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Size matters: smart copolymeric nanohydrogels: synthesis and applications

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1. ABSTRACT

In this work the synthesis of smart nanoparticles capable of respond to external stimulus (pH and temperature variations) is reported. To avoid post-polymerization modification, functionalized monomers able to respond to pH and temperature changes were and then polymerized. The synthesized monomers have the capability for coupling with folic acid which is the target molecule. For this reason their polymers can be used as targeted drug delivery systems. Smart polymeric nanoparticles were prepared by direct and inverse microemulsion polymerization of the synthesized monomers. The nanoparticles were charged with drugs and their release kinetic was studied.

2. INTRODUCCIÓN

Since the early twentieth century science advanced greatly to the point of being able to modify the structure of a large number of molecules, polymers among them, which nowadays are everyday materials and have great industrial and technological importance. Thanks to these advances, current polymer research is centered not from the perspective of inert materials, but in developing materials for applications that are beyond its typical use.

The discovery of the microscopic world has enabled science to deepen its knowledge of materials at smaller size. The understanding of the transition from micro to nano scale will provide significant improvements

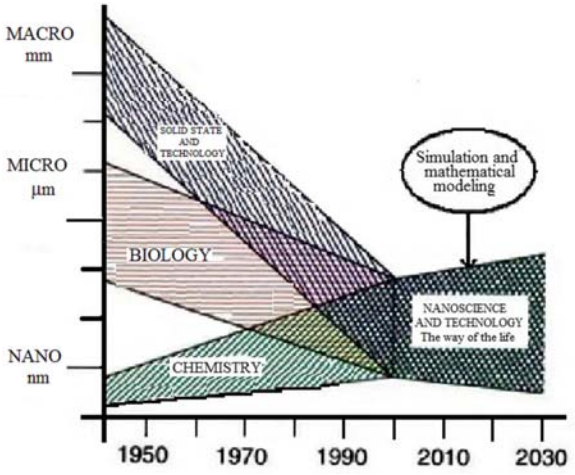

Figure 1. Multidisciplinary nature of nanotechnology.

in the understanding of matter and its applications (1). The seek for this knowledge has increased the number of investigations which, in turn, have generated a more complete knowledge about the surrounding environment and the development of new sciences that attempt to explain behavior at a microscopic scale. These new sciences have emerged due to the need for new technological breakthroughs. Nanotechnology is one of the predominant ones.

Nanotechnology is a field of applied science devoted to the control and manipulation of matter at a scale smaller than one micrometer, i.e. at the level of atoms and molecules (nanomaterials). Usually such manipulation occurs in a size range varying between one and one hundred nanometers (a nanometer is a billionth of a meter, i.e. 1 nm = 10^{-9} meters).

The word "nano" is a Greek prefix that indicates a measure not an object, so that nanotechnology is a multidisciplinary field characterized by the scale of the matter at which one works. Among the fields that cover nanotechnology we can mention: nanoscale engineering, mechanical, nanomolecular systems or nanofabrication systems, however, the term that encompasses all the fields is more properly known as molecular nanotechnology or simply MNT (the English acronym of molecular nanotechnology).

Richard Feynman, Nobel Prize for physics in 1965, was the first to point out the potential of nanoscience and nanotechnology in the famous speech he delivered five decades ago in the California Institute of Technology (Caltech) on 29 December 1959 entitled “There's Plenty of Room at the Bottom"(2), among other things he said: "The principles of physics, as I see it, do not talk about the possibility of maneuvering things atom by atom. This is not an attempt to violate any law; it is something that can be done in principle but in practice has not been done because we are too big. Many problems of chemistry and biology could be solved if we develop our ability to see what we are doing and to do things at the atomic level. Also at that conference proposed to manufacture products based on a rearrangement of atoms and molecules. That same year he wrote an article analyzing how computers working with individual atoms could consume very little energy with amazing calculation rates.

Nanotechnology was driven, in principle by the electronics industry, which needed urgently to overcome the size limits that marked the use of silicon in integrated circuits. Originally, though this approach was for applications, now the nanotechnology is being developed independently in the field of science. Its aim is essentially to create materials, devices and systems with new properties and functions as a result of their small size. Nanotechnology requires a new interdisciplinary approach to both research and in manufacturing processes. To obtain nanomaterials two routes are considered: the first is the miniaturization of microsystems and the second mimic nature by building structures starting from atomic and molecular levels. From the second route arises nanotechnology applied to biomedicine, science which is directed to the study of biological systems, which is largely based on the science of polymers.

Many materials, once they are reduced below 100 nanometers, have a number of unique features (much better in comparison with the macro scale). Due to the effects of quantum mechanics, the materials on this scale may be able to achieve better heat transfer and present better mechanical properties. As shown in Figure 1, the practical sizes used in engineering, biology and chemistry have been converging over the years up to the order of nanometers. This evolution clearly demonstrates the multidisciplinary nature of nanotechnology.

Synthetic polymers are within the field of biomaterials, the most diverse and have significant advantages over other materials. Due to its versatility, its use has spread rapidly in many fields of medicine. When designing a synthetic polymer it is generally aimed at satisfying a need, there is no single definition of smart polymers; however the most accepted definition is a polymer that under an external stimulus undergoes changes in its physical and/or chemical properties. The first time that the term "smart polymer" was used was in a newspaper article of the year 1982. This paper described how a group of researchers of the University of Michigan used electro rheological fluids to create smart materials. These fluids have the potential to change viscosity almost instantly in response to an electrical current. The fact revealed the existence of a new type of materials capable to modify its properties under an external stimulus. Two years later, in 1990, Mamada *et al.*, published an article in which they reported photo induced phase transitions of a gel (3). A year later, in 1991, appeared a review article on functional conducting polymers, which envisioned its potential application as smart materials inteligentes (4).

Special types of polymeric biomaterials are the hydrogels. Generally, the polymeric gels consist of monomers that are polymerized in long chains that crosslink chemically or physically to form a three-

dimensional network. An important feature of the hydrogels is that its soft consistency is similar to that of living tissue. However, obtaining this type of compounds at nano level is still under research. Currently, one method that has not been fully exploited to obtain nano-sized hydrogels is the microemulsion polymerization. Microemulsion polymerization is a synthetic procedure which permits to obtain particles with sizes smaller than 100 nm. One of the main problems of this method is the high amounts of surfactants used to stabilize the system, but nevertheless it is a useful tool for the synthesis of chemically designed nanogels that can be modified in post-polymerization processes.

The so-called stimulus-response nanogels or "smart" have emerged as a promising new class of materials with pharmaceutical applications. In these systems, small changes in some environmental variable such as temperature, pH, ionic strength, leads to a reversible phase transition in the structure of the gel. The poly(N-isopropylacrylamide) (PNIPA) hydrogel presents a well-defined Lower Critical Solution Temperature (LCST) in water near 32°C, above this temperature the gel structure collapses resulting in a sharp swelling, which is reversible if the system temperature returns to below 32ºC. Furthermore, when these materials have ionizing functional groups are sensitive to changes in pH. However, when these do not possess any ionizing functional group, pH has no effect on its swelling. The pH affects these systems similarly to temperature, so that a given change in pH of the medium makes the nanogel to swell, leading to an increased pore size of the polymer network, this facilitate molecules migration toward outside of the nanogel. This process is known as "liberation" and is the principle governing the current drug dispensing systems. Within the drug dosing systems we can find two basic types, which are the starting point for designing new mechanisms of transport and drug delivery, these are called "controlled release" and "targeted release".

Controlled release systems are those that try to eliminate or reduce side effects of a particular therapeutic drug producing an adequate concentration of the drug in the body. Its primary objective is to achieve a kinetic zero-order release to avoid changes in the concentration of the drug in the body, which does not occur with conventional dosing systems. Targeted delivery systems are devices that try to ensure that the active ingredient is released into the specific place, and that no release occurs elsewhere in the body. A good example of this type of system is the specific action devices for anticancer drugs delivery. Various investigations in recent decades have concluded that in different types of cancerous tissue, folic acid receptors are found in amounts significantly higher than those found in normal tissues (5-8). Consequently, an interesting idea is to take advantage of such biological markers for the design of targeted delivery systems of anticancer drugs using folic acid in the hydrogel molecule (9-11).

The current therapy against cancer is based mainly on chemotherapy, a procedure based on the administration of drugs capable to destroy cancer cells, but they also affect healthy tissues. The progress of recent years in this therapy show promising results for cancer patients. However, today traditional chemotherapy cures only a few types of cancers and has important secondary effects on the patient.

Harmful side effects of chemotherapy could be reduced considerably by using targeted release drugs. In this context, specific systems targeted release of antineoplastic agents, besides having a guide molecule toward cancer cells, requires that affects only the diseased tissue. Micro and nanogels sensitive to external stimuli could be used if they were able to respond to physical or chemical differences between healthy and tumor cells. Recent studies show that folic acid-functionalized systems are introduced into the interior of cancer cells through a process of receptor-mediated endocytosis of folic acid (12,13). These receptors (folate receptors) after binding with folate initiate a process of endocytosis where vesicles are formed[14]. Furthermore, this research shows that most of the endosomes that are introduced into the cell by the folate-receptor complexes have a pH between 4.7 and 5.3, being the average value of 5.0 (15). These pH values, which differ of blood pH (7.4), are precisely the crucial point in designing a delivery system for an antineoplastic drug. Then precise delivery can be achieved through a controlled-release drug device capable of making a pH dependent release, so that at the acidic pH inside the endosomes the drug is delivered, but not at the physiological pH.

The synthesis of nanoparticles chemically modified, so that their properties allow them to react to different stimuli by the environment around them, such as pH, temperature or ionic strength, is one of the most promising areas in current scientific research. Polymeric nanoparticles can be used as carriers of specific active ingredients, since they can protect them from unwanted chemical conditions (found in the body) and then release them under defined chemical changes. Features such as the potential functionality, rapid response to external stimuli, and its large surface area, make nanogels excellent candidates in various biomedical applications.

3. OBJETIVES

This chapter aims to show that smart polymeric nanoparticles can be synthesized, able to respond to external stimuli, specifically to changes in pH and temperature. To achieve this objective were consthree stages. The first, synthesizing reactive monomers i) a reactive monomer capable of being used in post-polymerization reactions to produce functional nanogels which combine both the ability to respond to pH and temperature. In this case, the monomer chosen was nitrophenyl acrylate or NPA. This monomer has a very "versatile" leaving group capable to react with nucleophilic reagents producing functional derivatives, ii) Synthesizeidered two monomers that are functionalized to produce the sensitivity to pH and temperature without having to be modified by post-polymerization processes. Furthermore, these monomers need to allow coupling of the guide molecule, folic acid, in order to be used as targeted delivery

Figure 2. Photograph of a poly(acrylic acid) (PAA) hydrogel: A dry (xerogel), and, B swelled.

systems through endocytic processes of the ligand-receptor type. The second stage; once synthesized the monomers, the next stage is to obtain nanoparticles using a microemulsion polymerization process. A reverse microemulsion polymerization is used for nanogels containing NIPA and a direct microemulsion polymerization for nanogels containing monomers chemically modified to obtain pH and temperature-sensitive nanohydrogels. N-isopropyl acrylamide, which presents a LCST close at 32ºC, is used as the base monomer. Third stage: Once the nanogels are synthesized and purified, the folic acid (guide molecule) is incorporated into the polymer matrix. This incorporation is achieved by using the method known as EDC (16). This method involves prior activation of the folic acid that will allow the covalent union to the nanogel in order to act in a mechanism type "*Trojan horse*", participating in the process of receptor-mediated endocytosis, as has been previously described. Another objective is to study the ability to release the drug from the smart nanohydrogel.

4. HYDROGELS

Hydrogels are crosslinked polymeric materials that form a three-dimensional network which can be natural or synthetic and that swell in contact with water to form soft and elastic materials. The flexibility of the polymer chains enables the entry of solvent molecules causing the hydrogel to swell, while its crosslinked structure prevents the dissolution of the hydrogel (17,18) (Figure 2).

Gels depending on the nature of the unions of the three-dimensional network can be classified into two types:

a) Physical gels. They have a three-dimensional network formed by unions that are not stable. Generally, the unions are of the van der Waals type forces, which are much weaker than covalent bonds. The three dimensional structure could be destroyed if the environmental conditions change (temperature, pH, concentration, etc.) (19).

b) Chemical gels. In this case the network is formed through covalent bonds. This type of bond is very strong and the three dimensional structure is not breakdown if the environmental conditions are modified. The appearance of a gel depends on its liquid/solid ratio, as this ratio increases the hydrogel becomes more soft (20). The hydrogels present a number of special features such as: i) They are hydrophilic due to the presence in its structure of water-soluble or ionizable groups (-OH,-COOH,-$CONH_2$,-CONH,-SO_3H), ii) Because of the existence of a three-dimensional polymer network structure they are water insoluble, iii) They have a soft and elastic consistency which varies depending on the monomer used and on the crosslinking density, and iv) They swell in water until reaching a thermodynamic balance, but without losing its shape. The dry form is called xerogel.

The hydrogels also have other types of interactions such as electrostatic forces, both attractive and repulsive and hydrophobic ionic interactions (21).

Hydrogels can be classified in various ways depending on which particular characteristics or properties are considered.
Based on the nature of the side groups they can be classified: in neutral or ionic (anionic, cationic, ampholytes) hydrogels. Depending on the number of monomer used in its preparation in: homopolymer, copolymer, or multipolymer.

Finally, they can be classified based on the physical structure of the hydrogels in amorphous, semi-crystalline, interpenetrated network and hydro-colloidal aggregates.

The hydrogels may also have a swelling behavior dependent on the external environment, and they are called "smart" hydrogels. Some of the factors affecting the swelling of such hydrogels include pH, temperature, ionic strength and electromagnetic radiation (22).

4.1. Hydrogels properties

There is a direct relationship between the properties of a hydrogel (or a polymer) and its structure. Therefore, when planning the properties of a hydrogel it should be considered several parameters (23) such as those shown in Figure 3 and discussed below.

This property affects others, such as permeability, mechanical properties and surface biocompatibility. The water content at equilibrium of a hydrogel is affected mainly by the nature of the hydrophilic monomer or monomers, the type and density of crosslinking and other factors such as temperature, ionic strength and pH.

Surface wettability and critical surface tension. The surface wettability depends on composition; hydrogels have low surface tension in aqueous or biological mediums.

Oxygen permeability. The rate of transport of low molar mass compounds through hydrogels is an important parameter for many applications. For example, oxygen permeability is of fundamental importance in contact lens applications. The oxygen permeability is governed by the equilibrium water content. When the hydrogels have water contents less than or equal to 30% oxygen permeability depends on the polymeric structure that determines the proportion of bound water and free water. However, with equilibrium water contents higher oxygen permeability is proportional to the logarithmic of the water content of the hydrogel (24).

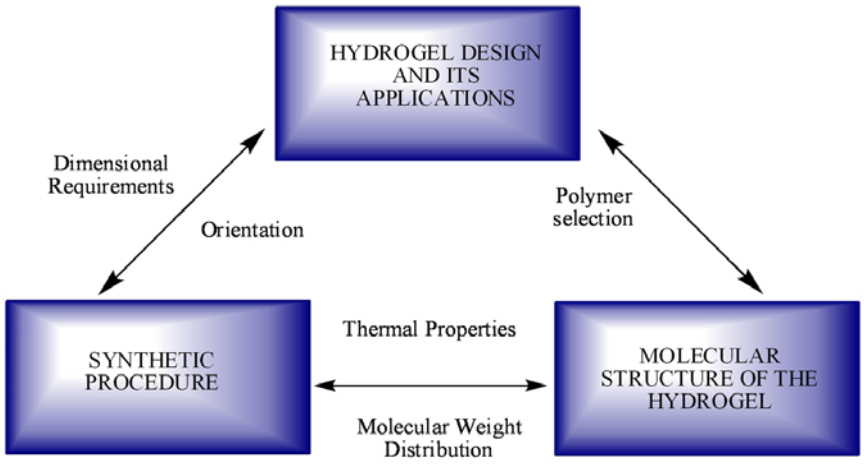

Figure 3. Planning scheme for the preparation of a hydrogel

Permeation selectivity. Hydrogel membranes have low surface tension in aqueous or biological fluids and their associated water content can control the permeability. Thus, it appears that the transport of ions across the membrane depends not only on its pore size, but on the water content, which is the factor that determines the pore size. Given that the water content depends on the molecular structure, hydrogels can be designed with differently membrane pore size and thus allow selective passage of different ions.

Optical properties. The refractive index of the hydrogels depends on their chemical composition, swelling degree and nature of the solvent that causes the swelling.

Mechanical properties. The mechanical strength is the ability of a material to withstand the action of a force without a break and is generally characterized by the stress that is induced. The response of a material to the action of a force can oscillate between two extreme behaviors:

Viscosity. Any force applied to the body causes it to flow, so that when the force stops the deformation is irreversible. The energy supplied is lost as heat.

Elastic. Once the applied force ceases the induced deformation disappears, recovering the work done. Between the both ideal models the actual behavior of the hydrogels is intermediate, viscoelastic behavior, which is defined as a combination of both behaviors.

4.2. Biocompatibility

In the field of biocompatible polymers the term refers to two aspects: a) the tolerance that the body has to foreign agents, and, b) to the chemical and physical during the time that it is in contact with the body. Since the hydrogels were introduced in the field of biomedicine they have shown to have great potential as biomaterials because of its good biocompatibility. This is because the physical properties of hydrogels are similar to those of living tissue which are its relatively high water content, soft and elastic consistency and low surface tension.

4.3. Hydrogels synthesis

In the synthesis of a hydrogel besides the usual elements in any polymerization such as solvent, monomer or monomers and the initiator, it requires a crosslinking agent, who will be responsible for the crosslinked structure (25-29). For synthesizing a hydrogel a large number of monomers can be used and they can be divided in three categories (30):

1) Monomer with no lateral ionizing groups: in this category are included acrylamide, N-vinylpyrrolidone, 2-hydroxyethyl methacrylate, etc.

2) Monomers with ionizable functional groups: acrylic, methacrylic, itaconic, sulfonic acids, and amines. When using these monomers hydrogels that absorb large amounts of water and have poor mechanical properties are obtained.

3) Zwitterionic monomers: in this case, the lateral substituent consists of two charged groups attached to the main chain. Its main characteristic is that swelling is higher in an acidic or basic solution than in purely aqueous.

There are several methods for preparing crosslinked hydrogels such as:

a) Radiation, in this case it is used emission of electrons, gamma rays, X-rays or ultraviolet light to excite the polymer and produce the crosslinking,

b) Chemical Reaction: This method is a copolymerization and crosslinking reaction between one or more monomers and multifunctional monomers which is present in very small quantities. Initiation systems that can be used are those used in conventional polymer synthesis: thermal decomposition of an initiator, temperature, ionic initiators, gamma radiation or redox.

Also it is possible to obtain crosslinking by the polymerization of a concentrated solution which can cause self-crosslinking through the elimination of hydrogen atoms in the polymer backbone, followed by combinations

of radicals. The choice of the crosslinking agent is essential to optimize the properties of the hidrogel (31).

Heterogeneous polymerizations. There are five types of heterogeneous polymerizations, i.e. where different phases coexist: emulsion, microemulsion, suspension, and dispersion and precipitation polymerization (15). Of these techniques only emulsion polymerization, microemulsion polymerization and precipitation polymerization allow obtaining particles of colloidal size.

Recently there have been reports of the synthesis of microgels using a new polymerization technique, microemulsion polymerization, which allows for smaller particle sizes (15-40 nm) than those obtained by emulsion polymerization (32).

Microemulsion polymerization. Microemulsion polymerization is an alternative to existing processes to produce latex containing polymer of high molar mass but with particle sizes smaller than those obtained by emulsion polymerization (33,34).

Microemulsions are fluid phases, microstructured, isotropic, optically transparent or translucent, at thermodynamic equilibrium, containing two immiscible fluids (usually water and oil), contrary to emulsion which are milky, opaque and thermodynamically unstable. An important difference between emulsion and microemulsion is that the amount of surfactant needed to stabilize the microemulsions (> 10% wt.) is much greater than that used in the emulsions (1 to 2% wt.). This greatly restricts the potential use of microemulsions in most applications, since it is required to use a formulation as cheap as possible (35). However, since by microemulsion polymerization it is possible to obtain monodisperse spherical microgels with diameters less than 50 nm (36-38) there is a promissory future for this technique.

The most important part of a microemulsion is the surfactant. Usually mixtures of surfactants are used to take advantage of each of them and their sinergy (39). Surfactants are organic compounds that are amphiphilic because they have hydrophobic groups (tails) and hydrophilic groups (heads). Therefore, they are soluble in both organic solvents and water.

There are four types of surfactants: a) Anionic, b) Cationic, c) Non ionic and, d) Amphoteric. Increasing the concentration of surfactant causes the formation of microstructures, which are aggregates of colloidal dimensions that exist in equilibrium with individual surfactant molecules. The concentration at which these microstructures (micelles) are formed is the critical aggregation concentration (CAC). The micellization phenomena is a cooperative process in which a large number of surfactant molecules associate to form a closed aggregate. When forming the micelles, the critical aggregation concentration is called critical micelle concentration (CMC). The critical micelle concentration depends on the number, length, type, branching or substitution of the hydrophobic chain and the nature of the polar group. The effects favoring micellization produce a decrease in the critical micelle concentration and vice versa.

Structure of a microemulsion**.** A microemulsion may have different structures depending on the relative amount of its constituent phases (aqueous phase and oil phase) of the surfactant and its HLB, besides the environmental temperature. The most general classification is as follows: a) oil in water microemulsion (O/W): globular structure consisting of oil droplets dispersed in the aqueous phase. The polar parts are oriented towards the outside of the micelle while the hydrophobic chains are oriented inward. The continuous medium is the aqueous phase. b) water in oil microemulsion (W/O): globular structure consisting of aqueous droplets dispersed in the oil phase. The polar parts are oriented inwards and the hydrophobic chains are directed outwards. The continuous medium is oil. c) Bicontinuous microemulsion: it consists of domains of both phases that are interconnected. In general, one can say that a water in oil microemulsion is obtained when the ratio water phase/oil phase is small and the HLB of the surfactant is low (1 to 3). The structure and formation of microemulsions is affected when a monomer is introduced into the environment. This is due to the co-surfactant role of some monomers such as acrylamide, or to the reduction that occurs in the solubility of the surfactant in both phases. Most water-soluble monomers reduce the interfacial tension when acting as co-surfactants and produce an increase in the zone of stable microemulsions. Thus, one can obtain microemulsions with low surfactant content and high amount of dispersed phase. When the amount of dispersed phase increases occurs a change in structure from globular to bicontinuous (see Figure 4). The structure of the microemulsions, influence markedly the polymerization rates (40).

When is added to the medium a salt or an ionic monomer, latex stabilization is achieved (41). It is known that the addition of an electrolyte to an aqueous solution produces a variation in the cloud point, i.e. the point at which the solubility changes. When this addition causes a migration of surfactant molecules into the oil phase, increasing the packing of it at the interface, it favors the formation of the microemulsion, due to an increase in the solubility by the presence of salt (salting out). If instead there is a decrease in the cloud point, there is a decrease in solubility by the presence of the salt (salting in). These phenomena are usually related to changes in the water structure around the ions (42) which modify the interactions between water and the surfactant. Ions such as Na^+ and K^+ decrease the surfactant polar head, while ions such as SCN^- and I^-, favor the solvation of the surfactant making it more water soluble (43). In general, the introduction of an electrolyte with salting out effect causes a change in the hydrophilic-lipophilic (HLB) balance of the surfactant, shifting the optimum HLB to form a microemulsion towards higher values. Regarding the preparation method, there is a difference between these two types of dispersions, which focuses on the order of addition of components. In the emulsion case the addition order is very important, contrary to what happens in the formation

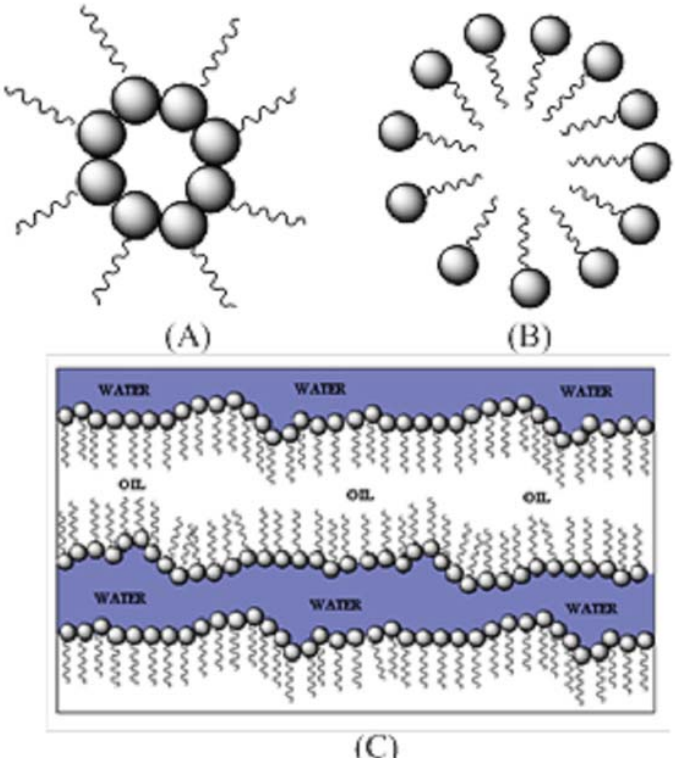

Figure 4. Microemulsion structures (A) reverse microemulsion, (B) direct microemulsion (C) bicontinous microemulsion.

of microemulsions, where it is not important.

Inverse microemulsion polymerization. The inverse microemulsion polymerization consists on polymerization of a microemulsion system where the continuous phase is the oil and the disperse is an aqueous solution of the monomer(s)

The inverse microemulsion polymerization of monomers soluble in water is a particularly suitable method for preparing high molar mass polymers at fast reaction rates (44), due to the high local concentration of monomer within each particle and that there is only a radical per particle which prevents growth termination by combination.

Polymerization in inverse microemulsion (W/O) is more interesting than the direct microemulsion polymerization (O/W), since they can produce latex with high solids content using moderate surfactant concentration (45). This is due to the surface activity of the water-soluble monomers which decreases the surfactant necessary for the formation of the microemulsion. The products have characteristics of water inversion, not requiring the addition of another reverse surfactant to get it. Moreover, the latex formed with this type of microemulsion has a direct application as flocculating agents in waste water treatment and water purification (46).

Globular microemulsion polymerization. Most studies have been conducted in globular microemulsions oil/water (O/W or direct) or water/oil (W/O or inverse). One of the most studied systems is formed by acrylamide in toluene, using AOT (sodium dioctyl sulfosuccinate) as surfactant. With this system it has been tried to understand the polymerization mechanism of such microemulsiones (47). During polymerization there is an increase in the number of micelles that have a radical (particles) indicating that grow by adding monomer from other inactive micelles, by monomer diffusion through the continuous phase. According to Candau, throughout the reaction there is an excess of surfactant which stabilizes the micelles and particles (48). Two populations appear, particles with diameter less than 50 nm and micelles with diameters around 10 nm. The number of particles continuously increases during the reaction due to particle nucleation by entry of radicals into micelles.

5. SWELLING THEORY

A three-dimensional polymer network can absorb large amounts of water or other compatible fluid. During swelling, the network chains are elongated but this expansion is accompanied by the appearance of a retractile force in opposition to the process of swelling until a balance between the two forces is reached (maximum or equilibrium value). Therefore, there is an analogy between swelling equilibrium and osmotic equilibrium.

The theories that describe the equilibrium swelling of gel/solvent systems are an extension of those that define the phase equilibrium in solutions of linear polymers. The

thermodynamic equilibrium is reached when the chemical potential (μ) of each component i, is the same inside (α) and outside (β) of the gel, i.e. when there is no net transfer of solvent through the interface (49)

$$\mu_i^{\alpha} = \mu_i^{\beta} \quad (1)$$

Therefore, the osmotic pressure (π) in a gel must be zero when equilibrium is reached. When i represent the solvent (component 1), Equation (1) is written in the form:

$$\Delta\pi_s = \frac{\mu_1^{gel} - \mu_1^{ex}}{V_1} = 0 \quad (2)$$

where $\Delta\pi_S$ is the osmotic pressure and V_1 is the solvent molar volume.

By the Flory-Rhener theory (50), $\Delta\pi_S$ can be expressed in terms of the elastic contributions of the deformed polymer chains from its reference state ($\Delta\pi_{el}$), the osmotic pressure of the polymer/solvent mixture ($\Delta\pi_{mix}$) and of the electrostatic effects ($\Delta\pi_{ion}$) (in the case of ionic microgels, The latter term is also known as the Donnan effect

$$\Delta\pi_S = \Delta\pi_{el} + \Delta\pi_{mix} + \Delta\pi_{ion} \quad (3)$$

Each one of the terms is calculated by:

$$\Delta\pi_{el} = \frac{N_c kT}{V_0}\left[\left(\frac{\varphi}{2\varphi_0}\right) - \left(\frac{\varphi}{\varphi_0}\right)^{1/3}\right] \quad (4)$$

$$\Delta\pi_{mix} = \frac{N_A kT}{V_s}\left[\varphi + \ln(1-\varphi) + \chi\varphi^2\right] \quad (5)$$

$$\Delta\pi_{ión} = \frac{fN_c kT}{V_0}\frac{\varphi}{\varphi_0} \quad (6)$$

where N_A is the Avogadro's number, N_c is the effective number of chains, k the Boltzmann constant, T the temperature, Vs is the molar volume of the solvent, φ_0 and V_0 are the volume fractions of polymer and no-swollen gel respectively ($V_0 = \pi d_0^3/6$), φ is the volume fraction of polymer in the swollen gel, χ is the polymer-solvent interaction parameter and f the number of counterions per polymer chain. Assuming that the swelling is isotropic, we can establish the following relation between the diameter of a spherical microgel and the polymer volume fraction

$$\frac{\phi}{\phi_0} = \frac{V_o}{V} = \left(\frac{d_0}{d}\right)^3 \quad (7)$$

where d_0 and d are the collapsed and swelled diameters respectively.

The terms and Δπion commonly favor the entry of the solvent and are a function of the polymer-solvent interaction parameter (χ) and the number of charges respectively. However, Δπel opposes to the entry of solvent. This is the solvent will penetrate the hydrogel until the sum of the terms in equation 3 becomes zero (equilibrium point).

The Flory-Rehner model uses several assumptions that have been object of many discussions and for this reason more realistic models have been developed, particularly for describing the effect of mixing polymer/solvent, However, these models are mathematically more complex.

5.1. Factors affecting the swelling

The crosslinking ratio is one of the most important factors affecting the swelling of hydrogels and is defined as the ratio of moles of crosslinking agent to the monomeric repeat units. Highly crosslinked hydrogels have a more compact structure and swell much less than the same hydrogel with a lower amount of crosslinks. The molecular structure of the polymer can also affect the swelling behavior. Hydrogels containing hydrophilic groups swell to a greater extent than those containing hydrophobic groups, since these groups collapse in the presence of water, minimizing their interaction with water molecules and resulting in hydrogels with less capacity to absorb water (51).

6. SMART NANOGELS

Smart nanohydrogels show volume changes in response to changes to external conditions. The polymer network can change its volume in response to a change in the environment such as temperature, pH, solvent composition, electric stimulation, the action of electric fields, etc. Over the past decade most research focused on the effects produced on the polymer networks due to changes in pH and temperature, since these variables are crucial in physiological, biological and chemical systems. Table 1 shows the different stimuli-response to various external factors.

The combination of molecular interactions such as van der Waals forces, hydrophobic interactions, hydrogen bonding and electrostatic interactions, determine the degree of swelling of the hydrogel at equilibrium.

Applications of smart hydrogels: Drug delivery, separation processes, bioreactions, artificial muscles, enzyme immobilization (52).

7. NANOGELS SENSITIVE TO CHANGES IN pH

When a gel contains ionizable groups is pH-sensitive, since the ionization degree is determined by the pH in terms of the ionization equilibrium constant. Changes in pH induce changes in the degree of ionization of the

Table 1. Effect of Different External Stimuli on the release of Bioactive Molecules from Smart Nanohydrogels

Stimulus	Hydrogel Type	Release Mechanism
pH	Acidic or basic hydrogel	Change in pH-swelling-release of drug
Ionic Strength	Ionic hydrogel	Change in ionic strength-change in concentration of ions inside the gel-change in swelling-release of drug
Chemical species	Hydrogel containing electron-accepting groups	Electron-donating compounds-formation of charge-transfer complexes-change in swelling-release of drug
Thermal	Thermo-responsive hydrogel	Change in temperature-change in polymer-polymer and water-polymer interactions-change in swelling-release of drug
Enzyme substrate	Hydrogel containing immobilized enzymes	Substrate present-enzymatic conversion-product changes swelling of gel-release of drug
Electrical	Polyelectrolyte hydrogel	Applied electric field-membrane charging-electrophoresis of charged drug-change in swelling-release of drug
Magnetic	Magnetic particles dispersed in microspheres	Applied magnetic field-change in pores in gel-change in swelling-release of drug

Table 2. Chemical groups sensitive to changes in pH

Anionic groups	Cationic groups
- COO-	- NH^+ -
- OPO^-_3	- NH^+
- OSO_3^-	- NH_2^+
- SO_3^-	- NH_3^+
- OCS_2^-	- $NRNH_2^+$
- PO_3^{2-}	- NR_2H^+
- PO_2^{2-}	- S^+
- SiO_2^{2-}	- P^+ -

hydrogel ionizable groups and, therefore, a change in the degree of swelling of the hydrogel. Table 2 shows the ionizable groups that can produce changes in the swelling of the hydrogel by changes in pH.

8. THERMOSENSITIVE NANOGELS

Recent studies show that it is possible to produce hydrogels with a particular transition temperature or even to synthesize hydrogels with various transition temperatures (53).

One of the most studied polymers, which respond to temperature changes in the external environment, is the poly(N-isopropyl acrylamide) (PNIPA). This polymer undergoes a phase transition in water at 32°C. It goes from a hydrophilic state (swollen) below this temperature to a hydrophobic state (shrunk) above it. In recent years, phase transitions and critical phenomena in polymer gels have attracted much attention. Poly(N-isopropyl acrylamide) (PNIPA) occupies a central position in the field of temperature-sensitive hydrogels or commonly called thermosensive hydrogels due its well-defined Lower Critical Solution Temperature (LCST) in water at certain temperatures. The formation of LCST PNIPA gels is due to amphiphilic nature of the monomer unit itself. The special properties of PNIPA make it a good candidate material for many applications, such as artificial muscles, drug delivery systems, reversible surfaces, separation membranes, enzyme immobilization, catalysis substrates actuators and chemical valve (54,55).

Consequently, the LCST of a polymer can be adjusted by varying the content of the hydrophilic or hydrophobic co-monomers.

8.1. Nanogels sensitive to other variations

Electromagnetic radiation is a parameter easy to control. Two methods have been used to induce volume phase transitions in response to changes in light intensity: 1) ionization by ultraviolet light radiation, for example, gels made of copolymers of NIPA and photosensitive molecules. At an appropriate temperature gels swell in response to ultraviolet light radiation and collapse when they fail to being illuminated, 2) Heating by illumination with visible light. This phenomenon is characterized by a temperature increase within a thermosensitive gel. This is the case of a hydrogel formed by the thermosensitive NIPA monomer, and the chromophore Chlorophyll (56). In the absence of light the gel volume changed continuously by varying the temperature, while lighting the transition temperature decreases and beyond a certain threshold of irradiation, the volume phase transition is discontinuous, 3) Electric field sensitive gels. It was reported that the intensity of electric current and the composition of the gel affected the release mechanism of a drug (57). The most important effect seems to be the migration and redistribution of counterions and ions within the gel, and 4) Gels sensitive to biochemical reactions. A gel can undergo a phase transition when in the medium there are present biochemically active elements such as enzymes or biological receptors.

9. NANOGELS CONTAINING ONE SPECIFIC BIOLOGICAL RECEPTOR

An important tool in therapy for various diseases is development of polymeric complexes with bioactive properties i.e. that have the capacity to interact with cellular mechanisms, has had a considerable increase due to the multiple applications you can have by coupling the biological receptors within polymeric matrices. Among these biological receptors are: acetylcholine, cytokine, Insulin, T cell, transforming growth factor beta, phosphotyrosine phosphatase, guanylyl cyclase, Muscarinic receptors M1, M2, M3 and M4, Nicotinic, mineralocorticoid, etc.

One biological receptor that has attracted much interest from the scientific community is the folic acid

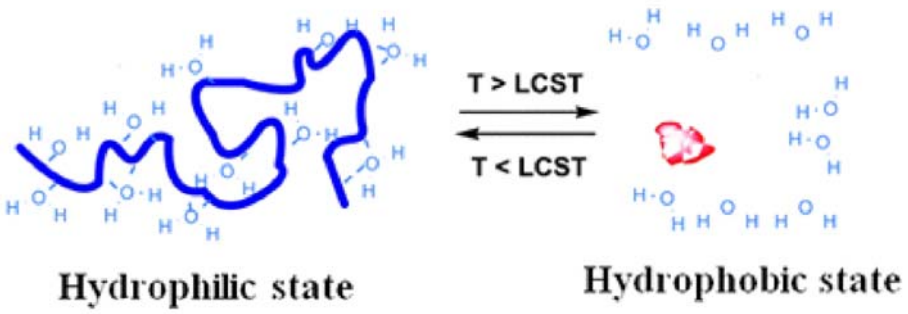

Figure 5. Diagram of the response to temperature by a smart polymer.

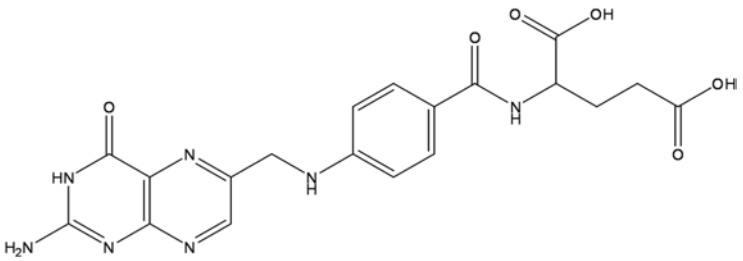

Figure 6. Molecular structure of folic acid.

receptor (58). The protein encoded by this gene is a member of the folate receptor family (FOLR). The members of this family of genes have a high affinity for folic acid and of various folic acid derivatives, in addition to mediate the delivery of 5-methyl tetrahydrofolate inside the cells. This gene has 7 exons, exons 1 to 4 encode the 5 'UTR and exons 4 to 7 encode the open reading frame. Due to the presence of promoters, there are multiple transcription sites and alternative exon splicing sites; also there are several variants of the transcription derived from this gene. The importance of the folate receptor is that in various diseases this gene is overexpressed on the cell surface which makes it possible to capture the folates through the cellular process of receptor-mediated endocytosis or RME (59). Folic acid, whose chemical structure is shown in Figure 6, is a natural vitamin required for transfer reactions in many metabolic processes and is now a promise in the vectorization of anticancer drugs. Several investigations in recent decades have concluded that folic acid receptors have a preferential expression in ovarian, endometrial, lung, kidney, colon cancers, among others, but very limited in normal tissues (60-63). This folate-cancer cells specificity is used for the design of anticancer systems using folic acid as the ligand molecule to direct the drug to the tumor cells (64-66). Folic acid, in addition to its high specificity towards tumor tissues, offers potential advantages, which include its small size, favorable pharmacokinetic properties, and reduced immunogenicity allowing repeated administration, high availability and safety. Moreover, folic acid is very stable at different temperatures (< 250º C) and in a variety of solvents, and in slightly acidic or basic media. Another important characteristic is that folic acid is cheaper than monoclonal antibodies which require careful handling to avoid denaturalization. All this, combined with its relatively simple chemical conjugation, make it an interesting and promising molecule in antitumoral specific therapies (57).

To determine at which pH these folate conjugates are subject to when passing into the intracellular environment, in studies it has been measured indirectly the pH of individual endosomes containing folate conjugates and it was found that although this value can vary considerably (4.7-5.3), the average pH is 5.0 (68, 69). This pH is markedly different of the physiological pH of the blood stream and of any healthy tissue (pH = 7.4).

10. RECEPTOR-MEDIATED ENDOCYTOSIS (RME)

Endocytosis is a cellular process by which the cell introduces large molecules or particles, and does so by including them in an invagination of the cytoplasm membrane, forming a vesicle that eventually breaks off and enters the cytoplasm. When endocytosis leads to the capture of particles is called phagocytosis, and when only portions of liquid are captured is called pinocytosis. Pinocytosis traps substances indiscriminately, while receptor-mediated endocytosis only includes those molecules that bind to the receptor being this type of endocytosis very selective. The RME allows cells to take specific macromolecules called ligands, such as proteins that bind insulin (a hormone), transferrine (a protein that binds to iron), cholesterol carriers and low density lipoproteins.

1.The RME requires specific membrane receptors to recognize a particular ligand and link to it,

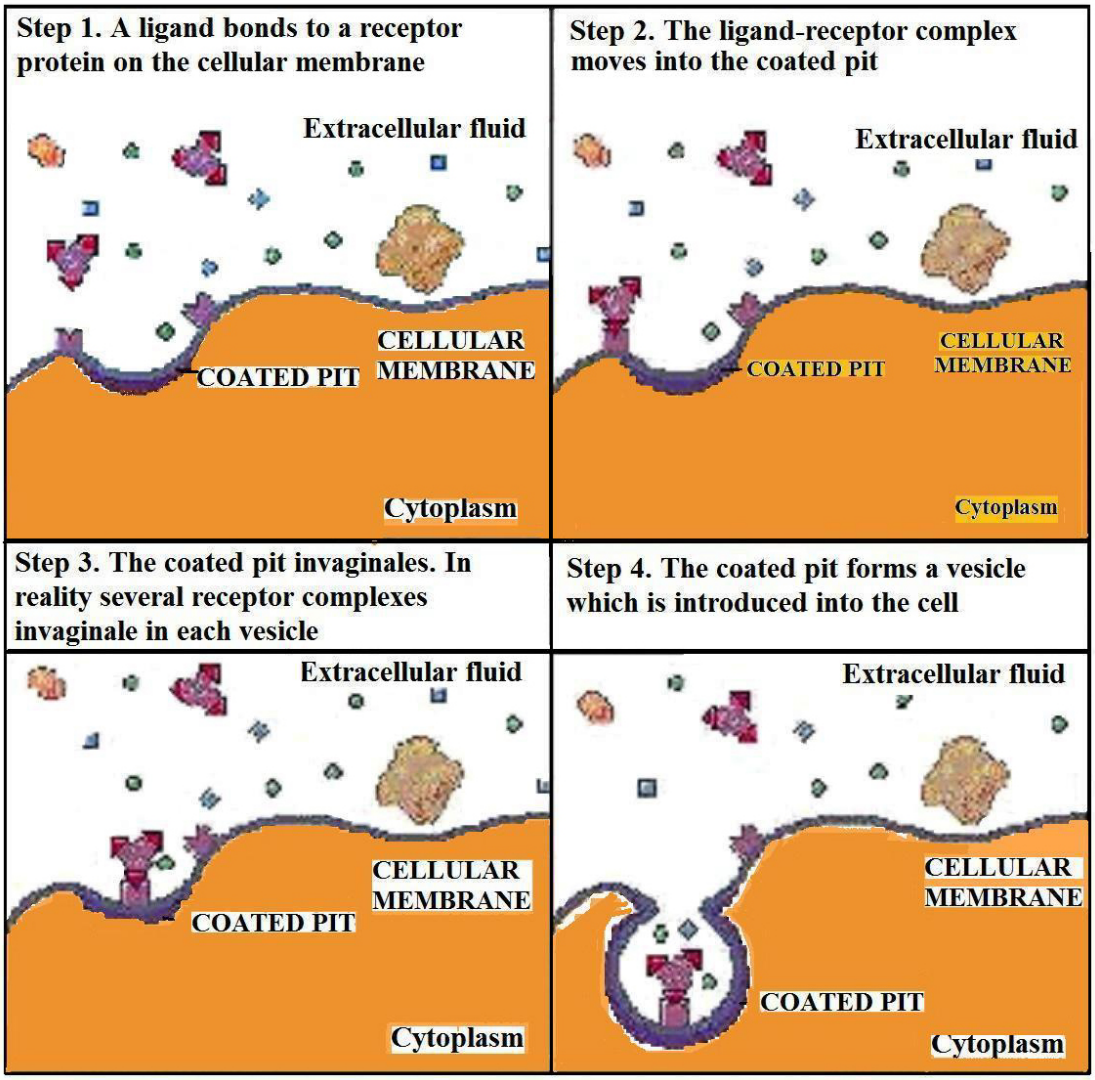

Figure 7. Schematic of receptor-mediated endocytosis process.

2. ligand-receptor complexes migrate along the surface of the membrane structures called coated pits. Just inside the cytoplasm, these pits are lined with a protein that can polymerize into a cage-shaped structure (membrane vesicle), and,

3. The vesicles move within the cytoplasm, taking the ligand from the extracellular fluid to within the cell. The materials bound to the ligand, such as iron or cholesterol, are introduced into the cell, then the empty ligand returns to the surface.

11. APPLICATIONS OF HYDROGELS

The importance of hydrogels as biomaterials is due to their physical properties, which are similar to those of living tissues. This similarity is due to its high water content and soft and elastic consistency. Nowadays the most important applications of hydrogels are: Contact lenses, Tissue prostheses, Human tubular prostheses, Coating of sutures, Surgery, Hemodialysis, Hemoperfusion, etc.

11.1. Application of smart nanohydrogels in controlled release of drugs

Devices for controlled drug release are particularly important applications that exploit the collapse-swelling properties of the polymers. In this field are particularly important hydrogels containing poly(N-isopropyl acrylamide) (PNIPA), which generate matrix that can exhibit thermally reversible collapse (70) (Figure 8).

Smart nanogels have the potential to be used with a variety of drugs and the loading and release of the active principles can be adapted to a wide range of environments (71-74). By a proper synthesis a delivery systems is prepared to respond to a pre-designated value of pH and/or temperature to release the drug

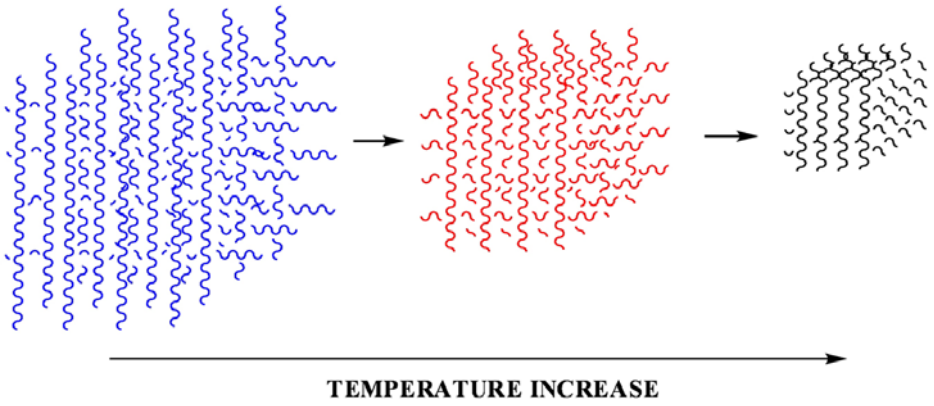

Figure 8. Behavior of a temperature-sensitive hydrogel.

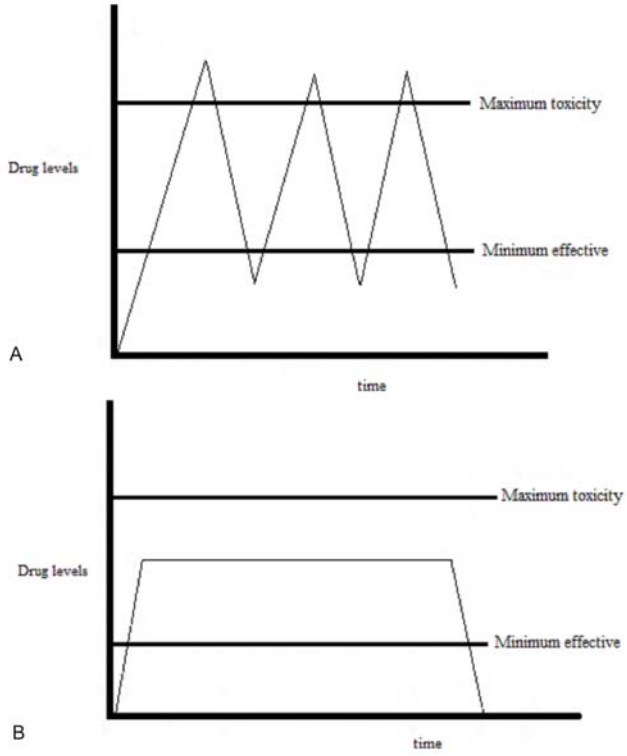

Figure 9. Comparison between a) the traditional delivery systems and b) controlled release systems.

In traditional drug administration using a single dose, the concentration of the drug in the body increases to a maximum value and then decreases due to excretion and/or metabolic conversion (Figure 9a). To achieve a therapeutic effective level for an extended period of time it is required high doses; however, but the drug concentration should remain below the toxic value to avoid causing harmful effects on the body (75).

A more effective approach to a constant drug concentration is by drug dosing at regular intervals of time. However, this procedure can be impractical and in many cases, may cause alternating periods of ineffectiveness and toxicity (76). By contrast, controlled drug release systems allow a dosage of active ingredient in a continuous and uniform way, and with a single dose the concentration of it is kept outside the limits of ineffectiveness and toxicity (Figure 9b).

11.2. Treatment of diseases by using smart nanogels.

One of the great advances in applied nanotechnology is the implementation of systems with polymeric matrices to combat chronic diseases and conditions which allow the use of specific markers to achieve a positive response to the administration of active ingredients. Two of the diseases that have attracted the attention of nanotechnology and biomedicine researchers are cancer and tuberculosis.

Cancer. Because traditional forms of advanced cancer adopt a variegated form with branches, which is similar to that of a marine crab, this illness is named "cancer"[77]. It is sometimes considered synonymous with the terms "malignant neoplasm" and "malignant tumor". Cancer treatments are based on: surgery, chemotherapy, radiotherapy and a new treatment called biological therapy which includes hormone therapy, immunotherapy, and non-cytotoxic targeted therapeutics, where among them are found polymeric devices have specific ligands. Cancer treatment is multidisciplinary where there is cooperation between different professionals. Given the current inability of medicine to cure more aggressive cancers in advanced stages of evolution, it is proposed to use vehicles that can dose the anticancer drug on site to have less aggressive treatment.

The chemotherapy term is usually reserved for drugs used in treating neoplastic diseases and have the function of preventing the reproduction of cancer cells. These drugs are called cytostatic or cytotoxic drugs. Antineoplastic therapy has a major limitation, which is its low specificity. The mechanism of action is to induce a cellular alteration in either nucleic acid synthesis, cell division or protein synthesis. The action of different cytostatic varies according to the administered dose. Chemotherapy usually is not the only cancer treatment; it is often combined with surgery and radiotherapy, a modality that is called combination therapy or multidisciplinary therapy.

There are currently more than 100 antineoplastic drugs that are commonly used:

1. alkylation agents: Their mechanism of action is to induce damage to cellular DNA (both neoplastic and healthy) by incorporating alkyl groups, and thus alter or prevent cell replication.

2. Antimetabolites: Substances similar to natural components: folic acid analog, methotrexate; purine analogues, 6-mercaptopurine; pyrimidine analogues, 5-Fluorouracil, Ara-C, taxol, etc.

Vinca alkaloids which are anti-mitotic and anti-microtubule agents. They are now produced synthetically and used as drugs in cancer therapy (78) and as immunosuppressive drugs. These compounds are vinblastine, vincristine, vindesine and vinorelbine. Periwinkle extracts and derivatives, such as vinpocetine, are also used as nootropic drugs.

Cancer immunotherapy attempts to stimulate the immune system to reject and destroy tumors. BCG immunotherapy for the first phase (invasive) of bladder cancer uses instillation of attenuated live bacteria in the bladder, and is effective in preventing recurrence in up to two thirds of cases.

The main problem with these drugs is their inability to differentiate tumor cells from healthy cells. Very often cytostatic drugs are dosed simultaneously. These combinations have the advantage that can reduce the resistance of tumor cells to cytostatic and can extend its efficacy by using drugs with different mechanisms of action.

Most of the cytostatics are administered intravenously because they are very toxic and is necessary dose adjustment as accurately as possible. Normally they are administered in cycles with rest periods. In these rest periods normal cells, such as bone marrow, recover from the toxic effect of cytostatic, while the tumor cells are recovered to a less extent. In this way, by repeating the cycles, is gradually reduced the number of tumor cells until to achieve complete eradication, while normal cells recover after each cycle.

Tumor markers: a window on the development of specific delivery systems. Tumor markers are substances in the blood coming from the cancerous tissues. There are two kinds of tumor markers:

1. The first group of markers is associated with the presence of cancerous tissue, and unfortunately tend to be of little help when making a diagnosis (due that a number of different tumors produce these markers). However, they can be useful in following the progress of the disease before the tumor could be found clinically or by image.

2. The second set of markers is associated with certain tissues that have developed cancer. Generally speaking, these substances are not specifically associated with tumors and may have elevated values when there is no cancer present, because of the presence of certain substances (drugs). But unlike the previous group, a high level targets as responsible a particular tissue. Examples of these markers are APE, β-HCG, AFP, and linfolytic marker. Thus, if PSA is elevated, we must seek for prostate cancer. When high levels of β-HCG or AFP are presented, indicate the possibility of testicular cancer or a malignant teratoma. While a high level of thyroglobulin indicates the possibility thyroid carcinoma. The PSA, or prostate specific antigen (an antigen is not prostate cancer specific), is produced by a normal prostate. It is a protein called serine protease enzyme which normally acts as an anticoagulant to keep the semen liquid, and only small amounts escape into the blood under normal circumstances. An enlarged prostate for benign prostatic hypertrophy leaks greater amounts leaks like a cancerous prostate. Of course, the only accurate method to confirm whether a high level of PSA is due to cancer is a biopsy of the prostate. New research has shown that folic acid specific ligand is over expressed in cancer cells also being able to classify it as a tumor marker. Also, as mentioned in this report, the folate receptor is one of the 25 receptors that mediate endocytosis by receptors. The advantage of this discovery allows us to design polymeric matrices that can be used as vehicles for controlled release of drugs which are able to detect and attack cancer cells without causing damage to the healthy cells (low toxicity).

Tuberculosis. Tuberculosis (TB) is a contagious

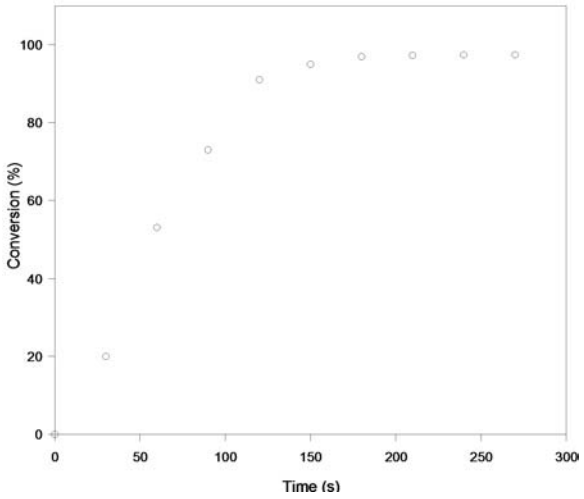

Figure 10. Plot of the monomer conversion as a function of time for the microemulsion polymerization of sample COP22.

lung disease that spreads through the air. When people with TB cough, sneeze, talk or spit, toss germs, known as tubercle bacilli. Simply inhaling a few bacilli is enough to become infected. However, not everyone infected with TB bacilli will become sick. The immune system kills the TB bacilli, or "walls off" where they can lie dormant for years. If the immune system does not control infection with TB bacilli, they multiply, producing the active form of the disease and damaging the body. Left untreated, each person with infectious TB will spread the germs to about 10 to 15 people every year. In 1999, WHO estimated at 3,689,833 the new cases of tuberculosis worldwide, although this organism estimate 8,500,000 total WHO reported in 2003, 8 million (140/100.000 inhabitants) of new TB cases, of which 674,000 (11/100.000 inhabitants) are co-infected with HIV. The prevalence of tuberculosis has a rate of 245/100.000 inhabitants and a mortality rate of 28/100.000. The 2006 WHO report estimated that 1.6 million people died from TB in 2005. The epidemiological trend of TB incidence is increasing worldwide, but the mortality rate and prevalence are decreasing (WHO-2003).

Tuberculosis treatment. Treatment of tuberculosis is performed with combinations of drugs, which are effective by six months of treatment. TB is curable, but early diagnosis is necessary (to obtain immediate medical attention) since it is a serious disease if not proper treated. Then, it is essential not to abandon the treatment because after cessation of treatment, the disease gets worse quickly and enhances the spread of drug resistant bacilli.

Recent research has demonstrated that within the pulmonary epithelium there is an associated receiver directly to the disease, the receptor lecitina128. The presence of a receiver capable of forming a polymer-ligand complex makes it a potential weapon in the treatment of tuberculosis through the design of devices capable of detecting this marker.

11.3. Smart targeted nanogels for cancer therapy

Smart nanogels (pH and temperature responsive) with dimensions ranging between 15 and 50 nm can be synthesized by microemulsion polymerization to be use in cancer therapy; above 50 nm the nanoparticles may cause clogging of the blood stream and below 15 nm are adsorbed by alveoli. The nanogels are vectored to target cancer cells using a guide molecule (folic acid).

The nanogels used in cancer therapy can be obtained following this synthetic procedure:

1.The microemulsion is prepared by dissolving in water the monomers: a thermo-responsive monomer (NIPA), a monomer with an ionizable group (N-pyridin acrylamide (NPAM)) (synthesized in our laboratory), a spacer monomer (2-acrylamidoethyl carbamate (2AAECM)) (synthesized in our laboratory) and N'N'-methylene bis-acrylamide (NMBA) as a crosslinking agent.

2. The aqueous mixture is added to the reactor which contained the organic phase (iso-parafinic hydrocarbon (ISOPAR M)) and the surfactants polyetoxylated sorbitan hexaoleate (ATLAS G-1086) and sorbitan sesquiolate (ARLACEL 83). To start the reaction, sodium disulfite (DSNa) is added to the microemulsion.

3. The initial temperature of the system is 25°C and rapidly increases to 40°C, due to the exothermic character of the reaction, returning to the initial temperature after 4 minutes.

4.After polymerization the nanogels are separated by precipitation, using reagent diethyl ether. The solvent is removed by decantation and the nanoparticles are dried in an oven at 50°C to constant weight.

5. Once the nanogels are purified, the next step is to incorporate the folic acid. First, a dissolution of folic acid is prepared by mixing it with 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDC) and tryethylamine, at 25°C, using magnetic stirring for one hour to produce activated folic acid. This mixture is dropped into a dispersion of nanogels in water to incorporate the guide molecule. The purification and the isolating procedure of the final product is carried out by dialysis using a phosphate buffer solution of pH = 7.4, and then distilled water. All of this procedure is performed in a dark environment to avoid degradation of the folic acid molecule.

Figure 10 shows that reaction rate is fast and high conversion is obtained (> 98%). Similar results were obtained for all the formulations.

Table 3 shows the formulations used for the polymerizations and the final average particle size of the nanogels. Particle size is measured by QLS using acetone as the dispersing media. In all cases the nanogels have average particle size in the nanometer range and narrow particle distribution (< 1.1). By increasing initiator concentration, particle size decreases because when more radicals are present more particles will be produced. When

Table 3. Particle size, D_p, of nanogels prepared using different formulations measured by quasielastic light scattering (QLS)

			Monomers composition (%)				
Sample	**%NMBA**	**%DSNa**	**NIPA**	**NPAM**	**2AAECM**	**%KNO_3**	**Dp (nm)**
COP20	5	1	95	5	5	0	45
COP21	5	2	95	5	5	0	43
COP22	5	3	95	5	5	0	40
COP23	5	4	95	5	5	0	38
COP24	5	5	95	5	5	0	36
COP25	4	5	95	5	5	0	40
COP26	3	5	95	5	5	0	42
COP27	2	5	95	5	5	0	41
COP28	1	5	95	5	5	0	41
COP29	5	3	95	5	5	0	42
COP30	5	3	95	5	5	0	40
COP31	5	3	90	2.5	7.5	0	42
COP32	5	3	85	7.5	2.5	0	41
COP33	5	3	85	10	5	0	40
COP34	5	3	95	5	10	2	35
COP35	5	3	95	5	5	4	33
COP36	5	3	95	5	5	6	30
COP37	5	3	95	5	5	8	28
COP38	5	3	95	5	5	10	28

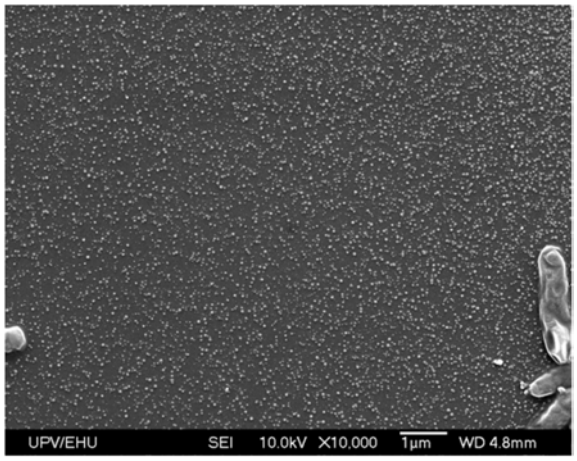

Figure 11. Scanning electron microscopy of a nanohydrogel obtained by microemulsion polymerization, and used in cancer therapy.

using water soluble salt there is a reduction on particle size. The incorporation of a salt reduces the size of the micelles by affecting the double electric layer and also reduces the monomer water solubility "*salting out*", which causes that smaller particles are formed. Similar results when using a salt were reported by Katime *et al.* (79) and Full *et al.*(80). Monomer composition and crosslinking agent concentration does not affect particle size.

In Figure 11 it can be observed the dimensions of spherical nanohydrogels obtained by SEM and used in cancer therapy. In this case the mean dimensions of the particles are less than 50 nm and with a narrow particle size distribution.

Figure 12 depicts swelling as a function of pH in aqueous solution. At pH below 4.0 the nanogels are in the swollen state with diameters between 50 and 60 nm, and as the pH increases the particles start to deswell and at a pH 5.5 are in the collapsed state, with diameters smaller than 22 nm. In this figure it can be observed that monomers composition affects the behavior of the volume phase transition of the nanogels. By increasing the concentration of pyridine groups, the volume phase transition shift at slight higher pH values. However, this shift does not affect the intended use since it is small and the swollen-deswelling behavior is in the needed particle size range. For those samples in which the composition was not modified, the pH at which the volume phase transition occurs was not modified.

Nanogels are loaded with 5-FU by preparing a nanogels dispersion in an aqueous phosphate buffer solution (pH = 4.0) under constant magnetic stirring for two hours. After this time, an aqueous solution of 5-FU (1000 ppm) is added and the agitation continued for 2 hours more. Once the nanoparticles are loaded with 5-FU, the pH media is changed to 7.4 causing the collapse of the particles. The nanogels are separated by decantation. The amount of 5-FU loaded is determined using the following equation:

$$C_{\text{Charge}} = C_{CS} + C_R \qquad (8)$$

where C_{Charge} is the concentration of 5-FU in the nanogels, C_{CS} is the initial concentration of 5-FU, and C_R is the concentration after the loading process.

Figure 13 shows the chromatographs of the 5-FU standards used to obtain a calibration curve to measure the amount of drug released from the smart nanogels.

Drug experiments are carried out at 37ºC (human body temperature) in an aqueous medium at pH 4.0 and 7.4, using the smart nanohydrogels (COP35-COP38) containing the folic acid. The amount of drug released (5-Fluorouracil) is determined by HPLC and UV-Vis, using Sink conditions. Figure 14 shows chromatographs of the aqueous solution after 24 hours of liberation, where it can be observed that at pH 7.4 a very small amount of 5-FU is released (t_r = 6.6 min; peak area = 59 arbitrary units), whereas at pH 4.0 the amount of 5-FU released is large (t_r = 6.6 min; peak area = 17.000 arbitrary units).

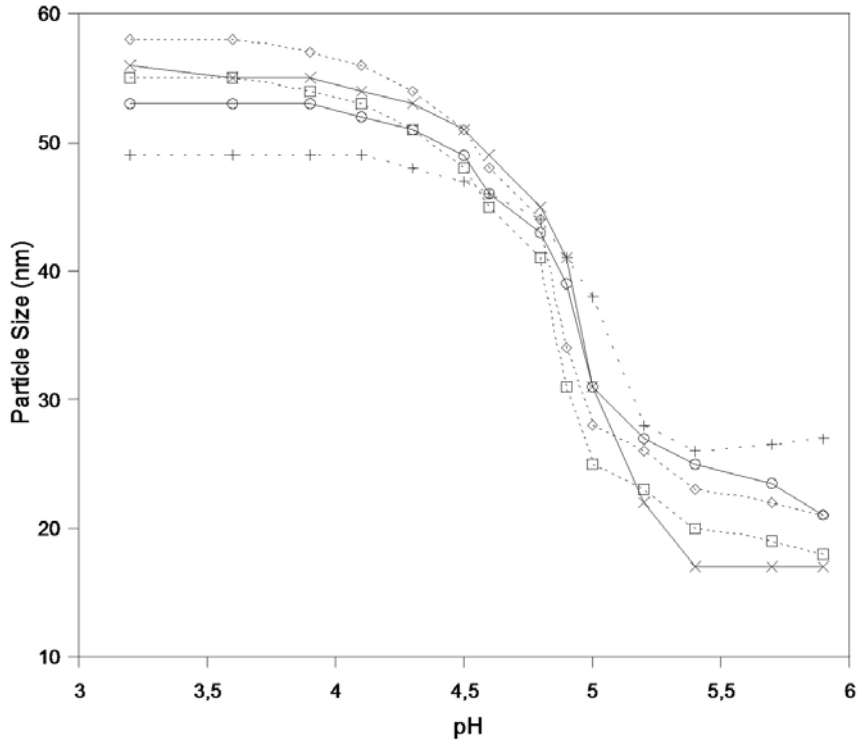

Figure 12. pH dependence of particle size of nanohydrogels with different proportions of ionizable groups.

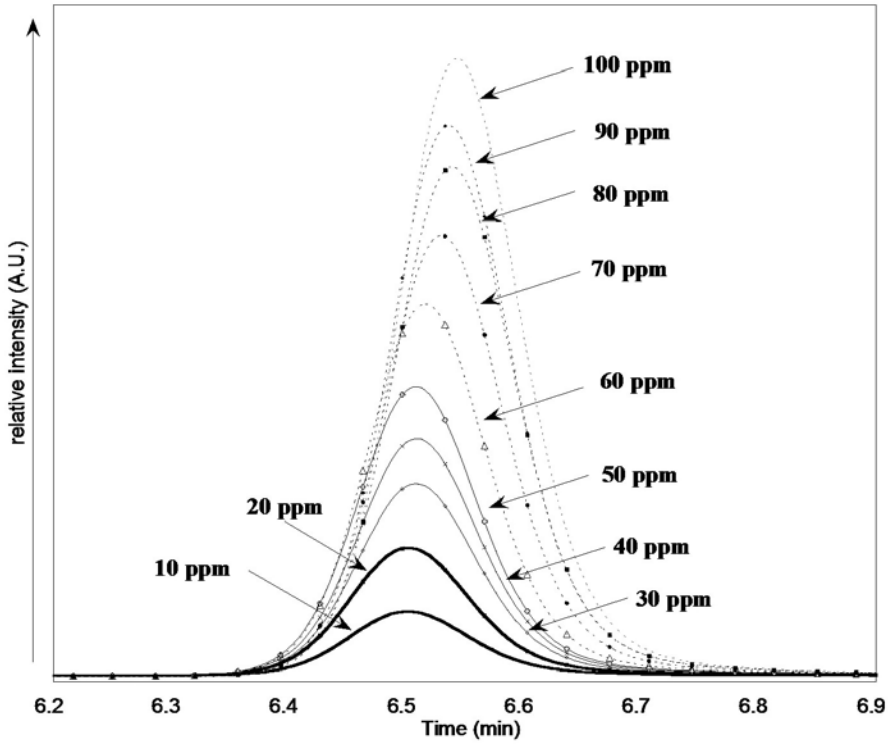

Figure 13. Chromatographs of the 5-Fluorouracil standards employed in the calibration of the HLPC.

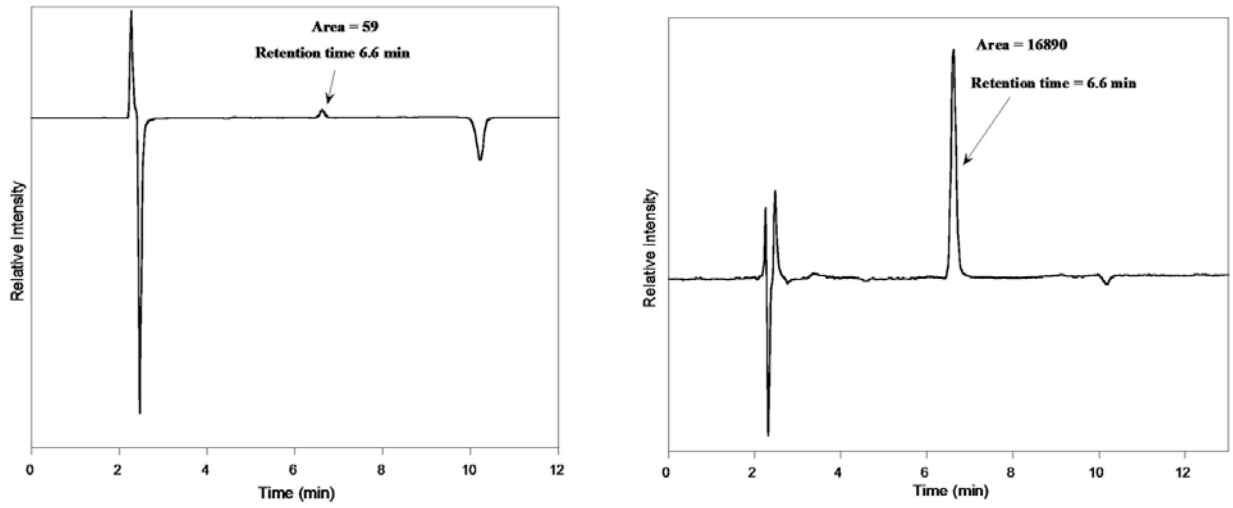

Figure 14. Chromatographs of the release of 5-FU from a smart nanohydrogel: a) at pH 7.4, and b) at pH 4.0.

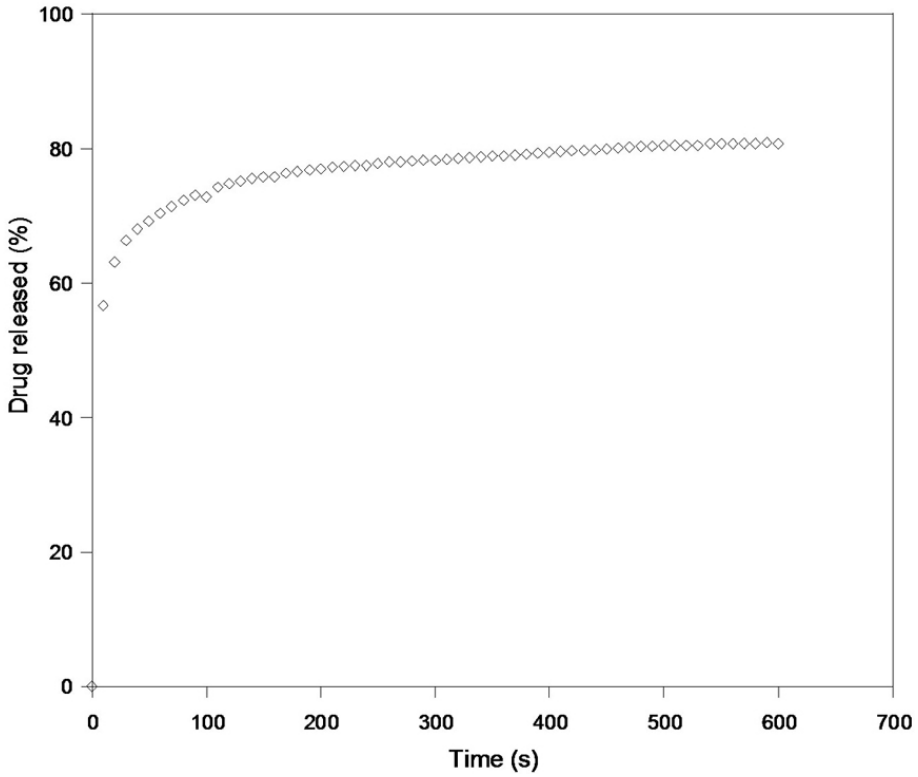

Figure 15. Drug release of 5-FU as a function of time from smart nanohydrogel at pH 4 and 37ºC.

Figure 15 shows the release of 5-FU, at pH 4.0 in aqueous solution at 37ºC. Drug release is fast after 600 s (10 minutes) around ≈ 80% is liberated. This fast release rate is favorable since once inside the cancer cell (where the pH is near to 5) the drug will be liberated and destroy it. This behavior is very important in this case, due to the fact that it is necessary to get, inside the cancer cell, a high antineoplasic concentration to be effective.

11.4. Smart nanogels for tuberculosis therapy

Smart nano-copolymeric matrixes are employed to release anti tuberculosis (anti–TB) drugs such as combined complexes of Ethambutol (EMB), Isoniazid (INH), Rifampicin (RMP) and Pyrazinamide (PZA).

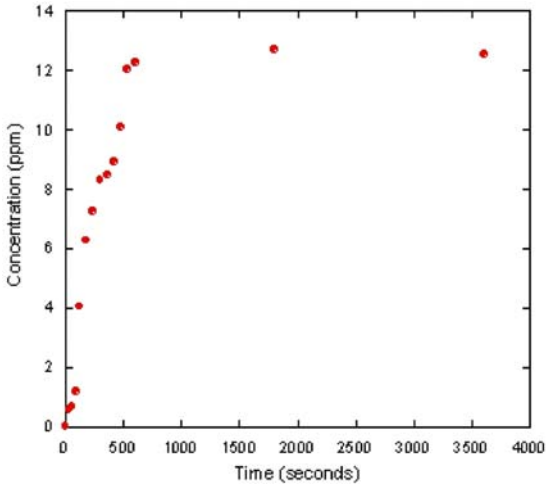

Figure 16. Anti-TB drugs release study from smart nanogels.

The nanogels are loaded using the following procedure:

1.TB drugs or ATD´s (Anti Tubercular Drugs) (INH, RIF, PZA and EMB) are dissolved in 10 mL of aqueous phosphate buffer solution (pH= 2.0) to obtain a total drug concentration of 10,000 ppm; the amount of each drug is that used in actual tuberculosis treatment (81, 82) (8.33, 16.67, 44.44, and 30.56% for INH, RIF, PZA and EMB, respectively). The mixture is maintained in constant agitation for five hours. Nanogels are suspended in 50 mL of phosphate buffer solution (pH = 2.0) and are maintained in constant agitation for five hours. Then, the solution of ATD's is added dropwise to the nanogels suspension and the mixture is kept under constant agitation for approximately 15 hours, then to collapse the nanohydrogels a few drops of NaOH solution (50% w/w) are added to obtain a pH of 7.4.

2.The nanogels are separated by centrifugation at 4500 rpm for 10 minutes. The loaded nanogels are washed with a phosphate buffer solution (pH = 7.4) and centrifuged again under the same conditions (4500 rpm for 10 minutes).

3.The nanogels loaded are placed in a Petri dish and dried in an oven at 50°C to remove any residual solvent.

The release of anti-TB drugs from the nanogels is carried out in 10 mL of phosphate buffer solution (pH = 2.0) at 37°C. At different times samples are taken to determine the amount of drug released. Measurements were done using a flow rate of 0.5 mL/min and a phosphate buffer solution of pH = 4.0 as the mobile phase, column temperature 50°C, and UV-vis detector at 200 nm. Figure 16 shows that drug release is fast and that after 600 seconds there are no significant changes on the amount of drug released.

Therefore, achieving the synthesis of a nanoparticle capable of responding to a specific change in pH and sensitivity to temperature which is capable of protecting an active substance inside the body and also capable to participate in cellular processes, such as the transport of substances and nutrients through the cell membrane, makes this system a novel development in cancer therapy which may be applicable, depending on their chemical structure and composition on the treatment of other diseases such as tuberculosis.

12. REFERENCES

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