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# Microfluidics potential for developing food-grade microstructures through emulsification processes and their application

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## ARTICLE INFO

## ABSTRACT

The food sector continues to face challenges in developing techniques to increase the bioavailability of bioactive chemicals. Utilising microstructures capable of encapsulating diverse compounds has been proposed as a technological solution for their transport both in food and into the gastrointestinal tract. The present review discusses the primary elements that influence the emulsification process in microfluidic systems to form different microstructures for food applications. In microfluidic systems, reactions occur within small reaction channels (1–1000 μm), using small amounts of samples and reactants, *ca.* 102–103 times less than conventional assays. This geometry provides several advantages for emulsion and encapsulating structure production, like less waste generation, lower cost and gentle assays. Also, from a food application perspective, it allows the decrease in particle dispersion, resulting in a highly repeatable and efficient synthesis method that also improves the palatability of the food products into which the encapsulates are incorporated. However, it also entails some particular requirements. It is important to obtain a low Reynolds number ($Re <$ approx. 250) for greater precision in droplet formation. Also, microfluidics requires fluid viscosity typically between 0.3 and 1400 mPa s at 20 °C. So, it is a challenge to find food-grade fluids that can operate at the micro-scale of these systems. Microfluidic systems can be used to synthesise different food-grade microstructures: microemulsions, solid lipid microparticles, microgels, or self-assembled structures like liposomes, niosomes, or polymersomes. Besides, microfluidics is particularly useful for accurately encapsulating bacterial cells to control their delivery and release on the action site. However, despite the significant advancement in these systems' development over the past several years, developing and implementing these systems on an industrial scale remains challenging for the food industry.

## 1. Introduction

An emulsion consists of two non-miscible liquid phases, usually water and oil. When an emulsion is formed is important to consider the ingredients we want to add to it, and, depending on this, they will be dispersed in the phase in which it is more soluble. Thus, lipid-soluble compounds such as fat-soluble vitamins, antioxidants and fat-soluble pigments are added to the oil phase. On the contrary, water-soluble vitamins, proteins, polysaccharides, phenolic compounds and water-soluble pigments are added to the aqueous phase (McClements & Li, 2010).

Depending on the size of the micelles generated, three different types of emulsions can be distinguished (Adjonu et al., 2014; Aswathanarayan & Vittal, 2019; Tadros, 2013, 2014), namely macroemulsions (>200

nm) and micro- and nanoemulsions (<200 nm) (Table 1). The stability of macroemulsions can be affected by even small variations in environmental conditions. In contrast, microemulsions are thermodynamically stables. As the surface to mass ratio is substantially increased, micro- and nanoemulsions are very useful for encapsulating and releasing bioactive compounds in a controlled manner, improving their solubility and absorption at the desired site in the gastrointestinal tract. They also have the advantage of forming transparent/translucent suspensions, which makes them very useful for incorporation into food without modifying its colour (Adjonu et al., 2014). Besides, smaller droplets or particles provide a better mouthfeel (Adjonu et al., 2014; Aswathanarayan & Vittal, 2019).

The emulsification process requires high mechanical energy to break up the aqueous phase and achieve the formation of droplets within the

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**Table 1**

Types of emulsions according to micelle size (Adjonu et al., 2014; Aswatha-narayan & Vittal, 2019; Tadros, 2013, 2014).

|  | Macroemulsions | Microemulsions | Nanoemulsions |
|---|---|---|---|
| Droplet size | > 1 μm | 4–200 nm | < 200 nm |
| Colouring | Variable according to formulation. Turbid | Transparent or translucent | Transparent or translucent |
| Formation | Not spontaneous. Mechanical formation or manual agitation | Spontaneous, generated by low energy (e.g. vortex) | Not spontaneous. Generated by high homogenisation pressure or ultrasound |
| Stability | Kinetically stables | Thermodynamically stables | Kinetically stables |
| Polydispersity | High | Low | Low |

oily phase, which can be supplied by simple tubes, static mixers, common agitators, colloidal mills, high-pressure homogenisers or ultrasound generators (Tadros, 2013). Many of the synthesis processes could cause a decrease in the bioactivity of certain encapsulated compounds. Besides, traditional emulsification methods present problems such as size polydispersion or a decrease in yield as the production volume increases (He et al., 2020). All these factors strongly condition the industrial application.

Microfluidics is a growing scientific discipline in research, based on the study of fluid transport at the microscale ($10^{-9}$ to $10^{-18}$ L), using channels with dimensions ranging from around 1–1000 μm in width or height (Niculescu et al., 2021; Nielsen et al., 2020). Microfluidics is currently being developed for application in many techniques, such as capillary electrophoresis, flow cytometry, biosensors, genetic analysis and drug detection (He et al., 2020). So, microfluidics can provide a solution to the drawbacks of traditional emulsification processes.

Microfluidics has several advantages (Andoyo et al., 2018; Sommer et al., 2008), including using smaller amounts of samples and reactants, $ca.$ $10^2$-$10^3$ times less than conventional assays. This means less waste, lower cost and more efficient assays; the channels have a higher ratio of surface area to sample volume, resulting in better heat transfer; the flow is laminar, which implies a low Reynolds number, allowing the sample to flow without turbulence; it involves a lower energy cost, as it has fewer components than the conventional system and better heat transfer; it allows simultaneous operations, as several tests can be carried out on the same chip; the sample is isolated from the outside, reducing operator errors, possible sample contaminations and sample processing; and most importantly for the food industry, it provides a smoother process in which highly monodisperse droplets are obtained.

There is a growing number of applications in the food industry, and more and more applications for microfluidics in food safety are being developed. A clear example would be the microfluidic chip developed by Li et al. (2019) for the fluorometric detection of nitrite in sausage. Or the ready-to-use chip designed by Xing et al. (2023) for multiplex detection of four common foodborne bacteria: *S. aureus*, *L. monocytogenes*, *E. coli*, and *S. enteritidis*. On the other hand, we can also find microfluidics applications in biocompounds detection and bioactive compound extraction. This is the case of a study in which high-performance liquid chromatography coupled to mass on chips was used to determine the flavonoid profile of soybean (Chang et al., 2012). However, applications for the synthesising micro- and nanoparticles for their incorporation in functional foods are still limited.

The so-called functional foods are those that can provide health benefits beyond nutrition. Sometimes it is necessary to fortify foods with bioactive compounds to adjust intake levels to the optimum. Fortifying food products with bioactive compounds raises many technological challenges, mainly due to the numerous reactions of these compounds with other food components during processing, storage and transport. Often, such compounds are administered in an aqueous medium,

increasing their palatability, acceptability and bioactivity. But these compounds are not always easy to disperse in water. They are generally susceptible to oxidation, so some encapsulation and transport system is required when they are to be incorporated into beverages or food products with high moisture content (Singh, 2016). In this sense, many researchers have focused their studies on developing new micro- and nanoparticles capable of transporting bioactive compounds and/or nutraceuticals. Fuciños et al. (2012, 2014) studied the synthesis of "smart" poly N-isopropylacrylamide nanohydrogels to encapsulate pimaricin (an antifungal widely used in the food industry). These nanoparticles allowed a controlled release of the encapsulated active compound (Fuciños, Amado, et al., 2017; Fuciños et al., 2015).

Moreover, in some cases, these particles can improve the organoleptic properties of the foods in which they are incorporated by masking unpleasant flavours. Estévez et al. (2020) synthesised spherical microcapsules with β-lactoglobulin using the spray-drying technique. These have been used to efficiently encapsulate bioactive peptides to protect them from degrading environments in food products and mask the intrinsic bitter taste of these compounds (Favaro-Trindade et al., 2010). Spray-drying was also used to encapsulate different bioactive compounds or ingredients, protecting them from degradation. Thus, Miranda-Linares et al. (2020) synthesise alginate-pectin nano- and microparticles by spray-drying to encapsulate lime and rosemary essential oils and α-tocopherol, Balakrishnan et al. (2021) used spray-drying to microencapsulate annatto extract using modified starch and gelatin blend, or Shelke et al. (2023) use this technique to encapsulate jamun juice powder employing various carrier agents: maltodextrin, gum arabic, whey protein concentrate, and waxy starch. These particles can improve the organoleptic properties of the foods in which they are incorporated by masking unpleasant flavours.

In other cases, the synthesised particles may be used to improve the texture of the food into which they are incorporated. Fuciños et al. (2019) and Fuciños, Míguez, et al. (2017) studied the synthesis of α-lactoalbumin nanotubes and their efficiency and loading capacity for caffeine encapsulation. Also, Fuciños et al. (2021) evaluated the functionality of these nanoparticles as bioactive agents and texture modifiers. de León-Zapata et al. (2017) developed a nanocoating based on candelilla wax and tarbush extract as an edible coating to retard Fuji apple senescence. Galindo-Pérez et al. (2015) and Zambrano-Zaragoza et al. (2017) also developed a nanoparticulate coating with good results preserving physicochemical characteristics of fresh-cut Red Delicious apples and fresh-cut melon, respectively.

In the specific case of functional foods supplemented with probiotics, their global market is expected to grow from $41.6 billion to $61.3 billion from 2022 to 2027, at an annual growth rate of 8.1% (BCC Publishing Staff, 2022). In addition, the increasing demand for non-dairy products means an interesting niche market for probiotic-supplemented non-dairy products. Many advances have been made to improve the bioavailability of probiotics in foods. In these advances, microencapsulation to protect and direct the release of probiotics is a powerful alternative to increase the viability and effectiveness of probiotics during food processing and through the gastrointestinal tract (Reque & Brandelli, 2021). The most commonly used methods for encapsulating probiotics are emulsification, coacervation, extrusion, freeze-drying or spray-drying. However, searching for methods that improve probiotics' encapsulation efficiency and bioavailability is a growing field of study.

In this work, we evaluate those parameters that are determinant in the application of microfluidic systems for the formation of food-grade emulsions, as well as the potential of these systems for the synthesis of microparticles for their application in the development of functional foods underlying their application for the encapsulation of probiotic bacteria, an aspect with great potential development because of their economic impact worldwide, focusing on the particular case of the usefulness of microfluidic systems for the encapsulation of probiotic bacteria.

## 2. Methodology

To achieve the proposed objectives of this work, we have applied different search methods. The main bibliographic sources, descriptors or keywords and a small quantitative analysis of the works published on microfluidics since it began to be developed will be detailed below.

### 2.1. Bibliographic sources

For the bibliographic search, we used the main scientific reference databases. These include Scopus, Wiley Online Library, Web of Science, and Science Direct. The Google Scholar search tool was also used as a resource. Mendeley (Elsevier) was used to manage the literature and generate the manuscript's bibliography.

### 2.2. Search strategy

The most frequently used keywords and descriptors: emulsions, emulsions and food, microfluidic, microfluidic and food, microfluidics application and food industry, lab-on-a-chip, lab-on-a-chip and food, biosensors and food safety, self-assembly, encapsulation, microparticle synthesis, or particle synthesis by microfluidics.

Several books were consulted to describe the detailed data related to emulsions, like Clausse et al. (2014). The information was completed with various articles from the different databases mentioned above.

Concerning the microfluidics information, the book by Seiffert & Thiele (2020) and several articles like those of Ushikubo et al. (2015) and Bonat Celli & Abbaspourrad (2018) were used as the main source to focus on the subject. We sought new information from them that was complemented by the most recent new studies.

### 2.3. Quantitative analysis

Using the "Analyse search results" tool provided by Scopus, we can see that the first scientific publications on microfluidics, according to Scopus (keyword: microfluidic), date back to 1970, but there is no relevant interest from the scientific community until around the year 2000. It can be seen that from 2000 onwards, the number of publications is increasing, reaching about 6000 by the year 2022. The main areas of interest are engineering (21.2%), chemistry (14.4%) and biochemistry, genetics and molecular biology (12.3%).

Regarding the application of microfluidics in food or food uses (keywords: microfluidic and food), the first studies date back to 1999, and since then, publications have continued, with increasing interest since 2004. In 2022, 247 articles were published. The main areas of interest within the food field are chemistry (20.3%), engineering (17.1%), and biochemistry, genetics and molecular biology (15.0%).
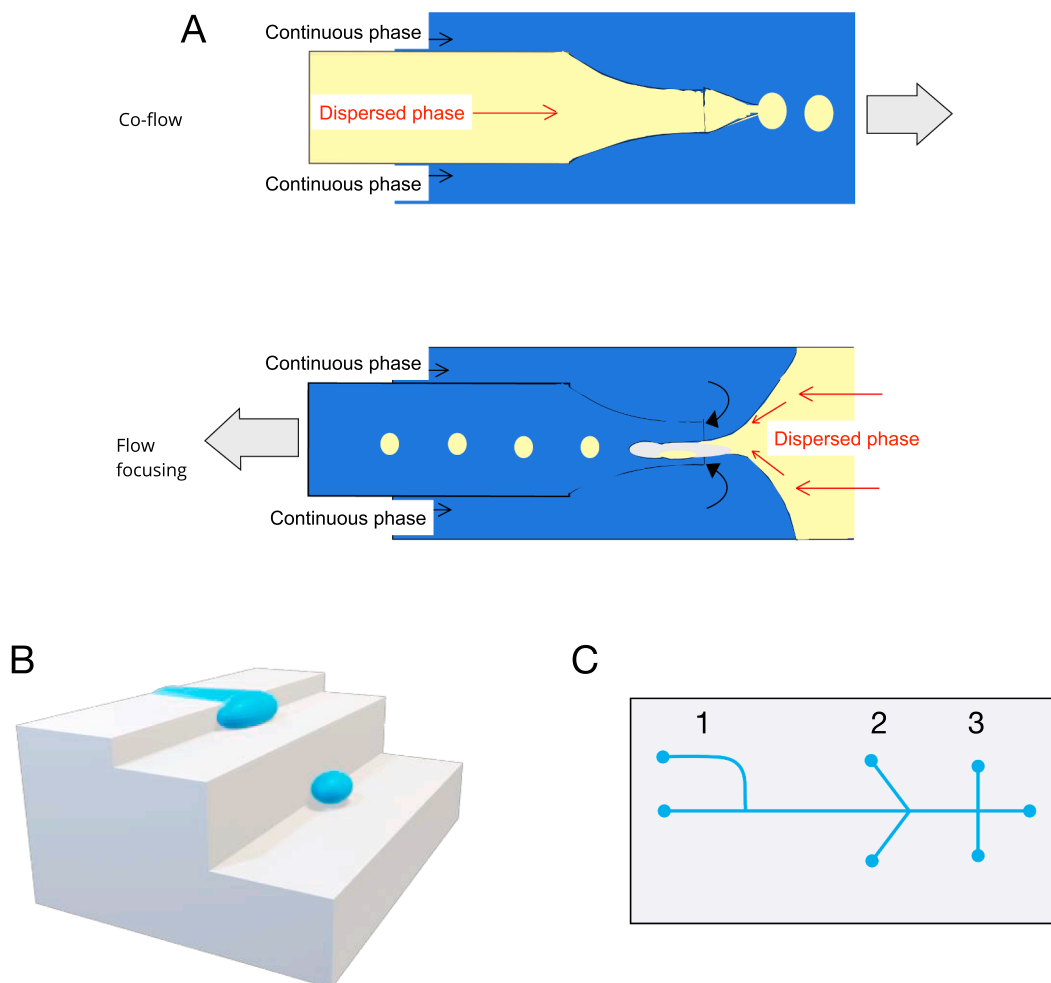


**Fig. 1.** Representation of the different methods of droplet generation using microfluidic devices: (A) capillaries; (B) terrace; and (C) planes with T-junctions (1), Y-junctions (2), and cross-junctions (3). Figures .
adapted from He et al. (2020).

## 3. Design of microfluidic systems for the synthesis of food grade emulsions

Traditional emulsion systems generate droplets of very dispersed size despite being very simple and fast. Microfluidics based on these conventional systems is also a simple technique, but, in contrast, it makes it possible to create droplets with a minimal size distribution (Zinchenko et al., 2014). Droplet generation methods fall into three categories: capillary, terrace and flat systems (Fig. 1) (He et al., 2020).

Capillary microfluidic devices control the flow velocity and direction by the positive pressure exerted by droplets at different inlets and outlets. There are various regimes of droplet generation, such as dripping, compression or jetting. In the latter case, when this pressure is high, the jet becomes so fine that it breaks into droplets. Two coaxial capillary microfluidic devices produce these trickle-jet regimes with different diameters. Coaxially oriented capillaries typically work through co-flow arrangements in which two fluids flow in the same direction (Fig. 1A) but in different capillaries (dispersed phase: inner capillary; continuous phase: outer capillary). But also, as flow focusing, the dispersed and continuous phase fluids flow in opposite directions in the outer capillary (Fig. 1A). In turn, both phases flow into the inner capillary, which consists of an open end inside the outer capillary where the continuous phase compresses the dispersed phase. The flow is broken as the jet or trickle droplets enter the inner capillary (He et al., 2020).

In terrace devices, the dispersed phase is placed in a microchannel, leading to a continuous phase-filled terrace area. Continuously, a disc-shaped droplet is formed, which will move to a deeper terrace region and take on a more thermodynamically favourable spherical shape (Fig. 1B).

Flat devices consist of rectangular cross-section channels manufactured with different techniques and materials and could have different geometries. These chips differ by the type of junction between the channels. Thus, the most common designs include T-junctions, Y-junctions, and cross-junctions (Fig. 1C). T-junctions are widely used because they allow droplet formation over a wide range of flow rates. In Y-junctions, droplet formation is more dependent on shear forces and interfacial tension. In cross-junctions, the flow in the side channels usually compresses the fluid flow in the main channel as a process of hydrodynamic flow-focusing. The latter junctions are the most commonly used for forming self-assembled structures. A higher shear is required to achieve droplet formation with this type of geometry due to the continuous phase flow on both sides of the dispersed phase stream. The droplets formed are smaller but more polydisperse (Ushikubo et al., 2014, 2015).

For the manufacture of microfluidic chips, the most common techniques are based on photolithography and soft lithography. Photolithography is used to generate the master moulds to manufacture the chip replicas. Soft lithography is a set of techniques based on using a soft elastomer material, usually polydimethylsiloxane (PDMS). This material is transparent, non-toxic and flexible. PDMS is a hydrophobic material, therefore, suitable for making W/O emulsions. The channel surfaces need to be hydrophilic to make microfluidic devices suitable for food emulsions (most of which are O/W emulsions), which can be achieved by chemical modification or by choosing a different material of construction (e.g. silicon or glass). The chemical modifications should consider that the treatments must be suitable for food applications. Searching for adequate wettability agents and, preferably, designing adequate chip geometries to avoid or reduce the use of chemicals is a challenge that could also be extended to other applications.

The general working cycle for the development of microfluidic devices is shown in Fig. 3 and includes the following steps:

1. Design of the chip using a computer program (e.g. AutoCAD® 2021).
2. Printing on a photomask using photolithography to replicate the printed channel structures on a substrate. This operation must be performed in a clean room to avoid the deposition of particles on the channels.
3. Reverse replicas are printed on a PDMS-based silicone elastomer base, in which holes are drilled for fluid inlet and outlet.
4. The chip is then bonded to a glass slide by treating the surfaces to be bonded with oxygen plasma for 30 s.

## 4. Fundamentals of microfluidics and parameters influencing the stability of food emulsions

Three categories of microfluidic devices are described in the literature: continuous flow, semi-batch and droplet devices. In continuous flow devices, the fluid flows continuously through the microchannels due to the action of hydrodynamic pumps. In semi-batch devices, specific quantities of fluid are bypassed from the main stream to another for further processing. Finally, droplet devices are characterised by the breakdown of the dispersed phase due to the action of the continuous phase flow, such that droplets are generated (Bonat Celli & Abbaspourrad, 2018) (Fig. 2). Droplet-based microfluidics is a powerful technology that produces uniform size and adjustable diameter. Each droplet can be considered a micro-scale version of the traditional chemical reaction flask, allowing identical composition and rapid mixing of reagents (Yu et al., 2016). The size of the droplets can be modulated by various parameters such as the system's geometry, flow conditions (flow rate) and the type of solvents used in the phases (Bai et al., 2019; Capretto et al., 2013; Seiffert & Thiele, 2020). Besides, microdrops can act as reactors or templates to form particles of smaller sizes (Capretto et al., 2013; Feng & Lee, 2019).

These droplet-based microfluidic systems are the most widely used in the food science field to prepare emulsions and microparticles, addressed in this work.

In contrast to macroscale processes, an increase in the surface area volume ratio occurs when a device or structure is realised at the microscale. Therefore, the dominant properties in microfluidic systems are surface tension, viscosity and diffusion (Selvaganapathy and RezaiP, 2017).

Surface tension is the cohesive force existing at the surface of the fluid molecules, which occurs at the interface between the liquid and the air. The relationship between the viscosity and the interfacial tension is given by the number of capillarity:

$$Ca = \frac{\rho \bullet v}{\gamma} \tag{1}$$

where $\rho$ is the fluid density, $v$ is the fluid velocity, and $\gamma$ is the surface tension of the fluid (Bonat Celli & Abbaspourrad, 2018). In this context, microfluidic microchannels provide low $Ca$ values. Droplet formation due to pressure is given at values of $Ca < 0.01$. When $Ca$ values are close to 1, droplet formation is associated with shear forces (Selvaganapathy and RezaiP, 2017).

Besides, the walls in the microchannels are very close to each other, which causes a reduction in the flow velocity because they avoid the flow of the adjacent fluid layers. The resistance caused by the microchannel to the fluid flow depends on the viscosity of the fluid and the dimensions of the channel, and it is given by the Reynolds number
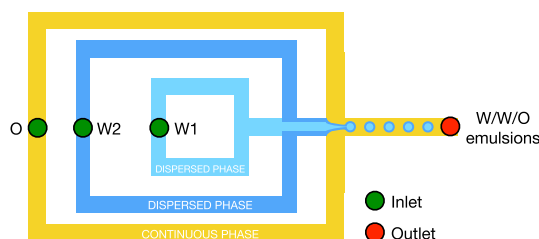


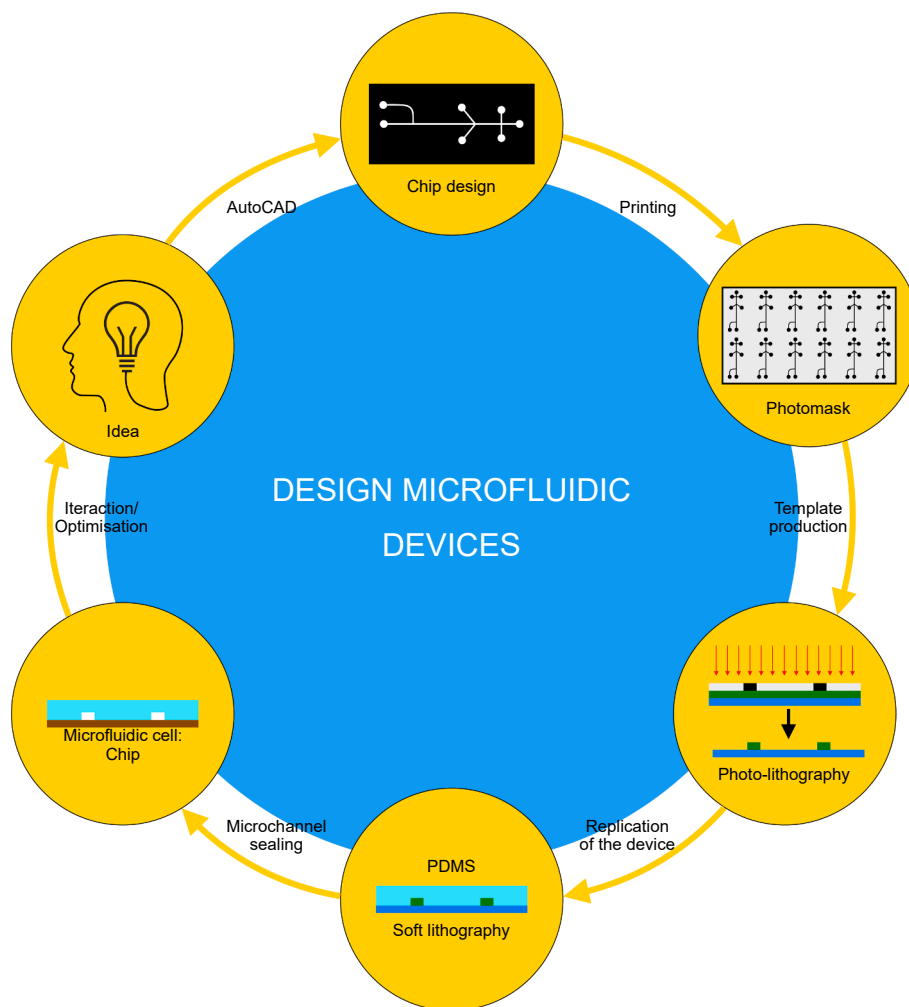**Fig. 2.** Schematic of droplet formation using a microfluidic system.

**Fig. 3.** Design of a microfluidic device using a combination of photolithography and soft lithography.
Adapted from Seiffert & Thiele (2020).

(dimensionless parameter):

$$Re = \frac{\rho \bullet v \bullet D_H}{\mu} \tag{2}$$

Where $\rho$ is the fluid density; $v$ is the fluid velocity; $D_H$ is the hydraulic diameter of the microchannel; and $\mu$ is the fluid viscosity (Selvaganapathy and RezaiP, 2017). In the case of microfluidics, it is important to obtain a low Reynolds number ($Re <$ approx. 250), as this this results in a laminar regime, which allows for greater precision in droplet formation (Selvaganapathy and RezaiP, 2017). Employing water as a fluid, flow velocities of $1$–$10^4$ µm s$^{-1}$ in typical channel radii of $1$–$100$ µm, give rise to $Re$ values between $10^{-6}$-10, i.e. laminar flow. Regarding viscosity, the fluids typically used in microfluidics range between 0.3 and 1400 mPa s at 20 °C, decreasing these values by increasing temperature (Seiffert & Thiele, 2020).

Finally, regarding the particle diffusion due to Brownian motion, this is also influenced by the microscale, as can be deduced from equation (3):

$$d^2 = 2 \bullet D \bullet t \tag{3}$$

Where $d$ is the distance a particle travels at a time $t$, and $D$ is the diffusion coefficient associated with the particle (Selvaganapathy and RezaiP, 2017).

In the case of food-grade emulsions, the viscosity of the water phase can be increased by adding glycerol. For the oil phase, alkaline oils with a low viscosity are often used to correctly form microdroplets in microfluidic systems. However, such oils are not food-grade. Therefore, the choice of suitable oil is decisive. Besides, the dimensions of the channels and the design of the microfluidic systems, as well as the process conditions used, have to be analysed and adjusted very carefully to obtain acceptable droplet sizes when working with food-grade oils (usually with high viscosity). Therefore, fluid velocity and, since viscosity is temperature-dependent, the temperature sensitivity of the fluids used will be two important parameters to ensure low $Re$ values and, thus, correct droplet formation (Muijlwijk, 2017; Schroen et al., 2021). Previous works have demonstrated the suitability of food-grade oils to obtain good results for emulsion formation employing a microfluidic system, like soybean oil (Quintana et al., 2021; Ulianova et al., 2020), sunflower oil (al Nuumani et al., 2020; Hughes et al., 2013), palm oil (Kim & Vanapalli, 2013), fish oil (Comunian et al., 2018), or corn oil (Comunian et al., 2017).

In addition to the parameters affecting microfluidic systems, there are factors affecting the stability of food emulsions. The main properties and parameters influencing emulsion stability are described below (Adjonu et al., 2014; Clausse et al., 2014; Vaclavik & Christian, 2014).

The droplet size of emulsions impacts their physical stability. Thus, in an emulsion made up of large droplets, droplet coalescence is more probable, and irreversible merge to form greater droplets that finally destabilise the emulsion. Therefore, working with small droplet sizes is preferable to obtain more stable emulsions.

The droplets could coalesce under high temperatures as the

temperature affects the oil phase. If the temperature is low, the oil droplets could solidify depending on the composition of the oil. In general, emulsions are not freeze-stable because of protein denaturation or the formation of ice crystals in the interfacial layer.

Besides, the oil can crystallise, mainly when the emulsion is stored (McClements, 2012), or can suffer from oxidation as its chemical structure contains many unsaturated bonds (Cornacchia & Roos, 2011). This could alter the functionality of the emulsion, which is especially important in oil-in-water (O/W) emulsions (Wang et al., 2020).

Changes in pH could also modify the interfacial tension and destabilise the structure of the emulsion. These pH changes are significant when proteins are used as surfactants.

To increase the stability of food emulsions, gums, pectins, or gelatines could be added to increase the viscosity so that it takes longer for the two phases, aqueous and oily, to separate. Besides, surfactants are added to form emulsions, playing two fundamental roles, namely to reduce interfacial tension and provide stability to the emulsion by forming a layer around each droplet. Sufficient surfactant is therefore required to give structural stability. The choice of surfactant is critical for the selection of the continuous phase. When working with two liquids, the one with the higher interfacial tension will form droplets, and the other liquid will form the continuous phase.

In food emulsions, biopolymers (proteins and polysaccharides) are commonly used to stabilise them. They stabilise emulsions by modifying the rheological properties of the main phase or adsorption at the oil–water interface, thus providing a steric or electrosteric barrier or a combination of both effects (Maphosa & Jideani, 2018). The stabilisation or destabilisation of the emulsion by a biopolymer depends on several parameters, such as the nature of the polymer, the concentration, pH, ionic strength, etc. Most polysaccharides behave as emulsion stabilisers by forming an extended network in the continuous phase that becomes highly viscous and may even form a gel. Only a few polysaccharide derivatives possess surface properties that allow their adsorption at the oil–water interface (Bouyer et al., 2012). So, their ability to stabilise emulsions will depend on the polysaccharide's nature and its molecular weight and concentration. So far, several polysaccharides have been used to stabilise emulsions for food applications, such as modified starch (Liu et al., 2018), pectin (Chen et al., 2018), gum arabic (Atgié et al., 2019) or chitosan (Li & Xia, 2011). Many proteins can also act as emulsifiers through their ability to adsorb to the oil–water interface, reduce interfacial tension and increase emulsion stability (McClements & Jafari, 2018). They are often used in O/W emulsions but not in W/O emulsions because they are soluble in water. The type of structure of a protein affects the properties of the emulsion (Clausse et al., 2014). Modifying proteins can improve their ability to stabilise emulsions, e.g. by partial denaturation, more hydrophobic groups can be exposed to the oil phase (Bouyer et al., 2012). Casein and whey proteins (Perugini et al., 2018; Xu et al., 2017; Zhou et al., 2020), gelatine (Zhang et al., 2020) and pea protein (Chen et al., 2019) are the most widely used surfactants in food due to their wide availability, lack of toxicity and natural origin (Bouyer et al., 2012; Clausse et al., 2014). On the other hand, the combination of polysaccharides and proteins, through the formation of complexes by covalent or electrostatic interactions, can be an excellent strategy to favour the stability of an emulsion and numerous studies support this. Some examples are whey protein isolate (WPI) with xanthan gum (XG) (Boonlao et al., 2020), xanthan gum (XG) with sodium caseinate (Long et al., 2013), WPI with chitosan (Lopes et al., 2021), WPI with carboxymethyl cellulose (Kaade et al., 2022), or soy protein hydrolysates with maltodextrin (Ding et al., 2021).

Some studies have demonstrated the usefulness of biopolymers as stabilisers of droplets formed by microfluidics. Quintana et al. (2021) used lecithin as an emulsifier to prepare W/O emulsions, and Jiao et al. (2022) used WPI and sodium caseinate as emulsifiers in the formation of O/W emulsions, using microfluidic systems.

A final type of emulsion stabiliser is small non-surfactant colloidal solids that adsorb to the surface of the droplets providing a physical barrier that delays or prevents coalescence. This would form what is known as Pickering emulsions (Bouyer et al., 2012). Such particles provide superior stabilisation compared to low molecular weight surfactants or proteins, as they act more quickly on the surface of the droplets (de Folter et al., 2012). They are effective in stabilising droplets by preventing coalescence for a short period. However, they do not generate structures with high viscosity and can be easily broken (Clausse et al., 2014). Besides, a relatively high particle concentration may be necessary to ensure good droplet stabilisation (de Folter et al., 2012). Cotton cellulose nanocrystals and calcium carbonate ($CaCO_3$) were successfully used to synthesise O/W Pickering emulsions in microfluidic systems (Marquis et al., 2016). Schröder et al. (2018) also present a study of the use of colloidal lipid particles to stabilise food-grade O/W Pickering emulsions in microfluidic systems.

## 5. Microfluidic applications in the food industry

Microfluidics has an emerging potential in the design of new structures of interest to the food industry. Thus, using these systems, it is possible to obtain structures capable of modifying the texture of foods or encapsulating particles capable of transporting bioactive compounds, providing a controlled release in the places where they best perform their function, and protecting them from the unfavourable conditions (enzymes, pH, and ionic strength) of the gastrointestinal tract. In addition, these carrier capsules can improve the organoleptic acceptance of certain bioactive compounds with unpleasant flavours (fish fatty acids, bioactive peptides, …) (Ushikubo et al., 2015).

As mentioned above, one of the main advantages of using microfluidic systems for synthesising these particles is the reduction of dispersion, obtaining a product with highly reproducible uniform characteristics. In addition, it also has a substantial economic advantage, as it reduces the cost and scale-up time of the synthesis processes (Ushikubo et al., 2015).

In the literature, we find multiple structure designs for different applications using microfluidic systems (Table 2). In the case of food applications, the development of emulsion-based structures has been of utmost interest. They can act as encapsulating systems (Feng & Lee, 2019) or intermediate systems, which operate as micro-reactors for synthesising micro- and nanoparticles (Capretto et al., 2013). These microfluidic reactors offer several potential advantages. In microfluidic systems, reactions occur within small reaction channels, generating a large surface-to-volume ratio. This allows for rapid, uniform heat and mass transfer, drastically improving yield and size distribution while reducing the formation of undesired by-products. In addition, recycling the synthesis solvents would be possible, providing a cost-effective and environmentally friendly technology (Capretto et al., 2013).

### 5.1. Emulsion synthesis

Emulsions play a crucial role in the food industry, both in the form of O/W and W/O. They can be used to form new food structures that provide textures or organoleptic characteristics to different foods (cheese, yoghurt or ice cream) or for the transport of bioactive compounds: hydrophilic, amphiphilic and lipophilic (McClements & Li, 2010; Ushikubo et al., 2015).

Conventional methods of emulsion formation (high-pressure homogenisers, colloid mills, high-speed or ultrasonic mixers) do not allow adequate particle size control, resulting in high dispersion. In addition, certain compounds can be affected by heating or shear generated during homogenisation leading to denaturation, in the case of proteins, or loss of activity, in the case of certain bioactive compounds. Microfluidic systems provide a gentler process in which highly monodisperse droplets are obtained (Ushikubo et al., 2015). Besides, microfluidic droplet synthesis allows high encapsulation efficiencies (al Nuumani et al., 2020).

We can find several studies on synthesising food-grade emulsions

**Table 2**
Food applications developed using microfluidics.

| Type of structure | Composition of the streams to form the emulsion | Application | Reference |
|---|---|---|---|
| Emulsion O/W | O: Hexadecane<br>W: pea protein solution | Design of plant protein-stabilised emulsions | Hinderink et al. (2020) |
| Emulsion O/W | O: astaxanthin in soybean oil<br>W: sodium dodecylsulphate in deionised water | Transport of astaxanthin (fat-soluble ketocarotenoid) used as a bioactive compound | Ulianova et al. (2020) |
| Emulsion W/O/W | $W_1$: NaCl and sucrose in water<br>O: polyglycerol polyricinoleate in sunflower oil<br>$W_2$: sucrose and waxy rice starch gelatinised in water | Improved perception of sweetness | Al Nuumani et al. (2020) |
| Emulsion W/O/W | $W_1$: MilliQ water with SDS or Tween 20<br>O: sunflower oil with PGPR<br>$W_2$: water with β-lactoglobulin in imidazole buffer | Study of double emulsion stability | Hughes et al. (2013) |
| Emulsion O/W | O: caffeic acid modified chitosan in acetate buffer<br>W: silkworm pupa oil isolate and Tween 20 | Generation of α-linolenic acid ethyl ester from silkworm chrysalis oil encapsulated in microparticles | Bai et al. (2019) |
| Solid lipid particles | O: palm oil<br>W: Tween 20 solution | Production of fat particles that could be used for incorporation into foods by modifying their rheology or in the transport of drugs | Kim & Vanapalli (2013) |
| Solid lipid particles | O: fish oil<br>W: protein aqueous solution (solutions of gelatin, casein and soy protein) | Encapsulation and protection of the fish oil for incorporation into food | Comunian et al. (2018) |
| Microgels | W: pluronic acid-poly(acrylic acid) copolymer<br>O: okara oil | Encapsulation of the probiotic *Lactiplantibacillus plantarum* CIDCA 83114 | Quintana et al. (2021) |
| Microgels | $O_1$: echium oil<br>W: sodium alginate and calcium-EDTA complex<br>$O_2$: soy lecithin and corn oil | Encapsulation of echium oil by ionic cross-linking microfluidic gelation | Comunian et al. (2017) |
| Microgels | $W_1$: dextran and protein (BSA or CAT)<br>$W_2$: Na-Alginate, PEG and protein (HRP or GOX)<br>O: paraffin oil | Encapsulation of bioactive molecules | Sun et al. (2019) |
| Microgels | $O_1$: sunflower oil<br>W: low acyl gellan gum, polyoxyethylene gellan gum, Tween 20<br>$O_2$: sunflower oil with calcium acetate and polyglycerol-polyglycerol-polyricinoleate | Production of gellan gum microcapsules containing hydrophobic compounds | Michelon et al. (2020) |
| Liposomes | $W_1$: PVA and dextran<br>O: soy lecithin and β-carotene in chloroform/hexane; ethyl acetate/hexane or ethyl acetate/pentane<br>$W_2$: PVA | Double emulsions to form β-carotene-filled liposomes | Michelon et al. (2019) |
| Liposomes | W: catechin and HEPES buffer<br>O: curcumin, dipalmitoylphosphatidylcholine, and cholesterol dissolved in isopropanol | Encapsulation of curcumin and catechin in liposomes for improved bioavailability | Hong et al. (2020) |
| Niosomes | O: Span 80 or Tween 85 with cholesterol and curcumin in etanol<br>W: distilled water | Encapsulation of curcumin | Obeid et al. (2019) |

W: aqueous phase; O: oil phase; EDTA: ethylenediaminetetraacetic acid; BSA: bovine serum albumin; CAT: catalase; HRP: horseradish peroxidase; GOX: glucose oxidase; PVA: polyvinyl alcohol; HEPES: 4-(2-hydroxyethyl)-1-piperazinetanesulfonic acid; Span 80: polysorbate 80; Tween 85: polyoxyethylene (20) sorbitan tri-oleate; SDS: sodium dodecyl sulfate; PGPR: polyglycerol polyricinoleate; Tween 20: polyoxyethylene (20) sorbitan monolaurate.

using microfluidic systems, such as those using proteins to stabilise O/W emulsions prepared with soybean oil (Saito et al., 2006) or O/W emulsions of polyunsaturated fatty acids using refined palm oil rich in ß-carotene (Neves et al., 2008). Table 2 provides a compilation of different applications developed by emulsion formation using microfluidic systems.

In addition, microfluidic systems are helpful for the synthesis of double emulsions, where a single water droplet is always formed inside an oil droplet, e.g. for W/O/W (Hughes et al., 2013). Double or multiple emulsions such as W/O/W and O/W/O are particularly interesting when designing encapsulation systems for hydrophilic and/or hydrophobic bioactive compounds (Ushikubo et al., 2015). Thus, it can achieve a controlled release of bioactive compounds in different parts of the gastrointestinal tract depending on where they need to exert their action (Muschiolik & Dickinson, 2017). Double emulsions also allow for the production of low-fat products due to the lower oil fraction proportion than single emulsions. However, the mouthfeel of multiple emulsions is similar to that of single emulsions (Muschiolik & Dickinson, 2017). Additionally, they allow masking flavours, prevent oxidation and improve the sensory characteristics of foods. Another application of double emulsions is the reduction of sodium content (Jiménez-Colmenero, 2013).

### 5.2. Formation of reactors for microparticle synthesis

The synthesis of solid particles to encapsulate a bioactive compound is another application of microfluidic systems. Encapsulation protects the bioactive compound from degrading conditions encountered in the environment, food or the gastrointestinal tract. Moreover, choosing the suitable polymer can exert a controlled release depending on an environmental stimulus (variation in pH, temperature, etc.).

As in emulsion synthesis, using microfluidic systems for microparticle synthesis allows for more uniform size distributions with more homogeneous mechanical properties (Ushikubo et al., 2015).

In the following sections, we will review the different types of particles developed so far using microfluidic systems and the applications that can be found for them in the literature.

#### 5.2.1. Solid lipid particles

When generating these particles in microfluidic devices, an O/W emulsion must be generated with the lipid containing the bioactive compound when used as encapsulants, followed by immediate cooling for the droplets to solidify (Feng & Lee, 2019). In this case, the bioactive compounds would be released once the melting temperature of the lipid is reached (Ushikubo et al., 2015).

Suspensions of solid lipid particles are coated with surfactants and dispersed in a continuous aqueous phase. The lipid structure that constitutes the particles solidifies partially or completely. The particles can be spherical or non-spherical in shape, depending on the crystals formed (McClements, 2016).

Solid lipid particles made of crystallisable fats are essential when producing ice cream or butter in the food industry. Additionally, they

enable encapsulation, protection and delivery of fat-soluble vitamins, carotenoids, and ω-3 oils and play a crucial role in food flavours and mouthfeel (Kim & Vanapalli, 2013; McClements, 2016).

Conventional techniques used to produce emulsions involving high shear forces (precipitation, spray drying or phase separation) for the production of microparticles result in polydisperse solid lipid particles, high variability in their structure and significant differences in encapsulation efficiency. Microfluidics allows the creation of multiple emulsions with a high degree of control, where the chemical composition of each flow can be varied, resulting in the formation of a wide variety of solid lipid particles (Duncanson et al., 2012), with the particular advantage of producing homogeneous particles (Kim & Vanapalli, 2013).

Despite the high potential and good results obtained in studies conducted so far, such as the one developed by Kim & Vanapalli (2013) to produce spherical and non-spherical fat particles with palm oil from a W/O/W emulsion (Table 2), the production of such particles by microfluidics and the consequent application in the food industry is not a widely explored field (Feng & Lee, 2019).

### 5.2.2. Microgels

A microgel is a microscopic three-dimensional network of hydrophilic polymers with both liquid and solid properties. Microgels absorb water or other liquid into their structure, leading to swelling, although the absorption characteristics are reversible. External stimuli, such as temperature, pH, ionic strength or type of solvent, can generate swelling and shrinking characteristics (Farjami & Madadlou, 2017; Ma et al., 2016).

The food industry uses a limited number of polymers that are "Generally Recognised As Safe" (GRAS). Most of the accepted polymers come from animal and plant sources (alginate, starch, cellulose, carrageenan and chitosan) and proteins (gluten, soy, zein, casein, whey protein, gelatine and collagen) and microbial (poly(hydroxyalkanoate) s) (Grujic et al., 2017; Niaounakis, 2013; Rhim, 2013).

Microgels are of particular interest in the food industry as they allow for reducing fat and energy density in processed foods and improving mouthfeel. Also allow for the decrease in cream formation during transport and storage, stabilisation of emulsions, and encapsulation of nutraceutical compounds directed to the desired release site (Shewan & Stokes, 2013). In addition, the outer membrane of microgel capsules allows the encapsulated material to be protected from harmful external stimuli (Duran et al., 2022).

Different methods can produce microgels: chemical/thermodynamic (e.g. phase separation) or mechanical/physical processes. There are those based on emulsions within the mechanical/physical methods, with the microfluidic technique being of particular relevance (Farjami & Madadlou, 2017).

The microfluidic technique allows the formation of monodisperse microgel particles, which helps to predict and adjust the release rate of the compounds of interest to achieve the desired final product. In addition, it is a reproducible method for the production of double emulsions, which is of interest for generating microgels (Shewan & Stokes, 2013).

The characteristics of microgels are relevant for the encapsulation of beneficial microorganisms. Incorporating them in miniaturised scale particles allows them to reach the target site more easily. In addition, they allow a better exchange of nutrients and oxygen between the inner capsule and the external environment. This also enables the control of the number of cells encapsulated inside the microgel (Mohd Isa, El Kadri, et al., 2021). This application will be dealt with in more detail in a subsequent section.

Water-in-oil (W/O) emulsion was necessary for the production of microgels by microfluidics, as it will serve as a template for producing the microgel (Sağlam et al., 2011). The aqueous phase of the emulsion will carry a polymer or multiple streams of the polymer in addition to the cross-linking agent. This aqueous stream is dispersed in the

continuous phase, which will be the oil, generating droplets.

Achieving gelation of the microgel particles requires the presence of some mechanism that induces gelation. Gelation can occur before or after the device's output (Farjami & Madadlou, 2017; Ushikubo et al., 2015). Ionic cross-linking of charged polysaccharides was one of the most common mechanisms for inducing gelation, whereby attractive forces occur between the polysaccharides and other species in solution, e.g. counterions, ionic surfactants and polymers. These attractive forces depend on pH, the isoelectric point of the polymer, ions present and salt concentration. In this case, a polymer such as sodium alginate (which is negatively charged) will be able to form a gel in the presence of calcium ions ($Ca^{2+}$) (Shewan & Stokes, 2013; Ushikubo et al., 2015). Another common gelation mechanism is covalent crosslinking, which involves the formation of covalent bonds for gel formation. A clear example would be the crosslinking of proteins such as gelatine in the presence of genipin (Shewan & Stokes, 2013; Ushikubo et al., 2015).

Several methods induce gelation inside the microfluidic device, either by ionic or covalent crosslinking: droplet coalescence, in situ mixing, internal gelation and external gelation (Ushikubo et al., 2015). Gelation by droplet coalescence comes from the collision of a droplet containing the polymer in solution, which collides with another droplet containing the crosslinking ion to form the gel (Fig. 4). This method does not produce microgels of homogeneous sizes (Liu et al., 2006; Ushikubo et al., 2015). The gelation provided by the in situ mixing is achieved by injecting the biopolymer and the crosslinking agent into different microfluidic device channels. When the two streams meet, they form a droplet that separates at the channel junction by the shear stress of the continuous phase (oil). The disadvantage of this method is that the polymer forms a gel at the channel junction (Ushikubo et al., 2015). The microgels' internal gelation is achieved by forming beads with polymers containing the crosslinking agent in an inactive form. A reaction initiator is diffused from the continuous phase through the droplets to activate the crosslinking agent and, thus, gelation (Fig. 5). The main drawback of this method is that it produces weak and unstable gels (Ushikubo et al., 2015). In external gelation, droplets of an aqueous solution containing the polymer are generated and form droplets when they meet another stream containing the dispersed crosslinking agent, initiating gelation (Fig. 5) (Ushikubo et al., 2015).

In the literature, we can find different applications, such as the one developed by Marquis et al. (2016), in which alginate microgels are obtained by internal gelation containing oil inside, which is an excellent alternative to encapsulating fat-soluble compounds. This study's starting point is an oil-in-water (O/W) Pickering emulsion stabilised by cotton cellulose nanocrystals. These emulsion droplets contain $CaCO_3$ on their surface, which acts as a cross-linking agent for the alginate (dispersed phase). Once the emulsion droplets are injected into the
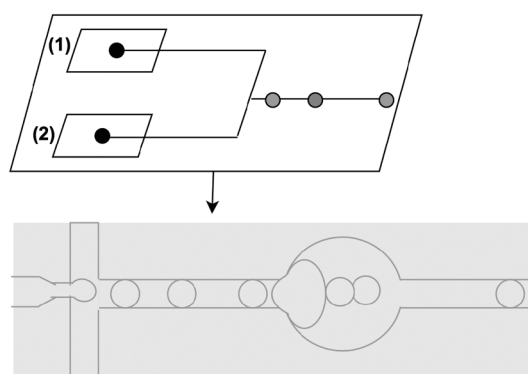


**Fig. 4.** Microfluidic system for the formation of microgels by coalescence. The figure above represents a diagram of the microfluidic pattern in which sodium alginate droplets are generated in the channel (1), and $CaCl_2$ droplets are generated in the channel (2).
Adapted from Liu et al., 2006.

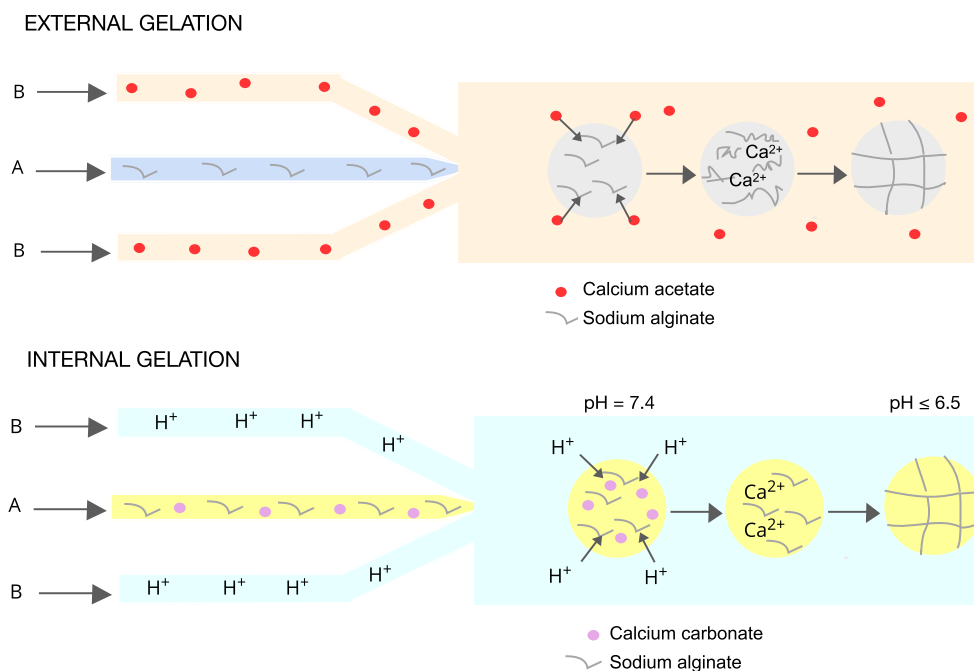EXTERNAL GELATION



INTERNAL GELATION

**Fig. 5.** Scheme of microgel generation by external and internal gelation using microfluidic systems.
Adapted from Zhang et al. (2007).

microfluidic device through one channel and meet the sunflower oil with acetic acid (continuous phase) injected through the remaining two channels, the acetic acid flows into the emulsion droplets. It causes a pH decrease inside the droplets, whereby calcium is generated due to the solubilisation of $CaCO_3$, which induces in situ gelation. Therefore, alginate microgels with several oil microdroplets are generated (Marquis et al., 2016). Further applications described in the literature for microgel synthesis by microfluidics are listed in Table 2.
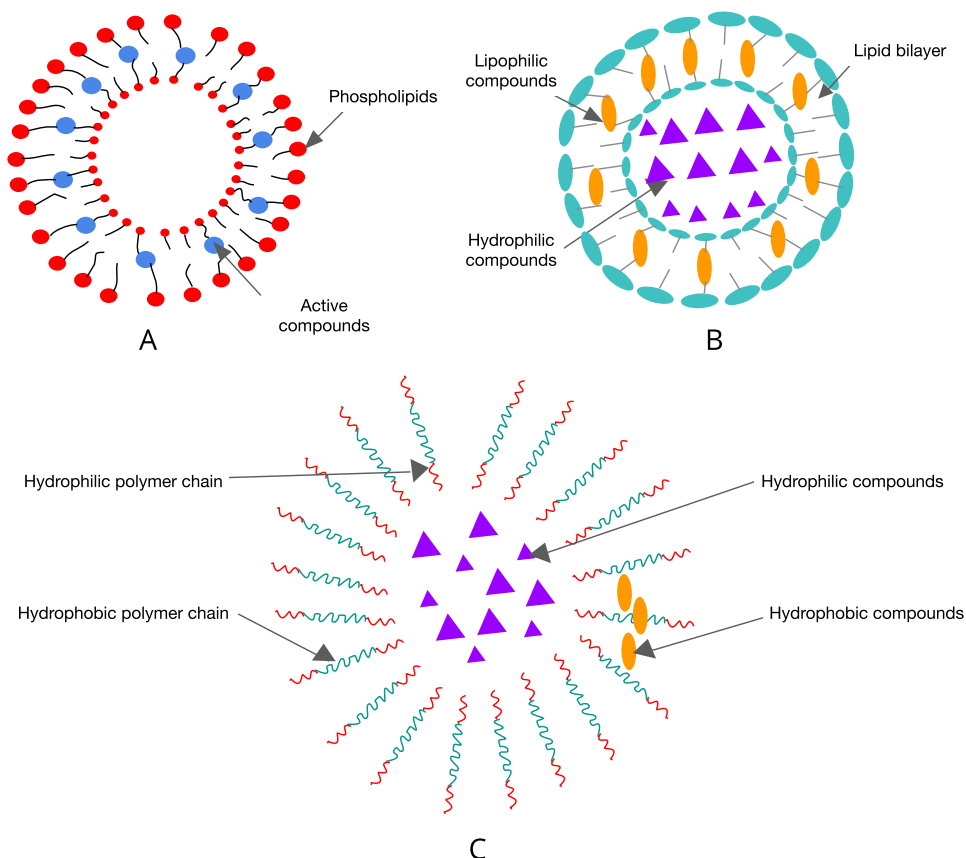


**Fig. 6.** Schematic representation of liposomes (A), niosomes (B) and polymerisomes (C). Adapted from Jhala et al. (2018) and Matos et al. (2019).

## 5.3. Synthesis of self-assembled structures

Self-assembled structures consist of surfactant molecules that are organised in different configurations depending on their concentration and the polarity of the medium (Ushikubo et al., 2015). In general, such structures are used to encapsulate hydrophilic and lipophilic compounds, e.g. flavourings, antioxidants, supplements and vitamins. They also protect bioactive compounds from detrimental environmental conditions such as temperature, pH, and chemical and enzymatic factors (Koubaa et al., 2018; Matos et al., 2019).

Microfluidics enables the production of these self-assembled structures so that, thanks to the small dimensions of the channels, faster and more uniform formation is facilitated (Ushikubo et al., 2015). Various self-assembled particles can be obtained using the microfluidic technique, including liposomes, niosomes and polymersomes (Ushikubo et al., 2015).

### 5.3.1. Liposomes

Liposomes are sphere-shaped vesicles consisting of a bilayer in which phospholipids self-assemble. The phospholipids consist of a polar head interacting with water and hydrophobic hydrocarbon tails moving away from water, creating the bilayer (Fig. 6A).

The microfluidic device produces liposomes using the flow-focusing method with cross-shaped geometry (Fig. 1C). For this purpose, an organic dispersion containing phospholipids is introduced into the central channel, and aqueous solutions, which compress the organic dispersion, are introduced into the adjacent channels. As a result, water molecules replace the organic molecules around the phospholipids, causing a solubility change in the system, which leads to the organisation of the phospholipids into bilayers and subsequently into spherical vesicles (Ushikubo et al., 2015).

Encapsulation of hydrophobic molecules is a challenge in the food industry because it is complex to incorporate a lipophilic bioactive compound into aqueous solutions. Furthermore, hydrophobic compounds have low bioavailability in the gastrointestinal tract. At this point, liposome encapsulation systems overcome this problem and are used as transport and release systems for bioactive compounds: hydrophilic, lipophilic and amphiphilic compounds (Matos et al., 2019; Toniazzo & Pinho, 2016). Nanoencapsulation of fish oils in nanoliposomes for subsequent application in yoghurt fortification is a clear example of their application (Ghorbanzade et al., 2017). They are also useful for modifying the texture of food components by developing new flavours and sensations (Ushikubo et al., 2015). However, there are still few studies of food applications of liposomes prepared by microfluidics (Table 2).

### 5.3.2. Niosomes

Niosomes are vesicles that self-assemble non-ionic surfactants in an aqueous solution, forming closed bilayers (Ge et al., 2019). Such structures can trap hydrophilic compounds in the aqueous compartments between the bilayers or lipophilic compounds, which are located within the surfactant bilayer (Sharma et al., 2015) (Fig. 6B). Niosomes have advantages over phospholipid compounds commonly used in liposomes in terms of lower production cost and higher storage stability (Kopermsub et al., 2011; Marianecci et al., 2014).

The use of microfluidics for the production of niosomes allows the formation of particles of controlled size and low polydispersion (Ushikubo et al., 2015). For their formation, in these systems, the main stream containing the surfactant mixture (sorbitan ester, cholesterol and diacetyl phosphate) in isopropyl alcohol is concentrated by the action of two adjacent streams of phosphate buffer. The mixture of alcohol and water facilitates the formation of niosomes (Lo et al., 2010).

Niosome systems have been used for different purposes in the food industry, e.g. encapsulation of carotenoids, capsaicin, curcumin, lactic acid, gallic acid or anthocyanins, or encapsulation of iron for yoghurt fortification (Elmi et al., 2020; Gutiérrez et al., 2016). However,

However, there are still a few food applications of these systems using microfluidics (Table 2).

### 5.3.3. Polymersomes

Polymersomes, or polymeric vesicles, consist of spherical vesicles whose structure is formed from amphiphilic copolymers self-assembled in an aqueous solution (Ushikubo et al., 2015) (Fig. 6C).

Polymersomes can be produced in microfluidics by two different techniques. The first consists of a mixing technique in a flat microfluidic device with flow-focusing geometry and perpendicularly crossed channels (Fig. 1C), in which a copolymer solution with ethanol is circulated to another stream in which water circulates. Another possible technique is the formation of a double W/O/W emulsion using a capillary-type microfluidic device, in which the solvent is evaporated from the emulsion, which is, in turn, stabilised by the copolymer. As the solvent evaporates, the polymer self-assembles and creates the polymersome (Ushikubo et al., 2015).

Microfluidics allows the creation of multi-compartment polymersomes that have the potential to store different compounds of interest and release them simultaneously (Duncanson et al., 2012).

As in the case of liposomes and niosomes, polymersomes are used for different encapsulation purposes within the food industry. A clear example is the encapsulation of anthocyanins from grape skins to obtain light mayonnaise (Constantin et al., 2020). However, it is still challenging to find food applications of polymersomes prepared using microfluidic systems.

## 6. Application of microfluidics for bacterial encapsulation

### 6.1. Suitability of microfluidics for the encapsulation of bacterial cells

Encapsulation is a widespread technology for protecting microorganisms against harmful environments, namely technological (e.g., dehydration, osmotic stress, high pressures, among others) and physiological processes (e.g., digestive juices, bile, acid pH). Among the different processes aimed at encapsulating microorganisms, emulsion droplets have several applications that have gained significant interest among researchers. Like microfluidic devices, microorganisms also have micrometric dimensions (ca. 1 μm in size). Therefore, they are suitable to be encapsulated using microfluidics (Niu & DeMello, 2012), providing an innovative method to control the process. Such control is associated with encapsulating individual cells as highly monodispersed droplets of controllable sizes and formats (e.g., multiple emulsions) in microdroplets containing a predetermined number of bacterial cells, increasing the efficiency of encapsulation. Full control of the microdroplets' generation can be achieved by modulating the diffusion rate across the oil phases (Zhang et al., 2013).

The stability of the generated emulsions is a challenge in microfluidics. In this regard, Mohd Isa et al. (2021) studied the effect of encapsulation on bacterial viability and metabolic activity, including the impact of bacteria on droplets' stability and the mechanism of release from double emulsions promoted by changes in the osmotic balance. Indeed, bacteria encapsulated in W/O/W emulsions, whereby the presence of nutrients in either of the aqueous phases (W1, W2 or both) can lead to changes in the composition of each phase. This indicates that nutrients can cross through the oil layer from W2 to W1, improving bacterial viability, considering that the oil layer acts as a protective barrier.

At this point, it must be noted that the destabilisation of W1/O/W2 emulsions can be beneficial for the controlled release of bacteria (or other food ingredients) in food products (Devanthi et al., 2018). A controlled release of bacteria can be achieved by changing the osmotic balance (e.g., changing the concentration of salts), which leads to the destabilization of emulsions under predefined conditions and stimulates the release of microorganisms (Mohd Isa, el Kadri, et al., 2021). For example, Kadri et al. (2016) were able to promote the release of E. coli

from the inner W1 phase into the outer W2 phase by changing the concentration of NaCl without affecting bacterial viability. The authors reported that both the concentrations of solute (in aqueous and oil phases) and the concentration of the hydrophilic and lipophilic surfactants strongly determined the release process. Different parameters can be modulated to adjust bacterial release, including the alteration of osmotic conditions (hyper or hypoosmotic, the latter promoting bacterial release) or the concentration of surfactants (lower concentrations promote bacterial release). A two-step mechanism for bacterial release includes splitting W/O/W droplets and releasing the W1 droplet (secondary emulsion formation); the collapse of W1 droplets promotes the release of bacteria into the W2 phase. Finally, besides the osmotic balance, a controlled release of bacteria can also be achieved by changing the pH and/or the temperature (e.g., freezing and thawing emulsion droplets).

Despite the potential of microfluidics to obtain fine-tuned stable emulsions, it has been scarcely used to encapsulate microorganisms in general. It remains relatively unknown in the world of lactic acid bacteria and probiotics.

### 6.2. Suitability of microfluidics for the encapsulation of probiotic bacteria

Probiotics are defined as "living microorganisms, which, when administered in adequate amounts, confer a health benefit to the host health" (Hill et al., 2014; WHO, 2002). They are consumed both incorporated into functional foods and as dietary supplements (Makinen et al., 2012; Okamoto et al., 2012) as pure or mixed cultures. It is desirable that bacteria safely arrive in the gut, the target organ, to exert their beneficial effects. This means they must overcome harmful environments, including processing, storage and passage through the gastrointestinal tract.

In this context, microfluidic encapsulation provides an exciting tool to protect probiotics against such harsh conditions, enabling their incorporation into the droplets with greater accuracy and ensuring high viability once they arrive in the gut. It also offers the possibility of incorporating additional materials (e.g., functional polymers such as pectins or inulin, among others) to enhance the protection of microorganisms when exposed to digestive conditions. Incorporating probiotics into polymers that are not hydrolysed along the gastrointestinal tract (e.g., inulin, pectins and even synthetically engineered polymers) represents a significant advantage in ensuring their safe arrival to the gut (Cassani et al., 2020; Ghibaudo et al., 2017, 2018, 2019). In this regard, in a recent work, Quintana et al. (2021) used a synthetic polymer composed of poly(acrylic acid) (PAA), a biocompatible and biodegradable anionic polyelectrolyte (pKa: 4.95), and pluronic® (PLU), composed of triblock copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) (PEO–PPO–PEO) (Fig. 7). The pluronic PEO block is hydrophilic and water-soluble, while the PPO block is hydrophobic and water-insoluble. These chemical characteristics provide an amphiphilic character to PLU (Quintana et al., 2017). The synthesis of copolymers containing PAA and PLU has been demonstrated to be a smart strategy to enable the safe arrival of drugs and bacteria into the gut. It also provides a polymer with less viscosity than others, which facilitates its use in microfluidic devices (Quintana et al., 2017, 2021).

To our knowledge, very few attempts have been carried out to encapsulate probiotic bacteria using microfluidic devices. In a recent report, Lactiplantibacillus plantarum CIDCA 83114 has been encapsulated using synthetically obtained polymers containing PAA and PLU. Besides the advantages mentioned above, using the copolymer as a disperse phase for encapsulating microorganisms in microfluidic devices provides an interesting system to control the delivery and release of probiotics delivery in the gut.

In another work, Lactobacillus and Bacillus subtilis were encapsulated into dual-core microcapsules in separate compartments through electrostatically-driven microfluidics. The alginate microcapsules were acid-resistant and retained the probiotic activity (lactic acid fermentation without interference among bacteria), fostering probiotics' proliferation and generating an anaerobic environment. The encapsulates also demonstrated to reduce inflammation, improve fat metabolism and restore intestinal barrier function, being suitable candidates for treating
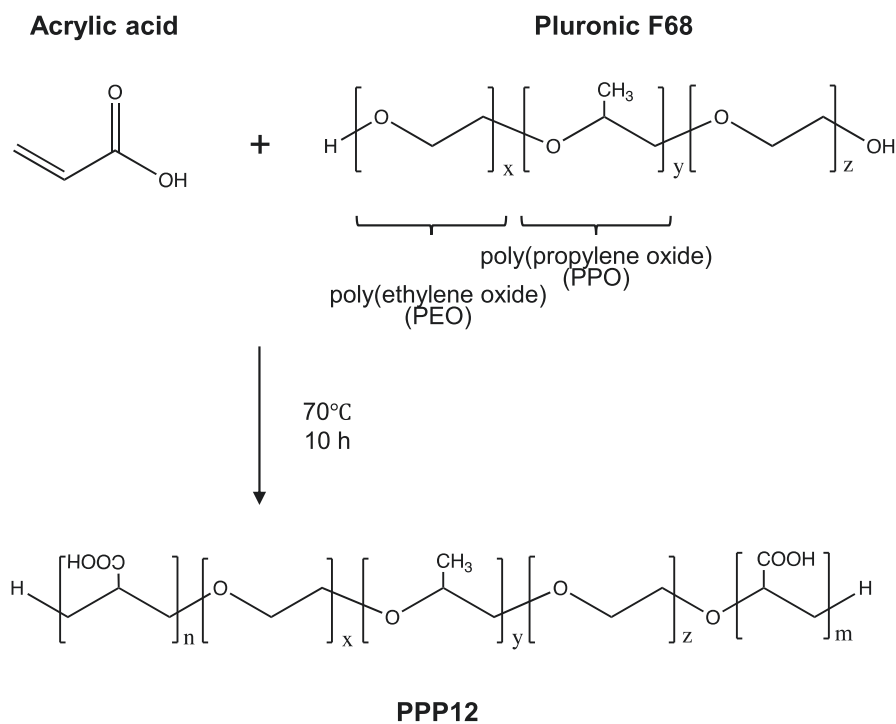


**Fig. 7.** Reaction scheme for the synthesis of PAA-PLU-PAA (PPP). $\times$ = number of ethylene oxide units; y = number of O-CH$_3$-CH$_2$ units; z = number of propylene oxide units, m, n = number of acrylic acid units.
adapted from Quintana et al., 2021.

metabolic syndrome and related diseases (Zhao et al., 2020).

## 7. Conclusions and future trends

Microfluidics presents several advantages for forming food-grade emulsions over traditional methods avoiding denaturation, in the case of proteins, or loss of activity in certain bioactive compounds incorporated in the emulsion. Microfluidic systems allow for reduced reaction volumes ($10^{-9}$ to $10^{-18}$ L) and minimise waste and more efficient and less costly reactions. Besides, microfluidics gives rise to a reduction in the size dispersion of the synthesised microparticles. The latter will be of great importance when incorporating these particles into food, as they provide better control of the texture and release compounds when these particles are used as encapsulating agents. Microfluidics provides a reproducible method for producing multiple emulsions (W/O/W or O/W/O), where the chemical composition of each flow can be varied, useful for encapsulating molecules different nature (hydrophilic and/or hydrophobic) in the same microparticle. This exhaustive control is particularly useful in applications like synthesising monodisperse microparticles containing probiotic bacteria with an exhaustive control of the number of cells encapsulated, protecting them against harmful environments and offering the possibility of incorporating functional polymers (e.g., soluble fibre as W phases) in different streams in the channels of the microfluidic system.

The evolution of research in applying microfluidic systems to the food field over the last 20 years has been considerable. However, this has not always been translated into developing new applications in the food industry. Commercial applications of microfluidics so far are more related to biosensors and the analysis and detection of different compounds. However, the development of scalable systems for the synthesis of new ingredients based on encapsulating particles or new food structures is still a topic with a wide field of exploration, where the search for food-grade fluids with physicochemical properties suitable for handling in microfluidic systems (viscosity between 0.3 and 1400 mPa s at 20 °C) is one of the critical points when transferring this technique to the industry. In turn, encapsulating bacteria using microfluidics still requires a thorough understanding of the effect of bacteria on the stability of droplets to expand their applications for both laboratory studies and industrial scale. Another limiting factor that requires further attention and research in the future is that working with such small sample volumes will require the implementation of multiple chips of very small sizes to make industrial production feasible. Finally, obstruction of the channels may require continuous production stoppages, increasing costs in the industry. All this means that there is still much research to be done on microfluidics applied to the food industry.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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