The effect of Ibuprofen–DEAE-Dextran nanoconjugates (surfactant solubilization) on the thermal properties and *in vitro* drug release kinetics of ibuprofen

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Abstract

Background: It has become increasingly desirable to overcome the low aqueous solubility of drug candidates and develop more novel and innovative formulation approaches to increase the dissolution rate of the poorly soluble drugs; due to significant difficulties presented by Active Pharmaceutical Ingredients (APIs) in drug product design and development. This work focused on the effect of stable amorphous ibuprofen-DEAE-Dextran nanoconjugates formulated in earlier studies via surfactant solubilization technique (organic solvent free process) on its physicochemical and drug release characteristics.

Methods: The nanoparticles were characterised via the Fourier Transform Infra-red (FTIR), Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), drug release profile and kinetics.

Results: The FTIR spectroscopic analysis revealed electrostatic, hydrophobic and hydrogen bonding interaction between solubilized ibuprofen and the cationic polymer (DEAE-Dextran) to form a new product (an amide). The DSC of the nanoconjugates exhibited broad and diffuse melting peaks which confirmed that the Ibuprofen-DEAE-Dextran nanoconjugates exist in amorphous state. Isothermal stability was suggested due to the disappearance of thermal decomposition peak of ibuprofen at 237.51 °C also disappeared in all the nanoconjugates. The TGA thermograms of the nanoconjugates exhibited two steps of weight loss profile due to the loss of free water and decomposition of the nanoconjugates. Marked enhancement of drug release was achieved by the nanoconjugates. The major mechanism of drug release from the nanoconjugates was by anomalous diffusion. **Conclusions:** This study therefore demonstrates

the improved drug release profile of amorphous Ibuprofen-DEAE-Dextran nanoconjugates with potential application in the delivery of poorly soluble drug.

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

Introduction

Research is ongoing on how to resolve the problems of poor solubility and dissolution characteristics which has remained unresolved over time. Therefore, formulation strategies to improve physicochemical properties, therapeutic quality of poorly soluble drugs and optimization of their delivery efficiency has been the focus[1,2]. Persson *et al*. studied the interaction of dextran sulphate and hydrophobicity of cationic amphiphiles in the order doxepin-HCl < amitriptyline HCl < chlorpromazine-HCl[3]. The data reported indicated that the polyelectrolyte-amphiphile interaction depended on both the hydrophobicity of the amphiphile and the charge density of dextran sulphate. The amorphous forms of highly water insoluble drugs are known to exhibit higher saturation solubility, enhanced dissolution and bioavailability as well as nanoscale formulation than the crystalline form[4]. Cheow and Hadinomoto prepared freeze-dried amorphous ciprofloxacin-dextran sulfate nanoplex via drug-polyelectrolyte complexation. The nanoplex powders exhibited about twice the dissolution rate (50% at 1 h) and solubility (0.3 mg/mL) when compared with raw ciprofloxacin crystals (25% at 1 h and 0.14 mg/mL respectively)[4]. The authors however acknowledged that the rapid crystallization of ciprofloxacin from the supersaturated solution could erode its solubility advantage. Therefore, to prolong the supersaturation period, crystallization inhibitors such as polyvinylpyrrolidone (PVP) have to be included.

Polymer-surfactant complexes have been reported to form nanoscopic core-shell structures above the critical micelle concentration (CMC)[5]. In this case poorly

soluble drugs are entrapped in the hydrophobic core while the hydrophilic part serves as the interface between the bulk aqueous phase and the hydrophobic domain. The polymeric micelles therefore serve as nanoscopic depot or stabilizers for poorly water soluble drugs[6]. Barreiro-Iglesias *et al.* studied the capability of pluronic conjugated with poly (acrylic acid) to enhance the solubility and stability of camptothecin[5]. The authors found that camptothecin solubility in polymer micellar solution was three to four-folds higher than in water at pH 5. A review of drug loaded polymeric micelles for the enhancement of the solubility of the drug has been presented by Jones and Leroux[6].

In our previous work, we reported the effect of Ibuprofen-Ddex nanoconjugates prepared by the drug-polyelectrolyte complexation technique (low energy and green process) on the physicochemical and in vitro drug release characteristics of ibuprofen[8]. The nanoconjugates containing low concentrations of Ddex up to 1.0 X 10^{-6} g/dm^3 exhibited enhanced dissolution efficiency for ibuprofen (81.32%) beyond which dissolution was retarded steadily. In another study, we investigated the direct impact of intermolecular attraction between ibuprofen (IB) and chitosan (CT) on crystal behaviour, saturated solubility and dissolution efficiency of ibuprofen[9]. We found that the IB-TC nanoplex exhibited both fast and extended release profiles of ibuprofen dictated by chitosan concentration.

The aim of this research was to study the effect of nanoconjugates formulated by another technique: surfactant solubilization in order to ultimately develop another drug delivery strategy for ibuprofen.

Materials and 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. The Ibuprofen-DEAE-Dextran nanoconjugates by solubilization method formed in earlier report were kept at room temperature prior to analysis[12].

Methods

Fourier Transform Infra-red

FTIR was conducted using Perkin-Elmer Precisely Spectrum One FTIR Spectrometer and a Universal ATR Sampling Accessory (Perkin Elmer, USA). The samples were mounted directly on the diamond surface and the arm was placed over it by applying enough pressure in the range of 100 to 120 units. The spectrum was recorded in the wavelength region of 4000 to 650 cm^{-1}. All spectra were then collected at an average of 16 scans at a resolution of 4 cm^{-1}. All measurements were taken in replicates of four determinations.

Differential scanning calorimetry (DSC)

DSC was performed using Perkin Elmer Precisely Jade DSC machine with a Perkin Elmer Intracooler SP cooling Accessory (Perkin Elmer, USA) to study the thermal behaviour of the pure ibuprofen, DEAE-Dextran, physical mixtures and ibuprofen nanoconjugates. The sample sizes in the range of 8 to 10 mg were heated in hermetically sealed aluminium pans under nitrogen flow (40mL/min) using a scanning rate of 20 ˚C/min from -50 to 300 ˚C. Empty aluminium pan was used as a reference. Indium was used as the standard reference material to calibrate the DSC instrument. All measurements were an average of four determinations and expressed as mean ± S.D.

Thermogravimetric analysis (TGA)

TGA was performed using Perkin Elmer Pyris 1 Thermogravimetric Analyser (Perkin Elmer, USA) to monitor the mass of the pure starting materials and

nanoconjugates as a function of temperature or time as the sample specimen is subjected to a controlled temperature program at atmospheric pressure. The weight of the empty reference pan placed in the crucible was zeroed and then removed. Samples of known weight in the range of 18 to 25 mg were placed in aluminium pans and measurements performed at a scanning rate of 10 ˚C/min in the range of 25 to 500 ˚C. All measurements were an average of four determinations and expressed as mean ± S.D.

Dissolution and drug release studies

Calibration curve (not shown) was constructed by using pure ibuprofen reference standard (secondary standard) dissolved in buffer solution (pH 7.4) within the concentration range 1.56 and 50 µg/mL (7.56 to 242.39 mM). The correlation was linear (r^2 = 0.9953) and the measurements at six levels of concentration were reproducible with limit of quantification (LOQ = 10 x standard error of the intercept/slope) of 0.37 µg/mL (1.79 mM). The concentrations of ibuprofen were derived from this calibration curve. Thereafter the dissolution profile of the ibuprofen-loaded nanoconjugates was studied using the USP dissolution method[13]. 90 mL of the ibuprofen-DEAE-Dextran nanoconjugates containing 1 mg/mL ibuprofen was put in a dialysis tubing (Visking Medicell Ltd London UK with diameter 18/32″ and exclusion size 12000 to 14000 Da). Both ends were tied. The dialysis tubing was suspended in 900 mL of PBS pH 7.4, rotated at 50 rpm and maintained at 37 ± 0.5 ˚C using Pharma Test PT DT7 USP Apparatus II Dissolution Test Instrument - paddle method (Pharma Test Ltd, Germany). At pre-determined time intervals 10, 20, 30, 60 min, 1, 2, 4, 6 and 24 h, 5 mL aliquots were withdrawn and replaced with 5 mL PBS pH 7.4. Drug concentrations were quantified using UV (ThermoFischer Evolution 60 UV-Visible Spectrophotometer, UK) after filtering through 0.45 µm filter (Sartorius, Germany) at 264 nm. All measurements were an average of four determinations and expressed as mean

± S.D. A calibration curve was produced using a series of dilution of reference standard ibuprofen in PBS pH 7.4 and analysed by UV at 264 nm. The calibration curve was linear at concentrations between 1.56 and 50 µg/mL with R^2 value of 0.99. The absorbance values obtained from the drug release of the nanoconjugates were correlated with the calibration curve and the amount of ibuprofen released was determined.

Drug release kinetics

Data obtained from *in vitro* release studies were fitted to various kinetic equations. The kinetic models used are zero order, first order and Higuchi equation.

The zero order rate equation (1) describes the systems were the drug release rate is independent of its concentration[12]. The cumulative % drug release vs. time plot is made.

$$C = K_0 t \qquad (1)$$

Where k_0 is zero order rate constant expressed in units of concentration/time and t is the time.

The first order rate equation (2) describes the release from the systems where release rate is concentration dependent[13]. The log cumulative % drug release vs. time plot is made.

$$Log\ C = Log\ C_0 - Kt/2.303 \qquad (2)$$

Where C_0 is the initial concentration of drug and K is first order constant.

Higuchi described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion: equation (3)[14]. The cumulative % drug release vs. square root of time is made.

$$Q = Kt^{1/2}$$

(3)

Where Q is the cumulative amount of drug released and K, is the constant and it reflects the design variables of the system.

Korsemeyer *et al.* derived a simple relationship which described drug release from a polymeric system: equation (4). To find out the mechanism of drug release, first 60 % drug release data was fitted in Korsemeyer-Peppas model:

$$M_t/M_\infty = Kt^n$$

(4)

Where M_t/M_∞ is a fraction of drug released at time t, K is the rate constant and n is the release exponent. The n value is used to characterize different release mechanisms[15]. The log cumulative % drug release vs. log time plot is made. The criterion for selecting the appropriate model is the highest R^2 value which indicates linearity of dissolution data[16].

Similarity factors

The similarity fit factor denoted f_2 was used to compare the dissolution profiles of the drug–polymer conjugates (test) and ibuprofen control (reference). The similarity f_2 factor is defined by equation (5) proposed by Moore and Flanner[17]:

$$f_{2=50\ Log\ \left\{(1+\frac{1}{n}\sum_{t=1}^{n}\ (R_t - T_t)^2)^{-0.5} \times 100\right\}}$$

(5)

Where n is the number of dissolution sample times, and R_t and T_t are the individual or mean percent dissolved at each time point, t, for the reference and test dissolution profiles, respectively. The f_2 value greater than 50 suggests that the two profiles are similar. The f_2 value of 100 suggests that the test and the

reference profiles are identical and as the value becomes smaller the dissimilarity between release profiles increases[18].

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 and Discussion

Fourier Transform Infrared

FTIR spectra of the samples were generated to identify any potential intrinsic molecular interaction between functional groups of ibuprofen and DEAE-Dextran or any local disturbances to the basic backbone spectrum of their structure due to neighbouring electrostatic effects. The assignments of peaks of drug and polymers used are summarized in Table I and the changes in peak positions and intensities of absorption bands are summarized in Table II and Figure I. The spectra of pure ibuprofen showed characteristic broad absorption peaks at 3020 and 2954 cm^{-1} corresponding to OH group usually from carboxylic acid which has strong tendency to form hydrogen bonded dimers and methyl CH-stretching respectively. Broad absorption bands at 3500 to 2500 cm^{-1} have been ascribed to OH group from carboxylic acid[19]. The hydrogen bonded dimer is also evident between 3309 and 2632 cm^{-1} (Figure I). This was consolidated by strong and sharp C-O stretching at 1230 cm^{-1} as well as strong and sharp carbonyl band absorption peak at 1706 cm^{-1} with high intensity due to large dipole moment of the carbonyl bond (C = O) stretching of the carboxylic acid (COOH) group. Major band in the region of 1750 to 1700 cm^{-1} have been reported to correspond to substances containing simple carbonyl compounds such as aldehyde, ketone,

ester or carboxylic acid[20]. It also includes absorption peaks at 2954 cm^{-1}, 2923 cm^{-1} and 2859 cm^{-1} which are characteristic for linear aliphatic C-H stretching as well as the presence of aromatic ring vibration (C = C) observed at bands 1507 cm^{-1} which supports the presence of aromatic structure of ibuprofen. DEAE-Dextran spectra contain characteristic broad absorption band at 3295 cm^{-1} corresponding to normal polymeric –OH stretching[20]. The N-H deformation vibration at 1641 cm^{-1} was assigned to tertiary amine (NH bend: 1650 to 1550 cm^{-1}) and C-N stretching at 1342 cm^{-1} (1340 to 1250 cm^{-1}). In the FT-IR spectrum of 1:1 physical mixture of ibuprofen and DEAE-Dextran (Figure I, Table II) the band features correspond to the bands in the individual components of the mixture however the carbonyl group peak at 1706 cm^{-1} shifted to 1716 cm^{-1} and a new peak appeared at 1844 cm^{-1} indicating a possible intermolecular interaction in solid state. Also absorption peaks at 3610, 3749 and 3902 cm^{-1} corresponds to monomeric hydroxyl groups which indicate a strong tendency to form intermolecular hydrogen bonding.

Table I Functional groups and vibrations assigned to the major peaks of pure components observed in the FTIR spectra displayed in Figure I

Material	Assignment / group	Group frequency Wave number/ cm^{-1}
Ibuprofen	Aromatic C-H asymmetric stretching	3088 and 3040
	Hydroxyl O-H stretching	3020
	Methyl –CH stretch	2954
	Methylene C-H stretching	2923
	Methyne C-H stretching	2859
	Carboxylic acid C=O stretching	1706
	Aromatic ring C=C-C stretching	1563, 1507, 1461 and 1450
	Methyl –CH bend	1462, 1450 and 1442
	Methyne C-H bending	1320
	Skeletal C-C vibration	1268, 1230, 1183
	Aromatic C-H in plane bending	1183, 1168, 1123, 1091, 1073, 1007 and 969
	Aromatic C-H bend out of plane bending	880, 865, 849, 834, 820, 779, 746 and 690
DEAE-Dextran	N-H stretching, O-H stretching	3295
	C-H stretching	2921
	N-H bending (C=O in amide group)	1641
		1456
	C-C stretching	1342

Methyne C-H bending	1144
C-O-C stretching	1007
C-N stretching	915
C-O stretching	760
C-Cl stretching	

DEAE-Dextran showed peaks at 3295 cm^{-1} (NH and OH), 2921 cm^{-1} (CH), 1641 cm^{-1} (NH), 1456 cm^{-1} (C-C), 1342 cm^{-1} (C-H), 1144 cm^{-1} (C-O-C), 1007 cm^{-1} (C-N), 915 cm^{-1} (C-O) and 760 cm^{-1} (C-Cl). Dermibilek and Dinc reported similar peaks for DEAE-Dextran at 3394 cm^{-1}, 2926 cm^{-1}, 1643 cm^{-1}, 1467 cm^{-1}, 1157 cm^{-1}, 1013 cm^{-1}, 918 cm^{-1} and 765 cm^{-1} [19]. The bands assigned as the finger prints of ibuprofen in literature include 2992 cm^{-1} (CH), 1706 cm^{-1} (C=O), 1230 cm^{-1} (C-C) and 779 cm^{-1} (CH)[22, 23]. Ibuprofen-DEAE-Dextran physical mixture showed peaks at 3610 cm^{-1} (OH), 2921 cm^{-1} (CH), 1716 cm^{-1} (CO), 1646 cm^{-1} (NH), 1319 cm^{-1} (CH), 1230 cm^{-1} (C-C), 1007 cm^{-1} (CN) and 778 cm^{-1} (CH) which are basically a combination of peaks present in ibuprofen and DEAE-Dextran with slight variations.

Table II FTIR spectral characteristics at various wavelengths for ibuprofen-DEAE-Dextran nanoconjugates: ibuprofen control, ibuprofen-reference, DEAE-Dextran-reference and ibuprofen-DEAE-Dextran physical mixture.

Formulation	Position of absorption band (Wavenumber) / cm^{-1}					
Ibuprofen-reference	3089	2859	1507	1329	1123	865
	3045	2727	1462	1321	1091	849
	3020	2632	1450	1305	1073	834
	2992	2542	1442	1268	1007	820
	2980	1911	1418	1231	969	779
	2954	1804	1379	1183	934	746
	2923	1706	1365	1168	880	690
DEAE-Dextran-reference	3295	2921	1641	1342	1007	760
			1456	1144	915	
Ibuprofen-DEAE-Dextran physical mixture	3902	3610	1844	1646	1524	1230
	3749	2921	1716	1555	1456	1007
			1675	1542	1319	778
IbTw80-control (IbD 1:0)	3496	2922	1735	1436	1296	946
		2836	1643	1349	1248	845
					1095	712
IbD1Tw80 (IbD 1:0.5)	3336			1635	1087	
IbD2Tw80 (IbD 1:1)	3337			1635	1086	
IbD3Tw80 (IbD 1:2)	3335			1635	1086	

IbD4Tw80 (IbD 1:4)	3342	1635	1086
IbD5Tw80 (IbD 1:8)	3343	1639	1086

Nanoconjugates derived from the surfactant (Tween 80) solubilization technique exhibited only two peaks at 3309 to 3334 cm^{-1} and 1634 to 1639 cm^{-1} at all concentrations of DEAE-Dextran. The characteristic aromatic and the fingerprint features completely disappeared while the hydroxyl group became a broad Gaussian-shaped peak at around 3334 cm^{-1} indicating intermolecular hydrophobic and hydrogen-bonded hydroxyl interactions respectively. Also the carbonyl group at 1706 cm^{-1} disappeared and a new peak appeared at 1634 cm^{-1} which corresponds to amide functional group as reported by Coates (1680 to 1630 cm^{-1})[20]. It was hypothesized that melt solubilization of ibuprofen (-COOH) in the presence of cationic DEAE-Dextran ($-N^{+}HR_2$) produced an amide probably through condensation reaction.

In the absence of DEAE-Dextran, the broad hydroxyl absorption became very weak and the aromatic as well as the fingerprint peaks of ibuprofen was more prominent. This effect was attributed to Tween 80, the non ionic surfactant. Also the intensity of the amide peak reduced and shifted to 1643 cm^{-1} while the carbonyl peak reappeared at 1735 cm^{-1} which may indicate the formation of ester or six-membered ring lactone[20]. A new peak also appeared at 1350 cm^{-1} suggesting the formation of carboxylic acid salt (carboxylate). It was opined that carboxylic acid salt, ester and amide as well as hydrogen bonding were formed during surfactant solubilization of ibuprofen in the absence of DEAE- Dextran.

Differential scanning calorimetry

The DSC technique was used to evaluate the complexation between ibuprofen and DEAE-Dextran in binary nanoconjugates. The DSC thermograms of the nanoconjugates and their components are presented in Figure II. The DSC

thermograms of Ibuprofen showed characteristic sharp endothermic peak at 80.07 °C suggesting its crystallinity, with enthalpy of fusion of 118.64 J/g. This melting peak is higher than the literature value for pure ibuprofen in the range of 75 to 78 °C[24]. This could be due to some impurity in the ibuprofen sample however in a similar study; Kumar *et al.* reported that the melting peak of ibuprofen was 82.76 °C[25]. The second peak of the pure ibuprofen was ascribed to the degradation of ibuprofen at 237.51 °C with enthalpy of fusion 2241.34 J/g. DEAE-Dextran showed a glass transition temperature (Tg) at 57.26 °C; a broad endothermic melting peak at 124.05 °C due to its amorphous characteristic; a small peak was noted at 201.21 °C probably due to the semi crystalline component of the polymer and a final peak at 268.50 °C attributed to the decomposition of DEAE-Dextran. The physical mixture of DEAE-Dextran and Ibuprofen showed peaks at 59.58 °C, 79.99 °C, 116.86 °C, 200.58 °C and 237.63 °C which represented peaks from individual components (ibuprofen and DEAE-Dextran). It was observed that the Tg of DEAE-Dextran increased by 2.32 °C in the physical mixture while the melting peak of ibuprofen did not change to any great extent (79.99 °C). However, the melting peak of the DEAE-Dextran decreased by 5.80% while the degradation peaks remained almost constant.

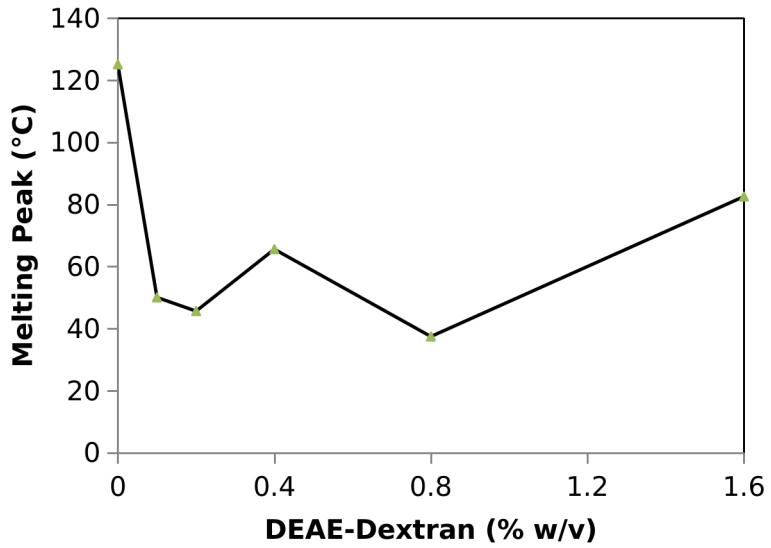

Figure I The DSC melting peak profile of Ibuprofen-DEAE-Dextran nanoconjugates.

The nanoconjugates exhibited three peaks in the range of -14.15 to 8.64 °C; 37.47 to 82.55 °C and 104.45 to 106.31 °C. The melting temperature of the first peak was observed at -14.00 °C in the absence of DEAE-Dextran but increased with increasing concentration of DEAE-Dextran to a maximum value of 6.35 °C at ibuprofen/DEAE-Dextran weight ratio of 1:2 beyond which it became unpredictable. The second peak did not show any particular pattern with increase in the concentration of DEAE-Dextran but exhibited two minima values of 45.65 °C and 37.47 °C at ibuprofen/DEAE-Dextran weight ratios 1:1 and 1:4 respectively suggesting formation of multiple complexes. The broad and diffused peaks with reduced melting temperatures of the nanoconjugates produced in this technique also signify the amorphous state of the new product. There was a shift of melting peak of ibuprofen to a range of 37.47 to 82.55 °C (Figure I). The thermal decomposition peak of ibuprofen at 237.51 °C also disappeared in all the nanoconjugates suggesting isothermal stability.

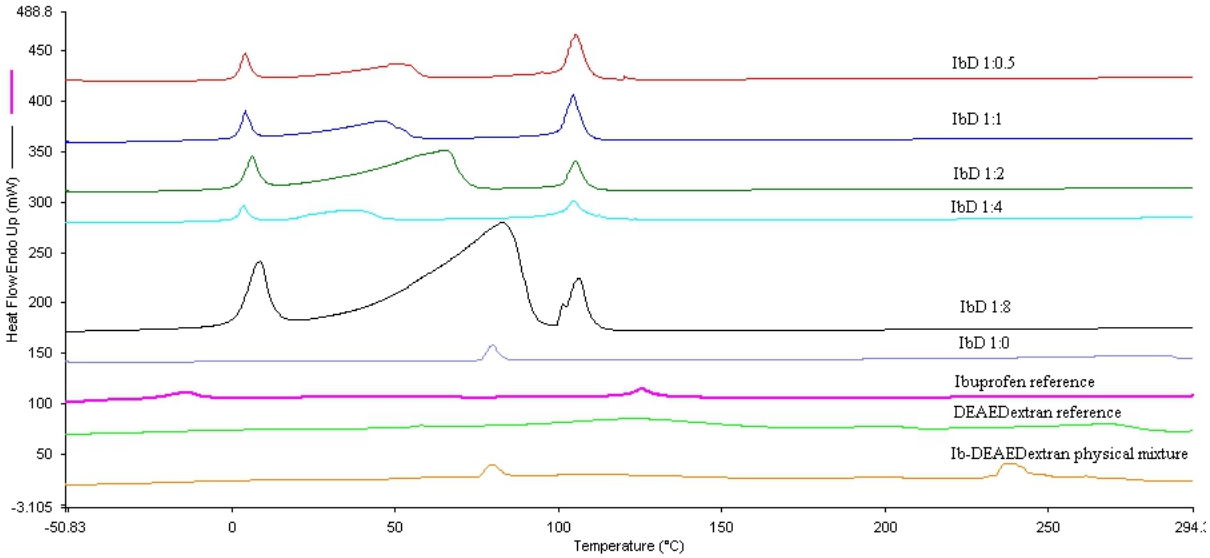

Figure II DSC thermograms of ibuprofen-DEAE-Dextran conjugates: ibuprofen control, pure ibuprofen, DEAE-Dextran and physical mixture of ibuprofen and DEAE-Dextran.

Thermogravimetric analysis (TGA)

The TGA thermograms of pure ibuprofen and ibuprofen control (processed ibuprofen without polymer) showed one step of weight loss of 99.5% and 99.3% at 240.76 °C and 104.52 °C respectively in Figure III. DEAE-Dextran showed two steps of weight loss of 6.94% and 67.61% at 24.94 °C and 272.15 °C respectively while the ibuprofen-DEAE-Dextran physical mixture showed three steps of weight loss of 4.13%, 47.92% and 36.17% at 126.01 °C, 218.05 °C and 253.12 °C respectively. The pure ibuprofen exhibited almost 100% degradation. DEAE-Dextran exhibited 88.2% degradation, a residue of 11.88% remained after degradation.

TGA thermograms of nanoconjugates obtained from surfactant solubilization technique are presented in Figure III. The nanoconjugates showed two steps of weight loss at a range of 104.34 to 130.96 °C and 379.29 to 405.62 °C which was close to 429.7 °C, the reported weight loss temperature due to decomposition of Tween 80[24]. The nanoconjugates showed two steps of weight loss at a range of 104.34 to 130.96 °C and 379.29 to 405.62 °C. The first weight loss was due to the loss of free water in the conjugates while the second weight loss was due to

the decomposition of the nanoconjugates[27]. The higher temperature of decomposition of the nanoconjugates compared to ibuprofen was due to the effect of Tween 80 on the nanoconjugates.

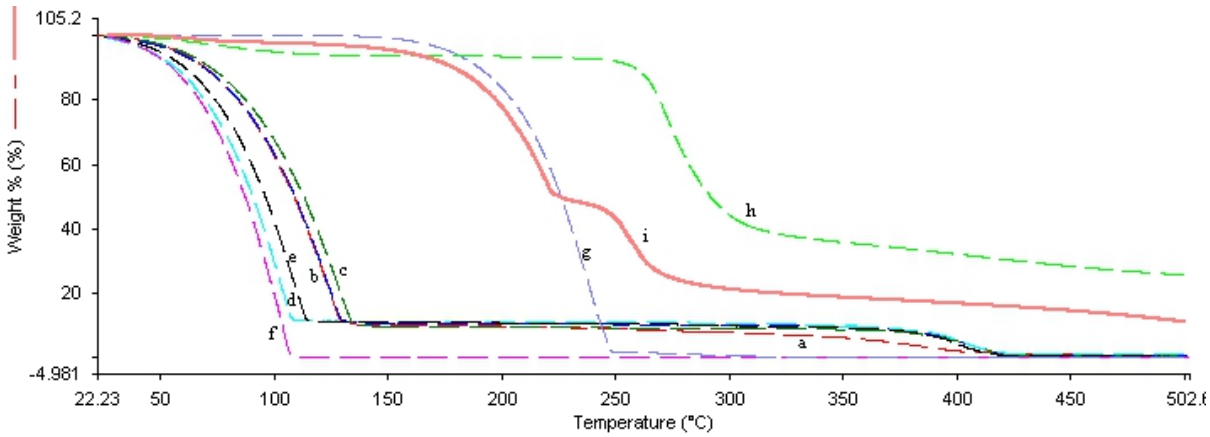

Figure III TGA thermograms of ibuprofen-DEAE-Dextran nanoconjugates (a) IbD1Tw80, (b) IbD2Tw80, (c) IbD3Tw80, (d) IbD4Tw80, (e) IbD5Tw80, (f) IbTw80-control, (g) ibuprofen-reference, (h) DEAE-Dextran-reference and (i) ibuprofen-DEAE-Dextran physical mixture.

Drug release studies

The *in vitro* release profiles of Ibuprofen-DEAE-Dextran conjugates and ibuprofen control in PBS pH 7.4 at 37 °C are shown in Figure IV. The release of Ibuprofen from nanoconjugates was in the range of 74.67 to 85.53%, which was significantly higher ($p < 0.05$, $n = 4$) than the release by ibuprofen control (30.72%) at the end of 24 h (Figure V). The order of release of ibuprofen from the nanoconjugates at 24 h is as follows: IbD4Tw80 > IbD2Tw80 > IbD5Tw80 > IbD3Tw80 > IbD1Tw80 > IbTw80-control. Increasing concentration of DEAE-Dextran increased the release of ibuprofen from 74.67 to 85.53%. However the increase was not statistically significant ($p > 0.05$, $n = 20$). Purcaru *et al.* studied the drug release of nimesulide from Tween 80 solutions in combination with buffer (pH 7.4)[28]. They reported that the release from 1% Tween 80 was instantaneous with drug release of 78 to 79% after 60 min. However, in this study the release of ibuprofen from nanoconjugates was 17% after 60 min. It was

opined that the affinity between the DEAE-Dextran and ibuprofen retarded the release. The solubilizing effect of tween 80 may have been hindered by increased degree of disorderliness (entropy) in the mixture due to the presence of the nanoconjugates.

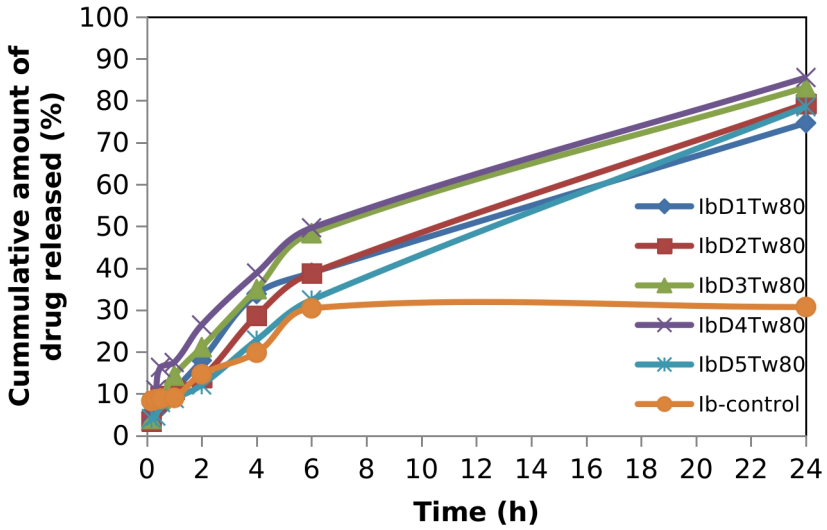

Figure IV Release profile of ibuprofen from DEAE-Dextran nanoconjugates. Each data point represents mean ± SD (n = 4).

Drug release kinetics

Kinetic or mathematical models are model – dependent methods useful in comparing *in vitro* dissolution profiles. Methods of drug release from nanoparticles include desorption of drug bound to the surface; diffusion through the nanoparticle matrix; diffusion through the polymer wall of nanocapsules; nanoparticle matrix erosion; or a combined erosion-diffusion process. To analyze the drug release mechanism in the complexes, the release data was fitted into different kinetic models: zero order, first order, Higuchi plot and Korsemeyer-Peppas (first 60% release)[15]. The parameters of the models were obtained by linear regression.

Based on Korsemeyer-Peppas equation, values of n = 0.5 refers to Fickian diffusion which indicates drug release by diffusion and n = 1 refers to Case II transport which indicates drug release by the process of swelling[29]. Values of n

between 0.5 and 1 refer to anomalous transport mechanism which indicates the superposition of diffusion and swelling processes. These extreme values of n are valid for slab geometry (thin films). For spheres which represent the particles in this study, different n values have been derived[30, 31]. Values of $n < 0.43$ refers to Fickian diffusion; $0.43 < n < 0.85$ refers to anomalous transport; while $n = 0.85$ refers to Case II transport.

Table III Regression coefficient (r) values of different kinetic models and diffusion exponent (n) of Korsemeyer-Peppas models for the release of ibuprofen from DEAE-Dextran conjugates.

Formulation	Zero order R^2	First Order R^2	Higuchi R^2	Korsemeyer-Peppas R^2	n
IbTw80-control	0.61	0.56	0.80	0.88	0.32
IbD1Tw80	0.91	0.65	0.99	0.96	0.60
IbD2Tw80	0.95	0.66	0.99	0.93	0.60
IbD3Tw80	0.89	0.59	0.99	0.98	0.65
IbD4Tw80	0.89	0.58	0.99	0.96	0.56
IbD5Tw80	0.98	0.74	0.98	0.96	0.57

The data were best fitted with Higuchi kinetic models for all the nanoconjugates. The n values obtained (Table III) indicated that for all the nanoconjugates, the release mechanism was anomalous (non-Fickian) diffusion where diffusion exponent (n) is $0.43 < n < 0.85$.

Similarity factor

The f_2 similarity factor was used to compare the drug release profiles of processed ibuprofen control (reference) with ibuprofen-DEAE-Dextran nanoconjugates (tests) and the mean f_2 data. The f_2 values obtained for the nanoconjugates were lower than limit value of 50 (f_2 values in the range of 35.60 to 40.38) suggesting dissimilarity of the drug release profiles of nanoconjugates compared to the control. This implied that the nanoconjugates were not identical with the control.

Conclusions

This study investigated the effect of Ibuprofen-DEAE-Dextran nanoconjugates formulated by the surfactant solubilization technique on the thermal and *in vitro* dissolution characteristics of ibuprofen. FTIR confirmed the interaction between ibuprofen and DEAE-Dextran to form an amide. The DSC thermograms of the nanoconjugates revealed broad and diffused peaks with reduced melting temperatures of the nanoconjugates which signified the amorphous state of the new product. The TGA thermograms exhibited two steps of weight loss. Higher temperature of decomposition exhibited by the nanoconjugates when compared to pure ibuprofen was due to the effect of Tween 80 on the nanoconjugates. The release of ibuprofen from nanoconjugates was 17% after 60 min. The affinity between the DEAE-Dextran and ibuprofen may have retarded the release. While at the end of 24 h, the release of Ibuprofen from nanoconjugates was in the range of 74.67 to 85.53%, which was significantly higher ($p < 0.05$, $n = 4$) than the release by ibuprofen control (30.72%). Fickian and non-Fickian anomalous mechanisms were deduced for the *in vitro* drug release of ibuprofen from the nanoconjugates. In conclusion the surfactant solubilization technique successfully enhanced the *in vitro* drug release of ibuprofen without the use of organic solvents.

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