DESIGN OF THE MANUFACTURING CONDITIONS TO INCREASE CURCUMIN LOADING IN POLYANHYDRIDE MICROPARTICLES

JULIE BORREAU [1*], KONRAD KWIECIEŃ [2], ANNA KUSIBAB [2], ELŻBIETA PAMUŁA [2]
[1] CY Cergy Paris Université, CY Tech, Department of Biotechnology and Chemistry, Av. du Parc, 95-160 Cergy, France
[2] AGH University of Krakow, Faculty of Materials Science and Ceramics, Department of Biomaterials and Composites, A. Mickiewicz Av. 30, 30-059 Kraków, Poland
* e-mail: borreaujulie@gmail.com

Abstract

Curcumin is a natural polyphenol with anti-inflammatory and anticancer potential, but its poor aqueous solubility and limited incorporation into polymeric carriers hinder clinical translation. Covalent or ion-paired conjugation with a polymeric matrix may enhance drug retention and loading, thereby improving delivery efficiency. This study investigated whether introducing acidic conditions to oil-in-water emulsification would promote curcumin–polyanhydride conjugation and improve encapsulation efficiency (EE) and drug loading (DL) in poly(sebacic anhydride) (PSA) microparticles (MPs). The results obtained by fluorimetry show that encapsulation outcomes strongly depended on the acid used. Trifluoroacetic acid (TFA) yielded negligible curcumin incorporation, with EE ranging from 1.7 ± 0.1% to 19.7 ± 0.6% and DL between 0.28 ± 0.01% and 3.3 ± 0.1%. In contrast, hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) produced results comparable to non-acidified controls, with EE around 30–35% and DL around 5–6%. A markedly different outcome was observed with 2% aqueous trichloroacetic acid (TCA). Condition X21 exhibited the best performance with 53.6 ± 0.5% (EE) and 8.9 ± 0.1% (DL), while another TCA-based formulation (X20) also showed high encapsulation values (EE = 45.5 ± 1.1%, DL = 7.6 ± 0.2%). However, despite this significant improvement in curcumin incorporation, TCA-derived MPs compromised L929 fibroblasts viability, whereas HCl- and H_2SO_4-treated formulations were cytocompatible. These findings demonstrate that low-concentration aqueous TCA enhances curcumin incorporation into PSA MPs, likely via conjugate formation, but compromises cytocompatibility due to residual TCA. Further optimization of TCA concentration and purification steps will be necessary to balance drug loading with safety for biomedical applications.

Keywords: *poly(sebacic anhydride), curcumin, drug delivery system, polymeric microparticles, trichloroacetic acid*

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Introduction

Curcumin, a natural polyphenol extracted from turmeric (*Curcuma longa*), has been extensively studied for its wide range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects [1,2]. Despite these promising properties, the clinical application of curcumin remains limited due to its poor aqueous solubility, chemical instability, rapid metabolism, and low systemic bioavailability [2,3]. Encapsulation of curcumin within polymeric carriers has emerged as a promising strategy to overcome these challenges, as it can enhance solubility, protect the compound from degradation, and facilitate sustained or targeted release [1].

Among biodegradable polymers, polyanhydrides are of particular interest due to their biocompatibility, degradation via surface erosion, and potential to form conjugates with bioactive molecules [5,6]. However, encapsulation efficiency (EE) and drug loading (DL) of curcumin in polyanhydride MPs generally remain low. At an equivalent curcumin concentration in the oil phase, the drug loading typically varies between 2% and 3% [4]. This limitation hinders the development of effective delivery systems and highlights the need for process optimization.

Other biodegradable polymers, such as poly(lactic-co-glycolic acid) (PLGA) and poly(lactic acid) (PLA), have also been extensively investigated for curcumin delivery. PLGA nanoparticles, for instance, have demonstrated enhanced curcumin stability, improved cellular uptake, and sustained release profiles, with encapsulation efficiencies often reaching 50–80% depending on the preparation method and polymer composition [7,8]. Similarly, PLA-based systems have been shown to protect curcumin from photodegradation and extend its release over several days, although drug loading typically remained lower than in PLGA carriers [9]. Other strategies include surface modification with poly(ethylene glycol) (PEG) or targeting ligands, which can further improve bioavailability and therapeutic efficacy [10]. These studies highlight the versatility of polymeric encapsulation approaches and provide benchmarks for assessing polyanhydride-based systems.

One possible strategy to enhance encapsulation in polyanhydrides relies on acid-catalyzed esterification between curcumin's phenolic hydroxyl groups and the anhydride bonds of poly(sebacic anhydride) (PSA). Acidic environments can potentially favour these interactions, thereby increasing curcumin retention within the polymer matrix. Yet, the effect of specific acids on encapsulation remains poorly documented. In particular, organic acids such as trifluoroacetic acid (TFA) and trichloroacetic acid (TCA) have been utilized in biomaterials processing, whereas inorganic acids like hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) are more conventional catalysts [3,4]. To the best of our knowledge, their comparative impact on curcumin encapsulation performance and cytocompatibility has not been systematically evaluated so far.

In this context, the present study investigated whether acidic conditions during oil-in-water emulsification could enhance the incorporation of curcumin into PSA MPs. We specifically assessed the effect of HCl, H_2SO_4, TFA, and TCA on encapsulation parameters and curcumin–polymer conjugation, as determined by fluorometry and mass spectrometry. Additionally, a curcumin-zinc complex was prepared to determine its impact on encapsulation outcome. Cytotoxicity of the resulting MPs was evaluated using L929 fibroblast cultures using Alamar Blue and Live-Dead fluorescence staining tests.

Materials and Methods

Chemicals and Reagents

Curcumin (≥94%) and poly(sebacic acid) (PSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trifluoroacetic acid (TFA), trichloroacetic acid (TCA), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), and dichloromethane (DCM) were obtained from Chemland (Poland). Poly(vinyl alcohol) (PVA, Mowiol 4-88), dimethyl sulfoxide (DMSO), and absolute ethanol were purchased from VWR International (Radnor, PA, USA). Zinc chloride ($ZnCl_2$) was obtained from Merck (Germany). Phosphate-buffered saline (PBS) and foetal bovine serum (FBS) were supplied by PAN-Biotech (Germany). Dulbecco's modified Eagle's medium (DMEM) was from Gibco, USA. All chemicals were of analytical grade and used without further purification. L929 murine fibroblasts (ATCC CCL-1) were used for cytotoxicity testing.

Instrumentation and Equipment

In the study the following equipment was used: freeze dryer (Martin Christ Alpha 1-2 LDplus, −50 °C, <0.1 mbar), centrifuge (MPW-351R, 4,000 rpm and 15,000 rpm), sonicator (SONICS Vibra cell, 40% amplitude), fluorometer (FluoStar OMEGA, BMG LabTech, 96-well black plates), fluorescence microscope (ZEISS Axiovert 40 CFL with ZEISS HXP 120 C metal halide illuminator), mass spectrometers (Electrospray ion trap, Amazon SL, Bruker Daltonics and Orbitrap Exploris 240, Thermo Scientific) and Ultimate 3000 chromatography system (Thermo Scientific).

Preparation of Curcumin–Zinc Complex

A curcumin–zinc complex was synthesized as described by Patel et al. (2015). Briefly, 2 mg/mL curcumin was dissolved in a 50% (v/v) ethanol–water mixture under stirring at 40 °C. $ZnCl_2$ (0.4 molar equivalent, 10 mM) was added and stirred for 2 h at ambient temperature. The product was centrifuged (4,000 rpm, 10 min), washed with the same solvent, and lyophilized (−50 °C, <0.1 mbar, 24–48 h) to obtain a dry solid complex.

Manufacturing of Microparticles

MPs were prepared using a conventional oil-in-water (O/W) solvent emulsification method. The organic phase consisted of 2% (w/v) PSA dissolved in DCM, to which curcumin or the curcumin–zinc complex was added. Acidified formulations involved either the addition of TFA or TCA (1–15% w/v) to the oil phase, specifically in DCM before mixing with curcumin, or the incorporation of HCl or H_2SO_4 (20% w/v) into the aqueous phase during emulsification, following preliminary tests in 100 µL increments to determine the maximum feasible amount.

The aqueous phase consisted of 8% (w/v) PVA solution in Milli-Q water, precooled to 0 °C. The organic phase was added dropwise under vigorous stirring (1,500 rpm), followed by sonication (40% amplitude, 1 min) to ensure homogenization. Emulsions were stirred for 2 h in a fume hood to allow complete DCM evaporation.

MPs were recovered by centrifugation (15,000 rpm, 20 min), washed three times with MilliQ water, frozen in liquid nitrogen, and lyophilized (-50 °C, <0.1 mbar, 24–48 h). In the study, 25 formulations X1-X25 were prepared (TABLE 1).

TABLE 1. Codes of the PSA microparticles produced

Code	Formulation of each batch type
X1	Control (mean of 6 batches)
X2	HCl 20%
X3	H_2SO_4 20%
X4	Curcumin-zinc complex
X5	TFA 1%
X6	TFA 1.5%
X7	TFA 2%
X8	TFA 5%
X9	TFA 7.5%
X10	TFA 10%
X11	TFA 15%
X12	TCA + DCM 1%
X13	TCA + DCM 1.5%
X14	TCA + DCM 2%
X15	TCA + DCM 5%
X16	TCA + DCM 7.5%
X17	TCA + DCM 10%
X18	TCA + DCM 15%
X19	TCA + H_2O 1%
X20	TCA + H_2O 1.5%
X21	TCA + H_2O 2%
X22	TCA + H_2O 5%
X23	TCA + H_2O 7.5%
X24	TCA + H_2O 10%
X25	TCA + H_2O 15%

Encapsulation Efficiency and Drug Loading

Fluorometry was used to quantify curcumin content within MPs. Lyophilized MPs were dissolved in DMSO (1 mg/mL) and diluted tenfold. Fluorescence was measured in triplicate in 96-well black plates (excitation 485 ± 12 nm; emission 510–590 nm). Encapsulation efficiency (EE) and drug loading (DL) were calculated relative to control formulations without acid treatment.

$$EE\ (\%) = \frac{Amount\ of\ drug\ encapsulated}{Initial\ amount\ of\ drug\ added} \times 100$$

$$DL\ (\%) = \frac{Weght\ of\ drug\ encapsulated}{Total\ weight\ of\ microparticules} \times 100$$

Mass Spectrometry

Mass spectrometry (MS) analyses were performed to assess the conjugation of curcumin with PSA in MPs. MPs were dissolved in chloroform at 1 mg/mL and then diluted 100-fold in a chloroform–methanol mixture. A 0.5 µL aliquot of each diluted sample was injected into the mass spectrometer via the autosampler of the Ultimate 3000 chromatography system (ThermoScientific, USA) at a flow rate of 30 µL/min. Analyses were carried out on an electrospray ion trap mass spectrometer (Amazon SL, Bruker Daltonics, Bremen, Germany) operated in positive ionization mode. Characteristic

sodium-cationized curcumin–PSA conjugate peaks were detected at m/z ≈ 575.3. The ionization voltage was set at 4.5 kV, with a mass scan range of m/z 100–1000. Relative ion intensities of the detected peaks were used as semi-quantitative indicators of curcumin incorporation.

Cell Culture and Cytotoxicity Assays

L929 fibroblasts were maintained in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. Cells were seeded at 1×10^4 cells/well in 96-well plates and allowed to adhere for 24 h at 37 °C with 5% CO_2. After this initial 24 h adhesion period, the medium was removed, cells were washed with PBS, and fresh medium containing MPs at concentrations of 10, 50, and 100 µg/mL was added. Cells were then incubated for another 24 h under standard culture conditions (37 °C, 5% CO_2). On the third day of culture, cytotoxicity was evaluated using both the Alamar Blue and Live/Dead assays.

Alamar Blue Assay

For the Alamar Blue (AB) assay, a 10% v/v solution of AB was prepared in DMEM. After MP exposure (24 h), the culture medium was removed, and the cells were washed with PBS. The solution was added to the wells with 150 µL of AB solution and was incubated for 3 h at 37 °C with 5% CO_2.

Fluorescence was measured at excitation 560 nm and emission 590 nm. Results were normalized against controls: 0% reduction was defined as AB solution without contact with cells, and 100% reduction as AB solution incubated at 121 °C for 15 min. The heated blank (Alamar Blue solution incubated at 121 °C for 15 min) served as a 100 % reduction control to define the maximum fluorescence reference for data normalization.

Live/Dead Staining

For the Live/Dead assay, after MP exposure (24 h), the culture medium was removed, and cells were washed with PBS. A staining solution was prepared by diluting 10 µL of calcein AM and 10 µL of propidium iodide in 10 mL of PBS. The solution was added to the wells with 100 µL of this solution and was incubated for 15 min in the dark at 37 °C. Fluorescence microscopy was used to visualize viable (green) and dead (red) cells. The proportion of dead cells was estimated semi-quantitatively from fluorescence microscopy images using ImageJ. The ratio of red (dead) to green (live) cells was calculated in five random fields per sample and averaged. These data were consistent with the Alamar Blue viability results.

Statistical Analysis

All experimental data are presented as mean ± standard deviation (SD) from at least three independent replicates. Statistical comparisons between multiple groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post hoc test. All calculations and graphical representations were conducted using Origin2024 software (OriginLab 2024, Northampton, MA, USA). Differences were considered statistically significant at $p < 0.05$. Each data point represents the mean ± SD of three replicate measurements from the same batch. For cell culture assays, values were obtained from three independent wells, ensuring biological replication rather than repeated readings from a single well.

Results and Discussions

MPs fabrication

The fabrication of PSA curcumin-loaded MPs was successful, and the particles exhibited morphology and size comparable to previously reported systems [11]. The MPs were prepared in the combinations shown in TABLE 1. The presence of acid did not significantly alter the spherical shape or size distribution of the MPs. Particles prepared under optimal conditions were predominantly spherical and yellow due to curcumin encapsulation. Over 90% of the MPs fell within the size range (1.5–2.5 µm).

The method to evaluate the loading of curcumin in MPs by subtraction of the residual curcumin in supernatant tends to overestimate the actual drug content, particularly when the quantification is not validated against the solid particles themselves or by complementary analytical methods. In contrast, our direct measurements by fluorometric analysis, further validated by mass spectrometry, provide a more accurate and realistic evaluation of curcumin incorporation in MPs.

For clarity, the formulations were grouped as follows according to the type of acid used in microparticle manufacturing: Group A – Inorganic acids (HCl, H_2SO_4), Group B – Organic acids (TFA series), Group C – Organic acids in dichloromethane (TCA + DCM series) and Group D – Organic acids in aqueous media (TCA + H_2O series).

Fluorometric analysis

Quantitative fluorometric analysis revealed significant differences in encapsulation efficiency (EE) and drug loading (DL) among the various microparticle (MP) formulations (FIG. 1). The control formulation (X1) was prepared without acid addition. Six independent X1 batches were produced, and their average values were used as the fluorescence reference.

Unloaded microparticles, i.e., X1, served as control and exhibited an EE of 32.1 ± 11.1% and a DL of 5.3 ± 1.9%. A relatively high variability observed for X1 is attributable to measurements across six independent batches, including one extreme value, which contributed to the overall dispersion.

Microparticles X2 and X3 (HCl, H_2SO_4) displayed divergent performance relative to X1. X2 exhibited slightly higher encapsulation values (EE = 34.71 ± 0.03%, DL = 5.79 ± 0.01%) compared to X1, although the differences were not statistically significant due to control variability. In contrast, X3 demonstrated lower encapsulation efficiency (EE = 22.8 ± 0.3%) and drug loading (DL = 3.8 ± 0.1%), indicating that more acidic conditions may compromise curcumin encapsulation.

Microparticles X4–X11 (Curcumin-zinc complex and TFA series) showed the lowest encapsulation performance. EE values ranged from 1.7 ± 0.1% (X10–X11) to 19.7 ± 0.6% (X5), while DL did not exceed 3.3 ± 0.1% (X5). Within this group, X4 (EE = 5.4 ± 0.1%, DL = 0.90 ± 0.01%) and X6–X11 were notably poor, with significant differences relative to X1 from < 0.05 to < 0.001. By contrast, X5–X7 achieved intermediate performance, yet remained below that of the control.

Microparticles X12–X17 (TCA + DCM series) demonstrated stable and comparable encapsulation relative to X1, with EE values ranging from 30.7 ± 1.5% (X17) to 37.9 ± 4.9% (X13), and DL up to 6.3 ± 0.8% (X13). Formulation X18 exhibited a pronounced decrease in encapsulation parameters (EE = 16.6 ± 0.4%, DL = 2.8 ± 0.1%), suggesting the existence of a solvent concentration threshold beyond which encapsulation efficiency is markedly reduced.

The highest encapsulation efficiencies were observed in microparticles X19–X25 (TCA + H_2O series), all of which exceeded the control. X21 achieved the best performance with an EE of 53.6 ± 0.5% and a DL of 8.9 ± 0.1% ($p < 0.001$). X19 and X20 also exhibited improved performance (EE = 41.3 ± 1.2%, DL = 6.9 ± 0.2%; EE = 45.5 ± 1.1%,

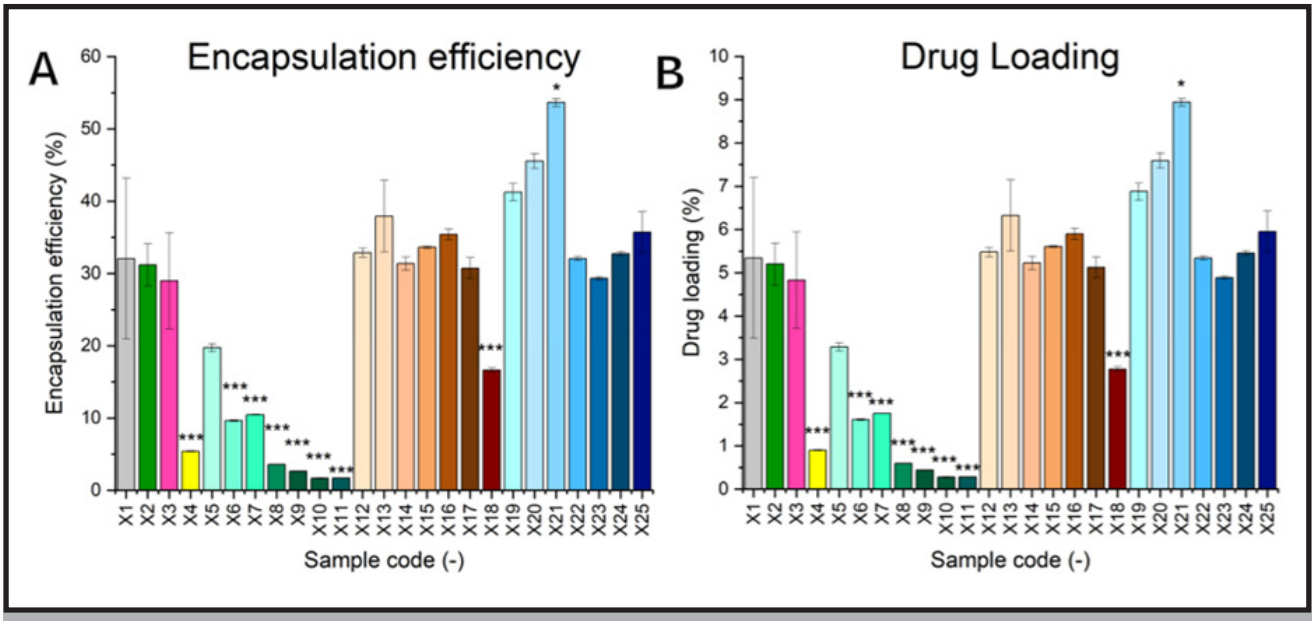

FIG. 1. Quantification of curcumin encapsulation efficiency (A) and drug loading (B) in PSA MPs based on fluorometry measurements. Statistical significance as compared to control unloaded MPs (X1) at *** $p < 0.001$, * $p < 0.05$. Drug loading (DL) values are directly proportional to encapsulation efficiency (EE), which explains the similarity of the statistical trends observed between Figures 1A and 1B.

DL = 7.6 ± 0.2%, respectively), although values remained slightly lower than X21. Microparticles X22–X25 maintained efficiencies comparable to X1 and X12–X17, with EE between 29.3 ± 0.3% and 35.7 ± 2.8%, and DL values between 4.9 ± 0.1% and 6.0 ± 0.5%.

Overall, X1 provided a robust reference for comparative analysis. X2 slightly outperformed the control, though without statistical significance; X3 exhibited lower performance; X4–X11 were generally poor to intermediate; X12–X17 were stable and comparable; X18 demonstrated a clear reduction; and X21 emerged as the most promising condition for curcumin encapsulation. Complementary mass spectrometry analyses confirmed the formation of polymer–curcumin conjugates.

This study was designed to maximize the encapsulation efficiency of curcumin within PSA microparticles, aiming to achieve the highest possible drug incorporation. Such optimization allows for reducing the amount of microparticles required to reach potential therapeutic levels, as excessive MP concentrations could lead to cytotoxic effects. The therapeutic concentration of curcumin was not evaluated in this work, but remains a target for future investigations.

Mass Spectrometry

Mass spectrometry was used to quantify curcumin encapsulated within MPs that underwent conjugation with the polymeric matrix (FIG. 2 and TABLE 2). The characteristic sodium-ionized curcumin peak was consistently observed at an average mass-to-charge (m/z) ratio of 575.3, in agreement with the expected value for a PSA monomer–curcumin conjugate ionized with Na^+ [11]. This experiment provides only relative concentration data, as the exact nature and distribution of the conjugate species obtained remain unknown. However, it may serve as a relative indicator of conjugation yield, as the same MPs concentration was used for all samples.

MPs X2 (HCl) exhibited higher ion intensity, whereas X3 and X8 (H_2SO_4 and TFA series) showed lower intensities compared to X1. These findings corroborate the fluorometry results, indicating limited encapsulation in the presence of these acids.

TABLE 2. Mass spectra intensity of the selected curcumin-loaded MPs after dissolution in chloroform

Batch	Intensity
X1	886087
X2	1282983
X3	598586
X8	594967
X19	1289708
X20	1123155
X21	2051931
X4	1229688

Conversely, X19, X20, and X21 (TCA + H_2O series) demonstrated a progressive increase in ion intensity with increasing TCA concentrations. X21 yielded the highest detected intensity, confirming the enhanced encapsulation efficiency under this condition. Interestingly, X4 presented a high ion intensity, exceeding that of X1 and comparable to X19 and X20. This observation contrasts with the fluorometry data, which suggested lower encapsulation for this formulation. This discrepancy may be attributed to interference from zinc ions in X4, potentially altering curcumin's fluorescent properties and thus affecting the accuracy of fluorometric measurements. This indicates that the influence of using the curcumin-zinc complex on EE should be further investigated, as the results of both experiments are not coherent.

Overall, mass spectrometry largely confirms the trends observed by fluorometry, validating X21 as optimal for curcumin encapsulation. Additionally, it highlights potential limitations of fluorometric quantification in specific cases, particularly for formulations containing metal salts. Each

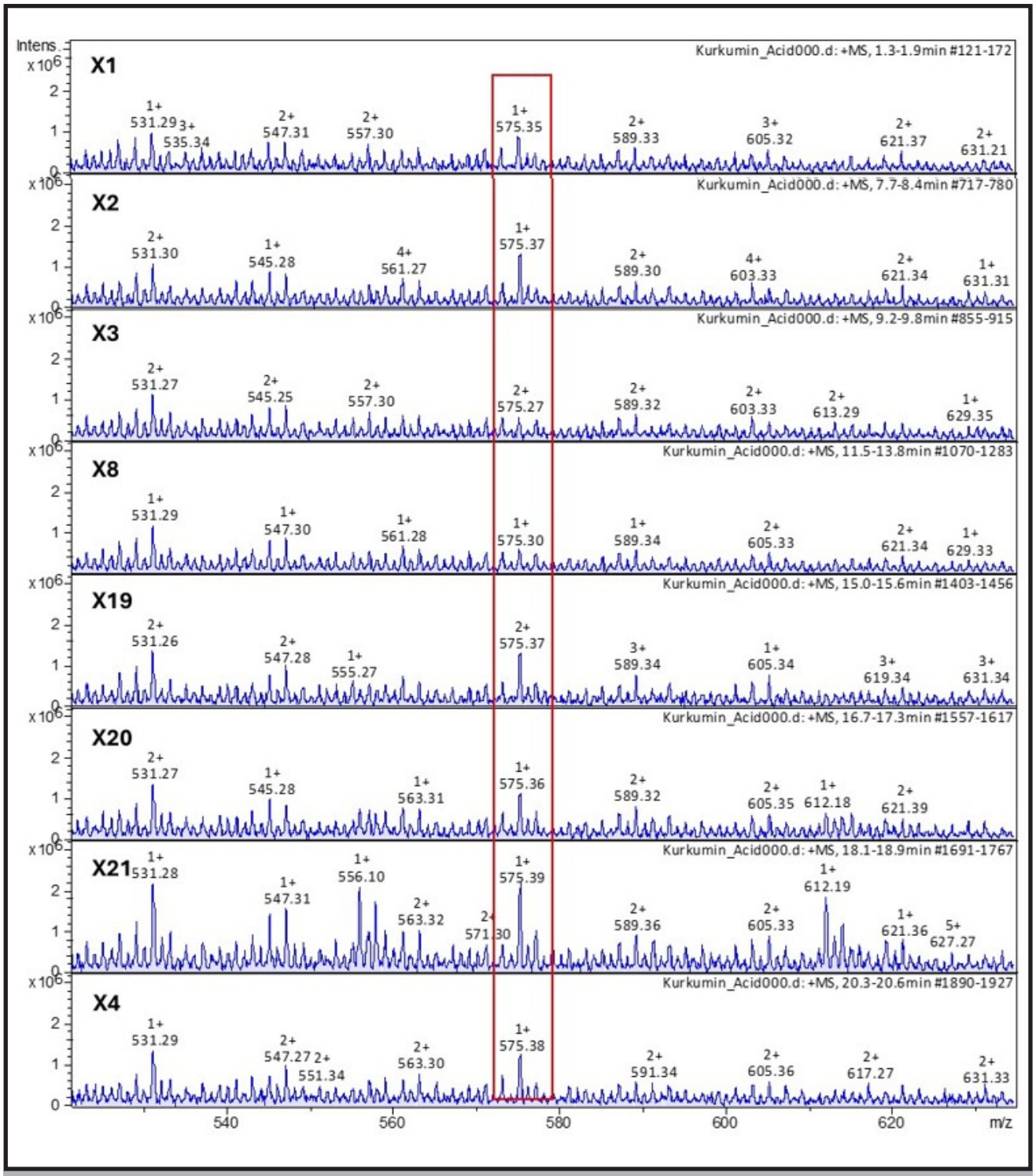

FIG. 2. Mass spectra of the selected curcumin-loaded MPs after dissolution in chloroform. Sodium-cationized curcumin–PSA conjugate peaks is detected at m/z = 575.3

mass spectrum was recorded once per condition as a semi-quantitative confirmation of fluorometric trends; therefore, no standard deviation was calculated.

Alamar Blue and Live/Dead Tests

The viability of L929 fibroblasts exposed to curcumin MPs prepared under various acidic conditions was assessed using the Alamar Blue assay (Figure 3A) and Live/Dead staining (FIG. 3B). The latter assay enabled direct visualization of live cells (green fluorescence) and dead cells (red fluorescence). Overall, a high cell density was observed under all conditions, indicating good initial cell adhesion and growth. However, notable variations in the proportion of dead cells were observed depending on the treatment. Viability of L929 cells cultured without contact with any MPs was used as a control and set as 100 ± 14.3% relative viability.

Unloaded microparticles (X1) slightly reduced cell viability; however, according to ISO 10993 5, viability remained above 70%, so the formulation should be considered non-cytotoxic [6]. Although cell density was generally high, some wells showed lower confluence and a moderate proportion of dead cells.

MPs X2, (HCl) produced under HCl conditions, exhibited overall good cell viability, comparable to X1 (108 ± 17% at 100 µg/mL, 98 ± 4% at 50 µg/mL, 124 ± 22% at 10 µg/mL).

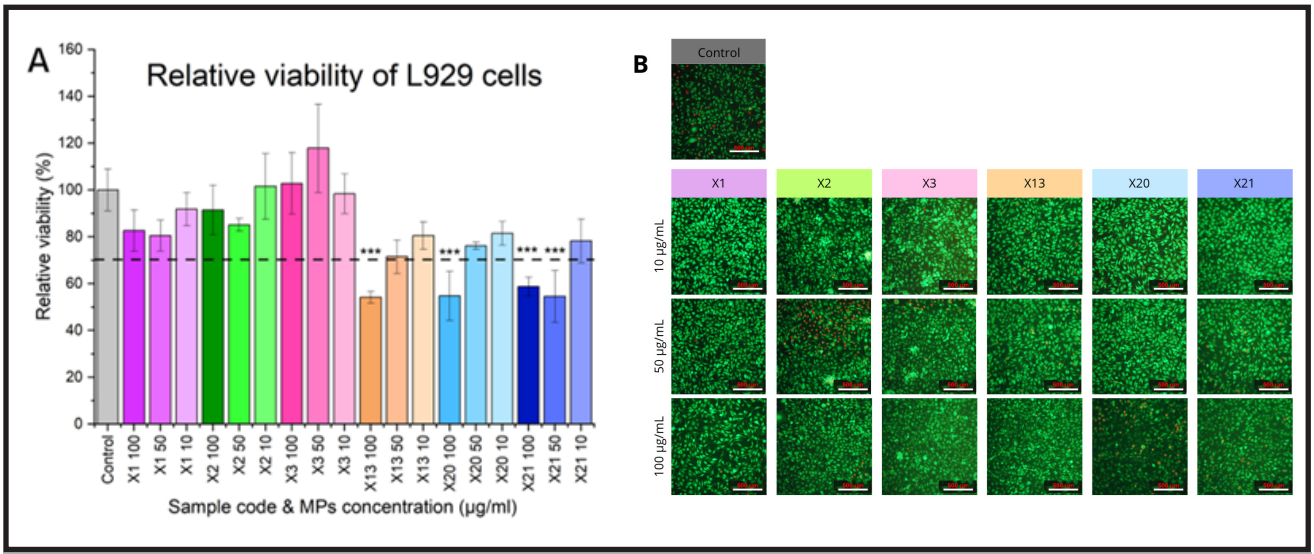

FIG. 3. A) Alamar Blue reduction (%) in L929 fibroblasts in standard conditions (Control) and treated with curcumin-loaded MPs at 10, 50, and 100 µg/mL for 24 h. * significant difference at p < 0.001; B) L929 cell viability by Live/Dead staining after 24 h of culture in standard conditions (Control) and treated with 10, 50, and 100 µg/mL MPs. Scale bar on each picture corresponds to 500 µm**

MPs X3 (H_2SO_4) produced under H_2SO_4 conditions demonstrated excellent cytocompatibility, with high cell density and a very low proportion of dead cells at all concentrations tested (126 ± 21%, 150 ± 30%, 119 ± 13%). Live/Dead staining results were consistent with Alamar Blue results, showing no cytotoxicity and suggesting either a stimulatory effect on the cells or possible interference with the assay. None of these differences was statistically significant.

In contrast, MPs X13, X20, and X21, produced under TCA-containing conditions, induced a marked decrease in cell viability, particularly at 100 µg/mL, where the percentage of viable cells dropped to 48 ± 4% (X21) and 56 ± 6% (X13), i.e., below the 70% threshold indicating cytotoxicity. Treatments with X13, X20, and X21 caused a noticeable increase in cell death, although overall cell density remained high. For X13 and X20, viability was relatively preserved at 10 and 50 µg/mL (X13: 87 ± 15% and 56 ± 6%; X20: 49 ± 17% and 49 ± 18%), but dropped significantly at 100 µg/mL. X21 induced a substantial decrease in cell viability at both 100 µg/mL and 50 µg/mL (48 ± 4% and 76 ± 11%). These findings suggest that trace amounts of TCA may remain in the MPs despite repeated washing, affecting fibroblast viability.

Overall, these data indicate that the cytocompatibility of curcumin MPs strongly depends on the acidic treatment used, with marked cytotoxic effects observed for X13, X20, and X21 (TCA conditions). In contrast, X2 and X3 (HCl, H_2SO_4) preserved high cell viability.

The objective of this study was to optimize curcumin-loaded PSA MPs under acidic conditions, evaluating encapsulation efficiency (EE), drug loading (DL), stability, and cytocompatibility. Our results demonstrate that the nature of the acidic solvent strongly influences curcumin incorporation. Formulations X5–X11 exhibited very low EE and DL, likely due to poor curcumin–polymer interactions or degradation under harsh acidic conditions, rendering them unsuitable for biomedical applications. In contrast, formulations X19–X25, particularly X21, achieved significantly higher EE and DL, highlighting the role of solvent polarity; water likely enhanced curcumin solubility during particle formation, improving retention within the MPs. A very low EE and DL were also measured for X4, indicating that curcumin-zinc complexation does not result in better encapsulation parameters.

These findings are consistent with previous reports on PSA-based MPs, where curcumin loading ranged between 2–3% for conventional preparation methods [5], and more optimized PSA formulations obtained 4.5–11% DL with tests on the supernatants [6]. Compared to PLGA and PLA carriers, which can reach EE up to 50–80% [7,8] but often lower DL, our optimized PSA MPs offer a favourable balance between payload and encapsulation parameters, particularly when acid-catalysed interactions are employed. This suggests that careful selection of acidic catalysts can enhance curcumin–polymer conjugation, as proposed in previous studies on polyanhydrides [5,6].

Mass spectrometry confirmed curcumin incorporation within the MPs (m/z = 575.3), in agreement with fluorometric trends in X19–X21. The anomalous high intensity in X4, not reflected in fluorometry, indicates potential interference from zinc ions, consistent with prior observations that fluorometric assays may be affected by sample composition [3]. This emphasizes the importance of complementary analytical techniques for accurate assessment of encapsulation efficiency and drug loading.

Cytotoxicity assays revealed low toxicity for untreated MPs X1 and those produced in an acidic environment, i.e., X2 and X3. On the other hand, X13 and X20-21 showed higher cytotoxicity at 100 µg/mL, likely due to residual acid, polymer denaturation, or byproducts of fabrication. These observations align with the cytocompatibility profile of PSA MPs [6], which were safe up to 10 µg/mL but exhibited decreased cell viability at higher concentrations. Notably, curcumin incorporation tended to mitigate cytotoxic effects, in line with previous findings where curcumin-loaded MPs reduced cellular stress compared to empty carriers [1,8].

From a physicochemical perspective, the observed differences between the acids can be attributed to their polarity, acidity, and interaction with both curcumin and the polymer matrix. Organic acids such as TFA and TCA possess strong electron-withdrawing groups that increase protonation of curcumin's phenolic hydroxyls [2]. In the case of TFA, the very low polarity of the medium and the high acidity promote curcumin degradation, explaining the poor encapsulation efficiency [1,11]. In contrast, TCA, being partially miscible with water, enhances curcumin solubility during emulsification and can facilitate esterification with PSA anhydride groups,

leading to higher curcumin retention [6,11]. However, its limited volatility allows traces to remain in the microparticles, which negatively affects cytocompatibility [6]. Inorganic acids such as HCl and H_2SO_4 mainly act as transient catalysts in the aqueous phase and are fully removed during washing, resulting in good encapsulation performance combined with high cell viability [6,11].

Overall, our optimized curcumin MPs achieve higher drug loading and satisfactory EE while maintaining acceptable cytocompatibility. Compared to prior PSA systems [5,6] and PLGA/PLA carriers [7–9], they offer a promising approach for biomedical applications, particularly when acid-catalysed interactions are leveraged to enhance curcumin retention. Future work should focus on further improving EE, minimizing cytotoxicity, and exploring co-encapsulation with therapeutic agents for combinatorial treatments [6].

Conclusions

In summary, low-concentration aqueous TCA employed during oil-in-water emulsification markedly improved curcumin encapsulation and loading within PSA MPs, likely via curcumin–polymer conjugation. Formulation X21 (MPs TCA+H_2O 2%) achieved the highest EE and DL values, whereas HCl and H_2SO_4 treated samples (X2, X3) exhibited the best cytocompatibility. However, this enhancement was accompanied by increased cytotoxicity, which was evaluated after 24 hours of cell exposure. As ISO 10993-5 recommends longer exposure times (up to 72 hours), these cytocompatibility results should be considered preliminary. Further optimization of TCA concentration, purification steps, and extended culture assays will be necessary to achieve a favorable balance between drug incorporation and biological safety of PSA microparticles. These findings suggest that appropriately engineered PSA microparticles constitute a viable platform for curcumin delivery in biomedical applications.

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