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# Potential doxorubicin delivery system based on magnetic gelatin microspheres crosslinked with sugars

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**Abstract:** The preparation and characterization of magnetic microspheres based on gelatin for use in drug delivery systems are reported. Sugars were employed as crosslinking agents and type A gelatin and type B gelatin were compared to prepare microspheres by water-in-oil emulsion. The influence of gelatin and sucrose concentration, temperature and stirring speed on microbeads' characteristics was studied. The gelatin concentration and stirring speed were the parameters directly associated with the particle sizes. We found no relevant difference between the use of type A and type B gelatin. In addition, the gelatin crosslinking study revealed that sucrose is not a crosslinking agent but fructose can crosslink the protein chains when the reaction medium has pH 9. The size of the microspheres varied from 5 to 60  $\mu\text{m}$  as measured by optical microscopic images. Doxorubicin adsorption and release were successfully performed using the microspheres crosslinked with fructose under the action of an external magnetic field. It was observed that the microspheres absorbed 69% of the doxorubicin that was in solution. After 24 h, about 45% of the DOX was displaced from microspheres to saline medium in the free form in the solution.

**Keywords:** gelatin microspheres, magnetic properties, sugar crosslinking.

## 1. Introduction

Gelatin is a mixture of water-soluble proteins obtained by hydrolysis of collagen from the skin, bones and connective tissues of animals<sup>[1]</sup>. There are two types of gelatins and they are characterized by their mode of manufacture. The Type A gelatin (pH 3.8-6.0; iso- electric point 6-8) is obtained from acidic hydrolysis of pork skin and the Type B gelatin (pH 5.0-7.4; isoelectric point 4.7-5.3) is obtained from basic hydrolysis of bones and animal skin<sup>[1]</sup>.

Attributable to the excellent biocompatibility and biodegradability<sup>[2,3]</sup>, gelatin has been widely used in biomedical materials for controlled drug release. In this application, can be found gelatin in different forms: films<sup>[4-6]</sup>, disks<sup>[7]</sup>, hydrogels<sup>[8,9]</sup>, sponges<sup>[10]</sup> and frequently microspheres<sup>[7,9,11-14]</sup>. Microspheres are usually prepared by water-in-oil emulsion. However, the main preparation parameters vary widely in the literature. When microspheres are produced, these parameters can

influence particle size and the microsphere's size is very important to define the administration route<sup>[15,16]</sup> and the liberation rates<sup>[16]</sup>. Because of this, in this work, we designed experiments to determine the most important parameters that can influence particle size.

Because gelatin is a water-soluble polymer, its must be modified for application in the human body (where the medium is aqueous). Thus, gelatin hydrogels can be prepared as three-dimensional hydrophilic networks that are able to release drugs at the controlled rates. Such networks can be physical as those obtained by gelatin mixed with other polymers such as sodium carboxymethyl cellulose<sup>[17]</sup>, hydroxyethyl cellulose<sup>[18]</sup> and carboxymethyl guar gum<sup>[19]</sup> to form interpenetrating polymer networks (IPNs) or chemical as those obtained by using chemical crosslinking agents<sup>[2]</sup>. The chemical crosslinking agents are bifunctional or polyfunctional compounds that act by binding to carboxylic or amino groups of adjacent molecules of gelatin. Examples of this type of crosslinker include formaldehyde, glutaraldehyde, glyceraldehyde, imines, ketones, saccharides, dyes, calcium carbonate, carbodiimides, genipin and other bifunctional compounds<sup>[1]</sup>.

There are many chemicals that can be used for gelatin crosslinking, but the crosslinking process in biomedical materials must be done with reagents that, like the polymer, are biocompatible and biodegradable. Most of these crosslinking agents can cause some cytotoxic effects because of unreacted fractions<sup>[7]</sup>. To avoid undesirable reactions, some studies have investigated the use of sugars as crosslinking agents<sup>[7,10,11,20]</sup>. Among the studied sugars, calls our attention the fact that the researchs conclude that sucrose is a crosslinking agent able to significantly reduce the gelatin water solubility. Additionally, sucrose is biocompatible, easy to obtain and inexpensive, making it a good candidate for use in controlled drug release. For these reasons, we will use it as a crosslinking agent in this work.

Besides the main features of biocompatibility, biodegradability and low water solubility, the device designed by us should possess the ability to be transported inside the human body directly to target cells. To this end, in one of the phases of this study, magnetite is incorporated in the microspheres produced. Magnetite will confer magnetic properties to the device. Thus, it can be injected into the patient's circulatory system and, with the aid of an external magnetic field, it is possible to concentrate the drug/biocomposite complexes at a specific target site in the body where the particles have entered the bloodstream<sup>[21]</sup>. Once the biocomposite is concentrated at the target, the drug can be released to act on the target cells by enzymatic activity or changes in pH, temperature or magnetic field. These magnetic drug delivery systems have many advantages over normal, non-targeted methods, such as: ability to target specific locations in the body; reduction of the drug quantity needed to attain a particular concentration in the vicinity of the target and reduction of the drug's concentration at non-target sites, minimizing side effects<sup>[22]</sup>.

The above described characteristics are essential for obtaining optimum system for controlled drug release. Despite its potential

applications, few studies have investigated the use of sugars as a crosslinking agent to obtain gelatin microspheres and there is no literature about magnetic gelatin microspheres sugar crosslinked. Thus, in order to obtain a similar device, we decided to evaluate the effect of gelatin type, sucrose concentration, magnetite concentration and crosslinking time on the physical properties of the microspheres based on gelatin, sucrose and magnetite.

## 2. Materials and Methods

### 2.1 Materials

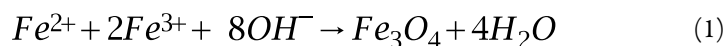
Type B gelatin (225 bloom), type A gelatin (300 bloom), sucrose, fructose, corn oil and doxorubicin were purchased from Sigma-Aldrich Co. Acetone, and sodium hydroxide were acquired from B. Herzog Varejo de Produtos Químicos Ltda. Ferric chloride, sodium chloride and ferrous sulfate were purchased from Proquímios Comércio e Indústria Ltda. All chemicals were analytical grade and used as received.

### 2.2 Preparation of gelatin microspheres

Microspheres were produced by thermal gelation. Briefly, 10 mL of 10% w/v gelatin solution preheated to 60 °C containing 40% w/w of sucrose was added dropwise to 40 mL of corn oil to form an emulsion by stirring with a two-paddle stirrer (1000 rpm). As the emulsion was obtained, the temperature was kept at 60 °C for different time periods and then lowered to 5 °C by rapid cooling in an ice bath. The microspheres formed were maintained in this condition for 30 minutes. Then, to completely solidify the droplets of the dispersed phase, 50 mL of precooled (5 °C) acetone was added and the mixture was stirred for another hour. The microspheres were filtered, washed with cool acetone (5 °C) and rapidly dried.

### 2.3 Preparation of magnetite

Magnetite nanoparticles were synthesized using an adaptation of a previously described co-precipitation method<sup>[23]</sup>. This involved adding 100 mL of an aqueous solution of sodium hydroxide (concentration of 10 mols/L) dropwise to a mixture of iron salts with Fe<sup>2+</sup>/Fe<sup>3+</sup> molar ratio of 1/2, forming an immediate dark brown/black solution. The solution was stirred for 1 h at room temperature and then was heated at 90 °C for another 1 h, which resulted in the formation of a black colloidal magnetite solution. Subsequently, the dispersion was cooled to room temperature and was washed several times with distilled water until neutral pH. The magnetite formed was separated by magnetic decantation/separation and was dried in an oven at 60 °C for 24 h. The Fe<sub>3</sub>O<sub>4</sub> nanoparticles' precipitation happened according to the Equation 1 below:



#### *2.4 Preparation of the magnetic gelatin microspheres*

Magnetic microspheres were produced by the same method described before. The magnetite was added in the gelatin solution and this mixture was then added dropwise to corn oil in order to form an emulsion.

#### *2.5 Size particle distribution and average diameter*

These analyses were performed using a method described by Allen<sup>[24]</sup>. In this method, optical microscopic images were used to measure the diameter of 625 microspheres of each sample.

#### *2.6 Magnetic properties*

The magnetic properties (saturation magnetization, residual magnetization and coercivity) were analyzed by using a Lake Shore series 7400 vibrating sample magnetometer (VSM).

#### *2.7 DSC and FTIR analysis*

The thermal properties of the gelatin microspheres were analyzed by using a Perkin-Elmer Pyris 1 differential scanning calorimeter. The melting temperature ( $T_m$ ) of the microspheres was determined under nitrogen atmosphere. Samples were scanned in aluminum pans, under static air atmosphere, at a heating rate of 20 °C/min in the temperature range of 50-200 °C.

FTIR spectra of microspheres were measured by the KBr pellet method using a Perkin Elmer Spectrum One spectrophotometer.

#### *2.8 Morphological analysis*

The gelatin microspheres' morphology was determined by observation of the samples with a FEI Inspect 550 scanning electron microscope. The samples were coated with gold in an argon atmosphere for 120 s and the images were captured using acceleration voltages of 5 kV and 20 kV.

#### *2.9 Swelling ratio*

Gelatin microspheres in the dry state were put on filter paper and weighed. Then the microspheres were immersed in distilled water at room temperature. Subsequently, the weight of the swollen microspheres was

determined after 60 minutes. The swelling ratio ( $R_{sw}$ ) of each test sample was calculated as follows (Equation 2):

$$R_{sw} = ((W_s - W_d) / W_d) \times 100 \quad (2)$$

where  $W_s$  denotes the weight of the test sample after swelling and  $W_d$  is its initial weight in the dry state.

### 2.10 Atomic absorption spectroscopy

The iron concentration of the gelatin microspheres was determined by atomic absorption spectroscopy. About 10 mg of each sample was heated in a flat-bottomed flask with 20 mL of aqua regia at reflux temperature for 24 h. Then the solution was cooled to room temperature, filtered into a 100 mL volumetric flask and the volume was completed with distilled water. The solution was analyzed by a Perkin Elmer Analyst 300 spectrometer.

### 2.11 Doxorubicin loading and in vitro doxorubicin release

The doxorubicin (DOX) loading and in vitro DOX release were determined using gelatin magnetic microspheres made with 50% magnetite and fructose crosslinked at pH = 9. The loading of DOX was performed by allowing the magnetic gelatin microspheres (50 mg) to contact a freshly prepared DOX solution (200 ppm) for 1 hour. Then, the amount of free DOX in the solution was quantified by UV-Vis spectroscopy (Fentom 600S) at 480 nm. The DOX loading efficiency (DL)(%) was calculated using the following Equation 3 :

$$DL(\%) = \frac{\text{Total amount of DOX} - \text{Free amount of DOX}}{\text{Total amount of DOX}} \times 100 \quad (3)$$

The *in vitro* DOX release experiments were carried out in phosphate buffer saline (PBS) (pH 7.4, 1.2 mM  $\text{KH}_2\text{PO}_4$ , 1.15 mM  $\text{Na}_2\text{HPO}_4$ , 2.7 mM KCl, 1.38 mM NaCl) in presence of constant magnetic field (6000 gauss) using a magnet. In order to determine the released amount of the DOX, 0.1 g of DOX-loaded magnetic gelatin microspheres was added to 8 mL of PBS (release medium, pH 7.4). The resulting suspension was gently shaken under a constant magnetic field of 6000 Gauss for predetermined time period. After shaking, 3 mL of supernatant was withdrawn and assayed for DOX spectrophotometrically (Fentom 600S at 480 nm). Each experiment was carried out in triplicate.

### 3. Results and Discussions

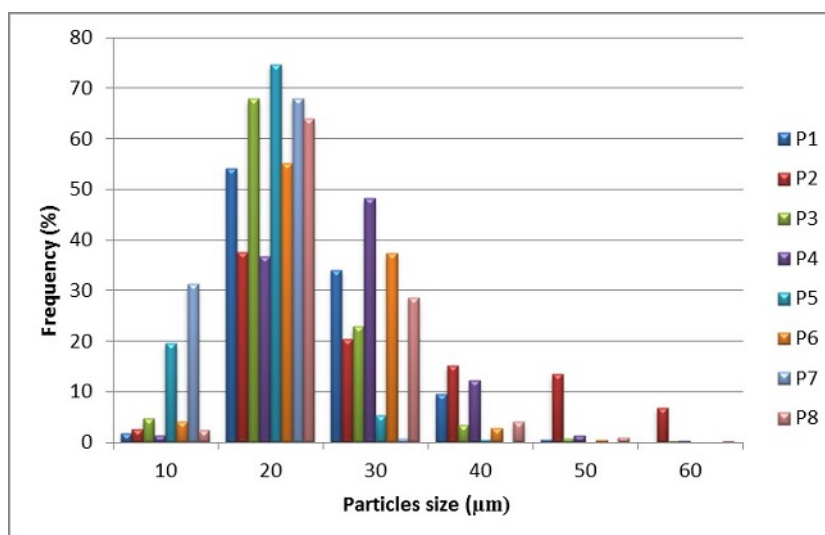
#### 3.1 Influence of gelatin and sucrose concentration, temperature and stirring speed on particle size of materials obtained

A full factorial design at two levels,  $2^3$ , was applied to evaluate the main effects. The variables considered and the levels studied are shown in Table 1. The experiments involved fixing the oil phase (corn oil), aqueous phase/oil phase ratio (1/4), heating time (10 minutes), cooling time (30 minutes) and acetone cooling time (1 hour). In order to replace cytotoxic crosslinkers, sucrose was chosen as crosslinking agent because it is a well-known biocompatible reagent.

**Table 1.**  
Experimental conditions in the  $2^3$  factorial design to evaluate their effects on particle size of gelatin microspheres.

Experiment	Gelatin concentration (%)	Sucrose concentration (%)	Temperature (°C)	Stirring speed (rpm)
P1	10	0	40	500
P2	10	0	40	800
P3	10	0	40	1000
P4	10	0	60	500
P5	10	0	60	800
P6	10	0	60	1000
P7	20	0	40	500
P8	20	0	40	800

The particle size distribution showed in Figure 1 revealed that the diameter of the microspheres produced in all experiments ranged from 5 to 60  $\mu\text{m}$ . However, there was predominance in the range from 11 to 30  $\mu\text{m}$ . For application in drug delivery systems, gelatin microspheres should have sizes below 5  $\mu\text{m}$  for intravenous administration and should be smaller than 125  $\mu\text{m}$  for arterial administration<sup>[15]</sup>. Thus, the particles obtained in all experiments were adequate for use in drug delivery by the arterial route.



**Figure 1.**  
Particles size distribution of gelatin microspheres obtained according to Table 1.

The analysis of variance (ANOVA) was used to analyze the effect of gelatin concentration, sucrose concentration, stirring speed and



temperature on gelatin microspheres' particles size As can be seen in Table 2 , with a 95% confidence level, the parameters that are directly associated with the particle sizes are the gelatin concentration and stirring speed. The data evaluation shows that smaller diameters are obtained with solutions of low gelatin concentration and higher stirring speeds. Because of this, the microspheres made afterward were prepared with a 10% gelatin solution and 1000 rpm stirring speed.

Table 2.

### Analysis of the effects of variables on particles size by ANOVA.

	1	2	3	4
Constant (intercept)	2.8 (2.7)	2.8 (2.7)	3.2 (3.1)	3.0 (2.9)
Dependent variable (log)	-0.2 (-0.2)	-0.2 (-0.2)	-0.2 (-0.2)	-0.2 (-0.2)
Industry, year dummies	Yes (2.7)	Yes (2.7)	Yes (2.7)	Yes (2.7)
Observations	6,741	6,741	6,741	6,741
R <sup>2</sup>	0.248	0.248	0.248	0.248
F	6.601	6.601	6.601	6.601
Prob > F	0.000	0.000	0.000	0.000

### 3.2 Gelatin type influence

Manufacturers offer a wide variety of gelatins that are simple combinations of type A and type B gelatins. Thus, it is important to know if the gelatin type will influence the properties of the final particles. In this study, microspheres were prepared with both gelatin types and their magnetic and solubility properties were compared.

The magnetic properties are extremely important for application in the device proposed in this paper. The saturation magnetization of the particles should be known in order to calculate the magnetic field strength that must be applied externally. Another very important characteristic is superparamagnetism. For application in the human body, this property is essential to prevent the particles' agglomeration, which can lead to clogging of blood vessels. For this reason, we assessed the effects of the magnetite concentration on the magnetic properties of the microspheres.

Table 3 shows the magnetic properties obtained. As might be expected, the saturation magnetization increased with rising magnetite concentration. Higher magnetization was observed for the microspheres obtained with type A gelatin when 50% was added during preparation, but this behavior was not observed for the other magnetite concentrations. All microspheres prepared in this experimental series had superparamagnetic behavior because remnant magnetization close to zero was observed.

Table 3.

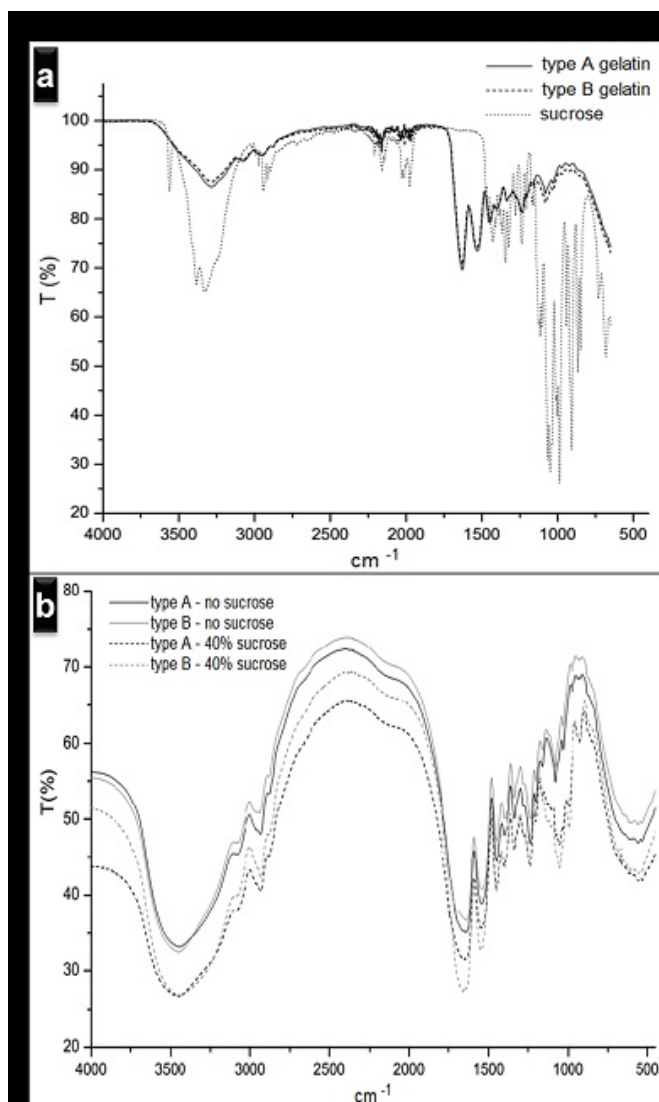
Influence of gelatin type and magnetite concentration on magnetic properties of materials.

Age	Gender	Height (cm)	Weight (kg)	Body Mass Index (kg/m <sup>2</sup> )	Estimated Energy Requirement (kcal/day)
18	M	175	75	24.5	2800
25	F	165	60	22.0	2200
35	M	180	85	26.9	3000
45	F	170	70	24.2	2400
55	M	175	75	24.5	2600
65	F	165	60	22.0	2000
75	M	170	70	24.2	2200
85	F	160	55	20.8	1800

The data show that the experimental values are all larger than the theoretical values. The most probable hypothesis for these experimental results is that magnetite in the pure state forms clusters but is evenly dispersed when placed in a gelatin matrix. The formation of clusters tends to decrease the saturation magnetization of the particles while homogeneous dispersion has the opposite effect<sup>[25]</sup>.



FTIR experiments were performed to find evidence of sugar-mediated crosslinking. Figure 2 a shows the spectra of raw materials used to produce gelatin microspheres (type A gelatin, type B gelatin and sucrose). According to Cortesi et al.<sup>[7]</sup>, the absorption band located at  $1450\text{ cm}^{-1}$  is characteristic of an aldimine stretching vibration, which provides evidence of the crosslinking of gelatin. However, this band is already present in the microspheres' raw material.



**Figure 2.**

FTIR spectra (a) raw materials (type B gelatin, type A gelatin and sucrose) and (b) gelatin microspheres.

Figure 2 b shows the FTIR spectra of type A gelatin and type B gelatin microspheres with no sucrose and with 40% (w/w) of sucrose. All microspheres showed the same peaks with similar intensities in the infrared region and no difference between type A and Type B gelatin was noted. Therefore, these spectra present no evidence of sucrose crosslinked gelatin.

In addition, water solubility tests were conducted to verify how the solubility was affected by addition of sugar. All the samples dissolved

completely in under 3 hours. These results indicate that the microspheres have a low level of crosslinking.

Type A and type B gelatin had similar properties with respect to solubility in water and minor differences in relation to magnetic properties. Thus, we decided to use only type B gelatin to produce microspheres from this point on.

### 3.3 Gelatin microsphere crosslink study

In order to optimize the efficiency of gelatin microspheres as drug carriers, their number of crosslinks in the polymer matrix should be evaluated. Because of this, we decided to observe how the heating time influences protein crosslinking in a series of experiments using type B gelatin. The obtained microspheres were characterized by differential scanning calorimetry (DSC), swelling analysis and scanning electronic micrography. The microspheres' composition, as well as swelling and DSC results are in Table 4.

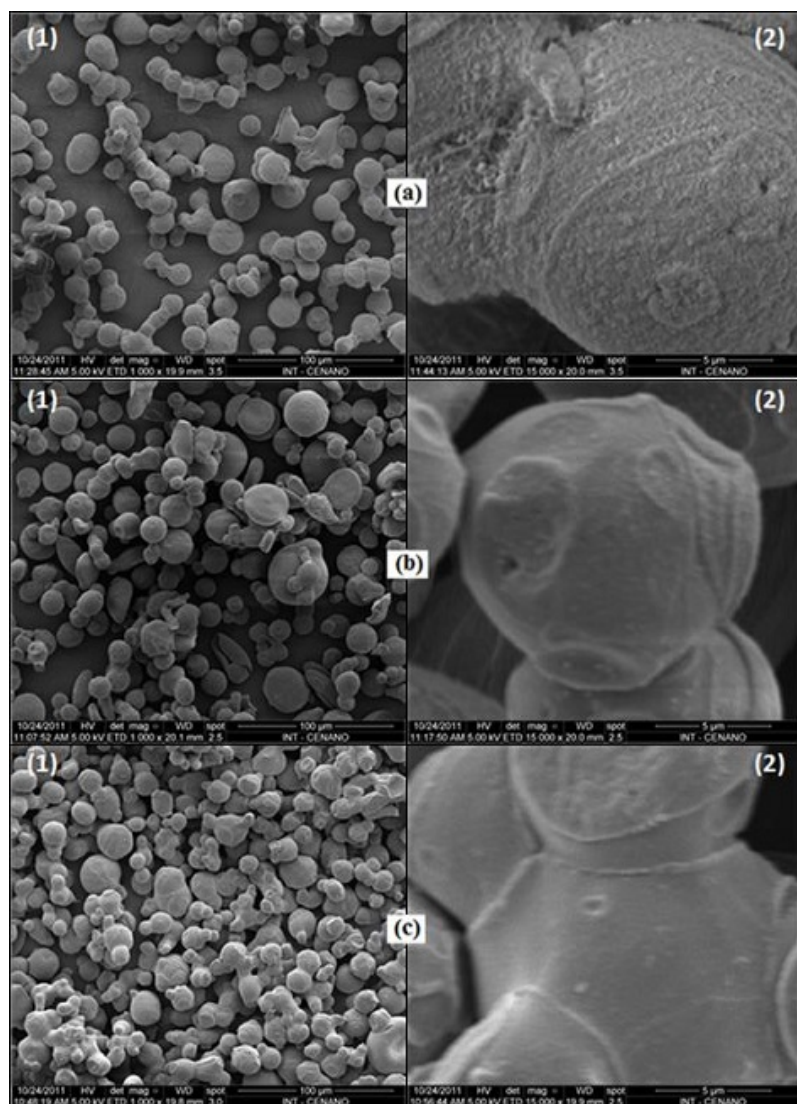
**Table 4.**  
Melting temperature and swelling ratio of the gelatin microspheres as a function of heating time and sucrose concentration.

Sample	Heating time (min)	Sucrose concentration (%)	T <sub>m</sub> (°C)	Swelling ratio
GB	10	0	31.5	1.1
GBt10	10	40	31.5	1.1
GBt2880	2880	0	32.5	1.0

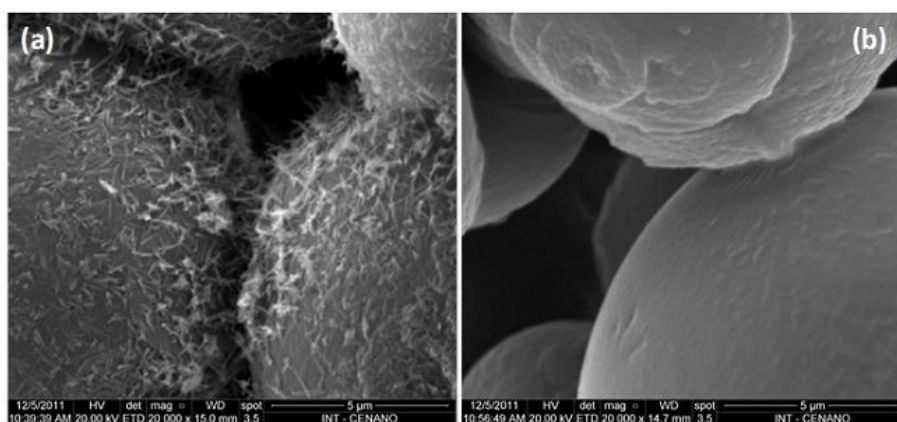
As can be seen in Table 4, the melting point of gelatin microspheres increased when the heating time increased. The only difference between sample GB and GBt10 is that in the last one, sucrose was added during the preparation. The melting points of samples GB (no sucrose) and GBt10 (40% sucrose) did not differ greatly and the sample without sucrose showed a slightly higher melting point than that in the presence of sugar.

We carried out swelling tests because they are a relatively easy way to measure the ability of gelatin microspheres to retain water. The results showed a significant decrease in the water retention with longer heating time. The values of T<sub>m</sub> and swelling ratio corroborate each other, confirming that longer heating time increases the number of crosslinks in the polymer matrix. In contrast, the presence or absence of sucrose in the microspheres had little influence on the data analyzed, which leads us to believe that sugar had little influence on the crosslinks formed.

Figure 3 shows scanning electron micrographs of microspheres of gelatin obtained at different heating times. As can be seen in Figure 3 (left side), no significant differences were observed. The particles present spherical morphology, but there are many agglomerates. Probably, these agglomerates are formed because it was not added a surfactant agent during emulsion preparation. Figure 3 (right side) shows the difference on particles' surface according the heating times. It can be observed that the surfaces became smoother when the heating time increased. With a higher magnification, this difference can clearly be seen when comparing the heating times of 10 and 2880 min (Figure 4).



**Figure 3.**  
Scanning electronic micrographs of the samples GB (a), GBt10 (b) and GBt2880 (c) with 1,000X (1) and 15,000X (2).



**Figure 4.**  
Scanning electronic micrographs of the samples GB (a) and GBt2880 (b) with 20,000X.

Based on these results, there are two possible explanations for these experimental observations. The first one is based on Russo's<sup>[26]</sup> paper. According to him, crosslinks can occur by intermolecular bonds (interstrand), which occur between arginine-lysine or arginine-arginine within the same strand, while amino acid residues from two neighboring strands can also interact and form intramolecular (intrastrand) crosslinked strands, providing strength to the gelatin. The second theoretical explanation is based on carbohydrate chemistry. Sugars commonly exist as cyclic molecules because alcohols react reversibly with aldehydes and ketones to give hemiacetals and hemiketals, respectively. However, in the equilibrium state, there is a mixture of carbohydrate isomers and a small fraction of aldehyde or ketone source. Although small, the fractions of aldehyde and ketone allow the occurrence of common reactions of these organic functions<sup>[27]</sup>. Sucrose is a disaccharide composed of one glucose and one fructose molecule, both reducing sugars. The link between the two monosaccharides (glycosidic bond) forming disaccharide prevents the opening of the cyclic-form portions of fructose and glucose, resulting in the absence of aldehydic and ketonic forms in equilibrium, so the common reactions of these functions do not occur<sup>[27]</sup>. Sucrose is liable only if there is a hydrolysis reaction of the molecule to form the start of monosaccharides, which can only happen with a strongly acidic medium or under the influence of catalysts or enzymes. Since the reaction medium for preparation of gelatin microspheres here did not provide the main conditions for hydrolysis of sucrose, the gelatin remained in its original form, i.e., unable to form crosslinking reactions. Considering the theoretical foundations presented and the results of thermal analysis and swelling, we assume that sucrose does not react with the gelatin chains, so the increase in melting point of the microspheres was only due to the crosslinks formed by intermolecular and intramolecular bonds, which were favored by increasing the heating time.

Although some authors<sup>[7,11,20]</sup> have indicated the use of sucrose as a biocompatible and biodegradable alternative to crosslink gelatin, we found no evidence of chemical reaction between the gelatin amino groups and sucrose. Thus, we decided to test fructose as crosslinking agent because the ketone functional group of fructose is more reactive than the aldehyde functional group of glucose. In order to compare this substance with a traditional crosslinking agent, microspheres crosslinked with glutaraldehyde were also prepared.

Preliminary solubility tests were performed comparing gelatin microspheres crosslinked with fructose (GBF sample) with gelatin microspheres crosslinked with glutaraldehyde (GBG sample). In these tests, a few milligrams of sample were left in contact with water for 24 h. The GBG sample was insoluble while the GBF was soluble. This result showed that the crosslinking of the protein chains' gelatin using sugar as crosslinking agent does not occur as easily under normal conditions, so more factors should be investigated. Therefore, we decided to modify the pH in order to change the equilibrium between cyclic and open

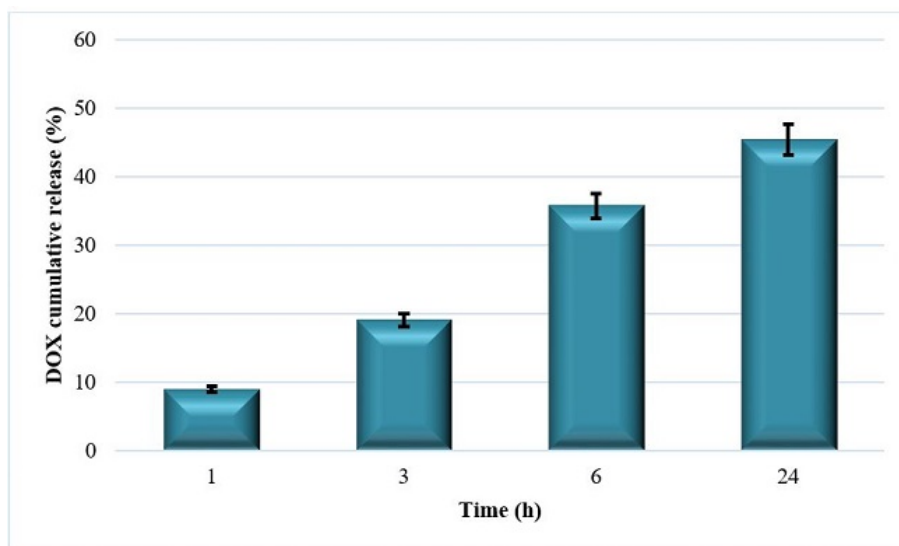
fructose forms and thus provide more ketone available for the formation of crosslinks. According to the literature, the kinetics of bond formation in chemical crosslinking of gelatin solutions is strongly affected by the solution's pH<sup>[28]</sup>, but at pH values higher than 9 and lower than 5 the denaturation enthalpy decreases, indicating that the triple helix amount is reduced<sup>[29]</sup>. Thus, gelatin microspheres were produced by varying the pH.

Preliminary solubility tests were performed and since the aim was to reduce solubility, the gelatin microspheres made with pH 9 solution (GBF9) were chosen.

The thermogravimetric analysis revealed an initial degradation temperature ( $T_{\text{onset}}$ ) of 288 °C for the GBG sample and 294 °C for the GBF9 sample. This analysis also showed that the GBF sample had a residual 10 percentage points higher than the GBG sample. Higher degradation temperatures indicate higher crosslinking degree because more energy is required to break chemical bonds. Likewise, a larger amount of residue confirms that the particle has more strongly linked protein chains. These results show that the fructose crosslinking was successful.

### 3.4 Preliminary drug release tests

We observed that the microspheres absorbed 69% of the doxorubicin that was in solution. If this value is compared in the literature for drug absorption by gelatin microspheres<sup>[13,14,30]</sup>, one can consider that a satisfactory amount of the drug was incorporated into the gelatinous matrix. Figure 5 shows the results of *in vitro* DOX release tests.



**Figure 5.**  
Release of doxorubicin from gelatin microspheres crosslinked by fructose.

The saline solution mimics the biological environment because it has similar pH and osmotic pressure. In these conditions, the gelatinous support gradually increased DOX release over the time. After 24 h, about 45% of the drug was displaced from microspheres to saline medium in



the free form in the solution. In this way, these preliminary release tests show that the method described in this study can be successfully used for the magnetic gelatin microspheres obtainment to incorporation and controlled release of doxorubicin.

#### 4. Conclusions

With the aim of obtaining gelatin microspheres with suitable properties for use in drug delivery systems, we evaluated the experimental parameters using a set of experiments. The statistical results showed that smaller particles can be prepared when low gelatin concentration and high stirring speed are used. By applying these parameters we obtained microspheres with appropriate size to use in arterial drug delivery systems. Because of the large variety of types available in the market, we decided to investigate whether there are significant differences between the use of type A gelatin and type B gelatin. The analyses showed no difference between the two types regarding crosslinking or adsorption of magnetic material in the gelatinous matrix. Furthermore, superparamagnetic samples were obtained with both gelatin types.

With respect to crosslinking of the protein chains, we analyzed whether use of sucrose is effective to make the beads more biocompatible. The microspheres obtained remained very soluble in aqueous media and, so sucrose is not a suitable sugar to crosslink gelatin. Nevertheless, the extent of crosslinking increased as a function of heating time periods. Because of this, we analyzed the use fructose in place of sucrose. Taken together the results obtained indicate that crosslinked gelatin microspheres can be prepared using fructose when the reaction pH is 9. The microspheres crosslinked with fructose were successfully used in preliminary tests of adsorption and release of doxorubicin (a drug that is widely used in the treatment of cancer patients). Thus, the material prepared in this paper has great potential for use in drug delivery systems.

#### 5. Acknowledgements

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