Gamma Radiation Induced Graft Copolymerization of Typical Monomers onto Poly (3-hydroxybutyrate).

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Abstract—The preparation of several graft copolymers obtained by radiation induced graft copolymerization of typical monomers onto poly (3-hydroxybutyrate) is reviewed. The general mechanisms by which graft copolymerization reaction proceed under various irradiation conditions are described. Main attention is given to the structure, properties, synthesis and characterization of the graft product, as well as the comparison of methods, advantages, basic fundamentals and the effect of reaction parameters on graft degree. Potential applications of the obtained products are also highlighted. Although the use of the graft copolymers is very promising, more efforts are needed to bring these copolymers to the pilot scale production and make them useful materials.

Keywords: poly (3-hydroxybutyrate); monomers; graft copolymerization; gamma radiation; graft degree.

I. INTRODUCTION

The radiation-induced graft copolymerization (RIGC) as a method of modification of polymeric materials has been extensively used over recent years. This is explained by the versatile and universal character of the radiation induced grafting. This is one of the more accepted methods for the syntheses of advanced materials to be used in the field of medicine and biotechnology. The application of RIGC for obtaining biocompatible polymeric materials is of great importance. It is well known that the graft allows obtaining products with a wide range of properties. The radiation-induced graft copolymerization, started last century (1950) with continuous research efforts from different groups with a clear goal to obtain modified materials for diverse applications. The main feature of interest is the potential to simplify the treatment process as a whole. Simultaneous irradiation method is the simplest irradiation technique for preparation of graft copolymers. In this method the polymer backbone is irradiated in the presence of monomer. Irradiation is carried out mostly in inert atmosphere or vacuum; sometimes the pre-irradiation method is used. In the latter method, the main chain of the polymer is irradiated to form active sites which act as macro-initiators; later the monomer reacts with these active sites of the irradiated polymer. To date, both techniques give grafted copolymers with improved properties and it is hard to decide which of them is better. Our research work is related with simultaneous irradiation methods through reasonably good results in the experimental conditions used. State of art report several works dealing with the RIGC’s processes. We are focused in the radiation induced graft copolymerization on polyhydroxyalkanoates (PHA), specifically in polyhydroxybutyrate (PHB). Our efforts are devoted to prepare various graft copolymers of gamma RIGC reaction. This was prompted by their great potential to be used in bioengineering and nanotechnology. The aim of the present review is to investigate the RIGC of typical monomers onto poly (3-hydroxybutyrate). PHB is known for its capability to be a biocompatible and biodegradable material, but somewhat brittle and hydrophobic. This investigation is focused in the syntheses and
characterization of a family of radiation induced graft copolymers based on PHB and typical monomers. \[^{[5,17]}\]

II. GRAFT DEGREE

Graft copolymerization is a process in which side chain grafts are covalently attached to a main chain of a polymer backbone (e.g. PHB) to form a grafted polymer. Fig. 1 display different kinds of formation for a graft copolymer based on topology (form), functionality (side groups) and composition (chemistry).

![Diagram of Topology, Functionality, and Composition of a Graft Copolymer](image)

The grafting degree (W) in the graft copolymerization reactions regularly is determined by the following equation

\[
W(\%) = \frac{m_g - m_i}{m_i} \times 100
\]

Where \(m_i\) is the initial weight of the original base backbone polymer and \(m_g\) is the graft copolymer weight after grafting. This variable is an alternative to elucidate the graft percentage by means of the mass increase. The grafting degree has also been determined by thermo-gravimetric analysis (TGA). However, it is important to note that both, the gravimetric determinations and thermo-gravimetric determinations are consistent in all cases; therefore, all discussions of this article is taken variable \(W\) calculated by TGA.\[^{[13-16]}\]

In this study, another parameter was introduced to measure the graft degree. The composition, C, calculated from the equation:

\[
C(\%) = \frac{m_i}{m_g} \times 100
\]

Where \(m_i\) is the mass of the analyzed component (the mass of monomer grafted onto PHB) and \(m_g\) is the total mass of the sample (grafted polymer). In this sense, there are no significant differences between \(W\) and \(C\) to calculate the graft degree. These parameters represent a measurement of the components’ mass distribution in the polymeric material.

Furthermore, the parameter that indicates the rate of graft (\(\xi_g\) g (%)) was also introduced in this work and is determined by the following equation:

\[
\xi_g(\%) = \left(\frac{\xi_{ci}}{\xi_{ct}}\right) \times 100
\]

Where \(\xi_{ci}\) is the number of grafted carbons (quaternary substitute carbons) and \(\xi_{ct}\) is the total number of tertiary carbons in the PHB backbone.

This allows the characterization of \(\xi_g\) (%) using Nuclear Magnetic Resonance (NMR). The above expression depends only on the structure of the graft copolymer. It has been expressed as percentage grafted carbons. Here the term degree of grafting is used to generalize all measurements using the parameters of grafting degree (\(W\ (%)\)), composition (\(C\ (%)\)), or the rate of graft \(\xi_g\) (%)).\[^{[14]}\]

III. MECHANISM OF THE RADIATION INDUCED GRAFT COPOLYMERIZATION OF TYPICAL MONOMERS ONTO PHB.

When the polyhydroxybutyrate is exposed to gamma radiation, many reactions take place, such as chain crosslinking causing an increase of the molecular weight and the network structure formation; the formation of small molecule product that bring other subsequent sub-product which are very difficult to characterize; the chain scission to bring graft copolymers of different molecular weights and graft degree. These reactions cause substantial changes in the irradiated polymer properties and the formation of small molecule byproducts. Whereas, it is very difficult to explain the complete reaction, we have attempted to explain the general mechanism according to the results obtained by other researchers and by our experience.

Irradiation chemistry of the PHB polymer was early investigated by Carswel.\[^{[5]}\] The spectra of the radicals species resulting from gamma irradiation of PHB has been obtained after radiolysis at 77 K and 303 K. The Electron Spin Resonance study showed that annealing over the
temperature range 300-350 K resulted in the loss of a triplet. This triplet has been assigned to a radical located on the carbon atom adjacent to the carbonyl group. This radical is believed to exist in two possible conformations. Radical decayed when the temperature was increased above 400 K.\[^5\]

Fig. 2 shows the most representative radicals formed on radiolysis of poly (hydroxybutyrate). It is assumed that the multilinie component obtained in the ESR study could result from the presence of a variety of different radicals. It seems that the tertiary radical is favored with respect to the rest, because their stability, according to the previous work carried out by Seville.\[^6\] However the mechanism of the RIGC of different monomers onto PHB implies the presence of stable radicals to form a variety of products (see number III and IV in the Fig. 2). In summary, after studying many research works, the conclusion obtained was that the hydrogen is lost from the carbon adjacent to the carbonyl group and this radical in believed to exist in two possible conformations. From our own experience we have concluded that the grafting occurs on methylic and methylenic group, depending of the monomer and the graft reaction conditions.\[^{13-16}\]

\[\begin{align*}
&\text{Radical I} \\
&\text{Radical II} \\
&\text{Radical III} \\
&\text{Radical IV} \\
&\text{Radical V} \\
&\text{Radical VI}
\end{align*}\]

Figure 2. Possible radicals formed on radiolysis of polyhydroxybutyrate.

All the macromolecular substances are produced by polymerization reactions from small molecules called monomers. In order to explain the RIGC mechanism we will use a general formula of the monomer (Fig. 3), where R1 is the functional group (for instance NH2, C=O, O-C=O, etc.)).

![Figure 3. General monomer.](image)

It is important to state that graft copolymerization is a process in which side chain graft are covalently attached to a main chain of a polymer backbone to form branched polymer. In our review the polymer backbone is the poly (3-hydroxybutyrate) (Fig. 4).

![Figure 4. Poly (3-hydroxybutyrate).](image)

The graft copolymerization takes place as a result of formation of active sites on the polymer backbone (PHB). From the different methods that could be used for graft copolymerization, the radiation induced was selected because of its advantages.
From the literature it is well known that radiation is useful to prepare and modify polymers, because firstly, it does not need any catalyst or initiator, so it is suitable to obtain products with high purity, and secondly, for the high energy of radiation, it can prepare polymeric products at low temperature, so as to restrain the destruction of the polymer caused by heat.\cite{4}

Radiation induced graft copolymerization involves several steps that can be represented as follows: a) Irradiation b) initiation c) propagation and d) termination.

As described above, in the initiation step, six different PHB radicals are formed when exposing PHB to gamma radiation. Any of them, could react to bring a new product. We will establish one of them (the more stable radical) with the purpose not to write the complete mechanism. However it is clear that all the possible radicals are competitive one each other and bring about a mixture of products, what is very difficult to determine because of the complexity of the graft reaction.

Anyway the CH radical has lower activation energy than CH$_2$, because the functional group creates steric hindrance for the CH$_2$ radical approximation. The minor activation energy leads to a prevalence of the CH radical of the monomer (see Fig. 5, 6).

![Figure 5. Irradiation step on the radiation induced graft reaction](image)

Another point to take into consideration is that in the initiation of the monomer, the CH radical is favored with respect to CH$_2$ radical, because the conformation is more stable. On one hand the graft reaction can be produced by coupling the CH radicals to produce the graft product. On the other hand, the reaction can be carried out by addition.

![Figure 6. Initiation step](image)

In the propagation reaction (Fig. 7), the homopolymerization occurs by the reaction of radical formed from monomer radiolysis with the remaining monomers molecules in solution. Equation 15 shows the addition of the monomer (i.e. AAm) to the active center of the initiated graft chain. In the propagation step, the product species, of the equations numbered 15 and 16, have the same nature. Both reactions are observed by the addition of the monomer through the ~CH2−, to give as reaction product R−CH•, which later react with P• to finally obtain the graft copolymer by the termination reaction. In this last step, the homopolymerization is favored as a characteristic of the simultaneous irradiation method, by the coupling or dismutation of the active centers of the monomer macro-radical $Mn + i •$. The change in
the solubility suggests that the crosslinked polymer reaction occurs in some extension (equation 17). All experimental evidences indicate that the favored reactions in the RIGC are the initiation of the monomer and polymer to bring stable radicals, the propagation of the macro-radicals of the monomer and the termination by coupling with preference to the homopolymerization with respect the copolymerization.

In the termination step, there is a high possibility of deactivation of the primary radical formed from the polyhydroxubutyrate by mutual recombination when the simultaneous irradiation method is used. So during graft reaction branching reaction is taking place simultaneously. The limitation of the RIGC by the simultaneous irradiation method; arise from the high level of homopolymerization. So, a number of conditions are adopted in order to increase the grafting level, such as the addition of good swelling agents (solvents), the decrease of the quantity of monomer added to the graft reaction, the selection of low dose rates and moderated doses of irradiation among others.

However, in spite of the optimization of the graft reaction, it is obtained a mixture of grafted PHB, semi-interpenetrated polymer networks, homopolymer, and other grafting byproducts obtained in the main chain scission while the backbone polymer was irradiated.

III.1 Solvents

In general, the solvents are basically used in the RIGC by the simultaneous method in order to bring about swelling of the base polymer (PHB), therefore enhancing the monomer accessibility to the grafting sites. The reaction mechanism of the solvent can be presented as follows.

\[ \text{PMn} + Sx \rightarrow \text{PMn}S + S \]  
\[ Mn + Sx \rightarrow MnS + S \]  

Where \( \text{PMn} \) is the graft growing chain of the polymer, \( Sx \) the solvent molecule, \( pMnx \) the terminated growing chain and \( x \) the solvent radical (e.g. \( H \) or \( Cl \)). \( Mn \) and \( Mnx \) are the growing chains of the homopolymer before and after termination, respectively. \[4\]
Figure 8. Termination step
IV. CRYSTALLINITY STUDIES.

The changes in the degree of crystallinity were observed by FT-IR spectroscopy. The inverse of the relative intensity of the band at 1185 cm\(^{-1}\) was analyzed, which displays the largest difference in intensity between the crystalline and amorphous states. The crystallinity index, \(CI\), was defined as:

\[
CI(\%) = \frac{A_{CH_3}}{A_{C-O-C}} = \frac{A_{1382m^{-1}}}{A_{1185m^{-1}}}
\]

Where \(A_{CH_3}\) is the intensity value (absorbance) of the band at 1382 cm\(^{-1}\) corresponding to bending vibrations (\(\delta(CH_3)\)), which is insensitive to the crystallinity changes and \(A_{C-O-C}\) is the intensity value (absorbance) at 1185 cm\(^{-1}\) corresponding to ester vibrations. The former band has no response while the latter is sensitive to the crystallinity changes in the material. This \(CI\) is not an absolute measure of the crystallinity degree, but it is useful as a comparison criterion.\(^{[13-16]}\) Several authors have investigated the polymer crystallinity by using the DSC. For example Martellini have calculated the crystallinity of PHB phase according to the following equation:

\[
X_{PHB}^{c} = \frac{\Delta H_{PHB}^m}{W_{PHB}^{m} \Delta H_{PHB}^{m,0}} \times 100\%
\]

Where \(\Delta H_{PHB}^{m}\) is the heat of fusion for the PHB phase in the films, \(W_{PHB}^{m}\) the weight fraction of the component in the film and \(\Delta H_{PHB}^{m,0}\) represents the heat of fusion for the 100% crystalline PHB, taken as 146 J/g for calculation.\(^{[17]}\) Finally, XRD is the most important technique in the crystallinity studies because it can provide an absolute criterion calculated from the areas under the reflection of the diffractograms.\(^{[13]}\)

V. PARAMETERS AFFECTING THE GRAFT COPOLYMER FORMATION.

The logical factors that affect the polymer grafting are solvent, monomer concentration and radiation dose.\(^{[10]}\) However, in general, there are many other parameters, which strongly affect the RIGC process such as the nature of the radiation, irradiation dose, dose rate, the nature of the monomer used in the graft reaction, the monomer concentration, the addition of diluents, the addition of a cross-linked agent, the grafting temperature and the addition of inhibitors in the radiation induced graft reaction. A combination of the mentioned parameters has to be adapted to achieve successful grafting reactions.\(^{[4]}\) In general, RIGC processes are very complex and difficult to explain. It depends on the monomer characteristics and the structure of the base polymer investigated. However from our experience in laboratory we have realized that radiation induced reaction has certain characteristics and patterns that allow inferring previously the result. Some of them will be after explained below such as the increase of graft degree with increasing dose etc.\(^{[13-16]}\)

From grafting point of view, the gamma radiation is preferable instead electron beam in terms of high penetration. On the other hand gamma irradiation dose rate is easily controlled by using suitable attenuator. Undoubtedly, if the experiment requires low dose rate with deep penetration, gamma radiation is of particular interest. High energy radiation such as electron beam, ions accelerator etc. are often used in surface modification of polymers. Most of the efforts are given to develop ion exchange or graft tailoring membranes.\(^{[2]}\)

The degree of grafting is strongly affected by the selection of the dose and the dose rate. The dose influences the amount of generated radicals in the grafting system. The dose rate is responsible of the survival as well as the termination of the radicals formed during the radiation induced graft reaction. It is well known that higher irradiation doses lead to higher degrees of grafting.\(^{[3]}\) In PHB irradiation studies doses are commonly about 10 kGy, because higher doses lead to constant graft degree.\(^{[13]}\)

On the other way, higher dose rates result in lower degrees of grafting. We suggest the use of dose rate minor than 1 kG/h when monomers are grafted onto PHB by the simultaneous irradiation method. This is attributed to the initiation, recombination and fast termination reactions when higher dose rate are used. In conclusion, we recommend the use of low dose and dose rate to obtain higher grafting degrees when using the simultaneous gamma irradiation method. The graft copolymerization of typical monomer onto PHB implies the increase of the number of free radical on the PHB compared with that of the monomer used to be grafted.\(^{[13-16]}\)

Unfortunately, in the simultaneous irradiation method the homopolymerization affects the efficiency to obtain the desired result (graft copolymer). When a monomer is chosen to be used in the RIGC onto PHB by the simultaneous irradiation method it is suitable to use a monomer with low viscosity and small dipole strength. The low viscosity enhances the monomer diffusion into the base polymer. It is recommended the used of
solvents if the monomer viscosity is high. The low dipole strength reduces the tendency of the monomer to form free radicals. However it is inevitable the monomer free radical formation and from grafting point of view it is favorable to find the best parameters combination to achieve the higher possible graft degree.\[15\text{--}\[16\]

The base polymer has to meet certain requirements in order to produce efficient grafting. PHB is suitable because its radiation chemistry is well studied. PHB hydrophobicity, high thermal, chemical and mechanical stability make it be an excellent option for radiation induced graft reaction by the simultaneous irradiation method. Moreover, its cristallinity degree permits that the amorphous region (about the 40 \%) of the trunk polymer be grafted and sometimes the cristallinity regions are grafted in some extend.\[7\]

The PHB can be used as films or powder, but the use of powder facilitates the monomer swelling and consequently its better diffusion into the trunk polymer. That is why we recommend the use PHB powder and the use of adapted solvent in the case of the use of poor swelling monomer. Another important parameter is the monomer concentration. This parameter is sometimes confused. It is recommended to study the monomer concentration as a function of the degree of grafting supporting the dose, dose rate and grafting time, in the same value in order to determine the efficiency in each case. In spite of the fact that an increase of monomer concentration generally causes an increase in the grafting degree, there is a tendency of grafting degree level off and reach saturation state.\[10\]

PHB grafting can also be achieved by the addition of solvents or bulk. The graft proceeds by front mechanism where it starts in layers close to the exterior surface then proceeds inwards towards the core of the polymer progressively through the swollen grafted layers. About the temperature, the analysis is somewhat controversial because on one hand the increase of the temperature in certain levels rises the degree of grafting because increases the diffusion of the monomer into the inner grafting sites but leading to shorter grafting chain because of the rapid termination reactions. On the other hand if lower temperatures are used the trapped radicals have longer lifetime and lower reactivity, what slows down the termination reaction leading to higher grafting degree. More efforts are necessary to obtain the ideal temperature for each system depending of the desired material.\[17\text{--}\[9\]

VI. GRAFT COPOLYMERIZATION

Mitomo was first to report on the preparation of PHB radiation induced graft copolymers.\[7\text{--}\[9\] It is worth to mention that most of the research work on the use of RIGC goes all the way back to the pioneering work of Chapiro on 1962. We consider Chapiro as one of the most important references for this kind of investigation.\[4\]

It is thought that radiation induced graft copolymerization started in 1950s. In relation with PHB, on 1996 Mitomo investigated the RIGC of acrylic acid onto PHB and PHBV copolymer by the pre-irradiation technique. It was used a \(^{60}\text{Co}\) gamma ray source. The PHB sealed in evacuated glass ampoules were pre-irradiated at 5 kGy, a dose rate of 10 kGy/h, and a temperature of \(-78\degree\text{C}\). After that, a 10\% AAc solution was introduced to the mentioned pre-irradiated polymer to produce the graft reaction. The experiment was performed in vacuum at constant temperature for various periods of time.\[7\]

The results showed that the PHB graft degree was higher than that of PHBV, although the PHB cristallinity is higher than that of PHBV. The grafting was mainly introduced into the amorphous regions of the samples, while the crystalline regions remain almost unchanged. Another interesting point is that the graft degree estimated by thermo-gravimetry was very close to that estimated from weight increase. This result agrees with our investigations described below.\[7\text{--}\[7\]

The higher PHB graft degree is frequently explained by the fact that lifetime of the radicals generated in crystalline regions is far longer than that in amorphous regions, so that former radical retardingly migrates to the crystal surface and acts more efficiently than the latter.\[7\]

In addition, the biodegradability steeply increased by introduction of small amount of grafted AAc because of the improvement of wettabillity between the polymer and the enzyme solution, and then decreased as graft degree increased much more. In other investigation, the biodegradation was suppressed by the introduction of MMA grafting which is hydrophobic. It can be concluded that the biodegradation is promoted by the introduction of hydrophilic monomers and suppressed by the introduction of hydrophobic monomers by RIGC reactions.\[7\]

Graft copolymerization of styrene (St) onto PHB and PHBV was also carried using simultaneous irradiation and pre-irradiation techniques with \(^{60}\text{Co}\) gamma rays. The graft degree obtained was lower than that in grafting methyl methacrylate (MMA).\[6\] Comparing with MMA, HEMA and AAC, the St monomer can be considered to polymerize onto the trunk polymer more linearly, i.e. without branches and crosslinks. The degree of grafting of the sample of 30\% St (St/PHB) increased steeply as the dose increased, but then leveled off at higher dose. The effect of the temperature was studied and the graft degree was increased with increasing temperature and the largest graft degree of 25\% was observed at 80\degree\text{C}.

\[\text{http://www.ijsat.com}\]
for a reaction time of 4 h. The graft copolymerization of styrene onto PHB according to the simultaneous method was carried out directly by irradiating the polymer in styrene monomer with gamma rays in an H-shaped vacuum glass ampoule, at a dose rate 5 kGy/h under reduced pressured (10⁻³ Torr). The graft degree increased after 1 h of grafting time (Dose = 5 kGy) and then level off. In conclusion, it was established that the PHB and its copolymers are remarkably degraded at irradiation above 20 kGy. As explained above, the maxima dose accepted to carry out the RIGC of typical monomers onto PHB is about 10 kGy. Bahari also investigated the Electron Spin Resonance (ESR). The results of these ESR measurements correspond well to the grafting behaviors with the formation of the radical (see number III and IV in the Fig. 2) discussed above. At a grafting temperature of 25 °C grafting starts actively.

The degradability of PHB and its copolymers grafted to styrene by RIGC was investigated by the same group of researchers. The thermal stability of the material obtained was improved. Due to the formation of a PST layer on the film surface of PHB, the penetration of the enzyme was prevented and consequently the enzymatic degradability was retarded. From application point of view, the grafting of St may become one method to control the degradability of PHB.

To improve the tenacity of PHB, isoprene was grafted by direct radiation by Ping Hu. The optimal conditions were determined, at which the grafting process occurred. In order to gain proper graft degree optimal grafting degree conditions are needed, so, efforts to understand the optimal conditions were made by the investigation of the added solvent, the monomer concentration and the radiation dose. In agreement with earlier investigation the graft degree increased its value with increasing radiation dose, but after 16 h (20 kGy) there were not more increase and the grafting degree remained steady. The study of the degradability of the obtained material would have been useful, but it was not performed. However the researchers totally dissolved the P (HB-g-IP) samples in hot trichloromethane, so the gel fraction in the material is nearly zero. Calorimetry showed that the enthalpies of grafted polymer had large increase compared to that of PHB, implying an increment in copolymers crystallinity. The analysis of the peaks under the areas of proton signal on Nuclear Magnetic Resonance analysis was in disagreement with Thermal Gravimetric analysis. It was concluded that due to the complexity of the graft reaction, there should be several kinds of grafting products because the Nuclear Magnetic Resonance analysis showed that probably branching reaction must have been taken place simultaneously.

The morphology and the mechanical properties were studied to confirm the obtaining of the graft copolymer and the increase of its tenacity and interpret the toughen mechanism of PHB grafting. When branching reactions are more efficient than graft reaction and only one of the polymer networks is covalently cross-linked, is presented a hydrogel system were the introduction of a linear polymer in a preformed network gives rise to a semi interpenetration polymer network. Martellini obtained semi-IPN of PHB and poly (ethylene glycol diacrylate) by gamma radiation technique. Thermal behavior of PHB showed no change in the melting temperature and small decreased in crystallinity with the increase in the amount of monomer in the mixture composition.
On the other side, the irradiation induced a decrease in both melting temperature and degree of crystallinity affecting the degradation rate of the material. These polymeric materials are often used for immobilization of biologically active substances.[18]

The radiation induced graft polymerization of maleic anhydride onto PHB by gamma RIGC has also been explored. Herom Ye focused on an effective way to improve the PHB thermostability.[19] Bulk reaction was performed by the simultaneous irradiation method in nitrogen atmosphere. The process was similar than graft polymerization of isoprene onto PHB but at a dose rate of 13.2 Gy/min or 8.76 Gy/min.[10] The ampoules were subjected to 60Co gamma ray source for 24 hours and later the product was purified as usual by soxhlet apparatus to remove the adhering homopolymers. The final product was then dried under vacuum at 40 °C to constant weight and the graft copolymer was characterized by different techniques.

One of the key concerns in any graft strategy is the graft determination and the demonstration of the formation of a new product. Ye demonstrated creativity by measuring the graft degree using chemical titration method.[19]

At this moment, Mitomo had reported much of their work about grafting monomers onto PHB. However no much effort was made to demonstrate the formation of the graft copolymer by direct techniques such as Nuclear Magnetic Resonance. Most of the results obtained were indirect and the PHB polymers structure analysis lacked of discussion. Ping Hu described the first Nuclear Magnetic Resonance (NMR), but the 1H-NMR not the 13C-NMR, and the results were not totally convincing because the intensity of the proton signals were very poor and broad. However the researchers realized the fact that probably the graft reaction occurs on the methane and methylene site of the PHB molecule according to the number of hydrogens that were found at these sites.[18,20]

On the contrary, Ye indeed showed clear 13C-NMR signals to deduce the structure of the graft copolymer. In agreement with the mechanism describes earlier, the MA was grafted by the tertiary PHB carbon after irradiation to form a quaternary substituted carbon. The calculation of the (εg (%)) parameter would have been possible but the researchers did not realize that the graft degree can also be obtained by the number of grafted carbons. Anyway, the results are quite interesting and described well the chemical displacements in accordance with the chemical environment of the grafting MA monomer.[19]

Another interesting detail was found when the chloroform solvent was reported as the better choice to obtain higher graft degree (compared with acetone solvent). There was no further discussion about this observation. We have obtained similar result when grafting AAm onto PHB by RIGC reactions and concluded that each system is different and in some cases chloroform can be very useful in spite of the formation of chloride radical and termination reactions. When isoprene was grafted onto PHB the chloroform was found no suitable to obtain graft, so the experimental study of the effect of solvent is necessary in each attempt of grafting monomers onto PHB.[21-23]

Back to the research performed by Ye, it was found that the increase on the monomer concentration increased the graft degree and the molecular weight in some extend. In general, it is concluded that the 10-20% of monomer in solvent or bulk reaction is suitable for this kind of reactions, depending of the characteristics of each polymeric system.[18]

The results of the calorimetric study provided by TGA and DSC analyses strongly imply that the extent of imperfect crystalization is increasing as a direct consequence of the introduction of MA groups. This is in well agreement with the research investigation obtained until this moment.

More recently, there have been various studies related with the biodegradability of graft copolymers obtained by RIGC of some monomers onto PHB. It is well known that practical application of PHB has been restricted by its brittleness and stiffness. So, the PHB graft copolymers obtained by RIGC must be controlled in order to enable utilization. Wada concluded that the biodegradability of grafted PHB would be affected by the hydrophilicity of grafted polymer.[20] From previous reports it was found that the enzymatic degradability of PHB powder grafted with MMA steeply decreased, whereas it was increased by the introduction of hydrophilic HEMA. Later investigations of the enzymatic degradability of grafted PHB by RIGC of acrylic acid were conducted by Wada. It was concluded that the biodegradability of PHB grafted with acrylic acid was considerably reduced with increase of degree of grafting, but it was recovered by thermal remolding.

Continuing this line of research, the synthesis, physicochemical characterization and evaluation for possible application of several grafted polymers obtained by RIGC of typical monomer onto PHB has been reported by our research group.[13-16]

A research article by Gonzalez reported the synthesis and characterization of a graft copolymer obtained by RIGC of vinyl acetate onto PHB. The monomer was grafted in order to improve the PHB hydrophilicity.[13] The graft degree was determined by different techniques. The irradiation experiments were carried out in an oxygen free system at room temperature. PHB film/vinyl
acetate ethanol solution (PHB (1g)/VAc (300 g) and PHB powder /vinyl acetate PHB (1g)/ VAc (6 g) were used. The samples were exposed to $^{60}$Co gamma ray source, with a dose rate about 1.57 kGy/h. In agreement with the earlier discussion the dose was selected at 10 kGy. The most important results reported in this paper was the verification of the synthesis by NMR and the grafting index ($\xi_4 (%)$) parameter determination. It was found that the grafting index was about 25 % in these experiments. These results show that an average of 5 VAc molecules per grafting was achieved. The short VAc oligomers reached more mobility to react with PHB radical. It is thought that some reaction occurred at methylenic groups absolute grafting index could be found from addition of the grafted PHB carbons divided by the total amount of carbons that can be grafted, but more efforts are necessary to describe this result.

The PHB was degraded in order to obtain an increase in graft degree. On the contrary, the graft degree decreases because the decrease in molecular weight resulted in an increase of crystallinity degree. The study of the pressure was not important, but it is necessary to eliminate the oxygen of the system in order to avoid the termination reactions that hinder the formation of the graft copolymer. Swelling behavior was also studied. The P (HB-g-VAc) revealed a water uptake increase tendency.

Later research completed the earlier work by the study of the effect of solvent in the synthesis of P (HB-g-VAc) by RIGC. Further saponification was carried out to obtain the P (HB-g-VAc) product that was characterized by different spectroscopic techniques. Solvents of low chain transfer constant were selected such as ethyl acetate, n-butanol, diethyl ether, hexane and acetone. For comparison, bulk reaction was also carried out. The results showed that the most favorable solvent to be used in the graft reaction was hexane W(%) = 26.27 %. Grafting degree was similar when using ethyl acetate and n-butanol (13, 21 % and 13.12 %), and it was fairly low in diethyl ether and acetone (W(%) = 7, 7 % and 6.54 respectively). However a much higher graft degree can be achieved in bulk. As vinyl acetate is good swelling monomer (10% swelling percentage on PHB), the graft reaction was favored with respect to the use of solvents in this case. The decrease in grafting degree when using solvents can be attributed to the suppression of monomer diffusion by the increase in viscosity due to the homopolymerization. On the other hand, the use of vinyl acetate monomer increases the polymer swelling and consequently enhances the diffusion of the monomer to the internal layers of the substrate and also increases the interaction between the internal PHB active sites and the monomer radicals formed by gamma irradiation.

It is not clear at this stage why hexane is the favored solvent in the graft reaction. Considering the complexity of the radiation induced graft reaction, it is possible that if any other variable, for instance the monomer concentration, is changed, then another solvent could play a better role in the light of grafting degree. For sure, it is possible to graft vinyl acetate onto PHB in some extend using the mentioned solvents or bulk reaction. It is recommended to use PHB powder to increase the graft reaction because the PHB films leads to the surface grafting.

An important calorimetric analysis showed that the graft degree for vinyl alcohol grafted onto PHB [P (HB-g-VA)] decreased with respect to the [P (HB-g-VAc)]. This is explained because the calculation was based on the mass balance. The saponification reaction implies a decrease in molecular weight. The result suggests that the graft degree W(%) is mass dependent. The grafting degree should be invariable because the hydrolysis works only in the vinyl acetate grafted chains and it does not create new graft sites. More efforts are necessary to determine the grafting index ($\xi_4 (\%)$) as it was obtained in this research. At this moment there is not a general formula for all polymers, but the variable can be deduced from the rate of grafted sites divided to possible grafted sites in each polymeric system, supported by NMR spectroscopy. 

At last, the experimental results showed that the introduction of vinyl acetate onto PHB by radiation induced graft reaction can strongly hinder the PHB crystallization and the PHB degree of crystallinity declines. As a result of the vinyl acetate graft chains the biodegradability decreases because PVAc grafted chains hinder the enzyme PHB depolymerize attack. However the material recovered the biodegradability after saponification. PVAc is not a hydrophilic polymer per se, but it is more hydrophilic than PHB. Anyway it was grafted because the objective was the synthesis of the later more hydrophilic P (HB-g-VA). The investigation in the looking for more hydrophilic materials continued. Typical monomers, methacrylic acid (MAc) and butyl methacrylate (BuM) were also graft polymerized onto PHB by RIGC reaction in order to improve PHB’s hydrophilicity. The irradiation conditions were similar of those used for VAc grafting. It was observed that in maleic anhydride grafting the chloroform was suitable solvent, so it was included joined with acetone and ethanol in the study of the effect of solvents of graft reaction for both MAc and BuM monomers radiation induced grafting reaction.

From the results it was concluded that methacrylic acid and butyl methacrylate were successfully grafted onto PHB. The grafting degree was quantified by thermo-gravimetric analysis. TGA
results showed that the more favorable solvent for graft reaction in both cases is ethanol, which presented the higher grafting degree. It was obtained graft copolymers for all graft conditions except when chloroform was used to obtain the P (HB-g-BuM) probably due to the termination reaction did not permit the formation of the graft copolymers. The extent of the grafting varied from 5% to 32% depending on the graft conditions. The swelling behavior was also studied, showing a great increase in PHB hydrophilic characteristics of the polymeric materials. The maximum swelling degree for P (HB-g-MAc) was 118 % and for P (HB-g-BuM) was 208 % showing incredible water absorbance what increase the possibility of using this grafted polymers in practical biomedical applications.\[15\]

The study was continued by the RIGC reaction of acrylamide onto PHB by the simultaneous irradiation method as well as the study of the effect of solvents on graft reaction. The investigation also looked to overcome the above mentioned PHB disadvantages.

The irradiation experiments were carried out in glass sealed vacuum ampoules at room temperature. The graft copolymers were prepared as follows: 400 mg of PHB were added to 1g of acrylamide, next 3 mL of selected solvent was added to the former solids. The mixture was exposed to $^{60}$Co gamma ray source at a dose rate of 0.83 kGy/h and a dose of 10 kGy. Different solvents such as ethanol, methanol, acetone, ethyl acetate and chloroform were used. From the results it was concluded that the grafting degree is similar when using polar monomer such as ethanol, acetone or methanol. Higher grafting degree can be achieved by using ethyl acetate. The highest value was obtained for chloroform solvent, the more favorable solvent to be used in graft reaction in spite of the chloride radicals, which leads to termination reactions. The AAm grafting to PHB backbone polymer was verified by Nuclear Magnetic Resonance, Fourier Transform Infrared, Scanning Electron micrograph, Thermogravimetric Analyses and Differential Scanning Calorimetry. From the spectroscopic and calorimetric techniques was supported that the degree of crystallinity decreased with increased graft degree and it was deduced that oligomers of PAAm were grafted onto PHB and the mechanism was probably by the methinic and methylenic PHB groups, but most on the methinic sites. The swelling behavior showed an increase in the PHB hydrophilic characteristics of the graft copolymers with increasing graft degree. A semi-interpenetrated polymer network was obtained when water was used as solvent in the radiation induced graft reaction.\[16, 21-23\]

Grondahl carried out the grafting of 2-aminoethyl methacrylate (AEMA) onto PHBV by gamma irradiation induced simultaneous method. The investigation aimed to introduce and characterize amine functionalities for tailoring purposes. Amine groups have previously been introduced onto PHBV substrate by many techniques such as RF ammonia plasma and chemical treatments buy arise the limitation related with the penetration on the substrate which is very shallow (plasma treatment) while the chemical treatment lead to extensive degradation.\[24\]

Radiation induced gamma successful grafting of AEMA onto PHBV was verified from XPS analysis, water contact angle and ATR-FTIR spectroscopy. The PHBV was used as a film, so the graft occurred on surface. The graft degree obtained was very low and varied with grafting conditions. Samples grafted in water displayed significantly lower values than those grafted in methanol. Graft reaction of AEMA onto PHBV was induced by $^{60}$Co gamma irradiation at a dose rate of 2.2 kGy/h. It is thought that the dose rate was very high, consequently the graft degree decreased with increasing dose, because the viscosity increased in the grafting system and it was not possible for PAEMA oligomers to continue the diffusion into the trunk of the polymer to reach the PHBV radical formed by the gamma induction. The dissolved oxygen was removed by streaming nitrogen gas through the solution (monomer /solvent of 2.5-4.5 %) for 5 minutes. An increased in AEMA concentration resulted in an increase of the graft degree when using methanol and the opposite trend when using water. It can be deduced that the result is connected with the swelling of the solvent on PHBV. In general, in the RIGC of typical monomers onto PHB the methanol solvent and ethyl acetate solvent resulted in graft product because they are good swelling solvents what permit the better diffusion of the monomer to the inner PHB layers. There was a significant increase of the water uptake of the obtained P (HBV-g-HEMA). It was successfully chemically modified the films surface of the graft copolymer in order to verify the graft reaction. In addition, it was demonstrated that AuNPs synthesizes using citric acid as the reducing and stabilizing agent were useful as a probe to assess the distribution of amine groups on grafted PHBV.\[24\]

VII. POTENTIAL APPLICATIONS

The polymeric materials obtained by RIGC of typical monomers onto PHB have a promising potential application because their properties are similar from PHB but more hydrophilic. The applications of polyhydroxyalkanoate (PHA) were reported by Zinn. Being entirely biocompatible and biodegradable, the PHB’s potential application includes drugs release, surgical sutures and bone
plates. A number of authors have investigated the potential of PHB to control or sustain drugs delivery. Graft copolymers obtained from PHB could have wider use in biomedical application. Graft copolymers may be used for the syntheses of granules with lower drugs release rates because of the decrease in the degree of cristallinity. Another possible application is the use of the graft copolymers as scaffold material in tissue engineering. Although several groups have investigated the potential applications of PHB, not much was discussed in the reviewed articles about the graft copolymers. In some cases the graft copolymer have been synthesized by grafting monomers onto a PHB film to test applicability to perm selectivity and separation processes or surface functionalization for tissue engineering applications. Bahari grafted St onto PHB to improve thermal stability because PHB and its copolymer have the disadvantage that they decompose rapidly by thermal degradation during moulding. Ping Hu investigated the graft of isoprene monomer onto PHB to improve the PHB tenacity but no application was suggested by the authors. Herong Ye also improved PHB thermo-stability by grafting maleic anhydride onto PHB but only was suggested that the results indicated that wide commercial application may be possible. The AAc was grafted by Mitomo and Wada, but lack of discussion about the applications. It is possible that the research work in this area has been somewhat limited by the cost of the polymers. Gonzalez dedicated most of their investigation to the syntheses of radiation induced graft copolymer for drugs delivery systems (VAc; VA, BuM, MA, AAm). From the results it was concluded that P (HB-g-VA) polymer was the best candidate for medical application and P (HB-g-AAm) was the worse candidate because there is a strong controversy about the PAAm biodegradability and blood biocompatibility. Lisbeth focused their attention on tailoring biomaterial surface properties probably for future applications. So, the research work in this area is still very poor and despite the continuous efforts to synthesize graft copolymer by RIGC of typical monomers onto PHB more efforts have to be undertaken to bring feasible applications.

VIII. CONCLUDING REMARKS

The radiation induced graft polymerization by the simultaneous method is a promising method that provides a suitable and economical technique to graft typical monomers onto polyhydroxybutyrate. Radiation is useful to prepare and modify polymers, because firstly, it does not need any catalyst or initiator, so it is suitable to obtain products with high purity, and secondly, for the high energy of radiation, it can prepare polymeric products under low temperature. RIGC involves several steps that can be represented as follows: a) Irradiation step b) initiation step c) propagation step and d) termination step. It was concluded that in PHB radiation chemistry at first the PHB radicals are formed and the most stable radical are methylic and methylenic radical, but it is thought that most of the reaction occurs on methylic PHB radical (\(p\bullet\)). The monomer also gives a reaction radical product \(R - CH\bullet\) when is exposed to gamma irradiation, to react with \(P\bullet\) to finally obtain the graft copolymer by the termination reaction. In the termination step, there is a high possibility of deactivation of the primary radical formed from the polyhydroxybutyrate by mutual recombination with the monomer radical. Many techniques have been used to determine the degree of cristallinity, such as FTIR, DSC and XRD, but XRD is the most important technique in the cristallinity studies because it can provide an absolute criterion calculated from the areas under the reflections of the diffractograms.

The obvious factors affecting grafting are solvent, monomer concentration and radiation dose and dose rate. From the results of the investigation in the field of radiation induced grafting onto PHB was concluded that a) there is not any rule related with the selection of the monomer, it is assumed that the selection of methanol or ethyl acetate is suitable compared with bulk reaction, b) the proper monomer concentration vary from 5 to 15 % v/v in solvent depending on the graft system, c) the dose would be about 10 kGy to obtain suitable graft degree and the dose rate would be as low as possible, for instance 0.5 kGy/h, for simultaneous irradiation method. This is attributed to the initiation, recombination and fast termination reactions when higher dose rate are used. The PHB should be used as powder to increase interaction with the monomer. It is suggested to remove the oxygen from the grafting solution by a vacuum system and seal completely the glass ampoule with fire. The graft degree can be obtained from mass balance but it must be calculated the grafting index from the structure calculating the ratio of the number of grafted sites and the number of sites that can be grafted.

The biomedical potential application of the graft copolymers obtained by RIGC reaction of typical monomers onto PHB is very promising but more efforts are necessary to find a clear application of the polymeric material. Recently most of the research has been focused in the application of graft copolymer as carriers for drugs delivery systems.
References


