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A review of the antimicrobial activity of thermodynamically stable microemulsions

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Abstract

Microemulsions are thermodynamically stable, transparent, isotropic mixtures of oil, water and surfactant (and sometimes a co-surfactant), which have shown potential for widespread application in disinfection and self-preservation. This is thought to be due to an innate antimicrobial effect. It is suggested that the antimicrobial nature of microemulsions is the result of a combination of their inherent kinetic energy and their containing surfactants, which are known to aid the disruption of bacterial membranes, . This review examines the contemporary evidence in support of this theory.

Introduction

Definition of "microemulsions"

The literature contains some apparently contradictory descriptions of microemulsions, which is further obscured by some papers describing nanoemulsions, which are in fact larger than the microemulsions we discuss here. Hence, for our purposes, we define microemulsions as clear, thermodynamically stable, isotropic liquid mixtures of oil, water and surfactant. They are frequently formulated in combination with a cosurfactant and have dispersed domain diameters varying from 1 to 100 nm, but most usually 10 to 50 nm (Prince, 2012; Chen *et al.* 2020). The aqueous phase may contain salt(s) and/or other ingredients, and the oil phase may be a complex mixture of different hydrocarbons and olefins (Rosano and Clausse 1987; Paliwal *et al.* 2019). Figure 1 shows the basic forms of oil-in-water (O/W) and water-in-oil (W/O) microemulsions.

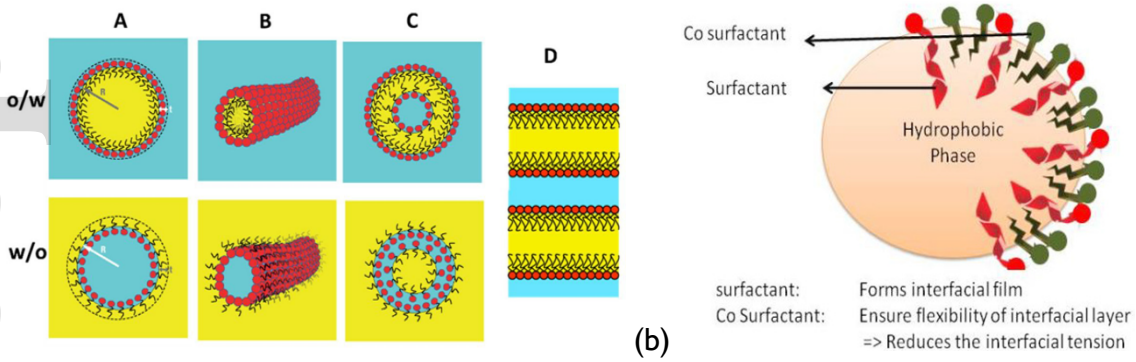

Figure 1: (a) Diagram representing potential self-assembled surfactant structures in microemulsions. Upper row oil-in-water, lower row water-in-oil. (A) Spherical micelles; (B) cylindrical micelles; (C) vesicles; (D) bicontinuous planar interfaces (Tartaro *et al.* 2020). (b) A schematic diagram of a water-in-oil single microemulsion droplet, showing the structure

relationship of surfactant, co-surfactants, oil (not labelled, but exterior to the droplet) and water (Mehta *et al.* 2015).

A brief history of the development of microemulsions

Microemulsions were first recognized as a special kind of colloidal dispersion by Hoar and Schulman (1943). They were first named microemulsions by Lewis (1954). However, they were probably accidentally discovered before then. Australian housewives have used a mixture of water, eucalyptus oil, soap flakes and white spirit to wash wool since the early part of the 20th Century (Langevin, 1988), which it has been suggested formed natural microemulsions subsequently used as cleaning agents. It was not until the 1970s that research into microemulsions revealed that they could be used to improve oil recovery (Shah, 1981). Microemulsions exist in a phase inversion region, and the interfacial film between oil and water micro-domains have a spontaneous radius of curvature close to zero (Auvray *et al.* 1984; Beckman *et al.* 2017). The structure is therefore lamellar-like. The reason the system is not macroscopically ordered has been explained by De Gennes and Taupin (1982). The bending elasticity of the surfactant film is small and the thermal fluctuations roughen the film, hence the lamellar order is rapidly lost. The dynamic properties of microemulsions have also been widely studied, but they are still poorly understood.

The ability for these liquids to solubilise both hydrophilic and lipophilic compounds has resulted in significant interest in their applications in a wide variety of fields such as pharmaceuticals, oil recovery and domestic cleaning products. The antimicrobial activity of microemulsions was first suggested by Anon. (1984), but was not previously shown in the literature until a study by Al-Adham *et al.* (2000). This review aims to look at the current status of the research in the area to produce evidence supporting a theory regarding the mode of action through which microemulsion antimicrobial action is achieved.

Current product applications of microemulsions

Early research into the uses of microemulsions focused upon their uses as pharmaceutical preparations (Tenjarla, 1999) and particularly as drug delivery systems (Solanki *et al.* 2012).

Partially due to studies exhibiting the antimicrobial nature of some microemulsion formulations, the focus has changed to their use as disinfectants. Broad internet searches suggest that microemulsions may be used in the following: dry cleaning processes, floor polishers and cleaners, personal care products, pesticide formulations, cutting oils, and drugs. However, specific examples are more limited as some current examples exhibit: (i) as crop care products (Anon. 2021a), (ii) microemulsion-based cleaning agents (Beisser and Hillerich, 2012), (iii) DOWSIL™ CE-1874 MicroEmulsion as a cleansing ingredient of shampoos and leave-in conditioners (Anon. 2021b), (iv) Cithrol™ 10GTIS a high-performance microemulsion facial cleanser (Anon. 2021c), and (v) the SeraShine™ range of facial cleansers available from KCC Beauty (Anon. 2021d). It is interesting to note that, whilst the role of these microemulsions is not specifically that of disinfectants, they are described as cleansing agents, a role associated with their antimicrobial properties.

Physico-chemical properties of microemulsions relevant to their antimicrobial action

For a thorough consideration of the physico-chemical properties of microemulsions readers are directed to the excellent review by Tartaro *et al.* (2020). For the purposes of our review on antimicrobial action we consider the following information sufficient to understand that which follows.

Like regular emulsions, microemulsions involve the dispersion of one solution in another solution with which it is normally immiscible. However, unlike normal emulsions microemulsions contain stabilising emulsification agents which lower the interphase tension between the two immiscible phases allowing the production of far smaller droplets in the dispersed phase and providing a thermodynamic stability unique to microemulsions (Lu and Gao, 2010). Due to the extremely small droplet size (~1-50nm, which is smaller than the wavelength of visible light, hence allowing it to pass through with minimal scattering), these solutions are optically transparent. Another feature unique to microemulsions is the fact that the high level of surfactant compounds present lowers the interphase tension to a point which facilitates spontaneous generation, with no input of energy (Singh *et al.* 2017). This spontaneous generation is one

criterion for distinguishing microemulsions from nanoemulsions, which contain much less surfactant and therefore, require a much higher input of energy to produce.

The internal structure of a microemulsion phase is not static, but is instead fluid, and its internal components are in constant and changing interaction with each another. Three unique forms of internal structure for a microemulsion have been elucidated: oil-in-water (O/W), water-in-oil (W/O) and bicontinuous (Nazar *et al.* 2009). A single microemulsion may take on each of the three morphologies and such transformations may occur if interrupted by changes in the environment or composition. Unlike other emulsion compositions a microemulsion will form spontaneously when the compositional elements are combined.

Microemulsions are categorised through the development of a ternary-phase diagram which balances the component parts against each other to place the microemulsion at its compositional state. An example of a typical phase diagram can be seen below (Figure 2).

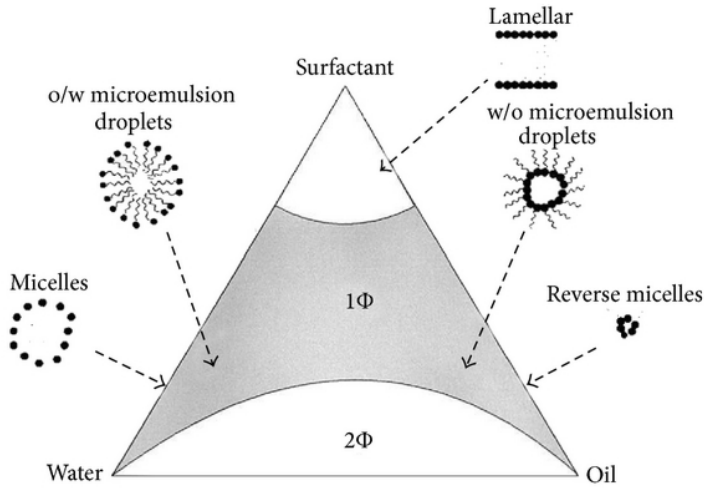

Figure 2: A model pseudo-ternary phase diagram, with the region of existence of O/W microemulsions, w/o microemulsions, micelles, reverse micelles, and bicontinuous two-phase system. The three corners representing oil, water, and surfactant (from Lawrence and Rees, 2000).

There has been significant ambiguity regarding terminology in the literature surrounding microemulsion research. A large amount of confusion is centred on the similarities and differences between *"microemulsions"* and *"nanoemulsions"*. This confusion is further increased by the use of these terms when referring to solutions with differing thermodynamics and droplet

sizes. Typically microemulsions lie in the range 1-100 nm (usually 10-50 nm), whereas nanoemulsions are larger at up to 600 nm (usually 20-200 nm). Therefore, it is necessary to determine unified definitions which facilitate clear distinctions between the colloidal systems in question. We propose such definitions in Table 1.

Table 1: Literature definitions identifying several related colloidal systems.

Colloidal System	**Scale (nm)**	**Definition**	**Source**
Micellar Solution	1	A colloidal solution containing a distribution of suspended amphiphiles, which form monolayer micelles varying greatly in size and morphology. *"Micellar Solutions have been shown achieving aggregate radii of 4-15 nm."*	Devised by the authors (Scherlund *et al.* 2000)
Microemulsion	10	*"a system of water, oil and amphiphile, which is a single optically isotropic and thermodynamically stable liquid solution"* *"(a) dispersion made of water, oil and surfactant(s) that is an isotropic and thermodynamically stable system with dispersed domain diameter varying approximately from 1-100nm, usually 10-50 nm."*	(Danielsson & Lindman, 1981) (Slomkowski *et al.* 2011)
Nanoemulsion	600	A colloidal system involving the stabilisation of the dispersed phase through the application of a surfactant compound. The surfactant is at low levels, hence ultimately lacking thermodynamic stability. Usually producing droplets of dispersed phase at ~600 nm. It should be noted that this system produces droplets of a larger size than microemulsions, which is at odds with the use of the prefix *"Nano"*.	Devised by the authors.

		"non-equilibrium systems with a spontaneous tendency to separate into constituent phases."	(Gutiérrez *et al.* 2008)
Macroemulsion (Emulsion)	1000-1000,000	*"(an) Emulsion in which the particles of the dispersed phase have diameters from approximately 1-100 μm (1000-100,000 nm)"*	(Slomkowski *et al.* 2011)
Liposomes		*"(an) association (of) colloids built up of amphiphilic lipid molecules that self-assemble in aqueous media into spherical self-closed structures"*	(Lasic & Needham, 1995)
	100-10,000 (500,000)	*"(a) form when phospholipids entropically self-assemble into vesicles in the presence of water, producing an aqueous medium surrounded by a lipid membrane"*	(Meure *et al.* 2009)
		"Liposomes have the potential to display sizes ranging from 0.1 μm to >10 μm (potentially reaching 500 μm)"	(Lesoin *et al.* 2011)

The effects of differing surfactants and co-surfactants upon the antimicrobial nature of microemulsions

Within the literature, groups working on the antimicrobial effects of microemulsions have almost exclusively utilised non-ionic surfactants from the Polysorbate (Tween) family, namely Tween 80, Tween 40 and Tween 20 (Zhang *et al.* 2010; Al-Adham *et al.* 2012; Kaur and Mehta, 2017). However, Brij™-35 (a nonionic polyoxyethylene surfactant that is most frequently used as a component of cell lysis buffers or a surfactant in various HPLC applications) has also been utilised in some studies (Al-Adham *et al.* 2000; Al-Adham *et al.* 2003). Literature review on these surfactants reveals a small number of studies indicating an inhibition of biofilm formation and even bactericidal activity from Tween 80 (Toutain-Kidd *et al.* 2009; Figura *et al.* 2012; Guo *et al.*

2020). No indications of antimicrobial activity can be found in the literature regarding polyoxyethylene (Brij™ 35) surfactants.

The most common co-surfactants incorporated into microemulsions with antimicrobial action are alcohols (e.g. Ethanol and Pentanol) with organic acids (e.g. Propionic acid) used in some instances. Alcohols (specifically Ethanol) are known to have growth inhibiting properties at high concentration and are used in antiseptic solutions (Oh and Marshall, 1993). The relatively low concentrations which co-surfactants comprise within a microemulsion would indicate that the antiseptic characteristic of alcohols do not account for the level of antimicrobial action exhibited by microemulsions (Erel-Akbaba *et al.* 2020).

Interfacial tension and stability of microemulsions

The interfacial tension in an O/W system is a reference to the energy comprising the boundary between the two phases. The energy is the result of hydrogen bonding of the water acting in a similar way to that in the development of "*surface tension*". The molecules at the outside of the system have fewer potential interaction partners than those in the bulk solution and thus interact more strongly with other water molecules. The oil molecules being much larger and unable to interact in the hydrogen bonding network, congregate in a separate phase. It is the energetic interaction between the molecules within each phase which acts against emulsification and generally outweighs the entropic bias towards mixing.

In microemulsions the addition of surface active, amphiphilic agents introduces potential interactions between molecules of the different phases by acting as an intermediary. This increase in interaction between molecules of oil and water results in a drop in the interfacial tension. When surfactant levels reach a sufficient point (known as the Critical Micelle Concentration, or CMC) the interfacial tension drops to a value at which it no longer sufficiently counteracts the *"entropy of mixing"*. The entropy of mixing is a force which favours emulsification as dispersion of one phase in another provides a larger number of potential molecular arrangements, than separate phases, and thus increases *"configurational freedom"* (entropy). It is the ability of the surface active agents (especially co-surfactants) to reduce the interfacial tension to below zero which bestows the spontaneous formation attribute on microemulsions, which results in prolonged physical stability of microemulsions. Even with a negative interfacial tension some solutions do not show spontaneous formation into microemulsions, but require gentle mixing.

This is likely due to other factors such as a slow speed of movement for non-polar molecules traveling through polar phases and vice versa (McClements, 2012).

Non-ionic surfactants used in microemulsions

The antimicrobial activity of the non-ionic surfactants (eg: Tween) in microemulsion formulations has been discussed by Kaur and Mehta (2017). They suggested that the mechanism of antimicrobial action of Tween microemulsions was a combination of the microbial requirement for water for growth and the structure of the microemulsion being harmful to the structural integrity of the microbial cell (Guo *et al.* 2020). Factors affecting the antimicrobial activity of microemulsions (eg: droplet size, synergistic effect and solubilisation) against resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus* have been studied (Guo *et al.* 2020). Results showed a 5-log reduction in bacterial titre of 45 mins and a LT90% of 15 s for both microorganisms. This confirms the role of Tween (Tween 80 concentration range of 15-25%), in that it can rapidly result in disruption of membrane structure, which results in irreversible cell damage (Al-Adham *et al.* 2000). This damage occurs in two phases; (i) initial periplasmic leakage where microemulsions leak through the space between the cell wall and the cytoplasmic membrane and subsequently (ii) cytoplasmic leakage, which causes cell death. The cell wall structure of the microorganism plays an important role in determining the exact mechanism of killing (Nielsen *et al.* 2016). Results showed that cytoplasmic membranes of *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* are adversely affected by microemulsions. Whereas, in Gram-negative *Pseudomonas aeruginosa* the outer membrane was affected initially and then the cytoplasmic membrane (Al-Adham *et al.* 2013). Different Tween microemulsion formulations have been assessed for their antimicrobial activity against different microorganisms (Zhang *et al.* 2010). For example, a cinnamon oil topical microemulsion has been studied against *Staphylococcus aureus* (Ghosh *et al.* 2013). Triton- and Tween 80-based formulations have been investigated against *Salmonella* spp., *Escherichia coli* 0157:H7 (VT-), *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Mycobacterium* spp. and *Listeria monocytogenes* (Teixeira *et al.* 2007). Other studies have investigated the antimicrobial effect of glycerol monolaurate loaded Tween microemulsions against *Aspergillus niger*, *Bacillus subtilis* and *Escherichia coli* (Fu *et al.* 2006). The outcomes of these studies appear to confirm the significant antimicrobial activity of Tween microemulsions. Most microemulsion research groups are agreed that the antimicrobial activity of microemulsions

results from the interaction between the microemulsion and the microbial membranes that disrupts the phospholipid bilayer and in turn causes the plasma membrane failure and increased membrane permeability. However, Tween alone (or in solution in water as a neutralising agent) has no significant antimicrobial activity, but as a surfactant it forms U-type microemulsions, which enhance membrane permeability and fluidity and disturb the functional properties of membrane resulting in cell death (Buranasuksombat *et al.* 2011; Kaur and Mehta, 2017).

The proposed mechanism of antimicrobial action of microemulsions

The proposed mechanism of antimicrobial action of microemulsions is the gross and irreversible disruption of microbial membranes resulting in cell lysis and death. This theory is based on two phenomena as follows:

(i) The similarity in forces involved in the structure and energetic maintenance of both microemulsions and bacterial membranes. Both systems are produced and maintained via a delicate balance of interfacial hydrophobic forces which rely on a delicate, but steady energy state. Therefore, it is suggested that the physico-chemical interaction of two such similar systems, such as a microemulsion and a bacterial cell membrane, may be expected to have a deleterious effect upon the forces holding the cellular membrane together. Microemulsions exhibit a greater level of kinetic activity than cell membranes, with a large and constantly changing variation in particle size and structure, resulting in constant energetic changes and fluid rearrangements of structure. These changes provide microemulsions with sufficient energy to interact with and grossly disturb the normally fluid, but stable bacterial membrane structure.

(ii) The essential presence of surfactants and sometimes co-surfactants in the structure for the microemulsion will necessarily bring these molecules into intimate contact with bacterial membranes. The ability of surfactants to disrupt and sometimes dissolve bacterial membranes is well understood, and indeed is often used by experimental microbiologists in the preparation of bacterial membrane and protein solutions (eg: for SDS-PAGE etc). One such example of this proposed mechanism for an amphiphilic copolymers is given in Figure 3.

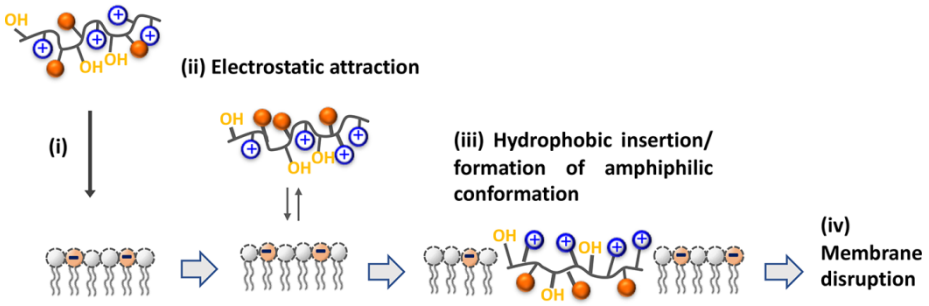

Figure 3. Proposed antimicrobial mechanism of cationic amphiphilic copolymers. (i) Cationic polymer chains are attracted to the anionic *E. coli* cell membrane surface by electrostatic interactions, and (ii) the polymer chains are associated with the membrane surface. (iii) The hydrophobic groups were inserted into the bacterial cell membranes, followed by the formation of membrane-active conformation and (iv) membrane disruption (MIC-determining step) (Mortazavian *et al.* 2018).

This indiscriminate interaction with the cell membrane will inevitably lead to loss of structure/function resulting in cell lysis. A significant volume of evidence has been produced to date in favour of this premise (Al-Adham *et al.* 2000; Al-Adham *et al.* 2003; Teixera *et al.* 2007; Zhang, *et al.* 2010; Al-Adham *et al.* 2012; Al-Adham *et al.* 2013; Alkhatib *et al.* 2013; Alkhatib *et al.* 2016).

Direct imaging

The most compelling evidence produced supporting the principle that microemulsions cause gross disruption of the microbial cell membrane is direct imaging, using Transmission Electron Microscopy (TEM), produced by Al-Adham *et al.* (2000), which exhibits a clear effect on bacterial membrane integrity. These images can be seen in Figures 4a (control) and 4b (microemulsion exposed).

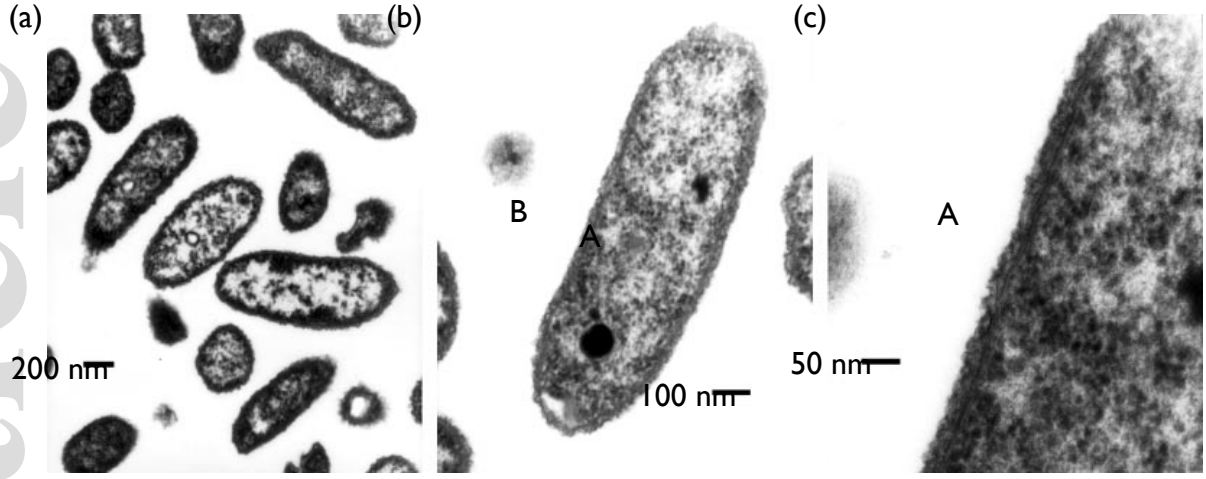

Figure 4a: Control images: Transmission electron micrographs of *Pseudomonas aeruginosa* ATCC 9027 exposed to water for 60 s and then prepared for TEM by the method of Al-Adham *et al.* (2000) and observed using a JEOL-1200 EX transmission electron microscope at 20,000X magnification (a), 50,000X magnification (b) and 100,000X magnification (c). Figure 3(b) shows normal cytosolic components (A) and an intact cell envelope (B). Figure 3(c) shows detail (A) of the cell envelope structure (from Al-Adham *et al.* 2000).

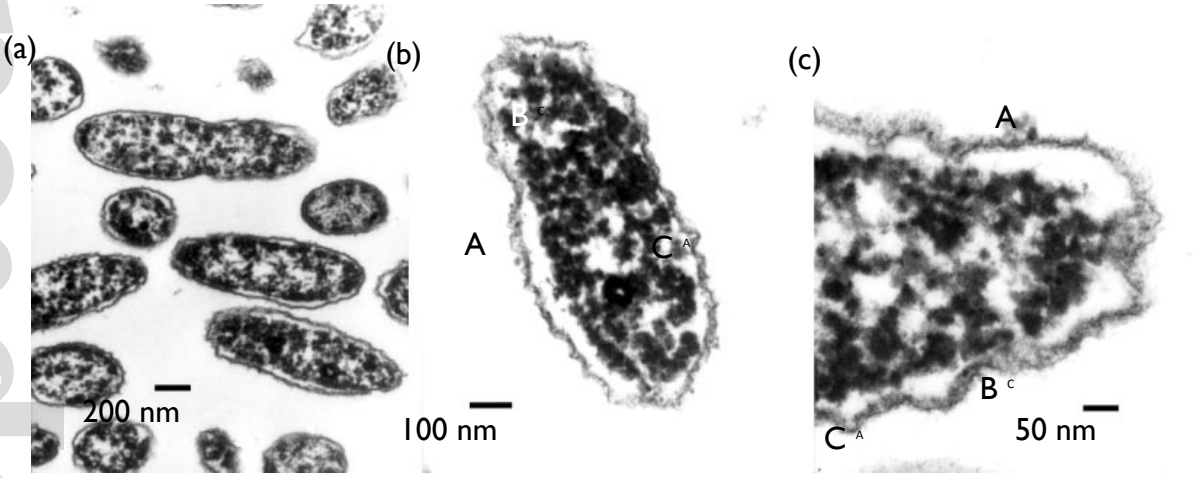

Figure 4b: Test images: Transmission electron micrographs of *Pseudomonas aeruginosa* ATCC 9027 exposed to a test microemulsion for 60 s and then prepared for TEM by the method of Al-Adham *et al.* (2000) and observed using a JEOL-1200 EX transmission electron microscope at 20,000X magnification (a), 50,000X magnification (b) and 100,000X magnification (c). Figure 4(b) shows crenation or separation of the cytoplasmic membrane from the cell envelope (A), coagulation of cytosolic components (B) and a disrupted outer membrane structure (C). Figure

4(c) shows membrane sloughing (A), membrane breaching (B) and `blebbing' (C) (from Al-Adham *et al.* 2000).

As can be seen from the TEM images, contact of a microbial cell with a microemulsion for 60 s causes rapid, serious and irreversible adverse effects, not just to the outer membrane, but also the intracellular components. The rapidity of the microemulsion effect in this study as well as in other killing rate studies (Al-Adham *et al.* 2013) are further evidence in favour of a mechanism of direct membrane disruption.

The studies by Alkhatib *et al.* (2013 and 2016) support the work above and provide evidence of significant disruption to bacterial cell membrane and cell wall structures. They observed that their *"formulations have affected St. aureus through changing the cell morphol. and cell wall composition, reducing the cell respiration and hydrophobicity, and enhancing the potassium leakage and cell permeability to the cytoplasmic constituents."* (Alkhatib *et al.* 2013*).* The same group also found that microemulsion formulations with cephalosporin *"affected the S. aureus through changing cell morphology, cell wall composition (sugar, protein and phosphorus), potassium leakage and cellular respiration."* (Alkhatib *et al.* 2016).

Hence, we propose a disruption model illustrated in Figure 5 below.

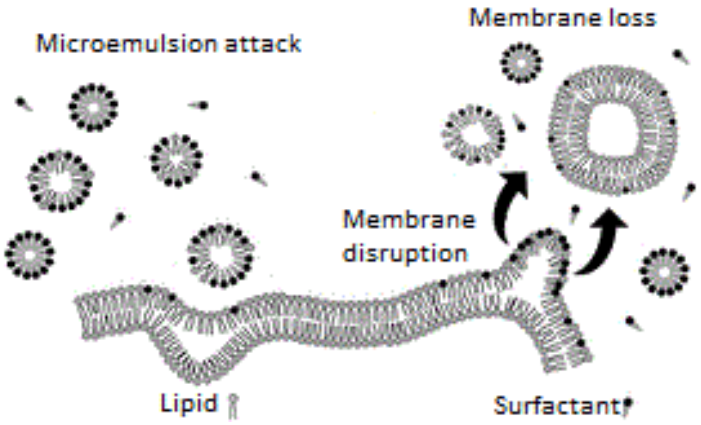

Figure 5. Schematic diagram of the propoised action of microemulsions against bacterial membranes (adapted from Fait *et al.* 2017).

Indirect supporting evidence

Studies have been performed which have identified an antimicrobial action of microemulsions towards a wide range of microbial species (some examples of which are given in Table 2). This evidence supports the hypothesis that the antimicrobial target of microemulsions is a cellular structure or function common to the several different microbial species and groups. Given the diversity of microbial groups exhibiting susceptibility to microemulsions, it is likely that this target is the cellular membrane(s).

Table 2: Some of the papers which have shown the antimicrobial effect of microemulsions (and nanoemulsions*) towards different types of microbes.

Microbe	**Studies showing evidence of anti-microbial action**
Bacteria	Al-Adham, *et al.* 2003; Zhang, *et al.* 2007; Zhang *et al.* 2010
Fungi	Hamouda, *et al.* 2001*; Zhang *et al.* 2010; Al-Adham, *et al.* 2013
Viruses	Donovan, *et al.* 2000*; Hamouda, *et al.* 2001
Bacterial Spores	Hamouda, *et al.* 1999*[1]; Zhang *et al.* 2010
[1]This study utilised a germinating agent resulting in the killing of the germinating spores and not those which remain in stasis. Hence, this does not constitute a true sporicide.	

A study performed by Al-Adham *et al.* (2012) provided evidence that the antimicrobial effect produced by a microemulsion was dependant on its position within the microemulsion stability zone (refer Figure 2 above). It was found that the antimicrobial effect was strongest towards the centre of the microemulsion's zone of stability. This is evidence that the anti-membrane effect is a product of the thermodynamic balance within the system and not a characteristic of any individual component(s). It should also be noted that earlier studies by Al-Adham *et al.* (2000 and 2003) tested all the components of the microemulsions for antimicrobial effects and found that any such effect present was negligable within the parameters of the experiment.

Evidence of a fungistatic, rather than fungicidal effect on *Candida albicans* supports the membrane disruption theory. It could result as a factor of the covalent bonds within the cell wall, which surrounds the plasma membrane of the cell, resisting disruption by the microemulsion and thus, the cells are not killed. However, growth is inhibited as the plasma membrane becomes exposed and susceptible to the microemulsion during the budding process (Van Hamme *et al.* 2006).

Ziani *et al.* (2011) showed that the nature of the surfactant in a microemulsion has effect upon the antimicrobial effect of that microemulsion. They observed that due to the negative charge of microbial membranes, anionic surfactants exhibited lower antimicrobial activity than cationic surfactants in an otherwise similar microemulsion preparation. This would be the result of differing charge interactions between the microemulsion and the bacterial cell membrane. This further suggests that the site of antimicrobial action is at the microemulsion/cell membrane interface, as suggested by the gross membrane disruption hypothesis.

Contemporary uses for microemulsions, including their antimicrobial uses

Due to their unique characteristics microemulsions have found many uses in a wide range of fields. One of the earliest applications of microemulsions was in oil recovery where they are able to solubilise the oil, hence allowing water removal from oil/water mixes with greater ease (Santanna *et al.* 2013). Microemulsions have also found applications within cosmetics (eg: hair-care products), drug delivery (eg: aceclofenac, meloxicam and indomethacin) and food production (Paul and Moulik, 2001; Boonme *et al.* 2011; Nastiti *et al.* 2017; Ja *et al.* 2019). It is interesting that the earlier, more general and delivery-focused use of microemulsions is being replaced by their antimicrobial and preservative applications. Some recent examples are given here.

Many workers have looked at the inclusion of plant extracted actives into microemulsions in order to elicit an enhanced antimicrobial action. These include: herbal microemulsions as anti-cariogenic biofilm agents (Ramalingam and Amaechi, 2020). Microemulsions prepared with essential oil from thyme (*Thymus vulgaris*) have been shown to be potent fruit disinfectants (Almasi *et al.* 2021a). Microemulsions have also been used as antiviral therapeutics and delivery systems (Franklyne *et al.* 2021). The enhancement of the natural antimicrobial activity of the

Asian ginger spice, galangal (*Alpinia galanga*) has been achieved by microencapsulation in a microemulsion system as shown by Khumpirapang *et al.* (2021). In addition, the antimicrobial activity of essential oils has been enhanced through their entrapment within microemulsions (Almasi *et al.* 2021b; Thakur *et al.* 2021).

Microemulsions have also been shown to have some anti-insect effects. In particular, turmeric (*Curcuma* spp.) containing microemulsions have been exhibited to have significant acaricidal effect against the red spider mite (*Tetranychus cinnabarinus*; Cheng *et al.* 2020).

In food production, microemulsions have shown considerable preservative effects (Yang *et al.* 2015), in that, microemulsions have been shown to improve the quality and safety of muscle-derived foods (eg: meat, fish, etc.; Das *et al.* 2020). Almasi *et al.* (2021b) have also applied the antimicrobial properties of microemulsion biopolymers in food production and preservation. Microemulsion-based edible coating for strawberries have an antifungal effect against black-spot of strawberry (caused by *Colletotrichum acutatum*), and hence extend the shelf life of the fruit (Dong *et al.* 2020).

In the pharmaceutical field microemulsions continue to elicit great interest and some recent developments include, microemulsions containing Amphotericin B for the treatment of fungal lung infections (Marena *et al.* 2020), the encapsulation of moxifloxacin into microemulsions for the treatment of skin conditions (Erel-Akbaba *et al.* 2020), and the development of a trans-dermal delivery system for the antispasmodic drug Eperisone Hydrochloride (Kumbhar *et al.* 2020).

Conclusions

Significant evidence exists supporting the theory that the antimicrobial activity of microemulsions is the result of gross disturbance of microbial membranes. It is posited that this disturbance is the result of a combination of (i) energetic exchange between the kinetically active microemulsion systems and the normally fluid, low energy, stable cytoplasmic membranes of microbial cells, and (ii) the presence of membrane-disruptive surfactants in the microemulsion structure. The physical contact between the two forms of normally stable structures results in a rapid and uncontrolled energy transfer from the microemulsion to the cytoplasmic membrane (and in the case of Gram-negative bacteria, the outer membrane) and subsequent breakage of the membrane. This process is aided by the presence of surfactants in the microemulsion structure.

Such breakage is supported by the evidence given by at least two research groups. Subsequently, the microemulsions may enter the microbial cell via the breached membrane and interact with the internal cellular components. The overall result of this activity is cell death. Hence, microemulsions are effective self-preserving agents for pharmaceutical systems and may be used as disinfectants.

Author contributions

ISIA-A, EAK and PJC undertook the original research investigating the antimicrobial nature of thermodynamically stable microemulsions. NJ, MA-R and FA-A gave essential advice concerning the medicinal chemistry of the microemulsion systems. ASAA-A and LBF contributed to our literature knowledge of the topic. All the authors contributed to the original idea behind the review and were involved in the writing and editing of the review.

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