ChemComm
Chemical Communications

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ROYAL SOCIETY OF CHEMISTRY

rsc.li/chemcomm

View Article Online
DOI: 10.1039/D1CC00819F

COMMUNICATION

Interfacial activity of modified Dextran polysaccharide to produce enzyme-responsive oil-in-water nanoemulsions

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Marcos Navascuez,[a,b] Raquel Gracia,[a] Marco Marradi,[a,c] Natividad Díaz,[a] Javier Rodríguez,[a] Iraida Loinaz,[a] Fernando López-Gállego,[b,d] Jordi Llop[b,e] and Damien Dupin[a,†]

Herein, we report the evaluation of Dextran (DXT) derivatives bearing hydrophobic or hydrophilic functional groups as stabilisers of Oil-in-Water (O/W) emulsions. All the investigated modifications conferred interfacial activity to produce stable O/W emulsions, being methacrylate(MA)-functionalised DXT the most promising stabiliser. A minimum amount of MA was required to obtain stable O/W nanoemulsions, which could be degraded in the presence of lipases.

Nanoemulsions (NEs) are heterogeneous systems based on the dispersion of nanodroplets (< 500 nm) of one phase emulsified in a second immiscible phase, resulting very attractive for encapsulation of active molecules in cosmetic and pharmaceutical formulations.[1,2] Low molecular weight surfactants commonly used to stabilise emulsions (e.g. sodium dodecyl sulfate) can exhibit undesired cytotoxicity,[3] while macromolecular PEGylated synthetic emulsifiers such as Cremophor, poloxamers and Triton X, have recently raised concerns due to Complement Activation-Related Pseudoallergy (CARPA).[4,5] Also, the lack of biodegradability of polyolefin based surfactant can cause detrimental environmental effect, as judged by increasing public concern.[6] Thus, there is an urgent need for sustainable emulsifiers from natural resources.

Polysaccharides represent a good alternative to PEG for their good water solubility, low toxicity, degradability and low interaction with living organism. Modified polysaccharides have been successfully used as Oil-in-Water (O/W) emulsion stabilizers to produce hydrophilic nanoparticles,[7–9] and emulsions.[10,11] For example, cellulose functionalized with sufficient hydrophobic alkyl chains acts as non-ionic emulsion stabiliser.[12] On the other hand, dextran (DXT), a natural biocompatible, neutral and cheap polysaccharide consisting of α-1,6 glycosidic linkages with α-1,3 ramifications, has been used as emulsifier to produce emulsions after functionalization with phenoxy or alkyl groups.[13–18] From our experience, DXT can be easily modified with methacrylate (MA) groups (DXT-MA),[19,20] and further undergo chemical cross-linking reactions to produce DXT-based hydrogels[20,21] or single chain polymer nanoparticles (DXT-SCPNs) of around 20 nm.[22,23] Remarkably, this synthetic protocol controls both the size and the functionality of SCPNs, making these nanoparticles promising drug delivery and imaging agents. However, to the best of our knowledge dextran derivatives and DXT-SCPNs have never been used as emulsifiers to produce O/W emulsions.

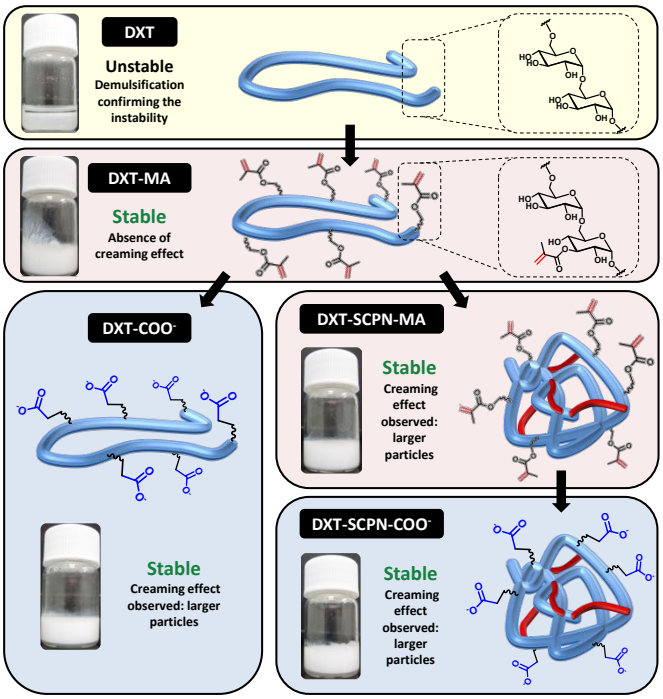

Figure 1. Schematic representation of the structure for pristine dextran (DXT) and dextran derivatives (DXT-MA, DXT-COO⁻, DXT-SCPN-MA and DXT-SCPN-COO⁻) combined with digital photographs of the resulting O/W emulsions prepared using 50wt% *n*-dodecane as the oil phase and 0.5wt% of stabiliser and sonicated during 4 min. Photographs obtained after storing for 24 hrs at 4°C. Pristine DXT emulsion showed phase separation after 1 hr.

In this work, we systematically investigated the capacity of DXT and DXT-SCPN functionalised with MA groups (DXT-MA and DXT-SCPN-MA, respectively) or carboxylate groups (DXT-COO⁻ and DXT-SCPN-COO⁻) to stabilise O/W emulsions produced by sonication (Figure 1, see ESI and Fig S1-S6).

[a] CIDETEC, Basque Research and Technology Alliance (BRTA), Parque Científico y Tecnológico de Gipuzkoa, Miramón Pasealekua, 196, Donostia-San Sebastián 20014, Spain.
[b] CIC biomaGUNE, Basque Research and Technology Alliance (BRTA), 20014 San Sebastián, Guipuzcoa, Spain.
[c] Department of Chemistry "Ugo Schiff", University of Florence, via della Lastruccia 13, 50019 Sesto Fiorentino (FI), Italy.
[d] IKERBASQUE, Basque Foundation for Science, María Díaz de Haro 3, 48013 Bilbao, Spain.
[e] Centro de Investigación Biomédica en Red, Enfermedades Respiratorias – CIBERES
[f] † e-mail: ddupin@cidetec.es
[g] Electronic Supplementary Information (ESI) available: Detailed experimental methods, additional characterisation data. See DOI: 10.1039/x0xx00000x

First, the most promising emulsifier was selected based on its surface tension (ST) value, the final droplet size of the emulsion produced, i.e. below 500 nm, narrow size distribution, and good emulsion stability (> 1 week), i.e. with no phase separation and unchanged hydrodynamic diameter (D_h), as judged by visual inspection and Dynamic Light Scattering (DLS), respectively. Next, the effect of the degree of substitution (DS) on the interfacial activity was investigated. Finally, envisaging the potential applications in the controlled delivery of hydrophobic drugs,[24] emulsion stability was evaluated in the presence of lipases, a family of enzymes that can hydrolyse ester bonds.

Pristine DXT did not show any interfacial activity, as already reported.[14] A clear phase separation was observed after sonication of *n*-dodecane or vegetable oils such as olive oil and sunflower oil, either at 10 or 50 wt%, with 0.5 wt% of an aqueous solution of DXT (See Table 1 and Fig. 1). On the other hand, stable O/W emulsions were achieved with our four selected stabilisers (from DXT-MA with DS = 52%) when *n*-dodecane was used as non-polar dispersed phase (Fig. 1). As expected, the presence of hydrophobic MA groups resulted in enhanced interfacial activity. Counter-intuitively, highly hydrophilic DXT-COO^- and DXT-SCPN-COO^- showed sufficient interfacial activities to stabilize O/W emulsions, despite the lack of hydrophobic groups in their structure that anticipated low affinity towards the oil phase. The unexpected stabilising capacity of both DXT-COO^- and SCPN-DXT-COO^- was first attributed to the relatively low pH of emulsification and the presence of COOH groups, at around pH 5.5. However, the same emulsions prepared at pH 7.4 exhibited smaller and stable droplets size (see Table 1 and ESI Table S1). Thus, the interfacial activity of DXT-COO^- and DXT-SCPN-COO^- is independent of the solution pH. Further works are currently under progress to understand such unexpected behaviour.

Moving towards potential biomedical applications, biocompatible oils (i.e. olive and sunflower oils) were next used to evaluate the interfacial properties of the emulsifier. Emulsions characterization was carried out by DLS to evaluate droplet D_h and Laser Diffraction (LD) to assess stability under recirculation and size distribution.[24] In all cases, stable emulsions were produced using 10wt% oil and 0.5wt% DXT derivatives by sonication (see ESI Table S2).

First, ST studies showed that the addition of functional groups significantly lowered ST compared to pristine DXT (68.0 mN/m).[15] The hydrophilic modification resulted in ST values of 53.9 and 55.9 mN/m for DXT-COO^- and DXT-SCPN-COO^- at pH 5.5, respectively, while the presence of hydrophobic MA groups decreased ST values to 45.1 mN/m for DXT-MA and 48.2 mN/m for DXT-SCPN-MA, similarly to ST reported for phenoxy-functionalized DXT.[15] Vigorous shaking of aqueous solutions of DXT derivatives at 0.5wt% resulted in foam formation, more abundant in the case of DXT-MA and SCPN-DXT-MA (see ESI, Fig. S7), confirming their higher surface activity (lower ST). Unlike previously reported for modified DXT,[16,18] the precise determination of the interfacial tension between oil and water in the presence of any of the DXT emulsifiers was not possible, confirming a slow adsorption at the oil/water interface, as already reported for amphiphilic DXT.[17]

Table 1. Summary of the hydrodynamic diameter (D_h), polydispersity (PDI), volume average diameter (D_v) and uniformity of O/W emulsions produced by sonication at 10wt% of vegetable oil (olive and sunflower) with an aqueous solution containing 0.5wt% of DXT, DXT-COO^-, DXT-MA, DXT-SCPN-COO^- or DXT-SCPN-MA at pH 5.5. All DXT derivatives proceed from the same batch of DXT-MA with DS=52%.

Emulsifier	Oil	Stability[a]	D_h[b] (nm)	PDI[b]	D_v[c] (d.μm)	Uniformity[c]
DXT	Olive	X	N/A	N/A	N/A	N/A
DXT-COO^-	Olive	OK	300	0.42	2.3	2.1
DXT-MA	Olive	OK	460	0.50	1.0	0.7
DXT-SCPN-COO^-	Olive	OK	230	0.50	0.9	1.3
DXT-SCPN-MA	Olive	OK	1180	>0.6	4.3	0.8
DXT	Sunflower	X	N/A	N/A	N/A	N/A
DXT-COO^-	Sunflower	OK	220	0.34	5.6	4.6
DXT-MA	Sunflower	OK	380	0.42	1	0.6
DXT-SCPN-COO^-	Sunflower	OK	310	0.46	1.5	0.9
DXT-SCPN-MA	Sunflower	OK	1620	0.52	1.9	1.3

N/A: Data not available due to the instability and rapid demulsification of the O/W emulsion; [a] stability after 1 week at T=4°C as judged by visual inspection and DLS; [b] obtained by DLS at 25°C; [c] obtained by LD at room temperature.

DXT-SCPN-MA-based emulsions resulted in large and highly polydisperse droplets as determined by DLS (>1 μm, PDI>0.5; Table 1). When DXT-MA was used, D_h values of 300 and 380 nm (olive and sunflower oils, respectively) with narrow uniformities (as judged by LD; Table 1) were obtained. These values are slightly larger than those reported for emulsions stabilised with phenoxy-functionalized DXT.[18] This result can be explained by the number of free MA groups on both stabilisers. In DXT-SCPN-MA, some MA groups are used for the cross-linking reaction, with the resulting decrease of the interfacial activity at the O/W interface and consequent formation of larger droplets. DXT-COO^- stabilised emulsions exhibited small droplet size (< 300 nm; Table 1) irrespectively of the oil used, as determined by DLS. However, broad and multimodal size distributions observed by LD suggest the presence of larger droplets, which were not detected by DLS due to the creaming effect (see ESI, Fig. S8). Unexpectedly, DXT-SCPN-COO^- also showed good properties as O/W emulsifier. Relatively small D_h values were obtained for both olive and sunflower oils (230 nm and 310 nm, respectively). However, broader uniformities (1.3 and 0.9 for olive and sunflower oils, respectively) than those obtained for DXT-MA (0.7 and 0.6, respectively) were observed. Also, DLS studies over time (See ESI, Table S2) showed higher emulsion stability with DXT-MA or DXT-SCPN-MA (constant D_h over 3 months) compared to DXT derivatives containing COO^- groups.

In view of D_h Values < 500 nm, low polydispersity and good stability, DXT-MA was selected as the most promising emulsifier. Besides its superior emulsifying properties, the preparation of DXT-MA reaches higher yields and is simpler, as it is the precursor used in the production of DXT-COO^-, DXT-SCPN-MA and DXT-SCPN-COO^-. Also, the presence of MA groups offers a reactive group that would allow further surface functionalization of the nanoemulsions.

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DOI: 10.1039/D1CC00819E

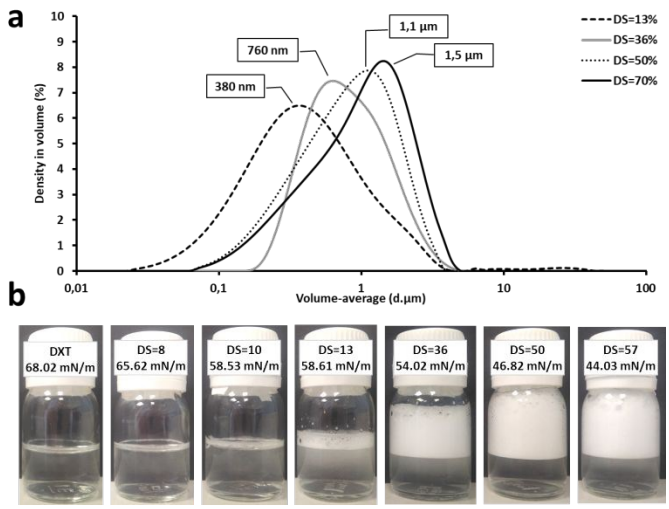

Figure 2.) Size distribution by LD of emulsions prepared at 10wt% of sunflower oil and 0.5wt% of DXT-MA with different DS (from left to right: 13%, 36%, 50%, and 70%). b) Surface tension values and digital pictures after being shaken vigorously for 20 seconds obtained for aqueous solutions of DXT-MA (DS: 0-57 %) at 0.5wt%.

Next, we explored the effect of DS for DXT-MA on the quality of the O/W emulsion produced. With that aim, DXT-MA with increasing DS values, i.e. 8, 10, 13, 36, 50 and 70%, were prepared (See ESI, Figure S9-S13) and evaluated as emulsifiers at 0.5wt% for the emulsification of sunflower oil at 10wt%. Stable emulsions failed using DXT-MA with DS values lower than 36%, suggesting that a minimum amount of MA groups is required to achieve sufficient interfacial activity. Actually, the emulsions prepared with DXT-MA at 10 and 13% DS remained stable for only one week; after this time, phase separation occurred.

Surprisingly, volume-average diameter obtained by LD showed that increasing DS values resulted in larger droplet size (Fig. 2a), which was attributed to a more compact conformation of DXT with higher DS promoted by the hydrophobic interactions, resulting in a lower surface coverage.[15] In view of these results, ST of aqueous solutions of DXT-MA at 0.5wt% was investigated. As expected, higher DS values, which increase the hydrophobicity of DXT chain, led to lower ST values (see Fig. 2b).[15] Vigorous shaking of an aqueous solution of DXT-MA with different DS at 0.5wt% clearly indicated a higher production of foam at higher DS values, confirming better adsorption at the air-water interface with lower ST values. Larger amounts of foam were observed for DS>36% (ST<54mN/m; Fig 2b) which correspond to the emulsifiers that can produce stable emulsions. Again, it was not possible to determine the O/W interfacial tension in the presence of DXT-MA, confirming the relatively slow adsorption of DXT-MA at the interface.[16,18] Also, the high distribution of droplet size, inherent to the sonication process, could be overcome using high pressure homogenizer, microfluidizer or tubular flow membrane contactor.[25]

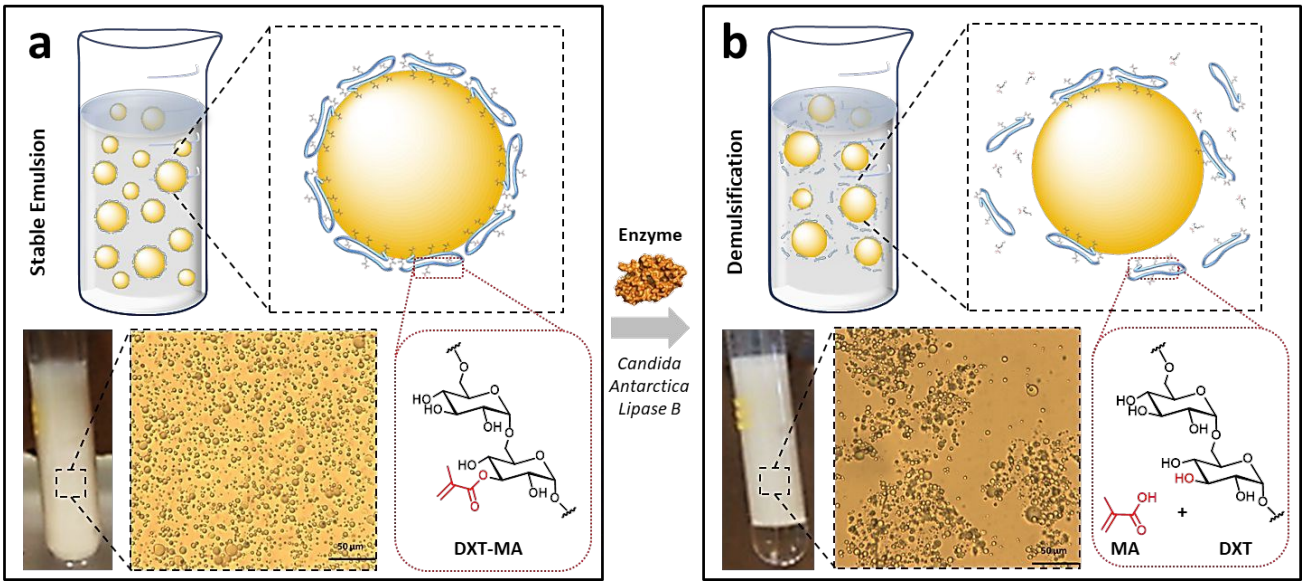

Figure 3. Schematic representation of sunflower oil-in-water emulsion at 40wt% using 0.5wt% DXT-MA (DS=52%) as enzyme-responsive materials. Scheme of emulsion destabilization triggered by *Candida Antarctica Lipase B* relying on the generation of methacrylic acid and pristine dextran together with digital pictures of macroscopic behaviour after 40h incubation correlated with optical microscope images after 20h incubation; before (a) and after (b) enzyme addition.

The presence of ester bonds generated during the production of DXT-MA, together with the absence of interfacial activity of the pristine DXT, suggests that lipases or esterases may catalyse their hydrolysis, compromising the stability of the resulting emulsion. Therefore, we anticipated that the action of these enzymes over DXT-MA-stabilised emulsions might trigger a selective and controlled release of the oil phase (Fig. 3), and consequently the delivery of an eventual drug confined into the hydrophobic oil phase.[26,27] As model lipase, we selected the *Candida Antarctica Lipase B* from (CALB) as this enzyme has been widely employed for oil hydrolysis in a diversity of biotechnological applications.[28] Experimentally, 2 mg of CALB were incubated towards 100 mg of a sunflower oil-in-water emulsion prepared at 40wt% oil and 0.5wt% of DXT-MA

(DS=52%) emulsifier. These experimental conditions were selected in order to obtain large droplets (>1 µm), visible by optical microscopy (OM). After 20h incubation, droplet aggregation and coalescence were observed, while creaming effect and phase separation were visually observed after 40h incubation (Fig. 3b). On the other hand, the same emulsion without the enzyme remained unaltered (Fig. 3a, See ESI Fig. S15). LD analysis confirmed the presence larger droplets underlying demulsification process upon the action of CALB for 40 hrs (See ESI, Fig. S15). These results confirmed that CALB triggers ester bond hydrolysis from DXT-MA, recovering pristine DXT with no interfacial activity, and ultimately compromising the emulsion stability and sustaining the demulsification process. The production of monodisperse droplets will be required to systematically investigate the demulsification kinetics.

In conclusion, we have demonstrated that a minor modification of natural DXT polysaccharide is sufficient to confer interfacial activity and stabilize O/W emulsions, even when functionalised with hydrophilic groups at either slightly acidic or neutral pH. Minimal functionalization with MA is required to produce stable emulsions. The resulting emulsions are enzyme-responsive thanks to the presence of ester bonds, inherent to MA functionalization. These properties open opportunities for encapsulation of hydrophobic drugs and/or active ingredients and triggered controlled release in the presence of biologically relevant enzymes, such as lipases.

Financial support for this work was provided by GV (ELKARTEK/BMG19/ref:KK-2019/00015), ELKARTEK/BMG21/ref KK-2020/00010). JL thanks the Spanish Ministry of Economy and Competitiveness (CTQ2017-87637-R). FLG thanks IKERBASQUE. MM thanks MIUR-Italy ("Progetto Dipartimenti di Eccellenza 2018-2022" allocated to the Department of Chemistry "Ugo Schiff"). Part of the work was performed under the Maria de Maeztu Units of Excellence Program from the Spanish State Research Agency (MDM-2017-0720).

Conflicts of interest

There are no conflicts to declare.

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