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"Synthesis, characterization and biomedical applications of microbial polymalic and polyglutamic acids derivatives."

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Nanoparticles made of microbial poly(γ-glutamate)s for encapsulation and delivery of drugs and proteins

Summary

This study was focused on the preparation and evaluation of nanoparticles made of alkyl esters of microbial poly(γ-glutamic acid) (PGGA) to be used as drugs and proteins carrier and delivery systems. Racemic poly(γ-glutamic acid) of bacterial origin was fully methylated or partially esterified to render non-water soluble polymers. A set of copolymers containing polyglutamic acid, and ethyl, hexyl, dodecyl and octadecyl glutamate units with alkyl contents of 50 and 75 % was prepared. Spherical nanoparticles with a diameter of 200-250 nm and a narrow distribution were generated from the alkylated polymers by the precipitation-dialysis method. These nanoparticles readily degraded hydrolytically upon incubation in simulated physiological medium at a rate dependable of the alkylation degree and the length of the alkyl group. All these nanoparticles were able to encapsulate efficiently erythromycin. Those made of carboxyl containing polyglutamates were also effective to load α-chymotrypsin. The release of such compounds from nanoparticles upon incubation proceeded essentially following the same profile that is followed in the hydrolysis of the corresponding substrate polymers. The loss of enzyme activity of the incubated protein diminished significantly upon encapsulation in these systems.

9.1. Introduction

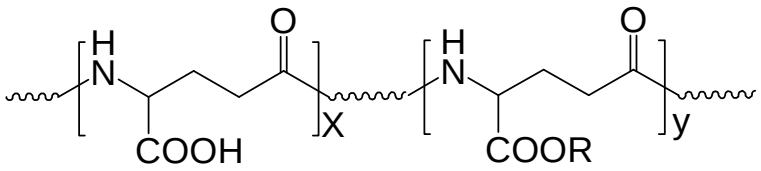
Polymer nanoparticles are outstanding devices for the adequate biological distribution of drugs, proteins or DNA, both at cellular and organ levels. The nanometer-size ranges of these systems offer distinct advantages as effective permeation through cell membranes and good stability in the blood flow. Polymers are very convenient materials for the manufacture of such nanoconjugates since they offer countless possibilities of molecular designs able to satisfy a wide span of needs. Amphiphilic block or graft copolymers are able to self-assembly forming polymeric nanoconjugates in aqueous solutions. When developing these formulations, the goal is to obtain systems combining optimized drug loading and release properties with long shelf life and low toxicity. These are the challenges that are being currently faced in this field of research.[1-4]

Several biodegradable polymers are quite suitable materials for preparing nano-sized particles for drug delivery applications. Aliphatic polyesters, such as poly(lactic acid), poly(glycolic acid), poly(ε-caprolactone) and their copolymers constitute the major family used in this regards. The degradation rate and therefore the drug release rate in these particles can be controlled by adjusting the molecular mass of the polymers and additionally, the composition and microstructure in the case of copolymers.[5-6] An important drawback in the use of these polyesters as DDS systems however is their lack of active functional groups to which biomolecules or drugs could interact and become firmly immobilized.

Poly(α,L-glutamic acid) (PALG) is a carboxylic functionalized polypeptide that has been object of intensive research for its possible applications in the biomedical and pharmaceutical fields.[7] Conversely, the other naturally-occurring isomer poly(γ-glutamic acid) (PGGA) has emerged as a promising material for DDS in the last few years.[8-9] PGGA is biodegradable, fully bioresorbable and highly hydrophilic. For a number of applications, as it is the case of drug delivery systems, water solubility of PGGA is a limitation that can be circumvented by chemical modification. Thus, amphiphilic poly(γ-glutamic acid)-*graft*-L-phenylalanine copolymers have been used to prepare biodegradable nanoparticles showing great potential as drug and protein carriers.[10-11] These and other nanoparticles made of analogous aminoacid-grafted PGGA have been reported to be efficient as antigen carriers.[12] The manufacture of polymer particles for protein delivery carrier systems requires special precautions because the complex structure of the protein molecule and its sensitivity to undergo conformational changes.

Protein aggregation or/and denaturation caused by either strong interactions with the polymer matrix or solvent effects during entrapping should be minimized without severe loss in the loading capacity. Thus, it has been recently shown that severe perturbations in the secondary structure of γ-chymotrypsin took place when this protein was encapsulated in PLGA using the solid-in-oil-in-water technique.

This study is focused on the use of hydrophobically alkylated PGGA (Scheme I) for producing nanoparticles suitable for carrier and delivery systems for drugs and proteins. Biotechnological PGGA produced by *Bacillus subtilis* with a racemic modification and high molecular weight is the starting polymer used in this work. Since alkylation has repeatedly proven to be an appropriate and simple procedure to render non-water soluble PGGA derivatives,[13-14] we have applied this method to prepare the polymers to be used for making the nanoparticles. The approaches applied consist of fully esterification with alkyl groups of small size and partial esterification with large alkyl groups. By these means the solubility of PGGA in water can be repressed without disturbing excessively the genuine properties of the polyacid. The hydrolytic degradation of the nanoparticles, their efficiency to entrap erythromycin and α-chymotrypsin and the effect of encapsulation on the activity of the enzyme, were evaluated.



R = $-CH_3$ (Me), $-CH_2CH_3$ (Et), $-(CH_2)_5CH_3$ (Hex),
$-(CH_2)_{11}CH_3$, $-(CH_2)_{17}CH_3$ (Octd)

Scheme I. Chemical formula of copolymers studied in this work.

9.2. Experimental

9.2.1. Materials. All chemicals were obtained commercially from either Aldrich or Merck. They were analytical grade or higher and used without further purification. Sodium poly(γ,DL-glutamate) with a D:L enantiomeric ratio of 59:41 and a weight-average molecular weight of about 300,000 which was kindly supplied by Dr. Kubota of Meiji Co. (Japan), was used in this work. The polymer was changed to the protonated form by acidification with 1M HCl followed by precipitation in 2-propanol.

The final sodium content of the acidified polymer was 5.1% w/w as determined by inductively coupled plasma (ICP) in a multichannel equipment Thermo Jarrell-Ash model 61E Polyscan. Erythromycin (assay 95% NT) and α-chymotrypsin (from bovine pancreas, 55 units·mg^{-1} protein) were supplied by Sigma and Aldrich, respectively.

9.2.2. Esterification of PGGA. Methylation of PGGA leading to poly(α-methyl γ,DL-glutamate) (PGGA-Me) was accomplished by applying a method recently reported by us, which is based in the reaction with diazomethane.[15] On the other hand, other partially alkylated PGGA's were prepared by reaction of PGGA with the corresponding alkylbromides in the presence of $NaHCO_3$ according to a common procedure previously described by several authors[13-14] with the appropriate modifications. In brief, a mixture of 200 mg (1.55 mmol) of PGGA in *N*-methylpyrrolidone (20 mL) was left under stirring at 80 ºC for 30 min.

The solution was then cooled to 60 ºC and added with $NaHCO_3$ (525 mg, 6.25 mmol). Then 8 mmol of alkyl bromide were slowly added for a period of 2 h and the mixture left to react for the adequate period of time to attain the desired esterification degree. After removing the NaBr generated in the reaction, the clean solution was poured into a suitable solvent in order to precipitate the resulting copolymers, which were recovered by centrifugation and finally dried under vacuum at 50 ºC.

9.2.3. Nanoparticles preparation and hydrolytic degradation. Nanoparticles of all copolymers were prepared by the precipitation-dialysis method. The copolymer (20 mg) was dissolved in 4 mL of NMP and the same volume of water added to yield a translucent solution. The particles were generated upon dialysis of the solution against distilled water for 24 h at room temperature using cellulose membrane tube (8,000 molecular weight cut-off). In this operation, the distilled water was replaced at 2, 8 and 12 h of treatment and the dialyzed solution was freeze-dried.

For hydrolytic degradation studies, nanoparticle samples were dispersed in phosphate buffered saline solution of pH 7.4 and incubated at 37 ºC. In all cases, aliquots were withdrawn at predetermined time intervals, and variations in the molecular weight of the nanoparticles were measured by gel permeation chromatography.

9.2.4 Erythromycin loading, encapsulation efficiency and release measurements. The copolymer (30 mg) was dissolved in NMP (5 mL) and then erythromycin (30 mg) was dissolved in the copolymer solution. The loaded nanoparticles were prepared by the precipitation-dialysis method described above. The amount of erythromycin incorporated into the nanoparticles was estimated by UV-vis spectrophotometry using the appropriate blank and calibration curve. For *in vitro* release evaluation, the nanoparticles were incubated in aqueous buffer under simulated physiological conditions and aliquots taken from the releasing media at scheduled times, the removed volume being replaced by fresh medium every time. The amount of released erythromycin was estimated by UV and the cumulative drug concentration was plotted against incubation time.

9.2.5. α-Chymotrypsin: loading, in vitro release studies and assays of activity. To prepare the enzyme-encapsulated nanoparticles, 10 mg of α-chymotrypsin were dissolved in 1 mL of PBS, pH 7.4, and 10 mg of copolymer were dissolved in 1 mL of NMP. The two solutions were mixed and the precipitated nanoparticles were collected by centrifugation. To remove the protein absorbed onto the surface, the nanoparticles were repeatedly rinsed with water and buffer saline solution of pH higher than the isoelectric point of the protein.

α-Chymotrypsin-encapsulated nanoparticles (10 mg) were poured into 5mL of 4% sodium dodecyl sulfate (SDS) aqueous solution to dissolve the nanoparticles, and the loaded protein content was determined by the Lowry method.[16] The α-chymotrypsin load, particle size and Z-potential were determinated by triplicate.

For releasing studies, α-chymotrypsin loaded nanoparticles (40 mg) were placed in 2 ml of PBS (pH 7.4) and incubated at 37 °C. At scheduled times the supernatant was removed and the concentration of released protein determined by absorbance measurement at 280 nm. The concentration of the released protein was used to construct cumulative release profiles. These experiments were performed by triplicate.

The enzymatic activity of entrapped α-chymotrypsin was determined using of benzoyl L-tyrosine ethyl ester (BTEE) as a substrate. The assay was performed as follows: A mixture of buffer (0.05 M Tris-HCl, pH 8.0; 1.8 mL), methanol solution of BTEE (0.3 $mg \cdot mL^{-1}$; 0.2 mL) and the released α-chymotrypsin solution (0.2 mL) was placed in a quartz cell and the absorbance changes taking place with time were followed in a UV-spectrometer. The specific activity of the released α-chymotrypsin was calculated taking as reference the activity of the free enzyme measured under the same conditions.

9.2.6. Measurements. NMR spectra were recorded on a Bruker AMX-300 NMR instrument with samples dissolved in $CDCl_3$ either pure or added with trifluoroacetic acid, and using TMS as internal reference. Sample concentrations of about 1% (w/v) were used for these analyses. Gel permeation chromatography (GPC) was performed using a Waters equipment provided with a refractive index detector and using 0.005M sodium trifluoroacetate-hexafluoroisopropanol (NaTFA-HFIP). Chromatograms were calibrated against PMMA standards.

Calorimetric measurements were performed with a Perkin-Elmer Pyris I DSC instrument operating under a nitrogen atmosphere and calibrated with indium. Sample weights of about 2-5 mg were heated or cooled at rates of ±10 ºC·min^{-1}. Thermogravimetric analyses were performed with a Perkin-Elmer TGA6 thermobalance at a heating rate of 10 ºC·min^{-1} under flowing nitrogen.

Scanning electron microscopy (SEM) was used to examine the surface morphology of the nanoparticles. Gold coating was accomplished by using a Balzers SDC-004 Sputter Coater. The SEM microphotographs were taken with a JEOL SSM-6400 instrument. Particle size distribution and Z-potential of nanoparticles in aqueous solution were measured by Non-Invasive Back-Scatter (NIBS) and electrophoresis M3-PALS respectively in a Zetasizer Nano ZS, Malvern.

Contact angles between water and solid surfaces were measured by means of an OCA 15+ contact angle measuring system supported by SCA20 software (Dataphysics, Germany). Angle values were registered after 30 seconds of dropping the water onto the polymer surface with a minimum of 10 measurements being made.

9.3. Results and discussion

9.3.1. Synthesis of polyglutamates. The alkylation reaction on PGGA leading to poly(α-alkyl γ-glutamate)s may be conducted by different methods reported in the literature. Reaction with diazomethane is a clean procedure that has been proved to be highly efficient to methylate PGGA without significant degradation of the polypeptide chain. We have applied here this method to obtain the hopolymer poly(α-methyl γ-glutamate) (PAAG-Me). On the other hand, *co*PGGA-(R_xH_y) copolymers, which are PGGA's partially esterified with alkyl groups other than methyl, were prepared by reaction of the polyacid with excess of the corresponding alkyl bromide under basic conditions.

This procedure allowed fine adjusting of the conversion by controlling the reaction time, the conditions appropriate to achieve the desired esterification degree being found by trial-and-error. The advancement of the reaction was monitored by ^{1}HNMR by quantitative comparison of the areas of the signals arising from the main chain CH_2 and the CH_3 of the alkyl side group.

The polyglutamates prepared in this work are listed in Table I where reaction times and esterification results are given for each of them along with some of their relevant features in connexion with this study. According to previous NMR study carried out by us on ethylation of PGGA by a similar method as it is done here, the copolymers are expected to have a random microstructure.[17] All these compounds were non-water soluble and their hydrophilicity and wetting properties were found to decrease with the increase in content and size of the alkyl group as revealed by the measured contact angles. The DSC analysis revealed that, at difference with PAAG-Me which is semicrystalline, the copolyglutamates do not display any sign of crystallinity. It should be noticed that homopolymers PAAG-Et and PAAG-Octd are known to crystallize readily in ordered tree- and two-dimensional arrays respectively.[18]

Table 1. PGGA Esterification results and polymer characteristics.

Polymer[a]	Esterification results[b]				Polymer characteristics			
	t (h)	S	X (%)	Y (%)	Mw[c] (g·mol^{-1})	PD[c]	CA[d] deg	^oT_d/ ^mT_d[e] (ºC/ºC)
PGGA	-	-	-	-	190,000	2.1	18	237/290
PAAG-Me	4	-	100	90	82,000	2.5	32	270/297
*co*PAAG-(Et $_{75}$H$_{25}$)	10	ether	76.2	82	151,000	1.7	37	232/290
*co*PAAG-(Hex$_{50}$H$_{50}$)	27	acid H_2O	49.3	91	162,000	1.8	62	233/290
*co*PAAG-(Dod$_{50}$H$_{50}$)	24	hexane	43.0	88	170,000	1.8	84	235/300
*co*PAAG-(Octd$_{50}$H$_{50}$)	24	hexane	50.2	85	173,000	1.9	101	236/304

[a]Polyacid and polyglutamates; in brackets, nominal copolymer compositions which have been rounded to 75/25 and 50/50 for simplicity. [b]Reaction time (t), conversion degree measured by NMR (X), solvent used for recovering the polymer (S) and yield (Y). [c]Weight-average molecular weight (Mw) and polydispersity (PD) measured by GPC. [d]Contact angle measured on films made by casting from NMP. [e]Decomposition temperatures: ^oT_d= onset corresponding to 5% of loss of initial weight; ^mT_d= maximum rate.

It is known also that the octadecyl side chains of copolymers *co*PAAG-(Et$_x$Octd$_y$) are crystallized for a fair range of copolymer compositions.[17] The fact that the whole set of *co*PAAG-(R$_x$H$_y$)'s appear unable to display ordering should be associated to the strong disturbing effect of the carboxylic group on the structure.

The detrimental effect of the carboxylic group on thermal stability is also remarkable. Although the TGA traces display a similar profile for all of them, the onset of thermal decomposition decreases about 30 ºC when such groups are present in the polypeptide chain.

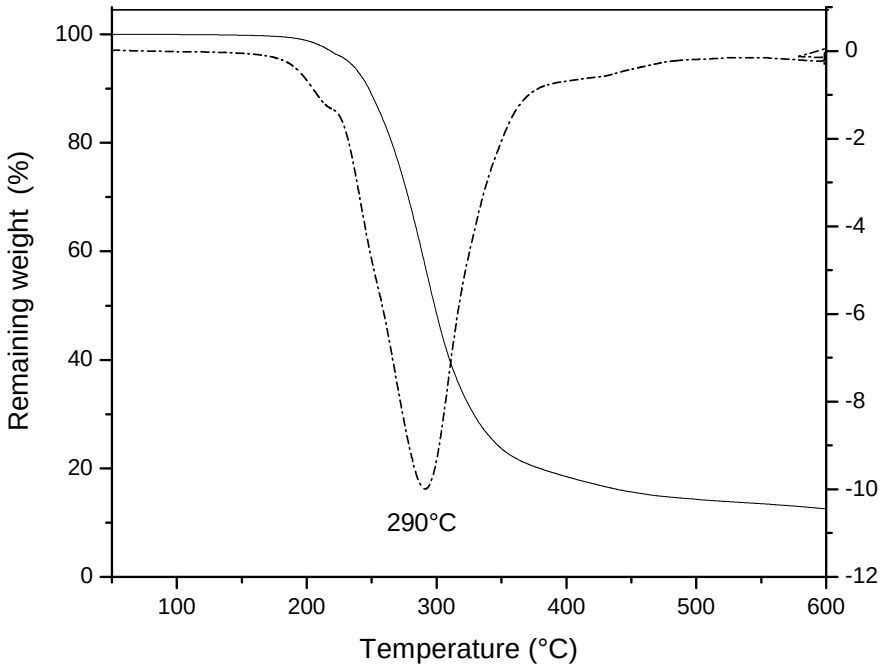


Figure 1. TGA traces of *co*PAAG-($Hex_{50}H_{50}$) recorded at the heating rate of 10 ºC·min^{-1}

9.3.2. Preparation, characterization and hydrolytic degradation of coPAAG-(R_xH_y) nanoparticles. The nanoparticles used in this study have been prepared applying the well established precipitation-dialysis method with NMP being the solvent of choice. Well-shaped spherical particles as those shown in Figure 4 were obtained by this method and the particle size distribution profiles for each case are compared in Figure 2. Fairly sharp distributions were obtained for all the cases, the average diameter ranging between 200 and 300 nm with the size increasing with the contact angle of the copolymer. As listed in Table 2, the zeta-potential measured for these particles ranged between near to 20 for the methyl ester up to about 35 for the copolyglutamate containing 50% of hexyl groups.

A decrease in both esterification degree and length of the alkyl group gives place to a decreasing in the Z-value as it should be expected for a less amount or diminished exposition of carboxyl groups located in the particle surface.

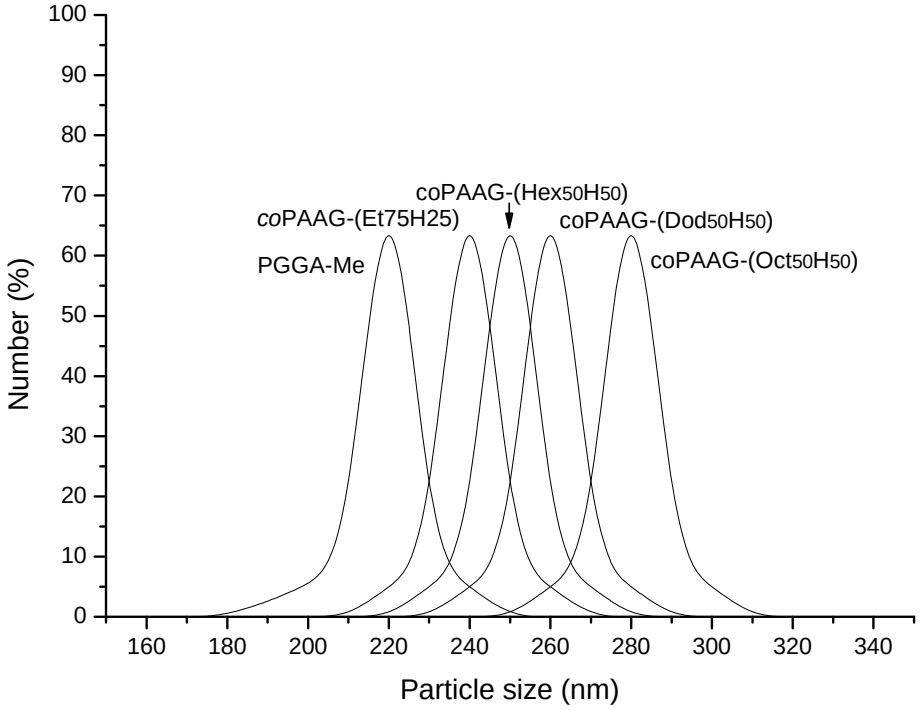


Figure 2. Particle size distribution of copolymer nanoparticles.

According to the blocky microstructure of these copolymers, the structure of these nanoparticles can be interpreted as if the short sequences of glutamic acid units were segregated to the outer part of the nanospheres whereas the hydrophobic alkyl glutamate segments were preferably located in the particle core.

Table 2. Nanoparticles properties and encapsulation efficiency.

			Encapsulation efficiency (% w/w)	
Polymer	Z-potencial (mV)	Particle size ± SD (nm)	Erythromycin	α-Chymotrypsin
PAAG-Me	-20.8 ± 1.8	220± 21	80	3
*co*PAAG-(Et $_{75}$H$_{25}$)	-25.1 ± 2.5	240± 24	75	20
*co*PAAG-(Hex$_{50}$H$_{50}$)	-33.9 ± 2.7	250± 22	61	25
*co*PAAG-(Dod$_{50}$H$_{50}$)	-30.2 ± 2.2	260± 20	57	22
*co*PAAG-(Octd$_{50}$H$_{50}$)	-22.8 ± 3.1	280± 21	43	18

The surface location of the carboxyl groups will facilitate their chemical modification as well as their ionic coupling with cationic species. Furthermore, a high zeta potential value is a favorable feature for nanoparticle stability because aggregation will be hindered by electrostatic repulsion.

PGGA is known to be hydrolytically degraded at pH 7.4 and 37 ºC near 60% in 2 days with generation of glutamic acid oligomers and monomer.[19] The process seems to be favoured by the local acidic environment provided by the carboxylic groups. On the contrary, we have observed that disks made of fully esterified polyglutamates are largely reluctant to hydrolysis without showing signs of degradation in months (unpublished results). As a previous step in the evaluation of the polyglutamate nanoparticles prepared in this work as drug delivery systems, their hydrodegradability under simulated physiological conditions was firstly examined. The results obtained from these experiments are displayed in Figure 3 where the decay of the molecular weight is plotted against incubation time for the five polymers under study.

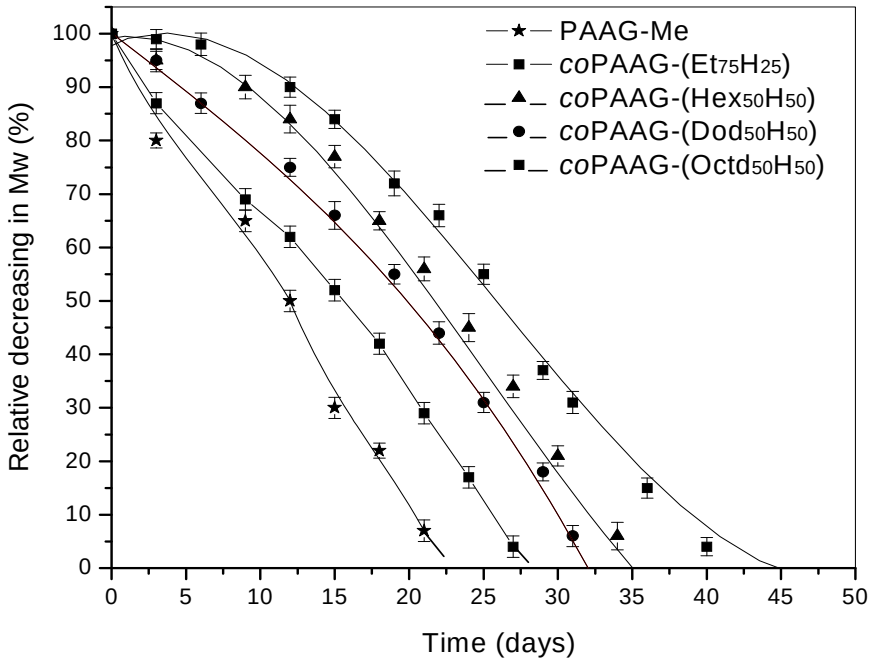


Figure 3. Hydrolytic degradation of nanoparticles at 37 ºC and pH 7.4.

What it is clearly shown by these plots is that all nanoparticles degrade following an almost linear profile to become fully degraded in a period of time that ranges from 20 to 45 days, depending on the polymer they are made of. As expected, degradation rate of copolymers is observed to increase with the content in carboxylic groups and to decrease with the length of the alkyl ester group. The fact that PAAG-Me appears to be the fastest degradable compound is rather striking since disks of PAAG-Et have been proved to be practically undegradable when subjected to similar conditions for months (unpublished results). The contrasting behaviour found in PAAG-Me may be explained taking into account both the exceptionally small size of the methyl group and the large specific surface of the nanoparticles.

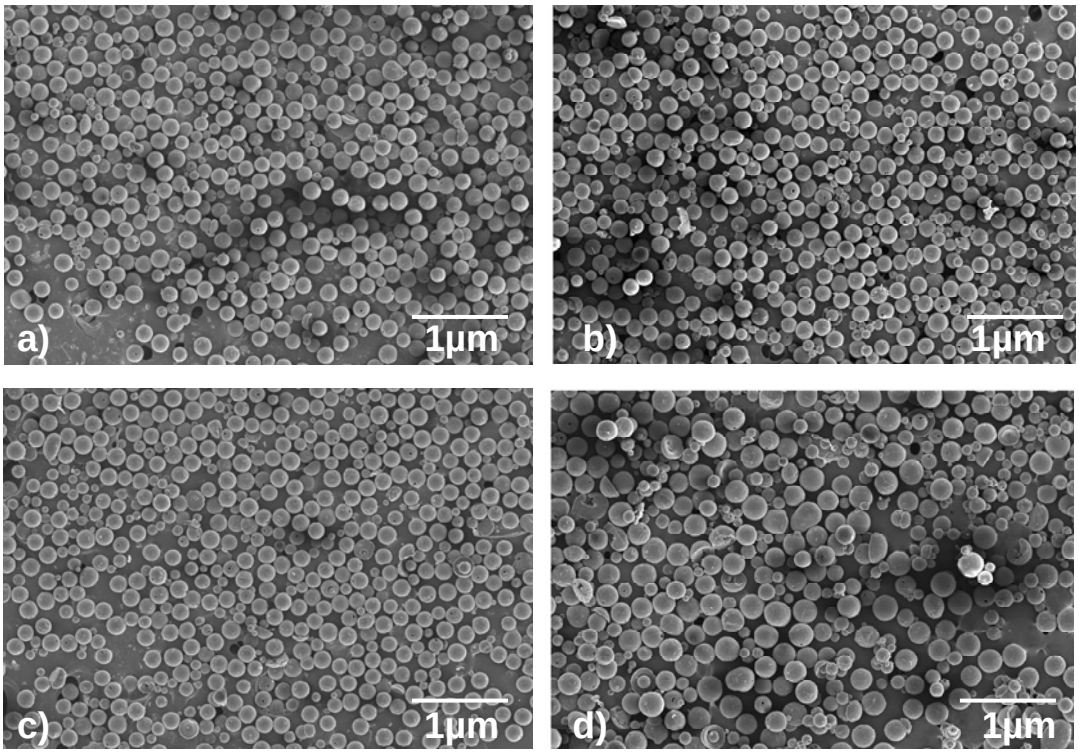


Figure 4. SEM images of nanoparticles made of a) *co*PAAG-(Et $_{75}$H$_{25}$); b) *co*PAAG-(Hex$_{50}$H$_{50}$); c) *co*PAAG-(Dod$_{50}$H$_{50}$); *co*PAAG-(Octd$_{50}$H$_{50}$).

9.3.3. Erythromycin encapsulation and release. When nanoparticles were prepared in the presence of equal amounts of erythromycin, the drug was encapsulated with an efficiency ranging from 43% for *co*PAAG-(Octd$_{50}$H$_{50}$) up to 80% for the homopolymer PAAG-Me (Table 2). Comparison of the encapsulation efficiency achieved for the 50% esterified copolymers leads to conclude that hydrophobic large groups disfavours the entrapment of the drug. The same effect seems to operate when carboxylic groups are present in the nanoparticle. Erythromycin is a non-water soluble dilactone macrolide with both hydrophobic and hydrophilic groups attached to the backbone ring.

It can be inferred therefore that a compensated combination of such groups in the nanoparticle will lead to a favourable interaction and consequently to a maximum entrapping efficiency. The case of PAAG-Me is exceptional since the methyl group is unique in providing an intermediate hydrophobic/hydrophilic character without requiring the presence of carboxylic units.

The releasing of erythromycin incubated under simulated physiological conditions as a function of time is plotted in Figure 5 for the whole set of polymers under study. In all cases, the liberation profile follows closely the degradation ones shown in Figure 3. However, a close comparison of these plots for every system reveals that the full release of the drug takes place in a period of time shorter than required for the complete degradation of the polymer. Such a shortening is more pronounced for polymers displaying higher degradation rates. These observations indicate that although the drug is released by a polymer degradation regulated mechanism; the delivering process becomes substantially modified by the precise constitution of the polymer matrix.

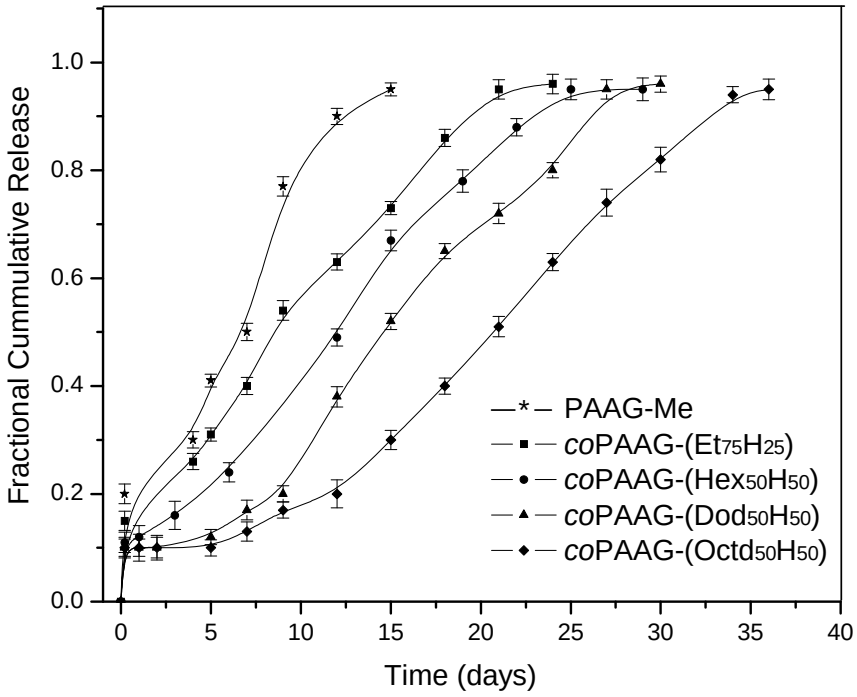


Figure 5. Erythromycin release profiles from nanoparticles upon incubation at pH 7.4 and 37°C.

9.3.4. Evaluation as protein carriers: α-Chymotrypsin encapsulation and release. In order to make a preliminary evaluation of the capacity of these nanoparticles as protein carrier systems, a study including encapsulation, release and enzyme activity retention was carried out using α-chymotrypsin. α-Chymotrypsin is a protein commonly used for exploratory studies of novel protein carrier systems, which has a molecular weight of 25,000 daltons and an isoelectric point of 9.1.

Among the different methods that are available for loading proteins on nanoparticles, encapsulation has been chosen in this work since our previous studies on polymalic acid nanospheres showed that best results were attained when this method was used.[20]

α-Chymotrypsin was encapsulated by coprecipitation upon mixing the aqueous solution of the protein and the NMP solution of the selected polymer. Efficiency results obtained in these essays are comparatively shown in Table 2. Similar efficiencies with values in the 20-25% range were achieved for the different copolymers. On the contrary, the amount of loaded α-chymotrypsin in non-carboxylic PAAG-Me was only 3%, indicating the determinant effect played by the carboxylic group in entrapping the protein. It is worthy noting also that a total correspondence is observed to exist between loading efficiency and the Z-potential of the nanoparticles. This corroborates the importance of the ionic interactions, not only in the coupling process taking place upon coprecipitation but also in the absorption of protein on the particle surface.

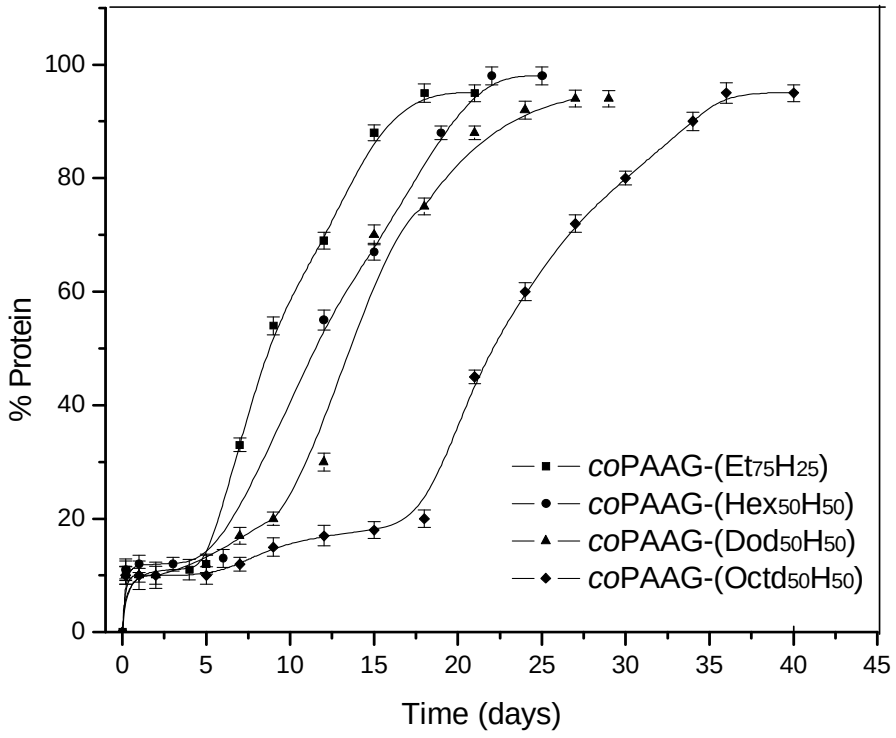


Figure 6. Protein release profiles at pH 7.4 and 37°C .

The releasing profiles obtained upon incubation in PBS at pH 7.4 and 37°C for the α-chymotrypsin loaded nanoparticles made of *co*PAAG-(R_xH_y) are compared in Figure 6. A common feature to all these plots is that proteins are delivered following similar profiles that are observed for the hydrolytic degradation of the nanoparticles indicating again a close connection between the two processes. At difference with what happened with nanoparticles loaded with erythromycin, the protein releasing and polymer degradation periods of times are comparable. However, a latency period characterized by almost absence of protein releasing is observed in the initials of the incubation for all four cases.

Such a lag time is substantially longer than that observed in the degradation profiles (Figure 3) arriving to last up to 15 days for the case of *co*PAAG-($Octd_{50}H_{50}$). The occurrence of a lag time in the releasing process indicates that polymer has to be degraded in a considerable extension for protein being able to leave the nanoparticle. A common fact observed for all the releasing profiles is that a small amount of protein (less than 10%) is released at the beginning of incubation; this initially released protein is that absorbed on the nanoparticle surface that was not removed by washing.

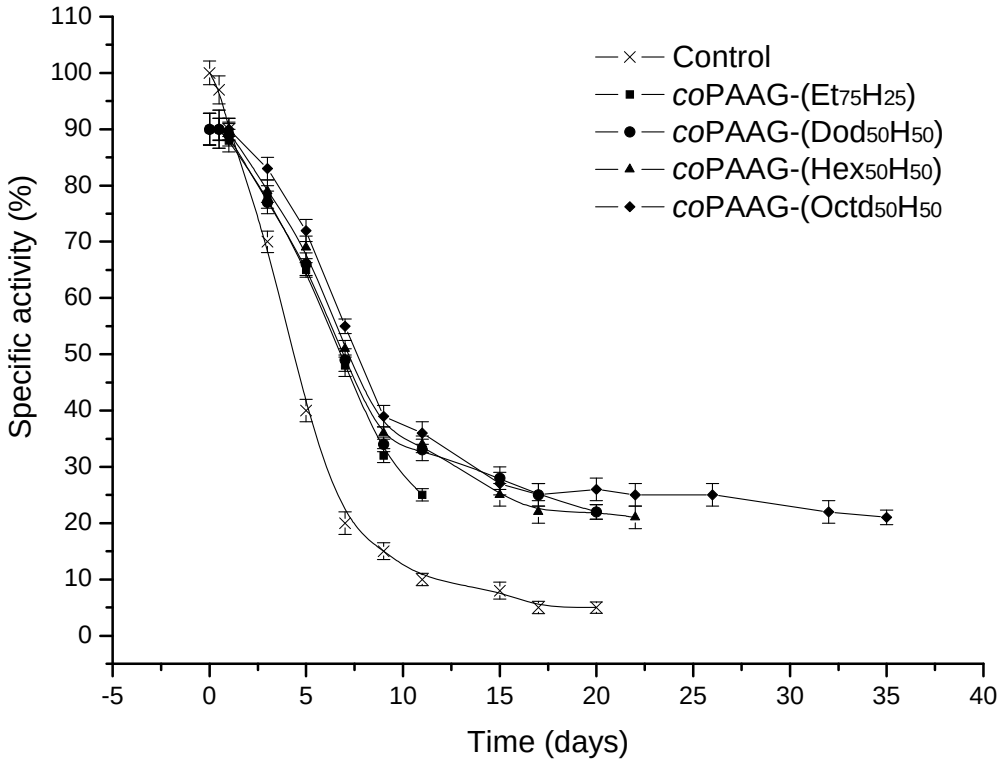


Figure 7. Decrease of the specific activity of α-chymotrypsin as a function of releasing time (values are corrected taking into account the amount of enzyme liberated at each time).

The loss of activity of functional proteins upon incubation in aqueous medium is a well-known fact that negatively affects storing and slow dosification of proteins in physiological fluids. Such loss of activity can be even enhanced when proteins are loaded on polymeric substrates if strong specific protein-polymer interactions occur. The activity measured for free α-chymotrypsin and α-chymotrypsin released from loaded nanoparticles as a function of time is plotted in Figure 7 for the four studied systems. Essays carried out with free α-chymotrypsin revealed that the activity of this enzyme decreased rapidly with time to about 20% of the initial value in five days to practically disappear after ten days of incubation. Results obtained for encapsulated α-chymotrypsin show differences worth of mention.

Comparison of the specific activity at time zero reveals that encapsulation has detrimental effect on enzyme activity with about 10% of activity loss. Nevertheless, further decay of activity takes place with a rate slightly lower for encapsulated α-chymotrypsin to attain a final value of about 20% which is maintained for longer incubation times. It can be inferred therefore that encapsulation helps to preserve the activity of the enzyme in overall. It should be stressed that for encapsulation the protein enters in contact with NMP used for dissolving the polymer. What can be inferred from results is that this solvent does not alter dramatically the conformation of the protein and that the encapsulating polymer contributes to its preservation in the aqueous environment.

9.4. Conclusions

The main conclusions drawn from this work are the followings: Fully methylated and partial alkylated poly(γ-glutamic acid) may be used for the manufacture of well-shaped spheroid nanoparticles with a diameter in the 200 nm range and a fairly narrow size distribution. These nanoparticles are readily degradable under simulated physiological conditions following almost linear profiles and taking place within periods of time of 15 to 40 days. The degradation rate is depending of both the length of the alkyl group and the esterification degree. Erythromycin can be very efficiently encapsulated in all these nanoparticles, the amount of entrapped drug being maximum (80%) in the case of fully methylated PGGA.

Nanoparticles made of polymers bearing free carboxyl groups are able to load considerable amounts of α-chymotrypsin (20-25%). Upon incubation, both erythromycin and α-chymotrypsin are released by a process that essentially encompasses polymer degradation. Loading of α-chymotrypsin in the nanoparticles helps to preserve the activity of the enzyme along time.

These systems attract exceptional interest since the liberation rate of the loaded compound could be tuned by adjusting the esterification degree and the constitution of the alkyl group. These nanoparticles offer therefore outstanding possibilities in the development of novel delivery systems for drugs, peptides and proteins.

9.5. References

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