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Emulsion Congealing Technique
– The optimal Parameters –

Monografia realizada no âmbito da unidade curricular de Acompanhamento Farmacêutico do Mestrado Integrado em Ciências Farmacêuticas, orientada pelo Professor Doutor João Canotilho, e apresentada à Faculdade de Farmácia da Universidade de Coimbra

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UNIVERSIDADE DE COIMBRA

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O Tutor da Monografia

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A Estudante

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Abstract

Microencapsulation technology is of interest to a wide range of industries, including pharmaceutical, food, agricultural, biotechnological, cosmetic, and other industries with various significant advantages [1]. A major application of microencapsulation technique in the pharmaceutical field is controlled or sustained drug delivery. In these past hear it was created various technologies that allowed the parenteral the administration of hydrophobic drugs, such as microencapsulation. One of these techniques is the Emulsion Congealing Technique. The objective of this experiment was to see the influence of different parameters in the shape and size of microparticles, using a hydrophobic drug for a parenteral administration; and to find an optimal protocol for this objective.

Keywords: microparticles, emulsion congealing, optimization, donut microparticles

Introduction

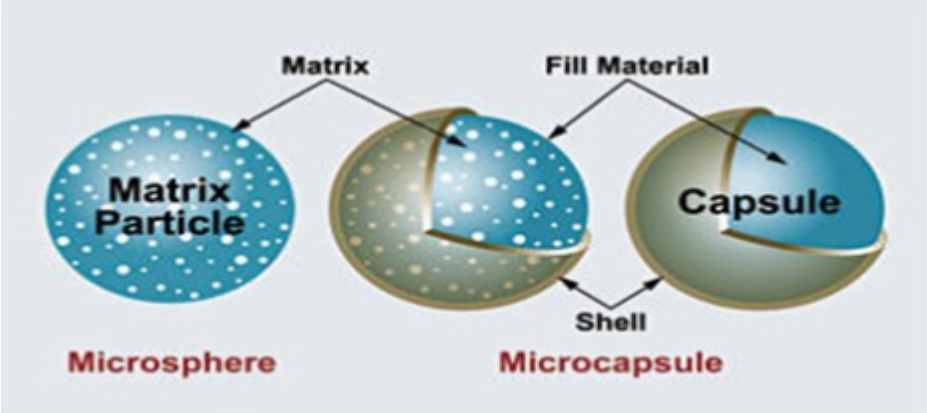
With the constant development of new drugs it became essential to create new type of ways to facilitate the administration of these new drugs. The main problem is that some for some drugs that the only possibility is a parenteral administration we have to create a way to transform the impossible to the possible. Parenteral route is the only choice for the administration of the hydrophobic drugs, and is also the most preferred route of administration in the case of emergency as it ensures very quick onset action [(2)]. In these past hear it was created numerous technologies that allowed the parenteral administration of hydrophobic drugs, such as microencapsulation. The technique that I chose to study was the Emulsion Congealing Technique, in part because it was an unknown technique for me, but also because it hasn't been found the optimal parameters for this technique.

Objective

Obtain an optimal formulation for the preparation of roundshape microparticles for parenteral use, using primarily the emulsion congealing technique.

1. Microencapsulation

Microencapsulation is the enveloping of liquid droplets or fine solid particles to form microcapsules, having an average diameter as small as 1 μm to 1000 μm. Microencapsulation technology is of interest to a wide range of industries, including pharmaceutical, food, agricultural, biotechnological, cosmetic, and other industries with various significant advantages, including: an effective protection of the encapsulated active ingredient against degradation; the possibility to control the release rate of the active ingredient [(1)]. Depending on the parameters such as type of solvent, drug loading, drug solubility and preparation technique a certain inner structure results whereby the microparticles can be divided into microspheres and microcapsules. Microspheres are one-block systems were the active ingredient can be dispersed or dissolve within the matrix. Microcapsules consist of a core which is surrounded by a shell [(3)].



Picture 1 - Microparticles structures

1.1 Application of microencapsulation

A major application of microencapsulation technique in the pharmaceutical field is controlled or sustained drug delivery. A wide number of pharmaceutical microencapsulated products are currently on the market, such as:

- Aspirin controlled release tablets (ZORprin®CR) are used to treat pain and fever, to relieve pain and inflammation associated with arthritis and other inflammatory conditions;
- Cephalexin (Ceff-ER) and Cefadroxl (Odoxil OD) antibiotic for bacterial infections;
- Microencapsulation of proteins and peptides has recently become a relevant alternative to develop novel drug delivery system.

The number of commercially available microsphere does not reflect the amount of research that has been carried out in this field, nor did the benefits that can achieve using this technology. Microspheres have also been found potential applications as inhalation or injection products [3].

Microparticles present various advantages as so:

- ☞ Improvement of the patient compliance; reducing the pain, irritation and thrombophlebitis (in rabbits, an emulsion formulation of diazepam caused significant reduction in local tissue reaction);
- ☞ Increase of bioavailability;
- ☞ Alter the drug release;
- ☞ Produce a targeted drug delivery (this approach has been recently extended to injectable lipid emulsions).
- ☞ Reduced Toxicity, reducing the reactivity of the core in relation to the outside of the environment;
- ☞ Improved Stability and Solubility (a number of drugs such as clarithromycin, demonstrated improved stability in emulsion formulation);
- ☞ Decrease evaporation rate of the core material (reduction of volatility);

Nevertheless, with these new technologies there's always some disadvantages that need to be solved or improved. Some of the major issues are:

- ☞ Certain solvents approved by the regulatory agencies are not necessarily good solvents of lipophilic drugs;
- ☞ The oil phase in the emulsion system generally does not exceed 30% causing drug-loading challenges for drugs with high dose requirements;
- ☞ Incorporated drugs may render the emulsion physically unstable during storage making formulation efforts challenging, and there are limited number of approved safe emulsifiers to stabilize the emulsion system. [4]

1.2 Administration of hydrophobic drugs

Parenteral route is the only choice for the administration of the hydrophobic drugs such as amphotericin B and paclitaxel, which are poorly absorbed by the oral route. Parenteral route is also the most preferred route of administration in the case of emergency as it ensures very quick onset of action. However, design of parenteral drug delivery systems is a critical and challenging task as the number of excipients approved for parenteral delivery is considerably small.[2][3][4]

Emulsions are preferred for the delivery of hydrophobic drugs due to advantages such as:

- ☞ Ability to solubilize considerable amount of drug;
- ☞ Ability to prevent hydrolysis of the drugs such as barbiturates;
- ☞ Ease of manufacture and scale-up and low cost as compared to the other colloidal carriers such as liposomes.

However, emulsions suffer from various disadvantages such as:

- ➢ Poor physical stability on long-term storage;
- ➢ Risk of emboli formation;
- ➢ The need for strict aseptic handling and rapid growth of microorganisms.

1.3 Microparticles Techniques

Microemulsions have evolved as second generation colloidal carrier systems and are being preferred over emulsions in several cases. Microemulsions are thermodynamically stable, transparent, low-viscosity colloidal dispersions consisting of microdomains of oil and/or water stabilized by an interfacial film of alternating surfactant and cosurfactant molecules [(2)].

There is many methods for microparticles preparation and that can be divide into:

- ➢ General Methods: Single emulsion technique and double emulsion technique;
- ➢ Physical methods: Phase Separation coacervation technique;
- ➢ Mechanical methods: Spray drying & congealing Fluidized Bed Technology Solvent evaporation Pan Coating Rotational Suspension Separation Extrusion Nozzle Vibration Technology Multiorifice Centrifugal Process.

The choice of technique depends on the nature of the polymer, the drug, the intended use and the duration of the therapy.

The melt dispersion technique (or Emulsion Congealing Technique) for the preparation of wax microparticles as an alternative to polymeric systems was first describe by Bodmeier et al. as an advantageous method due to its easy processability of low-viscosity melts thus obviating the need for organic solvents. Standard methods are the aqueous and the non-aqueous melting methods [(5)].

Procedures for making drug-load microparticles are based frequently on emulsification methods than can be classified into oil-in-water (o/w), oil-in-oil (o/o) and water-in-oil-in-water (w/o/w) (double emulsion) types. Their definition is self-explanatory,

such as lipophilic material dispersed as non-miscible droplets throughout an aqueous continuous phase is referred to as an o/w emulsion and so on [6]. The w/o/w emulsified system is perhaps more sophisticated than two-phase systems, whereupon a w/o emulsion is formed first and then further emulsified in another aqueous phase to form a double emulsion. The o/w method is straightforward, produces elegant, spherical MP, but is limit to inclusion of soil-soluble drugs and is not suited to the encapsulation of water-soluble counter-parts [6].

In the o/w – melting method the drug must be dissolved or particularly dispersed in the molten lipid. The drug-containing molten lipid phase is then emulsified into a heated emulsifier – containing external phase by vigorous mixing. Here, it is crucial to keep the temperature of the external phase above the melting point of the lipid in order to avoid premature congealing of the lipid and assure particle formation. The solution or dispersion can then be cooled down while high speed mixing whereupon the molten lipid hardens and forms particles. The microparticle suspension is sieved and washed and allowed to dry. A disadvantage of the o/w melt dispersion method is that highly water-soluble drugs cannot be encapsulated using this technique because the drug can be lost in the external aqueous phase during the emulsification process resulting in low encapsulation efficiencies.

1.4 Microencapsulation Process

In general the phenomenon of microemulsification is mainly governed by the factors such as: the natures and concentrations of the oil, surfactant, cosurfactant and aqueous phase; oil/surfactant and surfactant/cosurfactant ratio; temperature and pH of the environment and physicochemical properties of the drug such as hydrophilicity/lipophilicity, pKa and polarity. Usually the oil phase has the maximum solubilizing potential for the selected drug candidate which helps to achieve the maximal drug loading in the microemulsions. At the same time, the ability of the selected oil to yield systems with larger microemulsion existence region is also important. The choice of the oil phase is often a compromise between its ability to solubilize the drug and its ability to facilitate formation of microemulsions of desired characteristics.

The surfactant should favor microemulsification of the oil phase and should also possess good solubilizing potential for the drug. High HLSB surfactants such as Polysorbate 80 (Tween 80) are preferred for o/w microemulsion. Cosurfactants are needed to bring

about the surface tension close to zero. For parenteral microemulsions, short chain alcohols such as ethanol and benzyl alcohol can be employed as cosurfactants.

The aqueous phase, in parenteral microemulsions, should be isoosmotic to the blood which is achieved with the help of additives such as electrolytes (sodium chloride), glycerol, dextrose and sorbitol.

2. Characteristics of surfactants and emulsions

2.1 Surfactants

A surfactant possesses approximately an equal ration between the polar and the nonpolar portions of each molecule. When placed in an oil-water system, the polar groups are attracted to the water, and the nonpolar groups are oriented toward the oil. The surfactant lowers the interfacial tension between the oil and water phases. Surfactants are classified as cationic, anionic and nonionic based on the type of polar group on the surfactant. Cationic surfactants are often used as antibacterial agents because of their ability to disrupt the cell membrane of the microorganism. The ionized surfactants have relatively high water solubility and thus generally make oil in water emulsions. The nonionic surfactants, however, can be used to make either type of emulsion.

An o/w emulsion is generally formed if the aqueous phase constitutes >45% of the total weight and a hydrophilic emulsifier is used. Emulsions are used in many routes of administration. More typically, emulsions are used for topical administration. [7]

2.2 Emulsifying agents

Emulsions are stabilized by adding an emulsifier or emulsifying agents. Emulsifiers stabilize emulsions by reducing the interfacial tension of the system and by providing enough surface charge for droplet-droplet repulsion.

The choice of emulsifier is driven by its toxicity profile, intended site of delivery, and stabilizing potential. The small size of the microemulsions may result in higher blood circulation time which would be useful in certain cases. The excellent thermodynamic stability, high solubilization capacity, low-viscosity and ability to withstand sterilization techniques make microemulsions an interesting delivery system. The W/O microemulsions can be used for controlled delivery of the hydrophilic therapeutic actives such as aminoglycoside antibiotics. Several anti-cancer agents are required to be administered by parenteral route. However, poor water-solubility and high degree of toxic side effects limit their parenteral delivery. Most of the anti-cancer agents are formulated as a mixture of co-

solvents and surfactants and suffer from common problems associated co-solvent based parenteral formulations as described earlier.

All emulsifying agents concentrate at and are adsorbed on the oil/water interface to provide a protective barrier around the dispersed droplets. In addition to this protective barrier, emulsifiers stabilize the emulsion by reducing the interfacial tension of the system. Some agents enhance stability by imparting a charge on the droplet surface thus reducing the physical contact between the droplets and decreasing the potential for coalescence. Some commonly used emulsifying agents include tragacanth, sodium lauryl sulfate, sodium dioctyl sulfosuccinae, and polymers known as the Spans® and Tweens®.

Emulsifying agents can be classified according to:

1) Chemical structure: are synthetic, natural, finely dispersed solids, and auxiliary agents;

2) Mechanism of action: are monomolecular, multimolecular, and solid particle films.

Regardless of their classification, all emulsifying agents must be chemically stable in the system, inert and chemically non-reactive with other emulsion components, and nontoxic and nonirritant. They should be reasonably odorless and not cost prohibitive [(5)].

3. Experiment Analysis

Keeping all the theory in mind, the objective was to see the influence of different parameters in the preparation of microparticles for parenteral use. It was important to choose a technique that the optimal roundshape haven't be achieved yet and to use a hydrophobic drug that increases the difficulty of the preparation and administration. Consequently it was chosen the Emulsion Congealing Technique, using as emulsifier and stabilizer Tween 80, and changing numerous parameters as the amount of water, polymer (Dynasan 118), velocity of the mixer, type of the mixer, cooling process, etc. For these purpose it was designed numerous protocols.

The Emulsion Congealing Technique can produce microparticles W/O with much effortless. The real difficulty is to obtain all the microparticles with roundshape and in the same size range essential for parenteral administration (± 120 μm). The main problem with this technique is that when the microparticles are formed, somehow they don't have enough stability in their shape, that is provoke by the instability of the superficial tension between the fat phase (Dynasan 118) and the water phase. The first step was to design an experimental protocol just to see the influence of the parameters in the size, and shape of the microparticles.

Parameters: amount of tween 80, time of mixing and rotation speed.

Materials:

- Two 250 ml beakers
- Two heaters in a magnetic stirrer plate
- A propeller mixer
- One 500 ml beaker
- 45 µm sieve
- Glass vessels for collection of the samples
- Filtered paper
- An optical microscope for microscopic analysis

Constants used:

Dynasan 118	2 g
Water	100 ml
Temperature	>75°C
Cooling	Cold water
Mixer	propeller
Diameter flask	6,5 cm
High 250 ml beaker	9 cm
High mixer	15 cm
Distance mixer to the beaker	1 cm

Properties of:

- **Dynasan 118**: is a triglyceride derived from three units of steric acid. Insoluble in water with a melting point between 70-73 °C.
- **Tween 80**: hydrophilic nonionic surfactant that is widely an emulsifying agent in the preparation of stable oil in water (O/W) pharmaceutical emulsions. Soluble in water, with a melting point >180 °C.

The technique:

- In a beaker it was heated the Dynasan 118 over 75 °C until all sample was melted.

- In another beaker it was heated 100 ml of purified water with Tween 80 until the same temperature than the other beaker.
- When the two beakers were at the same temperature, the water phase was turn into the oil phase and the mixing started at the respective rotation speed and time.
- When the time ended I purred the result sample into a glace of cold water and let them rest until the temperature was inferior than the melting point of the Dynasan 118 (73 °C).
- The sample was filtered with a 45 µm sieve, and the dried in filtered paper.
- All the samples were analyzed in an optical microscope, measuring the diameter of the microparticles and capturing images.

In the first protocol, we just wanted to have a clear observation of how the particle size and shape of the microparticles could differ if we changed completely the parameters for a sample to another. The results of the first's samples are in table I

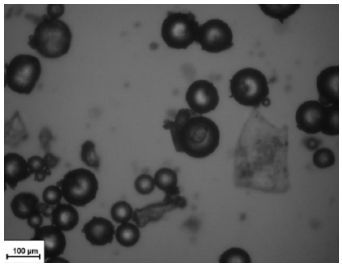
Table I - Size (µm) obtain with the different parameters

Sample	Tween 80 (ml)	Rotation	T (°C)	Mixer	Time (min)	Cooling	Average (µm)
G	0,25	3	>75	propeller	3	cold water	115,6702564
H	0,25	3	>75	propeller	6	cold water	110,3125
F	0,25	5	>75	propeller	3	cold water	99,77721519
A1	0,5	3	>75	propeller	1	cold water	139,0884298
A	0,5	3	>75	propeller	3	cold water	119,5485075
B	0,5	3	>75	propeller	6	cold water	133,2276119
E	0,5	5	>75	propeller	1	cold water	129,4641791
C	0,5	5	>75	propeller	3	cold water	91,71119403
D	0,5	5	>75	propeller	6	cold water	105,69403
I	0,5	3	>75	propeller	3	cold water	119,822488
J	0,5	5	>75	propeller	3	cold water	95,55365079
K	0,5	3	>75	propeller	3	cold water	117,7523962
L	0,5	5	>75	propeller	3	cold water	77,84482072
M	0,5	4	>75	propeller	3	Cold water	125,1883562
N	0,5	4	>75	propeller	3	Cold water	116,9101093
O	0,5	4	>75	propeller	3	Cold water	99,7784029
P	0,5	3	>75	propeller	3	Cold water	112,5079012
Q	0,5	4	>75	propeller	3	Cold water	121,5842767
R	0,5	5	>75	propeller	3	Cold water	85,28087367

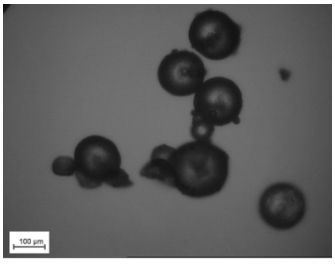
S	0,5	6	>75	propeller	3	Cold water	107,4354321
T	0,5	5	>75	propeller	3	Cold water	100,7391137
U	0,5	6	>75	propeller	3	Cold water	96,94144
V	0,5	6	>75	propeller	3	Cold water	113,2610048

It was our purpose to see if different times of the mixing could lead to a smaller microparticle and a better roundshape microparticle. If the sample as more time to be mixed so there will be a better contact between the tween 80 and all the surface of the microparticle with Dynasan 118, leading to a better stability. Also with more time of mixing we could obtain smaller microparticles since there's more time to the propeller "cut" the sample and obtain smaller microparticles.

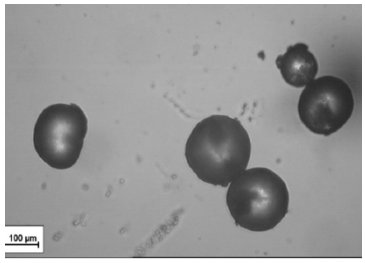
At the first glance, comparing the size particles at the same rotation and different times of mixing we could conclude that until a limit of time there's not much significance in the roundshape problem. For example if you look to samples C, D, E:



Picture 2 - Sample C



Picture 3- Sample D



Picture 4 - Sample E

In itch sample it was used 0,5 ml of Tween 80, rotation speed 5, and the time varied. The sample C had 3 min of mixing, the sample D had 6 min of mixing, and the sample E had 1 min of mixing. Comparing the samples C and D there's not much difference between the shapes of the microparticles, and also concerning the average of size. The size of sample C is 91,7 µm, and of sample D is 105,7 µm, so its observed a not expected small increase between the time of mixing 3 and 6. This increase is not significant since for the time used, in that sample, should be smaller particles. With this observation it was concluded that starting from 3 min there's not much difference in roundshape and size of microparticles. Comparing the sample E (1 min) with the others two, we can see that the microparticles are much bigger, 129,6 µm, giving the notion that is require for the sample to be mixed more time.

For the next protocol that was prepared, the objective was to see the influence of different rotations with the other properties constants. The parameters were the same that the previous protocol but it was tested different rotation speeds, rotation speed 3,4,5,6.

Table 2 – Protocol with different rotation speeds

Sample	Tween 80	rotation	Time (min)	Average (µm)	Ơ (µm)
1 (MO2801A)	0,530 g	3	3	**119,55**	54,88
2 (MO0102I)	0,530 g	3	3	**119,83**	42,72
3 (MO0402k)	0,530 g	3	3	**117,75**	58,11
4 (MO0502P)	0,530 g	3	3	**112,51**	68,99
5 (MO0402M)	0,530 g	4	3	**125,19**	41,4
6 (MO0402N)	0,530 g	4	3	**116,91**	42,98
7 (MO0402O)	0,530 g	4	3	**99,78**	43,91
8 (MO0502Q)	0,530 g	4	3	**121,58**	38,66
9 (MO2801C)	0,530 g	5	3	**91,71**	29,55
10 (MO0102J)	0,530 g	5	3	**95,55**	35,014
11 (MO0402L)	0,530 g	5	3	**77,84**	32,23
12 (MO0502R)	0,530 g	5	3	**85,28**	29,89
13 (MO0702T)	0,530 g	5	3	**100,74**	34,52

14 (MO0702S)	0,530 g	6	3	**107,44**	36,66
15 (MO0802U)	0,530 g	6	3	**96,94**	26,56
16 (MO0802V)	0,530 g	6	3	**113,26**	34,36

Table 3 – The results and observations with the firsts batches

Sample	Average (µm)	σ'(µm)	Observations
A1	139,0884298	64,26807293	Donut microparticles
A	119,5485075	54,88547986	Donut microparticles
B	133,2276119	49,49498705	Donut microparticles
C	91,71119403	29,6456584	
D	105,69403	31,30112	Some donut microparticles
E	129,4641791	52,12006584	Few donut microparticles
F	99,77721519	32,57962846	Few donut microparticles
G	115,6702564	54,76627035	Strange microparticles
H	110,3125	57,96148422	Few donut microparticles
I	119,822488	42,72289988	Few donut microparticles
J	95,55365079	35,0141435	Few donut microparticles
K	117,7523962	58,10938074	Few donut microparticles
L	77,84482072	32,23328642	Good
M	125,1883562	41,39601763	Very good
N	116,9101093	42,98402356	Good
O	99,7784029	43,90977636	Good
P	112,5079012	68,99056354	Donut microparticles
Q	121,5842767	38,66377423	Donut microparticles
R	85,28087367	29,88770453	Good
S	107,4354321	33,65533316	Some donut microparticles
T	100,7391137	34,52254358	Good (little microparticles at the surface of other bigger microparticles
U	96,94144	26,56326256	Some donut microp
V	113,2610048	34,36060409	Few donut microp

- The preparation of S,T,U,V, was obtain by putting the oil phase (Dynasan 118) into the water phase (water/tween 80) and then mix. After 3 min the emulsion was

cooled down in cold water and mixed at 300 rpm, and then waited. After we filtered using a filter paper we let it dried and passed the sample through a 45 µm sieve. The main problem for these batches (ST,U,V) was that when we putted the oil phase into the water phase there was a considerable loss of the oil phase, because it was in contact with cold air and then crystalized. So the ratio between the water phase/oil phase increased.

Other problem that was uncounted was the instability with the rotations speed 6 and 5, since sometimes the mixer wasn't stable, so the rotation of the emulsion wasn't homogeneous, and of course the instability was worst with rotation speed 6.

The main problems observed with the emulsion congealing technique, concerning these samples, were, as I already focus, the instability of the mixers when I increased the velocity, and the temperature.

It was impossible to fix the samples at a particularly temperature, and I only could get the two phases at >75°C and even so they were not at the same temperature. So this parameter was not used as a variable, as so we can't see the influence of the temperature in the changes of form and size of the microparticles. The temperature was measure with an IV digital laser thermometer for more accurate measurement of the temperature.

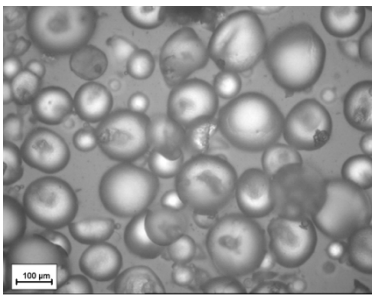
Picture 5- Digital laser thermometer

For different rotations speed it was conclude that with an increase of rotation we will obtain smaller microparticles, as expected, since an increase of rotation will increase the force facilitating the mixing between the oil phase and the water phase resulting in smaller microparticles.

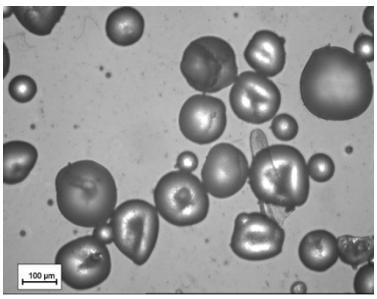
In theory Tween 80 (Polysorbate 80) is a hydrophilic nonionic surfactant that is used widely as an emulsifying agent in the preparation of stable oil-in-water pharmaceutical emulsions. Tween 80 provides a mechanical barrier and repulsive forces to stabilize the emulsion congealing. Reduce interfacial tension of the system and provide charge for droplet-droplet repulsion. So it is expected that when we use more concentration of tween 80 we will obtain smaller microparticles. Also with smaller concentration of Tween 80 it could lead to partial or minimal interfacial surface coverage by the emulsifier, and this would lead to an increase of the surface tensions and an increase in the droplet size. Tween 80 also was used as a stabilizer, so the use of larger amount of the emulsifier would create more round shape microparticles.

Considering the previous toughs, we tried to see the difference in microparticles size and shape using two concentrations of Tween 80: 0,25 and 0,5. The results were not as we

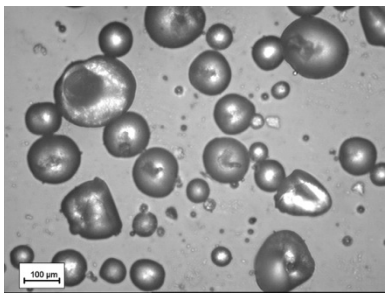
expected, in one sample (F) it was used less Tween 80 (0,25 g) and it was obtained smaller and rounder microparticles comparing with the samples that we used more Tween 80 (0,5 g) (for example sample A1 and B). Also with other samples, we didn't notice much difference between the samples that we used 0,5 g and 0,25 g of Tween 80. This could be easily explain, because in one hand maybe there is not much difference between the concentration 0,5 and 0,25 g of Tween 80 in the production of good round shape microparticles and size; and in another hand the droplet size is also affected by the ratio between the concentration of the oil phase and the Tween 80; so maybe the ratio 2 g Dynasan and 0,25 g of Tween 80 is more optimal that the other ratio (2g:0,25 g).



Picture 6- Sample F



Picture 7 – Sample A1



Picture 8 – Sample B

Considering this, it was design a new protocol with the objective of obtaining the optimal protocol for microparticles roundshape, less amount of tween 80, and size near 120 µm (optimal for parenteral use). For that purpose we used different concentrations of Tween 80, different stirrers, the presence of a baffle, and different amounts of Dynasan 118 and for the cooling process we use an ice bath, 500 ml.

The first step of this experience was a pretest to see the amount of temperature loss when the sample started to mix. So, we heated the water until 90°C and removed it from the heater and started to stir. We recorded the time at 85, 80, 75, 70 °C. This experience was done twice (table 4), to provide a more veracity on the results.

Tabela 4 – Amout of temperature loss in the samples

Sample	Time (85°C)	Time (80°C)	Time (75°C)	Time (70°C)
1	6 s	20 s	47 s	1 min
2	22 s	35 s	54 s	1,18 min

Observing these numbers, we were able to conclude that it's not possible to remove the beaker from the heating when we are going to proceed the mixing, because that at 90°C

it only takes 22 seconds to achieved 85 °C, and there is require at least 3 min of mixing. For this reason we mixed the sample at the same time that the heating was happening, in order to avoid the early crystallization of the Dynasan 118. This happens since the movement of the mixers decreases the temperature of the sample lower than her melting point.

With the next protocol we wanted to try a different cooling process to see if it has a large variable in the size and form of the microparticles. We thought that if we used ice has a cooling process, the shock between the two temperatures (of the sample and the ice bath) would be greater and could hardener more the microparticles avoiding the collapsing of the microparticles giving them the donut shape form. Also, we wanted to verify if the problem of non-homogenous mixing (that some time can occur with rotation 6 and 5) was truly responsible for the irregular shape of the microparticles. So we tried to use in some samples a baffler, who the all purpose was to disturb the continuous mixing. We too wanted to see the difference that we could obtain in the form of the microparticles using different mixers (propeller and dissolver). It was also tested different concentrations of tween 80, to discover the smaller quantity that could be used to avoid the formation of donut shape microparticles, and different ratios between Dynasan 118 and Tween 80 to see the optimal ratio and prove the conclusion that above was mention.

Tabela 5 – New protocol

Sample	Rotation Speed	Baffle	Tween 80	Stirrer	Dynasan 118 (g)	Water (ml)	Time (min)	T (°C)	Cooling
A1	4	no	0,50%	Propeller	2	100	3	80	Ice bath, 500 ml
A2	4	no	0,50%	Propeller	2	100	3	80	Ice bath, 500 ml
A3	4	no	0,50%	Propeller	2	100	3	80	Ice bath, 500 ml
B1	4	yes	0,50%	Propeller	2	100	3	80	Ice bath, 500 ml
B2	4	yes	0,50%	Propeller	2	100	3	80	Ice bath, 500 ml
B3	4	yes	0,50%	Propeller	2	100	3	80	Ice bath, 500 ml
C1	4	no	0,50%	Dissolver	2	100	3	80	Ice bath, 500 ml
C2	4	no	0,50%	Dissolver	2	100	3	80	Ice bath, 500 ml
C3	4	no	0,50%	Dissolver	2	100	3	80	Ice bath, 500 ml

D	4	no	0%	Propeller	2	100	3	80	Ice bath, 500 ml
E	4	no	0,10%	Propeller	2	100	3	80	Ice bath, 500 ml
F	4	no	1%	Propeller	2	100	3	80	Ice bath, 500 ml
G	4	no	0,10%	Propeller	5	95	3	80	Ice bath, 500 ml
H	4	no	0,10%	Propeller	10	90	3	80	Ice bath, 500 ml
I	4	no	0,10%	Propeller	25	75	3	80	Ice bath, 500 ml

Constants of the protocol:

Diameter propeller: 5 cm

Diameter dissolver: 2,58 cm

High beaker: 9 cm

Diameter beaker: 6,5 cm

Distance propeller: 15 cm

Distance dissolver: 16 cm

Distance propeller to the beaker: 1 cm

Distance dissolver to the beaker: 1 cm

800 ml beaker with ice bath

Magnetic stirrers

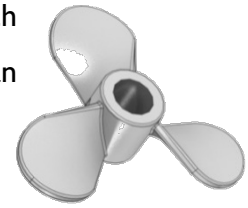
High of the baffler:

Mixing: 200 rpm

Mixing may be defined as the process in which two or more than two components in a separate or roughly mixed condition are treated in such a way so that each particle of any one ingredient lies as nearly as possible to the adjacent particles of other ingredients or components [8]. An emulsion is a negative mixture, and is formed when two immiscible liquids are mixed. This mixture is more difficult to prepare and require a higher degree of mixing with external force as there is tendency of the components of these mixtures separate out unless they are continuously stirred. Two immiscible liquids are mixed to effect transfer of a dissolved substance from one liquid to another. When two immiscible liquids are mixed together in the presence of an emulsifying agent an emulsion is produce. For the

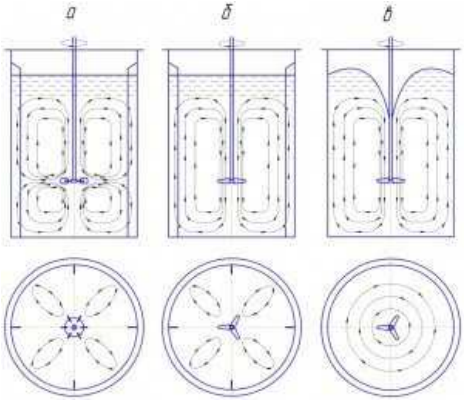
production of a stable emulsion, the mixing must be very efficient i.e. continuous without ceasing because the components tend to separate out if continuous work is not applied on them [8].

The propeller mixer is a device comprising a rotating shaft with propeller blades attached, used for mixing relatively low viscosity dispersions and maintaining contents in suspension. Propeller mixers are the most widely used form of mixers for liquids of low viscosity. It rotates a very high speed i.e., up to 8000 rpm due to which mixing is done in a short time. They are much smaller in diameter than paddle and turbine mixers. The most important disadvantage is that propeller is not effective with liquids of viscosity greater than 5 pascals per second for example glycerin and castor oil.



Picture 9 – Propeller mixer

Mixing fulfills many objectives beyond simple combination of raw ingredients. These include preparing fine emulsions, reducing particle size, carrying out chemical reactions, manipulating rheology, dissolving components, facilitating heat transfer, etc. So even within a single pharmaceutical product line, it is not uncommon to employ a number of different style mixers to process raw ingredients, handle intermediates and prepare the finished product [10].The importance of proper mixer selection and optimal operation can hardly be over-estimated. The High Shear Mixing and Emulsification are comprised of a rotor that turns at high speed within a stationary stator. As the blades rotate, materials are continuously drawn into one end of the mixing head and expelled at high velocity through the openings of the stator. The hydraulic shear generated promotes fast mixing, breaks down agglomerates and reduces the size of droplets. For emulsification to take place and remain in equilibrium, sufficient mixing energy is required. A common generalization is that the higher the shear put into creating the emulsion, the finer the droplets produced, and the more stable the emulsion. However, some emulsions are shear sensitive such that droplets start to coalesce past a certain level of mixing [9].

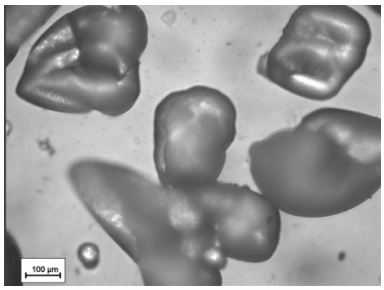


Picture 10 – Movement of different blades

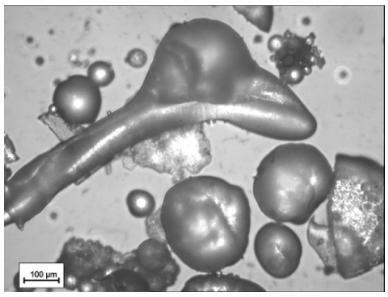
Table 6 – Results obtain with the previous protocol

Sample	Average (µm)	Ơ (µm)	T (°C) after addition	Observations
A1	114,9982721	32,72523282	15 °C	Many donut microparticles
A2	107,2238579	34,77915101	16°C	Many donut microparticles
A3	119,2389241	29,56072745	18°C	Many donut microparticles
B1	93,21196319	41,03171019	14 °C	Alot of microparticles with no round shape
B2	102,2795455	36,51302193	12°C	Many donut microparticles
B3	112,0819565	38,75445916	20 °C	Many donut microparticles
C1	185,6898305	53,78813706	12°C	Very few microparticles with round shape, the same microparticles have a big size.
C2	146,6985185	60,25783158	14°C	Very donut shape microparticles
C3	122,101005	56,13146365	14°C	Very donut shape microparticles
D	88,07916667	43,56438316	15°C	Very donut shape microparticles
E	77,05768116	37,44589603	14°C	Best sample until now, many small microparticles
F	104,9093079	35,42532187	14°C	donut microparticles
G	84,19926901	44,21620629	11°C	donut microparticles
H	86,61238532	41,59153285	13°C	Donut microparticles
I	75,25722022	42,89665693	15°C	Donut microparticles

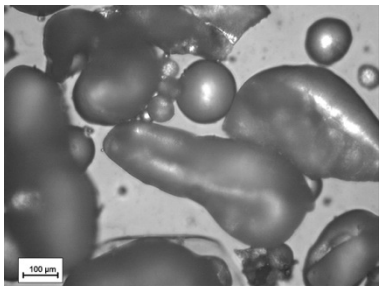
Observing the results and the images that are in following (C1,C2,C3) we could conclude that the use of a dissolver is not possible for obtaining round shape microparticles. When the dissolver mixer is moving it cuts de emulsion avoiding the production of a homogenous flow require to produce the microparticles. So, instead of evolving the emulsion cuts the microparticles.



Picture 11- Sample C1

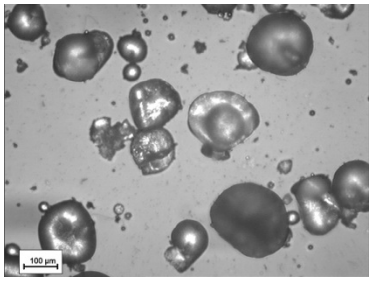


Picture 12- Sample C2

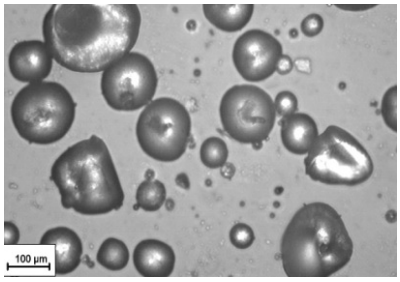


Picture 13 – Sample C3

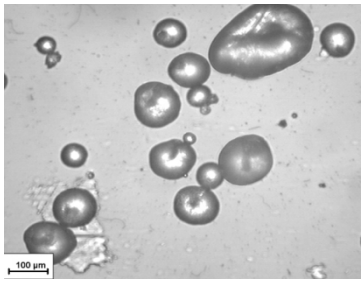
When we use a Baffler the turbine gives a radial circulation pattern, and the liquid is thrown out horizontally towards the surface of the vessel, and so there is more shear force. This could be more suitable for preparing emulsions, but it was prove that is not suitable for the preparation of microemulsions (microparticles). These require a more delicate flow and less shear stress to give the opportunity to form microparticles and not obtain an emulsion. When it was used a baffle in the preparation of the microparticles it was obtained in all the samples the donut shape form, as we can see in the next pictures bellow:



Picture 14- Sample B1



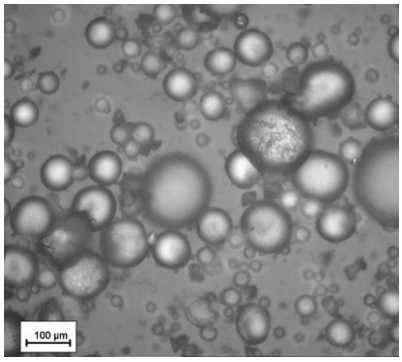
Picture 15- Sample B2



Picture 16- Sample B3

The ice didn't influence much the shape of the microparticles, comparing with the previous results, so we can conclude that the cooling process (cold water and ice) is not an important variable in the shape of the micropartices.

Observing the results the best sample was E (0,10 % Tween 80, Dynasan 118 2 g, water 100 ml) not taking into account the size of the microparticles that were relatively small. There were almost not donut microparticles, for that reason we believe that this formulation is the optimal one between the ration of the Tween 80 and the Dynasan 118. Of course is required more sampling to prove this conclusion.



Picture 17 – Sample E

Once the best formulation was discover it was necessary to see the influence of the drug that's going to be included in the microparticles formulation. For that, we needed to see the solubility of the drug in the fat phase (Dynasan 118) and if that solubility was affected by the increase of the temperature, because sometime happens in the preparation of the formulation. At first we made two samples, A and B, for witch sample we added 25 mg of the drug and recorded if it was dissolve in 75 °C. Than if it does we added more 25 mg of the drug and continued until the drug didn't dissolved. When this happened we increased at 80 °C to see if the drug dissolved and then added more 25 mg of the drug. This was made for these temperatures: 75, 80, 85, 90, 100, 110, 120, 130, 140 °C. With these results we

determined the maximum amount of the drug that could be dissolved in the fat phase for formation of microparticles, in which temperatures. This experiment was done twice A and B.

Table 7 – Results of the solubility of the drug in the fat phase (Dynasan 118)

Dynasan 118 mg	Total MO mg (75°C)	Total MO mg (80°C)	Total MO mg (85°C)	Total MO mg (90°C)	Total MO mg (100°C)	Total MO mg (110°C)	Total MO mg (120°C)	Total MO mg (130°C)	Total MO mg (140°C)	Total MO mg
25096	50,1		25	25,1	76,3	124,7	75,5	626,4	427,2	**1430,3**
25019	24,7		25	25,1	25	125,3	150,2	400,6	603,2	**1379,1**

It was tried to heat the same sample in different temperatures. The main problem was the difficulty to fix the sample in the exact temperatures, so it's not stable. When we tried to put the sample at 75, normally what occurred was that the sample kept changing between 73, 75, 77. Considering that the next fix temperature should be 80, the too samples (or too fixed temperatures) were very close. Other problem that was noticed, when we added the MO, was the temperature of the sample changed into an inferior temperature, that could be at a maximum of 10°C of difference. I couldn't conclude the reason for this to happen, because the quantity of the mass it's not sufficient to make a large difference on the temperature. Maybe the particles in the sample avoid an exact reading of the temperature or maybe some properties of the MO, or maybe the technique it's not optimal.

To check the crystal formation and fusion points and the difference in them when we use different cooling process in the preparation of microparticles, we heated the Dynasan 118 and dissolved the maximum of drug for 80, 100 and 120 °C, and then we cooled witch sample in two ways, the first was putting the sample in a beaker full of ice and the second was to let the sample cooling down at room temperature in the heater plate. For the 6 samples we screened using a DSC.

After testing the solubility in the fat phase we wanted to see the quantity of microparticles obtained and their size distribution using the same samples for the solubility test. The objective was to see if there was difference in the samples prepared at different temperatures. For that we joined the two samples prepared at 80°C, the two from 100 °C and the two prepared at 120°C. These 3 samples (MO13030401, MO13030402, MO13030403) were heated until 80°C (our fat phase with the drug) and we added the water with Tween 80 also heated 80°C. When we heated the two samples prepared at 120°C until 80°C, we noticed that the fat phase was white and not transparent as usual, who gave me

the proof that the quantity used of MO it's not soluble in 80°C, because some part of the drug is not dissolve in the solution. The white color was the remaining MO in the fat phase that didn't dissolved.

Table 8 - Size and shape of the microparticles obtained

Sample	Amount obtained (%)	Average (µm)	O' (µm)	Observations
MO13030401 (80°C)	76,54%	86,56197917	39,9781515	Some donut microparticles
MO13030402 (100°C)	55,45%	86,04804754	33,63556513	Some donut microparticles
MO13030403 (120°C)	75,88%	79,7621547	28,12018671	Some donut microparticles

The main difference between temperatures are showed in the size of the microparticles, as it appear that with a temperature > 120 °C there is a reduce of the size of the microparticles. The previous crystallizations didn't cause any difference in the form of microparticles, as you can see in the table above.

Conclusion

There is still a lot of research needed to achieve an optimal protocol for this technique. All the previous conclusions that were made are not sufficient for explaining the behavior of the microparticles form in the Emulsion Congealing Technique. The main conclusions made were that is essential for any protocol to achieve the optimal ratio between the fat phase and the water phase, because without it it's not possible to produce roundshape microparticles. Considering other parameter, the main one was the type of mixer, since I only experiment with two types: the dissolver and propeller. So it is needed to experiment with other mixers to see the influence between them.

The Pharmaceutical field is a constant changing field that continues to make progress, as the health of the population is a growing demanding. The microencapsulation techniques brought numerous advances in pharmaceutical field, but there's still allot to discover about new techniques, new parameters and new ways. For these reason is important to keep betting in the news technologies and new research in the Pharmaceutical Industry.

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