Independent co-delivery of model actives with different degrees of hydrophilicity from oil-in-water and water-in-oil emulsions stabilised by solid lipid particles via a Pickering mechanism

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DOI:
10.1016/j.jcis.2020.11.021

Independent co-delivery of model actives with different degrees of hydrophilicity from oil-in-water and water-in-oil emulsions stabilised by solid lipid particles via a Pickering mechanism: a-proof-of-principle study

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Abstract

Hypothesis

The development of vehicles for the co-encapsulation of actives with diverse characteristics and their subsequent controllable co-delivery is gaining increasing research interest. Predominantly centred around pharmaceutical applications, the majority of such co-delivery approaches have been focusing on solid formulations and less so on liquid-based systems. Simple emulsions can be designed to offer a liquid-based microstructural platform for the compartmentalised multi-delivery of actives.

Experiments

In this work, solid lipid nanoparticle stabilised Pickering emulsions were used for the co-encapsulation/co-delivery of two model actives with different degrees of hydrophilicity. Lipid particles containing a model hydrophobic active were prepared in the presence of either Tween 20 or whey protein isolate, and were then used to stabilise water-in-oil or oil-in-water emulsions, containing a secondary model active within their dispersed phase.

Findings

Solid lipid nanoparticles prepared with either type of emulsifier were able to provide stable emulsions. Release kinetic data fitting revealed that different co-delivery profiles can be achieved by controlling the surface properties of the lipid nanoparticles. The current proof-of-principle study presents preliminary data that confirm the potential of this approach to be utilised as a flexible liquid-based

platform for the segregated co-encapsulation and independent co-release of different combinations of actives, either hydrophobic/hydrophilic or hydrophobic/hydrophobic, with diverse release profiles.

Keywords: co-encapsulation; co-delivery; solid lipid nanoparticles; Pickering stabilisation; water-in-oil or oil-in-water emulsions; hydrophobic or hydrophilic actives

1. Introduction

The co-encapsulation and co-delivery of two or more active substances within the same vehicle has emerged as an attractive research area, with the majority of published work focusing on applications in combination drug therapy for cancer treatment [1,2], delivery of bioactives and nutraceuticals [3,4], and anti-ageing agents in the cosmetics sector [5]. Combining multiple actives within the same system diminishes the need for individual intake of different active ingredients, while co-delivery offers the benefit of improved physiological functions of the actives due to potential synergistic effects arising from co-administration [6,7]. Wang *et al*. [8] were able to simultaneously and independently deliver to a target tissue two water-insoluble drugs from single multicompartment polymeric hydrogel composed of PEGylated hydrocarbon (triblock copolymer based on PEG and poly(*ε*-caprolactone) (PECT)) and fluorocarbon (copolymer of mPEG and PPFEMA (PEPF)) nanoparticles, thus achieving improved tumour growth inhibition in cancer therapy over single drug treatment using the two actives individually. The benefits of multimodal drug delivery in cancer treatment were also demonstrated following a multicompartmental nanostructure approach, where polymeric PLGA (poly(lactic-co-glycolic acid)) nanocapsules incorporating a hydrophobic active were coated with a phospholipid bilayer encapsulating a secondary hydrophobic active, showing very high entrapment efficiencies and sustained release profiles [9].

Even though multi-delivery has been already successfully employed for solid dosage forms, realising such approaches in liquid-based formulated products, despite the benefits offered (e.g. dosage flexibility, better patient compliance, rapid absorption of the encapsulated actives at the target site), is much more challenging [10–12]. Simple oil-in-water (o/w) or water-in-oil (w/o) emulsions constitute a widely used liquid platform for the encapsulation and delivery of actives, as they can be designed to

offer protection against degradation, increased bioavailability of the actives, and encapsulation of both hydrophobic and hydrophilic molecules [13,14]. Further exploring their functionality, fabrication of multiple emulsions (e.g. w/o/w) has been shown to allow for controlled delivery of encapsulated actives as a response to changes in formulation parameters (e.g. type of oil) or environmental conditions (e.g. temperature), and oil-drug interactions [13,15,16]. In terms of co-encapsulation/co-delivery, a triple emulsion (water-in-oil-in-(oil-in-water)) with a highly compartmentalised structure was previously employed for the segregated co-encapsulation of three photosensitive compounds with varying degrees of hydrophilicity, resulting in their improved stability and protection [17]. Winkler *et al*. [18] employed biphasic PLGA/PCL (poly(lactic-co-glycolic acid)/polycaprolactone) Janus particles for the co-encapsulation of either two hydrophobic actives, using a single o/w emulsion method, or a combination of hydrophilic/hydrophobic compounds, using three different methods to enhance the solubility of the hydrophilic active; single o/w emulsion with partially water-miscible solvent, single o/w emulsion using a co-solvent or double w/o/w emulsions. Dual release was achieved, with high encapsulation efficiencies for the two hydrophobic compounds, while for the combination of hydrophilic/hydrophobic compounds, the double emulsion method yielded the highest encapsulation efficiency. In another study [19], sodium caseinate/chitosan (NaCAS/CS, protein/polysaccharide) co-precipitated complexes (containing a hydrophilic active) were used to stabilise oil-in-water emulsion droplets (containing a secondary hydrophobic active). This approach enabled the segregated co-encapsulation and independent co-release of two incompatible actives from a simple emulsion microstructure.

The present proof-of-principle work aims to demonstrate that the approach of Spyropoulos *et al*. [19] can be successfully applied to emulsions stabilised by solid lipid nanoparticles (SLNs). SLNs have been extensively utilised as single- and multi-drug delivery systems for actives with different characteristics [20–22], but also investigated for their capacity to stabilise (Pickering) emulsions [23–26]. As multi-drug delivery systems, SLNs offer limited control over manipulating the individual release profiles, due to the fact that the encapsulated actives have to migrate out of a crystalline network. As Pickering particles, thus far SLN-stabilised Pickering emulsions have only been utilised for the encapsulation of a single active within their included phase; in this case the lipid nanoparticles principally act as

stabilising agents [26]. Harnessing the advantages offered by the individual components of such a compartmentalised system, could potentially result in the development of an ideal delivery vehicle for the co-encapsulation of different combinations of actives, that could simultaneously address issues associated with controlled delivery. These advantages are introduced by the presence of two different phases that the incorporated actives can release from; a crystalline network offered by the SLNs and a liquid phase provided by the emulsion. In the current study, SLNs fabricated in the presence of two types of surface active species, are utilised for a two-fold purpose; encapsulating a model hydrophobic active (active 1), while at the same time stabilising w/o or

o/w emulsion droplets, containing a secondary model hydrophilic or hydrophobic active (active 2), respectively. The findings reported here offer evidence that depending on the type of emulsifier used during their fabrication, SLNs can provide significant stability to both w/o and o/w emulsions, while in tandem facilitating the generation of a variety of co-delivery profiles. By altering the surface properties of the lipid nanoparticles and in turn the properties of the stabilised emulsions, release kinetics can be controlled. This work not only proposes a novel method to achieve co-encapsulation and co-delivery of different combinations of hydrophobic and hydrophilic actives through the development of a compartmentalised vehicle, but also highlights the impact of the SLN characteristics upon obtaining stable Pickering emulsions with tuneable release profiles.

2. Results and Discussion

2.1 Effect of active encapsulation on the size and stability of SLN and SLN-stabilised emulsions structures

Tripalmitin SLNs were prepared in the presence of either a low molecular weight non-ionic surfactant (Tween 20; SLN1) or a globular protein (whey protein isolate, WPI, SLN2). SLN1 and SLN2 were subsequently used to stabilise w/o and o/w emulsions, respectively, via a Pickering mechanism. Based on theoretical calculations used to estimate the amount of SLN particles necessary to fully cover the surface of the emulsion droplets, it was found that there was excess of SLN particles present in both systems. The possibility of utilising Pickering emulsions for the co-encapsulation and co-delivery of

two segregated actives was assessed by incorporating actives, with different degrees of hydrophilicity, within both the SLNs and the emulsion droplets. Sudan III was used as the model hydrophobic active encapsulated within either type of SLNs (SLN1 or SLN2), while NaCl and dimethyl phthalate (DMP) acted as the model hydrophilic and hydrophobic actives encapsulated within the w/o and o/w emulsion droplets, respectively. Blank tripalmitin SLN1 and SLN2 have been employed in a previous study [25] as Pickering particles for the stabilisation of emulsions prepared with sunflower oil. SLN1 formed w/o emulsions, whereas SLN2 showed an inclination towards providing o/w emulsions. The latter was explained by the overall relative hydrophilic character of the particles, and the tendency of WPI to form a thick layer at the particle interface affecting the polymorphic state of the lipid crystals, and consequently the polarity of the particles. Similar observations were made in the current study when the same type of particles, but with Sudan III encapsulated within them, were assessed for their Pickering functionality. Size measurements of SLN1 and SLN2 showed that entrapment of Sudan III did not affect particle dimensions. Both types of particles were characterised by bimodal size distributions, which remained unchanged over a storage period of 9 weeks at 4°C. SLN1 showed two distinct size populations at 150 nm and 1.3 μm, while SLN2 displayed two peaks at 150 and 700 nm (*Figure 1A)*. The presence of the surface active species had a significant effect on both the average size of the particles, and their size distribution profile, which was further supported by SEM imaging (*Figures 1B & C)*. Stability of the SLN-formed Pickering emulsions was confirmed as the average droplet size, $D_{3,3}$ (measured using pulsed field gradient NMR) or $D_{3,2}$ (measured using laser diffraction) for the w/o or o/w Pickering systems, respectively, did not change significantly over a storage period of 50 days (*Figure 1D)*. The long-term storage stability of the SLN-stabilised emulsions could be the result of a synergistic mechanism between the surfactant and the colloidal lipid particles present in the system [27–29].

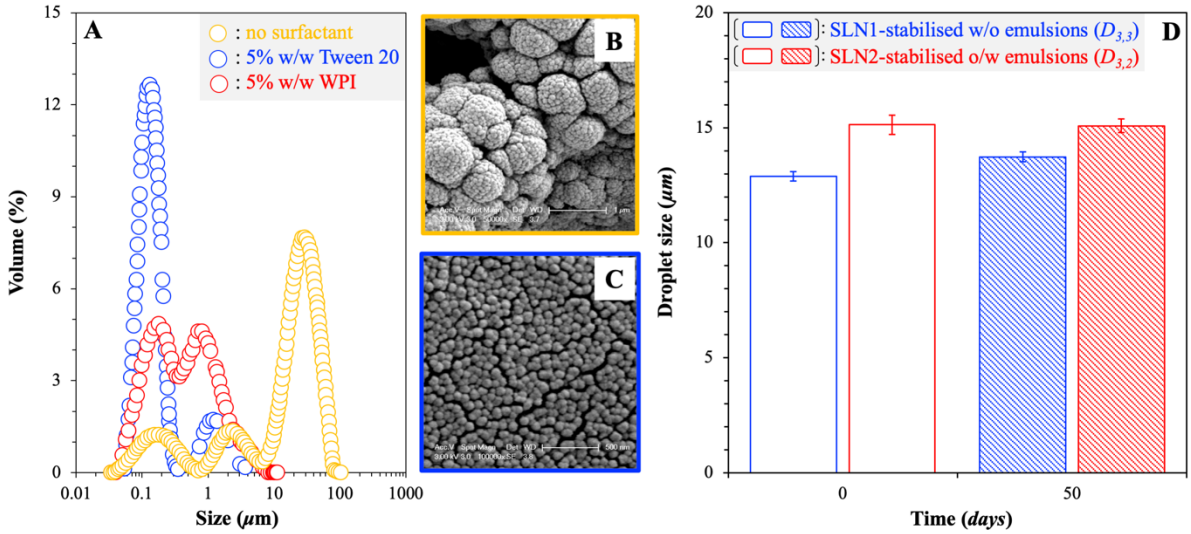

Figure 1. A. Particle size distribution of 5% w/w tripalmitin SLNs with no surfactant, and in the presence of 5% w/w Tween 20 (SLN1), and 5% w/w WPI (SLN2); Data reproduced from [25] - Published by The Royal Society of Chemistry. Cryo-SEM images of tripalmitin SLN particles fabricated with no surfactant (**B**), or in the presence of 5% w/w Tween 20 (**C**; SLN1). **D.** Droplet size measurements over a storage period of 50 days for SLN1-stabilised w/o Pickering emulsions ($D_{3,3}$; measured using pulsed field gradient NMR) and SLN2-stabilised o/w Pickering emulsions ($D_{3,2}$; measured using laser diffraction).

2.2 Co-delivery of hydrophobic/hydrophobic combination of actives

The co-release of Sudan III and DMP co-encapsulated within the SLN1-stabilised w/o emulsions, prepared using sunflower oil as the continuous phase, was investigated (*Figure 2A* and *B*). The discharge of Sudan III in sunflower oil serving as the dissolution medium, displayed a burst release with 96% of the active being liberated within the first 2-3 hours. The overall release percentage achieved corresponds to active discharge from both adsorbed and unadsorbed SLNs onto the water droplets' interface. Similar observations of burst release have been made in other studies [30,31], which were attributed to active enrichment of the particles' outer shell (suggesting a drug-enriched shell model) and the large surface area of the nanoparticles. Subsequent slow release could be the result of diffusion of the remaining active in the inner regions of the lipid matrix [32]. Fitting the release data into various empirical and semi-empirical kinetic models showed that the release of Sudan III is best described by the Weibull model (*Figure S2 & Table S2)*. According to a previous study [33] relating the α and β Weibull model parameters to diffusivity, it is expected that for low diffusion coefficient values, parameter α is also low, which was indeed the case here (*Table S2*). Regarding parameter β, also known as the shape parameter, the predicted value ($\beta > 1$) denotes that the position of the active near the leak boundaries allows for an increasing release rate, averting the creation of a depletion zone. The relatively

low *D* values calculated here align with the hypothesis that the burst release profile of Sudan III is potentially due to the presence of the active near the particles' surface.

Data fitting to the mechanistic models proposed by Crank [34] and Guy *et al*. [35], suggest that diffusion was the rate-limiting step for the release of Sudan III from SLN1; both models gave diffusion coefficient (*D*) values in the range of 10^{-14} cm^2 s^{-1}. Literature data regarding the diffusion coefficients of actives releasing from SLNs is limited. Emami *et al*. investigated the use of glycerin monostearate and cholesterol-based solid lipid nanoparticles for the delivery of budesonide. Fitting the Crank and diffusion-limited models used here to the release data reported for the optimised SLN (~218 nm) formulation in the latter study, give diffusion coefficients of 1.43×10^{-16} and 3.70×10^{-16} cm^2 s^{-1}, respectively. In another study with relevance to cosmeceutics [36], Tiyaboonchai *et al*. monitored the release of curcuminoids from stearic acid-based solid lipid nanoparticles (~450 nm). Fitting (by the current authors) the release data obtained after incorporating the curcuminoid-loaded SLNs into a cream formulation (as reported by Tiyaboonchai *et al*.) gave *D* values of 8.41×10^{-16} and 5.23×10^{-16} cm^2 s^{-1} for the Crank and diffusion-limited models, respectively. The larger *D* values determined for the systems studied here could be the result of poor distribution of Sudan III within the lipid matrix of the SLN1 particles. Overall, fitting of either type of kinetic model indicates that the previously hypothesised disproportional positioning of Sudan III near the particles' surface could be responsible for the exaggerated diffusion coefficients calculated by the models.

On the other hand, NaCl release (carried out in water as the dissolution medium and quantified by conductivity measurements over a period of 17 days) was negligible; only exhibiting 0.3% discharge at the end of the monitoring period (inset *Figure 2A*). This could have been caused by sintering of tripalmitin particles at the interface of the water droplets. As it has been previously shown [25], even though tripalmitin particles covered with Tween 20 were able to stabilise w/o emulsions over long storage periods, they could not provide sufficient steric protection against particle sintering. Consequently, formation of a tripalmitin solid lipid layer around the emulsion droplets could be the reason behind the release inhibition of the encapsulated active.

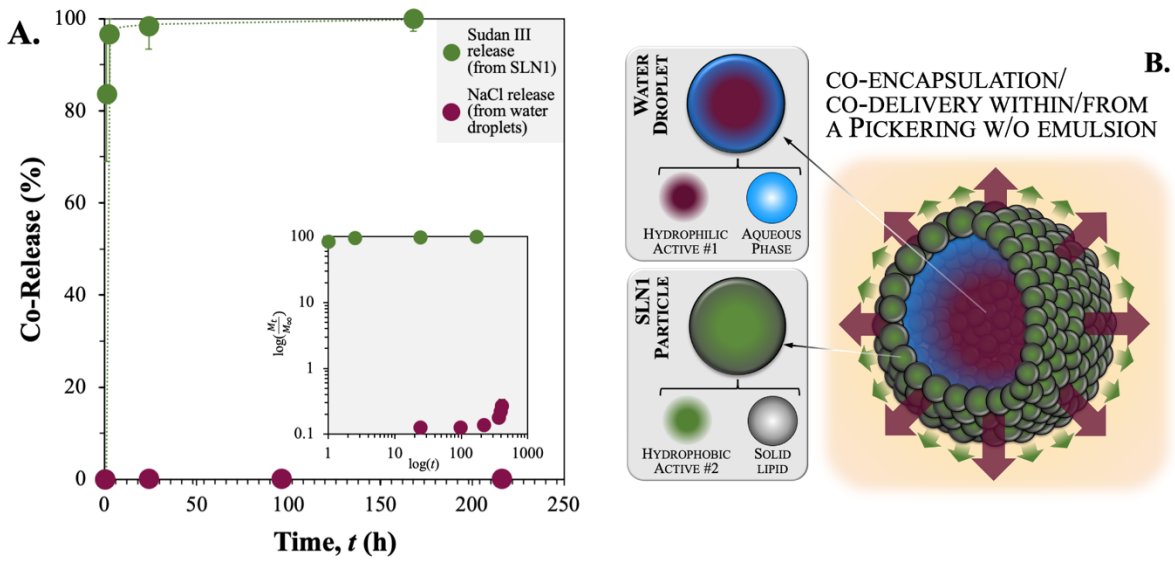

Figure 2. A. Co-release profiles of NaCl and Sudan III contained within the aqueous droplets of a w/o emulsion and the SNL1 lipid particles stabilising these, respectively. **B.** Schematic representation of the segregated co-encapsulation of a hydrophilic and a hydrophobic active within a lipid particle (SLN1) stabilised w/o Pickering emulsion microstructure.

Particle sintering could potentially emerge from emulsifier-related events at the surface of the lipid particles; such as adsorption of the surface active species to the tripalmitin crystals in such a way, that the non-polar groups of the emulsifier are incorporated within the lipid crystal structure, leaving the polar groups exposed [25]. Interaction between the polar groups of the emulsifier present onto neighbouring lipid particles, such as hydrogen bonding, can lead to effective Pickering stabilisation [37], and therefore also to release hindrance caused by the inability of the active to permeate the dense lipid layer barrier.

To further assess the effect of the type of oil on the release kinetics of the incorporative active from SLN1, three different oils with varying values of relative polarity were studied. Mineral and silicone oils have polarity indices of 43.7 and 26.6 mN m^{-1} respectively, while sunflower oil has a lower value of 19.3 mN m^{-1} [38]. w/o emulsions were prepared using either mineral or silicone oil as the continuous phase (in a similar manner to those formed with sunflower oil) and the release of NaCl was measured. The overall NaCl release percentage for all types of oils remained below 1% (*Figure S1)*. However, a slight increase in the rate of release could be observed for the two alternative oil types compared to that of the previously used sunflower oil, suggesting that choosing an oil with different properties (e.g. higher polarity) could potentially result in sustained release profiles. An enhancement of sintering

206 phenomena and the higher affinity of the Tween 20 covered tripalmitin particles for sunflower oil could
207 be responsible for the greater NaCl release reduction in this case. Heating the systems to a temperature
208 above 40°C, the melting point of the lipid particles, resulted in emulsion destabilisation and subsequent
209 complete release of the encapsulated NaCl to the external water phase.
210
211 **2.3 Co-delivery of hydrophobic/hydrophilic combination of actives**
212 In addition, the co-release of two model hydrophobic actives encapsulated within Pickering o/w
213 emulsions stabilised by the SLN2 particles was studied. DMP (0.2% w/w) served as a model
214 hydrophobic active encapsulated within the sunflower oil droplets of the o/w emulsions, while Sudan
215 III (secondary model hydrophobic active) was contained within the SLN2 particles. Sudan III release
216 into sunflower oil (serving as the acceptor phase) was shown to be practically non-existent; 0.2% of the
217 active was detected after 4 days (inset *Figure 3A*). DSC and microscopic analysis previously [25]
218 performed on SLN2 particles had indicated that WPI adsorbs onto the lipid particle interface, rather
219 than penetrating into the lipid crystal network, while there were no signs of particle sintering. The
220 presence of a thick layer of WPI around the particles, in combination with the α polymorphic form of
221 tripalmitin further supported the increased polarity of SLN2 compared to SLN1 [25,39]. The
222 hydrophilicity of the emulsifier used, which is effectively exerted onto the lipid entities it stabilises, is
223 an important parameter affecting the release kinetics of particle-enclosed species [40]. This, in tandem
224 with the presence of a thick WPI layer at the surface of the particles, enhances the barrier that the
225 encapsulated hydrophobic active needs to overcome in order to cross over into the external phase, and
226 thus significantly hinders the rate of its release [41]. The obtained release profile could also indicate a
227 drug-enriched core model regarding the incorporation and distribution of the active within the lipid
228 crystal matrix [42]. It is therefore apparent that altering the surface active component used in the
229 fabrication of the lipid particles, not only changes the type of emulsion that the latter can provide, but
230 also results in a profound shift in the release kinetics of the same encapsulated species (Sudan III).

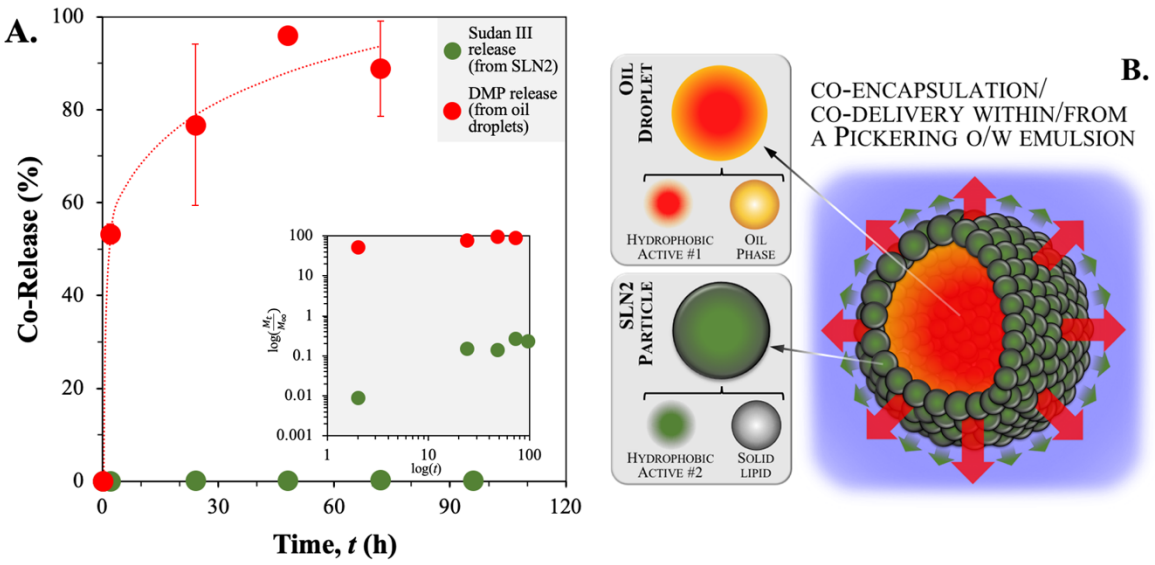

Figure 3. A. Co-release profiles of DMP and Sudan III contained within the oil droplets of an o/w emulsion and the SNL2 lipid particles stabilising these, respectively. **B.** Schematic representation of the segregated co-encapsulation of two hydrophobic actives within a lipid particle (SLN2) stabilised o/w Pickering emulsion microstructure.

Regarding the release kinetics of DMP contained within the oil droplets of the SLN2-stabilised o/w emulsions, the active was almost entirely discharged within 48 hours (*Figure 3A*). In current literature, active release from emulsion droplets has been discussed using two mechanisms; a diffusion-limited model or an interfacial barrier-limited model [35,43]. Application of the diffusion-limited and the Crank models gave diffusion coefficients in the range of 10^{-13} cm^2 s^{-1}. However, these values are significantly lower to the diffusion coefficient estimated using the Stokes-Einstein equation ($D = 8.5 \times 10^{-8}$ cm^2 s^{-1}). What is more, a study [44] on the release of tetracaine, a moderately hydrophobic molecule, from medium chain triglyceride emulsion droplets stabilised by lecithin reported a diffusion coefficient value of 5.46×10^{-7} cm^2 s^{-1}. Therefore, there is clear indication that DMP release in the current systems is not limited by diffusion (*Table S2 & Figure S3*). Instead, applying the interfacial barrier-limited model to the DMP release profile gave an interfacial rate constant (k_I) of 3.09×10^{-12} cm^2 s^{-1}, which is slightly higher but comparable to the value reported for DMP-loaded o/w Pickering emulsions stabilised by NaCaS/CS co-precipitates, which also followed an interfacial barrier controlled release [19], in addition to other literature on the release of hydrophobic actives from emulsions [40,43]. It is therefore evident, that the SNL2 fabricated in the presence of WPI offer a significant barrier for the passage of DMP into the external phase (*Figure 3B*).

3. Conclusions

In conclusion, the present proof-of-principle study demonstrates that the approach proposed by Spyropoulos *et al*. [19] can be applied to SLN-stabilised emulsions to enable the co-encapsulation and independent co-delivery of two segregated actives. The current work significantly extends previous research efforts in the area of compartmentalised multi-delivery of actives using liquid-based systems [8,9,17,18], by confirming that the employed co-encapsulation strategy is applicable to both w/o and o/w simple emulsions and can be utilised for the co-delivery of different combinations of actives with varying degrees of hydrophilicity/hydrophobicity. In addition, the current preliminary findings underline the effect of the emulsifier used during fabrication of SLNs on the attainment of different release profiles from the SLN-stabilised Pickering emulsions, a concept that has been previously described only for o/w emulsions [14]. Overall, this exploratory work greatly enhances the versatility and flexibility of the co-encapsulation/co-delivery simple emulsion strategy reported previously [19], and thus significantly aid research efforts towards the realisation of multi-active delivery approaches from liquid formulations. Further research should focus on confirming and fully understanding the release mechanisms proposed here, studying additional Pickering colloidal species that can be functionalised in the same manner and investigating means to expand the currently-realised co-release kinetics arsenal. The ultimate research goal is to be able to routinely and robustly fabricate such liquid-based formulations and to realise their potential applications in the foods, pharmaceutics, agrochemical and cosmetics sectors.

Acknowledgements

The authors would like to thank the Engineering and Physical Research Council (EPSRC, UK – Grant Number EP/L015153/1), Innovate UK (UK – Grant Number 100870) and Syngenta for funding the work presented here. The authors would also like to thank Pat Mulqueen at Syngenta (Jealott's Hill International Research Centre) for useful discussions.

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Supplementary Data for:

Independent co-delivery of model actives with different degrees of hydrophilicity from oil-in-water and water-in-oil emulsions stabilised by solid lipid particles via a Pickering mechanism: a-proof-of-principle study

S1. Experimental Section

S1.1 Materials

Tripalmitin (purity ≥85%), Sudan III, silicone oil, mineral oil, dimethyl phthalate (DMP, ≥ 99%), potassium sorbate (≥99%) and Tween 20 (HLB = 16.7) were purchased from Sigma Aldrich (UK). Whey protein isolate (WPI) was kindly gifted by Davisco (Davisco Foods International). Sodium chloride (NaCl, technical grade) was purchased from Fischer Scientific (UK). Commercially available sunflower oil was used for emulsions preparation. Distilled water (pH = 6.8, 1.63 μS cm^{-1}) was employed throughout this study. All materials were used without further purification. All compositions are reported as weight of the individual substance per weight of the total system; % (w/w).

S1.2 Methods

S1.2.1 Preparation of solid lipid nanoparticles

For the preparation of solid lipid nanoparticles (SLN), a melt-emulsification-ultrasonication method was followed that has been previously described elsewhere [S1]. SLN particles were fabricated in the presence of either Tween 20 (SLN1) or WPI (SLN2), both at 5% (w/w). Briefly, 5% (w/w) of tripalmitin used as the solid lipid was heated up to 75°C (approximately 10°C higher than the melting point of the lipid) and was added to the aqueous phase containing the surfactant heated at the same temperature, while mixed with a magnetic stirrer. The hot premix was then homogenised using a high intensity ultrasonic processor (Vibra Cell VCX 500, Sonics, USA) operating continuously for 3 min using a 12 mm probe (20 kHz, 95% amplitude). The dispersions formed were subsequently cooled down to ~4°C using an ice bath. SLNs were prepared with 0.05% (w/w) of the hydrophobic model active Sudan III. To ensure complete solubilisation of the active in the lipid phase, Sudan III and tripalmitin were stirred together above the melting point of the lipid for approximately 0.5 h 0.01% (w/w) of potassium sorbate was used as a preserving/antimicrobial agent in all formulations. To avoid phase inversion due to the presence of excess Tween 20, the aqueous SLN1 dispersions used as the included phase in the preparation of w/o emulsions were dialysed using distilled water by immersing the colloidal suspensions in pre-hydrated cellulose tubing (35 mm width, 12 kDa MW cut-off; Sigma Aldrich, UK). To ensure full removal of the excess surfactant, the distilled water was refreshed until a constant surface tension value was obtained. Unless otherwise stated, all SLN aqueous dispersions were kept at 4°C.

S1.2.2 Preparation of Pickering emulsions

All w/o and o/w emulsions were prepared using commercially available sunflower oil, silicone oil or mineral oil without further purification. Unless stated otherwise, sunflower oil was the oil phase used for emulsion production. For w/o emulsions, 20% (w/w) of the aqueous phase containing SLN1 were combined with 2% (w/w) of the model hydrophilic active NaCl (10% w/w solution) and 78% (w/w) oil phase. For o/w emulsions, 80% (w/w) of the aqueous phase containing SLN2 were combined with 19.8% (w/w) of oil phase and 0.2% (w/w) of the model hydrophobic active DMP. The mixtures were then homogenised using a high-shear mixer (Silverson L5M, Silverson Machines Ltd, UK) at 10,000 rpm for 2 minutes, while cooled in an ice bath to avoid shear-induced heating of the samples and melting of the lipid particles. The final composition of the SLN-stabilised Pickering emulsions is given in *Table S1*. Based on theoretical calculations, the quantity of SLNs used in either systems is enough to provide complete surface coverage of the emulsion droplets. Unless otherwise stated, all emulsions were stored at 4°C.

Table S1. Composition of Pickering stabilised w/o and o/w emulsions by tripalmitin solid lipid nanoparticles (SLN).

SLN1-stabilised w/o emulsions

Phase	Component	Composition (%)
SLN1-containing aqueous phase	*Tripalmitin*	1
	Tween 20	1*
	Sudan III	0.01
	Potassium sorbate	0.01
	NaCl	0.2
	Distilled H_2O	19.78
Oil phase	*sunflower, mineral, or silicone oil*	78

SLN2-stabilised o/w emulsions

Phase	Component	Composition (%)
Oil phase	*Sunflower oil*	19.8
	Dimethyl phthalate (DMP)	0.2
SLN2-containing aqueous phase	*Tripalmitin*	4
	WPI	4
	Sudan III	0.04
	Potassium sorbate	0.01
	Distilled H_2O	71.95

*The % composition of Tween 20 refers to the amount used for the production of SLN1 before dialysis.

S1.2.3 Microstructure characterisation

Characterisation of the SLN and emulsion microstructures formed in this work was carried out following the procedures used by Pawlik *et al.* [S1] *Size measurements*. Laser diffraction (Mastersizer® Hydro 2000SM, Malvern, UK) was performed to measure the size of the lipid nanoparticles and the droplet size of o/w emulsions, and pulsed field gradient NMR (Bruker Minispec, UK) to measure the size of the w/o emulsions. *Microscopy*. Images of the tripalmitin particle dispersions were obtained using a Phillips XL30 FEG Cryo Scanning Electron Microscope (SEM) equipped with a Gatan low temperature unit. An emulsion drop was placed on an analysis slide and dipped into nitrogen at −198°C.

The slide was then directly inserted into a preparation chamber at −180°C, where it was fractured and etched for 5 min at −90°C. The surface was subsequently coated in gold and imaged at −130°C.

S1.2.4 Release measurements

Release experiments for the lipophilic active Sudan III encapsulated within the SLN1 or SLN2 particles were carried out by placing a known quantity (40-45 g) of the SLN-stabilised Pickering emulsions, either the o/w or w/o systems, at the bottom of a beaker and gently topping it up with 50 g of excess (pure) oil (sunflower, silicone, or mineral oil) serving as the acceptor phase. Aliquots (~1 mL) were withdrawn at timed intervals from the acceptor phase (oil phase), passed through a syringe filter (0.2 μm pore size) and analysed using ultraviolet-visible (UV-VIS) spectroscopy (Thermo-Scientific®, UK) at λ = 510 nm. The release of NaCl from the SLN1-stabilised w/o emulsion droplets was studied by gently adding ~45 g of the emulsion on top of ~70 g of distilled water which were already placed in a beaker (external phase). A probe (S30 SevenEasy™ fitted with an InLab® 710 platinum 4-plate electrode, Mettler Toledo, UK), which was pre-positioned at the bottom of the beaker, was used to measure the conductivity of the external aqueous phase at regular time intervals. DMP release from the SLN2-stabilised o/w emulsion droplets was measured by placing ~10 g of the emulsion inside a pre-hydrated cellulose tube (Sigma Aldrich Company Ltd., UK, 35 mm width, M.W. cut-off of 12 kDa) and dialysed to a large quantity (~100 g) of water (acceptor phase) in order to create sink conditions. Aliquots of ~1 mL were withdrawn from the acceptor phase at regular time intervals, pressed through a syringe filter (0.2 μm pore size) and analysed using UV-VIS spectroscopy (at λ = 290 nm). During preliminary studies, it was shown that the DMP release rate from the oil droplets was controlled by transfer across the interface of the emulsion rather than limited by the passage of the active across the dialysis membrane. In all cases, the amount of active released into an acceptor phase was determined using previously obtained calibration curves. All release experiments were performed at room temperature.

S2. Results

S2.1 Effect of oil type on the release kinetics

The effect of the type of oil used as the continuous phase in the w/o emulsions on the release kinetics of NaCl encapsulated within the water droplets was investigated. A number of oils with different polarities were chosen; sunflower oil, mineral oil and silicone oil. NaCl release was monitored over a period of 17 days (*Figure S1*).

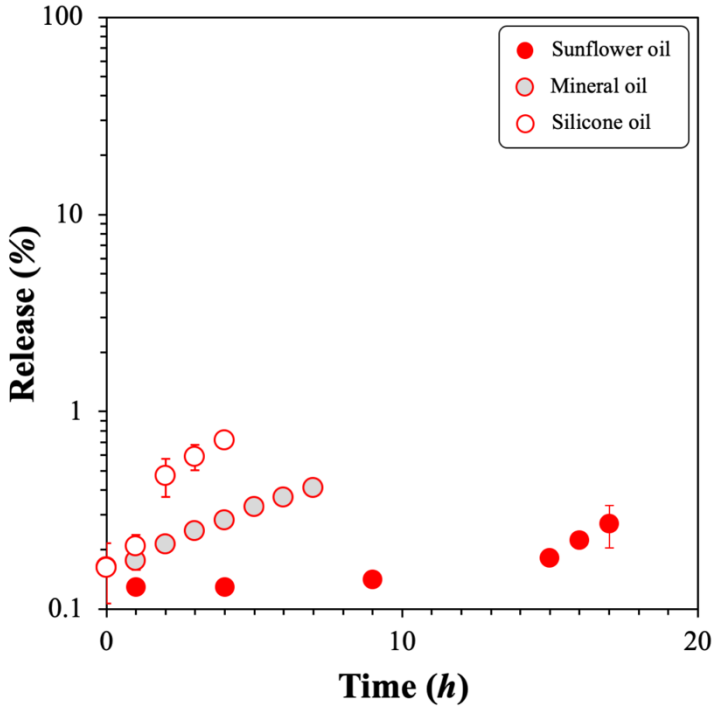

Figure S1. Release profile of model hydrophilic active NaCl from w/o Pickering emulsions prepared using three different types of oils, sunflower, mineral and silicone oil, stabilised by solid lipid nanoparticles (SLN1) containing a hydrophobic active (Sudan III).

S2.2 Modelling of release data

Various empirical and semi-empirical release kinetic models (First order, Higuchi, Hixson-Crowell, Baker-Lonsdale, Makoid-Banakar, Weibull) were evaluated for their capacity to describe the individual release profiles obtained for only those actives exhibiting appreciable release; these were Sudan III (encapsulated within the SLN1 particles stabilising w/o Pickering emulsion droplets) and DMP (encapsulated within the oil droplets of the SLN2-stabilised o/w emulsions). Fitting was performed using the Curve Fitting Toolbox™ 3.5.11 in Matlab® R2020a (MathWorks Inc., Natick, MA, USA). The coefficient of determination (R^2), the adjusted coefficient of determination (R^2_{adj}) and the root mean square error (RMSE) were used as model selection indicators. Only the Makoid-Banakar and Weibull models were deemed capable of providing satisfactory fits to the experimental data; the results for these models are presented in *Table S2*.

Mechanistic models were also examined for their ability to describe the Sudan III and DMP release profile data. Regarding the release of Sudan III from SLN1, the expression proposed by Crank [S2] gave ($R^2 = 0.99$) an effective diffusion coefficient (D) of 3.12×10^{-14} cm^2 s^{-1} (*Table S2*). Guy *et al.* [S3] developed a diffusion-limited model that assumes that the release of an active is predominantly governed by its diffusion through the matrix of the spherical enclosure containing it. The diffusion coefficient calculated in this case gave a D value of 3.11×10^{-14} cm^2 s^{-1} ($R^2 = 0.98$); thus very close to the diffusion coefficient determined by the Crank model (*Table S2).*

Release of actives encapsulated within emulsion droplets (as in the case of DMP discharge from the SLN2-stabilised o/w emulsions studied here) has been discussed using two limiting mechanistic models [S3]. According to these, release can either be limited by diffusion of the active through the phase that constitutes the emulsion droplet, or driven by transfer across the interfacial barrier present at the surface of the droplet [S3,S4]. Application of the diffusion-limited model (R^2 = 0.94) lead to a diffusion coefficient value of 6.98×10^{-13} cm^2 s^{-1} (*Table S2*); fitting the Crank model gave a similar value of 7.22×10^{-13} cm^2 s^{-1} (R^2 = 0.93). Fitting of the empirical and semi-empirical models revealed that the Weibull and Makoid-Banakar models were able to provide good fits to the experimental data (*Table S2*).

The release profiles for both Sudan III and DMP, as generated using the model parameters given in *Table S2*, are presented (together with the experimental release data) in *Figures S2* and *S3*, respectively.

Table S2. Best fit parameters to relevant empirical, semi-empirical and mechanistic kinetic models for the release of Sudan III (encapsulated within the SLN1 particles stabilising w/o emulsion droplets) and DMP (encapsulated within the oil droplets of the SLN2-stabilised o/w emulsions).

		Sudan III				***DMP***			
Model	**Equation**	**Parameters**	R^2	R^2_{adj}	**RMSE**	**Parameters**	R^2	R^2_{adj}	**RMSE**
Makoid-Banakar [S5]	$Q_t = k_{MB} t^n \exp(-kt)$	k_{MB} = 82.28 n = 0.08 k = 0.001	0.78	0.55	28.69	k_{MB} = 86.10 n = 0.21 k = 0.06	0.93	0.78	8.88
Weibull [S5]	$\frac{Q_t}{Q_\infty} = 1 - exp\left(-\frac{t^\beta}{a}\right)$	α = 0.55 β = 3.26	0.99	0.99	2.14	α = 0.59 β = 0.36	0.92	0.87	6.68
Crank [S2]	$\frac{Q_t}{Q_\infty} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-\frac{Dn^2\pi^2 t}{r^2}\right)$	D = 3.12×10^{-14}	0.99	0.99	15.57	D = 7.22×10^{-13}	0.93	0.93	11.67
Guy Diffusion-limited [S3]	$\frac{Q_t}{Q_\infty} = 1 - \frac{6}{\pi^2}\exp\left(-\frac{\pi^2 D}{r^2}t\right)$	D = 3.11×10^{-14}	0.98	0.98	20.80	D = 6.98×10^{-13}	0.94	0.94	6.18
Guy Interfacial barrier-limited [S3]	$\frac{Q_t}{Q_\infty} = 1 - \exp\left(\frac{-3k_I}{r^2}t\right)$	k_I = 5.62×10^{21}	0.34	0.34	45.88	k_I = 3.09×10^{-12}	0.94	0.94	20.76

R^2: coefficient of determination; R^2_{adj}: adjusted coefficient of determination; RMSE: root mean square error; Q_t: amount of active released at time t; Q_∞: total amount of active released when the formulation is exhausted; k_{MB}: release constant (*Makoid-Banakar*); n, k: empirical parameters (*Makoid-Banakar*); α: scale parameter (*Weibull*); β: shape parameter (*Weibull*); n: dummy variable (*Crank*); r: particle/emulsion droplet radius, D: apparent diffusion coefficient of the active within the system, k_I: interfacial rate constant. The values for D and k_I are given in cm^2 s^{-1}.

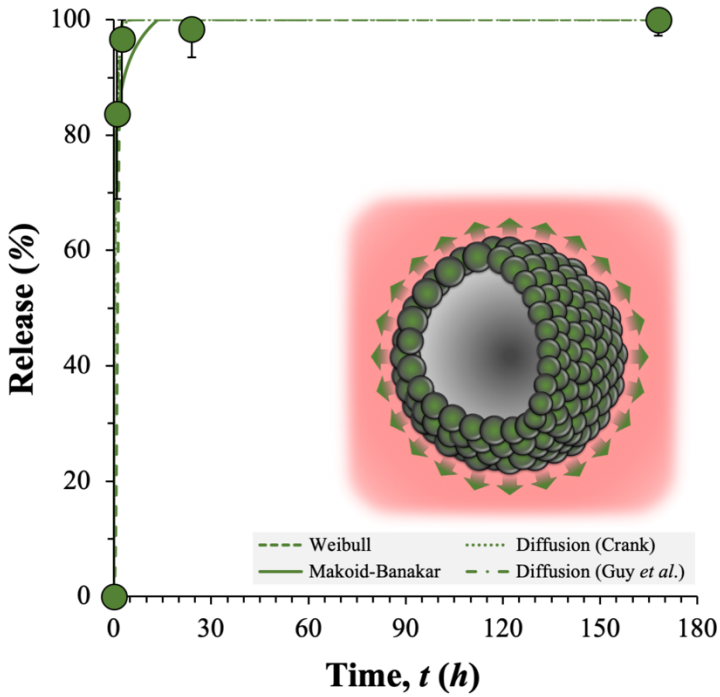

Figure S2. Release kinetic model fitting for Sudan III (encapsulated within the SLN1 particles stabilising w/o emulsion droplets) for the empirical and semi-empirical Weibull and Makoid-Banakar models, and for the mechanistic models for diffusion-controlled release proposed by Crank [S2] and Guy *et al* [S3].

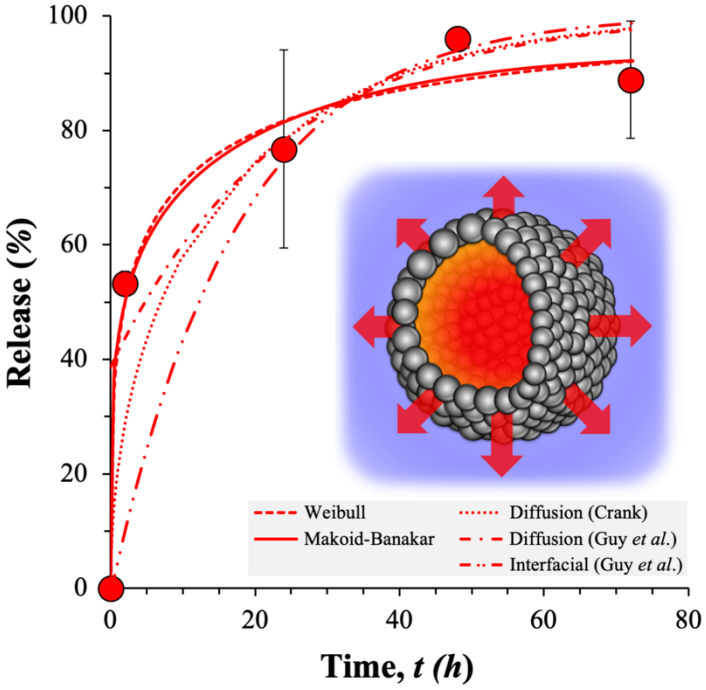

Figure S3. Release kinetic model fitting for *DMP* (encapsulated within the oil droplets of the SLN2-stabilised o/w emulsions) for the empirical and semi-empirical Weibull and Makoid-Banakar models, and for the mechanistic model for interfacial barrier-limited release proposed by Guy *et al.* [S3].

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