Universidade de Lisboa

Faculdade de Farmácia

The Influence of Preservative Systems in Cosmetic Gel Formulations prepared from Natural Rheological Modifiers

Cláudia de Matos João Pádua Santos

Mestrado Integrado em Ciências Farmacêuticas

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Orientadora: Doutora Paola Perugini, Professora Associada

Co-Orientadora: Doutora Aida Duarte, Professora Associada com Agregação

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Università di Pavia

Dipartimento di Scienze del Farmaco

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Resumo

Os geles são preparações semissólidas para uso farmacêutico com efeito protetor, terapêutico ou profilático. Os geles são sistemas semirrígidos de dois componentes, nos quais a fase contínua líquida é imobilizada por uma rede tridimensional reticulada constituída por partículas ou macromoléculas solvatadas na fase dispersa. As propriedades de coesão dos sólidos e as propriedades de difusão dos líquidos estão combinadas nos geles.

Os modificadores reológicos são adicionados às formulações com o objetivo principal de aumentar a viscosidade ou conferir um determinado perfil reológico. Também podem ter outras funções, como por exemplo, agentes gelificantes e emulsionantes.

Os conservantes são químicos naturais ou sintéticos que são adicionados às formulações para prevenir a contaminação microbiológica ou alterações químicas indesejáveis e também para aumentar o tempo de estabilidade do produto. Os sistemas de conservantes consistem na associação de dois ou mais conservantes para obter ao mesmo tempo uma atividade antibacteriana e antifúngica, originando, portanto, um espectro de ação mais alargado.

A estabilidade é referida como a ausência de separação da dispersão ao longo de um período de tempo. Na estabilidade de um produto cosmético, as propriedades dos produtos devem ser mantidas de forma a que o conjunto de características físico-químicas, organoléticas, microbiológicas e funcionais sejam adequadas ao fim a que se destinam. A estabilidade torna-se, portanto, um requisito essencial porque depende de outras condições essenciais que definem a qualidade do produto cosmético: segurança, conveniência, conformidade e eficácia para uso num sentido amplo.

Multiple light scattering é uma técnica utilizada para determinar o fluxo de luz transmitido e a retrodifusão de uma amostra. O valor obtido fornece informações sobre a homogeneidade da amostra. Além disso, este método permite detetar, compreender e prever fenómenos de instabilidade que ocorrem durante o envelhecimento ou tempo de prateleira.

O presente trabalho dedica-se ao estudo da influência dos sistemas de conservantes na estabilidade das formulações de geles para uso cosmético preparadas a partir de modificadores reológicos naturais. O objetivo é prever pelo método *multiple light scattering* se as formulações são estáveis no tempo. Se não forem estáveis, a finalidade é determinar o fenómeno de instabilidade.

Assim, foram preparados geles com diferentes concentrações de modificadores reológicos, conservantes e tensioativos. Os modificadores reológicos utilizados foram *Cellulose Gum* (CMC), *Sodium Carboxymethyl Betaglucan* (Beta-glucan) (Beta), *Carrageenan* (Car), *Acacia Senegal Gum & Xanthan Gum* (Solagum™ AX) (SAX), *Caesalpina Spinosa Gum* (SolagumTM Tara) (ST), *Microcrystalline Cellulose & Cellulose Gum & Xanthan Gum* (Sucrathix VX) (SVX)

e *Xanthan Gum* (XG), nas concentrações de 0,5%, 1% e 2%. Utilizou-se *Phenoxyethanol & Caprylyl Glycol* (Verstatil® PC) (PC), *Triethyl Citrate & Glyceryl Caprylate & Benzoic Acid* (Verstatil® TBG) (TBG) e *p-Anisic Acid* (dermosoft® 688 eco) (688) como conservantes e *Decyl Glucoside* (DG) e *Polysorbate 60* (PS) como tensioativos. Após a preparação, todos os geles eram homogéneos. Alguns eram claros e outros opalescentes devido aos componentes utilizados. Geralmente, a presença de Verstatil® TBG, decil glucosídeo, Solagum™ AX, Solagum™ Tara e Sucrathix VX tornam o gel opalescente.

Parâmetros como o pH e a viscosidade foram analisados. O pH foi medido no tempo zero (logo após a preparação) e após um mês, de forma a verificar se não houve alteração do valor de pH. O pH da maioria das amostras não alterou significativamente (variação de pH inferior ou igual a 0,5). A alteração de pH pode influenciar a eficácia dos conservantes que dependem do pH e a estabilidade do gel. A viscosidade foi medida por um viscosímetro rotacional com a agulha número 3 a 20°C. O valor de viscosidade foi obtido multiplicando o valor de leitura pelo fator e a curva de viscosidade traçada através do aumento da velocidade de 0,5 para 100 rpm. O perfil tixotrópico dos geles foi representado. As amostras com goma de celulose (CMC), Solagum™ AX (SAX), Sucrathix VX (SVX) e goma xantana (XG) têm um fluxo pseudoplástico, pois a viscosidade diminui quando a taxa de cisalhamento aumenta. Por outro lado, as amostras com beta-glucano (Beta), carragenina (Car) e Solagum™ Tara (ST), apresentam um fluxo dilatante, porque a viscosidade aumenta quando a taxa de cisalhamento aumenta.

A estabilidade dos geles foi monitorizada por avaliação organolética e pelo método *multiple light scattering* através do equipamento Turbiscan Tower. O Turbiscan é um inovador analisador ótico automatizado, trabalhando na região do infravermelho próximo com um modo de deteção dupla: transmissão e retrodifusão. Os geles foram transferidos para tubos de vidro cilíndricos e submetidos à análise de estabilidade pelo Turbiscan Tower. Para uma avaliação ótima, é importante que não existam bolhas de ar dentro da amostra e a amostra esteja homogeneamente distribuída. Para cada amostra foi realizado um ciclo a 20°C durante 6 horas, um ciclo a 4°C durante 6 horas e finalmente um terceiro ciclo a 20°C durante 6 horas. Para amostras transparentes, os valores de transmissão foram medidos; e para amostras opalescentes, o perfil de retrodifusão foi avaliado. De acordo com os resultados obtidos, a sedimentação, a separação de fases com clarificação e floculação são os fenómenos de instabilidade mais comuns nas amostras. As amostras tornam-se mais instáveis na presença de tensioativo e dermosoft® 688 eco. Além disso, amostras com menor concentração de tensioativo são mais estáveis. A maioria das amostras com Verstatil® TBG torna-se instável na presença de tensioativo. Amostras com beta-glucano e polisorbato 60, carragenina e decil glicosídeo, Solagum™ AX e decil glucosídeo, Solagum™ Tara e decil glucosídeo e goma

xantana e decil glucosídeo são instáveis. Todas as amostras com goma de celulose e tensioativo são instáveis. Por outro lado, as amostras com Sucrathix VX são as mais estáveis.

O controlo microbiológico foi realizado um mês após a data de preparação das amostras com a finalidade de avaliar se durante um mês, após a preparação da formulação, não houve desenvolvimento microbiano. Ao fim deste tempo, o conservante foi neutralizado com a adição de 9 ml de Eugon LT100 a 1 ml de amostra, retirou-se 1 ml e adicionou-se ao meio de crescimento para bactérias e outro 1 ml ao meio de crescimento para fungos. As culturas foram a incubar 48 horas a 37°C para bactérias e a 20°C por 3-5 dias para os fungos. Em todas as amostras não se verificou crescimento para os fungos, no entanto as amostras com o conservante dermosoft® 688 eco apresentaram crescimento bacteriano, um resultado esperado dado que, este conservante não é recomendado como eficiente para bactérias Gram+ e Gram-.

A análise fatorial é uma técnica estatística multivariada de dados exploratórios. O objetivo deste método é descobrir e analisar a estrutura de um conjunto de variáveis inter-relacionadas para construir uma escala de medição para fatores que de alguma forma controlam as variáveis originais. Variáveis com o valor do módulo maior que 0,15 têm significância na estabilidade do gel. Um valor de correlação negativa mostra que a variável contribui para a estabilidade do gel e um valor de correlação positivo causa a instabilidade do gel. A presença de goma de celulose, dermosoft® 688 eco, decil glucosídeo, polisorbato 60, combinação de Verstatil® TBG e decil glucosídeo, a combinação de Verstatil® TBG e polisorbato 60 e a variação do pH contribuem para a instabilidade do gel. No entanto, a presença de Sucrathix VX e Verstatil® PC contribui para a estabilidade do gel. Os resultados da análise fatorial são consistentes com os resultados observados.

O presente trabalho permitiu concluir que os sistemas de conservantes, utilizados nas formulações em estudo, têm influência na estabilidade dos geles, sendo que a presença de tensioativo foi o fator que mais contribuiu para a instabilidade do gel.

Palavras-chave: geles, modificadores reológicos naturais, conservantes, estabilidade, multiple light scattering

Abstract

Gels are two-component semi-rigid systems in which the liquid continuous phase is immobilized by a cross linked three-dimensional network consisting of particles or solvated macromolecules in the disperse phase. Gel have protective, therapeutic, or prophylactic effect.

Rheological modifiers are additives which are primarily used to increase the viscosity or impart a desired rheological profile to a formulation. They can sometimes be multifunctional and perform secondary roles such as gelling agents, emulsifiers, conditioners or film formers.

Preservatives are natural or synthetic chemicals which are added to several products as pharmaceuticals, cosmetics and food to prevent microbial contamination or undesirable chemical changes. Another purpose of the preservative addition is to prolong shelf life of the products. Preservative systems consist in an association of two or more preservatives to give a broader spectrum of activity.

Stability is referred as the absence of separation of the dispersion over a period of time. The stability of a cosmetic product is defined as the properties of the product to maintain the set of physico-chemical characteristics, organoleptic, microbiological and functional that made it responsive to its purpose of use. Stability becomes therefore an essential requirement because it depends on other key requirements that define the quality of the cosmetic product: security, agreeableness, compliance and effectiveness for use in a broad sense.

Multiple light scattering is a technique used to determine light flux transmitted through and backscattering from a product. The value obtained with this measurement gives information on the homogeneity of the sample and is characteristic of the dispersion. It enables to detect, to deep understand and to predict destabilization phenomena which take place during ageing or shelf-life tests.

The aim of the work is to evaluate the influence of preservative systems in cosmetic gel formulations prepared from natural rheological modifiers. In order to study this influence, it was prepared gels in different combinations of rheological modifier, preservative and surfactant. Then, organoleptic aspect, pH, rheology, stability and microbiological control were analysed.

The gels were prepared with different concentrations of rheological modifiers, preservatives and surfactants. After preparation, all gels were homogenous. Some were clear and other opalescent because of the components used. Generally, the presence of Verstatil® TBG, decyl glucoside, Solagum™ AX, Solagum™ Tara and Sucrathix VX becomes the gel opalescent.

Parameters as pH and viscosity were analysed. pH was measured at time zero and after 1 month. The pH of most of the samples did not change significantly (pH variation less than ±0,5). The pH change can influence the efficacy of the preservatives which are pH-depended and the gel stability. Viscosity was measured by rotational viscometer. For all viscosity

measurements, spindle number 3 was used. The viscosity values were obtained by multiplying the dial reading for factor. By increasing speed from 0,5 to 100 rpm., it is possible to trace a viscosity curve and thus obtain a rheological profile. All measurements were performed at 20°C. Samples with cellulose gum (CMC), Solagum™ AX (SAX), Sucrathix VX (SVX) and xanthan gum (XG) show a pseudo-plastic behaviour. Samples with beta-glucan (Beta), carrageenan (Car) and Solagum™ Tara (ST) show a dilatant flow.

The stability of the gels was monitored by organoleptic evaluation and by multiple light scattering by Turbiscan Tower. Turbiscan is an innovative automatized optical analyser, working in the near-infrared region with a double detection mode: transmission and backscattering. Gels for multiple scattering measurement were transferred into cylindrical glass tubes and submitted to Turbiscan Tower stability analysis. For each sample was performed one cycle at 20°C for 6 hours, one cycle at 4°C for 6 hours and finally a third cycle at 20°C for 6 hours. For clear samples transmission values were measured; for opalescent samples backscattering profile was evaluated. According with the results, sedimentation, phase separation with clarification and flocculation are the most common instability phenomena in the samples. The samples become more instable in the presence of surfactant and dermosoft® 688 eco.

Microbiological assay aims to evaluate if there is no microbial growth inside the sample and consequently if the preservative system has not been inactivated. Eugon LT100 broth (9 ml) was added to 1 ml of sample (9:1 broth:sample) in order to neutralize the preservative system. 1 ml of the previous mixture were transferred into culture medium: Tryptic Soy Agar for bacterial and Sabouraud Chloramphenicol Agar for fungi. Samples for bacteria were incubated at 37ºC for 48 hours and for fungi at 20°C for 3-5 days. For all samples tested, there were no fungal growth. On the other hand, samples with the dermosoft® 688 eco preservative showed bacterial growth because this preservative is fair for Gram+ and Gram- bacteria.

The factorial analysis is multivariate statistic technique of data exploratory. The purpose of this method is to discover and analyse the structure of a set of interrelated variables to construct a measurement scale for factors which somehow control the original variables. The factorial analysis calculates the correlation between the variables. Thus, variables with the modulus value greater than 0,15 have significance in the gel stability. A negative correlation value show that the variable contributes for the gel stability and a positive correlation value causes the gel instability. According to the results, the factors which the most influence the gels stability are the presence of cellulose gum, Sucrathix VX, Verstatil® PC, dermosoft 688® eco, decyl glucoside, polysorbate 60, the combination of Verstatil® TBG and decyl glucoside, the combination of Verstatil® TBG and polysorbate 60 and the pH variation.

Keywords: gels; natural rheological modifiers; preservatives; stability; multiple light scattering

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1. Introduction

1.1. Gels

1.1.1. Definition

The word ‘‘gel’’, introduced in the late 1800, is derived from ‘‘gelatin’’. (1,2)

According to the United States Pharmacopeia (USP), gels are defined as “semisolid systems consisting of either suspensions made up of small inorganic particles or large organic molecules interpenetrated by a liquid”. (1–3)

Gels are also defined as two-component semi-rigid systems in which the liquid continuous phase is immobilized by a cross linked three-dimensional network consisting of particles or solvated macromolecules in the disperse phase. (3,4) This disperse phase can be constituted by inorganic particles or organic macromolecules, primarily polymers. The inorganic particles are not dissolved but merely dispersed into the continuous phase; large organic particles are dissolved in the continuous phase, randomly coiled in the flexible chains. (4) The cross linking of the disperse phase in the gels can be established by via physical or chemical interactions. (3,4)

1.1.2. Physico-Chemical Characterization

Gels are systems with a crosslinked three-dimensional network of polymers dispersed in the liquid. After topical application, the liquid evaporates thereby leaving the drug in a thin gel film: forming matrix. (2) The presence of the crosslinked three-dimensional network gives the rigidity of gels. The structure of the network and the properties of the gels result from the nature of the particles and the type of the forces which are responsible for the linkages. (2,4) Spherical or isometric aggregates of small molecules or even single macromolecules constitute the individual particles of hydrophilic colloid. In linear macromolecules, the system is characterized from entangled macromolecules. The purpose of contact between which may either be generally small or consist of several particles aligned in a crystalline order. The force of attraction responsible for the linkage between gelling agent particles may range from strong primary valences to weaker hydrogen bonds and Van der Waals forces. A slight increment in temperature show the weak nature of the hydrogen bonds and Van der Waals forces because frequently causes liquefaction of gel. (4) Figure 1 represents the gels structure.

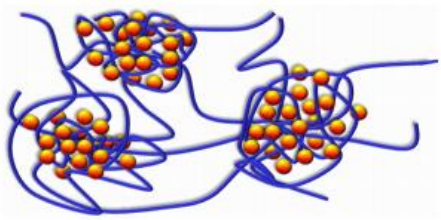

Figure 1: Gels structure (2)

Some gels are clear and others turbid because of the ingredients used. These ingredients have different solubility in the liquid of the continuous phase. (3) The appearance is like an elastic solid. Then, cohesive properties of the solids and diffusional properties of the liquids are combined in the gels. (5) In the steady state, the gel needs to be stressed or tense to flow. That is, the elastic modulus of gel, G', is greater than viscous modulus, G''. Thus, the gel is a solid but soft and squishy. Even though the polymeric network can be very dilute, it is enough to support shear stresses and thus gives the gel its solid-like material properties. (6)

Gels have several properties that will be described below. Ideally, gels should have a gelling agent for pharmaceutical or cosmetic application which should be inert and safe and it should not react with other components of the formulation. In addition, the solid-like nature of the gelling agent should be suitable to allow easy breaking when subjected to shear force either by agitation or topical application. Gels should also have preservatives to prevent microbial contamination. Regarding to topic gels, these should not be tacky and concerning to ophthalmic gels, these should be sterile. (2,4)

Gels have some characteristics such as swelling, syneresis, ageing, structure and rheology.

Firstly, swelling occurs when the solvent penetrates the matrix of the gel and is characterized by the absorption of the solvent by the agent, increasing the volume of the gel. Gel solvent interactions replace gel-gel interactions. The number of linkages between individual molecules of gelling agent and the strength of these linkages influence the degree of swelling.

Secondly, syneresis is the phenomenon which happens when gels contract spontaneously on standing and exude some fluid medium. The degree to which syneresis occurs, increases as the concentration of gelling agent decreases. The presence of syneresis shows the thermodynamic instability of the original gel. The contraction mechanism has been related to the relaxation of elastic stress developed during the setting of the gels. As these stresses are relieved, the interstitial space available for the solvent is reduced, forcing the liquid out.

Regarding to ageing, this process is related to the slow spontaneous aggregation performed by the colloidal systems. A gradual denser network of the gelling agent is formed by ageing. (2,4) Ageing is like the original gelling process and continues after the initial gelation, since fluid medium is lost from the newly formed gel. (2)

As regards the structure, as already mentioned, the cross-linking of the particles of the gelling agent forms a network that gives the rigidity to the gel.

Lastly, gels show rheological characteristics. Solutions of the gelling agents and dispersion of flocculated solid are pseudo-plastic because the viscosity decreases as shear rate increases, exhibiting a Non-Newtonian flow behaviour. By applying a shear force, the tenuous structure of the inorganic particles dispersed in the water is suppressed due to the breakage of the interparticulate association, exhibiting a greater tendency to flow. In the same way, if a shear force is applied in the macromolecules, the molecules align in the direction of the organic (single-phase system). (2,4)

1.1.3. Classification

The gels can be classified based on five parameters: colloidal phases, nature of solvent, physical nature, rheological properties and network configuration. (4,6) Table 1 summarize the gel classification.

According to the colloidal phases, there are single-phase gels and two-phase gels. Single-phase gels are characterized by organic macromolecules such as proteins, polysaccharides, and synthetic macromolecules, uniformly distributed on the continuous phase in such a way which no clear limits exist between the dispersed macromolecules and the liquid. (1,7) These type of gels is composed by natural gums or synthetic macromolecules, mentioned as gel formers. (7,4) They entangle randomly or bound by Van der Waals forces. (4) Mucilages are latter preparations. Although the continuous phase of the gels is usually water, oil and alcohol can also constitute this phase. For example, mineral oil can be combined with a polyethylene resin to form an oleaginous ointment base. Two-phase gels are characterized by a network of small discrete particles. The gel mass is mentioned as magma when the particle size of dispersed phase is relatively large. Two-phase gels may be thixotropic, forming semisolids on standing and becoming liquid on agitation. If gels and magmas have particles of colloidal dimension, they can be considered as colloidal dispersions. The accepted size range for colloidal particles is between 1 nm and 0,5 µm. The larger particle size of the dispersed phase in colloidal system is one difference between colloidal dispersions and true solutions. Other difference is in optical properties. True solutions look clear because do not scatter light and colloidal dispersions contain discrete particles scatter light. (7)

Concerning to the nature of solvent it is possible to classify gels as hydrogels, organic gels or xerogels. Hydrogels are composed by water on the continuous phase and a three-dimensional network of polymers or colloids as discontinuous phase. (4,6,8) Examples of these type of gels are bentonite magma, gelatin, cellulose derivatives, carbomer, and poloxamer gel. Organic gels contain a non-aqueous solvent, generally oil. Examples of organic gels are

plastibase (low molecular weight polyethylene dissolved in mineral oil & short Cooled), Olag (aerosol) gel and dispersion of metallic stearate in oils. Xerogels are solid gels with low solvent concentration. They are produced by evaporation of solvent or freeze drying, leaving the gel framework behind on contact with fresh fluid. They swell and can be reconstituted. Examples of these types of gels are tragacanth ribbons, acacia tear β-cyclodextrin, dry cellulose and polystyrene.

Related to the physical nature, the gels can be classified into elastic or rigid gels. Elastic gels show an elastic behaviour. The bonds which linked the fibrous molecules at the point of junction are relatively weak. Examples of these weak bonds are hydrogens bonds and dipole attraction. If the molecule possesses free –COOH group, then additional bonding takes place by salt bridge of type –COO-X-COO between two adjacent strand networks. Examples of elastic gels are alginate, Carbopol, pectin, agar and Guar gum. On the other hand, rigid gels can be composed by macromolecules in which the system linked by primary covalent bonds. Example of these type of gels is silica where silica acid molecules are held by Si-O-Si-O bond to give a polymer structure with a porous network.

According to the rheological properties, the gels generally exhibit a non-Newtonian flow and can be classified into plastic, pseudo-plastic and thixotropic gels. The rheogram of plastic gels is characterized from a yield value above which the elastic gel distorts and begins to flow. Bingham bodies and flocculated suspensions of Aluminium hydroxide are classified as plastic formulations. Pseudo-plastic gels show a pseudo-plastic profile. Viscosity decreases with increasing of shear rate, since long chain molecules of the polymers begin to align their long axis in the direction of flow with release of solvent from gel matrix. Dispersion of tragacanth, sodium alginate and Na-CMC are examples of pseudo-plastic gels. Thixotropic gels are characterized by weak bonds between particles which can be broken simply down by shaking. The resulting solution will revert back to gel due to the particles colliding and linking together again. Kaolin, bentonite and agar are examples of rheological modifiers with thixotropic behaviour. (4,8)

Gels can be characterized by chemical or physical interactions. Chemical gels are linked by permanent covalent inter-molecular bonds between cross-linked polymeric molecules. (5,6) Chemical gels are not affected by the time and/or temperature but it is affected by the electrolytes. (5) On the other hand, physical gels are connected by entanglements, ionic, hydrogen bonds, electrostatic interactions, dipole-dipole interactions, Van der Waals forces and hydrophobic interactions which are reversible secondary intermolecular forces relatively weak. (4,6). Some environment, as heating, can destroy gel structure, breaking inter-molecular interactions between polymeric chains. An example of physical gels is jellies. (5)

Table 1: Gel Classification (2,4)

Gel Classification	
Colloidal Phases	Single-phase Gel
	Two-phase Gel
Nature of Solvent Used	Hydrogel
	Organic Gel
	Xerogel
Physical Nature	Elastic Gel
	Rigid Gel
Rheological Properties	Plastic
	Pseudo-plastic
	Thixotropic
How Network is Held Together	Chemical Gel
	Physical Gel

1.1.4. Preparation of the Gel

Gels are relatively easier to prepare compared to emulsions. (9,10)

Gels are formed by aggregation of colloidal sol particles where the semisolid system is interpenetrated by a fluid (liquid or gas). The particles have between 1 nm and 0,5 µm of diameter and they link together to form a polymeric and colloidal network imparting rigidity to the structure and it is filled by a fluid. (5,11)

In order to formulate a gel, it is necessary the presence of the gelling agent in the aqueous phase that is the solid phase which form complexes composed by many molecules and are attached to each other. The gelling agents are natural or synthetic polymers which form linear (low yield gelling) and crosslinked structures. The keystone of all the proceedings is the polymerization of the gelling and the type of gelling agent. The system which obtains when a polymer is dissolved in a solvent is composed by individual macromolecules completely surrounded by solvent molecules with which are established more or less strong interactions. The more concentrated the solution, the more viscous it is. In concentrated solutions the further solvent addition forces the molecules to approach and the solution begins to show a transition from a concentrated solution to a gel because the branched chains (network) origin strong inter-molecular bonds (for example hydrogen bonds) in some places and then the opaque and rigid system becomes soft and transparent like the gels. (5)

On the other hand, there are other components necessary to produce a medicated gel such as actives, preservatives, stabilizers, dispersing agents and permeation enhancers. (10)

Generally, gels are prepared in industrial scale under room temperature. There are three methods for gel preparation: thermal changes, flocculation and chemical reaction.

Some gels are produced by thermal changes depending on the solubility of gelling agents. A gelling agent more soluble in hot water than in cold water when subjected to a decrease in temperature, undergoes a decrease in its degree of hydration, thus forming the gel. Examples of gels obtained by this procedure are gelatin gel, agar sodium oleate, guar gum and cellulose derivatives. On the other hand, the more soluble gelling agents in cold water than in hot water have hydrogen bonds with the water and when increasing the temperature, some hydrogen bonds may be broken which will cause the formation of the gel. This method cannot be used in all cases; therefore, it cannot be a general method.

Moreover, gels can be obtained by flocculation. In this method, the amount of salt added should be such as to cause precipitation leading to a state of age to form the gel and not an amount causing a complete precipitation. A rapid mixing must occur to avoid high local concentration of precipitant. The gels formed by flocculation have thixotropic profile. An example is ethyl cellulose: polystyrene in benzene is gelled by rapid mixing with suitable amounts of a non-solvent such as petroleum ether. However, adding salts to hydrophobic solutions don't form gels because coagulation occurs. Examples of these cases are gelatin, proteins and acacia gum which are not affected by the high concentration of electrolytes because of the salt out effect.

On the other hand, other gels are obtained by chemical interaction between solute and solvent. Examples are aluminium hydroxide gel, PVA, cyanoacrylates with glycidol ether (Glycidol), toluene diisocyanates (TDI), methane diphenyl isocyanine (MDI) that cross-links the polymeric chain. The aluminium hydroxide gel is obtained by interaction in aqueous solution of an aluminium salt and sodium carbonate: an increased concentration of reactants produces a gel structure. (4,8)

Generally gels are formulated by natural vegetable polymers such as gum arabic (from *Acacia senegal* exudate), Karaja gum (from *Sterculia urens* exudate), locust bean gum (extracted from seeds of *Ceratonia siliqua*), guar gum (extracted from seeds of *Cyamopsis tetragonolobus*), carrageenan (extracted from red algae *Chondrus crispus*), alginates (extracted from the family of *Laminarie* algae), xanthan gum (obtained from the fermentation of corn starch by the bacterium *Xanthomonas campestris*) and gellana gum (obtained from the fermentation of cultures of microorganisms of *Pseudomonas elodea*). These gelling agents are now much used in the food and more and more in small quantities in the cosmetic sector. However, cosmetic gel obtained with these natural polymers are not very pleasing to the customer due the sensory profile that it is not suitable for cosmetics. Furthermore, polymers have some disadvantages as gelling power and sensibility to the pH and electrolytes.

On the other hand, gels obtained by modified natural gelling agents (derivatives of cellulose modified as cellulose gum, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl methylcellulose), but also modified derivatives from guar gum (guar hydroxipropyl) show

particular lubricant properties. For this reason, this type of products is used in products for man (beard shaving products). In this group, there is also modified castor oil (hydrogenated castor oil) used for lipid gel.

Furthermore, the gelling agents include also synthetic acrylic derivatives (carbomer with different molecular weights 940-941-934), hydrocarbon derivatives (ethylene/propylene/styrene copolymer), and inorganic (smectite, clays, silicas). These gelling agents can be as liquid phase or solid powder. These gelling agents are technically better, with a compact and transparent structure, ease of preparation and good ability to deliver functional ingredients. (5)

1.1.5. Application

Gel represents a semisolid physical form for medical or pharmaceutical use, especially in the areas of cosmetics and food. (5) Gels are applied to the skin or mucosa with protective, therapeutic, or prophylactic effect. (12)

Gels are considered delivery systems which allow to administer drugs orally, topically or into body cavities and intramuscularly. (2,7,4) Gels can be used as long acting forms of drug injected intramuscularly.

Moreover, gelling agents are useful binders in tablet granulation, protective colloids in suspensions, thickeners in oral liquids, and suppository bases.

Cosmetically gels have been employed in wide variety of products, including shampoos, fragrance products, dentifrices, skin and hair care preparations. (2,4)

The gel containing anti-inflammatory steroids is used to treat scalp inflammations because the creams and ointments are too greasy for this location.

Gels have better potential as a vehicle to administer drug topically in comparison to ointment, because they are non-sticky, requires low energy during formulation, are stable and have aesthetic value. (2)

In according to gelling agent, it is possible to obtain different type of gels, such as soft/sliding, solid and siliceous-glyceric-gel (characteristics of toothpastes). The gels are contained in tubes or bottles.

In cosmetics field, gels as emulgel, hydrogel, hydroglicerin pastes and sticks have seen significant expansion for several applications, such as for the skin (face and body), for the tooth, for the hair and, nowadays, for the reconstruction of the nails. (5)

1.1.6. Advantages and Disadvantages

In Table 2 is resumed advantages and disadvantages of a topical gels.

Table 2: Advantages and disadvantages of a topical gel (2,13)

Advantages of a topical gel	Disadvantages of a topical gel
No gastrointestinal drug absorption and consequently no subjected to enzymatic activity and no drug interactions with food, drink, and drugs	Poor permeability of some drugs through the skin
No first-pass effect, possibly avoiding the deactivation by digestive and liver enzymes	Can be used only for drugs which require very small plasma concentration for action
A substitute for other routes of administration in cases as vomiting, swallowing problems, resistant children and diarrhoea	Larger particle size drugs not easy to absorb through the skin
Patient acceptability since is non-invasive and avoids the inconvenience of parenteral therapy	Vulnerable to microbial contamination and for this requires preservative addition
Reduction of doses as compare to oral dosage forms	Short duration of action due to rapid absorption
Ability to dissolve a wide range of medications with different chemical properties, making combination therapy with one transdermal cream possible	Possibility of allergenic reactions
Cooling effect due to evaporation	Enzyme in epidermis may denature the drugs
Can be used on macerated skin	Less stable, can crack
Drug therapy may be terminated rapidly by removal of the application from the skin surface	
Localized effect with the minimum side effects	
Gels have less additives	
Less greasy in nature and can be easily removed from the skin	
Cost effective	

1.2. Rheological Modifiers

1.2.1. Definition

Rheological modifiers are additives which are primarily used to increase the viscosity or impart a desired rheological profile to a formulation. (14) Furthermore, rheological modifiers are also commonly known as thickeners and they are added in limited proportion to another substance or mixture of substances in order to modify the rheological behaviour. (5,14) On the other hand, they can sometimes be multifunctional and perform secondary roles such as gelling agents, emulsifiers, conditioners or film formers. (14) Rheological modifiers are used to increase viscosity of suspensions and avoid particle sedimentation, and to increase the dispersion of insoluble substances and physical stability of emulsions and tensiolites. For example, a polymer can be used to stabilize a shower gel with exfoliate effect: polymer increases the viscosity of the system slowing down the surfacing or sedimentation of the exfoliating particles In particular polymer modifies rheological behaviour by nearly Newtonian

to pseudo-plastic profile with sliding threshold, in order to prevent the movements of the particles. (5)

A rheological profile can be classified as Newtonian or non-Newtonian. These last ones can be divided, according to shear, in pseudo-plastic, plastic and dilatant flow. (14) Figure 2 represents Newtonian and non-Newtonian flows.

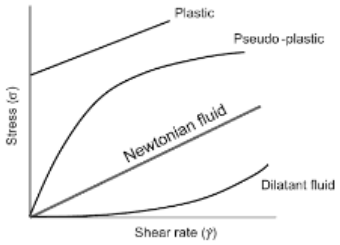

Figure 2: Newtonian and non-Newtonian flows (15)

A Newtonian flow has a constant viscosity independently from shear rate an ideal behaviour. (14) If graphically represented, with shear rate vs shear stress, it obtains a straight line through the origin of the Cartesian axes. The value of the coefficient represents the rate of viscosity. Since the viscosity is constant and independent of shear rate, is enough one measurement to completely characterize the system. (15) This type of flow is characteristics of water, glycerol, olive oil, and others solvents and mineral oils. (14,15)

Non-Newtonian flow is represented by the majority of the fluids. Plotting shear stress vs shear rate it obtains a curve. So, the coefficient of viscosity is different at each point on the shear stress versus shear rate curve. In this case it is possible to speak about “apparent” viscosity, determined at each point of the curve, using the tangent of the angle ϕ. The evaluation of apparent viscosity can be particularly important when the “thickening” behaviour of two high molecular weight water-soluble polymers are compared. One polymer may have a higher apparent viscosity than the other one at a low shear rate, but a lower apparent viscosity at higher rate. For this reason, the measurement of a single apparent viscosity has little significance for non-Newtonian fluid. It is not only necessary to measure the viscosity at more than one shear rate, but the values must be in the range which is important for the particular application. (16)

A pseudo-plastic flow behaviour is characteristic of many cosmetic products where viscosity decreases with increasing of shear rate. However, when the shear force is removed, the fluid immediately reverts back to its original viscosity. If the fluid returns to original structure with dilated time, it speaks about thixotropic behaviour time dependent.

Dilatant or shear thickening polymers show an increasing viscosity with increasing shear rates. Dilatant flow can be verify in dispersions with high solids content or high polymer concentrations. (14)

Figure 3 synthetises the types of flow behaviour.

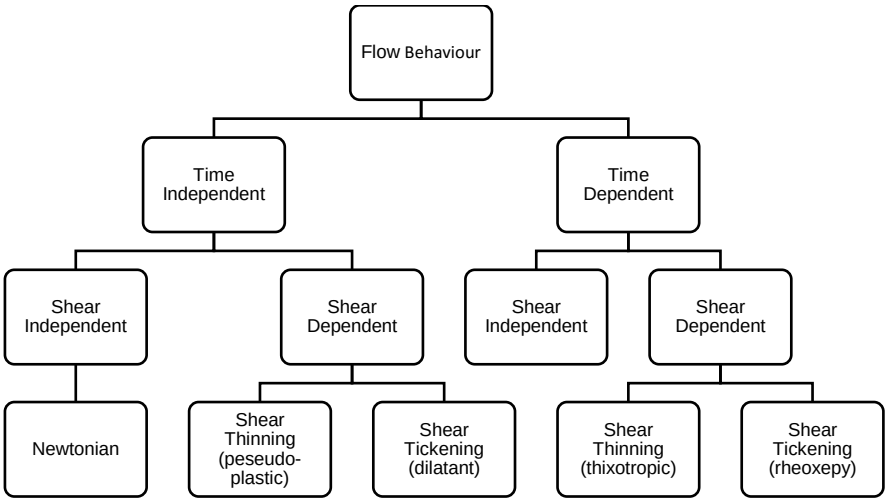

Figure 3: Types of Flow Behaviour (16)

1.2.2. Classification

The classification of the rheological modifiers can be based on a variety of schemes, including their ionic charge (anionic, cationic, non-ionic or amphoteric), their application in aqueous or solvent-based formulations, and their thickening mechanisms. Traditionally, these components are classified by their chemical nature and origin. Concerning to chemical nature, rheological modifiers can be organic or inorganic and related to the origin they can be natural, semisynthetic and synthetic.

Natural polymers are originated from plants, animals or microorganisms; they have larger chemical structures based on proteins or polysaccharides. (14) There are two types of problems related to the use of natural rheological modifiers: reproducibility, microbiological contamination and environmental contaminants. (5)

Semisynthetic rheological modifiers include modified celluloses such as carboxymethyl cellulose, methyl cellulose, hydroxyethyl cellulose, and hydroxypropyl cellulose. (15)

Synthetic rheological modifiers include more and more products, different for structure such as polyelectrolytic acrylic polymers and maleic anhydride copolymers. (5,15) It is possible to classify in acrylic derivatives, hydrocarbon derivatives, and amorphous silicas and silicates. (5)

1.3. Preservatives

1.3.1. Definition

Preservatives are natural or synthetic chemicals which are added to several products as pharmaceuticals, cosmetics and food in order to prevent microbial contamination or undesirable chemical changes. (17) The Regulation nº 1223/2009 defines preservatives as “are substances exclusively or mainly intended to inhibit the development of microorganisms

in the cosmetic product". (18) Another purpose of the preservative addition is to prolong shelf life of the products. It is essential to add preservatives to several products, specially to those that have higher water content, because this addition avoids the alteration and the degradation by microorganisms during storage. (17)

Preservative systems consist in an association of two or more preservatives in order to obtain at the same time an antifungal and antibacterial activity to give a broader spectrum of activity.

Some preservatives, due to their limited solubility in water, are difficult to add to formulations. For this reason it is better to pre-dissolve the preservative in an opportune solvent or use liquid preservative. (19)

1.3.2. Chemico-physical Characterization

Preservatives should have ideal properties. These type of formulation components should not be irritant or toxic. They should be physical and chemical stable and compatible with other ingredients used in formulation. Preservatives should be act as good antimicrobial agent and should exert wide spectrum of activity. They should be potent in order to use small concentration. Preservatives should maintain activity throughout product manufacturing, shelf life and usage. (17)

In addition, in order to be an ideal cosmetic preservative, it should have lack of irritation and lack of sensitization, should be stable at a wide range of temperatures, pH and dilated time, should be compatible with numerous ingredients and packaging materials, should be effective against numerous microorganisms and should not have odour or colour. (20)

In Table 3, the properties of an ideal preservative are reported. However, an ideal preservative does not exist. It is always better to use an association of preservatives. (19,20)

Table 3: Properties of Ideal preservative or preservative system (19)

Property	Explanation
Broad-spectrum activity	The preservative kills all types of microorganisms (yeast, mould, Gram-positive and Gram-negative bacteria). In general, molecules active against bacteria are not active again yeasts and moulds and vice versa.
Effective at low concentrations	Preservatives do not add a marketing value to products. In fact, they are really a form of insurance. Lower concentration levels reduce the irritation or other toxicity effects.
Water-soluble and oil-insoluble	Preservatives must be actives in the water phase since microorganisms grow in the water phase or at the water-oil interface.
Stable	The preservative should be stable under all temperature and pH conditions that it could encounter during the manufacturing of our cosmetics. However, no organic compound is stable in elevated heat or extreme pH conditions.

Colourless and odourless	Preservatives have not to add colour or odour to the product or react with other ingredients to form colours or odours.
Compatible	They should be compatible with all ingredients and not lose activity in their presence.
Shelf-life activity	The ideal preservative would function during the manufacturing and throughout the all life of the cosmetic.
Safety	It would be safe to use.
Easy to analyse	The preservative should be easy to analyse by popular analytical common methods. It would be even better to analyse for its anti-microbial activity this way. For example, it is easy to analyse paraben levels by HPLC. However, HPLC does not tell if parabens are totally inactive in the presence of Polysorbates or other inactivators.
Easy to handle	The ideal preservative would be easy to handle: liquids are easier to handle than solids; flakes or non-dusting or non-caking powders are easier than solid chunks. It also should be non-flammable or non-toxic as it is shipped.

1.3.3. Classification

The classification is based on mechanism of action and source. Related to mechanism of action, preservatives can be classified as antioxidants, antimicrobial agents and chelating agents.

Firstly, antioxidants prevent oxidation of actives. Examples of these type of preservatives are vitamin E, vitamin C, butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT).

Secondly, antimicrobial agents are active against Gram-positive and Gram-negative microorganisms, reason of degradation of the formulation. In addition, they are active at low concentration. Examples of antimicrobial agents are benzoates, sodium benzoate and sorbates.

Lastly, chelating agents prevent the degradation of formulation by forming a complex with other ingredients. Examples of these type of preservatives are disodium ethylenediamine tetracetic acid (EDTA), polyphosphates and citric acid.

Preservatives can also be classified as natural or artificial. In one hand, natural preservatives are obtained by natural sources such as plants, minerals, animals and others natural sources. Examples of these type of preservatives are neem oil, salt (sodium chloride), lemon and honey. Artificial preservatives are obtained by chemical synthesis and are active against various microorganisms in small concentration. Examples of these type of preservatives are benzoates, sodium benzoate, sorbates, propionets and nitrites. (17)

1.3.4. Mechanism of Action

The mechanism of action of a preservative is multiple and it is not always clearly identified. The bacteriostatic or bactericidal action could be affected by: destruction of the cell wall, modification of the cell membrane permeability or its destruction, denaturation of cytoplasmic or membrane proteins or enzymatic inactivation. (5)

The ethylenediaminetetra-acetic acid (EDTA) is a chelating agent which modifies the cell membrane permeability. This preservative acts in synergy with other chemical preservatives and this synergy interruptes the outer lipid layer of the cell membrane of Gram-negative bacteria. Then, the stability dependent of calcium and magnesium ions is altered, allowing more penetration of other antimicrobial agent into the bacteria cell.

A “self-preserved” formula is another method to preserve a product by using raw materials which not support the microbiological growing and optimize their relative content. Humectants like glycerin and sorbitol at specific levels decreses the water activity, increasing the formula resistance. Other ingredients have inherently antibacterial properties, contribuiting for a self-preservation of the product. Examples of these ingredients are alcohols, cationic detergents, fragance components, lipophilic acids (lauric and myristic acids), essential oils like tea tree oil or geraniol or eucalyptol. These ingredients are frequently used in cosmetic formulations. The physical factors which contribute to build a self-preserved product are pH and water activity. For example, the most of the microorganism living at pH around 5 to 8 and if the pH of the product is out of this range it is more difficult for bacteria to live. On the other hand, since water is essential for bacterial growth the decrease of water activity avoids bacterial contamination of the product. (21)

1.3.5. Microbiological Control

The microbiological control is described in Portuguese Pharmacopeia. There are five methods: microbiological examination of non-sterile products: microbial enumeration tests, microbiological examination of non-sterile products: test for specified microorganisms, efficacy of antimicrobial preservation, efficacy test of antimicrobial preservatives and microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.

In microbiological examination of non-sterile products: microbial enumeration tests, the determination of the total viable aerobic germs is performed by the membrane filtration method or by plaque determination. However, there are samples which cannot be analyzed by the membrane filtration method or by plaque determination samples. In these cases, the 'most likely number' method is used. The choice of the method depends on several factors, such as product nature and the expected number of microorganisms. All these methods are conventionally validated. These tests allow the determination of mesophilic bacteria and fungi

and yeasts that grown in aerobiosis. If the sample has antimicrobial activity, it is conveniently neutralized. If antimicrobial inactivators are used for this purpose, their efficacy and toxicity to the microorganisms in question is demonstrated.

For microbiological examination of non-sterile products: test for specified microorganisms, the selective media are used to specified search microorganisms. The microorganisms that have undergone subtheal lesions are not detected in any selective media. When using selective media, the procedures encompass a revival stage, since these microorganisms have an impact on the quality of the product. If the sample has antimicrobial activity, it is conveniently neutralized. The selective media for Enterobacteria and other Gram-negative bacteria, *Escherichia coli*, *Salmonella, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The neutralizing agents may be added to the product in order to neutralize any antimicrobial activity. These agents may be added to the buffered peptone solution with sodium chloride, pH 7,0, preferably before sterilization. In Table 4, there is an example of a composition of a neutralizing solution. Sterilize by autoclaving at 121°C for 15 minutes. If the neutralizing power of the solution is not sufficient, the polysorbate 80 or lecithin content may be increased, or other neutralizing agents may be added, such as sodium lauryl sulfate and sodium thioglycolate.

Table 4: Composition of a neutralizing solution

Composition	Quantity
Polysorbate 80	30 g
Egg yolk lecithin	3 g
Histidine hydrochloride	1 g
Meat or casein peptone	1 g
Sodium chloride	4,3 g
Monopotassium phosphate	3,6 g
Disodium phosphate dihydrate	7,3 g
Purified water	1000 ml

In efficacy of antimicrobial preservation, preservatives are added to pharmaceutical preparations when these preparations don’t have appropriate antimicrobial activity. They have the goal to avoid microbial proliferation under normal conservation conditions because microbial contamination could present a risk of infection for the patient and deterioration of the preparation, particularly in multi-dose containers. The effectiveness of a preservative depends on the active compound of the preparation, the composition of the preparation in which it is incorporated or the container and the mode of closure adopted. In the period of validity, the antimicrobial activity is evaluated to ensure that during that period there is no change in the antimicrobial activity. During the development stage of a pharmaceutical preparation, the antimicrobial activity of the preparation itself is checked or, if necessary, demonstrated that, when added with 1 or more suitable preservatives, it provides adequate protection against the

harmful effects which may result from microbial contamination or proliferation during the shelf-life and use of the preparation.

The efficacy test of antimicrobial preservatives consists of the artificial contamination of the preparation, if possible in the final recipient, by the inoculation of appropriate microorganisms, keeping the seeded preparation at a suitable temperature, collecting samples from the recipient at certain time intervals and carrying out a count of the microorganisms. Preservative properties are considered appropriate when, under the test conditions and after prescribed intervals of time and temperatures, there is a significant decrease or absence of an increase in the number of microorganisms in the inoculated preparation. As regards the reduction in the number of microorganisms as a function of time, the acceptance criteria vary for the various categories of preparations according to the desired degree of protection. The tests are carried out with 1 strain at a time. The specified microorganisms are supplemented with strains or species which constitute potential contaminants of the preparation.

In microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use, the manufacture, packaging, storage and distribution of pharmaceutical preparations shall be conducted in such a way as to ensure a satisfactory microbiological quality. Gels as they are local application belong to category 2. The acceptance criteria for category 2 are: a maximum of 10^2 viable aerobic germs (bacteria, fungi and yeasts) per gram or milliliter; a maximum of 10 Enterobacteria or other Gram-negative bacteria per gram or milliliter; absence of *Pseudomonas aeruginosa* in 1,0 g or 1 ml; and absence of *Staphylococcus aureus* in 1,0 g or 1 ml. (22)

1.4. Stability

Stability is referred as the absence of separation of the dispersion over a period of time. It is necessary to distinguish between colloidal stability from physical/mechanical instability. In colloidal stability, particles do not aggregate over the time and in physical/mechanical instability, the particles or droplets tend to sediment or cream under gravity over a period of storage. In this case, the particles or droplets may show no aggregation and the gravity force exceeds the Brownian motion. (23)

There are several theories which explain the stability of formulations. Firstly, it is necessary to define Stern layer. In the Stern layer, ions with opposite charge stay together around the charged surface. However, since the charge on the surface is not completely balanced, a second region, called the diffuse layer, balances the surface charge. (24)

In the 1940's, some scientists developed a theory about the stability of a colloidal system. This theory was called DLVO based on the attractive and repulsive forces present in a dispersion. (11,25,26) The total force between colloidal particles is obtained by adding together

the Van der Waals and electrical double layer forces. (25) The DLVO theory supposes that the dispersion as a diluted sample and that only two forces affect the dispersed particles: attractive and repulsive electrostatic forces. The electric charge and other properties are uniformly distributed over the solid surface and electrostatic forces, Brownian motion and entropy considerations determine the distribution of charged domains. Therefore, the DLVO theory explains the interaction between two particles as they approach each other.

Moreover, colloidal stability is then influenced by the energy of the attractive interaction due to Van der Waals forces and the energy of the repulsive electrostatic interaction. The particle energy can be expressed in according to Equation 1:

$$V_T = V_A + V_R \qquad (1)$$

where V_A represents the attractive forces, V_R the repulsive electrostatic interaction and V_T the particle energy. (11,26)

For spherical particles, the Van der Walls attractive energy is inversely related to the distance between the particles, while the electrostatic repulsive energy declines exponentially with distance. Usually, when the particles are at long distances from each other, a permanent phenomenon of coalescence/aggregation of the droplets/particles does not occur because the particles experience a minimal attraction. When the particles undergo an attraction at defined distances from 10 to 20 nm, form aggregates (secondary minimum in Figure 4) known as flocs, occurring then flocculation, reversible phenomenon.

The particles begin to experience some repulsion as they approach each other (primary maximum in Figure 4). The intensity of the force in the maximum primary determines whether the system will stay flocculated as it is. If the interaction energy at the primary maximum is high, the colloidal particles are stable and show no tendency to flocculate. If the energy of the interaction at the primary maximum is low, the particles can be forced together. This barrier may be overcome if the kinetic energy of the dispersion resulting from the normal thermal motion is sufficient for such. The particles will coalesce/aggregate permanently if the interaction energy reaches the primary minimum due to the separation of the particles sufficient for such (Figure 4). (24,26)

Summering, the balance of attractive and repulsive forces between the dispersed particles will determine whether flocculation/aggregation will occur. Repulsive interactions (which may be of electrostatic origin) between dispersed particles, which can be electrostatic origin, should be introduced in order to form a stable colloid. (26)

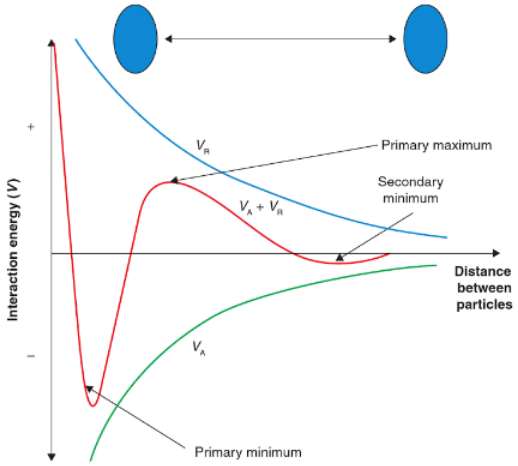

Figure 4: Variation in energy of the interaction between two particles as a function of distance (26)

DLVO theory are not able to explain all coagulation phenomena in natural colloidal systems because interactive forces and electrostatic repulsion, such as hydration, contribute also to maintain the system stability. Moreover, Gregory et al separated DLVO forces, such as Van der Waals force and electrostatic repulsion, from non-DLVO forces, such as hydration and hydrophobic interaction.

According to the DVO theory, before the collision and aggregation occur the potential energy barrier between particles must be reduced or removed. The addition of coagulant in water is a possible strategy to increase the concentration of counterions, which compresses the diffused electrical double-layer, lowering the surface potential and the energy barrier. The charge valence of the metal ions of the coagulant and the dosage are related to the compression of the electrical double-layer. The higher charge valence of the coagulant ion, less required dosage. Consequently, Al^{+3} and Fe^{+3} are better than Ca^{+2} and Na^{+} in the electrical double-layer compression. (27)

Similarly, Schulz-Hardy rule claims that the valence of ions having a charge opposite that of the hydrophobic particle determines the efficacy of the electrolyte on the aggregated particles. The value of aggregation for the efficiency increases with the increasing of the ions valence. The divalent ions are ten times more effective than monovalent ions whereas trivalent ions are thousand times more efficient than monovalent ions. Schulz-Hardy rule is only valid for systems in which there is no chemical interaction between the electrolyte that aggregates and the ions of the double layer of the surface of the particle. It should be noted that the forces promoting the aggregation are enough to overcome the electrostatic repulsion between the particles having identical charges. Concerning to the electrolyte solutions, a satisfactory aggregation is achieved at approximate concentrations of ions: from 25 to 150 mmol/l for monovalent ions, from 0,5 to 2 mmol/l for divalent ions and from 0,01 to 0.1 mmol/l for trivalent ions. The influence of ion valence and concentration on the aggregation of a suspended lyophobic particle can be determined experimentally by measuring the zeta potential change

or by observing the degree of aggregation in terms of a measurable parameter such as the height of the sediment.

Stokes' law describes the velocity of sedimentation of a uniform collection of spherical particles that is represented in Equation 2:

$$v = \frac{2r^2(\rho_1 - \rho_2)g}{9\eta} \qquad (2)$$

where v is the terminal velocity in cm/sec, r is the radius of the particles in cm, ρ_1 and ρ_2 are the densities (g/cm^3) of the dispersed phase and the dispersion medium, respectively, g is the acceleration due to gravity (980.7 cm/sec^2), and η is the Newtonian viscosity of the dispersion medium in poises (g/cm sec). Stokes' law holds only if the downward motion of the particles is not sufficiently rapid to cause turbulence. Micelles and small phospholipid vesicles are only settle if they are subjected to centrifugation.

If the particles are maintained in a deflocculated state, the sedimentation velocity can be reduced by decreasing the particle size. The rate of sedimentation is an inverse function of the viscosity of the dispersion medium. However, too high viscosity is unwanted, especially if the suspending medium is Newtonian rather than shear-thinning, because it then becomes difficult to redisperse material that has settled and it may be inconvenient to remove a viscous suspension from its container. It is verified random Brownian motion when the size of particles undergoing sedimentation is reduced to approximately 2 μm which does not corroborate the theoretical predictions of Stokes law regarding the sedimentation rate. The actual size at which Brownian motion becomes significant depends on the density of the particle as well as the viscosity of the dispersion medium. (3)

Then, in colloidal systems, instability phenomena such as sedimentation, aggregation/flocculation/coagulation and coalescence can occur. Sedimentation origins two separate layers because of the density difference between the disperse phase and the continuous phase. Aggregation/flocculation/coagulation occurs when two or more disperses particles clump together under the influence of Brownian motion and forms a single unit. Coalescence results of a formation of single larger droplets from aggregation and occurs until phase separation. (28)

Concerning to the cosmetic products, the stability of a cosmetic product is defined as the properties of the product to maintain the set of physico-chemical characteristics, organoleptic, microbiological and functional that made it responsive to its purpose of use. Stability becomes therefore an essential requirement because it depends on other key requirements that define the quality of the cosmetic product: security, agreeableness, compliance and effectiveness for use in a broad sense.

Related to the gels, their instability is shown by chemico-physical and organoleptic modifications. Modifications in transparency, turbidity, outcrop, viscosity, pH, crystallization,

conductivity, rheology, interactions between ingredients, functional ingredients and alcoholic degree title of the gels are connected to chemico-physical changes while modifications in colour, smell and taste are connected to organoleptic changes. (29) The stability parameters and methods for the gels are reported in Table 5.

Table 5: Stability parameters and methods for the gels (29)

Parameter	Method
Initial change in appearance in terms of transparency	Visual evaluation
Precipitation / formation of agglomerates	Visual evaluation
Crop	Visual evaluation
Separation	Visual evaluation
Crystallization	Visual evaluation
Smell	Olfactory evaluation: directed (by the bottle), indirect (on mouillette), on skin (on the skin application) or gas/mass chromatography
Colour	Visual evaluation, colorimetric (instrument)
Taste (particular cases)	Taste
Viscosity / rheological characterization	Viscometer / rheometer
pH	pH-meter
Alcohol content	Gas chromatography
Conductivity	Conductivity
Alteration of the title of specific substances such as preservative and functional ingredients	Titrimetry, gas chromatography, HPLC, TLC, UV, mass, IR (instrument)
Formation of unwanted species	Titrimetry, gas chromatography, HPLC, TLC, UV, mass, IR (instrument)
Alteration of the overall functional characteristics of the product	Application testing

1.5. Rheology

Rheology has origin in the Greek words rhéō (“flow”) and –logia (“study of”) and means the study of deformation and flow of matter. Flow is the continuous deformation of a material under the influence of external forces. When a force is applied to a liquid, it will flow to relieve the strain from this force. The measurement of this resistance represents viscosity which is the most frequently used as rheological parameter. Isaac Newton introduced the parallel-plate model which explains the flow measurement of a liquid. In this model, one plate is moving a constant speed while the other one is stationary. This model is represented in Figure 5.

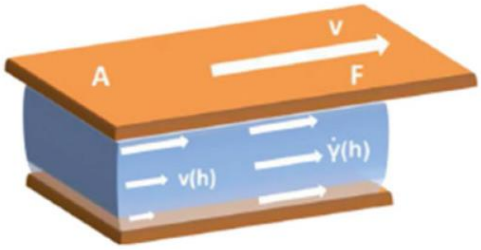

Figure 5: Simple Newtonian shear model (14)

Shear stress (τ) (Equation 3) and shear rate (γ) (Equation 4), can be derived from the model. Shear stress (τ) is the force (F) applied to the rectangular surface (A) when it is deformed by shear strain. The shear rate (γ) of the flowing fluid is defined by the velocity (v) and the displacement (h). (14)

$$Shear\ stress\ (\tau) = \frac{F}{A}\left[\frac{N}{m^2} = Pa\right] \qquad (3)$$

$$Shear\ rate\ (\gamma) = \frac{v}{h}\ [sec^{-1}] \qquad (4)$$

Viscosity which represents the resistance of the fluid to flow can be calculated from the shear rate and the shear stress according to the Equation 5. (14,16)

$$Viscosity\ (\eta) = \frac{\tau}{\gamma}\ [Pa\ sec] \qquad (5)$$

There are two ways to define viscosity which are “differential” viscosity and “apparent” viscosity. The “differential” viscosity is equal to the slope of the shear rates versus shear rate curve at some point (or the tangent of the angle θ). The “apparent” viscosity is equal to the slope of a line that connects the origin with a given point on the shear stress versus shear curve rate curve (or the tangent of the angle ϕ). The “apparent” viscosity is usually chosen. In fact, “apparent” viscosity is easily measured at one fixed shear rate while a “differential” viscosity requires measurements at several shear rates followed by measurement of the slope at the shear rate of interest. (16) “Differential” viscosity and “apparent” viscosity are represented in Figure 6.

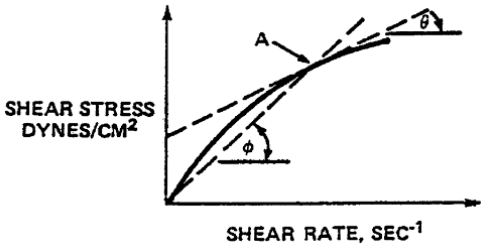

Figure 6: "Apparent" and "Differential" Viscosity (16)

1.6. Multiple Light Scattering

Multiple light scattering is a technique used to determine light flux transmitted (T) through and backscattering from a product. (30) This technique allows to measure the photon transport mean free path l^*. The value obtained with this measurement gives information on the homogeneity of the sample and is characteristic of the dispersion. (31) Moreover, multiple light scattering enables to detect, to deep understand and to predict destabilization phenomena which take place during ageing or shelf-life tests. (30)

In according to backscattering physical model, when a narrow light beam propagates into an optically thick dispersion contained in a glass measurement cell, backscattered light spot shown in displays two regions: a central part corresponding to short path photons, which undergo a few scattering events before escaping the medium; and a peripheral part corresponding to long path photons, which undergo a large number of scattering events before escaping the medium. The characteristic size of the backscattered spot light is representative of the photon transport mean free path l^*. The backscattered light flux BS measured through a thin detection area of thickness dh scales as $(dh/l^*)^{1/2}$ in agreement with experimental observations:

$$BS \approx \sqrt{\frac{dh}{l^*}} \qquad (6)$$

According to Mie theory, the transport mean free path l^* scales as particle mean diameter and the inverse of particle volume fraction:

$$l^*(d,\varphi) = \frac{2d}{3\varphi(1-g)Qs} \qquad (7)$$

where the asymmetry factor g and the scattering efficiency Q are derived from Mie theory.

In according to transmission physical model: the photon mean free path l represents the mean distance travelled by photons before undergoing a scattering phenomenon. The Lambert-Beer law gives an analytical expression of the transmission T, measured by the optical analyser as a function of the photon mean free path l:

$$T(l, r_i) = T_0\, e^{-\frac{2r_i}{l}} = T_0\, e^{-\frac{3r_i\varphi Q_S}{d}} \qquad (8)$$

where r is the measurement cell internal radius and $T0(nf)$ the transmission for the continuous phase. Therefore, the transmission T directly depends on the particle mean diameter d and particle volume fraction φ.

Multiple light scattering predicts the instability phenomena of the formulations, it is also able of know the mean diameter of the particles by the theories of Mie, Rayleigh and general optics. Because this value is obtained without any dilution, it enables to see the real state of the particles in the system. Therefore, for these reasons it is a simple and useful tool for quality control. (31)

2. Aim of the Work

The present work has the goal to determine the influence of different types of preservative systems in the stability of gel formulations prepared from natural rheological modifiers. In particular, different types of preservatives are considered: traditional and not traditional preservatives. In according to Regulation 1223/2009 it is possible to use preservatives listed in Annex V of the Regulation. However, the legislation and scientific research is always in evolution and some preservatives normally used, today they can be used only in determined condition (limited concentrations). For this reason, cosmetic company today prefer to use innovative molecules, or better natural molecules.

Rheological modifiers and preservatives represent two classes of ingredients that can be influenced from several parameters as pH.

In order to find this influence, gels were prepared and pH and viscosity were measured. After these measurements, samples were analysed by multiple light scattering technique. This technique can determine the stability of the formulations.

To sum up, the aim of to work is predict by multiple light scattering if the formulations are stable in time. If they are not stable, the purpose is to determine the instability phenomena and when it occurs.

3. Materials

3.1. Rheological Modifiers

3.1.1. Cellulose Gum (CMC)

Cellulose gum (CEKOL® Cellulose Gum, batch RV49683) was supplied by CPKelco. CMC is water-soluble polymer derived from cellulose by introducing carboxymethyl groups on the cellulose backbone. The formed anionic cellulose molecule hydrates and dissolves readily in water. CMC can impart viscosity to aqueous solutions. CMC is pseudo-plastic by nature and can show thixotropic and essentially non-thixotropic rheology. Besides controlling the rheology, CMC is known for its excellent water retaining capacity. (32)

CMC is soluble in hot and cold water. It is insoluble in organic solvents but it is miscible in ethanol and acetone. CMC viscosity does not increase with temperature. Viscosity ranges of the most CMC solutions at 1-2% are from 50–8000 mPa.s. Complete hydration is achieved faster by finer mesh grades but care in dispersion is required. Thixotropic behavior is seen with medium and high grades of CMC (DS of 0,4–0,7) but grades with “smooth-flow” characteristics are commercially available. (33)

CMC is incompatible with proteins and sodium caseinate. (34,35)

3.1.2. Sodium Carboxymethyl Betaglucan (Beta-glucan) (Beta)

Sodium Carboxymethyl Betaglucan (CM-Glucan granulate SD=0,85, batch 0713-039) was obtained by Mibelle Biochemistry. Beta-glucan is a derivate of ß-(1,3) and ß-(1,6) glucan, a natural yeast polysaccharide featuring immune-stimulating properties and other properties. Beta-glucan is insoluble in water and therefore not suitable for topical use. Consequently, CM-Glucan Granulate is a biologically active beta-glucan derivative that maintains the same biological activity as beta-glucan, is highly purified and also water-soluble. (36)

It has been demonstrated and confirmed a potential thermodynamic incompatibility between casein and beta-glucan. Other incompatibilities have been demonstrated with milk proteins and thermodynamics between polysaccharides and proteins. (37,38)

3.1.3. Carrageenan (Car)

Carrageenan (GENUVISCO® carrageenan CG-131, batch SK01396) was purchased from CPKelco. Carrageenan is a cell wall hydrocolloid found in certain species of seaweeds belonging to red algae (*Rhodophyceae*). Carrageenan is extracted with water under neutral or alkaline conditions at elevated temperature. There are three types of carrageenan: kappa, iota

and lambda. Kappa carrageenan forms firm gels in the presence of potassium ions while iota carrageenan forms elastic gels and thixotropic fluids in the presence of calcium ions. Finally, lambda carrageenan forms viscous, non-gelling solutions. Carrageenan is used as thickening, stabilizing, gelling and texturizing agent. (39)

Functional properties can be manipulated by cations addition: potassium and calcium increase the gel strength of kappa and iota carrageenan. However, the addition of sodium to carrageenan solution is not observed. Lower and higher pH values (under pH 4 and over pH 10), carrageenan gel loses its structure. Carrageenans generally require heat to become solubilized. Kappa and iota carrageenan, depending upon salt addition, solubilize at about 75°C. Gel takes place between 65°–45°C, dependently salt addition. Generally, the greater the addition of potassium or calcium, the higher the gel-set temperature. Kappa and iota carrageenan gels are thermo-reversible. Thermo-reversibility usually occurs at 10–15°C above gel-set temperature. The carrageenans associated with sodium salts are soluble in cold as well as hot water; but they are generally insoluble in alcohol and oils, (good solvents for carrageenan dispersions). It is observed that a high concentration of sugar prevents solubility below gel temperature. Instead, high amounts of alcohol might precipitate carrageenans out of solution. (40)

Carrageenan is incompatible with acid gelatin, amylose, amylopectine, casein and proteins. (41–45)

3.1.4. Acacia Senegal Gum & Xanthan Gum (Solagum™ AX) (SAX)

Acacia Senegal Gum & Xanthan Gum (Solagum™ AX, batch T91250) was supplied by SEPPIC. Solagum™ AX is a mixture of acacia senegal gum and xanthan gum; it is a natural thickening-stabilizing-texturizing polymer in the form of a non-dusty powder. Solagum™ AX has some properties: it dissolves quickly in hot or cold water and in wide pH range (between 3–12); it forms clear aqueous gels, it is resistant to electrolytes; it has good resuspension properties; it is a film forming agent. Finally, it is compatible with solvents, surfactants, AHAs, H_2O_2, sun filters and sunscreens, pigments. It is multifunctional gum: thickening, stabilizing and texturizing agent. (46)

3.1.5. Caesalpina Spinosa Gum (Solagum™ Tara) (ST)

Caesalpinia Spinosa Gum (Solagum™ Tara, batch 38553F, T23440) was obtained from SEPPIC. Solagum™ powder is a 100% natural gum. This non-ionic polysaccharide hydrates instantly in hot or cold water. Solagum™ Tara is ideal for medium or thick consistency formulations due to its high resistance to electrolytes. It acts as a texturizing agent by providing a structuring effect and a very soft feel. Solagum™ Tara has some properties: it dissolves

quickly in hot or cold water, cold or hot process, at wide pH range (between 3–12), it forms clear aqueous gels; it has an excellent resistance to electrolytes; it allows to obtain textures with medium or thick consistency; it has synergistic behaviour with Polyacrylate Crosspolymer-6 and Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer. Finally, it is compatible with many solvents, anionic and cationic ingredients, surfactants, AHAs, sun filters and sunscreens, pigments. (47)

3.1.6. Microcrystalline Cellulose & Cellulose Gum & Xanthan Gum (Sucrathix VX) (SVX)

Microcrystalline Cellulose & Cellulose Gum & Xanthan Gum (Sucrathix VX, batch 39505140718) was purchased from ALFACOS. Sucrathix VX forms creamy, soft gels that enhance the skin feel of a finished product and increases stability of a formulation. This component has some properties: it is stable to electrolytes, non-sticky and it is stable between pH 4,5-10 and with electrolytes up to 2%. (48)

3.1.7. Xanthan Gum (XG)

Xanthan gum (XANTURAL® Xanthan Gum, batch FO48624) was obtained from CPKelco. Xanthan gum is characterized by very high viscosity at low concentrations. Because of its pseudo-plastic nature, it imparts excellent stability to oil-in-water emulsions by preventing the coalescence of oil droplets. (49)

Xanthan gum is readily soluble both in hot and cold water. Solubility is achieved in wide range of pH values and salt concentrations. During xanthan gum addition, it is recommended that all dry ingredients be blended together and added to the liquid using high-speed agitation. The powder mixture should be added to homonogenaizer without entrapping air bubbles. Dispersibility can be improved by hydrating gum with a non-solvent such as alcohols or some oils. Hydration will also be slowed when introduced to a brine solution. Xanthan gum is stable in applications with a wide range of pH values (2–12). It has a tolerance to enzymes, salt, and heat. For instance, xanthan gum in a 1.1% citric acid/citrate solution at a pH 3,4 at 90°C for 24 hours showed excellent thermal stability. Xanthan gum also exhibits excellent freeze-thaw stability. Viscosity values are generally not affected by changes in pH, addition of salt and thermal changes for extended periods of time; whereas, other hydrocolloids break down under the same conditions. Xanthan gum also exhibits excellent synergy with galactomannans such as guar gum and locust bean Gum. Xanthan gum is a heteropolysaccarids of a high molecular weight (Mw-2.5, 106). D-glucose, D-mannose and D-glucuronic acid are monomeric units obtained by hydrolysis. The main chain of xanthan gum contains b-D-glucose units linked through the 1- and 4 positions. The side chain is a tri-saccharide occurring in every alternate

glucose residue. It consists of a D-mannose, b-D-glucuronic acid and a terminal b-D-mannose unit. (50)

3.2. Preservatives

3.2.1. Phenoxyethanol & Caprylyl Glycol (Verstatil® PC) (PC)

Phenoxyethanol & Caprylyl Glycol (Verstatil® PC, batch 480236) was supplied by Dr Straetmans. This preservative is used in a concentration between 0,8 and 1% and it is pH independent. Moreover, Verstatil® PC is water miscible, chemically stable with low impact on the stability of the product, pH independent, as said before, and broad antimicrobial performance. (51)

3.2.2. Triethyl Citrate & Glyceryl Caprylate & Benzoic Acid (Verstatil® TBG) (TBG)

Triethyl Citrate & Glyceryl Caprylate & Benzoic Acid (Verstatil® TBG, batch 475891) was obtained from Dr Straetmans. This product is derived from palm oil derivates. The effective pH and concentration are 4–6 and 1,0–1,5%, respectively. (52)

3.2.3. p-Anisic Acid (dermosoft® 688 eco) (688)

p-Anisic Acid (dermosoft® 688 eco, batch 110MAK0021) was purchased from Dr Straetmans. It is a naturally derived organic acid with fungicidal activity. The effective pH and concentration are 4–6 and 0,05–0,5%, respectively. (53)

3.3. Surfactants

3.3.1. Decyl Glucoside (DG)

Decyl glucoside (ORAMIX™ NS 10, batch 38957V T34859) was supplied by SEPPIC. It is a non-ionic surfactant derived from sugar, with good performance, innocuity and naturality. Decyl glucoside has some properties, like high foaming performances and cleansing agent and no skin aggressive. (54)

3.3.2. Polysorbate 60 (PS)

Polysorbate 60 (or Tween 60-LQ-(MV), batch 0000766724) was obtained by CRODA. Tween 60 is an ethoxylated (20) sorbitan ester based on a natural fatty acid (stearic acid). This ethoxylate is highly effective at forming O/W emulsions, particularly when used in combination with its non-ethoxylated derivative, Span 60. Its HBL is 14,9. (55)

4. Methods

4.1. Formulation of Gels

A series of basic gels was prepared from different rheological modifiers, glycerine and water.

Three different concentration of the rheological modifiers were considered: 0,5, 1 and 2%, C, A and B, respectively. The powder was transferred in a becker and, successfully, glicerin and water were added and mixed by magnetic stirring until complete dissolution of the rheological modifiers. If necessary, the gel was heated in order to enhance powder dissolution. At the end, preservative was added.

A series of gels was set up by adding surfactant in order to evaluate the possible interference onto preservative systems and gel. In this case, the gels were prepared according to same procedure of the previous set up viscous solutions, with the only exception that surfactant was added before of preservative system.

All formulations were stored at room temperature before to characterization.

4.2. Measure of pH

For the measure of pH, JENWAY 3510 pH Meter was used. Firstly, the instrument was calibrated by two different buffer solutions, pH 7 and pH 4. At the end of the calibration, the instrument was ready to measure pH formulations. The pH was controlled at zero time (t_0) and 1 month. At t_0, as Verstatil® TBG and dermosoft® 688 eco are pH-dependent, it was necessary to correct the pH to a value for which the preservative has activity. To increase the pH, hydroxide sodium was used and to decreased pH, lactic acid was used.

4.3. Rheology Measurements

Rotational viscometer, model BROOKFILED VISCOMETER RVT, was used in order to evaluate the viscosity profile of the preliminary set up viscous solutions.

The Viscometer can measure over a number of ranges since, for a given spring deflection, the actual viscosity is proportional to the spindle speed and is related to the spindle's size and shape. For a material of given viscosity, the resistance will be greater as the spindle size and/or rotational speed increase. The minimum viscosity range is obtained by using the largest spindle at the highest speed; the maximum range by using the smallest spindle at the slowest speed. (56)

For all viscosity measurements spindle number 3 was used. The viscosity values are obtained by multiplying the dial reading for factor. By increasing speed from 0,5 to 100 rpm, it is possible to trace a viscosity curve and thus obtain a rheological profile. Time-dependent viscosity curves were represented (thixotropic profile). All measurements were performed at 20°C.

4.4. Multiple Light Scattering

Turbiscan is an innovative automatized optical analyser, working in the near-infrared region with a double detection mode: transmission and backscattering (turbidity range from 0 to 50 000 NTU). (57,58)

The central part of the optical scanning analyser, Turbiscan, is a detection head, which moves up and down along a flat-bottomed cylindrical glass cell. The detection head is composed of a pulsed near infrared light source (λ = 880 nm) and two synchronous detectors. The transmission detector (at 180°) receives the light, which goes through the sample, while the backscattering detector (at 45°) receives the light backscattered by the sample. The detection head scans the entire height of the sample (55 mm), acquiring transmission and backscattering data every 40 µm. It can also be used in “fixed position” mode where the head is set at a fixed sample height and can make acquisitions every 0.1 seconds. This latter mode is of particular interest for monitoring very quick instability phenomena such as breaking of foam. (31)

It carries out step-by-step vertical scans of a tube (flat-bottomed cylindrical cell) containing a sample of a concentrated dispersion (such as emulsion, suspension or foam) and converts the macroscopic aspect of the mixture into graphics. Designed to work in the kinetic mode, it allows very early visualization of flocculation sedimentation, creaming and coalescence phenomena. (58)

The stability of the gels was monitored by organoleptic evaluation and by multiple light scattering by Turbiscan Tower manufactured (Formulaction, France). (59)

TSI parameter (Turbiscan Stability Index) was calculated by TowerSoft. This parameter represents the sum of all processes taking place in the sample (sedimentation, clarification, phase separation). Equation 9 represent the TSI formula.

$$TSI = \sqrt{\frac{\sum_{i=1}^{n}(x_i - x_{BS})^2}{n-1}} \qquad (9)$$

where x_i is the average backscattering for each minute of measurement, x_{BS} is the average x_i, and n is the number of scans. The higher TSI value, the less stable the gel.

Gels for multiple scattering measurement were transferred into cylindrical glass tubes and submitted to Turbiscan Tower stability analysis. For an optimal evaluation, it was important

that there were not air bubbles inside the sample and the sample was homogenously distributed. For each sample was performed one cycle at 20°C for 6 hours, one cycle at 4°C for 6 hours and finally a third cycle at 20°C for 6 hours.

For clear samples transmission values were measured; for opalescent samples backscattering profile was evaluated.

4.5. Microbiological Tests

Microbiological assay aims to evaluate if there is no microbial growth inside the sample and consequently if the preservative system has not been inactivated.

Instruments as glass, balance, mixer (vortex), spatulas and pipettings. were previously sterilized by ethanol 70%. Medium, agar and broth solution for microbiological assay were sterilized by autoclave at 121°C for 15-20 minutes.

The sample for microbiological evaluation was set up as follows. Eugon LT100 broth (9 ml) was added to 1 ml of sample (9:1 broth:sample) in order to neutralize the preservative system. The mixture was stirred for few seconds by vortex; 15 minutes were expected before to add broth solution into Petri dishes.

Finally, Agar medium, maintained at 55°C, and 1 ml of the previous mixture were contemporary transferred into Petri dishes. Tryptic Soy Agar for bacterial and Sabouraud Chloramphenicol Agar for fungi were used. Samples for bacteria evaluation were maintained 48 hours at 37°C; samples for fungi evaluation at 20°C for 3-5 days.

4.6. Factorial Analysis

The factorial analysis is multivariate exploratory statistic technique of the data. The purpose of this method is to discover and analyse the structure of a set of interrelated variables in order to construct a measurement scale for factors which somehow control the original variables. Thus, the common factor and the structural relationships that link factors to variables are estimated from the observed relationship between the original variables. (60) Summering, the aim of this statistical test is to simply complex sets of data. (61)

The generic formula of the model of factorial analysis is represented in Equation 10:

$$z_i = \lambda_{i1} f_1 + \lambda_{i2} f_2 + \cdots + \lambda_{im} f_m + n_i \ (i = 1, \dots, p) \qquad (10)$$

where $z_i = \frac{(x_i - \mu_i)}{\sigma_i}$, f_m represents the common factors ($m < p$), n_p represents the specific factors and λ_{ij} represents the weight of the variable i in the factor j (factor loadings) that means each λ_{ij} measures the contribution of the common factor j in the variable i. In this model, it is necessary to assume that: the common factors are independents (orthogonal) and equally

distributed with mean 0 and variance 1, the specific factors are independents and equally distributed with mean 0 and variance Ψ_j; and the common factors and the specific factors are independent. (60)

The factorial analysis gives the matrix of correlation. A correlation is a numerical measure of the degree of agreement between two sets of scores. It runs from +1 to -1: +1 indicates full agreement, 0 no relationship and -1 complete disagreement. (61)

The Kaiser-Meyer-Olkin Measurement of Sampling Adequacy (KMO) is a measure of adequacy of the data obtained by the ratio of the sum of the squares of the correlations of all variables divided by that same sum added by the sum of the quadrats of the partial correlations of all the variables. (62) The critical values are in the Table 6.

Table 6: Recommendation relatively to the factorial analysis based on the KMO values (60,62)

KMO value	Recommendation relatively to factorial analysis
1	Perfect
] 0,9 – 1 [	Excellent
] 0,8 – 0,9]	Good
] 0,7 – 0,8]	Reasonable
] 0,6 – 0,7]	Mediocre
] 0,5 – 0,6]	Bad but still acceptable
≤ 0,5	Inacceptable

Factorial analysis was performed by IBM® SPSS® Statistics version 24.

5. Results and Discussions

5.1. Formulation of Gels

The organoleptic aspect of the gels is described in Table 7.

Table 7: Organoleptic Aspect of samples

Sample	Preparation Date	Organoleptic Aspect
CMC_A / PC_0,8	14.02.2017	Clear and homogenous
CMC_A / TBG_1	24.02.2017	Opalescent and homogenous
CMC_A / 688_0,1	22.02.2017	Clear and homogenous
CMC_A / PC_0,8 / DG_10	01.03.2017	Opalescent and homogenous
CMC_A / TBG_1 / DG_10	06.03.2017	Opalescent and homogenous
CMC_A / DG_10 / TBG_1	09.03.2017	Opalescent and homogenous
CMC_B / DG_10 / TBG_1	09.03.2017	Opalescent and homogenous
CMC_A / TBG_1 / DG_5	09.03.2017	Opalescent and homogenous
CMC_A / PS_5 / TBG_1	10.03.2017	Opalescent and homogenous
CMC_A / PS_5 / PC_0,8	14.03.2017	Opalescent and homogenous
CMC_A / PS_10 / PC_0,8	14.03.2017	Opalescent and homogenous
CMC_A / 688_0,3	27.03.2017	Clear and homogenous
Beta_A / PC_0,8	14.02.2017	Clear and homogenous
Beta_B / PC_0,8	16.02.2017	Clear and homogenous
Beta_A / TBG_1	16.02.2017	Opalescent and homogenous
Beta_A / 688_0,1	22.02.2017	Clear and homogenous
Beta_A / PC_0,8 / DG_10	01.03.2017	Opalescent and homogenous
Beta_B / PC_0,8 / DG_10	06.03.2017	Opalescent and homogenous
Beta_A / TBG_1 / DG_10	06.03.2017	Opalescent and homogenous
Beta_A / PS_5 / PC_0,8	21.03.2017	Clear and homogenous
Beta_A / PS_10 / PC_0,8	21.03.2017	Opalescent and homogenous
Beta_A / PS_5 / TBG_1	21.03.2017	Clear and homogenous
Beta_A / PS_10 / TBG_1	21.03.2017	Opalescent and homogenous
Beta_A / 688_0,3	27.03.2017	Clear and homogenous
Car_A / PC_0,8	14.02.2017	Clear and homogenous
Car_A / TBG_1	24.02.2017	Opalescent and homogenous
Car_A / 688_0,1	01.03.2017	Clear and homogenous
Car_A / PC_0,8 / DG_10	01.03.2017	Opalescent and homogenous
Car_A / TBG_1 / DG_10	06.03.2017	Opalescent and homogenous
Car_A / DG_10 / TBG_1	10.03.2017	Opalescent and homogenous
SAX_A / PC_0,8	14.02.2017	Opalescent and homogenous
SAX_A / 688_0,1	23.02.2017	Opalescent and homogenous
SAX_A / PC_0,8 / DG_10	02.03.2017	Opalescent and homogenous
SAX_A / TBG_1	07.03.2017	Opalescent and homogenous
SAX_A / 688_0,3	27.03.2017	Opalescent and homogenous

ST_A / PC_0,8	14.02.2017	Opalescent and homogenous
ST_A / 688_0,1	03.03.2017	Opalescent and homogenous
ST_A / PC_0,8 / DG_10	02.03.2017	Opalescent and homogenous
ST_A / TBG_1	07.03.2017	Opalescent and homogenous
SVX_A / PC_0,8	14.02.2017	Opalescent and homogenous
SVX_A / 688_0,1	06.03.2017	Opalescent and homogenous
SVX_A / PC_0,8 / DG_10	03.03.2017	Opalescent and homogenous
SVX_A / TBG_1	07.03.2017	Opalescent and homogenous
XG_A / PC_0,8	01.03.2017	Clear and homogenous
XG_A / TBG_1	01.03.2017	Opalescent and homogenous
XG_A / 688_0,1	01.03.2017	Clear and homogenous
XG_A / PC_0,8 / DG_10	03.03.2017	Opalescent and homogenous
XG_A / TBG_1 / DG_10	08.03.2017	Opalescent and homogenous
XG_C / PC_0,8	14.03.2017	Clear and homogenous

After setting up, all gels showed homogenous. However, some samples were clear and others were opalescent. It had been observed that the addition of the Triethyl Citrate & Glyceryl Caprylate & Benzoic Acid (Verstatil® TBG), decyl glucoside, Acacia Senegal Gum & Xanthan Gum (Solagum™ AX), Caesalpinia Spinosa Gum (Solagum™ Tara) and Microcrystalline Cellulose & Cellulose Gum & Xanthan Gum (Sucrathix VX) causes an opalescent phenomenon.

5.2. Measure of pH

In Figures 7, 8, 9, 10, 11, 12 and 13 are reported the pH measurements of all samples at time zero and after one month of the preparation in order to evaluate a possible alteration of pH that could cause inactivation of certain preservatives or microbiological contamination.

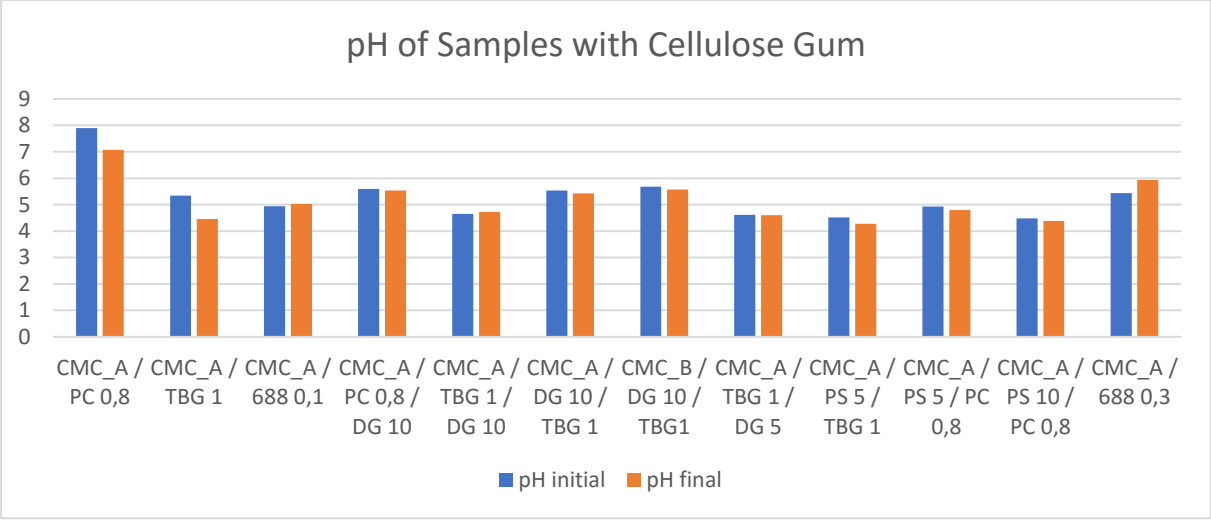

Figure 7: pH initial and pH final of samples with cellulose gum

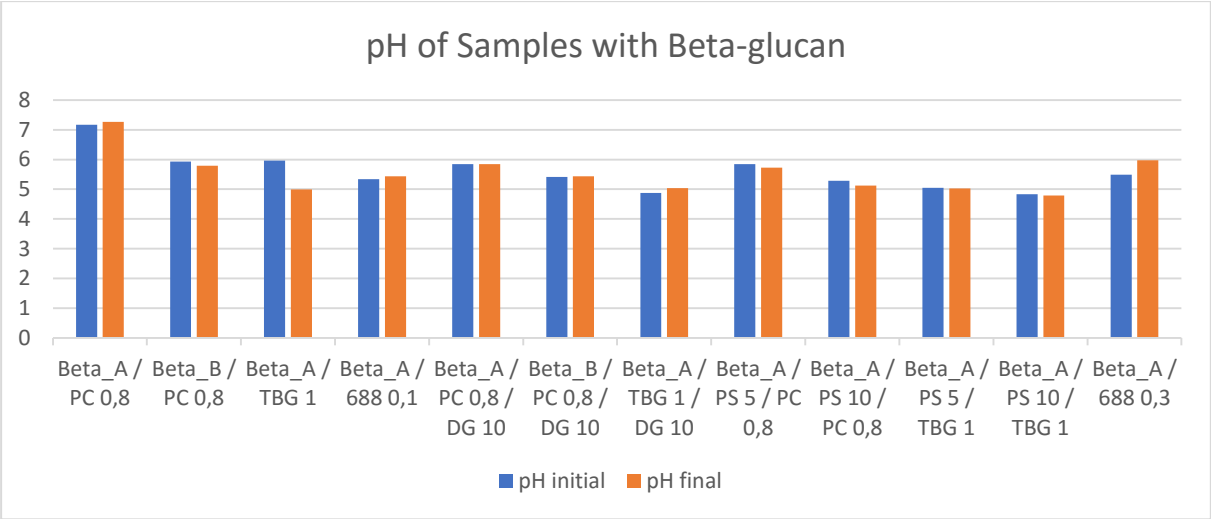

Figure 8: pH initial and pH final of samples with beta-glucan

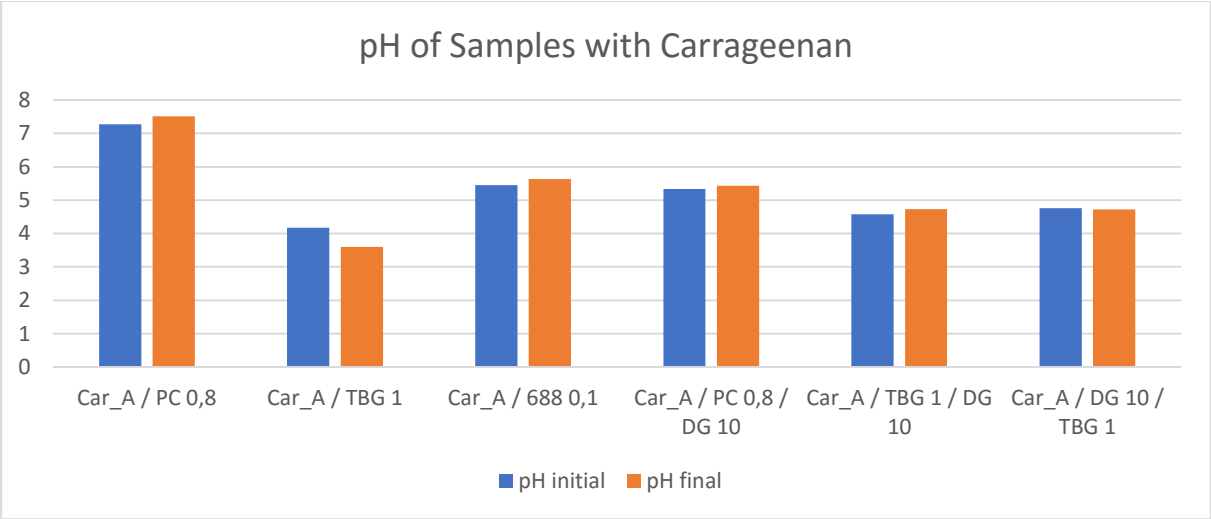

Figure 9: pH initial and pH final of samples with carrageenan

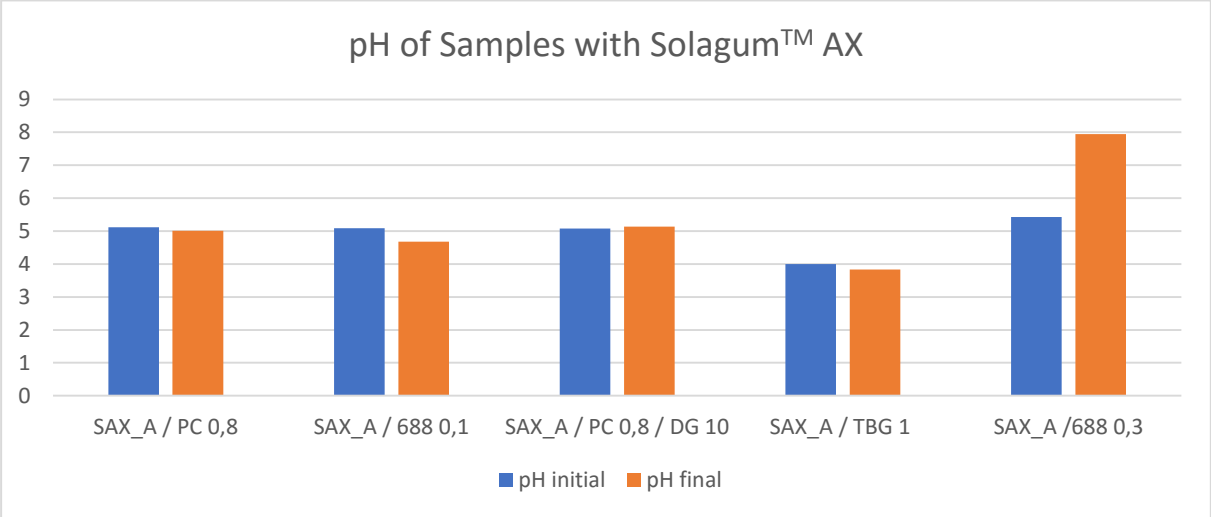

Figure 10: pH initial and pH final of samples with Solagum™ AX

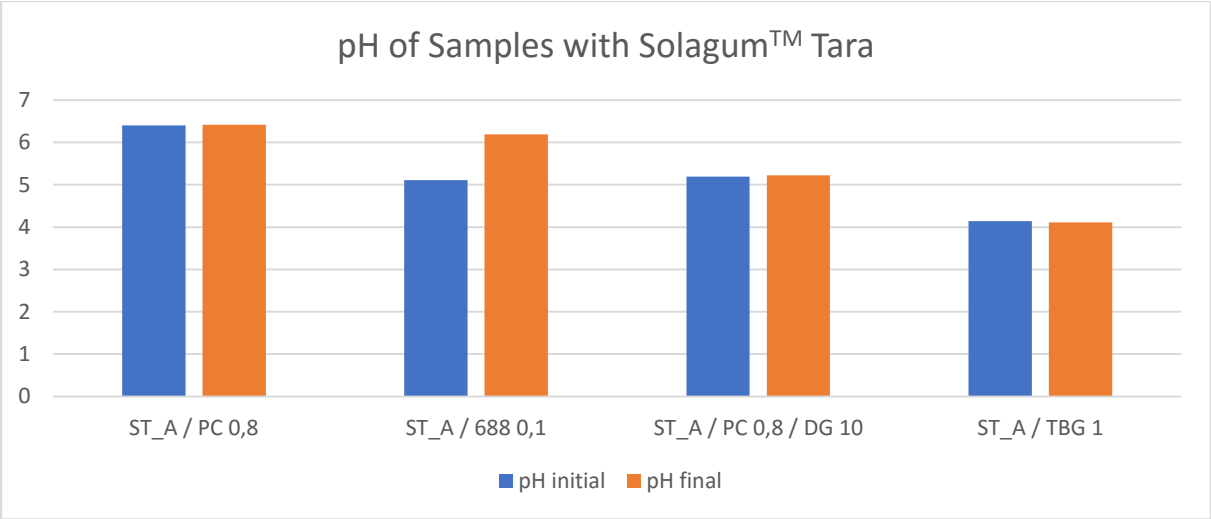

Figure 11: pH initial and pH final of samples with Solagum™ Tara

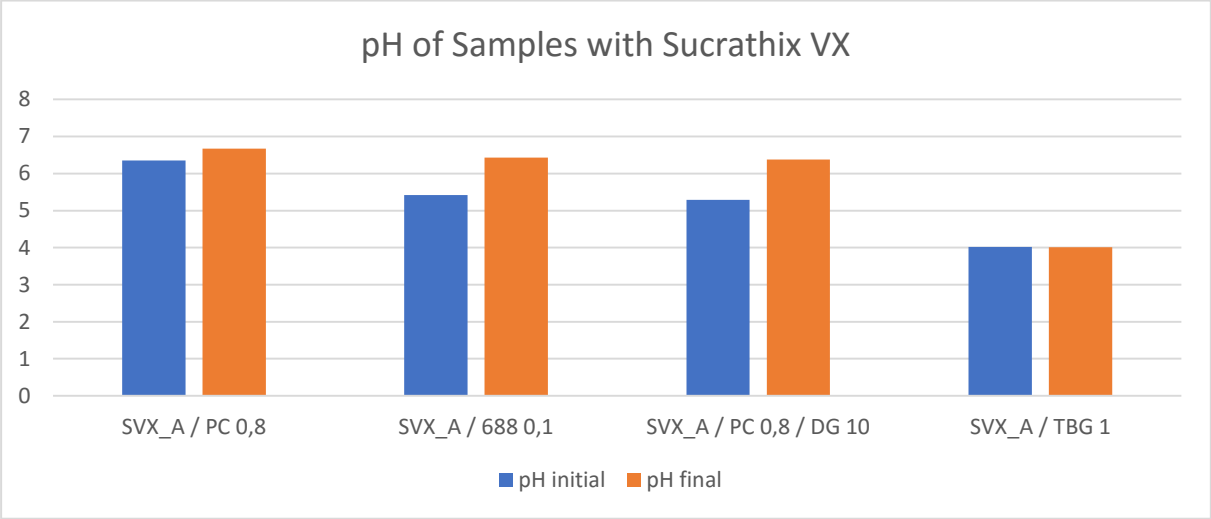

Figure 12: pH initial and pH final of samples with Sucrathix VX

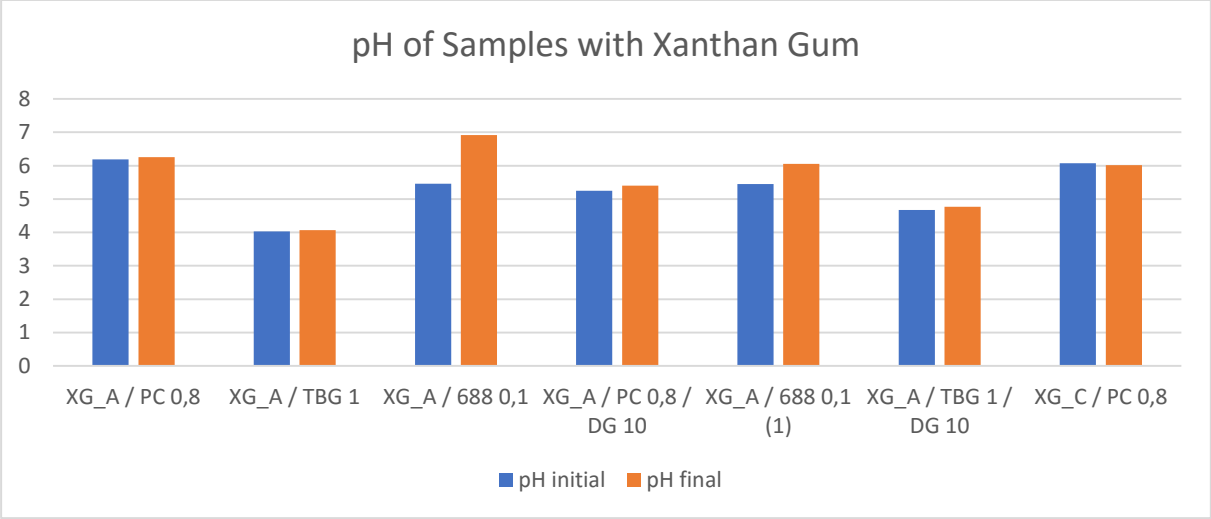

Figure 13: pH initial and pH final of samples with xanthan gum

From pH data, it is possible to make some considerations: many samples did not show significant variations of pH values (±0,5) and SAX_A / 688_0,3, ST_A / 688_0,1, SVX_A / 688_0,1, and XG_A / 688_0,1 showed an increasing of pH more than 0,5. Since dermosoft® 688 eco is a preservative based on p-Anisic Acid, it is important for its activity that pH solution maintained at 4,5-5,5 range. In fact, pH change can influence the efficacy of the preservatives which are pH-depended and consequently gel stability. An evaluation of pH of samples preserved with organic acid should be evaluated at 3 months also.

5.3. Rheology Measurements

The viscosity values of 10 r.pm. are reported in Table 8.

Table 8: Viscosity values of gels

Sample	Viscosity (mPa.s) at 10 rpm
CMC_A / PC_0,8	550
CMC_A / TBG_1	450
CMC_A / 688_0,1	500
Beta_A / PC_0,8	0
Beta_B / PC_0,8	0
Beta_A / TBG_1	0
Beta_A / 688_0,1	0
Car_A / PC_0,8	100
Car_A / TBG_1	50
Car_A / 688_0,1	150
SAX_A / PC_0,8	2200
SAX_A / TBG_1	2600
SAX_A / 688_0,1	2150
ST_A / PC_0,8	6200
ST_A / TBG_1	6750
ST_A / 688_0,1	6450
SVX_A / PC_0,8	700
SVX_A / TBG_1	400
SVX_A / 688_0,1	350
XG_A / PC_0,8	2000
XG_A / TBG_1	4850
XG_A / 688_0,1	5450
XG_C / PC_0,8	1200

Samples with cellulose gum (CMC), Acacia Senegal Gum & Xanthan Gum (Solagum^{TM} AX), Microcrystalline Cellulose & Cellulose Gum & Xanthan Gum (Sucrathix VX) and xanthan gum showed a pseudo-plastic behaviour, with increasing of shear rate was observed a decreasing

of viscosity. In samples with Sodium Carboxymethyl Betaglucan (beta-glucan), carrageenan and Caesalpina Spinosa Gum (Solagum™ Tara), the viscosity increased when the shear rate increases, showing a dilatant flow.

For samples with Sodium Carboxymethyl Betaglucan (beta-glucan) was measured the lowest viscosity and for samples with Caesalpina Spinosa Gum (Solagum™ Tara) the higher viscosity. Moreover, for some samples as Carrageenan, Microcrystalline Cellulose & Cellulose Gum & Xanthan Gum (Sucrathix VX) and Xanthan Gum, the preservative type seems to influence on viscosity. In particular, the addition of Phenoxyethanol & Caprylyl Glycol (Verstatil® PC) to Xanthan Gum gels determined a decreasing of viscosity (~2000 mPa.s vs ~5000 mPa.s).

5.4. Multiple Light Scattering

Multiple light scattering predicts the stability of the samples. So, it can detect instability phenomena such as flocculation, sedimentation, phase separation and phase separation with clarification. In Table 9 is reported the instability phenomena of samples. Detailed results of multiple light scattering analysis is in annex.

In Figures 14 and 15 transmission and backscattering profiles are reported. Profiles of the curves did not show significant variability over time confirming stability of the sample.

On the contrary in Figures 16 and 17 it is possible to observe a flocculation phenomenon in transmission profile and a sedimentation phenomenon in backscattering profile. In fact, the higher clarification (higher transmission percentage) determined in the middle of the sample corresponded to a sedimentation (and so higher backscattering percentage) on the bottom. Flocculation is a phenomenon in which the particles form flakes, increasing in size. In these cases, there are differences in the scattering of light. As it is possible to observe in the Figure 17 the flocculation phenomenon is represented from a series of horizontal parallel curves in the central part of the vial. The different colour of the curves represents a time scale. Sedimentation phenomena indeed is characterized from the migration of the particles to the bottom of the vial. Many times, sedimentation is a consequence of flocculation, as in this case.

In Figures 18 and 19 it is possible to see an association of two phenomena. In particular, Figure 19 shows a decrease in transmission in the bottom and/or an increase in backscattering in the bottom. Also in this case the overlapping coloured curves represent a particle migration.

In Figures 18 and 19, it is possible to see an association of two phenomena. In particular, Figure 19 shows a decrease in transmission in the bottom and/or an increase in backscattering in the bottom. Also in this case the overlapping coloured curves represent a particle migration.

Phase separation is the conversion of a single-phase system to a multi-phase system. Generally, one phase is clearer than the other one. In according to multiple light scattering,

two phase-sample shows a positive transmission and negative backscattering peaks in correspondence of a clearer phase. At the same time, the same sample shows a negative transmission and positive backscattering peaks in correspondence of an opalescent phase. In Figures 20 and 21, it is represented an example of phase separation. In detail, an increasing of the transmission on the top represents a clarification and overlapping curves in the middle of the sample represents a flocculation, preliminary mechanism of the future sedimentation, as it was observed in Figure 21 successively.

Finally, in the Figures 22 and 23, another example of phase separation is reported. In this case the clearer phase is in the bottom of the container. In fact, as transmission/backscattering profile reports, a transmission decreasing on the top and/or a backscattering increasing on the top. The overlapping curves can be present in the graphic represent the particle migration.

Table 9: Instability phenomena of samples predicted by multiple light scattering

Instability Phenomena	Samples
Flocculation	CMC_B / DG_10 / TBG_1
	ST_A / PC_0,8 / DG_10
Sedimentation	CMC_A / 688_0,1
	CMC_A / 688_0,3
	Beta_A / 688_0,1
	Beta_A / PS_10 / PC_0,8
	Beta_A / PS_10 / TBG_1
	Beta_A / 688_0,3
Sedimentation and flocculation	CMC_A / PS_5 / TBG_1
	CMC_A / PS_5 / PC_0,8
	CMC_A / PS_10 / PC_0,8
Phase separation	CMC_A / TBG_1 / DG_10
Phase separation with clarification	CMC_A / DG_10 / TBG_1
	CMC_A / TBG_1 / DG_5
	Car_A / PC_0,8 / DG_10
	Car_A / DG_10 / TBG_1
	SAX_A / PC_0,8 / DG_10
	XG_A / TBG_1 / DG_10
Phase separation with clarification and flocculation	XG_A / PC_0,8 / DG_10
Not homogenous	Beta_A / PS_5 / TBG_1
	Car_A / TBG_1 / DG_10
	SAX_A / 688_0,3
	ST_A / 688_0,1
	XG_A / PC_0,8
	XG_A / 688_0,1

Figure 14: Beta_A / PC_0,8 vial sample: gel formulation was homogenous

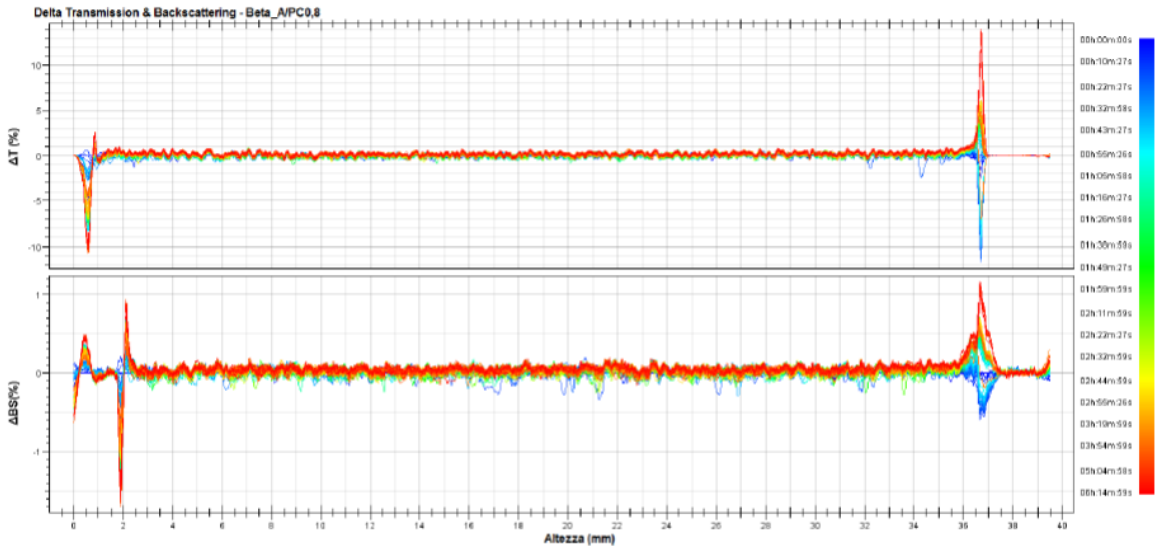

Figure 15: ΔBS and ΔT profiles of Beta_A / PC_0,8

Figure 16: Beta_A / PS_10 / PC_0,8 with sedimentation

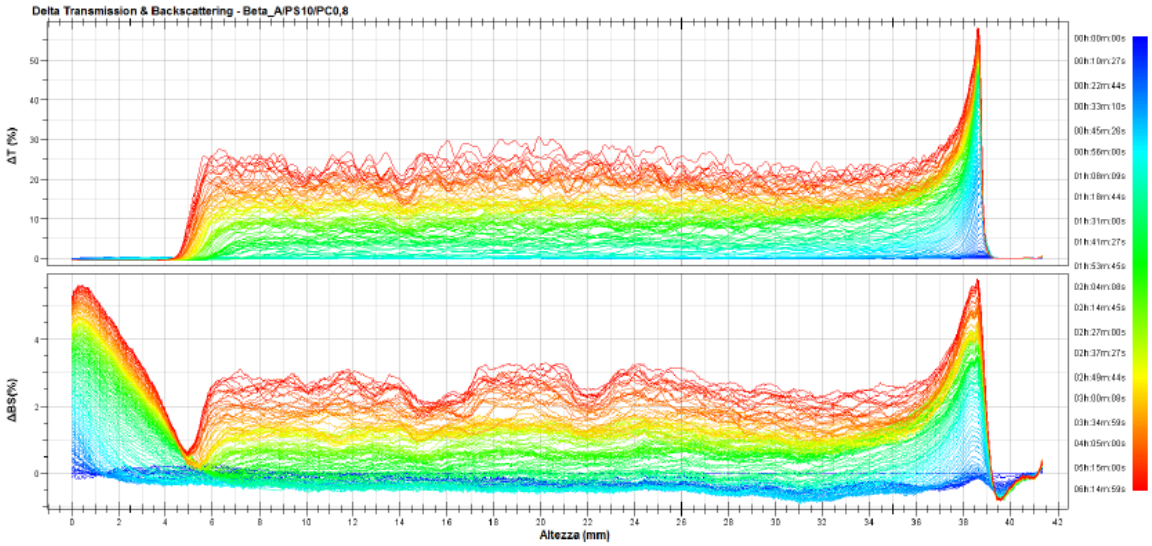

Figure 17: ΔBS and ΔT profiles of Beta_A / PS_10 / PC_0,8: sedimentation/flocculation phenomena was observed

Figure 18: CMC_A / PS_10 / PC_0,8 with sedimentation and flocculation

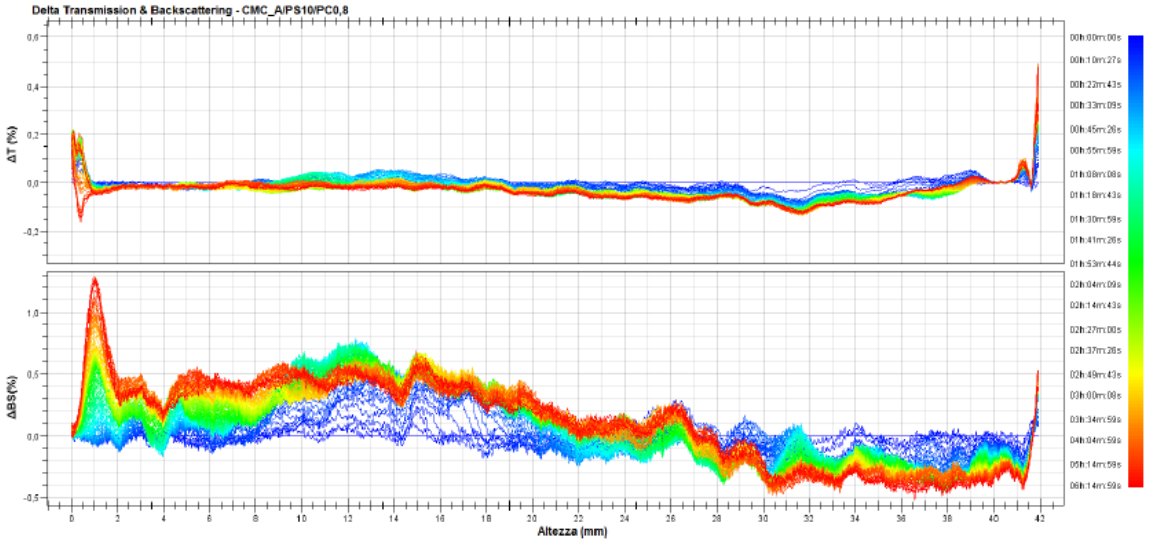

Figure 19: ΔBS and ΔT profiles of CMC_A / PS_10 / PC_0,8: a sedimentation and flocculation phenomena was reported

Figure 20: CMC_A / TBG_1 / DG_10 with phase separation

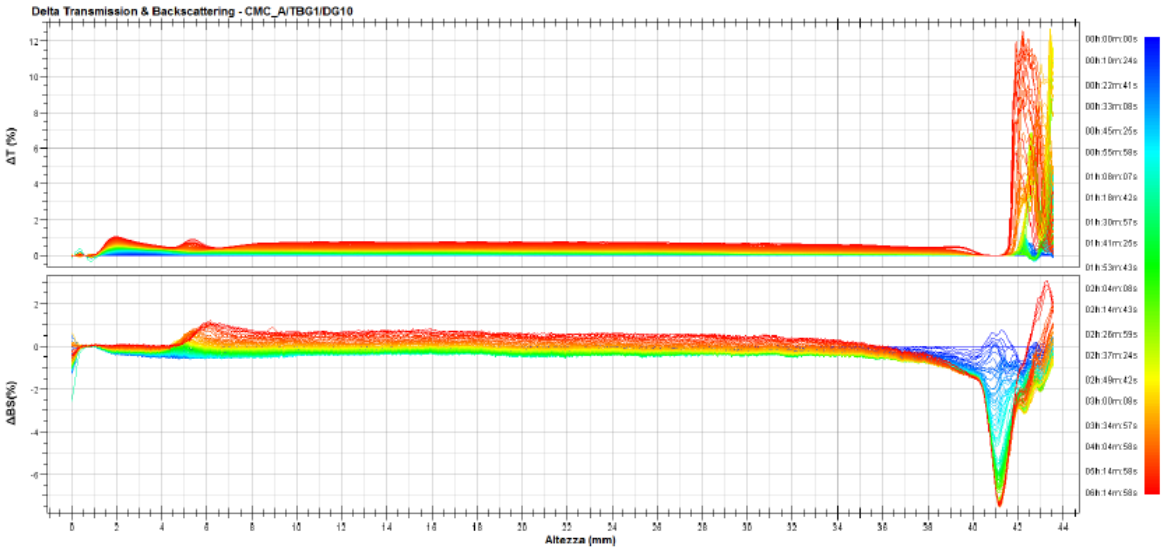

Figure 21: ΔBS and ΔT profiles of CMC_A / TBG_1 / DG:10: phase separation was observed

Figure 22: Car_A / DG_10 / TBG_1 with phase separation with clarification

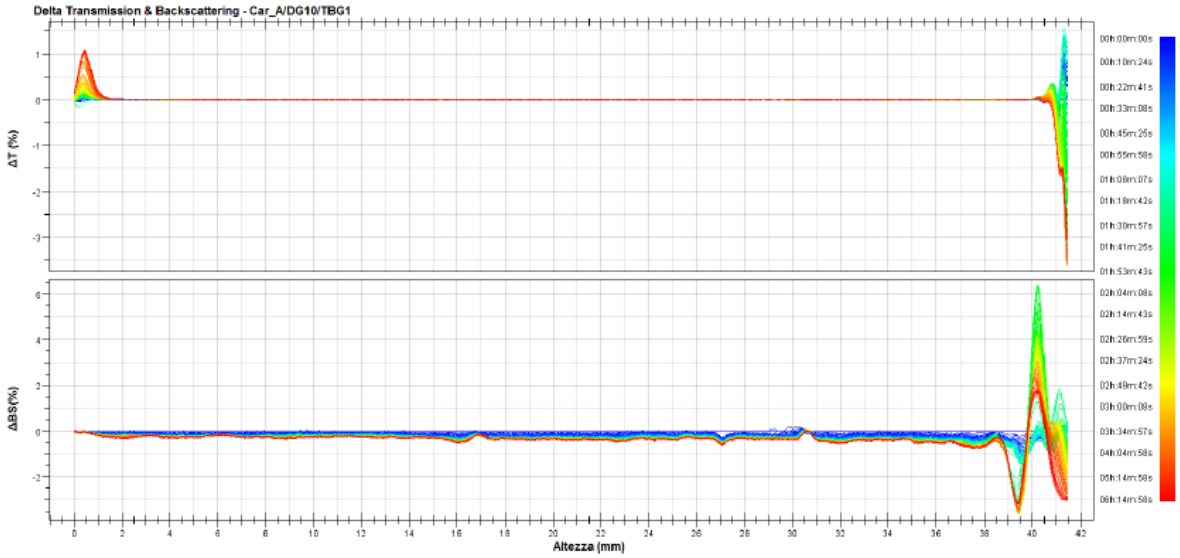

Figure 23: ΔBS and ΔT profiles of Car_A / DG_10 / TBG_1: phase separation with clarification was observed

According with these results, sedimentation, phase separation with clarification and flocculation are the most common instability phenomena in gels samples that were analysed. In detail, the samples become more instable in the presence of surfactant and p-Anisic Acid preservative (dermosoft® 688 eco). Moreover, samples with low concentration of surfactant are more stable. Many samples preserved by Triethyl Citrate & Glyceryl Caprylate & Benzoic Acid (Verstatil® TBG) became instable in the presence of surfactant. All samples with Cellulose Gum and surfactant are instable. Samples with Sodium Carboxymethyl Betaglucan (beta-glucan) and Polysorbate 60 are instable: two ingredients could be incompatible. Same consideration for Carrageenan and decyl glucoside association: all samples with these two ingredients demonstrated to be instable. The same behaviour happened with Acacia Senegal Gum & Xanthan Gum (Solagum™ AX) and Decyl Glucoside, Caesalpinia Spinosa Gum (Solagum™ Tara) and Decyl Glucoside and Xanthan Gum and Decyl Glucoside.

Finally, samples with Microcrystalline Cellulose & Cellulose Gum & Xanthan Gum (Sucrathix VX) are the most stable ones.

5.5. Microbiological Tests

Results of microbiological tests are reported in Table 10.

Table 10: Results of microbiological tests

Sample	Preparation Date	Test Date	cfu/ml for Bacteria Test	cfu/ml for Fungi Test
CMC_A / PC_0,8	14.02.2017	24.03.2017	<0	<0
CMC_A / TBG_1	24.02.2017	24.03.2017	<0	<0
CMC_A / 688_0,1	22.02.2017	24.03.2017	$>1.5x10^3$	<0
Beta_A / TBG_1	16.02.2017	24.03.2017	<0	<0
Beta_A / 688_0,1	22.02.2017	24.03.2017	$>1.5x10^3$	<0
SAX_A / 688_0,1	23.02.2017	24.03.2017	$>1.5x10^3$	<0
ST_A / 688_0,1	03.03.2017	18.04.2017	$>1.5x10^3$	<0
SVX_A / 688_0,1	06.03.2017	18.04.2017	$>1.5x10^3$	<0
XG_A / 688_0,1	01.03.2017	18.04.2017	$>1.5x10^3$	<0

For the samples tested, there is no fungal growth. Then, all preservatives are effective against mould and yeast in according to technical data sheet.

On the other hand, samples with the p-Anisic Acid preservative (dermosoft® 688 eco) have bacterial growth because the preservative is fair for Gram+ and Gram- bacteria. So, the preservative is not effective for bacteria. In order to avoid bacterial contamination it is needed to add another preservative or change to a preservative which is suitable to prevent bacterial contamination. In Figure 24, it is represented bacterial contamination.

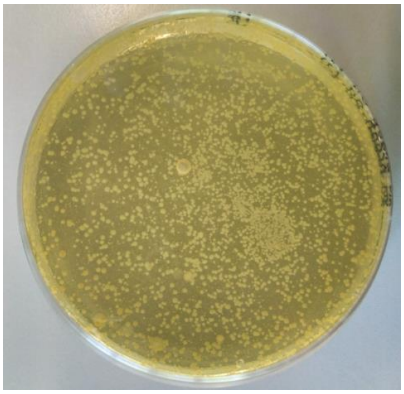

Figure 24: Microbiological test of XG_A / 688_0,1

5.6. Factorial Analysis

The results of the factorial analysis are reported in Figures 25, 26 and 27.

	Cellulose_gum	Beta_glucan	Carrageenan	Solagum_AX	Solagum_Tara	Sucrathix_VX	Xanthan_gum	MLS
Cellulose_gum	1,000	-,324	-,213	-,192	-,170	-,170	-,213	,273
Beta_glucan	-,324	1,000	-,213	-,192	-,170	-,170	-,213	-,107
Carrageenan	-,213	-,213	1,000	-,126	-,111	-,111	-,140	-,008
Solagum_AX	-,192	-,192	-,126	1,000	-,101	-,101	-,126	-,074
Solagum_Tara	-,170	-,170	-,111	-,101	1,000	-,089	-,111	-,006
Sucrathix_VX	-,170	-,170	-,111	-,101	-,089	1,000	-,111	-,304
Xanthan_gum	-,213	-,213	-,140	-,126	-,111	-,111	1,000	,117
MLS	,273	-,107	-,008	-,074	-,006	-,304	,117	1,000

Figure 25: Correlation between rheological modifiers and results of multiple light scattering

	Verstatil_PC	Verstatil_TBG	dermosoft_688_eco	Decyl_glucoside	Polisorbate_60	PC_DG	TBG_DG	PC_PS	TBG_PS	MLS
Verstatil_PC	1,000	-,660	-,439	,101	,118	,510	-,383	,344	-,221	-,224
Verstatil_TBG	-,660	1,000	-,386	,192	,052	-,337	,580	-,227	,335	,069
dermosoft_688_eco	-,439	-,386	1,000	-,353	-,207	-,224	-,224	-,151	-,129	,192
Decyl_glucoside	,101	,192	-,353	1,000	-,284	,634	,634	-,208	-,178	,247
Polisorbate_60	,118	,052	-,207	-,284	1,000	-,180	-,180	,730	,626	,283
PC_DG	,510	-,337	-,224	,634	-,180	1,000	-,195	-,132	-,113	-,009
TBG_DG	-,383	,580	-,224	,634	-,180	-,195	1,000	-,132	-,113	,322
PC_PS	,344	-,227	-,151	-,208	,730	-,132	-,132	1,000	-,076	,143
TBG_PS	-,221	,335	-,129	-,178	,626	-,113	-,113	-,076	1,000	,250
MLS	-,224	,069	,192	,247	,283	-,009	,322	,143	,250	1,000

Figure 26: Correlation between preservatives, surfactants and combinations of preservative and surfactant and results of multiple light scattering

	pH_Variation	Viscosity_very_low	Viscosity_low	Viscosity_medium	Viscosity_very_high	Pseudoplastic	Dilatant	MLS
pH_Variation	1,000	-,149	-,112	,225	,073	,115	-,115	,257
Viscosity_very_low	-,149	1,000	-,514	-,306	-,035	-,631	,631	-,107
Viscosity_low	-,112	-,514	1,000	-,486	-,304	,320	-,320	,064
Viscosity_medium	,225	-,306	-,486	1,000	-,181	,486	-,486	,038
Viscosity_very_high	,073	-,035	-,304	-,181	1,000	-,373	,373	-,074
Pseudoplastic	,115	-,631	,320	,486	-,373	1,000	-1,000	,101
Dilatant	-,115	,631	-,320	-,486	,373	-1,000	1,000	-,101
MLS	,257	-,107	,064	,038	-,074	,101	-,101	1,000

Figure 27: Correlation between pH variation, viscosities and rheology and results of multiple light scattering

The factorial analysis calculates the correlation between the variables. Thus, variables with the modulus value greater than 0,15 have significance in the gel stability. A negative correlation value show that the variable contributes for the gel stability and a positive correlation value causes the gel instability.

According to the results of the statistical analysis, the factors which the most influence the gels stability are the presence of Cellulose Gum, Microcrystalline Cellulose & Cellulose Gum & Xanthan Gum (Sucrathix VX), Phenoxyethanol & Caprylyl Glycol (Verstatil® PC), p-Anisic Acid (dermosoft 688® eco), Decyl Glucoside, Polysorbate 60, the association of Triethyl Citrate & Glyceryl Caprylate & Benzoic Acid (Verstatil® TBG) and Decyl Glucoside, the association of Triethyl Citrate & Glyceryl Caprylate & Benzoic Acid (Verstatil® TBG) and Polysorbate 60 and the pH variation. Of these factors, Cellulose Gum, p-Anisic Acid (dermosoft® 688 eco), Decyl Glucoside, Polysorbate 60, the association of Triethyl Citrate & Glyceryl Caprylate & Benzoic Acid (Verstatil® TBG) and Decyl Glucoside, the association of Triethyl Citrate & Glyceryl Caprylate & Benzoic Acid (Verstatil® TBG) and Polysorbate 60 and the pH variation contribute for gel instability. However, the presence of Microcrystalline Cellulose & Cellulose Gum & Xanthan Gum (Sucrathix VX) and Phenoxyethanol & Caprylyl Glycol (Verstatil® PC) contributes for the gel stability.

The results of the statistics analysis are consistent with the observed.

6. Conclusions

The aim of the work was to evaluate the influence of preservative systems in cosmetic gel formulations prepared from natural rheological modifiers. In order to study this influence, it was prepared gels in different combinations of rheological modifier, preservative and surfactant. Then, organoleptic aspect, pH, rheology, stability and microbiological control were analysed.

The results show that the factors which influence gel stability are: the type of surfactant and using of p-Anisic Acid preservative (dermosoft 688® eco). The gel instability is influenced by the surfactant. However, samples with Microcrystalline Cellulose & Cellulose Gum & Xanthan Gum (Sucrathix VX) are the most stable ones.

According to the microbiological tests, no sample has fungal contamination. However, samples with p-Anisic Acid preservative (dermosoft 688® eco) showed bacterial contamination due to the preservative that is not good for gram + and gram - bacteria.

Related to the statistics analysis, the factors which most influence the gels stability are the presence of Cellulose Gum, Microcrystalline Cellulose & Cellulose Gum & Xanthan Gum (Sucrathix VX), Phenoxyethanol & Caprylyl Glycol (Verstatil® PC), p-Anisic Acid (dermosoft® 688 eco), Decyl Glucoside, Polysorbate 60, the association of Triethyl Citrate & Glyceryl Caprylate & Benzoic Acid (Verstatil® TBG) and Decyl Glucoside, the association of Triethyl Citrate & Glyceryl Caprylate & Benzoic Acid (Verstatil® TBG) and Polysorbate 60 and the pH variation.

In conclusion, it is possible to say that preservatives can influence gel stability.

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Annex

Table 11: Aspect of Samples with Cellulose Gum

Aspect of Samples with Cellulose Gum			
Sample	**Organoleptic Aspect**	**Aspect after Turbiscan Analysis**	**Aspect at 1 month**
CMC_A / PC_0,8	Clear	Clear	Clear
CMC_A / TBG_1	Opalescent	Opalescent	Opalescent
CMC_A / 688_0,1	Clear	Clear with little sedimentation (0,3 cm)	Sedimentation
CMC_A / PC_0,8 / DG_10	Opalescent	Opalescent	Opalescent
CMC_A / TBG_1 / DG_10	Opalescent	Phase separation (1,3 cm)	Phase separation
CMC_A / DG_10 / TBG_1	Opalescent	Phase separation with clarification (0,8 cm)	Phase separation
CMC_B / DG_10 / TBG_1	Opalescent	Flocculation	Phase separation with clarification
CMC_A / TBG_1 / DG_5	Opalescent	Phase separation with clarification (0,7 cm)	Phase separation with clarification
CMC_A / PS_5 / TBG_1	Opalescent	Sedimentation and flocculation (0,7 cm)	Sedimentation not finished
CMC_A / PS_5 / PC_0,8	Opalescent	Sedimentation and flocculation (0,4 cm)	Sedimentation not finished
CMC_A / PS_10 / PC_0,8	Opalescent	Sedimentation and flocculation (0,8 cm)	Sedimentation not finished
CMC_A / 688_0,3	Clear	Sedimentation	Sedimentation

Table 12: Results of Multiple Light Scattering for Samples with Cellulose Gum

Results of Multiple Light Scattering for Samples with Cellulose Gum							
Sample	**Use**	**Bottom**	**Middle**	**Top**	**Turbiscan Stability Index (TSI)**	**Kinetic Profile**	**Discussion**
CMC_A / PC_0,8	Transmission	Positive peak (20%)		Negative peak (-50%)	<14, no stable until 3h, after 3h is stable		End of analysis there is no alteration of TSI → alterations due to air bubbles
CMC_A / TBG_1	Transmission				<0,2, very stable (not horizontal but almost)		Alterations due to particles in suspension which are not dissolved

CMC_A / 688_0,1	Transmission	Negative parallel curves (-20%)	Parallel curves	Parallel curves	<30, linear increasing due to the flocculation	Transmission: 5,72 mm/d from 0h39 to 1h39 Backscattering: 3,35 mm/h from 1h30 to 1h52 and 12,32 mm/h from 1h20 to 4h23	Because the separation is finished, became more stable in 20°C again Parallel curves due to phase separation with the clearer phase at the top → sedimentation, occurring particle migration from the top to the bottom, that is why the backscattering bottom is positive and transmission top is positive
CMC_A / PC_0,8 / DG_10	Transmission				< 35, linear increasing due to the alteration		Alterations due to air bubbles
CMC_A / TBG_1 / DG_10	Both	Parallel curves	Parallel curves	Positive peak (+12%) in transmission Negative peak (-8%) in backscattering	<5	Backscattering: 3,72 mm/d from 1h10 to 2h20	Only one cycle in 20°C Phase separation with the clearer phase at the top, that is why there is a positive peak in transmission and a negative peak in backscattering
CMC_A / DG_10 / TBG_1	Both	Positive peak (+50%) in transmission	Parallel curves	Parallel curves	<5	Transmission: 15,76 mm/d from 4h23 to 4h35 and 4,05 mm/d from 4h34 to 6h15	Only one cycle in 20°C Positive peak in transmission due to phase separation with the clearer phase at the bottom
CMC_B / DG_10 / TBG_1	Backscattering	Parallel curves at 4°C and 20°C	Parallel curves at 4°C and 20°C	Parallel curves at 4°C and 20°C	< 28, TSI increases from 1 to 28 when from 20°C to 4°C		Flocculation due to flakes formation at 4°C
CMC_A / TBG_1 / DG_5	Both	Positive in transmission	Parallel curves	Parallel curves	<2		Only one cycle in 20°C Positive at the bottom in transmission → phase separation with clarification
CMC_A / PS_5 / TBG_1	Backscattering	Positive peak (+3%)		Negative	<0,8, linear increasing due to the flocculation		Positive peak in backscattering due to phase separation with the more opalescent phase at the bottom → sedimentation that is why the backscattering bottom is positive and the backscattering top is negative

CMC_A / PS_5 / PC_0,8	Transmission				< 1,6		
CMC_A / PS_10 / PC_0,8	Both	Positive peak (1,5%) in backscattering		Negative in backscattering	< 8		Positive peak in backscattering due to phase separation with the more opalescent phase at the bottom → sedimentation that is why the backscattering bottom is positive and the backscattering top is negative
CMC_A / 688_0,3	Backscattering	Positive peak (4%)		Negative	< 1,5	Backscattering: 7,12 mm/d from 1h35 to 5h05 and 1,35 mm/d from 5h04 to 6h15	Two cycles Positive peak in backscattering due to phase separation with the more opalescent phase at the bottom → sedimentation that is why the backscattering bottom is positive and the backscattering top is negative

Table 13: Aspect of Samples with Beta-glucan

Aspect of Samples with Beta-glucan			
Sample	**Organoleptic Aspect**	**Aspect after Turbiscan Analysis**	**Aspect at 1 month**
Beta_A / PC_0,8	Clear	Clear	Clear
Beta_B / PC_0,8	Clear	Clear	Clear
Beta_A / TBG_1	Opalescent	Opalescent	Opalescent
Beta_A / 688_0,1	Clear	Clear with very little sedimentation (0,3 cm)	Clear with few flakes
Beta_A / PC_0,8 / DG_10	Opalescent	Opalescent	Opalescent
Beta_B / PC_0,8 / DG_10	Opalescent	Opalescent	Opalescent
Beta_A / TBG_1 / DG_10	Opalescent	Opalescent	Opalescent
Beta_A / PS_5 / PC_0,8	Clear	Clear	Sedimentation not finished
Beta_A / PS_10 / PC_0,8	Opalescent	Sedimentation (0,5 cm)	Sedimentation not finished
Beta_A / PS_5 / TBG_1	Clear	Not homogenous	Sedimentation not finished
Beta_A / PS_10 / TBG_1	Opalescent	Sedimentation (0,4 cm)	Sedimentation not finished
Beta_A / 688_0,3	Clear	Sedimentation	Clear

Table 14: Results of Multiple Light Scattering for Samples with Beta-glucan

Results of Multiple Light Scattering for Samples with Beta-glucan							
Sample	**Use**	**Bottom**	**Middle**	**Top**	**Turbiscan Stability Index (TSI)**	**Kinetic Profile**	**Discussion**
Beta_A / PC_0,8	Transmission	Negative peak (-10%)		Positive peak (15%)	< 2,5, TSI increases from 1,8 to 2,5 when 20°C to 4°C		Until 6h stay clearer in the top Alterations due to bubble airs
Beta_B / PC_0,8	Transmission	Negative peak (-5%)		Positive peak (20%)	< 2,3, no stable until 3h, after 3h is stable, TSI increases from 1,4 to 2,3 when 20°C to 4°C		Particle migration to bottom (top) along the time Alterations due to bubble airs
Beta_A / TBG_1	Transmission		Parallel curves at 4°C		< 8, no stable until 3h, after 3h is stable, TSI increases from 3 to 8 when 20°C to 4°C		Alterations due to the temperature change
Beta_A / 688_0,1	Transmission	Negative flocculation (-18%)		Positive (+20%)	< 3, TSI increases from 1,5 to 3 when 20°C to 4°C		
Beta_A / PC_0,8 / DG_10	Transmission				< 2,8, TSI matches at 20°C and 4°C		Alterations due to bubble airs
Beta_B / PC_0,8 / DG_10	Transmission		Parallel curves		< 7,5, TSI increases from 4 to 7,5 when 20°C to 4°C	Transmission: 18,62 mm/d from 4h04 to 4h44 and 0,92 mm/d from 4h45 to 6h14	Alterations due to bubble airs
Beta_A / TBG_1 / DG_10	Transmission				< 4,5		One cycle Alterations due to bubble airs
Beta_A / PS_5 / PC_0,8	Both				< 5, TSI matches at 20°C to 4°C		Alterations due to bubble air
Beta_A / PS_10 / PC_0,8	Both	Positive parallel curves	Parallel curves	Positive parallel	< 26, TSI decreases from 26 to 14 when 20°C to 4°C		Flocculation due to phase separation with the clearer phase at the top → sedimentation, occurring particle migration

		in backscattering		curves in transmission			from the top to the bottom, that is why the backscattering bottom is positive and transmission top is positive
Beta_A / PS_5 / TBG_1	Transmission	Parallel curves	Parallel curves	Parallel curves	< 12, TSI increases from 9 to 12 when 20°C to 4°C		
Beta_A / PS_10 / TBG_1	Both	Positive parallel curves in backscattering	Parallel curves	Positive parallel curves in transmission	< 26, TSI decreases from 35 to 20 when 20°C to 4°C		Flocculation due to phase separation with the clearer phase at the top → sedimentation, occurring particle migration from the top to the bottom, that is why the backscattering bottom is positive and transmission top is positive
Beta_A / 688_0,3	Transmission	Parallel curves	Parallel curves	Positive	< 90, TSI increases from 35 to 90 when 20°C to 4°C		Flocculation due to phase separation with the clearer phase at the top → sedimentation, occurring particle migration from the top to the bottom

Table 15: Aspect of Samples with Carrageenan

Aspect of Samples with Carrageenan			
Sample	**Organoleptic Aspect**	**Aspect after Turbiscan Analysis**	**Aspect at 1 month**
Car_A / PC_0,8	Clear	Clear	Clear
Car_A / TBG_1	Opalescent	Opalescent	Opalescent
Car_A / 688_0,1	Clear	Clear	Clear
Car_A / PC_0,8 / DG_10	Opalescent	Phase separation with clarification (0,3 cm)	Opalescent
Car_A / TBG_1 / DG_10	Opalescent	Not homogenous	Phase separation with clarification
Car_A / DG_10 / TBG_1	Opalescent	Phase separation with clarification (0,3 cm)	Phase separation with clarification

Table 16: Results of Multiple Light Scattering for Samples with Carrageenan

Results of Multiple Light Scattering for Samples with Carrageenan							
Sample	**Use**	**Bottom**	**Middle**	**Top**	**Turbiscan Stability Index (TSI)**	**Kinetic Profile**	**Discussion**
Car_A / PC_0,8	Transmission	Positive peak (+60%)		Negative peak (-65%)	< 12, no stable until 3h, after 3h is stable		End of analysis there is no alteration of TSI → alterations due to air bubbles
Car_A / TBG_1	Transmission				< 5	Transmission: 5,03 mm/d from 2h29 to 6h03)	Became more instable at 4°C (TSI increases from 1 to 5) because of the temperature change which causes molecules alterations
Car_A / 688_0,1	Transmission				<3,5		
Car_A / PC_0,8 / DG_10	Both	Positive (15%) in transmission	Parallel curves		< 9		Particle migration from the bottom to the top, few clarification
Car_A / TBG_1 / DG_10	Both				< 3,5		Became more instable at 4°C (TSI increases from 0,5 to 3,5) because of the temperature change which causes molecules alterations
Car_A / DG_10 / TBG_1	Backscattering			Positive peak (+2%)	< 0,8	Transmission: 1,15 mm/h from 3h24 to 3h51 and 0,69 mm/d from 4h05 to 6h15	Positive peak in backscattering due to phase separation with the clearer phase at the bottom One cycle

Table 17: Aspect of Samples with Solagum™ AX

Aspect of Samples with Solagum™ AX			
Sample	**Organoleptic Aspect**	**Aspect after Turbiscan Analysis**	**Aspect at 1 month**
SAX_A / PC_0,8	Opalescent	Opalescent	Opalescent
SAX_A / 688_0,1	Opalescent	Opalescent	Opalescent with flakes
SAX_A / PC_0,8 / DG_10	Opalescent	Phase separation with clarification (0,2 cm)	Opalescent
SAX_A / TBG_1	Opalescent	Opalescent	Opalescent
SAX_A / 688_0,3	Opalescent	Not homogenous	Not homogenous

Table 18: Results of Multiple Light Scattering for Samples with Solagum™ AX

Results of Multiple Light Scattering for Samples with Solagum™ AX							
Sample	**Use**	**Bottom**	**Middle**	**Top**	**Turbiscan Stability Index (TSI)**	**Kinetic Profile**	**Discussion**
SAX_A / PC_0,8	Transmission		Parallel curves at 4°C		< 1,1		Stable (at 4°C, it is the change of molecules in the gel due to the temperature change)
SAX_A / 688_0,1	Transmission		Parallel curves at 4°C		< 1,1		Stable (at 4°C, it is the change of molecules in the gel due to the temperature change)
SAX_A / PC_0,8 / DG_10	Transmission	Positive (+0,5%) at 4°C	Parallel curves at 4°C	0% at 4°C	< 1,7		Phase separation at 4°C; there is a positive bottom because there is clarification
SAX_A / TBG_1	Transmission		Parallel curves at 4°C		< 0,1		Very stable (at 4°C, it is the change of molecules in the gel due to the temperature change)
SAX_A / 688_0,3	Transmission		Parallel curves at 4°C		< 1,1		Stable (at 4°C, it is the change of molecules in the gel due to the temperature change)

Table 19: Aspect of Samples with Solagum™ Tara

Aspect of Samples with Solagum™ Tara			
Sample	**Organoleptic Aspect**	**Aspect after Turbiscan Analysis**	**Aspect at 1 month**
ST_A / PC_0,8	Opalescent	Opalescent	Opalescent
ST_A / 688_0,1	Opalescent	Not homogenous	Not homogenous
ST_A / PC_0,8 / DG_10	Opalescent		Flocculation
ST_A / TBG_1	Opalescent	Opalescent	Opalescent

Table 20: Results of Multiple Light Scattering for Samples with Solagum™ Tara

Results of Multiple Light Scattering for Samples with Solagum™ Tara							
Sample	**Use**	**Bottom**	**Middle**	**Top**	**Turbiscan Stability Index (TSI)**	**Kinetic Profile**	**Discussion**
ST_A / PC_0,8	Transmission				< 0,3		Very stable
ST_A / 688_0,1	Transmission				< 0,3		Very stable
ST_A / PC_0,8 / DG_10							
ST_A / TBG_1	Backscattering				< 0,3		Very stable

Table 21: Aspect of Samples with Sucrathix VX

Aspect of Samples with Sucrathix VX			
Sample	**Organoleptic Aspect**	**Aspect after Turbiscan Analysis**	**Aspect at 1 month**
SVX_A / PC_0,8	Opalescent	Opalescent	Opalescent
SVX_A / 688_0,1	Opalescent	Opalescent	Opalescent
SVX_A / PC_0,8 / DG_10	Opalescent	Opalescent	Opalescent
SVX_A / TBG_1	Opalescent	Opalescent	Opalescent

Table 22: Results of Multiple Light Scattering for Samples with Sucrathix VX

Results of Multiple Light Scattering for Samples with Sucrathix VX							
Sample	**Use**	**Bottom**	**Middle**	**Top**	**Turbiscan Stability Index (TSI)**	**Kinetic Profile**	**Discussion**
SVX_A / PC_0,8	Backscattering				< 0,3		Very stable
SVX_A / 688_0,1	Backscattering				< 0,3		Very stable
SVX_A / PC_0,8 / DG_10	Backscattering				< 0,9		Stable
SVX_A / TBG_1	Backscattering				< 0,3		Very stable

Table 23: Aspect of Samples with Xanthan Gum

Aspect of Samples with Xanthan Gum			
Sample	**Organoleptic Aspect**	**Aspect after Turbiscan Analysis**	**Aspect at 1 month**
XG_A / PC_0,8	Clear	Not homogenous	Opalescent
XG_A / TBG_1	Opalescent	Opalescent	Opalescent
XG_A / 688_0,1	Clear	Not homogenous	Not homogenous
XG_A / PC_0,8 / DG_10	Opalescent	Phase separation with clarification and flocculation (0,3 cm)	Opalescent
XG_A / TBG_1 / DG_10	Opalescent	Phase separation with clarification (0,2 cm)	Phase separation with clarification
XG_C / PC_0,8	Clear	Clear	Clear

Table 24: Results of Multiple Light Scattering for Samples with Xanthan Gum

Results of Multiple Light Scattering for Samples with Xanthan Gum							
Sample	**Use**	**Bottom**	**Middle**	**Top**	**Turbiscan Stability Index (TSI)**	**Kinetic Profile**	**Discussion**
XG_A / PC_0,8	Transmission				< 6		Became more instable at 4°C (TSI increases from 2 to 6) because of the temperature change which causes molecules alterations
XG_A / TBG_1	Backscattering				< 0,8		Became more instable at 4°C (TSI increases from 0,2 to 0,8) because of the temperature change which causes molecules alterations
XG_A / 688_0,1	Transmission			Negative peak (-35%)	< 3		TSI doesn't stabilize (continues to increase)
XG_A / PC_0,8 / DG_10	Both	Positive (0,5%) at 4°C in transmission	Parallel curves at 4°C	Parallel curves at 4°C	< 1,6		Particle migration to the top, occurring phase separation with clarification

XG_A / TBG_1 / DG_10	Backscattering		Parallel curves		< 0,4		Particle migration to the top, occurring phase separation with clarification; one cycle
XG_C / PC_0,8	Transmission	Positive peak (+20%)		Negative peak (-40%)	< 4	Transmission: 4,48 mm/d from 0h43 to 2h03	