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Ionic liquid forms of weakly acidic drugs in oral lipid formulations: preparation, characterization, in vitro digestion and in vivo absorption studies

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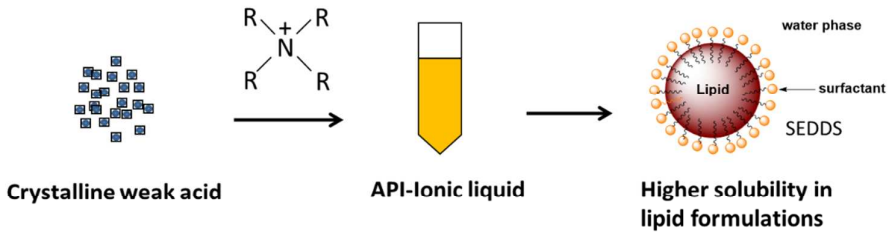


ABSTRACT

This study aimed to transform weakly acidic poorly water soluble drugs (PWSD) into ionic liquids (ILs) to promote solubility in, and the utility of, lipid-based formulations. Ionic liquids (ILs) were formed directly from tolfenamic acid (Tolf), meclofenamic acid, diclofenac and ibuprofen by pairing with lipophilic counterions. The drug-ILs were obtained as liquids or low melting solids and were significantly more soluble (either completely miscible or highly soluble) in lipid based, self-emulsifying drug delivery systems (SEDDS) when compared to the equivalent free acid. *In vivo* assessment of a SEDDS lipid solution formulation of Tolf didecyldimethyl ammonium salt and the same formulation of Tolf free acid at low dose (18 mg/kg, where the free acid was soluble in the SEDDS), resulted in similar absorption profiles and overall exposure. At high dose (100 mg/kg), solution SEDDS formulations of the Tolf ILs (didecyldimethyl ammonium, butyldodecyldimethyl ammonium or didecylmethyl ammonium salts) were possible, but the lower lipid solubility of Tolf free acid dictated that administration of the free acid was only possible as a suspension in the SEDDS formulation or as an aqueous suspension. Under these conditions, total drug plasma exposure was similar for the IL formulations and the free acid, but the plasma profiles were markedly different, resulting in flatter, more prolonged exposure profiles and reduced C_{max} for the IL formulations. Isolation of a weakly acidic drug as an IL may therefore provide advantage as it allows formulation as a solution SEDDS rather than a lipid suspension, and in some cases may provide a means of slowing or sustaining absorption. The current studies compliment previous studies with weakly basic PWSD and demonstrate that transformation into highly lipophilic ILs is also possible for weakly acidic compounds.

Keywords: ionic liquid, drug delivery, lipid formulation, SEDDS, in vitro digestion, lipolysis; poorly water soluble drug

INTRODUCTION

Increased emphasis on potency, and the application of contemporary drug discovery screening methods, continues to result in the increasingly frequent identification of drug candidates with high lipophilicity and poor water solubility.[1] The low water solubility of these drugs, often combined with high dose requirements, poses a major challenge for oral bioavailability due to poor absorption from the gastrointestinal (GI) tract. Several formulation approaches have been developed to enhance apparent drug solubility in the GI tract and to promote the absorption of poorly water soluble drugs (PWSD).[1] Lipid formulations provide one such approach and Self-Emulsifying Drug Delivery Systems or SEDDS are perhaps the most commonly applied class of oral lipid formulation.[2] SEDDS typically comprise drug dissolved in a mixture of lipids, surfactants and co-solvents, combinations of excipients that are chosen such that they emulsify spontaneously on contact with the GI fluids after oral administration.[3] SEDDS formulations enhance oral absorption of PWSD via two major mechanisms. Firstly, since drug is usually pre-dissolved in the formulation, traditional dissolution is avoided. Secondly, integration of the SEDDS formulation into endogenous lipid digestion processes leads to trafficking of coadministered PWSD into intestinal mixed micelles. Although this approach has been shown to be highly effective in a number of cases, a limitation is the identification of drugs with sufficient solubility in formulation lipids to allow the prospective dose to be dissolved in a quantity of formulation that can be readily filled into (ideally) a single capsule. Although lipid suspension formulations are possible, and may be effective, lipid solution formulations have additional advantages with respect to dissolution, and processing advantages such as increased physical stability, content uniformity and ease of capsule filling.

For PWSD, many of which are inherently hydrophobic, low lipid solubility usually arises as a result of strong intermolecular forces and high crystallinity in the solid state.[1] Here we explore the potential benefits of transformation of PWSD drugs, and in particular weakly acidic PWSDs, into ionic liquids (IL), with lower intermolecular forces and reduced crystallinity as a means of increasing lipid solubility and therefore increasing the applicability of SEDDS formulations.

ILs are defined as low melting organic salts (melting points below 100 ºC) which generally consist of bulky cations and/or anions with more charge delocalisation than simple ionic species.[4, 5] They are often referred to as "designer solvents" as the cation and anion can be varied widely to tune the physicochemical properties.[6] Accordingly, these multifunctional solvents have been the subject of widespread interest in a range of areas; for example as alternate green solvents, electrolytes or functional materials.[4, 7, 8]

However, until recently, the potential benefits of ILs in drug delivery have not been well explored.[9] The excellent solvent properties of ILs provide the opportunity to more readily dissolve active pharmaceutical ingredients (API) when compared to traditional excipients,[10-16] and the tunable solvent properties of ILs create the potential to tailor an IL to a particular drug. We have also recently demonstrated that formulations containing ILs as excipients may provide for sustained drug release following oral administration.[17] In addition to exploiting ILs as formulation excipients, the possibility of generating ILs from ionisable drugs (weakly acidic or weakly basic) to improve drug properties also exists.[18-25] These latter studies have mostly addressed the advantages of drug-ILs in overcoming the issue of polymorphism in the solid crystalline state or in decreasing melting point in order to increase *aqueous* solubility and dissolution rate. In contrast, our recent focus has been on using drug-ILs to promote drug solubility (and therefore drug loading) in lipid-based *(non-aqueous)* formulations (LBFs).[26, 27]

For example, we recently showed significant enhancement of non-aqueous solubility of drug-ILs formed from the weakly basic PWSD cinnarizine and itraconazole and a range of lipophilic counterions.[26] Promisingly, lipophilic drug-ILs in combination with LBFs resulted in up to 20-fold increases in oral exposure when compared to formulations containing the maximum quantity of the equivalent free base form. Similar results have been reported for the poorly water soluble weak base atazanavir.[28] We have also applied this approach to a range of weak bases where low water solubility was not the primary motivation for conversion to a LBF, but instead where LBF might be preferred to achieve a fast onset of action, facilitate taste masking or simply to provide alternate patient preference options.[27] To this point, however, the use of ionic liquids (or more broadly, lipophilic salts, where the latter definition allows for ionic complexes with lipophilic counterions, but where where the melting point of the salt is higher than the ionic liquid definition of 100 °C) to promote the solubility of weakly acidic drugs in lipid based formulations, has not been described.

Herein we investigate the applicability of drug transformation into lipophilic salts or ILs, in order to promote LBF utility, for the weakly acidic drugs, tolfenamic acid (Tolf), meclofenamic acid (Mec), diclofenac (Diclof) and ibuprofen (Ibu). IL forms of the acidic drugs were synthesised with a range of lipophilic counterions, the ILs characterised and their ability to increase lipid solubility assessed. Model lipidic SEDDS formulations containing Tolf-ILs (didecyldimethyl ammonium, butyldodecyldimethyl ammonium and didecylmethyl ammonium salts) were further evaluated using *in vitro* lipid digestion models to profile patterns of drug solubilisation during formulation digestion. Finally, *in vivo* drug absorption profiles were obtained for Tolf after oral administration of SEDDS formulations containing Tolf ILs in solution and compared to control formulations containing Tolf as a suspension of the free acid.

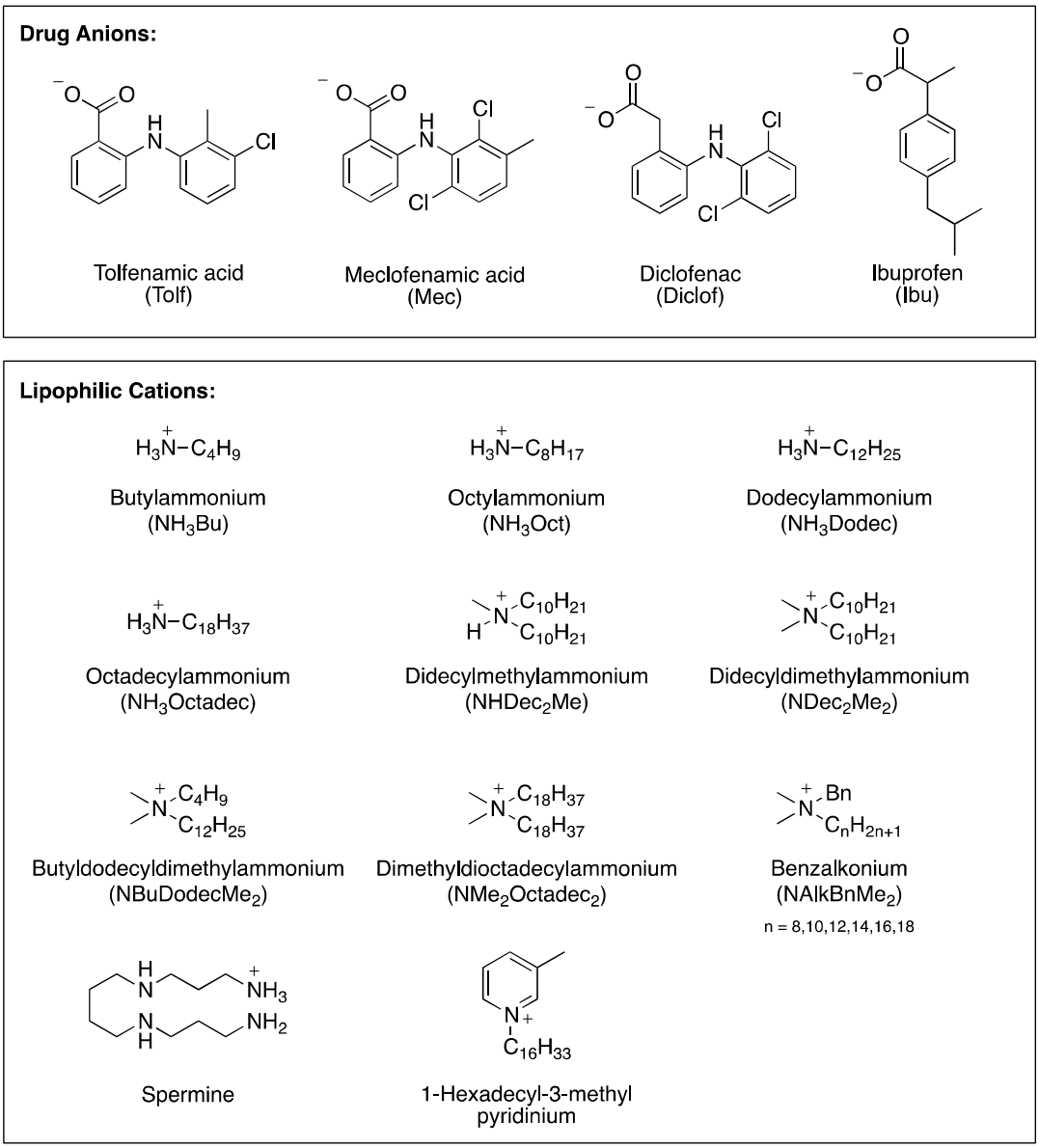


Figure 1 The weakly acidic drugs (shown as carboxylates) employed in this study (Tolf, Mec, Diclof and Ibu) and the lipophilic cations employed to form ILs.

EXPERIMENTAL SECTION

Materials

Tolfenamic acid, meclofenamic acid, meclofenamic sodium salt, diclofenac sodium salt, ibuprofen sodium salt, benzalkonium chloride, didecyldimethyl ammonium bromide, *N*,*N*-dimethyldodecylamine, octadecylamine, 1-butanol, soybean oil, sodium taurodeoxycholate (NaTDC), porcine pancreatin extract (8 X USP specification activity), 4-bromophenylboronic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Spermine was ordered from Alfa Aesar. Diclofenac acid, didecylmethylamine and dimethyldioctadecyl ammonium bromide were obtained from TCI (Tokyo, Japan). Lipoid E PC S (lecithin for egg, ~99% pure phosphatidylcholine (PC) was purchased from Lipoid GmBH, Ludwigshafen, Germany. Maisine™ 35-1 (Gattefossé, Saint-Priest, France); Kolliphor® EL (BASF Corporation, Ludwigshafen, Germany); 1.0 M sodium hydroxide (Ajax Finechem Pty Ltd, Sydney, Australia) were obtained from the indicated suppliers. Acetonitrile, ethanol, petroleum spirits, chloroform, dichloromethane, methanol, ethyl acetate, diethyl ether were purchased from Merck (Bayswater, Australia) and used without any pre-treatment. All other chemicals and solvents were of analytical purity or high performance liquid chromatography (HPLC) grade.

Drug and Counterion properties

Drug/counterion[a]	Molecular weight (Da)	pKa-1 (acid)	pKa-1 (base)	cLogP	cLogD7.4
Tolfenamic acid (Tolf)	261.71	3.9		5.49	2.26
Meclofenamic acid (Mec)	296.15	3.8		6.09	2.82
Diclofenac (Diclof)	296.15	4.0		4.26	1.10
Ibuprofen (Ibu)	206.29	4.9		3.84	1.34

Butylamine (NH_3Bu)	73.14		10.2	0.70	-1.91
Octylamine (NH_3Oct)	129.25		10.2	2.48	-0.13
Dodecylamine (NH_3Dodec)	185.36		10.2	4.25	1.65
Octadecylamine ($NH_3Octadec$)	269.52		10.2	6.92	4.32
Spermine	202.35		10.8 (10.2[b])	-1.45	-8.77
Didecylmethylamine ($NHDec_2Me$)	311.60		10.3	8.17	5.40

[a] Data for quaternary ammonium counterions not generated since they have a permanent positive charge
[b] pKa-2 for spermine in parentheses.
Data calculated using JChem for Excel (Ver. 16.4.1100.717), ChemAxon Ltd.

Characterisation

The ^{1}H and ^{13}C NMR spectra of the purified products were recorded in $CDCl_3$ (Cambridge Isotope Laboratories Inc., 99.8% D), CD_3OD (Sigma Aldrich, 99.8% D) or DMSO-d_6 (Cambridge Isotope Laboratories Inc., 99.9% D) on a Bruker Avance III Ultrashield Plus spectrometer at 400.13 and 100.62 MHz, respectively. High resolution mass spectra were obtained using a Waters LCT Premier XE time-of-flight mass spectrometer fitted with an electrospray (ESI) ion source and controlled with MassLynx software version 4.5. Low resolution mass spectra were obtained using an Agilent 1200 Single Quad LCMS.

For XRD analysis was performed on a Shimadzu XRD-7000 diffractometer (Shimadzu Scientific Instruments, Japan) with Cu K-alpha radiation. The applied voltage and current were 40 kV and 35 mA respectively. Samples were mounted on a stainless steel sample holder and were scanned in continuous mode between 2° and 40° (2θ), with a step size of 0.02° and a scanning speed of 2 s/step. A divergent slit width of 1° was employed for the

beam source, and a scattering slit width of 1° and receiving slit width of 0.3 mm were used for the detector.

General Methods for Drug-IL Preparation

Tolfenamic acid didecyldimethylammonium salt (Tolf•$NDec_2Me_2$)

Tolf (2.11 g, 8.06 mmol) was suspended in saturated sodium bicarbonate (100 mL) and 2M NaOH (20 mL) was added. The solution was stirred until all of the Tolf dissolved and a clear solution was obtained. Didecyldimethylammonium bromide (3.28 g, 8.06 mmol) was completely dissolved in a mixture of water (50 mL) and methanol (20 mL). The two solutions were mixed and oil droplets were immediately formed in the aqueous medium. The reaction mixture was stirred further for 30 min at room temperature. The oil phase was extracted with dichloromethane (3 x 100 mL) and the combined organic phases were washed with distilled water (3 x 100 mL) until a negative $AgNO_3$ test was obtained. The organic phase was dried (over anhydrous $MgSO_4$), filtered and evaporated to afford the desired product that was dried under high vacuum.

Yield 95%. ^{1}H NMR ($CDCl_3$) δ 8.09 (dd, J = 7.7, 1.5 Hz, 1H), 7.32 (dd, J = 7.8, 0.9 Hz, 1H), 7.16–7.07 (m, 2H), 7.01–6.93 (m, 2H), 6.73–6.69 (m, 1H), 3.38–3.34 (m, 4H), 3.31 (s, 6H), 2.38 (s, 3H), 1.60 (br s, 4H), 1.26–1.22 (m, 28H), 0.88 (2 x t, J = 6.9, 6H). ^{13}C NMR ($CDCl_3$) δ 172.9, 145.8, 143.5, 135.2, 132.2, 130.1, 127.6, 126.5, 123.8, 121.8, 117.3, 117.1, 114.2, 63.6, 51.0, 31.9, 29.5, 29.4, 29.3, 29.2, 26.3, 22.8, 22.7, 15.1, 14.2. HRMS +ve calcd 326.3787 found 326.3792 –ve calcd 260.0478 found 260.0477.

Tolfenamic acid didecylmethylammonium salt (Tolf•NHDec$_2$Me)

A mixture of Tolf (1.55 g, 5.92 mmol) and didecylmethylamine (2.27 mL, 5.91 mmol) in methanol (50 mL) was stirred at room temperature for 30 min. The methanol was evaporated (rotavap) and the resultant orange oil was dried further under high vacuum.

Yield 98%. ^{1}H NMR (MeOD-d_4) δ 7.95 (dd, J = 7.6, 1.6 Hz, 1H), 7.28 (dd, J = 7.6, 1.2 Hz, 1H), 7.17–7.21 (m, 1H), 7.02–7.10 (m, 2H), 6.97 (dd, J = 8.4, 0.8 Hz 1H), 6.70–6.74 (m, 1H) 2.99–3.03 (m, 4H), 2.77 (s, 3H), 2.35 (s, 3H), 1.65–1.72 (m, 4H), 1.23–1.37 (m, 30H), 0.89 (2 x t, J = 6.8 Hz, 6H). ^{13}C NMR (MeOD-d_4) δ 175.8, 147.3, 143.8, 136.3, 133.1, 129.3, 127.9, 124.0, 122.2, 119.8, 118.6, 115.3, 57.1 (2C), 40.3, 33.0 (2C), 30.6 (2C), 30.5 (2C), 30.4 (2C), 30.2 (2C), 27.7 (2C), 25.2 (2C), 23.7 (2C), 15.2, 14.5 (2C). HRMS +ve calcd 312.3630 found 312.3635 –ve calcd 260.0478 found 260.0489.

Tolfenamic acid butyldodecyldimethylammonium salt (Tolf•NBuDodecMe$_2$)

The precursor, butyldodecyldimethylammonium bromide was initially synthesized from 1-bromobutane and *N*,*N*-dimethyldodecylamine according to a slightly modified literature procedure.[17]

A mixture of *N*,*N*-dimethyldodecylamine (3.88 g, 5.00 mL, 18.2 mmol) and 1-bromobutane (2.99 g, 2.34 mL, 21.8 mmol) in toluene (60 mL) were stirred for 1 h at room temperature under nitrogen. The reaction was then refluxed overnight. The solution was cooled to room temperature and the toluene was evaporated *in vacuo*. The resultant yellow oily solid was triturated with diethyl ether (200 mL) for 2 h and washed further with diethyl ether (3 x 100 mL). Diethyl ether was removed by decantation and the resultant product was dried under high vacuum to afford butyldodecyldimethylammonium bromide as a white solid.

Yield 87%. ^{1}H NMR (DMSO-d_6) δ 3.27–3.22 (m, 4H), 3.00 (s, 6H), 1.64–1.58 (m, 4H), 1.34–1.24 (m, 20H), 0.93 (t, J = 7.3 Hz, 3H), 0.85 (t, J = 6.7 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 62.8, 62.6, 49.9, 31.3, 29.0 (2C), 28.9, 28.8, 28.7, 28.5, 25.8, 23.7, 22.1, 21.7, 19.2, 13.9, 13.5.

The title compound was synthesized using a slight modification of the procedure used in the synthesis of Tolf acid didecyldimethyl ammonium salt. In this case the reaction was carried out in water without methanol.

Yield 93%. ^{1}H NMR (DMSO-d_6) δ 7.88 (dd, J = 7.6, 1.6 Hz, 1H), 7.29 (d, J = 7.9, 1H), 7.11–7.03 (m, 3H), 6.91 (d, J = 7.8 Hz, 1H), 6.66–6.62 (m, 1H), 3.24–3.19 (m, 4H), 2.98 (s, 6H), 2.32 (s, 3H), 1.63–1.57 (m, 4H), 1.33–1.24 (m, 20H), 0.92 (t, J = 7.3 Hz, 3H), 0.85 (t, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 173.0, 145.9, 143.3, 135.2, 132.2, 130.3, 127.7, 126.5, 123.3, 121.9, 117.3 (2C), 114.2, 63.6, 63.5, 50.9, 31.9, 29.6, 29.5, 29.4, 29.3, 29.2, 26.2, 24.6, 22.7 (2C), 19.6, 15.1, 14.2, 13.6. HRMS +ve calcd 270.3161 found 270.316 –ve calcd 260.0478 found 260.0491.

Tolfenamic acid dimethyldioctadecylammonium salt (Tolf•$NMe_2Octadec_2$)

The title compound was synthesized by the procedure used in the synthesis of Tolf didecyldimethylammonium salt.

Yield 86%. ^{1}H NMR ($CDCl_3$) δ 8.08 (dd, J = 7.7, 1.5 Hz, 1H), 7.32 (dd, J = 7.9, 1.2 Hz, 1H), 7.16–7.08 (m, 2H), 7.01–6.93 (m, 2H), 6.72–6.68 (m, 1H), 3.37–3.33 (m, 4H), 3.31 (s, 6H), 2.37 (s, 3H), 1.58 (br s, 4H), 1.31–1.20 (m, 60H), 0.87 (2 x t, 6H, J = 6.9). ^{13}C NMR ($CDCl_3$) δ 172.9, 145.8, 143.5, 135.2, 132.2, 130.0, 127.5, 126.4, 123.9, 121.8, 117.3, 117.0, 114.2, 63.6, 51.0, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.2, 26.3, 22.7, 15.1, 14.2. HRMS +ve calcd 550.6291 found 550.6307 –ve calcd 260.0478 found 260.0485.

Tolfenamic acid benzalkonium salt (Tolf•NAlkBnMe$_2$)

The title compound was synthesized using a slight modification of the procedure used in the synthesis of Tolf didecyldimethylammonium salt. In this case the reaction was carried out in water only.

Yield 92%. ^{1}H NMR ($CDCl_3$) δ 8.14 (dd, J = 7.7, 1.5 Hz, 1H), 7.51–7.30 (m, 6H), 7.17–7.08 (m, 2H), 7.01–6.93 (m, 2H), 6.73–6.70 (m, 1H), 4.86 (s, 2H), 3.35–3.31 (m, 4H), 3.22 (s, 6H), 2.33 (s, 3H), 1.67 (br s, 2H), 1.31–1.20 (m, 21H), 0.88 (t, J = 6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 173.1, 146.0, 143.4, 135.2, 133.1, 132.3, 130.5, 130.3, 129.1, 127.7, 127.6, 126.5, 123.6, 122.0, 117.4, 117.3, 114.3, 67.6, 63.4, 49.5, 32.0, 31.9, 29.7, 29.6, 29.5, 29.4 (3C), 29.3, 26.3, 22.7, 15.1, 14.2. HRMS +ve calcd 332.3317 found 332.3321 –ve calcd 260.0478 found 260.0477.

Tolfenamic acid butylammonium salt (Tolf•NH$_3$Bu)

To the clear solution of Tolf acid (109.1 mg, 0.42 mmol) in methanol (25 mL) was added to *n*-butylamine (30.5 mg, 41.2 μL, 0.42 mmol) and the reaction mixture was stirred at room temperature for 30 min. The methanol was evaporated *in vacuo* and the resultant white solid product was dried further under high vacuum.

Yield >95%. ^{1}H NMR (DMSO-d_6) δ 8.38 (br s), 7.92 (dd, J = 7.7, 1.7 Hz, 1H), 7.31 (dd, J = 8.1, 0.7 Hz, 1H), 7.18–7.14 (m, 1H), 7.11 (t, J = 8.0 Hz, 1H), 7.04 (dd, J = 8.2, 0.8 Hz, 1H), 6.99 (dd, J = 7.9, 0.7 Hz, 1H), 6.69 (m, 1H), 2.79 (t, J = 8.0 Hz, 2H), 2.29 (s, 3H), 1.58–1.51 (m, 2H), 1.37–1.28 (m, 2H), 0.86 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 175.3, 147.5, 141.9, 135.6, 132.6, 132.2, 129.8, 126.9, 124.2, 120.6, 118.2, 117.2, 114.3, 39.7, 30.2, 19.9, 15.1, 13.5. HRMS –ve calcd 260.0478 found 260.0489.

Tolfenamic acid octylammonium salt (Tolf•NH_3Oct)

The title compound was synthesized by the procedure used for the synthesis of Tolf butylammonium salt.

Yield > 95%. ^{1}H NMR (DMSO-d_6) δ 8.15 (br s), 7.90 (dd, J = 7.7, 1.7 Hz, 1H), 7.30 (dd, J = 8.1, 0.7 Hz, 1H), 7.17–7.13 (m, 1H), 7.10 (t, J = 8.0 Hz, 1H), 7.03 (dd, J = 8.2, 0.8 Hz, 1H), 6.97 (dd, J = 7.9, 0.7 Hz, 1H), 6.69–6.65 (m, 1H), 2.77 (t, J = 8.0 Hz, 2H), 2.29 (s, 3H), 1.58–1.50 (m, 2H), 1.30–1.22 (m, 10H), 0.84 (t, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 175.3, 147.5, 141.9, 135.6, 132.5, 132.2, 129.7, 126.8, 124.1, 120.5, 118.4, 117.2, 114.3, 40.1, 31.8, 29.2, 29.1, 28.6, 26.8, 22.7, 15.1, 14.2. HRMS +ve calcd 130.1596 found 130.1598 –ve calcd 260.0478 found 260.0491.

Tolfenamic acid dodecylammonium salt (Tolf•NH_3Dodec)

The title compound was synthesized by the procedure used for the synthesis of Tolf butylammonium salt.

Yield >95%. ^{1}H NMR (DMSO-d_6) δ 7.89 (dd, J = 7.7,1.7 Hz, 1H), 7.30 (dd, J = 8.1, 0.7 Hz, 1H), 7.16–7.08 (m, 2H), 7.03 (dd, J = 8.2, 0.9 Hz, 1H), 6.96 (dd, J = 7.9, 0.6 Hz, 1H), 6.68–6.64 (m, 1H), 2.76 (t, J = 7.4 Hz, 2H), 2.28 (s, 3H), 1.56–1.48 (m, 2H), 1.27–1.22 (m, 18H), 0.85 (t, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 175.6, 147.4, 142.0, 135.6, 132.4, 132.1, 129.5, 126.8, 124.0, 120.2, 118.9, 117.2, 114.3, 39.9, 32.1, 29.8, 29.7 (2C), 29.5 (2C), 29.2, 28.3, 26.8, 22.9, 15.1, 14.3. HRMS +ve calcd 186.2222 found 186.2222 –ve calcd 260.0478 found 260.0486.

Tolfenamic acid octadecylammonium salt (Tolf•NH_3Octadec)

Tolfenamic acid (1.61 g, 6.17 mmol) and octadecylamine (1.66 g, 6.17 mmol) were heated at 65 ^{o}C in 50 mL methanol for 2 h, cooled to rt, and the solvent was removed *in vacuo*. The resulting solid was placed under high vacuum to give the desired product as a white solid.

Yield 98%. ^{1}H NMR (MeOD) δ 7.94 (dd, J = 7.8, 1.6 Hz, 1H), 7.29 (dd, J = 7.8 Hz, 1.2 Hz, 1H), 7.21–7.14 (m, 1H), 7.07 (t, J = 8.0 Hz, 1H), 7.02 (dd, J = 8.0, 1.2 Hz, 1H), 6.98 (dd, J = 8.2, 0.8 Hz, 1H), 6.75–6.69 (m, 1H), 2.92–2.86 (m, 2H), 2.35 (s, 3H), 1.68–1.59 (m, 2H), 1.44–1.22 (m, 30H), 0.90 (t, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 175.5, 147.4, 142.0, 135.6, 132.4, 132.2, 129.6, 126.8, 124.0, 120.3, 118.7, 117.2, 114.3, 40.0, 32.1, 29.9, 29.8 (2C), 29.7, 29.5 (2C), 29.2, 28.4, 26.8, 22.9, 15.1, 14.3, 1.2. HRMS +ve mode calcd 270.3161 found 270.3156. –ve mode calcd 260.0472 found 260.0480. m.p. 123-125 ^{o}C.

Tolfenamic acid spermine salt (Tolf•Spermine)

A mixture of Tolf (0.64 g, 2.45 mmol) and spermine (0.5 mL, 2.45 mmol) in methanol (35 mL) was stirred at room temperature for 30 min. The methanol was evaporated (rotavap) and the resultant oil slowly crystallised to give an off white waxy solid.

Yield 99%. ^{1}H NMR (MeOD) δ 7.84 (dd, J = 7.8, 1.6 Hz, 1H), 7.19 (dd, J = 7.8, 1.2 Hz, 1H), 7.08 (ddd, J = 8.4, 7.2, 1.8 Hz, 1H), 6.98 (td, J = 8.0, 0.4 Hz, 1H), 6.90 (ddd, J = 13.8, 8.2, 1.0 Hz, 2H), 6.62 (ddd, J = 7.8, 7.2, 1.2 Hz, 1H), 2.70 (t, J = 7.2 Hz, 4H), 2.62 (t, J = 7.2 Hz, 4H), 2.56 (t, J = 6.8 Hz, 3H), 1.64 (quin, J = 7.2 Hz, 4H), 1.52–1.42 (m, 4H). ^{13}C NMR (MeOD) δ 176.2, 147.2, 143.9, 136.2, 133.1, 132.1, 129.3, 127.9, 123.9, 123.0, 119.6, 118.6, 115.4, 50.0, 47.9, 40.2, 31.0, 27.7, 15.1. HRMS +ve mode calcd 203.2234 found 203.2229. HRMS –ve mode calcd 260.0469 found 260.0475. Complete melting by 121^{o}C.

Meclofenamic acid didecyldimethylammonium salt (Mec•$NDec_2Me_2$)

Sodium carbonate was added to distilled water (200 mL) to adjust the pH of the resulting solution to ~9. Me sodium salt (2.0 g, 6.30 mmol) was dissolved in 100 mL of this aqueous basic solution. Didecyldimethylammonium bromide (2.6 g, 6.30 mmol) was also dissolved in 100 mL of aqueous basic solution and 50 mL methanol. The two solutions were mixed and oil droplets were immediately formed in aqueous medium. The reaction mixture was stirred further for 30 min and the oil phase was extracted with chloroform (3 x 100 mL). The combined organic phases were washed with distilled water (3 x 100 mL) until a negative $AgNO_3$ test was obtained. The organic phase was then dried (anhydrous $MgSO_4$), filtered, evaporated and dried under high vacuum to afford the desired product.

Yield 90%. ^{1}H NMR ($CDCl_3$) δ 8.08 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.23 (d, *J* = 8.2 Hz, 1H), 7.07–7.03 (m, 1H), 6.97 (d, *J* = 8.2, 1H), 6.68–6.64 (m, 1H), 6.23 (dd, *J* = 8.1, 0.7 Hz, 1H), 3.37–3.33 (m, 4H), 3.31 (s, 6H), 2.37 (s, 3H), 1.58 (br s, 4H), 1.30–1.18 (m, 28H), 0.87 (2 x t, *J* = 7.0, 6H). ^{13}C NMR ($CDCl_3$) δ 173.0, 145.9, 137.6, 136.1, 133.4, 131.9, 130.3, 129.8, 127.6, 126.7, 122.3, 116.7, 112.7, 63.6, 51.1, 31.9, 29.5, 29.4, 29.3, 29.2, 26.3, 22.8, 22.7, 20.7, 14.1. HRMS +ve calcd 326.3787 found 326.3793 –ve calcd 294.0089 found 294.0087.

Meclofenamic acid 1-hexadecyl-3-methylpyridinium salt

The precursor, 1-hexadecyl-3-methylpyridinium bromide, was initially synthesized from 3-methylpyridine and 1-bromohexadecane according to a literature procedure.[17]

Yield 95%. ^{1}H NMR ($CDCl_3$) δ 9.39 (s, 1H), 9.25 (d, *J* = 6.0 Hz, 1H), 8.24 (d, *J* = 8.0 Hz, 1H), 7.99 (dd, *J* = 7.8, 6.2 Hz, 1H), 4.94 (t, *J* = 7.5 Hz, 2H), 2.64 (s, 3H), 2.09–1.93 (m, 2H),

1.46-1.12 (m, 26H), 0.86 (t, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 146.2, 144.7, 142.4, 139.2, 127.8, 61.0, 31.8, 31.1, 29.5 (2C), 29.4, 29.3, 29.2, 28.9, 25.9, 22.6, 18.3, 14.4.

The title compound was synthesized by the procedure used for the synthesis of Mec didecyldimethylammonium salt.

Yield 88%. ^{1}H NMR (DMSO-d_6) δ 9.05 (s, 1H), 8.95 (d, J = 6.0 Hz, 1H), 8.43 (d, J = 8.0 Hz, 1H), 8.04 (dd, J = 7.9, 6.1 Hz, 1H), 7.82 (dd, J = 7.6, 1.7 Hz, 1H), 7.39 (d, J = 8.2 Hz, 1H), 7.17 (dd, J = 8.3, 0.5 Hz, 1H), 6.96–6.92 (m, 1H), 6.55–6.50 (m, 1H), 6.00 (dd, J = 8.1, 0.9 Hz, 1H), 4.54 (t, J = 7.6 Hz, 2H), 2.50 (s, 3H), 2.35 (s, 3H), 1.93–1.88 (m, 2H), 1.28–1.23 (m, 26H), 0.85 (t, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 173.3, 146.2, 145.1, 144.6, 142.6, 139.5, 137.4, 136.2, 133.7, 132.0, 130.5, 130.2, 127.9, 127.7, 127.0, 121.6, 116.8, 112.7, 62.0, 32.0, 31.9, 29.8, 29.7 (2C), 29.6, 29.4 (2C), 29.2, 26.2, 22.8, 20.8, 18.6, 14.2. HRMS +ve calcd 318.3161 found 318.3162 –ve calcd 294.0089 found 294.0098.

Diclofenac didecyldimethylammonium salt (Diclof•$NDec_2Me_2$)

The title compound was synthesized by the procedure used for the synthesis of Tolf-didecyldimethylammonium salt.

Yield 91%. ^{1}H NMR ($CDCl_3$) δ 9.22 (br s, 1H), 7.29 (d, J = 8.0 Hz, 2H), 7.22 (dd, J = 7.4, 1.4 Hz, 1H), 6.97 (td, J = 7.8, 1.6 Hz, 1H), 6.89 (t, J = 8.0 Hz, 1H), 6.79 (td, J = 7.4, 1.2 Hz, 1H), 6.44 (dd, J = 8.0, 1.0 Hz, 1H), 3.73 (s, 2H), 3.22–3.18 (m, 4H), 3.09 (s, 6H), 1.53 (br s, 4H), 1.27–1.24 (m, 28H), 0.88 (t, J = 6.9 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 176.7, 143.3, 138.4, 130.5, 129.4, 128.8, 128.3, 126.1, 123.0, 120.4, 116.7, 63.4, 50.9, 43.5, 31.8, 29.4, 29.3, 29.2, 29.1, 26.2, 22.6, 14.1. HRMS +ve calcd 326.3787 found 326.3793 –ve calcd 294.0089 found 294.0087.

Diclofenac benzalkonium salt (Diclof•NAlkBnMe$_2$)

The title compound was synthesised by a slightly modified version of the procedure used for the synthesis of Mec didecyldimethylammonium salt. In this case the reaction was carried out in water without methanol.

Yield 93% ^{1}H NMR ($CDCl_3$) δ 9.08 (br s, 1H), 7.47–7.37 (m, 5H), 7.28 (d, *J* = 8.0 Hz, 2H), 7.22 (dd, *J* = 7.4, 1.3 Hz, 1H), 6.95 (td, *J* = 7.8, 1.5 Hz, 1H), 6.89 (t, *J* = 8.0 Hz, 1H), 6.77 (td, *J* = 7.4, 1.1 Hz, 1H), 6.44 (dd, *J* = 7.9, 0.8 Hz, 1H), 4.68 (s, 2H), 3.77 (s, 2H), 3.23–3.19 (m, 2H), 3.05 (s, 6H), 1.65 (br s, 2H), 1.32–1.23 (m, 22H), 0.88 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 177.2, 143.4, 138.5, 133.2, 130.6, 130.3, 129.3, 129.0, 128.8, 128.4, 127.6, 126.1, 123.0, 120.5, 116.9, 67.4, 63.1, 49.5, 43.5, 31.9 (2C), 29.7 (2C), 29.6, 29.5, 29.4 (2C), 29.3 (2C), 26.3, 22.8, 22.7, 14.2. HRMS +ve calcd 304.3004 found 304.3004 and 332.3317 found 332.3319 –ve calcd 294.0089 found 294.0099.

Ibuprofen didecyldimethylammonium salt (Ibu•NDec$_2$Me$_2$)

The title compound was synthesized by the procedure used for the synthesis of Tolf-didecyldimethyl ammonium salt.

Yield 96%. ^{1}H NMR ($CDCl_3$) δ 7.31 (d, *J* = 8.0 Hz, 2H), 6.97 (d, *J* = 8.0 Hz, 2H), 3.56 (q, *J* = 7.1 Hz, 1H), 3.25–3.20 (m, 4H), 3.13 (s, 6H), 2.38 (d, *J* = 7.1 Hz, 2H), 1.85–1.75 (m, 1H), 1.52 (br s, 4H), 1.43 (d, *J* = 7.1 Hz, 3H), 1.28–1.25 (m, 28H), 0.89-0.85 (m, 12H). ^{13}C NMR ($CDCl_3$) δ 179.2, 143.0, 138.2, 128.4, 127.5, 63.0, 50.8, 49.2, 45.1, 31.8, 30.2, 29.4, 29.3, 29.2 (2C), 26.2, 22.6 (2C), 22.4 (2C), 19.9, 14.0. HRMS +ve calcd 326.3787 found 326.3795 –ve calcd 205.1229 found 205.1225.

Formulation preparation

The formulations were based on long chain (LC) lipids and freshly prepared as homogenous mixtures of lipids, surfactant and cosolvent. The composition of the LC lipid containing self-emulsifying drug delivery systems (LC-SEDDS) utilized in the equilibrium solubility, *in vitro* and *in vivo* studies was 60% lipid (soybean oil:Maisine^{TM} 35-1, 1:1 w/w), 30% Kolliphor$^{®}$ EL and 10% ethanol. Drugs as the free acid (FA) or IL were subsequently dissolved or suspended in the formulations to the concentrations specified. Concentrations were confirmed by HPLC.

Equilibrium solubility studies

The equilibrium solubility of the drugs in the different formulations was determined by the addition of excess drug/drug-ILs to the LC-SEDDS formulation. In all cases, 0.5 g of drug or drug-IL was added to 0.5 g of formulation. Dissolution rates in viscous non-aqueous solvents are typically slow and therefore the formulations were incubated at 37 °C for 3-7 days in order to reach equilibrium. Samples were collected at regular intervals and centrifuged (21,000 x *g*, 37 °C, 10 min). An aliquot of the resulting particle-free supernatant was accurately weighed (15-40 mg) and dissolved in chloroform:methanol (5 mL, 2:1, v/v). Following further dilution with acetonitrile and mobile phase, samples were analysed for drug content by HPLC. All solubility tests were performed in triplicate and equilibrium solubility defined as the value attained when at least three consecutive solubility samples varied by ≤5%. For ILs that were effectively miscible or soluble in the formulations, the equilibrium solubility was quoted as greater than (≥) the measured concentration resulting from mixing a 1:1 w/w mixture of drug-IL and formulation (i.e. 0.5 g plus 0.5 g, nominally 500 $mg.g^{-1}$ of IL in the vehicle). To allow better comparison of solubilising capacity, results are quoted as $mg.g^{-1}$ of free acid equivalents in the vehicle (rather than $mg.g^{-1}$ of the IL) and are therefore

lower than the 500 $mg.g^{-1}$ nominal concentration of IL. Under these circumstances, the reported 'solubility' does not define the maximum solubility *per se* (since the systems were miscible) and differs for each IL by virtue of differences in the molecular weight of the counterions when compared to the free acid.

Drug solubilisation during in vitro lipid digestion

The methods employed utilised *in vitro* lipid digestion testing protocols recently published by the lipid formulation classification system (LFCS) consortium.[29] The specific methods are given below. Briefly, formulations were dispersed in simulated intestinal fluid (SIF) in a temperature controlled vessel (37 °C) for a period of 10-20 min and then digestion was initiated by addition of pancreatin. The pH was maintained at intestinal pH (6.5) via the conduct of experiments in a pH stat titrator. Digestion was allowed to continue for 60 min. At the end of the initial dispersion period and during the digestion period samples were taken and centrifuged to separate drug that precipitated out of solution and that which remained solubilised.

Three comparative formulations based on LC-SEDDS and loaded with Tolf•$NDec_2Me_2$, Tolf•$NBuDodecMe_2$, Tolf•$NHDec_2Me$, Tolf•$NAlkBnMe_2$ or Tolf free acid (FA) were freshly prepared as solutions. Formulations loaded with Tolf•$NDec_2Me_2$, Tolf•$NBuDodecMe_2$, Tolf•$NHDec_2Me$ or Tolf•$NAlkBnMe_2$ contained the same concentration of Tolf FA equivalents (~200 mg/g). The concentration of the formulation loaded with less lipid-soluble Tolf FA was 35 mg/g. Tolf content in formulations was confirmed by HPLC. The SEDDS formulation (~1 g) containing Tolf ILs or Tolf FA was subsequently dispersed in 39 mL of SIF (2 mM Tris-maleate, 150 mM NaCl, 1.4 mM $CaCl_2.2H_2O$, 3 mM NaTDC, 0.75 mM PC, pH 6.5, 37 °C).

After 5 and 10 min of dispersion, a 1 mL sample was removed, centrifuged (21,000 x g, 37 °C, 10 min) and the concentration of dissolved drug determined by HPLC. After the dispersion phase, 4 mL pancreatin extract (i.e. 1 mL containing ~10,000 TBU for every ~10 mL of digest) was added to initiate digestion. Digestion was continuously monitored over 60 min using a pH-stat titrator, which added 0.2 M NaOH into the reaction vessel in response to the decrease in pH stimulated by lipid digestion and fatty acid liberation. After 5, 15, 30 and 60 min of digestion, a 1 mL sample was transferred into an Eppendorf tube containing an enzyme inhibitor (4-bromophenylboronic acid, 5 μL/mL of a 1.0 M solution in methanol) to stop digestion and centrifuged (21,000 x *g*, 37 °C, 15 min). The centrifuged samples separated to form combinations of a pellet phase at the bottom of the tube containing insoluble precipitated material, a floating undispersed oily phase and a well dispersed, primarily micellar, aqueous phase. The aqueous phase, pellet phase and oily phase were separated, vortexed and diluted. The pellet phase was firstly dissolved in 150 μL 1.0M HCl, 100 μL chloroform/methanol (2/1:v/v) and 750 μL acetonitrile. The oily phase was first dissolved in 1 mL chloroform/methanol (2/1:v/v). These diluted samples, along with samples of solubilized drug in the aqueous phase were diluted further with acetonitrile and mobile phase and analysed by HPLC as below.

Analysis of drug concentrations in formulations and digestion samples

Solubility samples and *in vitro* digestion samples were assayed for Tolf, Mec, Diclof and Ibu content via HPLC, using an Alliance 2695 separation module and a 486 tunable UV absorbance detector (Waters Instruments, Milford, MA) and a Waters Symmetry C18 column (150 × 3.9 mm, 5 μm). For Tolf, Mec and Diclof assays, mobile phase A was 95:5 (v/v) water:acetonitrile with 0.01% (v/v) formic acid and mobile phase B was 95:5 (v/v) acetonitrile:water with 0.01% (v/v) formic acid maintained at a flow rate of 1 mL/min. For

Tolf, the mobile phase comprised an isocratic mixture of 10% of mobile phase A and 90% of mobile phase B. For Mec and Diclof, the mobile phase comprised an isocratic mixture of 20% of mobile phase A and 80% of mobile phase B. The injection volume was 50 μL and UV absorbance was monitored at 280 nm. The retention times were 2.4 min for Tolf, 2.7 min for Mec and 2.2 min for Diclof and the concentration range of the calibration standards was 0.8-50 μg/mL. For Ibu, the mobile phase comprised 35:65 (v/v) 5 mM aqueous potassium dihydrogen phosphate:acetonitrile and was maintained at a flow rate of 1 mL/min. The injection volume was 25 μL and UV absorbance was monitored at 230 nm. The retention time was 3.3 min and the concentration range of the calibration standards was 0.8-50 μg/mL.

Oral bioavailability studies

All experimental procedures were approved by the Monash Institute of Pharmaceutical Sciences Animal Ethics Committee. Experiments were conducted in fasted male Sprague-Dawley rats (270-310 g). A day prior to the study, rats were anaesthetised with isoflurane and the right carotid artery was surgically cannulated with polyethylene tubing (0.96 x 0.58 mm) to facilitate blood collection as described previously.[30] Animals were allowed to recover overnight and were fasted up to 12 h prior to and 8 h after dose administration with water provided *ad libitum*.

SEDDS formulations containing Tolf FA or Tolf ILs (Tolf•NBuDodecMe$_2$, Tolf•NDec$_2$Me$_2$, Tolf•NHDec$_2$Me) at high drug loadings (150 mg) were dispersed in 0.5 mL of water immediately prior to oral gavage to lightly anaesthetised rats, followed by a further 0.5 mL of water. Tolf ILs were dissolved in the SEDDS formulation to provide a dose of 100 mg/kg Tolf FA. To examine whether IL dose had an impact on absorption profiles, the Tolf•NDec$_2$Me$_2$ formulation was also administered at a lower dose (18 mg/kg). This was

achieved by dosing a lower quantity of the same formulation (27 mg of formulation dispersed in 0.5 mL of water, followed by 0.5 mL of water). Tolf FA was dissolved in the SEDDS formulation to give a dose of 18 mg/kg to match that of the low dose Tolf•$NDec_2Me_2$ formulation. Unlike Tolf IL, Tolf FA was not sufficiently soluble in the SEDDS formulation to provide a dose of 100 mg/kg in solution (to match the high dose ILs) and instead had to be suspended in the SEDDS formulation to provide a 100 mg/kg dose. Finally Tolf FA was dosed as an aqueous suspension at 100 mg/kg dose to evaluate the effect of an aqueous versus lipid suspension formulation. The aqueous suspension comprised 0.5% w/v sodium carboxymethylcellulose, 0.4% w/v Tween 80 and 0.9% w/v NaCl in water. Blood samples were collected via the carotid artery cannula at pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 10, 24, 28, 32 and 48 h post-dose. Blood samples were centrifuged at 6,700 x *g* for 5 min and plasma collected and stored at -80 °C until assayed for Tolf content by LCMS.

Quantification of drug concentrations in plasma

Tolf concentrations in rat plasma (except after administration of Tolf•$NHDec_2Me$ and Tolf•$NDec_2Me_2$ at 18 mg/kg) were assayed using LCMS. Calibration standards for Tolf were prepared by spiking blank rat plasma (50 µL) with Tolf standard solutions (5 µL) in acetonitrile to give plasma concentrations in the range of 50 to 10000 ng/mL. Plasma samples or calibration standards (50 µL) were spiked with 5 µL of internal standard (Diclof, 50 µg/mL in acetonitrile) and vortex mixed for 1 min. To precipitate plasma proteins, saturated ammonium sulphate solution (25 µL) was added and samples vortex mixed for 30 sec, followed by the addition of acetonitrile (90 µL) (and vortex mixed for a further 1 min). The samples were then allowed to stand at room temperature for 20 min. After centrifugation at 9,600 x *g* for 5 min at room temperature, 25 µL of supernatant was transferred into vials for analysis.

In most cases, Tolf plasma samples were analysed on a single quadrupole LCMS (Model 2010) with an electrospray ionization (ESI) interface (Shimadzu, Kyoto, Japan). Data acquisition and processing were performed using LCMS Solutions software (Shimadzu, Kyoto, Japan). Mobile phase A comprised of water containing 0.1 % (v/v) acetic acid and mobile phase B was acetonitrile containing 0.1 % (v/v) acetic acid. The mobile phase flow rate was 0.5 mL/min with the following gradient elution: 35 to 100% mobile phase B from 0-2.5 min; 100% B from 2.5-3 min; 100 to 35% B from 3-3.2 min and 35% B from 3.2-6 min. Each sample (5 µL) was injected onto a Kinetex C18 100A column (2.6 µm, 50 mm × 2.1 mm, Phenomenex, CA) held at 40 °C. The retention times of Tolf and the internal standard (Diclof) were 3.1 and 2.7 min, respectively. Tolf and the Diclof were detected in negative ion mode and selective ion monitoring (SIM) of m/z 260.00 and 294.00, respectively. The heat block and curved desolvation line (CDL) temperatures were set at 250 and 200 °C, respectively. Detector and interface voltages were 1.5 kV and 0.5 kV respectively. The nebulising gas flow rate was 1.2 L/min.

For Tolf•NHDec$_2$Me and the bioavailability study for Tolf•NDec$_2$Me$_2$ at the lower dose (18 mg/kg), unavailability of the Shimadzu 2010 instrument necessitated analysis to be undertaken using a Shimadzu 8030 LC-MS/MS system (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization source. Plasma sample preparation and LC separation conditions were consistent with the original Shimadzu 2010 LCMS method, however, it was necessary to reduce the injection volume to 2 µL. The the gradient elution was also modified as follows: 35 to 70% mobile phase B from 0-1 min; 70% B from 1-2 min; 70-95% from 2-2.5 min; 95% B from 2.5-3 min, 95-35% B from 3-3.5 min and 35% B until 4 min. The total run time was 4 min with Tolf and Diclof eluting at 1.6 and 1.4 min, respectively.

The MS conditions were optimised as follows: drying gas flow, 15 L/min; nebulizing gas flow, 2 L/min; desolvation line temperature, 200 °C; heat block temperature, 450 °C; and CID gas, 230 kPa. Tolf was monitored at *m/z* 259.90 > 216.10 with the first quadrupole (Q1) and third quadrupole (Q3) of the mass spectrometer set at 19.0 V and 15.0 V, respectively, and collision energy at 16.0 V. For the internal standard, Diclof was monitored at *m/z* 293.90 > 249.90 with the first quadrupole (Q1) and third quadrupole (Q3) of the mass spectrometer set at 23.0 V and 17.0 V, respectively, and collision energy at 13.0 V. The chromatographic data were acquired and analysed using the LabSolutions LCMS Version 5.4 software package for LCMS-8030 (Shimadzu).

Plasma concentrations in test samples were determined by comparison with a weighted (1/X) quadratic calibration curve of Tolf:internal standard peak area ratio plotted as a function of Tolf concentration. The assay was validated by analysis of n=3 spiked plasma quality control samples at low (50 ng/mL), medium (2000 ng/mL) and high (10000 ng/mL) concentrations. Intra-assay variability was acceptable and accurate to 94.2, 99.8 and 100.7% and precise to ± 2.5, 2.0 and 5.1% of the nominal QC concentrations at 50, 2000 and 10000 ng/mL.

RESULTS AND DISCUSSION

In the current study, a series of weakly acidic drugs were converted to highly lipophilic ILs, with low melting points and favourable solute-solvent interactions in lipids. This approach was taken to increase drug loading in LBFs and to promote the potential utility of LBF for weak acids with limited lipid solubility. We have previously described a similar approach for weakly basic PWSD,[26] but to this point the *in vitro* and *in vivo* behavior of LBF containing IL of weakly acidic drugs has not been described. Here weakly acidic drugs were converted into ILs using highly lipophilic basic counterions, many based on quaternary ammonium

structures similar to the widely used preservative benzalkonium. To address the potential for differential properties using quaternary versus tertiary ammonium salts, i.e. to assess the potential impact of a permanent positive charge versus pH dependent ionisation, data was also obtained for an equivalent tertiary ammonium salt of Tolf.

Tolf, Mec, Diclof and Ibu were chosen as model drugs (Figure 1). All are nonsteroidal anti-inflammatory drugs with relatively low water solubility, although in common with most weakly acidic drugs, solubility under neutral or slightly basic pH in the intestinal tract is significantly enhanced when compared to solubility in the stomach.[31-34] All four are relatively well absorbed after oral administration and as such the intent of the current studies was not to promote absorption *per se*, but rather to provide proof of concept data that IL could be employed to enhance drug loading in LBF, and to evaluate the impact of IL formulation on *in vitro* and *in vivo* behavior.

The selection of lipophilic counterions that are appropriate for use in ionic liquid preparations of weak acids is complicated since lipophilic cations are, in general, more toxic than the equivalent anions. We first examined a number of alkyl amines and spermine, a polyamine involved in cellular metabolism, since these are perhaps the simplest and most well defined.[35] As described below, these did form ionic complexes with the weak acids studied here, albeit complexes with melting points above 100 ^{o}C and therefore materials better described as lipophilic salts than ionic liquids. The advantages in lipid solubility for these materials, above the free acid, however, were limited. As such, we subsequently turned to bulkier and more branched lipophilic counterions. This was based on our previous experience with anionic counterions for basic drugs where bulky branched acids appeared to more effectively disrupt packing, reducing melting point and leading to greater increases in lipid solubility.[26] This led us to the quaternary ammonium compounds employed herein. These are amphiphilic

surfactants and biocides and are widely used as antiseptic and disinfectants in clinical and industrial environments. At low concentration benzalkonium (usually as the chloride) is commonly used as a preservative (e.g. in eye drops). The Registry of Toxic Effects of Chemical Substances (RTECS) at the Centers for Disease Control and Prevention in Atlanta GA, suggests that the lowest oral LD50 in rats for benzalkonium is 240 mg/kg (https://www.cdc.gov/niosh-rtecs/bo3010b0.html). Didecyldimethylammonium chloride is an antiseptic/disinfectant and has an oral LD50 in rats of 84 mg/kg (GESTIS substance database, Institute for Occupational Safety and Health of the German Social Accident Insurance) http://gestis-en.itrust.de. Didecylmethyl and butyldodecylammonium chloride are also disinfectants, however, acute toxicity data after oral administration could not be readily sourced. These materials are therefore less than ideal as pharmaceutically acceptable counterions, and as described below, some GI toxicity was evident in our studies. However, the intent here was to provide initial proof of concept with lipophilic cationic counterions, rather than to progress potential clinical or commercial candidates.

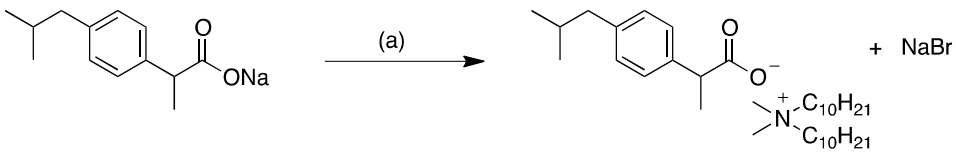
Drug-ILs were prepared by metathesis reaction between the sodium salt of the drugs and the desired quaternary ammonium halide salts, or via acid-base reaction between the drug free acid and the alkyl amine base. For Tolf, the sodium salt was not readily available and instead was formed *in situ* via deprotonation by sodium hydroxide. Metathesis reactions were carried out under basic conditions (sodium carbonate or sodium bicarbonate) at room temperature in water or in water/methanol mixtures where the solubility of the ammonium salt in water was limiting. Acid-base reactions between Tolf free acid and the alkyl amines (butyl, octyl, didecylmethyl, octadecyl, dodecylamine and spermine) were carried out in methanol.

The resulting water-immiscible drug-ILs formed immediately as oil droplets dispersed in the aqueous reaction medium. Drug-ILs were extracted with an organic solvent (dichloromethane

or chloroform) and purified via washing with distilled water. The resultant drug-ILs were isolated as liquids or low melting solids. By way of example, Scheme 1 presents the conversion of the sodium salt of Ibu, a crystalline white powder with a melting point of 190 °C via metathesis with didecyldimethylammonium bromide to form Ibu didecyldimethylammonium salt (Ibu•$NDec_2Me_2$), a low viscosity yellow oil. Synthetic and analytical details for all API•ILs are given in the methods and supplementary material.

Once drug-ILs were isolated, solubility in LBFs was assessed using a model long chain lipid-containing LBF. The LBF employed was a self-emulsifying drug delivery system (SEDDS) that disperses in aqueous media under very gentle agitation to form a nanoemulsion with particle size of 100-250 nm. The LBF comprised 60% w/w lipid (soybean oil:Maisine^{TM} 35-1, 1:1 w/w), 30% w/w surfactant (Kolliphor$^{®}$ EL) and 10% cosolvent (ethanol).

Scheme 1 Preparation of Ibu•$NDec_2Me_2$ from crystalline Ibu sodium and didecyldimethyl ammonium bromide



Reagents and conditions: (a) didecyldimethyl ammonium bromide, H_2O/CH_3OH.

The impact of IL conversion on the melting ranges and solubility of the drugs in the model LBF is shown in Table 1 and is detailed graphically for some examples in Figure 2.

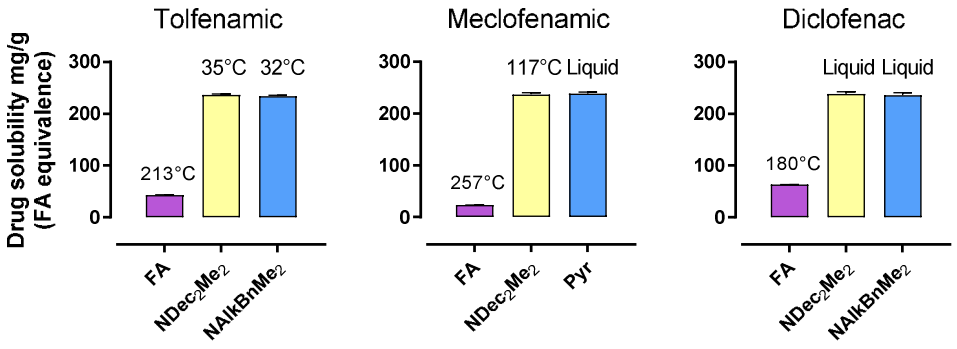


Figure 2 Comparison of the solubility of the free acid (FA) of Tolf, Mec and Diclof and selected drug-ILs in LC-SEDDS. Data shows the measured concentration after mixing a 1:1 w/w blend of formulation and IL. In all cases shown here ILs were effectively miscible at proportions of 1:1 w/w and as such a true solubility was not determined. The data shown are lower than the value of 500 mg/g that might be expected in a 1:1 mixture since the data are expressed as mg/g of the free acid, rather than mg/g IL. The molecular weight of the drug and the counterion were similar and as such values around 250 mg/g are evident. FA - free acid; $NDec_2Me_2$ – didecyldimethyl ammonium; $NAlkBnMe_2$ – benzalkonium; Pyr – hexadecyl-3-methyl pyridinium. The numbers above the bars are the melting point of the FA or IL.

Table 1 Melting temperature range and equilibrium solubility data (37 °C, mean ± SD, n = 3) for drug-ILs in a SEDDS formulation comprising (w/w): 60% lipid (soybean oil:Maisine™ 35-1, 1:1), 30% Kolliphor® EL and 10% ethanol.

Drug	IL Counterion	Melting Range of Drug-ILs (°C)	Solubility in LC-SEDDS (mg/g) Free Acid equiv.	Crystalline by XRD?
Tolfenamic (Tolf)	no counterion (FA)	213	42.7 ± 0.3	Y
	Butylammonium (NH_3Bu)	169-171	NA	NA[a]
	Octylammonium (NH_3Oct)	146-149	NA	NA[a]
	Dodecylammonium (NH_3Dodec)	127-129	54.7 ± 1.0	NA[a]
	Octadecylammonium ($NH_3Octadec$)	123-125	7.2 ± 0.2	NA[a]
	Spermine	121	82.0 ± 3.5	NA[a]
	Butyldodecyldimethylammonium ($NBuDodecMe_2$)	98-100	≥ 245 ± 5.1	Y
	Didecyldimethylammonium ($NDec_2Me_2$)	35-38	≥ 236.2 ± 3.4	Y
	Dioctadecyldimethylammonium ($NMe_2Octadec_2$)	55-57	≥ 162.8 ± 4.3	Y
	Didecylmethylammonium ($NHDec_2Me$)	liquid	>302.5 ± 9.2	N
	Benzalkonium ($NAlkBnMe_2$)	32-35	≥ 234.1 ± 3.1	Y
Diclofenac (Diclof)	no counterion (FA)	180	63.2 ± 0.5	Y
	Didecyldimethylammonium ($NDec_2Me_2$)	liquid	≥ 238 ± 7.6	NA[a]
	Benzalkonium ($NAlkBnMe_2$)	liquid	≥ 236 ± 7.7	NA[a]
Meclofenamic (Mec)	no counterion (FA)	257-259	23.4 ± 0.2	Y
	1-Hexadecyl-3-methylpyridinium	liquid	≥ 239 ± 4.1	NA[a]
	Didecyldimethylammonium ($NDec_2Me_2$)	117-120	≥ 237 ± 5.6	Y
Ibuprofen (Ibu)	no counterion (FA)	73-76	287.9 ± 4.0	Y
	Didecyldimethyl ammonium ($NDec_2Me_2$)	liquid	≥ 195 ± 5.2	NA[a]

[a] Not Attempted. Data were not generated for all the alkyl amine counterions since they provided little advantage in melting point depression or lipid solubility relative to the ammonium salts. XRD data were not attempted for the ILs that were liquids at room temperature, with the exception of Tolf•$NHDec_2Me$. XRD traces are provided in the supplementary information.

Drug solubility in any vehicle is a function of the strength of solute-solute interactions in the solid state (a property reflected by melting point), and the nature of solute-solvent interactions

in the vehicle. Hence, higher solubility in a particular solvent requires weaker intermolecular forces (usually indicated by a reduction in melting point) and favourable solute-solvent interactions. The strategy employed here was to synthesise drug-ILs using bulky counterions, in order to disrupt intermolecular forces in the solid state, and to employ highly lipophilic counterions to promote solute-solvent interactions in a SEDDS formulation. Formulations of this type spontaneously emulsify on contact with the gastrointestinal fluids and typically enhance absorption for drugs with solubility limited absorption.[36]

With the exception of the ILs generated with the alkyl amine counterions (Tolf•NH_3Bu, Tolf•NH_3Oct, Tolf•NH_3Octadec, Tolf•NH_3Dodec and Tolf•Spermine), all the IL synthesized, regardless of whether they were isolated as a solid or a liquid at room temperature were miscible with the SEDDS formulation on mixing in a 1:1 w/w ratio of drug-IL and formulation. In most cases, the results in Table 1 are therefore shown as ≥ the concentration measured in the formulation after 1:1 mixing and are expressed in $mg.g^{-1}$ of free acid equivalents in the vehicle.

To examine the effect of increasing alkyl chain length in IL based on alkyl amines, Tolf FA was first reacted with butyl, octyl, dodecylamine and octadecyl (Table 1). In all cases, isolation of the alkyl amine salt resulted in a reduction in melting temperature, and increasing the alkyl chain length of the counterion from C4 to C18 resulted in increased reductions in melting point from 213 °C (Tolf FA) to 123-125 °C (for Tolf•NH_3Octadec). However, measurement of the solubility of Tolf•NH_3Dodec in the SEDDS formulation revealed only slight increases in lipid solubility relative to the free acid for the C12 alkyl amine (Tolf•NH_3Dodec) and a reduction in melting point for the C18 variant (Tolf•NH_3Octadec). These trends are consistent with previous studies of ILs of weakly basic drugs with alkyl sulphate counterions, where for example the lipid solubility of cinnarizine octadecyl sulphate was lower than that of cinnarizine decylsulphate[26]. Tolf•Spermine was readily isolated, and solubility in LC-SEDDS was slightly

higher than Tolf FA, however, incorporation into LC-SEDDS resulted in significant discolouration of the formulation, consistent with chemical instability. As such, further measurements were not made in this series and attention turned to evaluation of alternate counterions. In an attempt to further reduce melting point and to better enhance solute-solvent interactions in the LBF, subsequent studies employed lipophilic, quaternary ammonium counterions where the quaternary ammonium centre was substituted with two methyl groups (in all cases) and two alkyl chains of varying chain length (C4-C18) (Figure 1). For Tolf, cations were employed with the two methyl group substitutions and either two further alkyl chains of the same chain length, e.g. two C10 chains (Tolf•$NDec_2Me_2$) or two C18 chains (Tolf•$NMe_2Ocadec_2$) or dissimilar chain lengths e.g. C4 and C12 alkyl chains (Tolf•$NBuDodecMe_2$). ILs were also made with benzalkonium (Tolf•$NAlkBnMe_2$). A comparator data set was also generated for Tolf•$NHDec_2Me$, the tertiary amine equivalent of Tolf•$NDec_2Me_2$. In all cases, the melting point of Tolf-ILs constructed with the alkylated quaternary (or tertiary) ammonium counterions was lower than that of the simple alkyl amines (C4, C8 and C12). Of these five ILs, Tolf•$NBuDodecMe_2$ had the highest melting point (98-100°C) and Tolf•$NHDec_2Me$ the lowest (liquid at room temperature). Within the Tolf quaternary/ternary ammonium IL set, there was evidence that shorter alkyl chains (<C12) and bulky aromatic groups were most effective in disrupting packing in the salt lattice. Irrespective of differences in melting point, all five ILs were miscible with the SEDDS formulation after mixing at a 1:1 (w/w) ratio of drug-IL to formulation. The stability of the SEDDS formulations was not formally assessed, however, SEDDS formulation containing the IL that were miscible with the formulation (ie those constructed with the quaternary ammonium counterions), were maintaned at 37^{o}C at the end of the solubility studies for several months, and no evidence of precipation was evident over that time.

Increases in the chain length of the symmetrically substituted cations from C10 to C18, resulted in an increase in melting point for the Tolf IL from 32-35 ℃ to 55-57 °C, presumably reflecting an increase in van der Waals solute-solute attractive forces in the higher molecular weight species.[37] This trend is consistent with our previous studies where the melting points of IL of basic drugs with alkyl sulphates typically increased for IL based on C18 alkyl sulphates when compared to C10 alkyl sulphates.[26] For Mec, IL comprising didecyldimethylammonium ($NDec_2Me_2$) (more appropriately classified as a lipophilic salt since the melting point was > 100 °C) and 1-hexadecyl-3-methylpyridinium cations resulted in a significant drop in melting point and corresponding increase in solubility in LBF when compared to Mec FA.

Across the three drugs, the melting points of IL formed with drugs that had lower melting points as the starting FA, were lower. For example, IL based on the $NDec_2Me_2$ cation resulted in the formation of room temperature IL for Diclo or Ibu (where the FA had a relatively low melting point), whereas for Tolf and Mec, where the melting point of the FA was higher, the $NDec_2Me_2$ ILs were solids at room temperature.

Having shown that IL with significantly lower melting temperatures and significantly higher solubilities in a model LBF could be formed using alkylated ammonium cations, subsequent studies sought to evaluate the behaviors of these formulations in an *in vitro* model of intestinal lipid digestion. The importance of lipid digestion in the performance of LBF has led to increasingly frequent use of *in vitro* lipid digestion models to mimic the likely events that occur on entry of LBF into the digestive environment of the small intestine.[29, 38-40] The basic premise of these studies is that maintaining drug in a solubilised state as a LBF is digested and as the digestion products are incorporated into bile salt/phospholipid micelles, is important in maximising the likelihood of drug absorption. To examine the possible impact of different counterions on drug solubilisation during *in vitro* digestion of LBF, model SEDDS

formulations containing Tolf FA, Tolf•NHDec$_2$Me, Tolf•NBuDodecMe$_2$, Tolf•NDec$_2$Me$_2$ or Tolf•NAlkBnMe$_2$ were dispersed in simulated intestinal fluid and then digested via the addition of a pancreatin extract (containing a range of intestinal esterases and lipases) as previously described (Figure 3).[29, 41]

The high solubility of Tolf•NDec$_2$Me$_2$, Tolf•NBuDodecMe$_2$, Tolf•NHDec$_2$Me and Tolf•NAlkBnMe$_2$ in the SEDDS formulation allowed the preparation of formulations at high drug loading (~200 mg/g Tolf FA equivalent). In contrast, the comparator Tolf FA formulation was limited to a drug load of 35 mg/g (80% of the saturated solubility of Tolf FA in the formulation). Interestingly, on dispersion in simulated intestinal fluid, the Tolf•NBuDodecMe$_2$ and Tolf•NAlkBnMe$_2$ formulations resulted in course emulsions that separated on centrifugation, resulting in recovery of the majority of drug in a phase separated oil phase. Since blank (drug free) formulations disperse readily to form a homogenous emulsion, the presence of the IL, especially at these high drug loadings, must have altered the colloidal properties of the dispersed formulation and reduced emulsification potential (Figure 3).

In contrast the LBF containing Tolf•NDec$_2$Me$_2$, Tolf•NMe$_2$Ocadec$_2$ and Tolf•NHDec$_2$Me dispersed well and drug was predominantly solubilised in the aqueous phase of the dispersed or digested formulation.

The differential behavior of the Tolf•ILs in the digestion test may reflect the symmetry (or otherwise) of the hydrophobic chains of the counterions as asymmetric double-tailed surfactants have previously been shown to less readily support micellisation, when compared to comparable, but symmetrical surfactants.[42] On digestion of the dispersed formulations, drug solubilisation patterns did not change dramatically. For the Tolf•NBuDodecMe$_2$ and Tolf•NAlkBnMe$_2$ formulations, addition of pancreatic enzymes led to transfer of a proportion of drug from the poorly dispersed oil/cream phase into the well dispersed aqueous phase, and

also resulted in an increase in drug recovery in the pellet phase. In contrast, initiation of digestion of the Tolf•$NDec_2Me_2$ and Tolf•$NHDec_2Me$ containing SEDDS resulted in little perturbation of drug solubilisation with negligible drug found in the pellet phase. The LBF containing Tolf FA, showed drug solubilisation patterns that were almost identical to that of the Tolf•$NDec_2Me_2$ and Tolf•$NHDec_2Me$ formulation, albeit at much lower drug load and therefore much lower solubilised concentrations. Effective solubilisation of Tolf FA by digested lipid formulations is consistent with previous detailed studies.[43, 44]

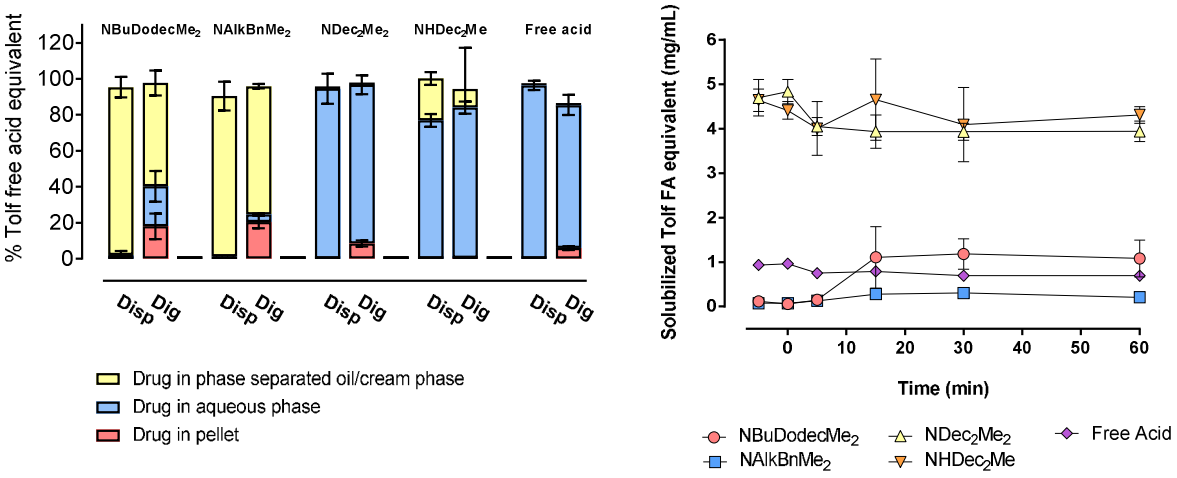


Figure 3 Fate of Tolf following dispersion and digestion of SEDDS containing Tolf FA, Tolf•$NDec_2Me_2$, Tolf•$NAlkBnMe_2$ or Tolf•$NBuDodecMe_2$ and Tolf•$NHDec_2Me_2$ ILs in SIF. **Left:** For Tolf FA, Tolf•$NDec_2Me_2$ and Tolf•$NHDec_2Me$, more than 75% of the incorporated drug remained solubilised in the colloidal species both on dispersion (Disp, left bars) and on digestion (Dig, right bars). In contrast for Tolf•$NBuDodecMe_2$ and Tolf•$NAlkBnMe_2$, a

significant proportion of the drug were recovered in an undispersed oil/cream phase. **Right:** Drug concentrations of Tolf ILs (Tolf•$NDec_2Me_2$, Tolf•$NBuDodecMe_2$, Tolf•$NAlkBnMe_2$, Tolf•$NHDec_2Me$) or Tolf FA in the aqueous phase of an *in vitro* digestion experiment following dispersion (-10 to 0 min) and digestion (0 to 60 min) of SEDDS containing Tolf-ILs (equivalent to ~200 mg/g Tolf FA) and 35 mg/g Tolf FA. Mean (n = 3) ± SEM.

In light of the very different behavior of the SEDDS formulations containing the IL formed with asymmetric counterions (Tolf•$NBuDodecMe_2$) when compared to the symmetrical counterion (Tolf•$NDec_2Me_2$ and Tolf•$NHDec_2Me$), an *in vivo* pharmacokinetic study was subsequently carried out in rats to assess the absorption of Tolf after oral administration of Tolf FA and the Tolf ILs formed with symmetrical and asymmetric counterions after formulation in the model SEDDS formulation. Tolf•$NHDec_2Me$ was also examined to probe the potential impact of permanent versus pH dependent ionisation.

Tolf FA has moderate lipid solubility, and as such it was possible to dose the free acid as a solution in the SEDDS formulation at 18 mg/kg. The higher lipid solubility of Tolf•$NDec_2Me_2$, Tolf•$NBuDodecMe_2$ and Tolf•$NHDec_2Me$, allowed increased drug loading in the SEDDS formulation, and administration at a much (> 5 fold) higher dose (100 mg/kg). To compare the Tolf-IL with Tolf FA at the same dose, a crystalline suspension of Tolf FA in the same SEDDS formulation and an aqueous suspension of Tolf FA were also administered at 100 mg/kg. Finally a lower dose (18 mg/kg) of the Tolf•$NDec_2Me_2$ was also administered to provide a direct comparison with the SEDDS solution formulation of Tolf FA. Formulations were administered to overnight fasted rats by oral gavage and drug concentrations in plasma was detected by LCMS. The data for Tolf plasma concentrations as a function of time and pharmacokinetic parameters are shown in Figure 4 and Table 2, respectively.

Despite the relatively low water solubility of Tolf FA, robust plasma exposure of Tolf was evident, even after administration of the aqueous suspension formulation of Tolf FA at 100 mg/kg. Formulation as a suspension in the SEDDS at the same dose resulted in a slightly lower C_{max}, but overall plasma exposure was not significantly different. Administration of Tolf FA as a solution in the SEDDS formulation resulted in significantly lower plasma exposure due to the need to administer at a much lower dose (18 mg/kg). Interestingly, dose normalization of the Tolf FA SEDDS solution data to 100 mg/kg revealed lower exposure than either the aqueous or SEDDS suspension. Previous studies[45, 46] suggest that the oral bioavailability of Tolf is high (60-75%) but in part limited by first pass metabolism. The data obtained here are therefore consistent with these previous findings showing good absorption after administration of aqueous and lipid suspension formulations, even at 100 mg/kg; and lower bioavailability after administration at a lower dose (18 mg/kg), presumably due to increased first pass metabolism. Formulation as a LBF therefore did not provide for increases in bioavailability under the current conditions and the absorption of Tolf FA did not appear to be solubility limited. As described above, this presumably reflects pH dependent solubility of the weak acid Tolf, and the attainment of sufficiently high concentrations of ionized Tolf under neutral or mildly basic conditions in the intestine.

Administration of the SEDDS formulation containing Tolf•$NDec_2Me_2$ IL at the same low dose as that achievable with Tolf FA (18 mg/kg) revealed similar plasma concentration-time profiles and exposure (Figure 5A). In contrast, administration of solution SEDDS formulations containing Tolf•$NDec_2Me_2$, Tolf•$NBuDodecMe_2$ or Tolf•$NHDec_2Me$ at the high dose (100 mg/kg) that was achievable by virtue of the increase in lipid solubility of the ILs, resulted in markedly different plasma profiles. In all cases, C_{max} was reduced and drug exposure seemingly sustained for up to 48 h. Figure 5B provides a direct comparison of the plasma profiles obtained after oral administration of a SEDDS solution formulation of 100 mg/kg Tolf•$NDec_2Me_2$ IL

with a suspension of Tolf FA in either water or the SEDDS formulation at the same dose. The data (Figure 5B, Table 2) reveal similar total exposure (AUC) across the two preparations but variable and seemingly slower Tolf absorption from the solution SEDDS formulation of the IL when compared to a suspension of the FA in the same formulation. The altered absorption profile is unlikely to be an intrinsic property of the IL as at the low dose (18 mg/kg) almost identical profiles were evident for the FA and IL. It seems more likely therefore that the combination of an increase in dose and the differentiation between a lipid solution formulation (IL) and a lipid or aqueous suspension (FA) formulation resulted in the altered profiles. The quantity of formulation administered (150 mg/rat), even at the high drug dose, was not extreme and many previous studies have shown typical drug absorption profiles after administration of this quantity of SEDDS formulation to rats. A combination of the formulation and IL form of the drug therefore appears to be necessary to drive extended exposure in the rat. The *in vitro* lipolysis studies suggest that drug precipitation is limited on GI processing for all formulations, and especially in the case of Tolf•$NDec_2Me_2$ and Tolf•$NHDec_2Me$ that the lipid formulation disperses well and IL solubilisation occurs within small, lipid colloids. It remains possible, however, that the very high lipophilicity of the Tolf•$NDec_2Me_2$ and Tolf•$NHDec_2Me$ ILs may result in slower partition of Tolf out of the dispersed lipid phase and that at high dose, where greater quantities of formulation must be processed, that this slows drug absorption.

Since the counterions here were quaternary ammonium compounds bearing a permanent positive charge, subsequent studies were performed to evaluate an analogue where Tolf•$NDec_2Me_2$ was altered to form the equivalent tertiary amine (where ionisation would be expected to be pH dependent). In this case (Tolf•$NHDec_2Me$), the extended release seen for Tolf•$NDec_2Me_2$ was less clear, but in general drug absorption profiles were similar (Figure 5C) and different to that exhibited by the Tolf-FA suspensions. The use of a quaternary ammonium complex versus a tertiary amine therefore does not appear to be a major driver of the altered

absorption kinetics, presumably reflecting the likely ionisation of Tolf•NHDec$_2$Me at intestinal pH. Administration of the asymmetric Tolf•NBuDodecMe$_2$ led to oral exposure slightly lower than that of Tolf•NDec$_2$Me$_2$ (Figure 5D) and similar to that of Tolf•NHDec$_2$Me, in spite of markedly different behaviour on *in vitro* lipolysis. Our initial hypothesis based on the *in vitro* lipolysis studies, was that drug absorption from the SEDDS formulation of Tolf•NBuDodecMe$_2$ might be slower than that from Tolf•NDec$_2$Me$_2$ due to clearer association of drug with the lipid phase of the formulation and less ready transfer into the aqueous phase of the digest. The *in vivo* data, however, suggest that even under conditions where the Tolf•NDec$_2$Me$_2$ formulation is highly dispersed and effectively incorporated into the digest aqueous phase, that drug absorption is slowed.

In light of the slowed absorption profiles of the SEDDS formulations containing the Tolf ILs at high dose, further experiments were condicted to explore the dispersion/dissolution behaviour of the SEDDS formulations in order to probe the possibility that altered dispersion *in vivo* may have slowed absorption. These experiments are described in the supplementary information. In short, the SEDDS formulations dispersed rapidly and completely, suggesting that poor dispersion, gelation or high viscosity were not likely to be contributors to the slowed absorption profiles. Finally, partitioning studies were conducted to probe whether the high lipophilicity of the ILs may have slowed partition out of the lipid phase of the SEDDS, into the aqueous phase of the GI fluids, thereby reducing the rate of absorption and prolonging the timescale of exposure. In these experiments (for details see supplementary information), Tolf•NDec$_2$Me$_2$ and Tolf•NBuDodecMe$_2$ (the Tolf quaternary ammonium ILs that were assessed *in vivo*), were loaded into a simple lipid solution formulation comprising the lipids that formed the core lipids of the SEDDS formulation employed here (ie a lipid solution of soybean oil/Maisine 1:1 w/w). This formulation was then dispersed into buffer, stirred, and equilibrated to a set pH using a pH stat titrator. A sample was then taken, centrifuged to separate the oil phase and the aqueous

phase and the concentration of Tolf in the aqueous phase measured by HPLC. The pH stat was then programmed to raise the pH by one unit and the process repeated. This was then replicated over a pH range of 2-9 to give an indication of the partitioning behaviour of Tolf ILs as a function of pH. The studies were repeated for Tolf free acid to allow comparison of the free acid and the ILs (realising the large differences in *in vivo* absorption patterns). For both Tolf FA and the Tolf ILs, partition out of the lipid vehicle increased with increasing pH, suggesting dissociation of Tolf and increased partition into the aqueous phase as ionisation increased above the pKa of Tolf (pKa 3.9). Consistent with the slow absorption of the ILs in vivo, however, at intestinal pH (6-7), the partitioning of the ILs out of the lipid formulation was significantly lower than the partitioning of the FA. It seems likely therefore that at least some of the reason for the slower absorption of the Tolf ILs may be due to reduced partitioning of Tolf from the lipid formulation into the aqueous GI fluids. Whether this reflects slowed dissociation of the IL complex, or reduced partition of the IL complex into the GI fluids is unknown. Whilst we expect the IL to dissociate in the aqueous phase of the GI tract at ~ pH 6.5 prior to absorption, it is possible that the slow rate of absorption of Tolf from the IL containing formulations reflects slow partitioning of the intact complex into and through the enterocyte. However, the similarity of absorption of Tolf FA and Tolf•$NDec_2Me_2$ at low drug dose, suggests similar absorption processes and a low likelihood that the complex is absorbed intact. The more likely explanation appears to be altered partitioning out of the formulation, and that this is more evident at high dose.

At the high dose administered here (100 mg/kg), administration of Tolf•$NDec_2Me_2$, Tolf•$NHDec_2Me$ and Tolf•$NBuDodecMe_2$ resulted in some diarrhoea, although this was not evident until later time periods (> 24 h). No evidence of intestinal irritation was apparent after administration of Tolf•$NDec_2Me_2$ at the lower dose. This is consistent with previous studies of ionic liquids comprising a combination of the artificial sweeteners saccharine and acesulfame

with the $NDec_2Me_2$ counterion. These ILs were synthesised to provide dual functionality (sweetener/antimicrobial) but were found to be acutely toxic in rats, albeit at higher doses (300-2000 mg/kg) than those employed here.[47] As such, it is possible that the continued absorption of Tolf and evidence of secondary peaks in the plasma level time profiles after administration of the higher dose (100 mg/kg) may have resulted, at least in part, in changes in GI physiology secondary to inflammation and irritation such as increasing fluid content or increased permeability. Further work is therefore required to better understand the potential toxicity of these novel cationic counterions.

Interestingly, these trends were not apparent previously for similar LBF containing ILs based on weakly basic drugs paired with alkyl sulphate counterions[26-28] and other recent studies performed in our laboratories. Further studies are therefore required to assess whether the effects seen here are common for IL of weakly acidic drugs, or whether this effect is specific for the alkylated ammonium counterions studied here.

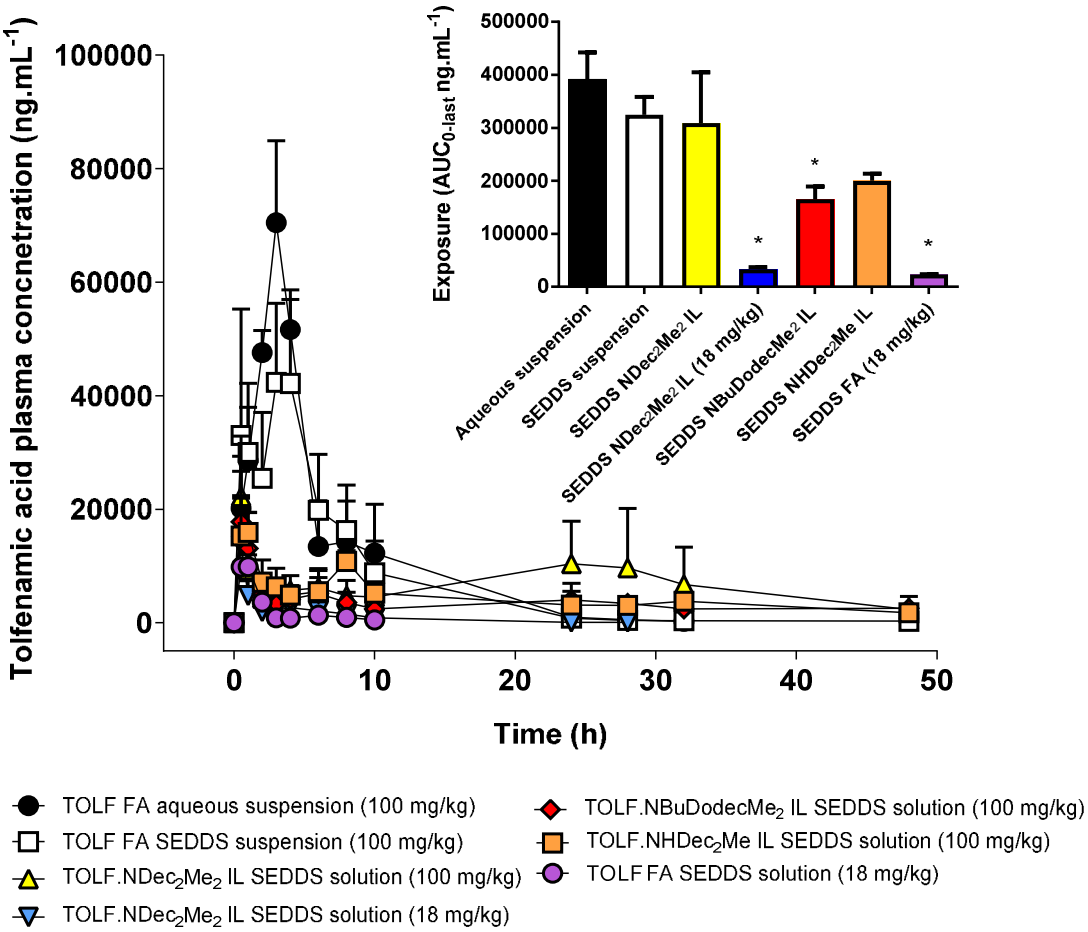
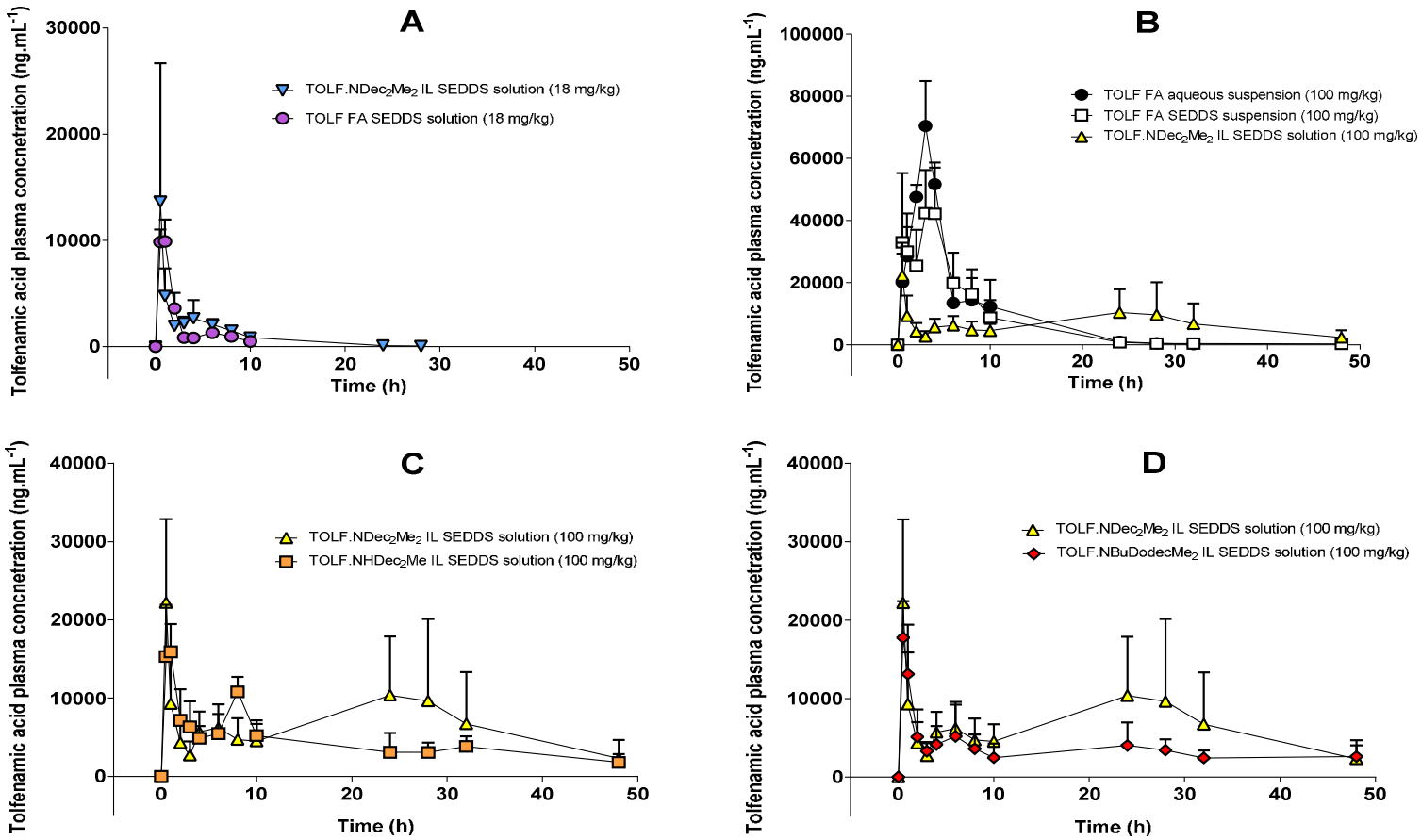


Figure 4 *In vivo* exposure of Tolf after oral administration of LBF containing Tolf FA or Tolf IL. Main: Tolf plasma concentration versus time data after oral administration of an aqueous suspension of Tolf FA (at a dose of 100 mg/kg) or a SEDDS formulation containing Tolf FA (as a solution at a dose of 18 mg/kg or a suspension at 100 mg/kg) or Tolf•$NDec_2Me_2$ IL (as a solution at 100 mg/kg and 18 mg/kg), Tolf•$NBuDodecMe_2$ (as a solution at 100 mg/kg) and Tolf•$NHDec_2Me$ (as a solution at 100 mg/kg). Mean (n = 4) ± SEM. Insert: Total Tolf exposure, expressed as $AUC^{0\text{-}48h}$ after administration of all formulations. Data expressed as Mean (n=4) ± SEM. (* statistically significant difference ($p < 0.05$) vs Tolf FA aqueous suspension).

Table 2 Pharmacokinetic parameters for Tolf FA after oral administration of Tolf FA or Tolf-ILs to rats. Values are expressed as means (n = 4) ± SEM.

	Dose[a] (mg/kg)	AUC $_{0\text{-last}}$ (µg.h/mL)	C_{max} (µg/mL)	T_{max} (h)	F%[b]
Tolf FA aqueous suspension	100	391.7 ± 50.4	70.5 ± 7.2	3.3 ± 0.3	100
Tolf FA SEDDS suspension	100	324.5 ± 33.8	54.9 ± 3.9	2.9 ± 0.8	82.8
Tolf FA SEDDS solution	18	23.0 ± 0.8 *	10.3 ± 0.8 *	0.8 ± 0.1	32.6[c]
Tolf•$NDec_2Me_2$ SEDDS solution	18	33.2 ± 3.5 *	13.7 ± 6.5 *	0.5 ± 0.0	47.0[c]
Tolf•$NDec_2Me_2$ SEDDS solution	100	308.9 ± 95.4	22.2 ± 5.3 *	0.5 ± 0.0	78.9
Tolf•$NHDec_2Me$ SEDDS solution	100	207.1 ± 20.3	19.7 ± 2.2 *	2.6 ± 1.8	52.9
Tolf•$NBuDodecMe_2$ SEDDS solution	100	165.0 ± 24.6*	18.0 ± 2.4 *	0.6 ± 0.1	42.1

[a] Tolf dose expressed in free acid equivalents. [b] F% is relative bioavailability compared to aqueous suspension formulation at 100 mg/kg dose. [c] After dose normalisation to 100 mg/kg. In all cases, the SEDDS is LC-SEDDS ((w/w): 60% lipid (soybean oil:Maisine™ 35-1, 1:1), 30% Kolliphor® EL, 10% ethanol). The aqueous suspension vehicle contained 0.5% w/v sodium carboxymethylcellulose, 0.4% w/v Tween 80 and 0.9% w/v NaCl in water. Statistical analysis undertaken using a one-way ANOVA with Tukey post-hoc HSD test. * Significant difference ($p<0.05$) when compared to Tolf FA aqueous suspension.


A
Tolfenamic acid plasma concnetration (ng.mL-1)
30000
20000
10000
0
0
10
20
30
40
50
Time (h)
TOLF.NDec2Me2 IL SEDDS solution (18 mg/kg)
TOLF FA SEDDS solution (18 mg/kg)
B
Tolfenamic acid plasma concnetration (ng.mL-1)
100000
80000
60000
40000
20000
0
0
10
20
30
40
50
Time (h)
TOLF FA aqueous suspension (100 mg/kg)
TOLF FA SEDDS suspension (100 mg/kg)
TOLF.NDec2Me2 IL SEDDS solution (100 mg/kg)
C
Tolfenamic acid plasma concnetration (ng.mL-1)
40000
30000
20000
10000
0
0
10
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40
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Time (h)
TOLF.NDec2Me2 IL SEDDS solution (100 mg/kg)
TOLF.NHDec2Me IL SEDDS solution (100 mg/kg)
D
Tolfenamic acid plasma concnetration (ng.mL-1)
40000
30000
20000
10000
0
0
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20
30
40
50
Time (h)
TOLF.NDec2Me2 IL SEDDS solution (100 mg/kg)
TOLF.NBuDodecMe2 IL SEDDS solution (100 mg/kg)

Figure 5 *In vivo* exposure (plasma concentration versus time data) of Tolf after oral administration of LBF containing Tolf FA or Tolf IL. Panel A, a SEDDS formulation containing Tolf FA or Tolf•$NDec_2Me_2$ as a solution at a dose of 18 mg/kg. Panel B, a SEDDS formulation containing a suspension of Tolf FA or a solution of Tolf•$NDec_2Me_2$ (at a dose of 100 mg/kg). Panel C, a solution SEDDS formulation containing Tolf•$NDec_2Me_2$ or Tolf•$NHDec_2Me$ (at a dose of 100 mg/kg). Panel D, a solution SEDDS formulation containing Tolf•$NDec_2Me_2$ or Tolf•$NBuDodecMe_2$ (at a dose of 100 mg/kg). Mean (n = 4) ± SEM.

CONCLUSIONS

Weakly acidic drugs have been converted into ILs using highly lipophilic cations based on alkyl amines or quaternary ammonium counterions. The resulting drug-ILs had reduced melting points (in some cases, below room temperature) when compared to the equivalent FAs and were significantly more soluble in a model LBF. This provided for significantly higher dissolved-drug loading capacities in lipid formulations and therefore the potential to reduce capsule size/number at the same dose. *In vivo* in rats, administration of low doses of LBF containing IL of Tolf resulted in similar drug absorption profiles to that obtained after administration of the FA in the same formulation. At the higher doses made possible by conversion to the IL, however, altered plasma profiles (and some GI irritation) were apparent when compared to the equivalent suspension formulations of the FA. Notably, maximum plasma concentrations were reduced and the time period of exposure extended up to 48 h post dose. Partitioning experiments suggest that this may reflect reduced partitioning/dissociation of the highly lipophilic ILs from the lipid formulation into the aqueous GI fluids. These absorption

trends are consistent with the possibility of utility for sustained or extended release, although there are many other well proven formulation approaches to controlled release that may provide a simpler means to the same end. Further studies are required to better understand the differences in exposure seen here for LBF containing IL of weakly acidic drugs, when compared to previous studies of LBF containing IL of weakly basic drugs where no GI irritation and more typical drug absorption profiles were seen.[26] In particular, further studies are required to evaluate whether the profiles observed here are common properties of ILs of weak acids, or whether they are a specific function of alkylated ammonium cations.

ASSOCIATED CONTENT

Supporting Information. NMR spectra and X-ray diffraction data is included in the supporting information that is available free of charge via the Internet at http://pubs.acs.org.

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DECLARATION OF CONFLICT OF INTEREST

This work describes, in part, intellectual property in the use of ionic liquids in drug delivery that has been assigned to Capsugel. H.D.W. is an employee of Capsugel.

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