Synthesis of Calcium Carbonate Micro Particles using Emulsion Membrane Process Applied for Drug Release Studies

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The aim of this work is the synthesis of calcium carbonate ($CaCO_3$) nano and microparticles and their application as biomaterials (vehicles) for the sustained release of doxycycline. $CaCO_3$ micro particles were synthesized by water-in oil (W/O) emulsion method using emulsion liquid membranes with bis (2-ethylhexyl) phosphate (D2EHPA) as carrier, Span 80 as surfactant, and toluene and kerosene as organic solvents. The aqueous phases contained 1 M $CaCl_2$, and 1 M Na_2CO_3, respectively. The Dynamic Light Scattering (DLS) data showed $CaCO_3$ particles with sizes ranging from around 100 nm to 3500 nm. The $CaCO_3$ particles with the average diameters around 600 nm attained an adsorbtion of doxycycline of maximum 97.9%, and a slow and steady release with a cumulative value of approximative 50% after ten days.

Keywords: calcium carbonate, nano and micro particles, doxycycline, drug release

In recent years, inorganic nano and micro particles were widely studied in modern pharmaceutical and medical applications. One of the main area of research is drug delivery, and many reviews and original articles have been reported in literature on using inorganic materials, such as calcium phosphate [1] colloidal gold [2,3] carbon nanotubes [4] porous silicon [5] iron oxide [6], clay minerals and double layered hydroxide (LDH) [7,8], as carriers for chemical and pharmaceutical substances in order to achieve a therapeutic response. Another promising inorganic material is calcium carbonate ($CaCO_3$), one of the most common mineral components in rocks and biological organisms, more extended to marine world (pearls, snails). Due to its biocompatibility, $CaCO_3$ was investigated for various medical applications, for example as carrier for drug delivery systems [9-15], or as implant for bone formation [16].

Several methods for nano and micro particles synthesis have been reported, the majority of them are being based on two main approaches: the biomimetic methods (precipitation and reverse emulsion) and the CO_2 bubbling method [17].

Water-in oil (W/O) microemulsion method has been widely used as an ideal system for inorganic particle synthesis. The specific interest in this method is due to the versatility of the preparation techniques that allows a good control of particle properties such as size, geometry, morphology, homogeneity and surface area [18-20]. One way to attain nanometer and micrometer $CaCO_3$ particles is via Emulsion Liquid Membrane (ELM) process. Although ELM method can be used to calculate and control the particle size [21], few reports and data are found for this approach.

This study was focused on the $CaCO_3$ micro and nano-sized particle synthesis and their application as carriers for the sustained release of doxycycline, using water-in oil (W/O) micro-emulsion technique with an ELM system. The drug loading and drug release behavior of $CaCO_3$ were investigated using doxycycline drug. The doxycycline was chosen as a testing model due to its variety of functional groups (hydroxyl, amino, amide). The drug release studies were made into stimulated body fluid at a *p*H value of 7.4. The size of the particles was determined by Dynamic Light Scattering (DLS) technique. The loaded and released doxycycline from $CaCO_3$ particles was detected by UV-Vis spectrophotometry. Pure doxycycline, synthesized $CaCO_3$ micro and nanoparticles, and doped doxycycline $CaCO_3$ biomaterials were characterized by spectroscopy.

Experimental part

Materials

$CaCO_3$ micro particles were synthesized using $CaCl_2$ (99.9%) and Na_2CO_3 (99.5%), from Aldrich. The Span 80 surfactant was obtained from Fluka, D2EHPA (95%) from Alfa Aesar, and doxycycline hyclate antibiotic (98%) from Aldrich. For in vitro drug release studies, a phosphate-buffered saline solution (PBS) with a *p*H value of 7.4 was used. The PBS was 0.2 M NaOH and 0.2 M H_2PO_4K. NaOH and H_2PO_4 K reagents were of analytical grade, from Scharlau.

Synthesis of calcium carbonate micro and nano particles

The calcium carbonate micro and nanoparticles were synthesized using a emulsion liquid membrane technique with Span 80 as surfactant. D2EHPA was used as carrier and toluene and kerosene were used as organic solvents. The aqueous phases contained 1 M $CaCl_2$ and 1 M Na_2CO_3, respectively.

In order to obtain $CaCO_3$ particles, three systems of emulsions were used (table 1). The primary emulsions were prepared by adding the receiving phase to the membrane phase (surfactant and carrier solubilized in the solvent), using a Heidolph RZR 2041 mechanical stirrer. The emulsification process was maintained for 60 min for the emulsions E1.1 and E1.2, and 120 min for the emulsions E1.3, E1.4 and E1.5.

The first system of liquid membrane emulsion was obtained by contacting two primary emulsions (E1.1 and E1.2) under stirring at 1300 rpm for one hour. The secondary emulsion was left for 72 hto allow the suspended particles to sink, and then the $CaCO_3$ particles were further separated by centrifugation at 5000 rpm for 30 min.

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The second system of liquid membrane emulsion was made of a primary emulsion (E1.3) and a solution (500 ml of 0.1 M $CaCl_2$). The contact was maintained for 120 min at 1300 rpm. The secondary emulsion was left to settle for 42 h and then was centrifuged at 5000 rpm for 30 min.

The third system of liquid membrane emulsion was realized by contacting the two primary emulsions E1.4 and E1.5 under stirring at 1900 rpm for 120 min. The obtained secondary emulsion was left for 42 h and the $CaCO_3$ nanoparticles were isolated by centrifugation at 5000 rpm for 30 min.

In order to remove traces of surfactant and organic solvent, the obtained $CaCO_3$ particles were washed several times with ethanol, and then dried in an oven at 110°C.

The drug loading

To investigate the drug loading efficiency, 0.5 g of $CaCO_3$ particles were contacted with 75 mL doxycycline solution, having initial concentration of 0.1 mg/mL. The resultant suspension was stirred at 250 rpm for 30 h. In order to monitor the adsorption process, at predetermined time intervals (6, 24 and 30 h), three samples were withdrawn from suspension and centrifuged at 5000 rpm for 30 min. The doxycycline concentrations in supernatants were determined by UV-Vis (Spectrophotometer CINTRA 202). The drug loading efficiency was calculated using the equation:

$$\text{Encapsulation efficiency} = [(a-b)/a] \times 100 \quad (1)$$

where a is the initial drug concentration and b is the drug concentration in the supernatant.

The obtained particles were lyophilized for 24 h. The drug adsorption onto $CaCO_3$ particles was highlighted by FTIR.

In vitro drug release

Studies of antibiotics release were performed into simulated body fluid (*p*H = 7.4) under agitation (200 rpm) at room temperature for 242 h (approximately 10 days). In order to determine the drug release profile, 0.1 g of the $CaCO_3$ loaded with doxycycline was contacted with 50 mL of PBS. Every day, samples of 5 mL were extracted with a pipette, centrifuged at 5000 rpm for 30 min, and replaced with 5 mL of fresh PBS. The amount of doxycycline release was determined by UV-Vis at 350 nm wavelength.

Characterization

The particle size was measured by DLS method using a Nano ZS (Red badge) instrument. In order to perform the DLS analysis, 0.01 g of dried and ground $CaCO_3$ particles were suspended in 25 mL of distilled water and then placed in an ultrasonic bath for 5 min. The DLS measurements were done at room temperature, immediately after the agitation process.

The chemical and structural compositions of pure doxycycline, nanostructurated $CaCO_3$ and doxycycline loaded $CaCO_3$ were studied with a Perkin Elmer GX FTIR spectrometer in the frequency range of 4000 - 400 cm^{-1}. The analyses were done by a 32 spectra average at 4 cm^{-1} resolution, using the transmission technique. The samples were finely ground to reduce scattering losses and band absorption distortions. About 5 to 10 mg of ground sample was incorporated in a KBr pellet. The present functional groups were identified and characterized by their spectral absorption bands.

Results and discussions

Calcium carbonate particles characterization

The $CaCO_3$ particles obtained in our experiments have sizes over a wide range, from around 100 nm to 3500 nm. The figures 1, 2 and 3 illustrate the DLS plots of particle sizes by intensity, and the table 2 shows the average diameters estimated by DLS from four counts. For system 1, when kerosene solvent was used, there are some aggregates, and the average diameter was 634.2 nm. When toluene solvent was used, system 3, a decrease in the sample stability was detected, with large aggregates, and the average diameter increased at 2395 nm. A pronounced aggregation phenomenon was observed for system 2, when a bimodal size distribution was noticed

Table 1

THE COMPOSITION OF THE PRIMARY EMULSIONS USED IN THE SYNTHESIS SYSTEMS

	No. emulsion	Carrier (% V)	Receiving phase (%V)	Solvent (%V)	Emulsifier (%V)
System 1 liquid membrane emulsion	E1.1	D2EHPA 0.25	$CaCl_2$ 1M 50	Kerosene 46.5	SPAN 80 3.25
	E1.2	D2EHPA 0.25	Na_2CO_3 1M 50	Kerosene 46.5	SPAN 80 3.25
System 2 liquid membrane emulsion	E1.3	D2EHPA 0.5	Na_2CO_3 1M 50	Kerosene 46.5	SPAN 80 3
System 3 liquid membrane emulsion	E1.4	D2EHPA 0.25	$CaCl_2$ 1M 50	Toluene 46.5	SPAN 80 3.25
	E1.5	D2EHPA 0.25	Na_2CO_3 1M 50	Toluene 46.5	SPAN 80 3.25

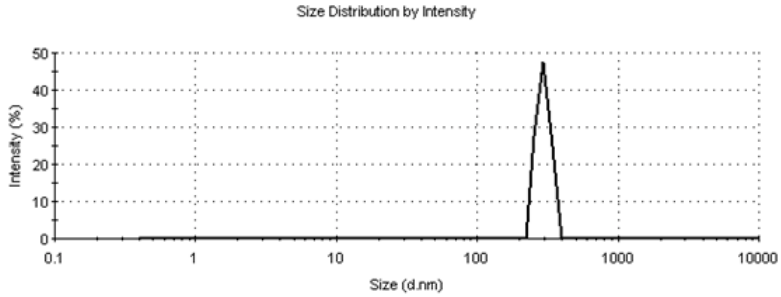

Fig. 1. The synthesized $CaCO_3$ particle size in emulsion system 1

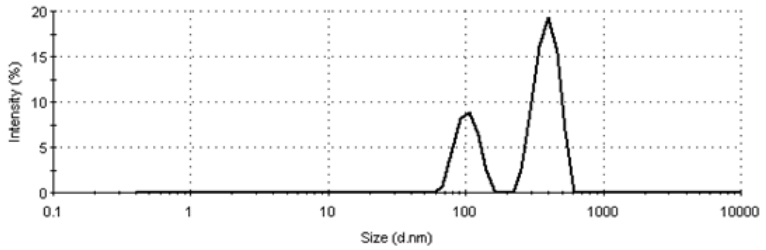

Fig. 2. The synthesized $CaCO_3$ particle size in emulsion system 2

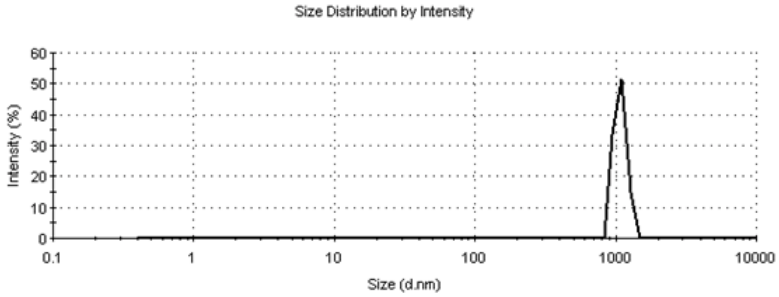

Fig. 3. The synthesized $CaCO_3$ particle size in emulsion system 3

Table 2
THE AVERAGE PARTICLE SIZE OF SYNTHESIZED $CaCO_3$

$CaCO_3$ particle sample	Average diameter (nm)
System 1	634.2
System 2	487.8
System 3	2395

(fig. 2). This is most likely due to synthesis process when the $CaCO_3$ particles were obtained by emulsifying only a single primary emulsion (E1.3). The small particle sizes seen for the two populations (around 100 nm and 400 nm) were attributed to kerosene solvent influence.

Although our experiments produce stable emulsions, the sedimentation and centrifugation stages in the preparation process have an evident impact on the size homogeneity of the final particles. However, no size control was attempted, the aim was to test the capacity of the $CaCO_3$ particles obtained by a simple ELM method as vehicles in drug delivery applications. The control of the particle size was achived by others [21], when the decrease in the concentration of CO_3^{2-} below 10^{-3} mol/L produced particles with sizes mostly between 50 and 100 nm in their experiments. Compared with ELM technique, the use of large surfactant quantities (more than 30 wt.%) in the reverse microemulsion can offer smaller particles, below 50 nm, as was observed by some authors [22]. A more interesting approach, especially for drug release applications, was published recently [23], where the preparation of $CaCO_3$ particles and the encapsulation of the drug was made in one-step at room temperature by in situ mineralization, and the results were nanospheres with uniform sizes and average diameters around 200 nm.

The FTIR spectra of pure doxycycline, nanostructurated $CaCO_3$, and doxycycline loaded $CaCO_3$ are given in figures 4 and 5.

As shown in figure 4, the main characteristic absorption bands of doxycycline are: 3670 cm^{-1} and 3413 cm^{-1} for –OH groups, 1667 cm^{-1} for C=O, and 1650 cm^{-1} for amide I group. The band at 1615 cm^{-1} is specific for -COOH group, and the bands at 1583 cm^{-1} and 1556 cm^{-1} are specific to amide II group. The bands at 1458 cm^{-1} and 1326 cm^{-1} are characteristic to the $-CH_2$ group. In the spectrum of doxycycline the vibration absorption band at 1243 cm^{-1} is ascribed to the amide III group.

The figure 5 displays the FTIR spectrum of doxycycline doped $CaCO_3$ compared with the FTIR spectrum of $CaCO_3$. Absorption bands at 2521 cm^{-1}, 1494 cm^{-1}, 1428 cm^{-1}, 875 cm^{-1}, and 712 cm^{-1} emphasize the presence of CO_3^{2-} anion in the spectrum of $CaCO_3$. In the doxycycline doped $CaCO_3$ spectrum there were identified characteristic absorption

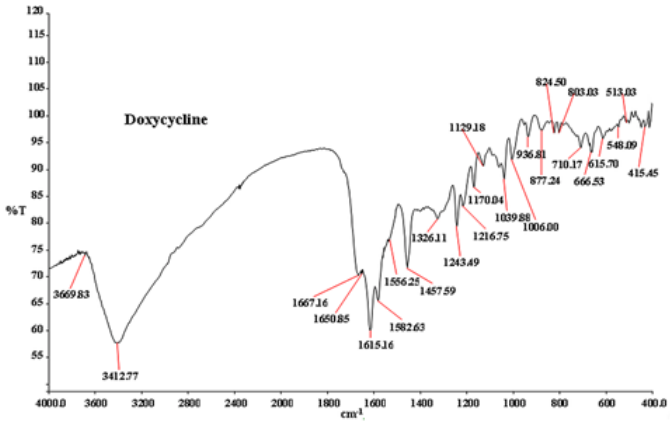

Fig. 4. FTIR spectrum of doxycycline

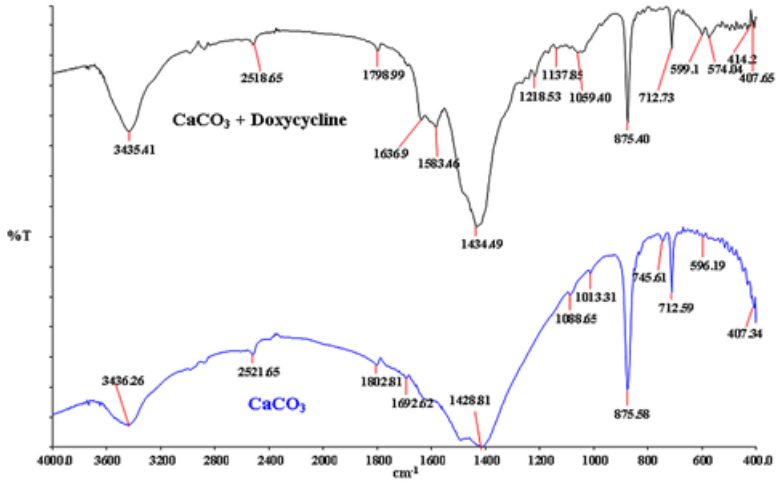

Fig. 5. FTIR spectrum of $CaCO_3$ and doxycycline doped $CaCO_3$

bands associated with doxycycline at 1636 cm^{-1}, 1583 cm^{-1}, 1475 cm^{-1}, 1243 cm^{-1}, 1218 cm^{-1}, 1170 cm^{-1}, 1138 cm^{-1}, 1059 cm^{-1}, 1040 cm^{-1}, 785 cm^{-1}, 767 cm^{-1}, 599 cm^{-1}, 574 cm^{-1} and typical absorption bands for $CaCO_3$ spectrum without any new bands. This indicates that doxycycline adsorption on the $CaCO_3$ nanoparticles is a physical process and the properties of doxycycline are preserved during the loading process, an important behaviour for a controlled drug release application.

In vitro loading and release of doxycycline

The loading efficiency for doxycycline was studied using $CaCO_3$ particles synthesized in the emulsion system 1 (average particle size ~650 nm) and system 3 (average particle size ~2500 nm).

Figure 6 shows the cumulative loading amount of doxycycline in $CaCO_3$ particles after 30 h contact. The initial doxycicline concentration was 0.1 mg/mL for both systems.

As seen, the drug loading percentage on $CaCO_3$ was around 95%, slightly better for system 1. A notable difference was in the length of contact time needed to attain the adsorption equilibrium. The amount of adsorbed doxycicline was almost at equilibrium after 5 h for system 1, in contrast to almost 30 h for the same amount attained by system 3. The particle size plays an important role on the time efficiency of the adsorbtion process, with little effect on the amount capacity for the same drug. The main interaction occurs between the drug and the surface of $CaCO_3$ particles by hydrogen bonding between the hydroxyl groups (OH) of doxycycline and the carbonyl groups of calcium carbonate. To form this bonds, a shorter time is necessary for a high number of small calcium carbonate particles to adsorb the drug, as is the case with system 1, compared with a small number of large particle (system 3).

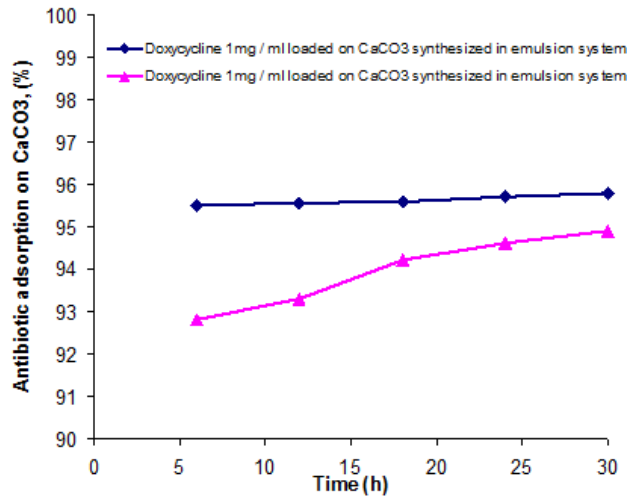

Fig. 6. Drug loading percentage onto calcium carbonate particles

The *in vitro release* study of doxycycline-loaded $CaCO_3$ was carried out at room temperature. The $CaCO_3$ particles synthesized in the emulsion system 1 were chosen due to the more effective time achieved in the drug loading experiments. The technique consists in the release of doxycycline from the surface of $CaCO_3$ particle into the PBS solution. Subsequently, the doxycycline can diffuse in the PBS where the drug concentration is determined. Data for the release profile at different intervals is presented in figure 7.

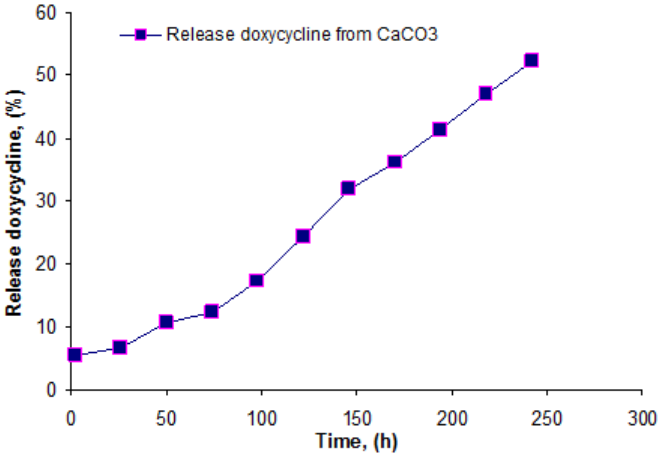

Fig. 7. The *in vitro* release of doxycycline from $CaCO_3$ particles

The desorption of doxycycline from $CaCO_3$ particles have a steady and slow drug release for a period of 242 h without an obvious initial burst release. After 50 h less than 10% of the doxycycline was released, and after 242 h (approximate 10 days) the release of doxycycline from $CaCO_3$ particles was around 50%. Current research in medicine for development of drug delivery systems has the aim to reduce the initial burst and to obtain a constant release rate. The stability of $CaCO_3$ – doxycycline system provides the opportunity for their use when a slow release over a long period of time is needed.

Due to their adsorption efficiency and effectiveness of slow release, the antibiotics - $CaCO_3$ particle biomaterials can be used as potential carriers for drug delivery applications.

Conclusions

$CaCO_3$ particles were synthesized by emulsion liquid membrane technique. Sizes from around 100 nm to 3500 nm were obtained, depending on the emulsion system and the solvent used.

The $CaCO_3$ particles have a high drug loading capacity, and the adsorption period depends on the particle size. The doxycycline loading percentage on $CaCO_3$ particles was around 95%, and the smaller particles have reached this amount at least 6 times faster than the larger ones.

The $CaCO_3$ particles with the average diameters around 650 nm attained an adsorbtion of doxycycline of maximum 97.9%, and a slow and steady release with a cumulative value of approximative 50% after ten days.

The results shows that the $CaCO_3$ particles synthesized by ELM technique can be used as potential drug carriers for the delivery of molecules over long periods of time. Further studies can be made for other drugs or various molecules to test this ability.

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