Formulation and physical characterization of Ibuprofen–DEAE-Dextran nanoconjugates via Surfactant Solubilization

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ABSTRACT

Background: The optimization of polymer-drug conjugate design is important in effective and efficient delivery of poorly soluble drugs.

Objectives: This work focuses on the formulation of novel amorphous ibuprofen-polymer nanoconjugates based on the polymer-drug complexation in order to improve its physical characteristics in the absence of toxic organic solvents.

Methods: Amorphous Ibuprofen – DEAE-Dextran nanoconjugates were prepared using surfactant solubilization method. Physical characterization of the nanoconjugates was carried out via conductivity, surface tension, viscosity, transmittance/turbidity, particle size measurement, zeta potential, conjugation efficiency and Scanning Electron Microscopy (SEM) techniques.

Results: A remarkably high loading capacity was achieved ranging from 89.05 to 96.34%. The conductivity measurements showed that the critical association concentration (cac) was exhibited at 2.34 mg/mL and critical micellar concentration (cmc) at 8.0 mg/mL. The presence of DEAE-Dextran decreased the cac of ibuprofen significantly ($p < 0.05$, $n = 6$) confirming the electrostatic interaction between DEAE-Dextran and ibuprofen. DEAE-Dextran showed surface activity and were adsorbed significantly at the water surface. The surface morphologies of the nanoconjugates singly and in aggregates were all spherical in shape. The formulation of ibuprofen-DEAE-Dextran conjugate reduced the size of ibuprofen (2.87 µm) significantly to 13.02 nm (268-fold) at 1.6% (3.2×10^{-2} mM) DEAE-Dextran concentration. The zeta potential values obtained were relatively low (-3.04 to +13.87 mV) indicating low repulsion stabilization.

Conclusion: This work shows the formulation of amorphous ibuprofen-DEAE-Dextran nanoconjugates via the surfactant solubilization with the potential of improving the delivery of ibuprofen.

Key Words: Ibuprofen, surfactant solubilization, DEAE-Dextran and nanoconjugate

Formulation et caractérisation physique des nano-conjugués Ibuprofènes–DEAE-Dextran au moyen de la solubilisation tensioactif

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RESUME

Contexte: L'optimisation de la conception du conjugué de médicament polymère est importante dans la livraison efficace des médicaments à faible solubilité.

Objectifs: Ce travail s'intéresse à la formulation de nouveaux nano-conjugués de polymère d'ibuprofène amorphe basés sur la complexation de médicament polymère afin d'améliorer ses caractéristiques physiques en l'absence de solvants organiques toxiques.

Méthodes: Les nano-conjugués d'Ibuprofène amorphe – DEAE-Dextran étaient préparés au moyen de la méthode de solubilisation tensioactive. La caractérisation physique des nano-conjugués a été conduite au moyen de la conductivité, la tension superficielle, la viscosité, la transmittance / turbidité, la mesure de la taille du particule, le potentiel zetâ, l'efficacité de conjugaison et les techniques de microscopie électronique à balayage (SEM).

Résultats: Une capacité de chargement très élevée fut réalisée entre 89,05 à 96,34%. Les mesures de conductivité indiquent que la concentration d'association critique (*cac*) fut exposée à 2,34 mg/mL et la concentration micellaire critique (*cmc)* à 8,0 mg/mL. La présence de DEAE-Dextran a réduit nettement la *cac* d'ibuprofène ($p < 0,05$, $n = 6$) confirmant l'interaction électrostatique entre le DEAE-Dextran et l'ibuprofène. Le DEAE-Dextran a montré une activité superficielle et a été nettement absorbé à la surface de l'eau. Les morphologies superficielles des nano-conjugués séparément et ensemble étaient toutes en forme sphérique. La formulation du conjugué d'ibuprofène-DEAE-Dextran a réduit la taille de l'ibuprofène (2,87 µm) considérablement de 13,02 nm (268-fois) à 1,6% ($3,2 \times 10^{-2}$ mM) de concentration de DEAE-Dextran. Les valeurs potentielles zetâ obtenues étaient relativement faibles (-3,04 à +13,87 mV) indiquant une faible stabilisation de répulsion.

Conclusion: Ce travail montre la formulation des nano-conjugués de l'ibuprofène amorphe -DEAE-Dextran au moyen de la solubilisation tensioactive avec le potentiel d'amélioration de la livraison d'ibuprofène.

Mots-clés: Ibuprofène, solubilisation tensioactive, DEAE-Dextran et nano-conjugué

INTRODUCTION

Ibuprofen is a weak organic acid amphiphile that is soluble in high pH aqueous environment in its ionized form.[1] This ionized species has propensity for electrostatic interaction with oppositely charged molecules. Hydrophilic cationic polysaccharides with glucosidic backbone have a particularly adequate structure to interact with amphiphilic molecules like ibuprofen; and the presence of charged or hydrophilic groups in their substituents provides strong affinity for the oppositely charged molecule or hydrogen bonding capacity.[2] A combination of spontaneous electrostatic and hydrophobic interactions between polyelectrolyte and the ionized drug underpins the formation of amphiphile-polyelectrolyte complexes of various structures and sizes depending on the type of amphiphile.[3,4] Literature is replete of reports on the role of polyelectrolyte-ionic surfactant (amphiphile) complexation in solubilization of poorly soluble drugs in a review by Langevin, however research on amphiphilic drug-polyelectrolyte complexation is limited.[5] Few reports found in literature include elucidation of the factors underpinning the complexation of amitriptyline, chlorpromazine and doxepin to polyelectrolyte in aqueous phase however the drug delivery and nanoscale applications were not evident from the report.[6-8] Hughert and Sunderlof studied the effect of polyelectrolyte counterion specificity, charge density and conformation on the interaction between carrageenan/furcelleran and amitriptyline by means of dialysis equilibrium technique but did not give a report on its drug delivery potentials. Our previous studies have been able to show that ionized ibuprofen species adsorb onto polymers through hydrophobic and electrostatic bonds (conjugation) with their aromatic ring and hydrophilic carboxylic groups respectively.[9-13]

The present study aims to investigate a novel drug-polyelectrolyte complexation technique of preparing amorphous ibuprofen nanoconjugate. This technique entails using non ionic surfactant (micellar solubilization) to solubilize ibuprofen. The respective solutions were mixed with a solution of oppositely charged polyelectrolyte to initiate drug-polymer interaction. Charge neutralization leads to spontaneous precipitation and formation of nanoscale drug-polyelectrolyte complex. The combination of strong electrostatic interactions between the drug and the polyelectrolyte as well as the spontaneous precipitation prevent the drug from reverting back into the ordered crystalline form hence amorphous drug-polyelectrolyte nanocomplex is formed. The cationic polyelectrolyte employed in this study is Diethylaminoethyl-Dextran (DEAE-Dextran) which is a natural biocompatible and biodegradable polycation.

Surfactant solubilization is one of the important methods of formulating by solubilising in surfactant solution above the critical micelle concentration (CMC) of 0.02 mM in the case of tween 80.[14] Surfactants tend to lower the surface tension and improve the dissolution of hydrophobic drugs in aqueous medium. Micelles are formed when the concentration of surfactants exceeds their critical micelle concentration. In this study, tween 80 also known as polysorbate 80, a non-ionic surfactant was used to entrap the drug within the micelle. Hence, polymer (DEAE-Dextran) was used to control the rheology of the formulations and surfactant (tween 80) was used to control the surface properties or wettability.

The intention of this research was to understand the intrinsic capacity of polymer-drug conjugates in terms of the physical parameters, which would shed light on the type of interaction (physical or chemical/agonist or synergistic), the best polymer-drug ratio, the capacity of the polymer to achieve the desired drug payload and other features that govern clinical risk-benefit ratio which are key indices to optimization of polymer-drug conjugate design and a consequent effective and efficient drug delivery. Presently there is not sufficient research information on the quantitative contribution of drug–polyion nanoconjugates to the biopharmaceutical characteristics of poorly soluble drugs justifying the need for this study to be carried out. Therefore the aim of this phase was to prepare a stabilized amorphous ibuprofen-polyion nanoconjugate using a fast, minimum energy and organic solvent-free technique.

METHODS

Materials

Ibuprofen was purchased from Fagron, UK while DEAE-Dextran hydrochloride (molecular weight 500,000 g/mol), pluronic F-68 and tween 80 were purchased from Sigma-Aldrich, UK. They were all used as received without further modification. Analytical grade sodium hydroxide was used.

Preparation of Ibuprofen-DEAE-Dextran nanoconjugates

Varying amount of DEAE-Dextran was dissolved and made up to 50 mL with 0.1% w/v pluronic solution to produce double strength of the required ratio ranging from 0.2 to 3.2% w/v solutions and heated to 80 °C

under magnetic stirring on a hot plate (Jenway 1000 stirrer). 200 mg ibuprofen was dissolved in 10 mL of tween 80, made up to 50 mL with 0.1% w/v pluronic solution and added drop-wisely into equal volume of the varying concentrations of DEAE-Dextran in 0.1% w/v pluronic solution at room temperature under continuous magnetic stirring for 10 min (Jenway 1000 hotplate and stirrer). The pH of the drug polymer complex mixture was adjusted to 6.0 (Mettler Toledo pH meter) using 1 M sodium hydroxide (NaOH).[15] The Ibuprofen-DEAE-Dextran nanoconjugate prepared were dialysed and labelled as IbTw80-control, IbD1Tw80, IbD2Tw80, IbD3Tw80, IbD4Tw80 and IbD5Tw80 respectively. The nanosuspensions formed were kept at room temperature prior to analysis.

Measurement of conductivity

The conductivity measurements of the nanoconjugate suspensions were determined before centrifugation using conductivity meter (Oakton CON 110 Series) with a cell constant of 1 cm^{-1} at 25 ˚C. The measurements were an average of at least six determinations.

Surface tension determination

The torsion balance for surface and interfacial tension measurements with rings of circumference 4 cm (White Elec. Inst, Co. Ltd) was used to determine the surface tension at 20 ˚C. The force required to detach a platinum wire ring immersed at the liquid surface was measured, which was proportional to the surface tension. The measurements were an average of at least six determinations. The Gibbs equation was used to calculate the surface excess (equation 1):

$$\Gamma = -\frac{C}{RT}\left(\frac{d\gamma}{dC}\right) \qquad (1)$$

In which Γ is the surface excess concentration of DEAE-Dextran; R is the Gas constant (8.314 J $mol^{-1}K^{-1}$; T is the temperature in kelvin; C is the concentration of DEAE-Dextran in mol/dm^3; dγ is the change in surface tension and dC is the change in concentration.

Measurement of viscosity

Viscosity of the nanoconjugates was measured by SV 10 Vibro Viscometer (A&D Company Ltd Japan). Viscosity was measured at 25 ˚C. 10.0 g of the sample was poured into the cup until the surface reached between the level gauges. The cup was attached on the table along guides. The grips were pinched and the sensor was gently lowered above the sample surface. The lever was lowered to secure the sensor plates. The knob of the table was turned so as to adjust the surface of the narrow part of sensor plates. The start key was pressed to start the viscosity measurement till 25 ˚C was reached and the reading was taken. The measurements obtained were the average of at least six determinations.

Measurement of transmittance/turbidity

The absorbance/transmittance of the nanoconjugates was measured at 420 nm (ThermoFischer Evolution 60S UV Spectrophotometer) against a blank of distilled water. The measurements obtained were the average of at least six determinations.

Determination of particle size and zeta potential of nanoconjugates

The mean particle size and zeta potential of the nanoconjugates were determined by ZetaPlus Zeta Potential Analyser (Brookhaven Instruments Corporation). Samples were diluted appropriately with distilled water and injected into the sample cell. Particle size measurements were carried out at a scaling angle of 90˚ and a temperature of 25 ˚C. Apparent z-average hydrodynamic diameter and polydispersity index (PI) were obtained. Zeta potential was determined by measuring the electrophoretic mobility of the dispersed particles in a charged field. Zeta potential measurements were done in aqueous solutions at 25 ˚C and the electric field strength was about 14.95 V/cm. All measurements of individual samples were a mean of six determinations for particle size and 10 runs for zeta potential.

Drug conjugation capacity/efficiency

Conjugation efficiency (equation 2) is the mass percentage of ibuprofen that forms the Ibuprofen complex relative to the initial amount of ibuprofen added. The amount of ibuprofen that forms the ibuprofen complex was calculated as the difference between the amount of ibuprofen added and the amount of non-conjugated ibuprofen in the supernatant after centrifugation. The ibuprofen concentration was measured by UV (ThermoFischer Evolution 60 UV Spectrophotometer, UK) after passing it through 0.45 µm filter (Sartorius, Germany) at 264 nm wavelength. [16] All measurements were an average of six determinations.

$$Conjugation\ efficiency = \frac{M_o - M_i}{M_o} * 100 \qquad (2)$$

Where M_0 is the initial amount of ibuprofen added and M_i is the amount of non-conjugated ibuprofen in the supernatant after centrifugation.

Morphology and size - scanning electron microscopy

The surface characteristics of the reference compound and nanoconjugates were examined by Carl Zeiss SEM EVO High Definition 15 Scanning Electron Microscope (Carl Zeiss, Germany) using variable pressure technology at low voltages with beam deceleration and high definition backscattered electron (BSE) imaging. The samples were mounted on double sided carbon tabs that were previously secured to aluminium stubs and then analysed at different magnifications and a pressure of 10 Pa. The accelerating voltage was 10 KV with probe current of 400 pA. The particle sizes of the reference compounds and the nanoconjugate images were determined using the SmatTiff software.

Statistical analysis

Quantitative data are presented as mean ± standard deviation. The significance of the differences between means was assessed using Analysis of Variance (ANOVA) and Post hoc Tukey Test with a statistical significance level set at $p < 0.05$ using IBM SPSS (Statistical Package for Social Science) 20.

RESULTS

Conductivity

The conductivity measurements of ibuprofen in distilled water from preliminary studies ranged from 0.10 to 0.50 mS/cm decreasing with increasing concentration (2.5 to 40.0 µg/mL) of ibuprofen. Conductivity of the nanoconjugates ranged from 3.16 to 12.78 mS/cm presented in Figure 1 and Table 1. The critical micelle concentration (cmc) of ibuprofen was obtained from the plot of conductivity as a function of drug concentration. The cmc values were taken as the break point intercepts of the linear portions on the graph.

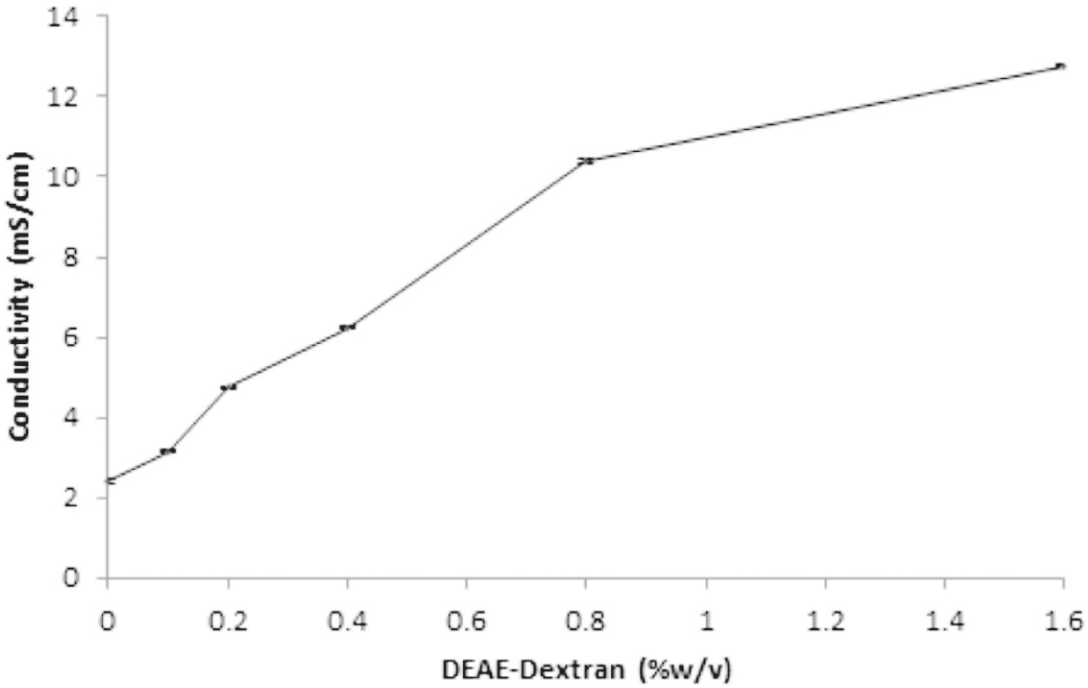


Figure 1: Conductivity of Ibuprofen-DEAE-Dextran nanoconjugates based on DEAE-Dextran content. Each data point represents mean ± SD (n = 6).

Table 1: Physicochemical properties of ibuprofen-DEAE-Dextran conjugates (mean ± SD, n = 6).

Formulation	Conjugation Efficiency (%)	Conductivity (mS/cm)	Surface tension (N/m)	Viscosity (mPaS)	Absorbance/Transmittance	Particle size (nm)	Polydispersity Index	Zeta Potential (mV)
IbTw80-control	-	2.43±0.07	47.25±1.22	2.39±0.05	0.075±0.001	2872.12±128.9	0.67±0.08	-7.251±1.3
IbD1Tw80	90.26±0.95	3.16±0.02	38.30±1.00	3.04±0.01	0.056±0.002	122.17±4.65	0.37±0.02	3.45±0.2
IbD2Tw80	90.26±1.03	4.76±0.03	39.68±1.04	4.01±0.02	0.047±0.004	104.08±4.99	0.37±0.01	3.30±0.46
IbD3Tw80	89.05±0.91	6.25±0.04	37.78±1.09	5.09±0.02	0.033±0.003	19.92±1.59	0.32±0.04	1.84±0.12
IbD4Tw80	91.30±1.35	10.40±0.08	40.65±0.39	7.57±0.03	0.033±0.003	13.02±0.92	0.16±0.14	1.37±0.16
IbD5Tw80	96.34±1.46	12.78±0.04	39.58±0.81	11.83±0.16	0.030±0.003	13.02±1.19	0.15±0.14	1.02±0.21

Surface tension

The surface tension of ibuprofen in distilled water obtained from preliminary studies ranged from 49.20 to 53.99 N/m decreasing with increasing concentration (2.5 to 40.0 µg/mL) of ibuprofen. The surface tension behaviour of Ibuprofen-DEAE-Dextran which ranged from 38.30 to 40.65 N/m is shown in Figure 2, exhibiting lower surface tension than the control (56.6 N/m). The profile showed an initial decrease till minima was reached at IbD2Melt (0.2% DEAE-Dextran), this was followed by a little increase which remained constant over the increasing concentration of DEAE-Dextran.

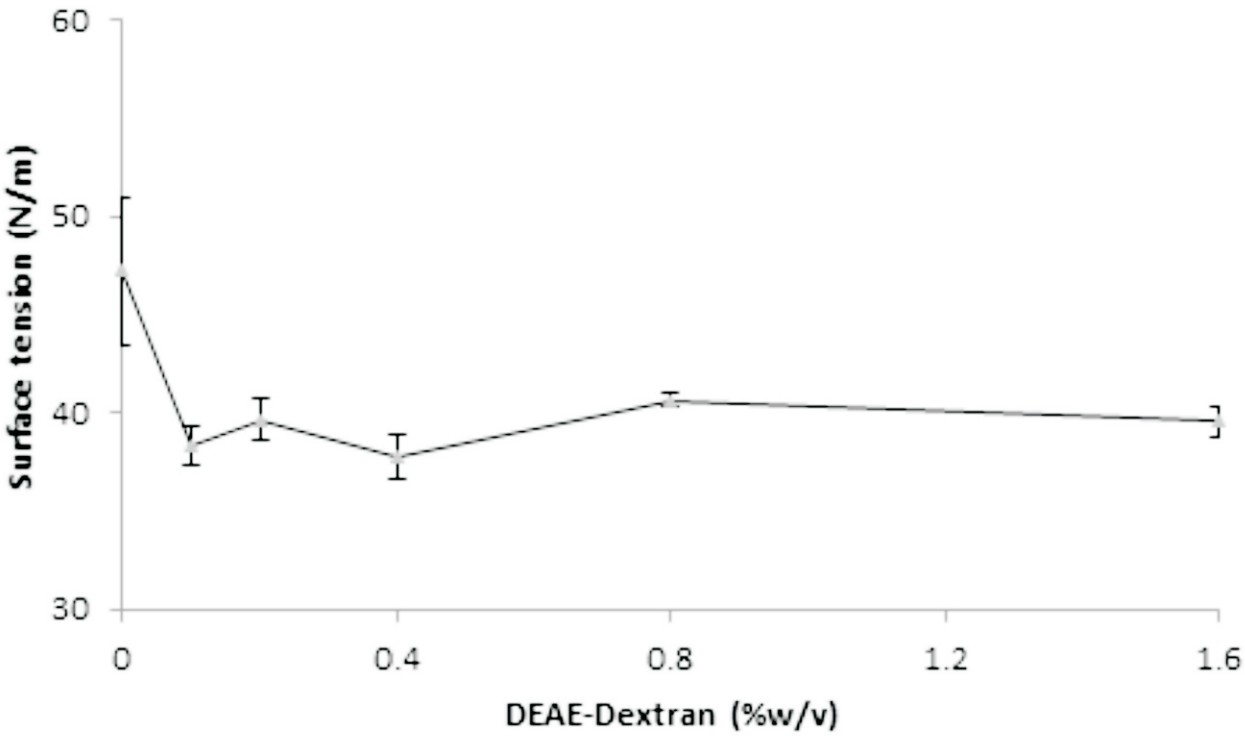


Figure 2: Surface tension of Ibuprofen-DEAE-Dextran nanoconjugates based on DEAE-Dextran content. Each data point represents mean ± SD (n = 4).

Viscosity

The viscosity of ibuprofen in distilled water obtained from preliminary studies ranged from 1.12 to 1.17 mPas decreasing with increasing concentration (2.5 to 40.0 µg/mL) of ibuprofen shown in Figure 3. The viscosity of DEAE-Dextran in distilled water ranged from 1.2 to 1.41 mPaS increasing slowly with increasing concentration (0.005 to 0.1% w/v). The viscosity of the nanoconjugates ranged from 3.04 to 11.83 mPas, exhibiting higher viscosity than the control (2.39 mPas). Viscosity increased gradually with increasing concentration of DEAE-Dextran. The intrinsic viscosity of IbDTw80 was 2.63 (R^2 = 0.99) mPas.

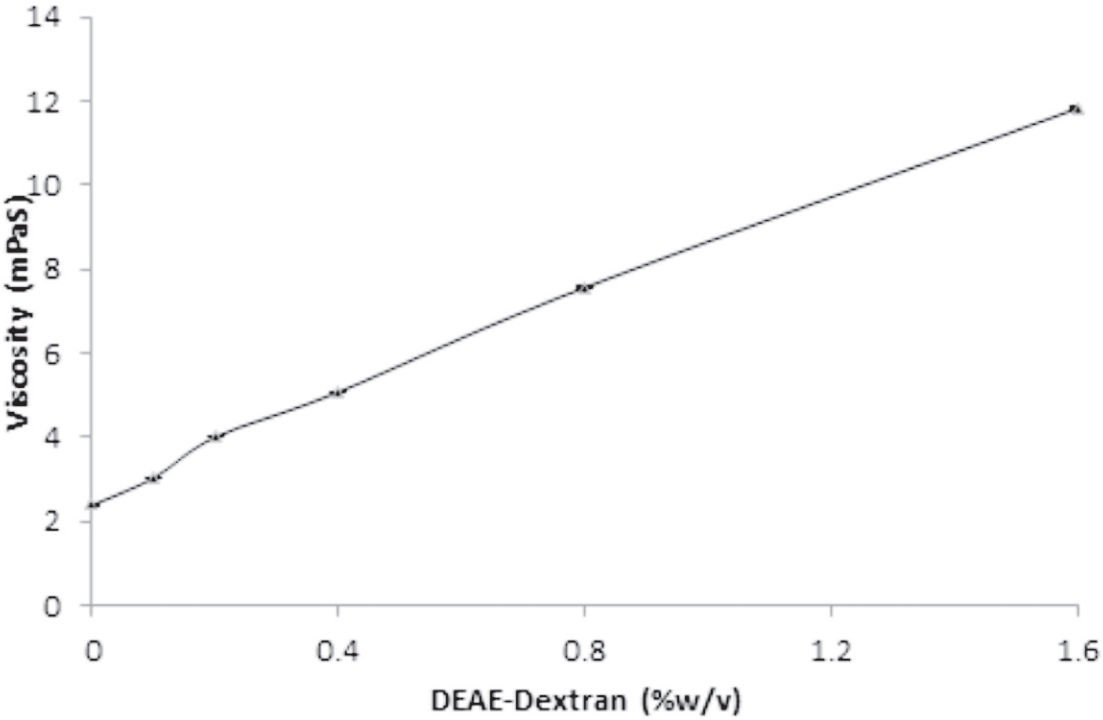


Figure 3: Viscosity of IbDTw80 conjugates based on DEAE-Dextran content. Each data point represents mean ± SD (n = 6).

Turbidity

The turbidity profile of the Ibuprofen-DEAE-Dextran nanoconjugates is shown in Figure 4. Turbidity profile of surfactant solubilized Ibuprofen-DEAE-Dextran conjugates decreased steadily to constant value of 0.033 at 0.4% DEAE-Dextran concentration.

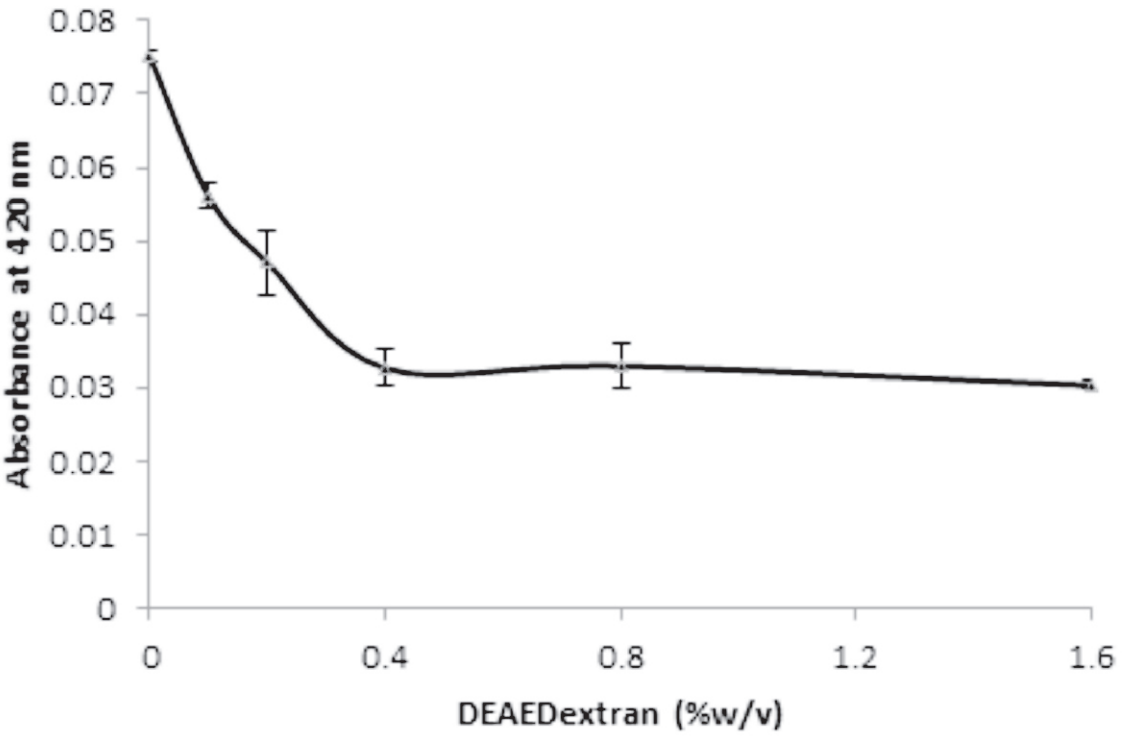


Figure 4: Absorbance at 420 nm of Ibuprofen-DEAE-Dextran nanoconjugates based on DEAE-Dextran content in the nanoconjugates. Each data point represents mean ± SD (n = 6).

Particle size, poly-dispersity index and zeta potential

The particle size of ibuprofen control (processed without polymer) was 2.87 µm. Miyadai et al. reported that raw ibuprofen has a mean particle size of 27 µm. [17] The particle size of the nanoconjugates ranged from 13.02 to 122.17 nm (Figure 5).

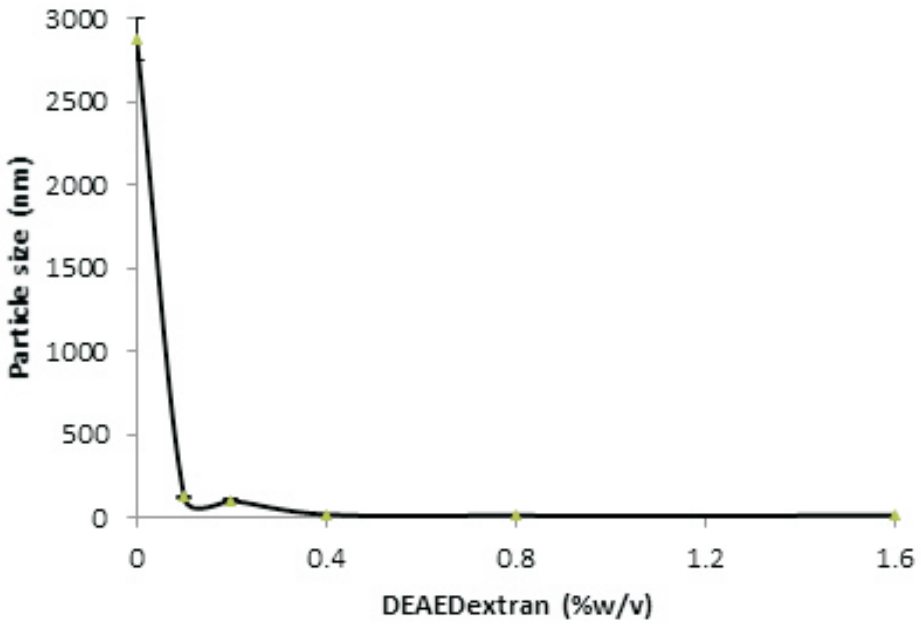


Figure 5: Effect of DEAE-Dextran concentration on particle sizes of the ibuprofen-DEAE-Dextran conjugates.

The PI of ibuprofen control was 0.67 (Table 1) which indicated a broad and non homogeneous size distribution while the nanoconjugates ranged from 0.15 to 0.37 shown in Figure 6.

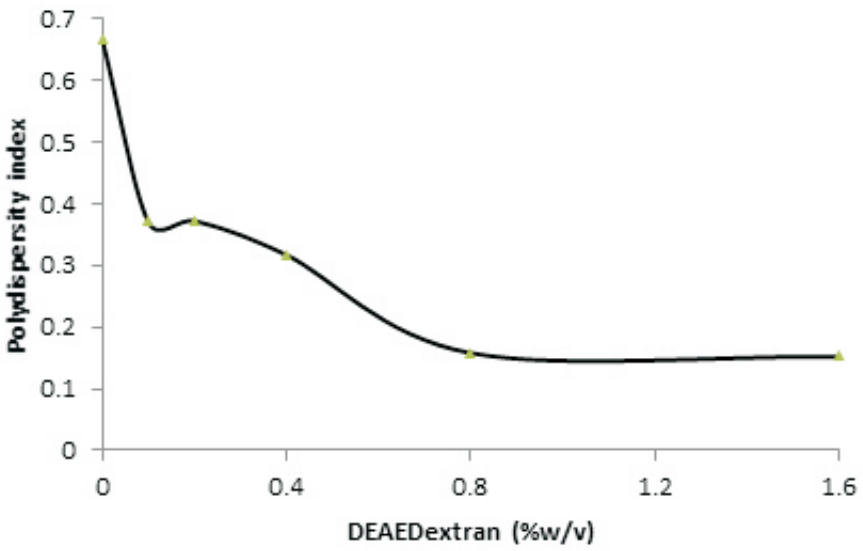


Figure 6: Effect of DEAE-Dextran on polydispersity indices of ibuprofen DEAE-Dextran nanoconjugates.

The zeta potential of the ibuprofen/DEAE-Dextran nanoconjugates increased from -7.251 mV in the control batch to steady maximum values of +3.45 and +13.87 mV shown in Figure 7.

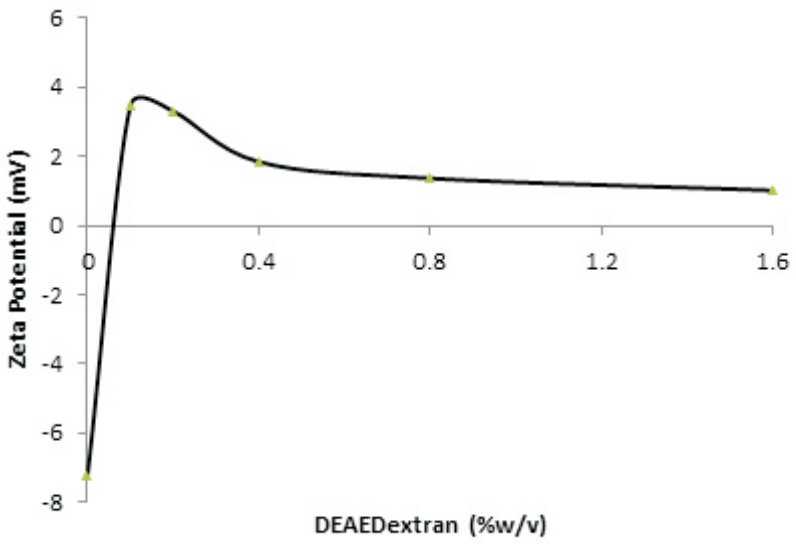


Figure 7: Effect of DEAE-Dextran on zeta potential measurements of Ibuprofen-DEAE-Dextran nanoconjugates

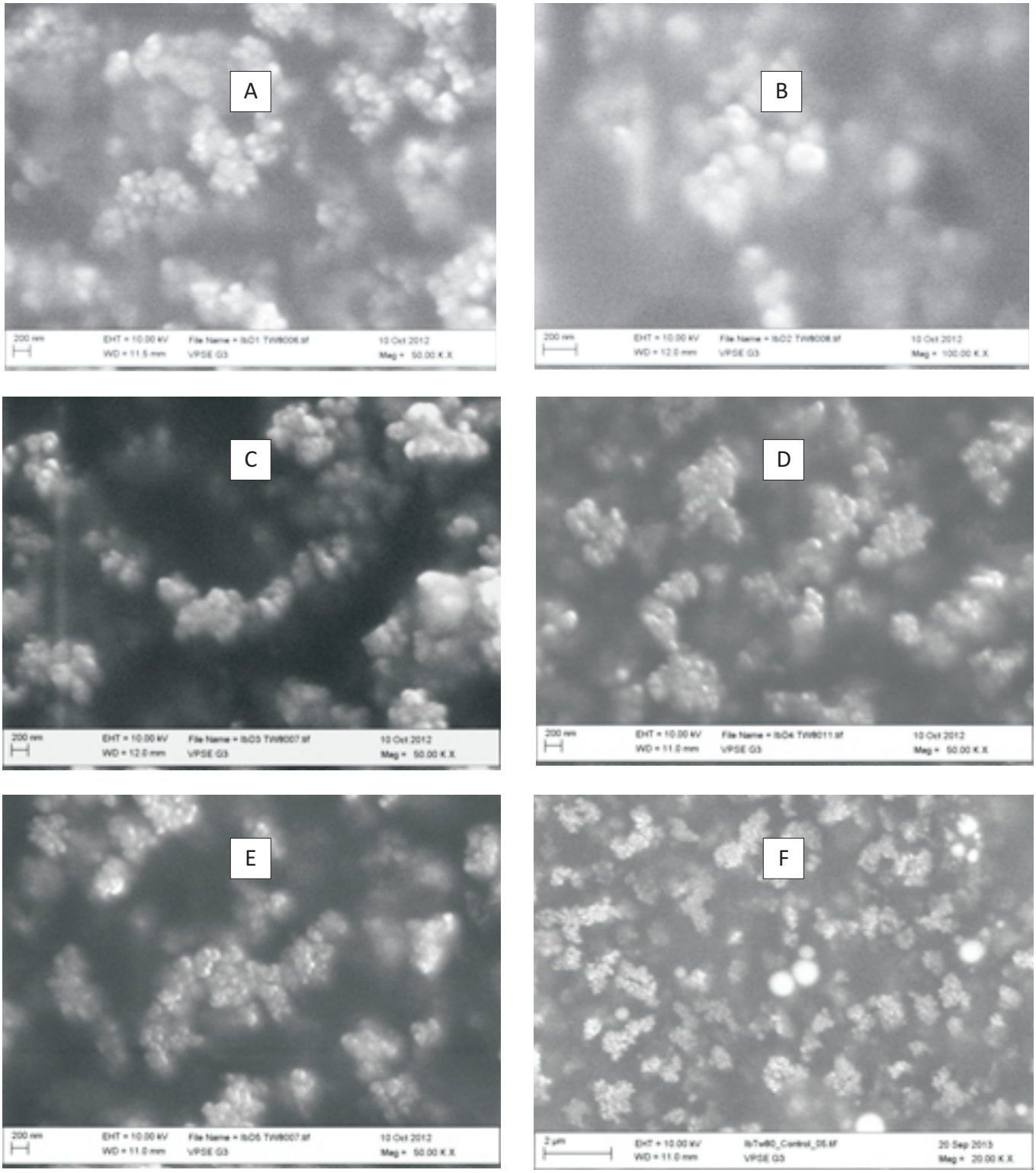
Drug conjugation capacity/efficiency

The efficiency of attaching drugs to the polymer carrier is very important for the success of polymer therapeutics. It is desirable to achieve higher conjugation efficiency for clinical applications. The conjugation efficiency of IbDTw80 conjugates was in the range of 89.05 to 96.33%.

Morphology and size – scanning electron microscopy

The SEM micrographs of ibuprofen, DEAE-Dextran, and the respective nanoconjugates are presented in Figure VIII. Ibuprofen had a distinct long needle-like crystalline structure and rough surface with particle size of 145.08 ± 56.63 x 32.82 ± 12.06 µm and aspect ratio (length to width of 4.42) in Figure 8G. This was similar to the SEM

image of ibuprofen crystals reported by Plakkot et al. with aspect ratio range of 4 to 6. [18] While DEAE-Dextran, had rough surface and irregular structures (Figure 8H). The surface morphology of ibuprofen control showed almost spherical shaped particles in aggregates (Figure 8F).



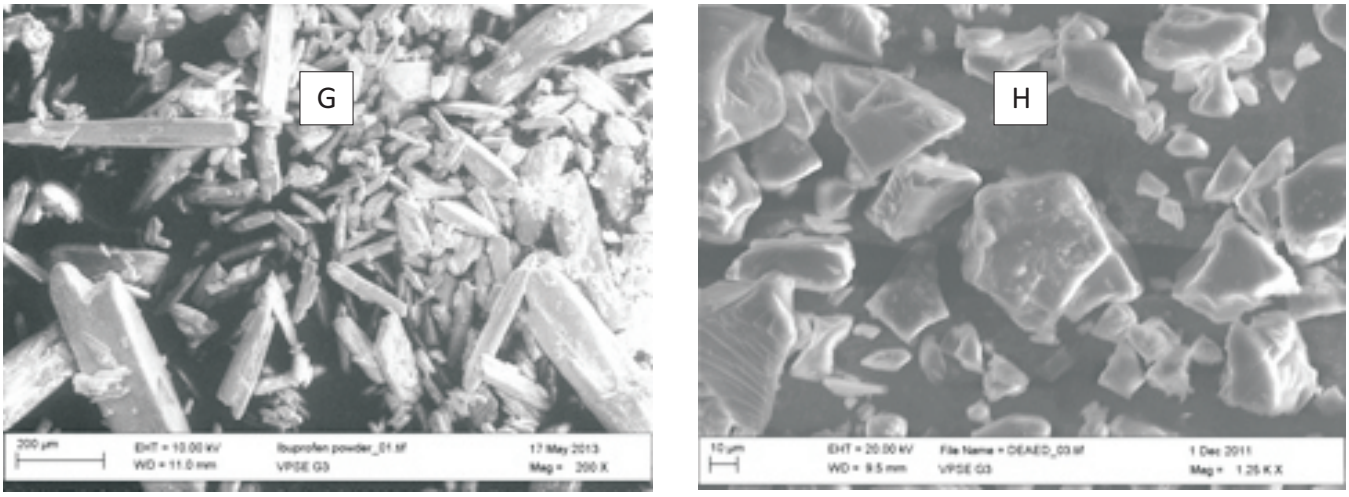


Figure 8: Scanning electron micrographs of ibuprofen-DEAE-Dextran conjugates (A) IbD1Tw80, (B) IbD2Tw80, (C) IbD3Tw80, (D) IbD4Tw80, (E) IbD5Tw80 and (F) IbTw80-control, (G) ibuprofen powder-reference and (H) DEAE-Dextran powder-reference.

DISCUSSION

Process optimization was based on preliminary studies carried out (results not presented) to determine the optimal formulation variables such as pH, concentration of polymers and drug, drug-polymer mixing ratio, mixing time, mixing speed, temperature and the order of polymer addition. Physicochemical properties of the drug, polymers and their binary conjugates such as surface tension, conductivity, contact angle, turbidity, viscosity and pH were evaluated to determine the critical association concentration and polymer saturation point which in turn was used to determine the appropriate drug-polymer ratio for the formulation of the nanoconjugates.

The final pH chosen for DEAE-Dextran (polycation) formulation based on the preliminary studies was pH 6.0. In a similar study Jiang et al. prepared ibuprofen nanoparticles stabilized by DEAE-Dextran by co-precipitation method at pH 6.0.[15] The binary conjugate (Ibuprofen-DEAE-Dextran) was prepared by surfactant solubilization method. The intention was to design a technique that can dissolve ibuprofen completely to facilitate maximum interaction with cationic polymer and ensure formation of conjugates of nano size dimension.

Reddy and Gudsookar prepared gliclazide-polyethylene glycol conjugate using the solid dispersion by solvent method.[19] The authors reported a reduction in particle size resulting in enhancement of surface area and increase in gliclazide wettability. However this method involved the use of solvent-ethanol to dissolve the drug and polymer followed by evaporation of the solvent. The method used in this study avoided the use of organic solvents due to safety issues and the increasing need to deliver safe drugs to patients. It also avoids the laborious process of evaporation.

Ibuprofen can either interact (electrostatic) with the end groups of DEAE-Dextran or can be encapsulated in the interior of the polymers (weak interactions).[15] It is expected that the ibuprofen with a carboxylic group may form a complex with the amine ($–NH_2$) groups of DEAE-Dextran.

An increase in the concentration of DEAE-Dextran increased the specific conductivity with two break points which signify two types of aggregation phenomena. The first break at lower DEAE-Dextran concentration was assigned the critical association concentration (cac) of DEAE-Dextran where the interaction between ibuprofen and DEAE-Dextran starts while the second break point was assigned the critical micelle concentration (cmc) which is attributed to the saturation of the polymer with the drug (polymer saturation point, psp). As concentration of the drug increases beyond the psp polymer-free aggregates are formed. It was noted that the presence of DEAE-Dextran decreased the cac of ibuprofen significantly ($p < 0.05$, $n = 6$) confirming the interaction between DEAE-Dextran and ibuprofen. The cac was exhibited at 2.34 mg/mL and cmc at 8.0 mg/mL. In a similar research, Khan et al. reported that a cationic polymer, hydroxyethyl cellulose ethoxylate quaternized (HECEQ), reduced the cac of ibuprofen sodium and was credited to strong affinity for ibuprofen and evidence of interaction between them.[20] Therefore the electrostatic interaction between DEAE-Dextran and ibuprofen was evident.

The surface activity of the drug-polymer was studied by

the evaluation of the surface tension measurements of the nanoconjugates as a function of polymer concentration. Addition of DEAE-Dextran caused a reduction in surface tension at the air/water interface indicating its ability to adsorb at the air/water interface in preference to the bulk of the medium. In theory, when surface active agents adsorb at the water interface they replace some water molecules thereby reducing the intermolecular (cohesive) forces of attraction between water molecules hence surface tension is reduced.

The amount of DEAE-Dextran present at the water surface, in excess of those in the bulk for the Tween 80 (non-ionic surfactant) solubilization as 7.23 x 10^{-5} calculated from the Gibbs equation.

Two break points were also observed in this technique. It was opined that the adsorption of Tween 80 was preferentially favoured until the surface was saturated leading to the first cmc at lower concentration of DEAE-Dextran. The effect of DEAE-Dextran became prominent afterwards however the cac was increased to 0.008 mM. Contrary to this finding, Lee et al. have noted that micelle formation of polymers can compete with surface adsorption and that polymer/surfactant combination is often considered to have synergistic effect due to co-adsorption. [21]

It was evident that DEAE-Dextran showed surface activity and were adsorbed significantly at the water surface with high probability of forming micelles and reducing the surface free energy of the system which in turn could facilitate solubility.

The viscosity of the drug-polymer solutions is an important parameter due to its association with the hydrodynamic stabilization of the particles formed.[22] The viscosity behaviour of the drug-polymer nanoconjugates was studied by the evaluation of the viscosity measurements of the nanoconjugates as a function of polymer concentration which increased with increasing concentration of DEAE-Dextran.

Turbidity was used to measure polyelectrolyte complex formation. The high turbidity values exhibited may indicate formation of insoluble complexes which are usually irreversible while the low turbidity may be due to formation of soluble complexes which are reversible.

The nanoconjugate size decreased steadily to a minimum (13.02 nm) with increase in concentration of DEAE-Dextran confirming the synergistic effect of polymer surfactant combination which is consistent with surface tension findings. The introduction of DEAE-Dextran at 0.1% (2.0 x 10^{-3} mM) concentration formed conjugates which reduced the particle size significantly by a factor of 28-fold ($p < 0.05$, $n = 6$) to 122.17 nm. Further increase in DEAE-Dextran concentration decreased the conjugate size steadily (268-fold) to 13.02 nm at 1.6% (3.2 x 10^{-2} mM). Poorly soluble drugs are commonly associated with critical problems of slow dissolution and erratic absorption with a consequent low and variable bioavailability. Preparation of dosage forms with particle size of less than 1 µm (nano-medicines) has been identified as an approach to enhance the dissolution as well as the rate and extent of absorption for poorly soluble drugs.[18]

Polydispersity index (PI) is the size or width of particle size distribution of the sample and has a scale which ranges from 0 to 1. PI value of 0.1 to 0.25 which has been assigned to a narrow size distribution while PI value greater than 0.5 indicates a very wide or broad size distribution. [23, 24] High PI values indicate less homogenous size distribution hence PDI should be as low as possible for the long term stability of formulations. The nanoconjugates formed from the lower concentrations of DEAE-Dextran exhibited semi homogenous size distribution which may translate to medium stabilization of the particles formed. However the higher concentrations of DEAE-Dextran (1.6 x 10^{-2} and 3.2 x 10^{-2} mM) exhibited PI of 0.16 and 0.15 respectively indicating narrow particle size distribution, high homogeneity and stabilization.

The minimum zeta potential of ±30 mV is required and can be used to assure the stability by electrostatic repulsion of nanoparticulate suspensions.[25] However, if stability is based on electrostatic and steric stabilizer; the zeta potential of ±20 mV suffices. [26] Low zeta potential values can lead to decrease in electrostatic repulsion between the particles thereby increasing the probability of particle aggregation.[27] Zeta potential is therefore a measure of the charge of the particle which is an index for particle stability.

However the overall profile showed low values close to neutrality. The negative zeta potential in the ibuprofen control indicates electrostatic repulsion between ibuprofen molecules and therefore higher physical stability of the colloidal suspension in the absence of DEAE-Dextran. The positive values of zeta potentials suggest surface modification of the nanoparticles by the cationic DEAE-Dextran. The values of zeta potential observed in this study were low (-3.04 to +13.87 mV) indicating low repulsion stabilization probably because stabilization of the nanoconjugates is by steric effect rather than electrostatic repulsion as reported by Plakkot et al. [18]

It appeared that the concentration of DEAE-Dextran did not affect the conjugation efficiency of the melt

solubilization technique. The conjugation efficiency was relatively constant, thus independent of DEAE-Dextran concentration. High conjugation efficiency was achieved by this method. This was higher than the findings of Jiang et al. which stated a maximum entrapment efficiency of 72.20% for ibuprofen-DEAE-Dextran complex.[15]

The surface morphologies of the nanoconjugates were all spherical in shape and in aggregates. The particle sizes decreased with increasing concentration of DEAE-Dextran in the range of 38.76 to 111.6 nm. This was comparable to that observed with the particle size analyzer in this study. It was observed that the surfactant solubilization technique exhibited the highest tendency to coalesce when compared to other techniques.

This study utilized the surfactant solubilization technique to dissolve ibuprofen completely thereby facilitating maximum interaction with cationic polymer DEAE-Dextran and ensuring formation of conjugates of nano size dimensions. The Ibuprofen-DEAE-Dextran nanoconjugates prepared were characterized using conductivity, surface tension, viscosity, transmittance/turbidity, particle size measurement, zeta potential, conjugation efficiency and scanning electron microscopy (SEM) techniques. High loading capacity of 89.05 to 96.34% was achieved. Addition of DEAE-Dextran decreased the cac of ibuprofen significantly ($p < 0.05$, $n = 6$); this confirmed the electrostatic interaction between DEAE-Dextran and ibuprofen. The particle size of ibuprofen was successfully reduced by a factor of between 28 and 268-fold in nanometre ranges. Stabilization of the nanoconjugates by steric effect rather than electrostatic repulsion was suggested due to the low zeta potential values obtained.

CONCLUSION

Stabilized amorphous Ibuprofen-DEAE-Dextran nanoconjugate was formulated without the use of organic solvents with the potential of enhancing the delivery ibuprofen. This method can be applied to drugs with poor aqueous solubility.

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REFERENCES

1. Raffa RB (2005). Analgesic, Antipyretic and Anti-inflammatory Drugs., in Remington: The Science and Practise of Pharmacy. Troy DB, Editor, Lippincott, Williams & Wilkins: Philadelphia, PA: 2393.
2. Rodriguez R, Alvarez-Lorenzo C and Concheiro A (2003). Interactions of Ibuprofen with Cationic Polysaccharides in Aqueous Dispersions and Hydrogels. Rheological and Diffusional Implications. *European Journal of Pharmaceutical Sciences* 20(4-5): 429-438.
3. Ramos LS, Luan M, Mohwald Y, Brezesinski HG (2007). Electrostatic Interactions Between Polyelectrolyte and Amphiphiles in Two and Three-Dimensional Systems. *Colloid Surface* A 303: 79-88.
4. Taylor DJF, Thomas RK, and Penfold J (2007). Polymer/Surfactant Interactions at the Air/Water Interface. *Advanced Colloid Interface Science* 132: 69-110.
5. Langevin D (2009). Complexation of Oppositely Charged Polyelectrolytes and Surfactants in Aqueous Solutions. A Review. *Advanced Coloid Interface Science* 147-148: 170-177.
6. Caram-Lelham NH, Sundelof LO (1997). Adsorption of Charged Amphiphiles to Oppositely Charged Polysaccharides - A Study of the Influence of Polysaccharide Structure and Hydrophobicity of the Amphiphile Molecule. *Biopolymers* 41(7): 765-772.
7. Persson BH, Caram-Lelham NA and Sundelof LO (2000). Dextran Sulfate-Amphiphile Interaction: Effect of polyelectrolyte Charge Density and Amphiphile Hydrophobicity. *Langmuir* 16(2): 313-317.
8. Hugerth A and Sundelof LO (2001). The Effect of Polyelectrolyte Counterion Specificity, Charge Density, and Conformation on Polyelectrolyte-Amphiphile Interaction: The Carrageenan/Furcelleran-Amitriptyline System. *Biopolymers* 58(2): 186-194.
9. Abioye AO, Kola-Mustapha A, Ruparelia K (2014). Impact of in situ granulation and temperature quenching on crystal habit and micromeritic properties of ibuprofen-cationic dextran conjugate crystanules. *International Journal of Pharmaceutics* 462: 83-102.
10. Abioye AO, Kola-Mustapha A, Chi GT and Ilya S (2014). Quantification of in situ granulation-induced changes in pre-compression, solubility, dose distribution and intrinsic in vitro release

characteristics of ibuprofen-cationic dextran conjugate crystanules. *International Journal of Pharmaceutics* 471: p.453-477.

11. Abioye AO and Kola-Mustapha A (2015). Controlled electrostatic self-assembly of ibuprofen-cationic dextran nanoconjugates prepared by low energy green process – a novel delivery tool for poorly soluble drugs. *Pharmaceutical Research* 32 (6): 2110-2131.
12. Abioye AO and Kola-Mustapha A (2015b). Formulation studies on Ibuprofen sodium-cationic dextran conjugate: Effect on tabletting and dissolution characteristics of ibuprofen. *Drug Development and Industrial Pharmacy*. Early Online 1 - 21. http://informahealthcare.com/ddl
13. Abioye AO, Issa S and Kola-Mustapha AT (2015). Ex vivo skin permeation and retention studies on chitosan-ibuprofen-gellan ternary nanogel prepared by in situ ionic gelation technique – a tool for controlled transdermal delivery of ibuprofen. *International Journal of Pharmaceutics* 490 (1-2): 112-130.
14. Attwood D and Florence AT (1983). Surfactant Systems. New York: Chapman and Hall Ltd: 779.
15. Jiang BH, Gao CL and Shen J (2005). Ibuprofen-Loaded Nanoparticles Prepared By A Co-Precipitation Method And Their Release Properties. *International Journal of Pharmaceutics* 304: 220-230.
16. Cheow WS and Hadinoto K (2011). Factors Affecting Drug Encapsulation and Stability of Lipid-Polymer Hybrid Nanoparticles. *Colloidal Surfaces B 85: 214–220.*
17. Miyadai NH, Moribe KK and Yamamoto K (2012). Optimization and Characterization of Direct Coating for Ibuprofen Particles using a Composite Fluidized Bed. *Advanced Powder Technology* 23(1): 40-45.
18. Plakkot SDM, York MP, Saunders M and Sulaiman B (2011). Comminution of Ibuprofen to Produce Nano-particles for Rapid Dissolution. *International Journal of Pharmaceutics* 415: 307-314.
19. Reddy SJ and Gudsoorkar VR (2005). Solid Dispersions of Gliclazide. *The Indian Pharmacist* 32: 82-84.
20. Khan IAA, Ali KM and Kabir-ud SD (2011). A Comparative Study of Interaction of Ibuprofen with Biocompatible Polymers. *Colloids and Surface Biointerfaces* 88(1): 72-77.
21. Lee JC, and Park CH (2008). Characteristics of Polymer Enabling Nano-comminution of Water-Insoluble Drugs. *International Journal of Pharmaceutics* 355: 328-336.
22. Galindo-Rodriguez SA, Fessi E and Doelker HE (2004). Physicochemical Parameters Associated with Nanoparticle Formation in the Salting-out, Emulsification-Diffusion, and Nanoprecipitation Methods. *Pharmaceutical Research* 21(8): 1428-1439..
23. Patravale VBD, and Kulkarni, RM (2004). Nanosuspensions: A Promising Drug Delivery Strategy. *Journal of Pharmacy and Pharmacology* 56(7): 827-840.
24. Ali HS and Blangden PN (2009). Preparation of Hydrocortisone Nanosuspension through a Bottom-Up Nanoprecipitation Technique Using Microfluidic Reactors. *International Journal of Pharmaceutics* 375(1-2): 107-113.
25. Motwani SK, Chopra S, Talegaonkar S, Kohli K, Ahmad FJ and Khar RK (2007). Chitosan-Sodium Alginate Nanoparticles as Submicroscopic Reservoirs for Ocular Delivery: Formulation, Optimization and In Vitro Characterization. *European Journal of Pharmaceutics and Biopharmaceutics* 68: 513-525.
26. Arunkumar ND and Rani MC (2009). Nanosuspension Technology and its Applications in Drug Delivery. Asian Journal of Pharmaceutics 3(3): 168-173.

Gan QW, Cochrane TC and McCarron P (2005). Modulation of Surface Charge, Particle Size and Morphological Properties of Chitosan-TPP Nanoparticles Intended for Gene Delivery. *Colloids and Surface B Biointerfaces* 44(2-3): 65-73.