Preparation and characterization of quercetin-loaded polymethyl methacrylate microcapsules using a polyol-in-oil-in-polyol emulsion solvent evaporation method

Dong-Hwan Lee, Gwan-Sub Sim, Jin-Hwa Kim, Geun-Soo Lee, Hyeong-Bae Pyo and Bum-Chun Lee

Abstract

Flavonoids and related compounds exhibit a wide range of useful pharmacological properties but present challenges related to their stability and solubility in commonly available solvents. In this study, polymethyl methacrylate (PMMA) microcapsules were prepared using a novel polyol-in-oil-in-polyol (P/O/P) emulsion solvent evaporation method as a means of stabilizing the flavonoids, using quercetin as a model flavonoid drug. The morphology of the microcapsules was evaluated using a scanning electron microscope, revealing a spherical shape with a smooth surface. The cross-section image of the PMMA microcapsules prepared with an amphiphilic polymer in the inner polyol phase showed that the microcapsule was filled with several submicron microspheres. The mean diameter varied from 1.03 ± 0.12 μm to 2.39 ± 0.42 μm, and the encapsulation efficiency ranged from 12.7% to 26.9%. When free quercetin was stored at 42°C, the residual quercetin content gradually decreased to 18% over 28 days as a result of oxidation. However, when encapsulated in PMMA microcapsules with an amphiphilic polymer in the inner polyol phase, the residual quercetin content decreased to just 82%. In-vitro release studies indicated a sustained release pattern throughout the 36-h study. The release kinetics of the microcapsules with an amphiphilic polymer followed a diffusion-controlled mechanism and the microcapsule without amphiphilic polymer followed an anomalous diffusion behaviour. This study suggests that the novel P/O/P emulsion solvent evaporation method can be applied to the encapsulation of flavonoids.

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Introduction

Microcapsules exhibit a number of interesting characteristics. The primary applications for microencapsulation include controlled release of encapsulated drugs, protection of the encapsulated materials against oxidation or deactivation due to environmental conditions, masking of odour and/or taste of encapsulated materials, and isolation of encapsulated materials from undesirable phenomena (Yoshizawa 2004).

The solvent evaporation method is the most popular technique for preparing microparticles (Bodmeier & McGinity 1987; Herrmann & Bodmeier 1995a). The technique of emulsion solvent evaporation offers several advantages. Emulsion solvent evaporation is preferable to other preparation methods such as spray drying, sonication and homogenization, because it requires only mild conditions such as ambient temperatures and constant stirring. Thus, a stable emulsion can be formed without compromising the activity of the drug (Kim et al 2002). The procedure involves emulsifying a drug-containing organic polymer solution into a dispersion medium. Depending on the state of the drug in the polymer solution and the dispersion medium, it can be classified as an oil-in-water (o/w), water-in-oil (w/o), or water-in-oil-in-water (w/o/w) double emulsion method. Among these, an o/w emulsion solvent–evaporation method has fairly good encapsulation efficiency for hydrophobic compounds (Cavalier et al 1986). For effective use of these methods, the drug has to be at least partially soluble in an organic solvent in order to be encapsulated (Delie & Blanco-Príeto 2005). Otherwise, the formation of an o/w emulsion leads to rapid diffusion

of the drug from the organic phase into the aqueous phase, resulting in microspheres having low or even no drug loading at all (O'Donnell & McGinity 1997).

Unlike the o/w method, solvent evaporation methods, modified by the formation of multiple w/o/w emulsions, are suited to the encapsulation of hydrophilic drugs such as peptides (Herrmann & Bodmeier 1995b; Okada 1997), proteins (Esposito et al 1996; Spiers et al 2000; Han et al 2001; Li et al 2001), or vaccines (Sah et al 1995; Singh et al 1995). Certain water-soluble small molecules (Erden & Celebi 1996; Mandal & Tanjarla 1996; Reza & Whateley 1998) have also been prepared successfully using this method. However, the double emulsion solvent evaporation method is insufficient to encapsulate some pharmaceutically useful drugs having poor solubility in water and selected organic solvents.

Flavonoids and related compounds, a class of phenolic compounds widely distributed in plants, can protect an organism against reactive oxygen species (ROS). Flavonoids exhibit multiple biological effects, including liver protection, as well as antithrombotic, anticancer and immunostimulant activities (Bonina et al 1996; Santos et al 1998; van Acker et al 1998). However, in spite of exhibiting a wide range of pharmacological properties, flavonoids and related compounds present two obstacles to their pharmaceutical use.

The first problem is poor solubility in aqueous media and commonly available organic solvents (Moridani et al 2002). Therefore, encapsulation of these compounds, using the conventional o/w and w/o/w emulsion solvent evaporation methods, is problematic. Few studies exist concerning encapsulation of these compounds; most that do address this issue employ encapsulation by the liposomal method and molecular encapsulation by cyclodextrin (Goniotaki et al 2004; Tommasini et al 2004; El-Samaligy et al 2006; Pignatello et al 2006). The second problem is the sensitivity of flavonoids to oxidative degradation, resulting in poor stability in an aqueous aerobic environment (Makris & Rossiter 2000).

The goal of the present study was to develop a novel method by which to encapsulate flavonoids and related compounds, specifically by use of a polyol-in-oil-in-polyol (P/O/P) emulsion solvent evaporation method, and to characterize the effects of encapsulation on the stability of flavonoids in an aqueous aerobic environment. In order to investigate the characteristics of the microcapsules formed, quercetin was used as the model flavonoid drug.

Materials and Methods

Materials

The following chemicals were purchased from commercial sources and used as received. Quercetin dehydrate and polyvinyl alcohol, with an average molecular weight of 30–70 kD, were obtained from Sigma-Aldrich Cheme GmbH (Steinheim, Germany). Polymethyl methacrylate (PMMA), with a molecular weight of 500–1000 kD, was supplied by Nihon Junyaku Co., Ltd (Tokyo, Japan). Acrylates/C10-30 alkyl acrylate crosspolymers (Pemulen TR-2) were obtained from BF Goodrich Company (OH, USA). Sorbitan sesquioleate (Arlacel 83), a lipophilic surfactant, was purchased from Uniqema Inc. (DE, USA).

Preparation of PMMA microcapsules using a P/O/P emulsion solvent evaporation method

PMMA microcapsules were prepared using a modification of the emulsion solvent evaporation method developed in this laboratory according to the following protocol. Varying amounts of PMMA were dissolved at room temperature in dichloromethane (DCM) and cooled at 4°C. Quercetin was dissolved in a mixture of 1,3-butylene glycol and glycerin (50:50) containing, in some cases, 1% (w/v) Pemulen TR-2. The suspensions of quercetin (2%, w/w, 5 mL) were emulsified into 25 mL of the PMMA solution in DCM, containing a lipophilic surfactant (Arlacel 83) in order to obtain a primary polyol-in-oil (P1/O) emulsion, by homogenization at a rate of $10\,000\,\text{rev min}^{-1}$ using a homogenizer (T.K. Robomics, Tokushu Kika Kogyo Co., Ltd, Tokyo, Japan) for 15 min. After homogenization, the primary P1/O emulsion was emulsified into a 90% (w/w) glycerin solution (polyol phase two) containing 1% (w/w) polyvinyl alcohol, at a rate of $2500\,\text{rev min}^{-1}$ using the homogenizer to prepare the polyol-in-oil-in-polyol (P1/O/P2) emulsion. The volume ratio of the primary emulsion and the glycerin solution was typically 1:8. After emulsification for 15 min, the P1/O/P2 emulsion was agitated with a magnetic stirring bar for 20 h. After that time, the emulsion was evaporated using a rotary vacuum evaporator until complete removal of organic solvent. Subsequently, the microcapsules were washed successively with deionized water followed by collection using centrifugation at $69\,000 \times g$ and then lyophilization (Figure 1). PMMA concentrations in the oil phase were varied from 4% to 12%, and the concentration of lipophilic surfactant was varied from 0.5% to 4%, keeping the total volume constant. All formulations were prepared in triplicate.

Scanning electron microscopy

The external and internal morphology of the microcapsules were analysed using a scanning electron microscope (Hitachi S-2500C; Hitachi, Tokyo, Japan). The microcapsules were fixed on a brass stub using double-sided adhesive tape and were subsequently rendered electrically conductive by coating with a thin layer of platinum (approx. 3–5 nm) in a vacuum evaporator for 100 s at 30 W.

Particle size analysis

The size of the microcapsules was analysed by a laser particle size analyser (Malvern Mastersizer 2000; Malvern Instruments, Worcestershire, UK) using distilled water as a dispersant. The samples were then sonicated in a sonicator attached to the instrument throughout the process, and the time of sonication was kept constant for all samples. The obscuration ranged from 4% to 6%. The volume of distribution was plotted using a computer program supplied by the manufacturer. The particle size was expressed as the volume median diameter. Three replicates were analysed for each batch of microcapsules.
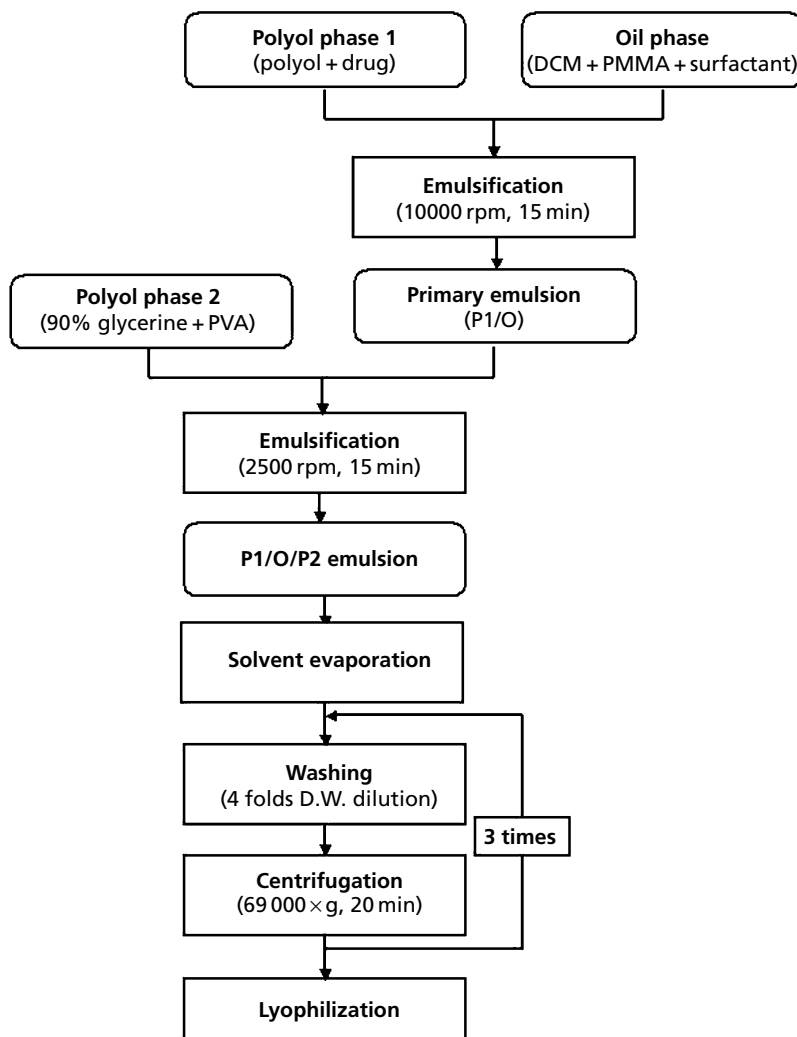
Figure 1 Preparation of polymethyl methacrylate microcapsules using a polyol-in-oil-in-polyol (P/O/P) emulsion solvent evaporation method.

Particle size distribution (polydispersity) was calculated by the following equation:

$$\text{polydispersity} = (D_{0.9} - D_{0.1})/D_{0.5} \tag{1}$$

where $D_{0.9}$, $D_{0.5}$ and $D_{0.1}$ are the particle diameters determined at the 90th, 50th and 10th percentile of undersized particles, respectively.

Encapsulation efficiency

A total of 100 mg of dried microcapsules was suspended in 10 mL MeOH. The quercetin was disintegrated by sonication. After suitable dilution, the samples were assayed for quercetin content using high-performance liquid chromatography (HPLC) as described below. Encapsulation efficiency was calculated as follows:

$$\text{encapsulation efficiency (\%)} = (\text{calculated drug}$$
$$\text{concentration/theoretical drug concentration}) \times 100 \tag{2}$$

Determination of quercetin concentration by HPLC

Quantification of quercetin was performed using a Waters 2695 Alliance HPLC system and a Waters 996 PDA detector (Waters, MA, USA). The compounds were separated on a Watchers 120 ODS-BP column (4.6×250 mm, 5 μm; Daiso Watchers, Osaka, Japan). Mobile phases consisting of 40% methanol, 20% acetonitrile and 40% acetate buffer solution (0.1 M, pH 4.5) were used. Quercetin in samples was identified and quantified using standards by comparing the retention time and UV detection at 370 nm.

In-vitro release studies

An in-vitro test for drug release from the microcapsules was performed on a Franz diffusion cell having a dialysis membrane (cut-off MW 12 000) using isotonic phosphate buffer solution (PBS, pH 7.4±0.2) as the dissolution medium. Polysorbate 80 (1.0 % w/v) was added to PBS to increase the solubility of quercetin for maintaining sink conditions. The

membrane was mounted between the donor and receptor compartments of Franz diffusion cells. The membrane was equilibrated before carefully dispersing the sample, equivalent to 3000 μg of drug, onto the donor side. The receptor compartment was filled with the dissolution medium, stirred at 100 rev min^{-1}, and maintained at 37°C ± 1°C using a circulating water bath. An aliquot of the dissolution medium was withdrawn at pre-determined time intervals for a period of 36 h and an equivalent amount of fresh medium was added. Samples collected were analysed using a UV/vis spectrophotometer (Cary 1E; Varian Inc., Victoria, Australia) at 370 nm to determine the amount of drug released from the microcapsules. The content of drug was calculated using the equation generated from the standard calibration curve. All experiments were replicated three times.

Drug release kinetics

The profile and kinetics of drug release are important because they correlate the in-vitro and in-vivo drug responses by comparing results of pharmacokinetics and dissolution profile patterns (Zahirul & Khan 1996). The kinetics of quercetin release from the microcapsules were determined by finding the best fit of the data obtained from in-vitro drug release studies to various kinetic models. The zero-order model (Equation 3) describes systems where drug release is a time-dependent diffusion process and independent of its concentration (Najib & Suleiman 1985):

$$Q_t = k_0 t \tag{3}$$

where Q_t is the amount of drug released at time t, k_0 is the zero-order rate constant expressed in units of concentration/time, and t is the time in hours. The first-order model (Equation 4) describes systems in which the release is dependent on its concentration:

$$\ln Q_t = \ln Q_0 - k_1 t \tag{4}$$

where Q_0 is the initial concentration of drug, and k_1 is the first-order constant. The Higuchi model (Equation 5) describes the release of the drug from an insoluble matrix to be linearly related to the square root of time and is based on Fickian diffusion:

$$Q_t = k_H \sqrt{t} \tag{5}$$

where k_H is the Higuchi's rate constant, and Q_t is the amount of drug released at time t. In order to authenticate the release model, dissolution data can further be analysed by the Korsmeyer–Peppas equation (Korsmeyer et al 1983; Equation 6):

$$M_t / M_\infty = K t^n \tag{6}$$

where M_∞ is the amount of drug released after an infinite time, K is a constant incorporating structural and geometric characteristics of the system, and n is the exponent characterizing the release process. The K value is a constant, which incorporates the characteristics of the macromolecular network/drug system and the dissolution medium (Peppas &

Khare 1993). The parameter n determines the dependence of the medium uptake or release rate on time. For spheres, if the exponent n = 0.43, then the drug release mechanism is Fickian release, and if 0.43 < n < 0.85, then it is non-Fickian or anomalous release. An exponent value of 0.85 is indicative of Case-II transport or typical zero-order release (Ritger & Peppas 1987).

Determination of quercetin stability

In order to determine the influence of encapsulation on quercetin stability in aqueous solution, free quercetin and encapsulated quercetin were incubated at 25°C and 42°C, respectively, during a 28-day period. An amount of the microcapsules equivalent to 10 mg of quercetin was suspended in 20 mL of PBS (pH 7.4 ± 0.2), containing Polysorbate 80 (1.0% w/v). The samples were collected at pre-determined times for the evaluation of the residual quercetin concentration as described above. All samples were analysed immediately using HPLC.

Statistical analysis

Statistical analysis of the effects of increasing concentrations of PMMA and lipophilic surfactant on the particle size, polydispersity and encapsulation efficiency was performed using a non-parametric Kruskal–Wallis test. After the Kruskal–Wallis test showed significance, post-hoc Nemenyi's tests were used to perform multiple comparisons. The effect of formulation type on the rate of drug release at each time point was analysed using the Kruskal–Wallis test. The Kruskal–Wallis test was also performed to test differences in the quercetin stability in aqueous solution at each time point between the various formulations. In all cases, post-hoc comparisons of individual differences between the various formulations were performed using Nemenyi's test. All statistical analyses were carried out using IGOR Pro 6.0 software (Wavemetrics Inc., OR, USA). $P \le 0.05$ was considered statistically significant.

Results and Discussion

Preparation of PMMA microcapsules using a P/O/P emulsion solvent evaporation method

PMMA microcapsules were prepared using a novel P/O/P emulsion solvent evaporation method using quercetin as a model flavonoid drug. In selecting a polyol as an inner polyol phase medium, it is imperative that the polyol can solubilize quercetin and be immiscible with the PMMA solution in a non-polar organic solvent such as DCM. 1,3-Butylene glycol is a good solvent for quercetin but is miscible with the PMMA solution in a non-polar organic solvent such as DCM. With polyol as an inner polyol phase, use of 1,3-butylene glycol alone as an inner polyol phase medium did not ensure formation of a stable emulsion, and glycerin was included to increase stability of the primary P/O emulsion.

In this study, a mixed solvent system composed of 1:1 proportions of glycerin and 1,3-butylene glycol was used as an inner polyol phase medium. Microcapsules were used to

study the influences of the polymer concentration in the oil phase, the lipophilic surfactant concentration in the oil phase and addition of Pemulen TR-2 in the inner polyol phase on the mean size of microcapsules, as well as on quercetin encapsulation efficiency. The results are given in Table 1.
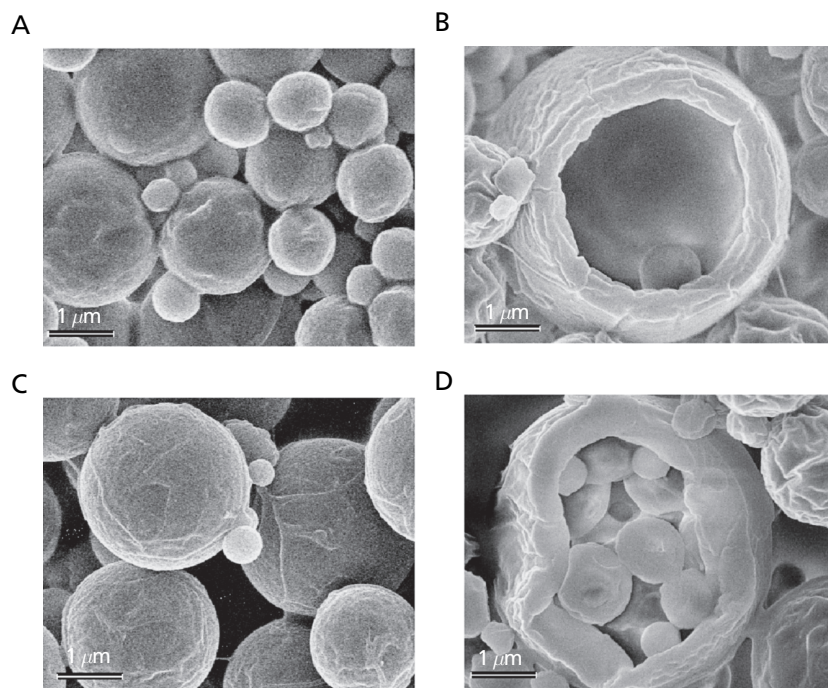
Morphology

Scanning electron micrographs of PMMA microcapsules are presented in Figure 2. The scanning electron micrographs of the microcapsules produced by the P/O/P emulsion solvent evaporation method show that they were spherical, nonporous and uniform with a smooth surface. No significant differences were observed in the outer surface morphology of

microcapsules prepared by the P/O/P emulsion solvent evaporation method with or without Pemulen TR-2 in the inner polyol phase (Figure 2A, C). In the case in which PMMA microcapsules were prepared without Pemulen TR-2 in the polyol phase, both the outer and inner shell surfaces were smooth and non-porous (Figure 2B). A cross-sectional image of the microcapsule prepared without Pemulen TR-2 demonstrated that the interior structure of the PMMA microcapsule was hollow. This observation is in agreement with the findings of Blanco-Prieto et al (1994) and Rafati et al (1997) who suggested that freeze-fractured micrographs of these particles illustrated alveolar structures specific to this process.

In the case in which PMMA microcapsules were prepared in the presence of Pemulen TR-2 in the inner polyol phase,

Table 1 Polymethyl methacrylate (PMMA) microcapsule yield, mean particle size and encapsulation efficiency

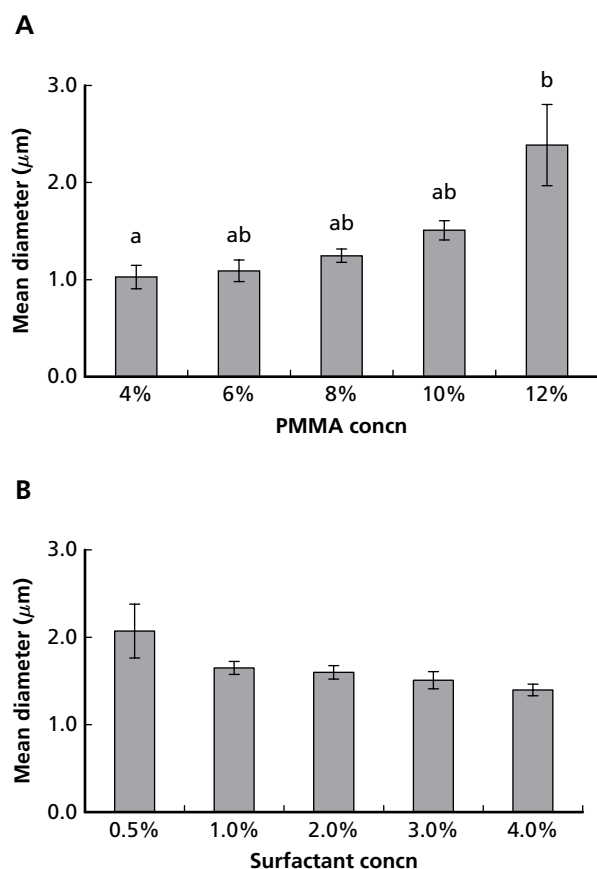| PMMA in oil phase (%, w/w)	Surfactant in oil phase (%, w/w)	Mean particle size (μm)	Polydispersity	Encapsulation efficiency (%)
4.0	3.0	1.03 ± 0.12	0.93 ± 0.13	12.7 ± 2.6
6.0	3.0	1.09 ± 0.11	0.96 ± 0.06	19.4 ± 2.0
8.0	3.0	1.25 ± 0.07	0.82 ± 0.01	23.0 ± 3.2
10.0	3.0	1.51 ± 0.10	1.59 ± 0.31	26.9 ± 2.5
12.0	3.0	2.39 ± 0.42	4.83 ± 0.95	26.7 ± 1.2
10.0	0.5	2.07 ± 0.31	2.45 ± 1.33	18.0 ± 1.5
10.0	1.0	1.65 ± 0.07	1.09 ± 0.04	19.0 ± 1.5
10.0	2.0	1.60 ± 0.08	1.11 ± 0.01	24.2 ± 2.3
10.0	4.0	1.40 ± 0.07	0.95 ± 0.06	26.4 ± 2.7

Figure 2 Scanning electron microscopy images of polymethyl methacrylate microcapsules prepared using a P/O/P emulsion solvent evaporation method: surface morphology (A) and cross-sectioned image (B) of the microcapsule without Pemulen TR-2 in the inner dispersed polyol phase; surface morphology (C) and cross-sectioned image (D) of the microcapsule with Pemulen TR-2 in the inner dispersed polyol phase.

the outer shell surface was smooth and non-porous (Figure 2D). However, the cross-sectioned image of the microcapsule prepared with Pemulen TR-2 illustrated that the microcapsule was filled with several submicron microspheres inside of the microcapsule.

Particle size analysis

The mean particle size ranged from $1.03 \pm 0.12\,\mu$m to $2.39 \pm 0.42\,\mu$m (Table 1). The mean diameter of microcapsules was significantly affected by the PMMA concentration in the oil phase (Kruskal–Wallis: $\chi^2 = 12.581$, $P < 0.05$). The increased PMMA concentration resulted in slightly increased mean particle size (Figure 3). The Nemenyi test showed that the differences in mean particle size between the 4% and 12% PMMA groups were statistically significant ($P < 0.05$). The particle size distribution was also significantly affected by the PMMA concentration in the oil phase (Kruskal–Wallis: $\chi^2 = 11.833$, $P < 0.05$). The increased PMMA concentration resulted in slightly broad particle size distribution (Table 1). The size of the microcapsules, expressed as volume median
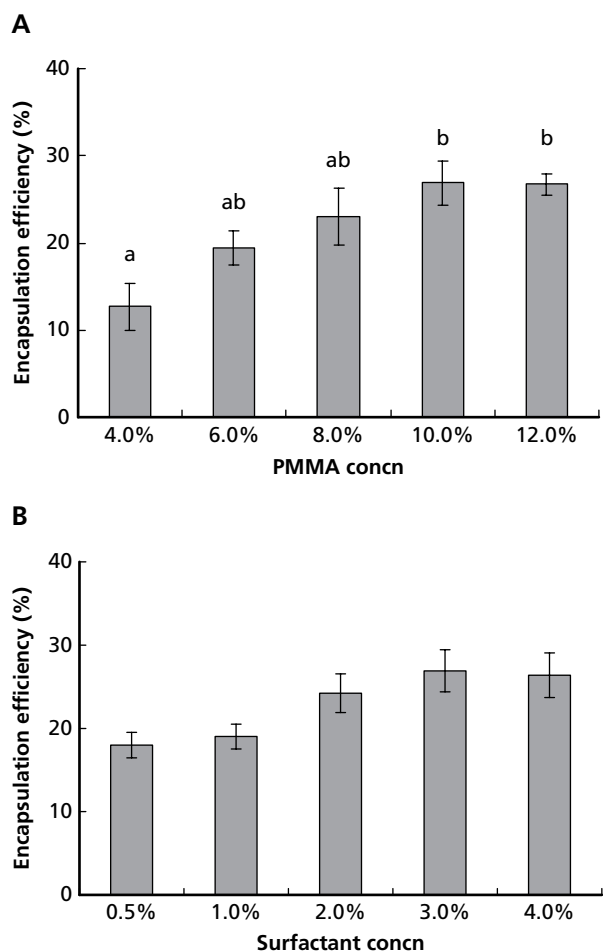

A

B

Figure 3 A. Mean diameter of microcapsules formed at different polymethyl methacrylate (PMMA) concentrations in the oil phase. B. Mean diameter of microcapsules formed at different surfactant concentrations in the oil phase. The size of microcapsules is expressed as the mean geometric diameter ($\pm$ s.d., n = 3). Treatments with the same letter are not significantly different (Nemenyi test, $P > 0.05$).

diameter ($\pm$ s.d., n = 3), ranged from 1.03 ± 0.12 to $2.39 \pm 0.42\,\mu$m. Increased polymer concentration significantly increased the viscosity, thus leading to increased emulsion droplet size and finally a larger microparticle size (Rodriguez et al 1998; Lee et al 2000). This result is in agreement with the findings of Jeffery et al (1991) who suggested that greater concentrations of the polymer in the sample led to an increased frequency of collisions, resulting in fusion of semi-formed particles and producing an overall increase in the size of the microspheres. The influence of the lipophilic surfactant concentration in the oil phase on size distribution (Figure 3) was also evaluated, and it was observed that the mean diameter of microcapsules was obviously affected by the surfactant concentration in the oil phase (Kruskal–Wallis: $\chi^2 = 11.900$, $P < 0.05$). The increased surfactant concentration decreased the mean diameter of microcapsules, reaching a minimum at 4.0%. The particle size distribution was also significantly affected by the surfactant concentration in the oil phase (Kruskal–Wallis: $\chi^2 = 12.367$, $P < 0.05$). The increased surfactant concentration resulted in slightly narrow particle size distribution (Table 1). The size of microcapsules, expressed as volume median diameter ($\pm$ s.d., n = 3), ranged from 1.40 ± 0.07 to $2.07 \pm 0.31\,\mu$m. Increased surfactant concentration significantly decreased the droplet size and increased the uniformity of the primary emulsion, thus leading to a decreased microcapsule size. Addition of Pemulen TR-2 in the inner polyol phase resulted in no significant size difference and the mean diameter of microcapsules was $1.62 \pm 0.12\,\mu$m.

Encapsulation efficiency

The encapsulation efficiency of the microcapsules was significantly affected by the PMMA concentration in the oil phase (Kruskal–Wallis: $\chi^2 = 11.700$, $P < 0.05$). Increased PMMA concentration in the oil phase resulted in slightly increased encapsulation efficiency (Figure 4). The Nemenyi test showed that the differences in encapsulation efficiency within the 4%, 10% and 12% PMMA groups were statistically significant ($P < 0.05$). The encapsulation efficiency ranged from 12.7% to 26.9%. The encapsulation efficiency of the drug depended on the quantity of microcapsules that encapsulated drug in the continuous phase. Increased concentration of polymer in a fixed volume of organic solvent resulted in an increased quantity of microcapsules (Youan et al 2001). For this reason, increasing the PMMA concentration in the oil phase resulted in an increase in the encapsulation efficiency. The influence of the lipophilic surfactant concentration in the oil phase on encapsulation efficiency was also evaluated (Figure 4), and it was observed that the encapsulation efficiency was affected by the surfactant concentration in the oil phase (Kruskal–Wallis: $\chi^2 = 11.267$, $P < 0.05$). However, the Nemenyi test showed that the encapsulation efficiency showed no significant differences with varied surfactant concentration ($P < 0.05$). The encapsulation efficiency (Table 1) ranged from 18.0% to 26.9%. Increased surfactant concentration in the oil phase decreased the droplet size and increased the uniformity of the primary emulsion, thus leading to a decreased probability of effluence of the inner polyol phase into the continuous polyol phase and leading to the increased encapsulation efficiency. Addition of Pemulen TR-2 to the

A

B

Figure 4   A. Encapsulation efficiency of microcapsules formed at different polymethyl methacrylate (PMMA) concentrations in the oil phase. B. Encapsulation efficiency of microcapsules formed at different surfactant concentrations in the oil phase ($\pm$ s.d., n = 3). Treatments with the same letter are not significantly different (Nemenyi test, $P > 0.05$).

inner polyol phase increased the encapsulation efficiency to 32.6%. The encapsulation efficiency was increased after addition of Pemulen TR-2 as a result of the increased inner polyol phase viscosity. Increased viscosity of the inner polyol phase may result in greater stability of the primary P/O emulsion as well, and thus impede diffusion of quercetin from the inner polyol phase into the outer polyol phase. Pemulen TR-2 has both hydrophilic and hydrophobic sections in the molecular backbone. The presence of Pemulen TR-2 caused adsorption of the hydrophilic section at the oil–polyol interface and swelling of the hydrophilic portion in the inner polyol phase. The hydrophilic portion in the inner polyol phase may interact with quercetin and prevent quercetin from diffusing out, resulting in greater encapsulation efficiency.

In-vitro release studies

The cumulative percentage release profile of quercetin from the prepared microcapsules is illustrated in Figure 5. The release of quercetin was prolonged when incorporated in
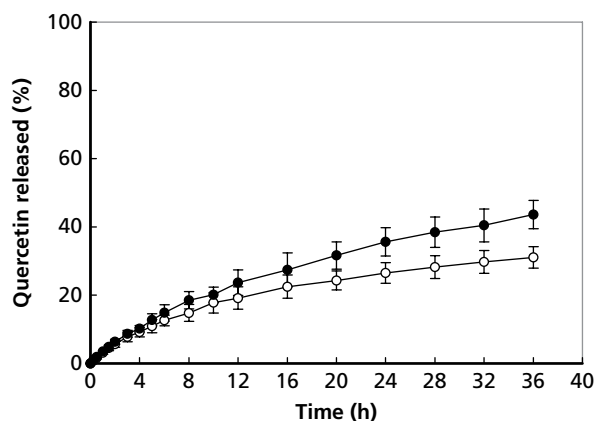
Figure 5   Quercetin release profile of polymethyl methacrylate microcapsules prepared using the polyol-in-oil-in-polyol emulsion solvent evaporation method: microcapsule without Pemulen TR-2 in the inner dispersed polyol phase (●); microcapsule with Pemulen TR-2 in the inner dispersed polyol phase (○). Error bars represent the s.d., n = 3.

microcapsules. It was found that after 36 h, the microcapsule having Pemulen TR-2 in the polyol phase released up to 31% of the drug, whereas the microcapsule without Pemulen TR-2 released 44% of the drug. Addition of Pemulen TR-2 to the inner polyol phase significantly affected the release profile of quercetin from the microcapsules ($P < 0.05$). Compared with that of microcapsules with Pemulen TR-2 in the inner polyol phase, the release rate of the microcapsules without Pemulen TR-2 was a little higher. These differences between the microcapsules can be explained by the fact that addition of the Pemulen TR-2 to the inner polyol phase was more viscous, providing a barrier for drug diffusion, and resulting in a slower drug release rate (Liu et al 2001). Additionally, the hydrophilic section of Pemulen TR-2 may interact with quercetin and prevent quercetin from diffusing from the microcapsules, resulting in the decreased drug release rate.

Mechanism of drug release

The kinetics of quercetin release from the microcapsules were determined by finding the best fit of the data obtained from in-vitro drug release studies to various kinetic models. The rate constants and n values of each model were calculated by linear regression analysis using Microsoft Excel 2000 software. Coefficients of correlation (r^2) were used to evaluate the accuracy of the fit. The coefficients of correlation, rate constants and n values are given in Table 2. It was found that the in-vitro drug release of the microcapsule having Pemulen TR-2 in the polyol phase was best explained by Higuchi's equation, which gave the best value for a linear kinetic model ($r^2 = 0.9928$), followed by the Korsmeyer–Peppas model ($r^2 = 0.9776$), first-order kinetics ($r^2 = 0.9385$) and finally zero-order kinetics ($r^2 = 0.9142$), suggesting that drug transport out of the microcapsule was driven mainly by a diffusion-controlled mechanism. According to Higuchi's model, an inert matrix should provide a sustained release over a reasonable period of time and yield a reproducible straight line when the amount of drug release is plotted versus the square

Table 2 Release kinetics of the polymethyl methacrylate (PMMA) microcapsule

Microcapsules	Zero-order		First-order		Higuchi		Korsmeyer-Peppas			Mechanism of drug release
	r^2	k_0 (h^{-1})	r^2	k_1 (h^{-1})	r^2	k_H $(h^{-1/2})$	r^2	n	k_{kp} (h^{-n})	
Microcapsule without Pemulen TR-2	0.9545	1.2158	0.9799	0.0160	0.9932	7.8199	0.9942	0.73	0.0358	Anomalous release
Microcapsule with Pemulen TR-2	0.9142	0.8655	0.9385	0.0105	0.9928	5.6870	0.9776	0.71	0.0302	Diffusion controlled release

root of time. The best fit model in case of the microcapsule without Pemulen TR-2 could have followed either the Korsmeyer–Peppas model ($r^2 = 0.9942$) or Higuchi's equation ($r^2 = 0.9932$). The drug release data were further analysed based on a power law, and the results ($n = 0.73$, $k = 0.0358$) confirmed that the microcapsule without Pemulen TR-2 followed non-Fickian diffusion, which meant that the release of quercetin from the microcapsule is an anomalous type (i.e. more than one type of release phenomenon could be involved).
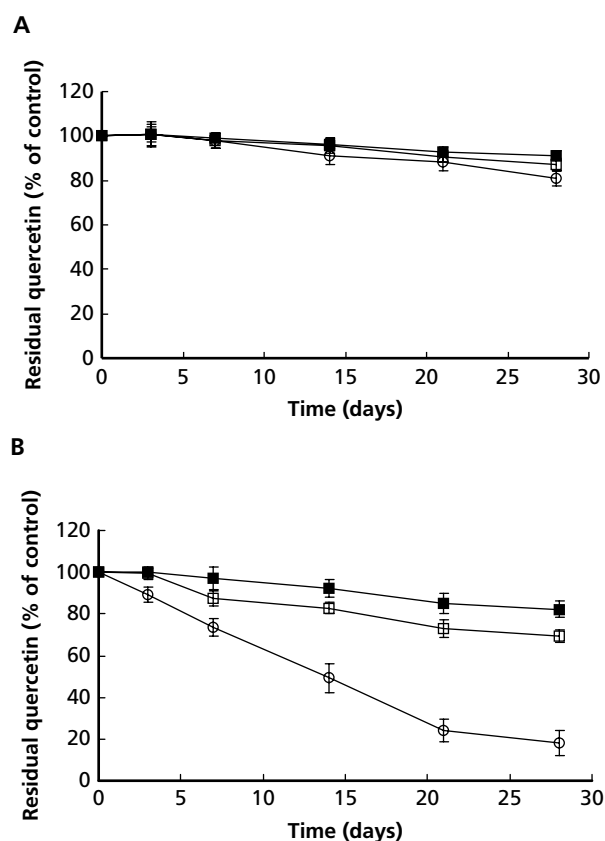
Influence of encapsulation on quercetin stability in aqueous solution

In order to determine the influence of encapsulation on quercetin stability in aqueous solution, free quercetin and encapsulated quercetin were incubated at 25°C and 42°C, respectively, during a 28-day period. The samples were collected at pre-determined times for evaluation of the residual quercetin concentration.

In the case in which free quercetin was stored at 25°C, the residual quercetin content gradually decreased to 81% over 28 days. The residual content of encapsulated quercetin gradually decreased to about 90% over 28 days, with or without Pemulen TR-2 in the inner polyol phase when stored at 25°C. The Kruskal–Wallis test showed that no significant differences existed in the stability of quercetin between free quercetin and the encapsulated quercetin when stored at 25°C (Figure 6A). Rapid degradation of quercetin was observed over 28 days for all samples stored at the higher temperature (42°C). The residual content of quercetin encapsulated in PMMA microcapsules without Pemulen TR-2 in the inner polyol phase decreased to approximately 70% over 28 days. For quercetin encapsulated in PMMA microcapsules with Pemulen TR-2 in the inner polyol phase, the residual quercetin content showed 80% retention at 42°C over 28 days. However, for the free quercetin stored at the higher temperature (42°C), the residual quercetin content gradually decreased to approximately 18% over 28 days (Figure 6B). The Nemenyi test showed that there were no significant differences in the stability of quercetin at each time point between free quercetin and encapsulated quercetin in the microcapsules without Pemulen TR-2. However, differences between free quercetin and encapsulated quercetin in the microcapsules with Pemulen TR-2 were statistically significant after 7 days ($P < 0.05$).

Conclusion

In this study, PMMA microcapsules were prepared using a novel P/O/P emulsion solvent evaporation method in which

Figure 6 Effect of encapsulation on the quercetin stability in aqueous solution when stored at 25°C (A) and 42°C (B): free quercetin (○); microcapsule without Pemulen TR-2 in the inner dispersed polyol phase (□); microcapsule with Pemulen TR-2 in the inner dispersed polyol phase (■). Error bars represent the s.d., $n = 3$.

quercetin was tested as a model flavonoid drug. The morphology of the microcapsules demonstrated a spherical shape with a smooth surface. Cross-sectional images of PMMA microcapsules having Pemulen TR-2 polymer in the inner polyol phase showed that individual microcapsules were filled with several submicron microspheres. The mean diameter varied from 1.03 ± 0.12 to 2.39 ± 0.42 μm and the encapsulation efficiency ranged from 12.7 to 26.9%. Upon storage of free quercetin at 42°C over a period of 28 days, the residual quercetin content gradually decreased to 18%, whereas the residual content of quercetin encapsulated in PMMA microcapsules with Pemulen TR-2 in the inner polyol phase decreased to 82%. The release kinetics of the PMMA microcapsules with

Pemulen TR-2 followed a diffusion-controlled mechanism and the microcapsule without Pemulen TR-2 followed an anomalous diffusion behaviour. In conclusion, this study suggests that the novel P/O/P emulsion solvent evaporation method can successfully be applied to the encapsulation of flavonoids.

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