Développement et caractérisation d'un nouveau procédé d'émulsification non dénaturant par transduction piézoélectrique de hautes fréquences

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À mes très chers parents auxquels je dois ce que je suis,

À mes chers frères, mes sœurs et leurs petites familles,

À mon très cher et tendre époux.

Abréviations

H/E Emulsion huile dans eau

E/H Emulsion Eau dans huile

H/E/H Emulsion huile dans eau dans huile

E/H/E Emulsion eau dans huile dans eau

DHA Docosahexaenoic Acid

EPA Eicosapentaenoic acid

ALA α-linolenic acid

DLS Dynamic Light Scattering

HLB Hydrophilic- Lipophilic Balance

CPG Chromatographie en phase gaz

FTIR Spectroscopie infrarouge à transformé de fourrier

^{1}H NMR Proton Nuclear Magnetic Resonance

PDI Polydispersity Index

PUFA Polyunsaturated Fatty Acid

SAXS Small Angle X-ray Scattering

SANS Small Angle neutron Scattering

HFU High frequency ultrasounds

LFU low frequency ultrasounds

HPH High pressure homogenization

LEC Colza Lecithin

CoQ$_{10}$ coenzyme Q_{10}

f fréquence

P pression

CMC critical micelle concentration

NTA Nanoparticles tracking analysis

SUR surfactant

v/v Volume/ Volume

w/w Weight/ Weight

Valorisation scientifique

Publications

- **Kaci, M.**, Meziani S., Arab-Tehrany E., Gillet G., Desjardins I., Desobry S. (2014). "Emulsification by high frequency ultrasound using piezoelectric transducer: Formation and stability of emulsifier free emulsion. Ultrasonics Sonochemistry 21(3): 1010-1017.
- **Kaci, M.**, Arab-Tehrany E., Gillet G., Desjardins I., Desobry S. (2015). Emulsifier free emulsion: Comparative study between a new high frequency ultrasound and standard emulsification processes. Chemical Engineering Journal (Soumis).
- **Kaci, M.**, Arab-Tehrany, E., Dostert, G., Gillet, G., Velot, E., Desobry, S. (2015). Efficiency of emulsifier free emulsions and emulsions containing rapeseed lecithin as delivery system used for vectorization and release of coenzyme Q10: physico-chemical properties and in vitro evaluation. Journal of Nanobiotechnology (Soumis).
- **Kaci, M.**, Arab-Tehrany, E., Michaux, F., Gillet G., Desjardins I., Desobry S. (2015). Structural analysis of emulsifier free nanoemulsions made with ultrasonic emulsification. (En cours de finalisation).

Communications internationales orales

- **Kaci M**., Arab-Tehrany E., Gillet G., Desjardins I., Desobry S. (2013). New emulsification process with high frequency ultrasound, Oral presentation, World biotechnology congress, Boston (USA).
- **Kaci M**., Arab-Tehrany E., Gillet G., Desobry S., (2014). Emulsifier free emulsion: comparative study between a new emulsification process using high frequency ultrasound and classic emulsification methods, Oral presentation, V Congreso Internacional de Ciencia y Tecnología de Alimentos, Cordoba (Argentina).
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- **Kaci M.**, Meziani S., Arab-Tehrany E., Gillet G., Desjardins I., Desobry S., (2012). Surfactant free emulsions produced by piezo-electric transduction with high frequency ultrasound, Food factory, Laval (France).
- **Kaci M.**, Arab-Tehrany E., Gillet G., Desjardins I., Desobry S., (2013). New emulsification method using piezoelectric transduction without addition of additive, Cosmminov, 2013, Orléans (France).
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Introduction et objectifs de la thèse

Introduction et objectifs de la thèse

Les émulsions sont omniprésentes dans notre quotidien et leurs domaines d'utilisation sont multiples. Elles sont retrouvées dans le domaine pharmaceutique où les émulsions simples et doubles sont largement utilisées (topiques, vaccins...). Aussi, on les retrouve abondamment dans le secteur alimentaire où la plupart des aliments naturels et traités par des procédés industriels sont sous forme d'émulsion comme le lait, la crème, les sauces, le beurre ou encore la mayonnaise. Elles sont également présentes dans les peintures, c'est le cas par exemple des émulsions de latex. Les émulsions sont prépondérantes dans le domaine cosmétique où 90% de ces produits sont issus des émulsions. Les crèmes, laits, gels, gommages, masques, fond de teint, … sont tous des émulsions.

Elles représentent une vaste gamme de produits du fait de leurs fonctionnalités, leurs textures et de leurs légèretés très appréciées par les consommateurs. Une émulsion est un milieu hétérogène et sa structure de base se compose d'une dispersion d'une phase dans une autre, dans laquelle la phase dispersée possède une échelle de longueur typique. La gamme de tailles des gouttelettes des émulsions peut varier considérablement, passant de quelques nanomètres à quelques dizaines de micromètres. C'est pour cela que beaucoup de travaux sont accès sur ces dernières car les gouttelettes sont si petites qu'elles se répartissent de façon homogène. Le mélange peut alors rester liquide ou prendre la consistance d'une pâte ou d'un gel.

Au sein des émulsions, nous retrouvons les nanoémulsions qui, à l'heure actuelle, présentent un grand intérêt pour les industriels du fait de leur grande stabilité.

Les émulsions sont des systèmes cinétiquement et thermodynamiquement instables et ses deux phases tendent à se séparer (à coalescer) car pour atteindre l'état le plus stable, elles tendent à réduire leur interface de contact et donc à se séparer entièrement, c'est pour cela que la présence d'agents tensioactifs est nécessaire pour assurer la stabilité de ces dernières. Ces tensioactifs s'accumulent aux interfaces l'huile-eau et contribuent à la stabilisation colloïdale des émulsions.

Conventionnellement, une émulsion est préparée avec un tensioactif qui joue un rôle dans la réduction des tensions interfaciales et qui crée des forces électrostatiques répulsives et stériques qui limitent les phénomènes de coalescence entre les gouttelettes de la phase dispersée. Différentes méthodes sont utilisées pour la fabrication d'émulsions telles que

l'homogénéisation à haute pression, l'homogénéisation à haute vitesse (type ultra-turrax) ou la sonication.

Contrairement aux émulsions, les nanoémulsions présentent une cinétique de dégradation tellement lente qu'elles sont dites stables et cela grâce à leur caractéristique de taille inférieure au micron. Dans ce cas, l'ajout de tensio-actif peut être réduit, voire supprimé.

Au cours des dernières décennies, beaucoup de recherches ont été effectuées sur les systèmes auto-émulsionnables générant des nano-gouttelettes. Cependant, ces dernières contiennent pour la plupart des agents de stabilisation pour le maintien de ces émulsions.

Dans cette thèse Cifre une nouvelle méthode d'émulsification brevetée par la société GENIALIS a été développée. Elle consiste à utiliser des ultrasons de hautes fréquences pour éviter les effets mécaniques violents de la cavitation acoustique aux basses fréquences et de stabiliser ces émulsions sans addition de tensioactifs.

L'émulsification ultrasonore est une technique d'émulsification basée sur l'application d'ultrasons à basses fréquences appelées ultrasons de puissance ; l'émulsification dans ce cas est due aux phénomènes de cavitation. Cette technique d'émulsification utilise des transducteurs munis de céramiques piézoélectriques qui vibrent sous l'effet d'un champ électrique avec des mouvements oscillatoires.

Ce phénomène repose sur une succession de pressurisation/dépressurisation conduisant à chaque cycle à la croissance des bulles de vapeur d'eau formées par vaporisation additionnelle à chaque dépression. La bulle de vapeur d'eau peut ainsi atteindre plusieurs microns avant d'éclater (collapse) sous l'action de la pression.

Au sein de la gamme d'ultrasons, la puissance disponible est inversement proportionnelle à la fréquence. Les ultrasons de puissance (16 - 100 kHz, et, dans une moindre mesure, 100 kHz - 500MHz) sont capables d'interagir avec la matière, produisant des modifications physiques et chimiques dues aux phénomènes de cavitation.

En effet, l'application d'ultrasons de basses fréquences provoque la formation et l'effondrement de microbulles par les fluctuations de pression d'une onde sonore simple. Chaque éclatement de la bulle provoque des niveaux extrêmes de turbulences très localisées. A l'intérieur des bulles de cavitation, des températures de plusieurs centaines de degré Celsius et des pressions supérieures à 800 bars sont atteintes conduisant à une dégradation des composés sensibles présents dans l'émulsion.

Au cours de la présente thèse, la méthode d'émulsification développée consiste à utiliser des ultrasons de hautes fréquences (>1MHz, ultrasons de diagnostics de faible puissance) pour éviter les effets mécaniques violents de cavitation. A ces fréquences, l'émulsification par

ultrasons se produit par deux mécanismes. Tout d'abord, l'application d'un champ acoustique produit des ondes déstabilisant les interfaces. Ce phénomène entraîne l'irruption de la phase huileuse dans le milieu aqueux sous forme de gouttelettes de l'ordre de 70µm. En second lieu, le mouvement global de la phase continue provoque le passage des gouttelettes à proximité du transducteur piézoélectrique conduisant à leur cisaillement et leur fractionnement vers des diamètres finaux de l'ordre de 1 micron.

Lors de notre étude, plusieurs points affectant l'émulsification à ultrasons à hautes fréquences ont été abordés. Ces points concernent essentiellement le cœur du processus : Comment se fait l'émulsification par hautes fréquences? Quels sont les mécanismes mis en jeu? Quels sont les paramètres influents ?…..

Le but de ce présent travail est de mettre l'accent sur la possibilité de développement d'une nanoémulsion cinétiquement stable dépourvue d'agents stabilisants donc ce travail sera accès sur la suppression de tensio-actifs dans les nanoémulsions.

La partie bibliographique (chapitre I) résume les notions principales sur les émulsions. Les différents procédés d'émulsification existant sont développés et principalement le procédé d'émulsification acoustique ainsi que les mécanismes de stabilisation des émulsions.

Des caractérisations physicochimiques des émulsions sans émulsifiant ont été faites dans le chapitre II et l'influence du temps de procédé sur les propriétés physicochimiques a été étudiée.

Dans le chapitre III, une étude comparative des propriétés physico-chimiques des émulsions de différents types produites via le procédé d'émulsification par ultrasons de hautes fréquences (UHF) développé par la société GENIALIS et via différents procédés d'émulsification standard tels que l'homogénéisation à haute pression (HHP) et la sonication par ultrasons de basses fréquences (UBF).

Le chapitre IV représente une application du procédé. Lors de leur utilisation en cosmétologie, la liste des ingrédients de formulation d'émulsion sans tensioactifs est réduite, ce qui limite l'apparition d'allergies ou autres désagréments. Une étude d'encapsulation d'un principe actif témoin «coenzyme Q10» a été réalisée avec des émulsions contenant un tensioactif (lécithine) et des émulsions sans tensioactif obtenues par le procédé d'émulsification par hautes fréquences.

Enfin, une étude poussée des émulsions sans émulsifiants par diffusion des rayons X aux petits angles (SAXS) et par résonance magnétique nucléaire (RMN) a était réalisée et comparée à des émulsions contenant des émulsifiants.

Chapitre I : Partie bibliographique

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1.1.Les émulsions

1.1.1 Définitions

Les émulsions sont des dispersions de deux phases non miscibles où l'une des phases est dispersée sous forme de gouttelettes dans l'autre. La phase qui est présente sous forme de gouttelettes est dénommée « phase dispersée » et la phase qui constitue la matrice dans laquelle se trouve les gouttelettes en suspension est appelée la « phase continue » (Bourrel and Schechter 1988; Pal 1994). Les deux phases non miscibles de l'émulsion n'ont pas la même solubilité. L'une est hydrophobe ou lipophile, désignée couramment : phase hydrophobe. L'autre est hydrophile, dite phase aqueuse (Chevalier, Bolzinger et al. 2014). La phase continue est parfois appelée la phase externe, milieu de dispersion ou milieu de suspension (Rao and McClements 2011; Chen, Liu et al. 2013). Ces systèmes sont des cas particuliers des dispersions colloïdales ayant une taille variant de 10 à 1000 nm (Goutayer 2008). Les émulsions contiennent aussi généralement un troisième composant, appelé l'agent émulsionnant ou émulsifiant**.**

Les émulsions sont des systèmes instables sur le plan thermodynamique, car la séparation des deux phases mène à une diminution de l'énergie libre du système. La stabilité du mélange est alors assurée par un agent émulsifiant formant un film interfacial autour des globules de la phase dispersée (Mason, Wilking et al. 2006). Conventionnellement une émulsion est préparée par des moyens mécaniques tels que homogénéisateur, mixer, ...

1.1.2. Structure des émulsions

Les ingrédients de l'émulsion s'auto-organisent sous l'effet de différents facteurs: physiques (forces intermoléculaires ou inter-gouttelettes), chimiques (Formation de liaisons covalentes) et biologiques (fermentation, etc.). Comme les émulsions sont des systèmes métastables, leur structure initiale évolue avec le temps, pendant le stockage et/ou le transport, suite aux traitements thermiques et aux contraintes mécaniques (Leal-Calderon, Schmitt et al. 2007).

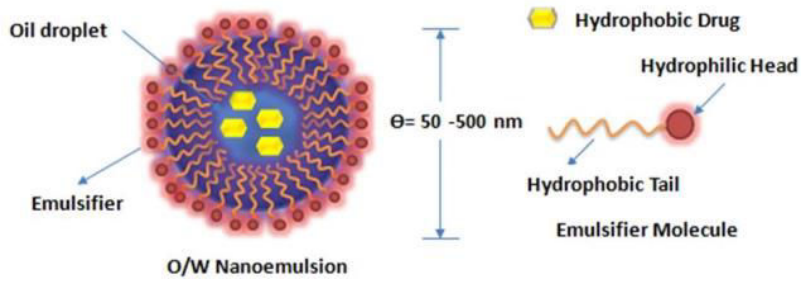


Figure 1 : Schématisation de la structure d'une gouttelette d'huile dans une nanoémulsion huile dans eau stabilisée par un tensioactif (Sivakumar, Tang et al. 2014)

Il est d'une importance primordiale de fabriquer des structures qui ne sont pas seulement bonnes en termes de perception sensorielle, mais aussi suffisamment stables pour être commercialement viable. Les exigences relatives à la composition et aux propriétés des gouttelettes d'émulsion sont différentes selon l'utilisation finale. Le comportement des émulsions alimentaires est déterminé par les trois parties du système: la phase huileuse, la phase interfaciale et la phase aqueuse (Figure 1). L'interface peut être éliminée dans le cas d'émulsions acoustiques sans tensio-actif.

Comprendre les propriétés structurales des émulsions exige une connaissance précise des comportements de ces trois constituant de l'émulsion, à la fois individuellement et en mélange (Schramm 2006).

1.1.3. Composition des émulsions

Les émulsions sont composées généralement de deux phases distinctes : une phase hydrophile généralement de l'eau, une phase hydrophobe généralement une huile, et d'un émulsifiant à l'interface de ces deux phases.

1.1.3.1. Phase hydrophile

Composée essentiellement d'eau, ou de macérât hydro glycériné, la phase aqueuse peut également intégrer une gomme, une résine ou d'autres ingrédients selon le produit fabriqué, elle peut être additionnée à d'autres substances humectantes ou à des principes actifs rendant le milieu plus ou moins impropre au développement de micro-organismes.

La phase aqueuse peut contenir des ions, des biomolécules telles que des polysaccharides ou des protéines, qui peuvent exercer des effets stabilisateurs ou déstabilisateurs.

1.1.3.2. Phase hydrophobe

C'est la phase qui conditionne les caractéristiques et propriétés du produit fini. La phase hydrophobe peut être de nature lipidique et sert souvent de matrice de vectorisation des

principes actifs hydrophobes utilisés dans les domaines des cosmétiques ou des pharmaceutiques. La phase hydrophobe peut être constituée de triglycérides mais aussi d'huile essentielle (terpènes) ou d'actifs hydrophobes. Concernant cette huile végétale, dans l'agroalimentaire il peut s'agir de l'huile de tournesol, colza, huile d'olive et autre huiles comestible. En cosmétique, il peut s'agir soit des huiles végétales ayant des vertus comme, nourrir et apporter confort et protection à la peau ou aux cheveux, comme exemple : huile d'amande ou l'huile d'argan. Il peut s'agir des huiles essentielles provenant de différentes plantes aromatique et présentant de nombreux avantages que ce soit dans le domaine cosmétique, pharmaceutique ou encore dans l'agroalimentaire. Les huiles essentielles ont été largement utilisées pour leurs propriétés antibactérienne, antifongique et insecticide (Bakkali, Averbeck et al. 2008).

Généralement, la phase dite huileuse est composée d'huile végétale, huile essentielles, vitamines, antioxydants et agents bioactifs ; la phase aqueuse comprend l'eau, les tensioactifs, les gommes, ou des sels.

1.1.3.3. Emulsifiant

Un émulsifiant peut être soit un tensioactif, une protéine, un polymère amphiphile, ou une combinaison du polymère et du tensioactif. La stabilisation d'émulsions par des particules solides est aussi possible (Figure 2).

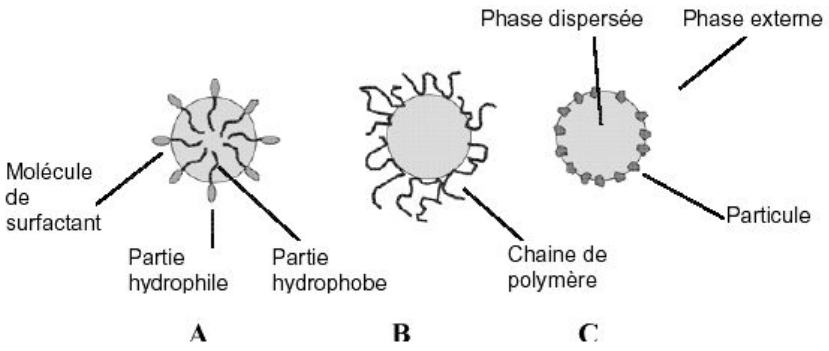


Figure 2 : Les mécanismes de stabilité des émulsions : A- Par les tensioactifs monomériques, B- Par les chaines polymériques, C-Par des particules solides (Frelichowska 2009)

Les émulsifiants peuvent être des molécules amphiphiles, c'est-à-dire qu'elles présentent deux parties de polarité différente, l'une lipophile « la queue » (qui a une affinité avec les

matières grasses), l'autre hydrophile « la tête » (qui a une affinité avec l'eau) (McClements 1999; Sjoblom 2005).

Généralement les agents tensioactifs appelés aussi agents de surfaces tensioactifs ou surfactants sont les plus utilisés. Ils ont la capacité de diminuer la tension interfaciale entre l'eau et l'huile et permet ainsi le mélange homogène des deux liquides non miscibles. Grâce à ce caractère amphiphile, les tensioactifs vont venir se fixer à l'interface des phases hydrophiles et lipophiles, ce qui va abaisser la tension de surface et donc stabiliser l'émulsion (Reddy and Fogler 1980).

Les tensioactifs sont caractérisés par leur balance hydrophile lipophile ou HLB qui traduit l'équilibre entre les groupements hydrophiles et les groupements lipophiles. Lorsque la HLB est comprise entre 1 et 9, le tensioactif est lipophile et favorise les émulsions eau dans huile E/H. Lorsque le HLB est comprise entre 11 et 20, le tensioactif est hydrophile et facilite les émulsions huile dans eau H/E. Cette balance est un facteur important dans le choix du tensioactif suivant le type d'émulsion souhaitée. La nature du tensioactif est également importante suivant ses propriétés (Brochette 1999).

Pour les tensioactifs, la stabilisation est aussi effectuée par la création d'une barrière stérique, mécanique et électrique entre les gouttelettes, ce qui empêche leur coalescence. Il existe quatre types de composés tensioactifs, qui sont regroupés selon la nature de la partie hydrophile (Figure 3) :

- Les tensioactifs anioniques : la partie hydrophile est chargée négativement.
- Les tensioactifs cationiques : la partie hydrophile est chargée positivement.
- Les tensioactifs zwitterioniques ou amphotères : la partie hydrophile comporte une charge positive et une charge négative, la charge globale est nulle.
- Les tensioactifs non ioniques : la molécule ne comporte aucune charge nette.

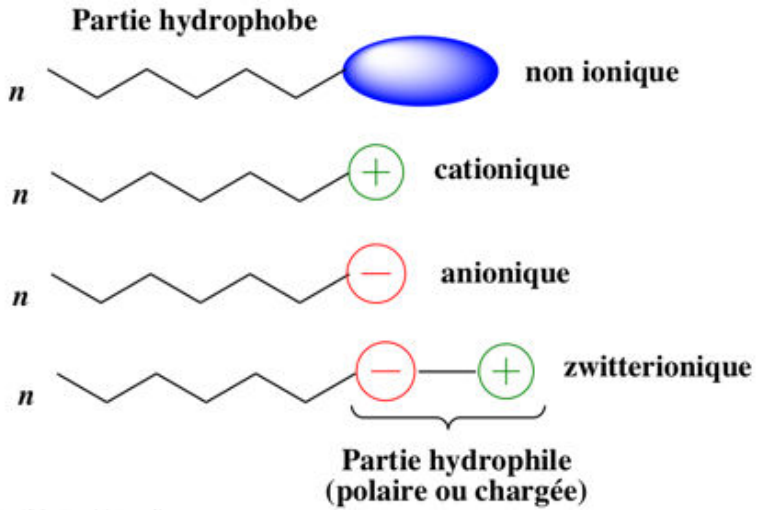


Figure 3 : Schématisation des différents types de tensioactifs

1.1.4. Les différents types d'émulsions:

Il existe différents types d'émulsions. En effet, selon la nature de la phase dispersée et le nombre de phases différentes émulsions subsistent. Les émulsions peuvent être classées en deux grands groupes:

1.1.4.1. Émulsions simples ou uniques

Dans le cas d'émulsions simples, des gouttelettes d'une phase liquide sont dispersées dans une autre phase liquide non miscible (Figure 4) (Pal 2011).

Les émulsions sont nommées suivant la phase dispersée en «huile-dans-eau» ou «eau-dans-huile». Les émulsions « huile dans eau » sont composées de gouttelettes d'huile dispersées dans une phase aqueuse alors que les émulsions eau dans huile ont une disposition inverse, c'est-à-dire que les gouttelettes d'eau sont dispersées dans une phase huileuse (Pal 2011).

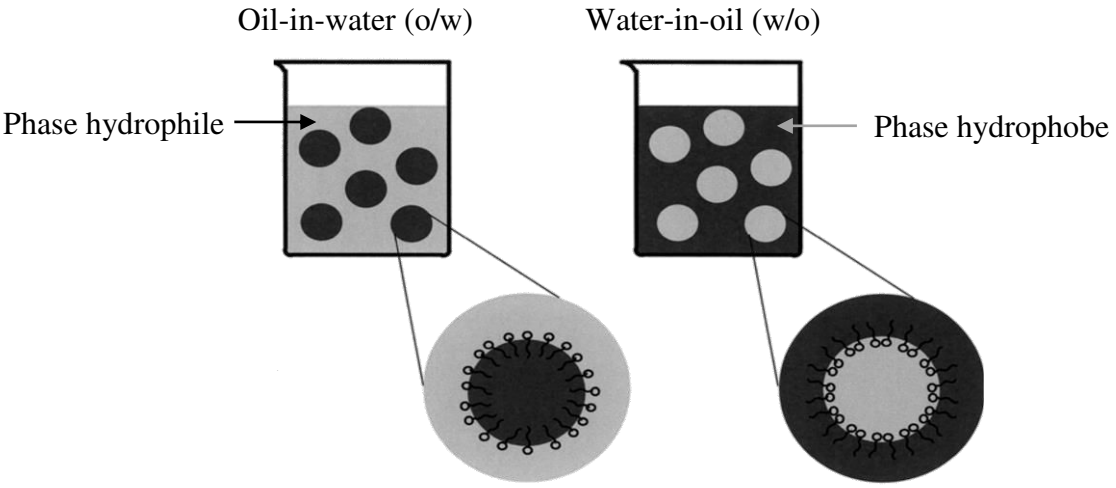


Figure 4 : Schématisation de la structure des émulsions huile dans eau et eau dans huile (Schramm 2006)

1.1.4.2. Émulsions doubles ou multiples

Les émulsions multiples sont des systèmes complexes, appelés «émulsions d'émulsions» (Garti 1997). Deux principaux types d'émulsions doubles peuvent être distingués (Figure 5) (Leal-Calderon, Schmitt et al. 2007):

- Emulsion eau dans huile dans eau (E/H/E) où une émulsion E/H est dispersée sous forme de gouttelettes dans une phase aqueuse.
- Emulsion huile dans eau dans huile (H/E/H) où une émulsion H/E est dispersée dans une phase huileuse.

Les émulsions E/H/E sont plus fréquente que émulsions H/E/H. Les émulsions doubles contiennent plus d'interface et sont encore plus thermodynamiquement instables que les émulsions simples (Van der Graaf, Schroën et al. 2005). Les émulsions doubles présentent beaucoup de possibilités intéressantes pour la libération contrôlée de substances chimiques initialement piégées dans les gouttelettes internes. Diverses industries, y compris l'alimentaire et les cosmétiques, manifestent un intérêt évident dans le développement technologique de ces systèmes complexes. Le domaine des produits pharmaceutiques est la principale zone d'application.

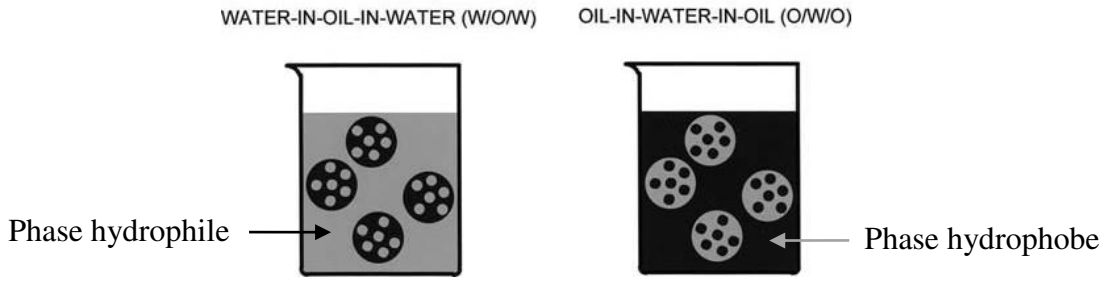


Figure 5 : Schémas d'émulsions doubles.

1.1.5. Classification des émulsions

Selon la taille de leurs gouttelettes, les émulsions peuvent être classées en trois types : Macroémulsions, nanoémulsions et microémulsions. Contrairement à ce que l'on pourrait penser, dans la littérature, les microémulsions sont les émulsions ayant la granulométrie la plus fine et leur taille de gouttelettes est plus petite que celle des nanoémulsions.

1.1.5.1. Macroémulsion

Regroupe des dispersions liquide/liquide, avec une taille de gouttelettes comprise entre 1 et 100 µm (voire jusqu'à 500 µm) correspondant à la dimension minimale accessible par agitation mécanique. La grande taille des gouttelettes augmente l'effet de la gravité sur ces dernières ce qui rend le système instable thermodynamiquement (Nielloud 2000).

1.1.5.2. Microémulsion

Les microémulsions sont des systèmes homogènes, thermodynamiquement stables, transparents et peu visqueux. Elles sont transparentes, parfois bleutées (par effet de Tyndall), en raison de leur faible tailles. Les phénomènes de diffusion de la lumière visible (de longueur d'onde 0,4 à 0,8µm), qui provoquent l'opacité des émulsions, n'interviennent plus pour cette échelle de taille deux cent fois plus petite. Les microémulsions sont des systèmes monophasiques dans lesquels un tensioactif rend possible la coexistence des phases eau et huile. Les microémulsions présentent des micro-domaines de petite dimension, typiquement de l'ordre de 10 à 100 nm, fluctuant rapidement dans le temps et dans l'espace. Contrairement aux macro/nanoémulsions (Salager, Anton et al. 2001).

1.1.5.3. Nanoémulsion

Les nanoémulsions peuvent être définies comme des dispersions d'un diamètre bien inférieur à l'ordre du micron (Cortés-Muñoz, Chevalier-Lucia et al. 2009). Les nanoémulsions sont très intéressantes en raison de la taille extrêmement faible des gouttelettes, entre 20-500 nm (Uson, Garcia et al. 2004). La plupart d'entre elles peuvent apparaître transparentes ou translucides grâce à leurs gouttelettes aux dimensions plus petites que la longueur d'onde de la lumière (D ~50 nm) rendant la diffusion de lumière relativement faible (Tadros, Izquierdo et al. 2004; Mason, Wilking et al. 2006; Qian and McClements 2011). Les nanoémulsions sont aussi connues sous le nom miniémulsions (El-Aasser, Lack et al. 1984), émulsions ultrafines, émulsions submicroniques (Benita and Levy 1993), microémulsions instables (Solè, Maestro et al. 2006).

En raison de leur petite taille des gouttelettes, certaines nanoémulsions ressemblant à des microémulsions transparentes ou translucides (Uson, Garcia et al. 2004). Cependant, les nanoémulsions, contrairement aux microémulsions, ne sont pas thermodynamiquement stable. Néanmoins, elles peuvent avoir une grande stabilité cinétique parce que leur petite taille de gouttelettes les rend stables contre la sédimentation et le crémage (Tadros, Izquierdo et al. 2004; Uson, Garcia et al. 2004; Lawrence and Warankanga 2006; Mason, Wilking et al. 2006).

Les nanoémulsions sont généralement formulées par les méthodes dites «haute énergie», utilisant des dispositifs spécifiques (comme les générateurs d'ultrasons ou homogénéisateurs à haute pression) en mesure de fournir suffisamment d'énergie pour augmenter l'aire interfaciale eau/huile et générer des gouttelettes submicroniques. Les méthodes dites "faible énergie" permettent également l'élaboration de nanoémulsions, mais par émulsification spontanée qui ne nécessite aucun dispositif, ni l'énergie (Anton and Vandamme 2011).

Pour que les nanoémulsions affichent un grand rapports zone interfaciale/volume, une concentration suffisamment élevée de tensio-actif (émulsifiants) doit être ajoutée pour permettre à la nouvelle surface de se développer pour permettre aux nanogouttelettes d'être rapidement revêtues lors du processus d'émulsification, ce qui devrait éviter ou limiter le cisaillement et la turbulence induite par coalescence, et assurer une meilleure stabilité physique de l'émulsion pour une longue période (Mason, Wilking et al. 2006; Cortés-Muñoz, Chevalier-Lucia et al. 2009).

- **Intérêt des nanoémulsions**

La préparation de ces émulsions avec une petite taille de gouttelettes est d'un intérêt particulier. Les gouttelettes de taille petite aboutissent généralement à une sensation en bouche plus crémeuse et une plus grande stabilité d'émulsion (McClements 2004; Kentish, Wooster et al. 2008).

En outre, la réduction des tailles des gouttelettes d'huile en dessous de 100 nm a le potentiel de fournir une émulsion translucide qui peut être intégrée facilement dans les boissons et gels alimentaire sans perte de transparence des produit finis (Tadros, Izquierdo et al. 2004).

Tableau 1: Les principales propriétés et caractéristiques des différentes classes d'émulsions a : (Jafari, Assadpoor et al. 2008), b :(Uson, Garcia et al. 2004). c : (Salager, Anton et al. 2001)

Propriétés	**Macroémulsions[a]**	**Nanoémulsions**	**Microémulsions**
Apparence	opaque	Transparente à opaque [ab]	Transparente
Méthodes de préparation	Homogénéisation classique	Haute énergie (pression)	Emulsification basse énergie
Taux de Tensioactif	Faible (<5%)	Moyen (5-10%)	Assez élevé (10-20%)
Taille des gouttelettes	0,5-100µm	20-500nm[b]	10-100nm

Stabilité thermodynamique	Instable Stabilité cinétique	Instable Stabilité cinétique	thermodynamiquement Stable [c]

1.1.6. Mécanisme d'émulsification

La préparation des émulsions nécessite généralement l'utilisation d'agents tensio-actifs (surfactants) et/ou de polymères amphiphiles et de l'énergie appliquée au système (homogénéisateurs ; ultrasonicateurs). Le procédé de préparation influence les propriétés de l'émulsion (la taille des gouttelettes , la stabilité, etc.), mais la nature de la dispersion finale (les phases constitutives) est identique, même si le procédé de préparation utilise un cisaillement élevé (en énergie externe, les méthodes de dispersion) ou l'énergie chimique stockée dans le système (méthodes de condensation) (Buzza and Cates 1993; Webster and Cates 2001; Mason, Wilking et al. 2006; Gutiérrez, Gonzalez et al. 2008; Sakai 2008).

Une interface est une région où les deux phases non miscibles d'une émulsion sont en contact. En raison des différences dans les interactions attractives entre les molécules des deux phases liquides, une tension interfaciale γ existe entre les deux liquides en contact. Cette tension interfaciale peut être réduite de manière significative par addition de molécules amphiphiles tensioactifs ou « surfactants », qui sont très solubles dans au moins l'une des phases liquides (Mason, Wilking et al. 2006).

La formation d'émulsion est non-spontanée et de l'énergie est nécessaire pour produire des gouttelettes (Tadros, Izquierdo et al. 2004; Delmas, Piraux et al. 2011; Tadros 2014). Ce mécanisme peut être compris à partir d'une étude de l'énergie nécessaire pour étendre l'interface, ($\Delta A\gamma$ où ΔA est l'augmentation de l'aire interfaciale de l'huile en passant de l'état complètement séparé de la phase aqueuse (avec une aire $A1$) à l'état dispersé ou on a production d'un grand nombre de gouttelettes (avec une aire $A2$) et donc la création de gouttelette fait que l'aire $A2$ est largement supérieure à l'aire $A1$ ($A2>>A1$), et γ est la tension interfaciale) (Tadros, Izquierdo et al. 2004). Comme γ est positive, l'énergie nécessaire pour étendre l'interface est positive et grande. Ce terme d'énergie ne peut pas être compensé par la faible entropie de dispersion TΔS (qui est également positive).

ΔG représente l'énergie totale de la formation d'une émulsion

$$\Delta G = \Delta A\gamma + T\Delta S \quad (1)$$

La formation de gouttelettes de grande taille (quelques micromètres) comme c'est le cas pour les microémulsions nécessite peu d'énergie et donc des agitateurs à grande vitesse tels que l'Ultraturrax ou Silverson Mixer sont suffisants pour produire l'émulsion.

En revanche, la formation de gouttelettes submicroniques, comme c'est le cas avec les nanoémulsions est difficile et nécessite une grande quantité d'énergie et/ou un surfactant. L'énergie nécessaire à la formation des nanoémulsions peut être estimée par la loi de Laplace p (qui représente la différence de pression entre l'intérieur et l'extérieur de la gouttelette) (Tadros, Izquierdo et al. 2004; Meleson 2008).

$$p = \gamma \left(\frac{1}{\mathrm{R1}} + \frac{1}{R2}\right) (2)$$

où R_1 et R_2 sont les rayons de courbure principaux de la gouttelette. Pour une gouttelette sphérique, $R=R_1=R_2$

$$p = \frac{2\gamma}{R} (3)$$

Pour fractionner une gouttelette d'émulsion en gouttelettes de plus en plus petites, elle doit être fortement déformée, cela peut être observé lorsqu'une gouttelette sphérique se déforme en une forme ellipsoïde allongée. L'augmentation de la tension interfaciale fait augmenter p. Par conséquent, le stress nécessaire pour déformer une goutte est plus élevé lorsque la taille des gouttelettes est faible. Ce stress est généralement transmis par le liquide environnant via l'agitation. Un stress plus élevé a besoin d'une agitation vigoureuse, et donc de plus en plus d'énergie est nécessaire pour produire de plus petites gouttelettes. Les tensioactifs jouent un rôle majeur dans la formation des nanoémulsion; en abaissant la tension interfaciale, p est réduit et donc le stress nécessaire pour fractionner une gouttelette est réduit. Les surfactants empêchent la coalescence des gouttelettes nouvellement formées (Tadros, Izquierdo et al. 2004). Différents phénomènes surviennent au cours de l'émulsification, à savoir le fractionnement des gouttelettes, l'adsorption de tensioactifs et la collision des gouttelettes (qui peut ou pas conduire à la coalescence). Chacun de ces processus se produit de nombreuses fois au cours de l'émulsification et l'échelle de temps de chaque processus est très court, généralement une microseconde, ce qui montre que le processus d'émulsification est un processus dynamique.

1.2. Procèdes de fabrication des émulsions

Selon la littérature plusieurs méthodes peuvent être appliquées pour la préparation d'émulsions et de nanoémulsions (couvrant la gamme des gouttelettes de taille rayon de 50 à 200 nm) (Tadros, Izquierdo et al. 2004). Les procédés consistent seulement à mélanger deux phases liquides. Dans l'une des deux phases un agent tensioactif hydrophile ou lipophile est solubilisé pour former un liquide homogène (Anton and Vandamme 2011). Une fois ces deux liquides mis en contact, l'utilisation d'un procédé d'émulsification est nécessaire (homogénéisateurs à haute pression, méthode de l'émulsification à faible énergie à température constante ou l'application de la température d'inversion de phase « PIT », émulsification acoustique), ce qui permettra ainsi l'obtention de nanoémulsions immédiatement stabilisées par les amphiphiles présents dans l'une des phases de l'émulsion (Tadros, Izquierdo et al. 2004; Anton and Vandamme 2011). La taille des gouttelettes des nanoémulsions est facilement contrôlable en fonction de rapport en l'huile/tensioactif et par l'énergie apportée au système.

Plusieurs procédures peuvent être appliquées pour améliorer l'efficacité de l'émulsification lors de la production de nanoémulsions:

- Il faut optimiser l'efficacité de l'agitation en augmentant la densité de puissance nette et en diminuant le temps de dissipation.
- L'émulsion est préparée de préférence avec une fraction volumique élevée de la phase dispersée diluée par la suite. Cependant, une grande fraction de la phase dispersée peut entraîner la coalescence au cours de l'émulsification (Tadros, Izquierdo et al. 2004).

1.2.1. Emulsification mécanique

1.2.1.1. Homogénéisateur à haute vitesse (rotor/stator)

L'homogénéisation des dispersions doit permettre de conférer au produit fini la granulométrie et la stabilité requises, au moyen d'outils à très fort taux de cisaillement, la taille des gouttes passant de l'ordre de 10 à 100 µm à une valeur inférieure au micromètre.

Le principe de fonctionnement de l'homogénéisateur à haute vitesse est le système rotor-stator qui est le système le plus couramment utilisé. C'est un équipement muni d'un stator percé d'orifices ou de fentes plus ou moins serrées et d'un rotor tournant à grande vitesse. Cet appareil exerce une dispersion/homogénéisation grâce à une vitesse élevée.

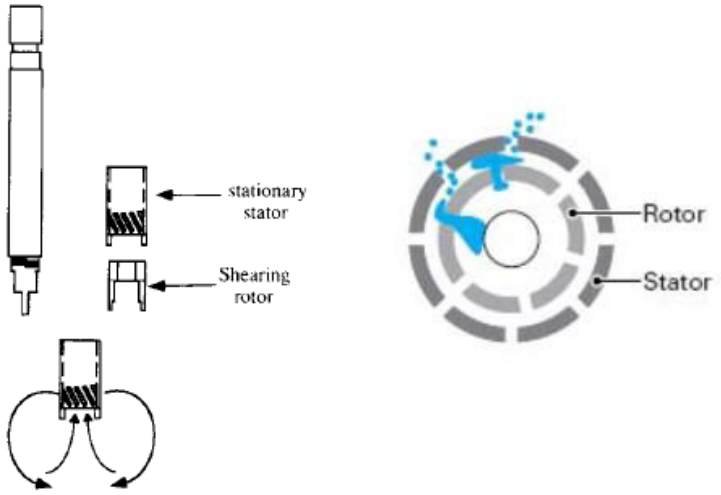


Figure 6 : (a) Représentation schématique de la configuration de l'homogénéisateur rotor/stator (Maa and Hsu 1996) (b) sonde de rotor/stator vue d'en bas

Le fluide est aspiré au centre dans le sens axial et passe à travers le rotor (partie mobile) où il est cisaillé, puis il est expulsé à travers les fentes du stator (partie fixe) (Figure 6) permettant un fort cisaillement du fait du faible entrefer entre le rotor et le stator et de la vitesse très élevée ce qui permet son homogénéisation.

1.2.1.2. Homogénéisation à haute pression

L'homogénéisation à haute pression est un traitement physique qui met en jeu un produit liquide ou pâteux projeté à forte pression (30 à 1000 bar) à travers une tête d'homogénéisation ayant une conformation particulière (Roustel 2010). Ce dispositif consiste à réduire la taille des particules (< 1µm) et permet d'avoir une émulsion fine et stable (Maherani, Arab-Tehrany et al. 2011).

L'homogénéisation à haute pression (microfluidisation) est largement utilisée pour produire des émulsions laitières et alimentaires. Les homogénéisateurs à hautes pressions sont particulièrement adaptés pour la production continue d'émulsions finement dispersées et sont donc d'un intérêt dans les domaines alimentaire et pharmaceutique (Leal-Calderon, Schmitt et al. 2007; Cortés-Muñoz, Chevalier-Lucia et al. 2009).

C'est un processus purement mécanique, qui consiste à forcer un produit fluide ou un pré-mélange grossier à passer à travers un orifice étroit (buse d'homogénéisation) sous haute pression (de 150 à 200 MPa, ou de 350 à 400 MPa pour une homogénéisation ultra-haute pression) (Figure 7). Une force de cisaillement extrême est créée sous ces conditions produisant des gouttelettes dont la taille est de quelques nanomètre (Dumay, Chevalier-Lucia et al. 2013).

La stabilité physique des nanoémulsions diminue avec l'élévation de la température, mais augmente avec la pression (jusqu'à 100 MPa) et les cycle d'homogénéisation (jusqu'à trois cycles) (Yang, Gu et al. 2008).

La diminution des tailles particulaires s'effectue par le passage forcé, à l'aide d'un piston, de l'émulsion à travers une valve d'homogénéisation dont l'ouverture est réglable. Plus l'ouverture de la valve est faible, plus la pression est élevée et plus les contraintes appliquées à la solution sont importantes. La diminution de taille est ainsi la résultante de forces d'impacts dues à d'intenses collisions interparticulaires, d'importantes forces de friction et des forces de cavitation. Ces dernières sont les plus prononcées. Le passage de la solution s'effectue à grande vitesse et de façon continue. En accord avec la loi de Bernoulli qui établit que le flux d'un liquide est constant pour une section donnée dans un système clos, la diminution du diamètre à travers lequel passe la microémulsion provoque simultanément une augmentation de la pression dynamique et une diminution de la pression statique au niveau des pistons (Roustel 2010).

Ces variations conduisent la pression statique en dessous de la valeur de la tension de vapeur du liquide à la température ambiante, provoquant ainsi le début de l'ébullition de l'eau avec la formation de bulles de gaz (bulles de cavitation). Quand les microémulsions quittent les pistons et que la pression initiale est restaurée, ces bulles implosent et la grande énergie qui accompagne ce phénomène est responsable de la cassure des particules.

Les fluides subissent une combinaison de flux d'allongement, de cisaillement et une cavitation. Malgré la complexité des mécanismes impliqués, les distributions de taille sont généralement reproductibles avec une taille moyenne allant de 50 nm à 5µm.

La taille de particule minimale réalisable dépend du type d'homogénéisateur (Figure 7 a-b), les conditions de fonctionnement de l'homogénéisateur (l'intensité de l'énergie, le temps et la température), la composition de l'échantillon (par exemple, le type d'huile, le type d'émulsifiant, et les concentrations relatives), et les propriétés physico-chimiques des composants des phases (tension interfaciale, viscosité) (Qian et McClements 2011).

Il existe divers types d'homogénéisateur à haute pression. Leur classification peut être basée sur la géométrie de la buse et leur conception dépend de la direction d'écoulement du fluide. Ils peuvent être subdivisés en diffuseurs radiaux, disséminateurs de jet, microfluidiseurs, et en valves à orifices (Schulz et Daniels 2000; Jafari et al. 2008b).

(a) (b)

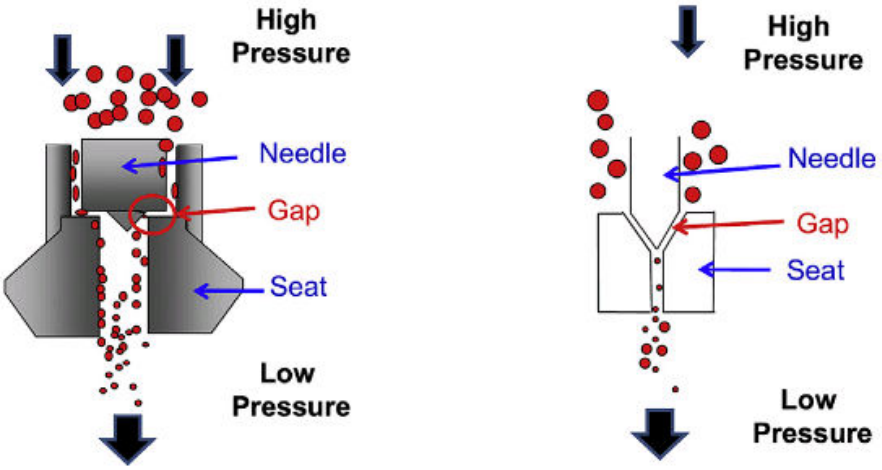


Figure 7: (a) Représentation schématique de la configuration de l'homogénéisateur à haute pression à forte angle -vanne de Stansted ™. (b) Représentation schématique de la configuration de l'homogénéisateur à haute pression -vanne de forme Y. (Dumay, Chevalier-Lucia et al. 2013).

Un saut de la température de 17-21 °C par 100 MPa est noté lors du traitement de lait entier ou émulsions H/E avec une température initiale de 4°C donnant une température de 24°C à la sortie (Thiebaud, Dumay et al. 2003; Picart, Thiebaud et al. 2006; Cortés-Muñoz, Chevalier-Lucia et al. 2009; Dumay, Chevalier-Lucia et al. 2013).

1.2.1.3. Emulsification par membrane

L'émulsification membranaire consiste à forcer la phase dispersée à s'imprégner dans la phase continue à travers une membrane ayant une distribution uniforme de taille des pores. La phase dispersée est enfoncée perpendiculairement à la membrane tandis que la phase continue coule tangentiellement à la membrane (Figure 8). Bien que facile, en principe, l'émulsification par membrane dépend de nombreux paramètres tels que les propriétés de membrane, les flux et la formulation influencent la distribution de la taille de l'émulsion. Pour obtenir une émulsion monodisperse, les pores de la membrane doivent eux-mêmes avoir une distribution de taille étroite. Habituellement, la taille des gouttes est proportionnelle à la taille des pores. Le choix de la porosité de la membrane est le résultat d'un compromis: si la densité des pores est trop grande, la coalescence des gouttes fraîchement formée est susceptible de se produire au même titre que l'augmentation de polydispersité, à l'inverse, si la densité des pores est trop faible, le taux de production est insuffisant. Aussi, la phase dispersée ne doit pas mouiller le revêtement de membrane et par conséquent une membrane hydrophile doit être utilisée pour produire une émulsion huile-dans-eau (H/E).

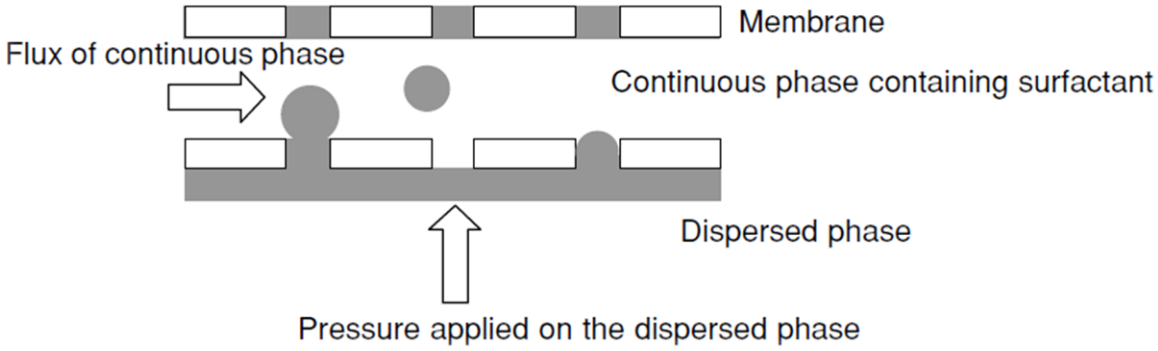


Figure 8 : Schématisation du principe de l'émulsification membranaire (Leal-Calderon, Schmitt et al. 2007)

1.2.2. Les méthodes d'émulsification à faible énergie

Il s'agit de méthodes d'émulsification faisant usage de l'énergie chimique stockée dans les composants. Appelée aussi condensation à basse consommation, elle reçoivent une attention accrue actuellement (Sadurni, Solans et al. 2005).

Une étude du comportement de phase des systèmes eau/huile/tensioactif a démontré que l'émulsification peut être réalisée par trois différentes méthodes à faible énergie :

- l'addition progressive d'huile au mélange eau/surfactant;
- l'addition progressive d'eau à une solution de tensioactif dans l'huile,
- mélanger tous les composants dans la composition finale, pré-équilibrage des échantillons avant émulsification.

Dans ces méthodes, les nanoémulsions sont obtenues à la suite des transitions de phase produite pendant le processus d'émulsification effectué, en général, à température constante avec changement de la composition ou à composition constante et avec évolution de la température, ou bien par la méthode PIT. Dans la pratique, une combinaison des méthodes d'émulsification de haute énergie et de faible énergie s'est révélée être un moyen efficace d'obtention des nanoémulsions avec de petites gouttelettes très uniformes (Sadurni, Solans et al. 2005).

Les résultats de cette étude ont montré que les nanoémulsions avec des tailles de gouttelettes de l'ordre de 50 nm n'ont été formées que lorsque l'eau a été ajoutée à des mélanges de tensioactif/ huile (Tadros, Izquierdo et al. 2004).

1.2.2.1. Méthodes d'émulsification spontanée et émulsification par inversion de phase

L'émulsification spontanée est un processus qui se produit sans apport d'énergie externe lorsque deux fluides non miscibles, avec une très faible tension interfaciale, sont mis en contact, alors que l'augmentation de la zone interfaciale nécessite généralement un apport d'énergie, donc l'émulsification spontanée est un phénomène intriguant, comme en témoigne l'abondante littérature qui lui est consacrée. Il est à noter que dans les procédés industriels, la cinétique de ce type d'émulsification, appelé également auto-émulsification, est accélérée par un apport d'énergie. L'émulsification par inversion de phase est également souvent considérée comme un procédé d'émulsification spontanée car elle nécessite une alimentation énergétique faible. Son avantage réside dans la possibilité de produire des émulsions concentrées (Clarence 2005; Leal-Calderon, Schmitt et al. 2007)

Cette faible consommation d'énergie « émulsification spontanée » est en fait une méthode efficace permettant la formation de gouttelettes d'émulsion cinétiquement stable et potentiellement concentrée d'une taille allant de 10 nm à 300 nm (Anton and Vandamme 2011).

Les méthodes de faible énergie profitent des propriétés physico-chimiques intrinsèques des composants afin de générer des gouttelettes submicroniques (Anton and Vandamme 2011).

- **Emulsification par inversion de phase**

L'émulsification par inversion de phase est souvent utilisée industriellement, notamment dans les produits cosmétiques. Son intérêt réside dans l'apport d'énergie réduit et les émulsions obtenues sont généralement fines (diamètre moyen inférieur ou de l'ordre de 1 µm) et monodisperses. Comprendre le mécanisme d'inversion de phase reste un défi, mais plusieurs études donnent un aperçu de ce phénomène étonnant et parfois spectaculaire. L'inversion de phase, se produit lorsque la structure de l'émulsion s'inverse, c'est à dire lorsque la phase continue devient la phase dispersée, et vice versa. Cela peut se produire avec un changement de toutes variables comme la température, la pression, la salinité, l'utilisation d'un co-tensioactif, ou la proportion d'huile et d'eau (Leal-Calderon, Schmitt et al. 2007).

Cette méthode s'appuie sur la formation spontanée de minuscules gouttelettes d'huile au sein des systèmes Huile/Eau lorsque la solution ou les conditions environnementales sont modifiées (Tadros et al. 2004a; Qian et McClements 2011).

Le concept de la PIT (température d'inversion de phase) consiste en l'utilisation de la capacité spécifique de tensioactifs, généralement non ioniques, comme les tensioactifs polyéthoxylés, à modifier leurs affinités pour l'eau et l'huile en fonction de la température, et donc de subir une inversion de phase. Cette inversion de phase dite transitoire de l'émulsion se produit lorsque, à composition fixe, l'affinité relative de l'agent tensioactif pour les différentes phases (huileuse, aqueuse) est modifiée par l'augmentation progressive de la température (Anton, Benoit et al. 2008).

L'inversion de phase dans les émulsions peut être de deux types:

- inversion de transition induite par l'évolution des facteurs qui affectent la HLB (Balance hydrophile lipophile) du système, par exemple la température et/ou la concentration d'électrolytes.
- Inversion catastrophiques, qui est induite par une augmentation de la fraction volumique de la phase dispersée.
- Inversion de transition peut aussi être induite par la modification de la HLB (Balance hydrophile lipophile) du tensioactif à température constante en utilisant des mélanges de tensioactifs (Tadros, Izquierdo et al. 2004).

1.2.3. Emulsification acoustique

L'émulsification acoustique est une technique d'émulsification basée sur l'application d'ultrasons et de vibrations généralement de basses fréquences, aussi appelés ultrasons de puissances. Les émulsions acoustiques sont préparées par application d'onde ultrasonores (>16kHz) aux deux phases non miscibles. L'émulsion acoustique donne de très petites particules avec un diamètre moyen de l'ordre de 0.2 µm et une distribution étroite de la taille des gouttelettes. Dans une émulsion acoustique, la taille des gouttelettes et leur distribution peuvent être facilement variées par le changement du temps d'application des ultrasons. Les courts temps produisent plus de grandes gouttelettes avec une large distribution de tailles alors que les temps de sonication les plus longs produisent des particules plus petites avec une distribution de taille très étroite. Ces émulsions ont été jugées comme étant très stables et cela même en l'absence de tensioactif (Reddy and Fogler 1980). En comparant les procédés d'agitation mécanique aux ultrasons de basses fréquences, Tadros et al. (2004) ont constaté que pour un diamètre donné désiré, la quantité de tensioactif nécessaire a été réduite, la consommation d'énergie (par la perte de chaleur) était plus faible et les émulsions ultrasonores

sont moins polydisperse et plus stables que les émulsion faites par voie mécanique (Tadros, Izquierdo et al. 2004; Kentish, Wooster et al. 2008).

1.2.3.1. Les ultrasons

1.2.3.1.1. Classification des ultrasons

Les Ultrasons couvrent les fréquences d'environ 20 kHz jusqu'à 10 MHz au-delà de l'audition humaine. Dans la pratique, trois gammes de fréquences sont rapportées pour trois usages distincts des ultrasons: les basses fréquences ou les ultrasons de puissance (20-100 kHz), les ultrasons de fréquence moyenne (300 à 1000 kHz) et les ultrasons de diagnostic ou de hautes fréquences ou élevée (1-40 MHz) (Figure 9) (Poux and Canselier 2004; Vajnhandl and Majcen Le Marechal 2005).

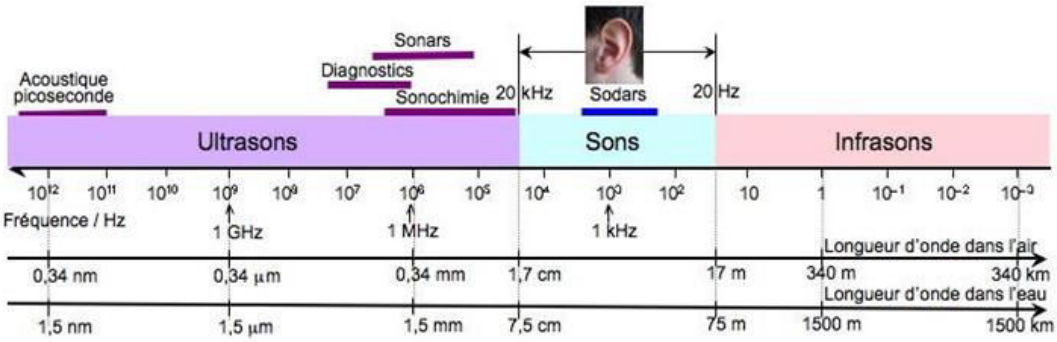


Figure 9 : Positionnement des ultrasons dans la gamme de fréquences

Les ultrasons de puissance (20 - 100 kHz, et, dans une moindre mesure, 100 kHz - 1 MHz) interagissent avec la matière et produisent des modifications physiques et chimiques des composés. Ces modifications sont en particulier dues aux phénomènes de cavitation (Canselier, Delmas et al. 2002; Jafari, Assadpoor et al. 2008).

1.2.3.1.2. Émission des ultrasons

Les ultrasons sont généralement émis par une surface plane vibrant de façon sinusoïdale autour de sa position d'équilibre, à la fréquence « *f* » et avec une amplitude « A » (de l'ordre du µm) (Poux and Canselier 2004).

L'émulsification acoustique est basée sur l'utilisation de transducteurs piézoélectriques en céramique, qui vibrent sous l'effet d'un champ électrique avec des mouvements oscillatoires (Figure 10).

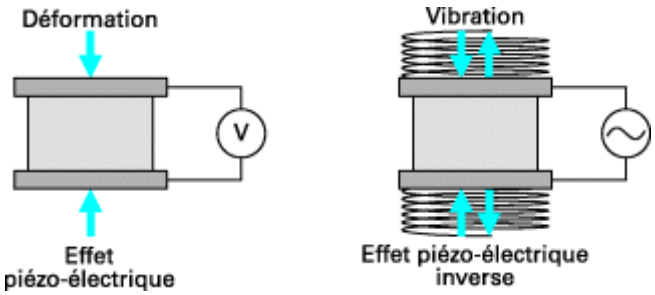


Figure 10: Schématisation de l'effet de piézoélectricité (Petrier, Gondrexon et al. 2008)

1.2.3.1.3. Propagation de l'onde

L'onde se propage en milieu solide, liquide ou gazeux avec une célérité c, qui dépend des propriétés physiques du milieu, et une longueur d'onde λ ($\lambda = c/f$). En milieu liquide, on suppose que l'onde est plane et longitudinale. La pression acoustique P_A ou surpression par rapport à la pression d'équilibre et l'intensité transportée par l'onde I (puissance par unité de surface) peuvent s'écrire en fonction des caractéristiques de la source et du milieu (Poux and Canselier 2004):

$$P_A = AZ2\pi f_I \quad (4)$$

$$I = \frac{P_A^2}{2Z} \quad (5)$$

Avec : P_A : pression acoustique (Pa)

I : puissance par unité de surface ($kg \cdot s^{-3}$)

Z : impédance acoustique ($kg \cdot m^{-2} \cdot s^{-1}$)

A : amplitude (m)

f : fréquence (Hz ou s^{-1}).

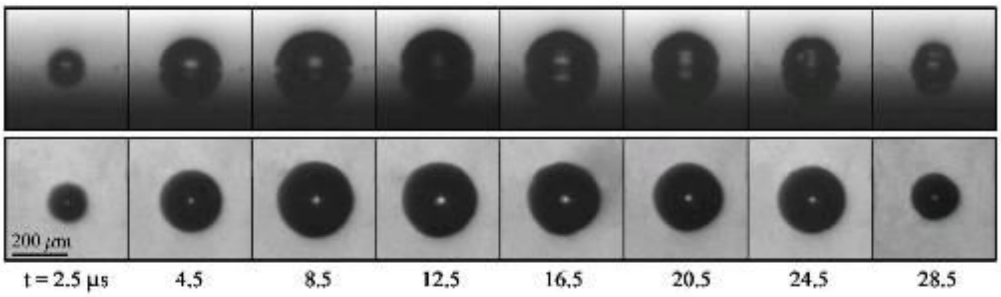
L'impédance acoustique Z du milieu traversé a des valeurs très différentes suivant le milieu de propagation : 410 $kg \cdot m^{-2} \cdot s^{-1}$ dans l'air, $1{,}5 \times 10^6$ dans l'eau et $1{,}5 \times 10^7$ dans le verre.

À l'interface entre deux milieux d'impédances acoustiques très différentes, l'onde se réfléchit et conduit à une onde stationnaire de période $\lambda/2$. À la traversée de milieux visqueux ou diphasiques denses, une partie de l'énergie acoustique va se dégrader en chaleur sous l'effet des frottements visqueux, et l'amplitude de l'onde diminue donc avec la distance à la source (Poux and Canselier 2004).

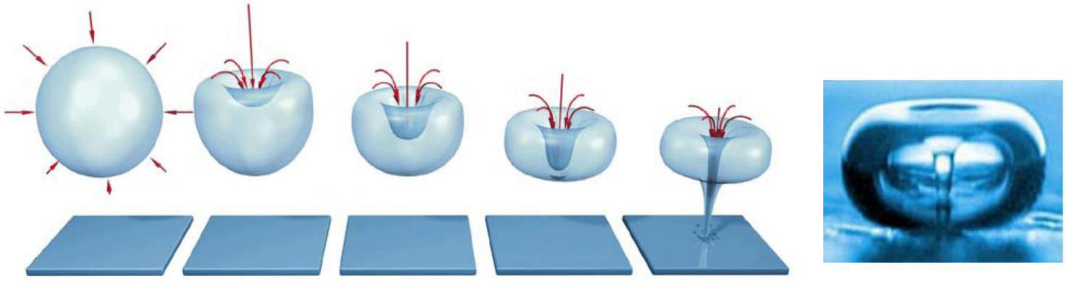
1.2.3.2. Emulsification par ultrasons de basses fréquences et cavitation

1.2.3.2.1. Cavitation acoustique

En effet, l'application d'ultrasons de basses fréquences provoque la formation et l'effondrement de microbulles par les fluctuations de pression des ondes sonores simples (Li and Fogler 1978a; Kentish, Wooster et al. 2008). Chaque éclatement de bulles de cavitation provoque des niveaux extrêmes de turbulences très localisées (Kentish, Wooster et al. 2008). À l'intérieur des bulles de cavitation, des températures de plusieurs centaines de degrés Celsius et des pressions supérieures à 800 bars (Figure 11,12) sont atteintes conduisant à une dégradation des composés sensibles présents dans l'émulsion. Les bulles de cavitation se développeront sur quelques cycles en piégeant de plus en plus de vapeur à partir du support pour atteindre une taille critique de l'implosion des bulles. Durant leur vie, les bulles de cavitation sont alternativement sujettes à des dilatations et à des contractions (Poux and Canselier 2004). Cependant, en moyenne, leur diamètre s'accroît : c'est ce que l'on appelle la « diffusion rectifiée ». À faible intensité acoustique, l'amplitude de variation du rayon des bulles est faible, et la durée de vie de la bulle est longue (cavitation stable). À forte intensité, le rayon de la bulle peut atteindre plusieurs fois sa valeur d'équilibre, et la bulle implose très vite, dès le premier maximum de pression acoustique (cavitation transitoire) (Poux and Canselier 2004). Le rayon des bulles de cavitation avant l'effondrement lors de l'application d'ultrasons à 20 kHz est de l'ordre de plusieurs centaines de micromètres. En résumé, le phénomène de cavitation comprend les répétitives distinctes et trois étapes: formation (nucléation), croissance rapide (expansion) au cours des cycles de compression /raréfaction jusqu'à ce que les bulle de cavitation atteignent une taille critique. Après cela, ces bulles de vapeur d'eau subissent l'effondrement violent (implosion) dans le liquide (Suslick 1990; Pang, Abdullah et al. 2011). La figure 11(a) représente l'accroissement et l'effondrement d'une bulle de cavitation vue d'en haut et de face à une pression de 2MPa, d'après Bremond et Arora (2006), dans ces conditions, une bulle de cavitation peut atteindre 200µm de diamètre avant de commencer son effondrement, ainsi sa durée de vie est de 28µs.



(a) Vue de face et de haut de l'expansion et du collapse d'une bulle de cavitation isolée sujette à une pression négative de 2MPa (Bremond, Arora et al. 2006).



(b) Photo d'une bulle de cavitation et la schématisation de son évolution jusqu'au collapse

Figure 11 : Formation et collapse de bulles de cavitation

- **Seuil de cavitation et fréquence**

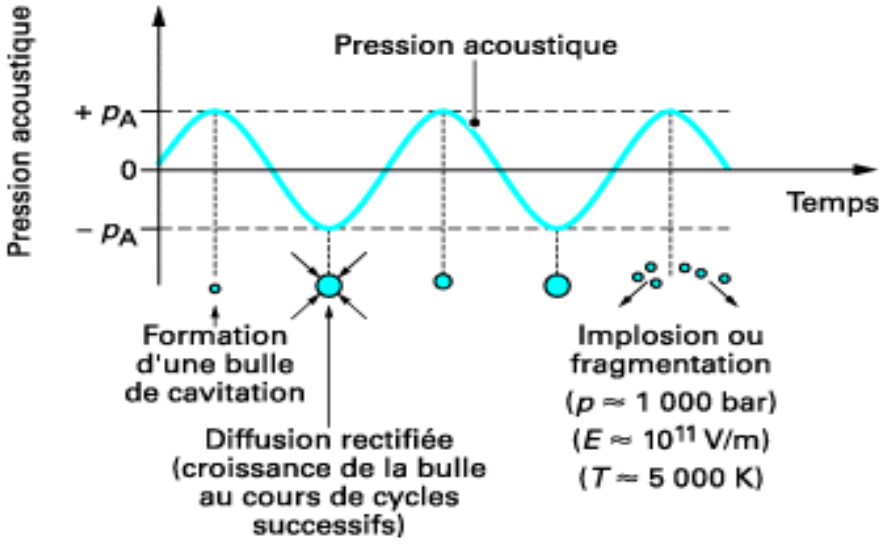
En plus de son influence sur la dynamique de la bulle, la fréquence de l'onde excitatrice agit également sur l'initiation de la cavitation. Un seuil de cavitation est la pression nécessaire à faire apparaître les premières bulles. Selon l'application visée, certaines études présentent ce seuil de cavitation en fonction de l'énergie (intensité ou puissance acoustique) qui est calculée à partir de la puissance. le seuil de cavitation dépend de la fréquence : plus la fréquence est grande, plus l'amplitude de l'onde doit être importante pour pouvoir initier la cavitation comme montre les résultats, obtenus dans l'eau et dans le sang (Saletes 2009).

1.2.3.2.2. Mécanisme d'émulsification par ultrasons de basses fréquences

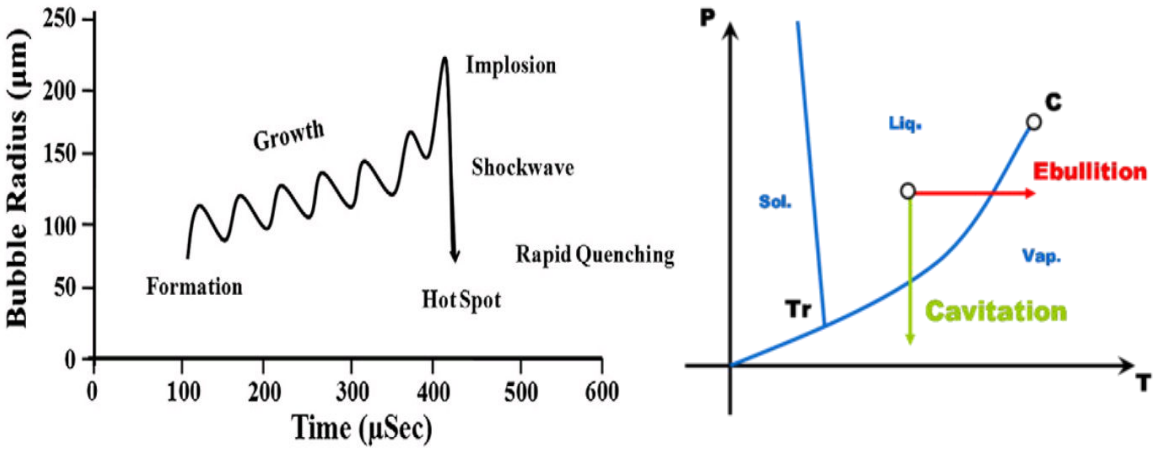
L'émulsification par ultrasons de basses fréquences se fait par deux mécanismes: instabilité interfaciale et cavitation. Tout d'abord, l'application d'un champ acoustique produit des ondes interfaciales qui induisent l'instabilité de ces interfaces, entraînant finalement l'éruption de la

phase huileuse dans le milieu aqueux sous forme de gouttelettes de l'ordre de 70µm (Li and Fogler 1978a; Li and Fogler 1978b; Abismail, Canselier et al. 1999). En second lieu, l'application d'ultrasons basses fréquences provoquent une cavitation acoustique, c'est-à- dire la formation et l'effondrement de microbulles par les fluctuations de pression d'une onde sonore simple.

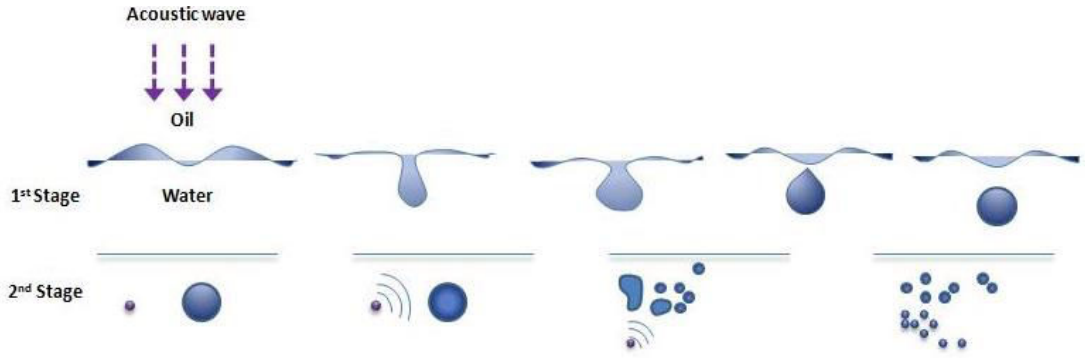
L'émulsification des solutions est produite grâce au fort cisaillement provoqué par le phénomène de cavitation. Ce dernier est dû à la succession de pressurisation/dépressurisation qu'entraine l'application de l'onde acoustique (Figure 12). Cette succession de cycles conduit à la croissance des bulles de vapeur d'eau formées par vaporisation additionnelle à chaque dépression. Les bulles de gaz peuvent ainsi atteindre plusieurs microns avant d'éclater (collapse) sous l'action de la pression. Chaque événement d'éclatement d'une bulle (une implosion à l'échelle microscopique) entraîne des niveaux extrêmes de turbulences très localisées. Ces turbulences (micro-implosions) sont très efficaces pour la rupture des gouttelettes primaires dispersées de taille inférieure au micron (Figure12) (Li and Fogler 1978a; Li and Fogler 1978b; Reddy and Fogler 1980; Abismail, Canselier et al. 1999; Kentish, Wooster et al. 2008). A l'intérieur des bulles de cavitation, des températures (1000 plusieurs °C) et des pressions élevées (> 800 bars) sont atteintes (Lavigne, Takeda et al. 2011) d'où la probabilité de dégradation des composés présents dans l'émulsion. Ainsi les ultrasons de basses fréquences produisent une cavitation plus violente, menant à des températures plus élevées et des pressions localisées sur le site de la cavitation.



(a) Mécanisme de cavitation ultrasonore (Poux and Canselier 2004)



(b) Augmentation et implosion des bulles de cavitation dans un milieu aqueux avec une irradiation ultrasonore (Pang, Abdullah et al. 2011) et diagrame de phase de l'eau



(c) Déstabilisation des interfaces et émulsification ultrasonore (Sivakumar, Tang et al. 2014)

Figure 12 : Mécanisme de cavitation et d'émulsification par ultrasons de basses fréquences

Les impacts de traitement par ultrasons de basses fréquences sur l'huile de tournesol ont été évalués par Pingret et al. (2012). L'étude a montré une augmentation de radicaux formés dans des huiles traitées aux ultrasons, ainsi que les modifications des paramètres physico-chimiques, en particulier l'oxydation des huiles (Figure 13) (Pingret, Durand et al. 2012).

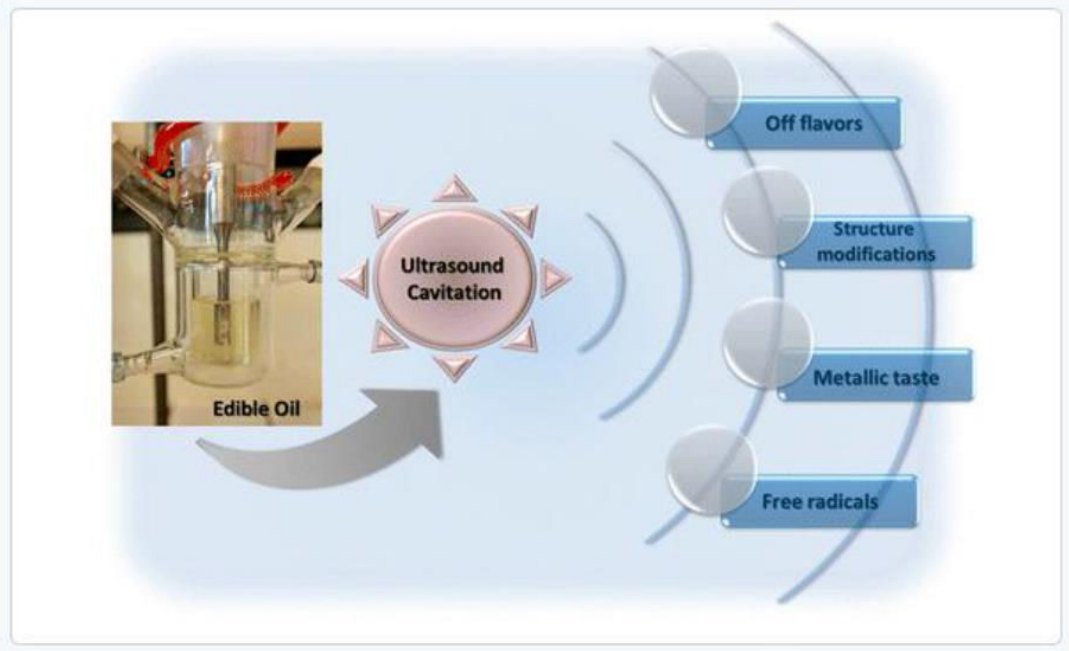


Figure 13 : Effet de la cavitation ultrasonore sur les propriétés des huiles alimentaires

1.2.3.1. Mécanisme de la cinétique sonochimique des molécules d'eau

La sonolyse de l'eau ou l'oxydation sonochimique peut s'effectuer en présence de tout gaz tels que l'air, l'azote, l'argon et l'hydrogène [29]. La présence d'oxygène dissous améliore les réactions sonochimiques. Les ultrasons induisent la séparation des molécules d'eau avec la présence d'oxygène dissous et provoque des réactions (1)-(13) [9,29]. Dans ces réactions, le signe «)))» désigne l'irradiation ultrasonore. La dissociation thermique de l'eau et les molécules d'oxygène dissous dans les cavités sont converti en espèces réactives comme le •OH, les atomes hydrogène (•H), atomes •O et les radicaux hydroperoxyles (• OOH) dans la bulle de cavitation (réactions (1) - (5)) (Pang, Abdullah et al. 2011).

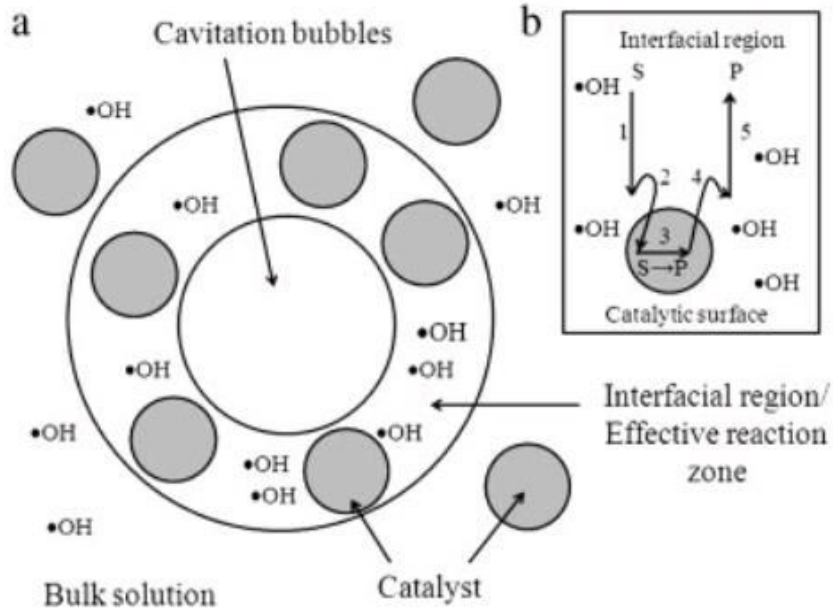


Figure 14 : Schématisation de (a) zone de réaction effective dans la bulle de cavitation et (b) 5 étapes dans la réaction catalytique hétérogène au niveau de la région interfaciale (Pang, Abdullah et al. 2011).

Après cela, les radicaux réactifs peuvent entrer dans une variété de réactions chimiques dans le gaz de la bulle de cavitation et/ou dans la solution en vrac. En l'absence de solutés, ces radicaux primaires peuvent se recombiner pour former du H2O, •O et de l'oxygène (O2) qui sont ensuite libéré dans la solution en vrac (réactions (6)-(8)). Du H_2O_2 est formé à l'extérieur des bulles chaudes ou à l'interface froid du fait de la recombinaison du OH• et •OOH (réactions (9) et (10)). D'autre part, les •H et •OH et peuvent en outre réagir avec H_2O_2 comme indiqué dans les réactions (11)-(12). Les radicaux (•OH et •OOH) peuvent également atteindre l'interface liquide-bulle et peut passer dans la solution en vrac où ils peuvent réagir avec des solutés (Figure 14) (Pang, Abdullah et al. 2011).

$H_2O +))) \rightarrow \bullet OH + \bullet H$ (1)

$O_2 +))) \rightarrow 2\bullet O$ (2)

$\bullet OH + \bullet O \rightarrow \bullet OOH$ (3)

$\bullet O + H_2O \rightarrow 2\bullet OH$ (4)

$\bullet H + O_2 \rightarrow \bullet OOH$ (5)

$\bullet OH + \bullet H \rightarrow H_2O$ (6)

$2\bullet OH \rightarrow H_2O + \bullet O$ (7)

$\bullet OOH + \bullet OH \rightarrow O_2 + H_2O$ (8)

$2\bullet OH \rightarrow H_2O_2$ (9)

$2\bullet OOH \rightarrow H_2O_2 + O_2$ (10)

$\bullet H + H_2O_2 \rightarrow \bullet OH + H_2O$ (11)

$\bullet OH + H_2O_2 \rightarrow \bullet OOH + H_2O$ (12)

$2\bullet H \rightarrow H_2$ (13)

1.2.3.2. Emulsification acoustique par ultrasons de hautes fréquences

1.2.3.2.1. Les ultrasons de hautes fréquences et cavitation

La fréquence de l'onde ultrasonore a un effet significatif sur le processus de cavitation car elle modifie la taille critique de la bulle de cavitation (Mrowetz, Pirola et al. 2003; Tezcanli-Guyer and Ince 2003). A des fréquences très élevées, l'effet de cavitation est réduite, soit parce que (i) le cycle de raréfaction de l'onde sonore produit une pression négative qui est insuffisant dans la durée et/ou l'intensité permettant d'initier la cavitation ou (ii) le cycle de compression se produit plus rapidement ne laissant pas le temps à la microbulle de croitre et de s'effondrer. Cependant, la recherche actuelle indique que dans des réactions telles que des oxydations, des fréquences plus élevées peuvent conduire à des taux de réaction plus élevées. Cela est dû au fait que la fréquence plus élevée peut effectivement augmenter le nombre de radicaux libres dans le système parce que bien que la cavitation est moins violente, il y a plus d'événements de cavitation et donc plus de possibilités pour les radicaux libres à produire (Adewuyi 2001).

1.2.3.2.2. L'effet des changements de fréquence des ultrasons sur les effets mécaniques et chimique de la cavitation

L'exemple le plus évident des effets mécaniques de la cavitation peut être trouvé dans le domaine du nettoyage aux ultrasons. Des bains de nettoyage traditionnels fonctionnent à l'extrémité inférieure de la gamme des ultrasons, généralement autour de 40 kHz.

Cette fréquence est utilisée pour le nettoyage de bijoux et de petits composants métalliques mais aussi pour la majorité des pièces de poids lourds (Bulat 1974; Mason, Cobley et al. 2011). Toutefois, la cavitation induite à cette fréquence est suffisamment puissant pour endommager ces éléments.

Francony et Pétrier (1996) ont observé que la dégradation par ultrasons du tétrachlorure de carbone a été amélioré et que la dégradation est plus rapides lors de l'utilisation d'une fréquence de 500 kHz au lieu de 20 kHz (Francony and Petrier 1996). Mais à de très hautes fréquences, le processus de cavitation est diminué. Entezari et Kruus (1996) ont étudié la vitesse de réaction d'oxydation sonochimique d'iodure à différentes températures (0-50 °C) et avec différents émetteurs d'ultrasons à basse fréquence (20 kHz) et de haute fréquence (900 kHz). Les résultats ont montré qu'à 900 kHz, la vitesse d'oxydation augmente jusqu'à 30°C à des niveaux de

puissance plus faibles tandis qu'à 20 kHz, la vitesse d'oxydation diminue lorsque la température augmente (Entezari and Kruus 1996).

Aux fréquences beaucoup plus élevées (autour de 1 MHz), le nettoyage est efficace sans dommage important de surface. Cette puissance acoustique utilisée est essentielle afin d'éviter ces dommages (Mason, Cobley et al. 2011).

Il ya très peu de travaux sur la relation entre la fréquence acoustique appliquée et les effets radicalaires et mécaniques résultants. Cependant Portenlänger et *al*., (1997) ont observé que pour la dégradation de dextrane par ultrasons, les effets mécaniques étaient plus importants dans le cas des basses fréquences (35 kHz) alors que seules les réactions radicalaires sont responsables de la dégradation à des fréquences plus élevées (> 500 kHz) (Portenlanger and Heusinger 1997). Dans les systèmes aqueux, l'augmentation de la fréquence des ultrasons diminue les effets mécaniques, mais les effets chimiques associés à la production de radicaux augmentent (Mason, Cobley et al. 2011).

1.2.3.2.3. Puissance acoustique et diamètres des bulles de cavitation

Au sein de la gamme d'ultrasons, la puissance disponible est inversement proportionnelle à la fréquence. Dans les travaux de Mason et Cobley (2011), cinq fréquences ultrasonores ont été utilisées pour traiter un ester de polyphénylène chargé de verre (Noryl HM4025) : 20 kHz, 40 kHz, 582 kHz, 863 kHz et 1142 kHz. Une estimation de la puissance réelle entrant dans le système a été déterminée par calorimétrie comme démontré par Kimura et al., (1996) (Kimura, Sakamoto et al. 1996). Les méthodes de laboratoire permettent de mesurer la puissance acoustique effective des système par calorimétrie (watts) (Kimura, Sakamoto et al. 1996) qui est convertie par la suite en puissance acoustique spécifique en divisant par le volume traité par ultrasons (Mason, Cobley et al. 2011) (Tableau 2).

Tableau 2 : Mesure de la puissance acoustique spécifique par calorimétrie faite par (Mason, Cobley et al. 2011)

Fréquences	Puissance par calorimétrie (W)	Intensité de puissance (W/dm3)
20	38.5	192.5
40	74.4	37.2
582	10.9	25.3
863	8.1	18.8
1142	7.5	17.4

Dans la littérature et comme le montre la Table 2, il est clairement établi que la puissance ultrasonore délivrée dans les systèmes traités est inversement proportionnelle à la fréquence de l'onde ultrasonore utilisée.

Le diamètre des bulles de cavitation est étroitement lié à la puissance de l'onde ultrasonore. En effet, comme le montre la Figure 15, le diamètre des bulles de cavitation est directement proportionnel à la puissance acoustique des ondes ultrasonores qui est elle-même inversement proportionnelle à sa fréquence (Tableau 2).

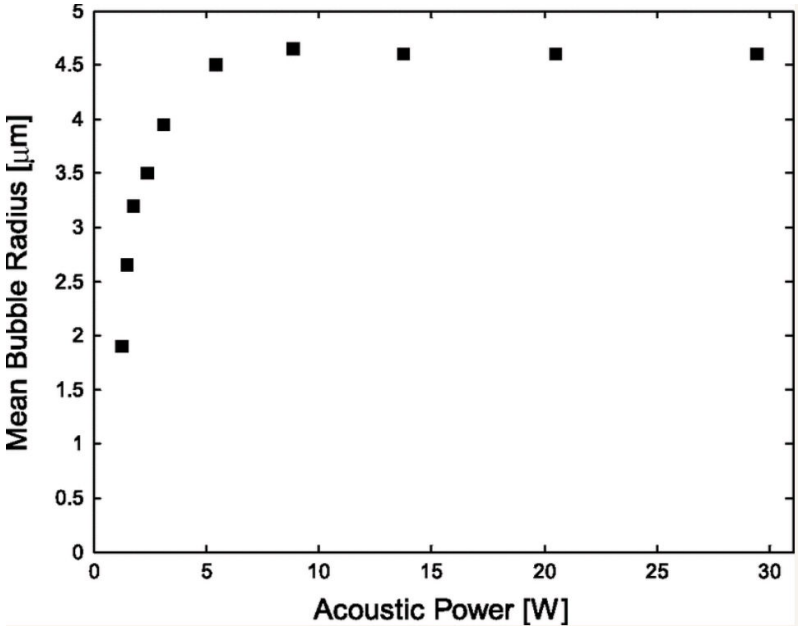


Figure 15 : Variation des rayons des bulles de cavitation (moyenne de la distribution de taille) sous sonication par ultrassons 1056 kHz de fréquence en fonction de la puissance acoustique. (Brotchie, Grieser et al. 2009).

1.2.3.2.4. Fréquences ultrasonore et diamètres des bulles de cavitation

Les dimensions critiques des bulles de cavitation dépendent du liquide et de la fréquence du son (Suslick and West 1986; Vajnhandl and Majcen Le Marechal 2005). Il est connu que la taille critique des bulles de cavitation formées dans l'eau est inversement proportionnelle à la fréquence des ultrasons (Tableau 3). Par exemple, il a été rapporté que la taille des bulles de cavitation sont entre 100 et 170 µm à 20 kHz et de 3,3 µm a une fréquence de 1 MHz (Suslick and West 1986; Adewuyi 2001; Vajnhandl and Majcen Le Marechal 2005; Pang, Abdullah et al. 2011).

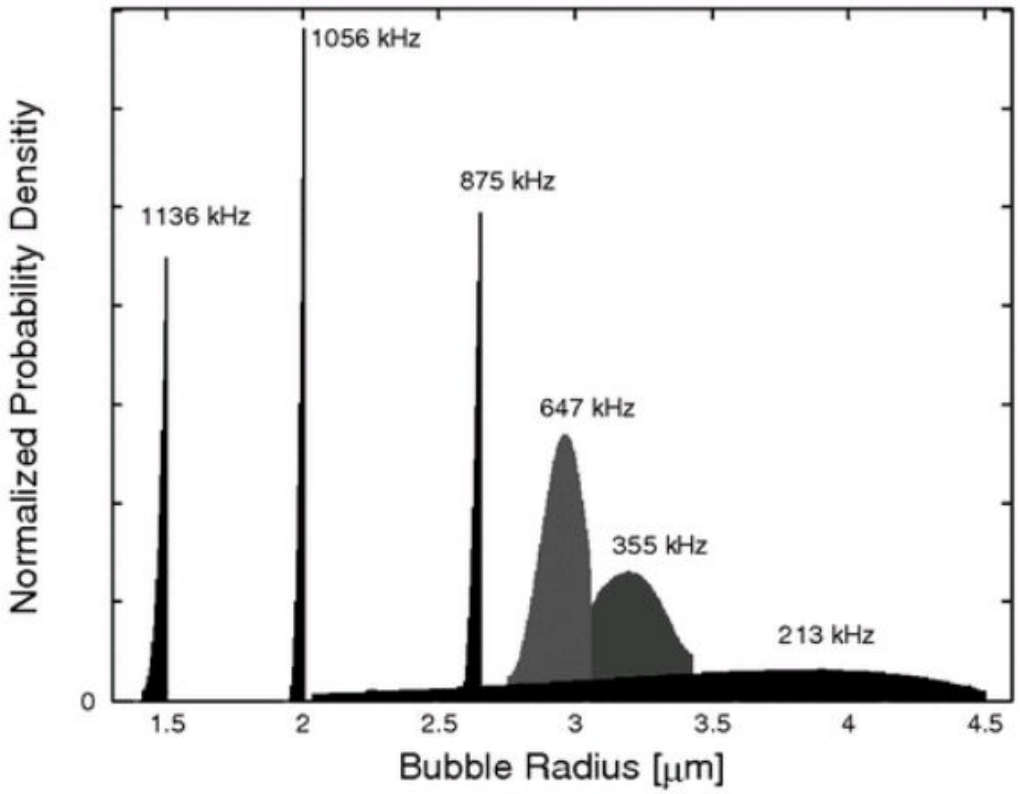


Figure 16 : Représentation des rayons des bulles de cavitation en fonction des fréquences ultrasonores appliquées. La puissance acoustique de l'ensemble des fréquences (213 kHz, 355 kHz, 647 kHz, 875 kHz, 1056 kHz, et 1136 kHz) est de 1,5 ± 0,4 W. les données pour 875 kHz, 1056 kHz, et 1136 kHz ont été réduite d'un facteur 4. les fréquences 1136 et 1056 kHz avait une grande précision avec un écart type de moins de 5%, alors que la taille déterminée à la fréquence inférieure (213 et 355 kHz) avait un écart-type d'environ 25% (Brotchie, Grieser et al. 2009).

En effet, comme le montre l'étude comparative de Brotchie, Grieser et al. (2009) (Figure 16) menée sur six fréquences ultrasonore différentes ayant la même puissance, les diamètres des bulles de cavitation sont inversement proportionnels à la fréquence ultrasonore de l'onde.

Cette diminution de diamètre des bulles de cavitation engendre une baisse des phénomènes de cisaillement dans les fluides traités entrainant pour les émulsions une baisse de la vitesse d'émulsification et donc une augmentation des temps de traitement ce qui explique l'utilisation des ultrasons de basses fréquences dans les industries.

Tableau 3: Valeurs théoriques et expérimentales des rayons de bulles de cavitation à différentes fréquences acoustiques

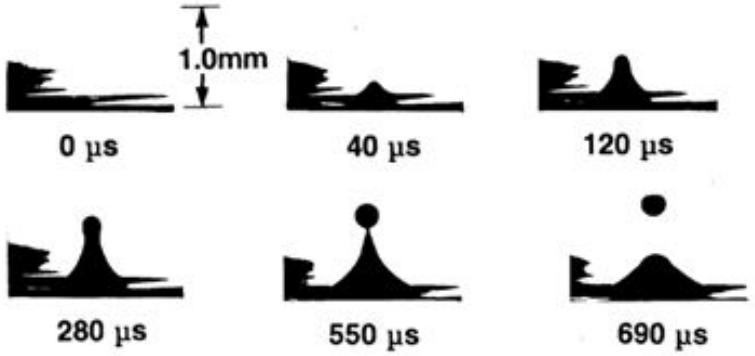
Fréquences (kHz)	Rayon de résonance linéaire (μm)[a]	Rayon théorique (μm)[b]	Rayon mesurée de la bulle et la largeur à mi-hauteur (μm)[c]	Valeurs expérimentales précédentes (µm)
20	150	0.1–100	···	3–25[d]; 2–5[c] ; 100–170[g]
140	21	0.1–10	···	···
213	14	···	3.9 (1.6)	···
355	8.5	···	3.2 (0.6)	···
515	5.8	···	···	2.8–3.7[e]
647	4.6	···	2.9 (0.2)	···
875	3.4	···	2.7 (0.02)	···
1000	3.0	0.1–3	-	3.3[g]
1056	2.8	···	2.0 (0.04)	···
1100	2.7	···	···	0.9, 1.4[f]

a Calcul à l'aide de l'équation de Minnaert's: $R_{rés} \approx 3/f$. (Brotchie, Grieser et al. 2009)
b Référence (Yasui 2002).
c Référence (Brotchie, Grieser et al. 2009).
d Référence (Burdin, Tsochatzidis et al. 1999).
e Référence (Lee, Ashokkumar et al. 2005).
f Référence (Chen, Matula et al. 2002).
g Référence (Adewuyi 2001; Vajnhandl and Majcen Le Marechal 2005; Pang, Abdullah et al. 2011)

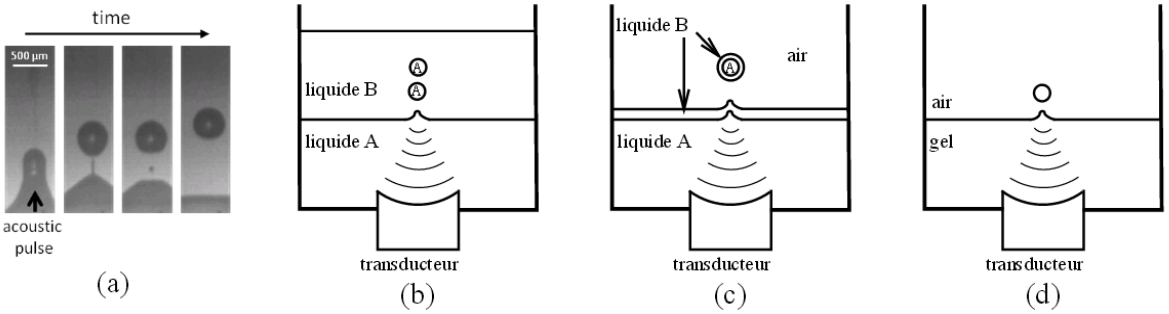
1.2.3.2.5. Emulsification par transduction piézoélectrique par ultrasons de hautes fréquences (Brevetée par la société GENIALIS)

Les ultrasons de hautes fréquences sont généralement utilisés pour mesurer rapidement et de façon non destructive des propriétés telles que la composition des aliments, la structure, le débit, l'état physique et propriétés moléculaires (McClements and Gunasekaran 1997).

La méthode d'émulsification développée consiste à utiliser des ultrasons de hautes fréquences, dont la fréquence est supérieure à 1MHz (1 à 3MHz). L'utilisation de telles fréquences permet d'éviter les effets mécaniques violents de la cavitation (Lavigne, Takeda et al. 2011).



(1) Photo de L'évolution de la formation d'une gouttelette dans l'air au court du temps avec une fréquence ultrasonore de 5MHz. Chaque image représente la superposition de 30 gouttelettes succesives, la qualité de l'image atteste sur la stabilité du processus de formation des gouttelettes (Elrod, Hadimioglu et al. 1989)



(2) (a) Ejection d'une goutte d'une surface libre induite par une impulsion acoustique incidente depuis le liquide. Configuration expérimentale (b) d'émulsification ultrasonore, (c) d'encapsulation, (d) d'éjection de gouttes de gel.

Figure 17 : Déstabilisation des interfaces et formation de gouttelettes par les ultrasons de hautes fréquences

Tout d'abord à ces fréquences, l'application d'un champ acoustique produit des ondes déstabilisant les interfaces. Ce phénomène entraîne l'irruption de la phase huileuse dans le milieu aqueux sous forme de gouttelettes de l'ordre de 70µm (Figure 17). En second lieu, le mouvement global de la phase continue provoque le passage des gouttelettes à proximité du transducteur piézoélectrique conduisant à leur cisaillement et leur fractionnement vers des diamètres finaux de l'ordre de 1 micron (Desjardins-Lavisse and Desobry 2010).

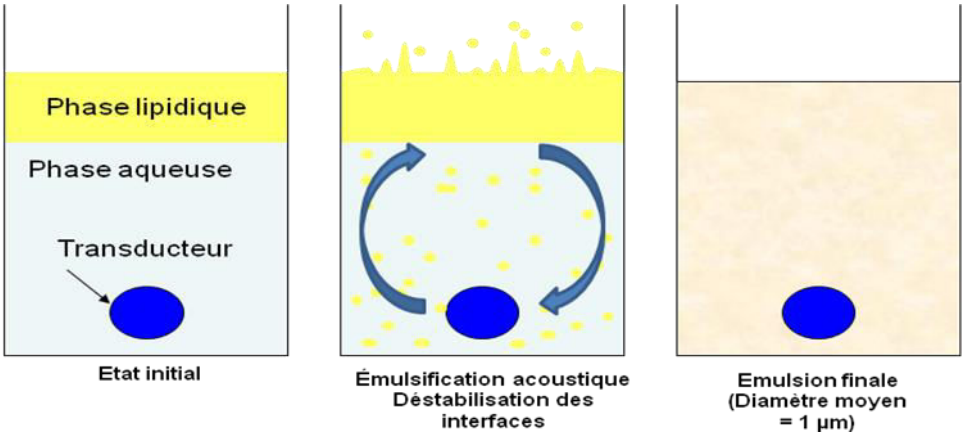


Figure 18 : Principe de l'émulsification hautes fréquences (Desjardins-Lavisse and Desobry 2010)

Sans les effets mécaniques de la cavitation, la rupture des gouttelettes ne sera pas due à l'effondrement des bulles par cavitation. En effet, après la formation des gouttelettes d'huile par déstabilisation des interfaces, le mouvement d'ensemble de la phase continue permet le passage des gouttelettes à proximité du transducteur piézoélectrique provoquant, de ce fait, le cisaillement et le fractionnement jusqu'à des diamètres finaux de 1µm (Figure 18)

Le Tableau 4 résume la différence entre les procédés d'émulsification précédemment cités.

Tableau 4: Comparaison entre les différents systèmes d'émulsification (Jafari, Assadpoor et al. 2008)

Système d'émulsification	systèmes rotor-stator	systèmes haute pression	systèmes à ultrasons	systèmes membranaires
Exemples	Mixeurs, agitateurs	Diffuseurs radicaux, disséminateurs de jet, microfluidiseurs	Sonotrodes (sonication)	Membranes en verre/céramique
Mécanismes de disruption des gouttelettes	Cisaillement en écoulement laminaire et/ou cisaillement et stress d'inertie en écoulement turbulent	Cisaillement et stress d'inertie en écoulement turbulent ; cavitation en écoulement laminaire	Cavitation en écoulements micro-turbulents	Flux de la phase dispersée
Taille minimale des gouttelettes (µm)	1.0	0.1	0.1-0.2	0.2-0.5
Limite optimale de viscosité	Faible à élevée (20-5000mPa)	Faible à moyenne (1-200mPa)	Faible à moyenne	Faible à moyenne
Applications	Laboratoire/industrielle	Laboratoire/industrielle	Laboratoire	Laboratoire
Intensité d'énergie	Faible-élevée	Moyenne-élevée	Moyenne-élevée	Faible-moyenne

1.3. Stabilité des émulsions

1.3.1. Modes de dégradation général des émulsions

Les émulsions sont thermodynamiquement instables et présentent un risque de floculation, de crémage ou de coalescence lors des interactions dynamiques des gouttelettes. Elles tendent vers la séparation de phase par le biais de transfert de masse, et autres mécanismes. Comme le montre la Figure 19, les différents modes de dégradations des émulsions sont les suivants :

1.3.1.1. Floculation

Lors de la floculation, les gouttelettes de la phase dispersée forment des agrégats et chacun de ces agrégats conserve encore son identité; l'agrégation est réversible

1.3.1.2. Coalescence

C'est un phénomène irréversible qui résulte de la rupture du film interfacial entre les gouttes de la phase dispersée et donc formation de grosses gouttelettes. La coalescence débute par une floculation, s'ensuit une fusion des gouttelettes.

1.3.1.3. Sédimentation et crémage

Ces deux phénomènes résultent de la différence de densité entre la phase continue et la phase dispersée. Si la phase dispersée à une densité plus élevée que la phase continue, il en résultera une sédimentation, cependant si la phase dispersée présente une densité moins importante que la phase continue ce qui aboutit à un crémage. Théoriquement, le crémage et la sédimentation sont des phénomènes réversibles, car il suffit en principe d'agiter modérément les préparations pour redistribuer les particules de façon homogène.

1.3.1.4. Mûrissement d'Ostwald

Le mûrissement d'Ostwald représente le flux de matière des petites vers les grosses gouttes, au travers de la phase continue. Les petites gouttes se vident au profit des grosses, et la granulométrie se modifie puisque les classes de faible taille disparaissent. Le mûrissement d'Ostwald intervient, en permanence, dès que des interfaces courbes sont présentes (Capek 2004).

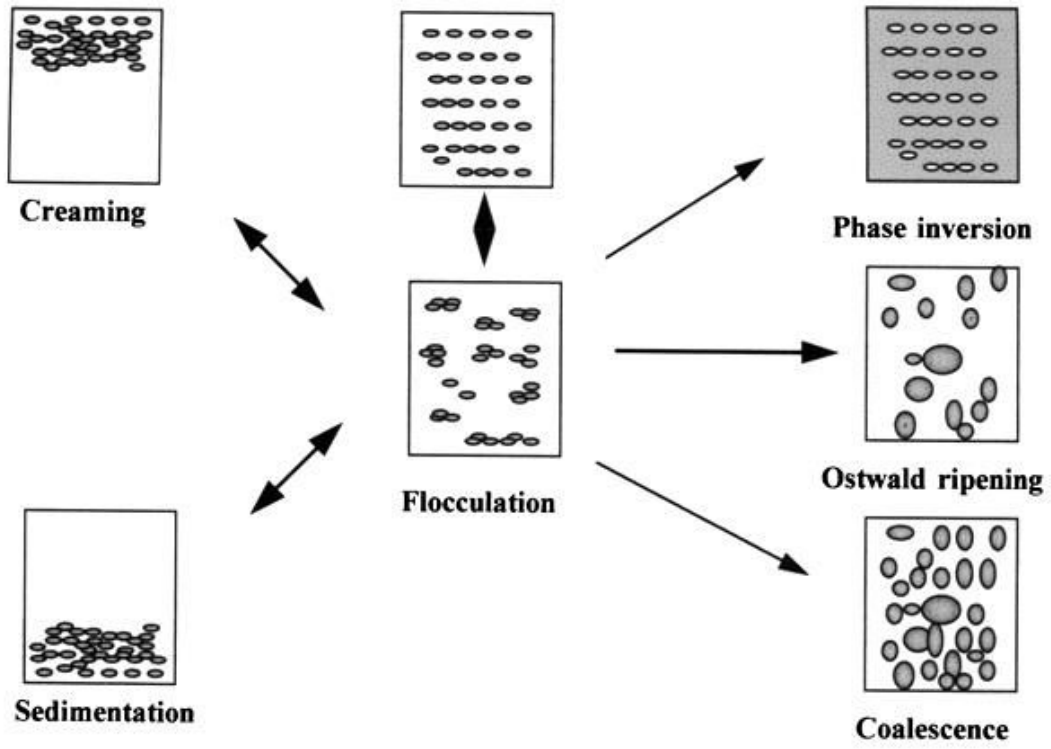


Figure 19 : Phénomènes de déstabilisation des émulsions (Abismail, Canselier et al. 1999)

En raison de leurs caractéristiques de taille, les nanoémulsions ont une grande stabilité contre le crémage (Cortés-Muñoz, Chevalier-Lucia et al. 2009).

La cinétique de déstabilisation de nanoémulsions est tellement lente (mois) qu'ils sont considérés comme cinétiquement stable. Ceci est principalement dû à leur très petite taille, induisant à une prévention contre la floculation et la coalescence des gouttelettes et donc la maturation d'Ostwald seule va régir le processus de déstabilisation (Anton and Vandamme 2011).

1.3.2. Modes de stabilisation des émulsions

Il est connu que les caractéristiques de l'huile affectent les propriétés de l'émulsion (par exemple, la taille des gouttelettes, la stabilité colloïdale), en présence de tensioactifs et / ou polymères amphiphiles.

La stabilité colloïdale des suspensions (tel que les émulsions) résulte des force attractive de Van der Waals et les forces électrostatiques répulsives. En l'absence de ces forces répulsives opposées aux forces de Van der Waals, les gouttelettes en suspension s'agrègent et Coalescent et l'émulsion est déstabilisée. Ces forces de stabilisation sont de 3 types (Rodrigues Rojas 2007; Sanhes 2008) :

- ***la stabilisation électrostatique*** par des anions et des cations adsorbés à la surface

- ***la stabilisation stérique*** ajouter de macromolécules de type nonionique, qui s'adsorbent à l'interface des gouttelettes, tels que les polymères amphiphiles. Ces couches macromoléculaires agissent en créant une barrière physique qui augmente la distance entre les gouttes.
- ***la stabilisation électrostérique :*** combine les effets stériques et électrostatiques. C'est une autre alternative à l'utilisation de surfactants ioniques ou de tensioactifs. Ces molécules possèdent un groupement polaire générant une répulsion électrostatique et une longue chaîne latérale lipophile créant une stabilisation stérique.
- **Stabilisation LBL:** Layer by layer stabilisation (LBL) ou stabilisation couche par couche représente une organisation successive de couches de tensioactifs chargés et de polymères (Figure 20)

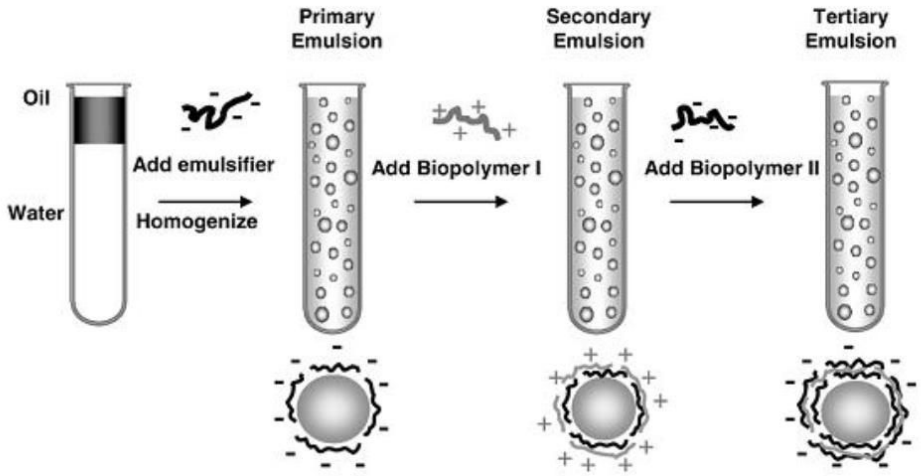


Figure 20 : Utilisation du mécanisme LBL pour la production d'émulsion H/E (Guzey and McClements 2006)

Actuellement, la stabilisation stérique (par polymère amphiphiles) et électrostérique (tensioactif) sont les deux modes les plus utilisés pour la stabilisation des émulsions.

1.3.3. Stabilisation des émulsions sans émulsifiant

Les tensioactifs introduisent une complexité pour comprendre les rôles de l'huile sur les propriétés des émulsions en raison de l'interactions des tensioactifs avec l'huile et l'eau (Sakai 2008). À cet égard, des émulsions sans tensioactifs (H/E sans aucun tensioactif) sont l'un des systèmes les plus adéquats pour l'étude des propriétés de ces émulsions. Pour cela une étude préalable des forces de stabilisation des émulsions est nécessaire.

Comme le montre des études précédentes de Lavigne et *al*., (2011), le passage de l'onde acoustique de haute fréquence (1,64 MHz) donne lieu à des phénomènes de dissociation de l'eau (électrolyse) , ainsi l'eau H_2O se retrouvera sous la forme OH- et H+ (Lavigne, Takeda et al. 2011).

Les ions se trouvant dans la solution peuvent se placer à l'interface huile/eau forment une double couche ionique (ions et contre ions) autour de la particule (Friberg, 2003), à l'origine d'une stabilisation électrostatique (Figure 21).

Si le potentiel électrique associé est suffisamment grand, alors la répulsion électrostatique empêche l'agglomération des particules (Stasrkey Ott, 2007).

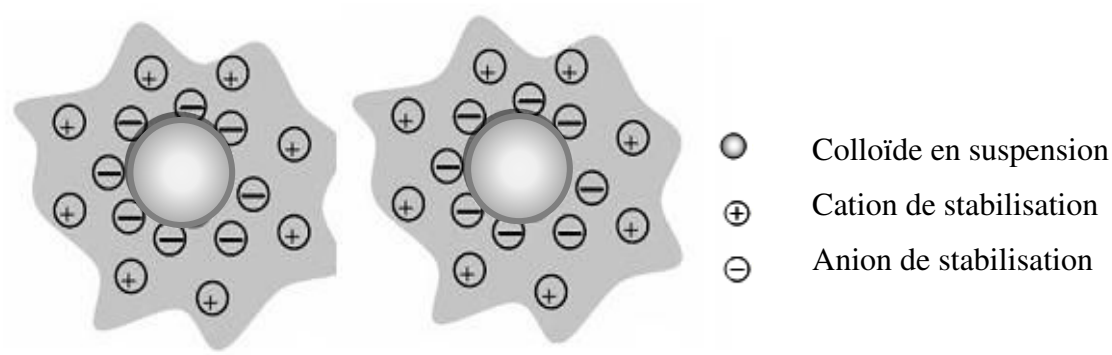


Figure 21: Représentation schématique de la stabilisation électrostatique de nanoparticules (couche de stern)

Les méthodes de synthèse des particules stabilisées de façon électrostatique utilisent principalement des sels comme agents stabilisants et plus récemment des liquides ioniques ont été largement utilisés (Reetz, 1996).

Comme le montre de nombreux travaux (Dickinson 1941; Reddy and Fogler 1980; Beattie and Djerdjev 2004), l'addition de OH- dans l'émulsion permet de stabiliser les gouttelettes d'huile et d'augmenter l'interface en créant des gouttelettes plus petites ce qui explique la charge négative de surface des gouttelettes d'huile de l'émulsion.

Hormis l'adsorption des ions OH- à l'interface des gouttelettes d'huile, Des travaux sur les interfaces huile/eau sans stabilisants en s'appuyant sur des travaux précédents ont supposés qu'une structuration des molécules d'eau à l'interface huile-eau pourrait produire un potentiel électrique en raison du moment dipolaire moléculaire (Figure 22). (Christenson, Claesson et al. 1989; Christenson, Fang et al. 1990; Marinova, Alargova et al. 1996)

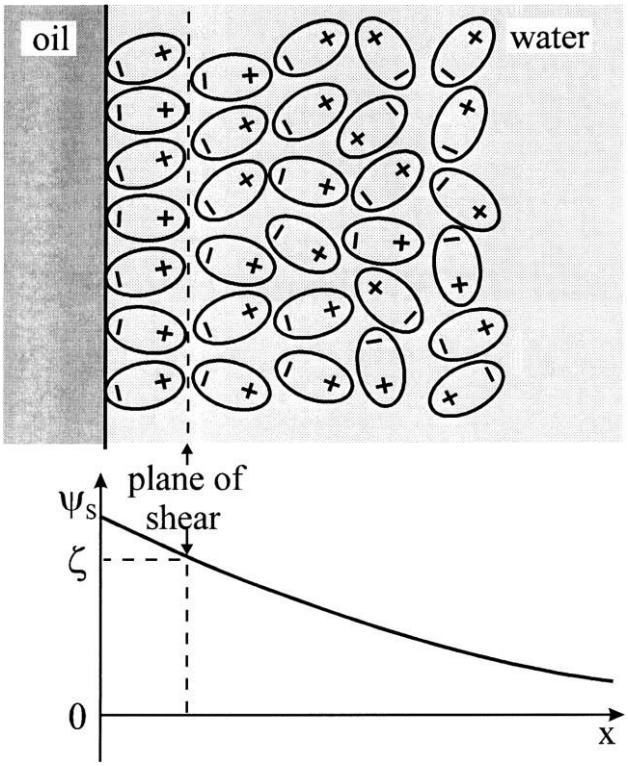


Figure 22 : Représentation schématique d'une structuration imaginaire à long terme des molécules d'eau à proximité de l'interface huile-eau qui pourrait produire un potentiel électrique en raison du moment dipolaire moléculaire (Marinova, Alargova et al. 1996).

Les travaux de Marinova et Alargova (1996) n'ont pas confirmé cette hypothèse mais leurs résultats permettent de conclure que les ions hydroxyle, libérés par l'équilibre de dissociation-association des molécules d'eau, sont adsorbés à l'interface huile-eau.

Il est connu que dans les fractions de phases aqueuses en vrac des liaisons hydrogène entre les molécules d'eau sont brisés en raison de leur mouvement brownien intensive. Par conséquent, l'adsorption spécifique pourrait résulter de restrictions dans le mouvement des molécules d'eau dans la couche interfaciale qui permet des liaisons hydrogène de l'ion OH- et des molécules d'eau voisins plus prononcée, accompagnés d'un gain d'énergie libre respective (Croxton 1986; Marinova, Alargova et al. 1996).

Résultats Expérimentaux

Chapitre II

Caractérisation physicochimique des émulsions sans émulsifiants faites par transduction piézoélectrique aux ultrasons de hautes fréquences

Ultrasonics Sonochemistry (2014) publié

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Chapitre II : Caractérisation physicochimique des émulsions sans émulsifiants faites par transduction piézoélectrique aux ultrasons de hautes fréquences

Dans la première phase d'étude, plusieurs points affectant l'émulsification par ultrasons de hautes fréquences ont été abordés. Ces points concernent essentiellement le cœur du processus : comment se fait l'émulsification par hautes fréquences? Quels sont les mécanismes mis en jeu? Quels sont les paramètres influents ? Quels sont les intervalles d'action de ces paramètres ? Etc….. Des essais ont été effectués et des réponses ont été apportées, mais cela ouvre la porte à d'autres interrogations qui nécessitent une étude plus approfondie. L'objectif de ce chapitre est de voir l'effet du temps d'émulsification par ultrasons de hautes fréquences sur les propriétés physicochimiques des émulsions. Plusieurs temps de traitement (2h, 4h, 6h, 8h, 10h) ont été testés avec un pilote d'émulsification par ultrasons de hautes fréquences (1,7MHz). L'émulsification a été faite avec différentes proportions d'huile végétale (5%, 10%, 15%). Une fois l'émulsion préparée, une caractérisation des propriétés physicochimiques a été faite afin de comprendre le mécanisme d'émulsification. Pour la caractérisation physicochimique, nous avons procédé à l'étude de la distribution granulométrique, du potentiel zêta, du pH, de la turbidité, de la conductivité ainsi que de la composition en acide gras de la phase huileuse émulsionnée. Les résultats ont montré une diminution de la taille des particules en fonction du temps de traitement. Les gouttelettes d'huile émulsionnées sont chargées négativement ce qui entraine une répulsion électrostatique de l'émulsion. Cette répulsion permet de prévenir les phénomènes de coalescence et donc empêche la déstabilisation de l'émulsion. Une diminution du pH est enregistrée ce qui soutient l'hypothèse de formation de couche de OH- autour des gouttelettes d'huile.

Emulsification by high frequency ultrasound using piezoelectric transducer: Formation and stability of emulsifier free emulsion

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Abstract

Emulsifier free emulsion was developed with a new patented technique for food and cosmetic applications. This emulsification process dispersed oil droplets in water without any emulsifier. Emulsions were prepared with different vegetable oil ratios 5%, 10% and 15% (v/v) using high frequency ultrasounds generated by piezoelectric ceramic transducer vibrating at 1.7 MHz. The emulsion was prepared with various emulsification times between 0 and 10 hours. Oil droplets size was measured by laser granulometry. The pH variation was monitored; electrophoretic mobility and conductivity variation were measured using Zêtasizer equipment during emulsification process. The results revealed that oil droplets average size decreased significantly ($p<0.05$) during the first six hours of emulsification process and that from 160 µm to 1 µm for emulsions with 5%, 10% and from 400 µm to 29 µm for emulsion with 15% of initial oil ratio.

For all tested oil ratios, pH measurement showed significant decrease and negative electrophoretic mobility showed the accumulation of OH^- at oil/water interface leading to droplets stability in the emulsion. The conductivity of Emulsions showed a decrease of the ions

quantity in solution, which indicated formation of positive charge layer around OH^- structure. They constitute a double ionic layer around oil particles providing emulsion stability. This study showed a strong correlation between turbidity measurement and proportion of emulsified oil.

Keys words: Emulsifier free emulsion, high frequency ultrasound, particle size, pH, electrophoretic mobility, conductivity.

Introduction

Emulsions are generally prepared with surface active agents (surfactant) [1-3] or amphiphilic polymers [2] to reduce interfacial tension. Emulsion stability is due to adsorption of stabilizer at oil/water interface creating electrostatic repulsion [1] and steric repulsion for emulsions containing polymers [4]. When emulsifier is added, it may interact with the other formulations compounds creating new emulsion properties. Therefore, it becomes difficult to study the role of oil phase alone on emulsion properties. The fraction of emulsifier in materials will greatly influence the purification and performance of the products [5]. Emulsifier absence should expose essential features of oil droplets themselves [2].

In ultrasonic emulsification, the sound ranges used for food can be divided into high-frequency/low-energy ultrasounds and low-frequency/high-energy power ultrasounds [6].

High frequency ultrasounds are usually used as a nondestructive, rapid, easy-to-automate, and relatively inexpensive analytical technique for quality assurance and process control with particular reference to physicochemical properties, such as composition, structure, physical state and molecular properties of foods [6]. As well as being used as an analytical instrument in the laboratory, ultrasound can also be used for continuous monitoring of food properties on-line during processing [7].

High frequency ultrasounds go through solid or liquid media without affecting their structure and are used as diagnosis tools e.g. analysis and medical imaging [8]. HF ultrasounds were used in different applications, such as quality control of food, commercial cooking oils, bread and cereal products, bulk and emulsified fat based food products, food gels, aerated and frozen foods, improvement in mass transfer, food preservation, support of thermal treatments, texture improvement and food analysis [9, 10].

These low energy ultrasounds have frequencies above 1 MHz at intensities below 1 $W \cdot cm^{-2}$. They have been used for non-destructive action on genetic improvement programs; it is also used for evaluating compositions of raw and fermented meat products and for evaluating composition of fish and poultry. On the other hand, high power (low frequency) ultrasounds

induce mechanical, physical and chemical changes through cavitation, which induce many food processing operations such as extraction, emulsification and inactivation of food bacteria [11, 12]. In ultrasound system, the droplet disruption is due to the physical shear force provided predominantly by the process of acoustic cavitation [13]. The use of low frequency ultrasound for emulsion formation is well established, mostly on an analytical laboratory scale [14]. Nano/micro-emulsions with the desirable droplet size can be easily generated from a variety of materials with ultrasound using only a fraction of the power of conventional mechanical devices [15]. Within ultrasound range, the power available varies inversely with the frequency and only powerful ultrasounds (16-100 kHz, and, to a lesser extent, 100 kHz-1 MHz) interact with matter, producing physical and chemical changes, essentially by cavitation phenomena [8] that is formation and subsequent collapse of micro-bubbles by pressure fluctuations of a simple sound wave causing extreme levels of highly localized turbulence "high pressure and high temperature" [11, 16, 17].

These ultrasounds (low frequency) use intensities higher than 1 $W \cdot cm^{-2}$ and frequencies between 20 and 500 kHz, which are disruptive and induce effects on the physical, mechanical or chemical and biochemical properties of foods [9].

The impacts of ultrasound treatment on sunflower oil using two different ultrasound arrangements (titanium and Pyrex) were evaluated by Pingret et al. (2012). The study showed an increase of formed radicals in sonicated oils, as well as modifications of physicochemical parameters, particularly oils oxidation [18].

The aim of this work was to use high frequency ultrasounds and low energy emulsification to create a non-denaturing and non-destructive emulsification process avoiding chemical changes in emulsions without emulsifier. This study evaluated applicability of new emulsification process using high frequency ultrasounds for generation of emulsifier-free emulsions with different oil ratios from: 5%, 10% and 15% (v/v).

1. Materials and methods

1.1. Material

Distilled water and commercially available sunflower oil were used as aqueous and oil phases, respectively, for all prepared emulsions. Sunflower oil was used for emulsification and (vegetable oil, Lesieur) purchased from a local supermarket (Nancy, France). Aqueous solution pH was adjusted at 10.0 before emulsification process with 1N NaOH solution. The NaOH was purchased from Fisher Scientific (France). The higher emulsified oil ratio was observed when an emulsion aqueous phase was adjusted at pH 10.0. Beattie and Djerdjev (2004) [19] produced

emulsions with several emulsification steps and maintained a basic pH (pH=9) throughout all step of emulsification process. After each step, NaOH was added to maintain a constant pH, allow a supply of OH^- and provide an electrostatic stabilization for emulsion. In our study, the pH was not maintained during the emulsification process, but initially adjusted to pH 10 and pH changes was followed during the emulsification process. All materials were used as received, without further purification.

A piezoelectric transducer was supplied by Hydro factory, Ltd. (Sarcelles, France). The volume of emulsion reactor was 6 liters. The piezoelectric transducers were equipped with a piezoelectric ceramic with 20 mm diameter vibrating under electric field influence. The ultrasonic frequency was chosen as 1700 KHz. Operation voltage was 24V and electric current intensity was between 0.82-0.90A.

1.2. Preparation of oil droplet dispersions

Emulsion was generated according to Genialis patent (N149668A1). Ultrasonication dispersed oil droplets in water without any surfactant. Emulsification was made with piezoelectric ceramics vibrating at high frequency ultrasounds with maintained temperature at 25°C.

Water and oil were placed in a thermostated reactor. For each liter, two transducers were added. Twelve piezoelectric transducers were placed at the bottom. Water and sunflower oil were exposed to emulsification process with high frequency ultrasounds during 10 Hours. Samples were taken every two hours for analyses. Various sunflower oil ratios were tested: 5%, 10% and 15% (v/v) and emulsion volume was 6 liters.

1.3. Size measurement

The mean distribution of oil globules was measured using a laser light scattering particle size analyzer with Mastersizer S (Malvern Instruments Ltd. UK) equipped with a He-Ne laser, with a beam of light of 360 nm, and wet sample analyzer that allowed emulsion measurements. The system was able of detecting particles in size ranging from 0.05 µm up to 900 µm. Measurements were carried out in ten replicates for each emulsion. Results were reported as typical globules size distribution in µm, the volume-weighted mean globule size D (4,3):

$$D(4,3) = \sum n_i \, d_i^4 / \sum n_i \, d_i^3$$

Where n_i was the number of particle i; d_i was the diameter of the particle i (µm). Oil globule size distribution was determined from the best fit between experimental measurements and

prediction using the Mie light scattering theory [20]. The D(4,3) index was chosen instead of D(3,2) since it is very sensitive to the presence of small amounts of large particles.

1.4. Electrophoretic mobility measurement

The electrophoretic mobility (µE) was measured every 2 hours during emulsification using Malvern Zetasizer Nano ZS (Malvern instrument, Worcestershire, UK). Measurements were performed on standard capillary electrophoresis cell equipped with gold electrodes. Electrophoretic mobility of emulsion droplets evaluated the surface charge of oil droplets. The mobility of emulsion droplets was measured by diluting emulsion (0.05%) ultrafiltrated water. The measurements were an average of 20 runs (equilibration time: 10s, number of run: 20, run) and quoted result is the average of 3 different measurements. Measurements were carried out at 25°C. Measurements were performed directly in the diluted emulsions and results were averages of triplicate analyses.

1.5. Emulsions pH

Emulsion pH was measured every 2 hours using a pH meter Lab 850 (Schott instrument, Deutschland, Germany) for all oil concentrations. Each measurement was performed on five different samples.

1.6. Turbidity measurement:

Turbidity measurement was performed with a turbidimeter (Analyte NEP 160 VanMc Instruments turbidimeter, Mulgrave, Australia) equipped with a probe-type CIP160-3 and counter anal NEP260. The probe was immersed in emulsion to measure mixture turbidity; the measurement was made in triplicate.

1.7. Emulsification capacity measurement

As emulsion destabilization for oil extraction from emulsified product was impossible to reach, the proportion of oil in emulsion was calculated at the end of emulsification process by measuring the difference between oil initially put in emulsion and non-emulsified oil remaining at emulsion surface. Measurements were carried out in five replicates. The oil ratio in emulsion was calculated as follows:

$$EC = (IV - FV) * 100/EV$$

EC: Emulsification capacity, IV Initial oil volume, FV: Floating oil volume, EV: Emulsion volume.

1.8. Conductivity measurement

Emulsion conductivity was measured each 2 hours during emulsification, using the Malvern Zetasizer Nano ZS (Malvern instrument, Worcestershire, UK). The conductivity value was used to indicate the type of emulsion produced: oil in water (O/W) or water-in-oil (W/O) [21]. The measurement of conductivity also allowed studying the electrolytes quantity evolution in the continuous phase.

1.9. Fatty acid composition

Fatty acid methyl esters (FAMEs) were prepared according to Akman (1998) [22] The separation of the FAMEs was carried out on a Perichrom™ 2000 gas chromatograph (Perichrom, Saulx-lès-Chartreux, France), equipped with a flame-ionization detector. A fused silica capillary column was used (50 m, 0.25 mm i.d. × 0.25 μm film thicknesses, CP 7419 Varian, Middelburg, Netherlands). The initial temperature of column was 120°C for 2 minutes, and then increased to 180°C at a rate of 2°C / min, then to a temperature of 220°C at 25min. The detector and Injector temperature was 250°C. Hydrogen was used as carrier gas at a flow rate of 40ml/min. 1μl of sample was introduced into the column for analysis. Standard mixtures (PUFA1 from marine source and PUFA2 from animal source; Supelco, Sigma–Aldrich, Bellfonte, PA, USA) were used to identify fatty acids (internal standard C21:0). The results were presented as duplicate analyses.

1.10. Statistical analysis

ANOVA analyses were processed with Excel software (Microsoft corporation version 2007). All tests were executed at 95% significance level to find out if the time of emulsification process and initial oil ratio had significant effects on volume-weighted mean globule size, D(4, 3).

2. Results and discussions

Emulsions was prepared according GENIALIS patent (N149668A1) (Fig.1). The invention relates to the method for preparing stable oil in water emulsion without adding an emulsifier. According to this method, a mixture of a lipid phase and aqueous phase is subjected to vibrating energy, in a sealed container, by applying a transducer operating at a frequency above 900 kHz and below 3MHz [23].

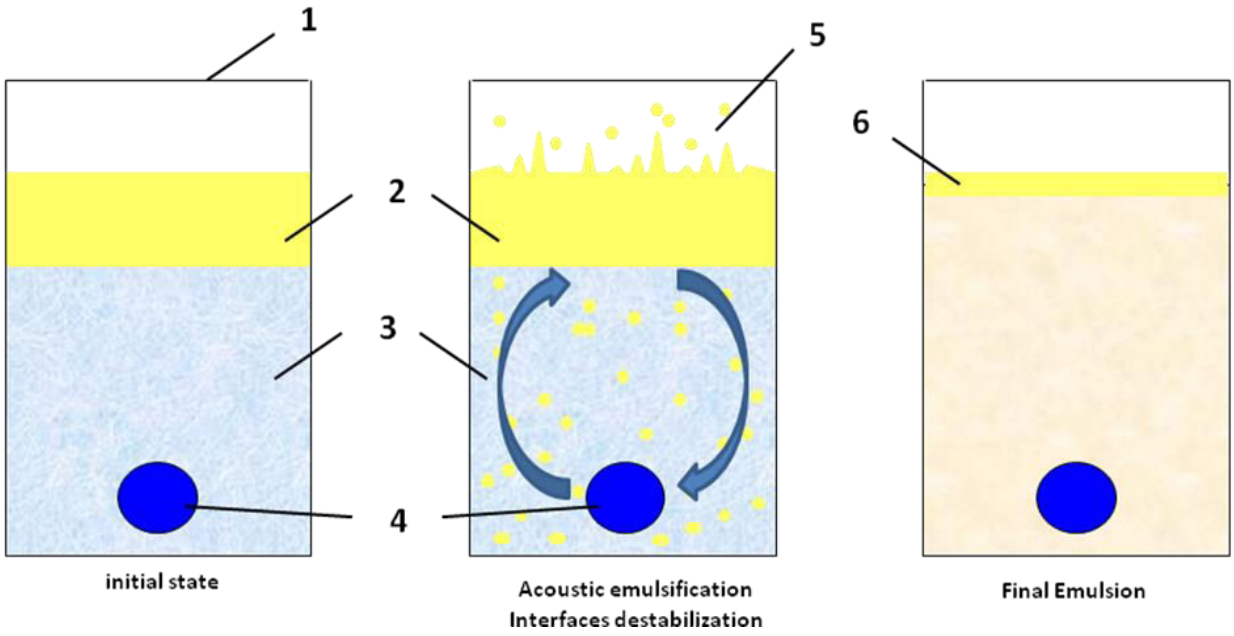

Fig.1: Diagram of high frequency ultrasounds emulsification using piezoelectric transducer (1: Tank, 2: Initial oil ratio, 3: Water, 4: Piezoelectric transducer, 5: Mist, 6: Non-emulsified oil) (Desjardins-Lavisse and Desobry 2010)

High frequency ultrasounds avoids violent mechanical effects of acoustic cavitation at low frequencies, because as Lavigne (2011) showed, choosing an appropriate frequency (> 1 MHz), cavitation mechanical effects may be limited at the expense of chemical effects represented by the dissociation of water. This emulsification technique uses piezoelectric ceramic transducers (Fig.1) which vibrates under influence of electric field with oscillating movements. These vibrations destabilize interfaces inducing an eruption of either phase in the other as nanosized droplets thereby forming nano-emulsions.

2.1. Effect of emulsification by high frequency ultrasounds on particle size

The evolution of the particle size distributions during emulsification process for three model emulsions with different initial ratio of vegetable oil was shown in Fig.2. After 2h of emulsification process, two peaks were registered for emulsions with 10% and 15% of oil with big droplets essentially. After 2 hours, the average diameter of the main population was 163 µm for emulsion with 10% of oil and 400 µm for emulsions with 15% oil with a second population at 1µm (peak 2). At the same time, emulsion prepared with 5% oil displayed one peak with a top at 163µm. Emulsification during 4 hours induced significant reduction in droplet sizes for emulsions with 5% and 10% oil ratio (Fig.2 a-b) with a peak range between

0.36–5.69 µm. For emulsion with 15% oil, the main pick had droplets size still above 100 µm (Fig.2c) while 5% and 10% emulsions had clearly a lower size (Fig.2 a-b). Application of high frequency ultrasounds on coarse emulsion induce splitting of big existing droplets. Between 2 and 4 hours of emulsification, a gap of 150 µm was registered for emulsions with 5 and 10%, and 250 µm for emulsion with 15% (Fig.2c), probably due to fractionation of existing oil drop. Ceramic vibration caused detachment of oil droplets at emulsion surface (in air), which induced an appearance of mist. The small mist droplets felt back in solution and took oil droplets in aqueous phase (Fig.1, Fig.4), this recirculation induced formation of new smaller droplets during emulsification process. In the 15% emulsion, a higher volume of large oil droplets required more shearing time for droplets splitting. After 6 hours of emulsification process, one single peak was observed at 1µm for emulsion with 5% initial oil ratio (Fig. 2a). At the same time, two peaks remained for emulsions with 10%: at 1.44 µm and at 14.22 µm (Fig. 2b), while for emulsion with 15% oil, the two peak values are 1µm and 140 µm (Fig.2c). Using the piezoelectric transduction process (emulsification by high frequency ultrasounds), a peak at 1µm was quickly appeared and increased from 2 to 10 hours. The peak at 1µm increased at the expense of peaks above 100 µm and a significant decrease ($p < 0.05$) in the D(4,3) was observed (Fig.3). These D(4,3) values were an average diameter that neglects size polydispersity but reflects progressive reduction of droplets sizes in the 3 systems.

After 6 hours of emulsification, monodisperse droplets population was obtained at 1µm for 5% emulsion. The bimodal distributions span has clearly decreased with transduction time, from 2 to 10 hours, and the droplet sizes were shift towards smaller values. This monodisperse size distribution required 10 hours to be achieved for emulsion with 10 and 15% initial oil ratio.

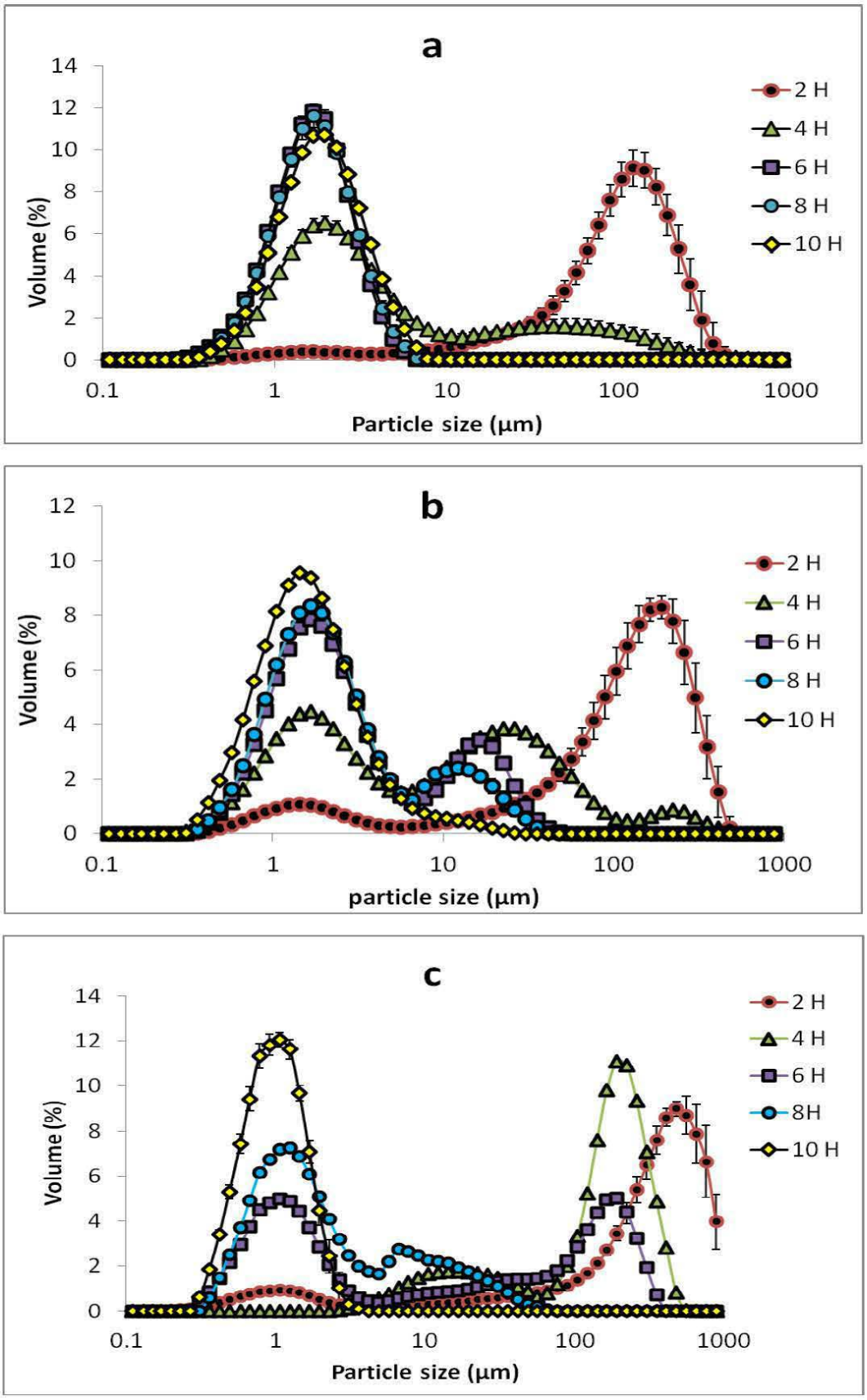

Fig.2: Particle size distribution of the emulsion containing different initial oil ratios (a: 5%, b: 10%, c:15%) as a function of emulsification time

Emulsion droplets diameter D(4,3) decrease during ultrasonic emulsification was shown in Fig.3. A significant reduction in the D(4,3) diameter was observed during the six first hours of process due to splitting of droplets above 100 µm. The decrease became relatively slow after 6 hours of transduction except for emulsion with 15% initial oil ratio reaching a D(4,3) diameter of 25 µm at 6 hours and 3 µm at 8 hours. Diameters variation was not significant after 8 hours of piezoelectric transduction. The high frequency ultrasound appeared very effective in reducing droplets size for all oil ratios.

In the literature, authors focused on low frequency (LF) ultrasounds. LF ultrasounds emulsification occurs by two mechanisms [11, 16, 24]. First, application of acoustic fields produces interfacial waves that induces interfaces instability, ultimately resulting in oil phase emergence in the aqueous phase as droplets of about 70µm. Secondly, application of LF ultrasound causes acoustic cavitation, that is, the formation and subsequent collapse of micro-bubbles by pressure fluctuations of a simple sound wave. Each bubble collapse (an implosion on a microscopic scale) event causes extreme levels of highly localized turbulence (high pressure and high temperature). Turbulent micro-implosions act as a very effective method of breaking up primary droplets of dispersed oil into droplets of sub-micron size.

Application of low frequency ultrasonic waves in emulsion led to formation of cavitation bubbles. The chemical and mechanical effects of ultrasound are due to the collapse of these cavitation bubbles. By choosing an appropriate frequency above 1 MHz, mechanical effects can be limited to the benefit of chemical effects which are represented by water hydrolysis [12].

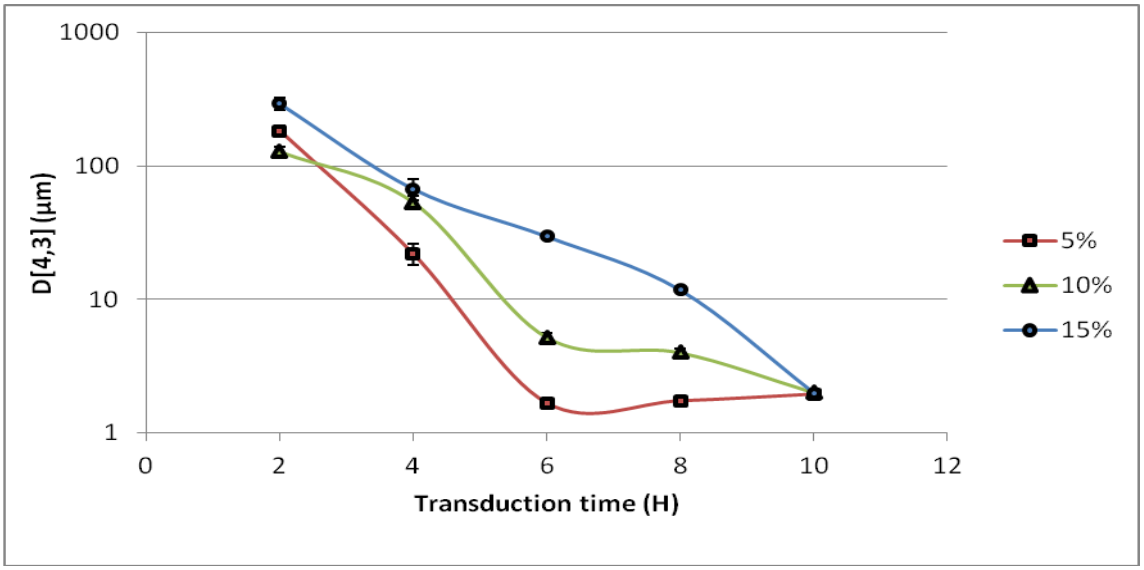

Fig.3. The effect of emulsification time with high frequency ultrasound (1,7MHz) on the particle size, D(4,3), of three vegetable oil emulsions with different initial oil ratio.

In the present study, the 1.7 MHz frequency used was above the interval of violent cavitation (formation and collapse of cavitation bubble). Without mechanical effects of cavitation, droplets breakage previously discussed cannot be due to cavitation bubbles collapse. After formation of big oil droplets by interfaces destabilization, the overall movement of the continuous phase allowed the droplets passage near to piezoelectric transducer causing shearing and splitting down to final diameters at 1 µm by high frequency vibrating movements (Fig.4). The 1.7 MHz frequency led to strong destabilization of the droplet interface and progressive decrease of droplets diameter.

In acoustic emulsification, particle size and size distribution can be easily varied with treatment processing time. Shorter treatment times produced larger particles with a wide multimodal distribution of sizes whereas longer times produced smaller particles with monomodal size distributions. Acoustic emulsions were found to be very stable without any surfactant to stabilize the system [1]. The stability of the emulsion was also influenced by the droplet size, the difference of the density between dispersed and continuous phases, the viscosity of the continuous phase, the van der Waals force and the electrostatic adhesion force between the droplets [25].

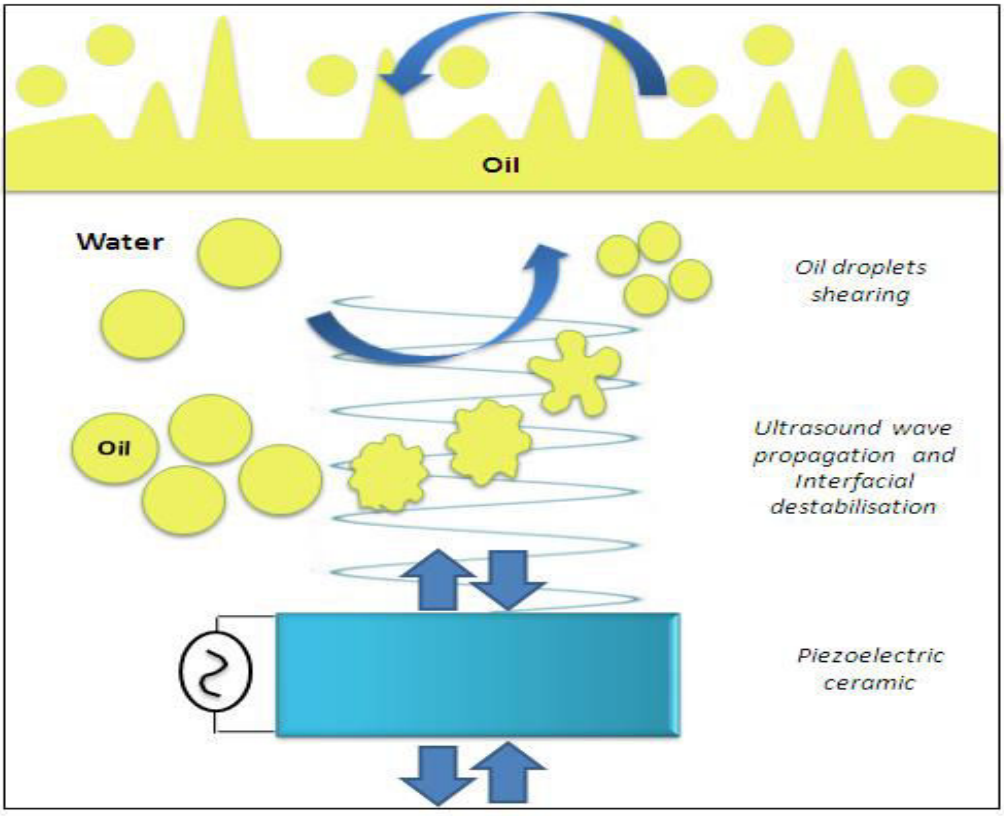

Fig.4. Decrease of droplet oil size during emulsification with high frequency ultrasound

2.2. Effect of emulsification with high frequency ultrasound on pH emulsion

One of the most important factors determining formation and characterization of electrical double layer of oil droplets was the solution pH that led to surface group's ionization and determined the final surface charge density. The electrical charge of emulsion droplets primarily resulted from adsorption of ionized species from the surrounding solution onto dispersed droplets surface, e.g., ionic emulsifiers, polyelectro-lytes, or mineral ions [3]. The magnitude and sign of the droplet charge therefore depended on the type and concentration of molecules adsorbed on the surface. One of the most important factors determining the formation and properties of multilayer colloids was the solution pH, both during and after interface formation [3].

The pH study showed evolution of ions concentration in solution which may explain the stability of these surfactant free emulsions (Fig.5).

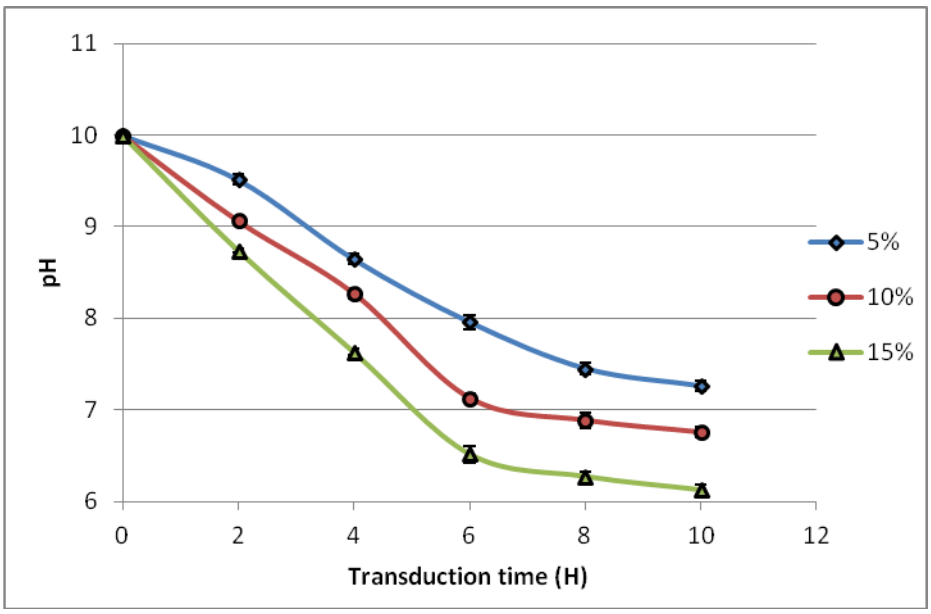

Fig.5. Effect of emulsification with high frequency ultrasound on pH of three vegetable oil emulsions containing different initial oil ratios.

A significant pH decrease was observed of the three emulsions and increased with the oil content (Fig.5). For these three emulsions, pH decrease versus time was statistically significant ($p<0.05$). As pH decreased the H_3O^+/OH^- concentration ratio increased in the hydrophilic phase of the emulsion.

As OH^- content in the continuous phase decreased with processing time, the first hypothesis was that hydroxide ions present in the aqueous phase were either placed and retained at oil /

water interface or the second hypothesis was that these ions dissolved in discontinuous phase at oil droplets.

Beattie and Djerdjev (2004) was shown an pH emulsion decrease after each emulsification process, addition of NaOH solution maintained the pH at a fixed value. They showed that amount of hydroxide ion added for maintaining the constant pH value on emulsion depended linearly on the oil/water contact surface area created [19].

1.3. Effect of emulsification by high frequency ultrasound on electrophoretic mobility

Droplets surface charge was determined (Fig.6). The emulsions prepared by high frequency ultrasound exhibited negative electrophoretic mobility and zêta potential. The electrophoretic mobility of the emulsions was between -4.8 to -5.7 µmcm/Vs that corresponded to -60 to -73 mV of zêta potential. Generally, higher absolute zeta potential values (above ± 30 mV) indicate a good stability against coalescence of dispersed droplets [26, 27]. The negative charge on the emulsions is possibly imparted by the free fatty acids present in oil phase [27, 28].

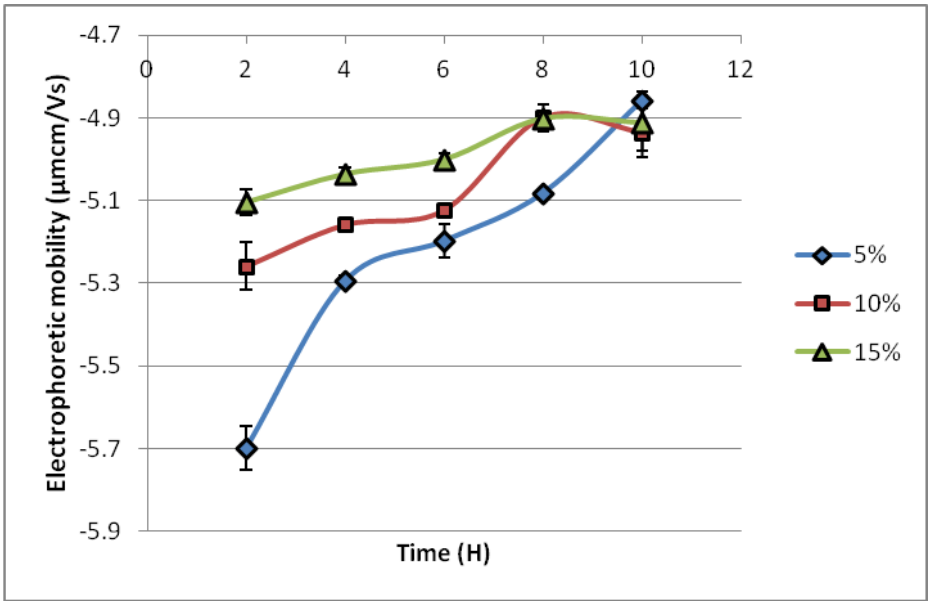

Fig.6. Effect of emulsification with high frequency ultrasound on electrophoretic mobility of three vegetable oil emulsions containing different initial oil ratios.

Vegetable oil was hydrophobic, coverage of oil droplets surface with hydroxide ions occurred in aqueous media due to hydrophobic interactions between oil and water [19]. So, it can be speculated that oil droplets accumulates more charges on the surface and forms thicker layer

[19]. When we consider the surface charges, it was observed that the surface charge is negative, where electrostatic interactions between oil droplets are repulsive.

According to Dickinson (1941), the mobility effect of the end group could be related to his "residual charge". This residual charge, which arise from the unequal division of electrons between the group concerned, and the rest of the molecule, results in a net charge of the end group, which attract or repels hydroxyl ions to the adsorption layer according to its sign [29]. The negative charge of oil droplets surface was due to hydroxide ions.

Negative value of electrophoretic mobility was observed after 2 hours emulsification, at the same time, a decrease in emulsion pH was recorded (Fig.5). It has long been known that the oil/water interface can acquire a negative charge in absence of surfactants [19, 29]. The pH decrease during emulsification process and the negatives values of electrophoretic mobility (Fig.6) suggested that oil droplets stabilization was related to hydroxide ions decrease in solution during emulsification.

The electrical charge of emulsion droplets primarily came from adsorption of OH^- from the surrounding solution into the surface, according with Siah Ying Tang and al. (2013) study, who found a negative charge of the oil droplet emulsion at basic pH (pH 10) [26]. In the presence of a greater fraction of oil, a larger number of suspended oil droplets in the emulsion were formed. In this case, oil/water interface was higher, whereby the hydroxide ions which surroundings each globule are less in the solution. When oil droplets number is higher, more hydroxide ions are needed for obtain the same charge. This suggests that for a same formula, when percentage of oil incorporated was higher, hydrodynamic radius was lower because the ions are shared between the existing droplets, therefore electrophoretic mobility and zeta potential had a lower absolute value.
During emulsification at high frequencies, a decrease in particle size was observed due to oil droplets split in formed emulsion (Fig.2). During droplets split, ions present around the particles were divided between the particles resulting from this division. Oil/water interface increase led to a decrease in the number of ions surrounding each one particle represented by the increase in the value electrophoretic mobility value (decrease of absolute value) (Fig.6).

According to the following formula, the electrophoretic mobility is directly proportional to the zeta potential, indicator of the stability of emulsions and colloidal suspensions.

$$\zeta = \eta u/\varepsilon\varepsilon_0 \text{ [30]}$$

Where η is viscosity of the continuous phase, u is electrophoretic mobility, ε is relative dielectric constant, ε_0 is dielectric constant in vacuum.

It has been suggested that zêta potential may serve as a partial indicator for the physical stability of the emulsion being formed. High absolute zeta potential values (above 30 mV) should preferably be achieved in most of the emulsions prepared in order the creation of a high-energy barrier against coalescence of the dispersed droplets [27].

This OH^- organization around oil droplets formed a clathrate structure inducing to electrostatic stabilization and prevented droplets coalescence.
For acoustic emulsions, there are currently two general theories for treating the stability of emulsions: steric and electrostatic stabilization. The steric stabilization, which is a generic term, encompasses all mechanisms by which non ionic macromolecules can impart colloid stability [1].

The electrostatic stabilization (DLVO theory), the most studies was proposed by Derjaguin, Landau, Venvey, and Overbeek (DLVO), the DLVO theory, inconjunction with the Smoluchowski treatment, quantitatively describes the electrostatic stability of an emulsion to flocculation [1].

1.4. Effect of emulsification by high frequency ultrasound on conductivity

Followed conductivity (Fig.7) allowed firstly to determine the type of emulsion (oil in water or water in oil) and secondly to see the evolution of ions solution. The conductivity of high frequency ultrasounds emulsion was measured during emulsification time, from 0 to 10 hours.

Conductivity evolution as a function of emulsification time was shown in the Fig.7. A decrease on conductivity is observed for all emulsion. On emulsion with 15% oil, the conductivity decreases from 0.079mS/cm at 2 hours to 0.024 mS/cm after 6 hours and 0.013 mS/cm at 10 hours. The same tendency is observed for the emulsion with 10 and 15% initial oil. The decrease of ions concentration in solution induces a decrease in conductivity value.

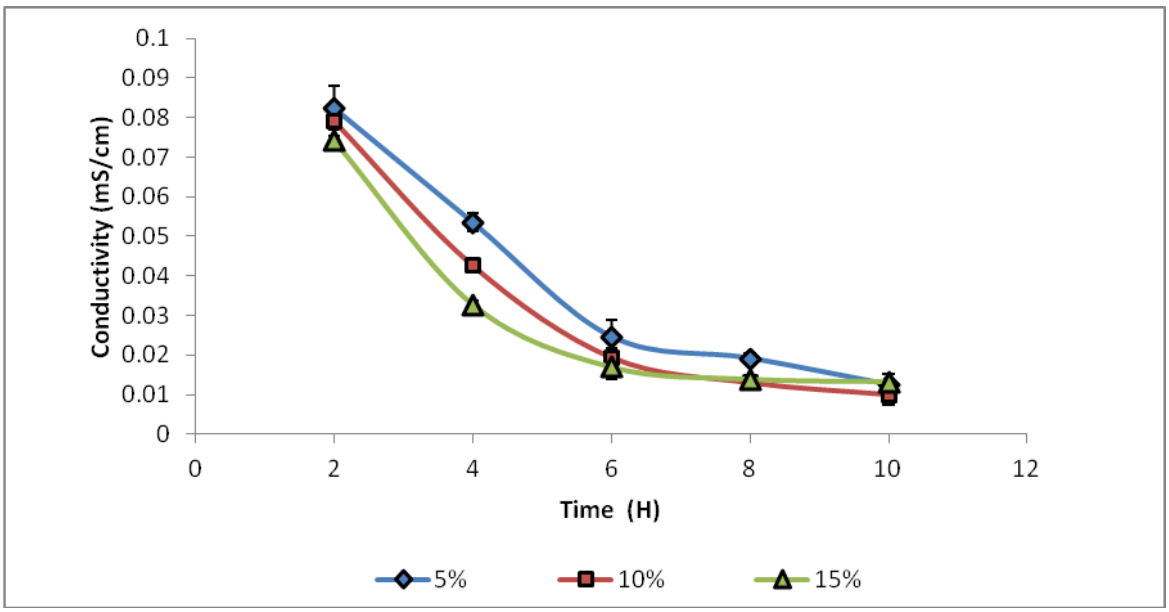

Fig.7. Effect of emulsification with high frequency ultrasound on conductivity of three vegetable oil emulsions containing different initial oil ratios.

During emulsification, an increase in oil/water interface was caused due to a decrease in the size of the oil droplets emulsion. Interface increase is also due to the incorporation of new droplets from non-emulsified oil phase present on emulsion surface.
When oil/water interface increase, hydroxide ions are placed at newly formed interfaces inducing diminution of hydroxide in solution and thus a decrease in the conductivity during the emulsification process.

The following of hydroxide layer formation, an attractive force is exerted on the Na+ solution ions causing their attraction to hydroxide layer and thus formation of a counter ions layer above ion hydroxide layer, the formation of the double ions layer around each particle explains conductivity decrease.

According to Beattie and Djerdjev 2004, most of the hydroxide ion surface charge is compensated by Na+ counter ions in the Stern or stagnant layer inside the shear plane. This double layer is essential to the electrostatic stabilization of surfactant free emulsion. Hence these emulsions are stabilized electrostatically by a high zêta potential with a relatively thick double layer on the order of 10 nm or more [19].

1.5. Effect of emulsification by high frequency ultrasound on Turbidity and emulsification capacity

Table 1 shows the value of turbidity and emulsification capacity according to initial oil ratio after 10 hours of emulsification by high frequencies ultrasound.

For turbidity, significant increase was observed when initial and final oil concentration increases.

Augmentation of initial oil ratio induces the increase of emulsified oil ratio. When oil ratio was higher, the first droplets formed have a higher volume. The passage of ultrasonic wave through the solution causes a splitting of the droplets formed and therefore a larger volume of oil emulsified which gives more concentrated emulsion.

According Reddy & Fogler, (1981), the turbidity of an emulsion is function of the concentration of particles and their sizes, emulsion stability can be determined by measuring the change in turbidity over time.

Table1: Summary of the oil percentage in the three emulsions containing different oil proportions.

Initial oil ratio (%)	Emulsification capacity (%)	Turbidity (NTU)
5	3.18 ± 0.11	819.67± 15.00
10	5.25 ± 0.18	1396.70± 21.50
15	10.99 ± 0.32	1890± 11.50

Therefore, the change in turbidity indicates the change in particle size and concentration. The turbidity of suspensions containing very coarse particles varies inversely with particle size. The turbidity of an emulsion containing very fine and polydispersed particles is a complex function of particle size and concentration [31].

Regarding the emulsions particle size, after 10 hours emulsification, the particle size is around 1µm whatever the initial oil ratio (5%, 10% and 15%) (Fig.2).

In this case, the increase in turbidity is not due to the variation of the particle size but it is due to the increase of the oil concentration in the emulsion.

1.6. Effect of emulsification by high frequency ultrasound on fatty acid composition of sunflower oil

The fatty acid oil composition produced from lyophilization of native and emulsified sunflower oil is showed in table 2.

As showed in the table 2, the composition of emulsified and native sunflower oil is similar. Fatty acid composition showed four principal compounds: two saturated fatty acids were identified, i.e. palmitic acid (C16) with 4,5% and stearic acid (C17) with 4% ; a mono-unsaturated fatty acid, i.e. oleic acid C18:1 n-9 (omega 9) with 22% ; and a polyunsaturated fatty acid, i.e. linoleic acid C18:2 n-6 (omega 6) with 65%. Emulsification with high frequency ultrasounds do not causes any change or degradation of fatty acid profile of used oil.

Table 2: Fatty acid composition of emulsified and native sunflower oil

Fatty acid composition	Oil before emulsification	Oil after emulsification
C14	0.053 ± 0,02	0.1 ± 0,01
C16	4.561 ± 0,21	4.476 ± 0,12
C17	0.030 ± 0,001	_
C18	3.9 ± 0,45	4.3 ± 0,25
C20	_	0.3 ± 0,02
C22	0.780 ± 0,012	0.8 ± 0,007
C24	0.233 ± 0,02	0.2 ± 0,016
C16.1	0.107 ± 0,01	0.1 ± 0,02
C17.1	_	_
C18:1 n-9	22.820 ± 0,53	22.1 ± 0,17
C18:2 n-6	65.966 ± 0,27	65.6 ± 0,54
C18:3 n-3	_	0.5 ± 0,04
C20:1 n-9	0.265 ± 0,041	0.2 ± 0,021
C22:1 n-9	_	0.1 ± 0,01

Conclusion

Using high frequency ultrasound to produce acoustic emulsion was a successful method. The study of high frequency ultrasounds effect on formation of oil emulsions droplets showed a decrease in suspended oil particles size formed due to deformation and breakage of oil droplet during propagation of acoustic wave. pH measurement showed a significant decrease and negative electrophoretic mobility was recorded, which showed the formation of hydroxide layer by adsorption of OH^- at oil/water interface leading to of droplets stability on emulsion. Conductivity measurement showed a decrease of solution ions, which indicates the formation of a double layer around oil particles assuring electrostatic stabilization. A strong correlation between turbidity measurement and emulsified oil ratio was observed. For a given particle size, an increase in turbidity is indicative of the increase of emulsified oil ratio. We believe that these findings and concepts on emulsifier-free-emulsions will lead to further development on new colloidal systems.

Acknowledge

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Chapitre III

Etude comparative entre le procédé d'émulsification par ultrasons de haute fréquence et des procédés d'émulsification standards pour des émulsions sans émulsifiants

Chemical Engineering Journal (Soumis)

Chapitre III : Etude comparative entre le procédé d'émulsification par ultrasons de haute fréquence et des procédés d'émulsification standards pour des émulsions sans émulsifiants

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L'objectif de cette partie a été de comparer les propriétés physico-chimiques d'émulsions de différents types produites via le procédé d'émulsification par hautes fréquences (UHF) à celle d'émulsions obtenues par des procédés d'émulsification standards tels que l'homogénéisation à haute pression (HHP) et la sonication par ultrasons de basses fréquences (UBF).

Ce travail a consisté à préparer deux types d'émulsions avec et sans principe actif. La granulométrie des émulsions a été mesurée ainsi que la mobilité électrophorétique, la tension de surface et les propriétés rhéologiques. La stabilité des émulsions a été suivie pendant 30 jours à 37°C et l'oxydation des phases hydrophobes des émulsions a été évaluée par spectroscopie infrarouge à transformée de Fourier (FTIR). Les résultats ont montré que la fraction nanométrique est plus importante pour les émulsions faites par les ultrasons de hautes fréquences. Elle représente plus de 90% (v/v) des gouttelettes de l'émulsion UHF et représente entre 50 et 70% (v/v) pour celles faites par HHP et UBF. Une bonne corrélation entre la taille des particules et la viscosité de l'émulsion a été observée par une étude rhéologique. Les émulsions UHF sont stables pendant 30 jours à 37°C, contrairement aux autres émulsions.

Les résultats de l'analyse FTIR ont montré que les huiles des émulsions avaient un même spectre que l'huile d'origine (non émulsionnée) ce qui démontre l'absence de dégradation de ses constituants.

Emulsifier free emulsion: Comparative study between a new high frequency ultrasound and standard emulsification processes

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Abstract

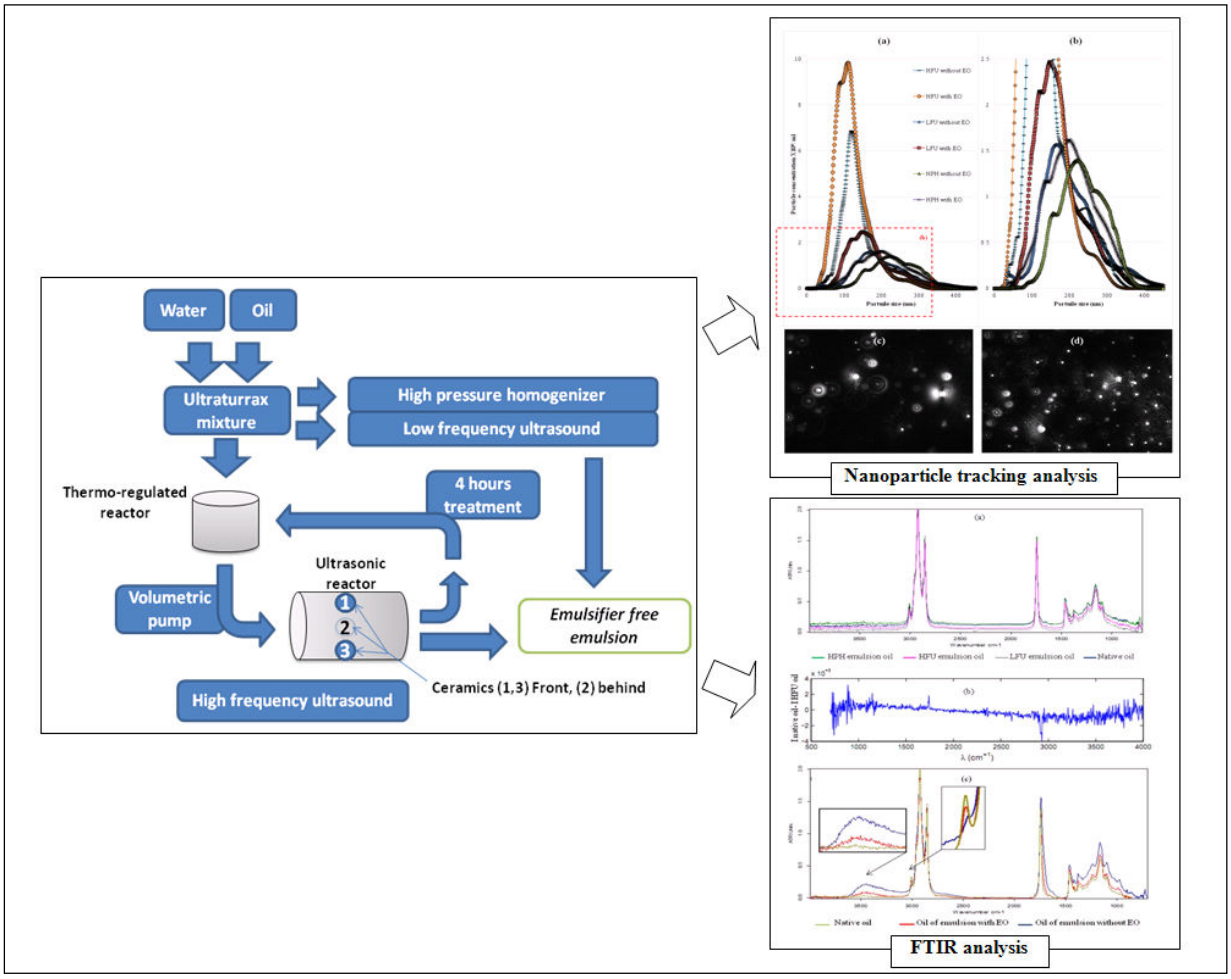
Emulsifier-free-emulsion was developed with a new technique for food applications. This method uses high frequency ultrasounds (HFU) generated by a piezoelectric ceramic transducer vibrating at 1.7 MHz. HFU emulsions were compared with other emulsions made low frequency ultrasounds (LFU) and high pressure homogenization (HPH) used as food standard emulsification.

The effect of essential oil addition on emulsifier-free-emulsions with edible oil was expected. Two emulsion types were prepared. First emulsions group was made using sunflower oil. For second emulsions group, orange essential oil was added for sunflower oil. Oil droplets size was measured by static light diffraction and nanoparticles tracking analysis (NTA) for characterization of micrometric and nanometric droplets fraction, respectively. Electrophoretic mobility, surface tension and rheological properties were measured. Emulsions stability and

oxidation were monitored during 30 days at 37°C. Using size distribution and particles concentration obtained by NTA analysis, nanometrical fraction was calculated and compared with the total emulsified oil ratio. More than 90% (v/v) of emulsion droplets were nanometric for HFU emulsions. Only 60 and 75% (v/v) of the droplets were nanometric for emulsion made with HPH and LFU, respectively, due to emulsion instability and fast coalescence phenomenon. HFU emulsions were stable against coalescence during 30 days at 37°C unlike other emulsions. Oily phases of emulsions analysed by FTIR showed that the processes don't affect the oil composition and orange essential oil decrease edible oil oxidation. The monitoring of surface tension indicates stable values for HFU emulsions.

Keys words: Emulsifier-free-emulsions, ultrasounds emulsification, vectorization, essential oil, nanoparticle tracking, oxidation.

Graphical abstract



Introduction

Emulsions are systems containing two immiscible liquids, one dispersed as droplets (dispersed phase) throughout the other (continuous phase) [1, 2]. They concern a wide range of foods.
Oil-in-water type emulsions are widely used in the food industry because they are vehicles that allow increased retention and stability of the active compounds [3]. Emulsions were defined mainly by the volume ratio of the two liquids, their order of addition and the nature of emulsifier [4]. Generally, emulsions are prepared with surface active agents (surfactant) [5-7] or amphiphilic polymers [7] which decrease interfacial tension. Emulsion stability is due to stabilizer adsorption at oil/water interface creating electrostatic repulsion for emulsions containing surfactants [5] and steric repulsion for emulsions containing polymers [8]. The size distribution of the oil droplets is another important factor in the stabilization of emulsions because larger sizes accelerate destabilization, and the method employed in emulsification can be directly related to this response [3]. Emulsions can be processed by mechanical means such as "colloid mill, homogenizer, mixer" [4, 5] or low frequencies ultrasonic generator [4]. Within the ultrasound range, the power available varies inversely with the frequency and only power ultrasounds (16-100 kHz, and, to a lesser extent, 100 kHz-1 KHz) is able to interact with matter, producing physical and chemical changes, essentially by cavitations phenomena [9]. On the contrary, higher frequency ultrasounds go through solid or liquid media without affecting their structure and are used as non-destructive characterization or diagnosis tools [9]. Only ultrasound frequencies ranging between 20 kHz and 1 MHz produce cavitations and there is a theoretical relationship between frequency and emulsion drop size, as a drop size reduction with increasing frequency [10].
The frequencies ranges employed can be divided into high-frequency (low-energy) diagnostic ultrasounds and low-frequency (high-energy) power ultrasounds. The former is usually used as a nondestructive, rapid, easy-to-automate, and relatively inexpensive analytical technique for quality assurance and process control for composition, structure, and physical state of foods [11]. Homogenization process is another process for emulsification. It has been largely introduced in the food industry and allows production of food emulsions with improved texture, taste, flavor and shelf-life, especially for dairy products like milk, cream and ice cream and also enhanced consumer acceptance of some products [12].
All this emulsification processes can produce the degradation and oxidation of edible oil phases, especially by temperature increase and cavitation phenomenon. In food industry, beverage

emulsions may contain a variety of different hydrophobic components, including flavour oils, essential oils, triacylglycerol oils, oil-soluble vitamins, nutraceuticals, weighting agents, and ripening inhibitors [13]. Lipid oxidation is one of the most important quality deterioration processes in food systems and has strong effects on the development of bad smell, unusual colour and harmful compounds [14]. Chemical reactions can occur within the oil droplets that are induced by water-soluble ionic species, such as oxidation of ω-3 fatty acids by transition metals [13].

To prevent these oxidation phenomena, antioxidant is usually added in oily phase. Essential oils are widely used in food and pharmaceutical industries where they encounter major concerns more likely insolubility and instability [15]. Plant essential oils are interesting natural antimicrobial agents to be incorporated into the edible films due to these plant extracts exhibit additional characteristics, such as antimicrobial and antioxidant effects [16]. Besides these antioxidant properties, orange essential oils are used as natural aromatic compounds and flavors for food products.

The aim of this work was to study the effect of emulsification processes and essential oil addition on physico-chemical properties and oxidation of emulsifier-free-emulsions and the ability of processes to provide stable emulsifier-free-emulsions against physical destabilization phenomena. The role of orange essential oil against edible oil oxidation was expected after one month storage. Different physico-chemical measurements of emulsions were realized in order to understand the effect of manufacturing methods on physico-chemical properties and stability of emulsifier-free-emulsions.

Materials and methods

1. Material

Distilled water and commercial sunflower oil were used as aqueous and oily phases respectively of all prepared emulsions. The sunflower oil was purchased from a local supermarket (Nancy, France). Essential oil (EO) supplied by Laboratoires Mathé (Maxeville, France). All materials were used as purchased without further purification.

2. Method

2.1.Emulsions preparation

To understand the behaviour of colloidal systems and assess the effects and interactions between different emulsions components, six emulsions were prepared with three different methods with and without essential oil. Emulsions without active ingredient contain 95% of aqueous phase and 5% of oily phase (v/v). Emulsions pH was maintained at 7.5 during all emulsification processes by NaOH 1N for avoid acidification of emulsions and incorporate hydroxide ions [17, 18].

"Active" emulsions contained 0.5% orange essential oil (v/v) in the oily phase.

For all emulsions, oily phase was added into aqueous phase and then a premix was made with high-speed homogenization (Rotor-stator) using Ultra-Turrax (T-25 Basic, IKA-Werk, Staufen, Germany) during 5 min at 13500 rpm rotation speed and 20°C.

After premix, three methods were used for emulsion preparation.

1. **High pressure homogenization (HPH):** This first method used a high pressure dynamic homogenizer (EmulsiFlex C3, Avestin). The ultra-turrax emulsion was produced by 6 homogenization cycles at 1500 bars.
2. **Low frequency ultrasound (LFU):** Short sonication times at LFU are known for the generation of highly stable emulsions. After Ultra-turrax process, the emulsion was manufactured at 40 kHz and 40% of full power during 120s to achieve a homogeneous solution [19], with a temperature regulation at 25°C.
3. **High frequency transduction (HFU):** The third method was high-frequency ultrasound emulsification using reactor equipped with three piezoelectric ceramics vibrating under the influence of an electric field [18, 20]. Shortly, vibration frequency of ceramics was 1700 KHz, processing temperature was 25°C. Ultra-turrax emulsion was placed in a thermo-regulated cell and recirculated to ultrasonic reactor using a volumetric pump. The ultrasonic treatment duration was 1 hours (Fig.1).

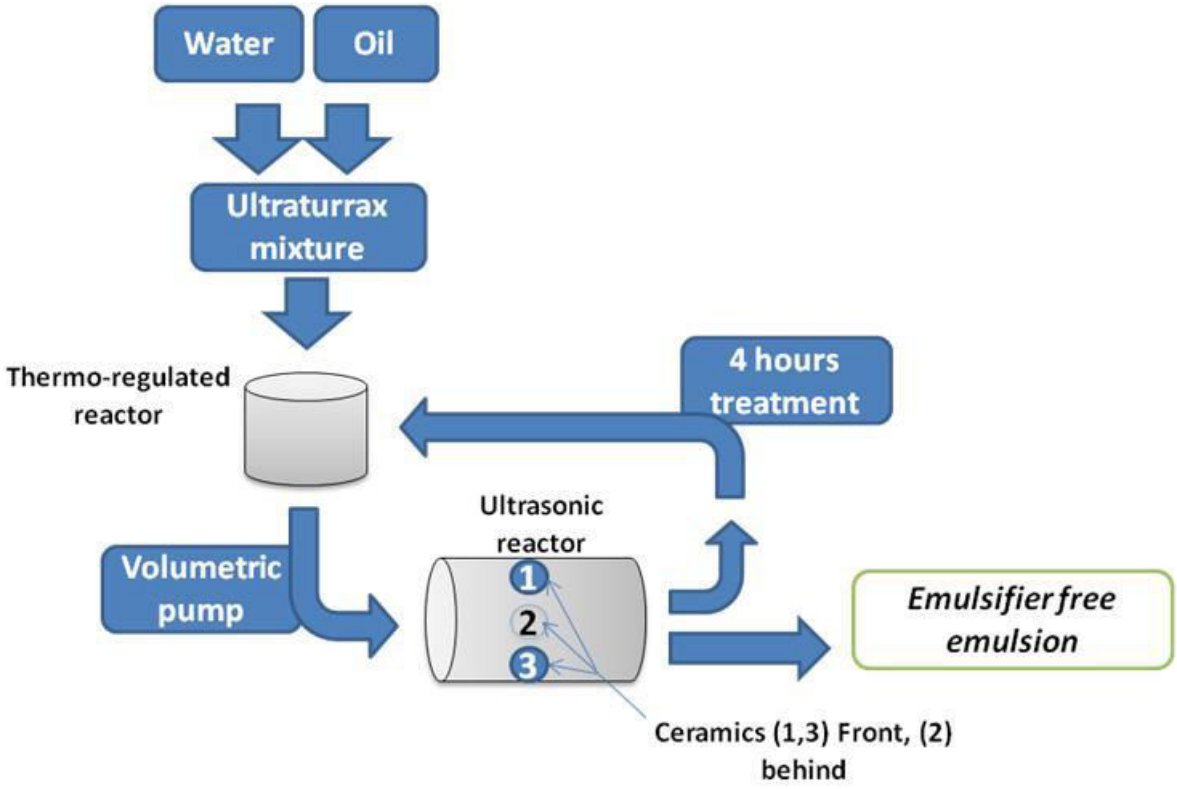


Fig. 1: Emulsification process diagram using high frequency ultrasound, operating frequency was 1.7MHz, temperature was maintained at 20°C during process and the treatment time was 4hours.

2.2. Size measurement

The mean oil globule size distribution was measured using a laser light scattering particle size analyzer (Mastersizer S particle size, Malvern Instruments Ltd, Nancy, France) equipped with a He-Ne laser (360 nm) and a wet sample system. The system was able to detect droplets ranging from 0.05µm to 900 µm. Measurements were carried out in ten replicates. Interval between each measurement was 10 seconds.

The D10, D50 and D90 were measured. D10 diameter is the diameter at which 10% of a sample's mass is comprised of smaller particles, the D50 and D90 is the diameter at which 50% and 90% of a sample's mass is comprised of smaller particles respectively.

2.2. Nanoparticles Tracking Analysis (NTA)

Nanoparticles tracking analysis (NTA) is a nanoparticles visualization technique that provides size, count and concentration measurements. NTA delivers true size distributions, even when the systems are complex and polydisperse, and supports the exclusive numerical data with

corroborating images. The measured diameter can be between 10 and 1000nm, beyond this value (1µm) this equipment cannot measure the size distribution of colloidal suspension.

NTA experiments were performed using a digital microscope LM10 System (NanoSight, Salisbury, UK). Samples were diluted in distilled water and introduced into the cell with a syringe. Video images of particles movement under Brownian motion were analysed by the NTA analytical software version 2.1. The measurements were made at 25°C and each video clip was captured for 60 s. Simples were diluted (1-25000) for measurement. From the number of particles/ml, particle size distribution, simples dilution and oil density, a volume fraction between 1 and 1000 nm was estimated and compared with ratio measured by emulsion lyophilized. Nanometrical fraction was calculated as the following equation:

$$N_f = \sum_{r=0}^{n} \frac{4\pi r^3 * C * D}{3 * 10^{19}}$$

where Nf: oil volume fraction of droplets between 1nm until 1000 nm (% v/v), r: oil droplets radius (nm), C: concentration of nanodroplets per 1 ml of diluted emulsion, D: dilution factor.

2.3. Zêta potential measurements

Zêta potentials were calculated from electrophoretic mobilities determined using the Malvern Nano ZS instrument (Malvern instrument, Worcestershire, UK). Measurements were averaged of 20 runs using dilute dispersions (1-400). Triplicate measurements were done with 10 seconds of equilibration time at 25°C. Particles electrophoretic mobility was converted to the zeta potential using Smoluchowski equation. The average of 10 measurements was taken to represent the measured potential. The applied voltage during the measurements varied in the range of 50–100 mV.

2.4. Emulsification capacity

Emulsions were prepared with 5% (v/v) of oil ratio. Without emulsifier, this 5% was not completely stabilized on emulsion and some residual oil phases was not formed into emulsions and was accumulate at the emulsion surface [17, 18]. The oil volume present in the emulsion was measured from freeze-dried emulsion. 5ml of each emulsion was frozen and lyophilized for obtain emulsified oil. Measurements were carried out on five replicates. Oil was weighted and oil/water ratio was calculated as follows:

$$EC = \frac{EOW}{EW * d} * 100$$

EOW: Emulsified oil weight (g), d: oil density, EW: Emulsion weight (5ml).

2.5. Emulsion stability

Emulsion stability was assessed by measuring the change in droplet sizes during storage at 37°C. The size measurement was made every three days for 1 month.

2.6.Fourier transform infrared (FTIR) spectroscopy

Composition of freeze-dried emulsions, native oil composition and oxidation products were followed by FTIR spectroscopy in total attenuated reflection mode (ATR-FTIR). Measurements were performed at 25°C with a Tensor 27 mid-FTIR Brüker spectrometer (Brüker, Karlsruhe, Germany) equipped with a deuterated triglycine sulphate (DTGS) detector (Brüker, Karlsruhe, Germany). A ZnSe ATR sampling accessory from Spectra Tech (Shelton, CT) was used for Total Attenuated Reflection measurements. The scanning rate was 10kHz and 256 scans were performed both for reference and sample from 4000 to 700 cm^{-1} with 2 cm^{-1} of resolution. The diaphragm was set to 4 mm. All data treatments were carried out using OPUS software (Brüker, Karlsruhe, Germany). Raw absorbance spectra were smoothed using a nine-point Savitsky–Golay smoothing function. Elastic baseline correction was applied to spectra then centered and normalized. Emulsion sample was frozen and lyophilized at different storage days. The measurement was made in two stages: T_0 corresponds to manufacturing day and 30 days (T_{30}) of storage at 37 °C. Results at T_0 were compared with native oil composition to study the effect of emulsification process on oil composition and effect. For measurement, 40 µl oil samples were poured onto the attenuated total reflectance (ATR) ZnSe crystal. After each measurement, the crystal was thoroughly cleaned up with ethanol, washed in water and then dried under nitrogen gas. Four separate experiments were done for each emulsion.

2.7. Surface tension measurement

Surface tension kinetics of each sample (20 mL in 40 mL capped bottles) were measured using a Krüss K100 tensiometer during 500s (Krüss GmbH; Hamburg, Germany). All the measurements were taken in triplicate at 25.0°C ± 0.5 °C.

2.8. Rheological behavior

The rheological behavior of emulsions was determined with Malvern Kinexus rheometer (Malvern Instruments, Worcestershire, UK) using co-axial cylinder sensor at 25°C. Flow curves were obtained after resting the sample in the sensor for 5 min at 25 °C to allow structure recovery and temperature equilibration. The shear stress was measured as a function of shear rate from 0.01 to 100 s^{-1} ; up and down curves was obtained. Complex modulus (G*) and complex viscosity (η^*) were determined as function frequency in the linear viscoelastic region, where rheological properties did not depend on frequency. Each measurement was performed in triplicate.

2.9. Statistical analysis

The statistical analyses (ANOVA) were processed with Excel software (Microsoft corporation version 2007). All tests were executed at 95% significance level to find out if the process and essential oil addition influence significantly the physico-chemical properties of emulsions.

Results and discussion

1. Particle size distribution

Particle size distribution of emulsions was made by static light scattering (SLS) by laser granulometer and dynamic light scattering by Nanoparticles Tracking Analysis (NTA). The SLS showed particle size dispersion in the micrometer range. The NTA measurement showed a nanometric size population (under 1µm). The data provided by the Nanosight enable us to calculate a proportion of particles having micrometrical and nanometrical size.

Figure 2 shows the particle size distribution of vegetable oil emulsions with and without orange essential oil, made using three different emulsification processes that are high-pressure homogenization (HPH), low frequency ultrasounds (LFU) and piezoelectric transduction with high frequency ultrasounds (HFU) made with SLS. Emulsions containing essential oil showed narrower particle size distribution than emulsions without essential oil (Fig.2) and peaks heights were smaller compared to emulsions without essential oil. The largest difference was recorded in the case of LFU emulsification where a distribution was from 0.3µm to 15µm for the emulsion without essential oil (EO), while it was between 0.3 and 12µm when essential oil was added.

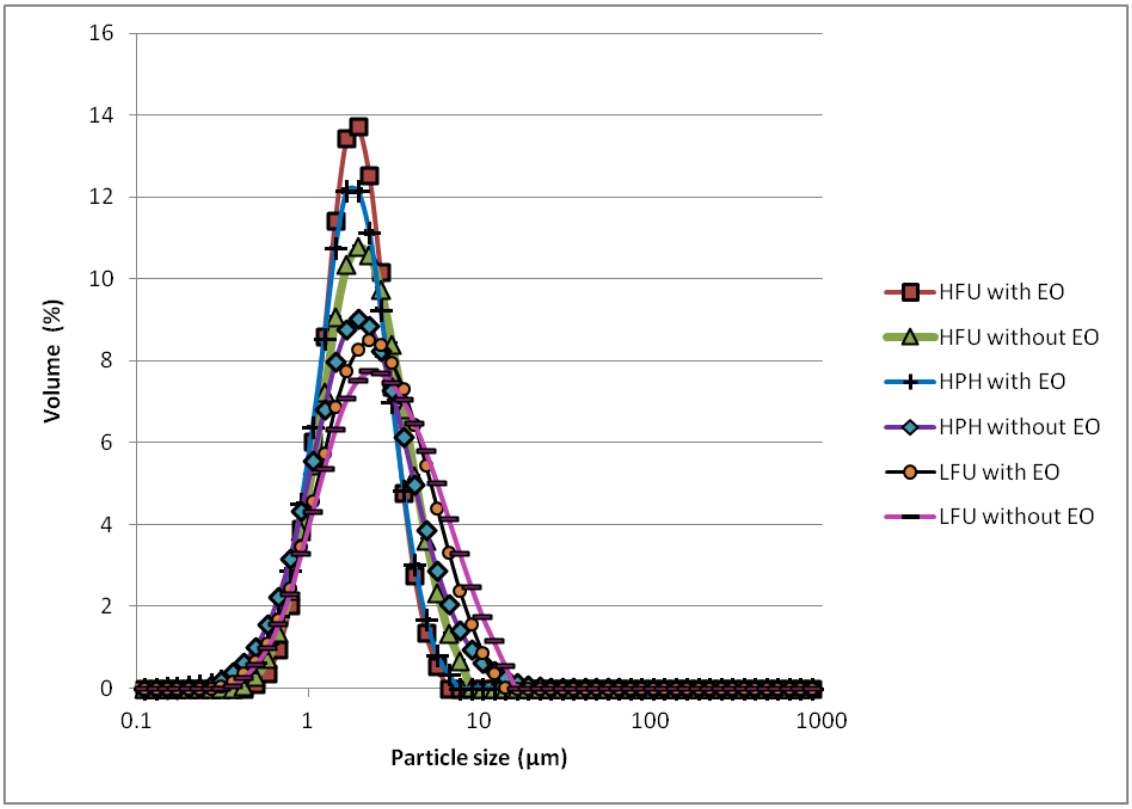


Fig.2: Particle size distribution (SLS) of emulsions made with three different process: high-pressure homogenization (HPH), low-frequency ultrasounds (LFU) and piezoelectric transduction with high frequency ultrasounds (HFU). These methods have been applied to emulsions with sunflower oil, containing or not orange essential oil (EO) without emulsifier.

For a same formulation (with and without EO), the emulsification process showed a variable size distribution. The low frequency ultrasounds emulsification gives an extensive distribution with high size average. The emulsions made by high pressure homogenizer (HPH) and with high-frequency ultrasound (HFU) has a peaks top at 1.95 µm, however, a narrower distribution was recorded of emulsion made with high frequency ultrasounds.

In essential oil emulsion, the peaks top in figure 2 was 14 and 12% of droplet volume (%) for emulsions made with HFU and HPH, respectively. This was also available for emulsions without essential oil where the distribution peaked at 11 and 9% of volume proportion for HFU and HPH, respectively.

LFU emulsification occurred by two mechanisms [4, 21, 22]. First, application of acoustic fields produced interfacial waves that induced interfaces instability, ultimately resulting in emergence of oil droplets of about 70µm in aqueous phase. Secondly, application of LFU caused acoustic cavitation, which was, the formation and subsequent collapse of micro-bubbles by pressure

fluctuations of a simple soundwave. Each bubble collapse (an implosion on a microscopic scale) event causes extreme levels of highly localized turbulence (high pressure and high temperature). Turbulent micro-implosions acted as a very effective method of breaking up primary droplets of dispersed oil into droplets of micro and sub-micron sizes. The LFU duration applied for emulsion manufacturing was 2 min to prevent oil degradation. According to Chemat et al., 2004, the sonication at low frequencies 20 kHz and 47 kHz induced flavor and composition deterioration in sunflower oil (degradations caused by high temperature and pressure at cavitation regions)[23]. The short times of LFU applications prevent a long times of cavitation and allow formation of big oil droplet (>1µm).

The HPH process did not allow mixing of two separated immiscible phases and did not incorporate the oil which not premixed by Ultra-turrax. This emulsification process (HPH) enables splitting droplet already existing and give a smaller droplets due to the transition of coarse emulsion through the hole with small diameter using high pressure. According Canselier et al, 2002 and Walstra 1983, the size of the emulsions can vary depending on the emulsification process used. D (4.3) obtained when emulsions was made with an Ultra-turrax were bigger than those obtained by LFU, and emulsions made by HPH has a smallest particle diameter compared to Ultra-turrax and LFU process [9].

2. Nano-particle size distribution, surface tension and emulsified ratio:

Table 1 shows the particle size average, D10, D50, D90, particles number per ml of dilution, nanoemulsion fraction calculated from data analysis and measured ratio of emulsions with and without essential oil, made using three different emulsification processes (HPH, LFU, HFU). Fig. 3a shows the particle size distribution of the same emulsion made with nanoparticles tracking analysis (NTA).

For all emulsification process, the emulsions containing the essential oil showed significant difference on particle size average compared to emulsions without essential oil (Table 1). The particle size distribution was wider without essential oil and conversely the particles size average was lower when essential oil was added.

Measurements of surface tension showed lower values for the essential oil compared to vegetable oil. This difference could explain the lower particle size with essential oil, because when surface tension of dispersed phases is low, the energy required to split oil droplets is reduced.

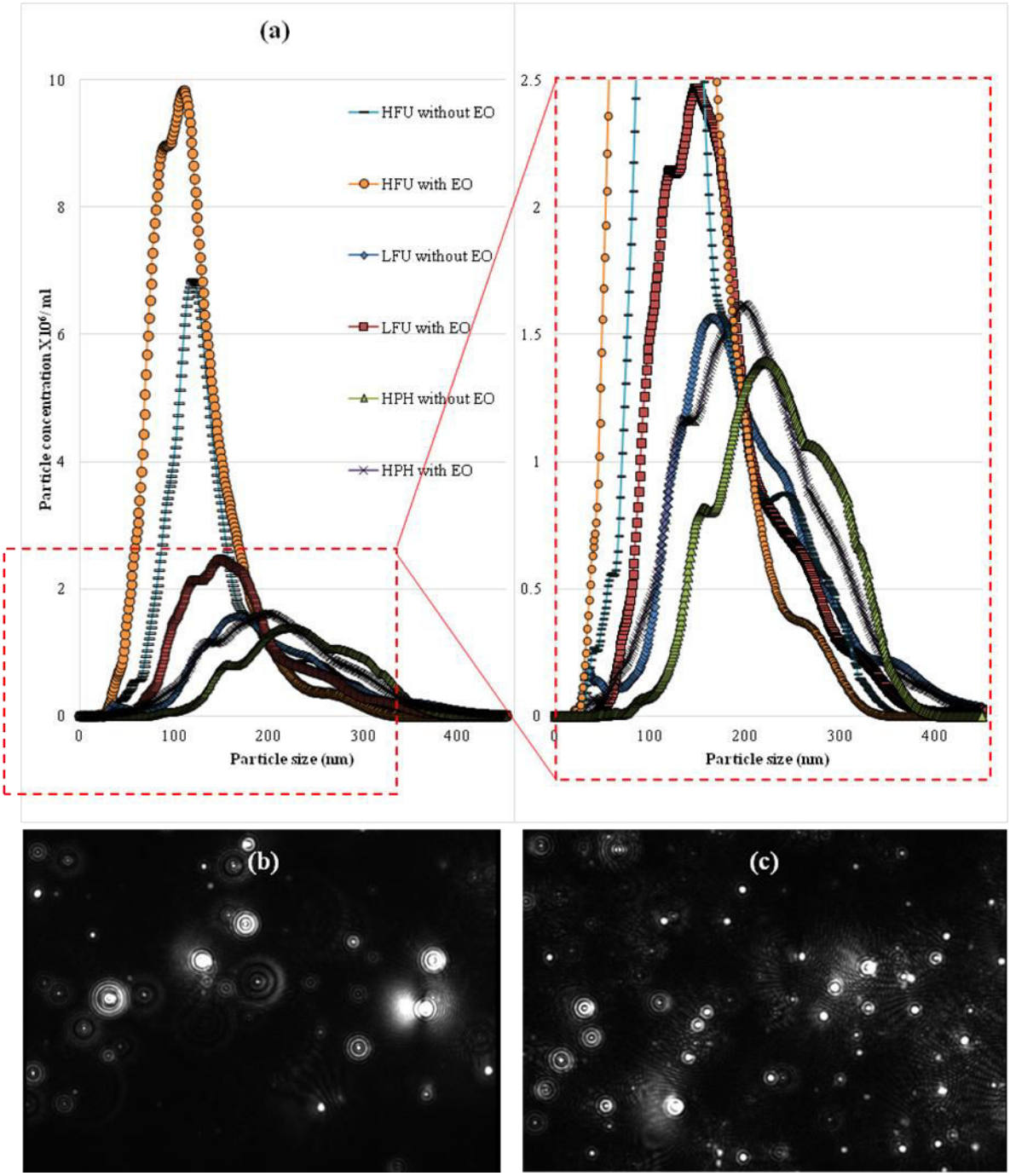


Fig. 3: (a) Particle size distribution of emulsions nanoparticles fraction made with three different processes: high-pressure homogenization (HPH), low-frequency ultrasounds (LFU) and piezoelectric transduction with high frequency ultrasounds (HFU). These methods have been applied to emulsions with sunflower oil, containing or not orange essential oil in formulation without emulsifier (b,c) the light halos produced by nanoparticle of HPH and HFU emulsion with EO respectively.

Table 1: Particles charge, surface tension, average, D10, D50, D90, particle concentration, calculated and measured oil ratio for emulsifier free emulsion obtained with different emulsification process high-pressure homogenization (HPH), sonication with low-frequency ultrasounds (LFU) and piezoelectric transduction with high frequency ultrasounds (HFU). These methods have been applied to emulsions with sunflower oil, containing or not orange essential oil in our formulation and without emulsifier. Measurement was made on diluted emulsions (1-25000). Nanometric droplets fraction was reported on measured Ratio (By lyophilization) for obtain the nanometric proportio

Emulsion	Surface tension (mN/m)	Zêta potential (mV)	Average (nm)	D10 (nm)	D50 (nm)	D90 (nm)	Particle concentration (Particle E+8/ml)	Calculated Nanometric droplets fraction (%)(v/v)	Measured Ratio (By lyophilization) (%)	Nanometric proportion (%)(v/v)
water	71.7 ± 0.1	-	-	-	-	-	-	-	-	-
Sunflower oil	33.4 ± 0.2	-	-	-	-	-	-	-	-	-
Orange essential oil	28.1 ± 0.1	-	-	-	-	-	-	-	-	-
HPH without EO	56.9±0.1^{a}	-46.1± 0.3^{a}	230±5^{a}	175±8^{a}	227±4^{a}	313±10^{a}	2.18±0.13^{a}	3.45 ±0.4^{a}	4.6±0.4^{a}	75.0
HPH with EO	52.0±0.8^{b}	-41.9 ± 0.8^{b}	218±5^{b}	144±4^{b}	202±2^{b}	265±12^{b}	2.76±0.04^{b}	3.54± 0.2^{ab}	4.7±0.2^{a}	75.3
LFU without EO	54.4 ± 0.2 ^{c}	-52.9 ± 0.5^{c}	207±13^{b}	118±3^{c}	175±5^{c}	255±11^{c}	2.48±0.11^{c}	2.86 ± 0.2^{ac}	4.1±0.2^{b}	69.7
LFU with EO	52.0 ± 1.1 ^{b}	-48.9 ± 0.4^{d}	182±8^{c}	103±5^{d}	157±6^{d}	210±7^{d}	3.20±0.08^{d}	2.53 ± 0.3^{cd}	3.9±0.1^{b}	64.9
HFU without EO	56.9 ± 0.1^{a}	-50.3±0.6^{e}	154±7^{d}	97±5^{e}	149±12^{d}	175±5^{e}	5.34±0.19^{e}	2.53 ± 0.2^{cf}	2.8±0.1^{c}	90.4
HFU with EO	53.3 ± 0.9 ^{b}	-47.9±0.3^{f}	125±4^{e}	72±2^{f}	111±4^{e}	145±7^{f}	8.76±0.24^{f}	2,31 ± 0.4^{df}	2.5±0.3^{c}	92.4

a-f: Different superscripts within the same column indicate significant differences between formulations and process containing or not orange essential oil ($p < 0.05$).

At nanoscale, emulsions made by low or high frequency ultrasounds show a narrower distribution than emulsions made with HPH and the average size distribution was lower for LFU and HFU.

For a given formulation (with or without EO), the emulsions showed a different size distribution due to the different emulsification process. The LFU and HPH emulsification give an average upper than 180nm. The HFU emulsions had an average smaller than 160nm, and a narrower distribution was recorded. D50 showed on the table 1 indicate that emulsion made with HPH and LFU had 50% of population with diameter under 250nm and the D90 indicate that 90% of emulsion droplets had a diameter under 350nm. Using HFU the D50 and D90 was lower than 250nm and 400nm, respectively.

The Fig.3b-c shows the light halos produced by nanoparticles of HPH and HFU emulsion with EO respectively. The observations of nanoparticles figures confirm the results in Table 1. Nanoparticles on HPH emulsion seems bigger and less then HFU emulsion.

The emulsification process using high pressure allowed having mono-dispersed particle sizes below 1 µm. On LFU emulsion, violent cavitation phenomena allowed nanometer size droplets especially for oil droplets in the vicinity of the ultrasounds probe.

When high frequency ultrasounds waves were used, the emulsion had smaller average size in spite of the absence of violent cavitation phenomena. According to Juliano et al. (2011), applying ultrasounds stationary wave at high frequency on milk samples containing fat, induced creaming of a milk fat fraction. The analysis of fat droplets size present in milk showed that only particles having a diameter lower than 1µm remained in suspension, other oil particles upper than 1µm were destabilized, coalesced and were found on the walls of the vessel [24]. Emulsions were prepared with 5% of oil ratio (v/v). This 5% was not completely dispersed on emulsion. Lyophilization was used for measurement of emulsified oil ratio. HFU caused coalescence of oil droplets having a higher 1µm [24], this lead to a decrease of emulsified oil ratio comparing to HPH and LFU emulsion ratio (Table 1). From the number of particles/ml, particle size distribution, simples dilution and oil density nanometer fraction was estimated and compared with ratio measured by emulsion lyophilized (table 1). For HFU emulsion, the results showed that calculated proportions represent more than 90% (v/v) of the total emulsified oil measured by emulsions lyophilisation, and between 60 to 75% (v/v) for LFU and HPH. The remaining proportion is in the upper part and 1µm which was showed in Fig.2. These results indicated that the majority of oil droplets in the HFU emulsion had a size lower than 1µm which

indicates the presence of nano-emulsion according to the literature [25] that are thermodynamically more stable than micro-emulsions.

3. Particle charge (zêta potential)

Particle charge (zêta potential) of sunflower oil emulsion containing or not essential oil and manufactured with different emulsification process (HPH, LFU and HFU) is shown in the table 1. The electrical charge on the oil droplets determines the stability of the droplets to aggregation due to its influence of the magnitude, range and sign of electrostatic interactions and it determines the interactions of droplets with other charged species in an emulsion e.g., ions (such as calcium or iron)[13]. The study of particles surface charge was very important because it affected the stability of emulsions, especially in the studied emulsion without emulsifier. Emulsifier free emulsions prepared with all emulsification process (HPH, LFU and HFU) exhibited negative charge at droplets surface. The zêta potential of emulsions was between -39 and -49 mV. In general, higher absolute zêta potential values (above ± 30 mV) indicate a good stability against coalescence of dispersed droplets [26, 27]. Emulsions negative charge is possibly imparted by free fatty acids present in oil phase [27, 28]. Vegetable oil was hydrophobic, coverage of oil droplets surface with hydroxide ions occurred in aqueous media due to hydrophobic interactions between oil and water. So, it can be speculated that oil droplets accumulates more charges on the surface and formed thicker layer [17]. Globally, surface charge is negative and electrostatic interactions between oil droplets are repulsive. The emulsion pH was regulated By NaOH addition. This pH decrease was due to the arrangement of hydroxide ions between water molecules and hydrophobic fatty acids [17, 18].

The results showed that there was a significant difference between emulsions with and without EO. Orange essential oil incorporation slightly decreased the absolute value of particle charge which agreed with particle size changes. Addition of essential oil decreased droplets charge because droplets were smaller. The essential oil incorporation reduced surface tension of oily phases which induce to a greater exchange surface. The increased exchange surface between oil and water lead to more incorporation of OH-ions by NaOH addition to obtain the same zêta potential.

4. Surface tension

As expected, the surface tension of emulsions solutions was lower in comparison with pure water whose surface tension is 72 mN m^{-1} [29]. The emulsion "water, sunflower oil and essential oil" surface tension was monitored during 500 seconds at 25°C (Fig.4a).

Differences between surface tension values with and without EO were found. For vegetable and essential oils, surface tension data showed lower values than water (table 1). Essential oil addition decreased significantly the emulsion surface tension, this diminution was probably due to lower surface tension of essential oil. For HFU emulsions, values show the ability of surface charges to form stable emulsions without emulsifier.

LFU and HPH emulsions showed an important decrease of surface tension values during monitoring that could be explained by the creaming process. On the contrary, HFU emulsion had a stable surface tension. The surface tension stability of colloidal systems during the monitoring indicated the absence of destabilization phenomena (especially creaming phenomenon). The creaming results from migration of the dispersed phase to the top because dispersed phases density is lower than continuous phase density. Creaming phenomena induced a progressive accumulation of oil particle on the sample surface that induces the surface tension diminution (Fig.4a).

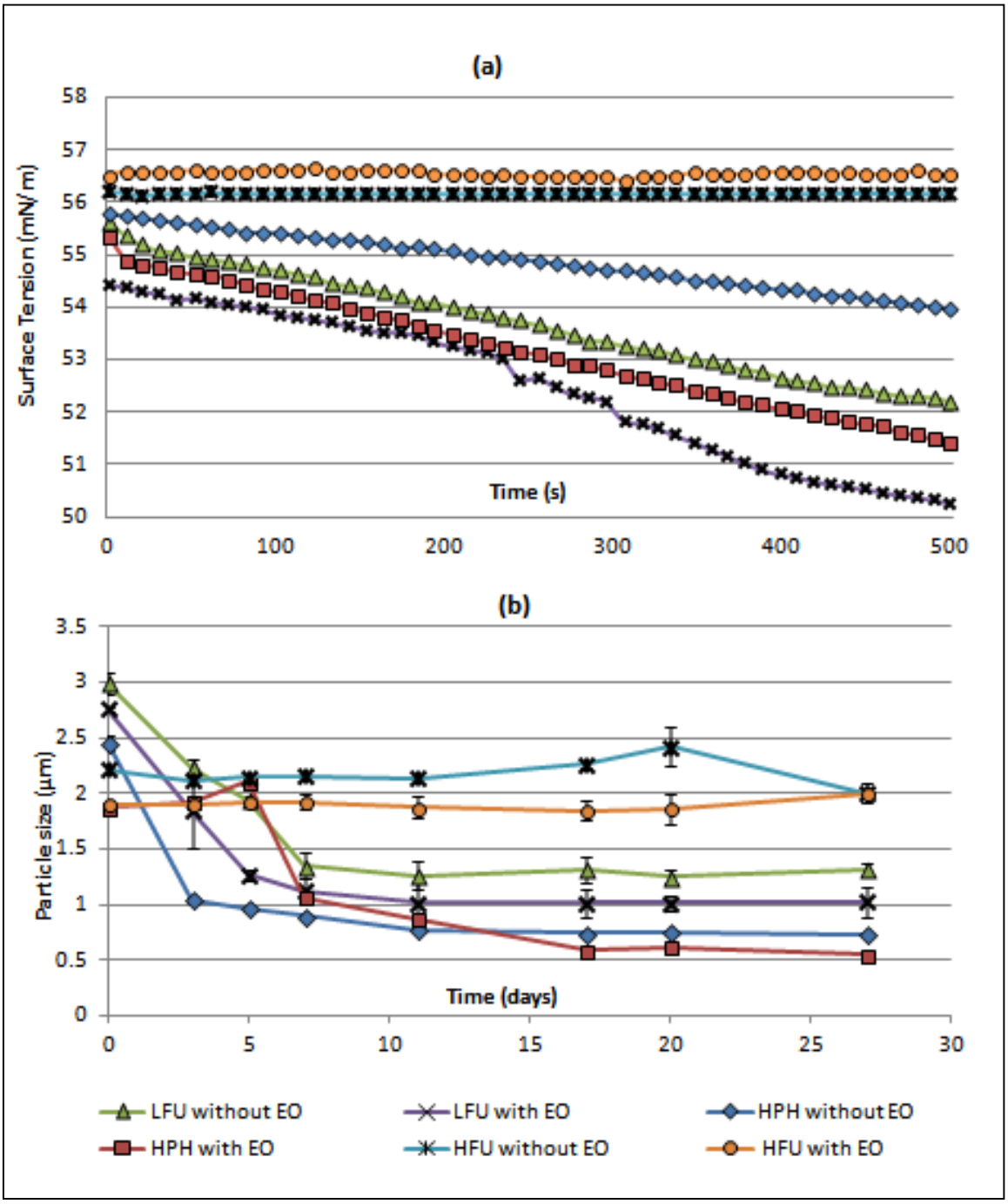


Fig. 4: (a) Surface tension monitoring of emulsions made with three different processes: high-pressure homogenization (HPH), low-frequency ultrasounds (LFU) and piezoelectric transduction with high frequency ultrasounds (HFU). These methods have been applied to emulsions with sunflower oil, containing or not orange essential oil without any emulsifier. (b) Droplets average diameter monitoring of emulsions made with three different processes: high-pressure homogenization (HPH), low-frequency ultrasounds (LFU) and piezoelectric transduction with high frequency ultrasounds (HFU).These methods have been applied to emulsions with sunflower oil, containing or not orange essential oil without emulsifier at 37°C for 1 month.

5. Emulsion stability

The average droplets size monitoring at 37°C for 1 month is shown in Fig.4b. For HPH and LFU emulsions, a significant size decrease ($p < 0.05$) was recorded from 0 to 16 days. The same observation was made for "HPH without EO" emulsion. This size reduction occurs with emulsion phases separation and apparition of oil at the surface of emulsion. This dephasing began to appear few hours after emulsification. In this size range (>1µm) and when emulsifier was not added, emulsions were not stable and droplets tended to coalesce. In this case, a phase separation was observed in the next few hours after emulsification. Phase separation is due to coalescence of big droplets inducing the formation of oil at the emulsion surface. The big droplets represented in Fig.4b disappear from emulsions creating oil at the surface (phases separation). oil at the surface was removed before measurement and size measurements were made for stable fraction of emulsions. This stable fraction contains only the smaller stable droplets. The relatively short LFU time (2 min) was not sufficient for reduce of size for all oil droplet in emulsions. Part of droplets had particle sizes bigger than 1µm not able to resist to thermodynamic forces which lead to coalescence phenomena and phases separation. Therefore for LFU emulsions, electrostatic stabilization was insufficient for avoid destabilization emulsions. Regarding HPH emulsion, a size decreasing was registered until day 16 due to a coalescence of big droplets. Droplets formed by high pressure homogenizer were smaller than LFU. During HPH, whole emulsion volume passes through a low-diameter hole under high pressure that provides low particle size and narrow distribution. These droplets need more time for coalescence phenomenon begin because it was thermodynamically more stable than LFU particle emulsion. It can suggest a slow coalescence phenomena inducing emulsion size increasing due to droplets merging during several days.

Concerning LFU emulsions, destabilization phenomenon occurs on the 7 first storage days, when big unstable droplets coalesced and formed oily phase at the surface. These coalescence phenomena occurred slowly until the droplets solution remaining had a size (small) and charge (high) necessary to obtain a colloidal suspension which will be not affected by the density difference of the two phases. This will present colloidal suspension with sufficient charge to keep droplets away from another. This phase separation was caused by a quick coalescence facilitated by emulsifier absence at oil / water interface. Oil droplets coalescence gave rise to bigger thermodynamically unstable droplets that ascends to the surface (phase separation). Size decrease (Fig.4b) was due to the coalescence and separation phases of big droplets initially

present on emulsions. Big droplets separates from the emulsion leaving only the smaller droplets and finally inducing size decrease for LFU and HPH emulsions.

HFU emulsions showed a stable particle size average during storage. High frequency ultrasound appeared very effective in reducing the droplet size of emulsions with or without orange essential oil and provide more stable emulsifier free emulsion [18] than LFU and HPH. The stability of HFU emulsion was partly due to the fact that 90% (v/v) of emulsified droplets had a nanometrical size, the nanometric fraction is between 60 to 75% (v/v) for LFU and HPH. Micrometrical fraction is more unstable than nanometrical, which explain the better stability of HFU emulsion. The high particles charge also participates to emulsion stability (Table 1). High frequency ultrasounds induced the formation of double layer (stern layer) structures surrounding droplets which provided a high stability against coalescence phenomena [18].

6. FTIR spectroscopy analysis

FT-IR spectroscopy is a rapid and non-destructive technique with minimum samples preparation [30, 31]. Fig.5a shows infrared spectra of native and emulsified oil of the three tested processes (HPH, LFU and HFU) directly after emulsification (t_0). The emulsified oil was extracted by freeze-drying of emulsion. FT-IR spectra are identical for the emulsified oils (HPH, LFU and HFU) and native oil. Oils were not degraded during emulsification. The subtraction of the values of the HFU oil curve from native oil curve value shows very low difference (near from zero) (Fig.5b). Oxidation of stable emulsions (HFU emulsions with and without EO) was measured 30 days after manufacturing and storage at 37°C (Fig.5c). This figure shows the effect of essential orange oil on the prevention against edible oil oxidation during storage in favoring conditions of oxidation (37°C). Oxidation was monitored by the growth of hydroxyl region absorption band (H_2O, ROH and ROOH), between 3100 and 3600 cm^{-1} [32], and the decreasing of the band around 3006 cm^{-1} assigned to the C–H stretching vibration of the *cis*-double bonds [31] (Fig.5c). Oxidations phenomenon was registered in the both emulsions with or without EO. The stretching vibrations were observed between 3100 and 3700 cm^{-1}, due to the accumulation of OH from moisture, hydroperoxides (ROOH) and their breakdown products alcohols (ROH) [33].

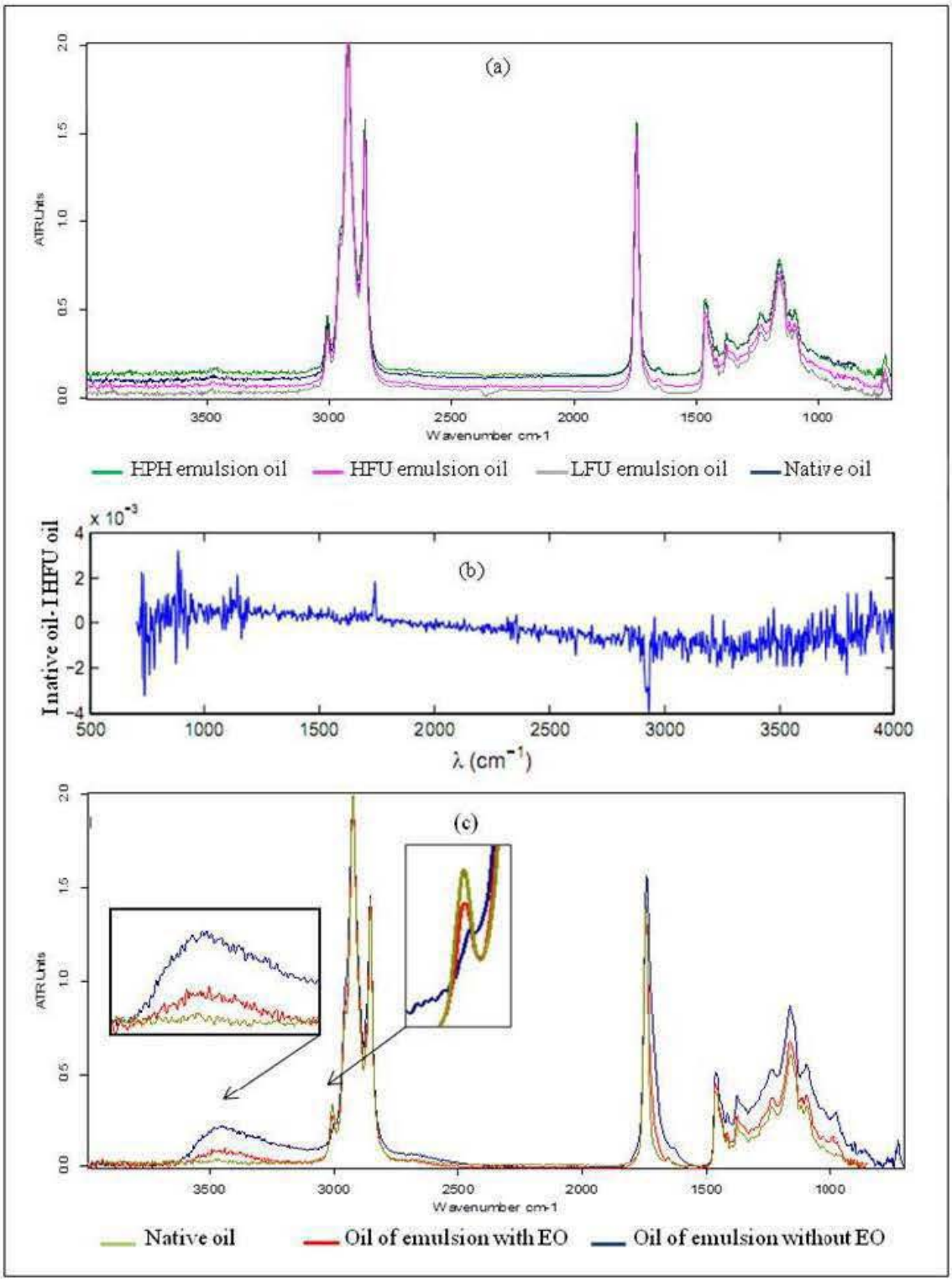


Fig. 5: (a) FTIR spectra at 0 days for native oil and emulsion oil made with three different processes: high-pressure homogenization (HPH), low-frequency ultrasounds (LFU) and piezoelectric transduction with high frequency ultrasounds (HFU).These methods have been applied to emulsions with sunflower oil and without emulsifier. (b) Difference between FTIR spectra of the native oil and HFU oil. (c) FTIR spectra of oil emulsions made with high frequency ultrasound with and without EO at 1 month storage at 37°C.

Here, the C–H stretching vibration of the *cis*-double bond (=C) varies from 3009 to 3006 cm^{-1} according to Vlachos et al. [34]. The frequency of the band in this study appeared approximately at 3010 cm^{-1} [35]. These oxidations are lower when EO was added; this is due to antioxidant properties of orange essential oil (Peng & Li, 2014).

7. Rheological behavior:

Measurements of viscosity (η^*) and complex modulus (G*) at fixed shear rate, shear stress and 1Hz frequency are plotted for all samples in Table 2. Study of emulsion rheological behavior is of interest to have information about fluid structure and particles interactions. Rheological properties of emulsifier free emulsions showed a shear Newtonian behavior.

HFU emulsion had a higher viscosity than LFU and HPH. This can be due with a smaller size of oil droplets on LFU emulsions. According to pal (1996), the droplet size has a strong influence on emulsion rheology. Fine emulsions (water-in-oil or oil-in-water) have much higher viscosities and storage moduli than the corresponding coarse emulsions [36].

Table 2: Rheological characteristics of emulsifier free emulsions made with three different processes: high-pressure homogenization (HPH), low-frequency ultrasounds (LFU) and piezoelectric transduction with high frequency ultrasounds (HFU).These methods have been applied to emulsions with sunflower oil, containing or not orange essential without emulsifier.

Sample Description	Shear modulus (complex component)(Pa)	Shear modulus (elastic component)(Pa)	Shear modulus (viscous component)(Pa)	Shear viscosity (complex component)(Pa s)
HPH without EO	5.848	0.802	5.792	0.931
HPH with EO	6.073	0.602	6.043	0.967
LFU without EO	3.859	3.851	0.254	0.154
LFU with EO	3.671	3.862	0.263	0.156
HFU without EO	7.366	1.280	7.254	1.172
HFU with EO	6.267	1.094	6.171	0.997

Conclusion

Emulsifier free emulsions are very difficult to stabilize. It can easily destabilize by different way: Creaming, sedimentation, coalescence, Oswald ripening ... the following study allows us to have stable emulsion with and without active agent and without emulsifier addition by using an emulsification process based on the high frequency ultrasound as emulsification process.

Emulsions done by high pressure homogenizer and low frequency ultrasounds showed coalescence and phase separation phenomenon due to the absence of emulsifier. The study of particle size by static light scattering (SLS) and Nanoparticles tracking analysis (NTA) revealed the presence of two types of population-wide micro and nano-emulsions. However, the nanometrical fraction represents a higher proportion of HFU emulsions (> 90% v/v) which explain the very stable emulsion during storage at 37°C. The FTIR analysis showed that the processes don't affect the oil composition and orange essential oil decrease edible oil oxidation. A strong correlation between emulsion viscosity and particle size was observed. Fine emulsions have much higher viscosities and storage moduli than the corresponding coarse emulsions.

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Chapitre IV

Etude de l'efficacité des émulsions sans émulsifiant et des émulsions contenant de la lécithine de colza utilisées comme système de vectorisation et de libération de Coenzyme Q10: propriétés physico-chimiques et application aux cellules humaines

Partie soumise à Journal of Nanobiotechnology

Chapitre IV : Etude de l'efficacité des émulsions sans émulsifiant et des émulsions contenant de la lécithine de colza utilisées comme système de vectorisation et de libération de Coenzyme Q10: propriétés physico-chimiques et application aux cellules humaines

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Dans cette partie, un exemple d'application des émulsions sans émulsifiants dans le domaine cosmétique menée dans la troisième partie de ce travail une stabilité physicochimique lors de leur utilisation comme vecteur de coenzyme Q_{10}.

Lors de leur utilisation en cosmétologie, la liste des ingrédients de formulation est réduite, ce qui limite l'apparition d'allergies ou autres désagréments. Une étude d'encapsulation d'un principe actif «coenzyme Q10» a été réalisée avec des émulsions contenant un tensioactif (lécithine) et les émulsions sans tensioactif faites par le procédé d'émulsification par hautes fréquences. Sans émulsifiants, lors de l'utilisation du principe actif «coenzyme Q10», nous obtenons des résultats satisfaisants permettant de garantir des nanoémulsions stables, ayant un profil rhéologique attendu et ne présentant aucune forme de toxicité démontrée à l'aide des tests in-vitro sur cellules humaines. Ces derniers tests ont pu mettre en évidence la non toxicité du vecteur (émulsion sans principe actif) et en plus l'efficacité du principe actif. Le coenzyme Q10 en l'absence de l'émulsifiant a permis une meilleur prolifération cellulaire et une augmentation de l'activité métabolique qui sont très recherchées lors de l'élaboration d'une crème cosmétique ayant un effet anti-âge.

Efficiency of emulsifier free emulsions and emulsions containing rapeseed lecithin as delivery system used for vectorization and release of coenzyme Q_{10}: physico-chemical properties and *in vitro* evaluation

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ABSTRACT

To improve vectorization and release of coenzyme Q_{10} (CoQ_{10}), nano-emulsions composed of Miglyol oil and CoQ_{10}, were developed as a cosmetic matrix, with or without rapeseed lecithin as emulsifier. Emulsifier free emulsions developed with a new emulsification process by high frequency ultrasound (HFU) at 1.7 MHz for CoQ_{10} vectorization. This HFU emulsions was compared with CoQ_{10} emulsion containing emulsifier manufactured with the same emulsification technique and also with another emulsification process: low frequency ultrasounds coupled with high pressure homogenization (LFU+HPH) emulsification. Emulsions physico-chemical properties were determined by measurement of average droplets size with nano-droplets tracking analysis, droplets surface charge with ζ potential measurement, surface tension and rheological behaviour. Emulsions made by LFU+HPH with emulsifier showed lower droplets size due to cavitation generated by HFU process. The surface tension results showed that there was no significant difference between emulsions containing lecithin emulsifier whatever the preparation process, with or without CoQ_{10}. *In vitro* biocompatibility

tests were performed on human mesenchymal stem cells to assess potential emulsions toxicity. This study showed successful results about stability and efficiency of CoQ_{10} vectorization in cosmetics matrix. *In vitro* tests proved that the vectors were harmless for cells. Furthermore, CoQ_{10} provided high rate of cell proliferation and metabolic activity especially when no emulsifier was added (with HFU emulsification). These results showed that CoQ_{10} effect was directly linked to the matrix in which it was incorporated.

Keywords: nano-emulsions, emulsifier free nano-emulsions, ultrasounds emulsification, coenzyme Q_{10}, vectorization and release, bioavailability.

INTRODUCTION

Many foods, cosmetics and pharmaceuticals industry need to deliver lipophilic functional components in hydrophilic matrix. In many cases, it is advantageous to deliver bioactive lipids in an aqueous medium because this increases their palatability, desirability, and bioactivity. Nevertheless, there are often technical challenges that need to be overcome before a lipophilic component can be successfully incorporated within an aqueous-based delivery system [1]. Oil-in-water (O/W) emulsions are currently among the most widely used methods of encapsulating bioactive lipids, but they are often susceptible to breakdown over time or when exposed to certain environmental stresses [1, 2].

Nano-emulsions have the ability to greatly increase the bioavailability of highly lipophilic substances encapsulated within them [3] and have many advantages on active agent vectorization and drug delivery. Their properties and stability depend on various factors such as rheological properties, droplets size and charge and interfacial tension. Emulsions are easily manufactured by mechanical means such as "rotor-stator systems, homogenizer" [4, 5] or low frequencies ultrasonic generator [5]. Besides efficient droplet disruption, stabilization of the newly formed interface is of great interest. If a poorly stabilizing emulsifier is used, coalescence of droplets immediately after droplet disruption may partially revert the result of size reduction [6].

Emulsions are generally prepared with surfactants for reducing of interfacial tension and permit stability over time due to stabilizers adsorption at oil/water interface creating electrostatic

or/and stearic repulsions for emulsions containing active agents [5, 7, 8]. Surfactants interact with the other ingredients and bring new properties to emulsions. Their quantity depends on the required efficiency and stability of the final product. Emulsifiers stabilize interfaces and help avoiding coalescence. They can influence the result in both steps of the emulsification process. It is possible that surface active agents facilitate droplet deformation and disruption because they have a lower interfacial tension [6]. However, surfactants would introduce complexities for understanding the roles of oil characteristics on emulsion properties because of their interactions with oil and water, and their incorporation into oil and water [8]. Oil-in-water emulsions are the most commonly used for coenzyme Q_{10} (CoQ_{10}) vectorization. CoQ_{10} also known as ubiquinone or ubidecarenone [9] is a fat-soluble component which plays a fundamental role in generating cellular energy through the mitochondrial respiratory chain and is also a major antioxidant in the organism [2]. However, due to its insignificant solubility, CoQ_{10} has poor bioavailability and poor delivery properties, and it is better absorbed in the presence of lipid [10]. According to McClement *et al.* (2007), there are a number of characteristics that an edible delivery system must have if it is going to be suitable for utilization by the food and other industries such as: protection against chemical degradation, releases of the bioactive lipid at a particular site-of-action and bioavailability/bioactivity [1]. A new patented methods for emulsifier-free-emulsions manufacturing was developed [11, 12] using high frequency ultrasounds (HFU) and without any emulsifier. The HFU used to produce acoustic emulsion showed a decrease in suspended oil droplets size formed due to deformation and breakage of oil droplet during propagation of acoustic wave. The pH decreases significantly and a negative surface charge was recorded, which showed the formation of hydroxide layer by adsorption of OH^- at oil/water interface leading to droplets stability on emulsion [12].

The aim of this work was first to study the systematic preparation and characterization of emulsifier free nano-emulsions and nano-emulsions with natural emulsifier (rapeseed lecithin). Secondary, effects of CoQ_{10} encapsulation on the physico-chemical properties of the emulsion such as droplets size, and physico-chemical stability were studied to find an optimal formulation and determine *in vitro* the effect of emulsifier and preparation methods on human mesenchymal stem cells behaviour.

Materials and Methods

1. Materials

Coenzyme Q_{10} (purity 98%) was provided by Sigma Aldrich (Lyon, France). Rapeseed lecithin was acquired from Solae Europe SA society (Geneva-Switzerland). Distilled water and Caprylic/Capric Triglyceride (Miglyol 812, SASOL, Paris, France) [13] were used as the aqueous and oily phases, respectively, for all emulsions. All materials were used as received, without further purification.

2. Emulsion preparation

Six emulsions were prepared with two different methods with and without coenzyme Q_{10} (active ingredient). Emulsions without active agent contained 90% of aqueous phase and 10% of oily phase (w/w). Oily phase was made up of Miglyol (100%) for emulsifier free emulsion, 76.6% and 23.33% of Miglyol and rapeseed lecithin respectively for emulsion with emulsifier. 1,64% of CoQ_{10} was added to emulsion as active agent [10]. For emulsion containing lecithin (LEC), rapeseed lecithin was added to oily phase by vortexing at 50°C and then added to water phase before emulsification process. Emulsions pH was maintained at 7.5 during all emulsification processes by NaOH 1N to avoid acidification of emulsions and incorporate hydroxide ions [12, 14].

- **Low frequency ultrasound (LFU) and high pressure homogenization (HPH):** the phases were sonicated at 40kHz and 40% of full power during 120s (1s "on" and 1s "off") to achieve a homogeneous solution [15], with a temperature regulation at 25°C. High pressure homogenization was used to obtain a narrow size distribution using a high pressure dynamic homogenizer (EmulsiFlex C3, Avestin). The emulsion was produced by 5 homogenization cycles at 1500 bars at 20°C.
- **Rotor-stator with Ultra-Turrax® (UT) and transduction with high frequency ultrasound (HFU):** these methods consist to apply high-frequency ultrasounds using ultrasonic reactor equipped with three piezoelectric ceramics vibrating under the influence of an electric field. The ceramics characteristics and emulsification process were completely described on previous papers [11, 12]. Shortly, vibration frequency of ceramics was 1700 KHz, processing temperature was 30°C.

The oil phase was added into aqueous phase and then a pre-emulsion was made with high-speed homogenization using Ultra-Turrax® (T-25 Basic, IKA-Werk, Staufen, Germany) during 5 min at 13500 rpm rotation speed at 20°C. After this premix process, the emulsion was placed in a thermo-regulated cell and recirculated to ultrasonic reactor using a volumetric pump. The ultrasonic treatment duration was 1 hours.

Different emulsions using high and low frequency ultrasounds were studied:

1. LFU-HPH-LEC emulsion: emulsion made with low frequencies process and high pressure homogenization, with rapeseed lecithin and without CoQ_{10}.
2. LFU-HPH-LEC-CoQ_{10} emulsion: emulsion made with low frequencies process and high pressure homogenization, with rapeseed lecithin and with CoQ_{10}.
3. UT-HFU-LEC emulsion: emulsion made with high frequencies process with Ultra-Turrax® premix, with rapeseed lecithin, without CoQ_{10}.
4. UT-HFU-LEC- CoQ_{10} emulsion: emulsion made with high frequencies process with Ultra-Turrax® premix, with rapeseed lecithin, with CoQ_{10}.
5. UT-HFU emulsion: emulsion made with high frequencies process with Ultra-Turrax® premix, without rapeseed lecithin, without CoQ_{10}.
6. UT-HFU-CoQ_{10} emulsion: emulsion made with high frequencies process with Ultra-Turrax® premix, without rapeseed lecithin, with CoQ_{10}.

3. Size measurement by Nanoparticles tracking analysis (NTA): particles

Nanoparticles tracking analysis (NTA) is a Nanoparticles visualization technique that provides size, count and concentration measurements. NTA delivers true size distributions, even when the systems are complex and polydisperse, and supports the exclusive numerical data with corroborating images. The measured diameter can be between 10 and 1000nm. NTA experiments were performed using a digital microscope LM10 System (NanoSight, Salisbury, UK). Samples were diluted in distilled water (1:25000) and introduced into the optic cell with a syringe. Video images of droplets movement under Brownian motion were analysed by the NTA analytical software version 2.1. The measurements were made at 25°C and 60s. Hydrodynamic radius (R_H) was calculated with Stokes Einstein relation:

$$R_H = {}^{k_B T}/_{6\pi\eta D} \qquad (1)$$

with k_B : Boltzmann's constant,

T : Absolute temperature,

η : Dynamic viscosity

D : Transverse diffusion coefficient

4. Emulsification capacity

Emulsions were prepared with 10% of oil ratio. For emulsifier free emulsion, this 10% was not completely emulsified [12]. The oil volume present in the emulsion was measured from freeze-dried emulsion. 5g of each emulsion was frozen and lyophilized for obtain emulsified oil. Measurements were carried out on five replicates. Oil was weighted and oil/water ratio was calculated as follows:

$$EC = \frac{EOW}{EW} * 100 \quad (2)$$

EOW: Emulsified oil weight; *EW*: Emulsion weight (5g).

5. ζ potential measurement

ζ potentials were calculated from electrophoretic mobility using the Malvern Nano ZS instrument (Malvern instrument, Worcestershire, UK). Measurements were averaged of 20runs using dilute dispersions (1-400). Triplicate measurements were done with 10 seconds of equilibration time at 25°C. Droplets electrophoretic mobility was converted to the ζ potential using Smoluchowski equation. The average of 10 measurements was taken to represent the measured potential. The applied voltage during the measurements varied between of 50-100mV.

6. Surface tension measurement

Surface tension measurements were carried with Krüss K100 tensiometer (Krüss GmbH, Hambour, Germany) with Wilhelmy plate method. The temperature was set at 25°C. The measurement was carried out in triplicates during 500s and the blade was immerged at 2mm from the surface.

7. Rheological characteristics

Emulsions were analysed with Malvern Kinexus rheometer (Malvern Instruments, Worcestershire, UK) using PMMA-titanium cone-plane (0°59′ cone angle, 40mm diameter, 27µm gap) at 25°C. Viscometry and oscillation were used for rheological study [16]. The rheological behaviour was studied between 0.01 and 100Pa.s^{-1} shear stress. The viscoelastic behaviour was investigated by performing dynamic measurements. Each oscillation was performed at 1Hz frequency for a determination of linear viscoelastic region (LVER) [23]. For each emulsion, shear stress value included on LVER region was then selected and applied to obtain viscoelastic properties. Each measurement was performed in triplicate.

8. *In vitro* tests

In vitro tests were made with human primary mesenchymal stem cells. The cells were harvested and expanded as previously described [17]. All emulsions samples were adjusted to the same oil ratio and tested with 3 different concentrations in alpha-MEM culture medium: 0.5, 1 and 2 mg/mL. Untreated cells are use as control (CTL). The cells were seeded at a density of 3000 cell/cm^2 and cultured in complete medium containing the different treatments. After three time-points, D1 (day 1), D3 (day 3) and D7 (day 7), biocompatibility tests were performed.

To evaluate the impact of the treatments on cell behaviour, different parameters were estimated: cytotoxicity was performed by LDH assay using Cytotoxicity Detection Kit: LDH (Roche, Cat No. 11644793001) according to manufacturer's instructions; cell metabolic activity was measured thanks to MTT ([4,5 dimethylthiazol-2-yl] 2,5 diphenyltetrazolium bromide) assay as explained elsewhere [18] cell proliferation was assessed by Hoechst assay, which allows cell DNA quantitation, was performed as previously described [19].

9. Statistical analysis

ANOVA analyses were processed with Minitab software. All tests were executed at 95% significance level to find out if the different methods of emulsification and formulation influence the stability and the quality of the emulsions. All data are presented as mean ±

standard deviation (SD). Statistical significance was determined by one-way ANOVA with p-value lower than 0.05.

Results and Discussion

1. Droplets Size of emulsions

Oil droplets size was determined for emulsions containing or not CoQ_{10}, stabilized by rapeseed lecithin or without emulsifier and are represented (Table 1). Smaller droplets were obtained by combination of low frequency ultrasounds and high pressure homogenization (HPH). In HPH emulsification process, sonication by low frequency ultrasounds was followed by high pressure process which permitted to obtain smaller droplets and homogenous solution. Belhaj *et al.* (2012), showed that the sonication step led to the formation of oil nano-droplets with large polydispersity index. By adding homogenization step, the mean droplets size decreased in both control sample and CoQ_{10} nano-emulsions [10].

Table 1: Average, droplets charge (ζ potential) and emulsification capacity of (i) emulsions containing rapeseed lecithin (LEC) and/or coenzyme Q_{10} (CoQ_{10}) obtained by two emulsification processes: "sonication with low-frequency ultrasounds (LFU) + process high-pressure homogenization (HPH)" and "Ultra-Turrax® (UT) + High frequency ultrasounds (HFU)", and (ii) emulsifier free emulsions with/without CoQ_{10} obtained by "Ultra-Turrax® (UT) + High frequency ultrasounds (HFU)".

Emulsion	Oil droplets size average (nm)	ζ potential (mV)	Emulsification capacity
LFU-HPH-LEC	143 ± 12^{a}	-45.1 ± 0.6^{a}	97.3±0.2%
LFU-HPH-LEC-CoQ_{10}	138 ± 02^{a}	-47.7 ± 2.5^{bc}	97.38±0.7%
UT-HFU-LEC	232 ± 22^{bc}	-50.9 ± 0.3^{d}	99.29±0.1%
UT-HFU-LEC-CoQ_{10}	203 ± 17^{bd}	-47.9 ± 2.0^{dc}	99.37±0.2%
UT-HFU	220 ± 30^{ec}	-60.5 ± 1.5^{e}	62.41±0.8%
UT-HFU-CoQ_{10}	216 ± 19^{ed}	-65.9 ± 2.1^{f}	62,55±0.5%

According to our results and for LFU-HPH emulsion, there was no significant difference in emulsions average size with addition of CoQ_{10} which indicates that the CoQ_{10} has no effect on the system when it was added at 1.64% amounts. On the contrary, Jiménez *et al.* (2014) shows that the addition of hydrophobic compounds seems to favour the compactness of dispersion by improving the orientation of amphiphilic molecules of lecithin through the interactions with the oil compounds [20]. Some studies described that during nano-emulsification, the encapsulated drug precipitates in the oil-water interface which may increase the emulsification time and droplet size [21].

The same conclusion was made for UT-HFU emulsion with and without CoQ_{10}. UT-HFU emulsions containing CoQ_{10} don't show a significant difference between the emulsions with and without surfactant (HF with lecithin and CoQ_{10} and HF without lecithin and with CoQ_{10}). It means that for the same emulsification process, rapeseed lecithin and 1.64% CoQ_{10} addition don't influence the average droplets size obtained for emulsion.

The different emulsification processes (LFU+HPH) and UT-HFU don't allow obtaining the same size average. LFU emulsification combined with HPH emulsification gave smaller droplets sizes than the emulsification with high frequency ultrasounds combined with the rotor-stator premix system.

LFU emulsification combined to HPH emulsification provides smaller droplets sizes than the emulsification with high frequency ultrasound combined with the rotor-stator premix system.

Thus, it was clear that size droplets depends not only on such physical parameters as the amplitude of sonicator, but also on the composition of lecithin and the surface-active properties of the lecithin [18]. According to the literature, LFU emulsification occurs by two mechanisms [4, 22, 23]. First, application of acoustic fields produces interfacial waves that induces interfaces instability, ultimately resulting in oil phase emergence in the aqueous phase as droplets of about 70 µm. Secondly, application of LFU causes acoustic cavitation, that is, the formation and subsequent collapse of microbubbles by pressure fluctuations of a simple sound wave. Each bubble collapse (an implosion on a microscopic scale) event causes extreme levels of highly localized turbulence (high pressure and high temperature). Turbulent micro-implosions act as a very effective method of breaking up primary droplets of dispersed oil into droplets of sub-micron size. Application of low frequency ultrasonic waves in emulsion led to formation of cavitation bubbles. The chemical and mechanical effects of ultrasound are due to the collapse of these cavitation bubbles. By adding a HPH step on emulsion preparation,

droplets size average became smaller and distribution was narrow. In the other case, by choosing an appropriate frequency above 1 MHz for ultrasounds emulsification, mechanical effects can be limited to the benefit of chemical effects which are represented by water hydrolysis [24].

With high frequency ultrasounds (above 1 MHz), violent cavitation was prevented and big oil droplets was formed by interfaces destabilization. The overall movement of the continuous phase allowed the droplets passage near to piezoelectric transducer causing shearing and splitting down to obtain a nano-emulsions by high frequency vibrating movements. The 1.7 MHz frequency led to strong destabilization of droplet interface and progressive decrease of droplets average size [12].

Low frequency ultrasounds can get smaller droplets average when this process was followed by homogenization method at 1500 bars pressure. This pressure allows obtaining smaller droplets which normally induce more emulsion stability. The first emulsion preparation method (LFU + HPH) is a method bringing a high energy to the system, the premix made initially by low frequency ultrasound use the violent cavitation, it followed by HPH using high pressure that can split the bigger droplets into smaller droplets. This provides emulsions with very small size (lower than 150 nm).

According Canselier *et al.* (2002) [25], D (4.3) droplets diameter obtained when emulsions was made with an Ultra-Turrax® were bigger than those obtained by LFU, and emulsions made by HPH has a smallest droplets diameter compared to Ultra-Turrax® and LFU process [26].

For the second process, the Ultra-Turrax® premix give a coarse emulsion [25], which when treated with high frequency ultrasound is refined, but as mentioned earlier, the HFU provide a lower energy to the system then LFU and HPH and therefore does provide the same droplet size average (between 200nm and 250nm).

2. ζ Potential of emulsions

The droplets surface charge was determined for all studied emulsions and represented on Table 1 as ζ potential values. The surface charge study of the droplets is of interest since it affects the stability dispersions specially in the studied solutions in which the viscosity is too low and also for emulsifier free emulsion which owe their stability to electrostatic repulsion [5]. Emulsifier free emulsions prepared with the different emulsification process, with or without lecithin and coenzyme Q_{10} exhibited negative surface charge of droplets. The ζ potential absolute values

(above ± 30 mV) indicate a good stability against droplets coalescence [27, 28]. In this study, emulsions exhibited more than 45mV (absolute value) ζ potential. According to obtained results (Table 1) and those found by literature [18, 29], the anionic fractions of phospholipids such as phosphatidylserine, phosphatidic acid, phosphatidylgycerol, phosphatidylethanolamine and phosphatidylinositol in lecithin with 80% phosphatidylcholine were responsible for the negative surface charges [18, 29]. So, these anionic fractions are probably responsible for the negative surface charge [29, 30]. The electrostatic repulsive force has been increased in phospholipid-stabilized o/w emulsions by the addition of oleic acid, phosphatidylserine, phosphatidylglycerol, or phosphatidic acid [30]. The using high frequency ultrasounds process provided a higher absolute value of ζ potential, especially for emulsions without rapeseed lecithin. The results show that coenzyme Q_{10} addition influences significantly the emulsions ζ potential for LFU-HPH emulsion and UT-HFU emulsion without lecithin. Also when rapeseed lecithin was added, there is no significant difference between the two preparation methods (LFU + HPH) and (UT + HFU). However, there is a significant difference for emulsions with and without rapeseed lecithin, which may suggest that the lecithin prevents surface ionization of oil droplets by other hydroxyl ions present in solution. According with results obtained by Kaci *et al.* (2014) [12], a pH decrease and a negative surface charge are shown when high frequency ultrasound emulsification was used and thus suggest that the stability of oil nano-droplets is due to hydroxide ions decrease in solution. So, it supposed that oil droplets accumulates more charges on the surface and formed thicker layer [14]. The emulsions electric charge is mainly due to the adsorption on their surface OH^- hydroxide ions that present in solution, and acquires a negative charge. The small size and the droplet surface charge of emulsifier free emulsions provide an electrostatic stabilization of emulsions [5, 12].

3. Emulsification capacity of emulsions

Emulsion oil ratio was measured after each emulsification process by emulsion lyophilisation and the results are represented in Table 1.

Emulsification by the two different processes (LFU + HPH) and (UT + HFU) for emulsions containing rapeseed lecithin exhibited a high emulsification rate (over 99%). Indeed, these two emulsification processes in the presence of emulsifier are very effective in providing a good emulsification rates. High frequency ultrasounds process without emulsifier doesn't provide a high emulsification rate (62%) (Table 1). It is noted from these data that the processes

containing the surfactant used to obtain a greater amount of oil emulsified. In the presence of an emulsifier, the method used for emulsification has no significant effect on the rate of emulsification. Indeed, the surfactant is used for these capabilities to stabilize the emulsion and allows ease of emulsification. For a same emulsification process, the presence of rapeseed lecithin caused a significant increase of the emulsification ability ($p>0.05$). This high amount is mainly due to the presence of the surfactant to facilitate the emulsion and thus to encapsulate a higher oil content. The coenzyme Q_{10} addition does not affect the emulsification ratio. This analysis leads to the conclusion that within the same process, high frequency or low frequency, the presence of 1.64% coenzyme Q_{10} has no effect on emulsification rate.

4. Surface tension of emulsions

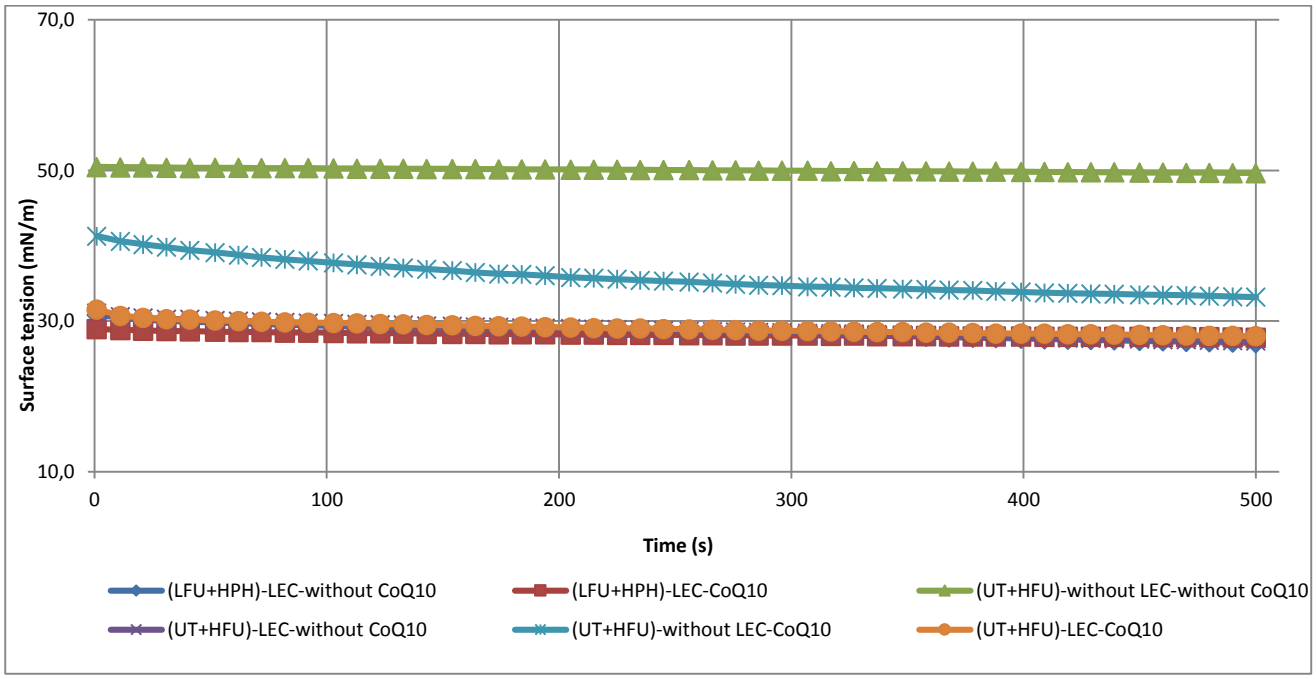

Figure 1: Surface tension monitoring of (i) emulsions containing rapeseed lecithin (LEC) and/or coenzyme Q_{10} (CoQ_{10}) obtained by two emulsification processes: "Sonication with low-frequency ultrasounds (LFU) + High-pressure homogenization (HPH)" and "Ultra-Turrax® (UT) + High frequency ultrasounds (HFU)", and (ii) emulsifier free emulsions with/without CoQ_{10} obtained by "Ultra-Turrax® (UT) + High frequency ultrasounds (HFU)".

The surface tension of prepared emulsions was followed during 500s at 25°C (Figure 1). The surface tension of oil-in-water emulsion decreased by increasing the emulsifier concentration

while the stability of emulsions increased [31]. As expected, the surface tension solutions were lower in comparison with pure water. The relatively high surface tension of water (72 mN/m) can be attributed to arrangement of hydrogen bonding in the interfacial zone [32]. The results reveal two emulsions groups having a different surface tension results: (i) emulsions with lecithin, (ii) surfactant-free emulsion. The emulsion with lecithin emulsifier showed a lower surface tension (~29 mN/m) and, in this case, emulsification process and coenzyme Q_{10} addition don't influence significantly the emulsion surface tension. For lecithin emulsions, the obtained values demonstrate the ability of lecithin to form stable nano-emulsions with and without CoQ_{10} in the dispersed phase [20] by reducing of surface tension.

The low surface tension values are explained by the lecithin surfactant action that decrease emulsion surface tension and make easier the emulsification process. When the surface tension is low, phase separation forces are lower and emulsions are more stable because all systems tend to have the minimum surface tension to be stable.

Thus UT-HFU formulations without surfactant (with or without CoQ_{10}), have a higher surface tension. These results are related to emulsions composition. Indeed, a very high surface tension (~50 mN/m) was found due to the sample composition. Emulsions without emulsifier contain more water than emulsion with lecithin, and also lecithin addition decrease the surface tension therefore surface tension of UT-HFU formulations tends to water surface tension value (~72 mN/m) (Figure 1).

The inclusion of CoQ_{10} did not produce any difference in surface tension for rapeseed nano-emulsions. However, the addition of these compounds significantly ($p>0.05$) reduced the surface tension of emulsifier free emulsions containing coenzyme Q_{10} (41 mN/m). This can indicate that the addition of hydrophobic compound affects the critical micellar concentration [20] and the corresponding minimal surface tension of hydrophobic phase.

The emulsifier free emulsion containing CoQ_{10} shows a decrease of surface tension value on the first 100 seconds and stabilizes at 33 mN/m. It could be explained by the creaming process. The creaming results from migration of the dispersed phase to the top because dispersed phases density is lower than continuous phase density. Creaming phenomena induced a progressive accumulation of oil droplets on the sample surface that induces the surface tension diminution. The surface tension stability of colloidal systems during the monitoring indicated the absence of destabilization phenomena.

The high surface tension of emulsifier free emulsions confirms to us that there is no emulsifier added on mixtures and reinforces our hypothesis about the stability of emulsifiers free emulsion that is due only to electrostatic forces fighting against the droplets coalescence in emulsions and therefore participates on stability of colloidal systems.

5. Rheological properties of emulsions

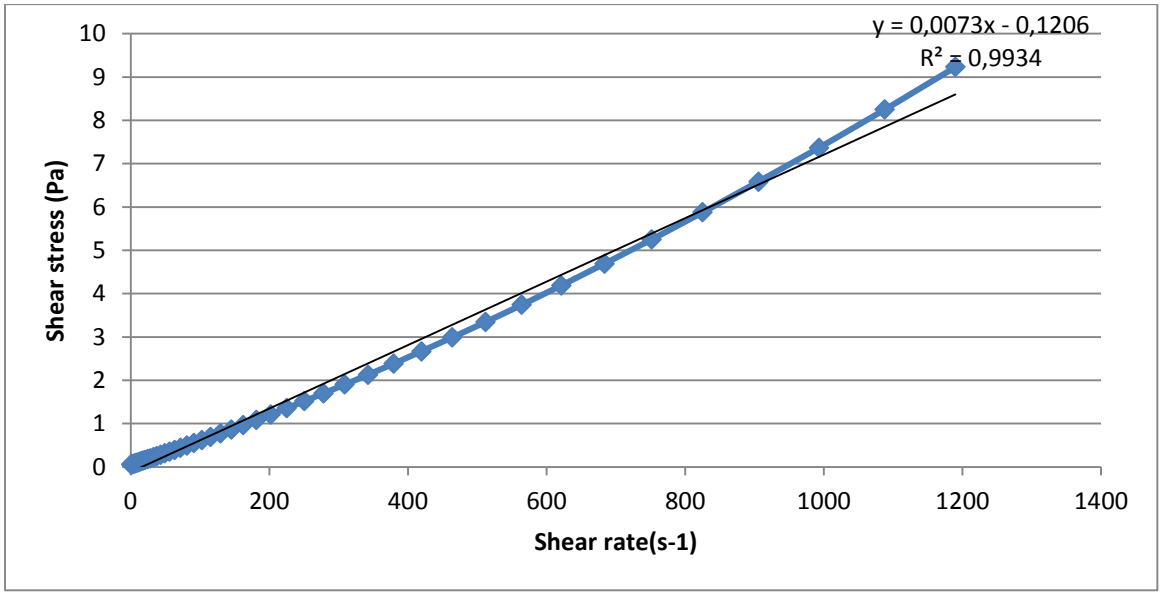

Figure 2: Rheological behaviour of emulsifier free emulsion made with high frequency ultrasounds and Ultra-Turrax® premix without coenzyme CoQ_{10}: variation of shear stress depending on shear rate.

The study of emulsion rheological behaviour is of interest to have information about fluid structure and droplets interactions. To define the rheological behaviour of studied emulsions, the stress was measured depending on applied shear rate. The results were showed in Figure 2, where a single emulsion was represented because the observed trend was the same for all studied emulsions, with a curve slope equal to y=0.0073x–0.1206 and a correlation coefficient was 0.99. Results lead to indicate Newtonian flow behaviour [3]. Newtonian liquids are characterized by a constant viscosity which is independent of the applied shear rate [16]. So, whatever the stress applied to the different formulations, it has no effect on their viscosity. These results suggest that lecithin does not affect the rheological properties of our emulsions [33]. This Newtonian behaviour of the emulsion is due to the water predominance in prepared emulsions.

- **Viscoelastic characteristics**

Strain sweep measurements ranging from 0.01 to 100% were carried out at 25°C and at a frequency of 1 Hz. These experiments enabled obtaining the linear viscoelastic region (LVR), corresponding to properties independent from the applied strain amplitude, the viscoelastic moduli then only depending on time or frequency [16].

Measurements of elastic modulus (G'), viscous modulus (G'') and complex modulus (G*) at fixed shear rate, shear stress and 1Hz frequency are given for all samples in Table 2. In rheological science, storage modulus G' is the real part of G*, G' is associated with the elastic response. G'' is G* imaginary part (loss modulus) and it characterizes the viscous response.

Table 2: Rheological characteristics of (i) emulsions containing rapeseed lecithin (LEC) and/or coenzyme Q_{10} (CoQ_{10}) obtained by two emulsification processes: "Low-frequency ultrasounds (LFU) + High-pressure homogenization (HPH)" and "Ultra-Turrax® (UT) + High frequency ultrasounds (HFU)", and (ii) emulsifier free emulsions with/without CoQ_{10} coenzyme obtained by "Ultra-Turrax® (UT) + High frequency ultrasounds (HFU)".

Sample Description	Shear modulus (elastic component) G' (Pa)	Shear modulus (viscous component) G'' (Pa)	Shear viscosity (complex component) G* (Pa.s)
LFU-HPH-LEC	0,107 ± 0,009	0,017 ± 0,006	0,013 ± 0,005
LFU-HPH-LEC-CoQ_{10}	0,105 ± 0,006	0,036 ± 0,006	0,017 ± 0,003
UT-HFU-LEC	0,061 ± 0,010	0,028 ± 0,08	0,010 ± 0,004
UT-HFU-LEC-CoQ_{10}	0,050 ± 0,003	0,020 ± 0,003	0,008 ± 0,002
UT-HFU	0,036 ± 0,003	0,026 ± 0,006	0,011 ± 0,004
UT-HFU-CoQ_{10}	0,211 ± 0,009	0,037 ± 0,010	0,041 ± 0,02

Table 2 show that all emulsions exhibited a predominant elastic behaviour (G′> G″) within the LVR. Elastic phenomena occured at all concentrations of dispersed phase (dilute, semi-dilute

and concentrated systems), and also additional variables such as processing conditions, composition and material properties of the constituents, affected the emulsion rheology [34]. According to Pozrikidis (1994), the effect of surface viscosity on rheology of a dilute emulsion was considered, and it was found that, in all cases, a dilute emulsion has some elastic properties [35]. Indeed, droplets deformation gave rise to an elastic interfacial force which revealed itself through a non-zero normal stress difference. Emulsions containing a lecithin showed much higher values of G′ than the emulsifier free emulsions and LFU+HPH emulsions showed much higher values of G′ than UT-HFU emulsions (Table 2). This is explained by the lecithin addition, but also by electrostatic stability of emulsifier-free-emulsions promoting repulsions between the droplets influencing rheological properties of emulsions. So, it is difficult to study the rheological properties of semi-dilute emulsion. Indeed, for semi-dilute emulsions of low viscosity fluids (*e.g.* oil/water systems), the normal stresses was below the detection limit of the available instruments [34].

6. *In vitro* biocompatibility of emulsions

The biocompatibility of emulsions made with different emulsification process on cells was measured by lactate dehydrogenase (LDH), colorimetric reduction test of tetrazolium salt (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide or MTT) and Hoechst assays.

a. Emulsions cytotoxicity assay

Extracellular lactate dehydrogenase (LDH) detection was used for cytotoxicity study and the results were collected at three time-points: 1, 3 and 7 days after bringing into contact emulsions and humans cells. LDH results are shown in Figure 3. A reference control (CTL), representing basal level of LDH release, was made with untreated cells. After 1, 3 and 7 days, there was no registered toxicity for manufactured emulsions compared to low cytotoxicity control. There was no relevant difference with LDH release between untreated cells or those treated by emulsions. Emulsions results was compared to this control and showed that there was no relevant ($p<0.05$) difference with tested emulsions. These observations showed that emulsions had not any toxicity for human mesenchymal stem cells.

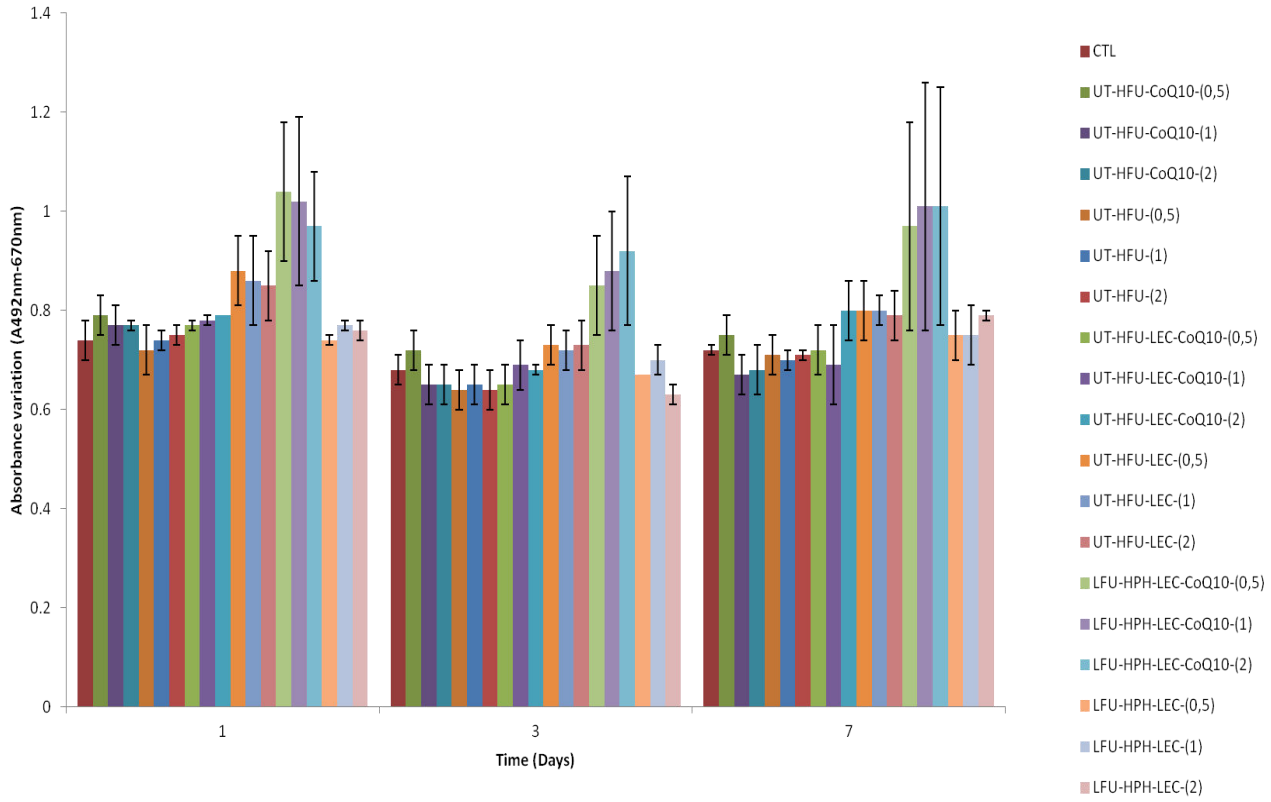

Figure 3: Lactate dehydrogenase activity of human cells after 3 days of treatment by: (i) emulsions containing rapeseed lecithin (LEC) and/or coenzyme Q_{10} (CoQ_{10}) obtained by two emulsification processes: "low-frequency ultrasounds (LFU) + process high-pressure homogenization (HPH)" and "Ultra-Turrax® (UT) + High frequency ultrasounds (HFU)", and (ii) emulsifier free emulsions with/without CoQ_{10} coenzyme obtained by "Ultra-Turrax® (UT)+ High frequency ultrasounds (HFU)". CTL: control meaning untreated cells. Emulsions were used at 0.5, 1 and 2 mg/mL. Results are mean +/- SD (n=3).

b. Emulsions effects on cell metabolic activity

MTT results are showed in Figure 4. The colorimetric reduction of tetrazolium salt (MTT) allows assessing cell mitochondria metabolic activity. It is based on the cellular respiration phenomena, where MTT assay measures the mitochondrial function activity of mitochondrial dehydrogenases [36]. The more metabolic activity is high, the more cellular functions are good. MTT is a yellow-coloured tetrazolium salt that is reduced to purple formazan at the expense of the reduction reaction products with concomitant oxidation of NADH and NADPH [37].

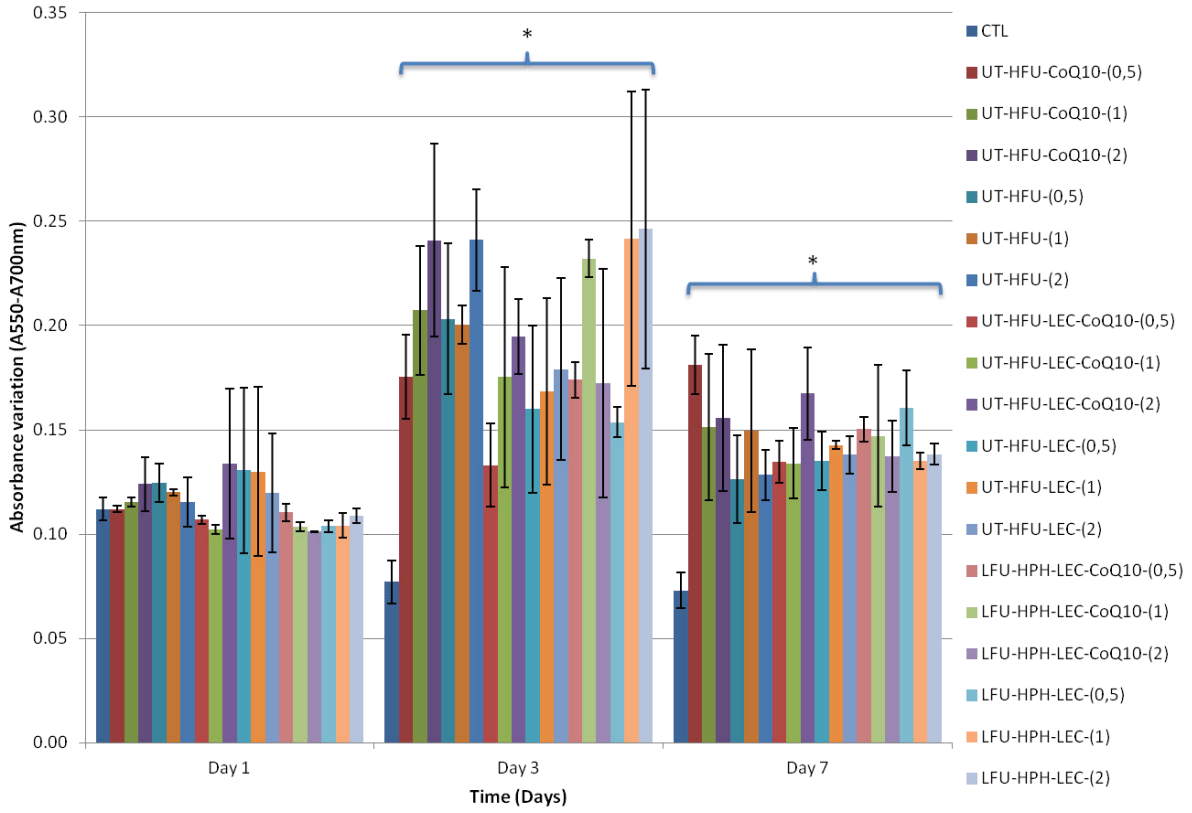

Figure 4: Human cell metabolic activity after treatments by: (i) emulsions containing rapeseed lecithin (LEC) and/or coenzyme Q_{10} (CoQ_{10}) obtained by two emulsification processes: "Low-frequency ultrasounds (LFU) + High-pressure homogenization" (HPH) and "Ultra-Turrax® (UT) + High frequency ultrasounds" (HFU), and (ii) emulsifier free emulsions with/without CoQ_{10} coenzyme obtained by "Ultra-Turrax® (UT) + High frequency ultrasounds" (HFU). CTL: control meaning untreated cells. Emulsions were used at 0.5, 1 and 2 mg/mL. Results are mean +/- SD (n=3). * means a significant difference of treatment compared to control ($p<0.05$).

After one day of culture (Figure 4), cell metabolic activity shows no significant difference ($p<0.05$) whatever treatment compared to untreated cells. At days 3 and 7, untreated cells metabolic activity remains unchanged compared to day 1 whereas emulsions increase cell activity significantly whatever treatment. Nevertheless, some of the emulsions treatments have a tendency to decrease activity at day 7 compared to day 3. This could be due to cell over confluence induced by cell proliferation, increasing the cells detachment probability accentuated by all treatments throughout the 7 days thus decreasing cellular metabolic activity. Another hypothesis could be cell phenotype changes, which would slow down cell activity. This should be verified by genomic tests or directly on protein.

The results obtained by MTT test at the 3rd day of culture indicate that the rapeseed lecithin presence which is rich in monounsaturated and polyunsaturated fatty acids helps metabolic activity of human mesenchymal stem cells [18] and also when coenzyme Q_{10}, which is a powerful antioxidant, was added [10]. A high metabolic activity was observed with high frequencies ultrasounds containing with coenzyme Q_{10} but without emulsifier (2mg/mL which is the maximum solubility of coenzyme Q_{10}). It is shown that for high frequency ultrasounds, coenzyme Q_{10} presence alone (without emulsifier) allows giving as much activity in the case of the presence of both of coenzyme and lecithin.
The combination of these two ingredients (coenzyme Q_{10} and rapeseed lecithin) seems to be unsatisfactory which suggests interference between the two compounds reducing the benefits of each.

MTT does not distinguish cell death between apoptosis and necrosis, nor takes account of possible increases in cell number in a cycling cell population. So, cell populations may remain constant or even increase as the cell cycle progresses [36, 38].

c. Influence of emulsions on cell proliferation

Hoechst assay results in figure 5 show the influence of the treatments on human mesenchymal stem cells proliferation with a progressive increase in DNA amount until the 7th day for the majority of the emulsions. At day 7, a very small increase was observed for emulsions made by low frequency (with emulsifier and with and without CoQ_{10}) which shows a weak value compared to untreated cells. Unlike LFU-HPH emulsions, UT-HFU emulsions exhibit higher DNA amount which significantly exceed the control value. This increase reflects a better cell proliferation based on the DNA quantity (Figure 5).

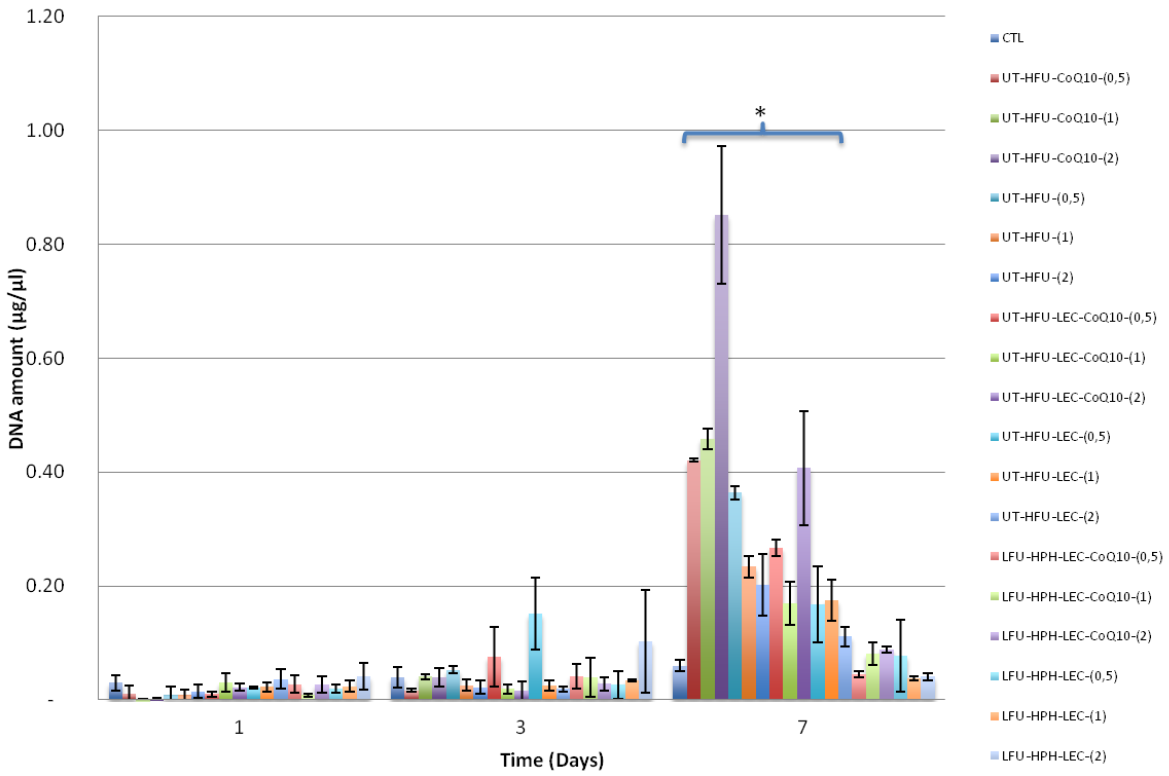

Figure 5: Human cell DNA quantification after treatments by: (i) emulsions containing rapeseed lecithin (LEC) and/or coenzyme Q_{10} (CoQ_{10}) obtained by two emulsification processes: "Low-frequency ultrasounds + High-pressure homogenization" annotated LFU and "Ultra-Turrax® (UT) + High frequency ultrasounds (HFU)", and (ii) emulsifier free emulsions with/without CoQ_{10} coenzyme obtained by "Ultra-Turrax® (UT)+ High frequency ultrasounds (HFU)". CTL: control meaning untreated cells. Emulsions were used at 0.5, 1 and 2 mg/mL. Results are mean +/- SD (n=3). * means a significant difference of treatment compared to control ($p<0.05$).

Cell proliferation is particularly important with formulations produced at high frequencies, without surfactant but using CoQ_{10}. the CoQ_{10} has a tendency to improve cell metabolic activity and it also enhances significantly cell proliferation [37]. Coenzyme Q_{10} is a powerful antioxidant and its beneficial effects may be largely attributed to its fundamental role in mitochondrial function and cellular bioenergetics [9]. The head and tail structure of CoQ_{10} takes different functions such that the head transfers electrons while the long chain holds it in a mitochondrial or cytoplasmic membrane [39, 40]. Improving the cellular energy metabolism, activation of the immune system and scavenging free radicals are some of its known functions

According to Bhagavan *et al.* (2007), the response following ingestion of solubilized formulations of CoQ_{10} is much greater indicating their superior bioavailability as compared with non-solubilized powder-based CoQ_{10} products (compressed tablets, chewable tablets, powder-filled capsules, and soft gels containing a suspension in oil) [9]. Several clinical studies confirm the fact that CoQ_{10} is used for the treatment of hypertension and heart disease [39, 41], breast cancer [39, 42] and Alzheimer's and Parkinson's diseases [39, 42]. There is a superior bioavailability of CoQ_{10} via oral ingestion [9, 39].

The results showed that the best formulation was HF without emulsifier and with 1 mg/mL of CoQ_{10}. These three assays (LDH, MTT and Hoechst) show that CoQ_{10} vectorized without emulsifier is harmless for human cells and promotes their proliferation. Its action is directly linked to the matrix in which it is vectorized. These results show that the combination of lecithin and CoQ_{10} slowed the action of CoQ_{10} coenzyme probably due to steric congestion hindering the release of the active agent and thus reduces its effect. According to McClement *et al.* (2007), a delivery system should enhance (or at least not adversely affect) the bioavailability/bioactivity of the encapsulated component [1].

Conclusion

Emulsions are systems widely used for vectorization especially for hydrophobic substances such as CoQ_{10}. The active agent release is very strongly related to the systems composition used for vectorization. The present paper studies the effect of emulsification process and CoQ_{10} incorporation on physico-chemical characters and human cells biocompatibility to compare these previous systems (without emulsifier) with the same systems containing a natural emulsifier (rapeseed lecithin). Low frequency ultrasound and high pressure homogenisation are usually used for emulsion production but these methods require emulsifier addition bringing the emulsions stability during storage time but the addition of surfactants induces the complexities in formulation and releases problem of molecules after vectorization. The emulsion made by LFU+HPH with emulsifier shows very low droplets size due to cavitation generated by this process while for UT+ HFU emulsions larger droplets sizes are recorded. This is explained by the absence of violent cavitations. ζ potential measurement showed high droplets charge for all tested emulsions due to negative charge of lecithin for lecithin emulsions and ionisation of oil droplets and formation of stern layer at oil/water interface. Surface tension monitoring shows a stable lecithin emulsion and emulsifier free emulsion without CoQ_{10}. The

addition of CoQ_{10} for emulsifier free emulsions induces creaming phenomena showed by surface tension diminution. Cell biocompatibility made with human mesenchymal stem cells shows no toxicity and an efficiency of CoQ_{10} vectorization in emulsions matrix. CoQ_{10} allows obtaining high rate of cell proliferation and metabolic activity especially when emulsifier was not added (with high frequency ultrasound emulsification). LDH and Hoechst assays show that CoQ_{10} promotes cell proliferation and that its effect is directly linked to the matrix in which it is incorporated. This work shows that combination of lecithin and CoQ_{10} slowed the action of CoQ_{10} due to steric congestion hindering the release of the active agent. Indeed, the emulsion with surfactant-free active ingredient obtained by high frequency ultrasounds allows obtaining better results from cellular proliferation and therefore this method is promising to the advance of the removal of surfactants in certain matrices vectorization.

Acknowledgement

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Chapitre V

Etude de la structure des émulsions sans émulsifiants comparées aux émulsions avec émulsifiants par SAXS et RMN

Partie en cours de finalisation

Chapitre V : Etude de la structure des émulsions sans émulsifiants comparées aux émulsions avec émulsifiants par SAXS et RMN

Les nanoémulsions présentent une grande interface de contact entre les deux phases non miscibles due à leur faible taille, donc une concentration suffisamment élevée de tensioactif doit être ajoutée pour permettre à la nouvelle surface de se développer et de permettre aux nanogouttelettes nouvellement formées d'être rapidement recouvertes de tensioactif pendant le processus d'émulsification. Une interface très impressionnante entre les phases dispersées et continues peut interférer dans la libération des actifs vectorisés.

Ce chapitre présente une étude plus approfondie de la structure de l'interface « phase dispersée / phase continue » et de la composition des émulsions sans émulsifiant faites par ultrasons de hautes fréquences avec un pré-mélange rotor-stator « Ultra-turrax » (UT-HFU) et des émulsions contenant un mélange de deux type d'émulsifiants (Polysorbate 80 et Lécithine de colza) faites par ultrasons de basses fréquences couplés avec l'homogénéisation à haute pression (LFU-HPH). La taille moyenne des gouttelettes des émulsions étudiées montrent que les deux procédés permettent la fabrication de nano-émulsions et que la présence d'émulsifiant n'a pas d'incidence sur la distribution de taille. Les procédés LFU-HPH couplés donnent une moyenne de taille plus petite que les procédés UT+HFU en raison de la puissante contrainte de cisaillement appliquée par les deux procédés LFU-HPH dues aux phénomènes de cavitation. La mesure du potentiel ζ montre que les gouttelettes ont une charge négative pour toutes les émulsions en raison de la charge négative de la lécithine pour les émulsions avec émulsifiant et due aux OH- adsorbés à l'interface huile/eau induisant une stabilité de l'émulsion dans le procédé par hautes fréquences. L'étude ^{1}H RMN montre l'absence d'interaction entre les différents composés et montre que le procédé de fabrication HFU ne provoque pas de dégradation chimique ou apparition de composés néoformés. L'analyse SAXS a montré une interface mince entre deux phases avec une densité électronique différente (eau / huile) pour les émulsions avec et sans émulsifiants. Aussi, un signal de diffusion des micelles de polysorbate 80 a été enregistré seulement dans le cas des émulsions avec émulsifiants.

La structure par diffusion des rayons X aux petits angles (SAXS) et par résonance magnétique nucléaire (RMN) a était réalisée et comparée à des émulsions contenant un ou plusieurs émulsifiants. L'étude SAXS montre la présence de micelles de tensioactifs pour les émulsions sans émulsifiants et les résultats de la RMN montrent l'absence de la signature des tensioactifs et des phospholipides dans les émulsions faites par ultrasons de hautes fréquences. Par ailleurs la RMN montre l'absence d'interaction entre les différents constituants de l'émulsion et aussi l'absence de structure néoformées ce qui montre que le procédé d'émulsification par hautes fréquences est non destructif. Il convient donc parfaitement aux applications cosmétiques et surtout pharmaceutiques qui utilisent des principes actifs couteux et sensibles et permettra d'augmenter les efficacités tout en réduisant les doses utilisées.

Structural analysis of emulsifier free nanoemulsions made with ultrasonic emulsification

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Abstract

Nanoemulsions are one of the most widely studied classes of drug delivery nanoscale systems. Nanoemulsions have a large interfacial area, so sufficiently high concentration of surfactant should be added to allow the new surface to expand and allow nanodroplets to be quickly coated during the emulsification process. An impressive interface between dispersed and continues phases can interfere on release of drugs at the active site. Emulsions with and without emulsifiers made with high frequency ultrasounds process (HFU) generated by a piezoelectric ceramic transducer vibrating at 1.7 MHz. Emulsion with emulsifier made with low frequency ultrasounds (LFU) coupled with high pressure homogenization (HPH) as emulsion control. Physicochemical properties of obtained emulsions were determined by measurement of average droplets size with nanodroplets tracking analysis, droplets surface charge with ζ potential measurement and surface tension. The average droplets size of studied emulsions show that the two processes allow nanoémulsions manufacturing and emulsifier addition does not affect the size distribution. LFU+HPH give smaller size distribution then UT+HFU due to powerful shear stress applied to LFU+HPH emulsion due to cavitations phenomena. ζ potential measurement showed negative droplets charge for all emulsions due to negative charge of lecithin for emulsions with emulsifier and for OH^- adsorption at oil/water interface inducing emulsion

stability. ^{1}H NMR analysis shows the absence of interaction between different compounds and shows that the HFU manufacturing process does not cause chemical degradation or neoformed compounds. SAXS show a thin interface between two phases with different electronic density (water/oil) for emulsions with and without emulsifiers and for emulsion with emulsifiers SAS show Polysorbate 80 micelles diffusion signal which doesn't appear in the emulsion without emulsifiers.

Keywords: Emulsifier free nanoemulsions, size distribution, ζ potential, ^{1}H NMR, SAXS,

Introduction

Emulsions are thermodynamically unstable systems. The addition of surfactants, solid particles (Pickering emulsion) or a mixture of surfactant with other amphiphilic polymers can kinetically stabilize the emulsions (Nikfarjam, Taheri Qazvini et al. 2015). Such systems possess a minimal stability, which may be accentuated by such additives as surface-active agents... Surfactants, in particular polysorbates, are commonly utilized excipients in the chemical and pharmaceutical industry. Due to their amphiphilic nature, polysorbate molecules are associated into micelles when the critical micelle concentration (CMC) is exceeded (Verbrugghe, Cocquyt et al. 2010). Lecithin is largely used as natural emulsifier. The main constituents of lecithin are phospholipids, which are amphiphilic molecules containing a water soluble, hydrophilic head group and a lipid-soluble, hydrophobic tail group.It is composed of various essential fatty acids such as α-linolenic acid (ALA) for vegetable lecithin, and eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) for marine lecithin.

One of the first industrial applications of high intensity acoustic energy was in the process of emulsification. When the interface of two immiscible liquids is ultrasonically irradiated, an emulsion is formed, i.e., tiny droplets of one liquid (dispersed phase) are scattered into the other liquid, which constitutes the continuous phase (Gaikwad and Pandit 2008).

Ultrasound emulsification was reported for the first time by Wood and Loomis (Wood and Loomis 1927). The ultrasound device has been found to produce o/w emulsion with very small particle sizes, more stable than those prepared, for instance, with mixer or other mechanical devices (Abismail, Canselier et al. 1999). When emulsifier is added, it may interact with the other compounds creating new emulsion properties. Therefore, it becomes difficult to study the role of oil phase alone on emulsion properties (Sakai 2008; Kaci, Meziani et al. 2014). Very stable o/w emulsion can be prepared with acoustic emulsification without any surfactant and stabilized by electrostatic stabilization due to preferential adsorption of OH^- ions at the

oil/water interface (Reddy and Fogler 1980; Marinova, Alargova et al. 1996; Kaci, Meziani et al. 2014). To the nanoemulsions have a large interfacial area, so sufficiently high concentration of surfactant (emulsifier) should be added to allow the new surface to expand to allow nanodroplets to be quickly coated during the process of emulsification this should avoid or limit modification induced by coalescence, and allow a better physical stability of the emulsion for a long time (Mason, Wilking et al. 2006; Cortés-Muñoz, Chevalier-Lucia et al. 2009).

The removal of adsorbed surfactant molecules from the surface of ultrafine particles is difficult. Correspondingly, introduction of surfactant-free emulsions is desired since the droplets are composed of oil molecules alone. Droplets in such emulsion are expected to have a clear surface and the interior with the highly hydrophobic nature of oil proper. (Kamogawa, Akatsuka et al. 2001). The Small-angle X-ray scattering (SAXS) analysis enable to evaluate the surfactant micelles and to estimate the thikness of oil/water interface The method is accurate, nondestructive, and usually requires only a minimum of sample preparation (Luykx, Peters et al. 2008; Maherani, Arab-tehrany et al. 2011). Proton Nuclear Magnetic Resonance (1H NMR) Spectroscopy is a powerful method used in the determination of the structure of unknown organic compounds. NMR has been used to study the structure and dynamics of lipid-water mixtures for many years (Groen, Goldhoorn et al. 1990).

In this work, two different emulsification processes were tested. Once of these processes (HFU process) was tested without emulsifiers addition. Physicochemical properties were determined for each process. 1H NMR analysis was used to determinate the chemical shift compounds and the study of interactions. SAXS analysis help us to understand the structure of emulsions specially emulsifier-free-emulsions

Materials and Methods

1. Materials

Distilled water, Caprylic/Capric Triglyceride (Miglyol 812, SASOL, Paris, France), rapeseed oil and apricot kernel oil were used as the aqueous and oily phases for all emulsions. All materials were used as received, without further purification. Polysorbate 80 (Tween 80) was provided by Sigma Aldrich (Lyon, France). Rapeseed lecithin was acquired from Solae Europe SA society (Geneva-Switzerland).

2. Emulsion preparation

Three emulsions were prepared with different methods. High frequency ultrasound process was tested with and without emulsifier addition. Low frequency ultrasound coupled with high pressure homogenization was used as control emulsion. Formulation was made according to Kabri et al., (2011) mixture design results. Prepared emulsions were compounds of 15% oily phase and 85% of aqueous phase. The oil mixture was compound of Miglyol (49%), rapeseed oil (49%) and apricot kernel oil (2%). For emulsion with emulsifier, the 6.5% of emulsifier was added to the mixture. Polysorbate 80 (4.4%) was added to water and rapeseed lecithin (2.1%) was added in oil mixture (Kabri, Arab-Tehrany et al. 2011). Emulsions pH was maintained at 7.5 during all emulsification processes by NaOH 1N for avoid acidification of emulsions and incorporate hydroxide ions (Beattie and Djerdjev 2004; Kaci, Meziani et al. 2014).

Emulsions were prepared with two different processes:

- **Low frequency ultrasound (LFU) and high pressure homogenization(HPH):** used for emulsion with emulsifier; the phases were sonicated at 40kHz and 40% of full power during 120s in pulsed mode (1s “on” and 1s “off”) to achieve a homogeneous solution (Kabri, Arab-Tehrany et al. 2011), with a temperature regulation at 25°C.High pressure homogenization was used to obtain a narrow size distribution using a high pressure dynamic homogenizer (EmulsiFlex C3, Avestin). The emulsion was produced by 5 homogenization cycles at 1500 bars at 20°C.
- **Rotor-stator (UT) and transduction with high frequency (HFU):** used for emulsion with and without emulsifier; these methods consist to apply high-frequency ultrasounds using ultrasonic reactor equipped with three piezoelectric ceramics vibrating under the influence of an electric field. The ceramics characteristics and emulsification process were completely described on previous papers (Desjardins-Lavisse and Desobry 2010; Kaci, Meziani et al. 2014). Vibration frequency of ceramics was 1700 KHz, processing temperature was 30°C.

  The oil phase was added into aqueous phase and then a pre-emulsion was made with high-speed homogenization using Ultra-Turrax® (T-25 Basic, IKA-Werk, Staufen, Germany) during 5 min at 13500 rpm rotation speed at 20°C. After this premix process, the emulsion was placed in a thermo-regulated cell and recirculated to ultrasonic reactor using a volumetric pump. The ultrasonic treatment duration was 1 hours.

Different emulsions using high and low frequency ultrasounds was studied

- LFU-HPH-SUR: emulsion made with low frequencies process and high pressure homogenization, with emulsifier (surfactant),
- UT-HFU-SUR emulsion: emulsion made with high frequencies process with Ultra-Turrax® premix, with emulsifier,
- UT-HFU emulsion: emulsion made with high frequencies process with Ultra-Turrax® premix, without emulsifier.

2 Size measurement - Nanoparticles tracking analysis (NTA):

Nanoparticles tracking analysis (NTA) is a nanoparticles visualization technique that provides size and concentration measurements. NTA delivers true size distributions, even when the systems are complex and polydisperse, and supports the exclusive numerical data with corroborating images. The measured diameter can be between 10 and 1000nm. NTA experiments were performed using a digital microscope LM10 System (NanoSight, Salisbury, UK). Samples were diluted in distilled water (1:25000) and introduced into the optic cell with a syringe. Video images of droplets movement under Brownian motion were analyzed by the NTA analytical software version 2.1. The measurements were made at 25°C and 60 s. Hydrodynamic radius (R_H) was calculated with Stokes Einstein.

3 ζ potential measurement

ζ potentials were calculated from electrophoretic mobility using the Malvern Nano ZS instrument (Malvern instrument, Worcestershire, UK). Measurements were averaged of 20runs using dilute dispersions (1-400). Triplicate measurements were done with 10 seconds of equilibration time at 25°C. Droplets electrophoretic mobility was converted to the ζ potential using Smoluchowski equation. The average of 10 measurements was taken to represent the measured potential. The applied voltage during the measurements varied between of 50–100mV.

4 Surface tension measurement

Surface tension measurements were carried with Krüss K100 tensiometer (Krüss GmbH, Hambour, Germany) with Wilhelmy plate method. The temperature was set at 25°C. The measurement was carried out in triplicates during 500s and the blade was immerged at 2mm from the surface.

5 Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy

In the present study, a rapid and simple experimental approach is reported using ^{1}H NMR to determine the composition, interactions and difference between emulsion with and without emulsifiers. Polysorbate 80 and rapeseed lecithin solution was analysed with ^{1}H NMR for the determination of emulsifiers compounds shift.

The NMR sample (600 µl) was prepared and placed in a 5 mm outer diameter NMR tube. D2O serves as the field frequency lock and all the spectra are referenced to water signal at $\delta = 4.7$ ppm, which is also used as an internal standard. The NMR experiments were performed at a magnetic field strength of 14.1 T (AVIII-600 spectrometer, Bruker Biospin Gmbh, Karlsruhe, Germany) and at 25 °C. An experiment was performed with spectral width of 8000 Hz (20 ppm), corresponding to 400 MHz proton resonance frequency. Tipping pulse was 30°, corresponding to 4.7µs pulse duration with 65536 acquisition points number, 16 accumulations number and 1s repetition time. Data were acquired and processed using Bruker Topspin Software, supplied by the manufacturer. The spectra are phased and the baseline corrected. The resulting spectra are aligned by right.

6 The Small-angle X-ray scattering (SAXS) analysis

Small Angle X-Ray Scattering (SAXS) data were collected on a SAXSess mc^2 apparatus (Anton Paar KG, Graz, Austria). This instrument is attached to a ID 3003 laboratory X-Ray generator (General Electric) equipped with a sealed X-Ray tube (PANalytical, $\lambda_{Cu\ K\alpha} = 0.1542$ nm) operating at 40 kV and 50 mA. Emulsions were introduced into a 1 mm capillary before being placed inside an evacuated chamber equipped with a temperature-controlled sample holder unit maintained at 25 °C. The 2D scattering patterns were detected by a CCD camera. Using SAXSQuant software (Anton Paar), the 2D images were integrated into the 1D scattering intensities I(q) upon the magnitude of the scattering vector q (Maherani, Arab-tehrany et al. 2011)

$$q = (4\pi/\lambda)\sin\theta$$

Where λ and θ are wavelength and scattering angle, respectively. All data were collected in the q range from 0.1 to 6 nm^{-1}. Scattering data, obtained with a slit collimation, contain instrumental smearing. Therefore, the beam profile has been determined and used for the desmearing of the scattering data. All data were corrected for the background scattering from the filled capillary

with solvent (water). The scattered intensities were evaluated on an absolute scale using water as a reference.

7 Statistical analysis

ANOVA analyses were processed with Minitab software. All tests were executed at 95% significance level to find out if the different methods of emulsification and formulation influence the stability and the quality of the emulsions. All data are presented as mean ± standard deviation. Statistical significance was determined by one-way ANOVA with p-value lower than 0.05.

Results and discussion

1. Effect of emulsification process on droplets size of emulsions

Oil droplets size was measured for emulsions with and without emulsifier made with two different processes and results was represented in Table 1. For UT-HFU emulsion, emulsifier addition doesn't influence significantly on average size.

Table 1: Droplets average size, droplets charge (ζ potential) and surface tension of (i) emulsions with emulsifier obtained by two emulsification process: "sonication with low-frequency ultrasounds (LFU) + process high-pressure homogenization (HPH)" and "Ultra-Turrax® + High frequency ultrasounds (HFU)", and (ii) emulsifier free emulsions obtained by "Ultra-Turrax® + High frequency ultrasounds (HFU)".

Emulsions	Droplets size (nm)	ζ potential (mV)	Surface tension (mN/m)
LFU-HPH- SUR	158 ±07[a]	-37.02 ± 0.27 [a]	35.35 ± 1.03 [a]
UT-HFU- SUR	179 ± 05[b]	-40.42 ± 0.71[b]	32.46 ± 1.65[a]
UT-HFU without SUR	185± 08[b]	-53.61 ± 1.95 [c]	42.81 ± 3.02 [b]

The results shown that the smaller droplets were obtained by combination of low frequency ultrasounds and high pressure homogenization. In this emulsification process, sonication by low frequency ultrasounds was followed by high pressure process which permits to obtain smaller droplets and homogenous solution.

The different emulsification process (LFU+HPH) and (UT+HFU) don't allow obtaining the same size average. LFU emulsification combined with HPH emulsification gave smaller droplets sizes than the emulsification with high frequency ultrasounds combined with the rotor-stator premix system. Thus, it was clear that the size of the droplets depends not only on such physical parameters as the amplitude of sonicator, but also on the composition of lecithin and the surface-active properties of the lecithin (Arab Tehrany, Kahn et al. 2012). Low frequency ultrasounds and high pressure homogenization process can get small droplets size. LFU use cavitations phenomena and HPH emulsification is due to impacts forces induced by intense interparticles collisions, significant frictional forces and cavitations forces. These LFU and HPH cavitations allow obtaining smaller droplets which normally induce more emulsion stability. The first emulsion preparation method (LFU+HPH) is a method bringing a high energy to the system, the premix made initially by low frequency ultrasound use the violent cavitation, it followed by HPH using high pressure that can split the bigger droplets into smaller droplets. It provides the emulsions with very small size (lower than 150 nm).

According Canselier *et al.* (2002), D (4.3) droplets diameter obtained when emulsions was made with an Ultra-Turrax® were bigger than those obtained by LFU, and emulsions made by HPH has a smallest droplets diameter compared to Ultra-Turrax® and LFU process (McClements and Gunasekaran 1997).

For the second process, the Ultra-Turrax® premix give a coarse emulsion (Canselier, Delmas et al. 2002), which when treated with high frequency ultrasound is refined, but as mentioned earlier, the HFU provide a lower energy to the system then LFU and HPH and therefore does provide the same droplet size average.

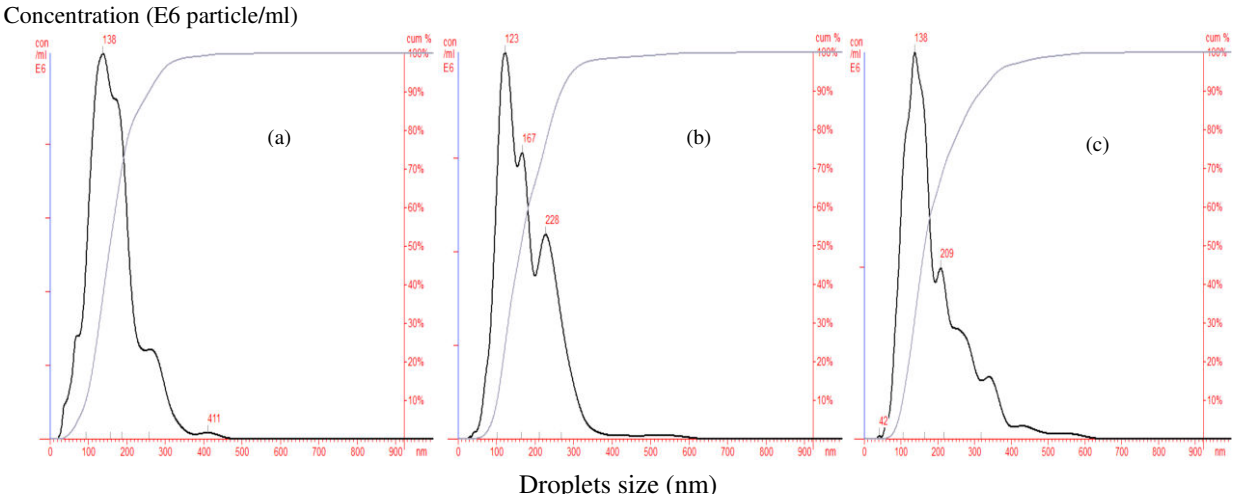

Figure 1: Particle size distribution of emulsions nanoparticles fraction made with three different processes: low-frequency ultrasounds (LFU) + high-pressure homogenization (HPH) with emulsifier or

surfactant (SUR) (a) and ultra-turrax (UT) coupled with high frequency ultrasounds (HFU) with emulsifier or surfactant (b) and without emulsifier (c).

2. Effect of emulsification process on droplets charges

ζ potential was determined for all studied emulsions and represented in Table 1. Emulsion droplets charges inform about emulsions stability. All emulsions show a negative surface charge.

The ζ potential absolute values (above 30 mV) indicate a good stability against droplets coalescence (Zhao, Wang et al. 2010; Tang, Shridharan et al. 2013). For emulsions with emulsifier, Negative charge was explained by rapeseed lecithin addition that contain an anionic fractions of phospholipids (Hung, Fang et al. 2007; Arab Tehrany, Kahn et al. 2012) , but this negative charge was less important compared to the previous study due to the absence of charge for polysorbate 80. In the previous study **(Chapter 4)**, emulsions with emulsifier exhibited more than 45mV (absolute value), ζ potential due to the presence of lecithin rapeseed alone that induce à high negative charge. ζ potential of emulsifier free emulsion was significantly higher ($p<0.05$) than the other emulsion with emulsifier. The absence of charge for polysorbate 80 in emulsion decreases the negative charge. Note here that oil droplets are negatively charged whereas no electrically charged substance in the dispersion. The initial levels were deep enough to allow better dispersion stability for the droplets. As showed, the ζ potential of oil droplets is negative, the following events that are possible to happen on the droplet surface would be responsible

- Carboxylic acid groups that have a permanent dipole and are weakly polar (C^+O^-) orient on the surface of oil droplets to induce a negative charge around them (Kamogawa, Akatsuka et al. 2001)..

- Hydroxyl ions generated in the dissociation of water are stabilized on the droplet surface due to the difference in dielectric constant between the oil droplet and aqueous phase (Schechter, Graciaa et al. 1998) (Croxton 1986; Marinova, Alargova et al. 1996; Kaci, Meziani et al. 2014) (Figure 2).

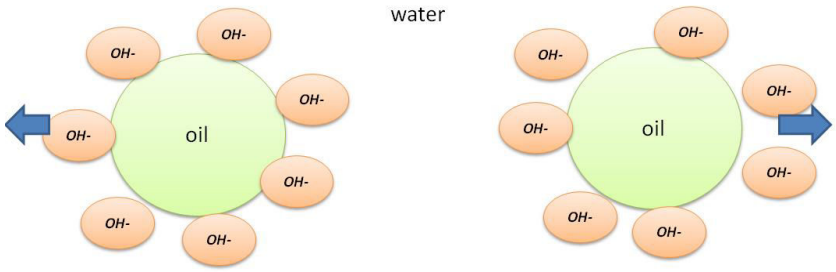

Figure 2: graphical representation of hydroxyl ions organisation at oil water interface (dimensions are not respected in this graphical representation).

- The permanent and induced dipoles are ordered following the structure of hydrophobic hydrated water molecules (Marinova, Alargova et al. 1996). This arrangement may induce the formation of clathrate hydrate structures around oil droplets surface that can explain negatives surface charges (Figure 3).

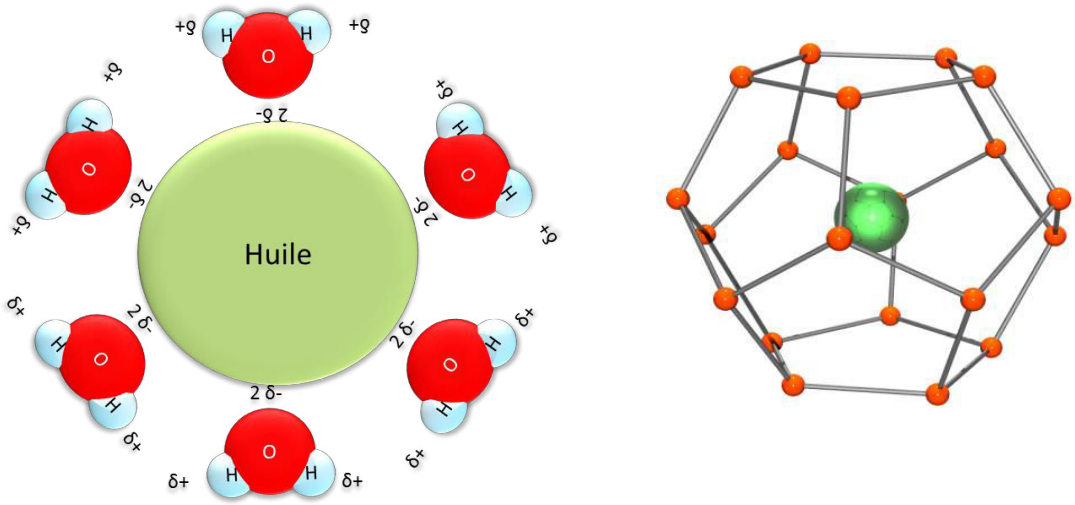

Figure 3: graphical representation of the water molecules arrangement in the oil/water interface of o/w emulsions (dimensions are not respected in this graphical representation).

3. Effect of emulsification process on surface tension

The surface tension of prepared emulsions was measured at 25°C and represented in table 1. The results reveal that emulsion with emulsifier had a lower surface tension induced by the surfactant addition. The surface tension of oil-in-water emulsion is decreased by increasing the emulsifier concentration while the stability of emulsions is increased (Azodi and Nazar 2012). The low surface tension values are explained by the lecithin surfactant action that decrease

emulsion surface tension and make easier the emulsification process. Emulsions without emulsifier had a higher surface tension than emulsion with emulsifier. In these emulsions there is no emulsifier or surfactant for decreasing the surface tension between phases but the presences of electrostatic repulsions induce these stability.

4. Effect of emulsification process on molecules chemical shift compounds by ^{1}H NMR analysis:

NMR spectroscopy has become the standard method for determination of composition and sample interaction. Its application for this purpose is based on the detection of an endogenous acid or base having a resonant frequency (chemical shift) sensitive to the molecules degree of protonation (Gasparovic, Barba et al. 1998; Maherani, Arab-Tehrany et al. 2012). ^{1}H NMR enables a rapid analysis of the overall polysorbate composition resulting in similar proton peak areas for both degraded and non-degraded polysorbate samples (Khossravi, Kao et al. 2002; Verbrugghe, Cocquyt et al. 2010).

Figure 5(a) displays the ^{1}H NMR spectrum of emulsions with and without emulsifier made with HFU. Qualitatively similar spectra were obtained with LFU emulsion and Figure 5(b) displays the ^{1}H NMR spectrum of polysorbate 80 sample and polysorbate 80 with rapessed lecithin.

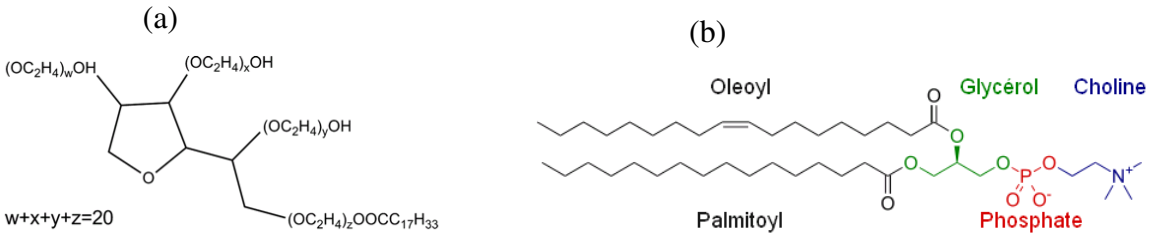

Figure 4: (a) Typival structure of polysorbate 80 (Zhang, Wang et al. 2012), (b) structure of lecithin.

Water signal is identified at ~4.7 ppm, and used as pic reference for treatement of specra. For polysorbate simple (Figure 5b), the ^{1}H NMR polysorbate spectrum in water shows the largest peak at ~3.7 ppm originates from the methylene (–CH_2–) groups of the ethylene oxide (EO) chains linked to the sorbitan ring (Zuriarrain, Zuriarrain et al. 2015).

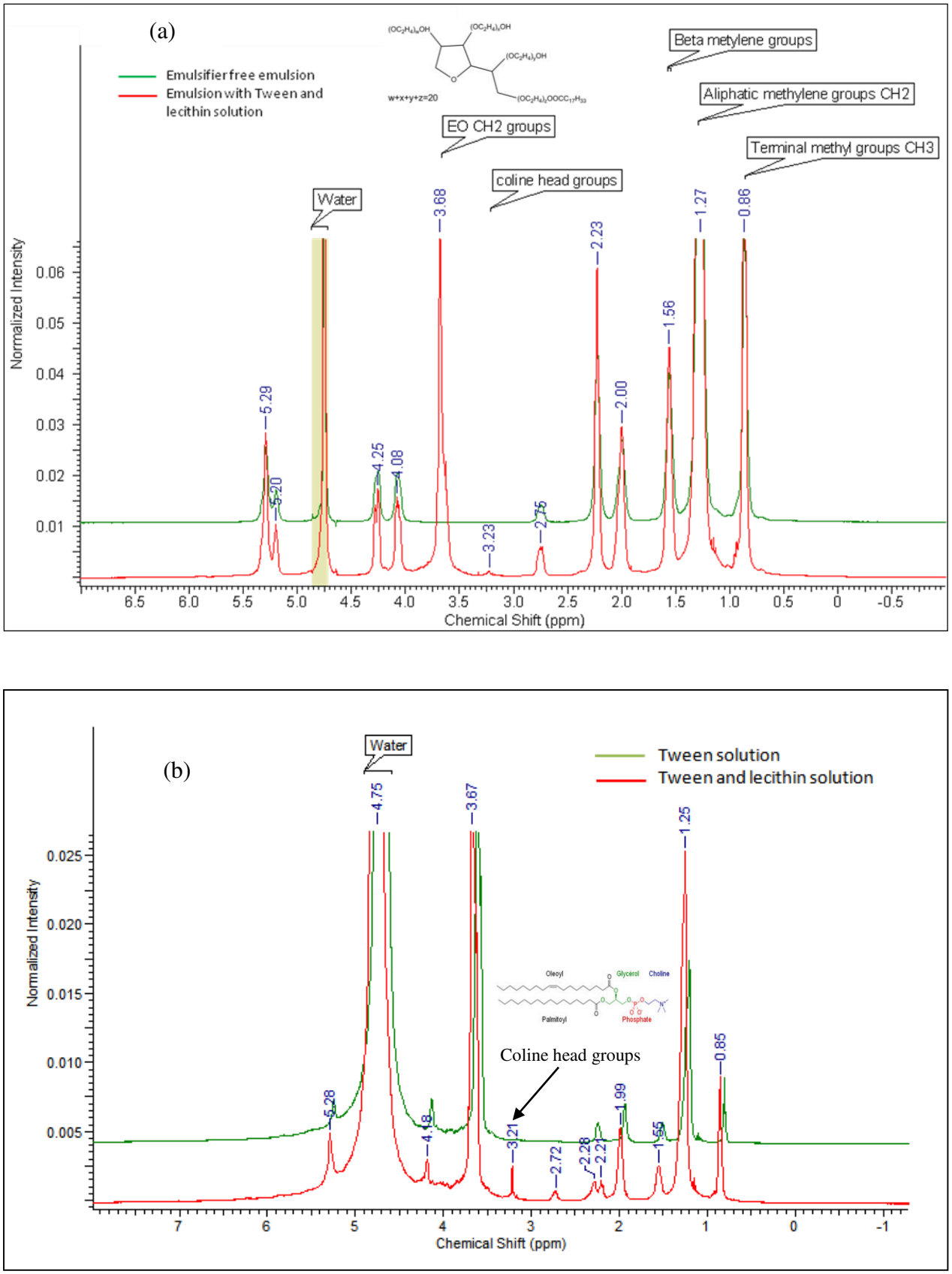

Figure 5: (a) ^{1}H NMR spectrum of emulsions with and without emulsifier made with HFU. (b) ^{1}H NMR spectrum of polysorbate 80 sample and polysorbate 80 with rapessed lecithin

The broadening of the peak is due to a partly overlapping signal coming from the protons of the sorbitan moiety. Most of the aliphatic methylene groups in the fatty acyl part contribute to the resonance at 1.3 ppm, while the terminal methyl group of the fatty acyl chain resonates at 0.9 ppm. The β-methylene groups proximal to the ester occur at 1.6 ppm, respectively. Finally, a relatively small resonance at 4.25 ppm originates from the methylene group of the ethylene oxide unit proximal to the ester bond (Verbrugghe, Cocquyt et al. 2010).

According to literature (Hung, Fang et al. 2007; Arab Tehrany, Kahn et al. 2012), lecithin contains the anionic fractions of phospholipids such as phosphatidylserine, phosphatidic acid, phosphatidylgycerol, phosphatidylethanolamine and phosphatidylinositol with 80% phosphatidylcholine

Two important ^{1}H NMR resonances was originate from lecithin (Stark, Gosselin et al. 1985): the choline head group (3.23 ppm) and the $(CH_2)_n$ (1.27 ppm) groups in the fatty acid moieties of the molecule are responsible for these resonances. These peaks can be used to quantify lecithin in the micellar fraction. No resolved signal in the spectrum could be assigned to cholesterol. Probably this is due to the fact that the concentration of micellar cholesterol is much lower than the concentration of taurocholate. Most resonances of cholesterol are over- lapped by taurocholate resonances as major parts of the molecules are identical (Stark, Gosselin et al. 1985; Groen, Goldhoorn et al. 1990).

^{1}H NMR of emulsion with emulsifier compared with emulsifier free emulsion (Figure 5a), show an identical chemical shift compounds with additional shift at 3.68ppm and 3.23 ppm characteristic of polysorbate and choline head group of lecithin respectively.

^{1}H NMR shows the absence of lecithin and polysorbate in emulsions without emulsifier unlike the emulsion with emulsifiers, and the absence of interaction between different compounds. I addition HFU manufacturing process does not cause chemical degradation or neoformed compounds.

5. Effect of emulsification process on emulsions structure by SAXS analysis:

SAXS is suitable for structural analysis of materials. It enables determination of the structure of objects such as dispersions, biological macromolecules solutions and emulsions. The scattering pattern of SAXS contains information about the structure, shape, and macromolecules size and the surface to volume ratio of particles.

Interface thikness also is a noticeable property of emulsions structure having a significant effect on emulsions application, since an increase in thikness could result in permeation release of the amphiphilic drugs from emulsions droplets with thin interface is greater than from thick interface with similar size (Maherani, Arab-tehrany et al. 2011).

As the structure extends further in two dimensions compared to its thickness, the form factor can be described as the separated product of the factor ~$1/q^2$ and the thickness scattering function, and the indirect Fourier transform is calculated for the thickness of a flat structure. The structure factor is calculated for a lamellar structure according to the modified Caillé theory (Zhang, Suter et al. 1994; Fruhwirth, Fritz et al. 2004; Maherani, Arab-tehrany et al. 2011).

The Figure 6 represents the small angle X-ray scattering of studied emulsions and used emulsifiers. The same signal was registered at the small angle q <0.2 nm-1. This signal corresponding to q^{-4} slop that indicates the diffusion of the droplets interface. Diffusion type I (q) = q^{-4} (Figure 1 (a,b) is characteristic of a thin interface between two phases with different electronic density (water/oil). this diffusion signal is characteristic of dispersion. The signal intensity at the small angles was higher for emulsions with surfactant due to diffusion of micelles added emulsion droplets. The signal of the interface (q <0.2 nm-1) is identical for emulsions with or without emulsifiers.

An intensity difference was registered from q> 0.2 nm^{-1} for emulsions with and without emulsifiers made by high frequency ultrasounds (Figure 6 (b)). Indeed, a higher intensity is recorded for the emulsion with emulsifier; this higher intensity was found in Figure 6(b) corresponding to X-ray diffusion of lecithin liposomes with polysorbate 80. A diffusion hump was recorded at q=1 nm^{-1} which corresponds to a distance of 6.3 nm which represents structure factor of Tween 80 micelles measured with DLS. These micelles with solvation layer have 8-9 nm size average (Figure 6 (c)).

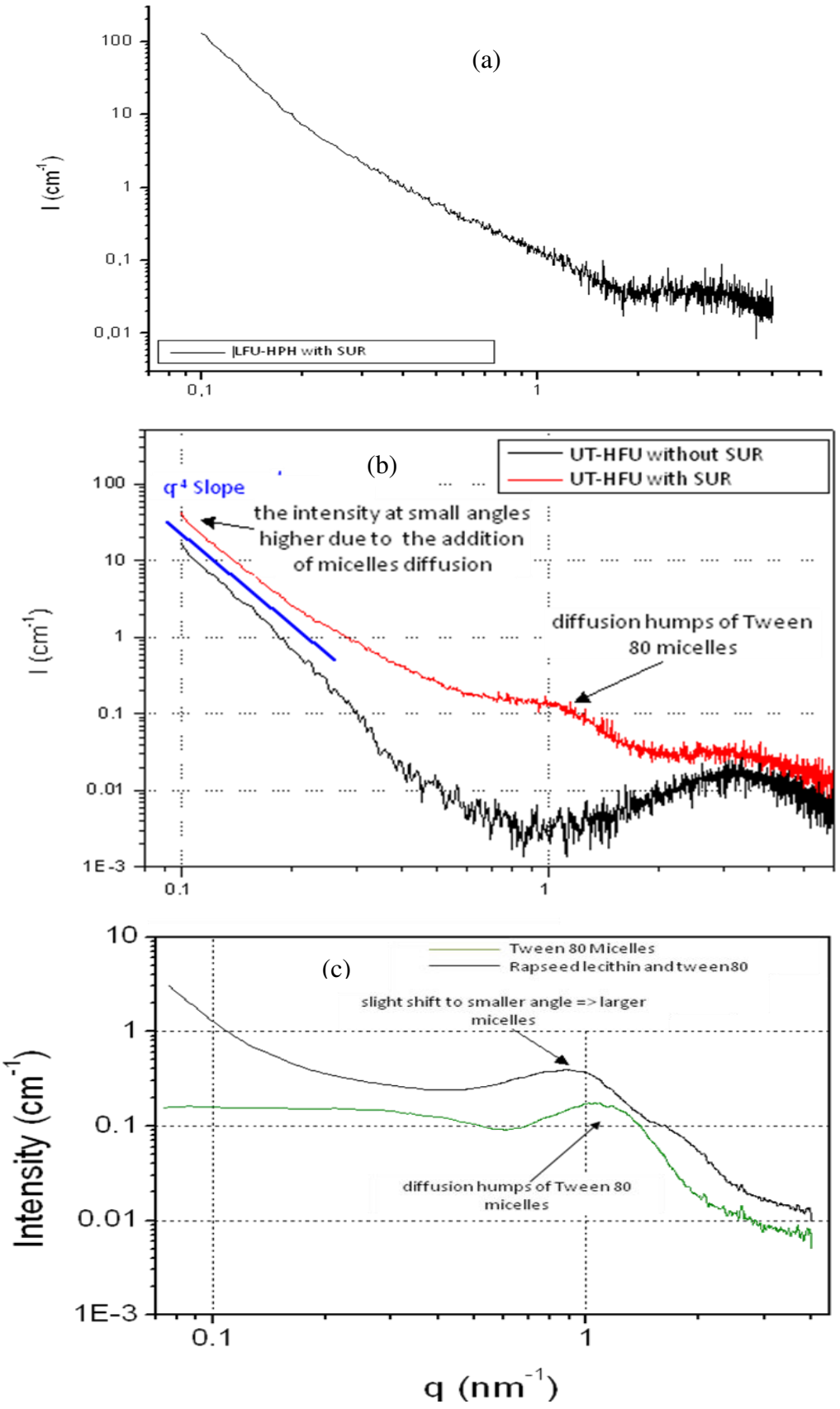

Figure 6: SAXS curves of (a) LFU-HPH emulsion with emulsifiers, (b) UT-HFU emulsion with emulsifiers and without emulsifiers, (c) Polysorbate 80 sample and Polysorbate 80 with rapeseed lecithin.

The lecithin added to Polysorbate 80 form liposome's which show a high intensity small angles q <0.2 nm-1 compared to Polysorbate 80 which shows a tray intensity at small angles.

The sample lecithin + Polysorbate 80 show an intensity at small angles and two bumps diffusion at 7 nm and 3.5Nm distances with ratio of 2, that suggest the presence of bilayers obtained with lecithin addition. Several equidistant broad peaks are also observed, suggesting a multilamellar stack of bilayers.

Conclusion

Ultrasonic emulsification was described as very efficient process enable to produce emulsions with very small droplets and allow obtaining a very stable oil/water emulsions without emulsifier addition. Physicochemical characterisation of studied emulsions show that the two processes allow nanoémulsions manufacturing. LFU+HPH give smaller size distribution then UT+HFU due to powerful shear stress applied to LFU+HPH emulsion due to cavitations phenomena. UT+HFU don't provide a powerful shear that induces using of longer process times but allows finely obtaining nanoémulsions with small and narrow size distribution without cavitations phenomena that induce molecules degradation. ζ potential measurement showed negative droplets charge for all emulsions due to negative charge of lecithin for emulsions with emulsifier and due to adsorption of OH^- at oil/water interface leading to of droplets stability on emulsion.

The study of composition and interactions by 1H NMR analysis shows the absence of interaction between different compounds and shows that the HFU manufacturing process does not cause chemical degradation or neoformed compounds.

Small angle X-ray scattering (SAXS) show the same signal at the small angle q <0.2 nm^{-1} for emulsion with and without emulsifiers corresponding to q^{-4} slop characteristic of a thin interface between two phases with different electronic density (water/oil) and emulsion with emulsifiers show Polysorbate 80 micelles diffusion signal which doesn't appear in the emulsion without emulsifiers.

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Conclusions et perspectives

Conclusions et perspectives

Les émulsions sont des systèmes présentant un grand intérêt dans divers domaines (alimentaire, cosmétique et pharmaceutique). Ainsi plusieurs procédés permettent l'obtention d'émulsions très fines ou nanoémulsions, nous citerons : homogénéisation à haute pression, méthodes d'émulsification spontanée, émulsification par membrane…etc., mais ces méthodes nécessitent l'utilisation de stabilisant pour le maintien de ces émulsions, afin d'éviter ou de ralentir les mécanismes de dégradation (coalescence, floculation,…). En ce qui concerne l'émulsification acoustique utilisant les hautes fréquences, cette dernière peut permettre l'obtention d'émulsions qui présentent une taille de gouttelettes faible et une stabilité importante au court du temps et surtout sans utilisation d'agents émulsifiants.

L'objectif de ce travail était le développement d'un nouveau procédé d'émulsification permettant l'obtention de nanoémulsions cinétiquement stables dépourvues d'agents stabilisants. Cette méthode d'émulsification brevetée par la société GENIALIS consiste à utiliser des ultrasons de hautes fréquences (1,7MHz) pour éviter les effets mécaniques violents de la cavitation acoustique aux basses fréquences.

Lors de la première étude, l'objectif était de voir l'effet du temps d'émulsification par ultrasons de hautes fréquences sur les propriétés physicochimiques des émulsions. Plusieurs temps d'émulsification (2h, 4h, 6h, 8h, 10h) par ultrasons de hautes fréquences (1,7MHz) ont été testés. L'émulsification a été faite avec différentes proportions d'huile végétale (5%, 10%, 15%). Pour la caractérisation physicochimique, nous avons procédé à l'étude de la répartition granulométrique, du potentiel zêta ζ, du pH, de la turbidité, de la conductivité ainsi que de la composition en acides gras de la phase huileuse émulsionnée. L'étude de la répartition granulométrique a montré une diminution de la taille des particules en fonction du temps de traitement. Les gouttelettes d'huile émulsionnées sont chargées négativement ce qui entraine une répulsion électrostatique des gouttelettes dispersées permettant de prévenir les phénomènes de coalescence et d'empêcher la déstabilisation de l'émulsion. Une diminution significative du potentiel zêta ($p<0,05$) soutient l'hypothèse de formation de couche de OH- autour des gouttelettes d'huile entrainant la formation de couche contre-ions donnant naissance aux couches de Stern.

La seconde étude consistait à comparer les propriétés physico-chimiques des émulsions sans émulsifiants obtenues par le procédé à ultrassons de hautes fréquences (UHF), aux émulsions obtenues par procédés d'émulsification standards tels que l'homogénéisation à haute pression (HHP) et la sonication par ultrasons de basses fréquences (UBF). L'influence de l'incorporation d'un principe actif qui est l'huile essentielle d'orange dans les émulsions sur les propriétés physico-chimique a été étudiée. Les résultats des mesures des répartitions granulométriques ont montré que la fraction nanométrique est plus importante pour les émulsions faites par les ultrasons de hautes fréquences. Elle représente plus de 90% des gouttelettes de l'émulsion UHF et ne représente que 50 à 70% pour les émulsions HHP et UBF. Les émulsions UHF ont montré une stabilité pendant une durée de 30 jours à 37°C contrairement aux autres émulsions. La mesure de la tension de surface a mis en évidence un crémage des émulsions HHP et UBF non présent pour les émulsions UHF. Les résultats de l'analyse FTIR ont montré que la fraction huileuse des émulsions a un même spectre que l'huile d'origine (avant émulsification) ce qui traduit une absence de dégradation.

Une étude de l'application des émulsions sans émulsifiants en domaine cosmétologique menée dans la troisième partie de ce travail montrent une stabilité physico-chimique lors de leur utilisation comme vecteur de coenzyme Q_{10}. Une étude d'encapsulation d'un principe actif «coenzyme Q_{10}» a été réalisée avec des émulsions contenant un tensioactif (lécithine) et les émulsions sans tensioactif faites par le procédé d'émulsification par hautes fréquences. En effet, lors de l'utilisation du principe actif «coenzyme Q_{10}», nous obtenons des résultats satisfaisants permettant de garantir des nanoémulsions, stables, ayant un profil rhéologique attendu. Des tests *in vitro* sur cellules humaines ont pu mettre en évidence l'absence de toxicité du vecteur (émulsion sans principe actif) et l'efficacité du principe actif. En l'absence de tensioactif, les émulsions ont permis une meilleure libération du coenzyme Q_{10}, et une meilleure prolifération cellulaire et une augmentation de l'activité métabolique des cellules.

Une étude approfondie des émulsions sans émulsifiant par diffusion des rayons X aux petits angles (SAXS) et par résonance magnétique nucléaire (RMN) a était réalisée et comparée à des émulsions contenant des émulsifiants. L'étude SAXS montre clairement l'absence de micelles de tensioactifs pour les émulsions sans émulsifiants. Les résultats de la RMN montrent l'absence de la signature des tensioactifs et des phospholipides dans les émulsions faites par ultrasons de hautes fréquences. Par ailleurs, la RMN montre l'absence d'interaction entre des différents constituants de l'émulsion et aussi l'absence de structures néoformées ce qui montre que le procédé d'émulsification par hautes fréquences est non destructif. Il convient donc

parfaitement aux applications cosmétiques et surtout pharmaceutiques qui utilisent des principes actifs couteux et sensibles, ce qui permettra d'augmenter leur efficacité tout en réduisant les doses utilisées.

Cette étude a permis d'ouvrir un large champ d'investigation autant sur le plan scientifique que sur le plan industriel. Cette étude a abouti à une compréhension globale de l'effet des ultrasons de hautes fréquences sur l'émulsification et la vectorisation d'actifs hydrophobes en traitant les mécanismes d'émulsification d'une part et la mise en place de pilote industriel d'autre part.

Cette thèse Cifre financée par la société GENIALIS a permis l'installation d'une unité industrielle. Un scale-up est en cours de réalisation pour la mise en place d'un pilote industriel et la production d'émulsions sans émulsifiant en continu. Des essais sont en cours de réalisation pour ajuster les paramètres de débit et de temps de séjour pour une stabilité optimale. Néanmoins de nombreux approfondissement restent à réaliser pour mieux appréhender l'effet du procédé sur la stabilité des émulsions sans émulsifiant.

- D'autres fréquences ultrasonores dans la gamme brevetée (1-3 MHz), ainsi que le positionnement et l'inclinaison des céramiques piézoélectrique feront l'objet d'études ultérieures. Le positionnement et la distance entre les membranes piézoélectriques est à approfondir pour permettre une meilleur focalisation des ondes ultrasonores de hautes fréquences lors de l'écoulement à travers les zones de traitement du pilote.
- Pour diminuer les temps longs de procédé dus aux faibles cisaillements subis par les émulsions faites par ultrasons de hautes fréquences, un procédé d'émulsification membranaire sera couplé avec ce procédé qui se chargera de stabiliser les gouttelettes nanométriques formées en amont pas la membrane d'émulsification.
- Des applications en parfumerie sont en cours d'étude pour l'obtention de parfum sans alcool permettant d'ouvrir des portes vers de grands marchés, dans le cadre d'un projet FUI (ESSENZ'O 2015-2017).
- Une optimisation de la partie électronique des céramiques piézoélectriques est en cours pour limiter les pertes d'énergie dus aux échauffements des composants.
- Afin de mieux comprendre les mécanismes de stabilisation des émulsions sans émulsifiant, une étude des interfaces huile/eau par SAXS avec un rayonnement synchrotron et avec un suivi de cinétique permettra d'avoir une meilleure résolution et une large fenêtre de vision des interfaces.

- Une étude par diffusion des neutrons aux petits angles (SANS) permettant la variation de contraste de la phase continue entourant les gouttelettes stabilisées mettra en évidence les phénomènes se produisant aux interfaces huile/eau.

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Emulsification by high frequency ultrasound using piezoelectric transducer: Formation and stability of emulsifier free emulsion

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Conductivity

ABSTRACT

Emulsifier free emulsion was developed with a new patented technique for food and cosmetic applications. This emulsification process dispersed oil droplets in water without any emulsifier. Emulsions were prepared with different vegetable oil ratios 5%, 10% and 15% (v/v) using high frequency ultrasounds generated by piezoelectric ceramic transducer vibrating at 1.7 MHz. The emulsion was prepared with various emulsification times between 0 and 10 h. Oil droplets size was measured by laser granulometry. The pH variation was monitored; electrophoretic mobility and conductivity variation were measured using Zêtasizer equipment during emulsification process. The results revealed that oil droplets average size decreased significantly ($p < 0.05$) during the first 6 h of emulsification process and that from 160 to 1 μm for emulsions with 5%, 10% and from 400 to 29 μm for emulsion with 15% of initial oil ratio.

For all tested oil ratios, pH measurement showed significant decrease and negative electrophoretic mobility showed the accumulation of OH^- at oil/water interface leading to droplets stability in the emulsion. The conductivity of emulsions showed a decrease of the ions quantity in solution, which indicated formation of positive charge layer around OH^- structure. They constitute a double ionic layer around oil particles providing emulsion stability. This study showed a strong correlation between turbidity measurement and proportion of emulsified oil.

1. Introduction

Emulsions are generally prepared with surface active agents (surfactant) [1–3] or amphiphilic polymers [2] to reduce interfacial tension. Emulsion stability is due to adsorption of stabilizer at oil/water interface creating electrostatic repulsion [1] and steric repulsion for emulsions containing polymers [4]. When emulsifier is added, it may interact with the other formulations compounds creating new emulsion properties. Therefore, it becomes difficult to study the role of oil phase alone on emulsion properties. The fraction of emulsifier in materials will greatly influence the purification and performance of the products [5]. Emulsifier absence should expose essential features of oil droplets themselves [2].

In ultrasonic emulsification, the sound ranges used for food can be divided into high-frequency/low-energy ultrasounds and low-frequency/high-energy power ultrasounds [6].

High frequency ultrasounds are usually used as a nondestructive, rapid, easy-to-automate, and relatively inexpensive analytical technique for quality assurance and process control with particular reference to physicochemical properties, such as composition, structure, physical state and molecular properties of foods [6]. As well as being used as an analytical instrument in the laboratory, ultrasound can also be used for continuous monitoring of food properties on-line during processing [7].

High frequency ultrasounds go through solid or liquid media without affecting their structure and are used as diagnosis tools e.g. analysis and medical imaging [8]. HF ultrasounds were used in different applications, such as quality control of food, commercial cooking oils, bread and cereal products, bulk and emulsified fat based food products, food gels, aerated and frozen foods, improvement in mass transfer, food preservation, support of thermal treatments, texture improvement and food analysis [9,10].

These low energy ultrasounds have frequencies above 1 MHz at intensities below 1 W cm^{-2}. They have been used for non-destructive action on genetic improvement programs; it is also used for evaluating compositions of raw and fermented meat products and for evaluating composition of fish and poultry. On the other

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Développement et caractérisation d'un nouveau procédé d'émulsification non dénaturant par transduction piézoélectrique de hautes fréquences

Résumé

Les émulsions représentent une large gamme de produits alimentaires, cosmétiques et pharmaceutiques. Pour assurer leur stabilité, une interface chargée de tensioactif est nécessaire. Cette interface constitue une barrière contre la coalescence mais gène la libération des principes actifs encapsulés. Dans cette thèse, une nouvelle méthode d'émulsification est développée. Elle consiste à utiliser des ultrasons à hautes fréquences (UHF) (1,7MHz) qui permettent d'avoir des émulsions stables sans émulsifiants tout en évitant les effets mécaniques violents de la cavitation acoustique présente aux basses fréquences. L'étude des répartitions granulométriques a montré une diminution significative de la taille des gouttelettes d'huile au cours du temps de traitement par ultrasons de hautes fréquences. Le suivi du pH des émulsions montre une forte diminution et une charge de surface importante des gouttelettes est enregistrée ce qui montre une accumulation d'ions OH^- à l'interface l'huile/eau conduisant à la stabilité des gouttelettes dans l'émulsion. La conductivité des émulsions diminue durant l'émulsification traduisant une baisse de la quantité d'ions en solution, ce qui indique la formation de la couche contre ions (charge positive) autour de la structure des OH^-. Les résultats montrent une stabilisation électrostatique des émulsions obtenue par la formation d'une double couche ionique autour des gouttelettes d'huile. Contrairement aux procédés d'émulsification standard, les émulsions faites par ce procédé montrent une stabilité de 30 jours à 37°C. L'utilisation des émulsions sans émulsifiant faites par UHF pour la vectorisation de CoQ_{10} montre une prolifération cellulaire plus élevée que dans le cas des émulsions avec émulsifiant. Une étude approfondie des émulsions sans émulsifiant par diffusion des rayons X aux petits angles (SAXS) et par résonance magnétique nucléaire (RMN) a été réalisée et comparée à des émulsions contenant un ou plusieurs émulsifiants. L'étude SAXS montre clairement l'absence de micelles de tensioactifs pour les émulsions sans émulsifiants et les résultats de la RMN montrent l'absence de la signature des tensioactifs et des phospholipides dans les émulsions faites par ultrasons de hautes fréquences. Par ailleurs, la RMN montre l'absence d'interaction entre des différents constituants de l'émulsion et aussi l'absence de structure néoformées.

Mots clés : émulsions sans émulsifiant, ultrasons de hautes fréquences, charge de l'interface, Vectorisation et relargage, biodisponibilité, FTIR, SAXS, RMN

Abstract

Emulsions are systems containing two immiscible liquids, one dispersed as droplets (dispersed phase) throughout the other (continuous phase). When emulsifier is added, it may interact with the other formulations compounds creating new emulsion properties. Therefore, it becomes difficult to study the role of oil phase alone on emulsion properties. In this thesis, emulsifier free emulsion was developed with high frequency ultrasounds (HFU) generated by piezoelectric ceramic transducer vibrating at 1.7 MHz. pH measurement showed significant decrease and negative electrophoretic mobility showed the accumulation of OH^- at oil/water interface leading to droplets stability in the emulsion. Emulsions conductivity showed a decrease of the ions quantity in solution, which indicated formation of positive charge layer around OH^- structure. They constituted a double ionic layer around oil particles providing emulsion stability. This study showed a strong correlation between turbidity measurement and proportion of emulsified oil. Unlike standard emulsification methods, emulsions made this process demonstrates stability for 30 days at 37 °C. The use of emulsions without emulsifier made by HFU for vectoring CoQ10 shows a higher cell proliferation in the case of emulsion without emulsifier. A study of emulsions without emulsifer by small angle X-ray scattering (SAXS) and nuclear magnetic resonance (^{1}H NMR) was performed and compared to emulsions containing emulsifiers. NMR analysis showed no interactions between different compounds and the HFU manufacturing process did not cause chemical degradation or neoformed compounds. SAXS showed a thin interface between two phases with different electronic density (water/oil) for emulsions with and without emulsifiers. For emulsion with emulsifiers SAXS showed surfactant micelles diffusion signal which doesn't appear in the emulsion without emulsifiers.

Keywords: emulsifier free emulsions, high frequency ultrasounds, interface charge, vectorization and release, bioavailability, FTIR, SAXS, RMN.