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Emulsions with unique properties from proteins as emulsifiers

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ABSTRACT

Many proteins are surface active molecules and form stable emulsions. In these emulsions, the protein covered oil droplets behave as sticky droplets even when they are ionically charged. As a result of the stickiness of the droplets the emulsions have gel-like properties. The stickiness is due to the multipolar nature of the proteins in contrast to the bipolar nature of surfactants or other amphiphilic compounds that form emulsions with repulsive droplets. Stable emulsions are also formed from particles like clays to which proteins are adsorbed. These hybrid compounds form even more stable emulsions with stronger elastic properties than clays and proteins on their own.

These so called pickering emulsions have paste-like properties and do not flow. The scaffolding network of the crosslinked protein bilayers on the droplets is so strong that both the water and the oil can be removed from the emulsions by freeze drying without collapse of the scaffold. The resulting sponge can be used again for the uptake of both water and oil. Emulsions which are prepared from different proteins differ mainly in their elastic properties.

Contents

1. Introduction

Emulsions are suspensions of oil in water (o/w-emulsion) or water in oil (w/o-emulsions) in which the droplets of the dispersed phase are stabilized by thin films, very often of monomolecular films of an emulsifier. Emulsions are thermodynamically unstable systems. They tend to separate with time into an oil and an aqueous phase. Emulsions can however be stable for long times. Three mechanisms are usually responsible for the separation of the systems [1]. The droplets of oil have normally a lower density than the aqueous phase and the droplets therefore up-cream to the top of the fluid phase. This process is well known in milk, one of the best known natural emulsions. During this process, the individual droplets can keep their identity. The small droplets can however also coalesce to larger droplets both in the bulk and in the top layer that finally leads to separated oil and water phases. Another mechanism for the instability is the aggregation of the droplets in the bulk phase that can lead to flocculation and, depending on the condition of the emulsion, to coalescence and phase separation. Instability of emulsions can also occur without coalescence of droplets, by the so called Ostwald ripening. This process is due to the fact that the oil molecules in the small droplets of the emulsion have a higher chemical potential than in the larger droplets. The chemical potential

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is controlled by the La Place pressure p in the droplets which is given by the radius r of the droplets and the interfacial tension σ between oil and water in the presence of the emulsifier.

$$p = 2s/r$$

The size of the droplets in emulsions can vary by several orders of magnitude from less than 100 nm to tens of micrometers [2]. The dimension depends mainly on the used emulsifier and on the shearing forces during the used emulsification process. Approximate dimension of the droplets can be estimated from the stress model in which the La Place pressure is set equal to the shear stress

$$2\sigma/r = \eta \cdot \gamma$$

where γ is the shear rate during the emulsification process and η is the viscosity of the oil that is dispersed in the aqueous phase. By using high pressure emulsifiers with $\gamma = 10^6\ \mathrm{s}^{-1}$ it is thus possible to reach diameters of 50 nm, that is dimensions of the same size as the droplets in microemulsions. Such emulsions do not look milky but can be rather transparent. In contrast to emulsions, microemulsions are thermodynamically stable phases and form spontaneously when water, oil and emulsifier are gently stirred [3–5]. In emulsions the interfacial tension between oil and water is in the range of a few mN/m while in microemulsions the interfacial tension is three orders of magnitude lower. It is usually minimized by using surfactant/co-surfactant mixtures, or by changing the salt concentration in the aqueous phase.

Emulsifiers are usually bipolar compounds. The compounds have a polar part that dissolves very well in the aqueous phase and an apolar part that is soluble in the oil phase. As a consequence these molecules like to adsorb at the interface between oil and water. Good emulsifiers are therefore simple surfactants [6], hydrophobically modified polyelectrolytes [7], phospholipids [8], or even small particles as in pickering emulsions [9].

It is also noteworthy to remember that a given emulsifier has a built-in curvature for the film. Depending on the sizes of the polar and hydrophobic groups the emulsifier likes to form o/w emulsions or w/o emulsions. It is for this reason that stable o/w emulsions can consist of 95% of oil and 5% of water in which water is the continuous phase. Such phases are called high internal phases or HIPE systems [10]. It is also possible to form w/o emulsions as HIPE systems [11]. HIPE emulsions usually have a high viscosity and a yield stress value. They have the structure of foams. It is noteworthy to mention however, that a thin liquid film of the continuous solvent phase exists between the dispersed polyhedral droplets and the interaction between these droplets is repulsive.

Stable emulsions can therefore be diluted with the solvent of the continuous phase without that the emulsions separate in two macroscopically separate phases. Technical and natural emulsions are usually optimized to have a long stability. Under these conditions the emulsifier should not be present in excess; otherwise a second phase might separate from the emulsion because of depletion forces [12]. Both repulsive electrostatic double layer forces and steric forces can be used to avoid aggregates of the droplets that are usually the prestate for coalescence. Emulsions are used for many cosmetic [13] and pharmaceutical [14] applications and in technical applications like emulsion polymerization [15]. The largest fraction of emulsions is used in food stuffs like milk, butter, mayonnaise and so on [16]. Emulsions exist as low viscous fluids or can have a paste-like property.

Proteins are often used to prepare emulsions or are present in natural emulsions as the emulsifier. Proteins are surface active polymers. In contrast to most of the normal emulsifiers that are bipolar compounds, proteins are multipolar compounds. This property has consequences as will be shown for the properties of the protein emulsions.

Hundreds of publications are published each year on various aspects of emulsions [17–20]. Summary articles are published from time to time [21]. Summary articles on protein emulsions have also appeared [22]. In most of these articles a distinction between the properties of protein emulsions and the properties of other emulsions was not made. It had not been recognized that protein emulsions have special features that emulsions from other amphiphilic compounds do not have. Protein covered droplets behave however as sticky droplets while surfactant covered droplets act as repulsive droplets. As a consequence of this difference, the different behavior of the two kinds of emulsions shows up particularly strong in the rheological behavior of the emulsions. Under equal conditions of preparation, equal amounts of oil and water, and of a few weight % of emulsifier the resulting emulsion from surfactants can be as fluid as water or as viscous as a toothpaste for proteins.

This article is about the special properties of emulsions that are prepared with the help of proteins. This difference between normal emulsions and emulsions from proteins has not been emphasized in most of the literature on emulsions.

Before the emulsions are discussed, the article will be started with a chapter on aqueous protein solutions. It will be shown in this chapter that the special properties of the proteins as amphiphiles with respect to those of surfactants are already evident in the properties of the protein solutions.

2. Properties of protein solutions

2.1. General information on proteins

Proteins are polymers from amino acids. While the main part of this article concentrates on the properties of emulsions prepared from proteins, properties of aqueous protein solutions are discussed in the first part. It is shown that protein solution in many ways behaves like surfactant solutions. Proteins are surface active molecules, they form monolayers [23] on aqueous solutions and interlayers between oil and water [24], they form clusters and micelles in the bulk [25–27], large supramolecular structures that can gelate [28,29] the aqueous phase, and they form emulsions with gel-like properties.

The aim of the article is to demonstrate that the special properties of the emulsions are a reflection of the properties of the proteins in the bulk solution. These properties are best recognized in the rheological properties of the protein emulsions.

As a consequence of the twenty amino acids that occur in nature, an infinite number of proteins are conceivable that differ in length and sequence of the amino acids. Today, the sequence of the amino acids can easily be determined, and is known for many natural proteins [30], like for insulin [31]. Amino acids are zwitterionic compounds that can carry negatively or positively charged side groups, or can have hydrophobic groups. As a consequence of the hydrophobic groups proteins are surface active molecules [32]. Many of them are negatively charged, some of them positively charged in water. The ionic charge depends on the pH of the solution. Many proteins have a point of zero charge (isoelectric point) around neutral pH [33]. The surface activity of proteins is manifested, and probably best known from the foam on beer. Proteins, also known as polypeptides, are linear polymers. In this state they have a persistence length lc which depends on the sequence of the amino acids. For contour lengths that are longer than lc the molecules could be present as statistical coils. This however is not the case. The polymers are folded into more dense states in a well defined conformation [34] in which the total energy is at an energy minimum. This conformation depends on the surroundings of the molecule like solvent composition, pH, salt concentration and so on. The total folding energy involves hydrogen bonds, hydrophobic interaction, and ion–ion interactions.

2.2. Proteins in the aqueous bulk phase

As a consequence of their composition and their conformation the molecules have a high or a low solubility in the aqueous phase. For concentrations above their solubility, proteins usually form a condensed