Food & Function

PAPER

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2014, **5**, 1218

Effect of interfacial composition on uptake of curcumin–piperine mixtures in oil in water emulsions by Caco-2 cells

İbrahim Gülseren,*[ab] Anilda Guri[ac] and Milena Corredig[a]

Received 1st November 2013
Accepted 15th March 2014

DOI: 10.1039/c3fo60554j

www.rsc.org/foodfunction

Encapsulation in lipid particles is often proposed as a solution to improve curcumin bioavailability. This bioactive molecule has low water solubility and rapidly degrades during digestion. In the present study, the uptake of curcumin from oil in water emulsions, prepared with two different emulsifiers, Tween 20 and Poloxamer 407, was investigated to determine the effect of interfacial composition on absorption. Piperine was added to the curcumin to limit the degradation of curcumin because it is known to inhibit β-glucuronidase activity. The emulsions were administered to Caco-2 cell cultures, which is used as a model for intestinal uptake, and the recovery of curcumin was measured. The curcumin uptake was significantly affected by the type of interface, and the extent of curcumin uptake improved significantly by piperine addition only in the case of oil-in-water emulsions stabilized by Poloxamer 407. This work provides further evidence of the importance of interfacial composition on the delivery of bioactives.

1. Introduction

Functional foods and bioactive food ingredients are increasingly researched due to their promise in the promotion of health. The extent of bioactivity exerted by these ingredients is largely defined by their cellular uptake and bioavailability. The bioavailability of such compounds may be limited by their low solubility in aqueous media, rapid degradation during processing and storage, interactions with the food matrix during digestion, and metabolic transformation.[1–3] Indeed, the bioactivity of individual metabolites may be more or less pronounced than that in the parent compound, and the bioactivity may be highly altered by cellular metabolism and molecular interactions following the uptake.

To enhance the delivery of bioactives with low solubility properties, encapsulation appears to be a viable solution. A well-designed encapsulation vector may be able to protect bioactive compounds during processing and storage and under the rapidly changing media conditions during digestion. Accordingly, the cellular uptake of the encapsulation vectors can be tailored by modulating composition, particle size and charge, and concentration of the medium and by controlling physicochemical stability, which in turn will affect the uptake and stability of the native bioactive.[4,5] In this research, curcumin is used as a model molecule for delivery in emulsion systems.

It has been shown that differences in interfacial composition may affect the stability of the emulsions and the kinetics of lipolysis;[4,6] however, much less is understood on the effect that different interfaces may have on the uptake of bioactives from small oil droplets by entherocytes.[7] Digestion occurs primarily in the upper intestine, and intestinal cells appear to uptake bioactive compounds from both digested and undigested oil droplets. A number of *in vitro* studies have been demonstrated differences in the lipolysis rate depending on interfacial composition.[1,8] Since undigested droplets can also be absorbed, and the rate of digestion and absorption may be tailored by altering the interfacial characteristics of emulsion droplets, the present research evaluates curcumin delivery from undigested emulsion droplets with varying interfacial characteristics. Two different emulsifiers were used to prepare oil in water emulsions: Tween 20, a small molecular weight emulsifier, and Poloxamer 407, which is often employed in medical nutrition.[9] Poloxamer 407 has also been previously demonstrated to inhibit pancreatic lipase activity[10] and to be incorporated in cellular membranes.[11] On the other hand, Tween 20 stabilized emulsions are rapidly digested and absorbed.[12] These emulsifiers were chosen for the present study because emulsions stabilized by these molecules have been reported under *in vitro* digestion conditions to have slower rates of fatty acid release and to be little affected by gastric conditions.[8,13]

Curcumin, a highly lipophilic bioactive molecule isolated from the rhizomes of turmeric (*i.e.*, *Curcuma longa*), displays a wide variety of health promoting activities including antioxidant, anti-inflammatory, anti-carcinogenic, and antimicrobial

[a]*Department of Food Science, University of Guelph, Guelph, Ontario, N1G 2W1, Canada*

[b]*İstanbul Sabahattin Zaim University (İSZÜ), Dept of Food Engineering, 34303, İstanbul, Turkey. E-mail: ibrahim.gulseren@izu.edu.tr; Fax: +90 (212) 693-8229; Tel: +90 (212) 692-9619*

[c]*Canadian Research Institute for Food Safety, University of Guelph, Guelph, Ontario, N1G 2W1, Canada*

activities.[14,15] In the majority of research studies on curcumin, the mixtures utilized were composed of at least three major curcuminoids (*i.e.*, Yu & Huang, 2011)[16] and possibly other compounds depending on the level of purity. Curcumin is rapidly metabolized in the intestines and in the liver, quickly β-glucuronidated, and reduced to form a family of related compounds,[15] which makes it very challenging to trace curcumin during absorption or in the bloodstream and excretion products.[17,18]

Because of its inefficient aqueous solubility,[19] for improving curcumin delivery, it is important to enhance its stability using encapsulation vectors that would also aid in the absorption of curcumin through epithelial cells. In the cases of food and nutraceutical applications, food-grade components should be used. Various formulations have shown the potential to improve delivery of curcumin such as liposomes,[20,21] oil-in-water emulsions,[22] protein particles[23,24] and through solid lipid nanoparticles.[12]

An additional approach suggested for enhancing the delivery of curcumin is the use of piperine.[25] This compound, isolated from black pepper, enhances curcumin absorption and has been shown to inhibit metabolizing enzymes, retard clearance of bioactives or nanoparticles from the body, and provide protection against oxidative damage.[26–29] Piperine co-administration with other bioactives has been shown to enhance the bioavailability of a number of therapeutic drugs and phytochemicals such as curcumin in animal and human intervention trials.[25,28,29]

In the present study, it was hypothesized that differences in the interfacial composition may affect the absorption of a bioactive molecule. Curcumin-loaded oil in water emulsions were prepared using two different emulsifiers, Tween 20 and Poloxamer 407, to determine differences in the uptake of curcumin by Caco-2. Piperine was added to the dispersed phase to better control the metabolic transformation of curcumin and to obtain higher recoveries during cell uptake. *In vitro* cell culture models, such as Caco-2 cultures, are an established model for testing drug or bioactive compound delivery.[15,29–31]

2. Materials and methods

2.1. Materials

Soy oil (S7381), curcumin (C7727, ≥94% curcuminoid content), piperine (P49007), Poloxamer 407 (16758), Dulbecco's Modified Eagle Medium (DMEM) (containing 25 mM glucose), HEPES buffer protease inhibitor cocktail (P8340), β-glucuronidase from *Helix pomatia*, and G7017 were obtained from Sigma-Aldrich Corporation (Oakville, ON, Canada). Triton-X-100 was purchased from Fisher Scientific, Fair Lawn, NJ, USA. MilliQ (Billerica, MA, USA) grade water was used for sample preparation. Fetal bovine serum (FBS) heat inactivated, nonessential amino acids (NEAA), 0.25% trypsin – 1 mM EDTA·4Na (1×), L-glutamine, penicillin–streptomycin (10 000 units of penicillin and 10 000 μg of streptomycin per ml), phosphate-buffered saline (PBS), and Hank's balanced salt solutions (HBSS), were purchased from Invitrogen (Invitrogen Canada Inc., Burlington, ON, Canada). A β-glucuronidase assay kit (I-2908) was purchased from Molecular Probes (Eugene, OR, USA). A CellTiter 96® reagent AQ_{ueous} One Solution Cell Proliferation Assay kit was purchased from Promega Corporation, Madison, WI, USA. Transwell permeable polyester (PET) clear inserts (0.4 μm) and 12-well cell culture plates were obtained from Corning.

2.2. Emulsion preparation

Soy oil (40% v/v) in water emulsions containing curcumin were stabilized with 4% Tween 20 or 4% Poloxamer 407. The physicochemical properties of emulsions prepared with these molecules have been previously reported.[32,33] Curcumin was solubilized in soy oil at 40 mg kg^{-1}, with or without addition of piperine at a 10 : 1 ratio (4 mg kg^{-1}). The soy oil phase was heated (approx. 60 °C) and stirred for 1 h with curcumin to ensure solubilisation, prior to homogenization. The mixture was pre-homogenized using a shear mixer at full speed (30 000 rpm) for 1 min (Power Gen 125, Fisher Scientific, Mississauga, ON, Canada). High-pressure homogenization was carried out at 750 bar for five passes using Emulsiflex C5, Avestin (Ottawa, ON, Canada). All the samples were freshly prepared and administered to the cell culture within 2 h of preparation.

2.3. Particle size and ζ-potential analysis

The particle size distribution of the emulsions was measured using static light scattering (Mastersizer 2000, Malvern Instruments, Worcestershire, UK) using water as the dispersing agent. The refractive indexes of soy oil and water were taken as 1.47 and 1.33, respectively. Dynamic light scattering (DLS) was used to measure the ζ-potential of emulsions (Zetasizer Nano, Malvern Instruments, Worcestershire, UK). The samples were diluted in MilliQ grade water (1 : 100) prior to all measurements.

2.4. Stability of emulsions

To ensure the stability of curcumin and piperine in the oil-in-water emulsions, the release of curcumin was assessed using dialysis. The samples were extensively dialyzed against a 50% ethanol solution (at a 60 : 1 ethanol solution : sample volume ratio) at room temperature. Samples were withdrawn every 30 min from the dialysis medium, and the amount of curcumin remaining in the sample was determined by subtracting the difference after measuring the concentration in the dialysate using a fluorescence spectrometer (Synergy H4 Hybrid Reader, Biotek, Fisher Sci.) at excitation and emission wavelengths of 430 and 549 nm, respectively. These values are based on literature data for curcumin fluorescence in ethanol, and the curcumin was quantified using a calibration curve in 50% ethanol.[34]

2.5. Cell culture

The Caco-2 cell line (CRIFS Culture Collection, Food Science, University of Guelph, ON, Canada) was maintained in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 1% NEAA and 1% antibiotic solution of penicillin–streptomycin and 25 mM HEPES buffer in a humidified atmosphere 5% CO_2 in a 37 °C incubator (Forma Series II Water-jacketed CO_2 Incubator, Model no: 3110, Forma Scientific, California, USA). Cells were

cultured in T-75 cm^2 tissue culture flasks (Fisher Sci., Mississauga, Ontario, Canada) harvested with trypsin–EDTA prior to seeding. In order to maintain relatively constant cellular phenotypes, cells of passages 26–38 were used throughout this study.

2.6. Cytocompatibility

Cytocompatibility was assessed after seeding Caco-2 cells at a density of 4×10^3 cells per well. The cells were allowed to attach by incubating for 24 h incubation at 37 °C in a 5% CO_2 atmosphere. The growth medium was removed and freshly prepared, Tween 20 or Poloxamer 407 (4%) stabilized oil-in-water emulsions (40% oil) were added to the medium at a dilution ratio of 1 : 5.6 (sample : medium; v/v). Diluted emulsions were prepared using HBSS buffer (1×).

The cells were incubated with media containing emulsion droplets for 24 h at 37 °C and 5% CO_2. The samples were removed from each well, the wells were rinsed twice with PBS, and cell viability was determined by a cellular metabolic assay. The CellTiter 96® reagent AQ_{ueous} One Solution a cell proliferation assay kit was then used following the manufacturer's instructions. NADPH production was monitored by reading the absorbance at 490 nm using an automated 96-well plate reader (Multi Detector Microplate Reader, Biotek Synergy HT Model, Vermont, USA). Results were compared with control wells that were not incubated with samples but only with media and the CellTiter 96® reagent.

2.7. Curcumin uptake through Caco-2 monolayers

Caco-2 cell monolayers were generated on insert filters of 12-well plates. A total of 0.5 ml of Caco-2 cell suspension was added onto the insert at a final concentration of 6×10^4 cells per well, whereas 1.5 ml of the medium was added to the basolateral compartment of the respective well. Uptake experiments were performed after 21 days of growth on the inserts when the Caco-2 cell monolayer was completely differentiated. The medium was changed every two days and the integrity of the monolayers was monitored by measuring the transepithelial electrical resistance (TEER) using an EVOM2 epithelial voltmeter (WORLD Precision Instruments, Sarasota, FL, USA).

TEER was calculated as follows:

$$\text{TEER } (\Omega\ \text{cm}^2) = [\text{TEER } (\Omega) - \text{TEER}_{\text{background}}\ (\Omega)] \times \text{area } (\text{cm}^2), \quad (1)$$

where TEER (Ω) is the electrical resistance across the monolayers directly read from the EVOM2 epithelial voltmeter, and $\text{TEER}_{\text{background}}$ (Ω) is that of the insert only (without cells). The surface area of the insert was 1.12 cm^2.

The cellular monolayer was washed once with HBSS, and 0.5 ml of HBSS at pH 6.5 was added on the donor compartment, whereas 1.5 ml of 0.4% BSA in HBSS (pH 6.5) was added onto the basolateral compartment. The cells were incubated for 45 min at 37 °C in order to equilibrate the monolayers prior to the uptake experiments. For the uptake studies A–B (apical to basolateral), freshly prepared emulsions were diluted with HBSS buffer (1×) to 8% oil volume fraction, and 20 μl were added to the apical compartment. Samples were incubated for 2 h at 37 °C in a 5% CO_2 incubator. TEER values were recorded before ($t = 0$) and after ($t = 2$ h) the permeation experiment to monitor the integrity of the monolayer.

2.8. Quantification of curcumin in the basolateral compartment

Aliquots (100 μl) withdrawn from the basolateral compartment of the transport system were diluted in ethanol (1 : 1; v/v), and the samples were transferred to a 96-well fluorescence plate (Corning Glass Works, Corning, Mississauga, ON, Canada). The samples were analyzed immediately by fluorescence spectroscopy as described in Section 2.4.

2.9. Cell lysis and enzyme assay

To evaluate the influence of piperine uptake and efficacy in controlling the breakdown of curcumin by β-glucuronidase, an enzymatic test based on β-glucuronidase activity was also performed. After the transport experiment, the cells were washed twice with PBS buffer at pH 7.4 to remove the residual media. Cell lysing was performed following the protocols of Palmgrén *et al.* (2005) with slight modifications.[35] Briefly, 250 μl of 1% Triton-X-100 solution was added to each well along with protease inhibitor cocktail previously prepared based on the manufacturer's instructions. Then the plate was kept in an ice bath for 5 min. After that, the cells were carefully scraped from the membranes, suspended by pipetting, sonicated briefly, and centrifuged for 10 minutes at 14 000 × *g* in a cold microfuge (Microfuge® 22R, Beckman Coulter, Mississauga, ON, Canada). The supernatants were further transferred to 96 well plates (Fisher Sci. Mississauga, ON, Canada), and enzyme activity was measured using a spectrofluorimeter. Excitation and emission wavelengths were 495 and 518 nm, respectively. Briefly, β-glucuronidase activity in the samples was tested using an appropriate assay kit (I-2908, Molecular Probes, Eugene, OR, USA). β-Glucuronidase from *Helix pomatia*, was used as an enzyme activity standard. Enzyme activity was expressed in sigma units, where 1 unit is defined as the amount of enzyme that will liberate 1.0 μg of phenolphthalein from phenolphthalein glucuronide in 1 h at 37 °C at pH 5.0. The duration of the enzymatic assay was 30 min.

3. Results and discussion

3.1. Physico-chemical characteristics of curcumin–piperine bearing emulsions

Curcumin and curcumin–piperine in the oil-in-water emulsions were prepared using high-pressure homogenization. The particle size was determined using integrated light scattering immediately after preparation. Table 1 summarizes the mean apparent diameter ($D_{3,2}$) and the ζ-potential for the two emulsions. No significant differences were noted between the emulsions, and all showed a monomodal distribution of oil droplets of 0.2 μm in diameter. The average diameter was not affected by the emulsifier type or by the presence of piperine. In

Table 1 Mean particle diameter ($D_{3,2}$) and ζ-potential of oil-in-water emulsions at the time of preparation as measured by a light scattering technique. Emulsions contained either curcumin or curcumin with piperine, as a β-glucuronidase inhibitor, and Tween 20 or Poloxamer 407 were used as emulsifiers. All of the samples were prepared in triplicate

Emulsion	$D_{3,2}$ (μm)	ζ-Potential (mV)
Curcumin–Tween 20	0.22 ± 0.03	−1.2 ± 1.6
Curcumin–Poloxamer 407	0.21 ± 0.07	−2.3 ± 0. 3
Curcumin–piperine–Tween 20	0.21 ± 0.02	−2.3 ± 2.4
Curcumin–piperine–Poloxamer 407	0.21 ± 0.07	1.3 ± 0.9

addition, the absence of curcumin and piperine from the oil phase did not alter the particle size distribution significantly (data not shown). Moreover, there were no changes in particle size distribution after 4 days of refrigerated storage (data not shown). These results demonstrate that all the emulsions were stable throughout the duration of the cell culture experiments.

Two different emulsifiers were utilized in emulsion formulations. Tween 20 is a surfactant with a small molecular weight that reduces interfacial tension rapidly upon homogenization and forms a surfactant monolayer at the interface.[12] Poloxamer 407 is a polymeric emulsifier often employed in nutritional products. Both types of emulsions have been widely characterized.[10] The concentrations of emulsifiers were sufficient for obtaining stable emulsions, and in both cases, surface charge was low, as demonstrated by ζ-potential. These emulsions were chosen because they have been reported to be show slow lipid digestion in *in vitro* digestion models.

The emulsions were also evaluated for their loss of curcumin during storage. Emulsions containing curcumin and those with curcumin and piperine were dialyzed against 50% ethanol at room temperature for 2 h. This length of time corresponded to the time of exposure of the emulsion in the cell culture experiments. Table 2 summarizes the amount of curcumin loss as a function of time for the various emulsions. This experiment was not fully equivalent to the stability of the emulsion in a cell culture medium; however, the system was chosen because curcumin is soluble in ethanol,[34] and a higher amount of release may be expected under these conditions compared with the cell culture environment. The losses in curcumin encapsulation (%) were determined spectrofluorimetrically at excitation and emission wavelengths of 430 and 549 nm, respectively.[34] In the majority of cases, percentage losses in curcumin were found to be less than 3% over 2 h. There were no significant differences in the extent of curcumin released over time (data not shown). In addition, the losses were not affected by emulsifier type or the addition of piperine. Based on these findings, it was concluded that the emulsions were an appropriate vector for the curcumin uptake studies and that very little leakage should be expected during the cell culture experiments.

Ethanol (50%) was utilized in order to evaluate the possible release of curcumin from the emulsion. It is clear that aqueous environments will remove a lower extent of curcumin from the samples because it is insoluble in water. Similar assays were used in previous studies to determine the stability of solid lipid nanoparticles.[36]

3.2. Caco-2 viability and monolayer integrity upon the administration of emulsions

The oil-in-water emulsions were administered to the Caco-2 cell culture to ensure that the samples were not cytotoxic at levels employed in this research and that the cell viability remained similar to that in control cells. As clearly shown in Fig. 1, the presence of emulsion droplets in the media enhanced the viability cells of the cells, demonstrating that the samples were not cytotoxic and thus suitable for the cellular uptake experiments. Decreasing the oil volume fraction resulted in a decrease in cell viability, clearly demonstrating that cell proliferation is a function of sample composition. It is important to note that in all cases with the same sample, medium dilution was carried out, as described in Section 2.

The influence of the emulsion bearing curcumin or curcumin–piperine administered to the Caco-2 culture on the integrity of the monolayer was measured by determining the transepithelial electrical resistance (TEER) values prior to and after the uptake experiment. There were no significant changes in the TEER (%) (Fig. 2), confirming what was already observed in Fig. 1 and again demonstrating that the presence of the emulsions had little influence on the cellular monolayer and that the integrity of the Caco-2 monolayer was well preserved during the experiment. The data clearly demonstrated that this setup was appropriate for curcumin uptake assays.[33]

3.3. Curcumin uptake

Recent uptake experiments with curcumin encapsulated in solid lipid nanoparticles or emulsions showed very little recovery in the basolateral fraction after absorption.[12] In the present work, in addition to the presence of piperine, higher

Table 2 Amount of curcumin recovered in the dialysate during a stability experiment. The emulsions were dialyzed against 50% ethanol as a function of time. Curcumin was quantified using fluorescence spectroscopy, and losses are reported as a percentage of the total curcumin. The analyses were carried out at least in duplicate

Storage time (min)	Curcumin–Tween 20, loss (%)	Curcumin–Poloxamer 407, loss (%)	Curcumin–piperine–Tween 20, loss (%)	Curcumin–piperine–Poloxamer 407, loss (%)
30	6.8 ± 1.2	3.0 ± 2.6	2.0 ± 0.3	1.3 ± 0.5
60	2.9 ± 1.4	1.3 ± 0.9	1. 7 ± 0.4	1.9 ± 0.9
90	3.7 ± 1.8	0.6 ± 0.4	2.2 ± 0.7	1.9 ± 0.1
120	1.8 ± 0.7	0.4 ± 0.2	1.3 ± 0.9	2.7 ± 0.8

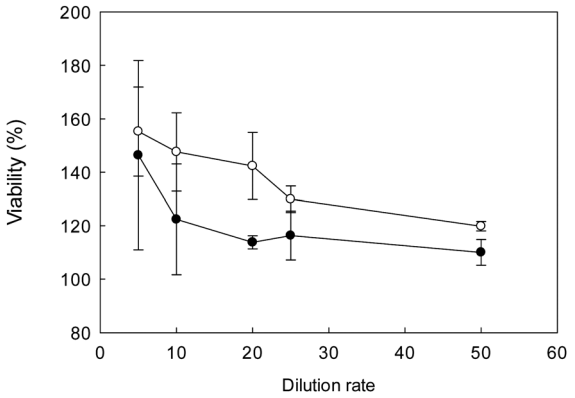

Fig. 1 Caco-2 viability (%, related to control) as affected by the administration of oil-in-water emulsions (40%) as a function of emulsion dilution. Emulsions containing Tween 20 (filled symbols) and Poloxamer 407 (empty symbols), both containing curcumin, were diluted from 1 to 50 times in HBSS buffer (1×) prior to their addition to the cells. Cells were incubated for 2 h in a humidified atmosphere at 37 °C and 5% CO_2. Control samples contained only the medium and are considered to have 100% viability. Results are the average of at least three independent experiments, and bars represent standard deviation.

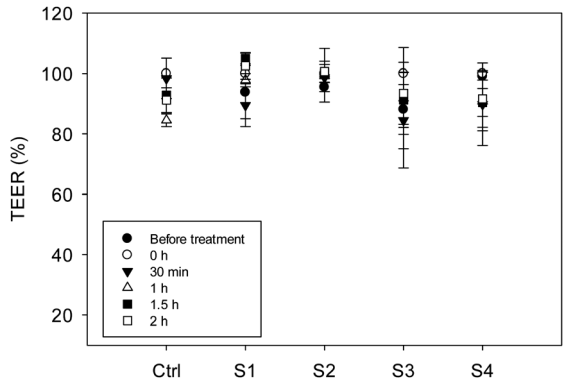

Fig. 2 Changes in transepithelial electrical resistance (TEER) (%) measured during the uptake of curcumin or curcumin and piperine emulsions in a Caco-2 cell culture as a function of time of incubation. Control is medium only. Emulsions were stabilized either with Tween 20 (S1 and S2) or Poloxamer 407 (S3 and S4) and contained curcumin or curcumin and piperine incorporated in the oil phase of all emulsion samples. Tween 20 or Poloxamer 407 (4%) were used in the stabilization of emulsions. The sample definitions are given in Table 1 for conciseness. All of the experiments were carried out at least in triplicate.

concentrations of oil were administered to the cells, and 0.4% BSA was added to the receiving buffer to better mimic the uptake environment of the cells, whereby serum proteins may act as carriers of hydrophobic molecules.[37] These changes improved the basolateral recovery of curcumin after absorption by the Caco-2 cell monolayer. The basolateral concentration of curcumin in the presence and absence of piperine was investigated as a function of incubation duration (2 h) and emulsifier type, as summarized in Fig. 3. In the case of Tween 20-stabilized emulsions (Fig. 3A), the extent of curcumin uptake was not significantly different from the initial values. However, the intake generally increased with time, accounting for about 20% of all curcumin present in the media after 2 h of incubation. These results clearly indicate that curcumin present in Tween 20-stabilized emulsions was absorbed by the Caco-2 cells and was recovered in the basolateral fraction. It is important to note that in the case of Tween 20 emulsions, the presence of piperine in the emulsion droplets did not induce a significant increase in basolateral curcumin uptake.

A different behaviour was shown for the Poloxamer 407-stabilized emulsions (Fig. 3B). In the case of emulsions containing only curcumin (without piperine), the uptake amount remained constant, at about 10% within the 2 h of incubation. On the other hand, in the presence of piperine, the uptake increased with time, and about 40% of the original curcumin was recovered in the basolateral fraction after 2 h. In this case, in the presence of piperine, there was a significant enhancement of uptake by Caco-2 cells. Unlike the case of Tween 20 emulsions, the presence of piperine improved the recovery of curcumin by affecting the rapid metabolism of this compound in the cell. The reason for the difference in the behaviour of the

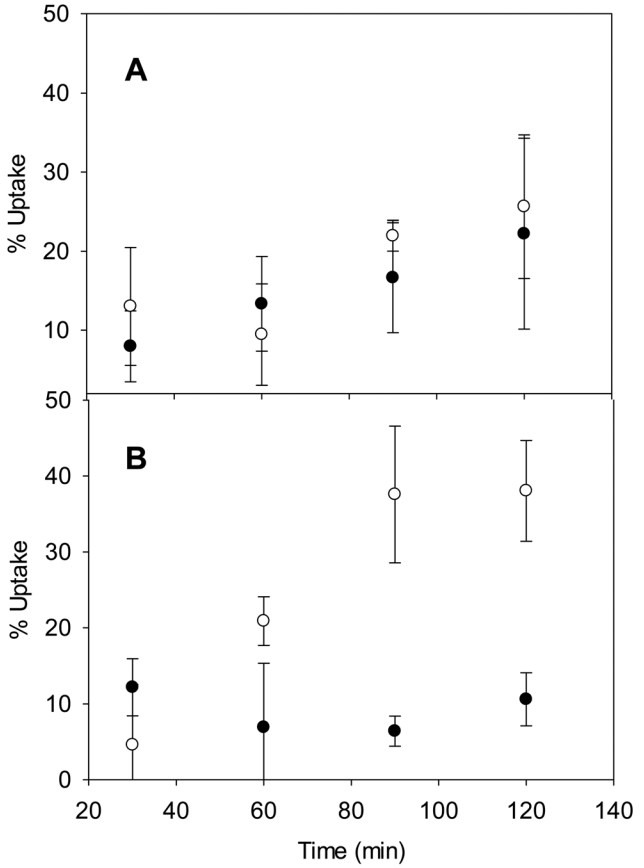

Fig. 3 Basolateral uptake (%) of curcumin by Caco-2 cells grown for 21 days on permeable Transwell® plates as affected by the administration of oil-in-water emulsions (8%) stabilized by (A) Tween 20 (4%), or (B) Poloxamer 407 (4%) as a function of incubation duration in the cell culture medium. Emulsions contained either curcumin (filled circles) or curcumin and piperine (empty circles). The experiments were carried out at least in triplicate.

emulsions in the presence of piperine between the two emulsifiers is unknown at this point as it does not seem to be related to the emulsion uptake but to the effect of piperine in the cell. Tween 20 may have created micellar structures that partitioned the compounds from the cell; however, the activity of β-glucuronidase was measured in all cell lysates, and no differences between piperine–curcumin and curcumin only emulsions were noted, regardless of the emulsifier type (data not shown).

4. Conclusions

Curcumin was employed as a model molecule to determine the ability of different emulsion systems to enhance the uptake of hydrophobic molecules. Since this compound is practically insoluble in water and is very much prone to oxidative and metabolic degradation, the biological effects evoked by curcumin in tissues other than the gastrointestinal tract may have to be attributed also to its metabolites. Nonetheless, efficient delivery vectors may be necessary to extend its stability during storage and to enhance its bioavailability upon consumption; therefore, measures to observe its cell uptake are needed. By using piperine, curcumin recovery may be enhanced. This work clearly showed that the co-administration of synergistic bioactive compounds can be an effective approach in the manufacturing of novel functional food products and that interfacial composition may be important in determining the kinetics of lipolysis and release of bioactives, in addition to their cell uptake and stability.

Acknowledgements

This work was partly funded by the Natural Sciences and Engineering Council of Canada (NSERC), through the Canada Research Chair in food nanostructures program.

References

1 H. Singh, A. Ye and D. Horne, *Prog. Lipid Res.*, 2009, **48**, 92.
2 L. Day, M. Golding, M. Xu, J. Keogh, P. Clifton and T. J. Wooster, *Food Hydrocolloids*, 2014, **36**, 151.
3 D. J. Mcclements and Y. Li, *Adv. Colloid Interface Sci.*, 2010, **159**, 213.
4 Y. Mao, M. Dubot, H. Xiao and D. J. McClements, *J. Agric. Food Chem.*, 2013, **61**, 5163.
5 A. Macierzanka, N. M. Rigby, A. P. Corfield, N. Wellner, F. Böttger, E. N. Clare Mills and A. R. Mackie, *Soft Matter*, 2011, **7**, 8077.
6 A. Malaki Nik, A. J. Wright and M. Corredig, *Colloids Surf., B*, 2011, **83**, 321.
7 D. J. McClements and Y. Li, *Adv. Colloid Interface Sci.*, 2010, **159**, 213.
8 S. Mun, E. A. Decker and D. J. McClements, *Food Res. Int.*, 2007, **40**, 770.
9 G. Dumortier, J. L. Grossiord, F. Agnely and J. C. Chaumeil, *Pharm. Res.*, 2006, **23**, 2709.
10 T. P. Johnston and I. J. Goldberg, *J. Pharm. Pharmacol.*, 2006, **58**, 1099.
11 E. V. Bartrakova and A. V. Kabanov, *J. Controlled Release*, 2008, **130**, 98.
12 A. Guri, İ. Gülseren and M. Corredig, *Food Funct.*, 2013, **4**, 1410.
13 M. Wulff-Pérez, M. J. Gálvez-Ruíz, J. de Vicente and A. Martín-Rodríguez, *Food Res. Int.*, 2010, **43**, 1629.
14 P. Anand, A. B. Kunnumakkara, R. A. Newman and B. B. Aggarwal, *Mol. Pharm.*, 2007, **4**, 807–818.
15 J. Lin, M. Pan and S. Lin-Shiau, *BioFactors*, 2000, **13**, 153.
16 H. Yu and Q. Huang, *J. Agric. Food Chem.*, 2011, **59**, 912.
17 M. Pan, T. Huang and J. Lin, *Drug Metab. Dispos.*, 1999, **27**, 486.
18 V. Ravindranath and N. Chandrasekhara, *Toxicology*, 1980, **16**, 259.
19 Y. Kaminaga, A. Nagatsu, T. Akiyama, N. Sugimoto, T. Yamazaki, T. Maitani and H. Mizukami, *FEBS Lett.*, 2003, **555**, 311.
20 N. B. Agarwal, S. Jain, D. Nagpal, N. K. Agarwal, P. K. Mediratta and K. K. Sharma, *Fundam. Clin. Pharmacol.*, 2013, **27**, 169.
21 C. Chen, T. D. Johnston, H. Jeon, R. Gedaly, P. R. Mchugh, T. G. Burke and D. Ranjan, *Int. J. Pharm.*, 2009, **366**, 133.
22 X. Wang, Y. Jiang, Y. Wang, M. Huang, C. Ho and Q. Huang, *Food Chem.*, 2008, **108**, 419.
23 S. Rahimi Yazdi and M. Corredig, *Food Chem.*, 2012, **132**, 1143.
24 A. Sahu, N. Kasoju and U. Bora, *Biomacromolecules*, 2008, **9**, 2905.
25 D. Suresh and K. Srinivasan, *Indian J. Med. Res.*, 2010, **131**, 682.
26 A. Khajuria, N. Thusu and U. Zutshi, *Phytomedicine*, 2002, **9**, 224.
27 D. V. Suresh, H. G. Mahesh, A. G. A. Rao and K. Srinivasan, *Biopolymers*, 2007, **86**, 265.
28 G. Shoba, D. Joy, T. Joseph, M. Majeed, R. Rajendran and P. S. S. R. Srinivas, *Planta Med.*, 1998, **64**, 353.
29 J. Shaikh, D. D. Ankola, V. Beniwal, D. Singh and M. N. Kumar, *Eur. J. Pharm. Sci.*, 2009, **37**, 223.
30 M. Markowska, R. Oberle, S. Juzwin, C. P. Hsu, M. Gryszkiewicz and A. J. Streeter, *J. Pharmacol. Toxicol. Methods*, 2001, **46**, 51.
31 B. Wahlang, Y. B. Pawar and A. K. Bansal, *Eur. J. Pharm. Biopharm.*, 2011, **77**, 275.
32 J. M. Rodriguez Patino, M. R. Rodriguez Nino and C. C. Sanchez, *Curr. Opin. Colloid Interface Sci.*, 2007, **12**, 187.
33 M. Wulff-Pérez, A. Torcello-Gómez, M. J. Gálvez-Ruíz and A. Martín-Rodríguez, *Food Hydrocolloids*, 2009, **23**, 1096.
34 C. F. Chignell, P. Bilskj, K. J. Reszka, A. G. Motten, R. H. Sik and T. A. Dahl, *Photochem. Photobiol.*, 1994, **59**, 295.
35 J. J. Palmgrén, A. Töyräs, T. Mauriala, J. Mönkkönen and S. Auriola, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2005, **821**, 144.
36 W. Tiyaboonchai, W. Tungpradit and P. Plianbangchang, *Int. J. Pharm.*, 2007, **337**, 299.
37 M. H. M. Leung and T. W. Kee, *Langmuir*, 2009, **25**, 5773.