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Grau de Química

Treball Final de Grau

Influence of electrolytes and pH on the phase behaviour of gelatin/maltodextrin aqueous mixtures, for water-in-water emulsion formation.

Influència dels electròlits i el pH en el comportament fàsic de les mescles aquoses de gelatina/maltodextrina, per obtenir emulsions del tipus aigua-en-aigua.

Jordi Aragón Artigas

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God made the bulk but the Devil created the surface.

Enrico Fermi

En primer lloc, m'agradaria agrair a l'Institut de Química Avançada de Catalunya (IQAC) i, en concret, al Dr. Jordi Esquena Moret, que m'hagin permès formar part del seu grup de recerca i participar en el projecte Europeu BIBAFOODS (FP7-PEOPLE-2013-ITN), on he pogut adquirir nous coneixements i aptituds i he pogut intercanviar visions amb investigadors d'arreu del món, convertint aquest Treball de Fi de Grau en una gran primera experiència en el món de la investigació, la qual ha sigut molt apassionant i molt profitosa. També m'agradaria donar les gràcies a la Dra. Maria Sarret Pons per oferir-me aquesta oportunitat, però sobretot per mostrar interès i dedicació en el seguiment del meu treball en tot moment.

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REPORT

CONTENTS

1. SUMMARY

The main objective of this work is to study the effect of different electrolytes and pH on the spontaneous formation of water-in-water emulsions. Water-in-water (W/W) emulsions are a less known group of emulsions which are based on the immiscibility of two compounds which are soluble in water. Although they are thermodynamically unstable, W/W emulsions could be kept for some time due to its kinetic metastability, without any surfactant. Subsequently, obtaining microgels from these emulsions could offer a new type of encapsulation with delayed release of active ingredients or drugs.

In this work, aqueous mixtures of gelatin and maltodextrin have been studied from its phase diagrams. Initially, the study of electrolytes has been made following the Hofmeister series, thereby, according to the capacity of hydration of ions, studying salts with different free energy of hydration ($CsNO_3$ i $LiCl$). It was expected that salts had opposite effect on the phase separation of the system, but it was observed that the two salts favour the miscibility of polymers and the precipitation of a coacervate.

Then, the effect of pH by adding acetic acid has been studied, observing an interesting result: acid medium favours the miscibility between the polymers, without precipitation. This phenomenon opens a new way: the neutralization of acid medium with sodium hydroxide could favour the spontaneous formation of emulsions. It has been verified that the addition of NaOH produces an instantaneous phase separation, however, obtaining emulsions from this phase separation has not been ensured. Due to the high system instability, many experimental factors could affect the formation of these emulsions. Future further work is required with more detailed studies.

Keywords: water-in-water emulsions, microgel, Hofmeister series, kosmotropic, chaotropic, Gibbs free energy of hydration, phase separation

2. RESUM

El principal objectiu d'aquest treball és l'estudi de l'efecte de diferents electròlits i del pH en la formació espontània d'emulsions aigua-en-aigua. Les emulsions aigua-en-aigua són un grup poc conegut d'emulsions basades en la immiscibilitat entre dos polímers que són solubles en aigua. Tot i ésser termodinàmicament inestables, es poden arribar a mantenir durant un cert temps degut a la seva metaestabilitat cinètica, en absència de tensioactiu. La posterior obtenció de microgels a partir d'aquestes emulsions podria oferir un nou tipus d'encapsulament amb alliberament retardat de fàrmacs o principis actius.

En aquest treball s'ha estudiat el sistema aquós amb mescles de gelatina i maltodextrina a partir del seu diagrama de fases. Inicialment, l'estudi de l'efecte dels electròlits s'ha realitzat seguint les series de Hofmeister, això és, segons la capacitat d'hidratació dels ions, estudiant l'addició de sals amb diferent energia lliure d'hidratació ($CsNO_3$ i LiCl). S'esperava que tinguessin un efecte oposat en la separació de fases del sistema, però s'ha observat que els dos afavoreixen miscibilitat entre els dos polímers i l'aparició d'una fase sòlida de coacervat.

Seguidament s'ha caracteritzat l'efecte de pH afegint àcid acètic, amb un comportament interessant: el medi àcid afavoreix la miscibilitat entre els dos polímers, però sense l'aparició d'una fase sòlida. Aquest fet obre una nova via: la neutralització d'aquest medi àcid amb hidròxid de sodi podria afavorir la formació espontània d'emulsions. S'ha comprovat que l'addició de NaOH produeix una separació de fases instantània, però no s'ha aconseguit assegurar que s'hagin obtingut emulsions a partir d'aquesta separació de fases. Degut a l'alta inestabilitat del sistema, molts factors experimentals podrien afectar la formació d'aquestes emulsions. Es requereix la continuació del treball, amb un estudi més detallat.

Paraules clau: emulsions aigua-en-aigua, microgel, sèries de Hofmeister, cosmotròpic, caotròpic, energia lliure de Gibbs d'hidratació, separació de fases

3. INTRODUCTION

Colloid science concerns systems in which one or more of the components has at least one dimension within the range of about 1 nm to 1 μm, in other words that systems containing large molecules and/or small particles. Another way of explaining it: colloid systems are "macrohomogeneous" and "microheterogeneous" systems, which are formed by two phases. These phases are differentiated by the terms dispersed phase (usually the phase forming the particles) and continuous phase (usually the medium in which the particles are distributed). The range of different colloidal systems of practical importance is very wide. For this reason, colloidal systems may be classified into three general groups:

  - Colloidal dispersions: they are thermodynamically unstable owing to their high surface free energy and are irreversible systems in the sense that they are not easily reconstituted after phase separation. Colloidal dispersions consist in which the dispersed phase and continuous phase have not affinity.

  - **True solutions of macromolecular material**: they are thermodynamically stable and reversible in the sense that they are easily reconstituted after separation of solute from solvent.

  - **Association colloids**: they are thermodynamically stable. Association colloids consist of a self-assembly of amphiphilic compounds [1], [2]. This work is focused on studying a specific type of colloidal dispersion. The particles in a colloidal dispersion have a sufficiently size for definite surfaces of separation to exist between the particles and the medium in which are dispersed. Therefore, simple colloidal dispersions are two-phase systems [3]. According to this, colloidal dispersions may be divided into 8 types, as it is seen in Table 3.1.

Table 3.1. Types of Colloidal dispersion.

Dispersed phase	Continuous phase	Colloidal dispersion	Examples
Solid	Solid	Solid sol	Ruby glass
Solid	Liquid	Sol	Paints
Solid	Gas	Solid aerosol	Smoke
Liquid	Solid	Solid emulsion	Pearl
Liquid	Liquid	Emulsion	Mayonnaise
Liquid	Gas	Liquid aerosol	Mist
Gas	Solid	Solid foam	Meringue
Gas	Liquid	Foam	Soap lather

.

The emulsion is the type of colloidal dispersion which is studied in this work.

3.1. EMULSIONS

This work will focus on liquid-liquid dispersions, emulsions, as seen in Table 3.1. Emulsions are mixtures of two immiscible or partially miscible liquids, from which one of them is the dispersed phase and the other is the continuous phase. The continuous phase is in the form of droplets, the diameter from which is, in general, above 0.1 µm [2]–[6]. This system is not thermodynamically stable due to the incompatibility of their compounds, but it could be kinetically stable. This minimum stability can be increased by using surfactants, polymers or small particles [4]. Emulsions may be classified based on two criteria: the nature of the phases (oil or water) and the number of phases which coexist (simple or multiple emulsion).

3.1.1. (W/O) AND (O/W) EMULSIONS

These two groups are simple emulsions which are based on the immiscibility between aqueous (polar compound) and oil phase (nonpolar compound). The system is called water-in-oil emulsion (W/O) when water droplets are dispersed in oil, and if oil droplets are dispersed in

water, the system is called oil-in-water emulsion (O/W). The formation of W/O or O/W depends on the composition of mixtures and the used emulsifier [6].

3.1.2. (O/O) EMULSIONS

This group of emulsions are based in the immiscibility of two oil compounds. The combination of oils with different polar behaviour, forming droplets of one oil which are dispersed in the other oil. An example of a couple of immiscible oils is hydrocarbons with fluorocarbons: fluorocarbons and hydrocarbons repel each other [6].

3.1.3. (W/W) EMULSIONS

Water-in-water (W/W) emulsions are a less known group of emulsions which is based on the immiscibility of two compounds which are soluble in water, so water-in-water emulsions can be produced by mixing diluted aqueous solutions of incompatible polymers. Many proteins-polysaccharides mixture experience this incompatibility and lead to formation of W/W emulsion, as seen in Figure 3.1 for a gelatin-maltodextrin mixture.
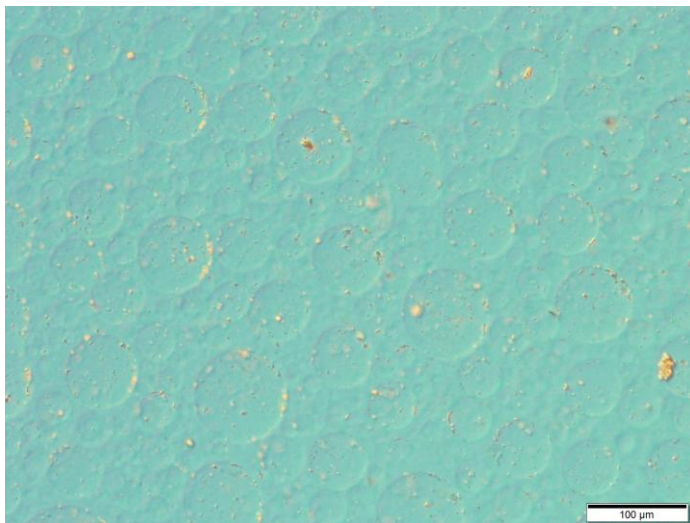

Figure 3.1. Example of W/W emulsion. Sample of 8% gelatin and 10 % maltodextrin.

W/W emulsions are highly unstable, despite the fact that interfacial tension between the two aqueous phase is usually rather low [7], [8]. Surfactant cannot be used to enhance stability, since their adsorption on a W/W interface is quite poor. Consequently, an advantage of (W/W) emulsions is that they not need surfactants to be metastable like (W/O), (O/W) and (O/O) emulsions. However, some stable Pickering W/W emulsions have been reported [7], for instance using globular proteins as stabilizers.

3.1.4. MULTIPLE EMULSIONS

These emulsions are formed by two or more immiscible phases which are separated from the other phase. Droplets of continuous phase could appear into droplets of dispersed phase, as it is seen in Figure 3.2. The emulsion was also divided into two categories: O/W/O and W/O/W emulsions. In a system O/W/O, the aqueous phase separates the two immiscible oil phase, while on a system W/O/W immiscible oil phase separates the two aqueous phases. Many of these emulsions were developed for the purpose of delaying the release of an active ingredient. In these emulsions in emulsions, any substance present in the internal phase has to cross the boundaries of the two phases to reach the external or continuous phase [6].
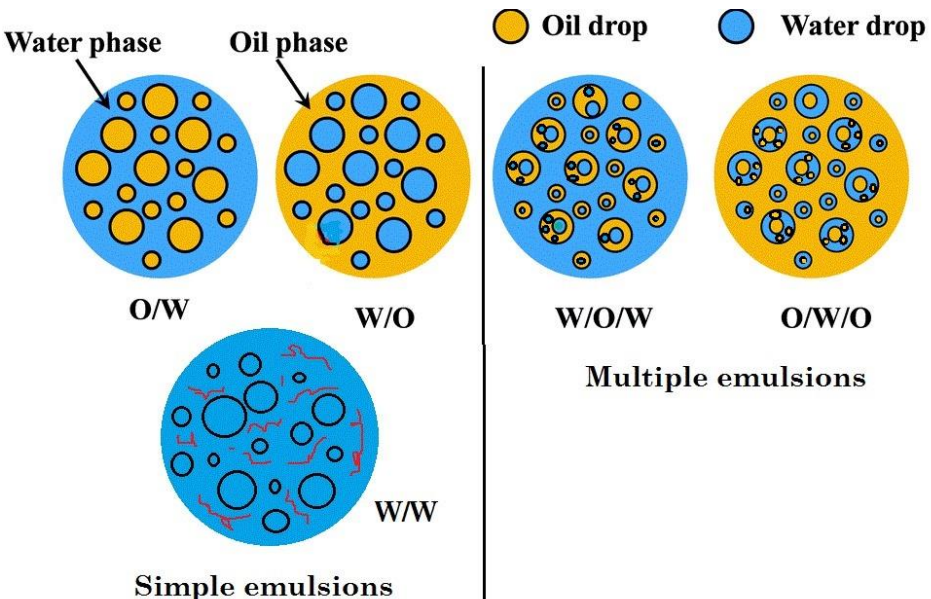

Figure 3.2. Classification of the most common emulsions types [5].

3.2. PHASE BEHAVIOUR IN MIXED AQUEOUS SYSTEMS

In aqueous mixtures of two hydrophilic water-soluble polymers, different behaviours can be observed [9], depending on the interactions between the polymers, as it is described in Figure 3.3.
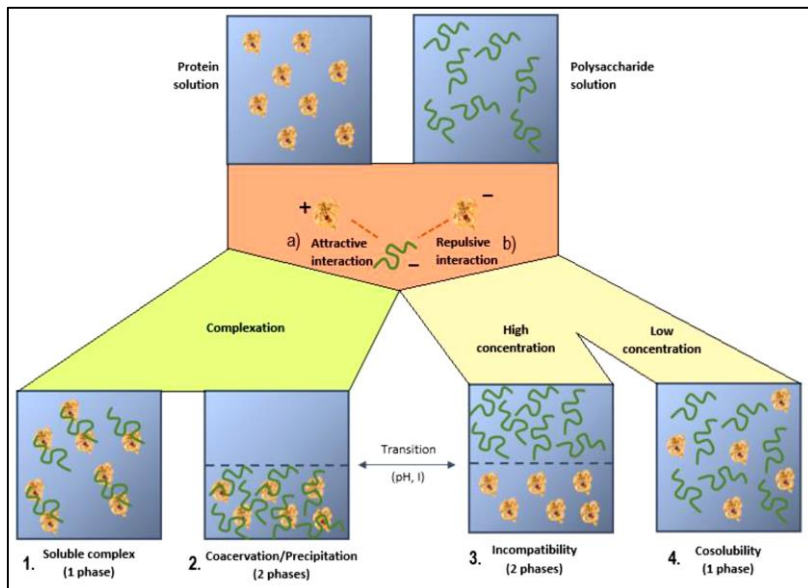
Figure 3.3. Possible interactions between two polymers in water [9].

There are two possible interactions between a protein and polysaccharide in water: attractive or repulsive forces. Depending on the type of interaction, the behavior of the system can be explained:

- **Attractive interactions:** complexing phenomena**.**
    - Soluble complexation: a complex is formed due to the attractive forces between the two polymers. In this case, one stable liquid phase is observed because the formed complex is soluble in water.
    - Coacervation/precipitation: a complex is formed due to the attractive forces between the two polymers. In this case, complexing leads the formation of a

                coacervate or precipitate, so two different phases are seen, one with the complex, and one without it. This situation is called "associative phase separation".

- **Repulsive interaction**: depending on concentrations.

    - High concentrations: Two different phases appear due to incompatibility of polymers. This situation is called "segregative phase separation".
   - Low concentrations: There is a low interaction between two polymers, consequently, only one phase is formed.

In order to form emulsions of two different polymers, repulsive interaction with high concentration of polymers is of interest. In this case, homogenising the mixture could lead to droplet formation within the emulsion (first method of formation of emulsions that it is seen). In biopolymer mixture systems the entropy is the main factor which determines the thermodynamic stability [10]. Abundant literature clearly shows that temperature, pH, ionic strength, charge density, chain conformation, etc. influence the phase separation behaviours of biopolymer mixtures, particularly of charged biopolymer mixtures. By changing these physicochemical parameters, a transition between associative and segregative phase separation can be obtained [11]. In this work the effect of salts and pH on the phase behaviour of a chosen system, will be analysed.

3.2.1. TRANSITION BETWEEN ASSOCIATIVE AND SEGREGATIVE PHASE SEPARATION: EXPERIMENTAL FACTORS

As explained in the previous section, some conditions or compounds could cause a transition between associative and segregative phase separation. In other words, some experimental factors might change the phase behaviour of a system. This work tries to study amongst others the effect of salts in the gelatin-maltodextrin system. Salts from the extremes of the Hofmeister series will be studied.

3.2.1.1. HOFMEISTER SERIES

Fran Hofmeister (1850-1922) first systematically reported specific ion effects in a series of papers. He studied the precipitation of egg yolk protein and of some other colloids in the presence of salts. From there emerged an ordering of the ions depending on their effectiveness, measured by concentration, to precipitate the protein. He ordered the salts according to their ability in promoting the precipitation (salting-out, left side of the series) or the solubility (salting-in, right side of the series) of a protein in aqueous solution [12].

$$HPO_4^{2-} > SO_4^{2-} > F^- > Cl^- > Br^- > NO_3^- > ClO_4^- > SCN^-$$

$$Cs^+ > NH_4^+ > Rb^+ > K^+ > Na^+ > Li^+ > Mg^{2+}$$
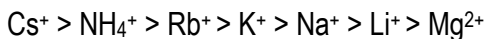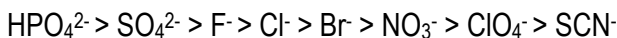
The 'conventional' cation series has a substantial difference compared to the case of anions. Indeed, salting-out cations are weakly hydrated and salting-in cations are strongly hydrated. Substantially, anions and cations behave in the opposite way. In fact, there is experimental evidence that the cations follow more complicated trends. Nonetheless, the understanding of the specific cation behaviour is even more interesting than that of anions, because many cations play a crucial role in fundamental biological systems [13] .

The effect of the addition of salts on solutions of nonelectrolytes is very complex, due to the different types of intermolecular interactions that involve the ions, the solvent, and the solute molecules. In hydration theories, ions attract and order surrounding solvent molecules forming hydration shells. This process implies the competition of ions and nonelectrolyte molecules for the same solvent molecules. The hydration and solubility of the nonelectrolyte could decrease[14]. Relating this concept with Hofmeister series, it is seen that salting-out ions are strongly hydrated, while salting-in ions are only weakly hydrated. The salts can also be reffered to as kosmotropic and chaotropic salts [13], [15]–[17]. Kosmotropicity of salt is the same that strongly hydrated, because a system with hydrated salts is more ordered (*kosmos* means order in old greek), water stays next to ions (Figures 3.4-3.5).
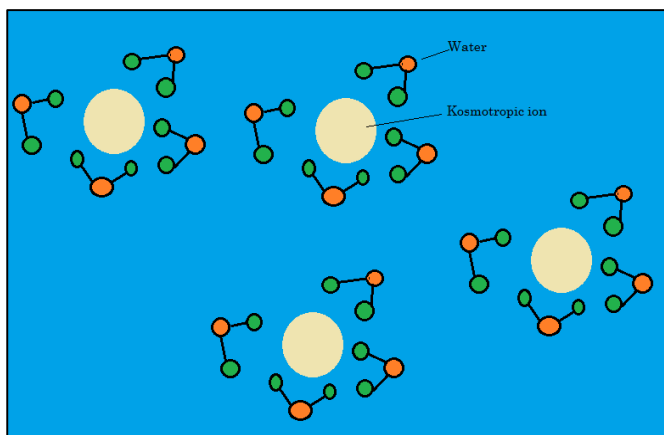
Figure 3.4. The picture shows how stronger hydration of kosmotropic ions "reorders" the solution.
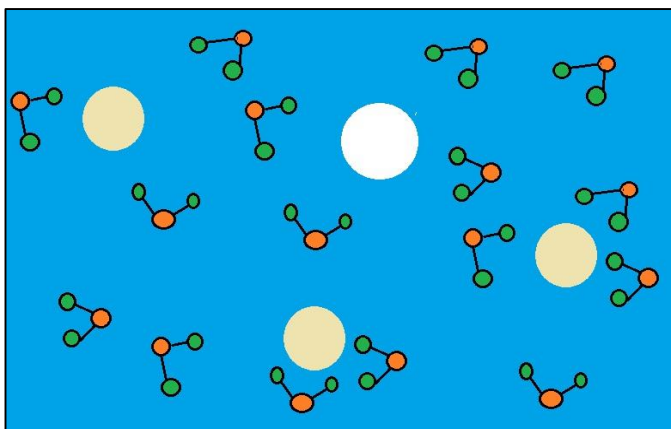
Figure 3.5. The picture shows how lower hydration of chaotropic ions does not "reorder" the solution.

A thermodynamic approach, utilizing the Gibbs free energy of hydration, ΔG_{hyd}, to quantify this series has been developed. ΔG_{hyd} is the change in free energy from an isolated ion into the gas phase to the aqueous solvated ion in solution. Kosmotropic ions have a large negative ΔG_{hyd}, due to the resulting structured water 'net' around the ion, while chaotropic ions have smaller negative or even positive values ΔG_{hyd} [15], [16] (Table 3.2).

Table 3.2. A list of examples for inorganic ions illustrating the kosmotropic and chaotropic salts. The ions in bold are those used in this work [16].

Cation	ΔG_{hyd} (kcal mol^{-1})	Anion	ΔG_{hyd} (kcal mol^{-1})
H$^+$	**-252**	ClO$_4^-$	-51.1
Cs$^+$	**-61.7**	TcO$_4^-$	-60.0
Rb$^+$	-67.1	**Cl$^-$**	**-82.9**
NH$_4^+$	-69.8	OH$^-$	-104
K$^+$	-72.7	F$^-$	-114
Na$^+$	-89.6	CrO$_4^{2-}$	-229
Li$^+$	**-115**	SO$_4^{2-}$	-261
Fe^{3+}	-446	SO$_3^{2-}$	-311
Cu^{2+}	-482	CO$_3^{2-}$	-353
Al^{3+}	-1083	PO$_4^{3-}$	-663

3.2.1.2 THE EFFECT OF PH

As is seen in the Hofmeister series, H$^+$ is a cation with a large negative ΔG_{hyd}, so it could be expected an important kosmotropic behaviour due to his large tendency to hydration. However, it is not the only effect expected by pH. Focusing on W/W emulsions formed by two polymers, an important point which determines their interaction is their charge. Polymers usually are big molecules with acid/base groups, as proteins. In this kind of compounds, the charge depends of acid/base groups. The isoelectric point (pI) is the pH at which a particular molecule carries no net electrical charge. The net charge on the molecule is affected by pH of it is surrounding environment and can become more positively or negatively charged due to the gain or loss, respectively, of H$^+$. In other words, an increase or decrease of concentration of H$^+$ (changing pH) could show another kind of effects in the mixture, changing the phase behaviour, due to an acid/base reaction that changes the charge of a compound and consequently, the interaction between the polymers will be different. The effect of charges of polymers is difficult to predict because a lot of other factors could intervene, causing a phase behaviour changes: large

polymers could have a different charge distribution beyond the molecule, the 3D structure could change due to the different charge [18]. For example, it is reported that the mixing of gelatin and carrageenan aqueous solutions, at pHs even above the pI, where gelatin has a negative overall charge, can lead to an associative phase separation, as the uneven distribution of cationic and anionic groups along the gelatin chain produces some positive patches that electrostatically attract carrageenan [11].

3.3. FORMATION AND STABILITY OF EMULSIONS

As it has been seen in the previous sections, emulsions are thermodynamically unstable, although two basic kinetic schemes are used for preparing emulsions just as they are for colloidal dispersion. It is used mechanical energy to mix all the emulsion ingredients or use physicochemical phenomena that could happen adding different compounds or changing conditions like temperature and pressure.

- **Dispersion methods**

The first methods consist of mixing the solution to form droplets. In other words, the particles, which could form emulsions, already exist as a starting material in the mixture. These methods include the idea of starting with macroscopic chunks of materials or agglomerates and employ methods to break them down to the desired colloidal dimension. For this reason, the mixing technique (homogenizer, vortexing, ultrasonic transducer or ultra.turrax stirrer) affects the size of droplets [19].

- **Condensation methods**

The second method uses the idea that a chemical nonequilibrium state can induce the emulsification. In other words, chemical energy could be used to form emulsions in several ways [4]. Pressure and temperature could change the stability of emulsion, but also adding compounds like salts or modifying pH, whose effect is seen belong.

Returning to the concept of kinetic stability, although droplets are obtained, phase separation will appear over time due to several breakdown processes. In other words, a freshly prepared emulsion changes its properties with time owing to series of different events that occur on a microscopic scale. Depending on the particular molecular characteristics involved, these initial events rapidly could lead to an equilibrium state with macroscopically separated phases [1], [3]–[6], [20]–[24]. Some of the breakdown processes are depictured in Figure 3.6.
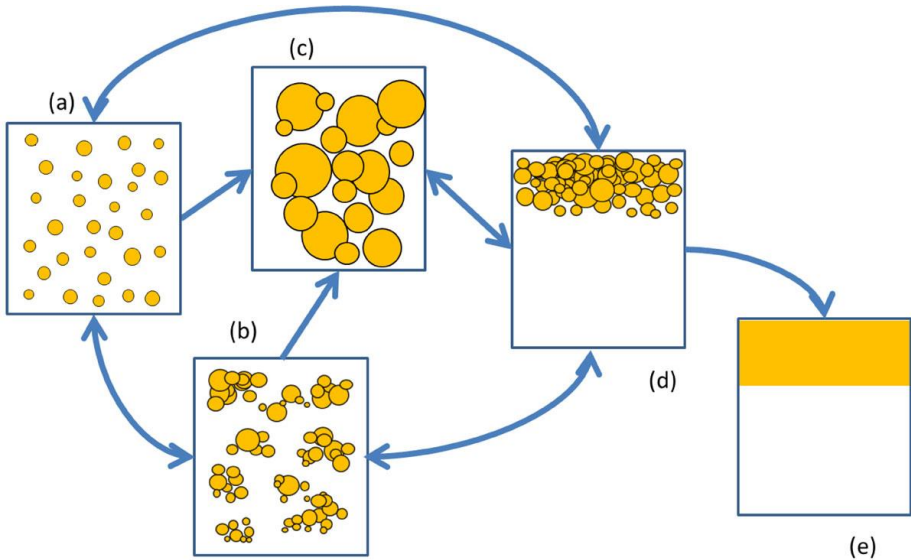
Figure 3.6. Types of breakdown processes of emulsions. Starting with a) a homogeneous dispersion of droplets in water, the main instability processes are b) flocculation, c) coalescence and d) creaming/sedimentation. Finally, e) the 2 phases are separated [21].

- **Flocculation:** It is the process where 2 or more droplets become associated with each other through attractive interactions, but maintain their individual integrity.
- **Coalescence:** It is the process of fusion between 2 or more droplets due to thinning and disrupting the liquid film, generating bigger droplets. This process leads the separation phase.
- **Creaming/sedimentation:** It is the migration of dispersed phase of emulsion, which leads the sedimentation of polymers and the phase separation. This process depends on an external force like gravity. If forces exceed the thermal motion of droplets (Brownian motion), a concentration gradient builds up in the system and droplets migrate.
- **Ostwald ripening:** It is the growth of larger droplets at the expense of smaller ones, which re-dissolve [22], in other words, this change in the morphology occurs as a result of small particles dissolving and transferring their mass to the larger particles.

The total energy of the two-phase system can be decreased via an increase in the size scale of one phase and thus a decrease in total interfacial area [25]. This phenomenon does not appear in Figure 3.5.

3.4. GELS

A gel is a non-fluid colloidal network or polymer network that is expanded throughout its whole volume by a fluid [26]. In other words, a gel is a semisolid system with a high liquid content, possessing a certain rigidity but also some elasticity. Systems which contain more than 10% of volume of the solid phase are referred to as pastes. The rigidity of a gel is ascribed to the formation of a three-dimensional network of colloidal particles in which a comparatively large quantity of dispersion medium is immobilized. Gelation thus represents a balance between precipitation and dissolution. The gels then possesses the property of thixotropy, becoming less viscous upon agitation but resolidify when resting [27]. Gelation may result from cooling of a colloidal solution (as e.g. emulsions) [3], that is why sometimes gels are named solid emulsions.

Gels may be classified in the following general groups, depending of nature of continuous phase [26]:

-   **Organogels**: the continuous phase is an organic solvent.
-   **Hydrogels:** the continuous phase is water.

3.4 .1. HYDROGELS

Hydrogels constitute a group of polymeric materials, the hydrophilic structure of which renders them capable of holding large amounts of water in their three-dimensional networks. Extensive employment of these products in a number of industrial and environmental applications is considered to be of prime importance. Natural hydrogels were gradually replaced by synthetic ones, due to their higher water absorption capacity, long service life, and wide varieties of raw chemical resources. Literature and consequently research on hydrogels has been expanding in the last decades. However, a number of publications and technical reports dealing with hydrogel products from the engineering points of view were examined to overview technological aspects covering this growing multidisciplinary field of research [28].

4. OBJECTIVES

Controlled and targeted release of drugs is a challenge for traditional drug administration [29]–[31]. Creation of gelified particles in the size of micrometers, microgels, on the basis of a W/W emulsion, could be an interesting novel way of conceiving a drug delivery system. For this reason, the main objective of this work is to study the spontaneous formation of W/W emulsions, based on biopolymer mixtures, by using different additives. Following the Master Thesis of Yoran Beldengrün [32], the studied system consists of aqueous mixtures of maltodextrin (an uncharged polysaccharide derived by hydrolysis from starch) and gelatin (a charged protein derived from collagen extracted from bovine skin).

The work plan is described below:

    a) The study in detail of phase behaviour of the gelatin-maltodextrin W/W system.

    b) The establishment of the relation between the Hofmeister series and the effect of ions on the studied system, comparing phase diagrams of the system in absence or presence of inorganic salts.

    c) The effect of changing pH (H^+ and OH^- are charged compounds too) on phase behaviour could let to control the system for obtaining or destabilizing emulsions.

    d) Formation and stabilization of the emulsions by gelification of the dispersed phase and the respective analysis of the microgels.

For the spontaneous formation of W/W droplets it is very important to control the phase behaviour of the system, and thus, to tune the phase boundary by adding the appropriate additives. The present work focuses in enlarging the regions where two aqueous liquids phases coexist.

5. EXPERIMENTAL SECTION

5.1. MATERIALS

1. Maltodextrin: Dextrose equivalent 4.0-7.0, molecular mass: 3.6 kDa molecular mass: 50-100 kDa (Sigma-Aldrich, ref: 419672-500G, CAS: 9050-36-6). Figure 5.1.

2. Gelatin from bovine skin: gel strength ~225 g Bloom, type B, molecular mass: 50-100 kDa , pI: 4.7-5-2 (Sigma-Aldrich, ref: G9382-500G, CAS: 9000-70-8). Figure 5.1.

3. Filtered deionized water: Milli-Q® water. Deionized water filtered by the ultra-pure Millipore water system, model Synergy Smart UV (resistivity at 25ºC: 18.2 MΩ·cm; conductivity 0.056 µS/cm, water quality: type I, ion concentration< 1µg/L).

4. Cesium nitrate: MW : 194.91 g/mol, hygroscopic, assay : 99% (Sigma-Aldrich, ref. 289337-50G, CAS : 7789-18-6).

5. Lithium chloride: MW : 42.39 g/mol, hygroscopic, assay : 99% (Sigma-Aldrich, ref. 793620-100G, CAS : 7447-41-8).

6. Tri-Lithiumcitrat-Tetrahydrat: MW : 281.99 g/mol, hygroscopic, assay : 99% (Merck, ref. 1056839024, CAS : 6080-58-6).

7. Urea: MW : 60.06 g/mol, hygroscopic, assay : 99% (Sigma-Aldrich, ref. U1250, CAS : 57-13-6).

8. Acetic acid: MW : 60.05 g/mol, assay : ≥99.7% (Sigma-Aldrich, ref. 320099, CAS: 64-19-7).

9. Sodium hydroxide: MW: 40 g/mol, BioXtra, assay : ≥98%, pellets (anhydrous) (Sigma-Aldrich, ref. 101204018, CAS : 1310-73-2).

10. Acetone: MW: 58.08 g/mol, CHROMASOLV® Plus for HPLC ≥99.9% (Sigma-Aldrich, ref. 650501, CAS: 67-64-1).
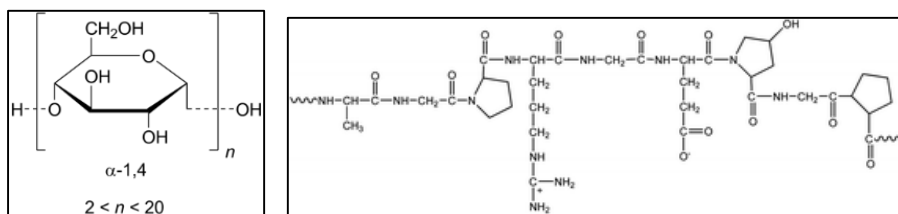

Figure 5.1. Molecular formula of maltodextrin (left) and gelatin (right)

5.2. EQUIPMENT AND INSTRUMENTAL

1. Optical Microscope: Olympus model BX51TRF-6, coupled to a digital camera Olympus DP73, controlled with an image/video capture software Stream Essential of Olympus.

2. Thermostated Bath: a 15 L of water bath of methacrylate with temperature controlled by HAAKE DC10 thermostat.

3. Vortex: IKA® VORTEX GENIUS 3.

4. Centrifuge: Eppendorf model 5804R, maximal velocity 5000 revolutions per minute (rpm) with a maximal working temperature of 40ºC.

5. Analytical balance: Mettler Toledo AB204-S/FACT balance with a precision of ±10-4 g (maximum capacity: 220 g).

6. Scanning Electron Microscopy, SEM: Hitachi TM-1000 Tabletop Microscope.

7. Freeze-dryer: Christ Alpha 2-4 LD Plus with a working pressure and temperature of ~0.03 mbar and -85ºC.

8. Heating magnetic stirrer: JP Selecta Model: Multimatic-5N (maximum temperature: 305ºC, maximum rpm: 1400 rpm).

9. Heating magnetic stirrer: IKA Model: RCT-basic (maximum temperature: 310ºC, maximum rpm: 1500 rpm).

5.3. METHODOLOGY

5.3.1. PREPARATION OF STOCK SOLUTIONS

The maltodextrin stock solutions (30 % w/w) were prepared by dissolving Maltodextrin into water Milli-Q and stirring 30 min at 95 °C. Stock solutions were prepared each day freshly. This process was repeated every day because Maltodextrin precipitates at room temperature, therefore it was impossible to keep it.

The gelatin stock solutions (25 % w/w) were prepared by dissolving gelatin into water Milli-Q stirring 30 min at 60ºC. The solution was stored in the refrigerator.

Stock salt solutions of cesium nitrate, lithium chloride, urea (non-ionic) and lithium citrate were prepared. A saturated solution of lithium citrate (40.3%) was prepared by dissolving lithium citrate into water Milli-Q. The concentration of all the others solutions was 18-19%.

The 20% acetic acid stock solution was prepared by diluting an ≥ 99.7% acetic acid solution into water Milli-Q. The 30% sodium hydroxide stock solution was prepared by dissolving NaOH pellets (≥98%) into water Milli-Q.

5.3.2. STUDY AND DETERMINATION OF PHASE DIAGRAMS

The phase diagram of this system was studied to know at which concentrations of maltodextrin and gelatin, two phases were present, and consequently, water-in-water W/W emulsions could be obtained.

These experiments were performed at different conditions to study the behaviour of the binodal line in response to physicochemical changes. Following modifications of the gelatin-maltodextrin system were tested:

- without any additive
- addition of salts of different extremes of the Hofmeister series
- addition of acetic acid.

Samples were prepared with concentrations of gelatin and maltodextrin expected to be close to the binodal line (studied in previous studies by the research group).

Moreover, mixtures with low concentrations of maltodextrin were prepared to study the coacervation/precipitation of the studied system.

5.3.2.1. Preparation of the samples

The mixtures were prepared in 5 mL vials following this order of addition: first water, then gelatin stock solution and finally maltodextrin stock solution. The samples were heated at 50ºC during 2 or 3 min before vortexing. After that, the mixtures were kept in a thermostated water bath at 50ºC during 5 days. Fixing the time to analyse the sample is important due to the thermodynamic instability of system, which was changing in time. Mixtures of maltodextrin and gelatin at different concentrations were prepared in order to determine the binodal line of the system. The range of gelatin and maltodextrin concentrations chosen, is seen in the results section (section 6). In the case of addition of salts, the samples were prepared in the same way as without salts and the salt solutions were added last.

In the case of gelatin/maltodextrin mixtures in presence of acetic acid (HAc), it should be considered that the isoelectric point of gelatin is 4.8-5.2. For this reason, the procedure of preparation of samples was changed a little compared to the previous cases. Samples were prepared as before, with a final concentration of 3.3% acetic acid, which lead to a pH of 3.5-4.5 (determined with universal pH indicator paper).

It should be emphasized that in both cases, adding salt or acid, they were added by titration and observed 24 hours after each addition. This allowed a fast screening of the phase behaviour with an additive.

5.3.2.2. Observation of the phase behaviour to build the phase diagram

After 5 days, phase behaviour of the samples was observed by eye. Mixtures will be indicated from now onwards as % gelatin / % maltodextrin. For example, 8/10 corresponds to 8% gelatin / 10% maltodextrin. It was followed the same procedure as the phase diagram without additive to observe the phase behaviour with salt or acetic acid. As mentioned above, mixtures of maltodextrin and gelatin at different concentrations were prepared in order to determine the binodal line of the system.

5.3.3. STUDY OF THE NEUTRALIZATION OF ACETIC ACID TO FORM W/W EMULSIONS

As will be seen, a new hypothesis was opened since the determination of the effect of H^+ in gelatin-maltodextrin W/W system: the neutralization of acetic acid of samples might allow a spontaneous formation of W/W emulsions due to a phase separation could be encouraged. NaOH was chosen to react with acetic acid. If NaOH led the phase separation of the mixture, the next step would be trying to get microgels by gelification of mixtures which at first only had one liquid phase but adding NaOH the phase separation was appeared.

5.3.3.1. Effect of NaOH on acidified mixtures

First of all, the amount of mixture was bigger than the previous section to observe clearly the changes of the system. Therefore, the experimental preparation of the mixture was exactly the same than the previous study, increasing the amount of the compounds (the studied mixture

was 4/13). The sample was heated in a heating magnetic stirrer at 50ºC during one day. The following day a necessary amount of NaOH stock solution to neutralize acetic acid was added dropwise.

5.3.3.2. Gelification, purification and lyophilization of mixtures

The experimental preparation of the mixture was exactly the same than the section "Effect of NaOH on acid mixtures". The mixture was heated and stirred in a heating magnetic stirrer at 50ºC, at 450 rpm during 20 min. After that, it was added dropwise the necessary amount of NaOH stock solution to neutralize acetic acid, keeping the same conditions (heating and stirring). It was kept 2 min in the stirrer, followed by rapid cooling in ice water during 1 min. The hydrogel was got. Purification of this hydrogel started diluting sample with 3x sample volume ice-cold Milli-Q water and vortexing to get a homogeneous suspension. Then, the samples was put into a tube, which it could be put into centrifuge, and it was centrifuged during 10 min at 10ºC at 2500 rpm. When centrifugation was finished, supernatant of sample was removed, and the previous process was repeated two or three times more: add again same amount of ice cold Milli-Q water, as amount that was removed, vortex strong and till al of sediment disperses again in the solution and centrifuge sample during 10 min at 10ºC at 2500 rpm. The obtained hydrogel could be stored on the fridge.

Freezing the sample with dry ice and acetone and subsequently lyophilising the hydrogel for approximately two days in order to inspect their microstructure by SEM was the final step of the process.

6. RESULTS AND DISCUSSION

6.1. PHASE BEHAVIOUR OF GELATIN-MALTODEXTRIN W/W SYSTEM

The phase diagram of gelatin-maltodextrin W/W system was studied at different conditions (without additive, with $CsNO_3$, with LiCl and with acetic acid) to predict the behaviour of the system, especially regarding its application to obtain emulsions.

The first phase diagram was obtained studying mixtures without additive, in a thermostated bath at 50ºC, during 5 days (Figure 6.1). As phase separation is a kinetic process, this time period was chosen to observe phase separation and also to compare the results between the experiments with different conditions. The mixture was kept at 50ºC because lower temperature leads to the precipitation of maltodextrin and the gelation of gelatin. Moreover, temperatures above 50ºC increase hydrolysis of gelatin.
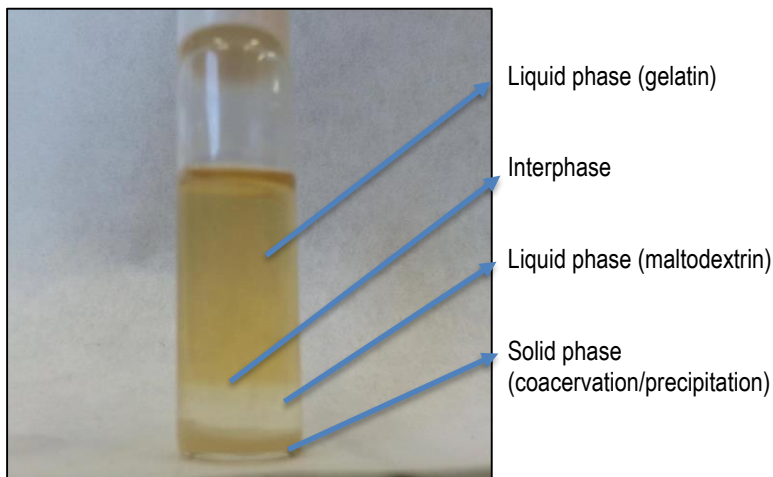

Liquid phase (gelatin)

Interphase

Liquid phase (maltodextrin)

Solid phase
(coacervation/precipitation)

Figure 6.1. An example of phase separation of a sample.

As seen in Figure 6.1 and Figure 6.2, different phase regions were observed in the phase diagram: one liquid phase (L), two different liquid phases (L_1+L_2), liquid phase + solid phase (L+S) and two different liquid phases + solid phase (L_1+L_2+S). The solid phase may have either originated from precipitated maltodextrin or from a coacervate which was formed between maltodextrin and gelatin. As previously reported by Kasapis et al. [33], [34], the precipitation of maltodextrin could have been favoured by the presence of gelatin, following a simple mass-action dependence on maltodextrin concentration, with gelatin driving the interaction by thermodynamic incompatibility and exclusion when sufficient concentrations of the two polymers.
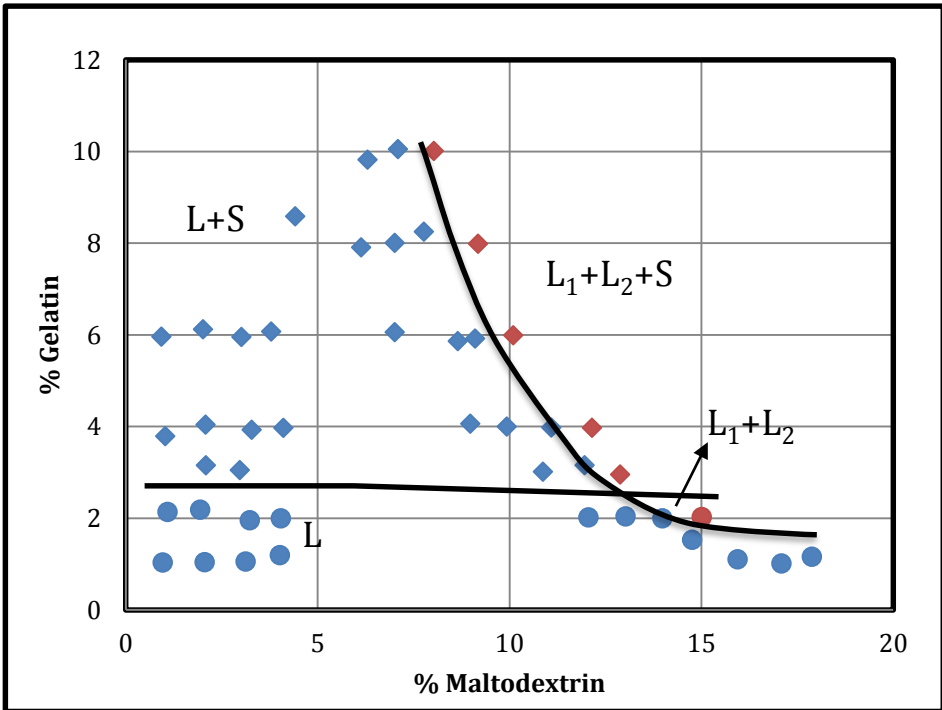

Figure 6.2. Phase diagram of gelatin/maltodextrin aqueous system at 50ºC, after 5 days.

The other explanation was the formation of a coacervate, which would imply an associative interaction between maltodextrin and gelatin; this initially seemed unlikely as maltodextrin is uncharged polysaccharide and gelatin a charged protein. Usually, the formation of a coacervate implies an electrostatic interaction, resulting from association of oppositely charged macro-ions [11]. However, some argue that weak energy interactions, especially hydrogen bonding but also hydrophobic interactions, can have a significant contribution to the formation of complexes and coacervates [35].

The influence of gelatin content is illustrated in Figures 6.3 and 6.4. Samples were prepared with 22% maltodextrin, and both in presence (3%) and absence of gelatin. In absence of gelatin, a single liquid phase (L) is observed. However, with adding 2% gelatin, two liquid phases (L_1+L_2) are observed, and also a precipitate is observed after sedimentation (S). Therefore, the formation of a precipitate is only observed if the gelatin concentration is above approximately 2.5 wt%.
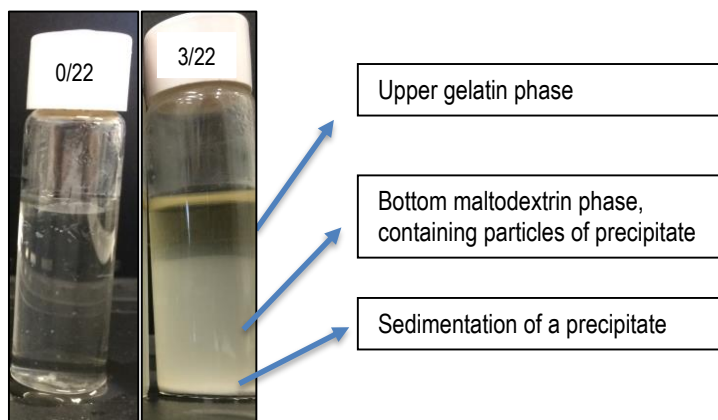

Figure 6.3. After one week: 0/22 (left sample) and 3/22 (right sample). The turbidity of bottom liquid phase of 3/22 sample is due to the formation of a precipitate.

After two weeks at 50ºC, some changes in phase behaviour are observed (Figure 6.4). The volume of the upper gelatin phase seems to decrease. This could be attributed to slow hydrolysis of gelatin.
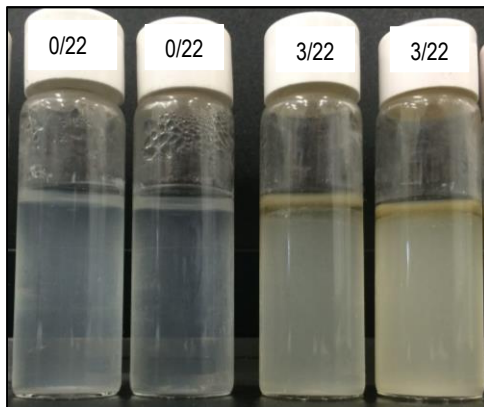
Figure 6.4. After two weeks: 0/22 (left couple) and 3/22 (right couple). The little turbidity of 0/22 samples means that precipitation is starting. The 3/22 samples are more turbid, and the volume fraction of the upper gelatin phase has greatly reduced. Each sample was prepared twice, to observe reproductibility.

Moreover, as seen in Figure 6.4., after two weeks, the onset of precipitation is observed in samples with 22% maltodextrin, in absence of gelatin, while those with gelatin looked cloudier and the two liquid phases almost disappeared.

Studying the phase behaviour of this system, as is seen in Figure 6.1, indicates that the solid phase is not present at concentrations under 2.5 wt% gelatin, "independently of % maltodextrin". It could be concluded that gelatin has an influence on the amount of solid phase which precipitates.

The approximate volume fractions of the gelatin and maltodextrin phases are indicated in table 6.1. Depending on the relative volume fractions of gelatin and maltodextrin phases, either gelatin-in-maltodextrin or maltodextrinin-gelatin can be obtained. If the contains high gelatin concentrations (gelatin concentration> 6 %), emulsions are composed of droplets that are enriched in maltodextrin, surrounded by a continuous solution that contains most of gelatin.

However, in the opposite situation, at low gelatin concentration (gelatin concentration< 4%), emulsions are composed of droplets that are enriched in gelatin, surrounded by a continuous solution that contains most of maltodextrin.

Table 6.1. Relative volume of the two separated liquid phases in the gelatin-maltodextrin W/W system.

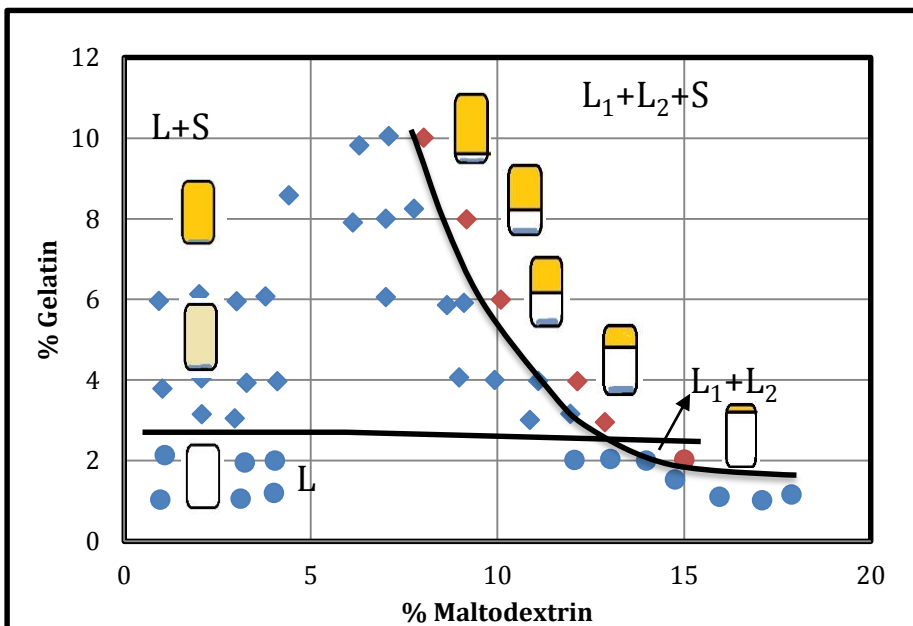| Sample	Composition	
	% upper phase (gelatin)	% bottom phase (maltodextrin)
10/8	86%	14%
8/9	76%	24%
6/10	56%	44%
4/12	32%	68%
3/13	13%	87%
2/15	10%	90%

Figure 6.5. Evolution of relative phase volumes (gelatin/maltodextrin aqueous system at 50ºC, after 5 days).

6.2. THE EFFECT OF SALTS IN THE PHASE BEHAVIOUR OF GELATIN-MALTODEXTRIN W/W SYSTEM

Starting from the phase behaviour of gelatin-maltodextrin W/W system, it is possible to study and compare the effect of additives in the system. First of all, salts were chosen according to Hofmeister series. As is seen in the experimental section, the studied salts were:

- $CsNO_3$: The two ions of the salt have a smaller negative ΔG_{hyd}, consequently, they are chaotropic ions.
- LiCl: The two ions of the salt have a large negative ΔG_{hyd}, especially Li^+, which is one of the monovalent ions with the largest negative ΔG_{hyd}. Consequently, LiCl is a kosmotropic salt. Small polyvalent ions have more negative ΔG_{hyd}, but they are not selected because they could complex gelatin [36].
- Lithium citrate: Trying to eliminate the effect of chloride in the previous salt to evaluate only the effect of Li^+. The problem is that citrate has 3 negative charges and it could ionically crosslink gelatin, which would not allow to observe the chaotropic/kosmotropic behaviour of the salts in an isolated manner.
- Urea: Although it is not an charged compound, urea has a larger negative ΔG_{hyd}, and it could change the phase behaviour of the system [13], [17].

The first hypothesis was the assumption that a kosmotropic salt could displace the binodal line to the left and a chaotropic salt would displace it to the right, as is shown in Figure 6.6.
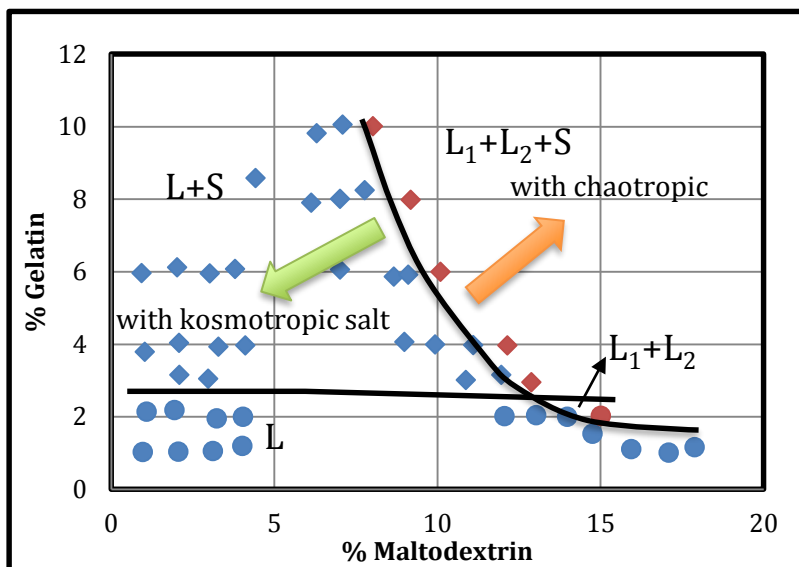
Figure 6.6. The effect of salts expected in gelatin/maltodextrin aqueous system at 50ºC.

First, some preliminary tests were carried out, in order to select the most appropriate electrolyte. The influence of $CsNO_3$ is shown in Figure 6.7. In absence of $CsNO_3$, at 6/10 gelatin/maltodextrin concentrations, two liquid phases (L_1+L_2) are observed. However, after adding 0.4 wt% $CsNO_3$, only one liquid phase (L) appears.
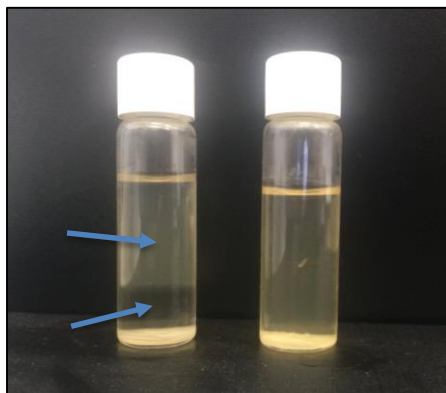

Figure 6.7. Left: sample without cesium nitrate (6/10). Right: sample with cesium nitrate (6/10, 0.4% $CsNO_3$). The blue arrows indicate two distinct phases, observed without $CsNO_3$.

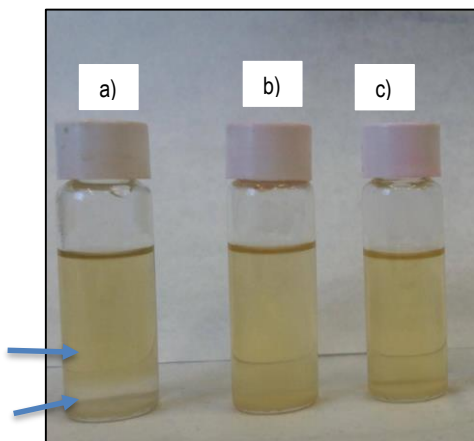The influence of lithium citrate and urea is shown in Figure 6.8.

Figure 6.8. a) sample 8/9 with a large amount of urea, b) sample 8/9 with 0.4 wt % LiCl, and c) sample 8/9 with 0.35 wt % lithium citrate.

Observing the previous figures, $CsNO_3$, LiCl and lithium citrate changed the phase behaviour of the system, but urea did not. Since it appeared that LiCl and lithium citrate have the same effect and considering that citrate could form complexes due to its multivalent nature, LiCl and $CsNO_3$ were used to study their influence on the phase diagram.

6.2.1 ADDITION OF CSNO$_3$

The influence of $CsNO_3$ on the phase diagram was studied adding a constant concentration ($\approx$ 0.4 wt %) of $CsNO_3$, under the same conditions as above. According to the "Hofmeister series", cesium is a chaotropic ion, which has smaller negative value for the Gibbs free energy of hydration [16]. Initially, it was though that cesium should ease the formation of one liquid phase versus two liquid phases, because the effect of disordering water could promote the coexistence of two incompatible solutions because a chaotropic salt does not "steal the water of hydration".
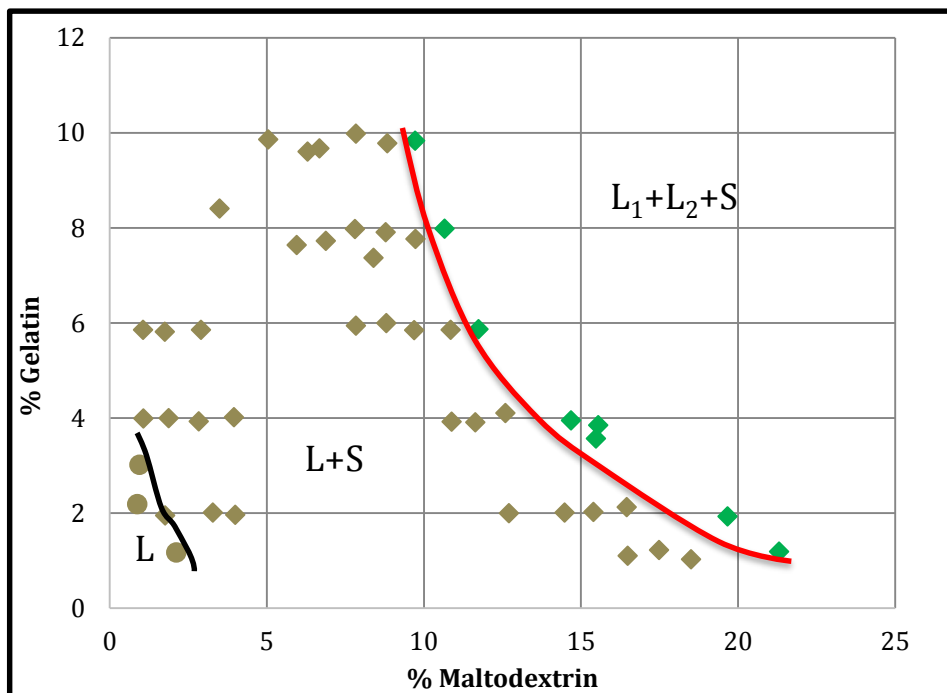
Figure 6.9. Phase diagram of gelatin/maltodextrin aqueous system with $CsNO_3$ at 50ºC, after 5 days.

Figure 6.9 shows the phase diagram after adding $CsNO_3$; all the mixtures had a concentration of $CsNO_3$ around 0.4%. The first evidence of the effect of $CsNO_3$ was the increase of the amount of solid phase in samples, decreasing the region of diagram without solid phase. Another evidence was observed: the binodal line was moved to the right, and means that the presence of $CsNO_3$ seems to favour the system to keep in one liquid phase against two liquid phases. An explanation of this last fact could be that maltodextrin and gelatin are more hydrated in presence of cesium ion as both a smaller negative value for ΔG_{hyd} [16]. Consequently, maltodextrin and gelatin do not interact easily in presence of Cs^+ and one liquid phase is enabled, following the previous hypothesis of coexistence of two incompatible solutions. On the other hand, the increase of amount of solid phase in samples could not be explained following this hypothesis. This fact responds better to an attractive interaction between polymers which is promoted by $CsNO_3$, where the precipitate could be a coacervate of maltodextrin and gelatin.

However, the objective in the present work was to achieve the opposite effect: finding an additive which could move the binodal line to the left of the diagram, enlarging the region in the phase diagram with presence of two aqueous phases. The importance of this idea is that it could be possible to encapsulate a compound inside a droplet of emulsion adding an additive which promotes the spontaneous formation of emulsion. Also, this effect lets to use lower % of polymers to get two phase behaviour.

6.2. ADDITION OF LICL

The phase diagram in Figure 6.10 shows mixtures after addition of LiCl, under the same conditions than before.
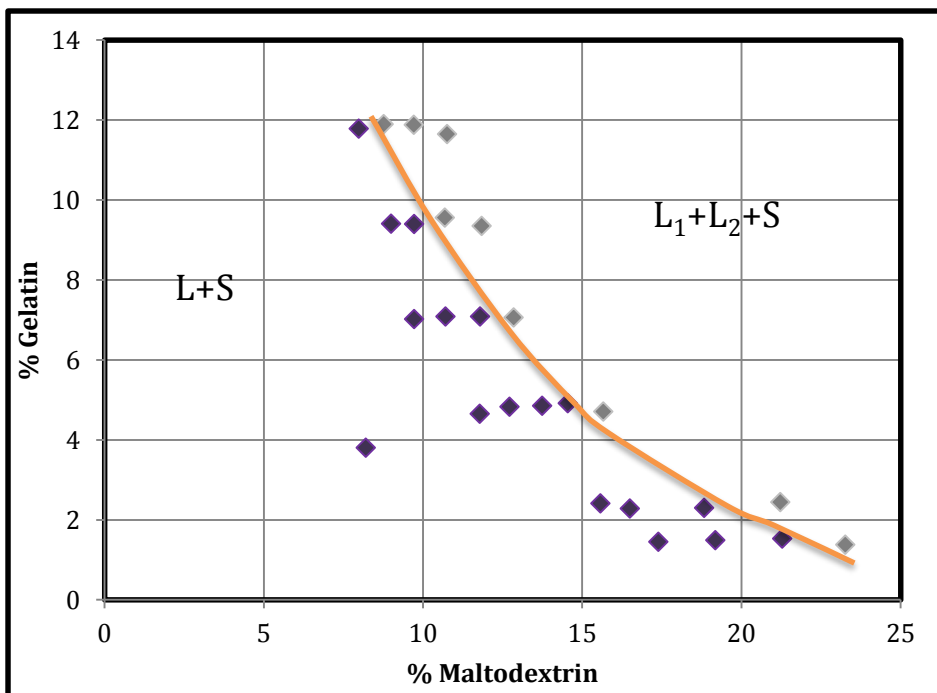

Figure 6.10. Phase diagram of gelatin/maltodextrin aqueous system with LiCl at 50ºC, after 5 days.

LiCl was chosen as lithium ion is a kosmotropic ion, which has a large negative value for the Gibbs free energy of hydration [16].

For this reason, lithium should be hydrated by water and as a consequence, maltodextrin and gelatin would be dehydrated and their mixture in aqueous solution would become unstable, leading to phase separation. However, this was not observed in our system.

All the mixtures had a concentration of LiCl around 0.4 wt % which is approximately the same concentration of $CsNO_3$ in the previous case. As it is seen in figure 6.10, there was not any mixture without solid phase and, as the previous case, the amount of solid was increased by adding LiCl. The hydration of lithium ion could favor the precipitation. However, the binodal line was moved to the right, which was the opposite expected behaviour. Another interesting result is that samples with LiCl had more precipitate than sample without additive or with $CsNO_3$ and, as is seen in Figure 6.10, all samples with Li^+ have a precipitate.

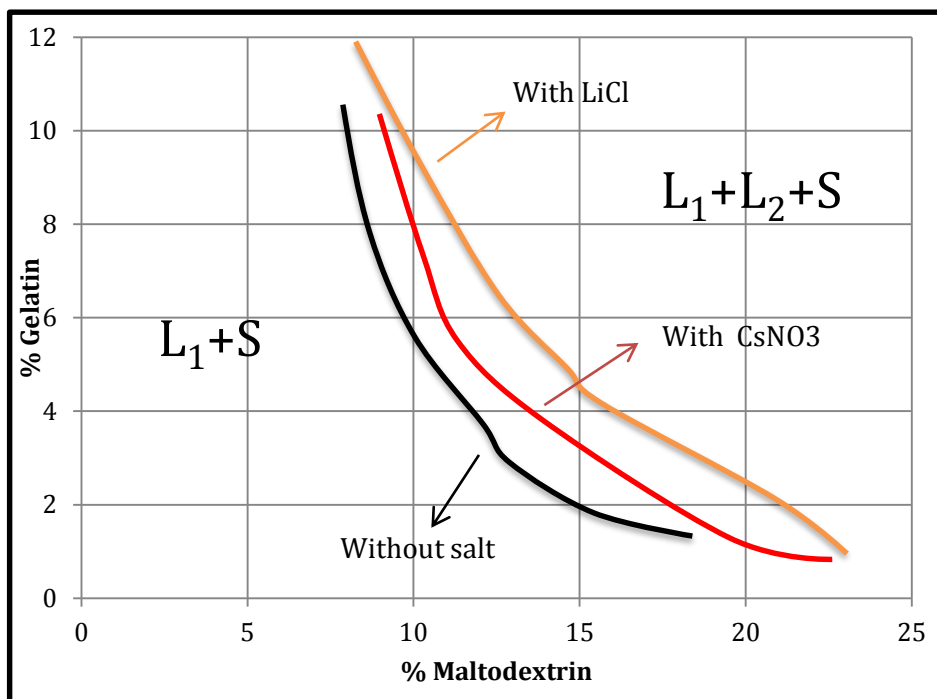Figure 6.11 summarizes the three previous experiments.

Figure 6.11. Comparison of the behaviour of liquid phases in the gelatin/maltodextrin W/W system with and without the presence of salts. The boundaries around solid phases are not represented.

The binodal line was moved more to the right for LiCl than $CsNO_3$. Therefore, both LiCl and $CsNO_3$ promoted the mixing of the two polymers, because the two electrolytes produce a reduction in the immiscibility region (L_1+L_2+S). Although it was expected that the behavior of the two salts was in opposite directions, the results show that the most important effect is that the salts have a higher affinity for water than polymers, and that the kosmotropic or chaotropic character only affects the relative displacement of binodal line (more negative is $\Delta Ghyd$, more to the right moves the bimodal line).

It might be concluded that the addition of both $CsNO_3$ and LiCl increases attractive interactions between the polymers: complexation between two biopolymers takes place, forming a coacervate, which is observed as a precipitate. Electrostatic interactions exist between macroions along the macromolecular chains, between oppositely charged side groups, and between other side groups. Net opposite charge, shape, size and flexibility of the macroions, and the reactivity of the side chain groups (amino acid residues in the gelatin) will control the interaction strength [24]. These facts might be changed by salts, which could change the charge density of amphoteric gelatin [11], favouring the attractive interaction between gelatin and maltodextrin. The formation of coacervate is promoted minimizing the effect of the solvent [37], which is the effect described by Hofmeister series. This coacervate formation implies as well that the effective concentration of the polymers in solution are lowered, implying a right shift of the binodal line. This effect is independent of chaotropic or kosmotroopic behaviour of the salts, and therefore would explain the right shift of the binodal line for LiCl and for $CsNO_3$.

6.3. THE EFFECT OF H^+ ON THE PHASE BEHAVIOUR OF THE GELATIN-MALTODEXTRIN W/W SYSTEM

To complete this research, the effect of H^+ was studied; H^+ has a value for ΔG_{hyd} between Cu^{2+} and Li^+. The chosen source of H^+ was acetic acid, as the acetate ion has a lower ΔG_{hyd} than sulfate ion or chloride ion from the commonly used acids H_2SO_4 or HCl.

The objective was to minimize the effect of counter ion, observing only the effect of H⁺. Gelatin is a charged protein with acidic and basic groups, with an isolectric point of 4.8-5.2. Acetic acid was added in a concentration to reach a pH below the isoelectric point and see the effect of H⁺ (at a pH between 3.5-4.5). As described in the introduction, pI describes the range of pH where the protein has a neutral net charge. Adding acid, an acid/base reaction occurs between H⁺ and the protein. This situation is like a buffer pH, and the effect of H⁺ in solution is not clear the influence of acetic acid and is shown in Figure 6.12.
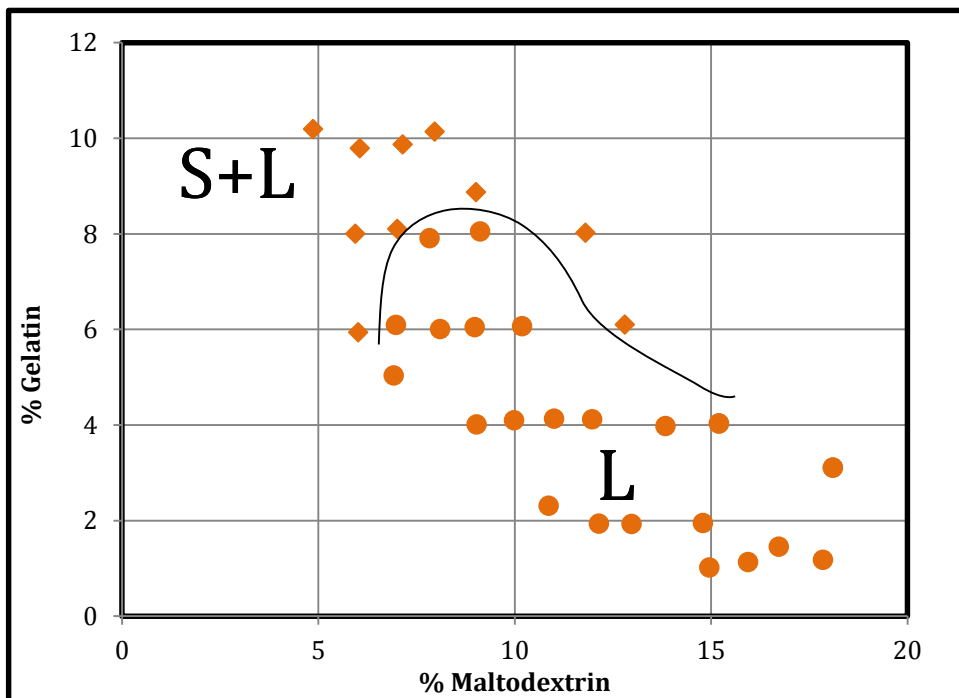

Figure 6.12. Phase diagram of gelatin/maltodextrin aqueous system with acetic acid at 50ºC, after 2 days (pH=3.5-4.5, aprox. 3.3% HAc in mixtures).

As seen in Figure 6.12, the system did not separate phases upon addition of acetic acid, but an interesting phase behaviour was observed: the solid phase disappeared in some mixtures with high percentage of gelatin (8/9 or 8/8 samples). This situation was not observed in the previous experiments. Acetic acid seemed to prevent the precipitation of maltodextrin or the formation of coacervate. This is an interesting phenomenon, as it is difficult to reverse the precipitation of maltodextrin due to its insolubility. The precipitation hinders in some cases the formation of emulsions, as the effective maltodextrin concentration in solution lowers, bringing it into the one phase region of the phase diagram.

As previously reported by Fang et al. [11], the mixing of gelatin and carrageenan aqueous solutions, at pHs even above the pI, where gelatin has a negative overall charge, can lead to an associative phase separation, as the uneven distribution of cationic and anionic groups along the gelatin chain produces some positive patches that electrostatically attract carrageenan. Carrageenan and maltodextrin could have a similar electrostatic behaviour because their monomers have similar structures. In this case, the opposite is suggested that could happen: the mixing of gelatin and maltodextrin aqueous solutions, at pHs below the pI, where gelatin has a positive overall charge, leads to some repulsive interactions, but low interaction (Case 4 in Figure 3.3). Therefore, no coacervation (attractive interaction) or phase separation (repulsive interaction) occurs.

A new hypothesis is proposed in view of the results of the effect of H^+ in gelatin-maltodextrin W/W system: having a mixture of the system with acetic acid which shows only one liquid phase, it could be possible to get a phase separation by adding a base and neutralizing the available H^+ of the system. Figure 6.13 shows the comparision of the phase boundaries, in presence and absence of acetic acid.
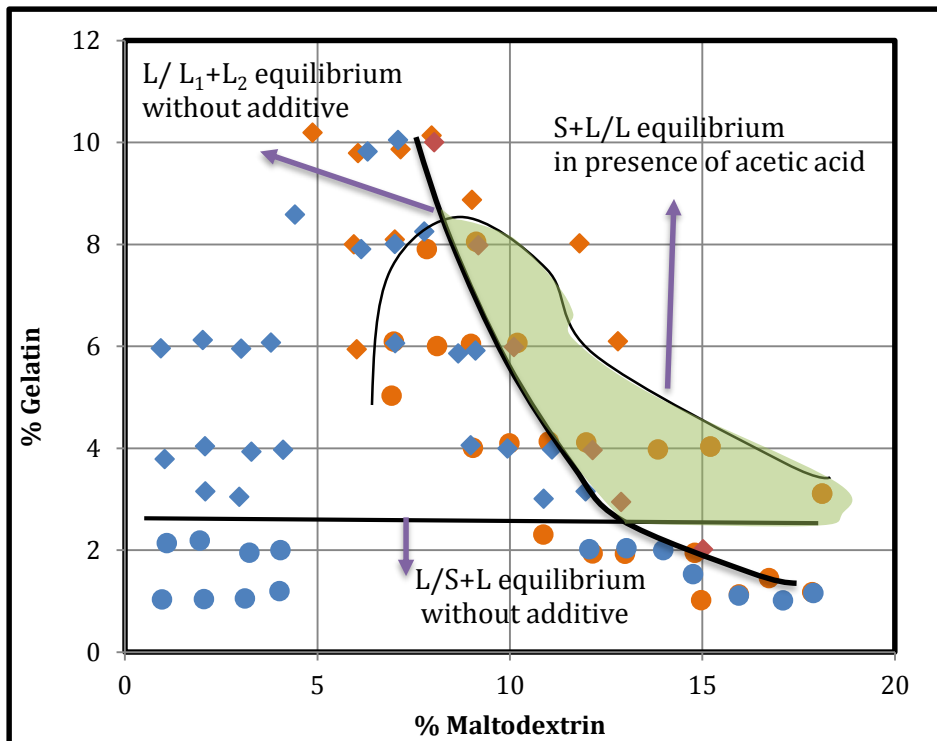
Figure 6.13 Comparision of the phase behaviour of gelatin/maltodextrin aqueous system without additive and with acetic acid at 50ºC.

The previous hypothesis was verified using samples of green zone. As seen in Figure 6.13, at this range of gelatin and maltodextrin, without additive, two liquid phases are observed. But with acetic acid only one liquid phase is obtained. Adding the necessary amount of base to neutralize the added acetic acid could lead to formation of spontaneous W/W emulsions by changing the charges of gelatin, and as explained above, thus also modifying its interaction with other polymers in solution. The used base was NaOH. The results of this experiment can be seen in Figure 6.14: adding dropwise the NaOH generated instantaneously a phase separation in the mixture.
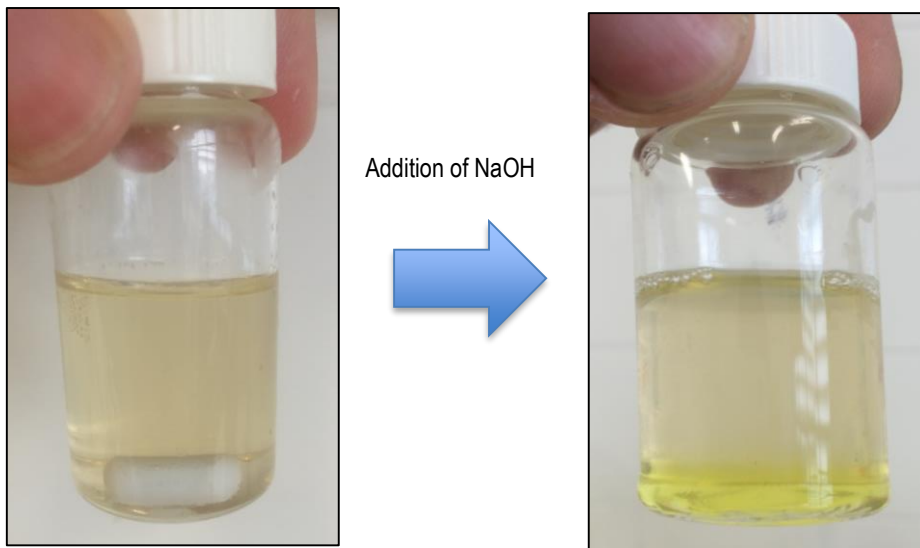
Figure 6.14. Left: the mixture before adding NaOH (4/13.8 with 4.4% HAc, pH=3.5-4.5). Right: the same mixture after adding NaOH without stirring (2.2% NaOH, pH=6.5-7.5). It was added approximately 2 g of 20% HAc stock solution and 0.9 g of 30% NaOH stock solution, achieving a HAc/NaOH molar ratio equal to 1. Temperature was kept constant at 50ºC.

First of all, it should be discussed if the phase separation is caused by the reaction with NaOH or by the concentration changes, because the addition of NaOH decreases the % of maltodextrin. A new experiment was designed to determine this: three samples were prepared as the previous one, with the same concentration of maltodextrin, gelatin and acetic acid. The additive of the following process was changed in each sample: water in the first sample, acetic acid in the second sample and NaOH in the last sample (this last mixture was like Figure 6.14). Phase separation was observed only in the last prepared mixture. These results allow to discard that the hypothesis that phase separation occurred due to concentration changes.

In Figure 6.14 can further be seen that the colour of the bottom phase was different than the other prepared mixtures in this work. In all previous mixtures, the upper phase was rich in gelatin and the bottom phase was rich in maltodextrin, indicating that gelatin solution has lower density than maltodextrin. Apart from the colour change, the addition of NaOH caused that the bottom phase had a lower volume, which would also indicate that this is the gelatin phase, so the bottom phase was rich in gelatin and the upper phase was rich in maltodextrin. Therefore, it could be assumed that the addition of NaOH changes gelatin properties enough to cause phase separation. The presence of gelatin in the bottom phase was detected by visual observation at room temperature, at which gelification occurred only in the bottom phase.

Getting a phase separation caused by adding NaOH means that a spontaneous formation of emulsions could be possible. This phenomenon may give a new method to obtain emulsions within a gelatin/maltodextrin aqueous system. Gelification keeps the microstructure of the emulsion droplets, avoiding phase separation with time. The microstructure of those gelified droplets, microgels, could be inspected by SEM, after purification and lyophilization of the sample. The lyophilization is a technique based on the sublimation of water maintaining the original structure of the system. A disadvantage of this procedure is that systems obtained in organic compounds, which usually have not good mechanical properties, end up collapsing. The different procedure to form emulsions (in the same system) could change its mechanical properties.

An experiment was performed under agitation as is seen in section 5.3.3.2. It was proceeded to gelify to form microgels from emulsions that obtained previously by adding NaOH. Before lyophilization of the hydrogel, it is attempted to check the formation of the emulsion, observing the gel under the microscope. Figure 6.15 does not show clear evidence of the emulsion formation, only the presence of solid is clear. This fact may show that following this experimental procedure emulsions are not formed.
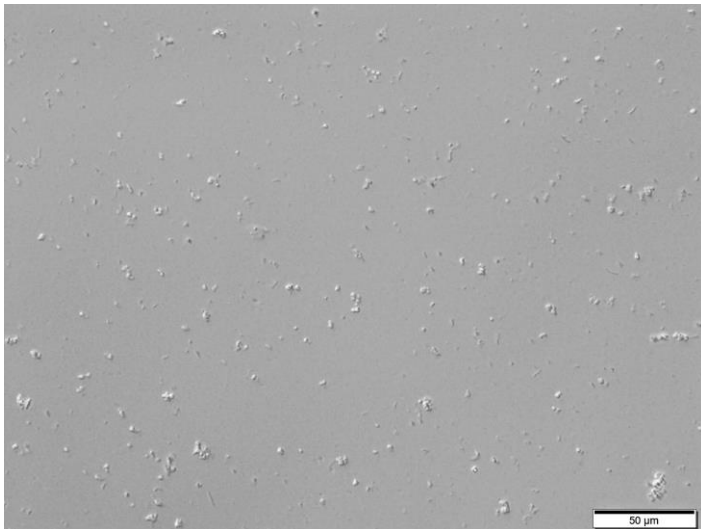
Figure 6.15. Optical microscopy image of the mixture ((3.65/15.07, 3.18% HAc and 2.21% NaOH, pH=6.5-7.5), after the gelification and dilution of sample, before its purification.

After the purification and lyophilization of the microgels, their microstructure was inspected by SEM. It could be possible that microemulsions were formed, but they were not observed by optical microscopy. Moreover, lyophilisation can lead to the collapse of the microgel sample. Figure 6.16 shows the microgels prepared in the present work (a-c) and those obtained in a similar system (4/11) without HAc or other additive (d-e) [32]. It was expected similar spherical or leaf-like shaped porous structures as images d) and e), but the microgels obtained in the present study do not show a so defined structure (collapsed). Image c) shows a clear structure, that it could be formed by aggregation of other smaller structures; it seems that in this case microgel was not so collapsed. Since emulsions had not been observed by the optical microscopy, the collapse in the lyophilization is an added problem to ensure that the designed procedure is useful to get spontaneous emulsions.

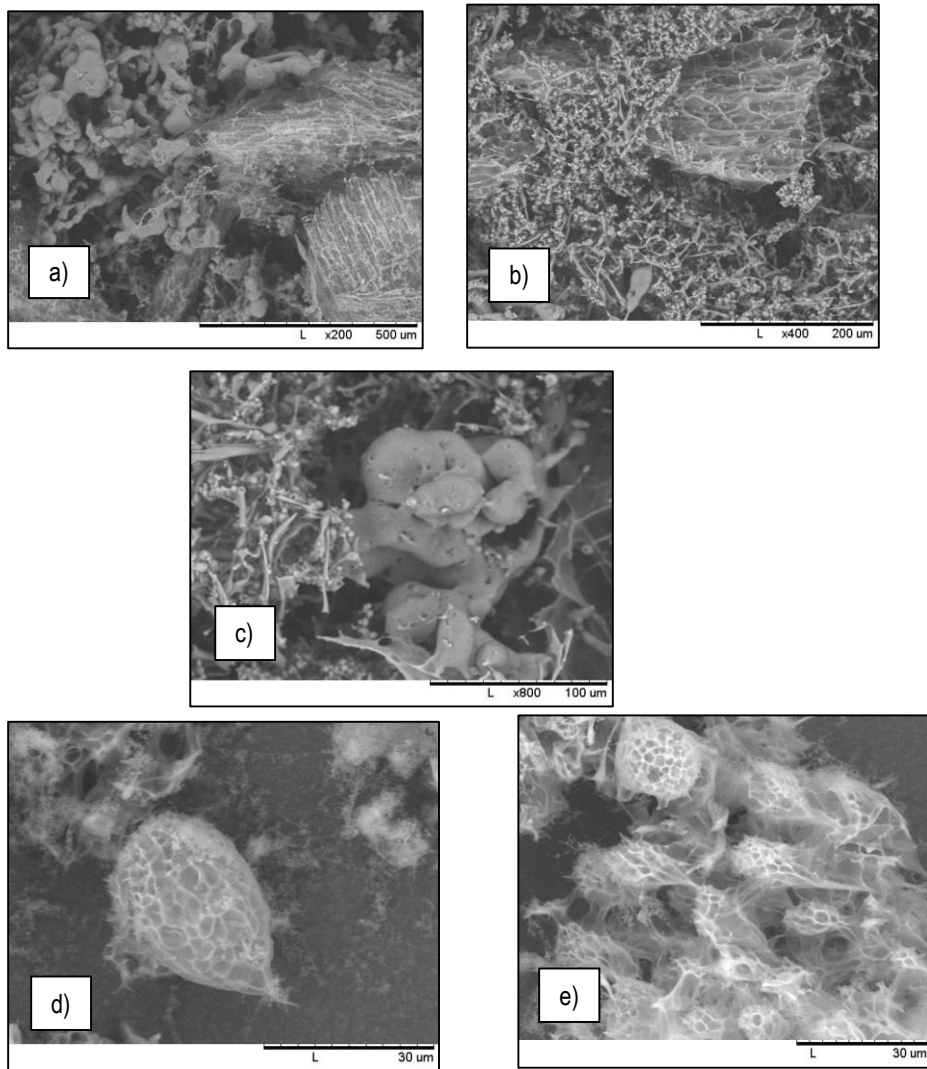**Figure 6.16.** a), b), c) were image by SEM of the microgels of mixture (3.65/15.07, 3.18% HAc and 2.21% NaOH, pH=6.5-7.5) after lyophilization (collapse). d) and e) are images by SEM of microgels of a mixture (4.5/11) in water without salts [32].

Summing up, emulsions are not observed clearly. Two hypotheses could be considered: on the one hand, the procedure followed to obtain emulsions is not useful and revising and modifying the experimental conditions (like time of stirring mixture with NaOH after his gelification, rpm of stirring...) could improve it. On the other hand, the emulsions were obtained, but they are not observed by the optical microscopy. The lyophilization was not successful and the collapse of the sample does not let to see clear microstructures by the SEM.

Consequently, it cannot be ensured that following this procedure emulsions are formed. Nevertheless, the phase behaviour observed when NaOH is added to mixtures containing HAc is a promising pathway for spontaneous formation of emulsions after phase separation of a one phase system. Further experimentation is needed to observe the emulsions of this phase separated system.

7. CONCLUSIONS

The main objective of this work consisted in the study of the phase behaviour of gelatin/maltodextrin W/W system, adding different additives, to observe their effect on phase behaviour. Conclusions can be drawn from the obtained results:

a) The phase behaviour of gelatin-maltodextrin W/W system has been studied at 50ºC, after five days, observing different regions in the phase diagrams: one liquid phase (L), two different liquid phases (L_1+L_2), one liquid phase and one solid phase (L+S) and two different liquid phases with solid phase (L_1+L_2+S). The presence of solid phase confirms the effect of gelatin on the precipitation of maltodextrin, reported elsewhere [33], [34].

b) Initially, it was expected that kosmotropic salt would displace the binodal line to the left and a chaotropic salt to the right. The results have shown, however, that both types of salts displace the binodal line to the right, promoting the mixing and compatibility of the two polymers. The difference between the salts is that LiCl (kosmotropic salt) displaces the binodal line stronger to the right than $CsNO_3$ (chaotropic salt). Moreover, the presence of these additives promoted the precipitation of maltodextrin. Both $CsNO_3$ and LiCl increase the attraction between the two polymers.

c) It has been seen that a pH=3.5-4.5 (adding acetic acid) promotes the mixing of the two polymers, in absence of solid phase. The neutralization of H^+ with NaOH favours the phase separation, opening a way to obtain emulsions.

d) W/W emulsions have not been observed by optical microscopy adding NaOH to an acid mixture. The dispersed phase was gelified, purified and lyophilized.

The study of procedure and conditions to obtain emulsions by neutralization of acidified mixtures can be the scope of future research.

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9. ACRONYMS

W/W (emulsion) – Water-in-water (emulsion)

W/O (emulsion) – Water-in-oil (emulsion)

O/W (emulsion) – Oil-in-water (emulsion)

O/O (emulsion) – Oil-in-oil (emulsion

W/O/W (emulsion) – Water-in-oil-in-water (emulsion)

O/W/O (emulsion) – Oil-in-water-in-oil (emulsion)

pI – Isoelectric point

MW – Molecular weight

rpm – Revolutions per minute

SEM – Scanning electron microscopy

wt % - Percentage by weight

HAc – Acetic acid