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STATE-OF-ART ON GEL TECHNOLOGIES FOR TRANSDERMAL DELIVERY OF BACITRACIN

Master Thesis in Tecnologias do Medicamento, supervised by PhD Eliana Maria Barbosa Souto and presented to Faculty of Pharmacy, University of Coimbra

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ABSTRACT

Gels have consistently been studied for their role in topical and transdermal drug delivery systems as a non-invasive technique, for pharmaceutical and cosmetics application. These formulations are semi-solid three-dimensional structures, porous, with unique characteristics, such as rigidity and elasticity at the same time. Because of their high aqueous phase content, gels permit a greater dissolution of drugs through the skin and enhance skin hydration by retaining a significant amount of transepidermal water, in contrast to creams and ointments. Conventionally, gels are differentiated into two different types according to the nature of their liquid phase: hydrogels, which contain a polar solvent (water) and organogels, which contain an organic/non-polar solvent, as external phase.

Hydrogels consist of polymeric materials that exhibit the ability to swell and retain a large amount of water or other biofluids in its structures. Despite its great affinity for water, they only possess a swelling behavior without dissolving in water. This proves its high flexibility, similar to natural tissue.

Organogels consist of a network of self-assembled molecules which forms a thermally reversible gel upon cooling, immobilizing a non-aqueous liquid. They are mainly composed by lipids (organic phase), so they easily interact with the lipid skin surface and enhance the drug permeation through the skin. The most widely used lipids are based in edible oils due to their high biocompatibility, such as soybean oil, sunflower oil, sesame seed oil or olive oil. Organogels form viscoelastic structures through non-covalent associations with gelling agents in low concentrations. The commonly used organogelators include sorbitan monostearate or sorbitan monopalmitate. These superstructures, often long fibers or needle-shaped structures, which entangle or form pseudocrystalline regions, immobilizing the liquid largely by surface tension and forming a gel of variable consistency. Lecithin organogels are a special type of organogels that do not require addition of any additional surfactant or penetration enhancer, as lecithin serves both the purposes.

Recent studies have reported other types of gels for dermal drug application, such as proniosomal gels, emulgels, bigels and aerogels, combining features of conventional hydrogels and organogels.

In conclusion, further studies in gel technologies are essentials to overcome the drawbacks of each gel system and for developing cost effective delivery systems for transdermal applications.

Keywords

Bacitracin; gel technologies; hydrogels; organogels; microemulsions; transdermal drug delivery.

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ABBREVIATIONS

AMPs	Antimicrobial Peptides
Asn^{12}	Asparagine in position 12 on aminoacids sequence
$D\text{-}Asp^{11}$	D-aspartic acid in position 10
$D\text{-}Glu^{4}$	D-glutamine in position 4
$D\text{-}Orn^{7}$	D-ornithine in position 7
$D\text{-}Phe^{9}$	D-phenylalanine in position 9
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic Acid
DOPE	Dioleylphosphatidyl ethanolamine
High sensitivity DSC	High-Sensitivity Differential Scanning Calorimeter
DSPC	Distearoylphosphatidylcholine
HCl	Hydrochloric acid
IPN	Multipolymer interpenetrating networks
$L\text{-}Cys^{2}$	L-Cysteine in position 2
$L\text{-}Ile^{1}$	L-Interleukin in position 1
$L\text{-}Val^{1}$	L-Valine in position 1
LMOG	Low Molecular Weight Organogelator
Log P	Octanol-water partition coefficient
Lys^{6}	Lysine in position 6
M.W.	Molecular weight
NaCl	Sodium Cloride
NMR	Nuclear Magnetic Resonance
O/W	Oil-in-water
pKa_1	Acid dissociation constant 1
pKa_2	Acid dissociation constant 2
PLO	Pluronic Lecithin Organogels
SANS	Small Angle Neutron Scattering
SAXS	Small Angle X-ray Scattering
SC	Stratum Corneum
SCVs	Small colony variants
cryo-TEM	cryo-Transmission Electron Microscopy
TEWL	Transepidermal Water Loss

UDP	Uridine diphosphate
UDP-GlcNac	Uridine diphosphate-*N*-acetylglucosamine
UDP-MurNac pentapeptide	Uridine diphosphate-*N*-acetylmuramic acid-pentapeptide
UMP	Uridine Monophosphate
Undecaprenyl-P	Undecaprenyl phosphate
Undecaprenyl-P-P	Undecaprenyl pyrophosphate
Undecaprenyl-P-P-MurNac pentapeptide	Undecaprenyl pyrophosphate-*N*-acetylmuramic acid pentapeptide
Undecaprenyl-P-P-(MurNac)-GlcNac	Undecaprenyl pyrophosphate-(*N*-acetylmuramic acid pentapeptide)- *N*-acetylglucosamine
UV/Vis Spectroscopy	Ultraviolet–Visible spectroscopy
W/O	Water-in-oil

1. TOPICAL AND TRANSDERMAL DRUG DELIVERY

Skin is one of the most accessible organ of the human body, with a large surface area, which makes it a focus of much research and product development for drug administration. Transdermal drug delivery is an easy and non-invasive route, well tolerated by patients, and which enhances transdermal delivery, avoiding local gastrointestinal toxicity and effects (pH changes, enzymatic deactivation, gastric retention) and first pass metabolism, however it acts as an impermeable barrier to the passage of exogenous substances [1].

The skin acts as a barrier between the environment and our tissues and internal structures. When intact, the skin protects against excessive water loss to the environment and blocks the passage of infectious agents such as bacteria and viruses or other harmful substances into the body. The outermost layer of skin and primarily responsible for the barrier function is the stratum corneum, which is characterized by a thin layer of squamous cells that package in a dense configuration with specialized lipids in the intercellular spaces, and an acid pH character.

The stratum corneum (SC) is comprised of layers of flattened cells called corneocytes. These cells start their life in the basal layer of the epidermis as keratinocytes and move over time up through the various layers of the epidermis. By the time they reach the skin surface, they have changed to non-living, pancake-flat cells that are tightly bound into sheets. Once the cells are in the very top layer of the stratum corneum, they are shed and the cycle begins again. The densely layered corneocytes (with a thickness of 15-20 μm) are responsible for providing physical barrier protection [2, 3].

The cells below the stratum corneum release lipids, which form a layer in and around the tightly stacked corneocytes. The types of lipids present are ceramides, essential fatty acids and their esters and cholesterol and its sulphates, that are structured in bilayers. This lipid layer is crucial in preventing water loss from the skin and prevent the entry of undesirable chemicals into the skin. Aggressive skin cleansers can remove parts of the lipid layer as well as affect the integrity of the lipid layer structure, causing continual barrier function disruption. Where the lipid layer is deficient, the skin becomes dehydrated, affecting normal shedding of cells from the surface of the stratum corneum and resulting in dry and flaky skin. Maintaining the normally acidic pH of the stratum corneum is essential for the correct formation of the lipid layer surrounding the corneocytes. It is also essential for maintaining normal bacteria on the skin surface and inhibiting the growth of disease causing bacteria, *P. acnes* (implicated in acne lesions) and the yeast that causes thrush. Increasing pH in the skin affects the normal shedding of cells from the stratum corneum, also leading to flaky skin [4].

It can be seen that the three components of normal skin barrier function are dependent on each other and adverse changes in one component will negatively affect the other barrier mechanisms. Where there is no underlying genetic reason for an impaired barrier function, barrier function can be improved by using skin care products that maintain an acid pH of the skin, cause limited disruption to the lipid layer, improve cell shedding and adequately moisturize the skin surface.

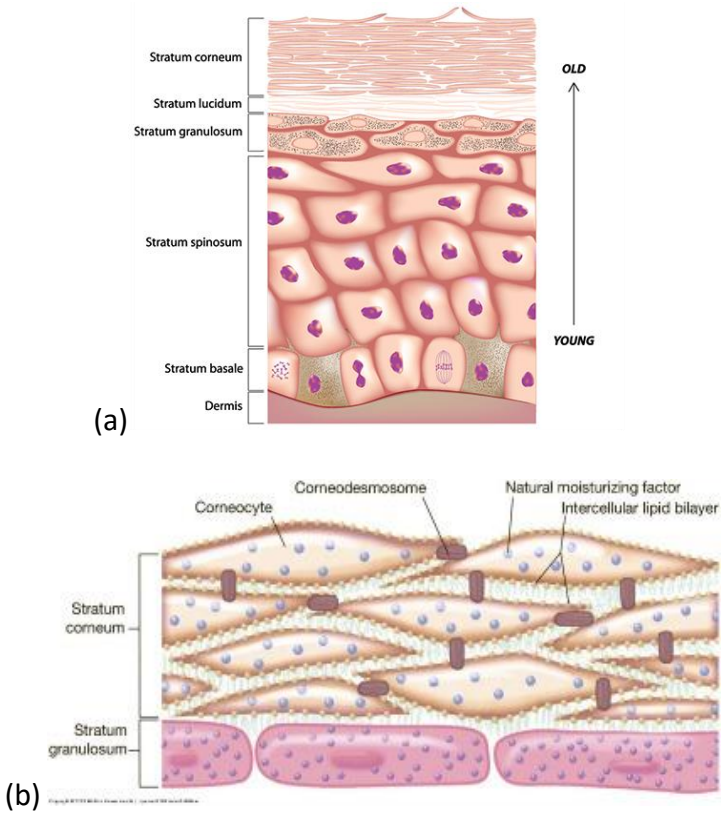


Figure 1 - (a) Structure of the various layers of the skin, since the formation of corneocytes in basal layer to the stratum corneum. (b) Structure of the stratum corneum. (Source: https://theraworx.com/wp-content/uploads/2012/07/Stratum-Corneum.png)

Topical or dermatological drugs consist of a set of products that are applied to the skin or at mucous membrane which potentiate or retrieve the basic function of the skin or pharmacologically alter the action of certain tissue. Lipophilic drugs can readily dissolve in the stratum corneum and diffuse through it at a rate that depends on molecular size and lipophilicity. Historically, it has been demonstrated that the skin tends to keep out drug molecules greater than 500 Da, especially those molecules of hydrophilic nature. After all, the main biological function of the skin is to deny entry to foreign substances. Therefore, bypassing the skin to allow drug entry is a necessary step to successful transdermal delivery.

Drug that is absorbed through the skin is delivered directly to the targeted site, is not susceptible to first pass metabolism by gut and liver, although some metabolism may occur in the skin itself.

Cutaneous permeation of the drug has several routes: the transcellular, intercellular or transappendageal (via the sweat glands or hair follicles). Since the appendages have a very small surface area, administration by this route is facilitated through the use of techniques such as iontophoresis (Fig. 2).

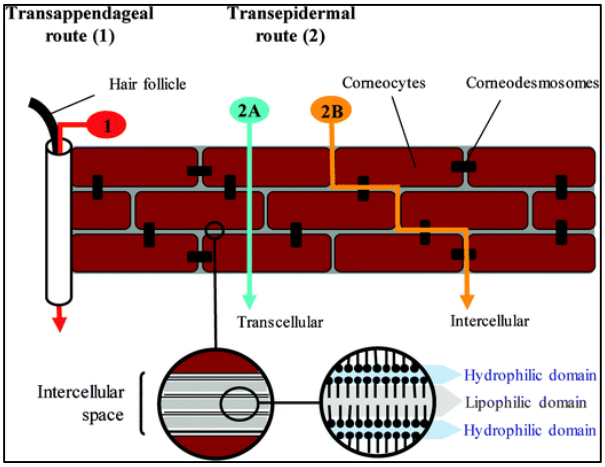


Figure 2 - Routes of permeation of a drug through the skin. (Source: Hadgraft et al., 2011 [5].)

After passing through the stratum corneum, drug encounters the more hydrophilic, viable epidermis and dermis, before being absorbed in capillaries perfusing the dermis, that is, skin permeation involves the following steps:

1st Formation of a thin film on the skin surface

2nd Drug dissolution and diffusion of the conveying system along the skin surface

3rd Partition of the drug along the epidermis

4th Diffusion of the drug by the inner layers of skin (dermis). Uptake by blood capillary cells and release of the drug into systemic circulation.

Transdermal permeation depends on skin's physiological factors such as the thickness of the skin, the lipid content, the density of hair follicles, the density of sweat glands, skin pH, blood flow, the moisturizing state of skin and its inflammatory conditions, and on drug's physicochemical factors, such as its partition coefficient, molecular weight, degree of ionization (which should be zero) and mobility and release's ability in the transport and drug delivery system [6].

Mechanisms to promote skin permeation of large and potentially hydrophilic peptides require a direct effect on the skin or some type of physical and/or chemical modification of the formulation, in order to change partition, diffusion or solubility of this molecules.

1) Direct effect on the skin
    a. Denaturation of intracellular keratin or change in its conformation cause swelling and increase hydration
    b. Effect on desmosomes (known as macula structures specialized in the cell-cell adhesion) that maintains cohesion between corneocytes
    c. Modification of lipid bilayers reduces resistance to permeation
    d. Changing the properties of SC solvents to modify their partitioning capacity
    e. Use of a solvent capable of removing lipids SC and decrease the resistance to permeation.
2) Formulation changes
    a. Adding a volatile solvent that achieves a state of supersaturation of the formulation with higher thermodynamic stability.
    b. Skin permeation enhancer molecules, which are good solvents for the drug. It improves the drug partitioning in the stratum corneum.
    c. Enhancer molecules which create a lipidic pool between bilayers, such as oleic acid, or molecules which disrupt bilayers uniformly, like Azone®. Azone® (1-dodecylazacycloheptan-2-one or laurocapram) serves as surfactant and enhances the skin permeation and transport of a wide variety of drugs including steroids, antibiotics and antiviral agents [7].
3) Iontophoresis

The combination of fatty acids with iontophoretic delivery enhances the permeation through the sweat glands and hair follicles. In this technique, a small electric current is applied between two electrodes placed on the skin, allowing the permeation of polar or neutral molecules, ionized, in a controlled manner due to the slight disruption caused in the lipid layer [8]. By combining fatty acids, which disrupt the SC lipids, and ions, which pass with lower resistance, the permeation is facilitated not only for low molecular weight molecules, but also high molecular weight ones, like proteins and peptides. This combination of methods allows to reduce the toxicity and irritation that are associated with the use of high levels of each. For example, Singh *et al*. found that iontophoresis in combination with R(+)-limonene, or palmitic, palmitoleic, stearic, oleic, linoleic or linolenic acids enhance *in vitro* permeation

of insulin through the skin, and the most efficient is the linolenic acid, either by passive transport or iontophoretic [9].

4) Other mechanical processes

Microneedle technology - Microneedle technology involves the use of small needles that create small pores in the skin, allowing drug passage across the outermost physical barrier. It's a non-invasive technique, which only breach the stratum corneum. Both the capillaries and nerve endings are avoided, leading to a painless feeling for the patient. Methods for drug permeation consist to create micropores and then deliver the drug topically; or to cover the microneedles with the drug in a simultaneous process of poration and absorption; or, on the other hand, encapsulate the drug in the microneedles, wherein the release occurs slowly as the needles degrade; a final method includes creating hollow needles, through which drug can be infused following puncturing of the skin [10].

Electroporation - utilizes very short pulses of high voltages (between 10 and 100 V) to perforate the skin. Breaches only the stratum corneum. This method targets the lipid bilayers surrounding the zone of the lesion, which by applying an electric current, disturb the structure of these lipid, allowing molecules to penetrate the skin. Increasing the voltage, number of pulses and duration of pulses may increase drug absorption.[11]

Sonophoresis - also referred to as cavitational ultrasound, relies on the application of sound waves to the skin to increase its permeability. Sound waves, generally between 20-100 kHz (low frequency sonophoresis), are believed to cause an increase in pore sizes on the skin (increased fluidity in these lipid bilayers), thus allowing drug penetration transcellularly through the stratum corneum. This method enable the administration of low molecular weight molecules and also macromolecules [12].

Thermal ablation - this technique relies on short pulses of high heat (approximately 100 °C) to create small, reversible channels in the micron size range. This permeates only the stratum corneum, avoiding a breach of the deeper capillary and nerve-containing tissue layers, so is a non-invasive technique. After the small breakages in the skin, drugs with low molecular weight may be primarily administered [13].

Chemical enhancement - use of biochemical molecules to enhance permeation of peptide drugs across the skin and remain nontoxic, non-irritating and non-allergenic. For example, one such peptide used to enhance skin permeability is magainin, a 23-amino acid peptide known to form pores in bacterial cell membranes [14].

Since the skin naturally functions as an environmental barrier, only a few drugs can penetrate it at an adequate rate by partitioning and diffusion. Generally, a drug molecule should be sufficiently lipophilic that it partitions into the stratum corneum, but sufficiently hydrophilic that it can also cross the viable layers.

1.1. ADVANTAGES OF TOPICAL DELIVERY SYSTEMS

Topical delivery of drugs is the best route of drug administration to treat skin diseases or disinfect the skin, because it avoids any systemic side effects. The main therapeutic advantages of drug delivery through the skin are avoid first-pass metabolism, caused by the transit in the gastrointestinal tract, as well as conditions associated with this (food intake, stomach emptying, motility intestinal and transit time); time duration of drug delivery is greater at a constant concentration, allowing to maintain constant the plasma concentrations of drug over time and decrease the frequency of drug dosing. In certain circumstances, the enzymatic hydrolysis may be used to enhance the permeation of certain hydrophilic drugs that are applied to the skin in the form of pro-drugs. If necessary, by observation for adverse effects, it is possible to immediately stop the treatment. This route is also an alternative when oral therapy is not possible in the case of nausea or vomiting. An advantage in terms of clinical practice is that these systems are easy to apply and remove, they are acceptable to the patient (patient compliance) and avoid the risks associated with intravenous administration.

1.2. LIMITATIONS OF TOPICAL DELIVERY SYSTEMS

Skin tends to keep out drug molecules with a molecular weight greater than 500 Da, especially those of hydrophilic nature. Drugs with a reasonable coefficient of partition and capable of solubilizing oil and water are ideal, since they have to diffuse through the stratum corneum lipophilic layer and through the hydrophilic layer of the epidermis, until they reach the systemic circulation. Very few drugs in their free form can overcome the various skin layers, thus, their administration is only enhanced by using percutaneous routes or permeation promoters. This route of administration is not feasible for drugs that irritate or sensitize the skin.

Topical drug delivery systems are relatively expensive compared to conventional dosage forms. They can contain large amount of drug, of which only a small proportion is used during the application period.

2. BACITRACIN

In antimicrobial therapy is important the treatment of infections caused by intracellular pathogens. The ability of some pathogens resist intracellularly and quickly multiply demonstrates why the infections that seem eradicated return a few weeks later [15]. *Staphylococcus aureus (S. aureus)* is a typical gram-positive bacterium and is the major pathogenic bacterium that causes chronic and antibiotic-resistant infections. It has been shown that small colony variants (SCVs) of this pathogen are present in the infections of bones, joints and skin, and are involved in the pathogenesis and manifestations of diseases such as chronic osteomyelitis, osteoarthritis and keratosis follicularis [16-18].

The main goal of antimicrobial therapy is to eradicate invading microorganisms by delivering an optimal amount of active drug to the site of infection. The ability of an antibiotic drug to reach effective concentrations at the site of infection is related to the physicochemical and pharmacological characteristics of the molecule [16]. Polypeptide antibiotics are potent antimicrobial agents, however they are not effective against facultative intracellular pathogens during their intracellular growth phase, because penetration of antimicrobial agents through the cell membrane into the cytoplasm is insufficient [19]. Nanotechnology presents a number of different drugs delivery systems that can help to overcome this obstacle and to promote the antimicrobial activity of these drugs. Some of the strategies consist of encapsulate antimicrobial agents in the material, allowing its release over time, or modify covalently the surface of materials with antimicrobial immobilizing agents such as antimicrobial peptides (AMPs), silver ions or polycationic groups, affording antimicrobial properties to the surface of the material [20].

Antimicrobial peptides are a group of active biomolecules which, depending on its composition in amino acids and conjugates (sugars, lipids, or other molecules), and their three-dimensional structure may have different mechanisms of action. In general, these peptides act through non-stereospecific mechanisms involving an initial connection to the extracellular side of the membrane / bacterial wall, through electrostatic interactions due to their cationic surface charge and amphiphilic structure, and then the insertion of the peptides in the hydrophobic interior of the lipid membrane, destabilizing it and leading to their rupture. This makes it difficult for pathogenic microorganisms to gain resistance. Some examples of AMPs are polymyxin, amphomycin, actinomycin, gramicidin, vancomycin, penicillin, cephalosporin, and bacitracin [21].

Bacitracin was discovered in 1945 by Meleney and Johnson and is produced by cultures of bacillus, *Bacillus subtilis* and *Bacillus licheniformis* [22, 23]. Its potent antibiotic activity mainly

acts against cocci and bacilli of Gram positive bacteria, including *Staphylococcus*, *Streptococcus*, and *Clostridium difficile*, but also against *Archaebacteria*, such as *Methanobacterium*, *Methanococcus* and *Halococcus* [22-24]. Bacitracin is one of constituent ingredients of various commercially available ointments for topical application in Portugal (together with neomycin and polymyxin B) as Polisulfadê®, Baciderma® or Bacitracin Labesfal®. They are used to prevent infection in shallow cuts and burn. Bacitracin is not administered systemically due to its nephrotoxicity, so this route of administration is used as a last resort. Its oral administration is possible, since the drug is not absorbed by the gastrointestinal system [25].

The structure of bacitracin is a cyclic dodecapeptide, which contains a thiazoline ring on the N-terminal of the polypeptide, formed by the condensation of the carbonyl group of L-Ile[1] or L-Val[1] aminoacids with –SH and $–NH_2$ groups of the aminoacid L-Cys[2]. The first five aminoacids adopt a tail configuration, near a cyclical heptapeptide formed by amide bond between the side chain of $–NH_2$ of Lys[6] and the C-terminal of Asn[12] and by four aminoacids (D-Glu[4], D-Orn[7], D-Phe[9] e D-Asp[11]) (Fig. 3). Bacitracin as a commercial compound is a mixture of cyclic congeners polypeptides whose aminoacid sequences differ at position 1, 5 and 8, in comparison to bacitracin A, which, due to slight changes in the tridimensional structures, exhibit different potencies as antibiotics (bacitracin A1 A2, B1, B2, B3, C, D1, D2, D3, E, F, H1, H2, H3, I1, I2, I3) (Fig. 3; Table 1). The chemical transformation of Bacitracin A into bacitracin F occurs by air oxidation in slightly alkaline aqueous solutions (Fig 4). In phosphate medium, at neutral or slightly alkaline solutions, there is a partial conversion of bacitracin A into bacitracin C. Bacitracin A and B are the most reactive in aqueous solution with formaldehyde and at pH 7, losing the characteristic absorption peak in the UV.

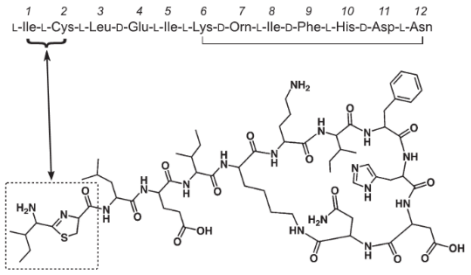
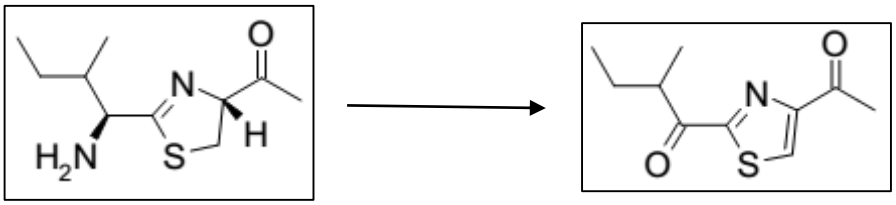


Figure 3 – Structure of Bacitracin A. (Source: Economou et al., 2013 [26])

Table 1 - Changes in the structures of the different congeners of Bacitracin in relation to bacitracin A.

	Bacitracin A	**Changes in the aminoacids sequence**	**M.W. (kDa)**
Bacitracin A	Ile^{1}; Ile^{5}; Ile^{8}		1422.7
Bacitracin B1	Ile^{1}	Val^{1}	1408.7
Bacitracin B2	Ile^{5}	Val^{5}	1408.7
Bacitracin B3	Ile^{8}	Val^{8}	1408.7
Bacitracin C		-Cys^{2}	1301.5
Bacitracin D1	Ile^{1}; Ile^{5}	Val^{1}; Val^{5}	1394.6
Bacitracin D2	Ile^{1}; Ile^{8}	Val^{1}; Val^{8}	1394.6
Bacitracin D3	Ile^{5}; Ile^{8}	Val^{5}; Val^{8}	1394.6
Bacitracin E	Ile^{1}; Ile^{5}; Ile^{8}	Val^{1}; Val^{5}; Val^{8}	1380.7
Bacitracin F	(Ile^{1}) aminomethylene-thiazoline moiety	(Ile^{1}) keto-thiazole moiety	1423.6
Bacitracin H1	(Ile^{1}) aminomethylene-thiazoline moiety	(Val^{1}) keto-thiazole moiety	1409.6
Bacitracin H2	(Ile^{1}) aminomethylene-thiazoline moiety; Ile^{5}	(Ile^{1}) keto-thiazole moiety; Val^{5}	1409.6
Bacitracin H3	(Ile^{1}) aminomethylene-thiazoline moiety; Ile^{8}	(Ile^{1}) keto-thiazole moiety; Val^{8}	1409.6
Bacitracin I1	(Ile^{1}) aminomethylene-thiazoline moiety; Ile^{5}	(Val^{1}) keto-thiazole moiety; Val^{5}	1395.6
Bacitracin I2	(Ile^{1}) aminomethylene-thiazoline moiety; Ile^{8}	(Val^{1}) keto-thiazole moiety; Val^{8}	1395.6
Bacitracin I3	(Ile^{1}) aminomethylene-thiazoline moiety; Ile^{5}; Ile^{8}	(Ile^{1}) keto-thiazole moiety; Val^{5}; Val^{8}	1395.6



Bacitracin A Bacitracin F

Figure 4 – Chemical transformation of bacitracin A into bacitracin F, by exposure to air. In this oxidation reaction, the aminomethylene-thiazoline group, resulting from condensation between Ile^{1} and Cys^{2}, aminoacids, is converted into a keto-thiazole group, in slightly alkaline aqueous solutions.

All different species of bacitracin can be separated by methods such as column chromatography and quantified by UV / Vis spectroscopy or NMR. Quantifying a commercial sample bacitracin, specie of bacitracin A corresponds to the major percentage of the sample, bacitracin B accounts for only ~30% of the sample and species D and I correspond to residual percentages. Thus, the biological effect (95%) of bacitracin is mainly due to the presence of species A and B in the mixture [27].

Craig, L. *et al.* (1952) observed that bacitracin A, the most abundant and biologically active specie, has a characteristic absorption peak between 250 and 255 nm in water or in slightly alkaline medium. However, if remaining in alkaline conditions for a few days, it is found a shift on the peak absorption for the 288/290 nm, that indicate the oxidative transformation of bacitracin into bacitracin F, which result from the formation of keto-thiazole ring. By placing bacitracin A in acid (HCl, 0.1 M), in the first minute the curve indicates the maximum absorption at 255 nm, however, after a few days it is no longer perceptible any peak of maximum absorption. By placing bacitracin C in water is obtained an absorption curve with peak at 290 nm, which means that, like bacitracin F, this compound also has no biological effect at neutral pH [25, 28].

Accordingly, bacitracin is a polar amphiphilic molecule with conditional stability. With a log P of -0.8, this molecule has a higher affinity for the oil phase than for the aqueous phase. It is stable under anhydrous bases such as paraffin, white wax and lanolin; It is not affected by the addition of hydroquinone, ascorbyl palmitate, cetyl alcohol, calamine, zinc oxide or benzocaine; it slowly becomes inactive at bases containing sterile alcohol, cholesterol, polyoxyethylene derivatives and sodium lauryl sulfate; it degrades rapidly at bases containing water, macrogol, propylene glycol, glycerol, cetylpyridinium chloride, benzalkonium chloride, ichthammol, tannic acid and phenol. With a pKa_1 of 3.19 and pKa_2 of 9.63, this protein is very soluble in water (water solubility of 50 mg/ml), methanol and dimethylsulfoxide, soluble in ethanol, poorly soluble in acetone, benzene or ether and practically insoluble in chloroform. Commercially, it is sold as a sterile powder that must be stored at temperatures between 2 and 15 °C and protected from sunlight. In slightly acidic solutions (pH 4) and neutral, at room temperature, the bacitracin is relatively stable and biologically active, but in solutions with pH higher than 9 at the same temperature, it degrades rapidly [25]. Thus, bacitracin can be applied on hydrophobic or hydrophilic carriers with controlled pH and temperature.

The congeners of bacitracin belong to a peptides class which are not synthesized on ribosome (NRAMPs, "nonribosomally synthesized peptides"). Their synthesis is catalyzed by a

large complex of peptide synthetases, that is does not require ribosomes because it occurs in the cytosol of bacteria or fungi [29, 30].

In free form, bacitracin is an antibiotic capable of inhibiting the action of certain proteases and other hydrolytic enzymes, it interferes with the formation of ubiquinone precursors, also in the biosynthesis of derived-membrane oligosaccharides or in cell division membrane-dependent processes. Moreover, in the presence of divalent ions, it produces redox agents that cause oxidative DNA breaks [31]. Structural changes in free bacitracin that reduce its amphipathic character (e.g., changes in aminoacid sequence), also reduce its biological potency and decrease the affinity to the cell wall [25].

Bacitracin is a metallo-antibiotic polypeptide, which means that its antibiotic effect relies on the presence of a metal. Bacitracin binds to divalent metal ions (such as zinc or cobalt), allowing its binding to extracellular pyrophosphates groups linked to the membrane, which enhances its affinity for bacterial cell wall (Fig. 5). During the last step of peptidoglycan synthesis, undecaprenyl-pyrophosphate (also referred to as bactoprenol, extracellular lipid carrier bound to the membrane) is released and hydrolyzed by a pyrophosphatase, which promotes its dephosphorylation to monophosphate. Specific binding of bacitracin to undecaprenyl-pyrophosphate prevent its hydrolysis / dephosphorylation, interrupting the flow of peptidoglycan precursors to the site of cell wall synthesis, inhibiting its synthesis and consequently causing bacterial death.

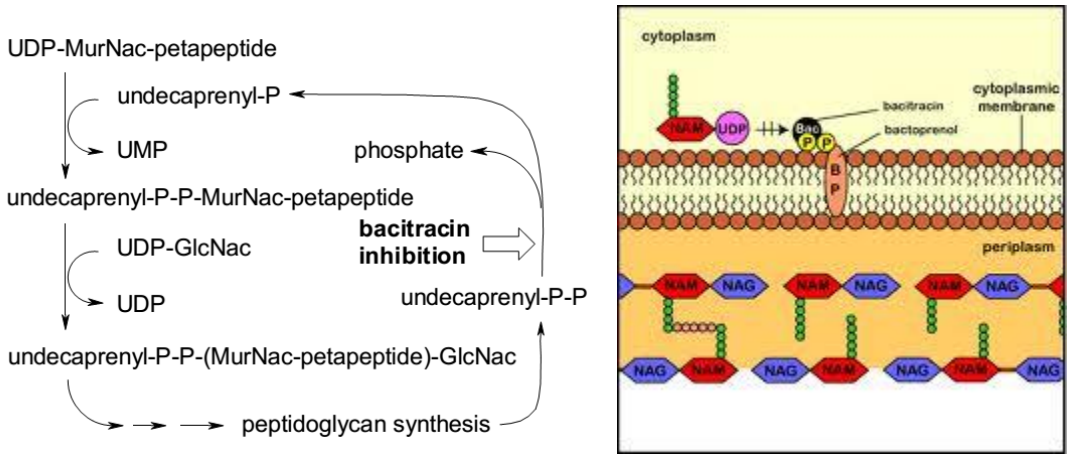


Figure 5 – Mechanism of cell wall synthesis and the mechanism of action of bacitracin in the inhibition of this process. (Source: Ming *et al.*, 2002 [25])

Bacitracin sequesters its target in an impressively efficient way, by using a scaffold that completely envelopes the target's pyrophosphate group (Fig. 6). This full involvement of the target protects pyrophosphate group from the contact with the solvent and from enzymatic

and non-enzymatic hydrolysis at the target site. By immobilizing the pyrophosphate between two metal ions strongly neutralizes the negative charge on the ligand [26].

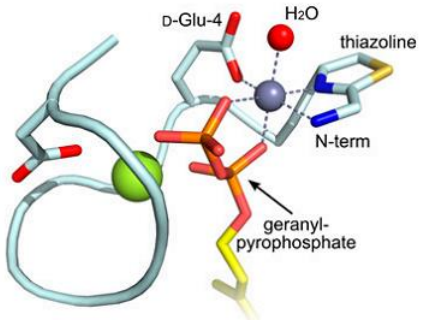


Figure 6 – Coordination of bacitracin with the zinc ion. Bacitracin is shown in a cyan ribbon representation, with atoms in side chains being colored gray (carbon atoms), red (oxygen), and blue (nitrogen); the sulfur of the thiazoline ring is colored gold. The pyrophosphate group of the ligand is colored red (oxygen) and orange (phosphorus); the geranyl chain is colored yellow. The sodium and zinc ions are shown as light green and light blue spheres, respectively. (Source: Economou et al., 2013 [26])

The amphipathic structure of bacitracin suggests that it may have an innate affinity for membranes, even in the absence of pyrophosphate ligand. However, it has been found experimentally on immobilized lipidic bilayers, similar to biological membranes, that without the zinc ion or the pyrophosphate ligand, do not occur considerable connections to inhibit the membrane synthesis. It was found that the percentage of connections to membranes is greater in the presence of zinc than in the presence of the ligand. Thus, it is concluded that zinc is an essential central ion for the organization of the tertiary structure in which the antibiotic and the pyrophosphate ligand group together and contributes to the specificity of bacitracin to membranes and target recognition (pyrophosphates groups on the membrane surface) [26].

Target recognition is a specific and necessary characteristic for antibiotics to bind to membranes. Despite bacitracin be able to break the integrity of the membrane, the concentrations needed for this to happen are much higher than the minimum concentration required to activate its effect while antibiotic, increasing its drug-safety.

3. GELS

A gel is a colloidal disperse system, semi-solid and soft, containing solid and liquid components in which the solid (gelling agent) form a network of aggregates that allows to immobilize the liquid component. Usually the gelling agent exists in the formulation in concentrations lower than 15%, but in the presence of a suitable solvent it can organize itself, through chemical or physical interactions, to form reticulate structures like rods, fibers, tubules or platelets. These structures incorporate the solvent, preventing it to flow as a result of surface tension that is generated between the two. Gels have simultaneously cohesive properties of solids and transport characteristics by diffusion of fluids. The rigidity of the gel is determined by the amount of fluid it entraps [1].

Gels can be classified according to different characteristics of its components, such as the nature of the solvent, the physicochemical properties of the gelling agents, the nature of intermolecular interactions, etc. The main distinction between gels is the nature of the solvent. In the case of hydrogels, the incorporated solvent is water or a polar aqueous fluid, and in the case of organogels, the solvent has nonpolar nature (oil, non-aqueous organic solvent). In addition to these conventional gels, have been developed more recently other formulations that combine aqueous phase and oil phase, such as the PLO, xerogels, aerogels, etc. (Table 2) [32].

Table 2 - Types, properties and applications of different gels.

Types and property of gels	Advantages and application of gels
Lecithin organogels ➢ Extracted from various plant and animal tissue part from the egg yolk. ➢ They have been found to have an isotropic structure	**Lecithin organogels** ➢ Thermodynamically stable, thermo-reversible (sol-to-gel) transition temperature at 40 °C) viscoelastic and non - irritant
Pluronic Lecithin Organogels (PLO) ➢ PLO is a soy lecithin-based organogels which consists of isopropyl palmitate or isopropyl myristate, water and pluronic F127 ➢ The apolar phase in the PLO represents 22% (v/v), hence is often regarded as microemulsion-based-gel.	**Pluronic Lecithin Organogels (PLO)** ➢ thermo-stable, viscoelastic and biocompatible in nature ➢ minimal skin irritation ➢ It has been used as a delivery vehicle for both hydrophobic and hydrophilic molecules for topical and transdermal application
Bigels ➢ Mix of hydrogels (aqueous systems) and organogels (lipophilic systems) without the addiction of a surfactant	**Bigels** ➢ Ease to prepare ➢ No skin irritation induced by surfactant ➢ Can deliver lipophilic and hydrophilic drugs

<table>
<tr><td>Niosomal and Proniosomal gels
Liposomes consisting of a nonionic surfactant, which can be of a hydrogel or organogel nature.
A proniosomal gel is a hydrated form of niosomes.</td><td>Niosomal and Proniosomal gels
great stability (proniosomes are more stable than niosomes)
Greater skin permeability
Suitable for both lipophilic and hydrophilic drugs
Expensive</td></tr>
<tr><td>Emulgels
Consist of a hydrogel or an organogel with o/w or w/o emulsion and a surfactant</td><td>Emulgels
Thixotropic, easily spreadable, easily removable, emollient, non-staining, water-soluble, long-term life, transparent and pleasing appearance
They can be used in the controlled release of drugs</td></tr>
<tr><td>Aerogels
Inorganic, composed of silica, and produced by supercritical drying</td><td rowspan="2">Aerogels and xerogels
High stability
Low thermal conductivity and thermally stable
Large surface area for drug carrying
They can be used for controlled drug delivery</td></tr>
<tr><td>Xerogels
Inorganic, composed of silica, produced by drying under normal pressure</td></tr>
</table>

A further distinction is the nature of the gelling agent (solid component), which may be polymers or low molecular weight molecules from natural, synthetic or semisynthetic origin, which crosslink and can be used as carriers for different pharmaceutical applications [33].

A gel can be divided into primary, secondary, and tertiary structure like a protein to understand the mechanism of gel formation. Primary structure (A° to nm scale) is composed of unidirectional aggregation of gelator molecules. The secondary structure (nm to μm scale) is nothing but the morphology of the aggregates like micelles, vesicles, fibers, ribbons or sheets [34, 35]. Whereas tertiary structure of a gel (μm to mm scale) involves the interaction of individual aggregates to form gel network [36].

Gels can be classified according to the nature of the linkages that hold the three-dimensional structure. Physical gels are formed by weak physical forces of attraction such as van der Waals interactions or hydrogen bonding. chemical gels are maintained by strong covalent bonds [37].

3.1. HYDROGELS

Hydrogels are crosslinked polymeric networks that swell in the presence of water. They are formed from a single reaction of one or more types of monomers. Another definition of hydrogels says that these polymer materials exhibit the ability to swell and retain a large amount of water or other biofluids in its structure, but despite its great affinity to water, they possess just a swelling behavior without dissolve in this medium, which shows its high flexibility, similar to natural tissue (Fig. 7) [38-40].

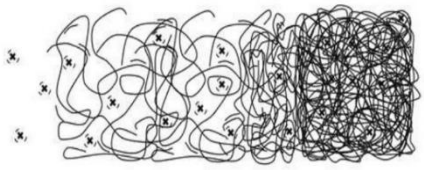


Figure 7 - Basic mechanism of drug release through a hydrogel. (Source: Hoffman *et al*., 2002 [41])

The ability of hydrogels to absorb water derives from hydrophilic functional groups (amines, hydroxyl and carboxyl groups) bound to the polymeric primary structure, while its dissolution resistance derives from the degree of cross-linking between chains of the same polymer or between different types of polymers. These latter interactions can be covalent linkages, hydrogen bonding, Van der Waals interactions, or interactions with certain cross-linking agents. Many materials, natural or synthetic, which have these characteristics, are considered ideal to form hydrogels [42].

Recently, gels have been defined as two or more component systems, consisting of a tridimensional network of polymer chains and water that fills the spaces between macromolecules. Depending on the properties of the polymers used as well as the nature and density of cross-linking, these structures, when in equilibrium, contain a large amount of water. Typically, in the swollen state, the mass fraction of water in a hydrogel is much larger than the mass fraction of the polymer. In practical terms, to achieve a certain degree of swelling, are used synthetic polymers water soluble even when they are not in reticulated form.

Hydrogels can be synthesized by various chemical processes of one or more steps. Single step procedures are, for example, the polymerization (sequential reaction in which repeating units bind onto each other and form progressively larger molecules) or cross-linking of multifunctional monomers. Multi-step processes involve the synthesis of polymeric networks resulting from interactions between reactive functional groups of the polymer chains, or resulting from the reaction of the polymers with suitable cross-linkers. Polymers engineering can design and synthesize polymeric networks by controlling the structure and the cross-

linking density, at molecular scale, and other properties such as biodegradability, mechanical strength and chemical and biological response to stimuli [39].

3.1.1. USE OF HYDROGELS

Since the development of the first synthetic hydrogels in 1954 by Wichterle and Lim, hydrogels technologies have grown. Due to its highly hydrophilic, biocompatible, flexible, non-toxic nature, these systems can be used for the manufacture of contact lenses, hygiene products, food additives, tissue engineering and regenerative medicine (muscles, artificial cartilage, etc.), diagnostic systems, wound dressings, separation of biomolecules or cells, barrier materials to regulate the biological fouling, biosensors and delivery and transport systems for drugs [38-40].

3.1.2. CLASSIFICATION OF HYDROGELS

Hydrogels can be classified according to the nature of the polymeric network, including the polymer's source, its basic chemistry, the polymer preparation method and the nature of chemical bonds responsible for the three-dimensional structure.

1) Classification based on the source

Hydrogels can be classified into two groups: natural or synthetic, depending on the nature of the polymer. For example, natural hydrogels consist of biomacromolecules such as proteins (like collagen, gelatin or fibrin) or polysaccharides (such as starch, alginate, dextran, chitosan, or sucrose). Synthetic hydrogels are obtained by chemical polymerization processes, or by interactions between different functional groups. Poly (vinyl alcohol) (PVA), poly (ethylene oxide) (PEO) and poly (acrylic acid) (PAA) are examples of hydrogel-forming synthetic polymers [39]. There are also hydrogels of hybrid nature, composed by natural and synthetic elements [43].

Table 3 – Examples of natural and synthetic polymers used for hydrogels formation (Source: [44]).

Natural polymers	Synthetic monomers/polymers
Chitosan	Hydroxypropyl methylcellulose (HPMC)
Alginate	Hydroxyethyl methacrylate (HPMA)
Fibrin	Polyvinylpyrrolidone (PVP)
Collagen	Poly(*N*-isopropylacrylamide) (PNIPAM)
Gelatin	Polyethylene glycol acrylate/methacrylate (PEGA /PEGMA)

Hyaluronic acid	Methacrylic acid (MAA)
Dextran	Poly (acrylic acid) (PAA)
	Poly (vinyl alcohol) (PVA),
	Poly (lactide-co-glycolic acid) (PLGA)
	Poly (ethylene oxide) (PEO); Poly (propylene oxide) (PPO)

Over the past three decades, natural hydrogels were being replaced by synthetic hydrogels, due to its characteristics, such as a longer half-life and greater ability to absorb water and to form a stronger gel. Synthetic gels have a well-defined structure that allow modifications according to desired function and degradability. Hydrogels can be synthesized from only synthetic components. Moreover, they are stable in sudden variations of temperature conditions [39].

2) Classification based on monomeric composition

Hydrogels can be composed of one or different types of monomers (structural units):

(a) Homopolymeric hydrogels: derived from a single type of basic monomer. Depending on the nature of the monomer and polymerization technique used, it can be obtained various crosslinked polymeric structures [39].
(b) Co-polymeric hydrogels: two or more different monomeric species, with at least one hydrophilic component, are disposed in a random configuration or in alternating blocks along the polymeric network chains (grafted block copolymers) [45].
(c) Multipolymer interpenetrating networks (IPN): composed of two or more independent polymers, synthetic and/or natural, that crosslink with each other, creating a network of higher complexity. In semi-IPN hydrogels, one of the polymers is self-crosslinked and the other one is not able to reticulate among itself [39].

3) Classification based on configuration

Depending on the physical and chemical composition, hydrogels can be classified into:

(a) Amorphous (non-crystalline, the network chains are randomly arranged)
(b) Semicrystalline (complex mixture of amorphous and crystalline phases)
(c) Crystalline

4) Classification based on the type of cross-linking and mechanical properties

(a) Chemical cross-linking – the polymeric network has permanent and tight junctions, based on covalent bonds between different macromolecular chains (interactions between polymer-polymer functional groups in the dry state or in solution) or by interactions with a cross-linking agent [41]. The polymeric network may be (1) chemically bonded and ideal; (2) non-ideal chemically-bonded, with self-coiled polymer chains or free terminals or (3) dual network, constituted by two different types of network which bind covalently and form an interpenetrating crosslinked structure. Depending on the nature of the functional groups on the surface of the structure, chemical or "permanent" hydrogels can be charged or uncharged. Charged hydrogels have changes in their swelling after pH variations and may change its shape when exposed to electric fields.

(b) Physical cross-linking – consisting of transitional junctions caused by physical interactions between polymeric chains, based on ionic interactions, hydrogen bonds or hydrophobic interactions. These gels are termed as "reversible" or physical gels once they lose their structure by dissolving or changes in environmental conditions such as pH, ionic strength or temperature in solution. This cross-linking does not require the use of cross-linkers and can be obtained by various techniques such as heating and cooling or polymer solution, heat-induced aggregation, etc. [39, 46, 47].

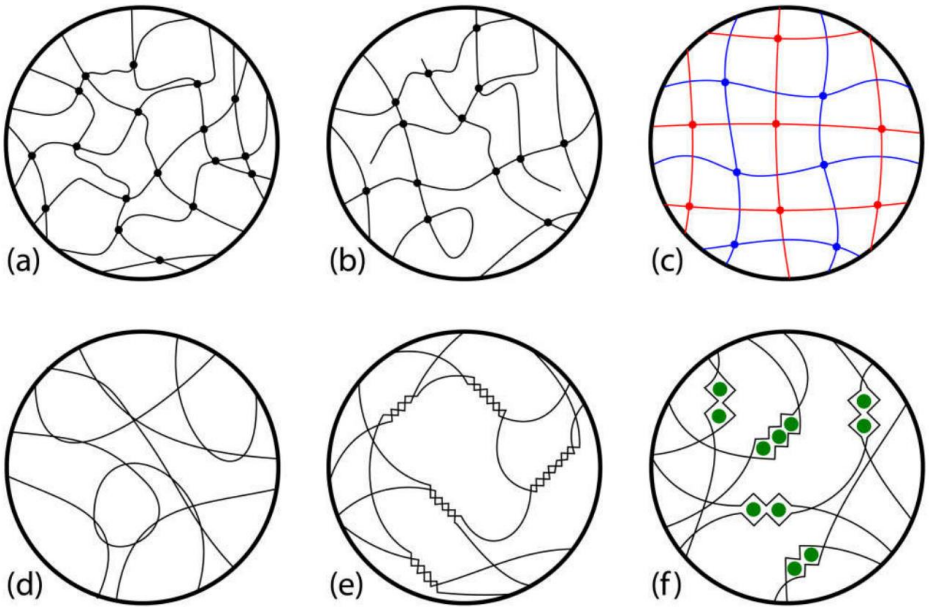


Figure 8 – Diagrams of the microstructure of the gels to define different types of cross-linking. (a) ideal network with tetra-functional links; (b) non-ideal cross-linking that includes loops and molecular ends; (c) gel with ideal dual reticulation; (d) physical cross-linking; (e) physical cross-linking with formation of helix; (f) alginate-type network that due to the divalent calcium ion, form local bridges between adjacent chains. (Source: Oyen *et al*., 2014 [48].)

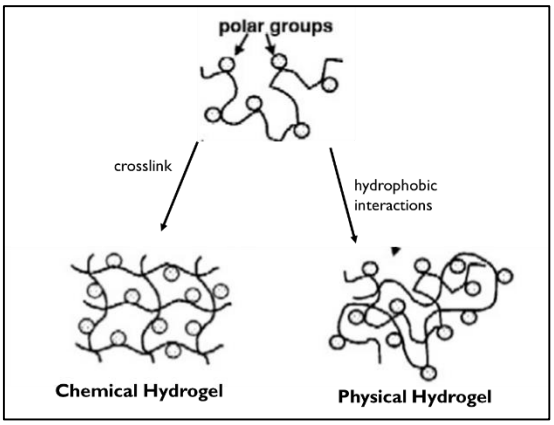


Figure 9 - Chemical vs. physical cross-linking on hydrogels networks. (Source: Hoffman *et al*., 2002 [46].)

5) Classification based on physical appearance

Depending on the polymerization process used for their preparation, hydrogels may be: matrix; film or membrane; spherical particles; tubules or clusters; emulsions.

6) Classification according to the electric charge of the functional groups

(a) Non-ionic (neutral total charge)

(b) Ionic (anionic or cationic)

(c) Amphoteric (containing both acidic and basic groups)

(d) Zwitterionic (containing both cationic and anionic groups in each repeating structural basic unit)

7) Classification according to a response to external stimuli

As already mentioned, hydrogels, as tridimensional networks, are capable of swelling, reversibly in water, and thus retain large quantities of fluid in the swollen state. Hydrogels can be designed with controllable swelling responses according to certain changes in external environmental conditions. Volume transitions (due to expansion or contraction of gels) depend on the presence of physical stimuli such as temperature (positive, negative or thermo-reversible response), electric field, magnetic field, light, pressure or ultrasound, chemical stimuli as pH (anionic or cationic), solvent composition or ionic strength, or biological stimuli such as the presence of enzymes or biomolecules. Variations in volume can be so drastic that they may correspond to a collapse of the structure or to a phase transition of the

hydrogel. These stimuli can also change the ability of gels to deliver drugs carried by them [39, 49, 50].

3.1.3. TECHNOLOGIES TO PREPARE HYDROGELS

Hydrogels, that have hydrophilic properties, form a resilient structure, not very rigid, but with sufficient cross-linkages so the polymer does not dissolve in water. They are generally prepared based on hydrophilic monomers, but hydrophobic monomers may also be used to adjusting the properties of hydrogels.

In general, polymers used can be of synthetic or natural origin. Synthetic polymers are hydrophobic in nature and chemically stronger than natural. Its mechanical strength results in a slower degradation rate, and therefore greater durability. Preparation of hydrogels from natural polymers can be used if the polymers have suitable functional groups or have been functionalized with radically polymerizable groups [39, 46, 47].

For preparing a hydrogel, there are three basic components: monomer, cross-linkers (join the monomer molecules) and a primer molecule (initiator). To control the heat of polymerization and the final properties of the hydrogels, diluents like water or other aqueous solutions, can be used. When the polymers do not form hydrogels naturally, cross-linkers are used to promote polymerization.

Then, the obtained hydrogel mass need to be washed to remove impurities like unreacted monomers, initiators, cross-linkers and other unwanted products.

Any technique capable of promoting the flexible cross-linking of a polymer and with swellability can be used to produce hydrogels. The choice depends on the final application of the hydrogel and its requirements, therefore the type of cross-linking can modify properties like thermal and chemical stability, structural rigidity, permeability, color, swelling, chelation capacity and efficiency or cellular immobilization [51]. Cross-linking of natural polymers can be made by:

(1) Linking polymer chains through a chemical reaction between complementary functional groups or by the use of enzymes.

(2) Applying ionizing radiation to generate free radicals of the backbone which can recombining as crosslinked junctions.

(3) Through physical interactions such as entanglement or branched, electrostatic interactions, or crystallization from amphiphilic polymers or co-polymers.

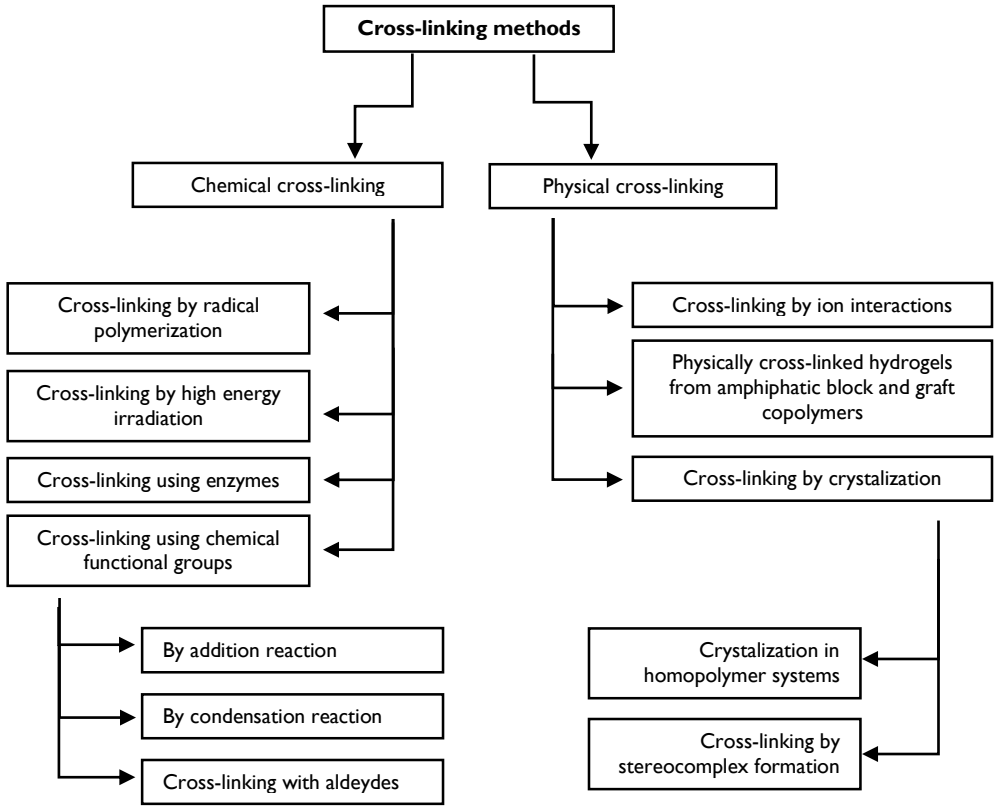


Figure 10 – Cross-linking methods to promote hydrogels formation. (Source: Hamidi *et al.*, 2008 [47].)

Chemical hydrogels can be prepared by two routes:

1) Tridimensional polymerization – a hydrophilic monomer is polymerized in the presence of a polyfunctional cross-linker. This polymerization can result in the formation of materials containing significant levels of residual monomers, so further purification is necessary since these free monomers can be toxic. Purification is carried out by extraction with excess of water and may take several weeks to be complete (Fig. 11).

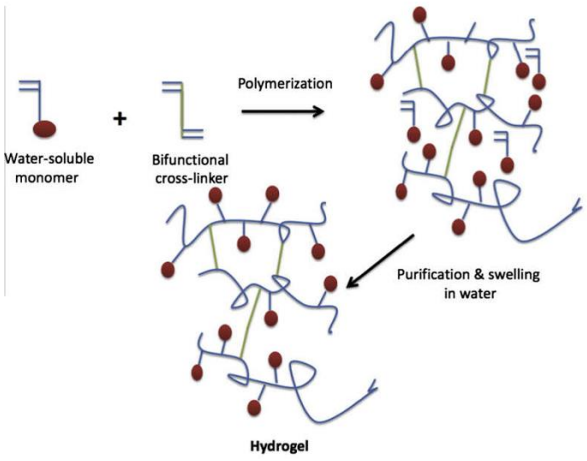


Figure 11 - Tridimensional polymerization for hydrogel synthesis. (Source: Caló *et al.*, 2015 [52].)

2) Direct cross-linking of water soluble polymers - induced by UV radiation, gamma radiation or incidence of electron beams, which are capable of generating free radical or polymerization initiator species.

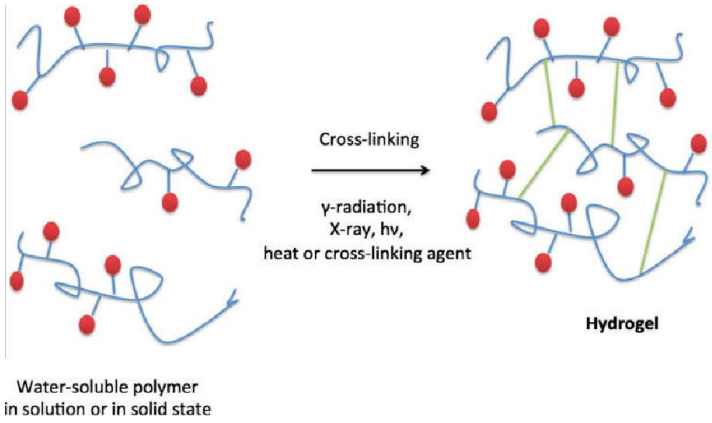


Figure 12 – Synthesis of hydrogels by cross-linking of water soluble polymers. (Source: Caló et al., 2015 [52].)

3.1.4. POLYMERIZATION TECHNIQUES

There are different polymerization techniques to form gels. [53]

1) Mass polymerization

This is the simplest type of polymerization, which involves only monomers and initiator molecules soluble in the monomers. It can be used one or more different types of low molecular weight monomers and the polymerization reaction begins with ultraviolet radiation or the use of chemical catalysts. Usually, it is added a small amount of cross-linking agent in any hydrogel formulation.

A high polymerization rate is obtained, due to the high reaction concentration of monomers. However, this process causes a reaction with high viscosity and consequently the heat loss increase. This polymerization technique produces a homogeneous and transparent vitreous matrix of polymer with high degree of hardness. When immersed in water, the glassy matrix swells, becoming soft and flexible.

2) Polymerization in solution

In these reactions, ionic or neutral monomers are mixed with a multifunctional cross-linker. The reaction is initiated thermally by UV irradiation or by a redox system. The presence of a solvent portion as a heat sink is the main advantage of solution polymerization, compared to mass polymerization. The solvents typically employed are water, ethanol, ethanol-water

mixtures and benzyl alcohol. After formation, these solvents can be removed by immersing the hydrogel in water. Phase separation occurs and the heterogeneous hydrogel forms when the amount of water during the polymerization is higher than the amount required to balance the swelling.

Hydrogels obtained must be washed to remove monomers, oligomers, cross-linkers in excess, initiators and other impurities [53].

3) Polymerization by dispersion or reverse-suspension

The polymerization by dispersion is an advantageous method because the products are obtained as a powder or granules (microspheres), and therefore, the grinding is not necessary. Once the process water-in-oil (W/O) is chosen instead of the commonly oil-in-water (O/W), the polymerization is referred as an "inverse suspension".

In this technique, the monomers and initiator are dispersed in a hydrocarbon phase as a homogeneous mixture. The viscosity of a monomer solution, mixing speed, rotor design and type of dispersing medium mainly regulates the size and shape of the formed resin particles.

The dispersion is thermodynamically unstable and require continuous stirring and simultaneous addition of a suspending agent with low hydrophilic - lipophilic balance [54].

4) Polymerization by grafting to a support

To improve the mechanical properties of a hydrogel, this may be grafted onto a coated surface on a strong support. This technique involves the generation of free radicals along the surface of the support and then the polymerization of the monomers to the surface, enhancing their covalent attachment to the surface of the support. It has been used several monomers to form hydrogels by grafting techniques.

5) Polymerization by irradiation

Radiation with high ionization energy, such as gamma radiation or electron beams, has been used for producing hydrogels from unsaturated compounds. By focusing radiation on the water molecules, hydroxyl free radicals are formed, which also attack the polymeric chains, resulting in the formation of macro-radicals. The combination of macro-radicals of various polymeric chains results in the formation of covalent bonds that leads to a crosslinked structure. Examples of polymers formed by this technique are poly vinyl alcohol (PVA), polyethylene glycol (PEG) or poly acrylic acid (PAA). The major advantage of this technique in

face of chemical initiation is the formation of hydrogels more pure and free from initiator molecules [55, 56].

3.1.5. POST-POLYMERIZATION PROCEDURES

The formation of polymeric hydrogels follows two well-established schemes: (a) polymerization of hydrophilic monomers and (b) modification or functionalization of existing polymers (natural or artificial).

Natural hydrogels are typically prepared by the addition of some synthetic parts on natural substrates, e.g. copolymerization of extract of vinyl monomers to polysaccharides monomers.

To generate the active centers of spread of the reaction, it can be used heat production methods (thermal initiators), light (photo-initiators), γ-radiation or electron beams, that promote the activation of the double bond carbons in vinyl groups.

These methods are also effective in the post-polymerization treatment, involving the conversion of more monomers, in order to avoid purification processes. Another solution to the purification involves the use of non-toxic polymers such as oligomers or macromonomers in the tridimensional polymerization (e.g. poly ethylene glycol dimethacrylate) [57].

Cross-linking of hydrossoluble polymers already prepared with poly (acrylic acid) (PAA), poly (vinyl alcohol) (PVA), polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), polyacrylamide and other polysaccharides are the most common systems used to form hydrogels. Since these polymers are biocompatible, it is not required to be removed from biological systems and therefore does not require the purification process during the synthesis.

3.1.6. TECHNICAL PROPERTIES OF A HYDROGEL

The functional characteristics of an ideal hydrogel are [39]:

- High absorption capacity in saline solution (maximum equilibrium of swelling)
- Desired absorption rate (having into account particle size and porosity) depending on the intended application
- The least amount of dissolved content and monomer residues.
- Lowest price
- High durability and stability in the swelling phase and during storage.
- The highest biodegradability without forming toxic degradation and species.
- Neutral pH after swollen in water
- Colorless, odorless and completely non-toxic

- Photostability
- Ability to re-moistening, if necessary. The hydrogel must be able to restore the embedded solution or keep it, depending on their applicability. (e.g. agriculture or hygiene)
- Easily removed with water
- Readily adhere to skin or mucous membranes
- Controlled or sustained delivery of drugs

It is impossible to fulfill and achieve the most of all these parameters simultaneously. Even at terms of their synthesis, components that are able to achieve the maximum level of some of these features lead to the inefficiency of others. Therefore, the production reaction parameters must be optimized in order to have a proper balance between the various characteristics. For example, hydrogels for sanitary products must have the highest rate of absorption, rewet lower and lower amount of residual monomers, whereas hydrogels for drug delivery systems must be porous and very sensitive to pH and temperature.

3.1.7. SWELLING - capacity to absorb water

Hydrogels can be considered as porous materials at a molecular scale in which pore spaces are occupied by water. In general, in first, is formed a chemical/covalent network and then when a hydrogel in its initial state (dry) is placed in aqueous solution, the water molecules will penetrate the polymeric network. These molecules will occupy gaps in the matrix, forcing it to expand, and allowing other water molecules continue to penetrate the polymeric network. This process gives the name of swelling [58].

Swelling is not continuous and depends on the strength of covalent bonds and / or physical interactions responsible for maintaining the structure of the matrix and the resultant osmotic pressure of the incoming water [14]. These two forces which act in opposite directions tend to reach equilibrium, leading to stability of the swollen state of the hydrogel. The resultant force of this equilibrium is referred as swelling pressure (P_{sw}), which is zero at equilibrium with pure water, and is given by the following equation:

$$P_{sw} = k \, . \, C^n,$$

where k and n are constants and C is the polymer concentration. The swelling can be described in terms of the weight, volume, or length. Thus, the mass of water absorbed by the matrix is given by:

$$m_{water} = \frac{m_{HG,w} - m_{HG,d}}{m_{HG,w}} \ ,$$

where $m_{HG,w}$ and $m_{HG,d}$ are the mass of wet and dry hydrogel, respectively. Also, while the percentage of swelling never exceeds 100%, the hydration rate does. Thus, the degree of swelling (G_{sw}) is given by:

$$D_{sw} = \frac{m_{HG,w}}{m_{HG,d}} \text{, with } D_{sw} \geq 1.$$

And the swelling ratio is given by:

$$R_{sw} = D_{sw} . \frac{\rho_0}{\rho_{sw}} = \frac{V_w}{V_d} \ ,$$

where ρ_0 is the density of the hydrogel in the dry state, ρ_{sw} refers to the density of the swollen gel, and V_w and V_d correspond to the hydrogel volumes in the wet and dry state, respectively [39, 59].

The cross-linking rate is one of the factors that affect the degree of swelling in hydrogels. It is defined as the ratio between the number of moles of cross-linking agent and the number of moles of repeating units in the polymer. The higher the cross-linking rate, the greater the amount of cross-linking agent incorporated in the hydrogel matrix. The swelling process occurs when a certain quantity of water penetrates the polymer network, by expanding the spaces between crosslinks and decreases the value of the enthalpy on matrix configuration.

The polymer network also has a contractive tensile strength exercising contraction in the network. Fundamentally, the osmotic pressure causes expansion of the matrix, while the tensile strength force its contraction. Thus, hydrogels with higher cross-linking rate will swell less, because the structure is more rigid and less flexible.

The degree of swelling can be obtained by the conventional theory of Flory-Huggins and is determined by the parameter of polymer-solvent interaction, the cross-linking density of the polymeric network and polymerization conditions. The presence of the drug in the solvent may also influence the degree of swelling [60].

In theory, there is no diffusion of the solute through the matrix when the particle size is equal to the pore size, since the particle (e.g. protein) stay encapsulated in the pore and does not diffuse. The size of pores depends on factors such the degree of cross-linking, the chemical structure of the monomer constituents and external factors (pH, ionic strength or temperature) (Fig. 13) [60].

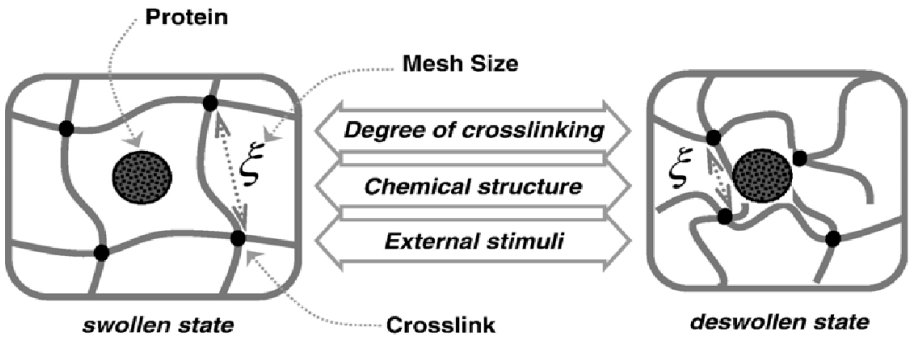


Figure 13 - Differences between the network structure in the dry and swollen state of the polymer in the presence of some protein. (Source: Lin *et al*., 2006 [60].)

The swellability derive from the presence of free hydrophilic groups in the polymer network (-OH, -COOH, -CONH-, -$CONH_2$ or –SO_3H). Due to the presence of these functional groups in the polymer network, swelling degree can achieve different degrees of hydration (sometimes over 90%). This can be quantified by the mass changes of the polymeric matrix. Factors such as chemical composition, the polymer network structure, quality and concentration of solvents, cross-linking rate or action of external stimuli, such as pH or temperature do vary the rate of swelling [39, 61].

Hydrogels with greater number of hydrophilic groups have higher water absorption capacity and higher degree of swelling. However, the higher the cross-link density, the higher the hydrophobicity, causing increased matrix hardness and lower elasticity. This leads to a decreased ability to absorb water, i.e., the degree of swelling of the hydrogel.

The swelling kinetics can be classified as swelling controlled by diffusion (following Fick's law) or controlled by relaxation (non-Fickian). When the diffusion of the water throughout the hydrogel occurs with greater speed than the relaxation of the polymer chains, it is said that the kinetics of swelling is controlled by diffusion.

3.1.8. HYDROGELS AS DRUG DELIVERY SYSTEMS

As mentioned above, hydrogels are tridimensional systems, with semi-solid morphology that allows the absorption of a large amount of water. The porosity depends on the density of cross-links present in the gel matrix and its affinity for the aqueous medium in which it is swollen. A high porosity allows encapsulation of drugs into the matrix and consequent release upon contact with the aqueous solution. Water promotes the expansion of the polymer matrix and subsequent release of drug at a given speed depending on drug diffusion coefficient into the polymeric network.

There are two methods for the adsorption of drug into the hydrogel. In the first method, during hydrogel synthesis, the addition of the drug along with the main monomers, the initiator and the reaction's cross-linking agent, in which will occur cross-linking of all the system. In the second method, the drug is dissolved in a solution placed in a container with the hydrogel to achieve equilibrium, then the system is removed from the solution and dried [62].

The major advantage of using hydrogels as drug delivery systems is related to pharmacokinetics. In this formulation, the drug elutes slowly, and remains at a constant and sufficiently high concentration in surrounding tissue, allowing to maintain the biological effect over an extended period of time.

Loading capacity per unit mass of the polymer can be described by the equations:

(a) Lower limit (maximum swelling phase)

$$\left(\frac{V_s}{W_p}\right) \times C_0$$

(b) Upper limit (swelling phase and polymer)

$$\left(\frac{(V_s + K\,V_p)}{W_p}\right) \times C_0$$

where Vs and Vp are the volumes of solvent absorbed and dry polymer, respectively. K is the partition coefficient between polymer chains and drug solution, and C_0 is the concentration of drug in solution [63].

The high porosity of hydrogels can be adjusted by controlling cross-linking density of the polymeric network and the affinity to water. This porous structure allows the encapsulation of drugs and their subsequent release, by controlled release and helps to maintain constant levels of release over a long period of time. The main mechanisms for the release of drugs from polymeric matrix are controlled by diffusion, by swelling, by chemical processes or as a response to environmental stimuli.

The most common mechanism of drug release is passive diffusion, wherein molecules of different sizes and characteristics diffuse freely out of the polymer matrix during the loading and storage of hydrogel, in response to a concentration gradient. The diffusion of the drug depends on the size of the matrix pores, the degree of cross-linking, chemical structure of monomers and the applied intensity of external stimuli [64]. Controlled release by diffusion can occur in the form of reservoir or matrix systems. The reservoir system includes a drug-containing core (capsule shaped or circular) coated with a polymeric hydrogel membrane. The concentration of drug is greater in the core, so the drug diffusion occurs primarily along the

hydrogel and then to the surrounding environment, allowing a constant rate of release [52, 60].

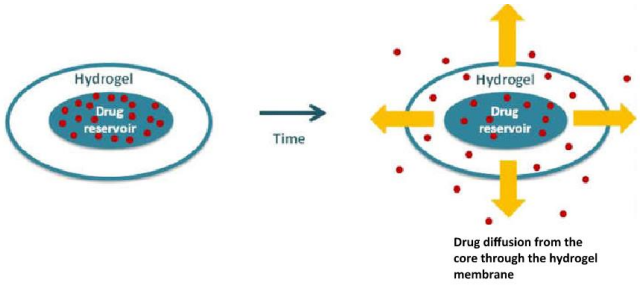


Figure 14 – Scheme of the mechanism of drug release by diffusion-controlled reservoir system. (Source: Caló *et al.,* 2015 [52].)

In diffusion matrix systems, drug is dispersed or dissolved uniformly in a three-dimensional hydrogel structure, in a compressed mixture of dry powder of the polymer. Drugs are released through the pores formed in the matrix network, when the pores are filled with water, at an initial rate of release proportional to square root of time, that is, time dependent and not constant, in contrast to what happens in the reservoir systems. The release is also dependent on the size of the pores within the matrix.

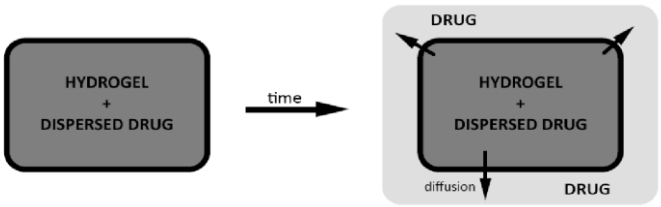


Figure 15 – Scheme of drug release mechanism by a diffusion matrix containing the dispersed drug. (Source: Lin *et al*., 2006 [60].)

In systems of controlled release by swelling, drugs are dispersed in a glassy polymer (dry state) which upon contact with aqueous / biological fluid begins to swell, expanding its size. While swelling occurs, the glass transition temperature of the polymer decreases, allowing relaxation of the molecular chains and establishing an elastic consistency, which subsequently permits the release of the drug out of the swollen area. This process exhibits a constant release rate independent of time.

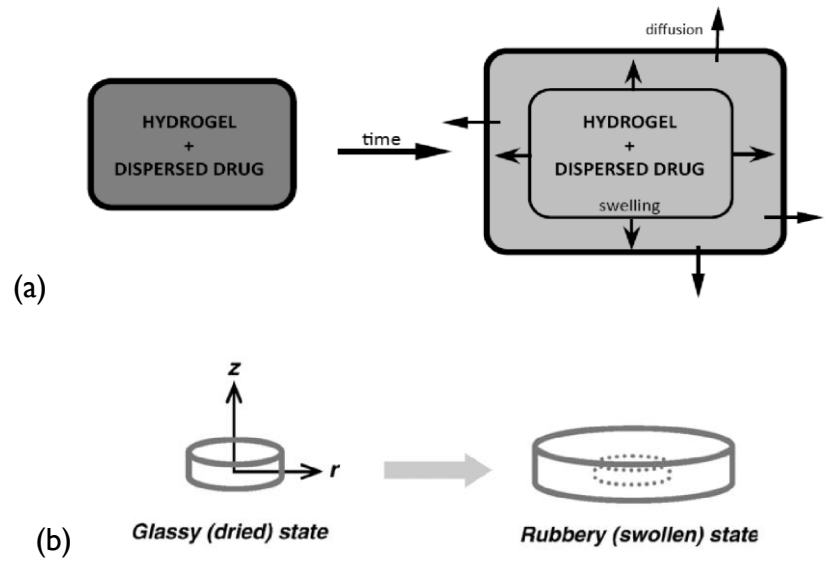


Figure 16 – (a) Scheme of a drug releasing system controlled by swelling. (b) Changes in size of a hydrogel in the dry and swollen state. (Source: Lin *et al.*, 2006 [60].)

A release system that combines diffusion and swelling processes is referred to as "abnormal transport" and depends on the size of the molecules to be transported. The gradient between the drug dispersed in the hydrogel and in the environment allows drug diffusion from local with higher concentration (hydrogel) to the local of lowest concentration. The molar flux in this case is proportional to the concentration gradient and the diffusion coefficient in the polymer and is given by Fick's Law.

$$J = -D \times \frac{\Delta C}{\Delta x},$$

where J is the molar flux (mol / $cm^2 s$), ΔC is the concentration gradient, C is the drug concentration in the polymer (mol / cm^3), D is the diffusion coefficient in the polymer (cm^2 / s) and Δx is the distance across the membrane/hydrogel (cm).

The rate of release is normally time-dependent, so, release kinetics is given by:

$$\frac{\partial c}{\partial t} = -\Delta J = \Delta \left(D \times \frac{\Delta C}{\Delta x} \right)$$

When the perimeter/ extension of hydrogel is constant (static drug delivery), the rate of release is given by:

$$\frac{\partial c}{\partial t} = -\Delta J = \Delta \left(D \times \Delta C \right)$$ [52, 58].

In theory, in these systems, the drug release kinetics is dependent on the rate of swelling of the hydrogel matrix. However, different polymer compositions allow to obtain different pore sizes of the hydrogel matrix which may vary between 5 to 100 nm (in swollen state), that is much larger than drugs molecular size they carry. As a result, the diffusion of these molecules

is not retarded by the effect of swelling. In this case, this mechanism is more desirable for the transport and release of macromolecules such as oligonucleotides, peptides and proteins [65, 66].

In release systems controlled by chemical processes, the drug can be released from the matrix by chemical reactions as hydrolytic or enzymatic cleavage of polymeric chains, reversible or irreversible reactions between the matrix and the drug, surface erosion of the hydrogel matrix, incorporation in the matrix of species that bind specifically to the drug and facilitating its release, respecting the specificity balance [67].

In response to various environmental stimuli, internal or external, hydrogels may change their swelling behavior, structure, permeability or mechanical behavior, in order to promote release of the drug. These hydrogels are known as "smart" systems. They can be sub-classified according to the nature of the stimulus applied (chemical or physical). Among the physical stimuli, the most used are the temperature, electricity, light, pressure, sound or magnetic field. As to chemical stimuli, the most common are the pH, solvent composition, ions or recognition of certain molecular species. The main advantages of using these smart polymers are the ability to deliver sufficient concentrations of a certain drug in a given time and place, reducing the adverse systemic reactions and increase the therapeutic patient compliance, allowing a reduction in the required dose of drug and consequently costs [68, 69].

For example, Huang and Lowe developed a dextran hydrogel to transport hydrophilic drugs capable of swelling and dehydrate repeatedly by a response of the medium temperature changes [70]. Zhang *et al.* developed a dextran hydrogel via pH sensitive membrane. The presence of carboxylic groups (-COOH) increases the porosity of the matrix in response to an increase in pH and ionic strength, and consequently promotes release of the drug by diffusion [71]. Lee *et al.* have created a polyamide-amine hydrogel sensitive to pH and temperature with a specific molecular structure in order to avoid the initial immediate release. This material can be produced through a single step process by coupling between secondary amine groups (-NHR_2) from diamine compounds (such as piperazine) and vinyl groups (CH_2=CH-) from an alkylene bisacrylamide compounds (such as *N,N'*-methylenebisacrylamide or *N,N'*-ethylenebisacrylamide). These hydrogel may be used for transport of different drugs, through different routes of administration [72].

Smart polymers based on block copolymers (contain two or more different monomeric units arranged in blocks) are hydrossoluble carriers with high viscosity and thermo-reversible (able to create, modify and break the links responsible for maintaining the polymeric network, in accordance with the temperature of the medium). Due to their amphiphilic character, form

micelles in an aqueous medium, which aggregate and form hydrogels when the medium temperature is above the critical gelation temperature [73]. Poloxamers are examples of such polymers and correspond to a family of non-ionic surfactants based on triblock sequences of polyoxyethylene – polyoxypropylene - polyoxyethylene (PEO_n-PPO_n-PEO_n), commercially known as Pluronics®, Kolliphor®, Synperonic® or Tetronics®. In these hydrogels, PPO (poly (propylene oxide)) is responsible for forming a hydrophobic central core via Van der Waals interactions between the methyl groups and the soluble substance, while the PEO (poly (ethylene oxide)) is responsible for the aqueous solubility through hydrogen links between the ether oxygen and water molecules. Because of these interactions, poloxamers are highly soluble in non-polar organic solvents and stable in aqueous solutions in the presence of acids, alkaline or metal ion species. Depending on the weight of propylene oxide and ethylene oxide and physical appearance at room temperature, the polymers are available in flake form (F), paste (P) and liquid (L). In the pharmaceutical industry, the most widely used is the poloxamer 407 (Pluronic® F-127) containing 70% PEO, and thereby it is very water soluble, low viscosity below 4 °C and forms a semi-solid gel at a temperature body. This polymer is more soluble in cold water than in hot water due to increased solvation and hydrogen bonds that form at low temperatures [33, 74].

3.2. ORGANOGELS

In recent years, an interest in organogels has grown dramatically due to the discovery and synthesis of several molecules capable of gelling organic solvents at low concentrations in the formulation.

Organogels are defined as semisolid, soft and viscoelastic structures that result from the immobilization of a non-aqueous liquid (fixed oil, mineral oil or organic solvent) within the spaces available in a tridimensional network by a gelator. They are formed through non-covalent associations, and usually depends on the nature of the gelling molecule - polymeric or low molecular weight organogelator (LMOG) - and its concentration (often <1%), the property of the oil and the protocol for preparing the organogel. A fine balance between aggregation and dissolution forces is critical in the formation of an organogel and the identification of organogelator-solvent combinations capable of forming these gels. Because of the easy method of preparation and inherent long-term stability, these products remain a challenge of considerable research and development on drug delivery, immobilization of enzymes for biocatalysis, synthesis and transformation of toxic wastes, separation technology, temperature sensors, flatbed displays, recovery of oil spills, templates for the creation of inorganic structures, etc. [75, 76].

Gelling agents based on polymers do not require high concentrations to occur the cross-linking and allow to immobilize the organic solvent through physical or chemical interactions. Chemical interactions are based on strong covalent bonds, dipolar interactions or coordination with metals and form a crosslinked matrix. Interactions of physical nature are stabilized by hydrogen bonds, Van der Waals forces and stacking / π-π interactions and form interlaced chains.

The process of gelation with low-molecular-weight organogelators (e.g., vegetable oils, sorbitan esters) depends only on physical interactions. These organogelator molecules have relatively low molecular weights (MW ~ 3000 Da) compared to the polymeric ones, used to form the gel networks in typical hydrogels, and correspond to the majority of the organogelators used. They are amphiphilic in nature and self-assemble in the presence of water to form fiber type structures, which physically interact with each other to form a crosslinked structure, when an appropriate balance exists between solubility and aggregation forces. Depending on the kinetic properties of the aggregates, that is, the three-dimensional network formation mechanism, these organogels may be classified as solid fibers matrix (strong interactions) or fluid fibers matrix (weak interactions). These latter links are reversible, requiring a higher concentration to form stable gels and polymeric viscosity equal [77, 78].

The aggregated molecules form superstructures, often long fibers, which entangle or form pseudocrystalline regions, immobilizing the liquid largely by surface tension and forming a variably consistent and thermally reversible gel upon cooling, characterized by a significant elasticity [78, 79]. The physical organogels maintain its structure by non-covalent forces and are considered thermo-reversible systems. On heating (above 40 °C), aggregates of gelator dissolve in the organic liquid and the gel melts until solution phase. On the other hand, by cooling the hot solution recover the high viscosity and returns to the gel phase. The temperature at which occurs the sol-to-gel and gel-to-sol transition is referred to as gelation temperature or phase transition temperature (PTT) [1].

3.2.1. TYPES OF ORGANOGELATORS

The organogelators can be differentiated in gelling agents of one or two components. Organogelator of two components depends on one or more compounds to gel the organic solvent. Organogelator of one component has the ability to gel alone, without the addition of any other compound [36].

According to their molecular weight [80]:

- **Polymerics**

This organogelators have high molecular weight and induce gelation even in low temperatures, through physical interactions between polymer molecules. Their ability as gelators may be controlled through chemical modification of the polymer chain structure, which can give linear, multi-branched or star-shaped polymers. The controlled drug release can be promoted by external stimuli such as temperature, light or the acidity of the medium. Gels developed from these polymers generally have a low gel-sol transition temperature (PTT) and are more resistant compared to those developed with low molecular weight organogelators. Some examples are the derivatives of L-lysine or L-alanine, besides the conventional polyethylene glycol, polycarbonate, polyesters or poly-alkalines.

Polymeric micelles obtained from the rearrangement of amphiphilic molecules have been used in oral administration since they increase the solubility of hydrophilic compounds in oil and can be used in the preparation of anhydrous peptides products [81, 82].

- **Low molecular weight organogelators (LMWO)**

These molecules have amphiphilic character and they are soluble in organic solvents at low concentrations upon heating, and gelling when cooled [83]. LMWO can produce gels of solid or fluid fibers matrices, depending on the intensity of intermolecular interactions. They form

physical gels, stabilized by relatively weak and efficient interactions, with various organic solvents at low concentrations. The solid fibers form when the heated mixture of organogelators is dissolved in the polar solvent and then cooled below the solubility limit of gelling agents. This results on the precipitation of the gelling agents as aligned fibers, which self-assemble. The fluid fibers result from the addiction of a polar solvent to a solution of amphiphilic molecules dissolved in a nonpolar solvent. These latter are present in the form of reverse micelles which, with the addition of water, rearrange themselves into tubular structures of reverse micelles. The tertiary structure of these organogelators may correspond to fibers, strings, strips or helices, due to the dimensional growth of the molecules [84]. amygdalin, carbohydrates, amino acids, soy lecithin, silicon dioxide, aluminum and zinc soaps and surfactants are some examples of LMW organogelators, which have amphiphilic and non-ionic character, with surface active properties and the ability to immobilize different solvents, such as vegetable oils or isopropyl myristate [85, 86].

3.2.2. METHODS TO PREPARE ORGANOGELS

Most organogels are prepared by heating the mixture of gelling agent (organogelator) with the nonpolar solvent, to allow dissolution of the organogelator in the liquid, obtaining an organic solution / dispersion, and then cooling it to form the gel. Lowering the temperature decreases the solubility of gelator in the liquid phase, and its expulsion from the solution phase. These, by interacting with each other, reorganize into well-defined aggregates such as tubules and fibers, which are self-assemble to form a three dimensional network which immobilizes the solvent and ensures gel strength. The intensity and nature of links between organogelators are important to gel formation. In its absence, it may be lost the gel form even if there several gelators aggregates.

There are two mechanisms of formation of organogels [87]:

A) Fluid-filled Fiber

First the mixture of surfactants and co-surfactants (organogelators) is dissolved in a nonpolar solvent, which promotes the formation of reverse micelles. Then, with the addition of water, are formed tubular reverse micelles. These elongated tubular micelles entangle among themselves to form three-dimensional structures capable of immobilizing the nonpolar solvent, as well as drugs dissolved in this medium (Fig. 17) [80, 88].

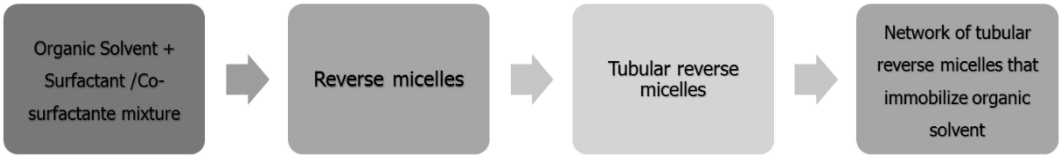


Figure 17 – Formation of organogels by fluid-filled fiber mechanism.

B) Solid Fiber

A nonpolar solvent and a solid gelling agent are heated to form a nonpolar solution of organogelators. After cooling to room temperature, the organogelator precipitates as solid elongated fibers and through physical interactions rearranges into a tridimensional structure, able to immobilize the organic solvent (Fig. 18) [80, 88].

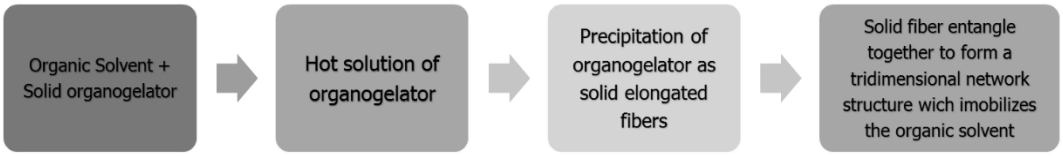


Figure 18 – Formation of organogels by solid fibers mechanism.

Gels prepared using the LMW organogelators are mainly stabilized by solid fibers. During cooling of nonpolar solution of organogelators, three situations are possible: highly ordered aggregation to form crystals; random aggregation resulting in an amorphous precipitate; or an intermediate process of aggregation between the two, which gives a gel (Fig. 19).

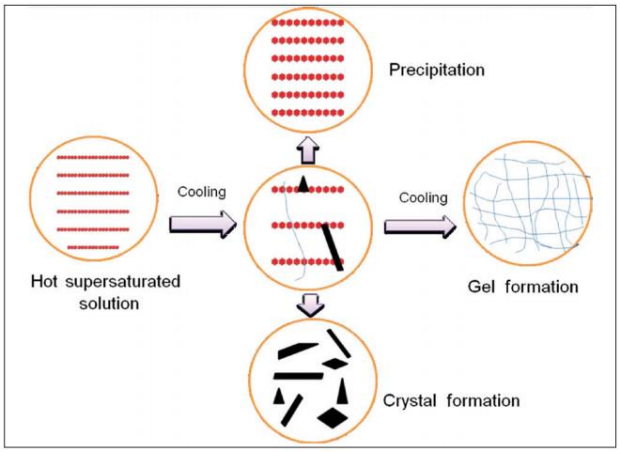


Figure 19 – Possible ways of aggregation of organogelators. (Source: Balasubramanian et al., 2012 [86].)

3.2.3. ORGANOGEL PROPERTIES

- Viscoelasticity – behavior of materials as viscous and elastic at the same time. The organogels behave like solids at a low shear rate. With increasing shear rate, decreases

the intensity of the physical interactions along the fibers, until they break, allowing the fluidity of organogels. This factor explains their pseudo-plastic behavior.

- Non-birefringence – They do not allow the passage of polarized light along the array (polarized-light microscopy, spectroscopy). Under these conditions, they reflect a black matrix, due to the isotropic nature of organogels.
- Thermo-reversibility - As gels are heated above the critical temperature, they lose their solid matrix structure and begin to flow due to the breakdown of the physical interactions between organogelator molecules. The reversibility is ensured when the solutions are cooled and some of the chemical bonds are restored, which can form the most stable gel configuration.
- Thermo-stability at room temperature - This may be due to the ability of gelling agents to self-assemble, which reduces the overall free energy of organogels system and its stabilization.
- They can be transparent or opaque, depending on its composition. E.g. lecithin organogels are transparent while the sorbitan ones are opaque in nature.
- Biocompatibility - use of natural origin components.
- Physicochemical properties [89]:

  - Charge - the presence of charged groups on the polymer promotes interaction with mucosal membranes. Polyanions, such as polycarboxylates, are preferable to polycations.

  - Solubility - mucoadhesive gels swell in contact with humidity, increasing mobility of the polymer molecules at the interface and exposing more active sites to form new bonds.

  - Molecular size / space configuration - increasing the molecular weight promotes the entanglement and more interactions into the polymeric network.

3.2.4. ADVANTAGES OF ORGANOGELS

- Transport of drugs with different chemical properties, such as solubility, molecular weight, size, etc.
- They are formed spontaneously due to molecular rearrangement of the surfactant/gelling molecules.
- Thermodynamically stable and its structural integrity is maintained for long periods of time.

- They are insensitive to moisture and as they are organic, they resist bacterial contamination. Thus, they are chemically more stable.
- They have a good hydrophilic - lipophilic balance (HLB), which enhances the partition coefficient with the skin and thus allows a better skin permeation and transport and delivery of several molecules topically.
- Biocompatible, biodegradable and non-immunogenic, making them safe for long term applications [87].
- Since they are constituted by hydrophilic and lipophilic components, they can incorporate hydrossoluble or lipophilic drugs, increasing the number of pharmacological applications.
- It is possible to use organic solvents of natural origin, such as vegetable oils, etc., enhancing their biocompatibility.
- Organogels can decrease the diffusion rate of the drug through the skin permeation because it is dissolved in the polymer and transported through the polymer chains.

3.2.5. DISADVANTAGES OF ORGANOGELS

- Unstable with variations of temperature
- Contraction of the gel due to prolonged exposure to body temperature causes the release of the entrapped solvent – syneresis
- The gelation process is impaired in the presence of impurities
- Primary materials, such as lecithin, are not available on a large scale
- Limited conditions of storage
- They can be greasy.

3.2.6. TYPES OF ORGANOGELS

- **Lecithin Organogels**

These organogels were first described by Scartazzini and Luisi, in 1988, when they investigated the conditions of reverse micelles formation from soybean lecithin aggregation. They are comprised by lecithin, a surfactant acting as a gelling matrix of the organogel, a non-polar organic solvent as a continuous phase and a polar solvent, typically water [90, 91].

Lecithin can be isolated from the degumming process of soybean oil, which consists in removing hydratable phosphatides of this oil, or from the phosphate portion of the egg yolk. Lecithin is the common name for 1,2-diacyl-sn-3-phosphatidylcholine (Fig. 20). As the name suggests, it is a natural mixture consisting of diglycerides of fatty acids linked to a phosphoric

acid choline ester, structures called phosphatidylcholines, which belongs to the class of organic phospholipids and phosphoglycerides, the main components of biological membranes. Thus, lecithin can be used as skin permeation enhancer, allowing greater adhesion of the PLO to skin and greater biocompatibility.

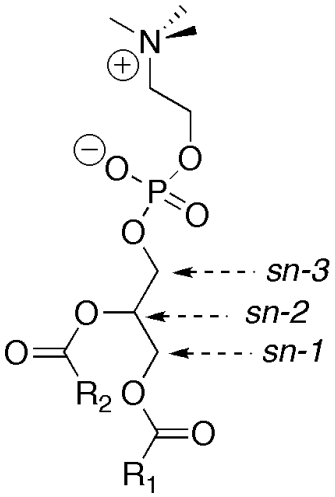


Figure 20 - The general structure of L-α-phosphatidylcholine (PC), with two hydrophobic fatty acid chains in the positions sn-1 and sn-2 of L-glycerol and the phosphate-containing polar head-group in sn-3 position. At the sn-1 position, it can be substituted with saturated acyl groups of 16 carbon atoms, while at the sn-2 position are added unsaturated groups of 18C. (Source: Chatgilialoglu *et al.*, 2010 [92].)

Lecithin organogels are formed when small amounts of water or other polar solvents, such as glycerol, ethylene glycol or formamide are added to non-aqueous solutions of lecithin. These nonpolar compounds self-organize in reverse spherical micelles, which self-assemble into a tridimensional network of crosslinked cylindrically reverse micelles, which immobilizes the non-polar organic continuous phase, being able to transform a liquid phase into a gel (organogels). They are transparent, thermally stable, thermo-reversible, with a sol-to-gel transition temperature of 40 °C, they are viscoelastic, biocompatible, and non-irritating and the self-assembly process is spontaneous, simple and easy to prepare. After gelling, lecithin aggregates keep the optical isotropy and transparency. They do not require the addition of surfactants, since lecithin alone serves as a surfactant and permeation enhancer. They are capable of maintaining skin hydration, in an environment rich in lipids, and thereby keep the skin bioactive state. They can be used to encapsulate hydrophilic and hydrophobic drugs - the hydrophobic ones are dissolved in the oil (lecithin plus organic solvent), while hydrophilic drugs are dissolved in water / aqueous solvent [93].

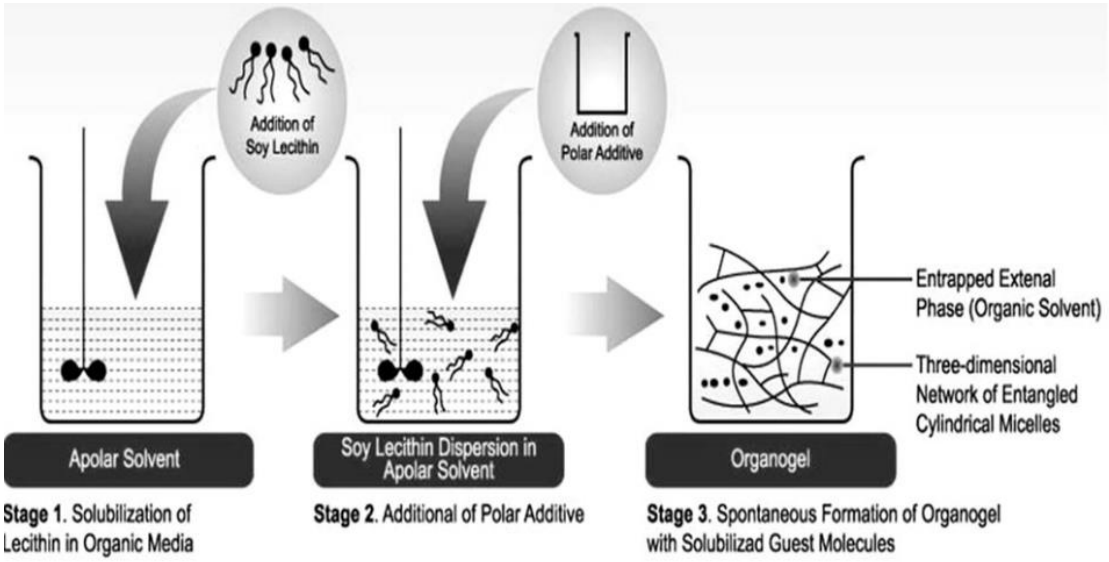


Figure 21 – Formation of organogels from soy lecithin and water. (Source: Sangale *et al*., 2015 [1].)

- **Pluronic Lecithin Organogels (PLO)**

These organogels are based on soybean lecithin with isopropyl palmitate or isopropyl myristate, water and pluronic F127. Pluronic F127 synthetic polymer is added as co-surfactant and stabilizer of the gel formulation, due to its capacity of gelling at body temperature and flow at refrigeration temperature (thermo-reversible). Isopropyl palmitate or isopropyl myristate are non-oleaginous emollients with a high capacity for spreading [94, 95]. The polar phase (water and polymer) corresponds to 20-30% (v/v) of the total formulation and the oil phase contains equal parts of lecithin and isopropyl palmitate or isopropyl myristate (about 20% (w/w) or 22% (v/v)), therefore PLO are designated as microemulsion-based gels. They were first developed in the early 90s, by Jones and Kloesel, as transdermal transport systems, who characterize them as "*an emulsion that has the appearance and feel of a gel*" [96].

With the addition of water, lecithin reverse micelles rearrange themselves into elongated tubular micelles, forming a stable tridimensional gel network, that incorporates the water in a w/o emulsion. This transition to a polymeric-like network is due to the formation of hydrogen bonds between the phosphate group of the lecithin molecules and the water molecules [97]. Thus, water acts as a stabilizing structure in the organogel formation process as well as solvent of polar molecules (pluronic F127 and hydrophilic drugs).

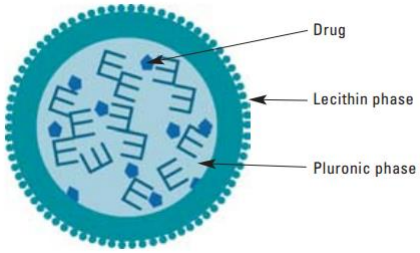


Figure 22 – Micelle of Pluronic Lecithin Organogel, incorporating hydrossoluble drugs. (Source: Sangale et al., 2015 [1].)

PLO form an opaque and yellowish solution. They are thermo-reversible, but thermodynamically stable, viscoelastic, non-irritating to the skin and biocompatible. They can be used as a transport system for hydrophilic and hydrophobic drugs for topical and transdermal application, because they are rapidly absorbed, however they can produce a skin minimal irritation. At body temperature, this structure is in thermo-reversible solid state (gel) and, as it cools, it returns to liquid phase. As the gelling temperature of the formulation is below normal skin temperature when the formulation is applied to skin this is in the gel state, with lower flow ability but more dispersibility [96, 98, 99].

They have a residence time on the skin longer than the time of microemulsions or sprays, allowing rheological properties that promote skin adhesion. The biphasic solubility (allows the incorporation of water and oil soluble ingredients) promotes the drug partition throughout the SC and the ability to change the skin barrier system due to amphiphilic nature of the phospholipids constituents of the organogel, promoting skin permeation properties. The non-polar tail of the phospholipid interacts with the lipids in the stratum corneum, disrupting its structure and allowing the passage and absorption of drugs by diffusion through the skin cells to the systemic circulation, facilitating its absorption [100, 101].

PLO is an ideal system for the transport of high molecular weight drugs, because they allow flexibility in dose, they are easy to prepare and have low cost, thus contributing to an increased drug residence time in the skin and sustained drug release.

- **Premium Lecithin Organogels (PrLO)**

These organogels derive from PLO, however they do not contain pluronic, which prevents skin irritation and intolerant reactions in the skin. Exhibit greater thermal stability due to the nature non-greasy and non-tacky of these and promote the permeation and bioavailability in the tissue, which provides more acceptable cosmetically.

- **Eudragit organogels**

They are a mixture of 30-40% Eudragit polymer (L or S) with a polyhydric alcohol, such as glycerol, propylene glycol or liquid polyethylene glycol. The viscosity of the gels increases with the concentration of Eudragit. They exhibit high stiffness and stability when the drug concentration is low. The viscosity decreases with increasing drug content.

- **Surfactants organogels (Amphiphilogels)**

In these organogels, both solid and liquid components are surfactants. Nonionic surfactants of sorbitan esters, sorbitan monostearate (Span 60®) or sorbitan monopalmitate (Span 40®) act as gelling agents (solid component) - form an organized tridimensional network, capable of immobilizing the liquid component. Moreover, polysorbates (Tweens®), and liquid esters of sorbitan (Span 20 and Span 80) are used as liquid components. When used Tween® 20, 40 or 80, it forms an amphiphilogel hydrophilic in nature. In the case of Span® 20 or 80, it forms a hydrophobic amphiphilogel. This characteristic depends on the hydrophilic or lipophilic character of the liquid surfactants. These gels are prepared by mixing the solid gelator (sorbitan monostearate or sorbitan monopalmitate) and the liquid phase (liquid sorbitan esters or polysorbates) and heating them at 60 °C to form a clear isotropic solution phase, and the cooling it to form an opaque semisolid at room temperature, that is, in these gels, one surfactant causes the gelation of another in a thermo-dependent way. These gels are formed by matrices solid fibers/tubules, and they are opaque and white in color [85].

Due to the non-ionic nature of surfactants, these gels do not cause skin irritation and therefore can be studied as delivery systems for poorly water-soluble drugs and antigens for transdermal administration. The liquid component of these organogels is responsible for the dissolution of the drug, thus, the hydrophilic or hydrophobic nature of surfactants defines the greater or smaller quantity of dissolved drug in the gels. As a result, the liquid component also influences drug release rates. Co-solvents can be included in the formulation of amphiphilogels, such as ethanol, propylene glycol or isopropyl alcohol, to increase the solubility of hydrophobic drugs in gel components [102].

- **Polyethylene Organogels**

They are formed from low molecular weight polyethylene that is dissolved in a mineral oil at temperature higher than 130 °C and then cooled causing a thermal shock which allows the formation of the gelled structure through physical interactions between the solid fibers. These

gels have been used as bases of ointments or patches. They are not irritating to the skin and have low sensitivity.

- **Fatty Acid Organogels**

Fatty acids molecules can also be used as organogelators, which, in aqueous solution and when the temperature of solubility decreases, form a water-in-oil emulsion. This results in the precipitation of organogelators and reticulated reorganization into tubules, which self-assemble and form the gelled structure [87].

3.2.7. FACTORS AFFECTING ORGANOGELS

- **Polar organic solvent**

  It induces the formation of spherical normal micelles of lecithin which can be associated with an increased cross-linking area of the polar region of lecithin, in which the solvent rearranges [103].

- **Phase Transition Temperature**

  It gives information about the nature of the microstructures present in the crosslinked gel network. For example, a narrow temperature range indicates that are formed homogeneous microstructures (fibers) on the gel, that is, crystallization occurs all at once.

  For determination of PTT, it can be used techniques such as hot stage microscopy, melting point apparatus or high sensitivity-DSC [85, 104].

- **Salt addition**

  The presence of salt attracts water, reduces the hydration of the polymer by increasing the intermolecular interactions between the polymers, which results in increased viscosity of the gel formed.

- **Temperature**

  The temperature effect depends on the chemical properties of the polymer and its way of interaction with the environment. If the temperature drops while the gel is in solution, the degree of hydration decreases and it gelation occurs due to the increase of intermolecular forces in the tridimensional network of gelling agent. The temperature effect is visible only in physical gels because gels resulting from chemical cross-linking are not liquefied by dilution or temperature changes.

- **Molecular weight**

  Polymers with lower molecular weight require higher concentrations to obtain a viscous gel also those composed of larger polymers.

- **Presence of surfactants**

  They can stabilize the tridimensional structure of the gel, as well as their interaction with the skin and enhance the skin permeation [105].

- **Drug incorporation in the formulation**

  It may alter the solubility of the drug and enhance its skin permeation, change the viscosity of the gels (e.g. viscosity of Eudragit or lecithin gels decreases with the increase of drug content) and increase the gelation temperature of the formulations and consequently, the stability of the gels [102].

3.2.8. CHARACTERISTICS TO EVALUATE IN ORGANOGELS [106-109]

- ✓ Physical appearance (color, consistency and separation phases)
- ✓ pH (it can help to determine if occurs skin irritation)
- ✓ Drug content (concentration of drug incorporated / adsorbed in the formulations)
- ✓ Homogeneity (evaluation of visual appearance and the presence or absence of aggregates / precipitation)
- ✓ Globular size and its distribution (mean particle diameter and polydispersity index)
- ✓ Critical Concentration of Gelation (a precise amount of organogelator is introduced into an ampule with an exact amount of organic solvent. The ampule is closed to form an internal pressure and increase the boiling point of the fluid. The interior is heated to promote complete fusion of content. Then the vial is allowed to cool for a sufficient time before being reversed. After reversing the ampule, the absence of fluid indicates gel formation) [36].
- ✓ Sol-gel Phase Transition Temperature (temperature at which the gel starts to flow, or vice versa. PTT depends on the physicochemical properties of the organogelator and solvent, as well as their physical or chemical interactions. It increases with increased concentration of organogelator. Permanent gels (chemical gels) does not show sol-gel transition, due to stronger interactions) [84, 108].
- ✓ Viscosity of the gels (rheological studies) [110].
- ✓ Spreadability (area of skin by which gel spreads after a uniform application. It is expressed as a function of time in seconds)
- ✓ Extrusion (ability to produce components in a semi continuous way from a certain template - weight in grams of gel to yield 0.5 g of gel tapes in 10 seconds)
- ✓ *In vitro* diffusion studies (Franz diffusion cells with the use the semipermeable cellulose membrane or skin extracts)

- ✓ Bioadhesion studies (force required to detach two surfaces of the mucosal when the gel formulation is placed between them)
- ✓ Skin irritation test (expose the organogel on the skin for 24 hours, examining erythema and edema)
- ✓ Anti-inflammatory studies (e.g. inhibition percentage of edema in animals with induced edema)
- ✓ Testing of physical and chemical long term stability (expose gels to extremes conditions of temperature and humidity - 0 °C and 40 °C - and evaluate after a few months their properties, pH, physical appearance, viscosity, content in drug and rate release) [109].

3.2.9. ORGANOGELS FOR TOPICAL AND TRANSDERMAL APPLICATIONS

The search of a vehicle to deliver the medicament into the skin layer (cutaneous delivery) or through the skin and into systemic circulation (percutaneous absorption) and to target the skin, varied kind of formulation systems and strategies have been evolved.

There are two ways for topical applications of drugs: internal and external topical. In the external topical application, the drug is spread, sprayed or dispersed throughout the tissue to cover the infected area. In the internal application, the drug is applied to the mucous membrane orally, vaginally or on rectal tissue for a local action [111, 112].

The importance of lipids has especially increased after realizing the utility of phospholipids. The use of natural biocompatible molecules with water can form diverse type of poly and supra molecular structures with retardant release in sustained release formulation. The topical delivery has been attempted and made successful using a number of lipid based systems via vesicular systems, lipid microsphere, lipid nanoparticles, lipid emulsion, polymeric gels, etc.

Organogels have already been used as drug delivery systems and have proved their ability to enhance the permeation of dermal and transdermal formulations, and to sustain the release of a drug, highlighting their potential as hydrophobic reservoirs [90, 113, 114]. They not only perform a local effect as they are also able to achieve a systemic effect via percutaneous absorption, in the presence of penetration enhancers and because of their lipophilic nature and occlusive effect. For an efficient transfer of semisolid formulations through the skin, it is necessary that they be highly permeable. The use of nonpolar solvents as skin permeation enhancers allows organogels to easily penetrate the skin. They do not cause skin irritation, they have easy spreadability, are easy to prepare, promote skin hydration by reducing Transepidermal Water Loss and have long term stability constituting a vehicle of choice for cosmetics and transdermal drug delivery systems [86, 115].

Table 4 - Examples of organogels for controlled drug delivery. (Source: Balasubramanian *et al.*, 2012 [86].)

Organogelator used	Organic solvent gelled	Model drug used	Pharmaceutical application
12-HAS (Hydroxystearic acid)	Soybean oil	Ibuprofen	Topical NSAID
Pluronic Lecithin Organogel	Water	Morphine	Topical analgesic for cancer pains
Gelatin contained microemulsion based gel	Isopropyl myristate and Tween 85®	Sodium Salicylate	Topical drug delivery through iontophoresis
Sorbitan monostearate	Sweet almond oil, alkanes like hexane, decane, vegetable oils, etc.	Propanolol, cyclosporin	Antihypertensive and immunosuppressant
Lecithin	Various organics	Diclofenac	Analgesic
Soybean lecithin	Isopropyl palmitate	Diclofenac, indomethacin	Analgesic
N-stearine-N0-stearyl-L-phenylalanina	Isopropyl myristate, Tween 80®, propylene glycol and water	Sodium salicylate	Anti-bacterial
1,3:2,4-Dibenzylidene-d-sorbitol (DBS)	Propylene glycol	5-fluorouracil	Antifungal
Betullin	Fixed oils (that are not volatiles)	Triterpene extract from birch bark	Organogel for actinic keratoses
Sorbitan monostearate	Almond oil		Topical application

3.3. MICROEMULSIONS

The first definition of microemulsion given by researchers Danielsson and Lindman in 1981 relates to microemulsion as a thermodynamically stable liquid solution, simple and optically isotropic of surfactants, water and oil [116].

A microemulsion is commonly known as O/W or W/O emulsion producing a transparent product having droplet size from 10 to 100 nm and that does not have the tendency to coalesce. The concept of "microemulsion" embraces thus:

(a) Aqueous micellar surfactant solutions containing a solubilized lipid, an "Oil-in-water microemulsion",

(b) Lipophilic micellar surfactant solutions containing solubilized water, so-called reversed micellar solutions. These are "water-in-oil microemulsions'.

(c) Systems where one has a continuous transition from an aqueous to lipophilic solution.

(d) The "surfactant phase" in non-ionic surfactant systems [116].

Microemulsion are characterized by physicochemical properties such as transparency, optical isotropy, low viscosity and thermodynamic stability [117].

Microemulsions are ideal vehicles for the transport of drugs since they present the main characteristics of fluid systems, such as thermodynamic stability (longer shelf life), easy formation (no interfacial tension and almost spontaneous formation), low viscosity with Newtonian behavior, high surface area (high solubilizing capacity) and very small particle size. The small particles are more likely to adhere to the membranes and transporting bioactive molecules in a more controlled manner. The microemulsions may be introduced into the body orally, topically through the skin or nasally, via a direct entry through aerosol in the lungs.

It is necessary to characterize microemulsions as well as drug locus in the loaded microemulsions. Due to the intermolecular interactions between the drug and microemulsions, the system microstructure can be changed. Some of the methods used to characterize these systems include the viscosity and conductivity, cryo-TEM, pulsed gradient spin echo (self-diffusion) NMR, DLS, small angle X-ray scattering (SAXS) and small angle neutron scattering (SANS) [118].

However, microemulsions contain a high amount of surfactant, frequently in combination with a cosurfactant, and in most cases, of alcohol, solvents and cosolvents that, as no active compounds, may involve a risk in the pharmaceutical field [119].

The use of microemulsions is advantageous due to easy preparation and low cost of these formulations and also the bioavailability. Up to date microemulsions have been shown to be

able to protect labile drug, control drug release, increase drug solubility, and reduce patient variability.

3.3.1. MICROEMULSIONS AS TRANSDERMAL DRUG DELIVERY SYSTEMS

There are many studies on microemulsions as topical drug delivery vehicles, and it has been shown that microemulsion formulations have improved transdermal and dermal delivery properties [120].

Microemulsions are constituted by:

(A) Oil Phase

Saturated and unsaturated fatty acids, alcohols, sulfoxides, surfactants or amides are used as the oil phase to enhance the skin permeation of different drugs.

Aungst *et al.* suggests that molecules with a saturated C10-C12 alkyl chain and a polar head or molecules with an C10 unsaturated alkyl chain, such as oleic acid are those with best capacity as skin permeation enhancers, and in the case of oleic acid, *cis* configuration disturbs more actively the lipidic layer of the skin that *trans* unsaturated configuration [121]. Since the SC is hydrophobic, the fatty acids can enter into the lipid bilayer, creating separate domains and induce highly permeable paths therebetween. Oleic acid should be used with some care, because it causes changes in Langerhans cells (located in superbasal layer of the epidermis) and plays an important role in initiating and coordinating the immune response of T-cells [122]. Other fatty acids such as linolenic acid, in combination with iontophoresis technique is capable of stimulating the lipid disorganization of SC and potentiate skin permeation of drugs adsorbed on microemulsions [8, 9, 123].

Other examples used as permeation enhancers are isopropyl myristate, isopropyl palmitate, triacetin, isostearilic isostearate, R(+)-limonene, as well as medium chain triglycerides [118].

(B) Surfactants

Since the discovery of the action of phospholipid molecules as promoters of cutaneous permeation by Kato et al., several studies have been conducted with use of these substances as non-toxic and biocompatible surfactants [124]. The phosphatidylcholine, one of the most common phospholipid constituent of the lipid bilayers of the body when it is in a fluid state and due to their physicochemical properties, can merge with the of SC lipids, disrupt its structure and facilitate the dermal and transdermal permeation drug [125].

When using lecithin as a source of phosphatidylcholine, it is necessary to add a co-surfactant such as ethanol. To form microemulsions, the required amount of lecithin increases the smaller the amount of ethanol. Ethanol is necessary to decrease the rigidity of the condensate film of lecithin and allow water solubility, allowing the formation of microparticles. Therefore, if on one hand an increase of the aqueous phase promotes the increase of particle size, on the other hand, for a constant aqueous phase, the particle size increases the smaller the amount of lecithin in solution [126].

Different sources of phosphatidylcholine may cause different effects on the drug permeation capacity. Kirjavainen *et al.* analyzed the effect of the drug partition into the lipid bilayers, using L-α-phosphatidylcholine from egg, L-α-phosphatidylcholine 60% from soybean seed, dioleylphosphatidyl ethanolamine (DOPE) and distearoylphosphatidylcholine (DSPC). They found that the first three are in the fluid state, allowing diffusion through the stratum corneum and drug permeation. In the case of DSPC molecules, they are in the gel state and therefore are not able to permeate through the SC [125].

Nonionic surfactants, such as Span 20® or Tween 20® affect SC lipids and make it more fluid, which is ideal for the diffusion of lipophilic molecules through the sc. Tween 20® allows polar molecules to distribute more readily for the SC, promoting permeation. These compounds enable the formation of micelles in an aqueous medium, which have a large capacity to extract skin lipids and to promote the permeation of hydrophilic compounds [127].

Caprylocaproyl macroglycerides, Plurol Isostearique® or Plurol Oleique® are examples of other surfactants that are barely irritating to the skin and are widely used in the production of microemulsions for topical administration [118].

(C) Co-Surfactants

Alkyl alcohols with simple chains are widely used as permeation enhancers. Ethanol is one of the most widely used because it increases the flow of various hydrossoluble drugs, since it promotes the solubility of the drug in formulation or changes the structure of the membrane, increasing the permeability of the drug. Ethanol can solubilize some of the stratum corneum lipids, increasing the drug flux there through [128]. Another mechanism is based on the fact that ethanol is a volatile compound, which when evaporate from the formulation, increases the drug concentration into a supersaturated state, which facilitates permeation. The permeation enhancer effect is dependent on its concentration in the formulation.

Other examples that can facilitate skin permeation are 1-butanol, decanol (as saturated fatty alcohol) or propylene glycol, that act similarly to ethanol [118]. Given several studies already conducted, it is concluded that it is necessary to take into account the concentrations

used, and the membranes in which the study is carried out, since drug permeation mechanism varies in a cellulose membrane or in a membrane pre-treated with additives, or in a dislipidic membrane or even in a extract of stratum corneum, and surfactants such as ethanol, phospholipids or propylene glycol known as enhancers, can act as drug permeation retardants, depending on its concentration and membrane used [118, 129].

(D) Aqueous phase

In most studies water is used as the aqueous phase. However, it can also be used phosphate buffer at pH 7.4 [118].

Microemulsions are advantageous encapsulation systems of lipophilic and hydrophilic drugs for topical administration. The drug permeation rate in microemulsions is superior to conventional emulsions (creams, lotions, etc.). In emulsions, the strong interactions between surfactants occurring in the interfacial film membrane, limit the mobility of the drug between the internal and the external phase of the formulation. In microemulsions, the co-surfactants function is to decrease the surface tension of the surfactant film and to allow the spontaneous formation of the microemulsion, promoting its thermodynamic stability [130].

Microemulsion has disadvantages like the need of a large amount of surfactant and cosurfactants for stabilization of nanodroplets, poor viscosity, and spreadabillity. Organogels are having better viscosity and spreadability than microemulsions [86].

Table 5 – Examples of transdermal drug delivery systems based on microemulsions. (Adapted from Kreilgaard. *et al*., 2002 [120].)

Drug	**Microemulsion**		
	Oil Phase	**Surfactants/Cosurfactants**	**Aqueous phase**
5-Fluorouracil	Isopropyl myristate	AOT (Aerosol-OT, dioctyl sodium sulfosuccinate)	water
Diclofenac	Isopropyl palmitate	Lecithin	water
Estradiol	Epicuron 200®, Oleic acid, Isopropyl myristate	Tween 20®, Tween 80®	water
Ketoprofen	Miglyol 812 N®	Lecithin/ *n*-butanol	water
Piroxicam	Isopropyl myristate	Hexadecyltrimethylammonium bromide	aqueous buffer (pH 5.5)
Prostaglandin E_1	Oleic acid	Labrasol®, Plurol Oleique®	water
Sodium fluorescein	Mygliol 812®, soybean oil	Brij 97®	water
Sucrose	Ethyl oleate	Labrasol®, Plurol Isostearique®	water, 154 nM NaCl

4. CONCLUSIONS

4.1. CONCLUSIONS ABOUT HYDROGELS

Gels need to be effective at enhancing permeation of a number of molecules to/ through the skin and cellular membranes, either low molecular weight molecules either peptides or proteins.

Due to its high water content, hydrogels allow to maintain a highly hydrated system around wounds, facilitating cellular immune activity essential for the healing process of wounds, as well as absorb exudates from wounds and protect it from secondary infections [131, 132]. Regarding mechanical behavior around the extracellular matrices, the hydrophilic surface of hydrogels exhibits low interface free energy when in contact with body fluids, reducing the tendency of cells and proteins to adhere to its surface. On the other hand, hydrogels have been reported to reduce skin irritation by absorbing moisture from the skin's surface and they are responsible for maintaining constant the Transepidermal Water Loss (TEWL). Thus, we can affirm that gels are biocompatible systems.

The hydrated environment around the wound may, however, facilitate microbial infection. Thus, gels capable of enhancing an antimicrobial action, in addition to their primary function (e.g. wound healing, drug delivery and transport, etc.) are required. This can be achieved by using polymers with antimicrobial activity, e.g. chitosan, or formulating gels to encapsulate or adsorb covalently therapeutic agents with this function, such as bacitracin, among others [20, 133].

The antimicrobial activity of the gels depends on their physicochemical characteristics. In acidic solutions, polymeric hydrogels become cationic and able to bind and kill bacteria through membrane disruption mechanism. Thus, in its cationic form, these gels exhibit antimicrobial activity. However, when exposed to neutral or alkaline medium, these gels return to their zwitterionic form and dead bacteria are released from the gel. This antifouling property prevents the accumulation of dead bacteria in the gel surface, since they can hinder the antimicrobial activity of the gel [20, 134].

Another advantage of hydrogels as transdermal systems is that they can be readily removed by simple washing with water. Over the materials based on hydrogels, with specific structures and characteristics of hydrophilicity/hydrophobicity, it is possible to obtain specific release kinetics and dissolution profiles, that allows the delivery of sensitive molecules, such as anticancer molecules, as well as the delivery of proteins and peptides.

It is not yet possible to find many hydrogels materials in the market as drug delivery systems, although the huge studies and the number of patents are increasing. This is derived from the high production costs of these materials.

4.2. CONCLUSIONS ABOUT ORGANOGELS

The organogels have interesting advantages over other formulations for transdermal drug delivery, including the easy preparation and administration. As they contain no water, their physical, microbiological and chemical stability is far superior compared to conventional topical bases. They have a high viscosity, making them viable for topical application and resistance to microbial contamination, due to the amount of the oil component and the absence of water. When compared with other lipid carriers, organogels have greater efficacy, stability and viability over time, making them a viable formulation to overcome the issue of cost-effective and productive search.

4.3. CONCLUSIONS ABOUT MICROEMULSIONS

Microemulsions can be formed by various types of oils, surfactants, co-surfactants and aqueous phases. They can solubilize lipophilic and hydrophilic drugs, allowing a greater drug accumulation in the active site.

These transport systems act as penetration enhancers, due to the surfactants and oily components, increasing the cutaneous absorption of the drug. The addition of a co-surfactant to the surfactant enhances this effect synergistically, as happens combining iontophoresis method and fatty acid molecules, allowing the permeation of either low molecular weight drugs, such as high molecular weight (e.g. peptides and proteins). However, these compounds are also recognized for may induce local skin irritation.

A major problem is that after application of the formulation on the skin, its composition can vary. For example, volatile compounds may evaporate, small molecules can be absorbed in its free form, and other components can interact with the skin and cause damage and irritation irreversibly. Thus, many drugs able to penetrate the skin and cause a therapeutic effect, still fail due to the incompatibility of the formulation in which they are inserted.

The structure and composition of the microemulsion facilitate solubilization of the drug relative to its individual components. is not well established yet the location of the drug formulation (core or interface) or the quantification of the maximum possible solubilization of the drug in the formulation and their behavior after dilution. The same components with different concentrations can form different vehicles and affect the rate of skin permeation of

the drug as well as the cumulative amount of it in the skin. In most cases, the lower the viscosity of the formulation, the greater the release rate of the drug [135].

4.4. GENERAL CONCLUSIONS

Recent developments in pharmaceutical science and technology have not only improved conventional gels (hydrogels or oleogels) as drug delivery systems, but also introduced new variations of semisolid vehicles particularly for transdermal delivery such as proniosomes and microemulsions incorporated into gels. All of gel systems present advantages over aqueous drug solutions because of their high viscosity, stability, biocompatibility, easy preparation and administration, and being compatible with hydrophilic or lipophilic drugs, making them suitable for topical/transdermal drug delivery systems.

In conclusion, further studies in gel technologies are essentials to overcome the drawbacks of each gel system and for developing cost effective delivery systems for transdermal applications.

5. REFERENCES

1. SANGALE, P. T.; MANOJ, G. V. - **Organogel: A novel approach for transdermal drug delivery system.** World J Pharm. Research, 4. 3, (2015), p. 423-442.
2. LEE, S. H.; JEONG, S. K.; AHN, S. K. - **An update of the defensive barrier function of skin.** Yonsei Med J, 47. 3, (2006), p. 293-306.
3. PROKSCH, E.; BRANDNER, J. M.; JENSEN, J. M. - **The skin: an indispensable barrier.** Exp Dermatol, 17. 12, (2008), p. 1063-1072.
4. ALI, S. M.; YOSIPOVITCH, G. - **Skin pH: from basic science to basic skin care.** Acta Derm-Venereol, 93. 3, (2013), p. 261-267.
5. HADGRAFT, J.; LANE, M. E. - **Skin: the ultimate interface.** Phys Chem Chem Phys, 13. 12, (2011), p. 5215-5222.
6. PELTOLA, S., et al. - **Microemulsions for topical delivery of estradiol.** Int J Pharm, 254. 2, (2003), p. 99-107.
7. WILLIAMS, A. C.; BARRY, B. W. - **Penetration enhancers.** Adv Drug Deliv Rev, 56. 5, (2004), p. 603-618.
8. JADOUL, A., et al. - **Modifications induced on stratum corneum structure after in vitro iontophoresis: ATR-FTIR and X-ray scattering studies.** J Control Release, 42. 2, (1996), p. 165-173.
9. RASTOGI, S. K.; SINGH, J. - **Effect of chemical penetration enhancer and iontophoresis on the in vitro percutaneous absorption enhancement of insulin through porcine epidermis.** Pharm Dev Technol, 10. 1, (2005), p. 97-104.
10. ARORA, A.; PRAUSNITZ, M. R.; MITRAGOTRI, S. - **Micro-scale devices for transdermal drug delivery.** Int J Pharm, 364. 2, (2008), p. 227-236.
11. HERWADKAR, A.; BANGA, A. K. - **Peptide and protein transdermal drug delivery.** Drug Discov Today: Technologies, 9. 2, (2012), p. e147-e154.
12. NANDA, S.; SAROHA, K.; SHARMA, B. - **Sonophoresis: an eminent advancement for transdermal drug delivery system.** Int J Pharm Technol, 3. 3, (2011), p. 1285-1307.
13. LEE, J. W., et al. - **Microsecond thermal ablation of skin for transdermal drug delivery.** J Control Release, 154. 1, (2011), p. 58-68.
14. KIM, Y. C., et al. - **Biochemical enhancement of transdermal delivery with magainin peptide: modification of electrostatic interactions by changing pH.** Int J Pharm, 362. 1-2, (2008), p. 20-28.

15. GODIN, B.; TOUITOU, E. - **Mechanism of bacitracin permeation enhancement through the skin and cellular membranes from an ethosomal carrier.** J Control Release, 94. 2-3, (2004), p. 365-379.
16. SCWAB, J. C. - **The importance of penetration of antimicrobial agents into cells.** Infect Dis Clin North Am, 3. (1989), p. 461 – 467.
17. VON EIFF, C.; PROCTOR, R. A.; PETERS, G. - **Small colony variants of Staphylococci: a link to persistent infections.** Berl Munch Tierarztl Wochenschr, 113. 9, (2000), p. 321-325.
18. VON EIFF, C.; PROCTOR, R. A.; PETERS, G. - **Staphylococcus aureus small colony variants: formation and clinical impact.** Int J Clin Pract Suppl, 115, (2000), p. 44-49.
19. PHILLIPS, O. A. - **Antibacterial agents: patent highlights July to December 2002.** Curr Opin Investig Drugs, 4. 2, (2003), p. 117-127.
20. VEIGA, A. S.; SCHNEIDER, J. P. - **Antimicrobial hydrogels for the treatment of infection.** Biopolymers, 100. 6, (2013), p. 637-644.
21. FOX, J. L. - **Antimicrobial peptides stage a comeback.** Nat Biotechnol, 31. 5, (2013), p. 379-382.
22. JOHNSON, B. A.; ANKER, H.; MELENEY, F. L. - **Bacitracin: A New Antibiotic Produced by a Member of the B. Subtilis Group.** Science, 102. 2650, (1945), p. 376-377.
23. MELENEY, F. L.; JOHNSON, B. A. - **Bacitracin.** Am J Med, 7. 6, (1949), p. 794-806.
24. MESCHER, M. F.; STROMINGER, J. L.; WATSON, S. W. - **Protein and carbohydrate composition of the cell envelope of Halobacterium salinarium.** J Bacteriol, 120. 2, (1974), p. 945-954.
25. MING, L. J.; EPPERSON, J. D. - **Metal binding and structure-activity relationship of the metalloantibiotic peptide bacitracin.** J Inorg Biochem, 91. 1, (2002), p. 46-58.
26. ECONOMOU, N. J.; COCKLIN, S.; LOLL, P. J. - **High-resolution crystal structure reveals molecular details of target recognition by bacitracin.** Proc Natl Acad Sci U S A, 110. 35, (2013), p. 14207-14212.
27. TSUJI, K.; ROBERTSON, J. H. - **Improved high-performance liquid chromatographic method for polypeptide antibiotics and its application to study the effects of treatments to reduce microbial levels in bacitracin powder.** J Chromatogr, 112. (1975), p. 663-672.

28. CRAIG, L. C., et al. - **The separation and characterization of bacitracin polypeptides.** J Biol Chem, 199. 1, (1952), p. 259-266.
29. GRUNEWALD, J.; MARAHIEL, M. A. - **Chemoenzymatic and template-directed synthesis of bioactive macrocyclic peptides.** Microbiol Mol Biol Rev, 70. 1, (2006), p. 121-146.
30. FROYSHOV, O. - **Enzyme-bound intermediates in the biosynthesis of bacitracin.** Eur J Biochem, 59. 1, (1975), p. 201-206.
31. PONS, M., et al. - **Conformational analysis of bacitracin A, a naturally occurring lariat.** Biopolymers, 31. 6, (1991), p. 605-612.
32. REHMAN, K.; ZULFAKAR, M. H. - **Recent advances in gel technologies for topical and transdermal drug delivery.** Drug Dev Ind Pharm, 40. 4, (2014), p. 433-440.
33. ALMEIDA, H., et al. - **Pluronic(R) F-127 and Pluronic Lecithin Organogel (PLO): main features and their applications in topical and transdermal administration of drugs.** J Pharm Pharm Sci, 15. 4, (2012), p. 592-605.
34. TERECH, P., et al. - **Structural variations in a family of orthodialkoxyarenes organogelators.** J Colloid Interface Sci, 302. 2, (2006), p. 633-642.
35. WANG, X., et al. - **Reversible organogels triggered by dynamic K+ binding and release.** J Colloid Interface Sci, 353. 2, (2011), p. 412-419.
36. MAITY, G. C. - **Low molecular mass gelators of organic liquids.** J Phys Sci, 11. (2007), p. 156-171.
37. DEBNATH, S., et al. - **Applications of organogels in drug delivery.** Indian J Res Pharm Biotechnol, 2. 1, (2014), p. 976-981.
38. KARAKATSANI, M.; DEDHIYA, M.; PLAKOGIANNIS, F. M. - **The effect of permeation enhancers on the viscosity and the release profile of transdermal hydroxypropyl methylcellulose gel formulations containing diltiazem HCl.** Drug Dev Ind Pharm, 36. 10, (2010), p. 1195-1206.
39. AHMED, E. M. - **Hydrogel: Preparation, characterization, and applications: A review.** J Adv Res, 6. 2, (2015), p. 105-121.
40. ADNADJEVIC, B.; JOVANOVIC, J. - **A comparative kinetics study of isothermal drug release from poly(acrylic acid) and poly(acrylic-co-methacrylic acid) hydrogels.** Colloids Surf B Biointerfaces, 69. 1, (2009), p. 31-42.
41. HOFFMAN, A. S. - **Stimuli-responsive polymers: biomedical applications and challenges for clinical translation.** Adv Drug Deliv Rev, 65. 1, (2013), p. 10-16.

42. VILA, M. M., et al. - **Development and characterization of a hydrogel containing nitrofurazone for antimicrobial topical applications.** Curr Pharm Biotechnol, 15. 2, (2014), p. 182-190.
43. PEPPAS, N. A., et al. - **Hydrogels in biology and medicine: from molecular principles to bionanotechnology.** Adv Mater, 18. (2006), p. 1345-1360.
44. LOPES, C. M.; FELISBERTI, M. I. - **Mechanical behaviour and biocompatibility of poly(1-vinyl-2-pyrrolidinone)-gelatin IPN hydrogels.** Biomaterials, 24. 7, (2003), p. 1279-1284.
45. YANG, L.; CHU, J. S.; FIX, J. A. - **Colon-specific drug delivery: new approaches and in vitro/in vivo evaluation.** Int J Pharm, 235. 1-2, (2002), p. 1-15.
46. HOFFMAN, A. S. - **Hydrogels for biomedical applications.** Adv Drug Deliv Rev, 54. 1, (2002), p. 3-12.
47. HAMIDI, M.; AZADI, A.; RAFIEI, P. - **Hydrogel nanoparticles in drug delivery.** Adv Drug Deliv Rev, 60. 15, (2008), p. 1638-1649.
48. OYEN, M. L. - **Mechanical characterisation of hydrogel materials.** Int Mater Rev, 59. 1, (2014), p. 44-59.
49. CHEN, G.; HOFFMAN, A. S. - **Graft copolymers that exhibit temperature-induced phase transitions over a wide range of pH.** Nature, 373. 6509, (1995), p. 49-52.
50. SUN, G.; ZHANG, X. Z.; CHU, C. C. - **Formulation and characterization of chitosan-based hydrogel films having both temperature and pH sensitivity.** J Mater Sci Mater Med, 18. 8, (2007), p. 1563-1577.
51. NETO, C. G., et al. - **Permeability studies in chitosan membranes. Effects of crosslinking and poly(ethylene oxide) addition.** Carbohydr Res, 340. 17, (2005), p. 2630-2636.
52. CALÓ, E.; KHUTORYANSKIY, V. V. - **Biomedical applications of hydrogels: A review of patents and commercial products.** Eur Polym J, 65. (2015), p. 252-267.
53. HENNINK, W. E.; VAN NOSTRUM, C. F. - **Novel crosslinking methods to design hydrogels.** Adv Drug Deliv Rev, 54. 1, (2002), p. 13-36.
54. ZHANG, Y.; ZHU, W.; DING, J. - **Preparation of thermosensitive microgels via suspension polymerization using different temperature protocols.** J Biomed Mater Res A, 75. 2, (2005), p. 342-349.

55. ROSIAK, J.; OLEJNICZAK, J. - **[Polymer materials for biomedical use obtained by radiation methods. IV. The therapeutic system for local release of prostaglandins].** Polim Med, 19. 3-4, (1989), p. 93-106.
56. KIZILEL, S., et al. - **Sequential formation of covalently bonded hydrogel multilayers through surface initiated photopolymerization.** Biomaterials, 27. 8, (2006), p. 1209-1215.
57. LIN-GIBSON, S., et al. - **Synthesis and characterization of PEG dimethacrylates and their hydrogels.** Biomacromolecules, 5. 4, (2004), p. 1280-1287.
58. GUPTA, P.; VERMANI, K.; GARG, S. - **Hydrogels: from controlled release to pH-responsive drug delivery.** Drug Discov Today, 7. 10, (2002), p. 569-579.
59. BAROLI, B. - **Hydrogels for tissue engineering and delivery of tissue-inducing substances.** J Pharm Sci, 96. 9, (2007), p. 2197-2223.
60. LIN, C. C.; METTERS, A. T. - **Hydrogels in controlled release formulations: network design and mathematical modeling.** Adv Drug Deliv Rev, 58. 12-13, (2006), p. 1379-1408.
61. CARR, D. A.; PEPPAS, N. A. - **Molecular structure of physiologically-responsive hydrogels controls diffusive behavior.** Macromol Biosci, 9. 5, (2009), p. 497-505.
62. SOUSA, R. G., et al. - **Dependence of copolymer composition, swelling history, and drug concentration on the loading of diltiazem hydrochloride (DIL.HCl) into poly[(N-isopropylacrylamide)-co-(methacrylic acid)] hydrogels and its release behaviour from hydrogel slabs.** J Control Release, 102. 3, (2005), p. 595-606.
63. KIM, S. W.; BAE, Y. H.; OKANO, T. - **Hydrogels: swelling, drug loading, and release.** Pharm Res, 9. 3, (1992), p. 283-290.
64. CANAL, T.; PEPPAS, N. A. - **Correlation between mesh size and equilibrium degree of swelling of polymeric networks.** J Biomed Mater Res, 23. 10, (1989), p. 1183-1193.
65. BELL, C. L.; PEPPAS, N. A. - **Water, solute and protein diffusion in physiologically responsive hydrogels of poly (methacrylic acid-g-ethylene glycol).** Biomaterials, 17. 12, (1996), p. 1203-1218.
66. CRUISE, G. M.; SCHARP, D. S.; HUBBELL, J. A. - **Characterization of permeability and network structure of interfacially photopolymerized poly(ethylene glycol) diacrylate hydrogels.** Biomaterials, 19. 14, (1998), p. 1287-1294.

67. PEPPAS, N. A., et al. - **Hydrogels in pharmaceutical formulations.** Eur J Pharm Biopharm, 50. 1, (2000), p. 27-46.
68. QIU, Y.; PARK, K. - **Environment-sensitive hydrogels for drug delivery.** Adv Drug Deliv Rev, 53. 3, (2001), p. 321-339.
69. AL-TAHAMI, K.; SINGH, J. - **Smart polymer based delivery systems for peptides and proteins.** Recent Pat Drug Deliv Formul, 1. 1, (2007), p. 65-71.
70. HUANG, X.; LOWE, T. L. - **Biodegradable thermoresponsive hydrogels for aqueous encapsulation and controlled release of hydrophilic model drugs.** Biomacromolecules, 6. 4, (2005), p. 2131-2139.
71. ZHANG, R., et al. - **A novel pH- and ionic-strength-sensitive carboxy methyl dextran hydrogel.** Biomaterials, 26. 22, (2005), p. 4677-4683.
72. NGUYEN, M. K.; PARK, D. K.; LEE, D. S. - **Injectable poly(amidoamine)-poly(ethylene glycol)-poly(amidoamine) triblock copolymer hydrogel with dual sensitivities: pH and temperature.** Biomacromolecules, 10. 4, (2009), p. 728-731.
73. HOFFMAN, A. S., et al. - **Founder's Award, Society for Biomaterials. Sixth World Biomaterials Congress 2000, Kamuela, HI,May 15-20, 2000. Really smart bioconjugates of smart polymers and receptor proteins.** J Biomed Mater Res, 52. 4, (2000), p. 577-586.
74. ESCOBAR-CHAVEZ, J. J., et al. - **Applications of thermo-reversible pluronic F-127 gels in pharmaceutical formulations.** J Pharm Pharm Sci, 9. 3, (2006), p. 339-358.
75. BOUDIER, A., et al. - **Evaluation of biocompatible stabilised gelled soya bean oil nanoparticles as new hydrophobic reservoirs.** J Microencapsul, 27. 8, (2010), p. 682-692.
76. TERECH, P.; ROSSAT, C.; VOLINO, F. - **On the Measurement of Phase Transition Temperatures in Physical Molecular Organogels.** J Colloid Interface Sci, 227. 2, (2000), p. 363-370.
77. VAN ESCH, J., et al. - **Low molecular weight gelator for organic solvents.** Springer Netherlands, 6. 3, (1999), p. 233-259.
78. TERECH, P.; WEISS, R. G. - **Low Molecular Mass Gelators of Organic Liquids and the Properties of Their Gels.** Chem Rev, 97. 8, (1997), p. 3133-3160.
79. VAN ESCH, J. H.; FERINGA, B. L. - **New Functional Materials Based on Self-Assembling Organogels: From Serendipity towards Design The Royal**

Netherlands Academy of Science is gratefully acknowledged for a fellowship for J.H.v.E. Angew Chem Int Ed Engl, 39. 13, (2000), p. 2263-2266.

80. VINTILOIU, A.; LEROUX, J.-C. - **Organogels and their use in drug delivery - a review.** J Control Release, 125. 3, (2008), p. 179-192.
81. CHATERJI, S.; KWON, I.-K.; PARK, K. - **Smart polymeric gels: redefining the limits of biomedical devices.** Prog Polym Sci, 32. 8, (2007), p. 1083-1122.
82. TAKTAK, F. F.; BÜTÜN, V. - **Synthesis and physical gels of pH-and thermo-responsive tertiary amine methacrylate based ABA triblock copolymers and drug release studies.** Polymer, 51. 16, (2010), p. 3618-3626.
83. HANABUSA, K., et al. - **Low molecular weight gelators for organic fluids: Gelation using a family of cyclo (dipeptide)s.** J Colloid Interface Sci, 224. 2, (2000), p. 231-244.
84. TRIVEDI, D. R., et al. - **Structure–property correlation of a new family of organogelators based on organic salts and their selective gelation of oil from oil/water mixtures.** Chemistry, 10. 21, (2004), p. 5311-5322.
85. JIBRY, N.; HEENAN, R. K.; MURDAN, S. - **Amphiphilogels for drug delivery: formulation and characterization.** Pharm Res, 21. 10, (2004), p. 1852-1861.
86. BALASUBRAMANIAN, R.; SUGHIR, A.; DAMODAR, G. - **Oleogel: A promising base for transdermal formulations.** Asian J Pharm, 6. 1, (2012), p. 1-9.
87. SAHOO, S., et al. - **Organogels: properties and applications in drug delivery.** Des Monomers Polym, 14. 2, (2011), p. 95-108.
88. MURDAN, S.; GREGORIADIS, G.; FLORENCE, A. T. - **Novel sorbitan monostearate organogels.** J Pharm Sci, 88. 6, (1999), p. 608-14.
89. SUZUKI, M., et al. - **L-lysine based gemini organogelators: their organogelation properties and thermally stable organogels.** Org Biomol Chem, 1. 22, (2003), p. 4124-31.
90. KUMAR, R.; KATARE, O. P. - **Lecithin organogels as a potential phospholipid-structured system for topical drug delivery: a review.** AAPS PharmSciTech, 6. 2, (2005), p. E298-310.
91. SCARTAZZINI, R.; LUISI, P. L. - **Organogels from lecithins.** J Phys Chem, 92. 3, (1988), p. 829-833.
92. CHATGILIALOGLU, C.; FERRERI, C. - **Biomimetic Chemistry: Radical Reactions in Vesicle Suspensions.** In: MUKHERJEE, A. Biomimetics, Learning from Nature. Italy: InTech, 2010. ISBN: 978-953-307-025-4. p. 117-136.

93. SHARMA, H.; GUPTA, P.; YADAV, A. - **Organogels: A Review.** Int J Res Pharm Life Sci, 1. 2, (2013), p. 125-130.
94. BARICHELLO, J. M., et al. - **Absorption of insulin from pluronic F-127 gels following subcutaneous administration in rats.** Int J Pharm, 184. 2, (1999), p. 189-198.
95. WILLIMANN, H., et al. - **Lecithin organogel as matrix for transdermal transport of drugs.** J Pharm Sci, 81. 9, (1992), p. 871-874.
96. JONES, M. - **The history of pluronic lecithin organogel: an interview with Marty Jones, BSPharm, FACA, FIACP.** Int J Pharm Comp, 7. (2003), p. 180–182.
97. SHCHIPUNOV, Y. A.; DURRSCHMIDT, T.; HOFFMANN, H. - **Electrorheological Effects in Lecithin Organogels with Water and Glycerol.** J Colloid Interface Sci, 212. 2, (1999), p. 390-401.
98. FRANCKUM, J., et al. - **Pluronic lecithin organogel for local delivery of anti-inflammatory drugs.** Int J Pharm Compd, 8. 2, (2004), p. 101-105.
99. MORALES, M. E., et al. - **Preparation, characterization, and in vitro release of new transdermal methimazole as alternative to oral delivery.** Drug Deliv, 16. 1, (2009), p. 1-10.
100. BA, W., et al. - **Optimization and evaluation of pluronic lecithin organogels as a transdermal delivery vehicle for sinomenine.** Pharm Dev Technol, 21. 5, (2016), p. 535-545.
101. AGRAWAL, V., et al. - **Preparation and evaluation of tubular micelles of pluronic lecithin organogel for transdermal delivery of sumatriptan.** AAPS PharmSciTech, 11. 4, (2010), p. 1718-1725.
102. JIBRY, N.; SARWAR, T.; MURDAN, S. - **Amphiphilogels as drug carriers: effects of drug incorporation on the gel and on the active drug.** J Pharm Pharmacol, 58. 2, (2006), p. 187-194.
103. PAL, K.; BANTHIA, A.; MAJUMDAR, D. - **Effect of heat treatment of starch on the properties of the starch hydrogels.** Mater Lett, 62. 2, (2008), p. 215-218.
104. TORO-VAZQUEZ, J., et al. - **Thermal and textural properties of organogels developed by candelilla wax in safflower oil.** J Am Oil Chem Soc, 84. 11, (2007), p. 989-1000.
105. PAL, K.; BANTHIA, A.; MAJUMDAR, D. - **Biomedical evaluation of polyvinyl alcohol–gelatin esterified hydrogel for wound dressing.** J Mater Sci Mater Med, 18. 9, (2007), p. 1889-1894.

106. REDDY, G., et al. - **Organogel: A Review.** Int J Pharm Technol, 2. 4, (2010), p. 584-602.
107. SANDHAN, V. R.; GONDKAR, S.; SAUDAGAR, R. - **Formulation Development of Ketoconazole Ophthalmic Formulation.** Res J Pharm Dosage Form Technol, 5. 6, (2013), p. 303-310.
108. PENZES, T., et al. - **Topical absorption of piroxicam from organogels--in vitro and in vivo correlations.** Int J Pharm, 298. 1, (2005), p. 47-54.
109. BAIBHAV, J.; GURPREET, S. - **Emulgel: A Comprehensive Review on the Recent Advances in Topical Drug Delivery.**Int Res J Pham, 2. 11, (2011), p. 66-70.
110. MACOSKO, C. W. - **Rheology: principles, measurements, and applications.** New York: Wiley-VCH, 1994. ISBN: 978-0-471-18575-8.
111. ASHARA, K. C., et al. - **Micro-emulsion based emulgel: a novel topical drug delivery system.** Asian Pac J Trop Dis, 4. (2014), p. S27-S32.
112. BHOWMIK, D. - **Recent advances in novel topical drug delivery system.** The Pharma Innovation, 1. 9, (2012), p. 12-31
113. MURDAN, S.; ANDRYSEK, T.; SON, D. - **Novel gels and their dispersions--oral drug delivery systems for ciclosporin.** Int J Pharm, 300. 1-2, (2005), p. 113-124.
114. VINTILOIU, A.; LEROUX, J. C. - **Organogels and their use in drug delivery--a review.** J Control Release, 125. 3, (2008), p. 179-192.
115. BAROLI, B., et al. - **Microemulsions for topical delivery of 8-methoxsalen.** J Control Release, 69. 1, (2000), p. 209-218.
116. DANIELSSON, I.; LINDMAN, B. - **The definition of microemulsion.** Colloids Surf, 3. (1981), p. 391-392.
117. BAROLI, B., et al. - **Microemulsions for topical delivery of 8-methoxsalen.** J Control Release, 69. 1, (2000), p. 209-218.
118. KOGAN, A.; GARTI, N. - **Microemulsions as transdermal drug delivery vehicles.** Adv Colloid Interface Sci, 123-126. (2006), p. 369-385.
119. KANTARCI, G., et al. - **Comparison of different water/oil microemulsions containing diclofenac sodium: preparation, characterization, release rate, and skin irritation studies.** AAPS PharmSciTech, 8. 4, (2007), p. E1-E7.
120. KREILGAARD, M. - **Influence of microemulsions on cutaneous drug delivery.** Adv Drug Deliv Rev, 54 Suppl 1. (2002), p. S77-S98.

121. AUNGST, B. J.; ROGERS, N. J.; SHEFTER, E. - **Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides.** Int J Pharm, 33. 1, (1986), p. 225-234.
122. TOUITOU, E., et al. - **Oleic acid, a skin penetration enhancer, affects Langerhans cells and corneocytes.** J Control Release, 80. 1-3, (2002), p. 1-7.
123. ALVAREZ-FIGUEROA, M. J.; BLANCO-MENDEZ, J. - **Transdermal delivery of methotrexate: iontophoretic delivery from hydrogels and passive delivery from microemulsions.** Int J Pharm, 215. 1, (2001), p. 57-65.
124. KATO, A.; ISHIBASHI, Y.; MIYAKE, Y. - **Effect of egg yolk lecithin on transdermal delivery of bunazosin hydrochloride.** J Pharm Pharmacol, 39. 5, (1987), p. 399-400.
125. KIRJAVAINEN, M., et al. - **Phospholipids affect stratum corneum lipid bilayer fluidity and drug partitioning into the bilayers.** J Control Release, 58. 2, (1999), p. 207-214.
126. ATTWOOD, D.; MALLON, C.; TAYLOR, C. J. - **Phase studies on oil-in-water phospholipid microemulsions.** Int J Pharm, 84. 2, (1992), p. R5-R8.
127. FANG, J. Y., et al. - **In vitro skin permeation of estradiol from various proniosome formulations.** Int J Pharm, 215. 1, (2001), p. 91-99.
128. MEGRAB, N. A.; WILLIAMS, A. C.; BARRY, B. W. - **Oestradiol permeation across human skin, silastic and snake skin membranes: the effects of ethanol/water co-solvent systems.** Int J Pharm, 116. 1, (1995), p. 101-112.
129. FANG, J. Y.; HWANG, T. L.; LEU, Y. L. - **Effect of enhancers and retarders on percutaneous absorption of flurbiprofen from hydrogels.** Int J Pharm, 250. 2, (2003), p. 313-325.
130. LEE, P. J.; LANGER, R.; SHASTRI, V. P. - **Novel microemulsion enhancer formulation for simultaneous transdermal delivery of hydrophilic and hydrophobic drugs.** Pharm Res, 20. 2, (2003), p. 264-269.
131. LAY-FLURRIE, K. - **The properties of hydrogel dressings and their impact on wound healing.** Prof Nurse, 19. 5, (2004), p. 269-273.
132. LOKE, W. K., et al. - **Wound dressing with sustained anti-microbial capability.** J Biomed Mater Res, 53. 1, (2000), p. 8-17.
133. AGREN, M. S. - **An amorphous hydrogel enhances epithelialisation of wounds.** Acta Derm Venereol, 78. 2, (1998), p. 119-122.
134. CAO, B., et al. - **Switchable antimicrobial and antifouling hydrogels with enhanced mechanical properties.** Adv Healthc Mater, 2. 8, (2013), p. 1096-1102.

135. TROTTA, M., et al. - **Influence of ion pairing on topical delivery of retinoic acid from microemulsions.** J Control Release, 86. 2, (2003), p. 315-321.

6. ANNEXES

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A STATE-OF-ART ON GEL TECHNOLOGY FOR TRANSDERMAL DRUG DELIVERY

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INTRODUCTION

Gels have consistently been studied for their role in topical and transdermal drug delivery systems as a non-invasive technique for pharmaceuticals and cosmetics application. Formulations for this drug delivery route must interact with skin, which is an anatomical and biological barrier, hindering the permeation of drugs to the circulatory system due to the extensive impermeable layer composed of dead cells, the *stratum corneum*. These formulations are semi-solid, porous, tridimensional structures, with unique characteristics, such as rigidity and elasticity at the same time [1]. Because of their high aqueous phase content, gels permit a greater dissolution of drugs through the skin and enhance skin hydration by retaining a significant amount of transepidermal water, in contrast to creams and ointments. To enhance the release of drugs through the skin, properties such as solubility of the drug, the size of the pores of the polymer matrix compared to the size of the active molecule and the chemical nature of the gel should be considered [2].
Conventionally, gels are differentiated into two types according to the nature of their liquid phase: hydrogels, which contain a polar solvent (water) and organogels, which contain an organic/non-polar solvent, as external phase [3].

HYDROGELS

Hydrogels are formulations consisting of polymeric materials that exhibit ability to swell and retain a large amount of water or other bio-fluids in their structures. Despite its great affinity for water, they only possess a swelling behavior without being dissolved in water. This proves their high flexibility, similar to natural tissue.

Hydrophilic natural or synthetic polymers can be chemically or physically crosslinked to produce hydrogels, that can be classified according to the type of bonds responsible for the crosslinking of gelling network.

- "reversible" or physical gels, if the molecular crossover and/or secondary forces such ionic strength, hydrogen bonding or hydrophobic forces represent the main support strength for the hydrogel structure. These gels rapidly lose their structure by dissolution, due to changes of environmental conditions, such as pH, ionic strength in solution or temperature [4];
- "permanent" or chemical hydrogels consist of a network of covalent bonds between different macromolecular chains and they can be obtained by linkages between different polymers in the dry state or in solution [4].

Depending on the nature of the functional groups on the surface of their structure, hydrogels can be charged or uncharged. Charged hydrogels exhibit alterations in their swelling behavior after pH variations and may change their shape when exposed to electric fields [5].

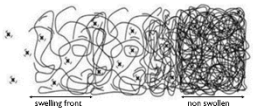

Fig. 1: Basic mechanism of drug release through an hydrogel.

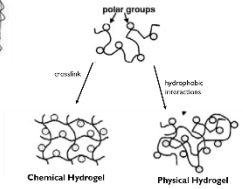

Fig. 2: Chemical vs. physical crosslinking on hydrogels networks.
Adapted from Hoffman, 2002 [6].

Table 1: Examples of different polymers to produce hydrogels.

Natural polymers	Synthetic monomers/polymers
Chitosan	Hydroxyethylmethacrylate (HEMA)
Alginate	N-(2-Hydroxy propyl)methacrylate (HPMA)
Pectin	N-Vinyl-2-pyrrolidone (NVP)
Collagen	N-isopropylacrylamide (NIPAAM)
Gelatin	Vinyl acetate (VAc)
Hyaluronic acid	Acrylic acid (AA)
Dextran	Methacrylic acid (MAA)
	Polyethylene glycol acryloxiethilmethacrylate (PEGAPEGMA)
	Polyethylene glycol diacrylate/dimethacrylate (PEGDA/PEGDMA)

ORGANOGELS / OLEOGELS

Organogels, or oleogels, consist of a network of self-assembled molecules which form a thermally reversible gel upon cooling, immobilizing a non-aqueous liquid. They are mainly composed of lipids (organic phase), so they easily interact with the lipid skin surface and enhance the drug permeation through the skin [7]. The most widely used lipids are based on edible oils, such as soybean oil, sunflower oil, sesame seed oil or olive oil, due to their high biocompatibility [8]. They do not cause irritation and possess long term stability. Organogels form viscoelastic structures through non-covalent associations with gelling agents in low concentrations, such as sorbitan monostearate (Span 60) or sorbitan monopalmitate (Span 40). These superstructures often form long fibers or needle-shaped structures, which entangle into pseudocrystalline regions, immobilizing the liquid largely by surface tension and forming a gel of variable consistency. They are transparent, allowing the use of spectroscopic methods to detect possible structural changes of the guest molecules.

OTHER TYPES OF GELS

Recent studies have reported other types of gels for dermal drug application, combining features of conventional hydrogels and organogels [9, 10].

Table 2: Types, properties and applications of different gels.

Types and property of gels	Advantages and application of gels
Lecithin organogels ➢ Extracted from various plant and animal tissue part from the egg yolk. ➢ They have been found to have an isotropic structure	**Lecithin organogels** ➢ Thermodynamically stable, thermo-reversible (sol-to-gel) transition temperature at 40 °C) viscoelastic and non - irritant
Pluronic Lecithin Organogels (PLO) ➢ PLO is a soy lecithin-based organogels which consists of isopropyl palmitate or isopropyl myristate, water and pluronic F127 ➢ The apolar phase in the PLO represents 22% (v/v), hence is often regarded as microemulsion-based-gel.	**Pluronic Lecithin Organogels (PLO)** ➢ thermostable, viscoelastic and biocompatible in nature ➢ minimal skin irritation ➢ It has been used as a delivery vehicle for both hydrophobic and hydrophilic molecules for topical and transdermal application
Bigels ➢ Mix of hydrogels (aqueous systems) and oleogels (lipophilic systems) without the addiction of a surfactant.	**Bigels** ➢ Ease to prepare ➢ No skin irritation induced by surfactant ➢ Can deliver lipophilic and hydrophilic drugs
Niosomal and Proniosomal gels ➢ Liposomes consisting of a nonionic surfactant, which can be of a hydrogel or oleogel nature. ➢ A proniosomal gel is a hydrated form of niosomes.	**Niosomal and Proniosomal gels** ➢ great stability (proniosomes are more stable than niosomes) ➢ Greater skin permeability ➢ Suitable for both lipophilic and hydrophilic drugs ➢ expensive
Emulgels ➢ Consist of a hydrogel or oleogel with o/w or w/o emulsion and a surfactant	**Emulgels** ➢ Thixotropic, easily spreadable, easily removable, emollient, non-staining, water-soluble, long-term life, transparent and pleasing appearance ➢ can be used in the controlled release of drugs
Aerogels ➢ Inorganic, composed of silica, and produced by supercritical drying **Xerogels** ➢ Inorganic, composed of silica, produced by drying under normal pressure	**Aerogels and xerogels** ➢ High stability ➢ Low thermal conductivity and thermally stable ➢ Large surface area for drug carrying ➢ They can be used for controlled drug delivery

CHARACTERIZATION OF GELS

The main properties to consider in the characterization of different gels are:

- morphology,
- over time stability,
- gelation kinetics,
- rheological properties (viscosity, stiffness),
- mechanical properties (texture analysis, shape recovery ability),
- thermal properties (differential scanning calorimetry, DSC),
- drug encapsulation (qualitative measurement by liquid chromatography, UV/Vis absorption spectroscopy or IR spectroscopy),
- *in vitro* drug encapsulation and release (Franz diffusion cells).

In general, the concentration of the gelling agent enhances the physical and mechanical properties of the organogels, such as the viscosity and consistency of gels, the resistance to deformation when *stress* is applied to the formulation, the boiling point and the thermodynamic stability of the system (entropy and enthalpy). However, in *in vitro* release assays, the presence of a higher concentration of organogelators negatively influence the release rate, because a higher network density formed by gelator can prevent the migration (diffusion) of the drug throughout the matrix [3].
By analyzing the *in vitro* release of the drug, it appears that the majority of the gels have a non-Fickian release behavior, characterized by a diffusion coefficient < 1. This indicates that drug release occurs mainly by diffusion or permeation throughout the hydrogel/organogel matrix.

CONCLUSIONS

Recent developments in pharmaceutical science and technology have not only improved conventional gels (hydrogels or oleogels) as drug delivery systems, but also introduced new variations of semisolid vehicles particularly for transdermal delivery such as proniosomes and microemulsions incorporated into gels. All of gel systems present advantages over aqueous drug solutions because of their high viscosity, stability, biocompatibility, easy preparation and administration, and being compatible with hydrophilic or lipophilic drugs, making them suitable for topical/transdermal drug delivery systems. In conclusion, further studies are essentials to overcome the drawbacks of each gel system and developing cost effective drug delivery systems.

References:
[1] Pal A. and Bey J., Langmuir, 27, 3401-8, 2011
[2] Chang R.K., Raw A., Lionberger R. and Yu L., AAPS J, 17, 1572, 2015
[3] Singh V. K., Pramanik K., Ray S. S. and Pal K., AAPS PharmSciTech, 16, 293-305, 2015
[4] Hoffman A. S., Adv Drug Deliv Rev, 65, 10-6, 2013
[5] Kurakazawa H., Dedhiya M. and Phakajaonsi F. H., Drug Dev Ind Pharm, 36, 1195-206, 2010
[6] Hoffman A. S., Adv Drug Deliv Rev, 54, 3-12, 2002
[7] Upadhyay R.K. et al., Drug Dev Ind Pharm, 33, 417-25, 2007
[8] Wright A.J. and Marangoni A.G., AOCS Press, Edible oleogels: structure and health implications, 81–99, 2011
[9] Mahler P., Mahler F., Duraz H., Ramazzina M., Liguori V. and Mautone G., Drugs Exp Clin Res, 29, 45-52, 2003
[10] Rehman R. and Zulfakar M. H., Drug Dev Ind Pharm, 40, 433-40, 2014

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