Quercetin Microspheres by Solvent Evaporation: Preparation, Characterization and Release Behavior

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ABSTRACT: Quercetin (Q) gastro-resistant microspheres were successfully prepared by solvent evaporation method using cellulose acetate phthalate (C-A-P), cellulose acetate propionate (CAP), or their mixtures in different ratios as matrices. The formulation and preparation conditions (stirring speed, polymer concentration, drug-to-polymer ratio, temperature) were optimized to obtain high encapsulation efficiency and production yield. The prepared microspheres were submitted to several chemical–physical analyses (light scattering, fluorescence and scanning electron microscopy, X-ray diffractometry, calorimetry, infrared spectroscopy), to obtain information about particle size distribution, drug loading, and morphology. Moreover, their release properties were investigated performing *in vitro* dissolution studies with a pH change method. The release tests evidenced that all samples exhibit a fairly gastro-resistance with a typical biphasic drug release trend, due to the pH-dependent solubility of the enteric polymers used as matrices. Moreover, the total amount of released quercetin strictly depends on the system composition, increasing with the C-A-P percentage in the formulation to such an extent that it is about complete ($\sim$ 90%) in the case of C-A-P microspheres. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 2994–3001, 2008

Key words: gastro-resistant microspheres; quercetin; cellulose acetate phthalate; cellulose acetate propionate; solvent evaporation

INTRODUCTION

Quercetin (Q, 3,5,7,3′,4′-pentahydroxyflavone) (Scheme 1), is a common dietary component occurring in various edible plant and herbal medicines marketed in Europe.[1–3] This flavonoid has shown antioxidant and anti-inflammatory properties in numerous mammalian cell systems, *in vitro* as well *in vivo*[4–7]; moreover, some of its derivatives are employed to treat cardiovascular chronic pathologies such as venous insufficiency, capillary fragility and permeability, hemorrhoids, and perivascular edema.[8] Although the variety of its biological effects, the Q bioavailability is an important unsolved problem. Q, in fact, is well absorbed by the small intestine but is unstable in gastric conditions; furthermore, it is poorly soluble and has inherently limited ability to permeate the gastric mucosa from oral dosage forms and to reach the bloodstream in efficacious quantity. Hence, it is usually used as i.p. administration.[9]

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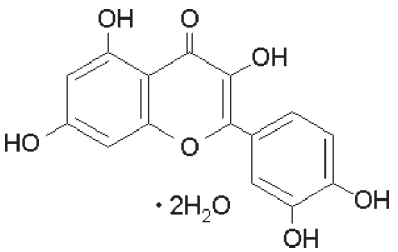
One strategy extensively used to ensure gastric protection and better deliver of a drug is its microencapsulation in gastro-resistant controlled delivery systems.[10–12] In a previous study,[13] we experimented this approach incorporating Q in gastro-resistant microspheres based on cellulose-derived polymers, such as cellulose acetate phthalate and cellulose acetate trimellitate, obtained by spray-drying. However, only an incomplete release of Q was observed from these formulations in simulated intestinal fluid, even if a good protection in gastric conditions was achieved.

The objective of this study was to prepare gastro-resistant microspheres of Q with improved release behavior. The systems were prepared by water-in-oil (W/O) emulsion solvent evaporation. Among the several microencapsulation methods, this is one of the most versatile and advantageous technique.[10,14] In fact, it requires only mild conditions, such as ambient temperature and controlled stirring; therefore, a stable emulsion can be formed without compromising the activity of core material. Moreover, opportunely selecting the preparation conditions (emulsion composition, stirring speed, temperature, surfactant, drug-to-polymer ratio, polymer viscosity,



Scheme 1 Chemical structure of quercetin.

etc.), it allows to microencapsulate both hydrophilic and lipophilic drugs with a good size control of the particles. However, the processing and formulation variables can greatly affect not only the physical characteristics of microparticles such as size, porosity, roughness, and morphology, but also properties such as drug loading, release kinetics, and targeting.[15–21] Consequently, extended experimental studies appear indispensable to better understand these relationships.

In this study, we prepared, by W/O emulsion solvent evaporation, gastro-resistant microspheres of quercetin using cellulose derivatives as matrix materials. In particular, two different cellulose derivatives, an enteric (cellulose acetate phthalate, C-A-P) and a rather impermeable polymer (cellulose acetate propionate, CAP), and their mixtures in 3 : 1, 1 : 1, and 1 : 3 ratios were tested. CAP has been used to control the drug release rate from theophylline microspheres[22] and to prepare impermeable outer shell of pulsatile delivery systems[23]; C-A-P, insoluble at low pH and soluble at slightly acidic pH values, provided some of the most effective solutions to pH-controlled release of NSAID drugs,[24,25] therapeutical peptides and proteins[26] and flavonoids[13,27] for delivering to the intestine. The obtained microspheres were characterized to obtain information about their particle size distribution, drug loading, and morphology. Moreover, their release properties were investigated performing *in vitro* dissolution studies, using a pH change method, and related to the morphology of the samples.

MATERIALS AND METHODS

Quercetin dihydrate 98% (Q), cellulose acetate propionate (CAP, $C_{76}H_{114}O_{49}$, FW 1811.69, $M_n \sim 25000$; 45 wt % combined propionyl, 2.5 wt % combined acetyl content) and cellulose acetate phthalate (C-A-P, $C_{116}H_{116}O_{64}$, FW 2534.12; 30–36 wt % combined phtalalyl, 21–26 wt % combined acetyl content) were purchased by Aldrich. Sodium dodecylbenzenesulfonate (SDBS, technical grade, purity: ~80%), used as surfactant, was purchased by Fluka. Petroleum ether 40–60°C (USP, BP and Ph. Eur. grade), acetone (technical grade), and vaseline oil (USP, BP and Ph. Eur. grade) were obtained from Carlo Erba Reagenti (Italy).

Preparation of microspheres

Cellulose-based microspheres loaded with quercetin and having different shell composition were prepared following a standard procedure involving solvent evaporation of water-in-oil emulsion (W/O). Typical preparation was performed dissolving 2.0 g of cellulose derivative (C-A-P, CAP or a mixture of them in 3 : 1, 1 : 1, and 1 : 3 ratios) and 0.500 g of Q to be encapsulated in 25 mL of acetone. Then, this hydrophilic phase was added with 0.2 g of SDBS and emulsified in 50 mL of vaseline oil using an IKA magnetic stirrer (1000 rpm, 15 min, 25°C). Once the emulsion was stabilized, agitation was maintained with a VELP mechanical stirrer (400 rpm, 30 min, 40°C), until the acetone evaporated after diffusing through the continuous phase, so creating solid microspheres suspended in the vaseline oil. Finally, to recover the prepared microspheres, the suspension was filtered through a standard sieve (400 mesh) and washed four times with 90 mL of petroleum ether, yielding a free-flowing powder. Following the same procedure, drug-free blank microspheres were also prepared, for comparison. The adopted samples nomenclature is detailed in Table I. The table also reports the viscosity values of the polymeric solutions in acetone (8% wt/v), measured according to the procedure described below.

Viscosity determination

Relative viscosities of polymeric solutions in acetone (8% wt/v of C-A-P, CAP or their mixtures in 3 : 1, 1 : 1, and 1 : 3 ratios) were determined using a Visco Basic Plus (Fungilab, S.A.) viscosimeter. The measurements were performed at 25°C, using the No. 3 spindle revolving at a speed of 100 r.p.m.

Drug content, microencapsulation yields, and encapsulation efficiency

Drug content was evaluated using a HPLC apparatus (Agilent 1100 series system) equipped with a Model G-pump and a DAD G-1315 A detector. The HPLC separation was achieved on a C18 μ-Bondapack column (150 mm × 3.9 mm i.d., loop 20 μL) using a mobile phase of MeOH/H_2O (1 : 1 v/v). The UV detector was operated at λ = 366 nm, and the column temperature was adjusted at 40°C.

TABLE I
Nomenclature Adopted for Q-Free and Q-Loaded Microspheres and Viscosity of the Dispersed phases (Cellulose solutions is Acetone at 8% wt/v)

Microspheres nomenclature	Matrix composition		Polymer/drug ratio	Dispersed phase viscosity (cP) ±0.5%
	C-A-P (%)	CAP (%)		
M1	100	0	–	7.9
M2	75	25	–	8.0
M3	50	50	–	8.1
M4	25	75	–	8.6
M5	0	100	–	9.0
M1-Q	100	0	4 : 1	7.8
M2-Q	75	25	4 : 1	7.9
M3-Q	50	50	4 : 1	8.1
M4-Q	25	75	4 : 1	8.6
M5-Q	0	100	4 : 1	9.2

Linearity

Reference standard solutions were prepared at five concentration levels (0.1–10 mg/mL) and were injected (20 μL) three times. The standard curve was analyzed using the linear least-squares regression equation derived from the peak area (regression equation $y = 3144.34x + 231.54$, $R^2 = 0.999$, where y is the peak area and x the concentration used).

Specificity

The peak associated with Q was identified by retention time, UV and MS spectra compared with standard and confirmed by coinjection.

Analysis of the microspheres

Samples (5 mg) of three batches of microspheres were dissolved in 5 mL MeOH, sonicated for 5 min, centrifuged for 10 min at 300 rpm. Q concentration was determined in the surnatant solutions using the same chromatographic conditions of standard Q. Each analysis was made in triplicate and the results were expressed as average value. The production yield was expressed as the weight percentage of the final product compared to the total amount of polymer and drug used in the microencapsulation experiment.

The encapsulation efficiency was calculated from the ratio of actual to theoretical drug amount in dry microspheres.

Size distribution of microspheres

Microcapsules were analyzed for their size distribution. Dried particles were dispersed in distilled water containing 1% by wt of Tween 20 surfactant and sonicated in water bath for 1 min before sampling. Particle size analysis was obtained using a Malvern laser light scattering granulometer (mod. Mastersizer 2600, Malvern, UK). The measurements were taken with a 100 mm lens, detecting particles from 1 to 100 μm.

Microscopy

Scanning electron micrographs (SEM) were obtained using a LEO 420 apparatus (LEO Electron Microscopy). The samples, sprinkled onto a double-sided carbon adhesive tape that had previously been secured on aluminum stubs, were coated with a AuPd alloy using a high vacuum sputter coater before analysis.

Fluorescence microscopy (FM) was performed by means of a Zeiss Axiophot fluorescence microscope, with a 63×/1.4 NA Plan Apochromat oil immersion objective (Carl Zeiss Vision, Germany). Prior to each observation, samples were stained with DAPI (4′,6-diamidino-2-phenylindole) that adsorbs violet radiation (λ_{max} = 372 nm) and emits a blue fluorescence (λ_{max} = 456 nm).

Infrared spectroscopy

FTIR measurements were carried out on neat microcapsule samples in the range of 4000–650 cm^{-1} using a Nexus ThermoNicolet spectrometer equipped with a SmartPerformer accessory for ATR analyses.

X-ray diffraction

X-ray diffraction spectra were recorded with a Rigaku RINT RAPID microdiffractometer having an imaging plate as detector, using a Ni-filtered Cu Kα radiation (40 kV, 20 mA). A Rigaku imaging plate, mod. *R*-AXIS DSBC, was used for digitizing the diffraction patterns.

TABLE II
Drug content Production Yields and Microencapsulation Efficiency of Q-Free and Q-Loaded Microspheres

Sample	Production yields (%)	Theoretical drug content (%)	Actual drug content (%)	Encapsulation efficiency (%)	Mean size (μm)	
					$D_{V0.5}$	span[a]
M1	90.2	–	–	–	32.4	0.89
M2	83.4	–	–	–	33.8	1.33
M3	80.3	–	–	–	31.1	1.56
M4	83.4	–	–	–	33.3	1.17
M5	80.8	–	–	–	35.1	0.88
M1-Q	97.8	20.0	19.3	96.5	24.6	0.83
M2-Q	99.3	20.0	20.0	100	24.7	0.56
M3-Q	97.2	20.0	19.8	99.0	29.6	0.72
M4-Q	95.4	20.0	18.5	92.5	30.1	0.75
M5-Q	84.5	20.0	17.9	89.5	27.1	0.67

[a] The relative span factor is defined by the equation $\Delta = (D_{V0.9} - D_{V0.1})/D_{V0.5}$ where the diameter $D_{V0.1}$, $D_{V0.5}$, and $D_{V0.9}$ are cumulative for 10, 50, and 90% volume fraction.

Thermal analysis

Differential scanning calorimetry measurements (DSC) were performed with a Mettler calorimeter mod. DSC 821 calibrated for temperature and enthalpy with metallic standards (indium and zinc). The thermograms were obtained from 4 to 8 mg samples, sealed in standard aluminum pans, under a nitrogen gas purge. The materials were heated to 105°C at 10°C/min and rapidly cooled to 0°C to give the samples the same thermal history; then, they were immediately reheated to 350°C at the same heating rate. The T_g was determined from the second heating run.

Thermogravimetric analyses (TGA) were performed with a TA Instruments Q500 apparatus, heating microsphere specimens up to 600°C in N_2 atmosphere at 20°C/min.

In vitro drug release

As we reported in a previous paper,[27] the solubility of Q was determined in biological fluids as follows: SGF (5.4 mg/L), SIF (28.9 mg/L), H_2O (7.7 mg/L). Q is a weak acid and its solubility is higher at high pH, as expected.

Release profiles of quercetin were determined providing sink conditions in a SOTAX AT Smart Apparatus (Basel, CH) on line with an UV spectrophotometer (Spectracomp 602, Advanced Products srl, Milan, Italy) and USP 28 dissolution test apparatus n.2: paddle, 100 rpm at 37°C. The pH change method (USP 28 drug release test, method A for Enteric Coated Articles) was used: 750 mL of HCl 0.1*N* (pH 1) from 0 to 2 h (simulated gastric fluid, SGF), then addition of 250 mL of tribasic sodium phosphate solution 0.2*M* to give a final pH 6.8 (simulated intestinal fluid, SIF). All the dissolution/release tests were made in triplicate; only the mean values are reported in graph (standard deviations < 5%). Samples of microspheres containing about 5 mg of Q were analyzed spectrophotometrically at $\lambda = 366$ nm.

RESULTS AND DISCUSSION

Q-free and Q-loaded gastro-resistant microspheres were prepared by water-in-oil (W/O) emulsion solvent evaporation method using cellulose acetate phthalate (C-A-P), cellulose acetate propionate (CAP) and a their mixture in three different ratios (3 : 1, 1 : 1, and 1 : 3) as matrix polymers. The obtained samples were preliminary characterized to evaluate the drug encapsulation efficiency, the microspheres yield and the particle size distribution. The results of these analyses are reported in Table II. The data show that all systems were produced with yield higher then 80%. However, the microencapsulation efficiency markedly depends on the microspheres matrix composition: it is close to the theoretical value for M1-Q, M2-Q, and M3-Q samples, containing al least 50 wt % of C-A-P in the matrix, and slightly decreases for the M4-Q and M5-Q, richer in CAP. This phenomenon may be related to the higher affinity of quercetin towards the cellulose acetate phthalate, having a partially aromatic structure. The particle size distributions were always unimodal, with mean sizes ranging from ~ 25 to 35 μm. In particular, the mean sizes slightly increase with the CAP amount in the microspheres matrix and don't feel much the drug inclusion effect. These results are in line with the relative viscosity of the cellulose solutions (see Table I): it is known, in fact, that polymeric solutions having higher viscosity favor the formation of particles with larger size.[19]

The morphology of the samples was examined by scanning electron microscopy (SEM) and FM. Figure 1 shows the SEM images of drug-free and drug-

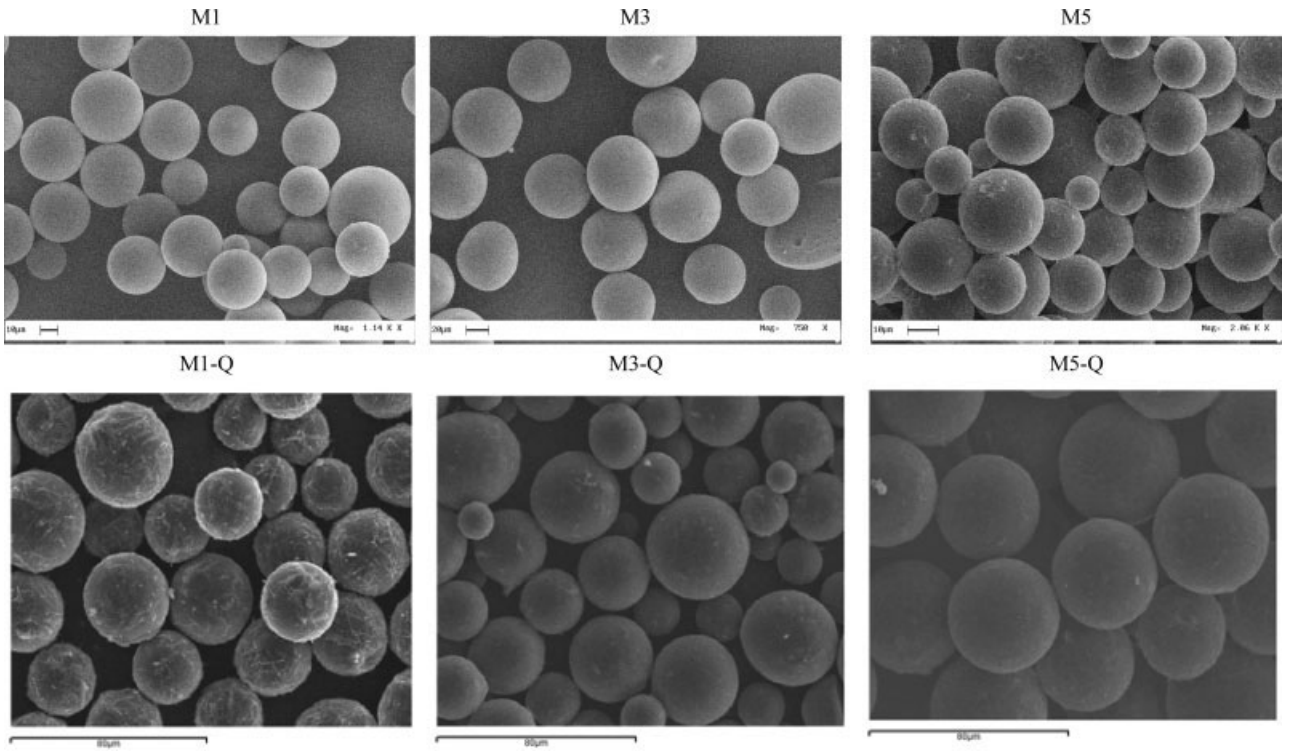


Figure 1 Scanning electron micrographs of Q-free (M1, M3, and M5) and Q-loaded microspheres (M1-Q, M3-Q, and M5-Q).

loaded microspheres having C-A-P, CAP and the C-A-P:CAP 1 : 1 blend as matrices. All samples exhibit regular geometry with a well defined spherical shape. The surfaces are quite smooth and nonporous and no coalescence phenomena are evident. Only the M1-Q sample evidences the presence of drug particles on the surface, which was responsible for the initial release of Q during the drug release tests in SGF dissolution medium. The phenomenon was better evident from FM images, reported in Figure 2. The photos clearly show few Q crystals (orange fluorescence), very little in size, adhering to the microsphere surface and not completely coated by the polymer, in the case of M1-Q and M3-Q samples. However, the drug remained stable during the microencapsulation process, as verified by means of infrared measurements (FTIR). The FTIR spectra of neat Q and Q-loaded microspheres were comparable and no peak shift or new peaks were observed (data not shown).

With the aim to investigate if the drug was molecularly dispersed in the microspheres, like a solid solution, X-ray diffraction (XRD) tests were performed. Figure 3 compares the XRD profiles of M1-Q, M3-Q, and M5-Q samples with that of crystalline neat Q. The diffractograms reveal that Q incorporation into the C-A-P and/or CAP matrices, in our experimental conditions, reduces the crystallinity of Q to practical insignificance, giving an amorphous drug/polymer blend. The obtainment of such a solid solution is a very important result since it has been proved to be an effective strategy for enhancing drug solubility

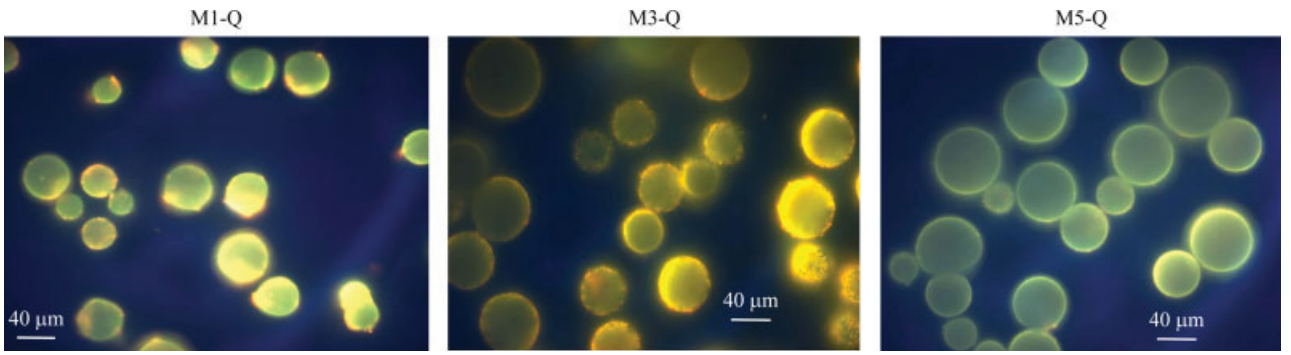


Figure 2 Fluorescence microscopy images of Q-loaded microspheres (M1-Q, M3-Q, and M5-Q). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

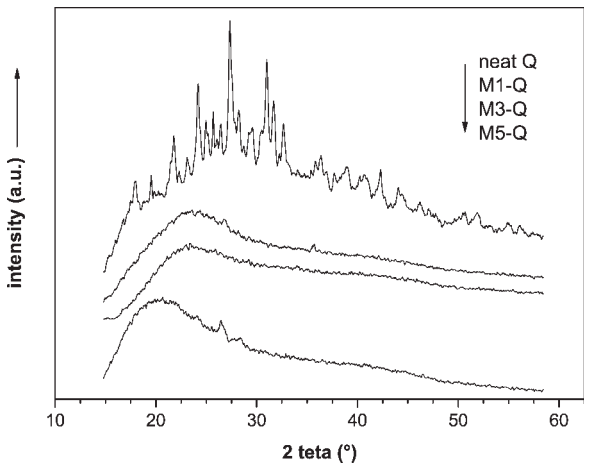


Figure 3 Comparison between the X-ray patterns of crystalline neat Q and Q-loaded microspheres (M1-Q, M3-Q, and M5-Q).

and bioavailability for other crystalline and poorly soluble drugs.[28]

To check the stability of these solid solutions, all the microsphere samples have been re-analyzed by XRD after storing at room temperature for 8 months: all systems were still amorphous, so demonstrating that no recrystallization phenomena occurred (data not shown).

Because of a so close dispersion of Q into the C-A-P and CAP matrices, the inherent bulk glass transition temperature (T_g) of the loaded polymers systems, measured by differential scanning calorimetry (DSC), significantly decreases respect to the unloaded ones, as shown from T_g data listed in Table III. This finding can be explained hypothesizing that Q may act as a lubricant for the cellulose derivatives, reducing the normal intermolecular forces among the macromolecules thus permitting them to slide over one another more freely; this results in a depression of T_g.[29]

The effect of Q microencapsulation on the thermal behavior of the prepared samples was better investigated by means of thermogravimetric analysis (TGA), which response is given in Figure 4(a,b) for the drug-free and drug-loaded microspheres. The TGA curve of neat Q is also reported for comparison. Figure 4(a) shows that the M1 and M5 drug-free microspheres exhibit a single stage decomposition pattern, starting to decompose at $T = 200$°C and $T = 300$°C, respectively. The M2, M3, and M4 microspheres, containing both cellulose derivatives in the matrix, have a double stage decomposition pattern with two different steps of degradation, one for each polymer, as predictable. Figure 4(b) shows that the Q loading into the microspheres significantly modify their TGA curves by changing the decomposition pattern of C-A-P. In fact, all M1-Q to M4-Q samples reduce their thermal stability starting to degrade at temperatures about 25°C lower than the unloaded ones; however, despite this unfavorable effect, at the end of this decomposition step a markedly lower weight loss percentage was registered. This result may be a consequence of the presence of strong interactions (such as hydrogen bonds) among the cocomponents in the solid state that delay or modify the C-A-P degradation processes.[30] Moreover, another step, due to the degradation of confined Q, appears in all TGA curves of Figure 4(b) in the temperature range of 300–460°C.

In vitro release studies for Q loaded microspheres were carried out using a pH change method according the USP 28 procedure to investigate the release behavior both in SGF and SIF medium. The dissolution profiles are plotted in Figure 5 together with that of neat Q, reported for comparison. As expected, Q is poorly soluble both in SGF and in SIF even if, being a weak acid, its solubility increases with pH, according to the dissolution profile shown in the graph.[13] All microsphere samples exhibit a pH-dependent biphasic drug release, typical of gastro-resistant dosage forms. In the SGF medium, after an initial Q release (about 20% for M1-Q and 15% for M3-Q and M5-Q samples in 30 min), which can be related to the solubilisation of the small drug particles adhering on the microspheres surface, only a very slow and incomplete release of the encapsulated drug occurs. So, a satisfactory gastric protection was gained in all cases. After the pH change, strong differences among the drug release behaviors of the three samples are present. In fact, the M1-Q microspheres release more than 90% of Q within 30 min after the pH change, since the C-A-P matrix is fairly soluble in SIF medium; on the other hand, the M5-Q ones release only 25% of Q at the same time and the M2-Q, M3-Q, and M4-Q ones have an intermediate behavior, since the CAP polymer is only slightly swelled at intestinal pH but remains insoluble. As a consequence, the drug must diffuse through the matrix to be released and only the drug near the surface undergoes to dissolution. So, the pH-dependent solubility of the two cellulose derivatives used as matrices, together with the amorphous state of the microencapsulated quercetin, as evi-

TABLE III
Glass transition Temperature of Q-Loaded and Q-Free Microspheres

Sample	M1	M2	M3	M4	M5	M1-Q	M2-Q	M3-Q	M4-Q	M5-Q
T_g(°C)	168	160	151	142	144	146	145	149	134	120

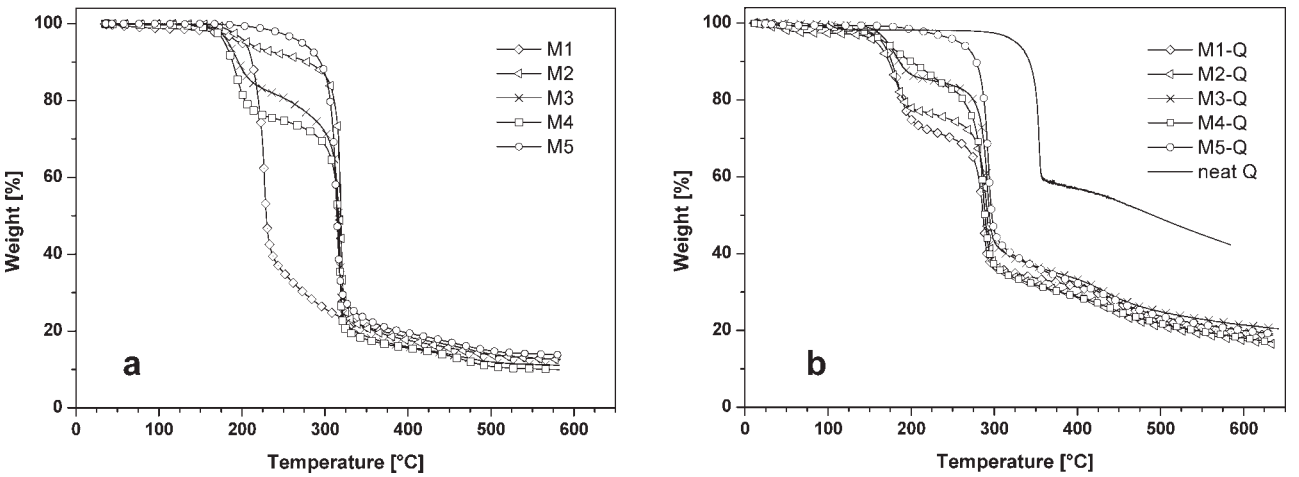


Figure 4 Thermogravimetric response of Q-free microspheres (a) and Q-loaded microspheres and neat Q (b).

denced from X-ray measurements, has a key role in determining the release behavior of the final microspheres. On the whole, from a comparison of all the release profiles reported in Figure 5, the M1-Q formulation appears as a very efficient system for controlled delivery of quercetin to the intestinal tract, by enhancing the solubility and thus the bioavailability of this drug.

CONCLUSIONS

The development of gastro-resistant microparticles containing very poor soluble drugs is a challenge issue. In this study quercetin loaded microspheres of appropriate particle size, well-defined spherical shape and great homogeneity were successfully prepared by solvent evaporation method, using C-A-P, CAP or their mixtures in different ratios as matrix polymers. The adopted microencapsulation procedure is shown to be simple, fast and with high entrapment efficiency. The drug delivery systems prepared in our experimental conditions were demonstrated to be an efficient way to target quercetin to the intestine following oral administration, since they have adequate properties to protect the flavonoid in the gastric medium and to incorporate it in an amorphous form so enhancing its bioavailability. However, the overall release behavior strictly depends on the system formulation. In fact, both C-A-P and CAP polymers partially protect quercetin at pH 1, limiting the release at 20 and 15%, respectively. Nevertheless, at pH 6.8 C-A-P almost completely releases Q (about 90% in 30 min after pH change), whereas CAP release only about 25% in 30 min after pH change. Besides, the systems based on C-A-P/CAP blends have an intermediate release behavior, both in SGF and in SIF.

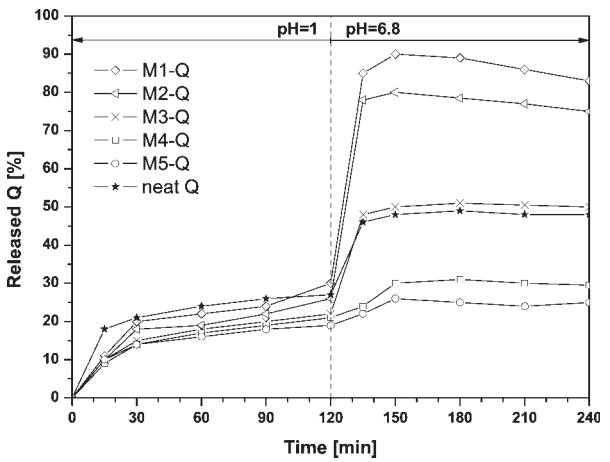


Figure 5 Dissolution profiles of Q-loaded microspheres, in comparison with the dissolution profile of the neat Q.

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