofen from reverting back into the ordered crystalline form. In theory, the smaller size of the nanoconjugates would lead to higher dissolution rate and saturation solubility. Therefore the focus of the present study was to evaluate the quantitative impact of this interaction on the physicochemical and dissolution profiles of poorly soluble ibuprofen.

Conjugation Efficiency

Conjugation efficiency (CE) increased from 91.60±0.1617% at 1:0.5 ibuprofen-Ddex ratio (9.70×10^{-3} g/dm^3 ibuprofen) to a maximum of 99.65±0.42777% at 1:4 weight ratio (4.85×10^{-3} g/dm^3 ibuprofen) followed by a steady decreased (Fig. 3a). This trend corresponds to decrease in particle size with increasing Ddex concentration as described in section 3.2. CE also increased consistently with Ddex concentration at all concentrations of ibuprofen studied (Fig. 3b) suggesting a potential scale-up application. Figure 3c shows a steady decrease in CE with increasing ibuprofen-Ddex weight ratio. However CE at 4.85×10^{-3} g/dm^3 exhibited the highest CE irrespective of the ibuprofen/Ddex ratio. It was opined that less Ddex was available to complex with increasing amounts of ibuprofen at higher ibuprofen/Ddex weight ratio. In corollary a relatively similar CE values at lower ibuprofen-Ddex ratio may be explained by the presence of excess amounts of Ddex. These CE values are remarkably high compared with the

Table I Physicochemical characteristics of ibuprofen-Ddex nanoconjugates

Ddex molar concentration	Physicochemical characteristics			
	Nanoconjugate size (nm)	Size of nanoconjugate aggregates (nm)	Zeta potential (mV)	Conjugation efficiency (%)
Pure ibuprofen (Aspect Ratio 5.16±1.15)	–	–	−7.25±1.3000	–
Ibuprofen/Ddex nanoconjugate (2.5×10^{-7} g/dm^3 Ddex)	157.10±10.0214	323.30±11.7144	0.96±0.2400	91.60±1.0053
Ibuprofen/Ddex nanoconjugate (5.0×10^{-7} g/dm^3 Ddex)	126.76±8.4918	376.80±10.8175	1.56±0.2304	97.94±0.8511
Ibuprofen /Ddex nanoconjugate (1.0×10^{-6} g/dm^3 Ddex)	107.18±8.1450	566.67±20.5555	2.56±0.2615	98.86±0.9108
Ibuprofen/Ddex nanoconjugate (2.0×10^{-6} g/dm^3 Ddex)	104.79±6.9301	591.43±25.0380	4.79±0.2488	99.32±0.9407
Ibuprofen/Ddex nanoconjugate (4.0×10^{-6} g/dm^3 Ddex)	**85.26±4.4461**	**1009.12±28.7991**	**9.08±0.8122**	**99.65±0.5619**

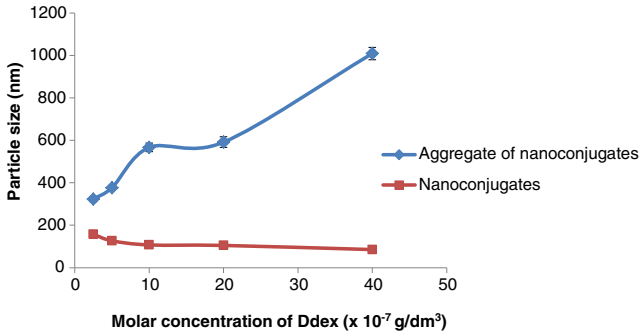
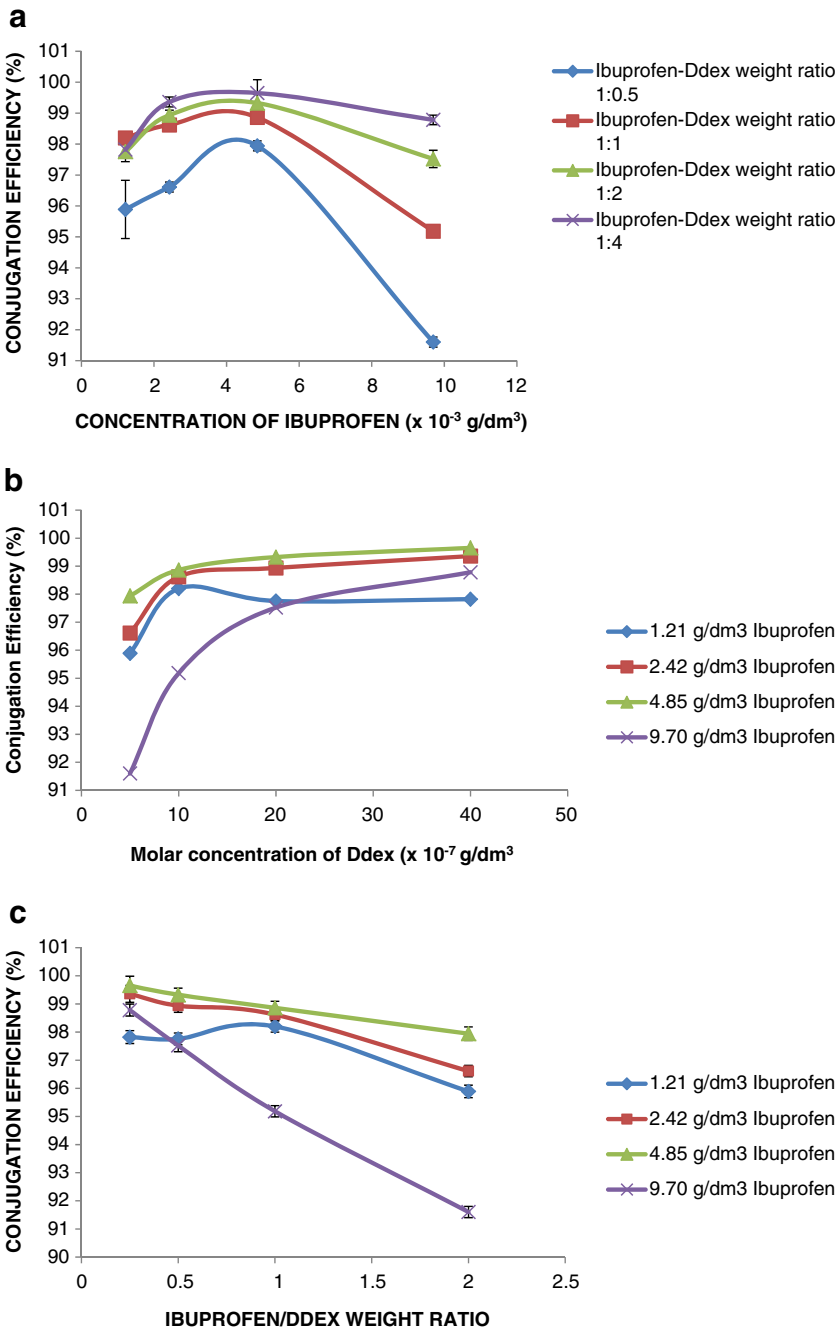


Fig. 2 Influence of Ddex concentration on particle size of the nanoconjugates ($n = 6$).

ibuprofen loading capacity reported in literature. For instance Hornig *et al.*, 2009 reported CE of 37 to 71% for a chemically synthesized ibuprofen-dextran (dextran ester) conjugates (29). The chemical reagents used in their technique such as dimethylsulfoxide (DMSO), N,N-dimethylacetamide (DMAc), N,N-dimethylformamide (DMF) and acetone may constitute some safety and environmental concerns. In similar studies 30% loading efficiency of ibuprofen in polymer-coated SiO_2 particles (30); 10% ibuprofen loading in lipid nanoparticles e.g. smectic cholesterol ester nanoparticles (31) and 9% ibuprofen in Eudragit polymeric nanoparticles (18) have been reported. The core-shell ibuprofen-Ddex nanoparticles prepared by complex

Fig. 3 Conjugation efficiency profiles of ibuprofen-Ddex nanoconjugates at varying molar concentrations of Ddex ($n = 6$).



coacervation technique also contained 32% ibuprofen (32). The remarkably high CE in this study could be explained by the simultaneous electrostatic and hydrophobic interactions between polyelectrolyte and amphiphilic drug (ibuprofen) as reported previously (5). The lower CE at higher concentrations of ibuprofen (9.70×10^{-3} g/dm^3) and lower concentration of Ddex (2.5×10^{-7} g/dm^3) (Fig. 3) could be due to incomplete neutralization of the electrostatic charges and insufficient hydrophobic groups respectively. It was opined that increasing concentrations of Ddex increased the number of hydrophobic groups available for interaction with ibuprofen molecules, leading to higher conjugation efficiency. However conjugation and internalization of ibuprofen in the nanoconjugate are limited at higher concentrations of ibuprofen especially above the polymer saturation point (*psp*) of Ddex (5) due to limited number of hydrophobic groups from Ddex. Therefore increase in ibuprofen concentration decreased CE.

Characterization of Ibuprofen-Ddex Nanoconjugate

FT-IR Spectra Analysis

The structural differences between pure ibuprofen, ibuprofen-Ddex physical mixture and ibuprofen-Ddex nanoconjugates were determined using the FT-IR measurements in the range of 400–4000 cm^{-1} (Fig. 4). The pure ibuprofen spectra exhibited the characteristic broad peaks at 3088 and 3019 cm^{-1} corresponding to OH group from carboxylic acid as well as the strong, sharp carbonyl peak (C=O); C-O stretching and aromatic ring vibration (C=C) at 1710; 1230 and 1507 cm^{-1} respectively (4, 5, 33). Other absorption peaks observed at 2992, 2980 and 2954 cm^{-1} are characteristic for linear aliphatic C-H stretching. Ddex spectra showed characteristic broad absorption band and N-H deformation vibration at 3521 and 1640 cm^{-1} corresponding to free non H-bonded OH and tertiary amine groups respectively (Fig. 4b). The spectra of Ibuprofen-Ddex physical mixture exhibited the features of both components however the ibuprofen's carbonyl (C=O) peak decreased to 1698 cm^{-1} probably due to resonance effect suggesting solid state interaction. New peaks were also observed at 1747 and 1675 cm^{-1} suggesting solid state synthesis of ester (1750–1725 cm^{-1}) and amide (1680–1630 cm^{-1}) respectively (4, 34). The ibuprofen-Ddex electrostatic interaction was amplified in the nanoconjugates as a new peak appeared between 1557 and 1567 cm^{-1} depending on the concentration of the Ddex (Fig. 4). Absorption band between 1560 and 1530 has been ascribed to amide II band which usually occurs due to coupling of the N-H bending and C-N stretching vibrations (34) suggesting the formation of amide product from ibuprofen-Ddex interaction. The characteristic carbonyl absorption peak at 1706 cm^{-1} in pure ibuprofen shifted to higher values in the nanoconjugate with increasing concentration of Ddex, to a maximum of 1721 cm^{-1} at 2.0×10^{-6} mM of Ddex followed by a decrease while the intensity of the signal decreased steadily (Fig. 4) which was attributed to the electrostatic interaction between ibuprofen and Ddex. The remarkable reduction in the intensities of all the characteristic peaks of ibuprofen and the disappearance of the Ddex characteristic peaks for secondary amine and polymeric hydroxyl groups in the nanoconjugates reinforces the propensity of electrostatic interaction between ibuprofen and Ddex as well as hydrogen bonding. We have previously reported hydrogen bonding, electrostatic and hydrophobic interaction between ibuprofen and Ddex (5).

^{1}H NMR and ^{13}C NMR Spectra Analyses

^{1}H NMR and ^{13}C NMR spectroscopy was utilized to further evaluate the interaction between ibuprofen and Ddex. Table II and Figs. 5 and 6 show the ^{1}H NMR spectra with the δ chemical shifts of the pure ibuprofen, Ddex and their nanoconjugate assembly. The spectra characteristics of pure ibuprofen and Ddex corresponded to the respective profile described previously (5). The broad OH-proton signal (from COOH group of ibuprofen) at 12.24 ppm; the weak ammonium proton signal at 6.0 and 8.15 ppm; the polymeric OH-group at 11.4 ppm and the amine groups (δ: 3.39 and 4.96 ppm), from the Ddex did not appear in the nanoconjugate samples suggesting the involvement of these species in the electrostatic interaction and hydrogen bonding. Splitting of the doublet signal of the ibuprofen C3 protons attached to the symmetry carbon (C2) indicate that they are likely to be in close proximity to the carboxyl group in conjugation. The appearance of new proton signals at the C2 (δ: 3.17 ppm); C3 (δ: 1.19 ppm) and C10 (δ: 0.98 ppm) in the nanoconjugates (Fig. 6b) supports the hydrophobic interaction while proton signals at δ: 4.1 and 4.2 ppm indicates formation of amide product (δ: 4–8 ppm). The (13) C chemical shifts for the carbonyl group in the nanoconjugate exhibited lower frequency (δ: 161.28; 144.66 ppm) compared with pure ibuprofen (δ: 175.49 ppm) (Table II; Fig. 7). The chemical shift δ: 161.28 ppm is within the resonance range for amides (δ: 160–175 ppm) confirming the findings from FTIR and ^{1}H NMR studies. The δ: 146.66 ppm is outside the resonance range of carboxylic derivatives (δ: 155–185 ppm) and the entire carbonyl chemical shift range (δ: 160–220 ppm) however it was close to the chemical shift range of oximes (δ: 145–165 ppm) (34) which could be a by-product of the interaction. The reduced intensity and up field chemical shifts in the aromatic carbon of the nanoconjugates suggest a reduced freedom of ibuprofen rotation and possibly involved in the hydrophobic interaction with the Ddex. It was opined that electrostatic and hydrophobic interactions as well as hydrogen bonding occurred between ibuprofen and Ddex in the nanoconjugate assembly. Figure 8 shows the 2D-NMR (heteronuclear proton-carbon chemical shift correlation spectroscopy (HETCOR)) of ibuprofen and its nanoconjugates with ^{1}H frequencies on horizontal axis and (13) C frequencies on vertical axis. Pure ibuprofen exhibited strong cross peaks correlation between each carbon and its directly

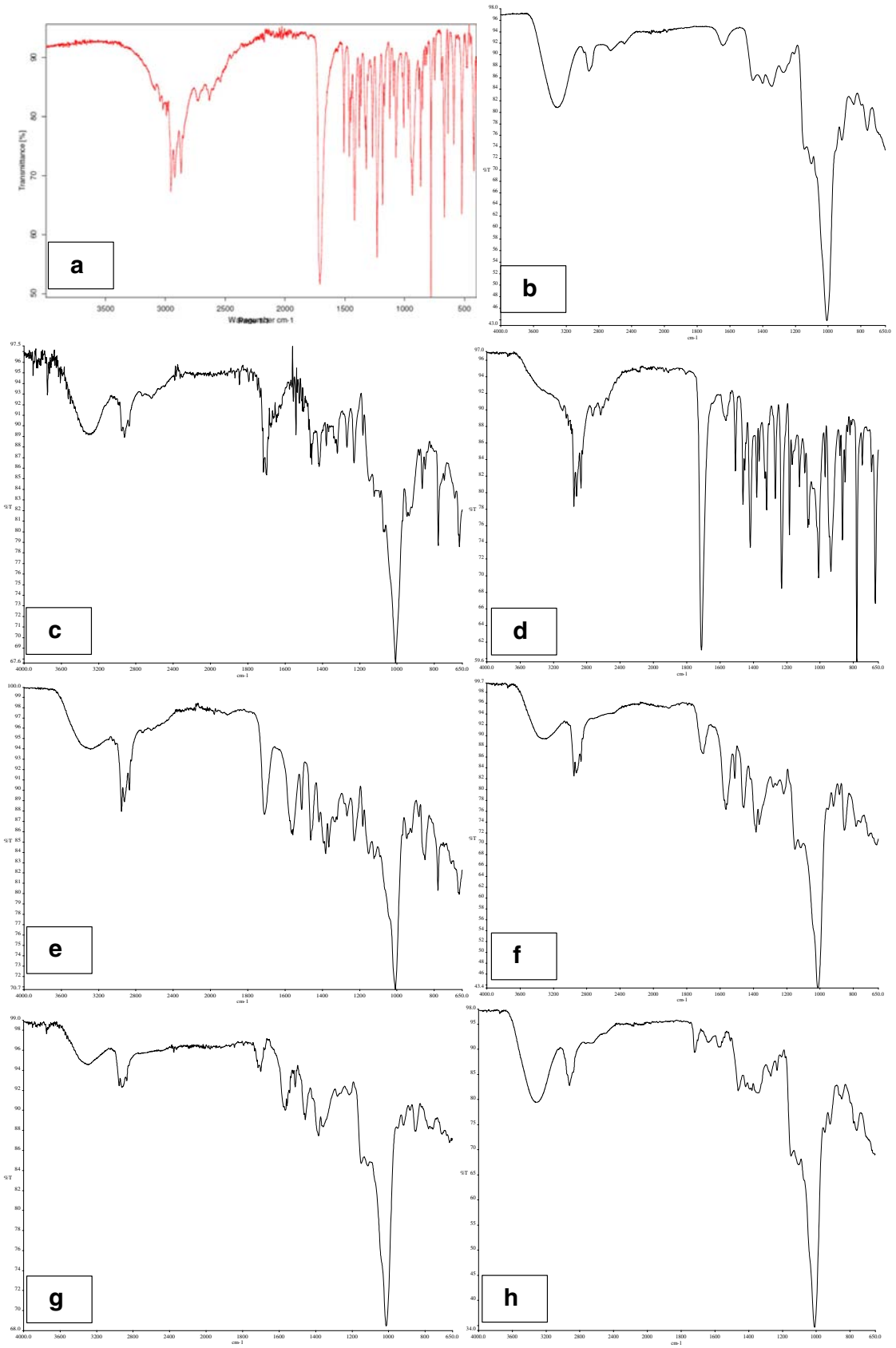


Fig. 4 Representative FT-IR Spectra of (**a**) pure ibuprofen sample; (**b**) Ddex alone; (**c**) physical mixture of ibuprofen and Ddex; Ibuprofen-Ddex nanoconjugate (**d**) 1:0.25; (**e**) 1:0.5 (**f**) 1:1; (**g**) 1:2 (**h**) 1:4.

Table II ^{1}H and ^{13}C Chemical Shift Assignment for Ibuprofen and its Nanoconjugates from the Representative Spectra

Carbon No.	Proton type / multiplicity	Ibuprofen		Ibuprofen-Ddex nanoconjugates	
		^{1}H Chemical shift (ppm)	^{13}C Chemical shift (ppm)	^{1}H Chemical shift (ppm)	^{13}C Chemical shift (ppm)
1	COOH	–	175.49	–	**175.49**; **161.28**; **146.66**; **78.42**
2	CH (Quartet)	3.63	44.33	3.56; 3.53[a] (Δδ: 0.07; 0.10)	**44.28**[d]
3	CH_3 (Doublet)	1.35	18.55	1.31[b] (Δδ: 0.04)	**18.46**[d]
4	C	–	138.52	–	**136.37**
5, 5′	CH (Doublet)	7.10	127.12	7.07 (Δδ: 0.03)	**127.10**
6, 6′	CH (Doublet)	7.20	128.97	7.18 (Δδ: 0.02)	**128.95**
7	C	–	139.56	–	**139.54**
8	CH_2 (Doublet)	2.41	44.21	2.40 (Δδ: 0.01)	**44.31**
9	CH (Multiplet)	1.83	29.64	1.81 (Δδ: 0.02)	**29.68**[d]
10	CH_3 (Doublet)	0.84	22.18	0.84 (Δδ: 0.00)	**22.18**
11	CH_3 (Doublet)	**0.86**	**22.18**	**0.86**[c] (Δδ: **0.00**)	**22.18**

[a] New signals appeared at 3.17 cm^{-1}

[b] New broad and weak signals appeared at 1.19 cm^{-1}

[c] New broad and medium signals appeared at 0.97; 0.98 cm^{-1}

[d] Negative intensity signal observed

bonded protons (Fig. 8a). From the HETCOR spectrum of the nanoconjugate (Fig. 8b), the strong cross peaks F1: 127.662, 129.685 and F2: 7.089, 7.190 ppm are (13) C and ^{1}H resonances from the aromatic ring. These values are close to the reported value (F1: 129.5; F2: 7.16 ppm) for *p*-substituted aromatic ring[34] confirming the para-di-substitution in the nanoconjugate and the direct bonding between aromatic carbons (C5,5′ and C6,6′) and their protons. The signal from the quartet C2 (F1: 45.116, F2: 3.625 ppm) corresponds to protons within the vicinity of a heteroatom (F1: 51.0; F2: 3.67 ppm) indicating direct connection at that point however signal overlap and up-field chemical shifts were dominant. Very weak new peaks observed in the nanoconjugate are circled in Fig. 8b. The interaction between ibuprofen and Ddex is represented in Scheme 2 as already validated previously (5).

Thermo-Analytical Characteristics of Ibuprofen-Ddex Nanoconjugates

The DSC and TGA thermograms as well as the thermo-analytical data of the pure ibuprofen, Ddex and their self-assembled nanoconjugates are presented in Figs. 9, 10 and Table III respectively. Pure ibuprofen exhibited a well-defined melting onset of 75.77 ± 0.42°C (melting peak of 81.22 ± 1.94) which conforms to 75–78°C reported in literature (35) suggesting a good degree of purity of the ibuprofen sample used in this study. The endothermic peak was ascribed to the melting of ibuprofen as no mass change was observed in the TGA thermogram (Figs. 9a and 10a). This was followed by single step decomposition peak. In a similar study Kumar *et al.*, (2012) (36) reported a melting peak of 82.76°C for ibuprofen which was slightly higher than 81.22°C observed in this study. The DSC curves of the amorphous Ddex did not show any melting endotherm however a decomposition peak was observed at 268.5°C which corresponded to a large mass loss on TGA curves at the 272.15°C (Fig. 10b). DSC curves of ibuprofen-Ddex physical mixture exhibited individual peaks of the components (Fig. 9c) while the ibuprofen-Ddex nanoconjugates exhibited single broad and diffuse peak. The endothermic melting peak of pure crystalline ibuprofen disappeared in the nanoconjugate suggesting their highly amorphous state due to the ibuprofen-Ddex conjugation. In corollary the thermal stability of the ibuprofen-Ddex nanoconjugates was evident by the absence of ibuprofen recrystallization peaks preceding the glass transition temperature and the exhibition of relatively high endothermic peaks during thermal analysis. We have reported similar binary amorphous ibuprofen-Ddex conjugate crystanules prepared by melt-*in situ* granulation-crystallizaton technique (5). Multiple steps of mass loss and decomposition temperatures in the nanoconjugates corresponded to derivative TGA peaks (Figs. 10 and 11)

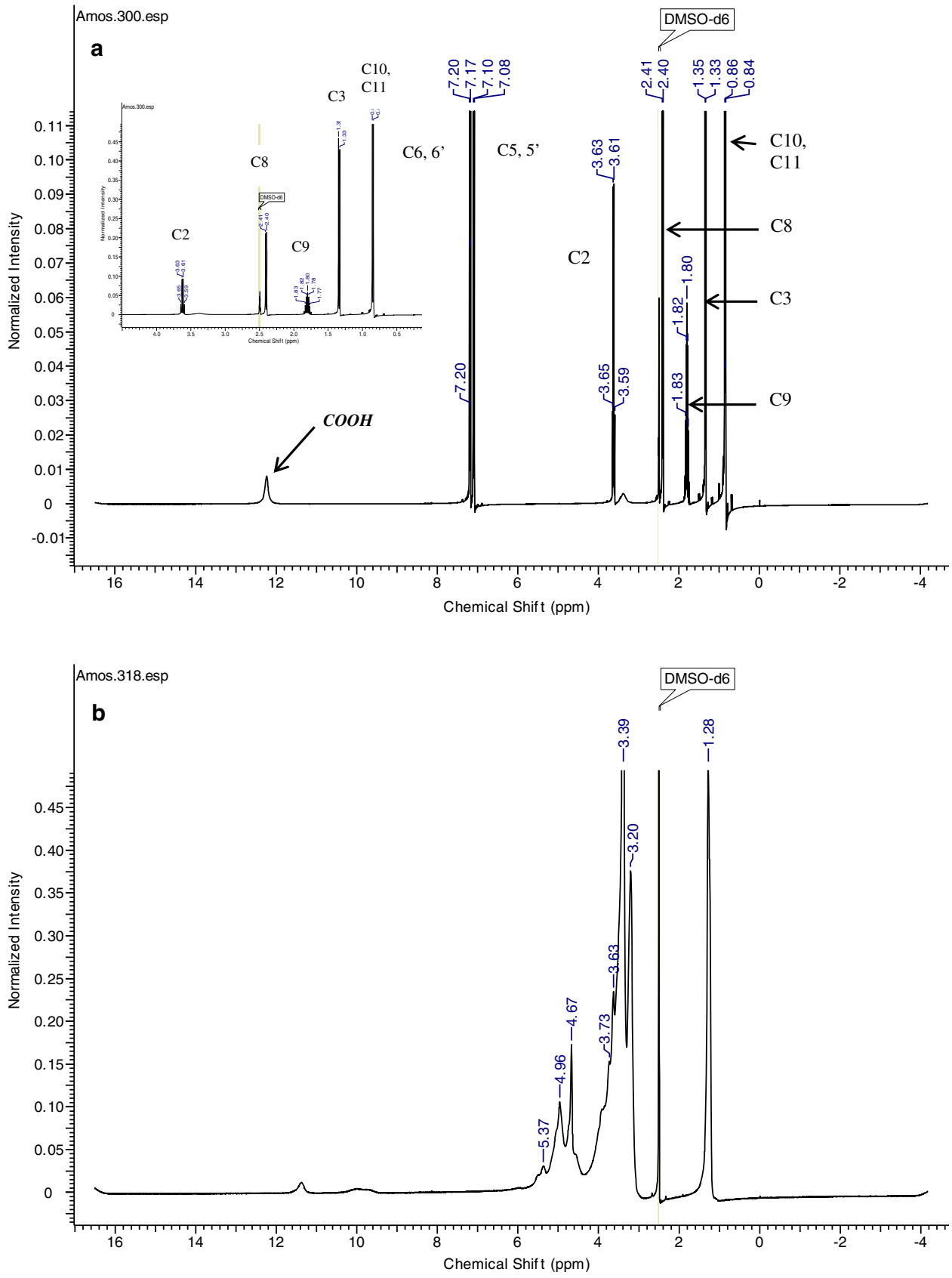


Fig. 5 Representative ^{1}H-NMR Spectra of pure ibuprofen (**a**); DEAE Dextran (**b**).

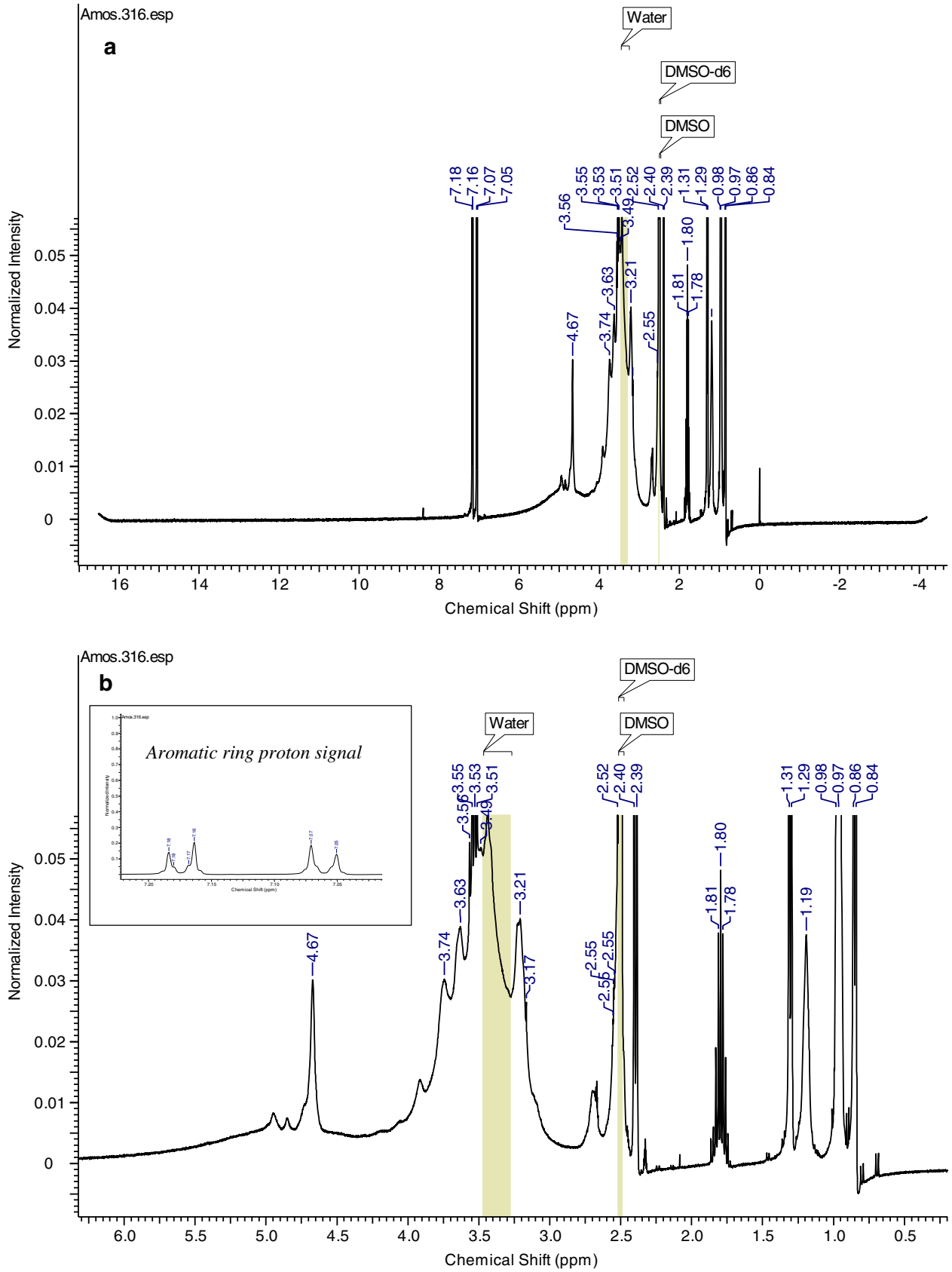


Fig. 6 Representative ^{1}H-NMR Spectra of ibuprofen-Ddex nanoconjugate (**a**) and expansion of the spectra showing the multiplicity of the proton signal.

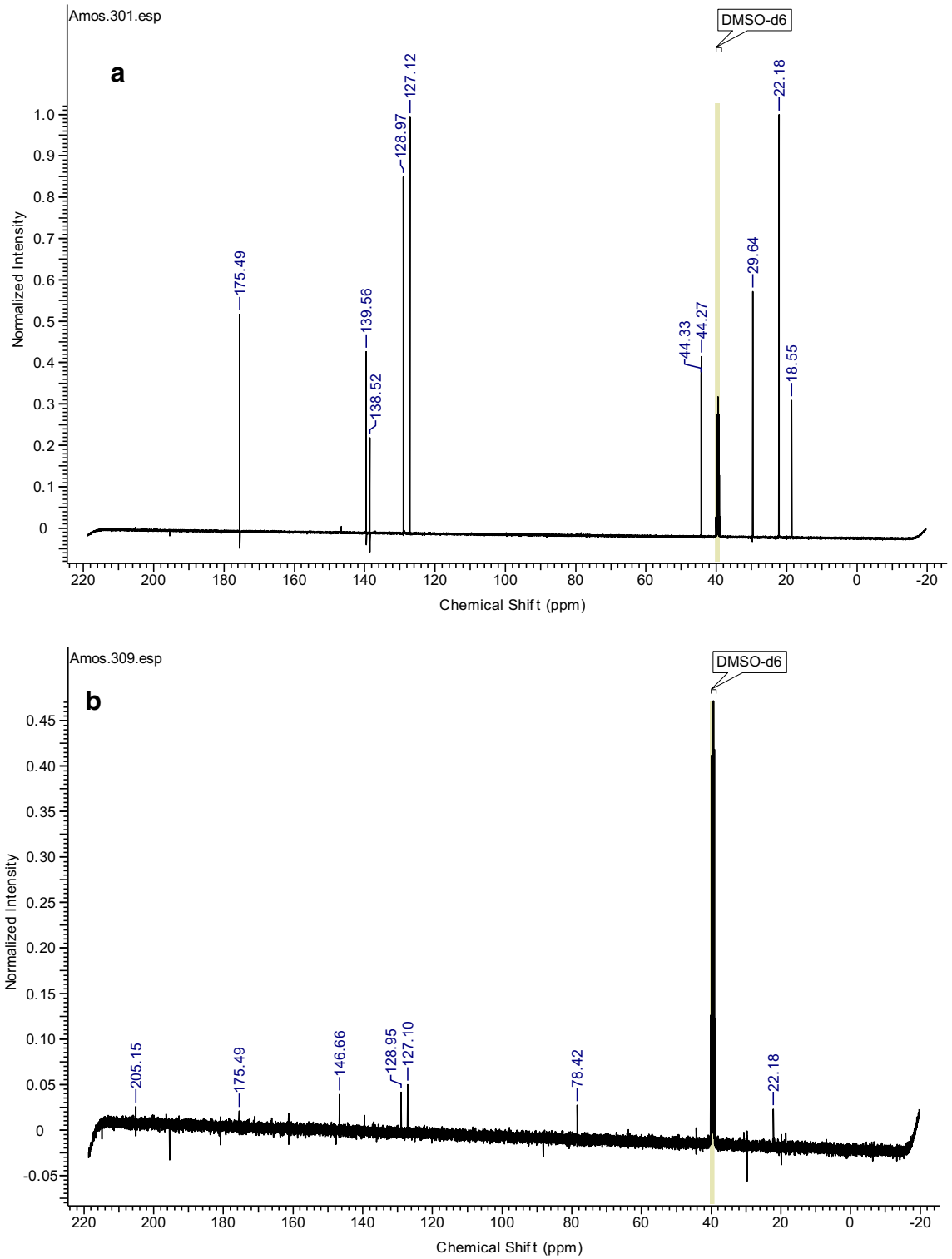


Figure 7 Representative (13) C-NMR Spectra of pure ibuprofen (**a**) and ibuprofen-Ddex nanoconjugate (**b**).

suggesting the breakdown of many complexes at different temperatures (multiple complexation). The TGA curves of the pure ibuprofen exhibited a one-step 100% mass loss at 240.76°C which was slightly lower than the 253.25°C degradation peak on the DSC thermogram suggesting the occurrence of evaporation prior to degradation. Derivative TGA plot (Fig. 10a) also shows a zero order degradation process which is consistent with the process described by Krupa *et al.*, 2010 (2). However the nanoconjugates exhibited second order degradation profiles in the TGA analysis showing different steps of mass

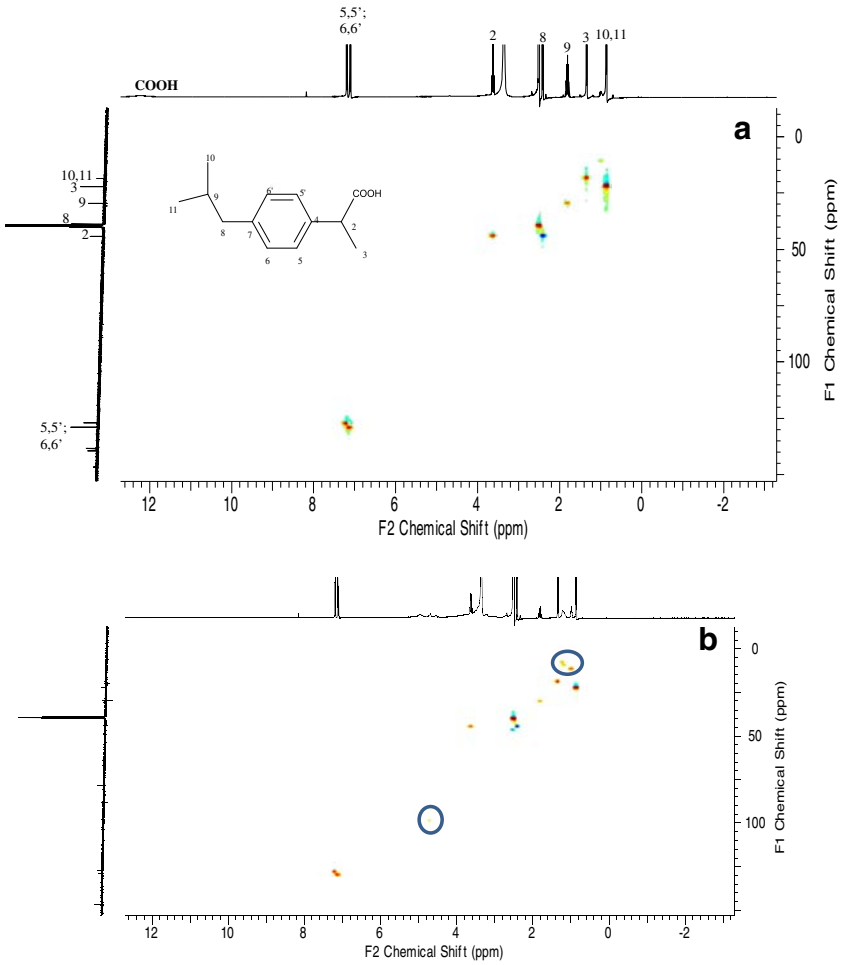
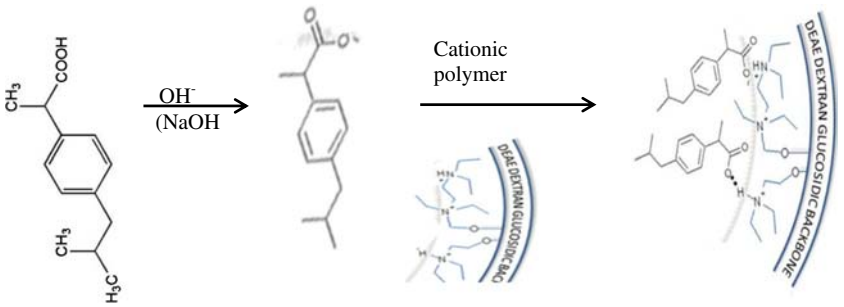


Fig. 8 The representative HECTOR spectra of (**a**) 2-[4 – (2 – methylpropyl) phenyl] propanoic acid and (**b**) ibuprofen-Ddex nanoconjugate.

loss supporting the multiple complexation phenomena which may have potential application in extended drug delivery system. The Tg of the nanoconjugates decreased steadily from 52.27°C in the physical mixture to a minimum of 36.96°C at 2.0×10^{-6} g/dm^3 of Ddex (Table III) beyond which the Tg increased steadily. The reduction in Tg indicates gradual transition from glassy to rubbery state in the presence of Ddex. At the rubbery state the polymer chain is more mobile and could have potential application in providing enhanced ibuprofen release profile from the nanoconjugate.

Dissolution Studies

The *in vitro* release profile of ibuprofen from the ibuprofen-Ddex nanoconjugate is presented in Figs. 12 and 13. The dissolution study was carried out without any release controlling barrier in order to determine the intrinsic dissolution rate of ibuprofen from the nanoconjugates. Initial burst release was evident in pure ibuprofen and the nanoconjugates containing lower concentrations of Ddex, however its extent decreased significantly from 49.92 to 18.13% ($p<0.05$; $n=6$) with



Scheme 2 Electrostatic interaction between ionized ibuprofen (anionic group) and cationic dextran

Ibuprofen-Ddex conjugate (Abioye et al., 2014a)

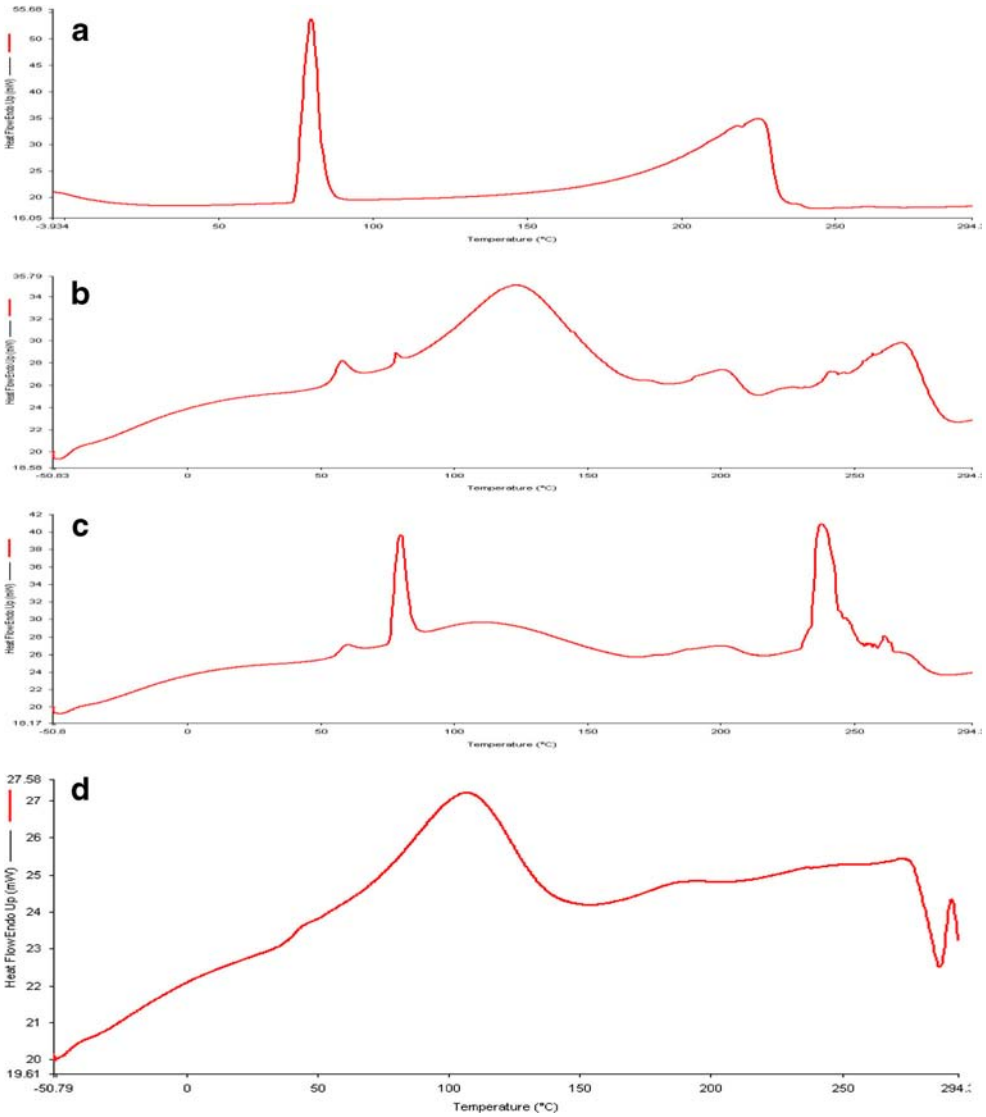


Fig. 9 Representative DSC Thermograms of (**a**) pure ibuprofen; (**b**) Ddex reference; (**c**) ibuprofen-Ddex physical mixture; (**d**) ibuprofen-Ddex nanoconjugate.

increasing concentration of Ddex due to strong interaction between ibuprofen and Ddex as evident from the FT-IR and NMR data in this study. The burst release of ibuprofen from the nanoconjugates containing lower concentrations of Ddex could be explained by the incomplete conjugation at lower concentration of Ddex than the critical conjugation concentration as reported previously (5). The rate of burst release increased to a maximum (17.57%/h) at 1.0×10^{-6} g/dm^3 of Ddex followed by a steady decrease. Pure ibuprofen exhibited 51.30; 52.37, 56.82 and 65.38% dissolution rate within 0.5; 1; 2 and 72 h respectively probably due to its low solublity and hydrophobic chacteristics. The plateau dissolution profile was ascribed to saturation of ibuprofen in the dissolution medium. On the other hand nanoconjugates containing low concentrations of Ddex (up to 1.0×10^{-6} g/dm^3) exhibited increased dissolution rate of 56.29; 60.13; 65.54 and 81.32% at 0.5; 1; 2 and 72 h respectively. The higher dissolution rate of the nanoconjugates relative to pure ibuprofen was ascribed to the smaller particle size (section 3.2) and amorphous state of the nanoconjugates (section 3.4.3). Higher concentrations of Ddex retarded dissolution rate steadily due to interaction between ibuprofen and Ddex as well as possible aggregation of the nanoparticles. For instance nanoconjugates containing 4.0×10^{-6} g/dm^3 Ddex exhibited a maximum of 32.34% dissolution rate at 72 h (Fig. 13). Although the particle size of ibuprofen was remarkably reduced by the process employed in this study, complete dissolution (100%) was not attained probably due to aggregation of the nanoparticles and increased ibuprofen-Ddex bond strength. Therefore the Null Hypothesis was rejected. It was concluded that the overall rate and extent of ibuprofen dissolution profiles cannot be explained exclusively by size reduction.

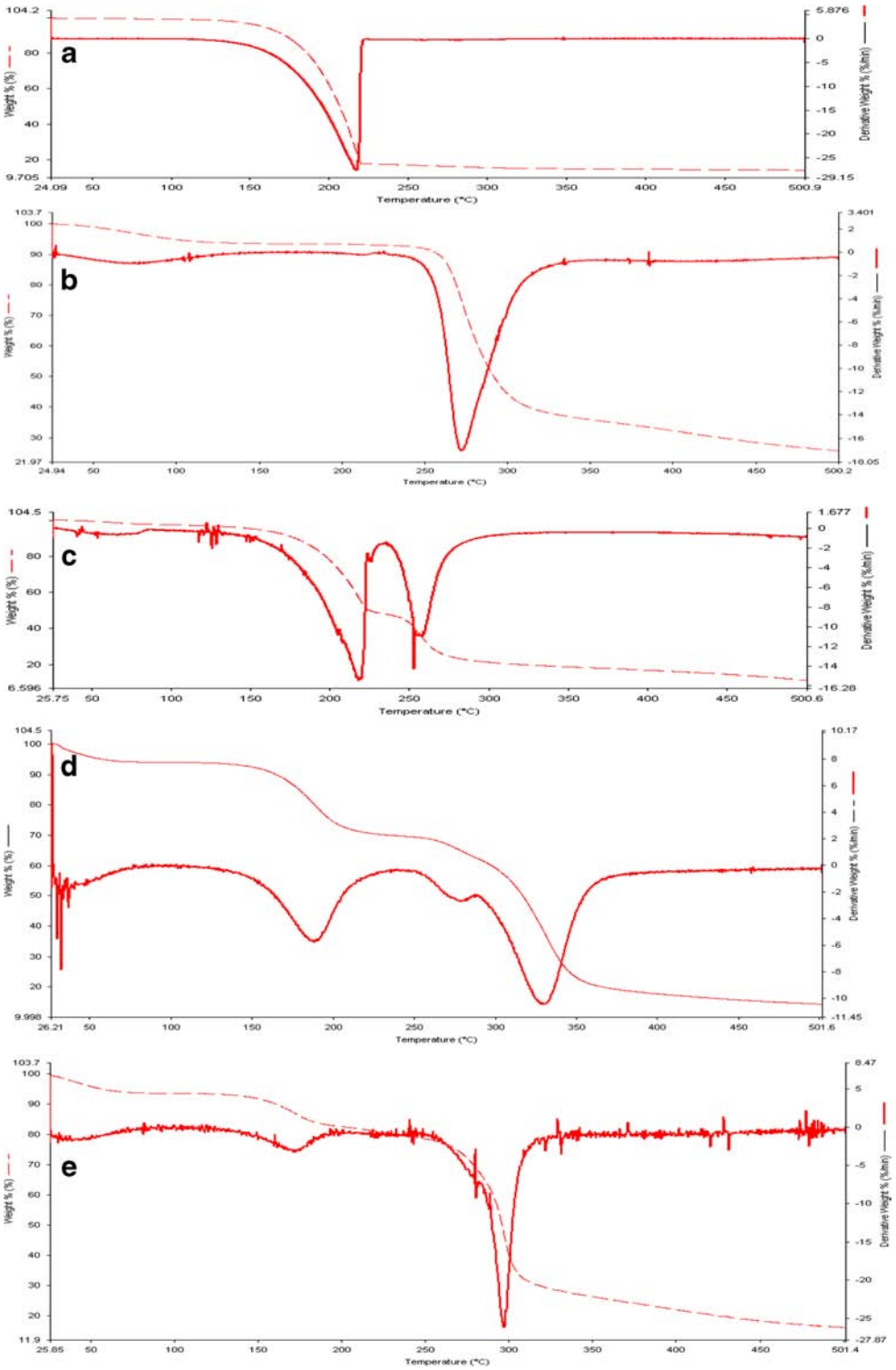


Fig. 10 Representative Derivative TGA of (**a**) pure ibuprofen; (**b**) Ddex reference; (**c**) ibuprofen-Ddex physical mixture and nanoconjugates with ibuprofen-Ddex weight ratios (**d**) 1:0.5; (**e**) 1:4.

Evaluation of System-Specific In Vitro Parameters and Mechanism of Ibuprofen Release from the Nanoconjugate

The time required for 50% dissolution (T_{50}) calculated from the regression equations of the linear region of the dissolution profiles were 16.10 ($R^2=0.9041$); 12.01 ($R^2=0.9111$); 0.82 ($R^2=0.9690$); 75.77 ($R^2=0.9164$) and 356.30 ($R^2=0.9563$) min for pure ibuprofen and nanoconjugates containing Ddex concentrations of 5.0×10^{-7}; 1.0×10^{-6}; 2.0×10^{-6} and 4.0×10^{-6} g/dm^3 respectively (Table IV). Nanoconjugates containing 1.0×10^{-6} g/dm^3 Ddex exhibited the shortest T_{50} (0.82 min) suggesting high drug release capacity with potential

Table III Thermo-Analytical Characteristics of IBUPROFEN, Ddex and their Nanoconjugates

SAMPLE / (Ibuprofen-Ddex weight ratio)	DSC				TGA			
	Onset melting (°C)	Melting point (°C)	Delta H (J/g)	Tg (°C)	Onset temp (°C)	Inflection point (°C)	Mass loss (%ΔY)	Total MASS LOSS
Pure Ibuprofen reference	75.77 ± 0.42	81.22 ± 1.94	140.83 ± 1.02	ND[b]	207.49 ± 7.89	240.76 ± 9.14	**100.058 ± 2.07**	**100**%
DEAE-Dextran-reference	53.54 ± 0.85 76.54 ± 1.22	57.86 ± 0.76 123.39 ± 4.16	5.22 ± 0.08 164.89 ± 5.64	56.58 ± 0.72	24.94 ± 0.7811 261.44 ± 7.89	24.94 ± 0.2245 272.15 ± 5.1198	6.54 ± 0.0966 61.58 ± 0.2549	68.12
	187.26 ± 8.23 245.83 ± 12.74	201.21 ± 10.58 268.50 ± 11.78	12.86 ± 0.84 70.84 ± 0.92					
Ibuprofen-DEAE-Dextran physical mixture	55.58 ± 0.76 [a]75.82 ± 1.40	59.58 ± 0.92 79.99 ± 1.84	2.69 ± 0.02 31.44 ± 0.95	52.27 ± 0.69	50.81 ± 1.0811 193.82 ± 3.1041	54.05 ± 0.4412 218.05 ± 2.9946	1.947 ± 0.0995 43.284 ± 0.1958	68.26
	90.69 ± 1.35	116.86 ± 2.76	35.51 ± 0.86		249.35 ± 6.1173	253.12 ± 2.0994	23.034 ± 0.0551	
	179.65 ± 7.43 233.64 ± 10.75	200.58 ± 10.68 237.63 ± 10.96	10.93 ± 0.22 67.67 ± 0.81					
Ibuprofen/Ddex nanoconjugate (5.0×10^{-7} g/dm^3 Ddex)	64.72 ± 1.01	101.34 ± 1.58	124.02 ± 10.87	47.42 ± 0.811	29.33 ± 0.2410 173.94 ± 2.9412	29.78 ± 0.1144 175.37 ± 1.1146	**6.680** ± 0.1144 16.315 ± 0.2516	**62.10**
					289.85 ± 6.0021	298.40 ± 4.9516	34.795 ± 0.1288	
					437.41 ± 7.8911	451.41 ± 5.1179	4.289 ± 0.0914	
Ibuprofen /Ddex nanoconjugate (1.0×10^{-6} g/dm^3 Ddex)	58.56 ± 0.98	100.23 ± 1.03	106.87 ± 10.91	40.45 ± 0.22	24.63 ± 0.1179 157.86 ± 2.7001	24.63 ± 0.1945 183.45 ± 1.8945	**5.108** ± 0.0889 15.33 ± 0.1199	**70.7**
					269.75 ± 4.1551	303.13 ± 4.2546	50.274 ± 0.1001	
Ibuprofen/Ddex nanoconjugate (2.0×10^{-6} g/dm^3 Ddex)	67.72 ± 0.96	106.89 ± 0.69	127.25 ± 1.08	36.96 ± 0.72	31.89 ± 0.1884 117.77 ± 1.0066	37.31 ± 0.1127 117.83 ± 1.8956	**6.84** ± 0.0258 21.135 ± 0.1164	**72.27**
					290.92 ± 3.4431	310.40 ± 3.3349	44.286 ± 0.09955	
Ibuprofen/Ddex nanoconjugate (4.0×10^{-6} g/dm^3 Ddex)	84.19 ± 1.08	119.61 ± 1.12	78.52 ± 1.20	46.04 ± 0.41	29.57 ± 0.1988 272.24 ± 4.7719	34.60 ± 0.1846 283.31 ± 4.1695	**7.283** ± 0.0849 50.297 ± 0.2831	**57.58**

[a] Endothermic onset of melting corresponding to ibuprofen in the ibuprofen-Ddex physical mixture

[b] *ND* Not determinable within the temperature range used in the study

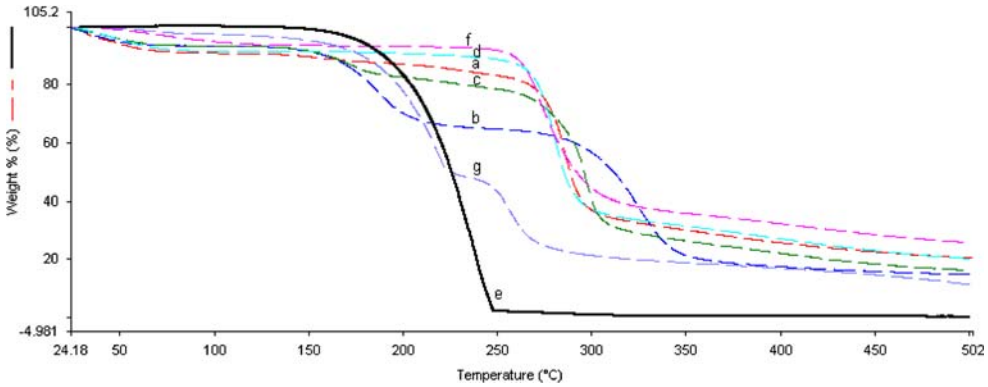


Fig. 11 Representative TGA Thermograms of nanoconjugates with ibuprofen-Ddex weight ratios (**a**) 1:0.5; (**b**) 1:1; (**c**) 1:2; (**d**) 1:4 and (**e**) pure ibuprofen; (**f**) Ddex and (**g**) ibuprofen-Ddex physical mixture.

application as rapid dissolving and fast acting formulation. Rapid dissolution and absorption of ibuprofen have been correlated with its serum concentration and enhanced analgesic effects (37). Higher concentrations of Ddex exhibited high T_{50} (356.30 min at 4.0×10^{-6} g/dm^3 Ddex) demonstrating potential application in controlled and extended release strategy for poorly soluble drugs. Mean Dissolution Times (MDT) of ibuprofen was calculated using Eq. 2.

$$MDT = \frac{\sum_{i=1}^{n} t_i \Delta M_i}{\sum_{i=1}^{n} \Delta M_i} \quad (2)$$

where i is the sample number; t_i is the midpoint time period between t_{i-1} and t_i calculated as $((t_i + t_{i-1})/2)$; n is the number of dissolution sample times and ΔM_i is additional amount of drug dissolved between t_i and t_{i-1}. Higher MDT values indicate lower drug releasing capacity of the nanoconjugate and *vice versa*

The MDT values of nanoconjugates increased with time and increasing concentration of Ddex in all cases relative to pure ibuprofen (Table IV) suggesting retarded dissolution rate due to the strong electrostatic interaction between ibuprofen and Ddex. In corollary the mean dissolution rate (MDR), calculated from Eq. 3, decreased steadily with time and Ddex concentrations supporting the retardation of dissolution rate by the Ddex.

Mean Dissolution Rate (MDR)

$$MDR = \frac{\sum_{i=1}^{n} \Delta M_i / \Delta t}{n} \quad (3)$$

where n is the number of dissolution sample times; i is the sample number; Δt is the time at midpoint between t_{i-1} and t_i calculated as $((t_i + t_{i-1})/2)$ and ΔM_i is additional amount of drug dissolved between t_i and t_{i-1}. Higher MDR values indicate higher drug releasing capacity of the nanoconjugate and *vice versa*

Dissolution efficiency (%DE) was calculated using Eq. 4. Lower concentrations of Ddex (up to 1.0×10^{-6} g/dm^3) increased percent dissolution efficiency (%DE) by between 6.32 and 24.38% however higher concentrations of Ddex reduced the DE steadily probably due to increasing bond strength between ibuprofen and Ddex.

$$DE(\%) = \frac{\int_0^t Y\, x\, dt}{Y_{100}\, x\, t} x\, 100 \quad (4)$$

where Y is the percentage of ibuprofen dissolved at time t

The dissimilarity (difference) factor (f_1) and similarity factor (f_2) values were calculated using Eqs. 5 and 6 respectively.

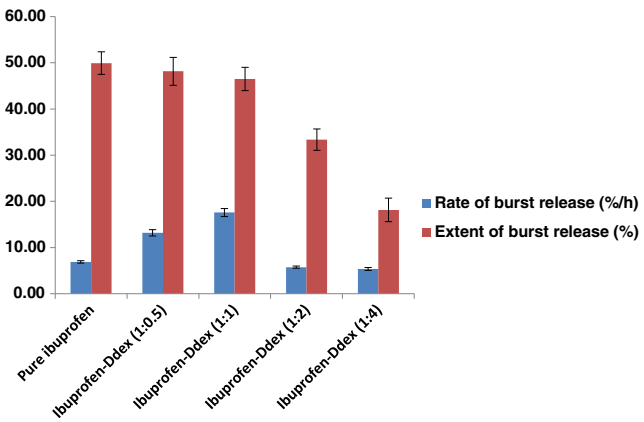


Fig. 12 Rate and extent of burst release of ibuprofen from ibuprofen-Ddex nanoconjugate.

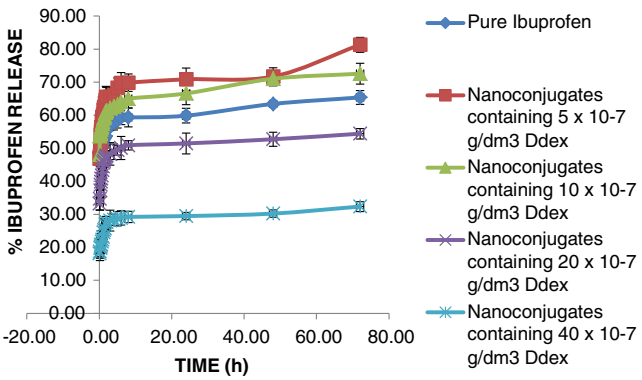


Fig. 13 *In vitro* drug release profile of ibuprofen from ibuprofen-Ddex nanoconjugates ($n = 6$).

Table IV Dissolution Parameters Calculated from Mathematical Equations and Statistical Moments for Pure Ibuprofen and Ibuprofen-Ddex Nanoconjugate

Ddex molar concentration	Dissolution Parameters of ibuprofen-Ddex nanoconjugate from one dissolution experiment																
	Mean Dissolution Time (MDT) (min)			Mean Dissolution Rate (MDR) ($\%min^{-1}$)			Dissolution Efficiency (DE) (%)			Dissimilarity and similarity factors compared with pure ibuprofen						Time required for50% 7dissolution
	MDT_{1h}	MDT_{2h}	MDT_{72}	MDR_{1h}	MDR_{2h}	MDR_{72h}	DE_{1h}	DE_{2h}	DE_{72h}	$f_{1\ (1h)}$	$f_{1\ (2h)}$	$f_{1\ (72h)}$	$f_{2\ (1h)}$	$f_{2\ (2h)}$	$f_{2\ (72h)}$	$T50\ (min)$
Pure Ibuprofen reference[i]	6.60	14.40	321.60	0.5522	0.1882	0.0847	52.37	56.82	65.38	–	–	–	–	–	–	16.10
Ibuprofen/Ddex nanoconjugate (5.0×10^{-7} g/dm Ddex)	9.60	16.20	588.0	0.202	0.1275	0.097	60.13	65.54	81.32	8.35	12.38	**14.25**	64.81	56.65	53.11	12.01
Ibuprofen /Ddex nanoconjugate (1.0×10^{-6} g/dm Ddex)	7.80	12.00	325.20	0.2012	0.1245	0.0915	58.12	61.39	72.57	8.52	9.62	**9.63**	66.49	63.67	62.71	0.82
Ibuprofen/Ddex nanoconjugate (2.0×10^{-6} g/dm Ddex)	10.80	16.20	249.00	0.1472	0.0922	0.0660	44.48	47.45	54.40	22.46	18.72	**17.50**	46.61	49.58	50.15	75.77
Ibuprofen/Ddex nanoconjugate (4.0×10^{-6} g/dm Ddex)	10.80	24.00	397.20	0.0778	0.0522	0.0382	23.05	27.70	32.34	59.39	56.10	**58.19**	26.03	26.68	26.68	356.30

$$f_1 = \frac{\sum_{i=1}^{n} |R_t - T_t|}{\sum_{i=1}^{n} R_t} \quad (5)$$

where R_t and T_t are the percent drug dissolved from the pure ibuprofen as reference

$$f_2 = 50\, log\left\{\left[1 + \frac{1}{n}\sum_{i=1}^{n} w_t (R_t - T_t)^2\right]^{-0.5} x\,100\right\} \quad (6)$$

Where n is the number of sample points, w_t is an optional weight factor, R_t is the reference assay at time point t and T_t is the test assay at time point t.

The similarity values (f_2) of the nanoconjugates increased to a maximum while the dissimilarity values (f_1) decreased to a minimum at the same Ddex concentration (1.0×10^{-6} g/dm^3) followed by a steady decrease and increase respectively (f_1= 8.35–22.46%; f_2=49.58–66.49%) as shown in Table IV. Values of f_2 between 50 and 100% indicate similarity between the dissolution profiles of pure ibuprofen and the nanoconjugates (38) however at 4.0×10^{-6} g/dm^3 Ddex, the f_2 values decreased to minimum of 26.03% while f_1 increased up to 59.39% suggesting increase in dissimilarity with increasing concentration of Ddex.

Mechanism of Ibuprofen Release from Ibuprofen-Ddex Nanoconjugate

Mechanism of ibuprofen release was elucidated by fitting the dissolution data into mathematical models including zero order, first order, Hixson-Crowell, Higuchi and Korsmeyer-Peppas equations (Table V). Pure ibuprofen reference fitted well into first order (R^2=0.9884); Higuchi (R^2=0.9933) and Korsmeyer-Peppas (n=0.43; R^2=0.9789) equations indicating diffusion mechanism of release (6). At low Ddex concentrations (up to 5.0×10^{-7} g/dm^3) all the models used in this study provided good fits for the nanoconjugates suggesting a combination of various release mechanisms. Nanoconjugates containing 1.0×10^{-6} and 2.0×10^{-6} g/dm^3 Ddex did not fit into any of the models except Korsmeyer peppas model with a release exponent value (n) of 0.64 and 1.18 respectively suggesting anomalous transport and super case II transport mechanisms respectively (38). Since the particle size of the nanoconjugates decreased with increasing concentration of Ddex (Fig. 2) the increased surface area-to-volume ratio and the potential risk of nanoparticles aggregation (Fig. 1) could explain the lack of fitness to zero order mechanism (39). However nanoconjugates containing 4.0×10^{-6} g/dm^3 Ddex fitted well into zero order model (R^2=0.9908) suggesting a constant slow (prolonged) release of ibuprofen from the nanoconjugate as shown in the dissolution curves (Fig. 13). Table V shows that all nanoconjugates fitted well into Korsmayer-Peppas model which was determined at less than 60% dissolution rate suggesting that the main mechanism of

Table V Mechanisms of *In Vitro* Release of Ibuprofen from the Ibuprofen-Ddex Nanoconjugates Using Mathematical Models

Dissolution Mathematical models	Zero order kinetics			First order kinetics			Higuchi model			Hixson-Crowell model			Korsmeyer-Peppas model		
Equation	$Q_t = Q_0 + K_0 t$			$Q_t = Q_0 e^{-K_1 t}$			$Q = K_H t^{1/2}$			$W_0^{1/3} - W_t^{1/3} = K_s t$			$Mt / M_\infty = at^n$		
Mechanism of release	Constant rate of release			Diffusion (Fick's first law)			Diffusion and permeability			Erosion release			Diffusion (semi empirical model)		
	[a]Q_0	[c]K_0	[b]R^2	[a]Q_0	[d]K_1	[b]R^2	[a]Q_0	[e]K_H	[b]R^2	[a]W_0	[f]K_s	[b]R^2	[g]n	[h]b	**[b]R^2**
Pure Ibuprofen reference[i]	50.04	0.0372	0.8896	1.6996	0.0003	0.9884	48.364	0.5212	0.9933	3.6855	0.0009	0.8888	0.4333	1.6608	**0.9789**
Ibuprofen/Ddex nanoconjugate (5.0×10^{-7} g/dm^3 Ddex)	46.48	0.2931	0.9111	2.2940	0.0013	0.9462	41.107	2.6271	0.9549	3.5999	0.0069	0.9002	1.145	1.5798	**0.9796**
Ibuprofen /Ddex nanoconjugate (1.0×10^{-6} g/dm^3 Ddex)	52.01	0.0983	0.7612	1.7161	0.0008	0.7286	48.44	1.2675	0.8634	3.7327	0.0023	0.7396	0.6430	1.6525	**0.9323**
Ibuprofen/Ddex nanoconjugate (2.0×10^{-6} g/dm^3 Ddex)	37.18	0.0882	0.7762	1.5564	0.0012	0.8337	30.744	1.6803	0.8677	3.3012	0.0033	0.8647	1.1800	1.4346	**0.9842**
Ibuprofen/Ddex nanoconjugate (4.0×10^{-6} g/dm^3 Ddex)	**18.29**	**0.0834**	**0.9908**	**1.2687**	**0.0016**	**0.9820**	**15.034**	**1.1352**	**0.9804**	**2.6440**	**0.0034**	**0.9857**	**1.413**	**1.1297**	**0.9248**

[a] Q_0, W_0 intrinsic dissolution; [b] R^2, coefficient of determination; [c] K_0 zero order release rate constant; [d] K_1 first order release rate constant; [e] K_H Higuchi's diffusion rate constant; [f] K_s Hixson-Crowell's diffusion rate constant; [g] n Korsmeyer Peppas' release exponent; [h] b Korsmeyer-Peppas' measure of burst effect; obtained from Abioye *et al.*, 2014b

ibuprofen release from the ibuprofen-Ddex nanoconjugates is diffusion from a controlled release polymeric system (38). Apart from nanoconjugates containing 1.0×10^{-6} g/dm^3 Ddex, all nanoconjugates exhibited *n* values of greater than 1 suggesting Super Case II transport mechanism of release. The specific release rate constant for the respective models decreased with increasing concentration of Ddex (Table V) supporting the retarded dissolution and controlled release phenomena. Only nanoconjugates containing 4.0×10^{-6} g/dm^3 Ddex fitted into Higuchi and Hixson-Crowell models suggesting diffusion mechanism due to increased solubility of the particles and discrete drug particle dissolution respectively (40). It was concluded that multiple mechanisms are involved in the release of ibuprofen from the nanoconjugates however diffusion and discrete ibuprofen nanoparticle dissolution are more prominent.

CONCLUSION

This study investigated the direct effect of controlled electrostatic self-assembly of ibuprofen (amphiphile) and cationic dextran on the physicochemical and *in vitro* dissolution characteristics of the resulting ibuprofen-Ddex nanoconjugate. The novel low energy and green technique, CAPESA, converted the rod-like and hydrophobic crystalline ibuprofen powder with average crystal size $453.88\pm29.8469\times97.12\pm5.4267$ μm [aspect ratio 5.16 ± 1.15 ($n=20$)] into spherical and amorphous nanoconjugates with particle size range of 85.2 ± 4.4461 to 157.10 ± 10.0214 nm corresponding to 2889- to 5327- folds reduction in particle size. However loose aggregates of the nanoconjugates were formed with size range between 323.30 ± 11.7144 and 1009.12 ± 28.7991 nm as stabilizing agent was not included in this study. Research on stabilization of the nanoconjugates is ongoing in our lab and would be published in due course. FT-IR as well as ^{1}H- and ^{13}C-NMR confirmed electrostatic interaction between ibuprofen and Ddex while DSC, TGA and SEM confirmed formation of amorphous nanoconjugate with evidence of multiple complexation and aggregation which was dictated by concentration of Ddex. Nanoconjugates containing low concentrations of Ddex up to 1.0×10^{-6} g/dm^3 exhibited enhanced dissolution efficiency for ibuprofen (81.32%) beyond which dissolution was retarded steadily. Multiple release mechanisms including diffusion; discrete drug dissolution; anomalous transport and super case II transport were noted. It was evident that concentration of Ddex and the extent of interaction with ibuprofen dictate the rate and extent as well as the mechanism of ibuprofen release from the nanoconjugate. It was concluded that the technique used in this study has potential practical application in the development of formulation-modulated delivery tool for poorly soluble drugs.

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