Ultrasonic Enhancement of Drug Release from Microspheres

M.M. Mohamed, M.A. El belbese, M. A. Kotb, N. M. Fikry and H.S. Ramadan
Biophysics Department, Medical Research Institute, Alexandria University, Egypt

INTRODUCTION

Targeting is a new therapeutic tool for malignant tumor as result of combining micro and nanotechnology with chemotherapeutics. Ultrasound has been shown to enhance degradation and drug delivery from biodegradable and non biodegradable polymeric devices. If a microsphere is partially filled with an entrapped drug substance, it is then able to transport the drug through blood vessels and release its load upon being triggered by an ultrasound pulse, which cracks the shell [1]. Drug targeting by ultrasound requires the use of sharp ultrasound waves focused on the tumor and does not require ultra-high ultrasound energies. It has also been shown that at constant frequency; drug release increases with increasing power density. Optimal power density of ultrasound waves ranges from 1 to 5 W cm–2, depending on the time, period of sonication which is typically 30 s up to a few minutes when continuous wave ultrasound is applied [2]. The present work aims to study the effect of ultrasonic waves and chitosan polymer microspheres loaded with 5-fluorouracil (5-FU) in improving drug release.

Experimental

The chitosan microspheres were prepared by chemical cross-linking method as described by Dubey and Parikh [3]. Briefly, 100 ml of paraffin oil was mixed with 1 ml of span 80. To this, 3 mL of chitosan solution was added dropwise with continuous stirring at 2000 rpm. After the complete addition of chitosan solution, 0.25 mL of glutaraldehyde was added to the mixture three times, twice after 1 hour and then after 2 hours, respectively. Suspension obtained was allowed to stand for 1 hour. Microspheres obtained as residue were washed 4 times with ether. After the final wash, microspheres were allowed to dry in air. The loading of 5-Fluorouracil on prepared chitosan microspheres was carried out by keeping 100 mg chitosan microspheres in 20 ml phosphate buffer solution (pH 5) containing 10 mg of 5-flurorouracil for 48 h. The amount of 5-flurorouracil loaded on microspheres was determined by recording the absorbance of the loading solution at λ = 257 nm using spectrophotometer. The prepared chitosan microspheres were characterized by analysis of particle size, scanning electron microscope, Fourier-transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD) and thermogravimetric Analysis (TGA). Encapsulation efficiency (EE) was calculated using the equation:

$$EE\,\% = \frac{Actual\ drug\ content}{Theoritica\ l\ drug\ content} x100 \quad (1)$$

Theoretical drug content was determined assuming that the entire drug present in the chitosan solution used gets entrapped in microspheres and no loss occurs.

The actual drug content in the 5-FU-loaded chitosan microspheres was quantitatively determined by immersing the dried microspheres (100 mg) in 20 mL of phosphate buffer saline pH 7.4. The solution was collected and the drug content entrapped inside the microspheres was determined by UV-Vis spectrophotometry at 257 nm.

To investigate the drug release behavior of prepared microspheres at 25 °C, microspheres loaded with 100 mg 5-Fu were kept in 20 ml phosphate buffer solution (pH 7). The amount of 5-Fu released in solution at different time intervals with different ultrasound intensities (Watt/cm2) was estimated recording absorbance at kmax=257 nm of filtered release medium using Shimandzu UV-VIS-1601 PC spectrophotometer.

RESULTS AND DISCUSSION

The particle size distribution curve of prepared chitosan microspheres (Fig 1) shows sharp distribution range of microspheres, with 90% of spheres in size range of 280–260 nm with average particle size of 422 nm and only 10% were oversized. The shapes of the dried microspheres were completely spherical, and the surface was rough and unfolded as shown by SEM (Fig 2).

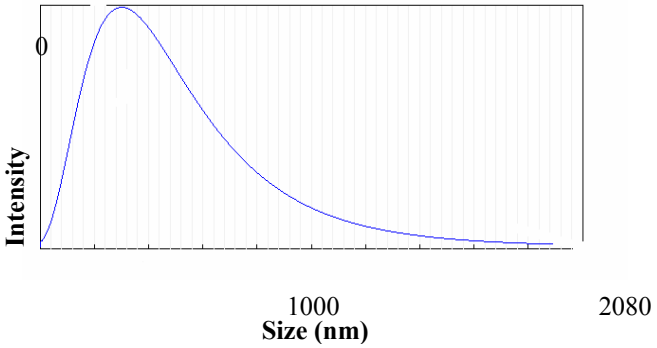

Fig 1: The particle size distribution curve for prepared hollow chitosan microspheres.

The FTIR spectra of chitosan and cross-linked chitosan (Fig 3) showed a characteristic band at attributed to NH_2 and OH groups stretching vibration and the band for amide I is seen in the infrared spectrum of chitosan. Whereas in the FTIR spectra of cross-linked chitosan two new peaks appears and others diaappeared.

XRD spectra of chitosan showed two prominent crystalline peaks (fig 4). In the case of cross-linked chitosan there was significant decrease in the intensity of characteristic peaks of chitosan. The distinct differences in the diffraction patterns of chitosan and cross-linked chitosan could be attributed to modification in the arrangement of molecules

in the crystal lattice, which might be due to amorphization

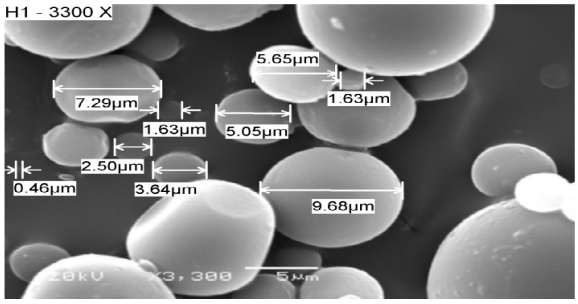

Fig 2: SEM image of chitosan microspheres (a) and Cross-section view of hollow microsphere showing cavity of chitosan microsphere (X 7000).

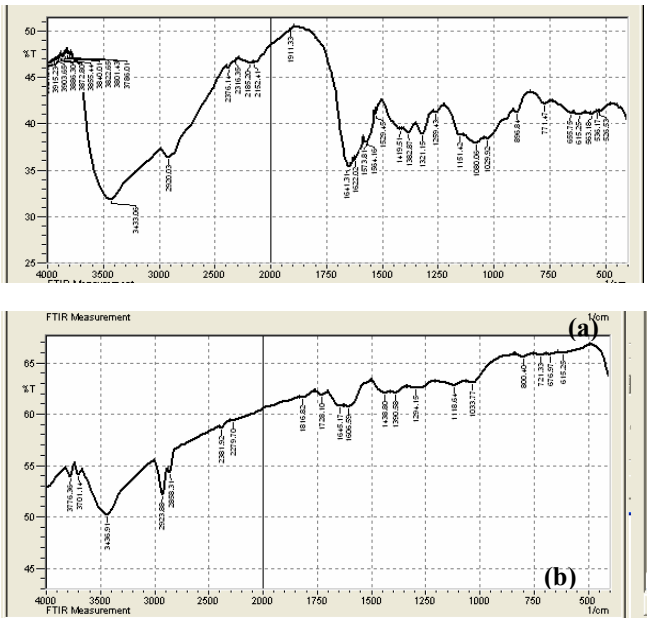

Fig 3: Fourier-transform Infrared spectra of chitosan powder (a) and crosslinked chitosan microspheres (b).

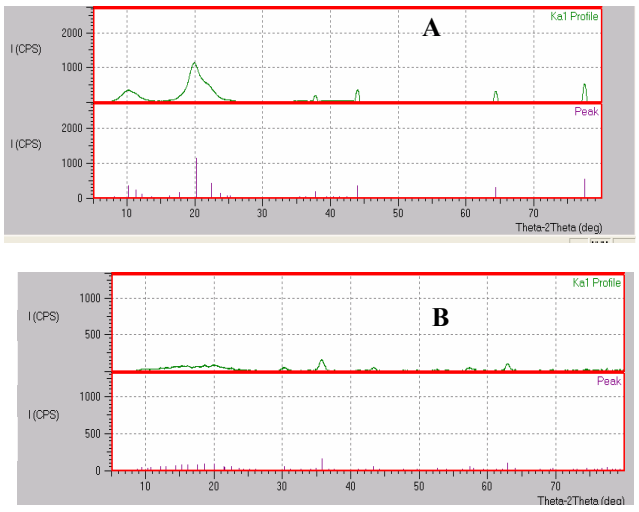

Fig 4: X-ray diffraction pattern of chitosan powder (A), cross-linked chitosan microspheres (B).

TGA (fig 5) showed that the chitosan powder and crosslinked microspheres showed strong transitions peaks were found at 210-230 ^{0}C and at 211-298 ^{0}C respectively. The percentage drug entrapment (PDE) was 33.09%. The amount of drug load was 132 µg drug/mg nanospheres. The percent of 5-fluorouracil released from chitosan microspheres was 97.73% after 30 min exposure for 0.8 MHz pulsed ultrasound waves at 1 W/cm^{2}. Sonication enhances drug release from microspheres, and subsequently increases the concentration of the free (non-encapsulated) drug.

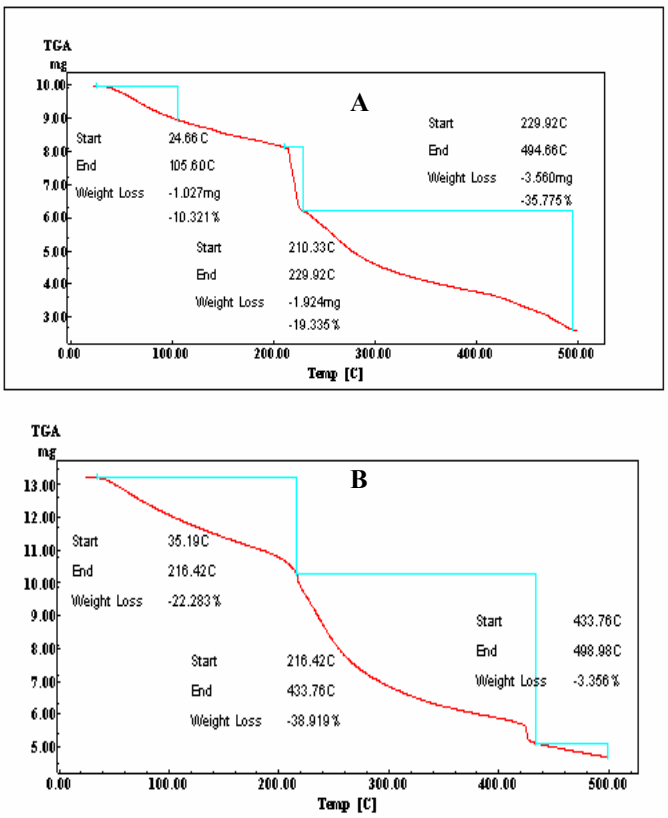

Fig 5: Thermogravimetric analysis of chitosan powder (left), cross-linked chitosan microspheres (right).

References:-

[1] NY Rapoport, D A Christensen, H D Fain , L Barrows and Z Gao. “Ultrasound-triggered drug targeting of tumors in vitro and in vivo”. Ultrasonics 2004; 42:943–50.

[2] A Marin, H Sun, G Husseini, W Pitt, D Christensen and N Rapoport. “Drug delivery in Pluronic micelles: Effect of high-frequency ultrasound on drug release from micelles and intracellular uptake.” J. Control. Release 2002; 84:39–47.

[3] RR Dubey and RH Parikh. “Two-Stage Optimization Process for Formulation of Chitosan Microspheres.” AAPS Pharm Sci Tech 2004; 5 Article 5 (http://www.aapspharmscitech.org).