UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Químicas e Ingeniería

TÍTULO: MODIFICATION OF COTTON GAUZES WITH pH-SENSITIVE POLYMERS BY GAMMA RADIATION

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

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DEDICATION

I want to thank God for giving me the strength and courage necessary to be at this moment in my life and for putting great people on my path who knew how to guide me along this process.

This thesis is dedicated to my parents, **Luis Carlos Romero** and **Sandra Janeth Fierro**, who are my greatest inspiration and pride, for their unconditional love, for the moral support they have shown me throughout my life, for their advice and confidence they have given me in difficult moments. Your example guides my steps, your words and lessons forged my character and your love illuminates my life. I love them.

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To my cousins **Salomé** and **Carlita**, who are like my younger sisters. This thesis is dedicated to you as a sign that dreams can come true. I love them with my soul.

To all my family, who are my greater motivation to improve myself day by day. Your moral support and love regardless of the circumstances were essential to complete this wonderful stage of my life. My eternal gratitude and effort will always be for you.

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To each person who contributed a grain of sand to make it possible. Thank you!

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RESUMEN

El interés en los polímeros sensibles al estímulo, que tienen una conformación dependiente de las variables fisicoquímicas de los medios, ha experimentado un crecimiento exponencial en los últimos veinte años debido al enorme potencial que contienen como componentes de los sistemas inteligentes de administración de fármacos. Entre ellos, aquellos que sufren cambios reversibles dentro de los rangos fisiológicos de variables como el pH, la temperatura o la fuerza iónica, son especialmente útiles como componentes de formas de dosificación con la capacidad de liberar fármacos en respuesta a variables fisiológicas o la aplicación de un estímulo externo. Es por esta razón que se ha estudiado la forma de modificar las superficies de los dispositivos biomédicos para combinar sus propiedades con las de los polímeros de estímulo sensibles. Una de las formas más exitosas ha sido la modificación superficial por radiación gamma. En este sentido, el objetivo principal de este trabajo fue sintetizar un biomaterial de algodón que contenga un polímero que responde al pH, mediante un injerto inducido por radiación gamma. Los ácidos acrílico y metacrílico, compuestos que tienen una respuesta de pH, se injertaron en la matriz polimérica de una gasa de algodón, por el método de preirradiación oxidativa. Para encontrar las condiciones de reacción ideales, se evaluaron los efectos de la dosis de irradiación, la temperatura de reacción, la concentración de monómero y el tiempo de reacción sobre el porcentaje de injerto. El injerto se verificó mediante caracterización instrumental mediante Espectroscopía Infrarroja (FTIR) y Microscopía Electrónica de Barrido (SEM). El análisis térmico se realizó mediante Análisis Termogravimétrico (TGA) y Calorimetría Diferencial de Barrido (DSC). Del mismo modo, se evaluaron las características físico-químicas, como el pH crítico, mediante potenciometría. A través de las técnicas de caracterización, es posible determinar que el injerto de ácido acrílico y ácido metacrílico inducido por radiación gamma, por el método de preirradiación oxidativa, en gasas de algodón, es factible.

Palabras clave: *Polímeros pH-sensibles, Celulosa, Radiación gamma, Ácido acrílico, Ácido metacrílico.*

ABSTRACT

The interest on stimuli-sensitive polymers, which have a conformation dependent on the physicochemical variables of the media, has experienced exponential growth in the last twenty years due to the enormous potential they contain as components of intelligent drug delivery systems. Among them, those who suffer reversible changes within the physiological ranges of variables such as pH, temperature or ionic strength, are especially useful as components of dosage forms, with the ability to release drugs in response to physiological variables or the application of an external stimuli. It is for this reason that the way of modifying surfaces of biomedical devices to combine their properties with those of sensitive stimulus polymers has been studied. One of the most successful forms has been the surface modification by gamma radiation. In this sense, the main objective of this work was to synthesize a cotton biomaterial that contains a polymer that responds to pH, by means of a graft induced by gamma radiation. Acrylic acid and methacrylic acid, compounds that have a pH response, were grafted into the polymer matrix of a cotton gauze, by the pre-irradiation oxidative method. To find the ideal reaction conditions, the effects of the irradiation dose, reaction temperature, monomer concentration, and reaction time on the graft degree were evaluated. The graft was checked by instrumental characterization by means of Infrared Spectroscopy (FTIR) and Scanning Electronic Microscopy (SEM). Thermal analysis was performed by means of Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC). In the same way the physical-chemical characteristics, like the critical pH, was evaluated, through the use of a potentiometer. Through the different characterization techniques, was possible to determine that is feasible the grafting of acrylic acid and methacrylic acid induced by gamma radiation, by pre-irradiation oxidative method, onto cotton gauzes.

Keywords: *pH-responsive polymers, Cellulose, Gamma radiation, Acrylic acid, Methacrylic acid.*

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ABBREVIATIONS

AAc	Acrylic acid
ATR	Attenuated Total Reflectance/Reflection
CO_2	Carbon dioxide
C=O	Carbonyl group
-COOH	Carboxylic group
°C	Celsius degree
°C/min	Celsius degree per minute
cm	Centimeters
^{59}Co	Cobalt-59
^{60}Co	Cobalt-60
DSC	Differential Scanning Calorimetry
FTIR	Fourier Transform-Infrared Spectroscopy
g	Grams
Gy	Gray
h	Hours
HCl	Hydrochloric acid
-OH	Hydroxyl group
J/g	Joule per gram
keV	Kiloelectron volt
kGy	Kilogray
kGy	Kilogray per hour
MeV	Megaelectron volt
MAAc	Methacrylic acid
$-CH_3$	Methyl group
µg	Micrograms
mL	Milliliters
mm	Millimeters
min	Minutes
M	Molar

^{60}Ni	Nickel-60
nm	Nanometers
%	Percentage
PAA	Poly(acrylic acid)
PMAA	Poly(methacrylic acid)
KOH	Potassium Hydroxide
SEM	Scanning Electronic Microscopy
TBq	Terabecquerel
TGA	Thermogravimetric Analysis
UV-Vis	Ultraviolet-Visible

1. INTRODUCTION

1.1. Polymers

The term polymer is defined as a very large molecule having high molecular mass, in the range of 10^3 to 10^7 units. Polymers are also referred to as macromolecules, which are composed by joining of repeating units on a large scale. These repeating units are derived from some simple and reactive molecules known as monomers, which are linked covalently. The formation of polymers from their respective monomers is known as polymerization and the total number of repeating units is known as degree of polymerization.[1] Figure 1 shows each definition.

A polymerization reaction can occur only if the reactants have functionality, which refers to the number of sites that a molecule has to bind to another under certain polymerization conditions. Functionality is defined according to the polymerization reaction that the compound is participating, because the functional groups of the molecules interact differently according to which they are subjected.

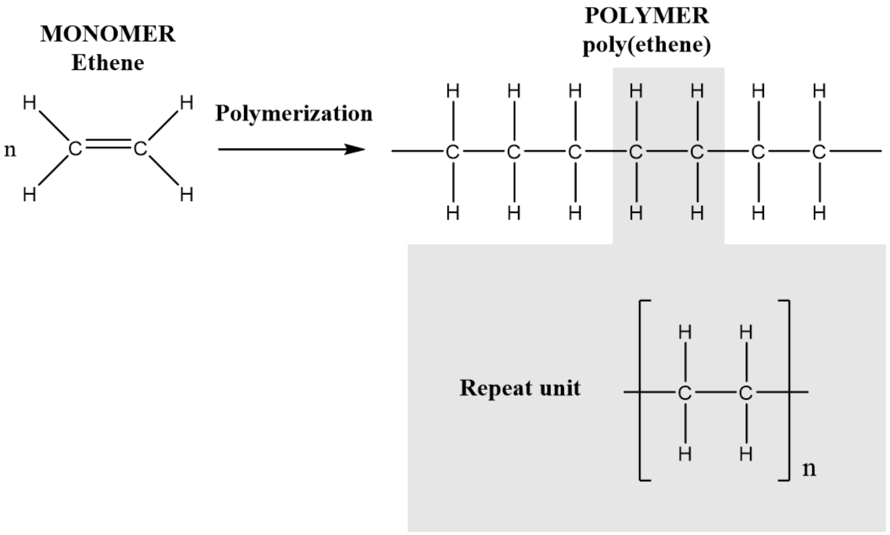


Figure 1. *Example of polymerization. Polymerization of ethene.*

1.1.1. Classification of polymers

There are several special considerations to take into account to classify polymers. For the purposes of this work, the classification according to the polymer structure, chemical composition and type of polymerization is studied in detail.

1.1.1.1. Classification according polymer structure

Monomers when joining generate different three-dimensional structures of polymers. This fact influences on its chemical and physical properties. Based on this criterion, polymers can be classified as: linear, branched and crosslinked polymers (Figure 2).

- **Linear Polymers:** Linear polymers are formed when monomer that originates it have two union points, so that, polymerization occurs in a unique direction, but in both senses.
- **Branched Polymers:** Branched polymers have three or more union points, so that, polymerization occurs in a three-dimensional way.
- **Crosslinked Polymers:** Crosslinked polymers are formed by bifunctional or trifunctional monomers with a strong covalent bond between various polymer linear chains, that is, monomeric units are crosslinked to form a three-dimensional network. Crosslinked polymers with a high degree of crosslinking will be fragile by nature.

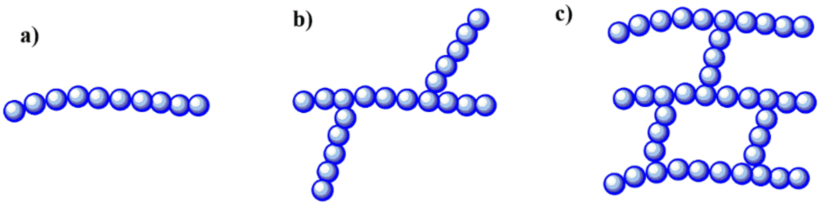


Figure 2. *Classification of polymers according structure. a) Linear polymers; b) Branched polymers; c) Crosslinked polymers*

1.1.1.2. Classification according to chemical composition

According to the number of monomers with different chemical composition that are present contained in the polymer and its arrangement in the chain, polymers can be classified as homopolymers or copolymers (Figure 3), according to the following categorization:

- **Homopolymer:** Polymer that is generated from the same repeating unit or monomer, regardless the functionality of the molecule.
- **Copolymer:** It is formed by the polymerization of, at least, two different monomers along all the chain, obtaining a material with different characteristics to the corresponding homopolymers. Depending on the polymerization process and the relative fractions of these repeat unit types, different sequencing arrangements along the chains are possible.

  Alternated Copolymer: This polymer has units of, at least, two monomers which are regularly alternated, that is, each repeat unit of type of monomer is linked to another type.

  Random Copolymer: This polymer has an aleatory disposition of two or more monomers.

  Block Copolymer: It possess a backbone which consists in very large sequences of different repeat units.

  Graft Copolymer: It is formed when one or more blocks of homopolymer are grafted as branches onto a main chain, called polymeric matrix, which composition is different to grafted side chains.

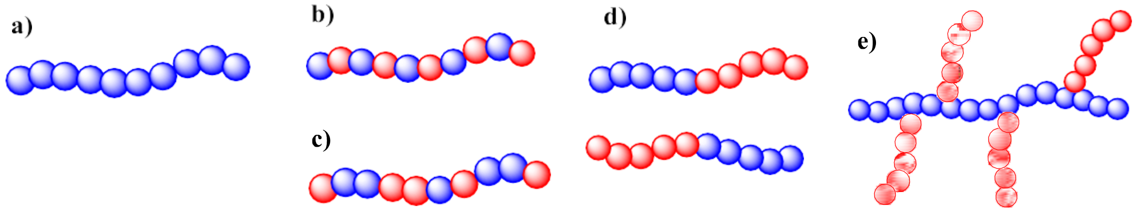


Figure 3. *Classification of polymers according chemical composition. a) Homopolymer; b) Alternated Copolymer; c) Random Copolymer; d) Block Copolymer; e) Graft Copolymer.*

1.1.1.3. Classification according to the type of polymerization

- **Polycondensation:** Condensation polymers are a kind of polymers where molecules join together (condensation), losing small molecules as by products, such as water or methanol.
- **Polyaddition:** Addition polymers are formed by repeated addition of monomer molecules. Monomers used in this polymerization are unsaturated hydrocarbons and they do not generate byproducts in the corresponding polymerization reactions.

  Free-radical Polymerization: It is any kind of addition polymerization, where polymer is formed by successive addition of building blocks to a propagating radical chain. This process involves, generally, several steps: initiation, propagation, chain transfer and termination. Polymerization can be initiated by several ways, but commonly includes the presence of species which can be decomposed into radicals, called initiators, which act as active centers.

  Ionic Polymerization: In this type of polymerization, active centers carries a cation or an anion. The chain reaction proceeds through either a cationic or anionic process.

  Coordination Polymerization: This polymerization typically uses coordination catalysts like Ziegler-Natta catalysts. In coordination polymerization, monomers with side groups are attached to a growing chain in a highly defined spatial arrangement.

1.2. Stimuli Responsive Polymers

Stimuli responsive polymers, or smart polymers, possess the ability to produce reversible changes in its conformation at the moment to be exposed to little variations in its environment (Figure 4). This is reflected into considerable changes in their microstructure, and in the chemical, physical, and physiological properties.[2] Smart polymers are able to respond to external stimulus by showing physical or chemical changes in its behavior.

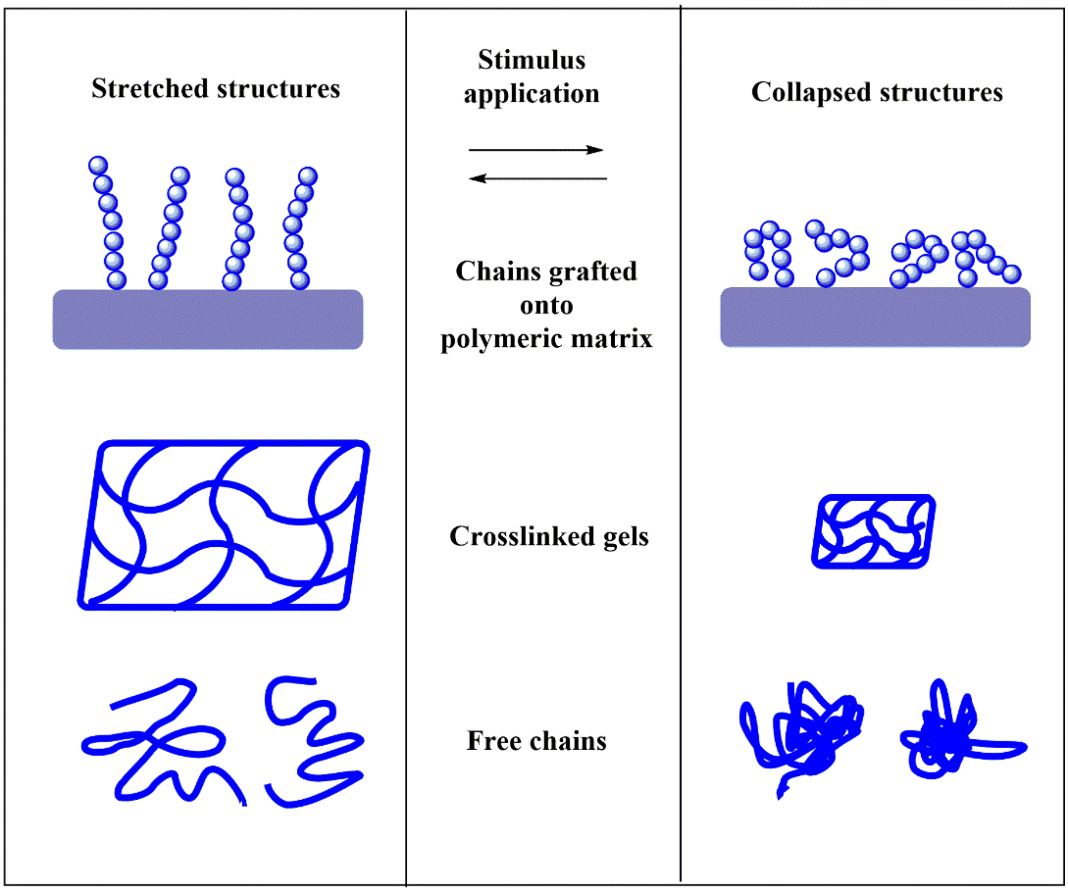


Figure 4. *Schematic representation of stimuli-responsive polymers behavior under certain conditions*

Stimulus can be physical like: temperature, light, magnetism, and electricity, biological like enzyme, protein, DNA, and glucose or chemical like: pH, ionic strength, organic solvent, electrochemical stimulus, redox or presence of any specific specie.[3] Stimulus can be controlled artificially or they can be promoted naturally by the internal physiological environment, through a feedback mechanism, which entails physical or chemical changes in the polymer network and allows the administration of drugs without any external intervention.[4]

Stimuli responsive polymers can be classified, according to the physical form, as free chains in solutions, chains grafted on a matrix, covalently cross-linked gels and reversible or physical gels.[5] The field of application of stimuli-responsive polymers comprises a wide range of proposes like: tissue engineering, extended drug delivery, usage as biosensors of different molecules, usage in disposable diapers providing the absorption of fluids, in breast implant, in drain tubes, in gauzes, in artificial tendons or cartilage and replacing vocal cords.

1.2.1. pH-responsive polymers

pH-responsive polymers are generally constituted by chains that contain ionizable groups, that either donate or accept free protons and have charges in response to the surrounding environmental pH. Under certain pH conditions, the functional groups present along the backbone and side chains of the polymer undergo ionization that leads to a conformational change in the polymer resulting in its swelling or dissolution.[6] Polyacid polymers will be unswollen at low pH, since acidic groups will be protonated and unionized, that is, when increasing the pH, a negatively charged polymer will swell. The opposite behavior is found in polybasic polymers, since the ionization of the basic groups will increase when decreasing the pH of the media. Polybasic polymers suffer swelling at low pH, and in its natural form, it is deswelling (Figure 5).

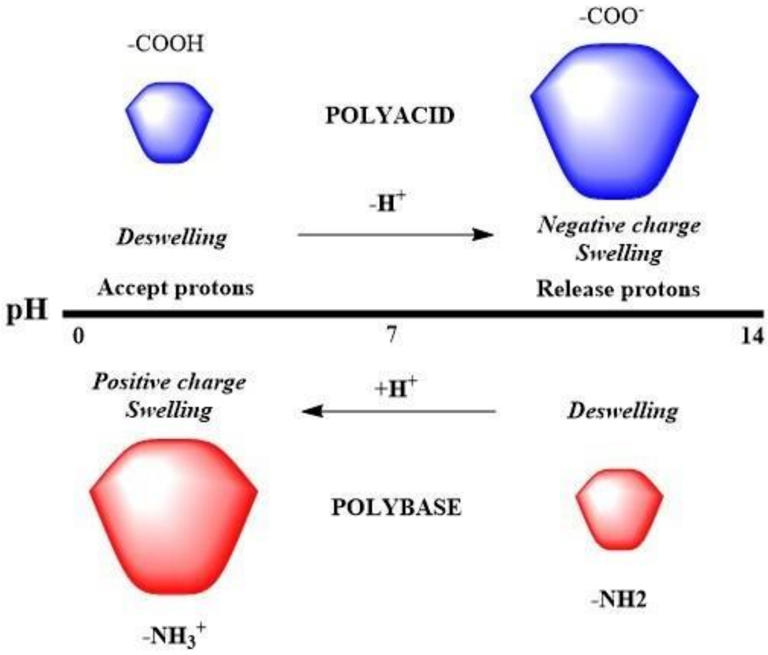


Figure 5. *pH-responsive polymers behavior under certain pH-conditions*

It is important to highlight some typical examples of pH-sensitive polymers with anionic groups like poly(carboxylic acids) (poly(acrylic acid) and poly(methacrylic acid)) and polysulfonamides . These polymers are weak polyacids, which present a pK$_a$ that varies from 3 to 11, depending on the electron removing nature of the functional group. In the other hand, pH-sensitive polymers with cationic groups like poly(4-vinylpyridine), poly(2-vinylpyridine), poly(vinylamine), poly(N,N-dimethylaminoethylmethacrylate) (PDMAEMA), and poly(2-diethylaminoethylmetahcrylate) (PDEAEMA) are protonated at high pH values and positively ionized at neutral or low pH, that is, in basic environments release protons and in acid environments gain protons.[4]

1.3. Ionizing Radiation

Ionizing radiation refers to radioactive particles that have enough energy to knock electrons from atoms and, thus, create ions. This radiation is capable of modifying the physical, chemical, and biological properties of matter. Radioactivity is a phenomenon whereby unstable atoms spontaneously release certain charged particles, neutral or very high energy photons, in order to transform into a more stable entity. The term "ionizing radiation" frequently covers a large number of different types of radiation, some of which are bundles of charged particles, which directly ionize the molecules of the irradiated medium. Ionizing radiation is located in the electromagnetic spectrum between $3x10^{15}$ and 10^{27} Hz.[7], and includes high energy electromagnetic waves, such as X- and γ-rays, and neutrons, being the γ-radiation the one that is of interest for this project.

1.3.1. Gamma Radiation

Gamma rays are high-energy electromagnetic radiation, with a wavelength of the order of 10^{-2} nm, shorter than that of X-rays. This radiation is produced by the transition of energy levels in the nucleus of the atom. This type of radiation is monoenergetic and depends on the radioactive isotope that emits it. Being photons, they do not easily interact with matter, so they have the greatest penetrating power of all ionizing radiation.

Gamma rays, like X-rays, have well-defined energies as they are produced by the transition between energy levels of the atom. Gamma rays are emitted by the nucleus, while X-rays result from energy transitions of electrons outside the nucleus or orbitals.[8]

The process by which a nuclide emits a gamma ray is called isomeric transition, during this transition the nuclide remains with the same atomic number and mass, but a lower energy level. The nuclide tends to stability using the rearrangement of subnuclear particles, thus changing their energy level. Nuclides that are in an excited state and need to emit energy in the form of gamma radiation to achieve stability are called metastable nuclides.[9]

Gamma radiation has attracted scientific interest as various applications have been found, ranging from sterilization of food or biomedical products,[10] to synthesis of inorganic nanoparticles and polymeric materials.[11–14]. For the synthesis of polymeric materials, such as graft copolymers, there are two methods that will be described later.

1.3.1.1. Cobalt-60

There are several sources of γ-radiation emission, but due to their characteristics such as half-life and availability, only some radionuclides are ideal for the radiation process, so Cobalt-60 has become the best γ-radiation source option.

The radioactive sources of Cobalt-60 are the most used, since it is available synthetically, it has moderate decay with production of gamma radiation of sufficient energy to ionize matter. The ^{60}Co is a synthetic radioisotope that is produced by bombing ^{59}Co with a neutron source; in the process additionally gamma radiation is produced ($^{59}\text{Co}(\eta,\gamma)^{60}\text{Co}$). This radioisotope has a half-life ($t_{1/2}$) of 5.24 years and a radioactive activity of 44 TBq per gram. Figure 6 shows the scheme of radioactive decay from ^{60}Co to ^{60}Ni.

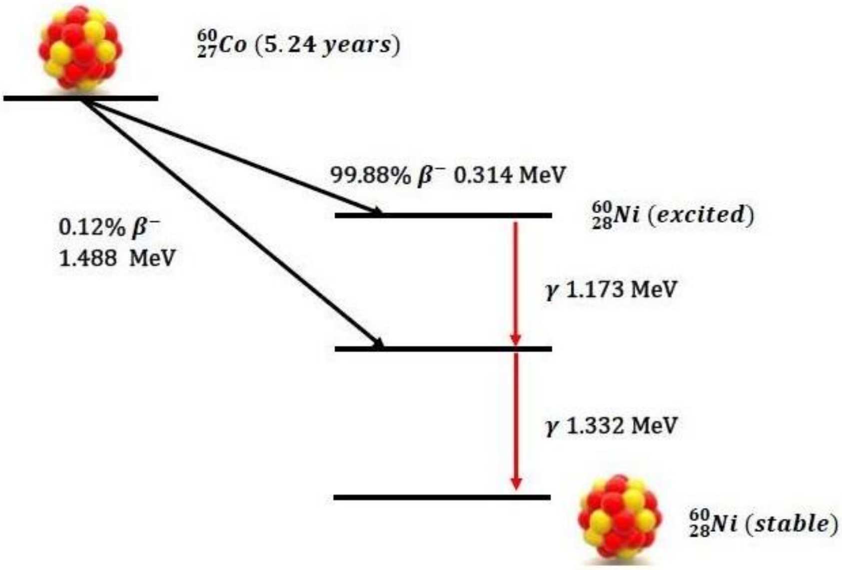


Figure 6. *Schematic representation of radioactive decay of Cobalt-60*

1.4. Ionization of matter by γ-radiation

A radioisotope emits radiation that is isotropic, which means that it goes in all directions. Radiation travels through matter, interacting with it and transferring energy from radiation to matter. The mechanism of interaction and energy transfer is quite different for photons and electrons due to the difference in their characteristics; an electron has charge and mass, whereas a photon has no charge and also has zero mass. Photons (γ-rays and X-rays) have, in essence, an infinite range because their interaction with matter follows an exponential law.[15]

A small fraction of the radiation energy that reaches the matter is absorbed by it. This fraction depends on its mass and composition, the type of radiation and the exposure time.[15] The absorbed dose is the measure of this absorbed radiation energy and is defined as the radiation energy (in Joules) absorbed by the unit mass (in kilograms) of matter; it is measured in units of gray.[16] Thus, 1 Gy = 1J/kg of the matter being studied.

The ionization of matter through γ-radiation is carried out by three different processes: the photoelectric effect, the production of pairs and the Compton effect.

1.4.1. Photoelectric effect

The photoelectric effect describes the case in which a low energy photon γ transfers its energy to an electron of the layers that are closest to the nucleus, which causes its expulsion with an energy equal to $h\nu - E_1$, where E_1 represents the energy of bonding of the ejected electron, the internal orbital of the atom is empty and the excited atom releases energy (E_1) in the form of radiation to return to its basal state, resulting in an ionized atom (Figure 7). The kinetic energy of the ejected electron is equal to the energy of the incident gamma photon minus the binding energy.

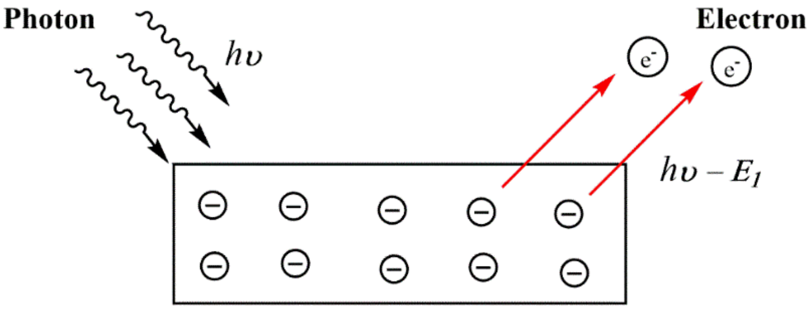


Figure 7. *Representation of Photoelectric Effect*

1.4.2. Production of pairs

This process does not involve the orbitals of the atom for ionization since a photon γ interacts with the magnetic field of the nucleus of an atom, becoming the mass of an electron-positron pair. At the end, the set of positrons is combined with a free electron, the two are annihilated and the entire mass of these two then becomes two photons of energy equal to the incident photon. Paired production requires that the incident energy exceed 1.02 MeV, because the resting mass of an electron corresponds to 0.51 MeV (Figure 8).

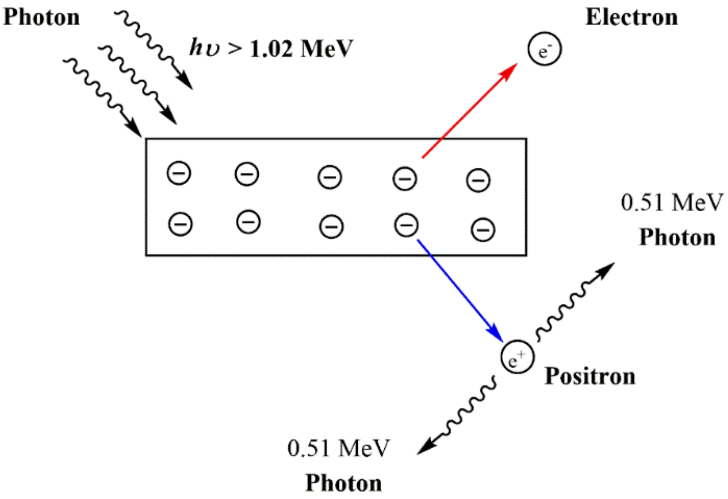


Figure 8. *Representation of Production of Pairs Phenomena*

1.4.3. Compton effect

The Compton dispersion, or Compton effect, is basically an elastic collision, where an incident photon γ loses energy due to its interaction with an electron that deflects it from its course, during this interaction, the photon yields a fraction of its energy to the electron inducing an expulsion of the photon (Figure 9). It is called "dispersion" because the energy of the original photon is emitted as a new lower energy photon γ whose emission direction is different from that of the incident photon γ.

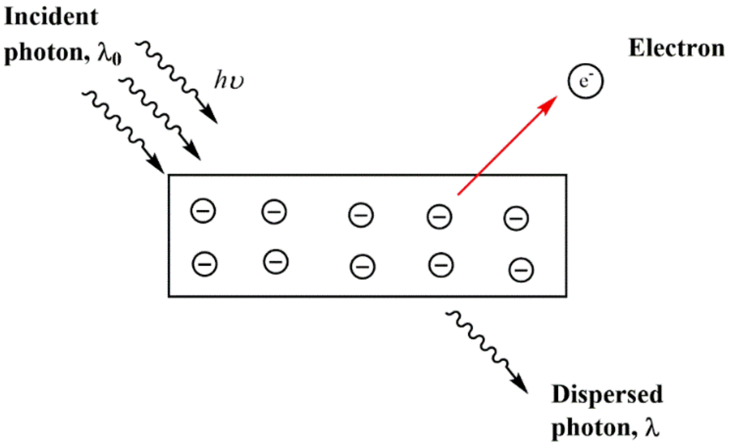


Figure 9. *Representation of Compton Effect*

1.5. Irradiation Methods

Radiation-induced graft copolymerization can be performed by two methods: (1) direct or simultaneous irradiation method and (2) pre-irradiation method, which in turn can be performed in the presence of high vacuum or in the presence of oxygen (pre-irradiation oxidative method)

1.5.1. Direct or simultaneous method

The polymer matrix is irradiated together with a monomer that can be in the form of steam, liquid or in solution. Irradiation is carried out in the presence of air, inert atmosphere or under vacuum, which causes the formation of free radicals or active sites both in the polymer matrix (P) and in the monomers (M). It is currently the simplest form of irradiation for graft copolymer formation.[7] The mechanism of this method is represented in the following scheme:

$$\begin{aligned} PH &\xrightarrow{\gamma} P^{\bullet} + H^{\bullet} \\ P^{\bullet} + M &\longrightarrow PM^{\bullet} \\ PM^{\bullet} + nM &\longrightarrow PM_{n+1}^{\bullet} \end{aligned}$$

Despite being the simplest, it presents a high possibility of deactivation of the active sites of the polymer matrix, due to mutual recombination. In the same way, the monomer units that have active sites can react with each other, starting a homopolymerization process,[17] represented in the following equations:

$$\begin{aligned} M &\xrightarrow{\gamma} M^{\bullet} \\ M^{\bullet} + nM &\longrightarrow M_{n+1}^{\bullet} \\ M_{n+1}^{\bullet} + M_{m}^{\bullet} &\longrightarrow M_{n+m+1}^{\bullet} \end{aligned}$$

For this reason, this method should only be performed when the irradiation product of the polymer matrix is much larger than the irradiation product of the monomer, which will favor copolymerization. Otherwise, homopolymer formation will be favored.[18]

1.5.2. Pre-irradiation method

For this method the polymer matrix is previously irradiated to generate the formation of active sites within it. Subsequently, the irradiated matrix comes into contact with the monomer. This method can be performed in the presence of a vacuum or air.

1.5.2.1. Pre-irradiation method in vacuum or inert atmosphere

When the radiation reaches the matter, the molecules are ionized and excited species are formed. These species form relatively stable free radicals, which in turn form active sites. The radicals generated by the irradiation are maintained in the polymer matrix and the grafting process begins in the presence of the monomer units introduced in the form of liquid, vapor or solution.[7]

The possibility of homopolymer formation is low because the monomer units are not irradiated and no radicals are formed in these units. On the other hand, its main disadvantages lie in its high cost due to the need to create an inert atmosphere, the low graft performance and the short lifetime of the generated radicals.[18]

1.5.2.2. Pre-irradiation oxidative method

This method, also known as alkylperoxy/hydroperoxy method, is carried out by irradiating the matter in the presence of oxygen from the air. In this way, the generated radicals react with oxygen forming peroxides and hydroperoxides. The monomer solution is introduced into the sample and heated in the absence of oxygen. Peroxides and hydroperoxides are homolytically broken by thermal decomposition and become initiators of copolymerization.[17] The basic scheme of the copolymerization process by the pre-irradiation oxidative method is detailed in the following scheme:

$$\mathrm{PH} \xrightarrow{\gamma} \mathrm{P}^{\bullet} + \mathrm{H}^{\bullet}$$

$$\mathrm{P}^{\bullet} + \mathrm{O_2} \longrightarrow \mathrm{POO}^{\bullet}$$

$$\mathrm{POO}^{\bullet} + \mathrm{PH} \longrightarrow \mathrm{POOH} + \mathrm{P}^{\bullet}$$

$$\mathrm{POOH} \xrightarrow{\Delta} \mathrm{PO}^{\bullet} + {}^{\bullet}\mathrm{OH}$$

$$\mathrm{PO}^{\bullet} + \mathrm{M} \longrightarrow \mathrm{POM}^{\bullet}$$

$$\mathrm{POM}^{\bullet} + \mathrm{nM} \longrightarrow \mathrm{POM}_{n+1}^{\bullet}$$

1.6. Cotton gauze

Cotton gauzes are medical devices that have traditionally been used in pads and bandages to cover and protect wounds without letting them come into contact with the air and all kinds of external agent. Due to its high absorption capacity and due to the aforementioned characteristics, cotton gauzes constitute basic materials in first aid cures. Medical gauze is made up of cotton threads that are obtained from cotton fibers.[19,20] These fibers are plant cells that are composed of non-cellulosic compounds which include: proteins (1.0-1.9%), pectins (0.4-1.2%), waxes (0.4-1.2%), inorganic substances (0.7-1.6%) and other (0.5-8.0%) in their first cell wall and in their lumen (4.5-12.0% of cell mass), and α-cellulose (Figure 10) in their second cell wall (88-96.5% of cell mass).[21]

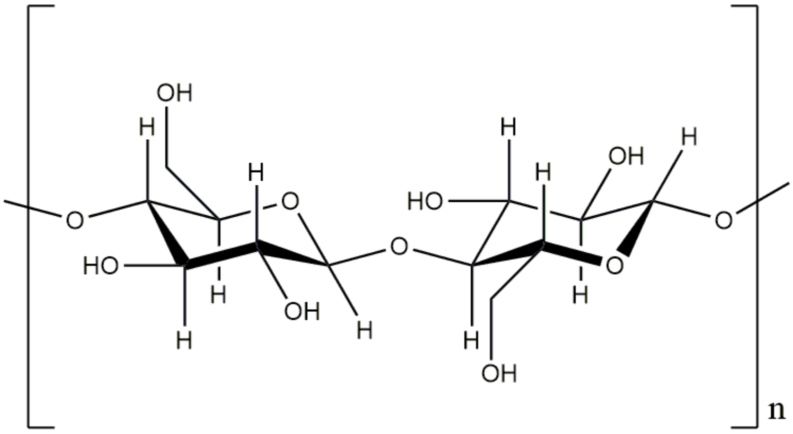
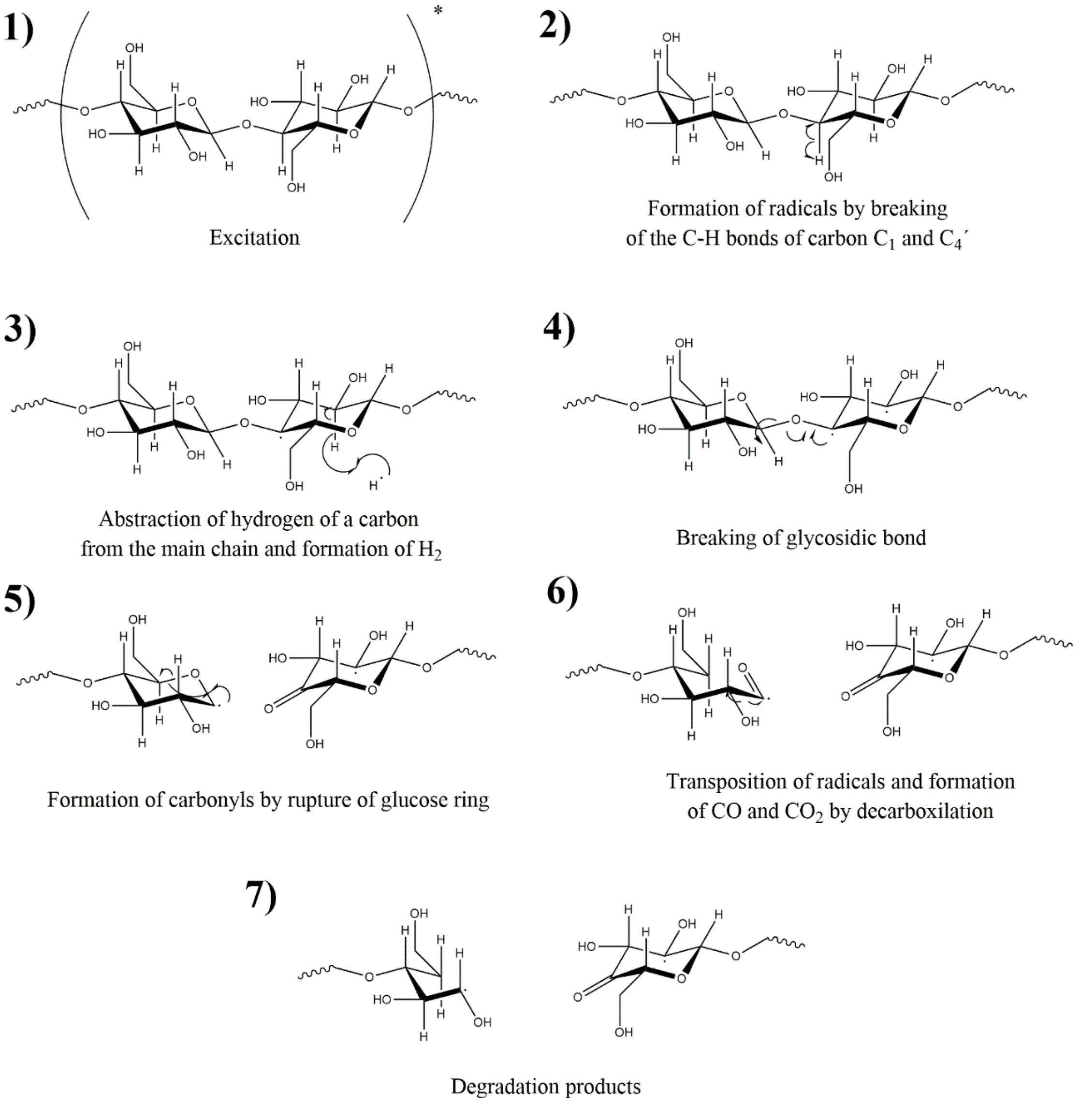


Figure 10. *Chemical structure of α-cellulose*

In its fibrous form, this natural polymer is capable of reaching high degrees of crystallinity (above 60%). In addition, having a large number of hydroxyl groups, the interactions of hydrogen bonds between chains are very strong, the polymer packaging is high and those groups are responsible for the hydrophilicity of the polymer. For these reasons, mechanical properties are invaluable in the textile industry and as a medical device.[19]

Cellulose, being exposed to ionizing radiation undergoes decomposition processes that have been widely studied due to the extensive use of the polymer.[20,22,23] For example, in its decomposition it produces glucose monomers that can then be used as raw material for other processes. According to several studies carried out on this subject, at higher doses of 40 kGy, decompositions are beginning to be observed due to the breakdown of the glycosidic bonds.[24]

Cellulose follows a degradation process due to the presence of a large amount of carbon-oxygen bonds.[25] This process is summarized in Figure 11.



1) Excitation

2) Formation of radicals by breaking of the C-H bonds of carbon C_1 and C_4'

3) Abstraction of hydrogen of a carbon from the main chain and formation of H_2

4) Breaking of glycosidic bond

5) Formation of carbonyls by rupture of glucose ring

6) Transposition of radicals and formation of CO and CO_2 by decarboxilation

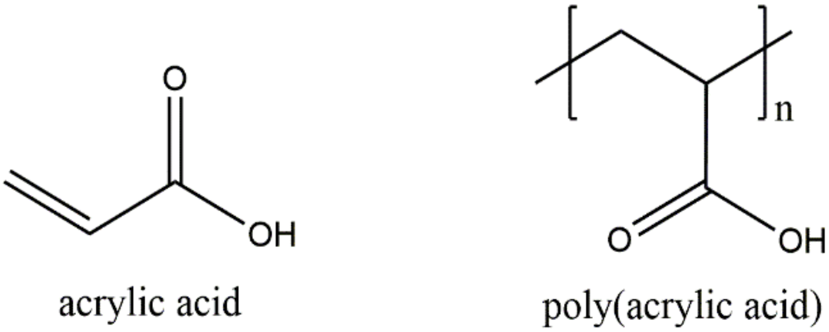
7) Degradation products

Figure 11. *Degradation process of cellulose by effect of ionizing radiation.*

1.7. Monomers

1.7.1. Acrylic acid

Acrylic acid (AAc), also known as propenoic acid, is an organic molecule that appears as a liquid with a characteristic pungent odor. It is an α,β-unsaturated monocarboxylic compound, that is, an ethene substituted by a carboxylic group.[26] It is a highly prone monomer to Michael-type reactions and can polymerize under exposure to fire or heat, forming poly(acrylic acid) (PAAc).[27] This reactivity is due to the presence of a vinyl group and a carbonyl group in its structure (Figure 12); double bonds are more reactive due to the nature of the π-bond. These reaction points will be essential to achieve the graft between the cellulose and the monomers.



acrylic acid

poly(acrylic acid)

Figure 12. *Chemical structure of acrylic acid and poly(acrylic acid)*

1.7.2. Methacrylic acid

Methacrylic acid (MAAc), also known as 2-propenoic acid, is a colorless, viscous liquid, is pungent in odor, and is miscible with most organic solvents.[28] It is an α, β-unsaturated monocarboxylic acid, that is acrylic acid in which the hydrogen in position 2 is replaced by a methyl group.[29] This liquid polymerizes easily by intense heating or by the presence of light, oxygen, and oxidizing agents, such as peroxides or the presence of traces of hydrochloric acid.[30] Like acrylic acid, it has two reactive points, the double bonds from vinyl and carbonyl group (Figure 13), which give it high reactivity and allow grafting.

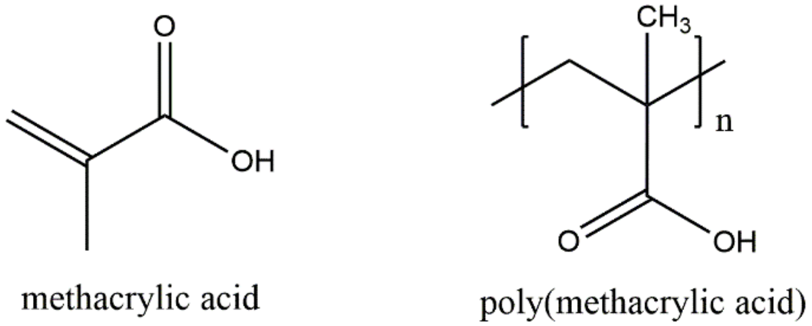


Figure 13. *Chemical structure of methacrylic acid and poly(methacrylic acid)*

1.8. Characterization Techniques

1.8.1. Determination of Grafting Percentage

The grafting percentage (GP) indicates the increase in weight of original cotton gauze subjected to grafting with a monomer and it is calculated generally through the following equation:

$$Grafting\ percentage\ (\%) = (\frac{Weight\ of\ polymer\ grafted}{Initial\ weight\ of\ backbone})\ x\ 100$$

$$Grafting\ percentage\quad (\%) = (\frac{W_g - W_0}{W_0})\ x\ 100$$

W_g is the final weight of the grafted gauze and W_0 is the weight of the original gauze without grafting. GP can be defined as apparent graft yield, which is a weight ratio of grafted polymer to original cotton gauze.

However, GP value given above is apparent or crude value, and it is not indicating the true values since they are calculated for the mixture consisting of true graft copolymer and the non-grafted cotton gauze.

1.8.2. Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy (ATR-FTIR)

The molecules are excited at a higher energy level when they absorb infrared radiation in a select frequency range. The radiation in this energy range includes the range of stretching and bending vibrational frequencies of the bonds in most covalent molecules. In the absorption process, the frequencies of the infrared radiation that binds with the natural frequencies of the molecule are absorbed, and the energy it absorbs serves to increase the amplitude of the vibrational movements of the covalent bonds of the molecule in question. However, it is important to note that not all links in a molecule are capable of absorbing infrared energy, only links with a certain dipole moment are capable of absorbing this type of radiation.[31]

Because each link has its own type of natural vibration frequency and that two links of the same type in two different molecules have slightly different environments, two molecules cannot present the same infrared spectrum. For this reason, this infrared absorption pattern or infrared spectrum of the molecule is used as a fingerprint. The most important use of this spectroscopic technique is to determine the structural information of a molecule by identifying its functional groups.[31]

The instrument that determines the infrared absorption spectrum of a molecule is the spectrophotometer. This instrument provides infrared spectra of the molecules in the range of 4000 to 650 cm^{-1}. Two types of spectrophotometers are commonly used in laboratories: dispersive instruments and Fourier transform, the latter providing the fastest information.[32,33]

Formation of the graft copolymer can be checked by infrared spectroscopy by comparing the spectra of the polymer matrix and the graft copolymer. The graft copolymer will show additional signals, both stretching and torsion, due to the atoms belonging to the grafted chains in the polymer matrix.[33,34]

1.8.3. Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry is a dynamic experimental technique which allow to determine the quantity of heat that absorbs or liberate any substance, when it is maintained under constant temperature, at determined time, or when it is heated or cooled at constant velocity, at a determined temperature range. DSC has an important role in Material Science due to its high degree of sensitivity and fast velocity of thermal analysis. On the other hand, the knowledge of thermal stability of materials, as well as complete characterization of its transitions, is of primary interest in materials with potential industrial or medical applications.[35]

In the experimental technique of DSC there are two capsules, one of which contains a sample to be analyzed and the other is generally empty and it is called reference capsule. Individual heaters are used for each capsule and a control system check if temperature differences occur between sample and reference. If any difference is detected, the individual heaters will be corrected in a such a way that temperature maintains equal in both capsules. That is, when there is an exothermic or endothermic process, the calorimeter compensates the needed energy to maintain the same temperature in both capsules.[35]

All changes, transformations or reactions where a change of energy is produced, can be measured through DSC. Among a great diversity of utilities of DSC, the most important are:

- Measurement of apparent calorific capacity (structural relaxation phenomena).

- Determination of characteristic temperatures of transformation or transition like: vitreous transition, ferrous-paramagnetic transition, crystallization, polymorphic transformations, fusion, boiling, sublimation, decomposition, isomerization, etc.
- Thermal stability of materials.
- Kinetics of crystallization of materials.

However, to identify the type of transformation that have place at a determined temperature, it is necessary to verify it through another complementary experimental techniques, that allow to corroborate the validity of conclusions extracted from DSC curves.[36]

1.8.4. Thermogravimetric Analysis (TGA)

Thermogravimetric Analysis consists in a continuous registration of the variation of mass of material under study as it varies the temperature at a constant thermal rate (lineal temperature change), usually under inert atmosphere. This thermogravimetric procedure is known as dynamic analysis. As result of the thermogravimetric analysis, it is possible to collect data about the change of mass with respect to temperature or time, and a thermogram, which graphically represents percentage variations in mass.[37]

This technique is widely used in quantitative and kinetic characterization of polymers. In relation to they, when polymers are exposed to a heat source with a constant increase, tend to lose mass, even though some can show an increase in mass before degradation in presence of an oxidant atmosphere and a low heat rate. Mass loss of the sample can be assumed like the evaporation of absorbed humidity, volatilization of solvent waste, oligomers of low molecular weight that generally volatilize, as well as other polymer degradation products.[37]

The balance is the key part of the instrument. It registers the initial mass of the sample, changes in mass during analysis and the final mass of the sample with a precision of ±1 μg. The balance has a thermal insulation that avoid to be affected by temperature. The infrared oven provides the needed temperature of analysis and regulates the variation rate of temperature. In the camera of the oven, there is a temperature sensor of high precision which register temperature constantly and avoid that the system exceeds defined temperature. For analysis, it is possible to perform measurements since 5 to 1000 °C, with a heat rate from 0 to 300 °C/min, with a precision of ±1%.

1.8.5. Scanning Electronic Microscopy (SEM)

Scanning Electron Microscopy is an analytical technique that uses an electron beam instead of light to form an image. To achieve this, the equipment has a device, a filament, which generates an electron beam to illuminate the sample and with different detectors the electrons generated from the interaction with the analyte surface are then collected to create an image. It reflects the surface characteristics of it, being able to provide information on the shapes, textures, and chemical composition of its constituents.[38]

Upon impacting the sample, the electron beam interacts with it and several effects occur that will be captured and processed depending on the equipment used.

- **Secondary electrons:** They occur when an electron in the beam passes very close to the nucleus of an atom in the sample, providing the energy needed to one or more of the inner electrons to jump out of the sample. These electrons are very low energy, so they must be very close to the surface to escape. This fact is the basis of this effect, as it provides valuable topographic information of the sample, and they are mainly used in scanning microscopy.[38]

- **Backscattered electrons:** They occur when an electron in the beam collides frontally with the nucleus of an atom of the sample being repelled in the opposite direction outside the sample. The intensity of this effect varies proportionally with the atomic number of the sample. For this reason, they are used to obtain a map with information on the surface composition of the sample.[38]
- **X-rays:** Excess energy can be balanced by the emission of X-rays. These are characteristic of each element of the sample, so they are used to obtain information about the composition of the sample. X-rays provide analytical information of a considerable volume of the sample.[38]

1.8.6. Potentiometric Titrations

Potentiometric titration is a process that involves the measurement of the potential of a suitable indicator electrode with respect to a reference electrode as a function of titrant volume. Potentiometric titration is performed when the detection of the end point of a titration is difficult employing a visual indicator. It is considered one of the most accurate to that purpose, because potential follow the real change of the activity and the end point directly coincide with the equivalence point.[39]

The main advantages of the potentiometric method are its applicability to cloudy solutions, fluorescent, opaque, colored, and when it is not possible to obtain suitable visual indicators.

Acid - base potentiometric titration method is based in the fact that hydrogen ions that are present in a sample as result of the dissociation or solute hydrolysis, are neutralized through titration with a standard alkali and vice versa. This process consists in the measurement and register of the cell potential (in millivolts or pH) after addition of reagent (standard alkali) known as titrant, using a potentiometer or pH meter. The main purpose is to find the concentration of analyte and, for this, a titration curve is constructed, plotting the observed values of pH against accumulative volume or titrant added (mL). The obtained curve must show one or more turning points, where slope of the curve changes of sign.[40]

Titration of a strong acid with a strong base is characterized by three main stages:

- Hydronium ions are in a greater quantity than hydroxyl ions before the equivalence point.
- Titrant is added and hydronium and hydroxyl ions are present in same concentrations, at the equivalence point.
- Hydroxyl ions are in excess, after equivalence point.

In the case of a titration of a weak acid with a strong base is characterized by some important stages:

- At the beginning, solution only contains hydronium ions and there a relationship between its concentration and its dissociation constant.
- After addition of some little quantities of base, series of buffer are produced, where there is a relationship between concentration of conjugated base and the concentration of the weak acid.
- At the equivalence point, the solution only consists of the conjugated form of the acid, that is, a salt.
- After equivalence point, there is an excess of base.

pH is a term that describes the intensity of acid or alkaline conditions of a solution. Acid conditions increases according pH values decrease, alkali conditions increase according pH increases and there are neutral conditions when pH is equal to 7. pH follows the relationship

$pH = -\log [H^+]$

where $\log$ is the base-10 logarithm and $[H^+]$ is the hydrogen ion concentration in units of moles per liter solution.

In the case of pH-responsive polymers is important to know its critical pH. Due to complexity of the graft copolymer and the nature of cotton gauzes, which is, due to its hydrophilicity is able to absorb large quantities of liquid, potentiometric titration is optimal to determine the quantity of acid groups grafted on the polymer matrix, critical pH, and the pK_a of the biomaterials.[41]

2. GENERAL AND SPECIFIC OBJECTIVES

2.1. General Objective

- To synthesize a cotton biomaterial containing a polymer that responds to pH, using the graft method induced by gamma radiation.

2.2. Specific Objectives

- To assess the effects of concentration, dose, temperature, reaction time, and graft percentage of acrylic acid and methacrylic acid on the synthesis of graft copolymers.
- To analyze modified cotton gauzes using analytical techniques such as Scanning Electronic Microscopy, Fourier-Transform Infrared Spectroscopy - Attenuated Total Reflectance, Thermogravimetric Analysis, and Differential Scanning Calorimetry.
- To study the pH-response effects of modified cotton gauzes by potentiometry.
- To evaluate the loading capacity of modified cotton gauzes for potential application as biomaterial.

3. HYPOTHESIS

In order to improve the properties of cotton gauzes, gamma radiation will chemically activate the surface of the gauze which will allow the formation of grafts with acrylic (AAc) and methacrylic acid (MAAc). The modified gauze will have pH-responsive properties and these alterations will also allow the loading of a drug such as vancomycin, which will provide the material with a potential antimicrobial application.

4. METHODOLOGY

4.1. Reagents, solvents, and materials

- Cotton gauzes (Lourdes TM size: 19x19 cm^2, thread thickness: 0.15 mm) produced by DIMACU S.A., Mexico City. (Mexico).
- The precursor monomers are acrylic acid AAc (99%) and methacrylic acid MAAc (99%) which were obtained from Sigma-Aldrich (United States).
- Ethanol (99.9%) was purchased from Baker (United States).
- Pyrex glass test tubes of 16 mm diameter and 150 mm height, and glass rods with a diameter of 4 mm and a height of 150 mm were used to make the ampoules.
- The source of gamma radiation used during the experiments is the ^{60}Co isotope that is contained in a Gammabeam 651-PT deep pool type irradiator with an activity of $2.35x10^{15}$ Bq, from the Institute of Nuclear Sciences of the National Autonomous University of Mexico, UNAM.
- Argon line.
- Ultraviolet Visible Spectrophotometer. Analytikjena Specord 200 Plus, Germany.

4.2. Preparation of reagents

The monomers used during the experiments were purified by distillation under reduced pressure to remove the hydroquinone monomethyl ether (MEHQ), stabilizer present in monomers, which is a compound that acts as a polymerization inhibitor. A quantity of 50 mL of monomer was placed in a round bottom flask inside a silicone bath, distillation equipment was mounted coupled to a vacuum pump and the liquid was kept under stirring with constant heating until a temperature of boiling under pressure of around 30 °C for both acrylic acid and methacrylic acid. Subsequently, the distilled monomer is kept refrigerated at a temperature of -6 °C until it is used.

4.3. Synthesis of Gauze-*g*-AAc and Gauze-*g*-MAAc by the pre-irradiation oxidative method

The grafting of the acrylic monomers AAc and MAAc onto cotton gauzes was carried out by the pre-irradiation oxidative method. For that, the gauze is deposited in glass ampoules and irradiated at certain doses at room temperature and in the presence of air. Then, 8 mL of aqueous dissolution of the acrylic acid and 8 mL of methacrylic acid monomers are added to the irradiated samples, in separated experiments, under required concentrations in a range of 5 to 20% v/v. Inert conditions are needed, for that oxygen is removed by means of air displacement with argon bubbling during 15 min. The samples are sealed with a blowtorch and heated in a water bath at required temperatures in the range between 45 and 75 °C during the necessary reaction times in a range of 1 to 4 h. Once the reaction time is over, ampoules are open and gauzes are deposited in a glass cup and are washed with an ethanol:water (50/50 v/v%) mixture, under constant stirring, during 4 h, in order to remove homopolymer and monomer residues that have not been grafted. After that, this mixture is removed, samples are dried in a conventional oven during 12 h at 70 °C, and for 4 hours in a vacuum oven at 55 °C. Finally, the graft percentage is reported and calculated by mass difference, method previously described. This methodology is graphically visualized in the Figure 14.

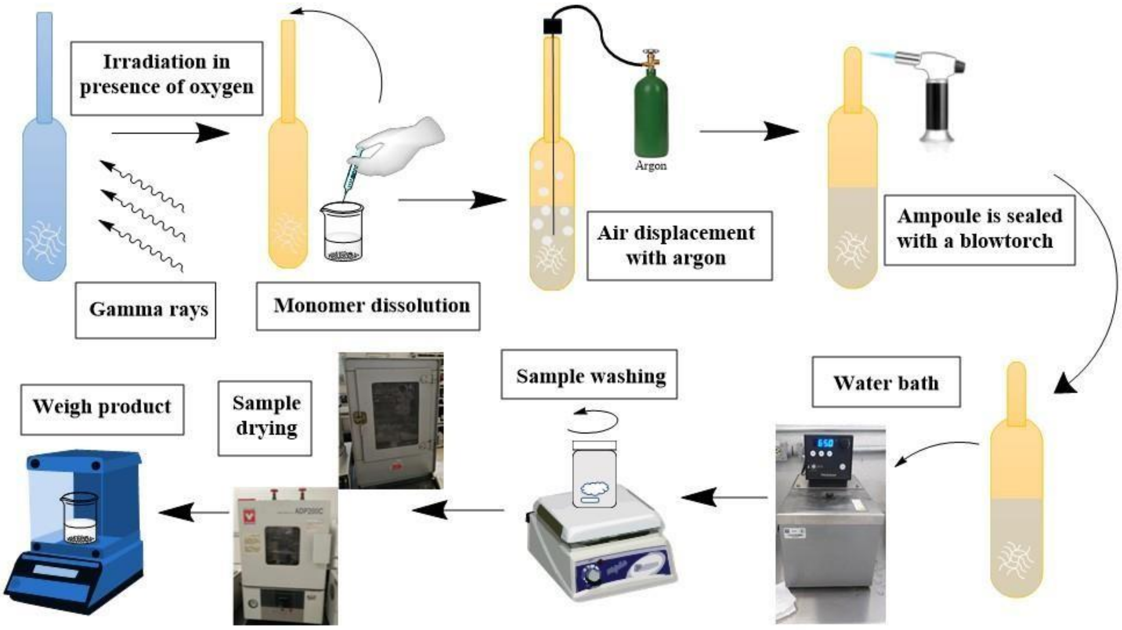


Figure 14. *Experimental stage for synthesis of Gauze-g-AAc and Gauze-g-MAAc by pre-irradiation oxidative method*

4.4. Evaluation of reaction conditions

4.4.1. Monomer concentration

To evaluate this parameter, the amount of acrylic acid or methacrylic acid that will dissolve in distilled water must be varied. For both monomers, graft percentage is evaluated at 5, 10, 15, and 20% v/v of aqueous solution. The reaction conditions used were at a temperature of bath of 65 °C, a radiation dose of 30 kGy, with a rate of 7.45 kGy/h and reaction time of 3 h for methacrylic acid and 4 hours for acrylic acid. The experiments were performed in duplicate.

4.4.2. Reaction time

For this parameter the reaction times that were evaluated were 1, 2, 3, and 4 h in which the ampoules sealed with the monomer dissolution and the sample were subjected to a 65

°C bath. The concentration of acrylic acid and methacrylic acid in this case was 10% v/v in water. The dose to which the samples were subjected is 30 kGy, at an irradiation rate of 7.45 kGy/h. The experiments were performed in duplicate.

4.4.3. Gamma irradiation dose

The effect of the gamma irradiation dose on the polymer matrix was evaluated. For acrylic acid, doses of 10, 20, 30, and 40 kGy are taken, at a reaction temperature for water bath of 65 °C and a reaction time of 4 h. For methacrylic acid, doses of 10, 15, 20, and 25 kGy are taken, at a water bath temperature of 65 °C and a reaction time of 3 h. The concentration of the aqueous solution of the monomers was 10% v/v for both acrylic acid and methacrylic acid. Experiments were performed in duplicate.

4.4.4. Temperature

For the evaluation of this parameter, the conditions that were established are aqueous dissolutions of acrylic acid and methacrylic acid with a concentration of 10% v/v. The dose used in each experiment was 30 kGy. For the acrylic acid the reaction time used was 4 hours and for methacrylic acid it was 3 h. The bath temperatures used in the experiments will be 45, 55, 65, and 75 °C. Experiments have been performed in duplicate.

4.5. Characterization of copolymer

4.5.1. Infrared Spectroscopy

Tests were performed with gauzes grafted with acrylic and methacrylic acid. A sample without grafting is taken, which will be our control sample and samples with different percentages of graft (48, 104, and 143%), in the case of AAc. For gauze grafted with methacrylic acid, those with graft percentages of 6, 56, 68, and 97% are taken. These samples must be completely dry before testing. For that, the samples are taken and placed in the oven at 70 °C during 6 h. Samples were analyzed by FTIR spectroscopy on a Perkin-Elmer Spectrum 100 spectrophotometer (Perkin Elmer Cetus Instruments, Norwalk, CT) with 16 scans, in the ATR module.

4.5.2. Thermogravimetric Analysis

Samples of cotton gauze grafted with acrylic acid with different graft percentages (45, 48, and 143%) Likewise, samples of cotton gauze grafted with methacrylic acid with graft percentages corresponding to 6, 87, and 136% are taken. For comparative purposes, in both cases, a pristine gauze as control sample are taken. These samples are dried for 6 h in the oven at 70 °C. Samples are weighed in the range between 5 and 6 mg. Each sample is placed on the platinum tray of the equipment and a heating ramp of 10 °C/min from 30 to 800 °C is used in a nitrogen atmosphere. The equipment used was the TGA Q50 from TA Instruments USA (Figure 15).

Figure 15. *TGA Q50 from TA Instruments USA*

4.5.3. Differential Scanning Calorimetry

Samples of cotton gauze grafted with acrylic acid with different graft percentages (45, 48, and 97%). Likewise, samples of cotton gauze grafted with methacrylic acid with graft percentages corresponding to 63, 79, and 190% are taken. A pristine gauze is taken to evaluate the differences with modified gauzes. These samples are previously dried in an oven at 70 °C for 6 h and 5 or 6 mg of sample are weighed. These quantities are deposited in aluminum trays that are sealed under pressure. The sealed capsule was placed in the equipment together with a reference (empty tray). For all the samples, two heating were carried out, the first with a ramp of 10 °C/min (to eliminate the thermal history) and the second 5 °C/min in a range of 20 to 300 °C, under nitrogen atmosphere. To perform this thermal behavior analysis, a Differential Scanning Calorimeter, TA Instruments equipment with aluminum trays was used (Figure 16).

Figure 16. *Differential Scanning Calorimeter, TA Instruments*

4.5.4. Scanning Electronic Microscopy

For the Scanning Electronic Microscopy test, a sample of unmodified cotton gauze was taken (pristine gauze), two gauze grafted with acrylic acid and two with methacrylic acid, with varying degrees of grafting. Minimal amounts of sample were cut and, with the help of a clamp, they were placed in a multiple sample holder. This sample holder must have an adhesive on which the samples are placed. The samples must be completely dry, so the sample holder with all samples was placed in an oven at 55 °C for 24 h. The images were taken using a scale of 200 μm. The analysis was performed using a Phenom XL G2 Desktop SEM.

4.5.5. Determination of critical pH of grafted gauzes

To evaluate the critical pH of grafted gauzes, titrations were performed. As samples to be evaluated, a non-grafted control gauze, three gauze grafted with acrylic acid with various graft percentages (25, 93, and 117%) and three gauze grafted with methacrylic acid with various graft percentages (6, 80, and 117%). The titrations were performed with a HANNA HI4212 potentiometer (Figure 17).

Figure 17. *HANNA HI4212 potentiometer*

In this experiment the titrant solution has been previously prepared at an approximate concentration of 0.1 M and a standardization must be performed to know its current concentration. For this purpose, a 0.04 M Potassium Hydrogen Phthalate solution is prepared, two drops of phenolphthalein indicator are added and titrated with the KOH (0.11M) solution. The objective is to titrate until the solution reaches a pink color. This experiment is performed by triplicate.

An experimental setup for a titration is prepared, as shown in the Figure 17. Approximately 0.3 g of each totally dried sample (pristine and modified gauzes) is weighed and deposited in a beaker. An amount of distilled water is added that completely covers the gauze. The pH of the medium is reduced to approximately 2 with a 1 M hydrochloric acid solution.

The beaker with the solution and the gauze should be under constant agitation, for which a magnetic stirrer is used. The pH of the medium is evaluated as titrant is deposited in the solution.

In the same way, reverse titrations of the systems that previously added the basic solution are made. For the reverse titration a solution of hydrochloric acid approximately 0.1 M will be used. This solution was standardized with a known solution of sodium carbonate (0.04 M). Two drops of methyl orange indicator were added and titrated with the HCl solution. It is added until the orange color becomes reddish-brown. This experiment is performed by triplicate.

The pH of the system was evaluated and a few drops of a 1 M KOH solution are added so that the system has a pH equal to 12. The experimental setup for titration was prepared, with the HCl solution as titrant. The pH of the medium was evaluated as the titrant was added to the solution. The system must be under constant agitation. For each direct titration system, reverse titration was performed.

4.5.6. Loading of Vancomycin

Samples of approximately 0.05 g of modified gauzes (Gauze-*g*-AAc and Gauze-*g*-MAAc) with various percentages of grafting, as well as pristine gauze, were placed in amber bottles with 4 mL of aqueous vancomycin solution at a concentration of 0.1995 mg/mL with constant stirring at room temperature for 24 h. For the quantification of drug that is loaded into the polymer, measurements were made after 0.5, 1, 2, 4, 6, 18, and 24 h of stirring, at 274 nm on the UV-vis spectrophotometer. Measurements were made in triplicate.

Calibration curve: for the quantification a calibration curve was made, for which solutions of concentrations 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, and 0.2 mg/mL were prepared. Measurements were made in triplicate, using distilled water as solvent (Annexes 1).

5. RESULTS AND DISCUSSION

5.1. Synthesis of Gauze-*g*-AAc and Gauze-*g*-MAAc

The cotton gauze was modified with acrylic and methacrylic acid, using the pre-irradiation oxidative method. The reaction mechanism is based on free radical polymerization, in which gamma ionizing radiation is the initiator. In the grafting process, gamma radiation breaks certain C_2-C_3 bonds in the cellulose chain, which causes the formation of active sites.[42] These active sites are highly reactive and react quickly with oxygen in the air, forming hydroxides and peroxides (Figure 18). These species, when subjected to a certain temperature, carry a homolytic breakdown of the peroxide (R-O-O-R´) and hydroperoxide bonds (R-O-O-H), again forming free radicals, which in turn reacts with the vinyl group of AAc and MAAc. In this way a chain reaction occurs, which is known as the propagation stage. The termination step is proposed to occur when two molecules that contain free radicals react and form the desired end product (Gauze-*g*-AAc and Gauze-*g*-MAAc). The proposed mechanisms for the grafting reaction of acrylic and methacrylic acids on cotton gauzes are illustrated in Figure 19 and Figure 20.

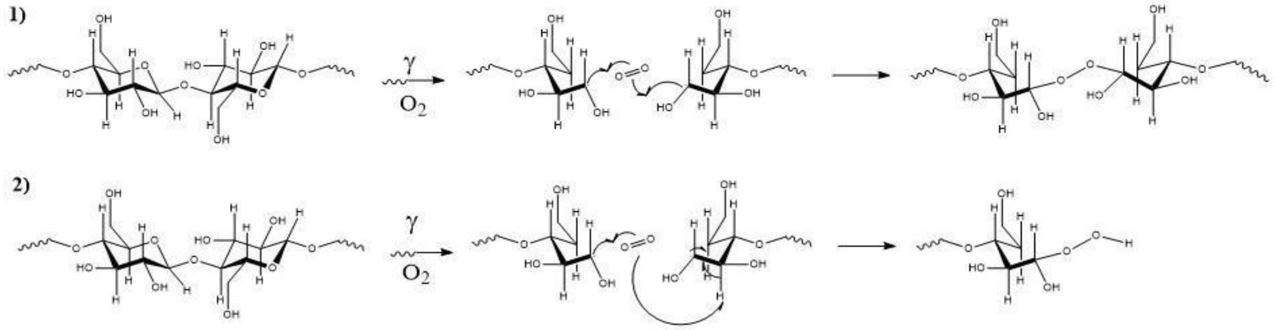


Figure 18. *Formation of peroxides (1) and hydroxides (2), due to gamma irradiation in the presence of oxygen from air*

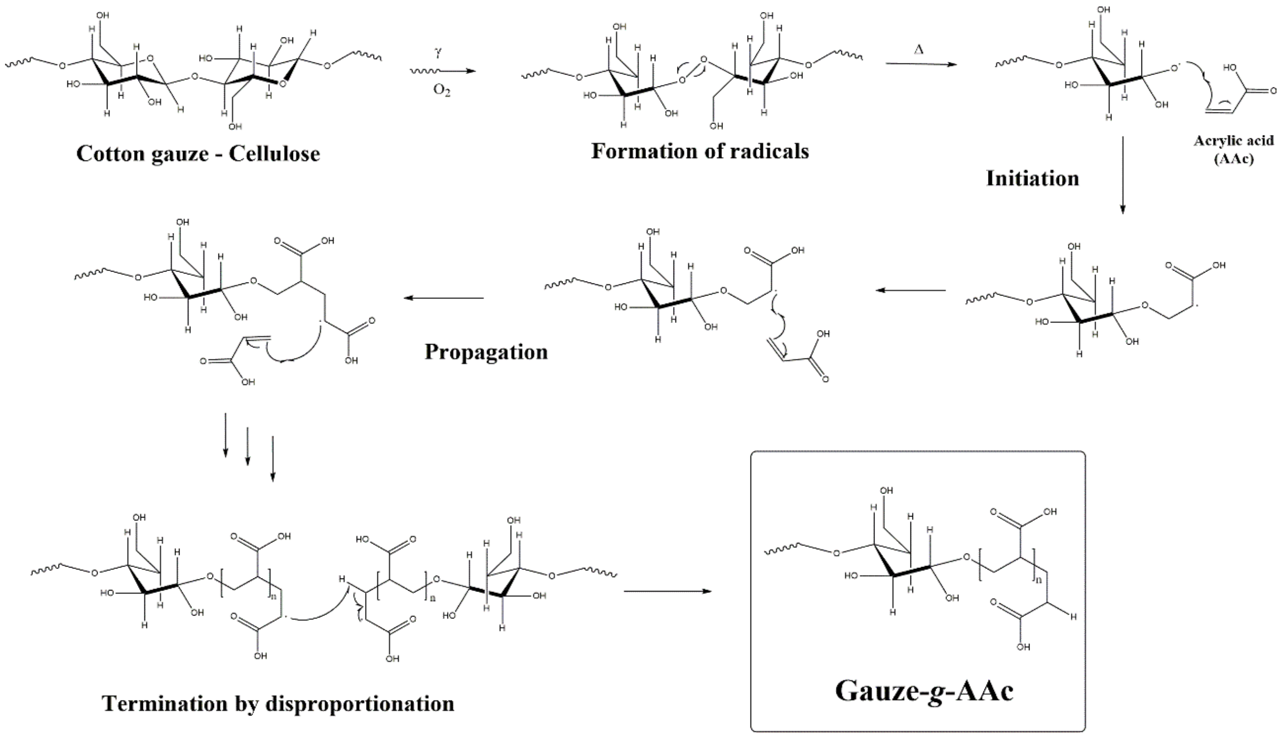


Figure 19. *Proposed mechanism of interaction between cellulose and acrylic acid*

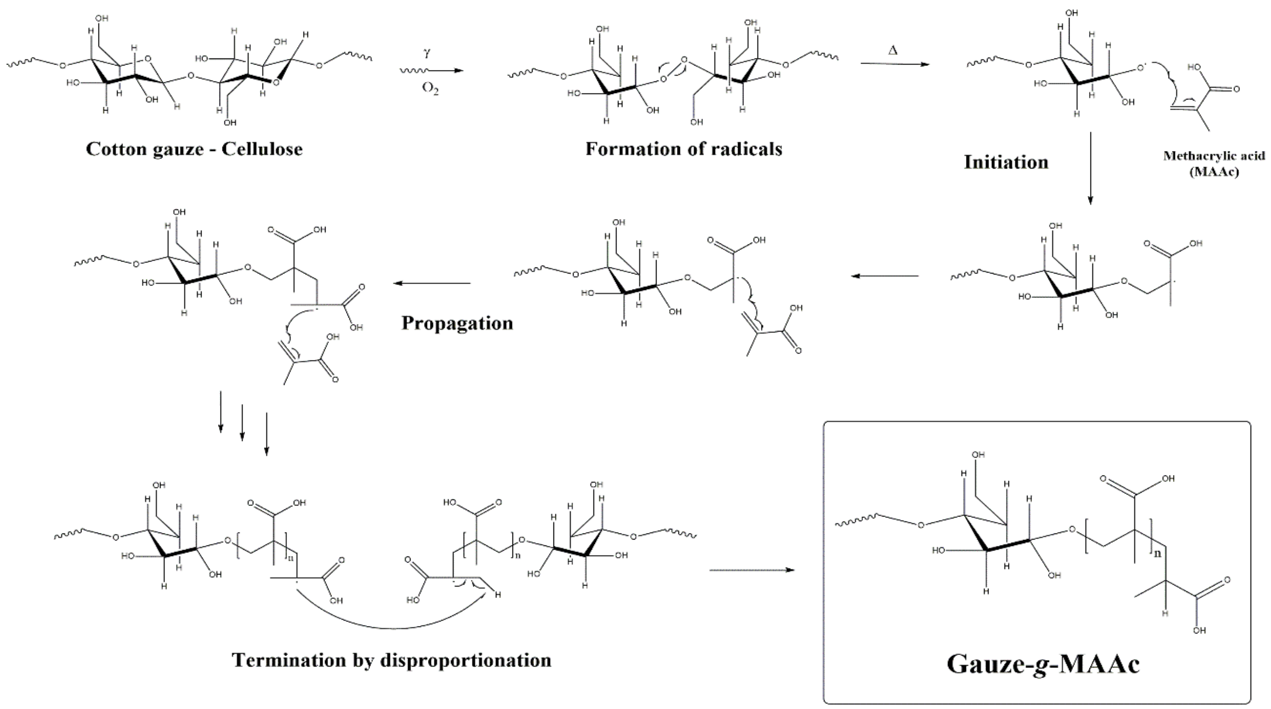


Figure 20. *Proposed mechanism of interaction between cellulose and methacrylic acid*

One of the first aspects that should be considered is that the percentage or degree of grafting should be controlled by changes in the reaction conditions. For this reason, a previous study should be carried out to determine the parameters that provide an optimal graft, that is, the one that has the desired properties and suitable for a potential biomedical application. The pre-irradiation oxidative method was selected for grafts of AAc and MAAc monomers due to the high reactivity of they. In this way, the amount of homopolymer formed during the synthesis of the copolymer was reduced. An experimental design based on previously mentioned methodology, was performed and the variables studied were monomer concentration, reaction time, gamma irradiation dose, and reaction temperature. Figure 21 and Figure 22 show a comparison regarding the organoleptic properties, such as texture an appearance, between unmodified cotton gauze and modified cotton gauzes.

Figure 21. *Pristine gauze, Gauze-g-AAc (26%), Gauze-g-AAc (52%), Gauze-g-AAc (143%) (From left to right)*

Figure 22. *Pristine gauze, Gauze-g-MAAc (22%), Gauze-g-MAAc (74%), Gauze-g-MAAc (116%) (From left to right)*

5.2. Effect of monomer concentration

The solutions of the AAc and MAAc monomers in aqueous solutions were studied in concentrations of 5 to 30% v/v with a variation of 5% v/v between each sample, with the established reaction conditions of: gamma irradiation dose of 30 kGy, water bath temperature of 65 °C and during a reaction time of 4 h. These conditions were selected through a bibliographic review and previous studies that have been carried out.

The grafting degree obtained in both monomers is presented in the following graphs (Figure 23 and Figure 24).

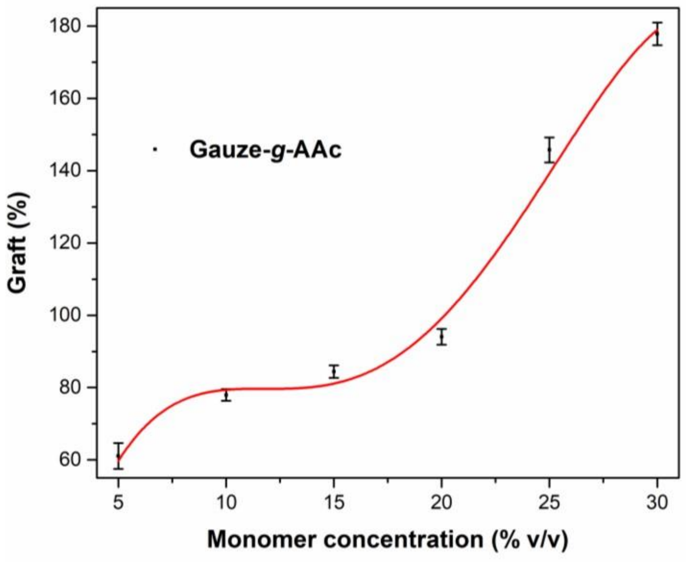


Figure 23. *Effect of AAc concentration on graft percentage in cotton gauzes*

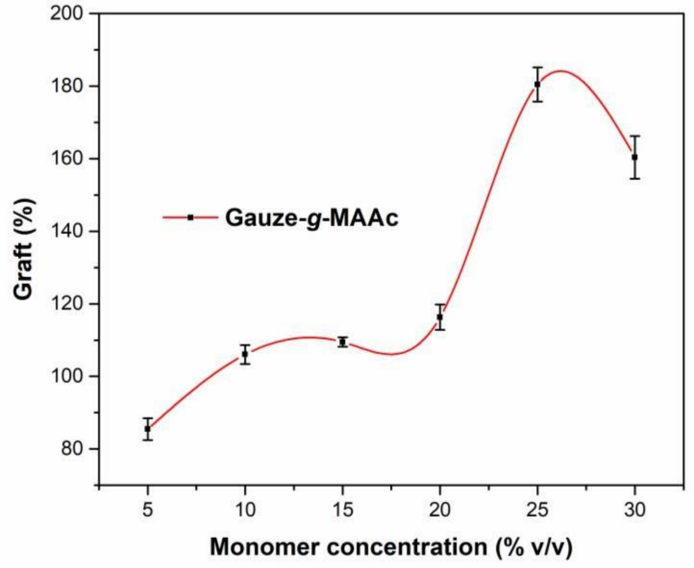


Figure 24. *Effect of MAAc concentration on graft percentage in cotton gauzes*

In the case of gauze grafted with AAc (Figure 23), the monomer concentration effect has an upward trend. According to the literature, an increase in the concentration of monomer induces an increase in the speed of reaction and the degree of grafting.[18] Likewise, when reaching high concentrations of monomer both variables have a constant value or even a possible decrease in the graft percentage due to problems in the diffusion of the monomer. However, as seen in the Figure 24, a graft of 180% is reached in the study interval and the monomer concentration is not high enough to induce diffusion problems.

In the case of grafts with MAAc (Figure 24), it can be seen that higher GP were present, in comparison with AAc grafts, due to the greater reactivity of the methacrylic acid monomer. In this sense it can be noted that, with a concentration of 5% v/v, a large amount of graft is obtained and that this behavior undergoes a considerable jump when the concentration that is stabilized in subsequent increases is increased. Also, a decrease in the percentage of grafting of methacrylic acid at higher concentrations can be seen. This can be attributed to a gel effect or Trommsdorff effect, because there is a higher concentration of molecules in the medium. Gel effect consists in an auto acceleration of the free-radical polymerization, produced by an increase on the viscosity from de medium which difficult the termination step.[43,44] The molecules will present diffusion problems and will begin to react with each other, forming gels, instead of grafting onto the polymer matrix.

In both cases of the AAc and MAAc grafts, when the graft percentages are higher, the cellulosic matrix becomes harder giving the system stiffness which represents a disadvantage to its potential use as biomaterial.

5.3. Effect of reaction time

The reaction time represents an important factor in the percentage of grafting in cotton gauzes. Due to the reactivity of the monomers to be grafted, short reaction times in the range of 1 to 4 h were chosen. The other variables remain constant (irradiation dose: 30 kGy, water bath temperature: 65 °C, and monomer concentration: 10% v/v). Figure 25 and Figure 26 show the results obtained from the relationship between the reaction time and the graft percentage.

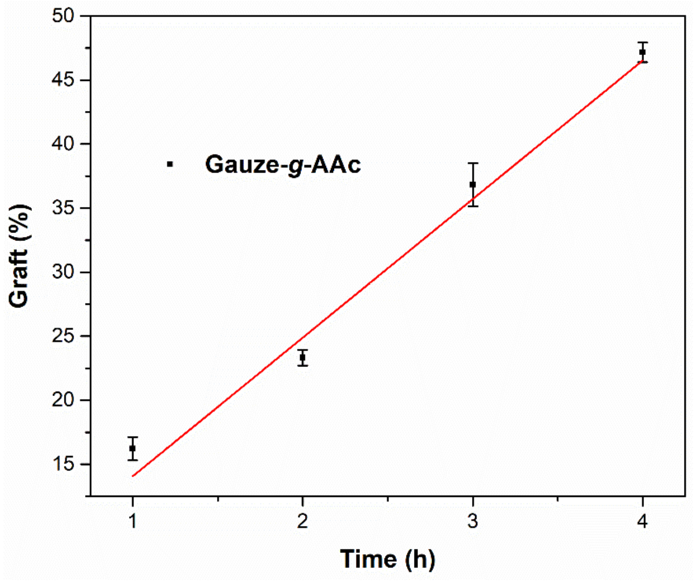


Figure 25. *Effect of reaction time in graft percentage of AAc onto cotton gauzes*

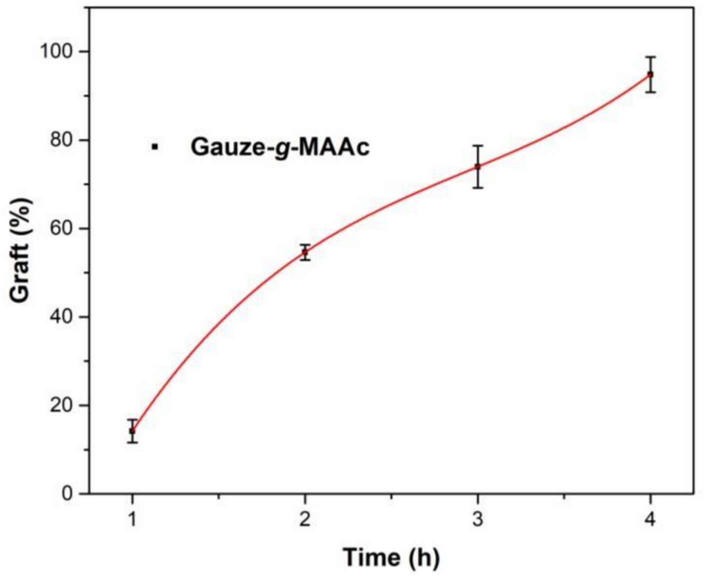


Figure 26. *Effect of reaction time in graft percentage of MAAc onto cotton gauzes*

In the case of grafting with acrylic acid (Figure 25) it can be seen that when the reaction time is short, the copolymerization reaction is not favored, which is reflected in the low graft percentage (15%). For grafting with MAAc (Figure 26), a similar behavior to that of AAc at short reaction times is noted. Methacrylic acid, being more reactive than acrylic acid, has higher graft percentages as it increases in reaction time.

In both cases the trend of growth can be explained because with more reaction time there is a greater amount of kinetic energy of the molecules, causing greater effective collisions between them and with the polymeric matrix, increasing the percentage of grafting in cotton gauzes.

5.4. Effect of γ-irradiation dose

The effect that the radiation dose will have on the graft percentage was studied. The polymeric matrix (cellulose) shows degradation in the presence of radiation whereby relatively low radiation doses were chosen, in a range of 10 to 40 kGy for grafting with AAc and 10 to 30 kGy for grafting with MAAc. The rest of the parameters are kept constant as mentioned in the methodology (water bath temperature 65 °C, monomer concentration 30% v/v, and reaction time 4 hours). Figure 27 and Figure 28 show the results obtained for grafts with AAc and MAAc, respectively.

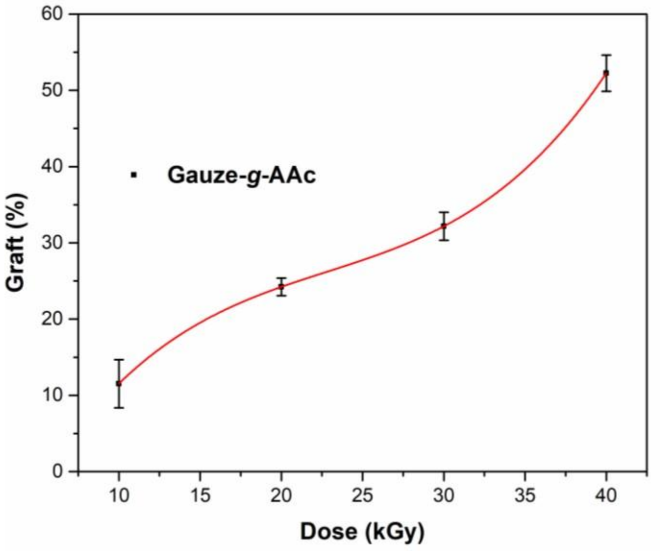


Figure 27. *Effect of γ-irradiation dose in graft percentage of AAc onto cotton gauzes*

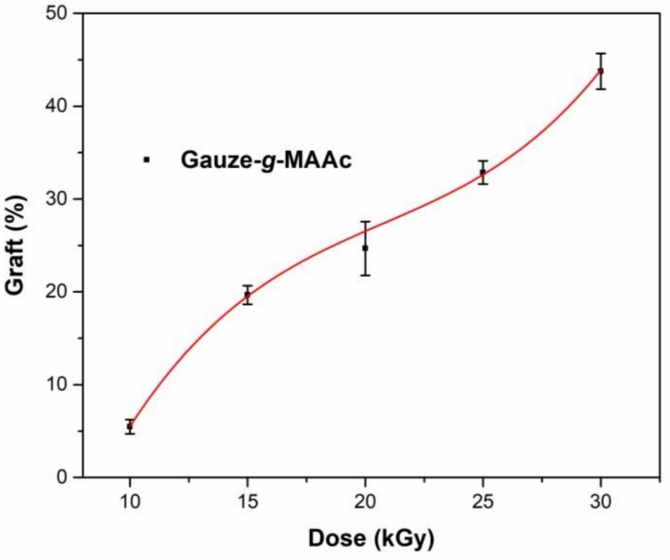


Figure 28. *Effect of γ-irradiation dose in graft percentage of MAAc onto cotton gauzes*

Ionizing radiation creates active sites in the polymeric matrix, both internally and superficially, by which the monomer can covalently bind to the polymeric skeleton. Thus, for both cases of grafts with AAc and MAAc, it can be observed that at very small irradiation doses, that is at 10 kGy, no greater graft amounts are obtained, which can be explained because these radiation rates are not suitable for generating sufficient active sites in the cotton gauze. In the case of the experiments with acrylic acid, the range between 10 and 40 kGy with an interval of 10 kGy was chosen due to the lower reactivity of the monomer, compared to the methacrylic acid monomer. The synthesis of cotton gauze with acrylic acid grafts greater than 40% is best carried out with doses greater than 25 kGy, this is without any deterioration of the polymeric matrix, which would affect the properties of the material at issue.

In the case of gauze with grafts of methacrylic acid (Figure 28), the tendency is practically the same as in grafting with acrylic acid, with the highest percentage of grafting being observed at a dose of 30 kGy. It was estimated that this is the appropriate dose because there is no deterioration, in addition to allowing adequate grafting percentages that do not allow the hardening of cotton gauze.

5.5. Effect of Temperature

To estimate the effect that temperature will have on the grafting percentage in cotton gauzes a range between 45 to 75 °C was determined. This range was selected due to the high reactivity of the monomers. The polymerization reaction for grafting with AAc and MAAc requires a very low activation energy, so the synthesis must be carried out at relatively low temperatures, for this reason the study interval of the effect of the temperature started at 45 °C, for both cases. Figure 29 and Figure 30 show the results of the experiments performed for the evaluation of the reaction temperature effect.

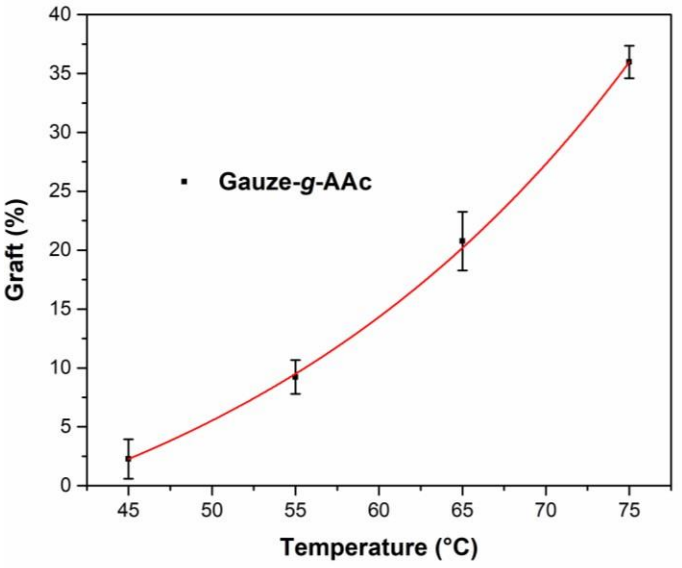


Figure 29. *Effect of reaction temperature in graft percentage of acrylic acid onto cotton gauzes*

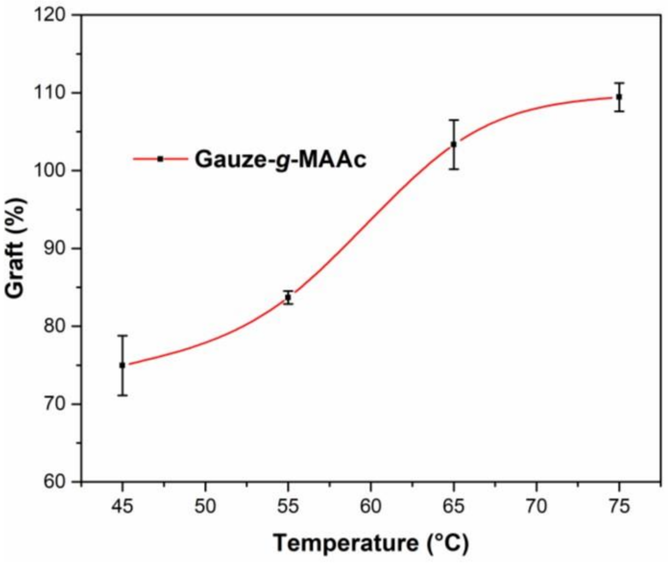


Figure 30. *Effect of reaction temperature in graft percentage of methacrylic acid onto cotton gauzes*

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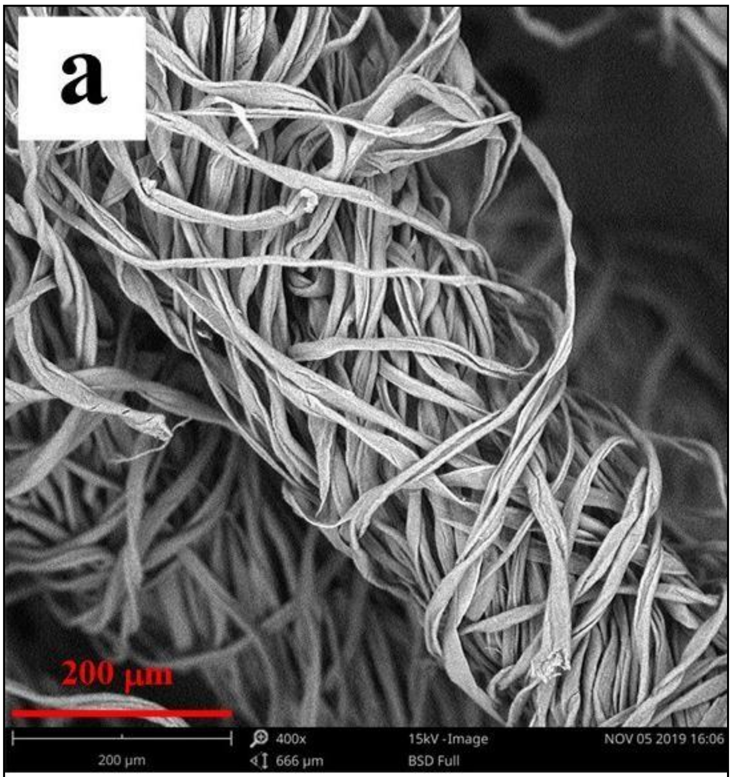
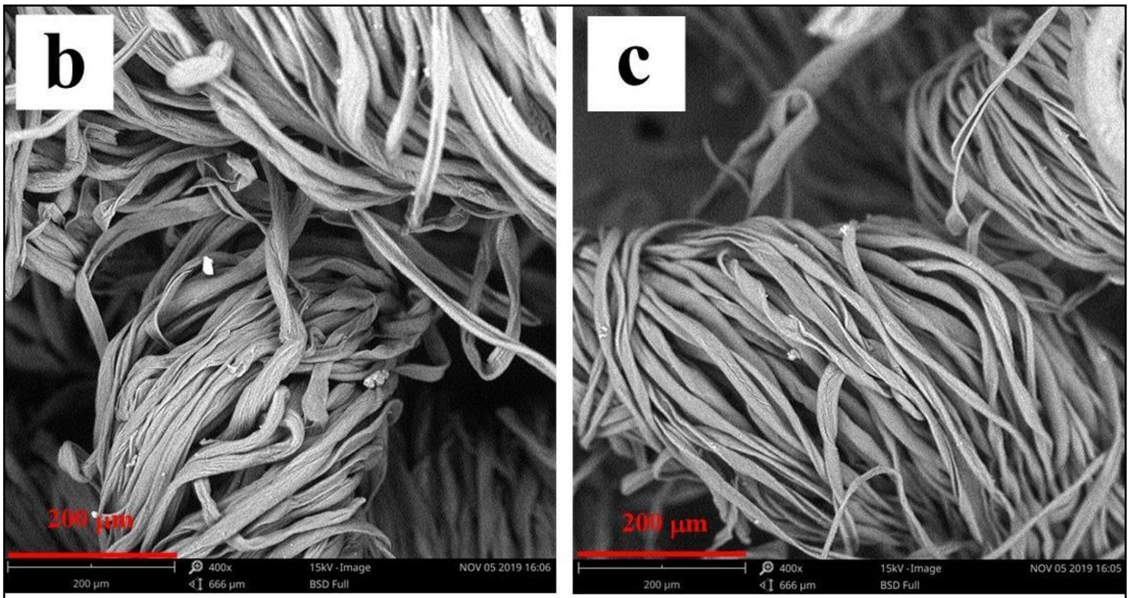
For grafts with acrylic acid (Figure 29), the trend follows an exponential behavior, without observing an absolute maximum graft. This behavior is due to the fact that at higher temperatures there is a greater kinetic movement of monomer particles which can be grafted onto the cellulosic matrix. In Figure 30, which represents the relationship between the reaction temperature and the degree of graft of methacrylic acid, greater grafts are observed due to the greater reactivity of the monomer, reaching a maximum peak at a temperature of 75 °C, with a 108% of graft. From this, possibly there is a decrease in the percentage of grafting at higher temperatures of 75 °C. This behavior could be due to the fact that the rapidity of the homopolymerization reaction of methacrylic acid, which is the reaction parallel to that of the copolymerization reaction, is much greater.

5.6. Scanning Electron Microscopy

The micrographs of a single gauze (pristine gauze), Gauze-*g*-MAAc (66%), Gauze-*g*-AAc (67%), Gauze-*g*-AAc (104%), Gauze-*g*-MAAc (97%), Gauze-*g*-MAAc (172%) and Gauze-*g*-AAc (180%) are shown in Figure 31.

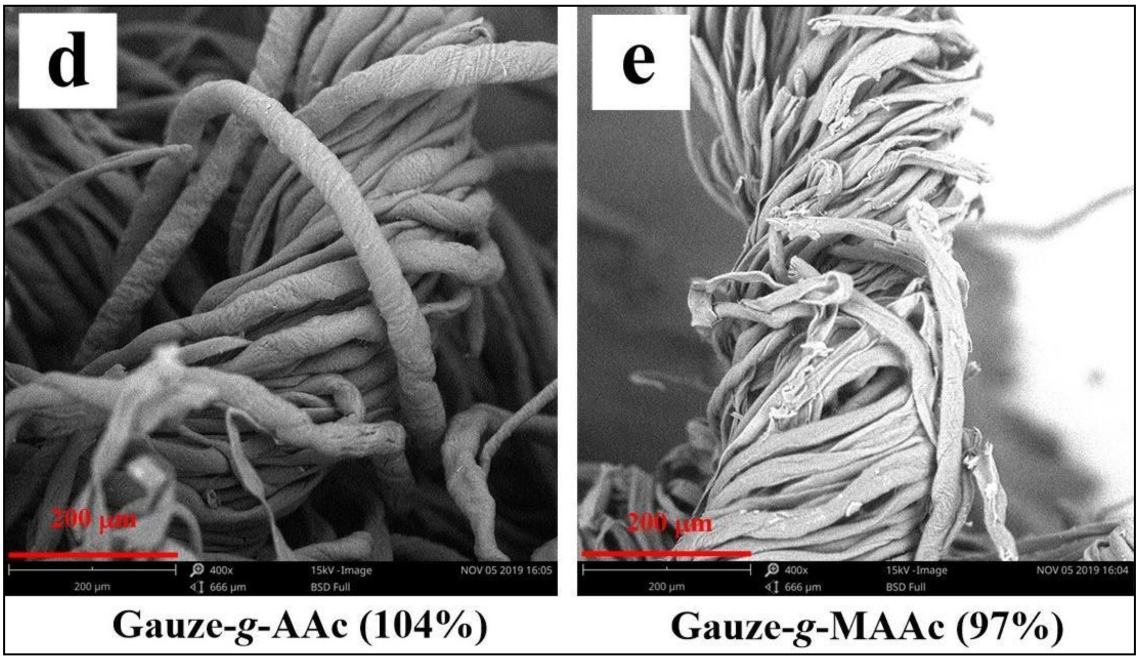


Pristine gauze

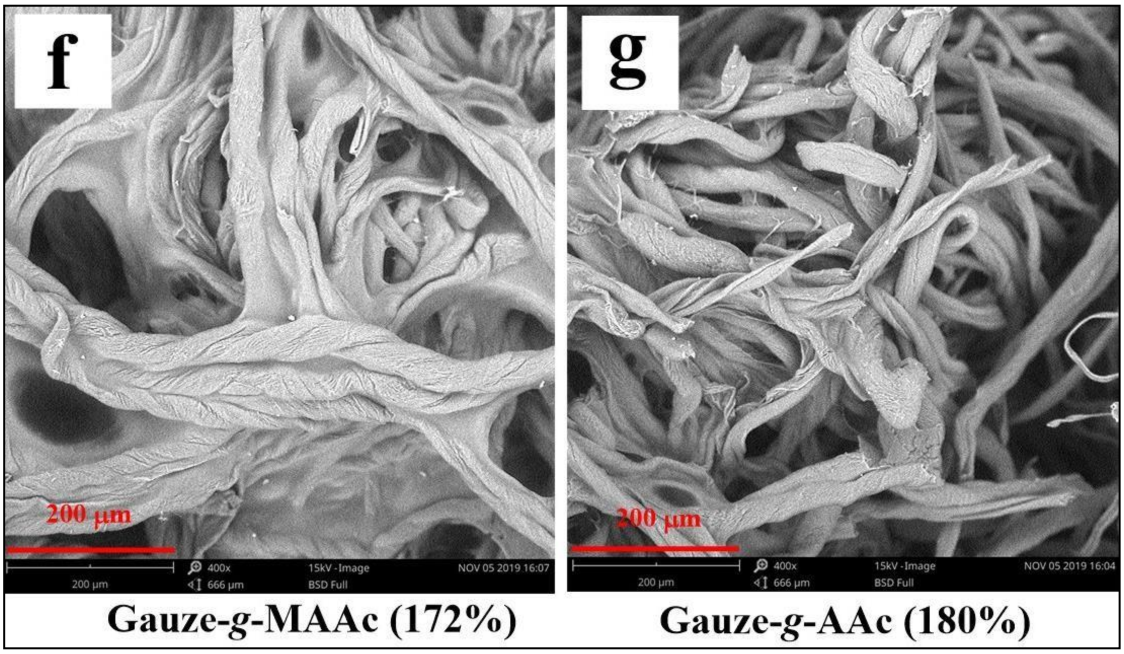


Gauze-*g*-MAAc (66%)

Gauze-*g*-AAc (67%)



Gauze-*g*-AAc (104%) Gauze-*g*-MAAc (97%)



Gauze-*g*-MAAc (172%) Gauze-*g*-AAc (180%)

Figure 31. *SEM micrographs of several samples of modified cotton gauzes with different GP*

The micrographs are useful to confirm that copolymerization occurred between the polymeric matrix and the pH-sensitive polymers. Figure 31a presents the sample of an unmodified gauze, it can be seen that the thickness of the thread is very thin.

Ionizing radiation is able to penetrate the polymeric matrix and generate random radicals both on the surface and inside (in bulk) of the material. The micrograph of Figure 31b corresponds to the graft of methacrylic acid in the gauze (GP: 66%). We can see a widening of the thickness of the thread and some small points on the surface of the material, product of the MAAc graft in the gauze. Figure 31c shows the grafting of acrylic acid in the polymer matrix (GP: 67%). When comparing with Figure 31b, it is noted that despite having similar percentages of grafting, the gauze that shows a greater thread widening is the one that has grafting with MAAc due to the greater reactivity shown by the monomer.

Cotton gauze grafted with AAc that has a graft percentage of 104% is displayed in Figure 31d. The micrograph shows a thickening of the thread due to the bulk grafting. In this case, radical formation predominated in the structure of the material and not so much in the surface. Thus, when comparing Figure 31d with Figure 31e corresponding to the gauze grafted with MAAc (GP: 97%), it can be seen that the copolymerization occurred superficially. It can also be inferred that the widening of the thread, due to bulk grafting, occurs in greater proportion when the cotton gauze is modified with MAAc.

Finally, gauze with graft percentages greater than 150% are shown in Figure 31f and Figure 31g. These gauzes show a certain stiffness, which can be seen in the structure of the material, so that it appears an interconnection between the threads of the cotton gauze. This also shows the bulk grafting within the polymeric matrix.

Thus, it has been confirmed that AAc and MAAc grafts were produced in cotton gauze both superficially and in bulk, within the glucopyranose structure of cellulose.

5.7. Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy

Figure 32 shows the results of the spectrum obtained for the pristine gauze sample and the modifications made to it with acrylic acid (Gauze-*g*-AAc) and methacrylic acid (Gauze-*g*-MAAc).

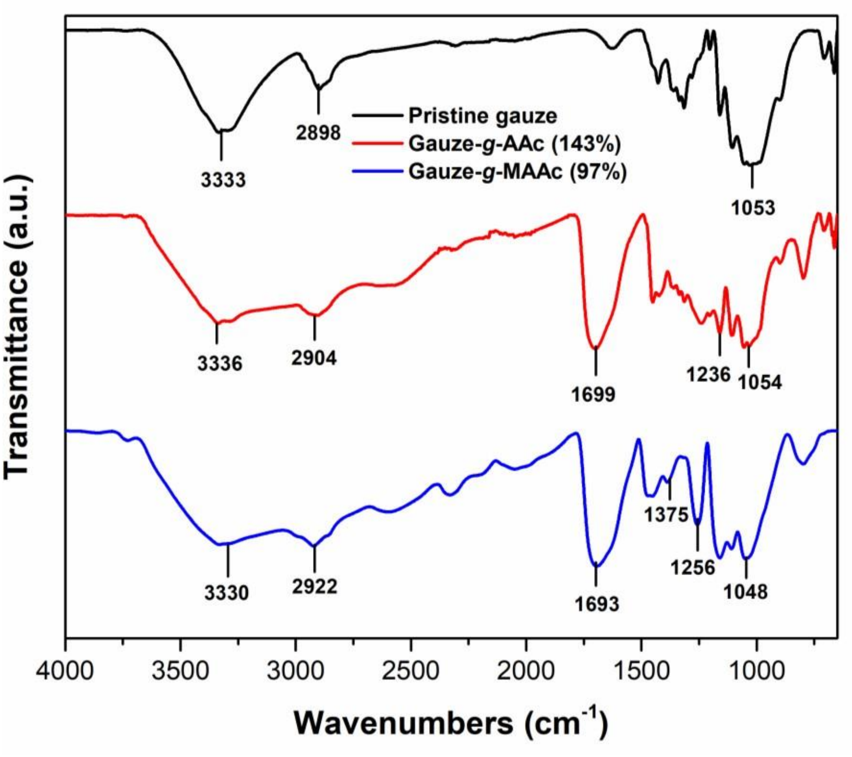


Figure 32. *ATR-FTIR Spectra for the samples of pristine cotton gauze, Gauze-g-AAc (143%) and Gauze-g-MAAc (97%)*

Due to its structure, the spectrum corresponding to cotton gauze, whose main component is cellulose, has intense bands in 3333 and 2898 cm^{-1}. The first signal is attributed to the hydrogen-bonded O-H stretch which is a broad peak in that region, and is due to the presence of O-H belonging to cellulose. The second band is due to the stretch of the C-H bond since C-H absorption always occurs at frequencies below 3000 cm^{-1}. It can also see a band in the region of 1300-1000 cm^{-1} that corresponds to the C-O stretch in the cotton fiber. The gauze, when modified, with the graft of AAc (Gauze-*g*-AAc), a more prominent and wider signal is observed in the region of 3400-2400 cm^{-1} that indicates the presence of COO-H acid groups. The C=O bond of the carboxyl group is clearly seen in the peak at 1699 cm^{-1}. This value can be shifted to lower frequencies due to conjugation effects.[45–47] Another important peak is the stretch of C-O bond which occurs, in this case, at 1236 cm^{-1}, with a medium intensity. Also, the stretch of the C-O bond at 1054 cm^{-1} of the cotton membrane is preserved. Grafting with AAc is corroborated because new peaks characteristic of the grafted compound is shown and the representative bands of cellulose are also maintained, corresponding to the pristine gauze.

Methacrylic acid is a compound that has a similar structure compared with acrylic acid. The only difference between the two is that in the methacrylic acid there is a methyl group present in its structure, specifically at C_2. Therefore, it can be ensured that the bands confirming the presence of acid groups will be maintained. For example, there is the presence of a broad band in the region of 3400-2400 cm^{-1}, associated with acid groups. This is confirmed with the band at 1693 cm^{-1}, corresponding to the carbonyl group, slightly displaced from the region of 1700 cm^{-1}, because the structure could appear conjugated. In addition, a band at 1375 cm^{-1} can be seen, which is attributed to the bending absorption of the methyl group.[45]

Figure 33 and Figure 34 show the spectra corresponding to unmodified gauze and grafted gauze with both acrylic acid and methacrylic acid, which have different graft percentages. The comparison shows that the intensity of the peaks as well as their location in the spectrum do not vary with the different graft percentages. This is because the infrared spectroscopy technique is a qualitative technique that indicates the presence of the functional groups present in the material. Thus, the reproducibility of the synthesis is represented since in all cases the grafts of acrylic acid and methacrylic acid in cotton gauze are confirmed.

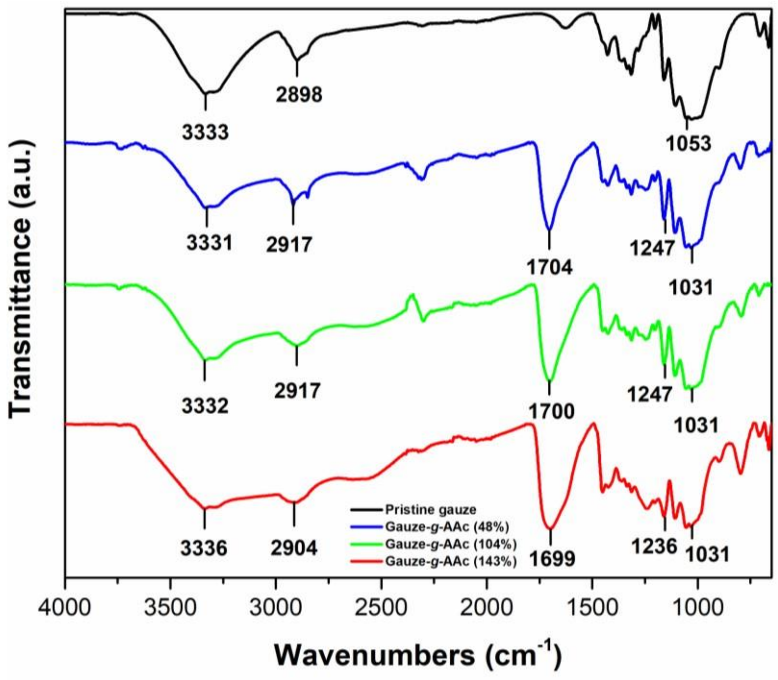


Figure 33. *ATR-FTIR Spectra for the samples of pristine cotton gauze compared to some Gauzes-g-AAc with different graft percentage*

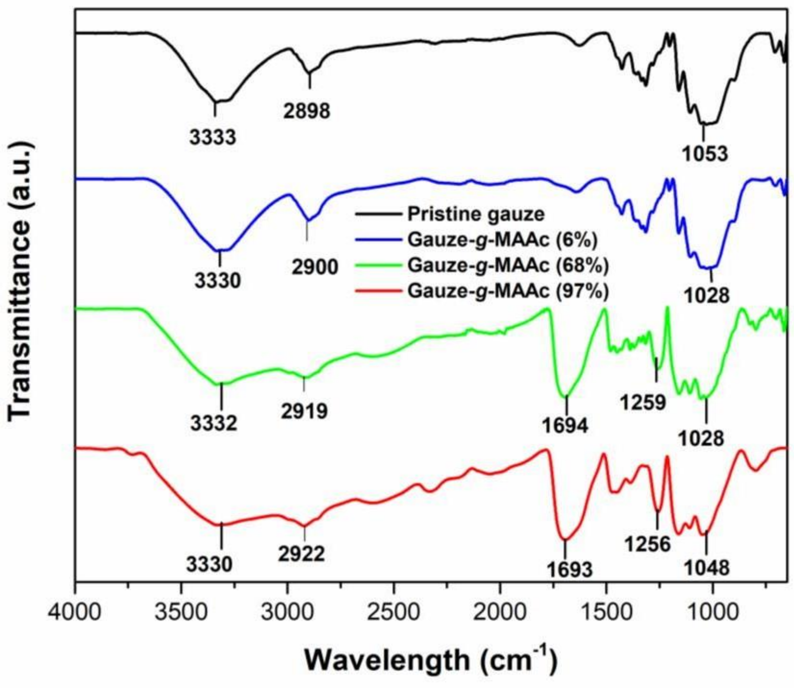


Figure 34. *ATR-FTIR Spectra for the samples of pristine cotton gauze compared to some Gauzes-g-MAAc with different graft percentage*

5.8. Thermogravimetric analysis

The thermogravimetric analysis of the samples allowed to determine its thermal stability. In the first instance, samples of a pristine gauze, Gauze-*g*-AAc (143%), and Gauze-*g*-MAAc (136%) were compared, and their results are shown in Figure 35 and Table 1.

Table 1. *TGA results of Gauze-g-AAc and Gauze-g-MAAc*

Sample	10% Weight Loss (°C)	Decomposition Temperature (°C)	% Char yield (800 °C, N_2)
Pristine gauze	295.08	356.17	9.63
Gauze-*g*-AAc (143%)	226.42	263.86, 350.26, 391.08	19.42
Gauze-*g*-MAAc (136%)	235.10	248.02, 362.75, 446.41	2.26

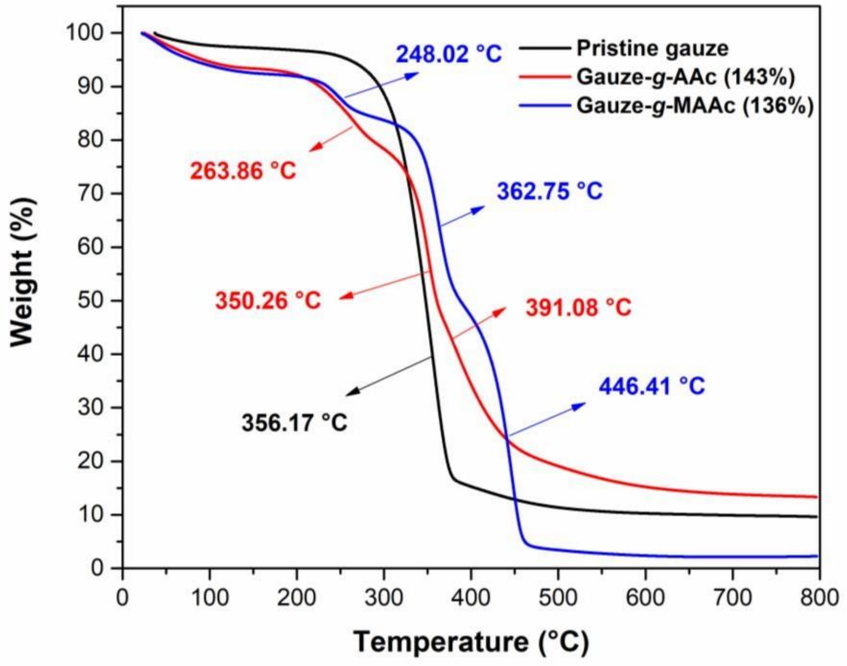


Figure 35. *Thermograms: pristine gauze (black), Gauze-g-AAc (red), Gauze-g-MAAc (blue)*

In the Figure 35, it can be seen that pristine gauze shows an abrupt weight loss in the range of 300 to 380 °C, with a maximum rate of decomposition at 356.17 °C. This fact is characteristic of polysaccharides and is due to the rapid breakdown of cellulose and the release of degradation products.[48] In addition, it can be noted that the unmodified gauze residue remains constant (around 10% weight) from 450 °C.

It is apparent that Gauze-*g*-AAc suffers weight loss in four decomposition steps. The first step occurs between 30 and 100 °C. An initial weight loss in this range can be attributed to the release of water absorbed by the polymer or by periods of dehydration that probably occur in the carboxylic groups of are present in the polymer.[49]

The second stage of decomposition of Gauze-*g*-AAc occurs in the range between 200 and 350 °C, with a maximum rate of decomposition at 263.86 °C. This behavior is attributed to a decarboxylation process (CO_2 removal) (Figure 36).[50] According to the literature, this stage is evident at temperatures above 200 °C and takes on greater importance above 250 °C.[49]

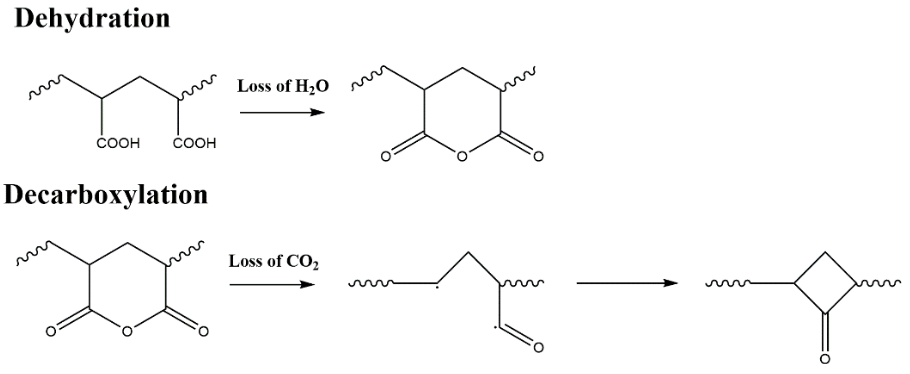


Figure 36. *Dehydration and decarboxylation processes of PAAc*

The third stage comprises a small range between 340 and 380 °C, with maximum decomposition at a temperature of 350.26 °C. This value is attributed to the decomposition of the polymeric matrix (cotton gauze) which was previously explained.

The last stage comprises a decomposition at a temperature of 391.08 °C. Above 350 °C, the acrylic acid polymer residue decomposes into cold ring fraction products, which are volatile at vacuum degradation temperature but not at room temperature.[50] These products consist of short chain fragments derived from two or more original repeating units, which may contain only anhydride rings or, in turn, both rings and structures containing carboxylic groups. Similarly, other volatile products, including carbon monoxide, also form at high temperatures, but in small quantities.[49]

In the case of Gauze-*g*-MAAc, processes very similar to those previously detailed with acrylic acid graft occur. The first stage lies in the range at early temperatures between 30 and 90 °C, which is attributed to the elimination of water molecules that have not been completely eliminated in the copolymer or by intramolecular cyclisation of adjacent monomer units to give six-membered anhydride ring structures. The second stage occurs between 210 and 350 °C with an abrupt drop in weight at a temperature of 248.02 °C, which is related to the processes of CO_2 removal or polymer anhydrization (Figure 37).[30] The third weight loss falls in the range between 320 and 380 °C, which is attributed to the decomposition of the cellulosic matrix. Finally, the fourth stage is in the range between 380 and 450 °C that corresponds to the formation of several products due to the cleavage of the polymer's main chain.[30]

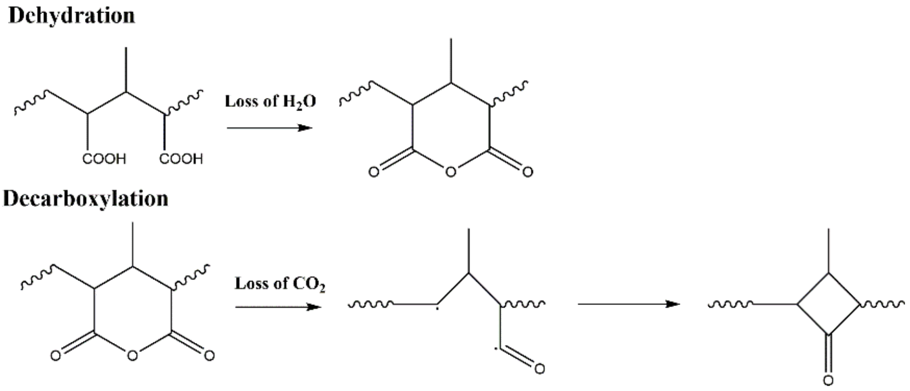


Figure 37. *Dehydration and decarboxylation processes of PMAAc*

Table 2 and Figure 38 show the results and thermograms obtained in the thermogravimetric analysis of gauze modified with acrylic acid with various graft percentages. Apart from the stages of decomposition that have been explained, in Figure 36, the reproducibility of the experiment is appreciated as it has very similar thermal behaviors when comparing all modified gauzes.

Table 2. *TGA results of Gauzes modified with AAc with different GP.*

Sample	10% Weight Loss (°C)	Decomposition Temperature (°C)	% Char yield (800°C, N_2)
Pristine gauze	295.08	356.17	9.63
Gauze-*g*-AAc (45%)	250.62	258.63, 350.98, 388.32	19.42
Gauze-*g*-AAc (48%)	245.63	254.67, 351.01, 387.45	19.20
Gauze-*g*-AAc (143%)	226.42	263.86, 350.26, 391.08	13.34

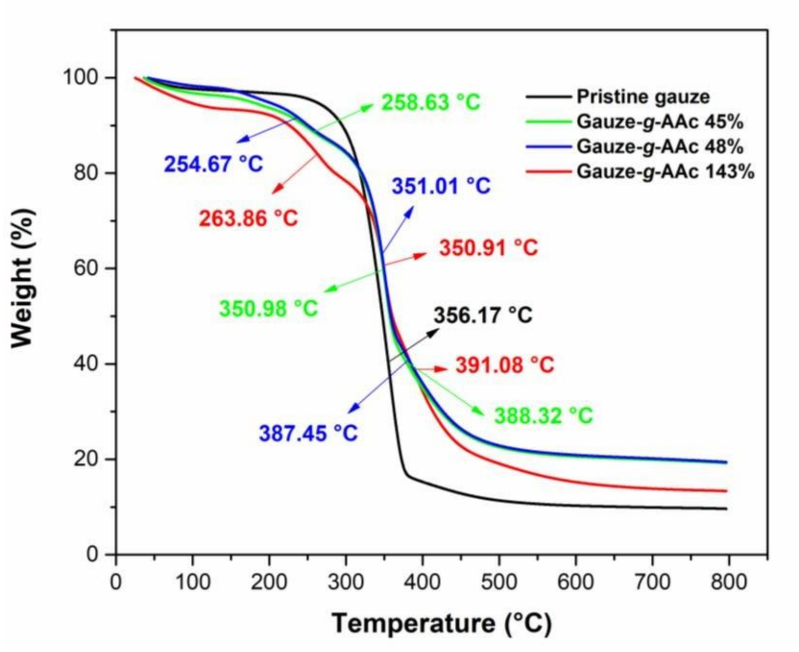


Figure 38. *Thermograms of cotton gauzes modified with AAc*

The same behavior of gauzes modified with AAc also occurs with the results shown in Table 3 and Figure 39 corresponding to grafts with methacrylic acid. The gauze with a low graft percentage (6%) shows a very similar behavior to that of pristine gauze, due to the decomposition of the products of the cellulosic matrix.[30]

Table 3. *TGA results of Gauzes modified with MAAc with different GP.*

Sample	**10% Weight Lost (°C)**	**Decomposition Temperature (°C)**	**% Char yield (800 °C, N_2)**
Pristine gauze	295.08	356.17	9.63
Gauze-*g*-MAAc (6%)	253.68	259.18, 350.91, 401.32	22.22
Gauze-*g*-MAAc (87%)	235.09	238.87, 353.08, 428.41	12.34
Gauze-*g*-MAAc (136%)	235.1	248.02, 362.75, 446.41	2.262

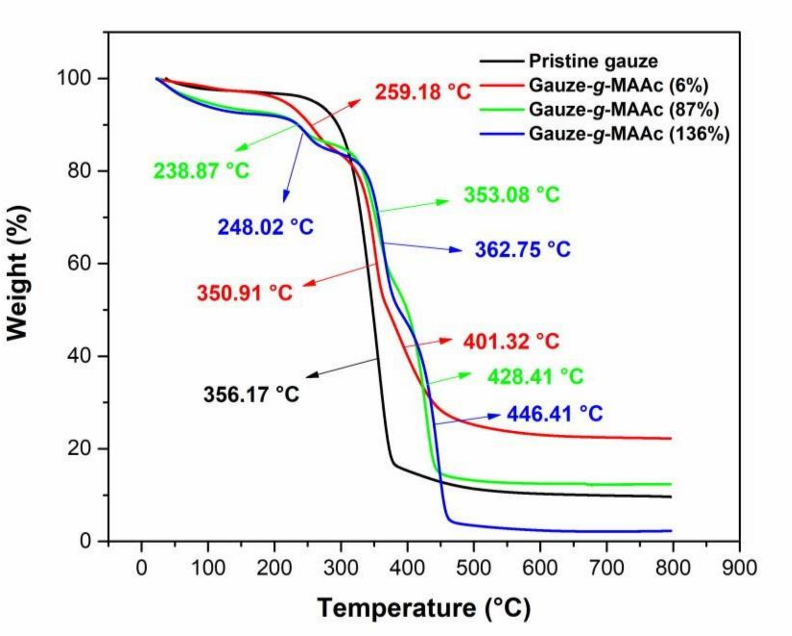


Figure 39. *Thermograms of cotton gauzes modified with MAAc*

From this study, it can be noted that the copolymers begin to degrade at a lower temperature than that of pristine gauze and this is due to the previously exposed dehydration and decarboxylation processes. It is important to take into account that all the modified materials have acquired greater complexity, which can be corroborated with the decomposition stages that the material has now. In the same way, the new materials present greater thermal stability, since the residues (char yield) at 800 °C are higher to pristine gauze. The difference in mass between the sample heated in the inert gas and air divided by the original sample weight is the car yield of the polymer. However, in the case of Gauze-*g*-MAAc (136%) the residue is much lower than that of pristine gauze at

800 °C; This is due to the conditions that were used for the synthesis of the material, specifically in the irradiation dose and reaction time, since a dose of 40 kGy and a reaction time of 4 hours were used, which affected the structure of the polymeric matrix.

5.9. Differential Scanning Calorimetry

Figure 40 displays the thermograms obtained by Differential Scanning Calorimetry and Table 4 shows thermal transitions obtained, where the thermal transitions of the different materials are observed, both of the pristine gauze and of grafted gauzes, observing the complexity that the material adopts when being modified with the polymers.

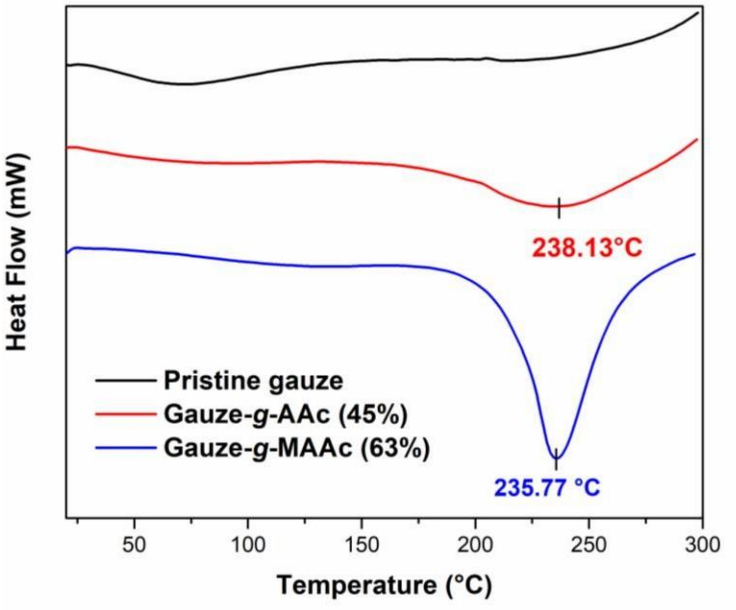


Figure 40. *Thermograms obtained by DSC for pristine gauze and modified cotton gauze*

Table 4. *Thermal transitions obtained using DSC*

Sample	Thermal transitions (°C)
Pristine cotton gauze	-
Gauze-*g*-AAc	238.13
Gauze-*g*-MAAc	235.77

The DSC thermograms displayed endothermic peaks corresponding to thermal transitions adopted by the modified material. In a study carried out by mixture of polyacrylic acid and with bone powder it was shown two endothermic transitions at 58 and 232 °C, at which the sample consumes energy values of 80.96 and 394.25 J/g, respectively, however no further explanation was given to the origin of these transitions. In the present case endothermic transition between 233 and 240 ° C, are observed for both polymers formed by AAc and MAAc grafted onto cotton gauze.[51] Further studies are required to elucidate the origin of these transitions observed in the present study.

Figure 41 shows the thermograms obtained by DSC and the complexity that acquires the material, and the reproducibility of the experiment is observed since the temperature values for thermal transitions are almost constant.

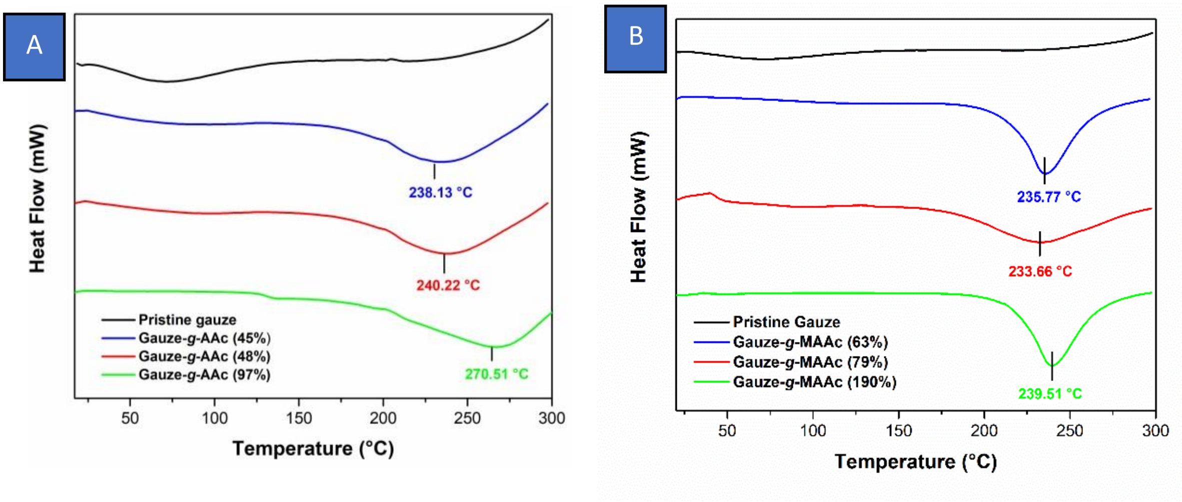


Figure 41. *Thermograms obtained by DSC for modified cotton gauze with different grafting percentages. A) Gauze-g-AAc. B) Gauze-g-MAAc*

5.10. Critical pH

Critical pH is a characteristic of pH-sensitive polymers and its value is an indicator of an abrupt conformational change in the material. In this case, the pH-response capacity of the material was studied by means of acid-base titrations and back titrations (Annexes 2 and Annexes 3). The determination of the end points in the titrations was made by calculating the first derivative in the Origin program.

Figure 42 shows the results obtained for direct titrations between the material modified with the titrant, which is a strong base (KOH 0.11M). Pristine gauze shows a single end point due to the titration between potassium hydroxide and hydrochloric acid that was used to reduce the pH of the medium. Three gauzes modified with acrylic acid that were selected for the experiment show a similar behavior, as they have three end points. The first end point corresponds to the pH at which the conformational change of the grafted polymer occurs, which was defined as critical pH. These values coincide with the literature that indicates that around a pH = 5, which is driven by the ionization state of the carboxylic groups,[52] as detailed in this expression: $R\text{-}COOH \rightleftharpoons H^+ + R\text{-}COO^-$

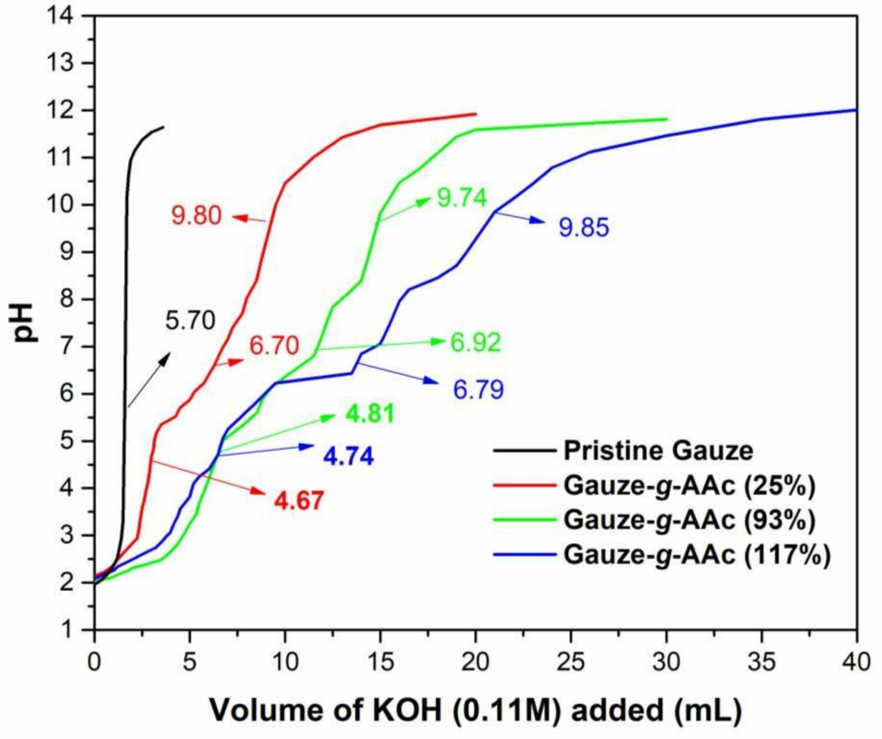


Figure 42. *Potentiometric direct titrations of pristine cotton gauze and modified Gauze-g-AAc. Determination of critical pH*

The second end point is related to the behavior of pristine gauze. The third end point, which is at an approximate pH of 9.8, is related to the free carboxylic groups and which is in total agreement with reported results.[53,54] In addition, it can be seen that as the graft percentage grows, a larger volume of titrant is needed to increase the pH. This is due to the greater number of acidic groups in the medium. From this analysis, it can be said, that, at low pH, the poly(acrylic acid) takes on a compact globular conformation (but not fully collapsed). As the pH increases, ionization occurs and the polymer adopts a fully solvated open coil conformation.[52]

Figure 43 shows the results obtained from titration of pristine gauze and gauzes grafted with methacrylic acid. It can be noted that the gauze that has a lower percentage of grafting (6%) has the most similar behavior to that of pristine gauze, since it does not have a greater number of carboxylic groups.

For gauze with a higher percentage of grafting, three end points are appreciated, the second being associated with the titration between the acid and the base. The poly(methacrylic acid) undergoes an expansion of its chain in a pH range between 4-6, when a charge density is attained.[55] This behavior can be seen in the first end point of the degree. At pH < 4, the polymer adopts a compact conformation that could be due to the hydrophobic interactions of the methyl groups and the hydrogen bonds between the carboxylic acid groups.[55] The third end point is associated with the titration of free acid groups in the middle.[53]

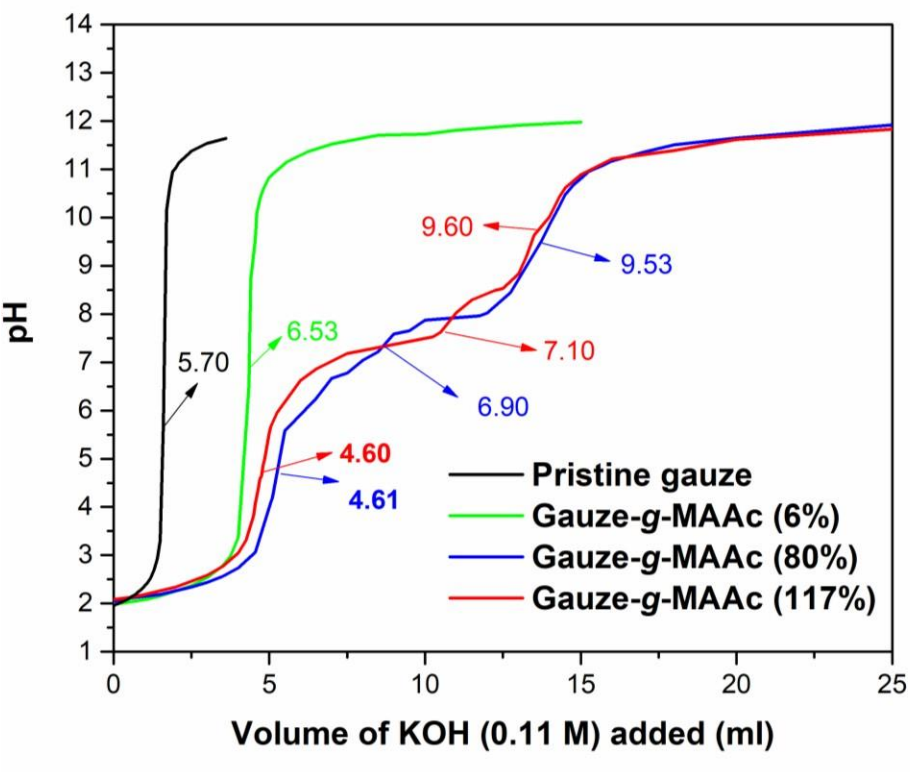


Figure 43. *Potentiometric direct titrations of pristine cotton gauze and Gauze-g-MAAc. Determination of critical pH*

5.11. Loading of Vancomycin

One of the objectives set forth in this project is the drug loading capacity of the new modified polymer matrix. This process will depend on the nature of both components. For this reason, the behavior of the modified gauze in the presence of an antimicrobial drug such as vancomycin was studied. In the same way, the relationship between the percentage of grafting and the amount of drug loaded in the polymer was evaluated.

Vancomycin (Figure 44) is a branched tricyclic glycopeptide that has antibacterial activity against many microorganisms and has a bacteriostatic effect on *Enterococci*. Its action is bactericidal and is exerted by inhibiting the synthesis of the bacterial cell wall.[56] Vancomycin binds with great affinity to the precursors of this cell compartment.

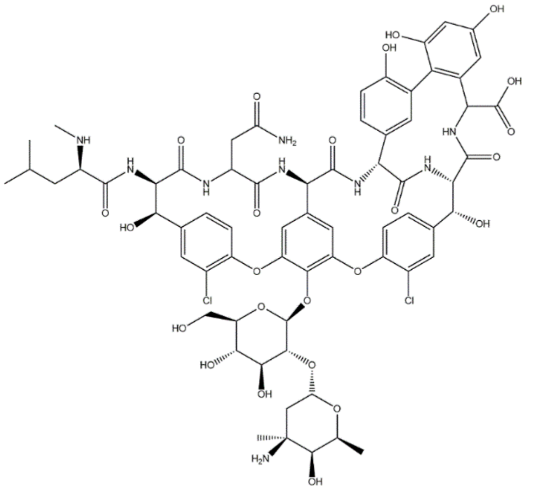


Figure 44. *Chemical structure of vancomycin*

Modified gauze samples with different graft percentages of AAc and MAAc are deposited in contact with an aqueous solution of vancomycin and the fuel-loaded drug quantified by UV-Vis. In Figure 45 and Figure 46 it can be seen that the pristine gauze does not have the carrying capacity, it does not show interactions with vancomycin. However, in Figure 45, which shows the behavior of gauzes grafted with AAc, a direct relationship is seen between GP with the amount of vancomycin loaded in the matrix. Thus, the gauze grafted with 47 and 67% loading, 1334 and 2351 µg/g of vancomycin, respectively. A maximum drug loading is observed after 2 hours of exposure, marking a trend thereafter.

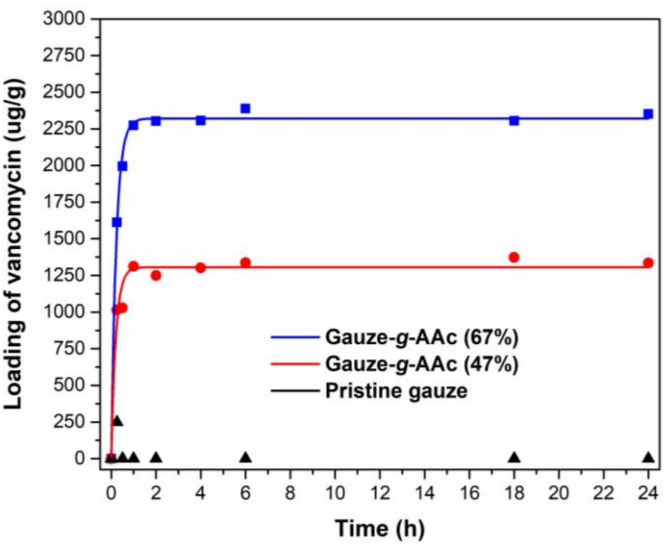


Figure 45. *Loading profile of vancomycin in cotton gauze modified with AAc*

In the case of gauzes modified with MAAc, a trend is shown after 2 hours of exposure to the aqueous vancomycin solution (Figure 46). Gauze-*g*-MAAc (10%) has loaded 6900 µg/g, Gauze-*g*-MAAc (27%) has loaded 7100 µg/g and Gauze-*g*-MAAc (97%) 8500 µg/g. Thus, it can be inferred that the vancomycin load is influenced by the ionic interactions of the cationic moiety of vancomycin (amino and amido groups) with the carboxylic groups present in both the PAAc and the PMAAc of the modified gauzes (Figure 47).[57]

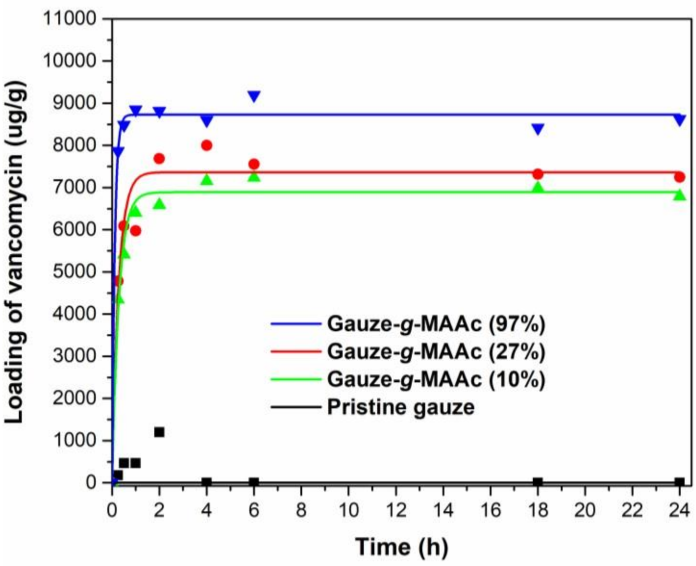


Figure 46. *Loading profile of vancomycin in cotton gauze modified with MAAc*

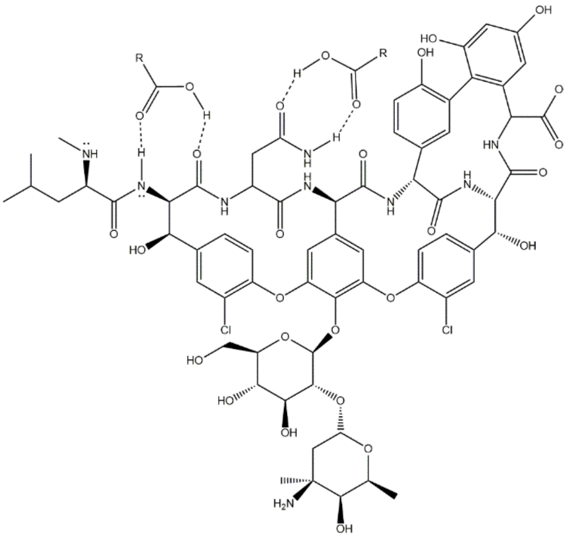


Figure 47. *Proposed hydrogen bond interactions between vancomycin and carboxylic groups*

6. CONCLUSIONS AND RECOMMENDATIONS

- The modification of cotton gauze was carried out by means of the use of ionizing gamma radiation. In this way, it was possible to graft pH-sensitive polymers into the cellulose structure. The process is reproducible and the percentage of grafting will depend on the reaction conditions that are used.
- Gamma radiation produces mechanical alterations in the cotton material, therefore irradiation doses higher than 40 kGy are not recommended as the functionality of the material is affected.
- SEM micrographs corroborated the graft, which shows that it was performed superficially and in bulk on the cellulosic structure.
- By means of the ATR-FTIR characterization technique used, the presence of AAc and MAAc in cotton gauzes is confirmed by the appearance of a broad band corresponding to carboxylic acid group, in the region of 3400 - 2400 cm^{-1}, another of carbonyl (C=O) signals near to 1700 cm^{-1}, and C-O stretch at 1236 cm^{-1}.
- Thermogravimetric analysis indicates thermal stability because the modified materials show four decomposition temperatures, which indicates the complexity that the materials have acquired. In addition, one of these stages of decomposition corresponds to that of cellulose, so the new material retains certain properties of the original material.

- The DSC technique allowed to observe thermal transitions associated to both polymers formed by AAc and MAAc grafted onto cotton gauze. However, it is necessary to establish new DSC heat-cool-heat protocols, with controlled variation of cooling rates, in order to erase the previous polymers thermal history and confirm these transitions.

- Grafted monomers have the property of pH-sensitivity, which is preserved after grafting onto cotton gauze. The critical value in which a change in the structure of the modified material occurs ranges from pH = 4.60-4.90 which coincides with the pK_a values of the monomers.
- The graft gives new functionalities to the cotton material. The modified material contains carboxylic groups that allow it to interact with drugs such as vancomycin.
- It is necessary to perform blank tests of polymerization reactions by direct radiation to the monomers to know the properties of the polymer to be grafted and to make better comparisons in the characterization of copolymer.
- In this project, the vancomycin loading capacity of the new material was evaluated, however it is necessary to carry out controlled drug release tests, as well as antimicrobial tests to evaluate its potential application as a biomaterial.
- More characterization techniques are required to better elucidate the structure of the copolymer. Thus, it can be carried out, for example, Nuclear Magnetic Resonance to know the arrangement of molecules in the structure.

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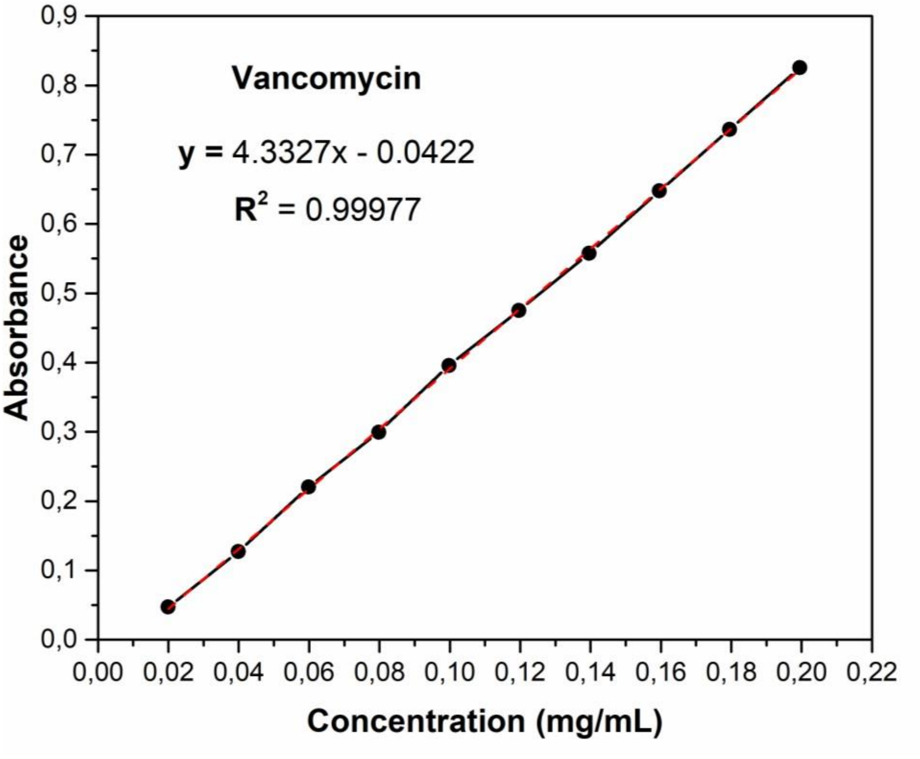
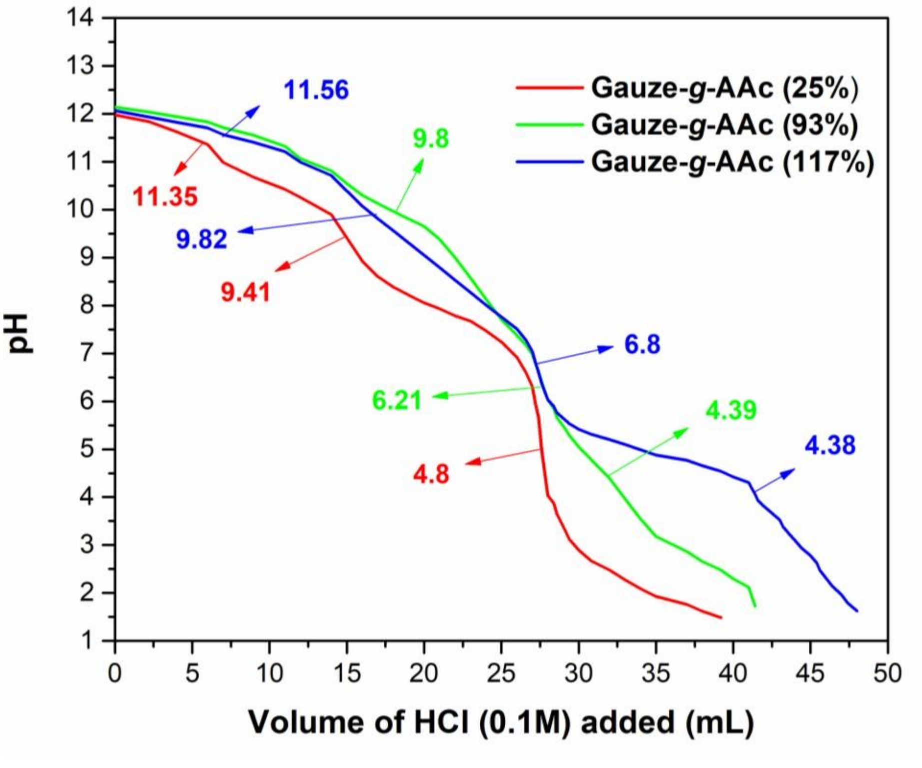
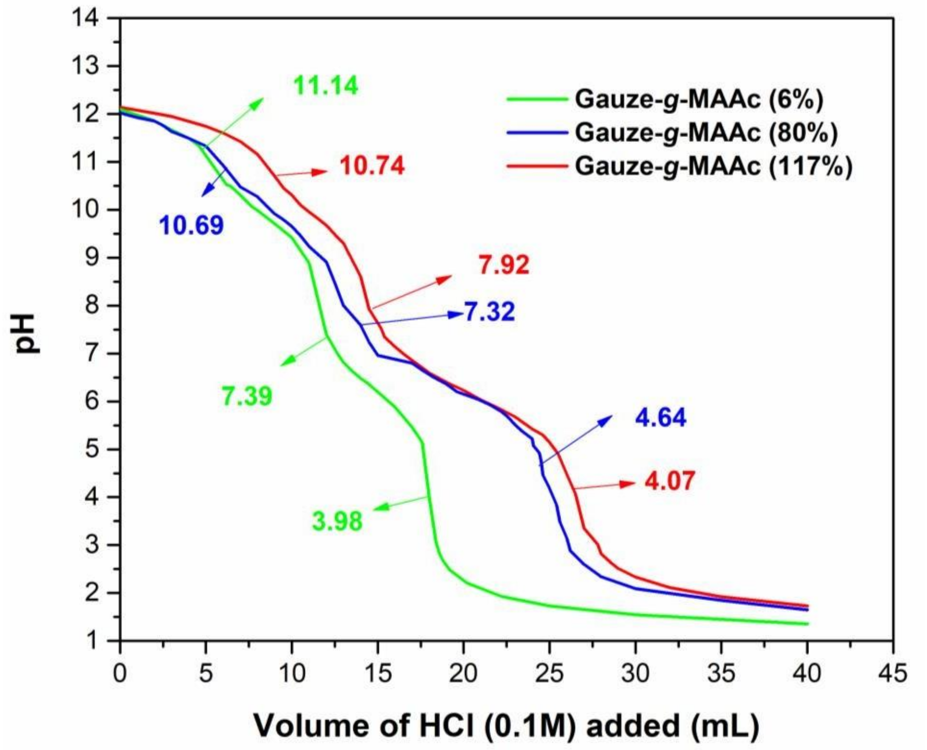
8. ANNEXES



Annexes 1. *Calibration curve used for loading of vancomycin*



Annexes 2. *Back titration of Gauze-g-AAc using hydrochloric acid (0.1M) as titrant*



Annexes 3. *Back titration of Gauze-g-MAAc using hydrochloric acid (0.1M) as titrant*