UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Biológicas e Ingeniería

TÍTULO: pH SENSITIVITY POLYMERS FOR POTENTIAL BIOMEDICAL APPLICATION OBTAINED BY GAMMA-RAY

Trabajo de integración curricular presentado como requisito para la obtención
del título de Ingeniero Biomédico

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Dedicatory

Thank God for allowing me to successfully complete this very important and satisfying stage in my life.

To my mother, for being my primary support throughout my life. For her unconditional love and advice in good times and even more in bad times.

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Resumen

El cuerpo humano experimenta cambios en temperatura y/o pH que son ampliamente estudiadas como un mecanismo propuesto para la liberación controlada de fármacos. En la actualidad, con el avance de la tecnología, es posible el diseño de materiales altamente biocompatibles tales como hidrogeles sensibles a estímulos basados en polímeros que muestran propiedades favorable cuando se experimentan estos cambios. Los hidrogeles son una clase única de materiales blandos que consisten en redes de polímeros hidrófilos y retienen grandes cantidades de agua (Vermonden & Klumperman, 2015).

El presente trabajo fue realizado en el Instituto de Ciencias Nucleares en UNAM, el consiste en la síntesis de hidrogeles a base de un compuesto de agar y acido acrílico, reticulados por rayos gamma. El estudio de diferentes parámetros tales como dosis, concentraciones de monómeros, la temperatura durante la mezcla y el tiempo de reacción para determinar las condiciones óptimas durante la síntesis. Las muestras obtenidas se caracterizaron por diferentes técnicas. Para determinar los grupos funcionales se utilizó la espectroscopia FTIR-ATR. DSC realizó técnicas térmicas para determinar la transición vítrea (Tg), mientras que TGA analiza la descomposición de la masa frente a la temperatura. Asimismo, se realizaron caracterizaciones fisicoquímicas como hinchamiento máximo, cambios cíclicos de pH, ángulo de contacto y pruebas mecánicas. Además, se sintetizaron Nanopartículas de Plata (AgNP) para proporcionar una actividad antimicrobiana al hidrogel. La carga y el suministro de AgNP y ciprofloxacina emplearon la espectroscopia UV-VIS para determinar su longitud de onda y absorbancia de acuerdo con la Ley de Lambert-Beer. El material final incorporado con agentes antimicrobianos se sometió a pruebas microbiológicas.

PALABRAS CLAVES

Polímeros, Agar, Acido Acrílico, Hidrogeles, Nanopartículas de Plata

Abstract

The human body experiences changes in temperature and/or pH that are widely studied as a proposed mechanism for controlled drug delivery. Nowadays, with the advancement of technology, it is possible to design highly biocompatible materials such as stimuli sensitive hydrogels based on polymers that show favorable properties when these changes are experienced. Hydrogels are a unique class of soft materials that consist of hydrophilic polymer networks and retain large amounts of water (Vermonden & Klumperman, 2015).

The present work was made in the Institute of Nuclear Sciences at UNAM, which consist of the synthesis of hydrogels based on a compound of agar and acrylic acid, cross-linked by gamma rays. The study of different parameters such as doses, monomer concentrations, the temperature during the mixture, and reaction time to determine the optimal conditions during the synthesis. The obtained samples were characterized by different techniques. To determine the functional groups was used FTIR-ATR spectroscopy. Thermal techniques were performed by DSC to determine the glass transition (Tg), while, TGA analyzes the decomposition of the mass against the temperature. Also, characterization physico-chemical as maximum swelling, cyclic changes of pH, contact angle and mechanical tests were performed. Additionally, silver nanoparticles (AgNPs) were synthesized to provide an antimicrobial activity to the hydrogel. The load and delivery of AgNPs and and ciprofloxacin employed UV -VIS spectroscopy to determine its wavelength and absorbance according to the Lambert Beer-Law. The final material incorporated with antimicrobial agents was subjected to microbiological tests.

KEYWORDS

Polymers, Agar, Acrylic Acid, Hydrogels, Silver Nanoparticles.

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Abbreviations

%	Percentage
mL	Milliliters
g	Grams
mg	Milligrams
ug	Micrograms
°C	Celsius degree
°	Degree (angle)
min	Minutes
cm	Centimeter
^{59}Co	Cobalt 59
^{60}Co	Cobalt 60
^{60}Ni	Niquel 60
NaOH	Sodium hydroxide
$C_6H_5Na_3O_7$	Sodium citrate
$AgNO_3$	Silver nitrate
$Na_3PO_4.12H_2O$	Dodecahydrate trisodium orthophosphate
H_3BO_3	Boric acid
$C_6H_8O_7$	Citric acid
KeV	Kilo electronvolts
kGy	KiloGrays
Tg	Glass transition
Tm	Melting Temperature
AAc	Acrylic Acid
PAAc	Poly (Acrylic Acid)
AgNPs	Silver nanoparticles
TGA	Thermogravimetric analysis
DSC	Differential scanning calorimetry
FT-IR	Fourier Transform-Infrared Spectroscopy
UV-VIS	Ultraviolet-Visible
-COOH	Carboxylic group
C=O	Carbonyl group
-OH	Hydroxyl group

CH_2	Methylene
CH_3	Methyl
E.coli	Escherichia Coli
MRSA	Methicillin Resistance Staphylococcus Aureus

1. INTRODUCTION

1.1 Polymers

Encyclopedia Britannica (2019) refers a polymer, any of a class of natural or synthetic substances composed of very large molecules, called macromolecules, that are multiples of simpler chemical units called monomers. The process by which the monomers are joined together to produce a polymer is called polymerization. The materials have unique properties, depending on the type of molecules being bonded and how they are bonded (Bradford, 2017). The chemical bonds and nature of the subunits also indicate an effect on the physical and chemical properties of the polymers.

1.1.1 Classification.

There are different ways to classify the polymers, belong the most important are the classification by the origin, composition, structure, methods of polymerization and thermo-responsive.

1.1.1.1 By origin.

Natural Polymers: They are those materials that encompass whole nature, even the human body. This type of materials exists from the beginning of the planet (see in Table 1).

Synthetic Polymers: They are recently discovered because they are created from raw materials by chemical reactions. The usefulness is almost unlimited and are applied mainly in the industry and medical field (see in Table 1).

Table 1. Examples of polymers based their origin.

	Monomer	Polymer
Natural	Monosaccharide Nucleotide Amino acids	Polysaccharide (Cellulose) Nucleic acids (DNA, RNA) Polypeptide (proteins)
Synthetic	Ethylene Propylene Styrene	Polyethylene Polypropylene Polystyrene

1.1.1.2 By composition.

Homopolymer: When the Monomers may be all alike such as ethylene and polypropylene.

-A-A-A-A-A-A-

Copolymer: It consists of two or more monomers presents in the polymer chain, arranged of diverse forms:

Alternating copolymer: When two different monomers are arranged one by one.

-A-B-A-B-A-B-A-B-

Block copolymer: It consists of a cluster of homopolymers joined together followed by another cluster of homopolymers and its end.

-A-A-A-B-B-B-A-A-A-B-B-B-

Random co-polymer: The polymeric chain has not a specific order within its monomers.

-B-A-B-B-A-B-A-A-

1.1.1.3 By structure.

Lineal chain polymer: It consists of straight polymeric chains formed by monomers linked by identical bonds.

Branch chain polymer: It consists of single polymeric chains with random points in the structure forming side chains. This structure does not closely package.

Crosslinking polymers: They also know as a network because of form a 3-D structure. The monomers are joined through covalent bonds (see Figure 1).

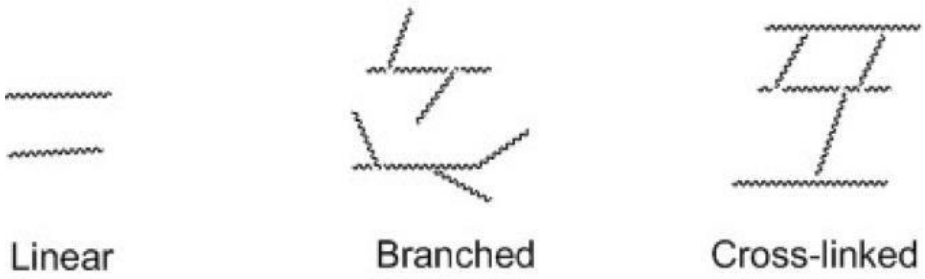


Figure 1. Structure of polymers

1.1.1.4 By methods of polymerization.

1.1.1.4.1 Polymerization by condensation.

This method, practically consists of at least two functional groups in the monomers to produce a chemical reaction (see Figure 2). This polymerization is of low molecular weight, slower than addition polymerization. The terminal functional groups on a chain remain active, so that groups of shorter chains combine into longer chains in the late stages of polymerization (Reusch, 2019). Also, this reaction may produce a little molecule like water, apart than macromolecule. Some examples are the polyester, polyamide, among others, which have in their structures functional groups following their names such as acid, ester, or amine group.

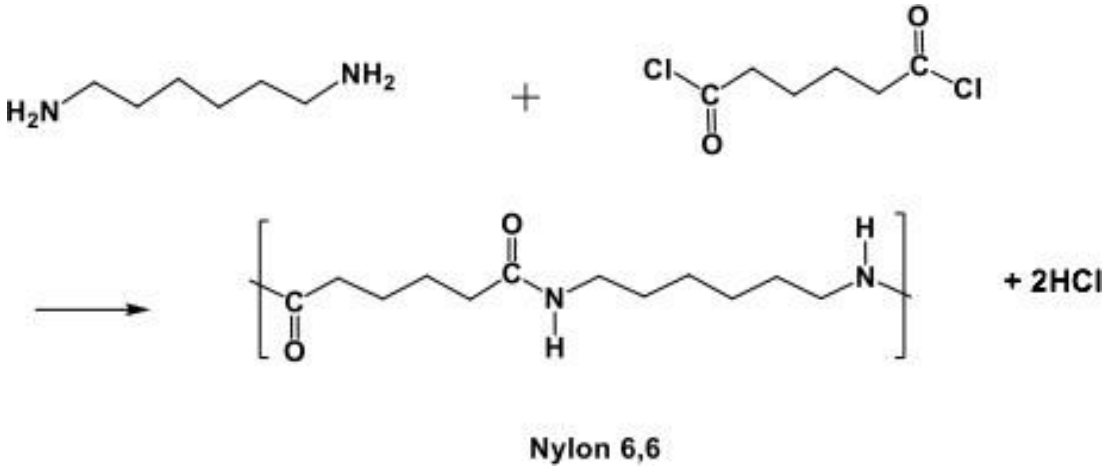


Figure 2. Example of polymerization by condensation to form Nylon 6.6 from adipoyl chloride and hexamethylenediamine.

1.1.1.4.2 Polymerization by addition.

Polymerization by addition, or also known as chain growth reaction is referring to alkenes monomers that have double bonds in their chemical structure, which is a way to carry out chemical reactions. The electrons present in the double bonds can be arranged to bind a new molecule forming single bonds (see Figure 3). This reaction must have an initiator to begin the process, producing a rupture of double bonds while appearing of one radical by which the reaction with another monomer will occur. However, as going on the process, the number of monomer decreases while the molecular weight rises, such as the vinyl groups present in the polypropylene, which are formed by a mean double bond, beginning the polymerization of big molecules.

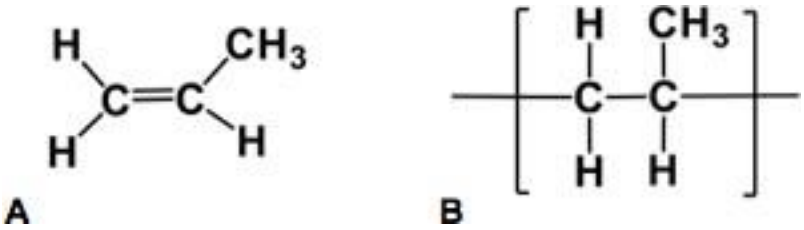
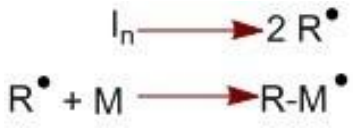


Figure 3. Example of polymerization by addition from: a) Propylene to b) Polypropylene.

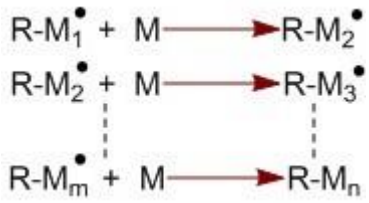
1.1.1.4.3 Free radical polymerization.

Free radical polymerization is a technique that consists of successive addition of monomers due to free radicals presents in the monomers, which are created by initiators. This polymerization is depicted by three phases, initiation, propagation and termination.

Initiation: It produces free radicals in different ways, the decomposition of the initiator using either chemical reaction (azobisisobutyronitrile), thermal decomposition (styrene) or by radiation. It consists of two steps, the first one creates free radical, while the second in the transference of this active species into the monomer. The velocity of reaction depends on either the nature of the decomposition and the temperature of the reaction.



Propagation: This step continues immediately after the initiation step, where it will add monomers to a radical chain, generating rapid growth. Once that the propagation occurs, it continues until there is not more monomers or by termination.



Termination: It consists of the inactivation of the growth. The termination step mainly consists of three processes to stop the polymerization: combination, disproportionation or impurities.

By combination: The ends chain of two molecules containing free radical is joined, stopping the propagation. It results in the increment of the long chain.

By disproportionation: It consists of the interaction of a free radical to hydrogen to get a double bond.

By impurities: It may act as inhibitors or delayed effects on the polymer. During the propagation, it can deactivate the growing up.

1.1.1.5 By thermo-responsive.

1.1.1.5.1 Thermoplastics.

Thermoplastics polymers consists of polymers bound by electrostatic bonds (Van Der Waals), which are weak links. Thermoplastic polymers readily soften when heated, allowing manufacturers to mold them into a wide range of shapes, then re-soften them and mold them again. This ability to reuse thermoplastic polymers indefinitely means they are highly recyclable (Mayer, 2018). Thermoplastics soften when heated and harden when cooled (Redwing, n.d.). The process can be used more times and it is easier than thermosets. Either linear and cross-linked polymer present this characteristic such as PVC.

1.1.1.5.2 Thermosets.

The property that presents this kind of polymers is opposite than thermoplastics. Thermosetting polymers (also called thermosets) are a family of plastics characterized by the fact that they are formed starting from a liquid solution that irreversibly leads to a solid material during a heating step (Saldívar-Guerra & Vivaldo-Lima, 2013). However, the transition from solid to liquid may be reversible at a specific temperature such as Bakelite that is applied during the fabrication of electrical insulation.

1.1.2 Thermal properties.

1.1.2.1 Crystallinity.

The polymers also present a structure either well-ordered (crystallinity), semi-ordered or amorphous as shown in Figure 4. The structure of amorphous materials cannot be described in terms of repeating unit cells such as that of crystalline materials; because of nonperiodicity, the unit cell of an amorphous material would comprise all atoms step (Saldívar-Guerra & Vivaldo-Lima, 2013). Both structures influence directly in the properties of the polymers. The amorphous regions give the material flexibility, while the crystalline regions give the material strength. Thus, many materials contain both crystalline and amorphous regions giving the material a balance between strength and flexibility (Carraher & Seymour, 2003). The main transitions of polymers are associated with glass transition temperature (Tg) and melting temperature (Tm). Some examples of not-ordered structures are the polystyrene or methyl acrylate, however, polyethylene and polyamides are crystalline polymers.

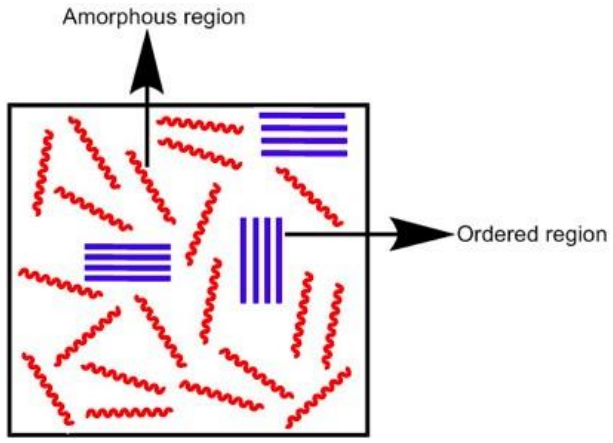


Figure 4. Crystalline and amorphous regions in a polymer.

1.1.2.2 Glass transition temperature.

The glass transition temperature of a noncrystalline material is the critical temperature at which the material changes its behavior from being “glassy” to being “rubbery” (Saldívar-Guerra & Vivaldo-Lima, 2013). The Tg allows determining how to affect the external factors during the physical changes of the material. Only, amorphous regions present Tg, because it corresponds to a solid-state not equilibrated that needs a major volume that crystalline.

1.1.2.3 Melting temperature.

The melting temperature is a property only reported by the crystal regions of a polymer, this temperature causes that the polymer passes from the solid state to the liquid. Widmann & Scherrer (1991) reported that the determination of the melting point using the differential scanning calorimetry (DSC) method has been satisfactorily used as a method of evaluating the degree of purity of a compound.

1.2 Radiation

Radiation can be defined as the amount of energy that is transmitted through particles or electromagnetic waves. Radiation is present every day and represents an energy form available for different applications. There are different examples of natural radiation such as solar light which is important for all species life. Ionizing radiation consists of enough energy to cross the medium and cause changes with the interacted matter like X rays and gamma rays. On the opposite side, it is known as non-ionizing radiation such as electro waves of TV and cellphones.

Additionally, irradiation term encompasses the process when an object is subjected to a controlled radiation source with great application in the sterilization of medical instruments and agriculture.

1.2.1 Sources of radiation.

The ionization radiation arises from a synthetic isotope which is ^{60}Co. However, according to Marsh (2017), the source of radiation to get ^{60}Co is the ^{59}Co, which is an isotope found in nature, usually in rocks, soil, water, plants, animals and humans and is non-radioactive. However, the ^{60}Co is artificial material which is made bombarding ^{59}Co with neutrons. This radionuclide decay transforming a neutron into a proton emitting two wavelengths of high-energy gamma-rays of 1.17 and 1.33 MeV- respectively. Then, the final result is obtaining ^{60}Ni (Eq. 1), which is a stable element as shown in Figure 5. The period of the decaying is 5.27 years.

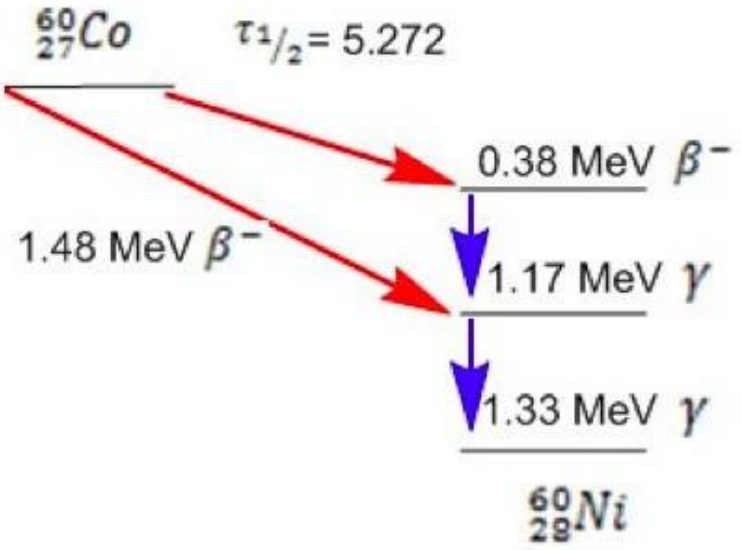


Figure 5. Disintegration of the ^{60}Co.

$$^{60}Co \rightarrow {}^{60}Ni + \beta + \bar{\upsilon} + \text{gamma rays} \qquad \text{Eq. 1}$$

β particles: The term beta particle is reversed for an electron that has been emitted during a nuclear decay process (Gilmore, 2011). This process is indicating the differences of this procedure in comparison than other methods of electrons emitted with defined energy. It is divided into β^- (or negatron) which is emitted during the transformation of a neutron to proton and β^+ (or positron) that is originated during the conversion of a proton into a neutron.

$\bar{\upsilon}$: antineutrins are particles with null charge and small mass emitted by a neutron during the beta decay.

1.2.2 Gamma radiation.

Gamma rays show a short wavelength which implies a major level of energy. So, the energy produced during gamma rays is released by the nucleus, for this reason, show major power than x-rays where the energy is released through electron interactions. This type of electromagnetic radiation is produced by radioactive elements or by the subatomic process. Also, it can be produced during high energy events in the space. Energies in the gamma radiation range are conveniently in keV (Gilmore, 2011).

1.2.3 Ionizing radiation.

Some isotopes are subjected to the emission of radiation or particles (α and β) and consequently the degradation, into another stable element. In the polymers, this radiation would produce degradation or crosslink.

1.2.4 Radiation Interaction with the matter.

An attenuation coefficient is a measure of the reduction in the gamma-ray intensity at particular energy caused by an absorber. The absorption coefficient is related to the amount of energy retained by the absorber as the gamma radiation passes through it (Gilmore, 2011). There are three types of interaction with the matter:

a) Photoelectric effect: It consists of the transference of energy from one photon to an electron, resulting in the disappearing of the first one. The electron acquires this energy and it becomes a free particle.

b) Compton effect: In this case, there is a collision between a photon and an electron resulting in the partial transference of energy from the photon. The rest of the energy is taken by another photon that presents minor energy.

c) Pair production: it consists in the conversion of a photon in an electron-positron pair when the photon is closest to nucleus field.

1.3 Stimuli sensitive polymers

Smart polymers are stimuli sensible polymers that can generate a response during external changes, or combine response due to temperature or pH effects as shown in Figure 6. In other words, smart polymers are able to respond to a stimulus by showing physical or chemical changes in its behavior as, for example, the delivery of the drug carried by itself (Gupta, Vermani & Garg, 2002). An important feature of these smart polymers is that the macroscopical changes are reversible, i.e., these systems are able to recover their initial state when the sign or stimuli ends (Stuart et al., 2010).

These class of polymeric materials can be used in the human body because it presents constantly changes to pH and temperature as a protection pathway. A great number of

polymeric materials has been studied. So, they remain on the ¨top¨ of biomaterials for drug delivery.

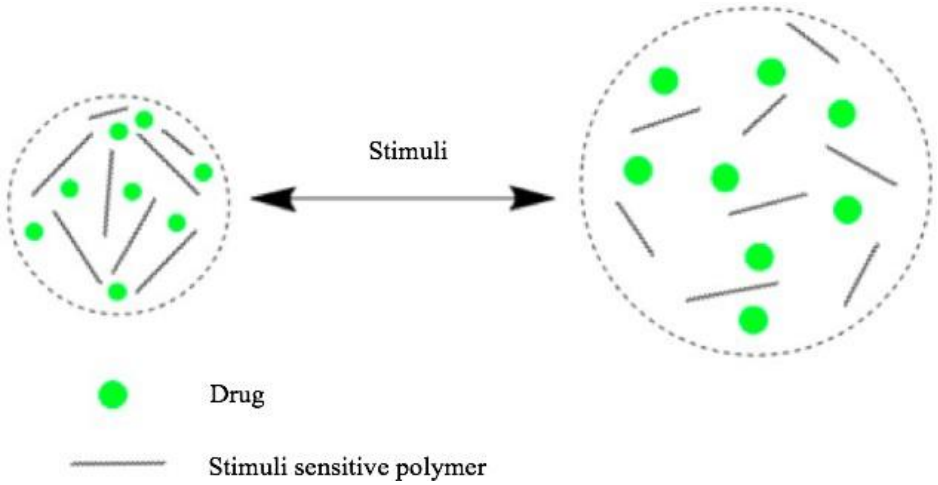


Figure 6. Representation of stimuli sensitive polymer.

1.3.1 Thermo-sensitive polymers.

Thermo-sensitive polymers are undergoing a structural change when are subjected a temperature transition. According to with Bajpai et al. (2008), thermo-responsive polymers present in their structure a very sensitive balance between the hydrophobic and the hydrophilic groups and a small change in the temperature can create new adjustments, is to say, these class of materials are constituted amphiphilically.

Phase transition of hydrophilicity or swelling degree present a middle point induced by the temperature, it is known as critic solution temperature (CST) (see Figure 7). If the polymer solution has one phase below a specific temperature, in other words, they become insoluble upon heating, they have a lower critical solution temperature (LCST) (Bawa et al., 2009), thus, this indicates a decreasing in the solubility as the temperature increase.

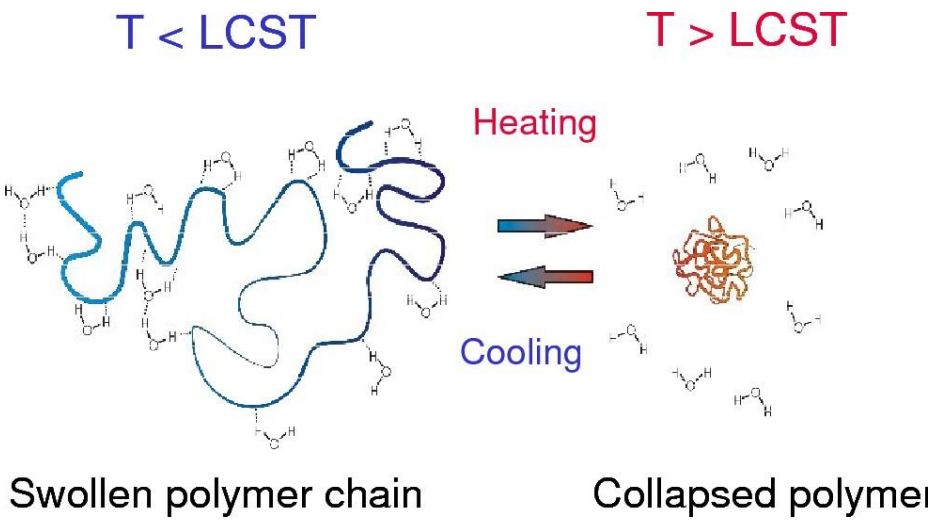


Figure 7. Representation of thermo-sensitive polymers (Vihola, 2007).

These kinds of smart polymers are widely studied in the human body, due to their biocompatibility, non-toxicity and the changes that they experience. Physiologically, thermal stimuli are very important e.g. during a fever there is an elevation of body temperature due to the presence of pyrogens (Bawa et al., 2009).

1.3.2 pH sensible polymers.

This type of polymers presents a challenge in the scientific community adapting their properties to potential biomedical applications. pH-sensitive polymers are polyelectrolytes that contain ionic groups that may be weak acidic or basic that are subjected to a transition phase with the external changes. A novel alternative to produce this type of biopolymers is through controlled free radical, meanwhile, the conventional method continues being an option.

The main characteristic of pH sensitive polymers is that they are able to suffer an ionization or not in response to pH changes. These polymers contain in their structure acid groups (carboxylic or sulphonic) or basic groups (ammonium salts) (You et al., 2010). These kind of groups allows them to accept or release protons.

pH sensitive polymers named as polyacids or polyanions, such as, poly(acrylic acid) (PAAc) or poly(methacrylic) acid (PMAA) are polyanions that have in their structure a great number of ionizable acid groups, like carboxylic acid or sulfonic acid (Grainger & El-Sayed, 2010). The carboxylic groups accept protons at low pH values and release protons at high pH values (Gil, Hudson, 2004). In this case, PAAc show major swelling at high values of pH (basic). The phase transition occurs in fast changes from 0.2 to 0.3 units of pH due to protonation/deprotonating process by the pH variations.

There are two types of pH sensitive polymers which are anionic and cationic as shown in Figure 8. Anionic polymer suffers a partial dissociation in aqueous solution such as –COOH, whose pH will depend of acid nature, conjugated base stability, size and polarity of the molecule. In contrast, cationic polymers such as $–NH_2$, are protonated at pH<pKa.

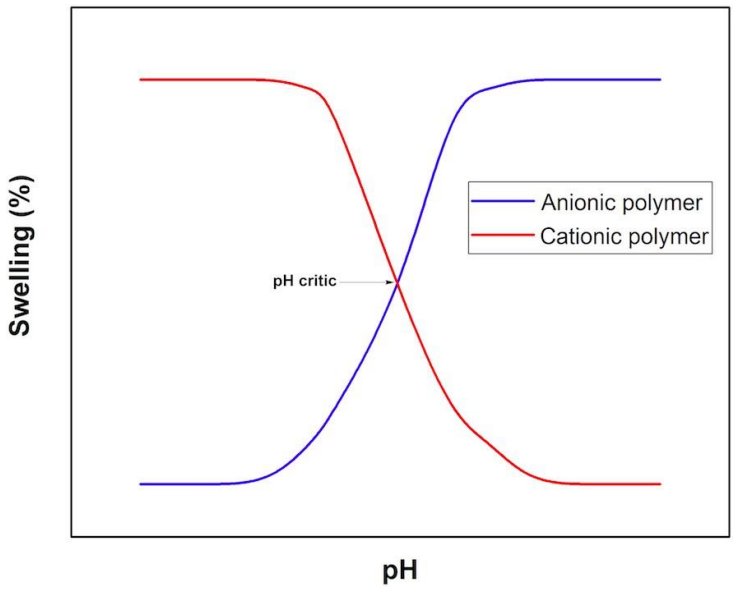


Figure 8. Representation of pH sensitive polymers and critical pH.

pH-sensitive polymers are biomaterials with potential application in the medical fields, taking into account that the human body presents a different range of pH depending on the type of organ affected or treated.

1.3.3 Hydrogels.

Polymers may form an arrangement three-dimensional, forming a cross-linked structure called hydrogel. Hydrogels areknown as synthetic biocompatible material (biomaterial) with a large capability of absorbing mainly water or another compounds depending on their nature. The ability of hydrogels to absorb water arises from hydrophilic functional groups attached to the polymeric backbone, while their resistance to dissolution arises from cross-links between network chains (Ahmed, 2015).

Therefore, the crosslinking of polymers may be performed by crosslinking agents (union of polymers) or radiation (free radicals). So, due to its structure and its hydrophilicity, this biomaterial presents a capacity of swelling from dozens to thousands of old times its initial size.

The properties of the hydrogels depending on the precursor's monomers and their properties. Many kinds of materials are used for preparing superabsorbents, but most of those materials are acrylic acid and acrylamide-based products (Li et al., 2012). The hydrogels may be composed of smart polymers whose change due to external excitation. Water-soluble polymers such as poly(acrylic acid), poly(vinyl alcohol), poly(vinylpyrrolidone), poly(ethylene glycol), polyacrylamide and some polysaccharides are the most common systems used to form hydrogels (Caló & Khutoryanskiy, 2015). These materials are used for biomedical applications because they present non-toxic properties. Also, the doses of radiation play an important role in the characteristics of these biomaterials, because it performs two activities on the polymers, the firsts are the crosslinking and the second one is the sterilization process involved during the irradiation.

Hydrogels depend on different factors to determine their characteristic properties. These properties give them the possible applications from tissue engineering in the formation of synthetic cell matrix to controlled drug delivery and implantable devices.

1.3.3.1 Biomedical Applications.

Hydrogels are widely applied to different parts of the human body. According to Anamica and Pande (2017) can be present different application such as, in Immunotherapy, Vaccine, Plastic surgery, Wound Healing, Electrophoresis, Proteomic, Tissue engineering (Bone regeneration, Cardiac, Dental), Drug delivery, Wound dressing and so on.

Contact lenses: It is an example of plastic surgery that is widely used in the whole world. According to Caló & Khutoryanskiy (2015), contact lenses are mainly classified as ‘hard’ or ‘soft’ according to their elasticity. The bibliography affirm that the first hydrogel employed for contact lens was based on poly-2-hydroxyethylmethacrylate (PHEMA).

Wound dressing: An important use of hydrogels is into skin burn. The skin is the bigger organ, which presents some layer that protects the body, being the first barrier of defense against some danger. A wound is a defect or a break in the skin which can result from trauma or medical/physiological conditions (Caló & Khutoryanskiy, 2015). Wounds are classified into chronic or acute, also depending on the affected layer into superficial, partial-thickness or full-thickness. The application of biomaterials for the treatment of wound healing increasing in the last years. The fabrication of dressing for the healing process. Hydrogels are widely used as

debriding agents, moist dressings, and components of pastes for wound care (Caló & Khutoryanskiy, 2015).

1.4 Monomers

1.4.1 Agar.

Agar is a hydrophilic polysaccharide with a complex structure, which is composed of agarose (70%) and agaropectin (30%) as shown in Figure 9. Formed of long chains of sugar molecules, agar is prized by microbiologists for its ability to form hard gels when mixed with water and growth nutrients (Callaway, 2015). It is insoluble in cold water but soluble in boiling water. According to McHugh (1987) at 34-43 °C it forms a firm gel which does not melt again below 85 °C. Such hysteretic behavior is reproducible and can be repeated a great number of times (Dea et al., 1972). The presence of agaropectin reduces the exclusion limit as these molecules do not participate to the gel network and obstruct the pores, which is the reason why electrophoresis gels are prepared with pure agarose rather than agar (Medin, 1995). Note that the thermal hysteresis is used in the food industry and makes agar much more suitable for applications in microbiology than the low-melting-point gelatin (25 to 30°C) (Armisen & Galatas, 2009).

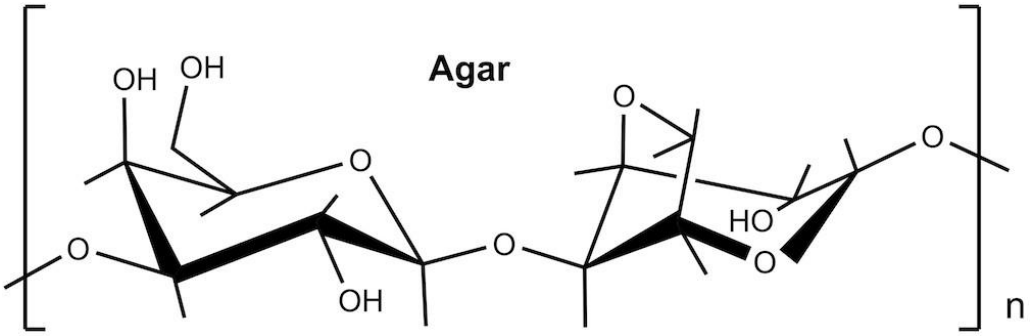


Figure 9. Chemical structure of agar monomer.

Agar may be considered as polymers made up of monomers of the sugar galactose. Due to its properties, agar has been used during investigations for its ability to form gels. The great presence of agarose allows them swelling so well due to its hydrophilic bonds.

1.4.2 Acrylic acid.

It is a clear liquid, which is soluble in water, however, presents a melting point at 12 °C. Acrylic acid (2-propenoic acid) is a highly reactive carboxylic acid that can react with itself to

form poly (acrylic acid), which is used as an absorbent in hygiene products (American Chemical Society, 2007) which is studied in the polymer field. Acrylic acid has two reaction points or functional groups required for polymerization process (Acrylic acid, 2017); the vinyl and carbonyl group as shown in Figure 10. The double bond in its structure allows them to form links with other polymers to present applications such as elastomers, adhesiveness among others.

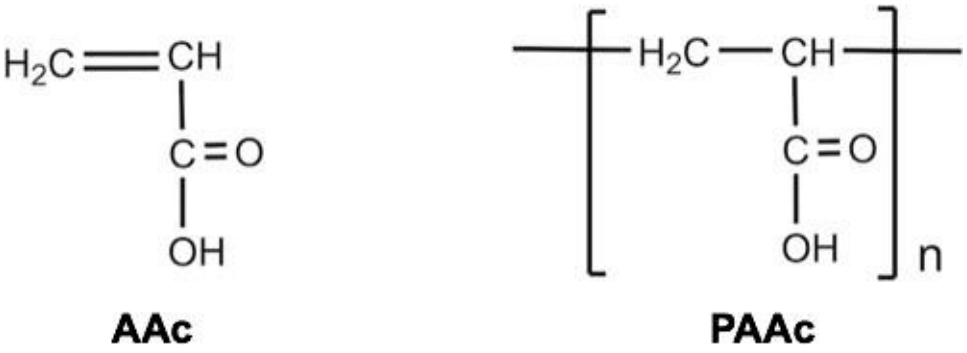


Figure 10. Chemical structure of AAc and PAAc.

1.5 Characterization techniques

The creation of new materials must be analyzed through different techniques that can help us to know the different properties and features such as chemical groups, thermal behavior, size, hydrophobicity, mechanical resistance among others.

1.5.1 FT-IR.

Fourier Transform Infrared Spectroscopy (FT-IR) is one of the techniques that are used today for measuring the intensity of infrared radiation as a function of frequency or wavelength (Naseska, 2016).

This technique used the infrared spectrum electromagnetic to identify some compounds through molecular rotational and vibrations. This region is subdivided into three subparts: near, medium, far (Table 2). The rotations and vibrations of the molecules are accompanied by a change in a dipole moment of the compound. In theory, any molecule with a permanent or inducible dipole will be IR active (Tschida & Lawson, n.d.).

Table 2. Wavelength of FT-IR regions

Infrared	**cm^{-1}**	**Characteristic**
Far	400-10	Low energy used in rotational spectroscopy
Mid (IR)	4000-400	Molecular structure Rotational-vibrational
Near	14000-4000	Overtones and harmonic vibrations

In the polymer field, this technique helps to improve the detection of the functional structure of the compounds to an appropriate classification. Infrared spectroscopy is most useful in providing information about the presence or absence of specific functional groups. The physical properties like crystallinity degree, size, orientation, mechanical behavior are dependent on the molecular structure. It is important to note that this technique has application only in covalent bonds.

1.5.2 Thermal analysis.

Thermal techniques allow to determine thermal properties. Techniques like DSC and TGA, demonstrate the physical properties of a material when subjected to variable temperature changes. The results depict information useful such as peaks, discontinuities, transitions, among others, that correspond to the observed sample.

1.5.2.1 Thermogravimetric analysis (TGA).

According to Groenewoud (2001), thermogravimetric analysis is the technique in which the mass of a sample is monitored against time or temperature based in a thermobalance. A thermobalance is a combination of a suitable electronic microbalance with a furnace, a temperature programmer and computer for control, that allows the sample to be simultaneously weighed and heated or cooled in a controlled manner (Brown, 2004). There are basically, two types of TGA analysis:

1. The non-isothermal thermal stability determination: it consists of measuring the mass change at a constant temperature.

2. The isothermal thermal stability determination: the temperature is stabilized while it produced mass changes.

TGA has several applications to determine the thermal conditions of a compound:

Thermal stability of materials, oxidative stability of materials, composition of Multi-component systems, estimated lifetime of a product, decomposition kinetics of materials, the Effect of Reactive or Corrosive Atmospheres on Materials, moisture and volatiles content of materials (Mohomed, 2016).

TGA is an analytic technique used in industrial fields. This thermal procedure only allows to analyze the change in the mass volume, however, it does not represent phase transitions. Therefore, TGA is a very common technique used in polymer research to determine thermal stability.

1.5.2.2 Differential scanning calorimetry (DSC).

Differential scanning calorimetry is a thermal analysis technique in which the heat flow rate (power) to the sample is monitored against time or temperature while the temperature of the sample, in a specified atmosphere, is programmed (Krakow, 2012). Also, The International Confederation for Thermal Analysis and Calorimetry (ICTAC) defines DSC as a technique where the heat flow rate difference into a sample and reference material is measured (Lever, Haines, Rouquerol, Charsley, & Eckeren, 2014).

This technique consists of two capsules, one as reference (empty) and the other as a sample to analyze. Each capsule is heated by itself heater and recorder by separated sensors. According to (Groenewoud, 2001) a second differential-control loop adjusts the power input as soon as a temperature difference starts to occur due to some exothermic or endothermic process in the sample. Therefore, the cells with the sample are recorded.

DSC technique encompasses temperatures ranges from the temperature of liquid nitrogen (195,8 °C) to 450 °C. Therefore, DSC is used to determine Tg, heat capacity jump at Tg, melting point, and crystallization temperatures, heat of fusion, characterization of thermosets and measurements of liquid crystal transitions (Menczel & Prime, 2009).

It allows us to determine the thermal transitions of different materials at this range. Specifically, the polymeric and pharmaceutic compounds present their phase transitions at this temperature range.

1.5.3 Contact angle.

Contact angle is a technique that consists in the angle formed during the interaction between the liquid and solid surface as shown in Figure 11. Typically, the contact angle is illustrated by a drop of liquid on the surface. (Adstamongkonkul, 2011). So, this angle tells us about the properties of the solid. When the angle is minor than 90°, depicts the hydrophilicity of the solid due to the liquid wet it, showing that the droplets are extended in the area of the solid. Meanwhile, the hydrophobic effect occurs when the angle is major than 90° so when the angle is even major than 150-180 ° the surface of the solid is so super hydrophobic, sometimes called a lotus effect. In this case, the liquid does not wet the material.

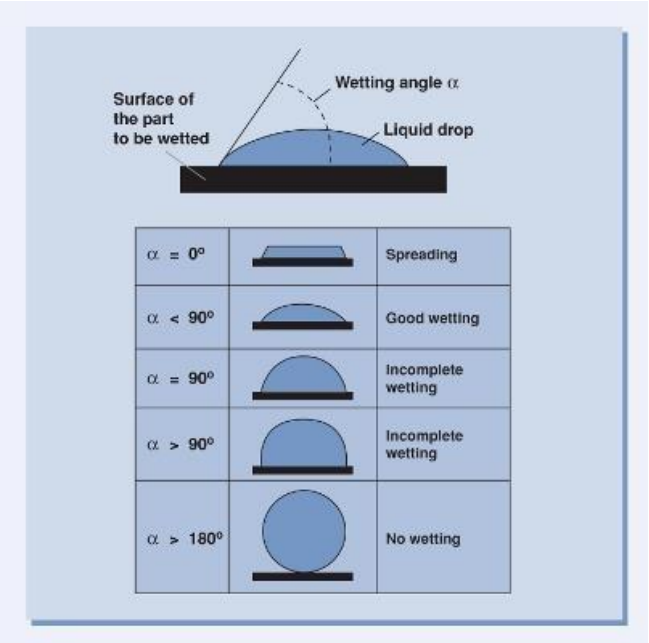


Figure 11. Contact angle measurement (Adstamongkonkul, 2011).

2. GENERAL AND SPECIFIC OBJECTIVES:

2.1 General

Synthesize hydrogels pH-sensitive based agar and acrylic acid, which was crosslink by gamma radiation.

2.1 Specific

- To determine the optimal conditions through variables such as radiation dose, monomer concentration, temperature and reaction time.
- To study the reaction of the hydrogel by changing variables such as radiation dose, monomer concentration, temperature and reaction time.
- To analyze the thermal behavior of the material using DSC and TGA.
- Structurally characterize the polymer network using FTIR-ATR technique.
- To study the behavior of the hydrogel at pH variations based on limit swelling for every sample.
- To determine the hydrophilic behavior of the hydrogel obtained as well as the degree of wettability through the contact angle characterization.
- To synthesize silver nanoparticles (AgNPs) due to its antimicrobial effect.
- To load the hydrogel with AgNPs and ciprofloxacin.
- To analyze the load and delivery of the antimicrobial compounds using UV-VIS spectroscopy.

3. METHODOLOGY

This chapter describes in detail the experimental methodology that was performed for obtaining of hydrogel employing agar and acrylic acid as monomers. These monomers formed a cross-linked structure material through γ-rays. Additionally, during the synthesis of hydrogels different parameter such as monomer concentration, reaction time, and irradiation doses was studied. Subsequently, the synthesis of AgNPs are presented. Therefore, the hydrogel was subjected to characterization techniques such as FTIR-ATR, TGA, DSC, contact angle, swelling, pH cycles, and mechanical properties meanwhile the AgNPs was subjected to UV-VIS spectroscopy. Finally, the load and delivery of AgNPs and ciprofloxacin that presents antimicrobial properties were evaluated using UV-VIS spectroscopy.

3.1 Reactive

Agar (Sigma-Aldrich): It was used as was received (see Table 3).

Table 3. Characteristics and properties of agar

Characteristics	**Value**
Physic state	White colored powder
Solubility	Boiling water
Molecular weight	336.337 g/mol
pH	6.8 to 7.0
Melting point	85 to 95 ºC
Gelation point	32 to 45 ºC

Acrylic acid: This monomer was purified using distillation to high vacuum employing a system as Figure 12, to eliminate the inhibitor and impurities. The pure monomer was stored in cooler (see Table 4).

Table 4. Characteristics and properties of AAc

Characteristics	**Value**
Physic state	Liquid
Solubility	Soluble
Molecular weight	72.06 g/mol
pH	3 (approximately)
pK_a	4.26 (at 25 ^oC)
Melting point	14.0 ^oC

Figure 12. Assembly for distillation of AAc under reduced pressure

3.2 Instrumentations and equipment

- Vacuum stove with heating.
- Potentiometer pH analyzer
- Freeze drying equipment, Freeze Dry System/ Freezone 4.5 LABCONCO.

- Differential Scanning Calorimetry, TA instruments, USA. Model Q100.
- Fourier Transform Infrared Spectroscopy, Spectrum 400, FT-IR/FT-NIR Spectrometer, Perkin Elmer.
- Thermogravimetric Analysis, TA-Instruments Q50, model Discovery TGA, USA.
- Ultraviolet Visible Spectroscopy (UV-VIS spectroscopy). Analytikjena Specord 200 Plus, Germany.
- Mechanical Test. SHIMADZU CORPORATION, model AGS-X, Japan.

3.3 Radiation sources

The monomers were irradiated simultaneously with gamma radiation (Gammabeam Irradiator 651 PT, MDS Nordion, Canada) from a source of ^{60}Co, which is found in the Institute of Nuclear Sciences from UNAM.

3.4 Synthesis of hydrogels

1. 0.8 g of Agar was dissolved into 50 mL of distilled water at 85 °C.
2. Different concentrations of each monomer were placed into a beaker with constant stirring until obtaining a homogenous solution.
3. The final solution was placed into a petri dish. It was changing each parameter: reaction time (10 to 30 min), monomer concentrations (see Table 5 and Table 6) and irradiation doses. The samples were situated into a stove at 65 °C by different times for the reaction process.
4. Subsequently, the petri dishes were placed into a box to be irradiated in the Gammabeam, at doses of 10, 15 and 25 kGy, at a doses rate of 7.2 $kGyh^{-1}$.
5. Then, the hydrogels obtained were situated in a closed beaker and washed 3 times by one hour every wash with constant stirring.
6. Finally, the samples were subjected to lyophilization (technique that eliminates the water present) based on liquid nitrogen to subsequent characterization (schematic representation in Figure 13).

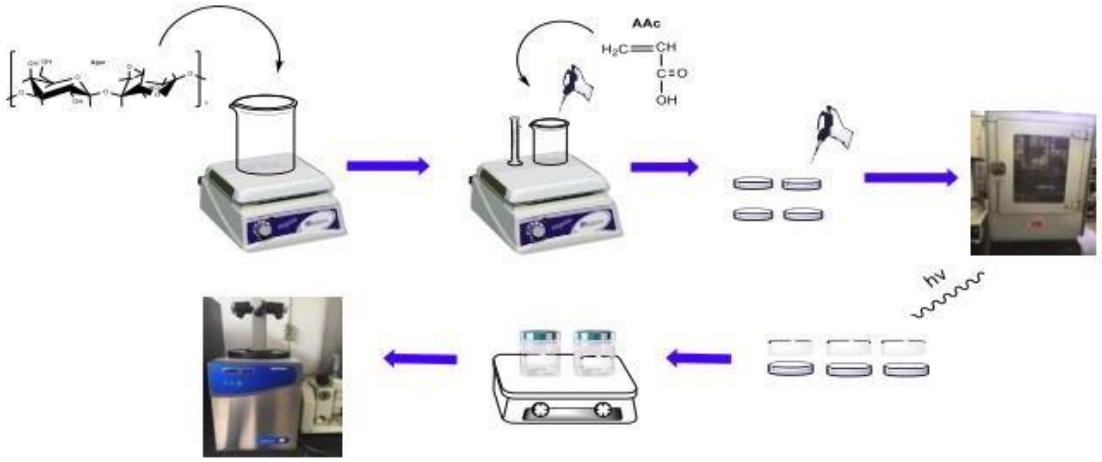


Figure 13. Graphical representation of the process followed to obtain hydrogel by gamma radiation.

3.5 Synthesis of AgNPs

It was performed using the reported method by Lee & Meisel (1982), which consists in using sodium citrate as reducing and stabilizing agent.

1. To reach a temperature close to boiling point of 250 mL of distillated water.
2. 45 mg of $AgNO_3$ was dissolved into 50 mL distillated water.
3. The solution was getting at pH 8, using NaOH at 0.1 M (two drops).
4. 5 mL of $C_6H_5Na_3O_7$ of a solution 1% of compound. Subsequently, mix both solutions with constant stirring.
5. To keep close to boiling temperature with a constant stirring by one hour (schematic representation in Figure 14)

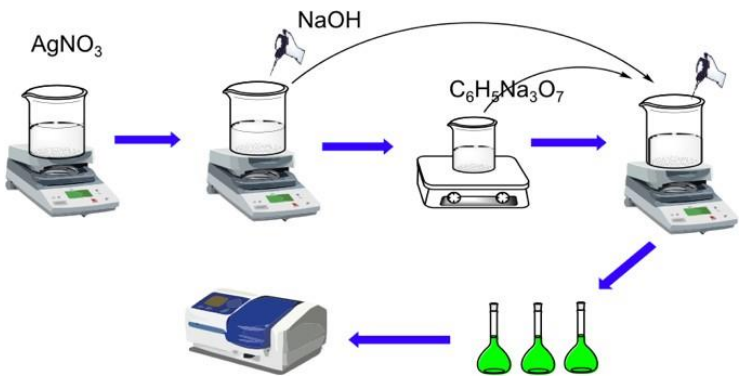


Figure 14. Graphical representation of the process followed to obtain Silver Nanoparticles.

3.6 Instrumental Characterization

3.6.1 Fourier Transformed Infrared Spectroscopy (FT-IR).

The hydrogel sample characterized by infrared spectroscopy was previously lyophilized. This method used a FT-IR/FT-NIR Spectrometer (Figure 15), Perkin Elmer with Fourier Transformed and diamond tip, applying 16 scans made to the samples, in order to determine the region from 3800 to 800 cm^{-1}.

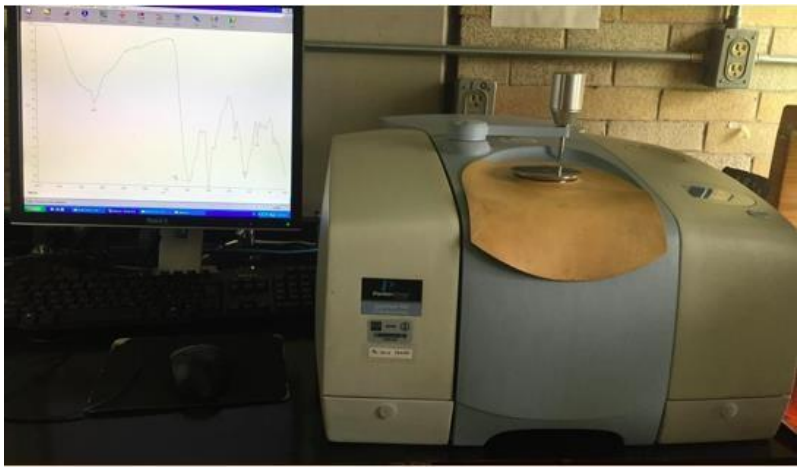


Figure 15. FT-IR equipment (FT-IR/FT-NIR Spectrometer).

3.6.2 Thermogravimetric Analysis (TGA).

For TGA analysis it used the TGA Q50, TA instruments, USA. The samples were dried previously to remove the moisture. The samples analyzed had a weight between 5-15 mg, they were placed in a platinum tray, which is a component of TGA Q50 (Figure 16). The analysis was performed at heat flow of 10°C/min from 25 to 900 °C in nitrogen atmosphere. It used PAAc (solid) instead that AAc (liquid) due to this process consist of the loss of solidified mass when is subjected at high temperatures.

Figure 16. Thermogravimetric equipment (TGA Q50).

3.6.3 Differential Scanning Calorimetry (DSC).

The samples of hydrogel and monomers were dried to remove moisture absorbed by the material. It used a DSC calorimeter 2010 (TA Instruments, USA) Model Q100 (Figure 17). The samples (5-10 mg) were encapsulated, then were placed in the equipment near to empty reference capsule. The analysis was carried out in two warming, the first from 20-100 °C to determine the Tg and remove some residual solvent, meanwhile, the second attempt from 25 to 300 °C to ensure the values and the transitions phases (Tm). The warm-ups had a heat flow of 5 °C/min in a nitrogen atmosphere. It used PAAc (solid) instead that AAc (liquid) due to this process consist of the loss of solidified mass when is subjected at high temperatures to evaluate different transitions that are present.

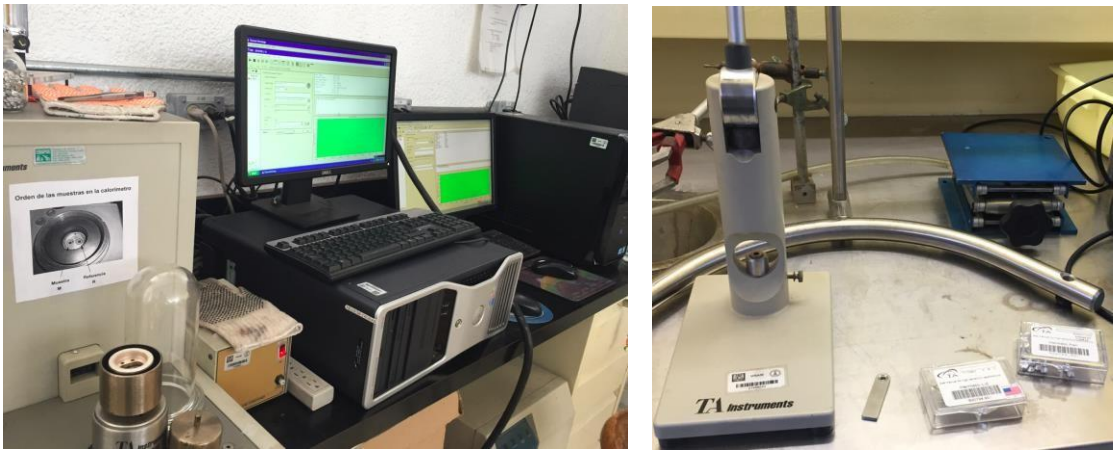


Figure 17. DSC equipment (DSC calorimeter 2010).

3.6.4 Contac angle.

To measure the contact angle, DSA 100 KrussGmb, Hamburg, Germany equipment was used (Figure 18). The sample must be as flat as possible and it is placed on a slide. A needle containing distilled water and then it drops a one drop on the sample. It used a software called ¨drop share¨ to determine the angle formed between the drop and the material surface. The measurements of each sample were made three times.

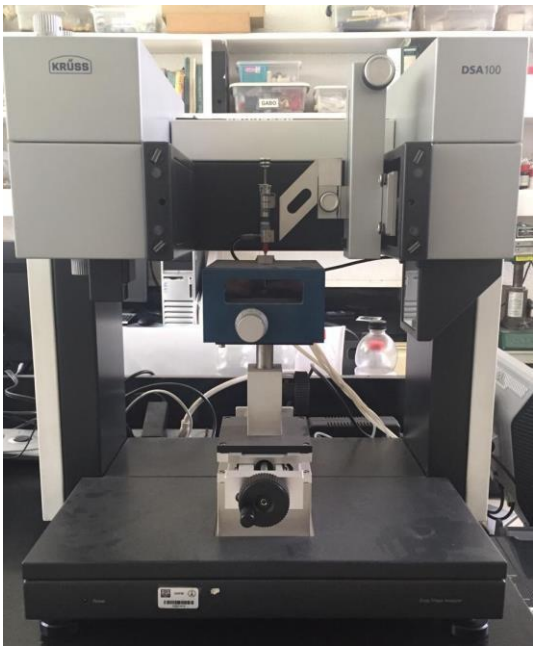


Figure 18. Contact angle equipment (DSA 100 KrussGmb)

3.6.5 Limit swelling.

Limit swelling is a technique that analyzes weight differences. Several samples were cut in a little circle, then they were dried at vacuum and weighted to determine its initial weight. Subsequently, the circle samples were immersed into distilled water at room temperature. At different time intervals, the samples were weighted to determine the need time while reaching the limit swelling (Figure 19). It is important to say that the hydrogels samples were made with different parameters and by triplicate to determine the average and standard deviation. Finally, following equation was employed:

$$Swelling\ (\%)\ = \frac{X^H - X_I}{X_I}\ x\ 100 \qquad Eq.\ 2$$

Where, X_H is the weight of the hydrogel swelled, while X_I is the weight of the hydrogel dry. The maximum swelling is determined when the weight of the hydrogel in at range of time is kept constant.

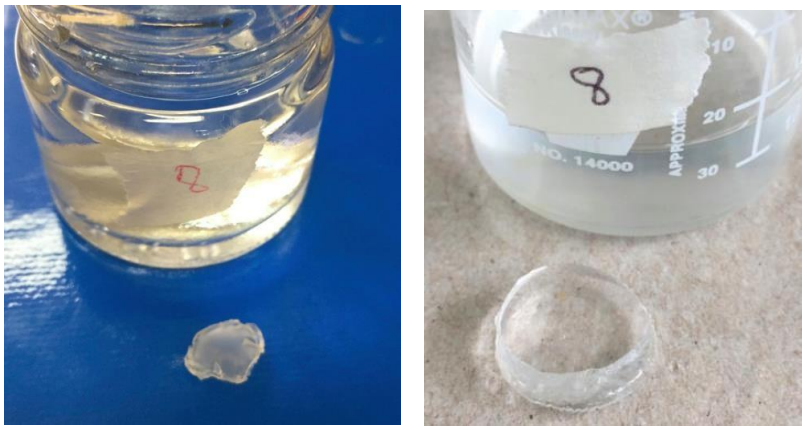


Figure 19. Example of maximum swelling.

3.6.6 Critical pH.

Buffer solutions were prepared in a pH (measurement of acidity and alkalinity of a solution) range from 2 to 12. The sample was washed and subsequently dried. Based in the time of maximum swelling, the time of each buffer was employed at room temperature. Then, it removed the excess of solution from the sample and it was weighted. The swelling percentage followed the Eq. 2, in every samples. The samples were subjected by triplicate at this tests to reduce the error.

3.6.6.1 Preparation of buffer solutions.

Two solutions were prepared. The firsts (solution 1) was prepared using boric acid (H_3BO_3) at 0.2 M and citric acid ($C_6H_8O_7$) at 0.05 M, while the second (solution 2) consisted of dodecahydrate trisodium orthophosphate ($Na_3PO_4 \cdot 12H_2O$) at 0.1 M. The solutions were mixed at different proportions depending the pH desired. The Annexes 1 depict the correct values for each pH solution.

3.6.7 Mechanical testing.

Sample films of poly (Agar-co-AAc) were subjected to tensile tests to determine the Young modulus, percentage of deformation. This technique aims to determine how different monomer concentrations and doses applied to the hydrogel affect. Immediately, after that irradiation process, the samples were cut as shown in Figure 19. The thickness of the sample was measured

in a range from 0.90 to 2.20 mm, at each end and in the middle, to obtain the average thickness. The samples were determined using the following conditions: 44% moisture, rate at 10 mm/min and at room temperature (Figure 20).

Figure 20. Equipment employed to mechanical test and the proper cut sample (model AGS-X-Shimadzu).

3.6.8 Ultraviolet–Visible Spectroscopy.

UV-VIS Spectroscopy used a spectrophotometer of Analytikjena Specord 200 Plus, Germany (Figure 21). The analysis of the AgNPs was made in a range from 190 to 500 nm (theoretical size). However, to determine the ciprofloxacin the range was performed from 190 to 350 nm. It performed two measurements, the first to determined the absorbance values and wavelengths and the second to ensure the first data.

Figure 21. UV-VIS spectrometer equipment.

3.6.9 Load of drugs.

It cuts a several little circle of the hydrogels dried with 1 cm of diameter and were immerse into 4 mL of AgNPs within a vial and other sample circle into an amber glass beaker containing 4 mL of ciprofloxacin due to is photo-sensible compound. The samples were subjected at constant stirring during 48 hours at room temperature. Each 10 hours, the absorbance values were measured. The amount of both drug loaded were determined by the following equation:

$$Drug\ absorbed\ (\%) = \frac{C^F - C_I}{W1}\ x\ V \qquad Eq.3$$

Where $C_F - C_I$ (final and initial concentration respectively) is the difference between absorbance of the compound as well as V depicts the volume applied of them. The wavelength of maximum absorption of each drug at 219 nm in AgNPs and at 266 nm in ciprofloxacin were followed for every sample.

3.6.9.1 Calibration curve.

Following the Beer Lambert Law to get a more accurate value, the calibration curve was made for each compound. The Annexes 2 and 3 depict the calibration curve following the Beer Lambert Law through UV-VIS spectroscopy of AgNPs and ciprofloxacin. The measurements were made by triplicate to ensure the values.

3.7 Antimicrobial effect

3.7.1 Determination of antimicrobial hydrogels on *E. coli* and MRSA.

1. Using 12-well plates, each of the hydrogels were placed (representation in Figure 22).

2. Previously, the inoculum of *E. coli* and MRSA was adjusted in soy tripticasein broth (BD, Bioxon) taking as reference the tube 0.5 of the McFarland scale ($1x10^8$ bacteria).

3. Subsequently, it was incubated at 37 °C in aerobic conditions for 18 hours.

4. After incubation, 100 µL of the culture medium of each of the treatments was taken and passed to a 96-well plate.

5. Subsequently, the absorbance at a wavelength of 600 nm was quantified using a microplate reader (Biotek, Winooski, VT).

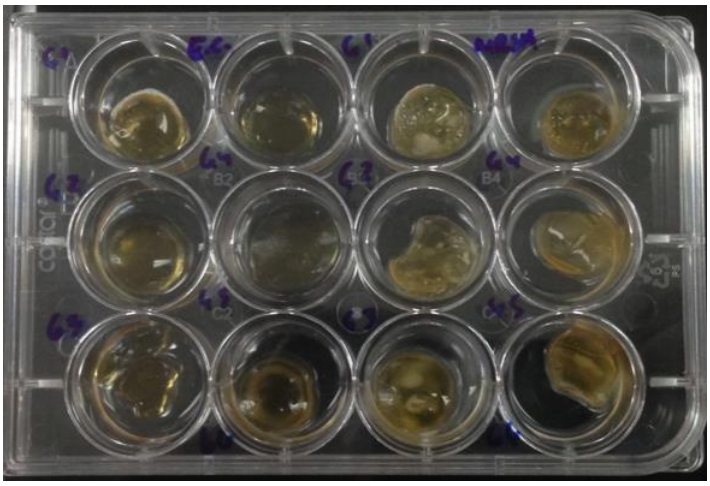


Figure 22. Determination of antimicrobial activity using E. coli and MRSA.

4. RESULTS, INTERPRETATION AND DISCUSSION

Agar and AAc formed a cross-linked structure to obtain a new biomaterial, hydrophilic and pH-sensitive. Besides, according with the bibliography, it is known that agar at low temperatures can solidify, resulting in a well defined but brittle compound. The AAc improve the mechanical properties, provided to the polymeric matrix adhesive properties and the pH-sensitivity.

On another hand, it is known that the other parameters influence the results such as the monomer concentration, reaction time and applied doses. For this reason, hydrogels at different parameters were examined.

During the first experiments, we focused on determining the appropriate conditions of the samples. Figure 23 show the different parameter to consider to obtain successful samples.

Figure 23. Unsuccessful vs successful results.

The unsuccessful samples were performed following different concentrations, reaction time and radiation doses as you can see in Table 5. The temperature during the mixture reached 68 °C. The last-mentioned parameter was changed until 85 °C and the different value of monomer concentration was studied to obtain visibly successful samples as you can see in Table 6. The successful samples were studied with reproducibility to ensure the results.

Table 5. Unsuccessful parameter.

Sample	1	2	3	4	5	6
Agar (%)	84	84	77	77	70	70
AAc (%)	16	16	23	23	30	30
Reaction (min)	30	30	30	30	30	30
Doses	15	20	15	20	15	20

Table 6. Successful parameter

Sample	M51-M52	M53-M54	M55-M56	M57-M58	M59-M60
Agar (%)	80	80	70	70	60
AAc (%)	20	20	30	30	40
Reaction (min)	15	20	15	20	15
Radiation (kGy)	20	25	20	25	20

4.1 Proposed mechanism of interaction monomers using gamma rays.

Agar and AAc was subjected a crosslinking using gamma rays. The radiation on the AAc affect mainly the vinyl group over the carbonyl group. The carbonyl group is not prone to polymerization by radical initiators because of its polarized nature (Odian, 2004). Two free radical will be formed into the vinyl group by which the monomer could self-polymerize and form linkages with free radical on the agar. On another hand, the polysaccharide agar will form free radicals on hydroxyl groups, which have been reported in other polysaccharide such as chitosan (similar structure). Also, due to its difference in electronegative values and binding dissociation energy (463 kJ/mol), hydroxyl group is readily deprotonated. Finally, based on the bibliography and the process performed, we proposed the mechanism with a new bond formed between carbon and oxygen (358 kJ/mol) as you can see in Figure 24.

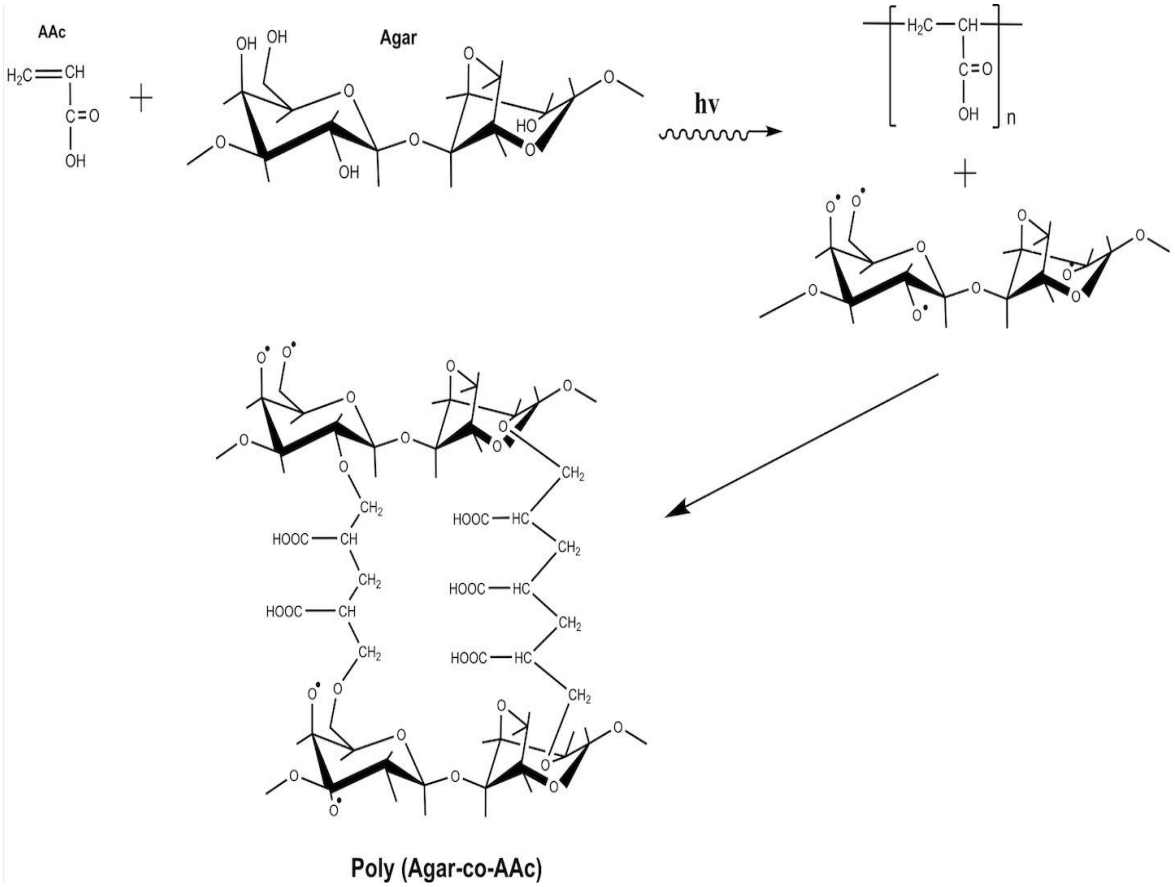


Figure 24. Proposed mechanism of interaction between agar and acrylic acid by gamma rays.

4.2 pH residue

AAc present a pKa= 4.26 at 25 °C. In this case the samples were subjected to several washes in an aqueous solution to determine the deprotonating of the acidic groups. The chemical behavior of the carboxylic acids is determined by the –COOH. Therefore, the carboxyl group consists of a carbonyl group (C=O) and hydroxyl group (-OH). So, -COOH will be deprotonated in presence of water as you can see in Figure 25. In this case, the –OH is the group which will suffer deprotonating (Eq. 3). The Annexes 4 depict the values of pH residue after three washes.

$$RCO(aq) + H_2O(l) \rightleftarrows RCOO^-(aq) + H_3O^+(aq) \qquad Eq.3$$

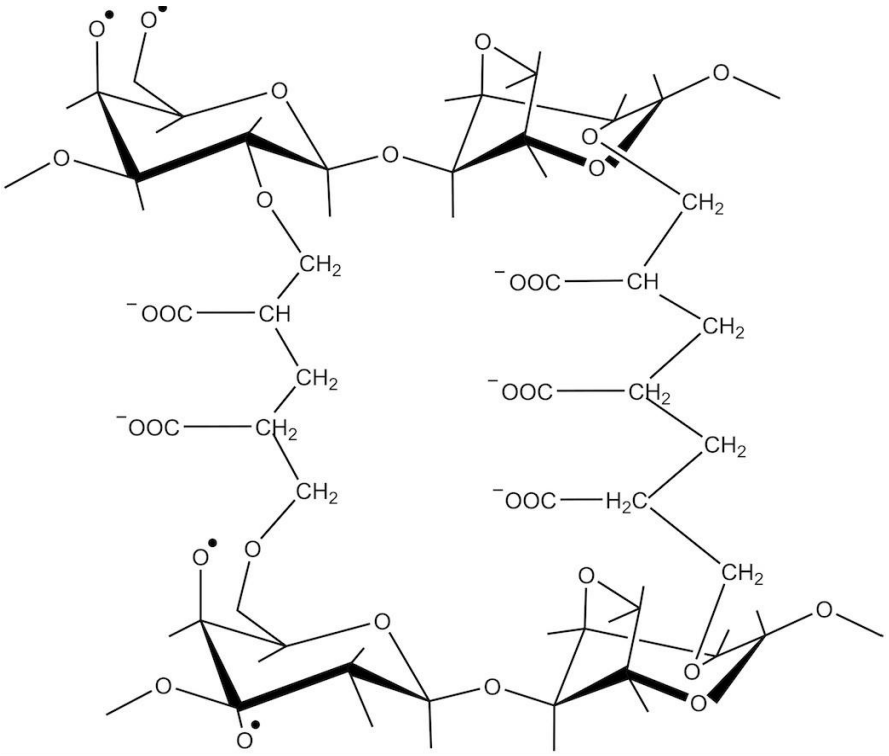


Figure 25. Deprotonated of the carboxyl group in presence of water.

4.3 Monomer concentration effect

The concentration of each monomer is important to take into account during the formation of new material. It was realized a study of the monomer concentration to determine how it affects the properties of the hydrogel. Figure 26 depicts the effect present in samples subjected at the same doses but different monomeric concentrations. Therefore, the hydrogel at 80% of agar presents major solubility to the water than 70 or 60%. The reason corresponds to the great amount of hydroxyl group in the agar structure which is readily forming hydrogen bonds with the surroundings than the groups present in the AAc. Therefore, this implies major swelling but with a brittle property, because AAc provides stiffness to the system.

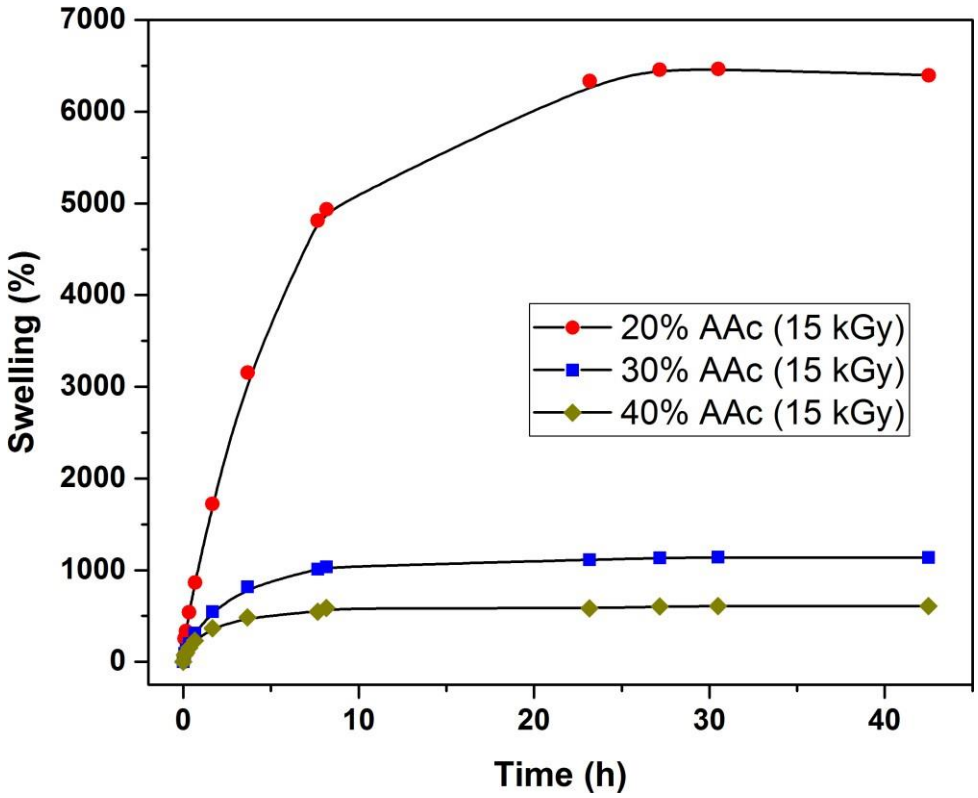


Figure 26. Monomer concentration based on agar concentrations

4.4 Dose effect

The polymerization rate increase at high irradiation doses, which implies a major number of free radicals. However, the end rate also depends on high-doses. In this case, it can observe that the doses also affect the polymeric matrix. So, when the hydrogel is subjected at high radiation doses, it increases the crosslinking of the chains, which results in a decrease of the solubility. Hydrogels at high irradiation doses present a major crosslinking, therefore the swell will be minor than at less irradiation doses. Figure 27 depicts a results of sample with the same monomer concentration but subjected at different irradiation doses. These samples were immersing in water and reported their values.

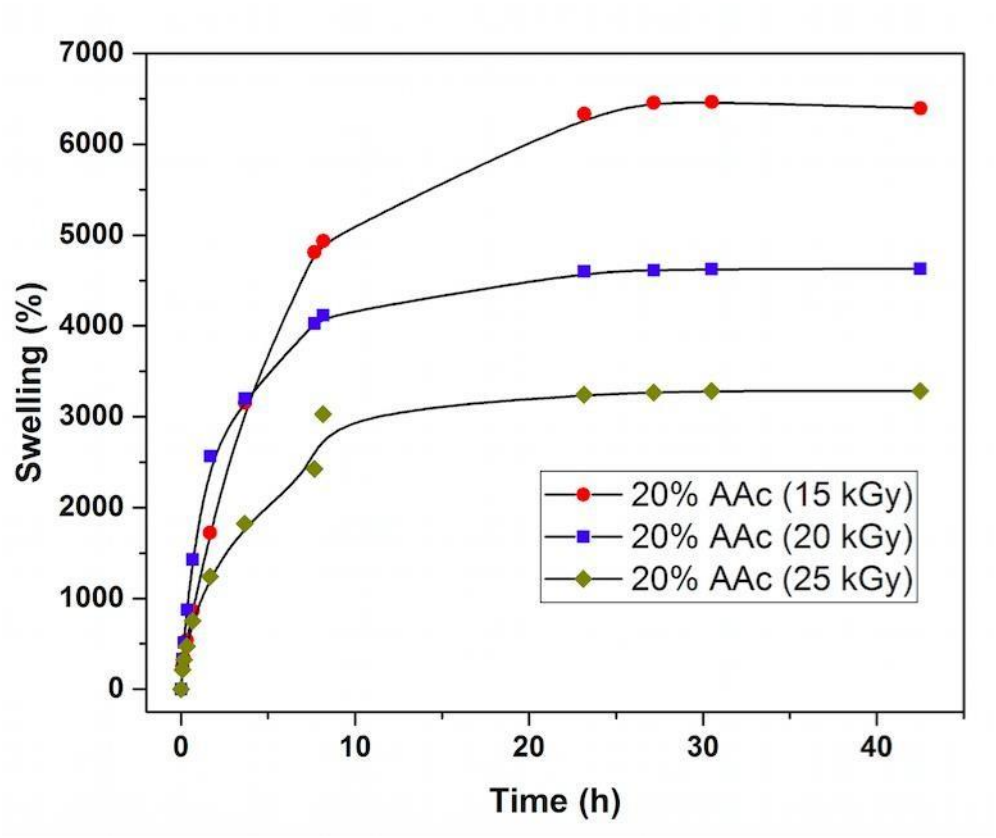


Figure 27. Doses effect of samples irradiated at different doses.

4.5 Characterization of polymeric compound poly(Agar-co-AAc): limit swelling, critical pH

These tests were performed making a comparison between different monomer concentrations and doses applied. For every case, it studied the effect on the hydrogel.

4.5.1 Limit swelling.

Limit swelling tests was performed at different monomer concentration (20, 30, and 40%) of AAc and agar (80, 70 and 60%). Additionally, it analyzed the effect of the irradiation doses (25, 20, 15 kGy) on each concentration. Figure 28 shows the rapid water absorption at the beginning and became reduced with increasing immersion time. The limit swelling varies in the samples, mainly in samples at major amount of agar (1), in this case approximately 25 hours to reach the maximum. However, the another samples (2 and 3) reach the maximum swelling approximately at 10-12 hours.

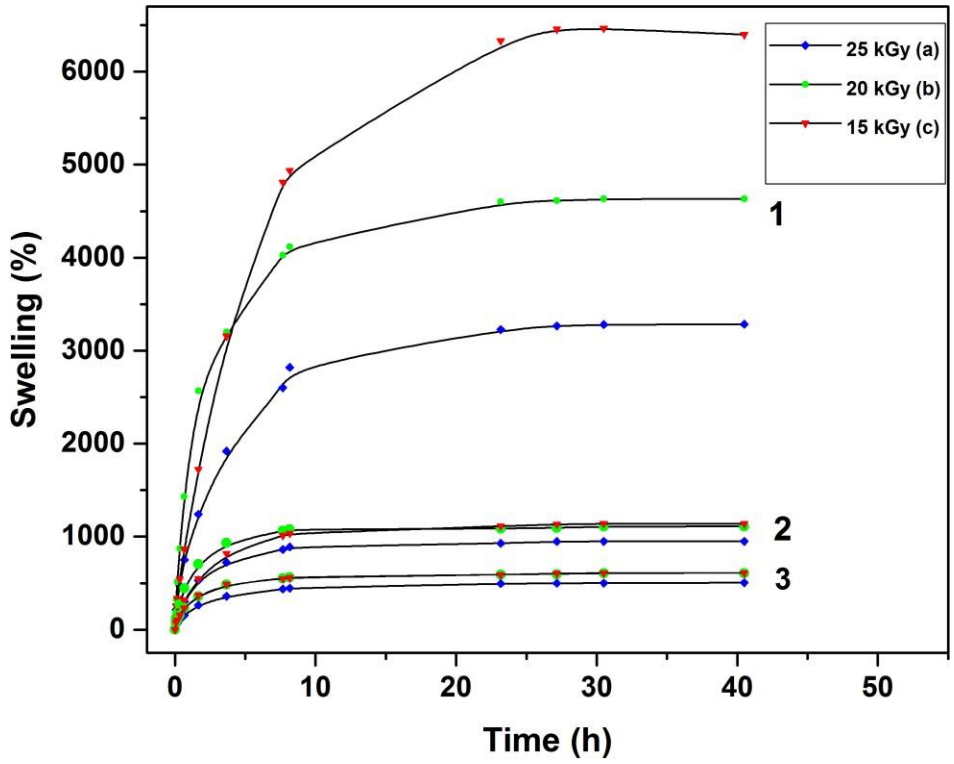


Figure 28. Determination of limit swelling of samples at different monomer concentrations and doses applied. 1) 20% AAc 2) 30% AAc. 3) 40% AAc.

The reason corresponds due to the present of hydrophilic groups (hydroxyl) in the composition of the agar, which has a great facility forming hydrogen bonds with the water. Although the carboxylic acids (–COOH) in the AAc also can form hydrogen bonds, the hydrophilicity of the hydroxyl groups (-OH) is greater. This implies, that a major AAc, the swelling will be minor.

Likewise, at minor doses applied to the system, the swelling is major in every sample. The reason is that the crosslinking is proportional to the doses applied, is to say, at major doses 25 kGy, the hydrogel will present a major crosslinking than a sample irradiated at 20 or 15 kGy. However, the crosslinking is disproportional to the solubility. This explains the reason every sample irradiated at 25 kGy, independent of the concentration of agar, show less swelling the others. The values of every sample are presented in the Annexes 5, 6, 7 and 8.

4.5.2 Critical pH and pH sensibility.

pH tests were based on the time of limit swelling of each samples, to switch every pH medium. The critical pH for poly (Agar-co-AAc) with different parameter was found to be 5.3

approximately (see Figure 29), finding the equation of each curve, and its subsequent derivative to determine the maximum peak and its second derivative to ensure the value. At pH values below 5.3 carboxylic acid groups of AAc are not ionized (collapsed state) diminishing the exposition to water molecules, meanwhile at pH values above 5.3 the ionization of the COOH groups leads to repulsions among AAc chains, which makes them to adopt a swollen conformation. Blank (1939) reported that the skin pH values between 4.2 and 5.6, while Zlotogorski (1987) emphasized for forehead pH is 4.0-5.6 and for the cheek it is 4.2-6.0. Additionally, the pH sensitivity presents a progressive increment inversely proportional to the amount of acid and this value can be observed at any dose. The obtained results seem to be according to the pH values previously reported by Zlotogorski and Blank. The -COOH group is a weak acid which is partially dissociated, but the OH group provides major hydrophilicity to the system, making the pH critic forward to basic values.

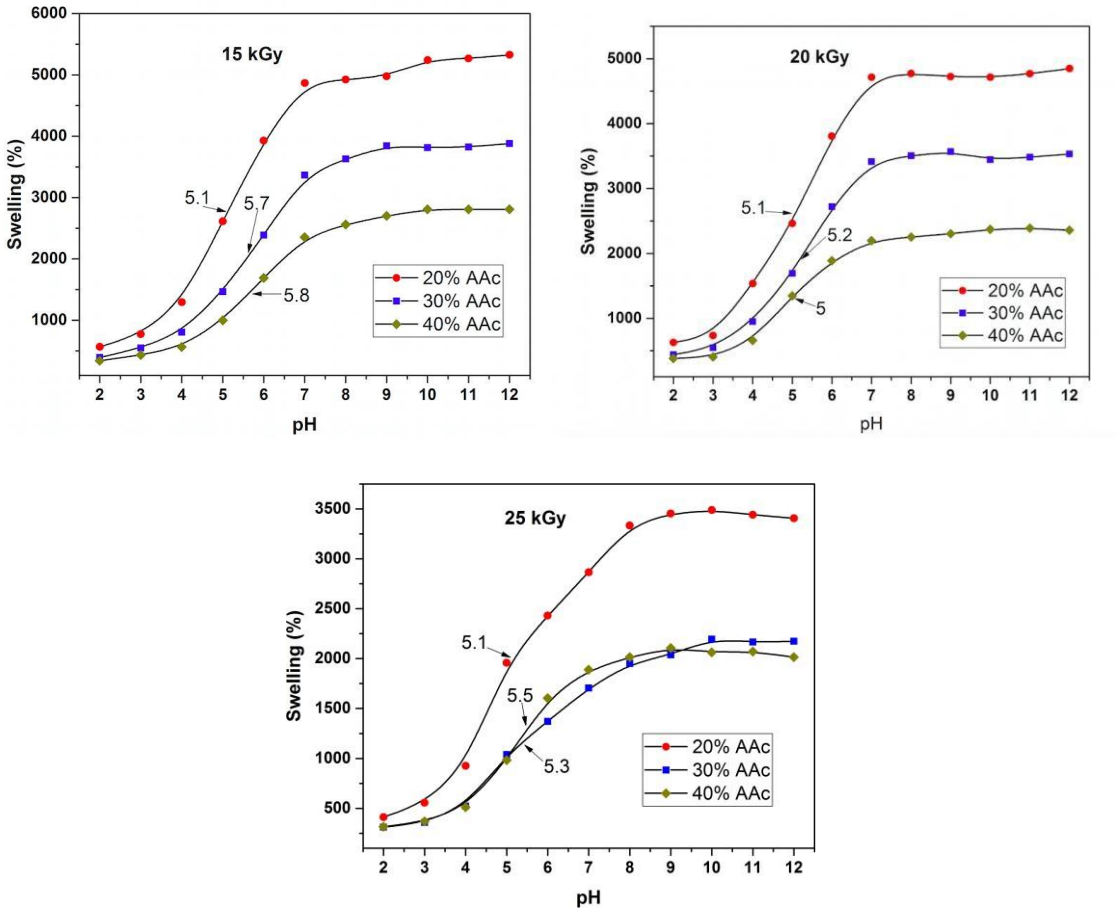


Figure 29. Determination of critical pH at different concentrations and doses of copolymer.

4.5.3 Contact angle.

Contact angle technique allows determine the hydrophilicity degree of the material. Figure 30 present the angle formed in two sample while, Table 7 present the angle values obtained. The adhesion that happens during the interaction between liquid and solid provokes that the cohesion force of the first one increment the volume in a bulk form and try to avoid it.

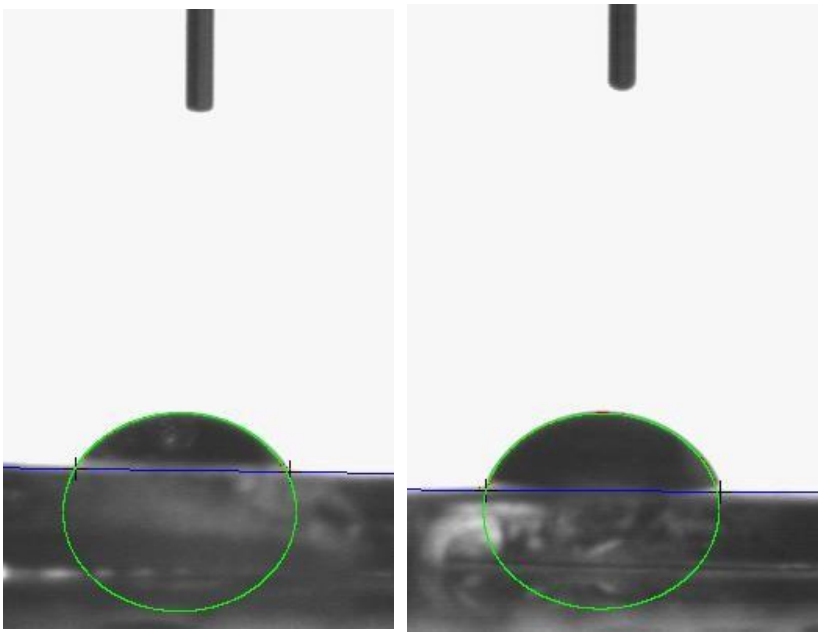


Figure 30. Determination of contact angle comparing two sample: 80% (left) and 60% (right) of agar at 20 kGy

Hydrophilicity is a characteristic proper of the hydrogels. Table 7 present the contact values formed between the surface our hydrogel at different concentrations of monomer and applied doses with a drop. The copolymer samples show angles minor than 90°, demonstrating the hydrophilic behavior. The increase in the order of hydrophilicity is proportional to the amount of the agar, due to this monomer present hydroxyl group which has a great affinity with the water and readily form hydrogen bonds. This results agree with the limit swelling based on the monomer concentration.

Table 6. Contact angle results of copolymer obtained.

Sample	Agar (%)	Contact angle (°)	Deviation
15 kGy	80	71.4	±0.33
	70	77.5	±0.13
	60	83.7	±1.59

20 kGy	80	72.8	±0.19
	70	75.9	±0.44
	60	76.7	±0.44
25 kGy	80	74.4	±0.56
	70	77.8	±0.31
	60	81.2	±1.12

4.6 Characterization by FT-IR

The characterization of polymeric materials was performed by FT-IR, by the comparison of the monomers precursors and the sample as show in Figure 31 and Table 8.

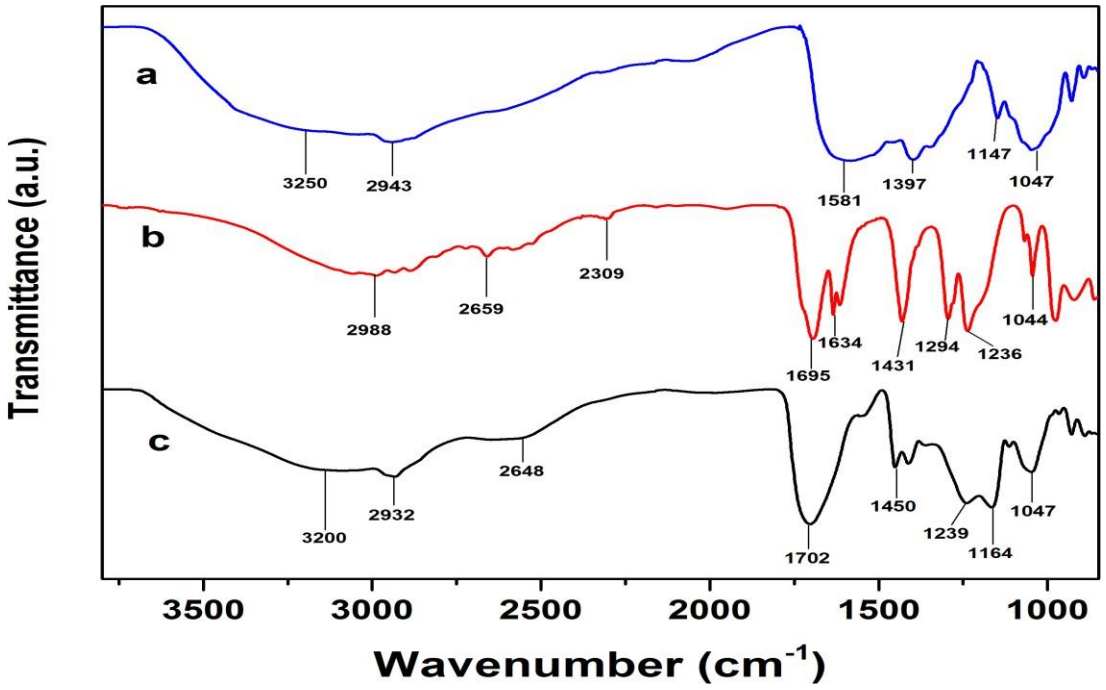


Figure 31. FT-IR spectrum of monomers and hydrogels: a) agar, b) AAc, c) poly (agar-co-aac).

The FT-IR spectrum of the agar monomer has a band at 3250 cm^{-1}, which corresponds to vibration of alcohol group, a little band at 2943 cm^{-1} that belong to C-H bond with sp^3 hybridization, then there is a band at 1581 cm^{-1} related with C-OH flexion in the plane, two more bands in 1397 and 1047 cm^{-1} which refers to C-C bending and C-O stretching respectively.

However, the FT-IR spectrum of the AAc monomer present characteristics bands at 2988 $cm^{-1}$of the alcohol group, an another band in 2659 cm^{-1} associated to CH_2 bond; so, how is

normal in the carboxylic acid presents a double bond at 1695 cm^{-1} corresponding to carbonyl group (C=O), at 1634 cm^{-1} it observes a band that represents the C=C acyclic of the vinyl group, in 1431 cm^{-1} there is another band associated to C-H bond, another two bands are observed at 1294 and 1236 cm^{-1} which are characteristics of C-O of the carboxylic acid that is asymmetric and symmetric respectively, finally the band at 1044 cm^{-1} correspond to C-O bond.

Therefore, the poly (Agar-co-AAc) present similar functional groups in its structure. So, the band of the vinyl group reported in the acid disappear, showing that the polymerization occurs in this linkage.

Table 7. Contact angle results of copolymer obtained

Functional group	**Agar (cm^{-1})**	**AAc (cm^{-1})**	**Hydrogel (cm^{-1})**
O-H	3250	------	3200
C-H	2943	2988	2932
$C-H_2$	------	2659	2642
C=O	------	1695	1702
C=C	------	1634	------
C-H	------	1431	------
C-O	1047	1044	------

4.7 Characterization by TGA

Thermogravimetric analysis is a technique that allows determine the thermal stability of the materials. For this technique, it compared monomer precursors (Agar and PAAc) and the final result poly (Agar-co-AAc) and loaded with AgNPs as show in Figure 32 and Table 8.

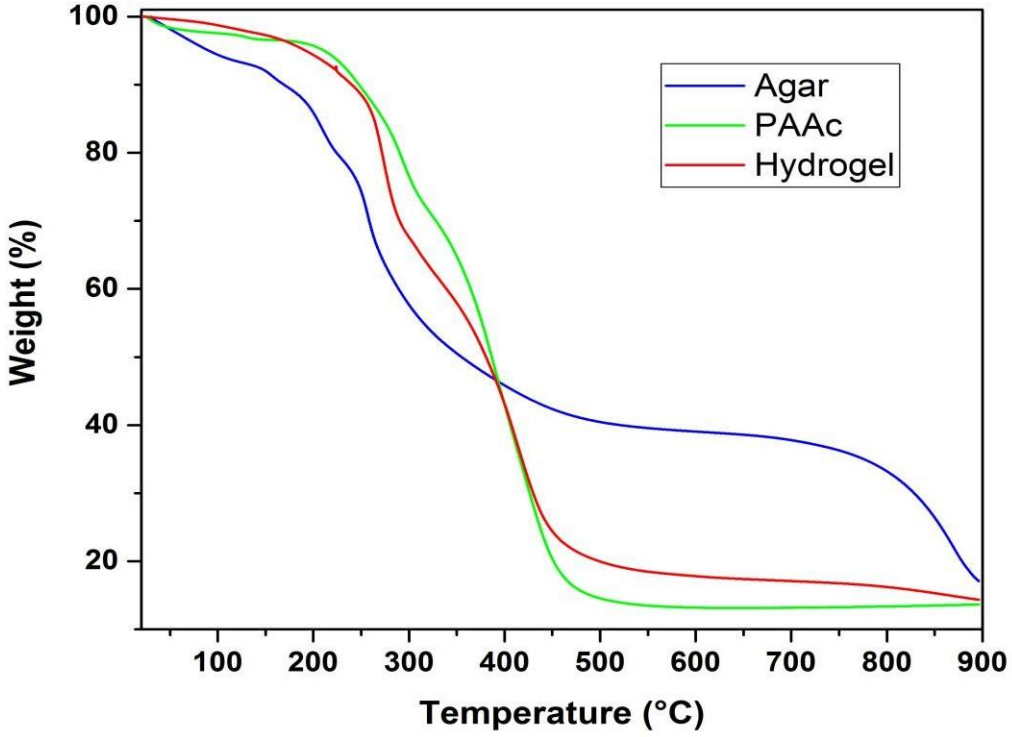


Figure 32. TGA results of monomers and the copolymer.

Agar loss the 10% of the initial mass at 196.04℃, also it presents several decomposition temperatures at 155.74, 209.40, 256.99 and 868.42℃ respectively. The larger decomposition of the agar is related with the agarose content, meanwhile the second one at 868.42℃, correspond to agaropectin. So, the large amount of agarose content (70%) against the small amount of agaropectin (30%) supports this argument, therefore agarose content is shown in Figure 32. On another hand, PAAc presents a better thermic resistance due to it loss the 10% of its initial mass at a 247.63℃ and its decompositions also occur in three steps, at 130.02, 293.53 and 393.72℃. Finally, the hydrogel at 240.05℃, loss the 10% of its mass, however, we could analyze a positive effect in the thermic resistance during the mixture between monomer due to it show two decomposition temperatures, at 273.5 and 413.78℃ respectively.

Table 8. Description of values by TGA

Sample	**10% mass loss (℃)**	**Temperature Desc. (℃)**	**Residue at 900 ℃ (%)**
Agar	196.04	155.74 209.40	28.04

		256.99 868.42	
PAAc	247.63	130.02 293.53 393.72	13.65
Hydrogel	240.05	273.5 413.78	14.35

4.8 Characterization by DSC

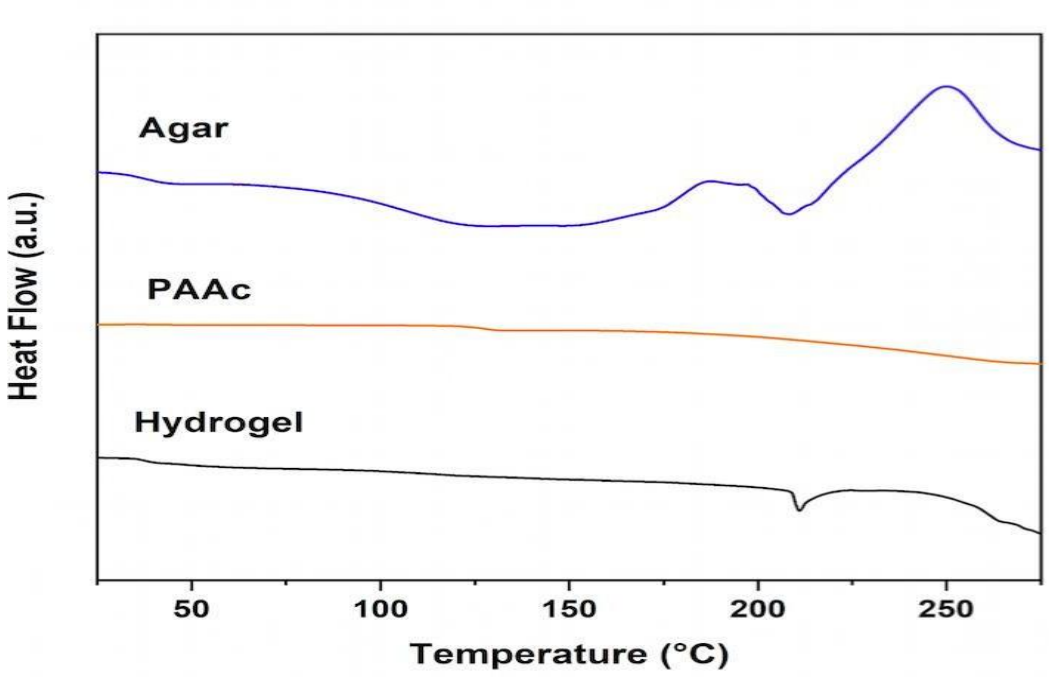


Figure 33. DSC results of monomers and copolymer.

DSC analysis was employed to determine the thermic transitions of the materials, mainly Tg as shown in Figure 33 and details Table 9. The Tg determine the transition from crystal or semi crystal to rubbery material.

Agar presents the Tg at 32.95℃, also at 207.76℃ present an endothermic peak while at 249.47℃ an exothermic peak. Meanwhile, PAAc present the Tg at 128.83℃. Finally, poly (Agar-co-AAc) present the Tg at 37.54℃, however, it also presents two endothermic peaks at 211.08 and 278.10℃ respectively, similar values than agar monomer.

Table 9. Description of values by DSC

	Glass Transition (℃)	Thermal Transitions (℃)
Agar	32.95	207.76 249.47
PAAc	128.83	-------
Hydrogel	37.54	211.08 278.10

4.9 Mechanical testing

Sample films of poly (Agar-co-AAc) were subjected to tensile tests to determine the Young modulus, percentage of deformation. The acrylic acid, present better mechanical properties because its viscosity, which provides a thick and sticky consistency to the hydrogel reducing their swelling capacity.

Samples with less amount AAc have better elastic properties, showing better resistance to breakage. Figure 34, the sample with 20% AAc and 15 kGy shows the best elastomeric behavior than the another samples.

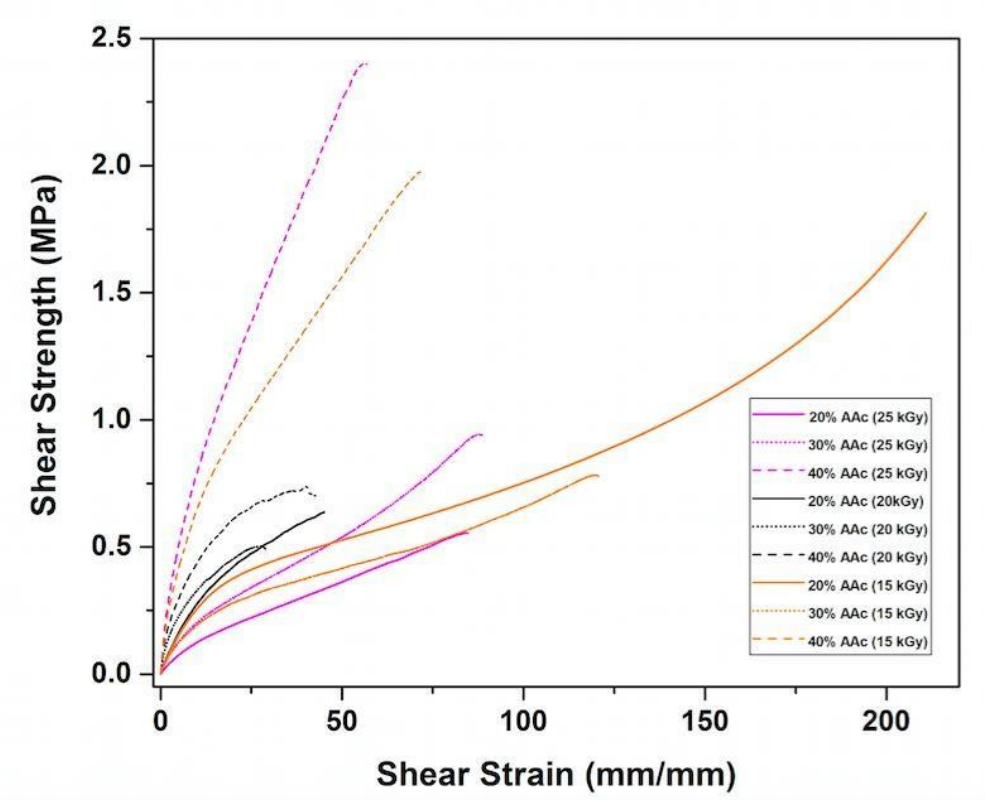


Figure 34. Results of tensile testing at different monomer concentrations and applied doses.

It is important to mention that Young's module is directly proportional to the rigidity of a material. In general, we can conclude that the concentration of AAc directly affects the stiffness of the material as shown in Table 10, determined the progressive increase of Young's modulus as the concentration of AAc increases, that is, a better resistance to elongation.

It is known that crosslinking of a polymeric matrix is proportional to the irradiation doses, providing a major interaction between the chains and stiffness. This criterion is agreeing with samples irradiated at 20 and 25 kGy, because according with the Figure 34, the stiffness is better. On the opposite side, with less irradiation dose, the crosslinking is not strengthened, resulting in elastomeric behavior. So, in general, elongation is inversely proportional than the irradiation doses and AAc concentration.

Table 10. Obtained values of Young Modulus and mechanical properties

Sample (hydrogel)	Radiation (kGy)	Young modulus (MPa)	Tensile Test (MPa)	Displacement prior to breakdown (mm)
20% AAcc	25	0.44 ± 0.35	0.665 ± 0.51	85.26
30% AAc	25	1.06 ± 0.80	1.335 ± 0.44	90.05
40% AAc	25	4.49 ± 2.16	2.460 ± 0.28	56.11
20% AAc	20	0.73 ± 0.13	1.03 ± 0.21	46.11
30% AAc	20	1.24 ± 1.13	0.60 ± 0.17	31.52
40% AAc	20	1.19 ± 0.24	0.76 ± 0.13	43.67
20% AAc	15	0.79 ± 0.22	2.56 ± 0.44	215.17
30% AAc	15	0.85 ± 0.31	1.11 ± 0.42	122.72
40% AAc	15	3.9 ± 1.29	2.16 ± 0.035	73.40

4.10 Load of antimicrobial compounds

The load of different drugs in any polymeric system will depend on the nature of all components involved. Therefore, the load of ciprofloxacin and AgNPs at different monomer concentration and doses applied is studied. A calibration curve was performed to determine the correlation between compound concentration with absorbance of it. Also, it is important to note that the assays were made by duplicate to ensure the correct values by reproducibility.

4.10.1 Load of ciprofloxacin.

Ciprofloxacin is a compound that contains in its structure carboxylic and amine groups (Figure 35), by which it can interact with donor and receptor groups of electrons.

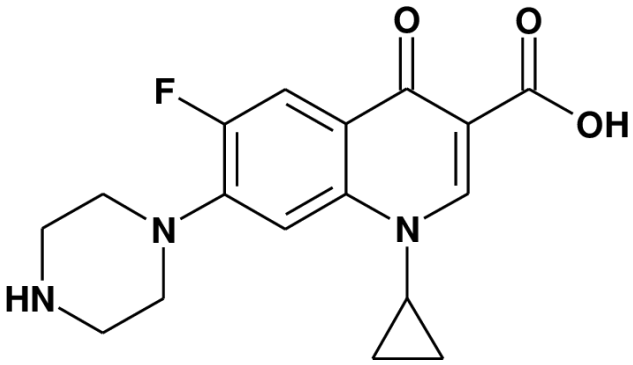
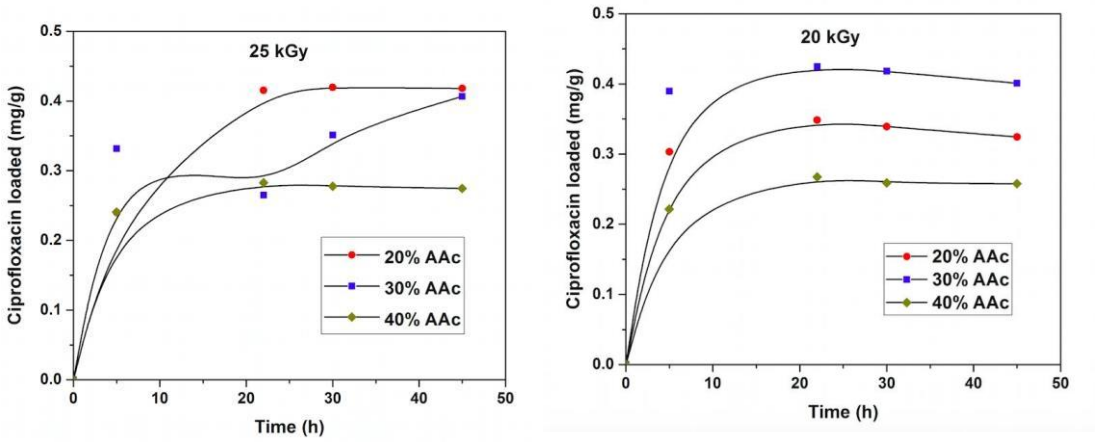


Figure 35. Chemical structure of ciprofloxacin (Diab, Shqair, Salim & Alsubu, 2014)

Samples at different monomer concentration and doses were load with ciprofloxacin. The concentration of ciprofloxacin was 0.012 mg/mL. Figure 36 shows the load, meanwhile, the calibration curve is shown in Annexes 2. Poly (Agar-co-AAc) at 30% AAc concentration demonstrate the major adsorption of the antimicrobial compound. Likewise, sample at 30% AAc and minor doses applied (15 kGy) present the major adsorption of the material, this could indicate that as minor crosslinking exist a better link (union between functional groups) with the compound. The values indicate that 30% of AAc has a great affinity to form the interaction drug-polymer.



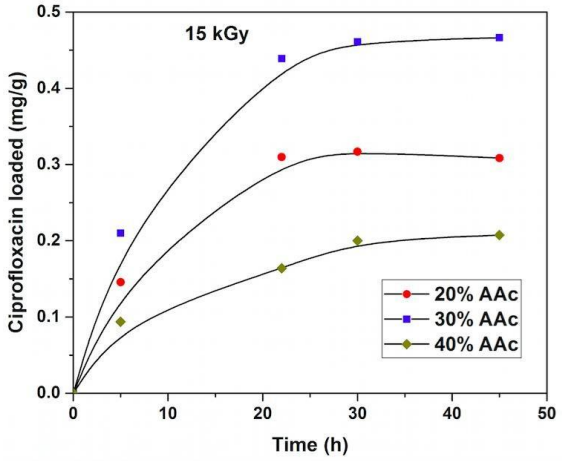


Figure 36. Load of ciprofloxacin. Condition: 25 ºC and 0.012 mg/ml. Constant stirring.

The specific percentage of load and delivery is described at Annexes 11. Likewise, hydrogel at 30% of AAc present a major affinity to the ciprofloxacin during the load, this is seen during the decrement in the absorbance (UV-spectroscopy). The assays of drug delivery of ciprofloxacin were carry out at controlled temperature and pH (T=37 ºC and pH=6 and saline solution at 0.9% m/v).

The delivery at pH=6 was choose, because the pH of skin round this value. These samples showed a great capacity to load the drug, however during the delivery the best sample were those with less amount of irradiation doses (20 and 15 kGy), with an efficacy of almost 70% in 40 hours. However, samples subjected to saline solution are not so effective, presenting a success of approximately 50% in 40 hours. In general, samples at major irradiation doses present the less amount of delivery as shown Figure 37.

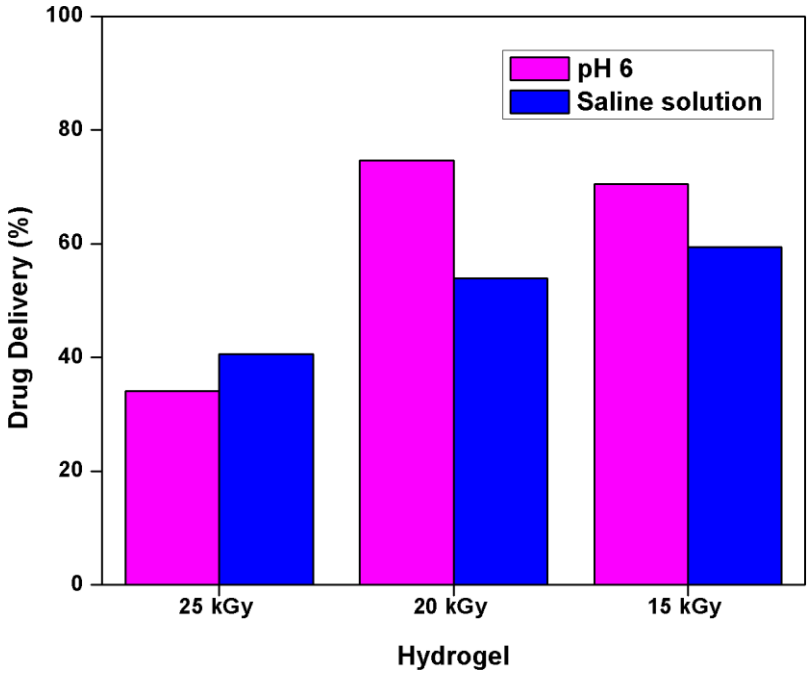


Figure 37. Percentage of drug delivery at different conditions. Constant stirring at 37 ºC

4.10.2 Load of AgNPs.

PAAc has a great affinity with some metals such as silver, copper, among others. Silver has been used as an antimicrobial compound or to the process of purification of water. Therefore, it proposed the load of AgNPs into the polymeric matrix. Figure 38 shows the load at a concentration of 0.0784 mg/ml into hydrogels at 20 and 25 kGy. The calibration curve is shown in Annexes 10. The hydrogels at 15 kGy and any monomer concentrations do not present affinity to load this antimicrobial compound after the 45 hours. Samples at 20 kGy present minimum adsorptions, demonstrating an improvement during the interaction between the copolymer and the compound as the crosslinking increase. Likewise, as the crosslinking rise due to an increment of doses applied, the nanoparticles present better adsorption between the drugs and the polymeric chains. Sample at 20, 30 and 40% AAc at 25 kGy has a significate load of 0.37, 0.33 and 0.31 mg/g respectively.

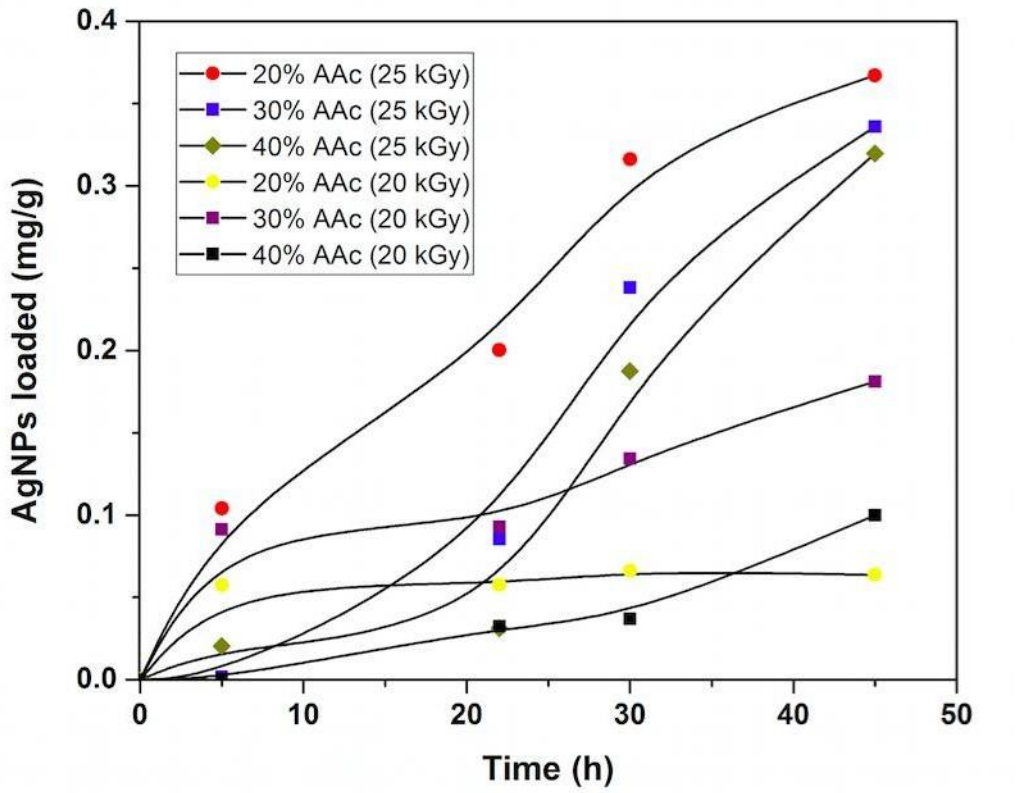


Figure 38. Load of AgNPs. Conditions: 20 ºC and 0.0784 mg/ml. Constant stirring

Following the election than ciprofloxacin, the load of AgNPs in hydrogels with 30% of AAc content were studied again at different doses. The annexes 9, show the percentage values performed during these tests, where likewise than the Figure 38 and 39, the load is proportional to the dose irradiation. The results affirm that the deposition of the AgNPs occur at a major crosslinking between the chains.

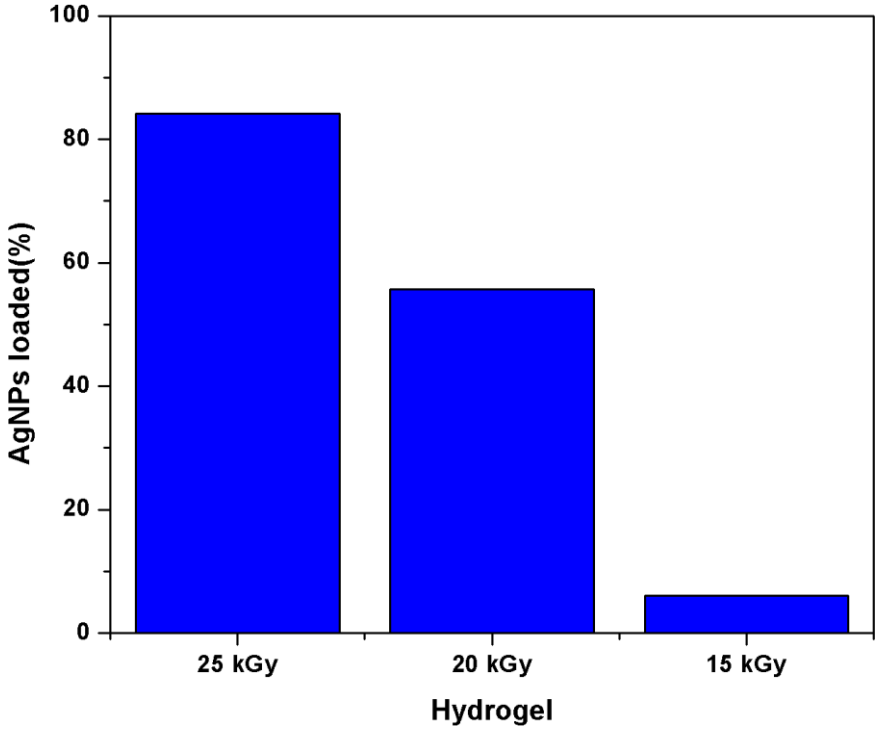


Figure 39. Comparison in AgNPs loaded at different irradiation doses.

4.11 Antimicrobial activity

This technique was determined by optic density at 600 nm of absorbance during 18 hours of incubation with the hydrogel loaded with antimicrobial compounds (Figure 40 and Table 11 were performed). Two types of bacteria were examined, *Escherichia Coli and Methicillin Resistance Staphylococcus Aureus* using samples at 70% of agar and 30% of AAc content and irradiated at 25 and 20 kGy.

Table 11. Results of sample loaded with ciprofloxacin and AgNPs for microbiological tests.

Sample/Bacterial survival (%)	**E.coli (%)**	**MRSA (%)**
Hydrogel-ciprofloxacin-25kGy (S1)	1.772928742	38.38194152
Hydrogel-Ciprofloxacin-20kGy (S2)	1.909307876	59.64252678
Hydrogel-AgNPs-25 kGy (S3)	1.750198886	51.64628896
Hydrogel-AgNPs-20 kGy (S4)	1.716104103	64.81656301

Figure 40 present the control sample where 100% of the bacterial growing was observed. The death bacterial depicts approximately 98% of efficacy against *E. coli*, which suggest that *E. coli* is highly sensitive to hydrogel loaded both AgNPs and ciprofloxacin. Antimicrobial activity against the *MRSA* decreased its effectiveness than with E. coli. Hydrogels loaded with AgNPs reduce from 40 to 50% (S3 and S4) the percentage of bacteria growing, meanwhile that

hydrogel load with ciprofloxacin present an inhibitory effect until 40% (S1 and S2) against the MRSA. The interesting point is that as the irradiated doses increase in the sample, it has an improved antibacterial effect.

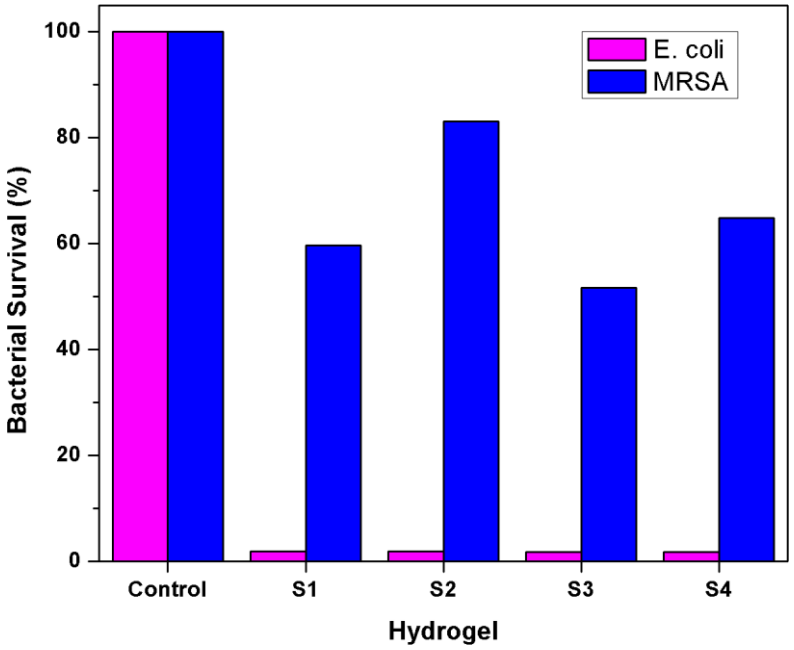


Figure 40. Antimicrobial effect against E.coli and MRSA.

5. CONCLUSIONS AND RECOMMENDATIONS

- The creation of hydrogels from Agar and AAc irradiated simultaneously is feasible using gamma radiation. The process is reproducible obtaining different properties depending on the monomer concentrations and doses applied.

- The degree of AAc significantly alters the properties of the material, including the swelling, pH behavior, and mechanical properties. The crosslinking increases as the doses rise, the change of properties also are affected by the crosslinking degree.

- Although both monomers are hydrophilic, during the increment in Agar concentration, the hydrogels present major swell behavior. Additionally, the swelling is inversely proportional with the increment of AAc concentration and doses applied.

- Critical pH does not show a great difference between monomer concentrations, keeping in the range from 5.0 to 5.8. However, the pH sensitivity is proportional to the amount of Agar concentration.

- The values obtained from the contact angle ensure the hydrophilicity of the material. Also, the homogeneity of the material was verified taking different points of the hydrogel and make the measurement.

- FTIR-ATR and thermal analysis allow ensuring the crosslinking, which increase the thermal resistance.

- Samples at 15 kGy do not present significate adsorption of AgNPs in comparison of 20 and 25 kGy. Likewise, every sample presents a good specificity for the ciprofloxacin, showing good adsorption values.

- Mechanical tests provide information about the stiffness and elasticity properties. The stiffness of the material is proportional than the amount doses applied, likewise, the elastomeric behavior is proportional to the amount of agar concentration.

- The hydrogel loaded with AgNPs and ciprofloxacin present a great efficiency against the different bacteria strains, showing a good specificity of the hydrogel against mainly *E.coli* than MRSA. Also, we can see an improvement as the degree of crosslinking increases.

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7. ANNEXES

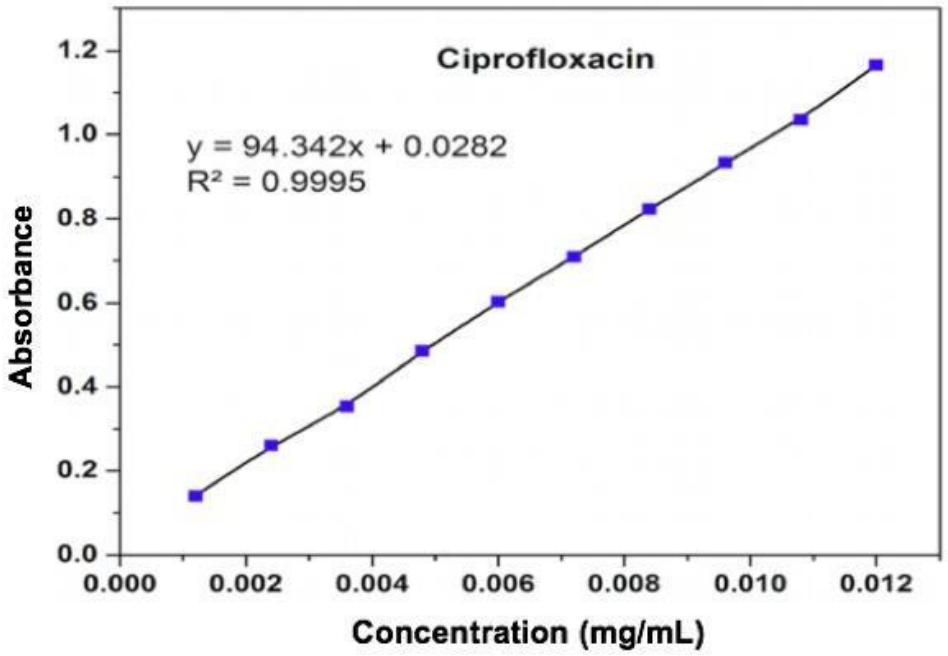
Annexes 1. Preparation of buffer solutions

pH	Solution 1 (mL)	Solution 2 (mL)
2	195	5
2.5	184	16
3	176	24
3.5	166	34
4	155	45
4.5	144	56
5	134	66
5.5	126	74
6	118	82
6.5	109	91
7	99	101
7.5	92	108
8	85	115
8.5	78	122
9	69	131
9.5	60	140
10	54	146
10.5	49	151
11	44	156
11.5	33	167
12	17	183

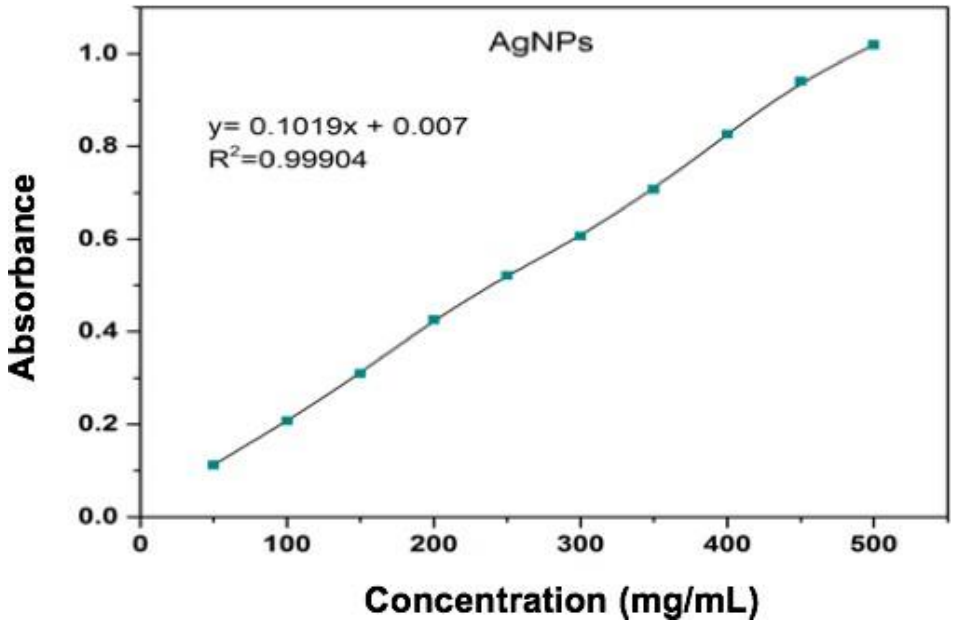
Solution 1: Boric acid (H_3BO_3) at 0.2 M and citric acid ($C_6H_8O_7$) at 0.05 M.

Solution 2: Dodecahydrate trisodium orthophosphate ($Na_3PO_4 \cdot 12H_2O$) at 0.1 M

Annexes 2. Calibration curve of ciprofloxacin.



Annexes 3. Calibration curve of AgNPs.



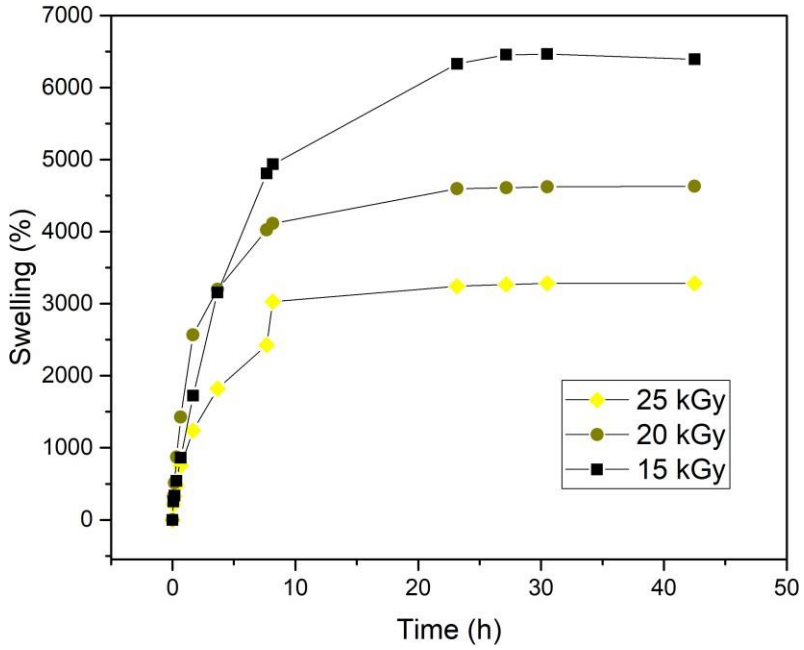
Annexes 4. pH of sample after three washes

Sample	First wash	Second wash	Third wash
M41	3.153	3.944	4.32
M42	3.165	4.08	4.25
M43	3.224	4.15	4.284
M44	3.233	4.04	4.203
M45	3.245	4.103	4.32
M46	3.27	4.07	4.22
M48	3.254	4.23	4.34
M49	3.13	4.165	4.275
M50	3.19	4.12	4.319

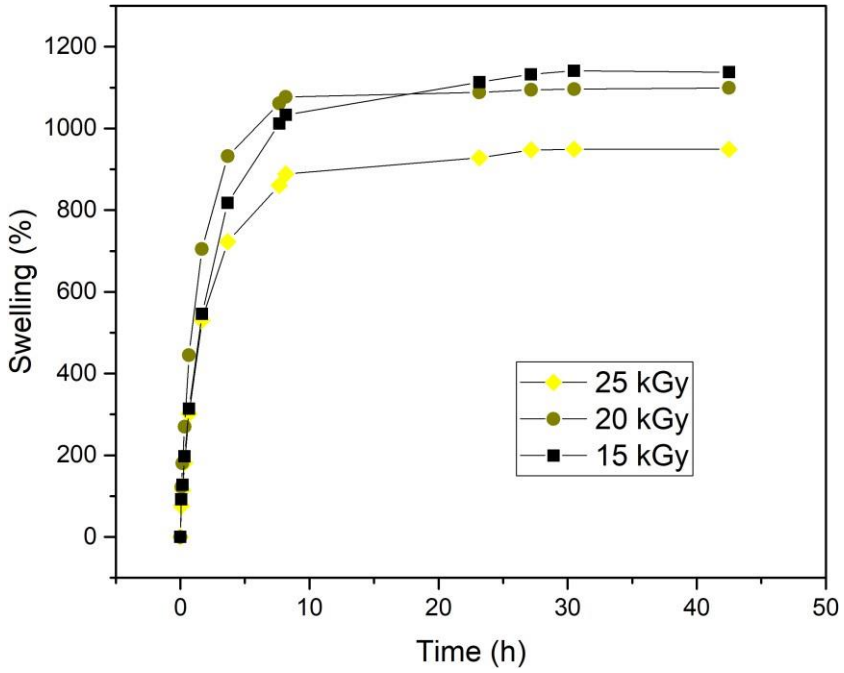
Annexes 5. Results of limit swelling.

AAc (%)	Agar (%)	Doses (kGy)	Final Swelling (%)	Standard Deviation
20	80	25	3282.90836	1342.872932
20	80	20	4630.7066	1856.052253
20	80	15	6396.168582	2719.07355
30	70	25	949.106455	383.9124475
30	70	20	1099.4961	437.1410137
30	70	15	1137.606032	462.2201449
40	60	25	502.12716	198.0244671
40	60	20	594.320934	230.4813265
40	60	15	607.712766	235.6757319

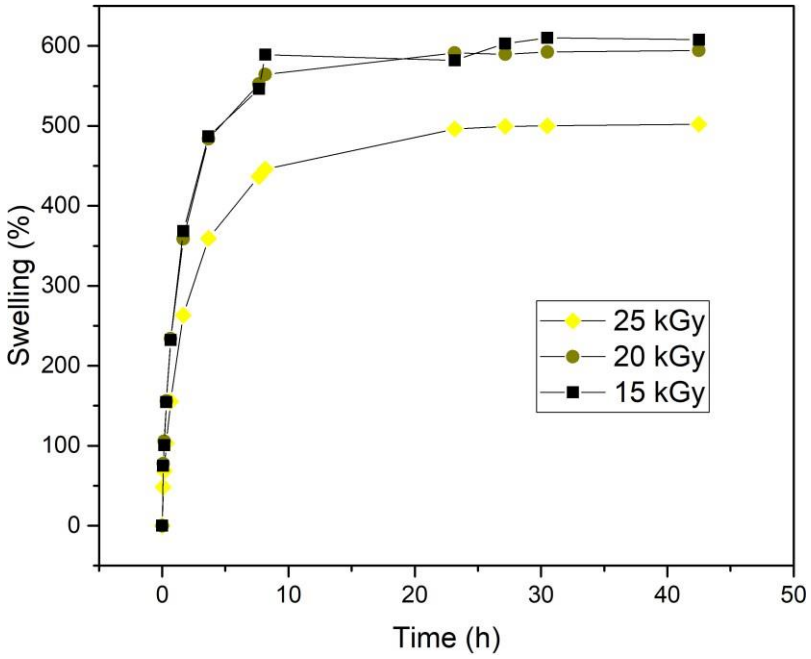
Annexes 6. Limit swelling using 80% agar at different doses



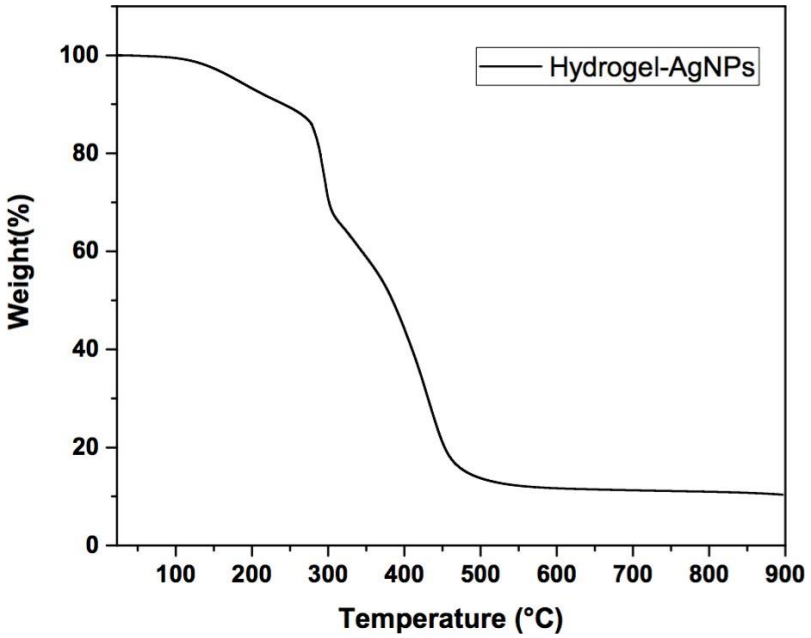
Annexes 7. Limit swelling using 70% agar at different doses



Annexes 8. Limit swelling using 60% agar at different doses



Annexes 9. TGA of hidrogel loaded with AgNPs..



Annexes 10. Description of values at 30% of AAc subjected to load and delivery of ciprofloxacin.

Ciprofloxacin	25 kGy	20 kGy	15kGy
Load (%)	86.65	77.09	89.68
Delivery (pH 6) (%)	34.12	74.67	70.46
Delivery (Sol. saline) (%)	40.58	53.94	59.42