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Chitosan and Poly (Vinyl Alcohol) microparticles produced by membrane emulsification for encapsulation and pH controlled release

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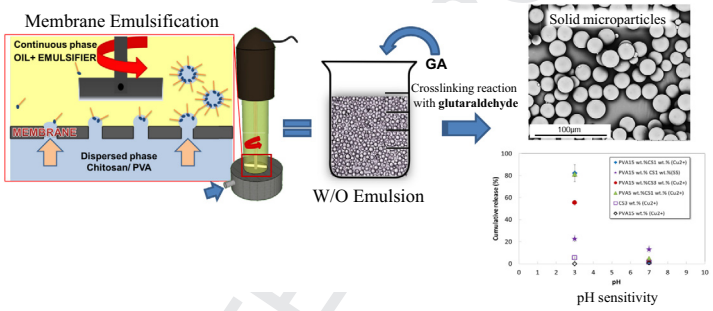
HIGHLIGHTS

- Membrane emulsification was used for the formulation of polymeric drops.
- Membrane with sharp pore openings produced smaller and more uniform drops.
- Glutaraldehyde was used for chemical crosslinking of the liquid droplets.
- Uniform and pH sensitive microparticles were produced using chitosan and PVA.
- Degree of crosslinking and chitosan–PVA blends influenced the release.

GRAPHICAL ABSTRACT



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ABSTRACT

The Dispersion Cell membrane emulsification technique was used for the production of w/o emulsions with controlled droplet size and narrow size distribution. The influence of the operating parameters of the process was investigated. Varying the dispersed phase flux (10–1250 L h^{-1} m^{-2}) and the shear stress (2–59 Pa), droplets between 30 and 280 µm were produced with *CV*'s as low as 18%. Nickel and stainless steel membranes were used for the membrane emulsification. Pore geometry influenced the droplet size as well as uniformity and a normally hydrophilic stainless steel membrane with sharp pore openings produced more uniform and smaller drops compared to a PTFE coated hydrophobic nickel membrane with a conical pore surface. For the dispersed phase 15 wt.% PVA or 1–3% wt.% chitosan as well as their blends in water were used. Surfactants PGPR and ABIL EM90 were tested to determine their capability to form stable emulsions in Miglyol 840. PGPR could not be used to stabilize the emulsion with chitosan as the dispersed phase, probably due to the chemical interference between the carboxyl group present in the PGPR and chitosan. Solid microparticles were obtained by chemical crosslinking with glutaraldehyde (GA) at different concentrations (1–50 vol.%). Particles crosslinked using less than 10 vol.% GA were able to swell and release encapsulated compounds. Acid sensitive particles were produced by blending the PVA and chitosan. Up to 80% of Cu^{2+} and 20% of sodium salicylate was released from the particles under acidic conditions. No significant release was determined under neutral conditions.

1. Introduction

Formulation of polymeric particles for the purpose of drug release systems has received increased interest over recent years [1]. Conventional oral drug administration methods, such as solutions, suspensions, tablets, capsules [2], often do not provide protection from the acidic environment present in the stomach [2]

Abbreviations: PVA, Poly (Vinyl Alcohol); PVAc, Poly Vinyl Acetate; *CV*, coefficient of variation; CS, chitosan; Miglyol 840, propyleneglycol dicaprylate/dicaprate; PGPR, polyglycerol polyricinoleate; ABIL EM 90, modified polyether–polysiloxane; GA, glutaraldehyde; AAS, Atomic Absorbance Spectrophotometer; SS, sodium salicylate.

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and do not provide a constant therapeutic blood concentration of the drug over time [3,4]. A re-administration of the drug is required and the possibility of drug reaching the toxic levels. Encapsulating the drug into a polymeric matrix makes it possible to tailor the release, obtaining a site-specific release, prolonged action and/or sustained release [5,6]. The use of polymers enables the production of formulations able to respond to specific environmental stimuli such as pH [7], temperature [8] and magnetic fields [9]. The capability to release a drug depending on the pH can be exploited to tailor the release in a specific part of the gastro-intestinal tract, the pH in the stomach varies from 1.5 to 3.5, while entering the intestine the pH varies from 5 to 7.5 [2]. pH dependent release can be very useful for the administration of medicines that have the stomach as the site of action and that can be potentially dangerous for the intestine [10]. Chitosan is a pH sensitive polysaccharide which has been extensively used for the tailoring of active compounds in the stomach [11], on its own or forming interpenetrating polymer network hydrogels [12] with other polymers [13–15]. Chitosan is the deacetylated form of chitin (poly-β-(1 → 4)-N-acetyl-D-glucosamine), the second most abundant polymer in nature [11,14]. The molecular weight and degree of acetylation determines the properties of chitosan [14]. Because of its biocompatibility, biodegradability, low cost and ability to open intercellular tight junctions, this polymer is a valuable excipient for oral drug delivery systems [16]. The presence of amino groups is the key factor that makes the polymer sensitive to pH variation: below their pKa (6.3) the amino groups of chitosan become positively charged, leading to swelling of the polymer in relation to two principal mechanisms: a *mass transfer* in the bulk of the polymer, and an *electrostatic repulsion* among the polymeric chains [15]. The process of swelling in acidic environment and the subsequent de-swelling in neutral-basic environment is called *dynamic swelling* [15]. These characteristics make chitosan an effective polymer for the production of pH sensitive formulations. Solid chitosan microparticles can be produced by crosslinking the polymer ionically or covalently [17]. Covalent crosslinking involves the formation of chemical bridges between polymeric chains; using bifunctional reagents such as glyoxal or glutaraldehyde. Berger et al. [17] reported a decrease in pH sensitivity of chitosan when covalently crosslinked due to most of the amino groups present in chitosan reacting with the crosslinker. The addition of another polymer able to react with the glutaraldehyde, can reduce the chitosan crosslinking density and increase the number of protonable amino groups available [17]. Poly (Vinyl Alcohol) is a water soluble, biocompatible and pharmaceutically approved [18] polymer that also reacts with glutaraldehyde producing a material with proven stability in both highly acidic and highly alkaline conditions [19]. Poly (Vinyl Alcohol) has a chemical structure with pendant hydroxyl groups originated from the hydrolysis of Poly Vinyl Acetate (PVAc) [20]. Crosslinked Poly (Vinyl Alcohol) on its own has the capability to sorb water and swell [20]; allowing drug dispersed within the polymeric matrix to diffuse out, but it does not show pH sensitivity [21,22]. Particles made of only Poly (Vinyl Alcohol) can be suitable for the release of active molecules with time [3] depending on the extent of the crosslinking reaction [20,22]. For the production of pH sensitive formulation, blends of chitosan and PVA can be produced, combining the Poly (Vinyl Alcohol) chemical, thermal and physical stability with the pH sensitivity of chitosan [14,15,23]. In the work presented here an alternative method for producing highly uniform particles with the desired dimensions was adopted: the particles were obtained by solidification of liquid polymeric droplets produced by *membrane emulsification*. Using this technique, emulsions are produced drop by drop injecting a dispersed phase through the pores of a membrane into the continuous phase using low shear. With this method it is possible to produce uniform sized droplets (*CV* of 10–20%) [24]; by modifying some of the operating parameters of the process [25,26]. Furthermore, membrane emulsification overcomes some of the disadvantages typical of the classical methods of emulsion production such as: unreliable scale-up, insufficient droplet uniformity, mechanical stress due to high forces applied (high shear), and poor batch to batch reproducibility [27]. The possibility to use low shear stress for droplet production has great advantage in pharmaceutical and biological applications when delicate and/or shear sensitive materials as well as thermosensitive substances should be emulsified. Compared to other methods for drop by drop generation: microchannels and microfluidics; which can produce drops with *CV* below 5% [24], membrane emulsification as a process has far greater productivity and can be scaled up [24,28].

Chitosan particles (but not blended with PVA) have been obtained by membrane emulsification [29] for the encapsulation of sensitive compounds such as insulin [30] or proteins [31]. For their production cross-flow membrane emulsification [32] in combination with tubular ceramic (SPG) membranes was used [32]. Cross-flow membrane emulsification, where the shear is induced by the recycled flow of the continuous phase, is not appropriate for the production of droplets larger than 20 μm, due to droplet break up in the pump. In addition, SPG membranes have highly tortuous pores [24] which can be fouled quite quickly and operate under very low transmembrane flux [24].

In the present work an alternative membrane emulsification system The Dispersion Cell [33,34] has been used for production of w/o emulsions in combination with microsieve membranes (metal foils with straight through pores) which are less prone to fouling compared to the SPG and ceramic membranes [24] and operate under higher transmembrane fluxes [24]. SPG and ceramic membranes are brittle and therefore their use is limited in food and pharmaceutical applications. In the literature the Dispersion Cell has been mainly reported for production of o/w emulsions [32,35–37]. Up to date only two papers deal with generation of w/o emulsions to produce silica [38], and alginate [8] particles therefore we investigate the most suitable microsieve membranes for production of w/o emulsions (hydrophobic nickel and stainless steel) in combination with formulation to obtain the most uniform polymeric droplets (chitosan (CS), Poly Vinyl Alcohol (PVA) or PVA-CS blends) in an oil continuous phase. A new strategy combining the crosslinking with emulsion generation has been developed to produce particles having different release profiles depending on the polymeric composition and the crosslinking.

2. Materials and methods

2.1. Materials

The oil phase in the w/o emulsion was produced by mixing Miglyol 840 (propyleneglycol dicaprylate/dicaprate SASOL, Germany) with oil soluble surfactants: 2 wt.% PGPR, polyglycerol polyricinoleate, (ABITEC, USA) or 2 wt.% ABIL EM 90, modified polyether–polysiloxane, (EVONIC industrials, Germany). The water phase in the w/o emulsions contained either pure Poly (Vinyl Alcohol) (PVA) (MW 13.000–23.000 g/mol, degree of hydrolysis 87–88% Sigma Aldrich, UK), chitosan (CS) (MW 50.000–190.000 g/mol Sigma Aldrich, UK) or PVA and CS blends. PVA solution was prepared by dissolving predetermined amounts of PVA in warm water (70–80 °C) 0.1 M HCl (Fisher Scientific, UK). CS is soluble is slightly acidic solutions with a pH below 6. The solubility of CS depends on the charge of the polymer: the acidic environment protonates the amino groups making the polymer water soluble, on the other hand, if the pH is increased the polymer loses the charge and becomes essentially hydrophobic [39]. CS was dissolved in warm (50–60 °C) 6 vol.% acetic acid. After solubilization the polymeric

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solutions were cooled to room temperature and blended to appropriate ratios. Final solutions were stirred constantly for at least 2 h. Where appropriate, the inner water phase contained 5000 ppm of Cu^{2+}; with 20,000 ppm of $CuSO_4 \times 5H_2O$ (MW = 249.685 g/mol) or 13,500 ppm $CuCl_2 \times 2H_2O$ (MW = 170.48 g/mol both supplied by Fisher Scientific, UK) as a Cu^{2+} source, or 3000 ppm of sodium salicylate (SS) (MW = 160.11 g/mol Sigma Aldrich, UK) was encapsulated as a model drug, in separate samples. As a crosslinker 50 wt.% glutaraldehyde (GA) was used (Sigma Aldrich, UK) diluted as appropriate. The densities of both dispersed and continuous phases were measured. The viscosity of both dispersed and continuous phases were measured using a Rheometer AR100-N (TA instrument, USA), at 20 °C, using a cone-plate configuration. The cone geometry was 4 cm in diameter, 1.59°, with a truncation of 56 μm. The equilibrium interfacial tensions at the water/oil interface were done by the Du Nouy ring method [40] using a White Electric Instrument tensiometer (model DB2KS). The physical properties of the surfactant solutions and the equilibrium interfacial tensions are listed in Table 1.

2.2. Methods

2.2.1. Dispersion Cell and membranes

The w/o emulsion was produced using the Dispersion Cell and flat disc metal membranes (Fig. 1(a)) supplied by Micropore Technologies Ltd. (Derby, UK). A two blade paddle stirrer (governed by DC power supply INSTEK, model: PR3060) placed above the membrane provided the rotation speed (300 –2000 rpm) and subsequently the shear (2 –59 Pa) needed for droplet detachment. Production of w/o emulsions using membrane emulsification is challenging and membrane wetting should be avoided therefore PTFE coated hydrophobic nickel and stainless steel membranes (Micropore Technology Ltd., Derby, UK), both having different top surfaces, have been investigated. Membranes with 10 μm pore diameter and 200 μm pore spacing were mainly used, except where reported differently.

2.2.2. Experimental procedure

The w/o emulsion was prepared using a stationary disc membrane placed in the bottom of the Dispersion Cell (Fig. 1(a)). The polymeric water phase was injected through the membrane using the syringe pump (World Precision Instrument Inc., AL-1000, UK) using fluxes between 12 and 1250 L h^{-1} m^{-2}. Previous work by Pan et al. 2012 [41] showed that the contact angle of the hydrophilic membrane can be changed so the membranes were pre-soaked in Miglyol (pure continuous phase) for at least 30 min to increase the hydrophobicity of the membrane surface. The initial volume of continuous oil phase in the cell was 100 cm^3 and the experiments were run until the dispersed phase concentration reached 10 vol.%. Once the desired amount of aqueous phase had passed through the membrane, both the pump and the agitator were switched off, the droplets were collected and analyzed. The membrane was cleaned initially 5 min in an ultrasonic bath using hot soapy water followed by 5 min washing in 2 wt.% citric acid. The membrane was then rinsed with distilled water and dried using compressed air and left in continuous phase to promote the hydrophobicity of the surface. To produce solid microparticles the liquid droplets were solidified by chemical crosslinking using GA. A secondary emulsion (composed by a predetermined concentration of GA in water dispersed in the continuous phase) was prepared in a separate beaker using a homogenizer (IKA® T 10 ULTRA-TURRAX®, Germany) operating at 30,000 RPM for 3 min (giving droplets sized between 5 and 20 μm). The volume ratio of GA solution and continuous phase in the secondary emulsion was 1:1. The volume ratio GA: polymer was 1:1. Primary emulsion of polymeric droplets produced in the Dispersion Cell was then mixed with secondary emulsion of GA using magnetic stirring in a separate beaker for 90 min (Fig. 1(b)). In order to observe the internal structure particles were mounted in a resin and cut into slices using a Base Slege Microtome (Leitz, Weitezer, UK). Particle cross section was observed under an SEM and revealed solid and homogeneous internal structure. When PVA solution was used as the dispersed phase, the reaction occurred at room temperature, while for the blends of PVA and CS as well as pure CS solution the temperature needed to be elevated to 75 °C. By homogenizing the crosslinker with the continuous phase at very high shear it was possible to obtain very small droplets of GA. In this way the surface area of GA droplets was increased promoting the GA diffusion through the continuous phase consequently reacting with the aqueous polymeric droplets. The secondary GA emulsion was gently mixed with the primary polymer emulsion using a magnetic stirrer (Fig. 1 (b)). Using this method it was possible to avoid the "extraction step" reported by other authors [42,43] which consists of the extraction of GA into organic solvents to make it miscible with the oil phase of the primary emulsion. After the solidification, the particles were washed with toluene or hexane, freeze dried and stored in air tight containers until further analysis. Due to the toxicity of GA it was important to determine that there is no residual GA left within the particles. To assay the presence of remaining GA, the polymeric particles were left in release medium (pH = 3 or

Table 1
Viscosities, densities and interfacial tensions measured for the different dispersed phase and continuous phase used for the production of the emulsions.

Continuous phase	Viscosity (mPa s^{-1})	Density (kg m^{-3})	Dispersed phase	Viscosity (mPa s^{-1})	Density (kg m^{-3})	Interfacial tension (mN m^{-1})
			PVA			
Miglyol	8.12	910	15 wt.%	99	1034	6.6
2 wt.% PGPR in Miglyol	11.28		15 wt.%	99	1034	2.2
			15 wt.% PVA + 1 wt.% CS	~500	1045	3.1
2 wt.% ABIL EM 90 in Miglyol	8.38		10 wt.%	24	1022	1.6
			15 wt.%	99	1034	2.1
			20 wt.%	588	1045	2.7
			25 wt.%	2550	1057	3.5
			Chitosan			
			1 wt.% CS	37	1008	1.7
			2 wt.% CS	33	1014	2.2
			3 wt.% CS	~60	1022	3.1
			15 wt.% PVA + 1 wt.% CS	~500	1045	2.6
			5 wt.% PVA + 1 wt.% CS	217	1015	2
			15 wt.% PVA + 3 wt.% CS	~15,000	1063	N/A

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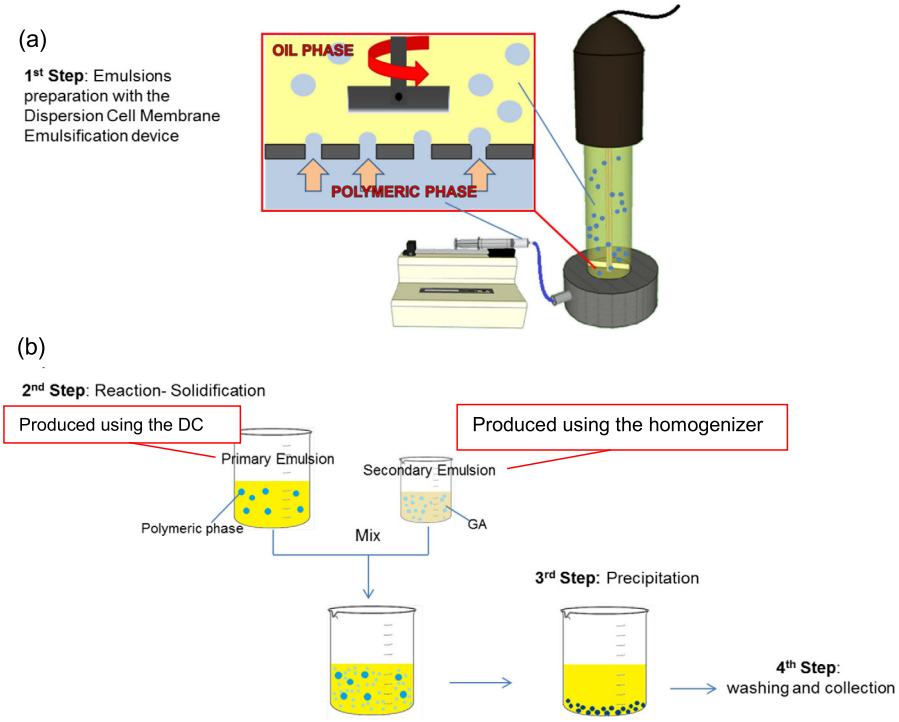

Fig. 1. Production of microparticles: (a) Dispersion Cell used for the emulsification of the polymeric phase. (b) Solidification step – converting the polymeric droplets into solid microparticles.

pH = 7) up to 5 days. The amount of GA present in the sample was analyzed with UV/VIS spectrophotometer operating at wavelength of 235 nm or 280 nm [44]. No signal corresponding to GA at those wavelengths was detected therefore it is believed that no unreacted GA was left in the particles. The sizes of freshly prepared droplets of dispersed phase in Miglyol, as well as solid particles were determined using a Leitz Ergolux optical microscope with an attached Pulnix TM-6CN monochrome camera. The droplets sometimes burst when placed on a microscopic slide due to surface tension effects, so photographs of the droplets had to be taken very quickly. For each experiment, numerous photos were taken and at least 300 droplets, or particles, were measured. As an indication of the droplet size distribution, the coefficient of variation (*CV*) was determined using the Java-based image processing package ImageJ. For each emulsion, three separate samples and measurements were produced and the mean average droplet size D_{av} is reported;

$$D_{av} = \sum_{i=1}^{n} \frac{d_i}{N} \quad (1)$$

where d_i is the *i*th diameter of the droplet, and N is the total number of the droplets counted. The coefficient of variation (*CV*) was calculated accordingly to (2) for each emulsion produced; and reported to express the degree of uniformity.

$$CV = \frac{\sum_{i=1}^{n}(d_i - D_{av})/N}{D_{av}} \times 100\ (\%) \quad (2)$$

2.2.3. Swelling of the microparticles and in vitro release

The swelling study was performed on the PVA particles cross-linked with different amounts of GA (1–50 vol.%). 0.35 g of PVA particles were mixed with 20 ml of swelling (release) media (phosphate buffer solution, pH = 7.3) and the samples were placed in a shaking water bath at 37 °C. After 24 h (no further change in particle size was observed) the excess of water was removed and the diameter of swollen particles was measured. For the in vitro release microspheres were produced using the polymeric solutions containing Cu^{2+} or SS. 0.35 g of now loaded microspheres (containing 350 mg of Cu^{2+} or 1000 mg of SS respectively) was placed in 20 ml buffer solutions with pH values of 3 or 7 containing small amount of 2 wt.% SDS (sodium dodecyl sulphate) added to the suspension to avoid particle agglomeration. At predetermined time intervals 5 ml of the release media was removed and replaced with 5 ml of fresh media to maintain the volume or the release media. The amount of Cu^{2+} released was assayed using an Atomic Absorbance Spectrophotometer (AAS) (Spectra AA-200 Varian, UK operating at wavelength of 244.2 nm); while the SS concentration in the sample was measured using a UV–VIS spectrophotometer (Lambda 35 Perkin Elmer, UK operating at wavelength of 300 nm). The amount of Cu^{2+} and SS was calculated from the corresponding calibration curves, made for each release media used. Samples in triplicate were averaged for each experiment. The cumulative release percentage was calculated as follows:

$$CR = \frac{V_t \sum_{i=1}^{n-1} C_i + V_o C_n}{m} \times 100\ (\%) \quad (3)$$

where V_o is the volume of the release media (=20 mL), C_n is the concentration of the compound determined at a specific time interval, V_t is the volume of the replaced media (=5 mL), and C_i represents the concentration of the encapsulated compound in the previous sample. m is the mass of the encapsulated compound in the sample.

3. Results and discussion

3.1. Effect of pore geometry and operating parameters on droplet size and uniformity

The effect of the dispersed phase flux as well as the shear stress on the formed droplets, their size and uniformity, was investigated using two metallic disk membranes: stainless steel and hydrophobic nickel. 15 wt.% PVA in water was used as dispersed phase and

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the continuous phase was 2 wt.% PGPR in Miglyol. *CV* and average droplet diameter are respectively shown in figures Fig. 2(a) and (b) as a function of the dispersed phase flux (12–1250 L h^{-1} m^{-2}) and the shear stress applied on the membrane surface (22–59 Pa). Increase of the shear stress over the membrane surface resulted in decrease of the average droplet diameter (D_{av}), while an increase of the D_{av} was observed with the flux increase as previously seen by other authors [34,36,45]. For the nickel membrane the biggest droplets (64 μm) were produced using the combination of the lowest shear (22 Pa) and 270 L h^{-1} m^{-2} as dispersed phase flux. Gradually the droplet size decreased down to 36 μm when the shear was 59 Pa, at the lowest flux applied (10 L h^{-1} m^{-2}). When the lower flux was used the emulsions had a *CV* below 40%. Gradually increasing the flux gave an improvement in the uniformity with the *CV* going down to 20%. It is possible that due to the low flux not all pores of the membrane were active [32,35].

The uniformity of the droplets improved using the steel membrane, at comparable shear and flux conditions. In addition the droplets produced using the stainless steel membrane were overall smaller and more uniform (lower *CV*) at the same shear conditions compared to those produced using the nickel membrane. The *CV*'s of the emulsions produces with the stainless steel were all below 25%. The lowest value of *CV* obtained was 18% applying an intermediate shear (35 Pa) and 270–430 L h^{-1} m^{-2} as dispersed phase flux. A *CV* ~ 35% is only observed for the emulsion produced under very high shear (59 Pa) and flux (430 L h^{-1} m^{-2}) but too large droplets can be broken under the high shear. It is also possible that due to the high flux the droplets grow too fast and the surfactant does not have enough time to reach the interface before droplet detachment [32].

In order to predict the droplet diameter for a different values of shear stress, a mathematical model (Eq. (4)) introduced by Kosvintsev [30] was used. This model takes into account the presence of two forces acting on a growing droplet at the membrane surface. These forces are: the capillary force (which is proportional to the value of interfacial tension between the two immiscible liquids) and the drag force: a function of the shear stress applied. Balancing these two forces it is possible to obtain the droplet size:

$$D = \frac{\sqrt{18\tau_{max}^2 r_p^2 + 2\sqrt{81\tau_{max}^4 r_p^4 + 4r_p^2\tau_{max}^2\gamma^2}}}{3\tau_{max}} \quad (4)$$

where D is the theoretical droplet diameter, τ_{max} is the value of shear stress, r_p is the pore radius and γ represents the interfacial tension. $\tau_{max} = 0.825\mu_c\omega r_{trans}/\delta$ where μ_c [Pa s] is the continuous phase viscosity, r_{trans} is the transitional radius and δ is the boundary layer thickness $\delta = \sqrt{\mu_c/\omega\rho_c}$. The experimental values of droplet diameter were compared to the theoretical ones determined using the Eq. (4). In Fig. 3 the dotted curve shows the theoretical values of the D_{av} calculated using Eq. (4) at different shear stress. In the graph are reported the D_{av} obtained using the two membrane types and at different dispersed phase fluxes. It is noticeable that at very low values of flux the experimental values are closer to the theoretical ones. The shear – capillary model does not consider the dispersed phase flux as having an influence on the final droplet size. However, an increase of the flux produces a gradually higher divergence between the experimental droplet size and the model predicted ones. The influence of the dispersed phase flux on the drop formation and detachment has been previously investigated by Schröder and Schubert (1999) [46]. According to Schröder and Schubert (1999) the droplets formed on the membrane surface are influenced by the number of the active pores on the membrane surface. The number of active pores within the model has to be estimated which is an additional fitting parameter which needs to be taken into consideration [45] and therefore correlating size with flux has been avoided in this work.

Contact angles were measured for water and 15 wt.% PVA on hydrophobic nickel and stainless steel membranes and the results are reported in Table 2. In addition the photographs of the droplets sitting on different membrane surfaces are reported within Fig. 3. According to the contact angle measurements the PVA droplet wets the stainless membrane far more than the nickel membrane (Fig. 3(a) and (b)). The stainless is more hydrophilic than the nickel and, therefore, one would assume that it is more likely to wet than the nickel with the dispersed phase. Hence, the smaller drops, and better size distribution, coming from the stainless membrane, cannot be attributed to the membrane surface wetting properties: the results are the wrong way round – nickel is more hydrophobic and

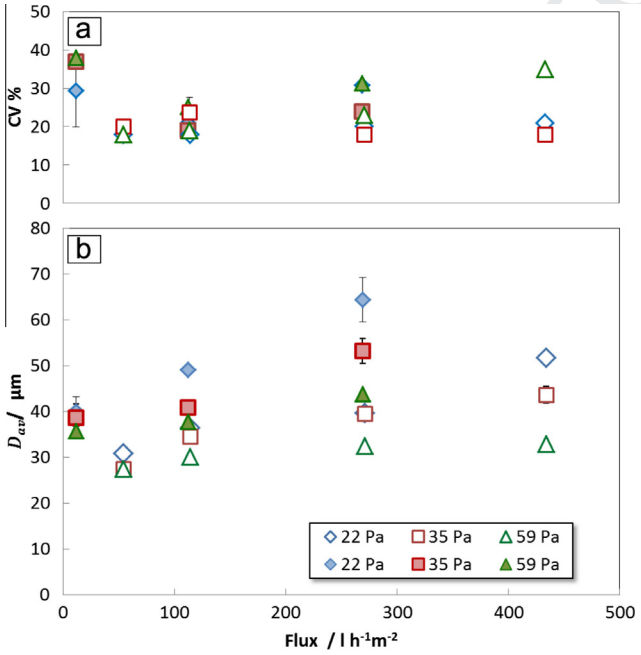

Fig. 2. Influence of the transmembrane flux and the shear stress: (a) coefficient of variation on the mean droplets diameter, (b) average droplet diameter (D_{av}); the hollow signs represent experiments performed using the 10 μm stainless steel membrane; the full signs represent experiments performed using the 10 μm nickel membrane. Dispersed phase 15 wt.% PVA; continuous phase 2 wt.% PGPR.

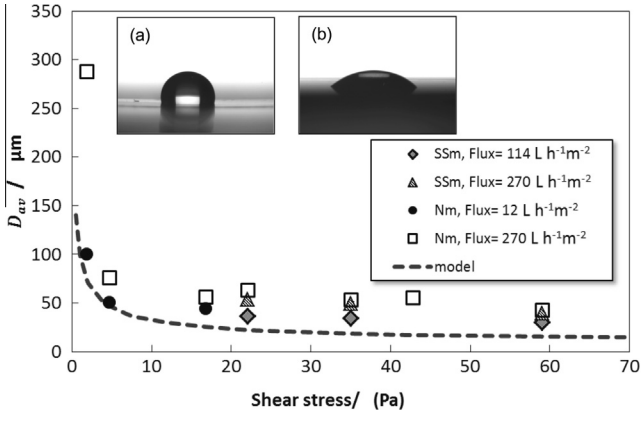

Fig. 3. Comparison between the theoretical droplet size calculated using the model (Eq. (4)) (dashed line) and the average droplet diameter obtained under different conditions of shear and transmembrane flux. Nm – nickel membrane, SS – stainless steel membrane dispersed phase (DP) 15 wt.% PVA; continuous phase (CP) 2 wt.% PGPR. (a) 15 wt.% PVA droplet on the hydrophobic nickel membrane. (b) 15 wt.% PVA on the stainless steel membrane.

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Table 2
Contact angle measurements (dispersed phase on the membrane surface).

Membrane	Dispersed phase	Theta(average)(deg)
Hydrophobic nickel	Pure water	122.2 ± 0.09
Hydrophobic nickel	15 wt.% PVA	102.7 ± 0.10
Stainless steel	Pure water	60.1 ± 0.72
Stainless steel	15 wt.% PVA	40.8 ± 0.39

should wet better with the oil phase. Fig. 4 shows both membranes with the nickel membrane showing a conical surface above the pores (lines within SEM image, Fig. 4(a–c)). This contrasts with the pores of the stainless membrane which have sharp openings without conical configuration (Fig. 4(d–f)). Thus the smaller and more uniform drops are more likely to be a consequence of the sharp pore opening; i.e. flat membrane surface. In addition all these polymeric phases are highly acidic therefore the use of the stainless steel as membrane material is advantageous as it is more resistant to acidic pH than nickel.

3.2. Effect of surfactant present in the continuous phase

Droplets stability is not greatly affected by the membrane type, but it is by the emulsifier used. PGPR and alternatively ABIL EM 90 were used to stabilize the aqueous phase in Miglyol. When pure PVA (15 wt.%) was used as a dispersed phase, 2 vol.% PGPR produced uniform emulsions with high uniformity (Fig. 5(a)); slightly less uniform, but stable emulsions were obtained using 2 vol.% ABIL EM 90 as stabilizer (Fig. 5(b)) and PVA (15 wt.%). Using the same composition of the continuous phase, PVA and CS in water (ratio 15:1) were used as dispersed phase and the resulting emulsion is shown in Fig. 5(c), it was unstable and the presence of very big droplets was observed. It is hypothesized that after the emulsification process the droplets start to coalesce due to interaction of CS with the stabilizer. The hydrophilic part of PGPR contains polyricinoleic acid, characterized by the presence of groups – COOH. An interaction between carboxylic group and the CS amino group in an acidic environment can occur, destabilizing the emulsifier and interfering with its action. Thus, a slight increase of the interfacial tension was measured for the system when PGPR was used as emulsifier (Table 1). The blend of PVA and CS (15:1) was emulsified with continuous phase composed by 2 vol.% ABIL EM 90 in Miglyol (Fig. 5(d)). In this case the resulting emulsion was more stable. The uniformity was not as good as in Fig. 5(a) and (b), but the stability was improved (no obvious coalescence found). It was not possible to use the 10 μm pore size stainless steel membrane for the emulsification of the polymeric phase composed by the two polymer blends (15:1) due to the high viscosity of this phase. This limited the work with the polymer blends to the nickel membranes with larger pore size (20 μm) (Fig. 5(b) and (d)).

3.3. Effect of crosslinker on final solid particles

The crosslinking reaction with GA is a condensation polymerization that links the polymeric chains. For higher degree of crosslinking the polymeric segments are more interconnected reducing the capability of the network to swell. The degree of crosslinking can be used to tailor the degree of swelling; the lower the degree of crosslinking the greater the degree of swelling [3]. Moreover, the amount of crosslinking has an influence on the release profile of a compound from the particles: the water is absorbed by the particles and due to the diffusion the encapsulated compound within the polymeric matrix is released. To study the influence of the crosslinker concentration on the swelling, microparticles with different degree of crosslinking were allowed to swell completely in water for 24 h in an oscillating bath at 37 °C. To produce the solid particles droplets were crosslinked using different amounts of GA (1–50 vol.%) to determine the lowest concentration required to obtain solid microparticles. To ensure that the solid particles are formed, after a maximum 90 min of reaction, a sample of particles still in the oil phase of the emulsion was taken. The sample was washed with acetone and left at room temperature for few seconds to dry. Acetone dissolves the oil and removes the excess water, thus establishing if the dispersion was of droplets, or solidified particles which do not dissolve. The sample of particles was than observed with the microscope. The lowest GA concentration needed for solidification of the particles was 1 vol.% of GA. The size of the dried particles crosslinked using different GA concentrations was measured and it was seen that the diameter was not affected by the amount of crosslinker used; the samples prepared with different GA concentrations had D_{av} = 32 ± 2 μm, CV = 20 ± 2%. Particles were prepared from emulsion with D_{av} = 53 ± 2 μm, CV = 20 ± 2%. According to Rathnbone et al. (2002) the average particle size of the microparticles used for oral drug delivery is between 20 and 120 μm and particles used are within the range of particles size commonly used for oral drug delivery [47]. The SEM analysis of dried particles showed the corrugated surface, more evident for less crosslinked particles (Fig. 6). No swelling was observed for PVA particles crosslinked with 10 vol.% GA or above. A cross section of the sliced particles observed on an SEM was solid and homogeneous therefore, it is believed that the GA can easily diffuse from the droplets of the secondary emulsion into the polymeric droplets of the primary emulsion. Having in mind that the droplets size is quite small GA molecules have quite short pathway for diffusion. Fig. 6 also presents the degree of swelling as a function of the amount of crosslinker used. Particle diameter was not influenced when the concentration of GA was between 10 and 50 vol.%. Reducing the GA concentration for crosslinking of the particles below 5 vol.% it was possible to produce particles which readily swell and able to incorporate water. At higher GA concentration the number of linkages between the polymeric chains is higher, reducing the ability of the polymeric network to absorb water.

3.4. Effect of polymers on Cu^{2+} release

To determine the release profile of a sample compound from the produced particles, Cu^{2+} was used. Cu^{2+} was chosen since it is a small ion that is easy to detect by AAS and previous papers have used it as a drug analogue molecule [48]. The use of AAS to determine the concentration of Cu^{2+} enables even small changes of Cu^{2+} concentration with time to be monitored. The behaviour of the particles was studied in acidic conditions (pH = 3) to mimic the gastric fluid, and neutral conditions (pH = 7) to mimic the intestinal tract. Normally, the gastric emptying time is about 3–4 h [49], after a normal meal. Thus, the release was assayed up to 3 h, required for possible drug absorption at stomach level. Fig. 7(a) shows the release of Cu^{2+} from 15 vol.% PVA particles as a function of the time depending on the amount of GA used. The release of Cu^{2+} decreases with the increase of the crosslinker concentration; for example the particles prepared with 25 vol.% of GA released less than 1% of Cu^{2+} after 3 h, while the particles prepared with the 1 vol.% of GA released up to 70% of Cu^{2+} in 3 h. High initial release of Cu^{2+} was observed from the PVA particles, giving the so called "burst release" [50]. "Burst release" of Cu^{2+} in the early minutes, could be attributed to a diffusion of Cu^{2+} from the particles surface. It is interesting to notice that no release from PVA particles was observed if GA concentration for crosslinking was 10 vol.% or above. But if the PVA was blended with CS even if the particles were crosslinked with 10 vol.% GA it was possible to tailor the release as can be seen from the Fig. 7(b). Fig. 7(b) shows the release

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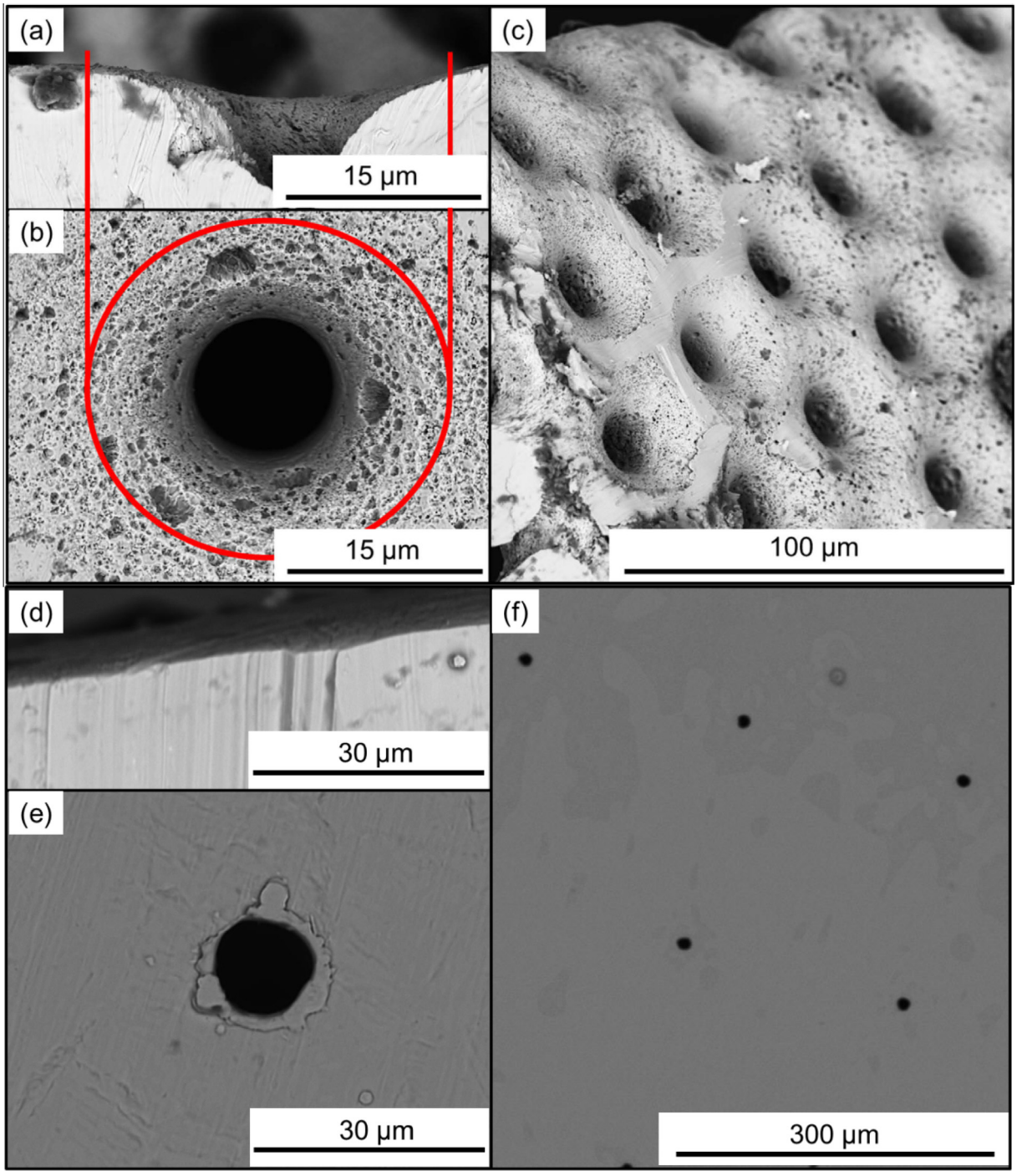

Fig. 4. Hydrophobic 10 μm nickel membrane: (a) pore cross-section. (b) Pore top view. (c) Membrane side view. Red lines mark the pore edges. Stainless steel 10 μm membrane: (d) Pore cross-section. (e) Pore top view. (f) Top view of the membrane surface. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

from particles made by either pure 15 vol.% PVA, pure 3 vol.% CS or PVA and CS blends. 10 vol.% of GA was used for crosslinking of all particles within Fig. 7(b) and it was the lowest required concentration of crosslinker which allowed production of particles which kept their shape after solidification and drying. Cu^{2+} was released over time reaching up to 80% after 3 h depending on the polymeric blend, 10% more than when 15 vol.% PVA was crosslinked with 1% GA (Fig 7(a)). When pure polymers were used almost no Cu^{2+} was released probably due to high crosslinking density. The sample made by the combination of 15 wt.% PVA and 3 wt.% of CS, gave intermediate release. Such behaviour could be attributed to the high viscosity of this polymeric phase (Table 1), which as a result produces more dense particles, less able to swell and release Cu^{2+}. Highest release up to 80% was achieved when PVA was blended with 1% CS. To investigate the pH sensitive release, the particles were tested at pH = 3 and pH = 7, and the results are shown in Fig. 8. Particles made with pure PVA or pure CS (crosslinked with 10 vol.% of GA) did not show any significant Cu^{2+} release no matter the pH. When PVA was blended with CS higher release of Cu^{2+} was obtained at pH = 3. Particles produced blending PVA with 1% CS demonstrated the highest release at acidic conditions. Such pH dependent release can be attributed to protonation

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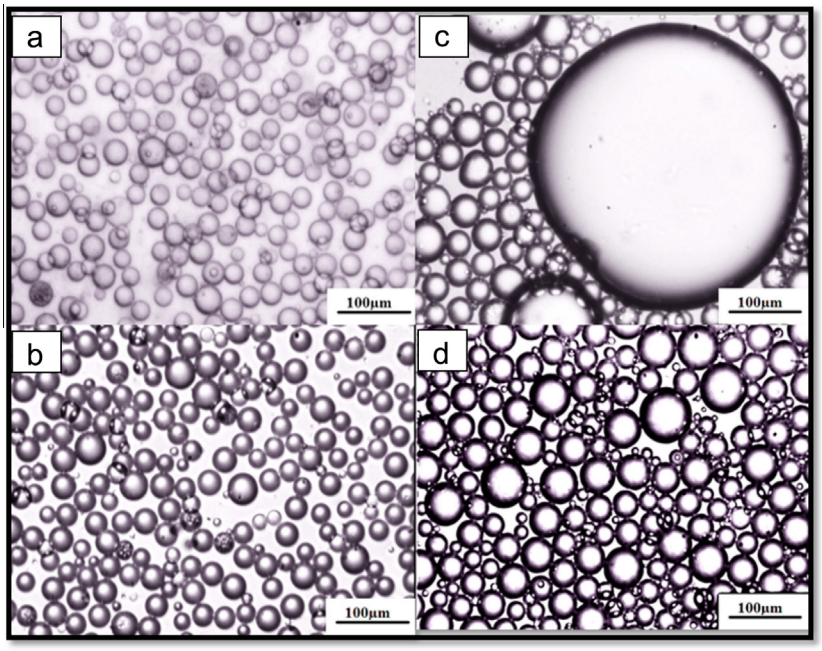

Fig. 5. Emulsions: (a) 2 wt.% PGPR in Miglyol as continuous phase (CP) and 15 wt.% PVA as dispersed phase (DP). (b) 2 wt.% ABIL EM 90 in Miglyol as CP and 15 wt.% PVA as DP. (c) 2 wt.% PGPR in Miglyol as CP and PVA:CS 15:1 as DP. (d) 2 wt.% ABIL EM 90 in Miglyol as CP and PVA:CS 15:1 as DP.

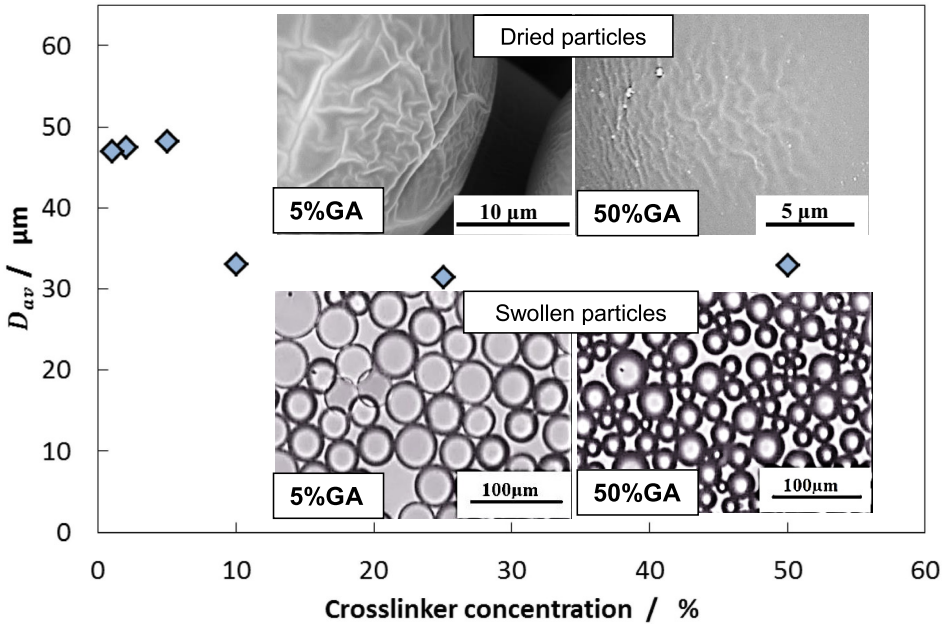

Fig. 6. Swelling proprieties of PVA particles crosslinked using GA solutions (1–50%), expressed as value of D_{av} of the swollen particles in water. Top images – SEM's of dry crosslinked particles. Bottom images – optical microphotographs of swollen crosslinked particles. 5 vol.% and 50 vol.% GA solutions used respectively.

of amino groups of chitosan with the decrease of pH [17]. This protonation leads to chain repulsion, diffusion of proton and counter ions together with water inside the gel and dissociation of secondary reactions allowing swelling [17]. None of the samples swelled significantly at neutral conditions.

3.5. Effect of the polymers on sodium salicylate release

To study the behaviour of a real drug molecule, sodium salicylate (SS) was encapsulated within the PVA-CS particles crosslinked with 10 vol.% of GA. The sample that gave the highest release of Cu^{2+} was chosen (15 wt.%PVA–1 wt.%CS). The release was tested at pH = 3 and 7. As shown in Fig. 7(b), the release of SS is very slow reaching only 23% after three hours. SS is an anionic drug [51] (MW = 160.11 g mol^{-1}) and it is hypothesized that in an acidic environment the ionic amino groups of CS can delay the release of SS, that can interact ionically with the charged group of CS. Comparing the release of Cu^{2+} with the SS, the delay of the SS release can be explained considering the drug–polymer ionic interaction [52] and the higher MW of SS. On the other hand, Cu^{2+} is a positively charged ion, which can be repelled from the CS polymeric matrix, facilitating release. In Fig. 8 the release of SS in acidic and

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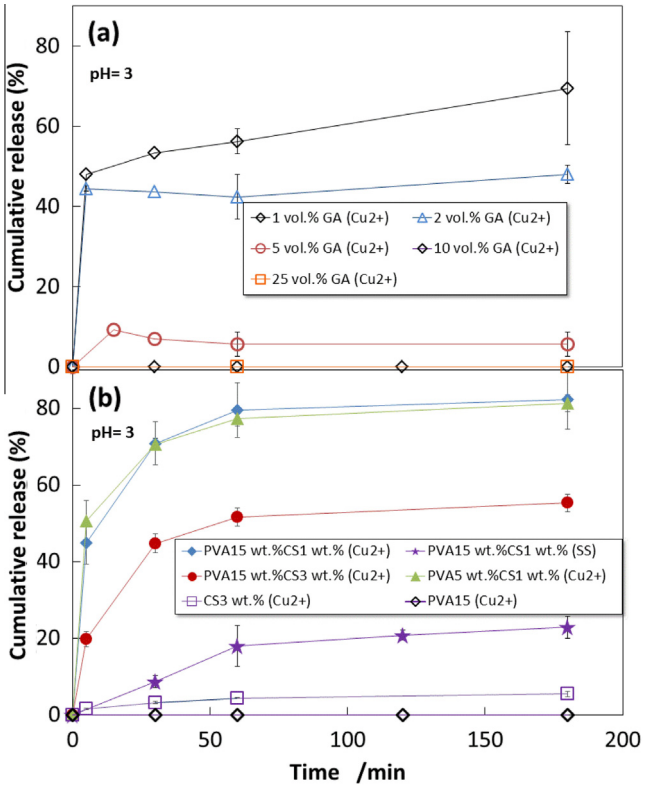

Fig. 7. Release study. (a) Influence of GA concentration on Cu^{2+} release from 15 vol.% PVA particles. (b) Influence of the polymeric blends (PVA nad Chitosan) on Cu^{2+} and SS (sodium salicylate) release (10 vol.% GA as crosslinker). In all experiments final particle size tested for all formulation was 30 μm *CV* = 20 ± 2%. All particles contained 5000 ppm of Cu^{2+} or 3000 ppm sodium salicylate where appropriate.

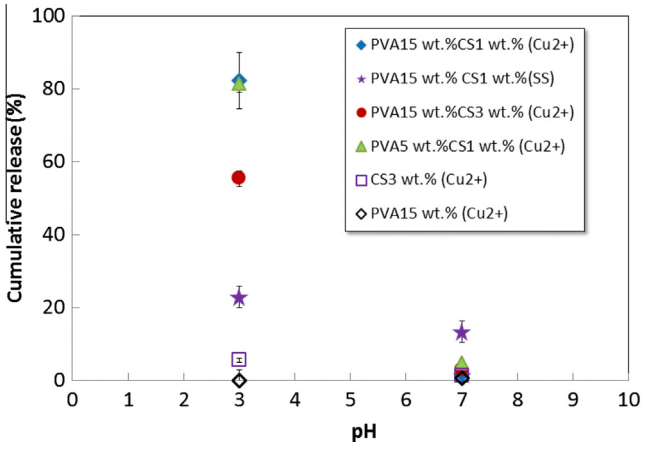

Fig. 8. Cu^{2+}or SS release in different pH conditions (10 vol.% GA as crosslinker is used for all the samples). In all experiments final particle size tested for all formulation was 30 μm *CV* = 20 ± 2%. All particles contained 5000 ppm of Cu^{2+} or 3000 ppm sodium salicylate where appropriate.

neutral environment is reported, and little difference exists between the two release media. A small amount of SS was released at pH = 7 from the PVA-CS particles after 3 h. However, this behaviour was expected since CS is not ionized at pH = 7 and the ionic interaction between the polymer and the SS do not occur. SS is released in a small quantity at pH 7 probably from the surface of the particles. It must be considered that for CS based particles the type of drug encapsulated plays an important role as well as the crosslinker amount and the pH of the release medium.

4. Conclusions

Uniform emulsions were produced with the Dispersion Cell membrane emulsification technique, with a *CV* of 18% under the best operating conditions (shear of 35 Pa and 270–430 L h^{-1} m^{-2} as dispersed phase flux). Modifying the operating parameters, it was possible to produce emulsions with droplets between 30 and 280 μm. Hydrophilic stainless steel membrane (pre-soaked in the oil continuous phase) produced smaller and more uniform droplets compared to a PTFE coated hydrophobic nickel membrane. Contact angle measurements confirmed the hydrophobicity of the nickel membrane therefore the pore surface (conical for the nickel and flat for the stainless steel membrane) had a greater influence on the size and uniformity – possibly with the drop forming on an area greater than the pore itself for drops produced using the nickel membrane. The minimum concentration of crosslinker needed for solidification was 1 vol.% GA. The amount of GA affected the capability of the particles to sorb water and swell. This behaviour to swell is directly correlated to with the ability of the particles to release the encapsulated compound. PVA particles prepared with 1 vol.% of GA released 70% of the encapsulated compound (Cu^{2+}) within 3 h. The release profile of Cu^{2+} from the PVA particles was characterized by an initial "burst release", due to the release of Cu^{2+} from the surface of the particles. Blending chitosan and PVA it was possible to increase the release of Cu^{2+} up to 80% in 3 h under acidic conditions. No significant release (less than 3%) was observed at neutral conditions. Due to the higher MW and the ionic interaction with the polymeric matrix the release of sodium salicylate was almost 4 times lower compared to Cu^{2+} after 3 h in acidic environment and around 10% in neutral environment. Thus, the encapsulation of negatively charged drugs (such as sodium salicylate) delays the release and affects the selective release in acidic pH.

Our future work will focus on drug and cell encapsulation and we will consider the possibility to blend chitosan with other polymers such us alginate and gelatin in the formation of biocompatible and permeable hydrogels to promote drug release in the intestine. Different polymer coatings (e.g. EUDRAGIT®) might be tested to provide stability in acidic environment followed by the release in intestine. Furthermore the emulsion production system will be scaled up in order to evaluate an increase in the productivity, and if the resulting particles are consistent with what is expected from the information obtained with the laboratory Dispersion Cell.

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