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REVIEW

Delivery of Curcumin Using Skim Milk or Oil in Water Emulsions: Effect of the Matrices on Cellular Uptake

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Abstract: To enhance the curcumin delivery in a variety of food grade matrices namely spray dried ethanolic curcumin in fresh skim milk (Spray dried Cu-SM), a fresh mixture of ethanolic curcumin and skim milk (Fresh Cu-SM) a powder mixture of curcumin and skim milk powder (Powder Cu-SMP) and oil in water emulsion (Emulsion) were studied. The cellular uptake of curcumin from the respective matrices was studied on Caco-2 cell monolayers. Spray dried Cu-SM showed higher encapsulation efficiency compared to a corresponding Powder Cu-SMP and an oil-in-water emulsion (40% oil) bearing curcumin. Furthermore, ethanolic administration of curcumin in spray dried form enhanced the cellular uptake of curcumin considerably higher than non-ethanolic samples (approx. 4 times). Overall, milk protein based vectors were found to perform better than emulsion samples. These findings highlighted the fact that curcumin uptake may be tailored by fine tuning of curcumin delivery vehicles which highlights possible application of powders as functional foods.

Key words: curcumin encapsulation, spray drying, skim milk powders, cellular uptake, Caco-2

1 Introduction

In order to deliver bioactive molecules using food delivery systems it is important to design matrices that can efficiently encapsulate compounds, maintain their bioefficacy during processing and storage, and ultimately facilitate the uptake by the intestinal cells. The encapsulation and delivery of hydrophobic molecules is further challenged by their low solubility. For example, curcumin is a hydrophobic molecule isolated from the rhizomes of the spice, turmeric (*Curcuma longa*) with proven bioactivity, that has shown great challenges to its delivery because of its low aqueous solubility rate[1)], as well as its rapid metabolic degradation inside the body. Among the food matrices proposed as delivery systems for curcumin in food systems, solid lipid nanoparticles[2)], nanoemulsions[3-5)], liposomal dispersions[6)], and casein dispersions[7)] have been reported.

Milk protein based nanovehicles have previously been shown to successfully encapsulate bioactive compounds including polyphenols (curcumin, EGCG) and minerals[7, 8-11)]. Since both whey proteins and caseins are known to be avid binders and carriers of bioactive compounds *in vitro* and *in vivo*, their utilization in encapsulation systems makes them appropriate for the delivery of curcumin. Various whey protein nanocarrier systems were successfully demonstrated to encapsulate, stabilize and deliver polyphenolic compounds in cancer cell culture environments[8)]. Native and high pressure treated casein micelle dispersions were shown to encapsulate considerable amounts of curcumin providing a dairy based platform for the addition of this highly hydrophobic and bioactive compound and many similar compounds[7)]. Since solubility of curcumin in ethanol is significantly higher (see for example, Wang *et al.*)[12)], ethanolic solutions provide an appropriate method of curcumin administration to water based encapsulation systems. Similarly, DMSO[13)] and other solvents[14)] can be utilized in solubilisation of curcumin. The present work evaluated the potential of spray dried milk as a matrix for delivery of curcumin which was initially solubilized in ethanol. Indeed, spray drying is recognized as an effective means of encapsulation of sensitive flavor compounds and bioac-

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tives[15–19], however, the use of SM as a delivery matrix for curcumin has not been reported to the best of our knowledge.

Spray-drying is a high temperature processing unit operation where a liquid product is atomized in a hot gas current to manufacture powdered materials. In this process, the liquid feed might be composed of a solution (aqueous or organic), an emulsion or a suspension and consequently very fine powders can be prepared. The size of the particles generated by spray drying is dependent on different variables like feed rate and temperature, gas flow rate and temperature, composition, and also other physicochemical properties[20]. Although the evaporation of the liquid phase will cause evaporative cooling of the samples, as evaporation is completed, temperature of the dried particles will rapidly rise to that of the drying chamber, especially due to large surface area of the particles[17]. Therefore, although some thermal losses in the sensitive compound content of particles might be observed during the spray drying of aqueous samples, the short treatment times observed and the possibility of rapid cooling limit the extent of losses. The objective of this study was to investigate the potential use of spray dried skim milk as a matrix for the delivery of hydrophobic bioactives. Curcumin was employed as model system, and a fresh ethanolic solution was mixed with fresh SM, and used fresh or immediately spray dried. The reconstituted samples were then compared to a dry mixture of SMP and curcumin, reconstituted, as well as freshly prepared oil in water emulsions. To test the efficacy of the systems in the delivery of curcumin, Caco-2 cells were employed as a model for the absorption studies. The human epithelial adenocarcinoma Caco-2 cell line is an appropriate model to be used and has been considered as a golden standard for pharmaceutical research[21].

2 Experimental

2.1 Materials

Fresh pooled bovine milk was obtained from the Research Station of University of Guelph (Elora, Ontario, Canada). Sodium azide (0.02% by wt) (Fisher Scientific, Fair Lawn, NJ, USA) was immediately added to minimize bacterial growth. Milk was skimmed by centrifugation (20 minutes at 18000 x g, 4℃) (supplied with a J2-21 rotor, Beckman-Coulter, Mississauga, Ontario, Canada), followed by vacuum filtration using 110 mm glass microfibre Whatman filters (934-AH™) (Florham Park, NJ, USA). SM was kept refrigerated (4℃) at all times. The remaining milk fat concentration was $<0.1\%$ in all cases. Low heat SMP was purchased from Parmalat Foods Inc. (London, ON, Canada). The protein content was approximately 36% wt and based on manufacturer information, the majority of milk proteins in this sample retain their native state. Curcumin (C7727, ≥ 94% curcuminoid content), soy oil (S7381), Tween 20 (P1379), Dulbecco's Modified Eagle Medium (DMEM) (containing 25 mM glucose) (D5796), HEPES buffer (83264), ethanol were obtained from Sigma-Aldrich Corporation (Oakville, ON, Canada). MilliQ (Millipore, 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 (10000 units of penicillin and 10000 μg of streptomycin per mL), phosphate-buffered saline (PBS), Hank's balanced salt solutions (HBSS) were purchased from Invitrogen (Invitrogen Canada Inc., Burlington, ON, Canada). Transwell permeable polyester (PET) clear inserts (0.4 μm) and 12-well cell culture plates and 96 well plates clear bottom were obtained from Fisher Sci. (Mississauga, ON, Canada).

2.2 Sample preparation

Different formulations of curcumin in various dairy matrices were prepared. Firstly, to maintain a protein content that is comparable to SM, a mixture of curcumin powder and SMP (12%) were reconstituted in MilliQ water and slightly heated (50℃, 1 h) and then refrigerated overnight at 4℃ to ensure complete hydration (i.e., no spray drying) (Powder Cu-SMP). A fresh mixture of SM and 5% ethanolic curcumin were prepared on the day of experiment (Fresh Cu-SM) and further diluted in the cell culture medium at a final ethanol concentration ≤ 1% in order to prevent compromising the cellular viability (data not shown). Alternatively as mentioned above, a mixture of SM-ethanolic curcumin (5%) was spray dried using a B-290 Buchi Mini Spray Dryer (Flawil, Switzerland) (Spray dried Cu-SM) equipped with standard glassware in co-current configuration and fitted with a two-fluid nozzle. The solution feed rate was 6 $mL.min^{-1}$ (20% of the maximum rate of peristaltic pump) while the inlet and outlet temperatures were 135℃ and 80℃ respectively. Gas spray flow rate was 630 $L.h^{-1}$. The powder was collected in air-tight containers, allowed to cool to ambient temperature and then stored at −20℃ until further usage. The spray dried sample was reconstituted to 200 mL with 2 h magnetic stirring at room temperature and a 30 s ultrasonic bath treatment to break loose aggregates. The reconstituted dispersion was analyzed for particle size distribution immediately after reconstitution procedures. Finally, curcumin bearing emulsion droplets (40% oil and 4% Tween 20, target for mean particle size = 0.15 μm) were prepared. The mixture was pre-homogenized with a high-speed blender (PowerGen 125, Fisher Scientific, Mississauga, ON, Canada) (15,000 rpm, 1 min at 25℃) and the homogenization was completed by passing the premix through a high pressure homogenizer (Emulsiflex C5, Avestin, Ottawa, Canada) (5 passes at 500 bars) (Emulsion). The curcumin concentration in all

Table 1 Mean particle size (μm), size span, ζ-potential and the extent of curcumin encapsulation in all formulations immediately after preparation. Each value represents the mean ± S.D. (n = 3). Each value represents the mean ± S.D. (n = 3).

Sample	Mean particle size $D_{3,2}$ (μm)	Span	ζ-potential (mV)	Encapsulation efficiency (%)*
Fresh curcumin-SM (Fresh Cu-SM)	0.10 ± 0.05	1.454	− 30.42 ± 0.66	22.39 ± 5.51
Spray dried curcumin-SM (Spray dried Cu-SM)	0.11 ± 0.03	1.477	− 35.38 ± 0.44	17.70 ± 0.40
Powder mixture (Powder Cu-SMP)	0.25 ± 0.05	1.118	− 26.5 ± 0.69	9.97 ± 3.19
Emulsion	0.22 ± 0.04	1.101	0.134 ± 0.39	4.68 ± 0.87

* Curcumin concentrations are not reported here since the curcumin concentrations are numerically equal to %EE (for example, 9.97 $mg.mL^{-1}$ also corresponds to 9.97% EE).

samples prepared was 0.02%. In all cases, encapsulation efficiency was determined based on ethanolic extraction of free curcumin from the samples. When necessary, centrifugation was also utilized (30 min at 5000 xg). Further details were explained in Guri *et al.*[4]. The sample names and abbreviations are listed on Table 1.

2.3 Particle size and ζ-potential analysis

The particle size distribution of the samples described above was measured using a laser diffraction technique (Mastersizer 2000, Malvern Instruments, Worcestershire, UK) and water was used as the dispersing agent. Refractive indices of soy oil, water and protein were taken as 1.47, 1.33 and 1.45, respectively. Where appropriate, ζ-potential of the samples was measured by means of dynamic light scattering (DLS) (Zetasizer Nano, Malvern Instruments, Worcestershire, UK). In this case, all samples were diluted in MilliQ grade water (1:100) immediately prior to the analysis.

2.4 Cell culture

Caco-2 adenocarcinoma cells were kindly provided from CRIFS culture collection (University of Guelph, Guelph, ON, Canada). These cells are able to create a tight monolayer with well-developed tight junctions once they reach full confluency, as a close representation of the epithelial layer in the human gut[22]. We have been successfully using this cell model either in isolate or in combination with HT-29-MTX cells as a valid approach to access the uptake of bioactives in the small intestine[4, 5, 23]. DMEM supplied with 10% FBS, 1% antibiotic solution (10,000 units/mL of penicillin and 10,000 μg/mL of streptomycin), 1% NAA and 0.25% HEPES, was used for propagation of the cells. Cells were weekly grown in 75 cm^2 flasks at 37℃ and 5% CO_2 at humidified atmosphere. The medium was changed every second day and cells were passaged weekly using 0.25% trypsin-EDTA solution. Cells from 26-36 passages were used throughout the study.

2.5 Cytotoxicity of curcumin formulations and their uptake on Caco -2 cells

Sulforhodamine B (SRB) assay was used to evaluate the biocompatibility of curcumin formulations (see Section 2.2 above for details) on the proliferation rate of Caco-2 cells before performing the uptake experiments. This test was performed according to Skehan and coworkers[24] with slight modifications. In detail, cells were seeded at a concentration of 4×10^3 $cells.well^{-1}$ on 96 well plates and allowed to adhere for 24 hours. The medium was discarded and cells were washed 3 × with phosphate buffer saline (PBS, pH 7.4). Afterwards, the water reconstituted, spray dried sample, powder dispersions, fresh dispersions and oil-in-water emulsion (see section 2.2 for details) were serially diluted (50 ×) in medium. The diluted samples were administered to the cells and incubated for another 24 h. A control of ethanolic curcumin (1%) (EtOH-Cu) in the cells were also tested. The optical density of plates was measured using an automated 96-well plate reader (Multi detector microplate reader, Biotek Synergy HT Model, Vermont, USA) at 570 nm and the results are expressed as % proliferation of non-treated and treated cells. The spray dried powder samples were also tested 15 days after storage at −20℃ to check if the cytotoxic effect on cells was retained. Liquid samples were kept refrigerated (4℃), since frozen storage could destabilize and/or negatively affect the liquid samples.

2.6 Curcumin uptake by Caco-2 monolayers

For uptake studies, Caco-2 cells were seeded on 12 well inserts at a concentration of 6×10^4 cells per insert. The medium was changed every other day until the cells will complete their differentiation in 21 days[25], and the permeation study was performed. The development of the monolayer was monitored throughout differentiation period by

means of transepithelial electrical resistance (TEER) which was also measured prior to and after uptake experiments using an EVOM2 (WORLD Precision Instruments, Sarasota, FL, USA).

Briefly the monolayers were gently washed with PBS 2× to remove any cell debris without disturbing the monolayer. Aqueous curcumin dispersions were added to the monolayers at a dilution ratio sample: medium 1:30 (v/v) along with the control EtOH curcumin solution and cells were incubated for 4 h at 37℃ and 5% CO_2. This dilution rate of the sample was chosen based on SRB results. Immediately after the incubation time, the basolateral medium was carefully collected in microcentrifuge tubes and kept at −80℃ until further analysis.

2.7 Liquid Chromatograpy / Mass Spectrometry (LC/MS)

The basolateral samples collected from the uptake experiments were rapidly lyophilized (Genesis 25L, Virtis, SP Industries, Warminster, PA, USA) and subsequently reconstituted in methanol. The dispersions were clarified by centrifugation (5000×g, 15 min) using a microcentrifuge (5415D, Brinkmann Instruments, Westbury, NY) and the supernatant was filtered using 0.22 μm nylon syringe filters (Fisherbrand, Ireland) and further analyzed by means of Liquid Chromatograpy/Mass Spectrometry (LC/MS). Samples were run through an Agilent 1100 LC-MSD unit (Santa Clara, CA, USA). The initial mobile phase conditions were 90% water (0.1% formic acid) and 10% acetonitrile (0.1% formic acid) then a single step gradient to 100% acetonitrile in 10 min was applied. The flow rate was maintained at 0.4 mL.min^{-1} and the injection volume was 20 μL. The mass spectrometer electrospray capillary voltage was maintained at 4.5 kV and the drying gas temperature of 280 ℃ with a flow rate of 10 L.min^{-1}. Nebulizer pressure was 40 psi. Nitrogen was used as both nebulizing and drying gas, helium was used as collision gas at 60 psi. The mass spectrometer was set on enhanced resolution negative-ion mode. The instrument was externally calibrated with the ESI TuneMix (Agilent). Quantitation of curcumin was determined using the QuantAnalysis software (Bruker Daltonics). Calibration curves were created various curcumin solutions (0.5, 1 and 2 μg/mL pure curcumin in methanol) on a daily basis and the curcumin concentration of basolateral samples was determined from the calibration curve. The major curcumin peak was obtained at an elution time of approximately 5.5 min.

2.8 Cell viability assay on the basolateral fractions

Instead of a quantitative method, a second approach to study the presence of curcumin after absorption in the basolateral fractions was performed using another set of cells[26]. The anti-proliferative activity of the basolateral fractions was tested using a Caco-2 model, following the same procedure previously described for SRB assay in section 2.5. Briefly, aliquots of 100 μL of the basolateral medium were transferred to 96 well plates containing Caco-2 cells, using the same concentration of cells and incubation times as mentioned earlier.

2.9 Statistical analysis

All experiments were conducted in three independent replicates and their average value with a standard deviation was reported. To determine the statistically significant differences between treatments in the cell viability assay for basolateral fractions, one-way analysis of variance (ANOVA) and a subsequent Dunnett's test for multiple comparisons versus the control in SigmaPlot software was used. Significant differences were considered at $p < 0.05$.

3 Results and Discussion

3.1 Sample characterization and encapsulation efficiency

A characterization of the particle size and charge was performed for all samples (see Section 2.2 and Table 1 for details). The mean diameters of Fresh Cu-SM and Spray dried Cu-SM were similar (0.10 and 0.11 μm, respectively). The mean particle diameter of the emulsion droplets (Emulsion) was comparable to that of the powder sample (Powder Cu-SMP) showing a larger diameter of about 0.22 and 0.25 μm, respectively. Larger mean size of the powder samples could be attributed to the high temperatures that might have occurred during the thermal processing of commercial SMP. Similarly, in their attempt to use spray dried curcumin encapsulated in liquid or reconstituted powder forms, Yan and coworkers[27] found that there were no differences in droplet size between the emulsion samples and reconstituted powder formulations, although their formulations have a different composition in terms of oil and surfactant and co-surfactant used to stabilize the emulsion.

In the current study, the zeta-potentials of Fresh Cu-SM and Spray dried Cu-SM and powder Cu-SMP were found to vary between −26.5 to −35.4 mV which indicated that the electrostatic stability of the particles were moderate[28]. In the case of emulsions stabilized by Tween 20 (i.e., a non-ionic surfactant), as expected, the emulsion droplets only carried residual charge (approx. −0.13 mV). The presence of curcumin in the samples stored either at −20℃ (for powder form) or 4℃ (for emulsions) did not significantly affect mean particle sizes up to 15 days (data not shown), all of which were indications of reasonably stable systems.

Since the encapsulation systems demonstrated reasonable stability, the extent of curcumin encapsulation was determined using an LC/MS method (Table 1). While the emulsions seemed to carry a lower extent of curcumin (4.68 mg.mL^{-1}) compared to our previous studies[4, 5] on encapsulation of curcumin in emulsions and solid lipid

nanoparticles (SLN), this could partly be attributed to the differences in emulsifiers used and the instrumental analysis techniques utilized (spectrofluorometry vs LC-MS). At all cases, the extent of increasing oil and emulsifier contents seemed to enhance the encapsulation efficiency (%) since it was possible to solubilize more curcumin in a more concentrated dispersed phase and also to stabilize the curcumin bearing droplets. Although it is difficult to compare the interaction of curcumin with oil droplets or milk protein based particles possibly due to the presence of a small amount of ethanol in the system, which is a better solvent for curcumin than water and soy oil. In general, it was possible to stabilize and encapsulate more curcumin in the milk protein based vectors.

Part of the losses in curcumin *inter alia* might be due to its partial insolubility at room temperature or during refrigeration, rapid crystallization, stresses observed during high pressure homogenization or partial micellar solubilization of curcumin in micelles formed by excess Tween 20[4]. Among the powder forms, the encapsulation of curcumin was the lowest in the Powder Cu-SMP sample (Table 1), which can be explained by extremely low water solubility of curcumin and consequently, the rapid sedimentation of most of the curcumin molecules prior to interacting with milk proteins. Certain manipulations like high hydrostatic pressure treatment of curcumin in SM have shown to enhance the binding of curcumin to caseins in SM[7]. Although the preparation of an ethanolic solution prior to spray drying enhanced the extent of encapsulation (Table 1), the highest efficiency was attained by the liquid mixtures (Fresh Cu-SM). This finding indicated that spray drying accounted for partial loss of curcumin although the process was carried out under mild conditions (135℃). Therefore, we can conclude that powdered systems are appropriate vehicles of curcumin encapsulation, while the method of curcumin addition to the system and following thermal treatments might affect the encapsulation efficiency.

3.2 Cytotoxicity of curcumin formulations on Caco-2 cells, curcumin uptake and bioactivity of basolateral contents

Prior to testing the curcumin absorption by Caco-2 monolayers grown on inserts for 3 weeks a screening experiment related to cytotoxicity induced by the treatment to the Caco-2 cells was performed. Powder Cu-SMP, Spray dried Cu-SM, Fresh Cu-SM and emulsion samples were incubated with Caco-2 cells for 24 h as well as a corresponding ethanolic curcumin solution (EtOH-Cu). As presented in Fig. 1, all treatments and controls showed a proliferative activity above 82% for a dilution rate in freshly warmed medium of 25×. Therefore, a final dilution ratio of 30× in the well was chosen to ensure the viability of Caco-2 cells during the uptake experiments. Especially since the uptake

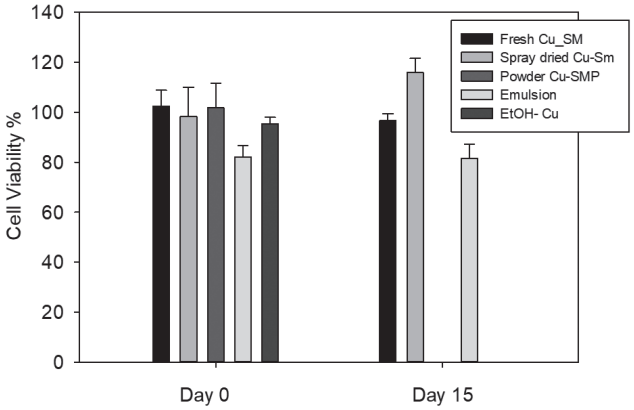

Fig. 1 The viability (%) of Caco-2 cells after 24 h exposure to freshly prepared and spray dried mixture of ethanolic curcumin and skim milk (Spray dried Cu-SM); freshly prepared mixture of ethanolic curcumin and skim milk (not spray dried) (Fresh Cu-SM); powder mixture (Powder Cu-SMP) and curcumin bearing oil-in-water emulsions (40%) (Emulsion) at day 0 and 15. A control sample of EtOH-curcumin solution was also tested. All samples were diluted in cell culture medium 25× (sample: medium v/v). The results are expressed as percentage of the OD values obtained from cells growing without any treatments. Each value represents mean ± S.D. (n = 3).

measurements were carried out with cells cultivated for 21 days, they can be safely anticipated to be less affected than the cells utilized in cytotoxicity experiments (24 h culturing only). Furthermore, we do not expect the sample to have the same efficacy on the monolayers consolidated after 21 days growth as to compare to cells grown for only 24 h. The anti-proliferative activity of curcumin on colon cancer cells and even more specifically the role of curcumin in suppressing Caco-2 proliferation are already well-known[29]. The cytocompatibility of all samples was tested after storage for 15 days at −20℃ (powdered samples) or 4℃ (liquid samples) to protect the stability of these samples and no changes on activity were observed for any of them, which renders the powders stable over the storage period (data not shown). This finding is in line with earlier results on particle size analysis (see section 3.1).

Cells were used for the uptake experiments only after the completion of the differentiation period (21 days) and the development of the monolayer was monitored by measuring the transepithelial resistance (TEER). After 21 days of growth the cells reached a resistance value of 1200 $\Omega \cdot cm^2$ and only the wells in this TEER range were chosen for the uptake experiments. As indicated above (see section 2.7), TEER measurements were carried out to ensure the integrity of the cellular monolayers prior to sample administration and after the uptake experiments and the curcumin

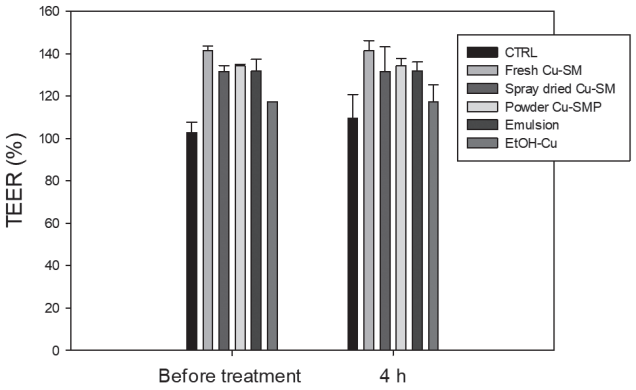

Fig. 2 Relative values of transepithelial electrical resistance (TEER) (%) of confluent Caco-2 monolayers measured immediately prior to the curcumin uptake experiment (t = 0) and after 4 h of treatment compared to fully differentiated cells at 21 days. Samples were diluted in medium and incubated with cells for a period of 4 h. Cells growing in medium without treatments were used as controls. All the experiments were carried out at least in triplicate. Results for similar samples as Fig. 1 are shown.

collected in the basolateral compartment was due to the uptake and not due the leakage of the monolayer. Results presented in Fig. 2 demonstrated that all the samples tested were biocompatible with the Caco-2 cells showing a TEER value above 100 ensuring the monolayer integrity during the cell culture experiments. Four hour of treatment of the samples did not negatively affect the monolayer integrity since the TEER values were relatively stable (Fig. 2).

The extent of curcumin uptake by Caco-2 cells was evaluated in Fresh or Spray dried Cu-SM formulations, their respective powder mixtures (Powder Cu-SMP) and curcumin bearing emulsions (Emulsion). After 4 h incubation of the samples with the confluent cellular monolayers, the basolateral medium was collected and the extent of curcumin uptake from various formulations was analyzed using LC/MS (Table 2). The highest curcumin recovery was reported for the liquid mixture of ethanolic curcumin-SM administered either from the reconstituted Spray dried Cu-SM samples or Fresh Cu-SM samples (40.51 ± 0.9 and 78.12 ± 19.2 $ng.mL^{-1}$, respectively).

When the amount of curcumin uptaken from the Spray dried Cu-SM was compared to the respective reconstituted Powdered Cu-SMP, the extent of uptake was almost 4 times higher for the spray dried sample which is coherent with the higher encapsulation efficiency observed for this sample (almost twice as high) (Table 2). The transport of curcumin through emulsion in the Caco-2 monolayers was comparable to some extent to the spray dried liquid mixtures; although the encapsulation efficiency recorded for

Table 2 Amount of curcumin recovered in the basolateral compartment during cellular uptake experiment on confluent Caco-2 monolayers for 4 h at 37℃ and 5% CO_2. Curcumin was quantified using LC/MS. Each value represents the sample mean ± S.D. (n = 3).

Sample	Basolateral curcumin concentration ($ng.mL^{-1}$)
Fresh Cu-SM	78.12 ± 19.24
Spray dried Cu-SM	40.51 ± 0.91
Powder Cu-SMP	11.32 ± 3.63
Emulsion	31.26 ± 6.55
EtOH - Cu	9.63 ± 1.93

the emulsion is significantly lower that in the case of the latter. This finding pointed out to the fact that emulsion delivery is a good proven vehicle for hydrophobic molecules through epithelial cells[4, 5]. On the other hand, we do not expect high losses on curcumin during uptake as we had previously shown that no more than 3% curcumin losses were observed for emulsion formulations stabilized by Tween 20[5]. This is more prominent when compared to the ethanolic curcumin references (EtOH-Cu), where the amount of curcumin delivered is 10 times higher although the recovered curcumin in the basolateral compartment is still in a low range which shows that the delivery of curcumin has to meet further obstacles prior to uptake[4, 5]. In any case, our findings point to the fact that the milk protein based systems could perform as highly efficient carriers of hydrophobic bioactive.

During further investigations, curcumin uptake after absorption and transport though Caco-2 cells was assessed. Viability of Caco-2 cells after 24 h exposure with aliquots of basolateral fractions from uptake experiments was measured relative to a control containing only medium from untreated basolateral fraction (Fig. 3). These findings indicated that the presence of curcumin or its bioactive metabolites in the basolateral fraction was capable of affecting the viability of the cells. Fresh Cu-SM showed the highest reduction in viability (>20%) which is in agreement with LC/MS results. The basolateral curcumin recovery in Fresh Cu-SM sample was more than double the amount detected in other samples, however these differences in concentration were not observed to the same extent in cell response. Spray dried Cu-SM and emulsion had a similar and lower response (<20%), with differences in concentration detected, based on chromatographic analysis, of almost 4 times between spray dried samples. There were no significant differences in viability between curcumin ethanolic solution fraction (EtOH-Cu) and control (CTRL), which supports the detection of lower extents of uptaken curcumin compared to other systems. Based on mass spec-

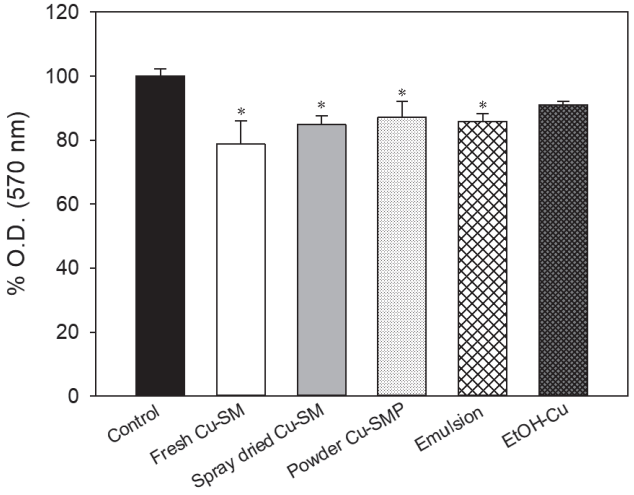

Fig. 3 Percentage cellular viability (%) of Caco-2 cells based on optical density (O.D.) after 24 h incubation with basolateral content from the uptake experiments. Values are the means of three independent experiments ± standard deviation. * Indicates statistical differences between control cells and treatments at $p < 0.05$.

troscopy, these results support our findings on the potential influence of basolateral curcumin on the viability of cells during cell culture assays[26].

As mentioned earlier, the encapsulation efficiency of the Spray dried Cu-SM or EtOH-Cu samples were higher in the case of than Powder Cu-SMP, which emphasized the importance of the solubilizing agent that brought curcumin more or less efficiently to the system. Similarly, Yan *et al.*[27] showed in an *in vivo* study on rats that after oral administration curcumin in the spray dried liquid formulations significantly improved curcumin absorption compared with that of curcumin powder. Although there are several factors that should be taken into account regarding curcumin solubility in different buffers[30] and its tendency to degrade in different environments[31]. The operation variables also affect the control of curcumin loss and stability[31]. On the other hand, milk proteins have shown capacity to encapsulate hydrophobic molecules due to their hydrophobicity and molecular structure[7, 8]. As per heat stability caseins are relatively heat stable and can be held at 140℃ for more than 1 hour without noticeable change in physicochemical functionality[32] which again reaffirmed the sustained activity of the Spray dried Cu-SM.

Overall, the encapsulation will be highly dependent on the individual characteristics of the bioactive molecules, their solubility and partial interactions with milk proteins without neglecting the physical parameters of the process operation like in case of spray drying.

Previously we have evaluated curcumin uptake by Caco-2 cells or co-cultures of Caco-2/HT-29-MTX cells. Encapsulation of curcumin in solid lipid nanoparticles (SLN) was shown to result in a low extent of curcumin recovery (mostly ng.L^{-1} level)[4]. Although when different stabilizers were used in attempt to deliver curcumin *in vitro* through emulsions formulations, the extent of curcumin delivery was closely related to the emulsifier type[5].

Albeit we focus on protecting the labile bioactive molecules, their bioavailability and bioactivity is maintained after uptake, and certain modifications on the structure could enhance a different uptake mechanism utilized by the cell itself either through protein mediated processes or simply through passive diffusion. The complexity of the curcumin both structurally and functionally opens new insights in the attempts to increase its bioavailability and biofunctionality.

4 Conclusions

The delivery of bioactive compounds could be enhanced by using different encapsulation formulations prepared through spray drying process. Curcumin solubilisation and hydration rate and its thermal stability could potentially influence the encapsulation efficiency which in turn could affect the uptake from the cells. We have shown here that curcumin uptake delivered through spray dried ethanolic curcumin and skim milk is more efficient compared to simply powder mixtures of curcumin and skim milk. The trend in curcumin recovery from spray dried liquid mixtures versus reconstituted powders, or oil in water emulsion, demonstrated that the cellular uptake of curcumin heavily depended on the design and processing history of the appropriate formulation and under suitable conditions, and powdered forms based on dairy derived ingredients could be instrumental in curcumin delivery.

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