Hybrid Chitosan-Gelatine magnetic polymer particles for drug release

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The chitosan-gelatin magnetic polymer nano/microparticles were synthesized by double crosslinking in reverse emulsion. In this method, sodium sulphate solution was added for ionic crosslinking and glutaraldehyde toluene extracted was added for covalent crosslinking of the polymers. The magnetite nanoparticles were synthesized by hydrothermal method and were used in the preparation process of magnetic polymer nano/microparticles as powder. The characterization of prepared magnetic polymer particles was performed by Fourier Transform Infrared Spectroscopy, Scanning Electron Microscopy, Transmission Electron Microscopy, laser diffraction analysis, Thermal Gravimetric Analysis and Vibrating Sample Magnetometry analysis. The *in vivo* acute toxicity of magnetic polymer particles was evaluated on rats. The swelling capacity in aqueous media of pH 7.4 and the ability to include and release 5-fluorouracil are influenced by the preparation parameters. The very low toxicity of these magnetic polymer particles makes them suitable for being used as potentials drug carriers.

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1. Introduction

Senyei and al. were proposed for the first time the idea of using magnetic particles as a carrier tool for drug delivery [1]. These magnetic particles can be surrounded with a layer of polymer or encapsulated in a polymer matrix that enables them to be functionalized easily. Coating the magnetic particles with a biocompatible polymer increases the circulatory half-life of the drugs from minutes to hours or days. The therapeutic agents can be dissolved, entrapped, attached to, or encapsulated within a polymer matrix with magnetic particle core or with dispersed magnetic nanoparticles. The magnetic particle therapeutic agent conjugate is administrated orally or injected in body. Under the guidance of a strongly external magnetic field, the magnetic polymer particles with the therapeutic agent are guided to the area of the targeted sites where the drugs are delivered [2-4].

The polymer matrix must accomplish several requirements such as biocompatibility, biodegradability, mechanical strength and ease of processing. The mixing of two polymers in preparing of particles is often studied, for improving the biological properties and the drug loading capacity [5-7].

Chitosan is made from the second most abundant natural polymer - chitin, by partial deacetylation in alkaline conditions. It is a biodegradable, biocompatible and non-toxic linear polysaccharide and has many reactive functional groups (amino and hydroxyl). Due to its functional groups, which allow crosslinking and binding of many therapeutic agents, chitosan is widely used in biomedical applications.

Gelatin is a natural protein made by hydrolysis of collagen, which is a fibrous material that exists in bones, skin and in connective tissues of animals [8]. Gelatin is a biocompatible, biodegradable and water soluble polymer. The macromolecular chains of gelatin present many amino and carboxylic groups, which assure an easily crosslinking with different crosslinking agents. For these reasons it is used for preparations of tablets, emulsions, surgical sponges, ointments, salves, jellies, suppositories, plasma substitute for medicines, dietary/health supplements, syrups, etc. in various pharmaceutical applications [9-12].

The preparation methods used for obtaining of polymer particles based on naturals or synthetic polymers or on their derivatives for drug delivery applications are: emulsion crosslinking, emulsion droplet coalescence, coacervation, spray-drying, ionic gelation, reverse micelle preparation, dispersion polymerization, emulsion and miniemulsion polymerization [13-18]. The particles based on chitosan or gelatin can be prepared by chemical crosslinking using glutaraldehyde [19]. The disadvantage of this method is the toxicity of this agent. Ionic gelation, on the other hand, uses non-toxic anions, such as sodium sulphate or sodium tripolyphosphate [20]. In this case the crosslinking occurs by ionic bonds and the main disadvantage is: the obtained particles are soft and pH dependent [21]. Combining the two types of crosslinking, the advantages of this double crosslinking method are: first

of all, the reduction of the amount of the covalent crosslinker (glutaraldehyde) by using instead the ionic crosslinker (sodium sulphate, in our case), less toxic and, the second one, the obtained network is stronger.

C.A. Peptu and Jatariu have already successfully obtained polymer nano/microparticles based on gelatin and chitosan, by using the same preparation method (double crosslinking in reverse emulsion method) [9, 12]. Those encouraging results led us to the idea of preparing magnetic polymer particles based on gelatin and chitosan. To the best of our knowledge such magnetic polymer particles have not been described in literature. The first purpose of our study was to prepare and to characterize the magnetic polymer particles based on chitosan, gelatin and magnetite using a reverse emulsion double crosslinking method. The magnetic polymer particles may be used for storage and controlled release of drugs and also as drug target carriers, especially for applications in cancer therapy. Because of that reason, the second objective of our work was the study of 5-fluorouracil (anticancer drug) inclusion and release.

2. Experimental

2.1. Materials

Chitosan low molecular weight (CS) (91.1% deacetylation degree), Gelatin (GEL), Toluene, Acetone, n-hexane, Sodium sulphate, Glutaraldehyde (25% aqueous solution) for synthesis (GA), Span® 80 and Tween® 80, $FeCl_3.6H_2O$, $FeSO_4.7H_2O$, KOH, 5-fluorouracil (5-FU) were purchased from Merck, Germany. Glutaraldehyde was extracted from toluene and then was used in preparation process of polymer magnetic particles. Glacial acetic acid p.a. grade was received from Chemical Company, Romania. All solutions were prepared with double distilled water. The chemicals used in this study were of analytical grade purity and were used without further purification.

2.2. Methods

2.2.1 Preparation of magnetite powders

Magnetite powders (Fe_3O_4) were prepared by hydrothermal method following the process: A mixture of $FeCl_3.6H_2O$/ $FeSO_4.7H_2O$ was dissolved in a beaker containing distilled water. Under stirring, KOH solution was added into the solution until the precipitate occurred and then transferred to a Teflon-lined stainless steel bomb, non-stirred autoclave and sealed. The reaction was conducted in suitable condition [22]: 3 hours and 150^{o}C. After reaction, the solid was taken out of the Teflon autoclave, then washed with distilled water to remove impurity ions and dried in an oven.

2.2.2 Preparation of chitosan-gelatin magnetic microparticles

Magnetic chitosan-gelatin microparticles were prepared by reverse (w/o) emulsion crosslinking method with two crosslinking agents, the ionic one (the sodium sulphate) and the covalent one (the glutaraldehyde).

The polymer solution (0.5% concentration) was prepared by dissolving chitosan and gelatin (1/1 w/w ratio) in acetic acid solution 2% by continuously stirring at 40°C and then stirred overnight until the complete homogenization. In this solution was added the magnetite powder and the obtained mixture was sonicated for 2 minutes. Then, the Tween 80 (2% concentration in aqueous phase) was added as hydrophilic stabilizing agent. This mixture, which is the aqueous phase, was sonicated again and then dropped in the organic phase (fourth times more than volume of aqueous phase), composed by toluene with 2% Span 80 (as hydrophobic tensioactive agent), by continuously stirring under ultraturaxation (4000-7000 rpm). After that, the ionic crosslinking agent (sodium sulphate solution 10 % w/v), was dropped in the obtained w/o emulsion with a syringe with sharp needle. After 10 minutes of ionic crosslinking, the mixture was added in a glass reactor equipped with a mechanical stirrer. Under vigorous stirring (after 50 minutes at 500 rpm), glutaraldehyde extracted from toluene, as covalent crosslinker, was added in dropwise and the crosslinking reaction was carried out for another hour at room temperature at 500 rpm stirring speed. Glutaraldehyde-saturated toluene was prepared by mixing equal volumes of 25 % (w/v) aqueous glutaraldehyde solution and toluene in a decantation funnel and, after stirring for 10 min, the mixture was allowed to separate. The magnetic polymer nano/microparticles were separated by centrifugation and washed repeatedly with acetone, water and with n-hexane to remove the toluene, the surfactants, the non reacted polymers and the excess of the crosslinking agents. The nano/microparticles were dried from n-hexane at room temperature. The influence of the following preparation parameters (Table 1) on the physico-chemical characteristics of the particles was studied: polymers amino groups-ionic crosslinker (sodium sulphate) molar ratio (1, 5 and 10), polymers-magnetite w/w ratio (1/2, 2/3 and 1/1) and speed agitation on turax (4000, 5500, 7000).

3. Characterization

3.1 Fourier transforms infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) was used for structural characterization of the magnetic polymer particles. The FTIR spectra were recorded for CS, GEL and magnetic polymer nano/microparticles, using a Thermo Nicolet Nexus FTIR spectrophotometer, to confirm the formation of interpenetrated structure and to demonstrate the presence of the magnetite into the prepared particles. FTIR spectra of plain CS, plain GEL, plain magnetite and magnetic polymer particles based on

gelatin and chitosan were obtained under identical conditions. The samples were ground with KBr and pressed into pellets at 200 bars for FTIR transmission measurements. Spectral scanning was done from 4000 to 400 cm^{-1}.

3.2 Scanning electron microscopy

Environmental scanning electron microscopy (SEM) (ZEISS SUPRA 40VP, Colonne Gemini) was used to examine the surface morphology as well as the size of the magnetic polymer particles and of the magnetite nanoparticles. The samples were spread in very thin layer on tape support covered with carbon and then were coated by gold which was carried out by the BALTEC SCD 005 SPUTTER COAT. The microphotographs were recorded at an accelerating voltage of 3.0 kV, at different magnification (5.000-30.000 times).

3.3 Transmission electron microscopy

Transmission Electron Microscopy (TEM) was used to determine the particles size and the distribution of the magnetite nanoparticles inside the polymer magnetic nano/microparticles. The morphology of the magnetic polymer particles was detected using a transmission electron microscope TECNAI F30 G^2 with linear resolution of 1Å. Samples for TEM measurement were prepared by dispersing the magnetic polymer particles in acetone at an appropriate concentration and a drop of aqueous dispersion was deposited on a carbon film coated copper grid before analysis.

3.4 Particles size analysis

The magnetic polymer particles were analyzed for their size and size distribution. Nano/microparticles were dispersed in acetone (to avoid the swelling) vortexed for 2 minutes and ultrasonicated for 60 seconds before sampling. The particles size was measured by laser diffraction (SHIMADZU SALD 7001, Japan) and plotted for size distribution using the software supplied by the manufacturer.

3.5 Thermo gravimetric analysis

The magnetite content of the magnetic polymer particles was determined by thermogravimetric analysis (TGA), using a SDT-Q600 analyzer under nitrogen atmosphere, (100 ml/min) between 25 and 900°C, at a temperature rate of 20°C/min. The weight of the samples was situated between 10 – 15 mg. The thermal tests were accomplished for gelatin, chitosan, magnetite and for magnetic polymer nano/microparticles, in order to calculate the magnetite content. The operation parameters were kept constant for all the samples, in order to get comparable data. Curve processing designed to determine the thermal and kinetic characteristics was done using the software developed by Q Series™ Thermal Analysis.

3.6 Magnetic properties

For drug delivery, the superparamagnetism is a characteristic required because, when the external magnetic field is removed, the magnetization need to disappear (negligible remanence and coercivity) and in this way the agglomeration of the particles and the possible embolization of capillary vessels are avoided. The magnetic properties of the magnetic polymers nano/microparticles were measured using a vibrating sample magnetometer (MicroMag, VSM - Vibrating Sample Magnetometer, Model 3900, Princeton Measurements Corporation, USA), at room temperature.

3.7 Toxicity of particles

To use the particles for drug administration is necessary to determine the toxicity of magnetic polymer particles. That was evaluated by the average lethal dose (LD_{50}), which is defined as mg of active matter per kilogram of adult animal body. To measure the LD_{50}, particles were administered on rats weighing 20±2 g, as a suspension in Tween 80, via the intraperitoneal way, as described by the Speerman–Karber method [23].

3.8 Swelling experiments

The swelling degree of magnetic polymer particles was gravimetrically determined by measuring the extent of swelling of the particles in phosphate buffer solution (PBS, pH = 7.4). To ensure complete swelling, the particles were suspended in PBS for 24 hours. After the time period, the samples were ultracentrifugated and excess water was then carefully removed by swabbing with filter paper. The samples were weighed to an accuracy of 0.01 mg with an electronic microbalance. The swelling degree (Q) was calculated with equation (1):

$$Q(\%) = [(m_s - m_0)/m_0]*100 \quad (1)$$

where m_s is the weight of swollen particles and m_0 is the weight of the dry particles. All the experiments were performed in triplicate and average values were considered for data treatment and calculations.

3.9 Drug loading

Drug loading was investigated by diffusion. A well known amount of swollen magnetic polymer nano/microparticles was kept for 24 h in a thermostatic batch, at 37 °C, in 1 ml of an aqueous solution containing 5-fluorouracil (10 $mg.ml^{-1}$). The particles used were previously swollen in order to avoid the competition between water and drug molecules. Then, the drug loaded particles were ultracentrifugated and dried by liophilization. The amount of the retained drug was determined by accounting for the amount of drug in the supernatant, using a previously made calibration curve at 265 nm wavelength.

3.10 The *in vitro* release studies

The *in vitro* drug release studies were realized by dialysis. The drug loaded magnetic polymer particles, precisely weighed, were introduced in the dialysis membranes (12000 Da) and, after that, were individually added to 10 ml phosphate buffer solution (pH=7.4 similar of blood), in flasks. All the flasks were shaken in a thermostat device at 37°C for all the release period. At regular time intervals the drug released and present in the medium was spectrophotometrically determined with the UV-Vis spectrophotometer NanoDrop and estimated using the same calibration curve at 265 nm wavelengths.

The release efficiency was calculated according to equation (2):

$$\text{Efficiency } (\%) = (m/m_t)*100 \quad (2)$$

where m is the amount of drug released from particles and m_t is the amount of the drug loaded in the particles.

Table 1. Preparation parameters for magnetic polymer nano/microparticles (Polymers amino groups/Ionic crosslinker, Agitation speed, Polymers/MNP), the average diameter and the magnetite (MNP) content from final particles CGMPs.

Sample code	Polymers amino groups/Ionic crosslinker* (molar)	Agitation speed (rpm)	Polymers/MNP (w/w)	D (μm)	Magnetite (%)
CGMPs1	10	4000	1/1	1.74	12.30
CGMPs2	10	5500	1/1	1.68	10.29
CGMPs3	10	7000	1/1	1.54	10.68
CGMPs4	5	7000	1/1	1.35	17.67
CGMPs5	1	7000	1/1	1.05	19.62
CGMPs6	1	7000	2/3	0.89	28.32
CGMPs7	1	7000	1/2	0.74	33.03

* sodium sulphate solution (10% w/v) as ionic crosslinker
CS/GEL (w/w) = 1/1 and polymers/GA (w/w) = 22.32, for all the formulations

4. Results and discussion

4.1 Fourier transforms infrared spectral study

FTIR spectra of GEL, CS and magnetic polymer microparticles are compared in Fig. 1. FTIR spectral data show the specific absorption bands of two natural polymers, proving that the ionic and covalent crosslinking has been realized and confirm the presence of the magnetite in microparticles.

In the case of magnetic polymer nano/microparticles, all the peaks appeared both in GEL and CS were observed. In addition, a new band was observed at 1657 cm^{-1}, indicating the C=N stretching vibration of the imine group of Schiff base. This band confirms the formation of crosslinking by reaction of amino groups of the gelatin and of the chitosan with carbonyl groups of the glutaraldehyde. The band at 1030 cm^{-1} is due to the presence of acetal group resulted from the reaction of GA with hydroxyl groups of CS. The band corresponding to ionic crosslinking (as it can be seen in the situation of polymer (CS-GEL) particles [12]) is superposed over the band characteristic to magnetite. Also, in the FTIR spectra of the magnetic polymer nano/microparticles, there is a band at 563 cm^{-1} which confirms the presence of the magnetite (Fe_3O_4) in the composition of the particles. Thus, FTIR data confirm the successful crosslinking of both GEL and CS to form interpenetrated network (IPN) structure in the presence of ionic and covalent crosslinkers.

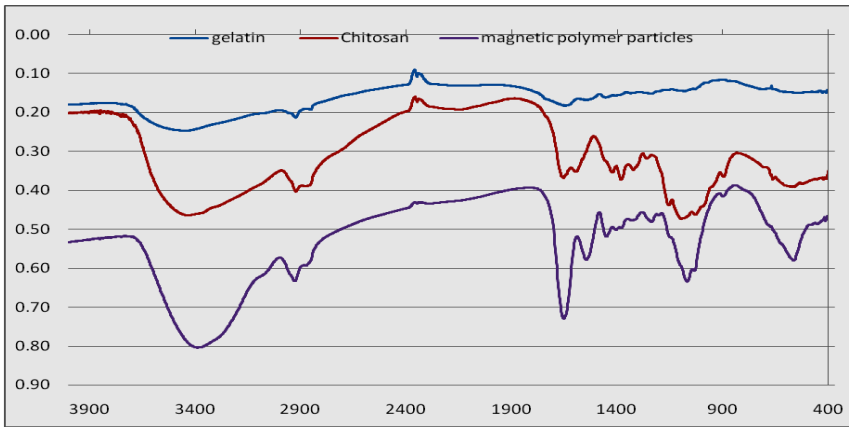


Fig. 1. The FTIR spectra for (GEL, CS) polymers and for magnetic polymer nano/microparticles, sample CGMPs1.

4.2 SEM

Scanning electron microscopy for magnetite nanoparticles presents a nearly spherical shape of freshly prepared magnetite particles with average diameter D = 40 nm, as it can be seen in Fig. 2, confirmed also by the dimensional analysis [22].

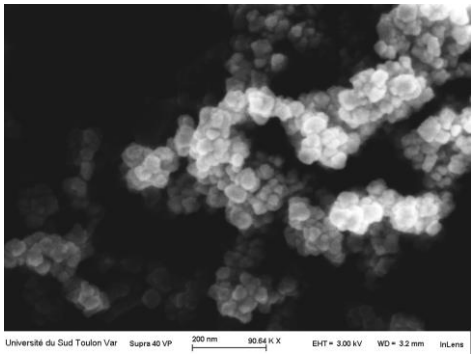


Fig. 2. The SEM microphotograph of magnetite nanoparticles (90.640 x).

The SEM microphotographs of magnetic polymer nano/microparticles (sample CGMPs3, Fig. 3) show the particles round in shape, whit harsh surface and compact in nature; also, a main particle population was observable, in the micron range and relatively homogenous as dimensions, and the average diameter medium being about 1.54 μm. The dimensions were confirmed by dimension analysis. As shown in the photograph for these types of particles, the particles are not very aggregated. They can be readily dispersed in water.

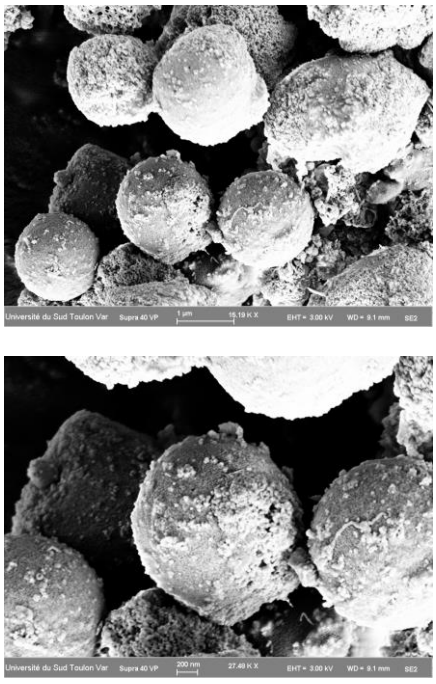


Fig. 3. The SEM microphotographs of magnetic polymer particles, sample CGMPs3 (15190 x and respectively 27480 x).

4.3 TEM

Fig. 4 presents the TEM microphotographs of magnetic polymer particles, sample CGMPs3. The particles' shape is spherical and the internal structure of the magnetic polymer spheres can be easily observed. The network polymer becomes transparent/clear. The small magnetite nanoparticles are easily identified, because they absorb the electron beam and the magnetite particles appear as dark spots within the polymer matrix.

The TEM images show no evidence for the presence of polymer particles without incorporated magnetite and no magnetite nanoparticles outside the polymer particles. Every nanoparticle of magnetite seems to be covered by polymer layer. At higher magnifications, the detailed structure of the magnetite nanoparticles within the polymer particles are clear, with darker spots in the center meaning thicker areas and lighter smaller spots on the edges meaning thinner areas of the particles.

As shown in Fig. 4a, in the smaller particle, the single magnetite entities seem to be still well separated. The dark grainy structures near the middle of the sphere, around 40 nm in diameter, appear similar in size to the individual magnetite nanoparticles (Fig. 2). The distribution of magnetite particles inside the polymer sphere seems to be rather homogeneous (Fig. 4b and Fig. 4a, the bigger particle), if the particles are higher and randomly distributed in smaller particles (Fig 4a, the small particle). The larger in diameter the particle, the darker it appears in the TEM photographs, which qualitatively suggests similar concentrations of magnetite nanoparticles relative to polymer within each particle.

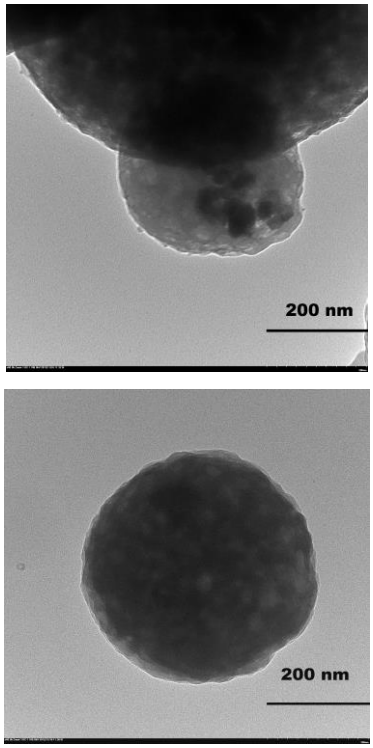


Fig. 4. The TEM microphotograph of sample CGMPsS3, a) 200 nm x 40.0 kV; b) 200 nm x 40.0k Zoom 1-HC 1 100.0 kV.

4.4 Size distribution

To limitate the swelling behavior, the magnetic polymer particles were dispersed in acetone, in order to obtain the same dimensions to those in dry state and then analyzed by laser diffraction granulometry. The obtained values for all preparations are presented in Table 1.

The size distribution profiles are presented in Fig. 5. The curves obtained present a monomodal distribution and the particles have a rather narrow size distribution. It has been noticed that particle size and size polydispersity are strongly dependent on polymers' molar ratio of amino groups/sodium sulphate, on stirring speed and on polymers/magnetite ratio. The average diameter of the magnetic polymer particles decreases with the increase of the stirring speed. The formulation prepared at 4000 rpm speed in ionic crosslinking step have a medium diameter of 1.74 μm, while formulation prepared at 7000 rpm have the medium diameter of 1.54 μm. This is due to formation of a smaller droplets in the emulsion prepared with higher stirring speed.

Another interesting observation is that particle size decreased with an increase in the amount of ionic crosslinking agent. It is observed that particle size of CGMPs5 (with 1/1 polymer amino groups/sodium sulphate molar ratio) is lower than that of CGMPs3 (with 10/1 polymer amino groups/sodium sulphate molar ratio), due to the formation of a network structure with a higher crosslinking density. The free amino groups of polymers (chitosan and gelatin) are able to ionic crosslinking almost instantaneously. That fact determines the polymer chain wrapping. Thus, the shrinking of polymer network is more pronounced, and consequently, the smaller the particles sizes as the polymers' NH_2/ Na_2SO_4 molar ratio the lower is.

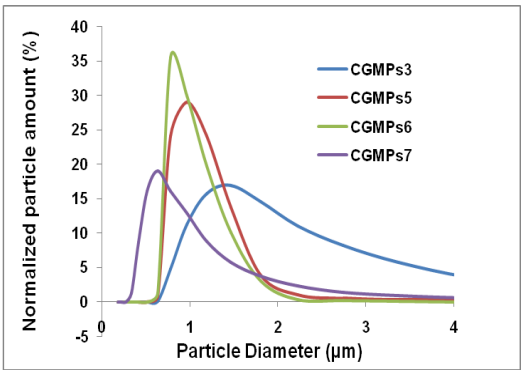


Fig. 5. Average size of magnetic polymer particles determined by diffractometry measurements.

The same behaviors were observed and reported in literature [24, 25]. A big amount of ionic crosslinker determine a higher crosslinking density so a smaller diameter of particles.

The particle diameter is also higher with the polymers mixture/magnetite ratio and it varies slowly between the 1.00 to 0.74 μm. Their size increased with polymer/magnetite ratio in relation with the increase in polymer content and decrease in magnetite one. The same behavior was found by Tataru and Balaita[24, 26].

4.5 TGA

Thermogravimetry is a good method for the mass loss determination of individual components. The TGA curves of magnetic polymer particles (not shown) indicate decomposition phenomena in the 30–900˚C temperature interval.

All the samples show three degradation steps and the residual amount after heating was situated in the range of 50-67%. The mass loss of magnetite by degradation is only 0.86%, in the 70–120 ˚C interval, characteristic for water elimination.

It may be estimated that the loss mass in the degradation process of the magnetic polymer particles is due to the degradation of the polymers and the particles residuum is composed by the magnetic material and the residuum of polymers.

The first step of the weight loss is around 8% in the 70–120 ˚C interval, characteristic for water elimination from particles. The second weight loss (around 40%) at 250– 600 ˚C and the third step at 800 ˚C are characteristic for the decomposition of polymer matrix. This indicates that the particles contain 10-33 % of inorganic magnetite. As we expect, the magnetite content of the magnetic polymer particles significantly increased with increasing of the magnetite in the initial mixture (or with the decrease of the polymers/magnetite weight ratio, Table 1). The high magnetite content of CGMPs7 sample indicates that they have a strong magnetic sensitivity under an external magnetic field, which has been found by the fact that the magnetic polymer particles move toward the outer field very quickly and obviously can be separated completely from acetone or water in very short time (Fig. 6).

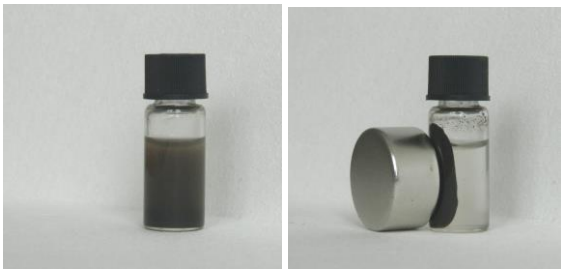


Fig. 6. The magnetic polymer particles in acetone: first image, in absence of magnetic field; second image, in presence of magnetic field generated by an external magnet.

4.6 Microparticles toxicity

The toxicity of the magnetic polymer particles was determined on the basis of the average lethal dose (LD_{50}). The results are presented in Table 2. The particle systems were compared with the pure polymers used for synthesis and with polymer particles (prepared without magnetite by Peptu [9], using the same preparation method).

Table 2. The values of lethal dose (LD_{50}) for the samples prepared with polymers only and with polymers and magnetite.

Sample	LD_{50} (mg/kg body mass)
Gelatin	8190
Chitosan	7700
gelatin and chitosan particles	7140
Magnetic gelatin and chitosan particles (sample CGMPs3)	5400

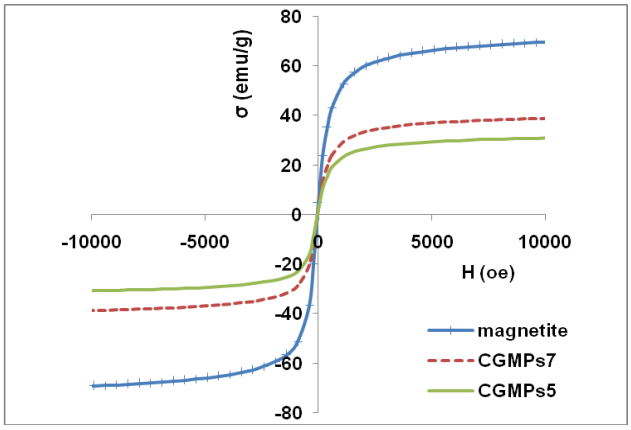
According to Hodge–Sterner toxicity scale [27], a LD_{50} value under 1 mg/kg body mass is considered extremely toxic, between 1and 50mg/kg body mass very toxic, between 50 and 500 mg/kg body mass moderately toxic, between 500 and 5000 mg/kg body mass low toxic, between 5000 and 15.000 mg/kg body mass is practically nontoxic, and above 15.000 mg/kg of body mass is considered nontoxic.

The values obtained did not vary much compared with pure polymers or with polymer particles; therefore, the magnetic polymer particle systems are in the category of practically nontoxic, so they can be used in biomedical applications.

4.7 VSM

All the prepared magnetic polymer particles were analyzed and exhibit similar superparamagnetic behavior at room temperature (no remanence in the absence of a magnetic field was observed). The magnetometer measurements confirmed the superparamagnetic behavior of the magnetic polymer particles.

The presence of the polymer (non-magnetic material) is obvious by the fact that saturation magnetizations of particles CGMPs are about one half of value for the used pure MNP (magnetite) (69.98 emu/g). The values of magnetization of saturation (Ms), for magnetic polymer particles are in a good agreement with magnetite content obtained from TGA measurements. For example (figure 7), the sample CGMPs5 show a saturation magnetization (M_s) of 31.12 emu/g and the content of magnetite is 19.62%, while the sample CGMPs7 present a saturation magnetization M_s = 37.24 emu/g and the content of magnetite is 33.03%. Polymers are not a magnetic material, consequently the increase of the saturation magnetization can be considered an indication of the polymers participation in particles' composition. The same behavior was found by Braconnot [28]. All the magnetic polymer particles present sufficient magnetization (about 30 emu/g) so, the magnetization properties of the magnetic polymer particles are strong enough for magnetic drug carrier. As expected, the polymer matrix added reduces the magnetite content, consequently, reduction in the saturation magnetization as reported in Fig. 7.



Fig, 7. Magnetizations curves for magnetite, CGMPs7 (at 1/2 polymers/magnetite ratio) and CGMPs5 (at 1/1 polymers/magnetite ratio).

4.8 Swelling studies

The loading and the release of drugs (by diffusion) from polymer network matrices are influenced by swelling of the crosslinked nano/microparticles. The swelling degree (presented in Table 3) is influenced by the preparation parameters of magnetic polymer particles and also, it is directly proportional with the particles dimensions. The correlation between particles dimensions and preparation parameters was already discussed. With increase of turaxation speed, the medium diameter decreases, the swelling degree decreases also, from 492% (sample CGMPs1) to 363% (sample CGMPs3). With increase of amount of ionic crosslinker agent (sodium sulphate) used for preparation process, Q decreased from 363% (sample CGMPs3) to 309% (sample CGMPs5). This reduction in swelling is due to the formation of a rigid polymer network with higher crosslinking density. The same behavior was found by other authors [25, 29]

The swelling degree for samples CGMPs5, CGMPs6 and CGMPs7 increased with the polymer/ magnetite ratio (Table 3). Considering that the polymer is the material that determines the swelling, the increase of the magnetite content determines the decrease of polymer content consequently, the decrease of the swelling degree.

4.9 Drug loading

The diffusion of drug in particles follows the swelling behavior. The amount of drug loaded into the nanoparticles is presented in the Table 3. The largest loaded drug amount was recorded for particles with the highest degree of swelling (CGMPs1 formulation) and the lowest at CGMPs7 samples, according to the swelling degree.

Table 3. Swelling degrees, amount of drug loaded and release efficiency for magnetic polymer nano/microparticles.

Sample code	Q (%)	5-FU/particles (mg/g)	Release efficiency (%)
CGMPs1	492	91	84±0.56
CGMPs2	415	85	82±0.82
CGMPs3	363	74	83±0.67
CGMPs4	331	69	87±1.03
CGMPs5	309	62	86±0.73
CGMPs6	277	57	85±1.11
CGMPs7	262	55	86±0.90

4.10 Release kinetic

In vitro release experiments were carried out in an alkaline environment, simulating the blood conditions (pH = 7.4) with phosphate buffer solution. The drug release efficiency is more than 80% for all the formulations. Average weight of cumulative release in time plots for drug loaded particles of CGMPs4 formulation (who has the highest release efficiency of drug loaded) are displayed in Figure 5, the red line. The drug release profile from the particles presents two phases: an initial rapid burst phase ("burst effect") for the first 400 minutes of release, followed by a slower phase for the drug release, for the next 1000 minutes. The burst phase is probably due to the release of drug molecules adsorbed on the particles' surface while the slower phase may be attributed to the drug entrapped in the particles matrix (IPN network).

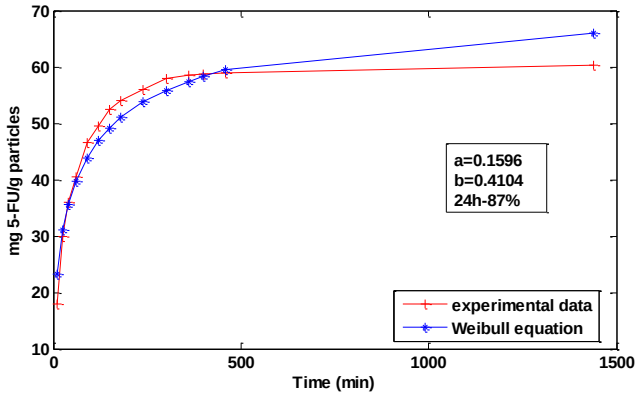


Fig. 8. The 5-FU release kinetic for CGMPs4 sample. Experimental data is presenting with red line and blue line is the corresponding fitting by Weibull model.

The data obtained from *in vitro* kinetic release were fitted to kinetic equation such as first-order Weibull model (see line blue in Figure 8). The results indicated that the drugs release from particles follow Weibull model. The Weibull equation (3) describes quite well the drug release data for all the samples:

$$Q_t/Q_\infty=1-\exp(-a\cdot t^b) \quad (3)$$

where *a* and *b* are constants and Q_t and Q_∞ are cumulative amounts of drug released at time t and infinite time, respectively [30, 31]. The value of b=0.4104 obtained show that the release follows the Fickian diffusion in fractal space.

For the first 60% released drug, the Korsmeyer-Peppas equation (4) was applied

$$Q_t/Q_\infty=k\cdot t^n \quad (4)$$

where Q_t / Q_∞ is fraction of drug released at time *t*, *k* is a kinetic parameter that represents drug-polymer interaction and *n* is an empirical parameter that depends on the system geometry and characterize the nature of the release mechanism. The *n* value (determined from the experimental data) informs on the validity of the Fickian transport mechanism.

The value of the release exponent obtained (n=0.4423) is lower but very close to the limit of 0.5 value, which indicates a predominantly Fickian behavior for the release of 5-FU from particles.

5. Conclusions

New double crosslinked magnetic interpenetrated network matrix nano/microparticles of gelatin and chitosan have been prepared and investigated for the controlled release of anticancer drug (5-fluorouracil). The SEM microphotographs of the magnetic polymer nano/microparticles demonstrated a spherical morphology and indicated a polydisperse size, which was confirmed by laser difractometry. The TGA results indicated that the magnetite content of CGMPs were between 10-33 % by weight. CGMPs are superparamagnetic, the magnetization of saturation is situated in the range of 30-37 emu/g. The specific saturation magnetization was found to be related to chemical composition of the particles and is proportional to the weight content of magnetic material. The swelling capacity, the 5-FU loading and respectively release can be controlled by modulating the preparations parameters (polymers/ionic crosslinking ratio, polymers/magnetite ratio and stirring speed) in preparation process. The 5-FU loading and *in vitro* release were shown to be related to the swelling behavior in aqueous environment. The prepared polymer magnetic nano/microparticles present superparamagnetic properties proving by the absence of hysteresis on the magnetization curve and the acute toxicity was similar to the individual polymers, practically nontoxic degree of toxicity for the particles.

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