Stabilization of carbon dioxide-in-water emulsions by proteins†

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At CO_2 pressures between 40 and 100 bar, we have succeeded in creating very stable carbon dioxide-in-water emulsions using a relatively inexpensive and innocuous protein (principally β-lactoglobulin) as the sole emulsifying agent.

Liquid and supercritical carbon dioxide are finding increasing use as solvents in various processes.[1–3] Because both liquid and supercritical CO_2 are not appreciably miscible with water, biphasic processes can be inefficient. However, potentially they can be greatly improved by emulsification, which increases the contact area and mass transport. However, a literature survey indicates that there are few effective surfactants available for emulsifying CO_2 in water. Normal hydrocarbon-based surfactants are known to be poor emulsifiers, presumably because the long-chain hydrocarbon moieties (beyond C_8 in chain length) of these have little affinity with the CO_2 phase.[4] Fluorocarbon surfactants have been used with some success to create CO_2-based emulsions[5] as well as microemulsions,[6] but some of the advantages of using CO_2 are compromised because of issues of sustainability, environmental acceptability, and expense of such surfactants.

Proteins are well known for their ability to adsorb at almost any interface: air–water, oil–water and solid–water, and even including fluorocarbon–water.[7] However, reports of proteins being used directly as stabilizers of CO_2 emulsions are limited. Ghenciu *et al.*[8] reported that the stability of CO_2 droplets was enhanced by protein (subtilisin) at the interface of the phases. Proteins are not likely to stabilize the inverse system (*i.e*, water droplets in CO_2), since Bancroft's Rule[9] would require the protein emulsifier to be more soluble in the less polar CO_2 phase, so that synthetic surfactants are probably still required for water-in-CO_2 emulsions. However, the results described herein demonstrate that proteins can indeed stabilize CO_2-in-water emulsions very effectively.

Pure samples of the proteins bovine β-lactoglobulin (β-L), bovine serum albumin (BSA) and bovine β-casein (β-C) were tested as emulsifiers in a pH 7 buffer and 0.1 mol dm^{-3} ionic strength. For comparison, the conventional nonionic surfactant Tween 20 (polyoxyethylene sorbitan monolaurate) and anionic surfactant sodium dodecyl sulfate (SDS) were also investigated.

To create the emulsions, 15 ml of aqueous buffered (pH 7) solution was introduced into a high pressure cell, which was sealed and pressurised with CO_2 until a pressure of 100 bar was reached. A clear liquid CO_2 layer was then visible on top of the aqueous phase with a CO_2 volume fraction of approximately 0.25 in the system. Maximum stirring using a magnetic follower created turbulent agitation (*i.e.* not a vortex) of the CO_2–aqueous interface. The above procedure was performed at room temperature (20–25 °C). The results for the various systems are summarized in Table 1.

With 1 wt% Tween 20 or 0.5 wt% SDS an emulsion of CO_2-in-water was formed above the aqueous phase. The estimated initial droplet size was <30 μm. A lower concentration of SDS was used than Tween 20 because at 1 wt% SDS the solution was quite turbid, which made visual observations of droplets difficult. However, both these surfactant concentrations are more than adequate to give stable hydrocarbon oil-in-water emulsions of at least 30 vol% oil. With Tween 20 the lower aqueous layer did not go clear until after 1 h, but with SDS this only took 5–6 min. After 3 h both kinds of emulsions showed the formation of a clear CO_2 phase on top of the upper emulsion droplet phase, though this was more marked for the SDS system than for the Tween 20 system. After 24 ± 2 h only a thin (1 mm) layer of droplets remained between a clear upper CO_2 phase and a lower aqueous phase in the Tween 20 system, but with the SDS system no droplets remained at all.

As soon as the CO_2 formed a fluid layer in contact with 1 wt% β-casein (β-C) solution, the latter started to go very cloudy. On application of stirring, this cloudiness increased, with what appeared to be extensive protein precipitation visible. A concentration of 1 wt% β-C is usually more than adequate to achieve stable fine emulsions containing 30 vol% hydrocarbon oil, its excellent emulsifying and stabilizing properties the result of its unusual conformation, which is very open and possesses little secondary structure.[9] Near the isoelectric pH of β-casein (*ca.* pH 5.2) the protein precipitates and is therefore useless as an emulsifier. However, measurement of the pH immediately before and after pressurization did not suggest any significant fall in pH of the buffered protein solutions due to the solubilization of CO_2 in them. The known interaction between CO_2 and accessible lysine residues (which would be favoured by the relatively open structure of β-C) forming a carbamate functionality[3] may explain the apparent precipitation of β-C, indirectly resulting in poor emulsifying performance.

With 2 wt% BSA an emulsion initially similar in appearance to that formed with the SDS and Tween 20 was formed, though after 4 h there was still no evidence of an upper clear CO_2 phase forming; what remained was a dense cream layer of droplets and a lower, completely clear aqueous phase.

With 2 wt% β-L, after agitation *via* the stirrer had ceased, the whole cell was filled with a turbid dispersion of droplets. After 30 min, a distinct cream layer started to become resolved and a less cloudy, lower aqueous phase developed. After 4 h the lower

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Table 1 Summary of CO_2-in-water emulsion stability for different system compositions

System	Stability	Droplet size range[a]	Time for separation[a,b]
Buffer only	Very poor	0.1–2 mm	<30 min
0.5 wt% SDS	Poor	<30 μm	<24 h
1 wt% Tween 20	Poor	<30 μm	<24 h
1 wt% β-C	Poor	ND	<1 h
2 wt% BSA	Moderate	20–30 μm	>24 h
2 wt% β-L	Good	6–17 μm	>72 h
2 wt% β-L, heated to 62 °C	Flocculated	ND	>24 h
2 wt% β-L + 0.8 wt% xanthan	Very good	80–130 μm	ND[c]

[a] ND = not determined [b] Meaning the time for the formation of a clear upper CO_2 phase with essentially no droplets remaining in the system. [c] No creaming was visible over the maximum observation time of 48 h.

aqueous phase was almost, but not quite, clear. The rest of the cell was filled with a creamed layer of droplets. Image analysis of 150–160 droplets in the cream layer gave an approximately log normal distribution of the number of droplets *vs.* droplet size, centred on 9.6 μm, with a d_{43} value of 16 μm and a d_{32} value of 15 μm.‡ Fig. 1(a) gives an example of the droplets within the cream layer, plus the measured and fitted log normal droplet size distribution.

In comparison with the behaviour of the SDS and Tween 20 systems, the appearance of the emulsion was essentially unchanged after 20 h, with no clear upper CO_2 phase appearing. Even after 3 days storage at 100 bar, on re-dispersal of the emulsion cream layer *via* the stirrer, the droplet size distribution appeared to be essentially the same, so that the system appeared to be completely stable over this length of time.

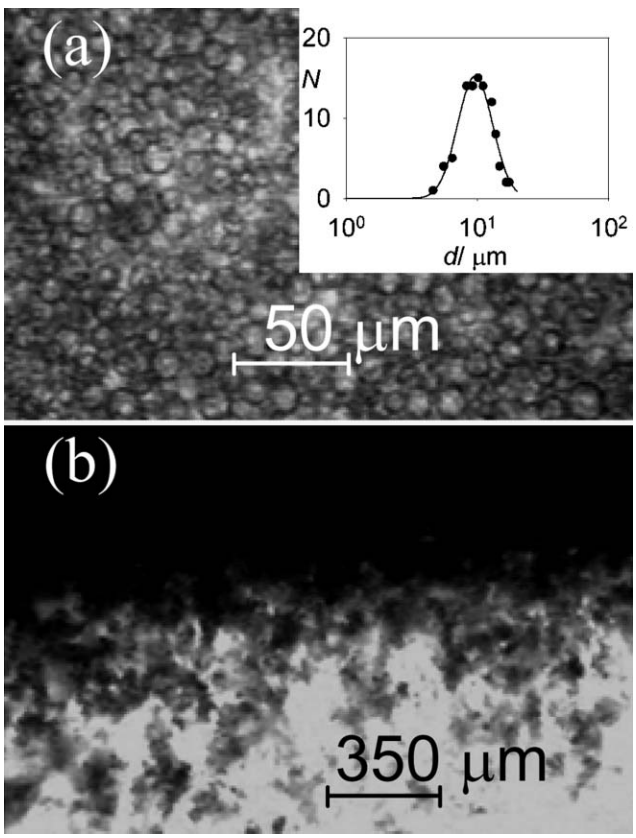

Fig. 1 Images of CO_2-in-water emulsions formed with 2 wt% β-L: (a) CO_2 droplets within the cream layer, with inset showing measured number frequency (N) of droplets *vs.* their diameter (d), with fitted log normal distribution; (b) emulsion after heating from room temperature (and 40 bar pressure) to 62 °C (and 100 bar pressure) and cooling to room temperature, showing the aggregated appearance of the emulsion.

The contrast in behaviour between the different proteins is noteworthy. β-L is a globular protein and its mode of emulsion stabilization is complex, the protein tending to unfold and slowly cross-link at the interface after adsorption.[9] At most fluid interfaces this gives rise to mechanically stronger films than with β-C, the latter behaving more like a lower molecular weight surfactant in terms of the higher fluidity of the adsorbed layer and its non-cross-linked nature.[10] Any slight fall in pH during pressurization due to the solubilization of CO_2 in the aqueous phase might actually aid the stability of the emulsions formed with β-L, by inducing thicker, more stable interfacial films as the isoelectric point of β-L (pH 5.3) is approached,[10] β-L remaining soluble at this pH. The isoelectric pH of BSA is slightly lower (pH 4.7) than β-L, but the BSA molecule is more rigid, due to a larger number of intramolecular disulfide bonds. Thus BSA is less able than β-L to unfold on adsorption and form intermolecular cross links at the interface.[9]

To overcome gravity-induced creaming, an experiment was performed with a mixed aqueous phase containing 2 wt% β-L + 0.8 wt% of xanthan. Xanthan gum is a non-surface-active polysaccharide that is commonly used to thicken food emulsions. When stirring ceased the whole cell was filled with a uniform dispersion of CO_2 droplets in the aqueous phase, and these were completely stable and exhibited no creaming over 48 h.

In one experiment a 2 wt% β-L system was heated from room temperature to 62 °C after the initial formation of the β-L-stabilized emulsion. Since β-L in bulk solution begins to become irreversibly denatured at around 60 °C, it was of interest to see if there was any change in the stability of the emulsion around these temperatures. Such heat processing might induce additional unfolding and cross-linking of protein films, in which case emulsion stability might be increased and the protein films formed might be more robust on release of the CO_2 pressure. In this experiment the dispersion was initially formed at 40 bar of CO_2 and room temperature (20–25 °C) and then heated to 62 °C, whereupon the pressure increased to 100 ± 5 bar. The process of heating took approximately 20 min. After reaching 62 °C the system was allowed to cool down to room temperature and 40 bar pressure, which took approximately 15 min. Throughout the heating and cooling cycle the system was stirred as before.

The dispersion formed at 40 bar and room temperature initially appeared to be slightly coarser than those formed previously with β-L (at 100 bar and room temperature) and it creamed more

rapidly. However, this is not surprising, as at 40 bar the CO_2 would be expected to have a vapour-like density ($\approx$0.1 g cm^{-3} assuming the phase behaviour can be extrapolated from that of pure CO_2[11]), whereas at 100 bar it would be liquid (density $\approx$ 0.86 g cm^{-3}). Thus CO_2 'droplets' formed at 40 bar will have considerably greater buoyancy than those at 100 bar. At 62 °C and 100 bar, the condition reached at the end of the heating stage, CO_2 would be in the supercritical phase (density $\approx$ 0.28 g cm^{-3}). Additionally, therefore, this meant that during the heating and cooling, the particles of the dispersed phase contracted and expanded, respectively. The main effect of heating was that the dispersed phase became increasingly aggregated, and this aggregation persisted after cooling down—see Fig. 1(b)—though there was no evidence of droplet coalescence.

For all the systems, after release of the pressure and boiling off the CO_2, the aqueous solution remaining in the cell was initially turbid. However, with the exception of the heated β-L system, the solution turned clear within 20–30 min. This transient turbidity was therefore presumed to be due to the slow release of CO_2 bubbles from the supersaturated solutions and/or, in the case of β-casein, re-solubilization of the protein.

In conclusion, it appears fairly straightforward to produce stable CO_2-in-water emulsions using an appropriate globular protein as the emulsifier. A protein, such as β-lactoglobulin, that is capable of forming a reasonably strong adsorbed film and/or that is resistant to carbamate formation *via* exposed lysine residues may be a prerequisite for emulsification. There seems little reason to suppose that much smaller droplets and/or more concentrated emulsions could not be formed using more vigorous agitation/homogenization. Also, the manufacture of smaller initial droplet sizes, plus heat processing and more controlled pressure release, may enable the creation of coherent collapsed adsorbed protein layers as encapsulating agents for use in controlled release.

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Notes and references

‡ The mean diameters d_{43} and d_{32} are the common parameters used to describe the particle size distribution of emulsions, defined by $d_{xy} = \Sigma N_i d_i^x / \Sigma N_i d_i^y$, where N_i is the number of droplets of diameter d_i.

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