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Biopolymeric microparticles combined with lyophilized monophase dispersions for controlled flutamide release

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ABSTRACT

Despite its short half-life, no controlled release formula of flutamide (FLT) was prepared until now. Therefore, 15 chitosan microparticle formulations were prepared for oral prolonged delivery of FLT via ionotropic gelation and emulsification-ionic gelation techniques then characterized for various parameters. FLT was successfully encapsulated into microparticles with loading capacity up to 39.98% and entrapment efficiency up to 97.16% using emulsification technique. Differential scanning calorimetry indicated that FLT was retained in a crystalline form in the microparticles prepared using ionotropic gelation whereas its crystallinity was significantly reduced using emulsification technique. Relationship between formulation variables and release behavior of FLT was explored. Chitosan microparticles prepared by ionotropic gelation showed a slower FLT release with a $T_{25\%}$ of 7.9 h whereas microparticles prepared by emulsification-ionic gelation under the same conditions showed a quick release profile with a $T_{25\%}$ of 0.3 h. Using 3 different hydrophilic carriers, immediate release FLT dispersions were prepared via lyophilization of monophase solution technique then combined with prolonged release chitosan microparticles to develop 6 controlled release formulae of FLT. A wide range of FLT release profiles were generated providing a prolonged release of drug after a suitable initial burst release.

1. Introduction

Prostate cancer has become one of the most common malignancies in the male population worldwide. Antiandrogenic agents are therapeutically effective for benign prostatic hypertrophy (BPH) and androgen dependent prostate cancer. Of the nonsteroidal antiandrogens, flutamide (FLT) is the only one presently recommended for monotherapy (Denis, 1995; Debruyna, 1996).

The low bioavailability of FLT after oral formulations may be due to poor wettability, low aqueous solubility and low concentration at the absorption surface (Nari, 1989). Therefore, developing novel formulations that mitigate solubility and dissolution will produce higher concentrations of FLT in solution at the absorption site and hence may overcome the solubility-mediated poor bioavailability.

Since FLT undergoes a rapid first pass hepatic metabolism after oral administration resulting in a relatively short half-life of 5–6 h, it is usually given 250 mg three doses per day. Therefore, a prolonged-release dosage form has been recommended in order to enhance patients' compliance and to reduce the incidence of local side effects such as nausea and diarrhea (Nari, 1989; Zuo et al., 2002).

Usually, there are two steps for the formulation of a controlled-release dosage form of a poorly water-soluble drug. First, different technologies such as reduction of particle size and/or solid dispersion of drug with polymers are utilized to improve the drug dissolution rate. Thenafter, a controlled-release technology can be applied to achieve a sustained release of the drug (Huang et al., 2006; Jingjun et al., 2006).

Although the pharmacokinetics and dosage characteristics of FLT make it a suitable candidate for the design of controlled-release delivery systems, to our knowledge, no controlled release formulation of FLT was prepared until now. In our previous studies, immediate-release FLT dispersions with cyclodextrins, hydrophilic polymers, polyols and aminoacids (Elgindy et al., 2010, 2011a, 2011b) have been prepared via lyophilization of monophase solution technique to enhance the dissolution rate of FLT. The dissolution rate of FLT could be enhanced to variable extent.

Chitosan microparticles have a potential application for preparing controlled release drug delivery systems (Sinha et al., 2004). Therefore, in this study chitosan microparticles as oral prolonged release vehicle for FLT were prepared utilizing simple ionic gelation and emulsification-ionic gelation techniques. The effect of various formulation variables on the release behavior of FLT from the prepared chitosan microparticles was also evaluated. In the second step, controlled release formulae of FLT were designed by combining immediate and sustained release components in a hard gelatin

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Table 1
Composition, loading capacity and entrapment efficiency of FLT-loaded chitosan microparticle formulations prepared by different formulation variables (values are the mean ± SD, $n = 3$).

Formula	Preparation method	D/P ratio	Chitosan (%w/v)	Crosslinker (%w/v)	Loading capacity (%w/w)	Entrapment efficiency (%w/w)
L_1	Ionic gelation	1:1	1	1% TPP	31.56 ± 4.34	95.30 ± 4.21
L_2	Ionic gelation	1:2	1	1% TPP	19.61 ± 1.34	85.00 ± 4.35
L_3	Ionic gelation	1:3	1	1% TPP	14.94 ± 2.70	83.83 ± 4.88
L_4	Ionic gelation	1:5	1	1% TPP	10.66 ± 1.47	79.34 ± 4.42
L_5	Emulsification	1:1	1	1% TPP	25.00 ± 1.36	80.10 ± 1.56
L_6	Emulsification	1:1	1.5	1% TPP	33.34 ± 1.35	92.30 ± 4.06
L_7	Emulsification	1:1	2	1% TPP	25.29 ± 2.48	61.40 ± 2.76
L_8	Emulsification	1:1	1	5% TPP	19.60 ± 0.76	97.16 ± 2.64
L_9	Emulsification	1:1	1	10% TPP	16.07 ± 0.96	66.00 ± 4.28
L_{10}	Emulsification	1:1	1	1% TPP + 6.25%GA	39.98 ± 2.64	88.34 ± 4.36
L_{11}	Emulsification	1:1	1	1% TPP + 12.5%GA	38.02 ± 2.53	86.23 ± 4.26
L_{12}	Emulsification	1:1	1	1% TPP + 25% GA	34.03 ± 1.48	85.19 ± 3.56
L_{13}	Emulsification	1:1	1	1%TPP + 1% SPA	22.93 ± 1.83	89.45 ± 3.35
L_{14}	Emulsification	1:1	1	1%TPP + 1%HPβCD	26.33 ± 2.74	84.27 ± 4.36
L_{15}	Emulsification	1:1	1	1%TPP + 1% SLS	23.09 ± 4.33	78.49 ± 4.24

D/P ratio: drug/polymer ratio; GA: glutaraldehyde; SPA: sodium polyacrylate.

capsule. Various combined formulae were evaluated as a controlled release dosage form of FLT using the pH gradient technique.

2. Materials and methods

2.1. Materials

Flutamide (FLT) was kindly donated by Archimica (Origgio, Italy). Highly viscous chitosan (CS), viscosity > 400 mPa s, 1% in acetic acid (20 °C), sodium triphosphate pentabasic (TPP), hydroxypropyl-β-cyclodextrin (HPβCD), glutaraldehyde solution, GA (25%, v/v in water), sodium polyacrylate (SPA) and tertiary butyl alcohol (TBA) were purchased from Sigma–Aldrich (St. Louis, USA). Pluronic F-127 was kindly supplied by Pharaonia Pharmaceuticals (Alexandria, Egypt). L-Arginine as purchased from Qualikems (India). Polyoxyethylene sorbitan monooleate (Tween 80) was from (Riedel-de Häen, Germany). Sodium lauryl sulfate (SLS) was from Oxford laboratory reagent (India). Methylene chloride was obtained from ADWIC, El-Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt). All other chemicals were of analytical grade and used without further purification.

2.2. Preparation of chitosan microparticles

2.2.1. Simple ionotropic-gelation technique

Chitosan microparticles were prepared by a capillary extrusion procedure (Bodmeier et al., 1989). FLT was dispersed in a 10 mL solution of chitosan (1%, w/v) in acetic acid (1%, v/v). Microparticles were formed by dropping the bubble-free CS/FLT dispersion through a glass syringe (a nozzle of 2 mm inner diameter) into 100 mL of a magnetically stirred (RH basic, Ika Labortechnic, Germany) solution of the crosslinking agent (TPP) for 30 min. The falling distance was 10 cm. The supernatant was decanted and the gelled MPs were washed with distilled water and then air dried for 24 h.

2.2.2. Emulsification ionic-gelation technique

Chitosan (1%, w/v) was dissolved in 10 mL 1% (v/v) acetic acid solution containing 2% (w/v) Tween 80. FLT was dissolved in 1 mL of methylene chloride and then emulsified with the aqueous phase by a MPW-120 homogenizer (MPW Med. Instruments, Poland) at 9000 rpm for 20 min. The ratio of oil and aqueous phase was 1:10 (v/v). The O/W emulsion was dropped into 100 mL of 1% (w/v) TPP solution through a glass syringe. After certain crosslinking time, the supernatant was decanted and the microparticles were washed with distilled water and then air dried for 24 h.

Chemically co-crosslinked microparticles were prepared by using TPP solution containing 2 mL glutaraldehyde solution (6.25, 12.5 or 25%, v/v). On the other hand, ionically co-crosslinked microparticles were prepared by using TPP solution containing 1% (w/v) of sodium polyacrylate, SLS or HPβCD.

The two techniques described above were used to prepare a total of 15 FLT-loaded chitosan microparticle formulations using different formulation variables (Table 1).

2.3. Design of FLT controlled-release formulations

FLT immediate-release lyophilized dispersions (LDs) with three different hydrophilic carriers were prepared using the lyophilization of monophase solution technique described in our previous studies (Elgindy et al., 2010, 2011a, 2011b). Flutamide was dissolved in TBA (100 mg/5 mL), an equal amount of carrier (HPβCD, Pluronic F127 or arginine) was dissolved in 5 mL water and mixed with the drug–TBA solution then lyophilized to give 1:1 FLT–carrier LD. Immediately after mixing, the vials were frozen at −80 °C for 4 h followed by placing them in a Cryodos-50 lyophilizer (Telstar Cryodos, Spain) with a condenser temperature of −70 °C. Lyophilization was performed at a pressure of 40 mbar and a shelf temperature of −40 °C for 1 day followed by a secondary drying at 25 °C for another day.

FLT controlled release formulae (F_1–F_5, Table 2) were prepared by simply mixing the immediate release lyophilized dispersion (equivalent to 65 mg FLT) and the prolonged release chitosan microparticles (equivalent to 60 mg FLT) and then filled into a hard gelatin capsule.

A microencapsulated lyophilized dispersion of FLT (F_6, Table 2) was developed as a novel controlled release formula. FLT–Pluronic 1:1 LD, equivalent to 125 mg FLT, prepared as described above

Table 2
The composition of flutamide controlled-release formulae prepared by mixing immediate and prolonged release components.

Formula	Prolonged-release component (equivalent to 60 mg FLT) + immediate-release component (equivalent to 65 mg FLT)
F_1	FLT-HPβCD 1:1 LD + L_1 (ionic-gelation CS MPs)
F_2	FLT-Pluronic 1:1 LD + L_1 (ionic-gelation CS MPs)
F_3	FLT-Arginine 1:1 LD + L_1 (ionic-gelation CS MP)
F_4	FLT-HPβCD 1:1 LD + L_7 (emulsification 2% CS MPs)
F_5	FLT-HPβCD 1:1 LD + L_{12} (emulsification CS 25% GA MPs)
F_6	Ionic-gelation CS MPs encapsulating FLT-Pluronic 1:1 LD 125 mg FLT

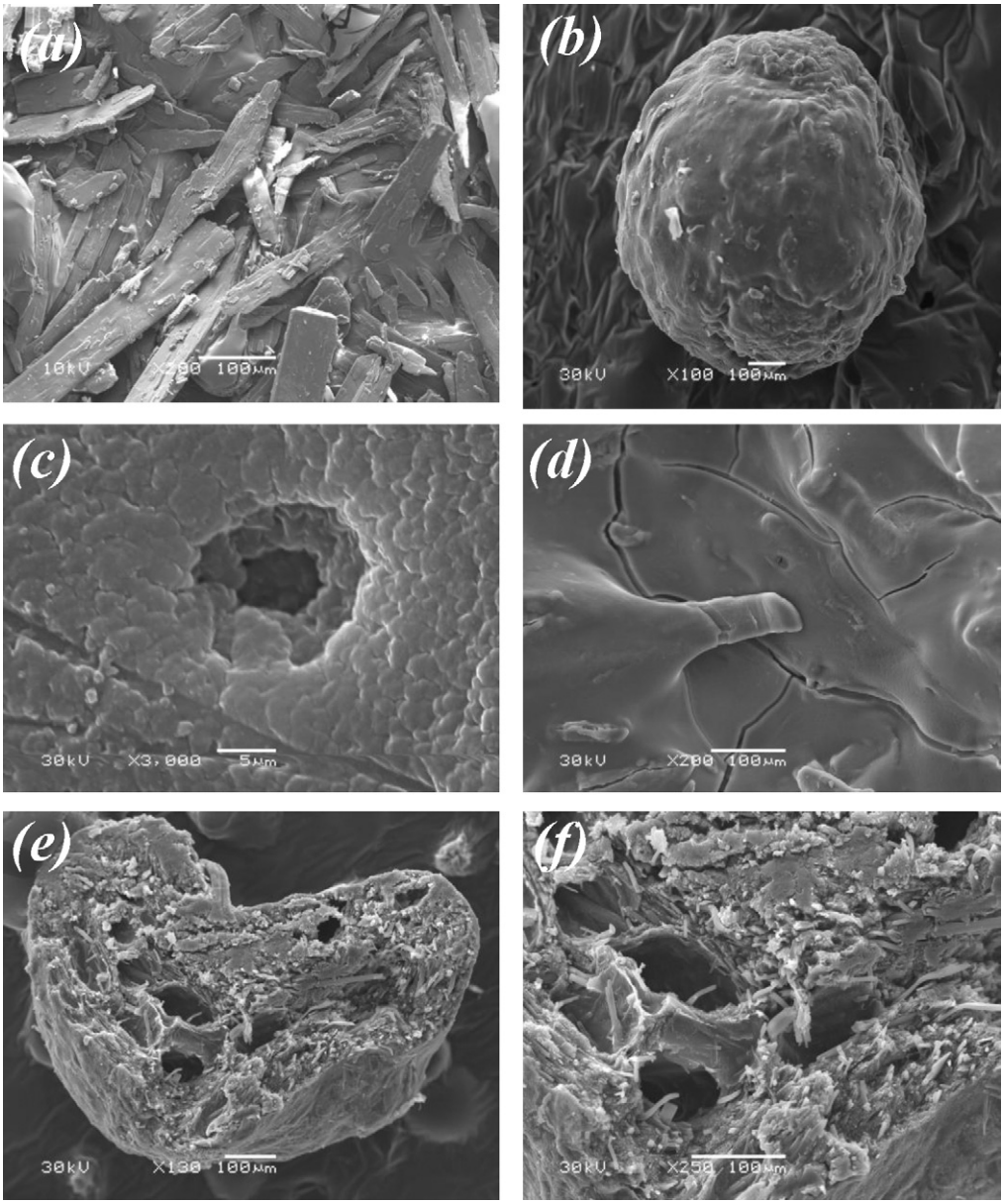

Fig. 1. Scanning electron micrographs of FLT and FLT-loaded chitosan microparticles showing: (a) morphology of pure FLT, (b) morphology of chitosan microparticles, (c and d) surface topography of chitosan microparticles, (e and f) cross-sectional morphology of chitosan microparticles.

was dispersed in 10 mL of chitosan solution and was used for the preparation of chitosan microparticles by simple ionotropic gelation technique.

2.4. HPLC assay for FLT

A reverse phase HPLC method was used for quantifying FLT (Umrethia et al., 2005). HPLC analysis was carried out with a Perkin Elmer series 200 chromatograph (Perkin Elmer, USA) using a Spheri-5, RP-18, 220 mm × 4.6 mm, 5 μm column (Perkin Elmer, USA) and a UV detector. An isocratic solvent system consisting of 75:25 (v/v) methanol–water was used at a flow rate of 1 mL/min, an injection volume of 20 μl and the peaks were detected at 304 nm. Under these experimental conditions the total run time was approximately 6 min and the retention time was 3.5 min. Calibration curves (peak area versus drug concentration) were linear ($R^2 > 0.999$) over the FLT concentration range of 0.6–60 μg/mL.

2.5. Characterization of chitosan microparticles

2.5.1. Drug loading capacity (%LC) and entrapment efficiency (%EE)

An aliquot of accurately weighed 15 mg of each batch of the MPs was triturated in a mortar and then transferred into a 50 mL volumetric flask containing 25 mL methanol. The flask was shaken vigorously for 15 min and then left for 8 h with intermittent shaking for complete drug extraction. The solution was then filtered through a 0.45 μm membrane filter and assayed for FLT content by HPLC at 304 nm. All the experiments were carried out in triplicate. The percentage loading capacity (%LC) and entrapment efficiency (%EE) for each formula were calculated using the following equations:

$$\%LC = \frac{\text{amount of drug entrapped in MPs}}{\text{total amount of MPs}} \times 100 \quad (1)$$

$$\%EE = \frac{\text{amount of drug entrapped in MPs}}{\text{initial amount of drug added}} \times 100 \quad (2)$$

2.5.2. Morphology observation

The surface topography of FLT-loaded CS MPs was examined using a JEM-100S scanning electron microscope (JOEL, Japan). Samples were mounted on metal stubs using double-sided adhesive tape then coated with approximately 10–20 nm gold film for 20 s under vacuum using a sputter coater and then examined. Cross-sections were obtained by cutting the microparticles with a razor blade in order to observe the core and internal structure of the MPs. Scans were performed at an acceleration voltage of 10 kV.

2.5.3. Differential scanning calorimetry (DSC)

DSC thermograms CS, FLT and CS FLT-loaded MPs were recorded by DSC 6 differential scanning calorimeter (Perkin Elmer, USA). Samples (2–4 mg) were placed in sealed aluminum pans and heated at 10 °C/min under a nitrogen atmosphere (flow rate 20 mL/min) in the range of 30–400 °C. An empty aluminum pan was used as a reference. The equipment was periodically calibrated with indium. Inert atmosphere was maintained by purging nitrogen at a flow rate of 20 mL/min.

2.5.4. In vitro release of FLT from chitosan microparticles

The release of FLT from CS MPs was investigated using the USP XXIV dissolution apparatus II (Pharmatest, Germany) with a paddle speed of 100 ± 2 rpm. A known weight of MPs (equivalent to 60 mg drug) was added to 900 mL PBS (pH 7.4) containing 0.2% (w/v) Tween 80 at 37 ± 0.5 °C. At appropriate time intervals, 5 mL samples were withdrawn and immediately replaced with an equal volume of prewarmed release medium at the same temperature till 12 h. All samples were run in triplicates, filtered through a 0.45 μm membrane filter and the amount of FLT released was analyzed by HPLC at 304 nm. The percentage cumulative amount of drug released at each time interval was plotted against time.

The release of FLT from different controlled release formulae (F_1–F_6) was investigated using the pH-gradient method (F.I.P., 1996). A known weight of the formula (equivalent to 125 mg FLT) was added to 900 mL 0.1 N HCl (pH 1.2) containing 0.2% (w/v) Tween 80 at 37 ± 0.5 °C. After 2 h, 30 g of sodium triphosphate was added to the dissolution medium to change the solution pH gradually from 1.2 to 7.4. The same sampling and analysis schedule was repeated (F.I.P., 1996).

3. Results and discussion

3.1. Drug loading capacity and entrapment efficiency

FLT was found to be efficiently entrapped into CS MPs using the preparation process. The relatively high efficiency of encapsulation could be explained on the basis of the low water solubility of FLT during crosslinking and hardening process. Therefore, the loss of FLT from MPs was minimal during the hardening and washing process (Ko et al., 2002).

The values of drug loading capacity (%LC) and entrapment efficiency (%EE) of FLT-loaded MPs prepared from various compositions are listed in Table 1. The %LC obtained ranged between 10.66 and 39.98% (w/w) corresponding to formulae L_4 and L_{10}, respectively, while %EE was in the range of 61.40–97.16% (w/w) corresponding to formulae L_7 and L_8, respectively.

Using ionic gelation technique, it can be seen that increasing the initial loading of FLT from 1:5 to 1:1 drug/polymer ratio, led to a corresponding increase in % LC from 10.66 to 31.56% (w/w) and % EE from 79.34 to 95.3% for formulae L_4 and L_1, respectively (Table 1). At L_1 (D/P 1:1), the high concentration of drug may cause a molecular abundance (crowding) between drug molecules within the chitosan droplets leading to a steric hindrance at the escaping sites, hindering escape of drug molecules out of the droplets and thus increasing %EE. Our work is in accordance with that of Govender et al. (2006), who reported that increasing the theoretical loading of tetracycline HCl in the formulation from 10–60% (w/w) led to a corresponding increase in drug content from 0.36 to 21.26% (w/w) and drug entrapment from 5.47 to 69.81%.

A number of reports have shown that the entrapment efficiency increases with an increase in CS concentration. This may be explained on the basis that the increase in viscosity of the CS solution with increasing its concentration prevents drug crystals from leaving the droplet (Ventura et al., 2008). Since greater amount of polymer is able to incorporate a greater amount of drug, increasing CS concentration from 1 to 1.5% (w/v) increased the EE from 80.1 to 92.3% (w/w). Using emulsification technique, Further increase in polymer concentration to 2% (w/v) leads to a reduction in EE to 61.4% (w/w) which may be due to poor homogenization efficiency of the highly viscous 2% CS solution. Our unreported data using the ionic gelation technique showed a gradual increase in %EE with increasing polymer concentration. On the other hand, using the emulsification-ionic gelation technique, FLT is present as fine solution droplets within the aqueous chitosan phase. The high chitosan solution viscosity (2%, w/v) may retard the coalescence of the oily drug solution droplets resulting in a small drug droplet size, increasing the surface area available for escape of drug molecules thus reducing the %EE to 61%.

It was also deduced that a lower EE of 66% (w/w) and LC of 16.07% (w/w) were obtained with high concentration of TPP (10%, w/v) compared to 80.1% (w/w) EE and 25% (w/w) LC when using only 1% (w/v) TPP. Similar pattern was obtained in the work of Anal et al. (2006). For MPs crosslinked with 6.25, 12.5 and 25% (v/v) of glutaraldehyde, entrapment efficiencies were 88.34, 86.23 and 85.19% (w/w), respectively, i.e. EE decreases as the glutaraldehyde concentration increases. Such a decreasing trend is due to an increase in crosslinking density where glutaraldehyde forms a covalent crosslink with CS which consequently makes the microparticle more rigid thereby reducing the free volume spaces within the polymer matrix (Babu et al., 2008). The drug loading capacity consequently decreased from 39.98 to 34.03% (w/w) upon increasing glutaraldehyde concentration from 6.25 to 25% (v/v).

3.2. Morphology observation

Pure FLT crystals were clear in Fig. 1a while FLT-loaded CS MPs showed a spherical shape with a particle size range of 0.63–1.13 mm (Fig. 1b). SEM images revealed that FLT-loaded CS MPs have a rugged and wrinkled surface showing cracks and pores that may be due to the evaporation of water during air drying (Fig. 1c and d).

The cross-sectional morphology of FLT-loaded CS MPs is shown in Fig. 1e and f. The SEM images confirmed the porous nature of the MPs due to interconnecting network of CS. Some crystals of FLT were observed in the internal structure of the MPs. Similarly, Dhawan and Singla (2003), observed the presence of nifedipine crystals in the internal structure of the drug-loaded CS microspheres.

3.3. Differential scanning calorimetry (DSC)

The DSC thermograms of CS, FLT and FLT-loaded MPs prepared by both simple ionic-gelation and emulsification-ionic gelation techniques were shown in Fig. 2. The thermal behavior of CS powder has a large endothermic peak over 55–86 °C which is mainly due to water evaporation, this is followed by a wide exothermic peak occurring in the range of 285–300 °C. This area can express the overall exothermic effect connected with decomposition of CS (Hekmatara et al., 2006).

FLT thermogram shows a clear melting endothermic peak at 113 °C reflecting its crystalline nature (Adel et al., 1997). Upon

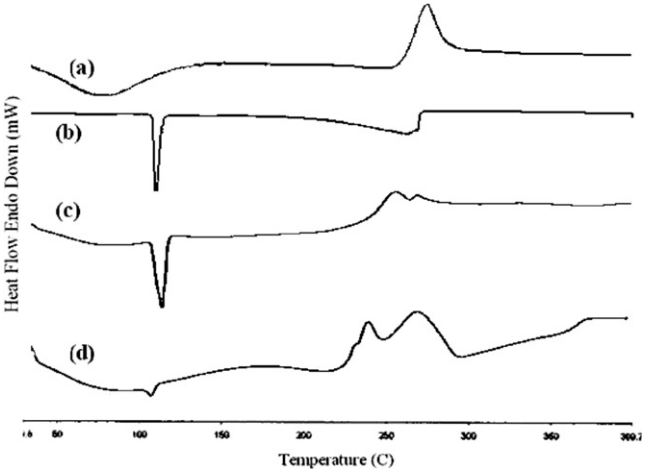

Fig. 2. DSC thermograms of chitosan (a), FLT (b), simple ionic gelation MPs (L_1) (c), emulsification-ionic gelation MPs (L_5) (d).

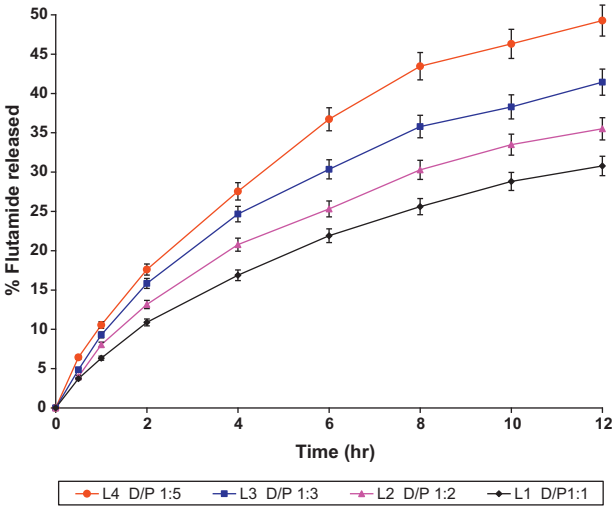

Fig. 3. The influence of drug/polymer ratio on FLT release from CS MPs prepared by ionic gelation technique in PBS (pH 7.4) at 37 °C (Table 1).

incorporation into the MPs, FLT peak was still detected in the thermogram of ionic gelation MPs (L_1) with a similar fusion enthalpy to pure drug indicating that FLT was retained in a crystalline form in these MPs. For the MPs prepared by emulsification-ionic gelation technique (L_5), the drug endothermic peak was slightly shifted from its original position with a very low fusion enthalpy reflecting that FLT crystallinity was significantly reduced indicating that the drug may be either dispersed molecularly or dissolved in the polymer matrix.

3.4. In vitro release of FLT from chitosan microparticles

3.4.1. Effect of drug/polymer ratio

The release profiles of FLT from CS MPs prepared by ionic gelation technique with different drug loadings in PBS (pH 7.4) at 37 °C are shown in Fig. 3. After 12 h, increasing FLT loading from 1:5 to 1:1 has reduced the (PR_{12}) % of drug released from 49.27 to 30.78%, respectively (Table 3). The decrease of drug ratio can make the drug well dispersed in the CS phase, followed by a decreasing size and increasing surface area of drug particles in the gel matrix. Thus, the decrease of drug content in bead may lead to increasing its release. Similar findings were recorded for the release of diclofenac sodium (Gupta and Ravi-Kumar, 2000) and indomethacin (Shiraishi et al., 1993) from CS MPs.

On the contrary, Dini et al. (2001) reported that the release of hydroquinone from CS microspheres increased, as the drug loading increased. This can be due to the enhancement of the driving force for diffusion inside the microspheres and outside in the release medium resulting in higher drug release rates.

3.4.2. Effect of chitosan and TPP concentration

The % FLT released after 1 h (PR_1) in PBS (pH 7.4) at 37 °C was 34.40, 19.64 and 12.34% from CS MPs prepared with 1, 1.5 and

Table 3
Release parameters of FLT from chitosan microparticles (L_1-L_{15}) in PBS (pH 7.4) and from different controlled release formulae (F_1–F_6) using the pH-gradient method (values are mean ± SD, $n = 3$).

Formula	PR_1[a]	PR_2[b]	PR_{12}[c]	$T_{25\%}$[d]	DE_8%[e]
L_1	6.32 ± (0.95)	10.88 ± (2.83)	30.78 ± (2.45)	7.9 ± (1.34)	16.70 ± (1.22)
L_2	8.04 ± (0.24)	13.15 ± (2.63)	35.50 ± (2.25)	6.3 ± (0.73)	19.00 ± (2.43)
L_3	9.29 ± (1.65)	15.83 ± (1.34)	41.43 ± (3.32)	4.3 ± (0.22)	22.44 ± (1.75)
L_4	10.54 ± (0.34)	17.67 ± (1.34)	49.27 ± (4.34)	3.5 ± (0.45)	25.87 ± (2.45)
L_5	34.40 ± (2.22)	36.68 ± (2.19)	50.77 ± (2.87)	0.3 ± (0.03)	38.07 ± (3.04)
L_6	19.64 ± (3.22)	25.64 ± (2.43)	39.88 ± (4.53)	1.9 ± (0.12)	29.32 ± (2.94)
L_7	12.34 ± (4.76)	15.58 ± (3.1)	30.55 ± (3.86)	8.3 ± (1.96)	18.66 ± (2.10)
L_8	25.70 ± (4.65)	30.30 ± (3.87)	47.32 ± (2.48)	0.9 ± (0.21)	33.86 ± (1.86)
L_9	22.80 ± (4.97)	26.98 ± (5.56)	42.83 ± (3.65)	1.8 ± (0.55)	29.94 ± (3.37)
L_{10}	36.78 ± (3.90)	40.68 ± (3.75)	58.54 ± (3.75)	0.3 ± (0.43)	43.39 ± (3.76)
L_{11}	15.29 ± (2.37)	21.18 ± (2.38)	43.35 ± (3.29)	3.1 ± (0.26)	26.61 ± (3.92)
L_{12}	12.17 ± (1.72)	17.82 ± (1.86)	37.76 ± (2.32)	4.4 ± (0.33)	22.51 ± (2.30)
L_{13}	18.74 ± (1.38)	28.21 ± (2.93)	40.33 ± (1.92)	1.8 ± (0.86)	30.15 ± (3.65)
L_{14}	33.70 ± (3.03)	37.88 ± (5.32)	58.44 ± (3.88)	0.4 ± (0.70)	42.68 ± (3.17)
L_{15}	24.83 ± (2.48)	29.52 ± (3.97)	45.64 ± (3.72)	1.1 ± (0.62)	33.09 ± (5.23)
F_1	52.83 ± (1.58)	56.85 ± (1.95)	68.20 ± (2.74)	0.28 ± (3.78)	58.56 ± (3.84)
F_2	22.23 ± (2.39)	37.44 ± (1.30)	54.44 ± (2.55)	1.25 ± (1.86)	41.67 ± (3.93)
F_3	35.35 ± (3.85)	46.08 ± (2.46)	61.20 ± (2.56)	0.50 ± (2.30)	48.11 ± (2.94)
F_4	50.18 ± (4.32)	51.37 ± (2.38)	57.13 ± (1.58)	0.22 ± (4.30)	50.91 ± (1.49)
F_5	38.19 ± (1.36)	41.20 ± (3.64)	51.01 ± (2.73)	0.42 ± (1.43)	41.77 ± (2.73)
F_6	36.82 ± (2.88)	49.75 ± (2.93)	65.23 ± (4.36)	0.72 ± (3.54)	53.39 ± (2.27)

[a] PR_1: % FLT released after 1 h.
[b] PR_2: percentage of FLT released after 2 h.
[c] PR_{12}: % FLT released after 12 h.
[d] $T_{25\%}$: time required to release 25% of FLT.
[e] DE_8%: % dissolution efficiency of FLT after 8 h.

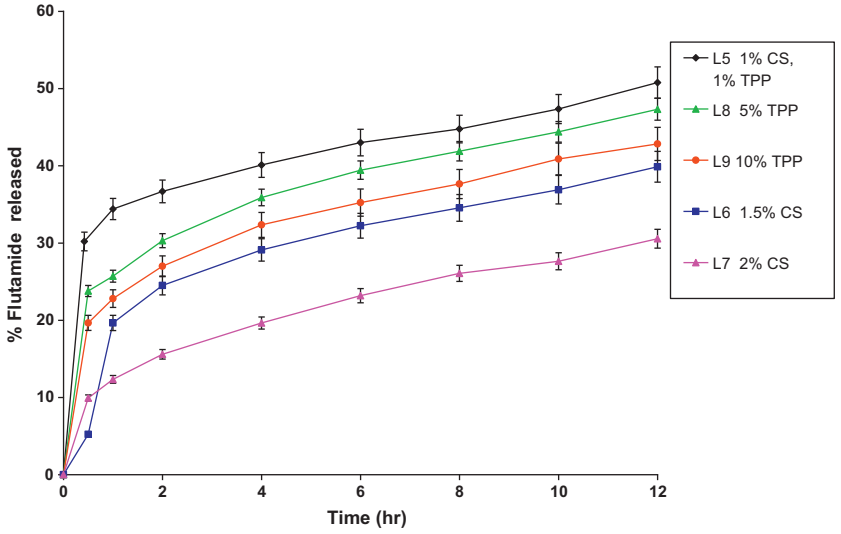

Fig. 4. The influence of chitosan and TPP concentration on FLT release from CS MPs prepared by emulsification-ionic gelation in PBS (pH 7.4) at 37 °C (Table 1).

2% (w/v) CS, respectively using emulsification technique (Fig. 4, Table 3). These results indicate that the release behavior of the drug is relative to the viscosity of CS solution. The increased viscosity of CS solution forms relatively strong walls of MPs upon interaction with TPP thus resulting in a slower drug release (Lim et al., 1997).

Fig. 4 also shows that the release of FLT from TPP/CS MPs decreased with the increasing TPP concentration. Only 20 min was required to release 25% of FLT ($T_{25\%}$) from 1% TPP-crosslinked CS MPs. Increasing TPP concentration to 10% increased the $T_{25\%}$ to 1.8 h (Table 3). This may be attributed to reduced swelling and erosion of CS MPs with the increased crosslinking of the matrices (Ko et al., 2002).

3.4.3. Effect of co-crosslinking

3.4.3.1. Chemical co-crosslinking. By increasing the concentration of glutaraldehyde as a chemical co-crosslinker from 6.25 to 25% (v/v), the $T_{25\%}$ of FLT from CS MPs prepared by emulsification technique increased from 2.5 to 4.4 h, respectively (Fig. 5, Table 3). At a high concentration of glutaraldehyde, the degree of polymer crosslinking increased and the available free space for drug diffusion decreased, resulting in slow drug release rates (Dini et al., 2001). Also a denser microparticle matrix might exhibit slower polymer degradation, thus resulting in slower drug release. This finding might imply that the CS MPs co-crosslinked with glutaraldehyde succeeded in the prolonging the drug release, which is very beneficial for maintaining the drug concentration in the therapeutic range.

3.4.3.2. Ionic co-crosslinking. Fig. 6 shows the release profiles of FLT from CS-TPP MPs prepared by emulsification and co-crosslinked with different ionic crosslinkers, namely; sodium lauryl sulfate (SLS) and sodium polyacrylate (SPA). Also hydroxypropyl-β-cyclodextrin (HPβCD) was used as a weakly ionic crosslinker (Maestrelli et al., 2006). After 8 h, HPβCD increased the % dissolution efficiency (DE_8) of FLT to 42.68% compared to 38.07% of TPP-crosslinked MPs. This may be attributed to the high solubilizing capacity of HPβCD. On the other hand, the DE_8 of FLT from SLS co-crosslinked MPs was reduced to 33.09% (Table 3). Elgibaly et al. (2003) attributed the slow release of melatonin

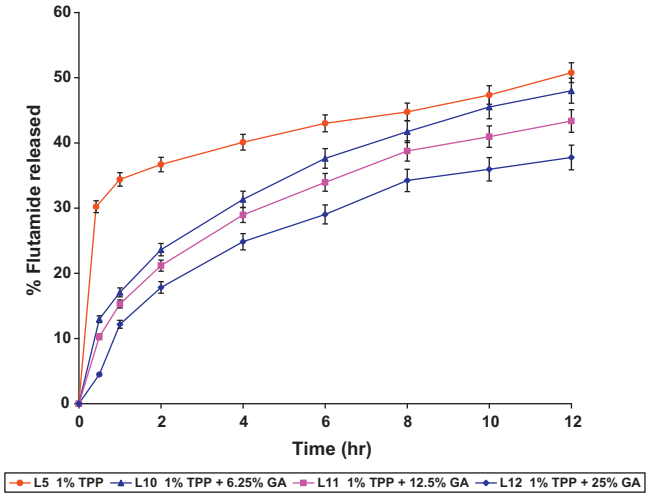

Fig. 5. The influence of glutaraldehyde concentration as a chemical co-crosslinker on FLT release from CS MPs prepared by emulsification-ionic gelation in PBS (pH 7.4) at 37 °C (Table 1).

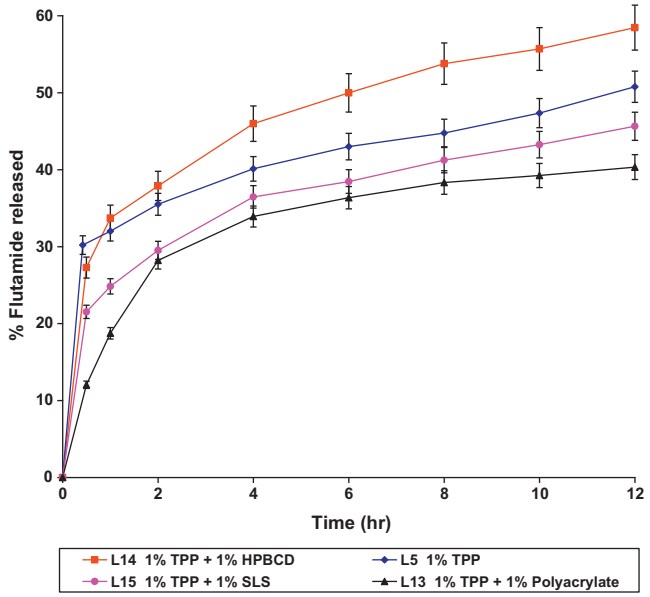

Fig. 6. The influence of type of ionic co-crosslinker on FLT release from CS MPs prepared by emulsification-ionic gelation in PBS (pH 7.4) at 37 °C (Table 1).

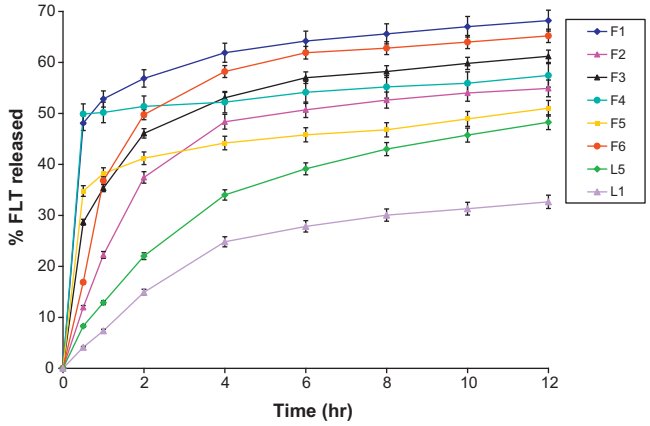

Fig. 7. The release profile of FLT from different controlled release formulations using the pH-gradient method (Table 2).

from SLS co-crosslinked microcapsules to their structured integrity. Additionally, the poor wetting ability and hydration of these microcapsules resulted in poor contact of drug in the particles with the dissolution medium. The reduction in drug release with systems containing SLS may be also due to the fact that the surface of the particles are rendered more hydrophobic by the SLS carbon chain protruding outside while the ionic head is linked to ammonium of the chitosan. Sodium polyacrylate (SPA) co-crosslinked CS/TPP-MPs showed the slowest release with a DE_8 value of 30.15%. SPA may form a high viscosity layer on the surface of chitosan particles thus reducing the drug diffusivity.

3.4.4. *Effect of preparation technique*

The effect of preparation technique on the release of FLT from CS MPs can be deduced by comparing FLT release from CS/TPP MPs prepared by simple ionotropic gelation (L_1, Fig. 3) and emulsification-ionotropic gelation (L_5, Fig. 4). CS/TPP MPs prepared by simple ionotropic gelation showed a slower FLT release with a $T_{25\%}$ of 7.9 h and 30.78% of drug was released after 12 h. Whereas MPs prepared by emulsification-ionic gelation under the same conditions showed a quick release profile with a $T_{25\%}$ of 0.3 h and 50.77% of drug was released after 12 h (Table 3). The fast release behavior of FLT from CS MPs prepared by emulsification-ionotropic gelation could be explained by the DSC results where FLT crystallinity was significantly reduced indicating that the drug was either dispersed molecularly or dissolved in the polymer matrix whereas it was present in a crystalline form in MPs prepared by simple ionotropic gelation.

3.5. *Evaluation of FLT release from controlled-release formulations*

The pH-gradient method was used for the evaluation of the in vitro release of FLT from different controlled-release formulae in order to simulate the conditions of human gastro-intestinal tract (GIT) (Fig. 7). Three different hydrophilic carriers namely HPβCD, pluronic F127 and arginine were chosen according to their ability to increase FLT release to prepare immediate release lyophilized dispersions (Elgindy et al., 2010, 2011a, 2011b) then these lyophilized dispersions were combined with chitosan microparticles in a capsule to prepare controlled release formulae of FLT.

From Fig. 7, it was noticed that chitosan microparticles prepared by both simple ionic gelation and emulsification-ionic gelation techniques (formulae L_1 and L_5) exhibited a prolonged drug release but without a significant initial burst release. In comparison, a faster initial drug release at pH 1.2 was observed for the first 2 h followed by a prolonged drug release for the remaining 10 h of the experiment was obtained with the controlled release formulae. This bimodal release behavior can be attributed to the addition of the immediate release lyophilized FLT dispersions which provided an initial fast drug release followed by a prolonged drug release for 12 h provided by chitosan microparticles. Percentage dissolution efficiency of FLT after 8 h (DE_8%) from the controlled release formulae, in an ascending order, was: F_2 (41.67%) < F_5 (41.77%) < F_3 (48.11%) < F_4 (50.91%) < F_6 (53.39%) < F_1 (58.56%). The % drug released after 12 h (PR_{12}) was in the range of 51.01–68.2% corresponding to formulae F_5 and F_1, respectively (Table 3).

It was evident that the FLT release rate could be critically modified by changing the immediate and prolonged-release fractions employed in the formulae. From inspection of the release profiles, among the various combinations, formulae F_1 and F_6 were found to comply with the secondary requirements for sustained release formulations suggested by Lang (1971), with regard to the release pattern and time. These formulae seemed to be the more promising ones since they showed an initial rapid release (about 50% of FLT after 2 h), where a sufficient release of drug in the early stage would be necessary to offer a more balanced bioavailability, followed by a sufficiently slow uniform release of the drug over 12 h.

4. Conclusion

In this work, controlled release formulae of FLT could be developed by combining the immediate release lyophilized monophase dispersions and the prolonged release chitosan microparticles in one capsule. FLT was successfully encapsulated with high loadings in chitosan microparticles prepared via simple ionic-gelation and emulsification-ionic gelation techniques. Studying the influence of different formulation variables on the release of FLT from the prepared microparticles indicated that a wide range of release profiles could be generated. The developed controlled release formulations were found to be effective in providing a prolonged release of drug for a long period of time after a suitable initial burst release.

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