New Dendrimer-Based Nanoparticles Enhance Curcumin Solubility

Authors Maria Cristina Falconieri[1], Mauro Adamo[2], Claudio Monasterolo[2], Maria Camilla Bergonzi[1], Marcella Coronnello[3], Anna Rita Bilia[1]

Affiliations
[1] Dipartimento di Chimica, Università degli Studi di Firenze, Sesto Fiorentino, Italy
[2] Department of Pharmaceutical and Medicinal Chemistry, Royal College of Surgeons in Ireland, Dublin, Ireland
[3] Dipartimento di Scienze della Salute, Sezione di Farmacologia Clinica e Oncologia, Università degli Studi di Firenze, Firenze, Italy

Key words
- *Curcuma longa*
- Zingiberaceae
- curcumin
- new polyamidoamine dendrimer-based spherical nanoparticles
- solubility

Abstract

Curcumin, the main curcuminoid of the popular Indian spice turmeric, is a potent chemopreventive agent and useful in many different diseases. A major limitation of applicability of curcumin as a health promoting and medicinal agent is its extremely low bioavailability due to efficient first pass metabolism, poor gastrointestinal absorption, rapid elimination, and poor aqueous solubility. In the present study, nanotechnology was selected as a choice approach to enhance the bioavailability of the curcuminis. A new polyamidoamine dendrimer (G0.5) was synthesized, characterized, and tested for cytotoxicity in human breast cancer cells (MCF-7). No cytotoxicity of G0.5 was found in the range between 10^{-3} and 3×10^{-8} M. Consequently, G0.5 was used to prepare spherical nanoparticles of ca. 150 nm, which were loaded with curcumin [molar ratio G0.5/curcumin 1 : 1 (formulation 1) and 1 : 0.5 (formulation 2)]. Remarkably, the occurrence of a single population of nanoparticles having an excellent polydispersity index (< 0.20) was found in both formulations. Formulation 1 was selected to test *in vitro* drug release because it was superior in terms of encapsulation efficiency (62 %) and loading capacity (32 %). The solubility of curcumin was increased ca. 415 and 150 times with respect to the unformulated drug, respectively, for formulation 1 and formulation 2. The release of curcumin from the nanoparticles showed an interesting prolonged and sustained release profile.

Abbreviations

AP-1:	activator protein
BCL 2:	B-cell leukemia protein
BCL2L1:	B-cell leukemia lipoprotein 1
COX:	cyclooxygenase
DLS:	dynamic light scattering
EE%:	encapsulation efficiency
IL:	interleukin
LC%:	loading capacity
LOX:	lipoxygenase
MCF-7:	human breast cancer cells
NP:	nanoparticle
PAMAM:	polyamidoamine
SRB:	sulforhodamine-B
TEM:	transmission electron microscopy

received Dec. 4, 2015
revised January 29, 2016
accepted February 4, 2016

Bibliography
DOI http://dx.doi.org/10.1055/s-0042-103161
Published online
Planta Med © Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943

Correspondence
Prof. Anna Rita Bilia
Department of Chemistry
Building of Pharmaceutical Sciences
University of Florence
Via Ugo Schiff 6
50019, Sesto Fiorentino, Florence
Italy
Phone: + 39 05 54 57 37 08
ar.bilia@unifi.it

Introduction

Curcuma longa L. is a rhizomatous herbaceous perennial plant of the Zingiberaceae family, originating from southeast India. The mainly bioactive constituents from the rhizomes are a mixture of three curcuminoids, principally represented by curcumin (diferuloylmethane), the "yellow coloring matter" discovered about two centuries ago, having a distinctly earthy, slightly bitter, slightly hot peppery flavor and a mustardy smell. In the last 50 years, hundreds of *in vitro* and *in vivo* experiments, from cell cultures to animal researches and clinical trials, indicated that curcumin may have potential as a chemopreventive and therapeutic agent in diseases such as inflammatory bowel disease, pancreatitis, arthritis [1], chronic anterior uveitis [2], and neurological disorders such as Alzheimer's disease [3]. Curcumin represents a very interesting natural constituent because it can modulate several molecular targets and inhibit transcription factors (NF-kB, AP-1), enzymes (COX-1, COX-2, LOX), cytokines (TNF, IL-1, IL-6), and antiapoptotic genes (BCL2, BCL2L1) [4]. Nevertheless, the manifold advantages of curcumin are counterbalanced by a pharmacokinetic pitfall, which is the major limitation of applicability of curcumin as a health promoting and medic-

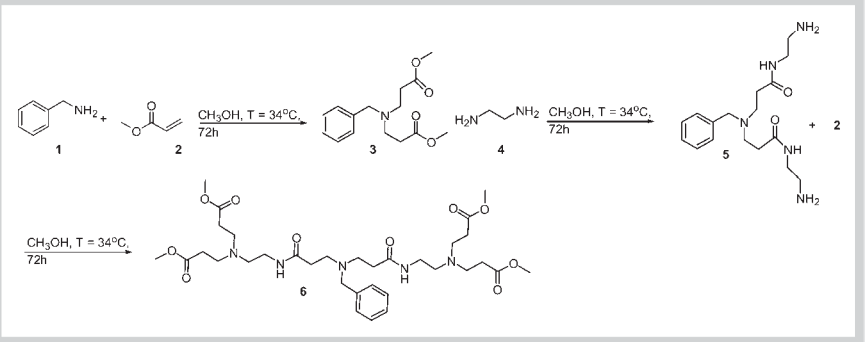

Fig. 1 Synthesis of the new G0.5 dendrimer (**6**).

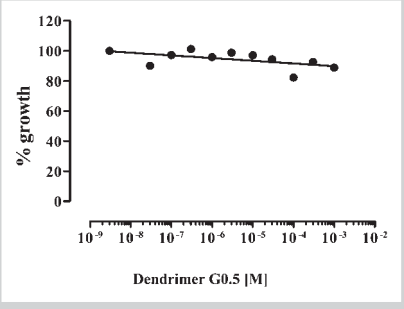

Fig. 2 Dendrimer G0.5 cytotoxicity on MCF-7 cells.

inal agent. The extremely low bioavailability is attributed to a scarce poor gastrointestinal absorption, an extensive first-pass metabolism, a rapid elimination, due to hepatic glucuronidation and sulfation of the compound, and to the poor aqueous solubility and stability [5]. The biomedical potential of curcumin could be enhanced by increasing the doses. In fact, curcumin does not show any dose-limiting toxicity when it is administered at doses of up to 8 g/day for three months, but the best approach exploited in the last years to increase its bioavailability is based on the strategy of the nanoparticulate drug delivery systems. Our research group has already developed several types of nanoparticles, such as phospholipid vesicles (liposomes) [6], micelles [7, 8], lipid nanovectors [9–11], and cyclodextrins complexes [12].

The present work focused on the development of nanoparticles to efficiently deliver curcumin for therapeutic use by enhancing its solubility and stability. The nanoparticles used in this study were based on the design of a dendrimeric vehicle. Dendrimers are core-shell nanostructures with precise architecture and low polydispersity, which are synthesized in a layer-by-layer fashion (expressed in "generation") around a core unit, resulting in a high level of control over size, branching points, and surface functionality. Three different parts are recognized in dendrimers, namely: (i) an initiator core, (ii) branches, and (iii) the terminal functional groups [13]. Therefore, dendrimers, possessing a modular structure, could be designed and then fine-tuned to either encapsulate or conjugate the desired drug. The design always follows an analysis of the drug molecular properties, which must be complementary to the dendrimer. To the best of our knowledge, the literature reports only non-covalent adducts of curcumin with high molecular commercial or pegylated-PAMAM dendrimers [14–17]. Considering the nature of the functional groups present, the interaction between the abovementioned dendrimers and curcumin occurred presumably via hydrophobic interactions, hydrogen bonds, and van der Waals interactions. On the bases of these reports, we posed the question of using a spherical and stable nanoparticle based on small dendrimers to deliver curcumin and we directed our attention to the preparation of dendrimer G0.5. It was hypothesized that spherical objects that could be engineered in size would offer the opportunity of delivering a quantum of curcumin as a function of the dendrimer size. In cascade, the dendrimer size could be controlled via the number of iterative polymerization steps and, in the end, by the number of branches.

Results and Discussion

The synthetic layout to provide a new dendrimer G0.5 (**6**) is reported below (**Fig. 1**). Hence, the initiator unit benzylamine **1** was reacted with methylacrilate **2** to generate two branches via an aza-Michael reaction. Resulting compound **3** was then reacted with ethylendiamine **4** to provide primary diamine **5**. This was further reacted with excess acrylate **2** in a consecutive aza-Michael reaction to give dendrimer **6**, as reported in **Fig. 1**.

The cytotoxicity of G0.5 dendrimer was evaluated *in vitro* on human breast cancer cells, MCF-7, at concentrations in the range between 10^{-3} and 3×10^{-8} M. This study showed that G0.5 held no cytotoxicity (**Fig. 2**) at the tested concentrations, reaching a maximum cytotoxicity of 10% at the highest concentration evaluated (10^{-3} M). The low cytotoxicity of the G0.5 is probably due to the presence of neutral methoxy ester moieties in place of amines, as in PAMAM. It is well known that PAMAM dendrimers, possessing a variable number of cationic residues, may exert cell toxicity by acting at the cell membrane level [18].

G0.5, a pale yellow oil, was dissolved in EtOH (1 : 9 v/v) and diluted with Millipore water (1 : 50) to obtain an aggregate, which was lyophilized and characterized by DLS and TEM. DLS analysis showed that G0.5 formed aggregates as NPs. A prevalent population (69.9 ± 2.07%) was present with a size of 176.7 ± 7.27 nm. The polydispersity index was 0.33 ± 0.06, a value indicating good homogeneity of the sample. The presence of aggregates of G0.5 in the form of NPs was confirmed by TEM analysis, which showed good homogeneity of the sample. The size of the G0.5 NPs evaluated by TEM was ca. 100 nm with a regular globular shape (**Fig. 3**). NPs dimension measured by TEM analysis was lower than that obtained by DLS, an effect we have explained considering the absence of water molecules in the samples analyzed by TEM. G0.5, containing several amides, could form hydrogen bonds with water, which increased the size of the aggregates. TEM analysis confirmed the presence of the basic G0.5 monomer in the NPs by imaging small structures of 5–7 nm (**Fig. 4**), which were compatible with the dimension of G0.5 in the monomeric form.

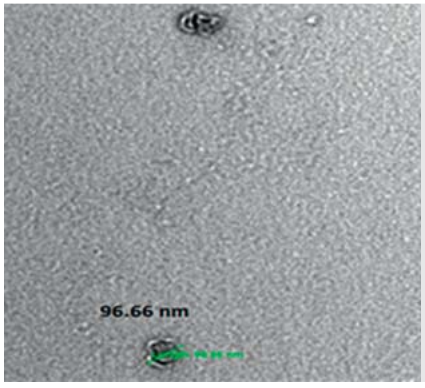

Fig. 3 TEM micrograph of NPs based on the new G0.5 dendrimer (Color figure available online only).

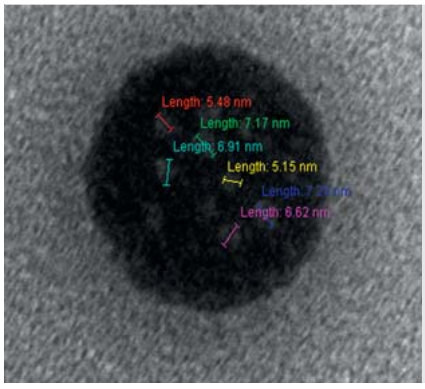

Fig. 4 Detailed TEM micrograph of a single NP based on the new G0.5 dendrimer (Color figure available online only).

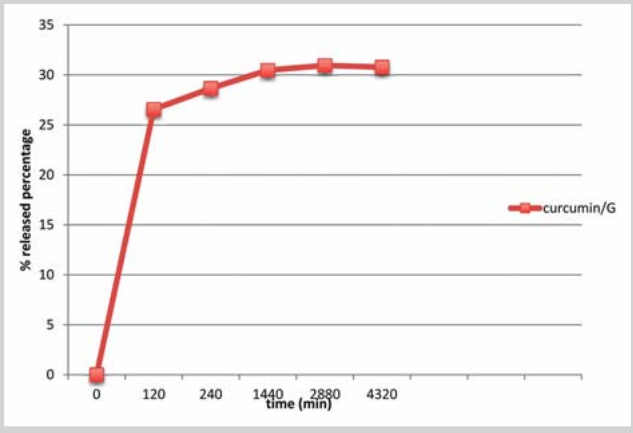

Fig. 5 *In vitro* release profile of curcumin from G0.5 NPs (Color figure available online only).

As a follow-up of this investigation, two formulations based on G0.5 NPs were prepared. In the first one (formulation 1), 37 mg of G0.5 were dissolved in 10 mL of EtOH and 20 mg (molar ratio 1 : 1) of curcumin were added to the resulting solution. In the second one (formulation 2), only 10 mg (molar ratio 1 : 0.5) of curcumin were added. The two solutions were evaporated and the residues treated with 10 mL of Millipore water to provide a precipitate of unloaded curcumin and a supernatant constituted of G0.5 NPs loaded with curcumin. A validated HPLC method was used to evaluate the content of unloaded curcumin. In addition, NMR analysis confirmed the absence of dendrimer G0.5 in the precipitate. EE% and LC% were obtained by an indirect method, subtracting the total curcumin used (*Total Drug*), namely 20 and 10 mg, while the portion of free curcumin (*Free Drug*) was obtained by calculating the curcumin present in the precipitate after centrifugation, according to the equations reported in the experimental part. The concentration of curcumin obtained in the precipitate (*Free Drug*) was determined through HPLC-DAD, using the external standard method. EE% and LC% of the two formulations are reported in **Table 1**. DLS analysis of G0.5 NPs loaded with curcumin showed similar dimensions of the unloaded NPs, with a mean diameter of ca. 150 nm. Remarkably, the occurrence of a unique population of NPs (100%) having an excellent polydispersity index (< 0.20) was found in both formulations. The solubility of curcumin in water at room temperature was determined to be ca. 3×10^{-3} µg/ml and taking into account the EE% of curcumin in formulations 1 (ca. 62%) and 2 (ca. 44%), the solubility of

Table 1 EE% and LC% of the two NPs G/curcumin formulations.

Molar ratio Curcumin/G0.5	EE%	LC%
Formulation 1 (1 : 1)	61.8 ± 0.3	32.1 ± 0.2
Formulation 2 (0.5 : 1)	43.9 ± 0.2	15.1 ± 0.2

curcumin was, respectively, 1.24 mg/ml and 0.44 mg/ml in the respective formulations. These values are in agreement with the curcumin solubility values increase of ca. 415 and 150 times with respect to the unformulated drug, respectively, for formulation 1 and formulation 2. Accordingly, formulation 1 was selected for further analyses because it was superior in terms of EE% and LC %, as reported in **Table 1**.

Formulation 1 was further evaluated for the *in vitro* release of curcumin. Hence, curcumin released from the G0.5 NPs was determined by HPLC during 72 h. To calculate the % of curcumin released in the medium at each time point, the following formula was used:

$$\%\ \text{curcumin released}_t = \frac{\text{mg curcumin}_t}{\text{mg curcumin}_{tot}} \times 100 \quad (1)$$

Where

- mg curcumin$_t$ = mg curcumin released for each time point t
- mg curcumin$_{tot}$ = total mg of curcumin loaded in NPs

Cumulative curcumin release versus time is plotted in **Fig. 5**. The curcumin maximum percentage released was 30.95 ± 0.001%, equivalent to 4.05 µg/ml. The curve trend is in agreement with a prolonged and sustained release of curcumin from the NPs.

A new G0.5 dendrimer related to PAMAM has been successfully synthetized. No cytotoxicity properties were evidenced for G0.5, which is an advantage over related PAMAM dendrimers, which hold, conversely, variable levels of toxicity. It was shown that G0.5 spontaneously formed NPs, which were fully characterized in terms of average diameter and polydispersity by DLS, while the morphological analysis was performed by TEM. Curcumin, a natural molecule with anti-inflammatory, antioxidant, and anticancer activities, was loaded in the new G0.5 NPs and it was shown that inclusion of the drug did not modify the structure of the formulation, giving excellent values in terms of average diameter and polydispersity. EE% and LC% were very satisfactory, in particular, for formulation 1 (molecular ratio curcumin/G0.5 was 1 : 1) with 62% EE and 32% LC. A remarkable increase of curcumin solubility (ca. 450 times more than the unformulated drug) was

found for formulation 1. Finally, the release profile of curcumin from this formulation showed an interesting prolonged and sustained release profile of curcumin, with the possible application as a drug delivery system.

Materials and Methods

Reagents and standard solutions

Benzylamine and ReagentPlus® 99% were from Sigma-Aldrich; Buffer saline (PBS) was from Euroclone Company; Cell line MCF-7 were from American Type Culture Collection. Culture medium DMEM, Gibco®, was purchased from European Division; Curcuma e.s 95% curcumin, PM = 368.39, was from Sigma-Aldrich; Dimethyl sulfoxide (DMSO, 99.9%) and bovine serum albumin were from Sigma-Aldrich. Ethylenediamine ≥ 99.5% by GC was from Sigma-Aldrich; FBS and glutamine were from the Euroclone Company; Isoton solution for the electronic cell counter was a Beckman-Coulter sample. Methyl acrylate 99%, containing ≤ 100 ppm monomethyl ether hydroquinone as an inhibitor, was from Sigma-Aldrich. Phosphotungistic acid and copper grid of 200 mesh coated with a carbon film was from Società Italiana Chimici. Potassium carbonate, ≥ 99.0%; Potassium permanganate, ACS reagent, ≥ 99.0%; Silica gel, Merk F-254; Single-use material for cell cultures were from Costar and Sarstedt. SRB and trichloroacetic acid were from Sigma Chemical Co. TRIS (hidroxymethylaminomethane) was from Merck.

Synthesis of dendrimer 6

The preparation of dendrimer **6** was carried out using a method reported in the literature [19] and modified according to our needs. In particular, product **3** (**Fig. 1**) was prepared starting from a solution of benzylamine **1** (0.88 mL; 8 mmol) in methanol (20 mL) to which methylacrylate **2** (2.6 mL; 28.8 mmol) was added in a dropwise fashion. The resulting reaction mixture was stirred under argon for 72 h at 34 °C. After this time, the solvent was removed under reduced pressure and the residue obtained confirmed to be pure **3** via ^{1}H NMR and was used in the following step without further purification.

Compound **5** (**Fig. 1**) was prepared starting from a solution of **3** (2.235 g; 8 mmol) in methanol (4 mL) to which ethylendiamine **4** (9.95 mL; 148.8 mmol) was added. The reaction mixture was then stirred for 72 h at 34 °C. The solvent was removed under reduced pressure and the residue obtained was confirmed to be the desired **5** by ^{1}H NMR and was used in the following step without further purification.

Compound **6** (**Fig. 1**) was prepared following the same procedure reported for the synthesis of product **3**. Hence, to a solution of **5** (459 mg; 1.37 mmol) in methanol (3.4 mL), methylacrylate **2** (1.24 mL; 13.68 mmol) was added dropwise. The reaction mixture was maintained at 34 °C for 72 h. After this time, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel 60 (Merck 70–230 mesh) using EtOAc/MeOH at different gradients, starting from (10/0.8), (10/1), (10/1.2). Pure **6**, so obtained, was characterized by ^{1}H NMR. All TLC (Merck F-254) were visualized via UV fluorescence.

Characterization of compounds 3, 5, and 6

Compound **3:** Weight: 2.235 g, yield: 99%, yellow oil; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.26 (m, 5 H), 3.64 (s, 6 H), 3.58 (s, 2 H), 2.79 (t, 4 H, J = 6.8), 2.47 (t, 4 H, J = 7.2).

Compound **5**: Weight: 2.341 g, yield: 99%, yellow oil; ^{1}H-NMR (400 MHz, CH_3OD) δ 7.25 (m, 5 H), 3.60 (s, 2 H), 3.22 (t, 4 H, J = 6.4), 2.76 (t, 4 H, J = 6.8), 2.70 (t, 4 H, J = 6.4), 2.40 (t, 4 H, J = 6.4).

Compound **6**: Weight: 0.8461 g, yield: 91%, yellow oil; TLC: AcOEt/MeOH (10/0.8) R_f = 0.4; AcOEt/MeOH (10/1) R_f = 0.2; AcOEt/MeOH (10/1.2) R_f = 0.1; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.21 (m, 5 H), 3.58 (s, 2 H), 3.56 (s, 12 H), 3.20 (m, 4 H), 2.74 (t, 4 H, J = 6.4), 2.68 (t, 8 H, J = 6.8), 2.44 (t, 4 H, J = 6), 2.34 (m, 12 H).

Cytotoxicity evaluation by the sulforhodamine-B assay

Cytotoxicity of dendrimer G0.5 was evaluated 72 h after exposure of the cells to various concentrations in a range between 10^{-3} and 3×10^{-8} M. The MCF-7 cells, in the exponential growth phase, were diluted in complete DMEM medium to obtain a density of 5000 cells/100 µL and distributed in plates of 96 wells. Subsequently, the cells were incubated for 24 h at 37 °C in a humidified atmosphere at 5% CO_2 to allow adhesion before exposure. All experiments were performed in triplicate. Inhibition of cell growth was assayed by sulforhodamine-B (SRB), which is capable of binding cellular proteins and developing a color directly proportional to cell viability [20].

Briefly, MCF-7cells were fixed with 10% trichloroacetic acid and incubated for 1 h at +4 °C; then the microplates were washed with tap water 5–6 times and air-dried. The fixed cells were stained with 0.4% SRB solution dissolved in 1% acetic acid and kept at room temperature for 30′. Unbound SRB was removed by washing with 1% acetic acid solution and the dye bound to the proteins was extracted with 10 mM unbuffered TRIS base in a volume of 150 µl/well at room temperature under stirring. Optical density was read in an automatic 96-well microplate reader interfaced with the software Microplate Manager/PC version 4.0 (Bio-Rad Laboratories) for automatized analysis of the results at 540 nm to maximize the value of SRB absorption. The results were processed using the program GraphPad 5 and were compared with those obtained from blank wells that represented the untreated control.

Morphological analysis of G0.5 nanoparticles by transmission electron microscopy

NP dispersions were analyzed in terms of morphology and mean diameter by TEM (Jeol Jem 1010). Ten µL of NP dispersion diluted 10-times was applied to a carbon film-covered copper grid. Most of the dispersion was blotted from the grid with filter paper to form a thin film specimen, which was stained with a phosphotungstic acid solution 1% w/v in sterile water. The samples were dried for 3 min and then were examined under a JEOL 1010 electron microscope and photographed at an accelerating voltage of 64 kV.

Characterization of the G0.5 NPs was done in terms of particle size, polydispersity index, and ζ-potential. The particle size of the developed NPs was measured by a DLS, Zetasizer Nano series ZS90 (Malvern Instruments) equipped with a JDS Uniphase 22 mW He-Ne laser operating at 632.8 nm, an optical fiber-based detector, a digital LV/LSE-5003 correlator, and a temperature controller (Julabo water-bath) set at 25 °C. Time correlation functions were analyzed to obtain the hydrodynamic diameter of the particles (Zh) and the particle size distribution (polydispersity index, PD) using ALV-60X0 software V.3.X provided by Malvern. Autocorrelation functions were analyzed by distribution, fitting a multiple exponential to the correlation function to obtain particle size distributions. In particular, polydispersity values were calculated for each peak as peak width/mean diameter. Scatter-

Table 2 HPLC method for curcumin quantification.

Time (min)	Solvent A (%)	Solvent B (%)
0.10	18.0	82.0
10.00	44.0	56.0
13.00	44.0	56.0
27.00	48.0	52.0
32.00	80.0	20.0
35.00	18.0	82.0

ing was measured in an optical quality 4 mL borosilicate cell at a 90° angle, diluting the samples 30-folds in PBS. ζ-potentials of the NPs were measured using a Malvern Instruments Zetasizer Nano series ZS90. For all samples, an average of three measurements at the stationary level was taken. The temperature was kept constant at 25 °C by a Haake temperature controller. The ζ-potential was calculated from the electrophoretic mobility, μE, using the Henry correction to Smoluchowski's equation.

Curcumin loaded G0.5 nanoparticles

Two different molar ratios were used to prepare formulation 1 and formulation 2, namely a molar ratio of 1 : 1 (20 mg; 0.054 mmol of curcumin plus 37 mg; 0.054 mmol of G0.5) and a molar ratio of 0.5 : 1 (10 mg; 0.027 mmol of curcumin plus 37 mg; 0.054 mmol of G0.5) dissolved in ethanol (10 mL). The solution was stirred for 24 h and then evaporated under vacuum in order to completely remove the solvent. Millipore water (10 mL, pH 6.52) was added and the mixture was stirred for 24 h in the dark. The separation of G0.5 NPs loaded with curcumin from unloaded curcumin was performed by centrifugation at 4000 rpm, at 4 °C for 30 min. The precipitate was represented by curcumin, while the complex was dispersed in the aqueous medium. The two precipitates were analyzed by ^{1}H NMR to assess the lack of a dendrimer; only curcumin was identified. ^{1}H-NMR (400 MHz, d_6-DMSO) δ 7.57 (d, 2 H, J = 7.6), 7.52 (s, 1 H), 7.32 (s, 2 H), 7.14 (d, 2 H, J = 8); 6.81 (d, 3 H, J = 8.4), 6.77 (s, 1 H), 6.73 (s, 1 H), 3.83 (s, 6 H).

High-performance liquid chromatography analysis for curcumin quantification

Curcumin was assayed by HPLC/DAD analysis performed using an HP 1100 liquid chromatograph (Agilent Technologies) equipped with an HP 1040 diode array detector (DAD), an automatic injector, an autosampler, and a column oven, and was managed by an HP 9000 workstation (Agilent Technologies). The UV-Vis spectra were recorded between 220–500 nm and the chromatographic profiles were registered at 420 nm. Separations were performed on a reversed-phase Luna C18 column (150 × 2 mm, 3 μm, Phenomenex) maintained at 25 °C with an injection volume of 20 μL. The eluents were H_2O at pH 3.2 by formic acid (Solvent A) and acetonitrile (Solvent B) at flux 0.2 ml/min. The multistep linear gradient applied is described on **Table 2**.

Calibration curve

A calibration curve was built with an exact quantity of curcumin and solubilized in methanol HPLC ranging from 0.2016 μg/μL to 2.016 μg/μL. The linearity of the calibration curve is expressed by R^2 that is 0.9935, as reported in **Fig. 6**.

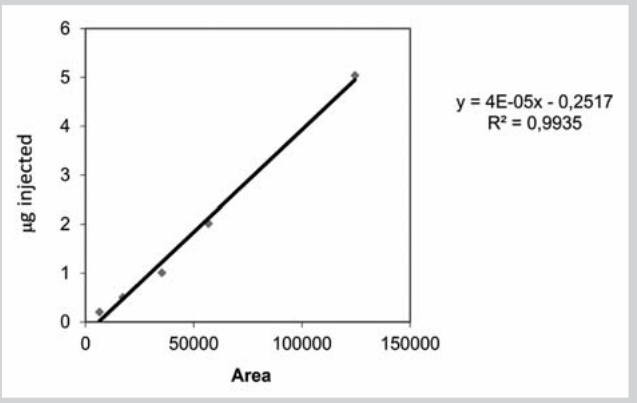

Fig. 6 Calibration curve of curcumin.

Encapsulation efficacy and loading capacity

EE% and LC% of G0.5 NPs were determined by an indirect method, by detracting the amount of free curcumin (*Free drug*) from the total amount of curcumin used in the preparation of NPs (*Total Drug*) to quantify the supernatant after purification of the NPs.

$$EE\% = \frac{(\text{Total Drug} - \text{Free Drug})}{\text{Total Drug}} \times 100 \quad (2)$$

$$LC\% = \frac{(\text{Total Drug} - \text{Free Drug})}{\text{Weight of G0.5}} \times 100 \quad (3)$$

In vitro release test

A dialysis bag method was performed to study the drug release using a mixture of distilled water and EtOH (20 : 80 v/v) as a dissolution medium. Release was monitored for 72 h. The dialysis bags were hydrated in distilled water before use. Formulation 1 (12.44 mg) was put into the dialysis bag. The bag was placed in a beaker containing 200 mL of dissolution medium, maintained at 37 °C, and stirred at a rate of 100 rpm. Aliquots of the dissolution medium were withdrawn at different time intervals (2 h, 4 h, 24 h, 48 h, 72 h) and were replaced with the same volume of fresh medium to maintain the sink conditions. The samples were suitably diluted and analyzed by HPLC. All of the operations were carried out in duplicate along with the controls.

Acknowledgements

This work was supported in part by the Università degli Studi di Firenze.

Conflict of Interest

The authors declare no conflict of interest.

References

1 *Ammon HP, Wahl MA.* Pharmacology of *Curcuma longa*. Planta Med 1991; 57: 1–7
2 *Pescosolido N, Giannotti R, Plateroti AM, Pascarella A, Nebbioso M.* Curcumin: therapeutical potential in ophthalmology. Planta Med 2014; 80: 249–254

3 *Kim MH, Kim SH, Yang WM.* Mechanisms of action of phytochemicals from medicinal herbs in the treatment of Alzheimer's disease. Planta Med 2014; 80: 1249–1258
4 *Sandur SK, Pandey MK, Sung B, Ahn KS, Murakami A, Sethi G, Limtrakul P, Badmaev V, Aggarwal BB.* Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and tumerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-indipendent mechanism. Carcinogenesis 2007; 28: 1765–1773
5 *Sharma RA, Steward WP, Gescher AJ.* Pharmacokinetics and pharmacodynamics of curcumin. Adv Exp Med Biol 2007; 595: 453–470
6 *Isacchi B, Bergonzi MC, Grazioso M, Righeschi C, Pietretti A, Severini C, Bilia AR.* Artemisinin and artemisinin plus curcumin liposomal formulations: enhanced antimalarial efficacy against *Plasmodium berghei*-infected mice. Eur J Pharm Biopharm 2012; 80: 528–534
7 *Lapenna S, Bilia AR, Morris GA, Nilsson M.* Novel artemisinin and curcumin micellar formulations: drug solubility studies by NMR spectroscopy. J Pharm Sci 2009; 98: 3666–3675
8 *Tahmasebi Mirgani M, Isacchi B, Sadeghizadeh M, Marra F, Bilia AR, Mowla SJ, Najafi F, Babaei E.* Dendrosomal curcumin nanoformulation downregulates pluripotency genes via miR-145 activation in U87MG glioblastoma cells. Int J Nanomedicine 2014; 9: 403–417
9 *Bergonzi MC, Hamdouch R, Mazzacuva F, Isacchi B, Bilia AR.* Optimization, characterization and *in vitro* evaluation of curcumin microemulsions. LWT-Food Sci Technol 2014; 59: 148–155
10 *Righeschi C, Bergonzi MC, Isacchi B, Bilia AR.* Solid lipid nanoparticles for oral delivery of curcumin. Planta Med 2013; 79: PN14
11 *Bergonzi MC, Righeschi C, Guccione C, Bilia AR.* Lipid nanovectors for oral delivery of curcumin and evaluation of their performance through PAMPA systems. Planta Med 2014; 80: P2N4
12 *Mazzacuva F, Guidelli G, Bergonzi MC, Bilia AR.* Enhanced water solubility and stability of curcumin by microinclusion in natural and semisynthetic cyclodextrins. Planta Med 2011; 77: PK9
13 *Kolhe P, Khandare J, Pillai O, Kannan S, Lieh-Lai M, Kannan RM.* Preparation, cellular transport and activity of polyamidoamine-based dendritic nanodevices with a high drug payload. Biomaterials 2006; 27: 660–669
14 *Mollazade M, Nejati-Koshki K, Akbarzadeh A, Zarghami N, Nasiri M, Jahanban-Esfahlan R, Alibakhshi A.* PAMAM dendrimers augment inhibitory effects of curcumin on cancer cell proliferation: possible inhibition of telomerase. Asian Pac J Cancer Prev 2013; 14: 6925–6928
15 *Cao J, Zhang H, Wang Y, Yang J, Jiang F.* Investigation on the interaction behavior between curcumin and PAMAM dendrimer by spectral and docking studies. Spectrochim Acta A Mol Biomol Spectrosc 2013; 108: 251–255
16 *Abderrezak A, Bourassa P, Mandeville JS, Sedaghat-Herati R, Tajmir-Riahi HA.* Dendrimers bind antioxidant polyphenols and cisplatin drug. PLoS One 2012; 7: e33102
17 *Shi W, Dolai S, Rizk S, Hussain A, Tariq H, Averick S, L'Amoreaux W, El Idrissi A, Banerjee P, Raja K.* Synthesis of monofunctional curcumin derivatives, clicked curcumin dimer, and a PAMAM dendrimer curcumin conjugate for therapeutic applications. Org Lett 2007; 9: 5461–5464
18 *Fant K, Esbjörner EK, Jenkins A, Grossel MC, Lincoln P, Nordén B.* Effects of PEGylation and acetylation of PAMAM dendrimers on DNA binding, cytotoxicity and *in vitro* transfection efficiency. Mol Pharm 2010; 7: 1734–1746
19 *Tianzhu Y, Liu X, Bolcato-Bellemin A, Wang Y, Liu C, Erbacher P, Qu F, Rocchi P, Behr J, Peng L.* An amphiphilic dendrimer for effective delivery of small interfering RNA and gene silencing *in vitro* and *in vivo*. Angew Chem Int Ed Engl 2012; 5: 8478–8484
20 *Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR.* New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 1990; 82: 1107–1112