

Original Research Article

Connection of poly (Propylene imine) dendrimer to curcumin and investigation into anti-cancer effects of its products

Keyhan Mohammadpour^a, Sabah Salahvarzi^{a,*}, Zeynab Dadgar^b

^a Department of Chemistry, Faculty of Science, Khorramabad Branch, Islamic Azad University, Khorramabad, Iran

^b Department of biology, Faculty of Science, Arak University, Arak, Iran

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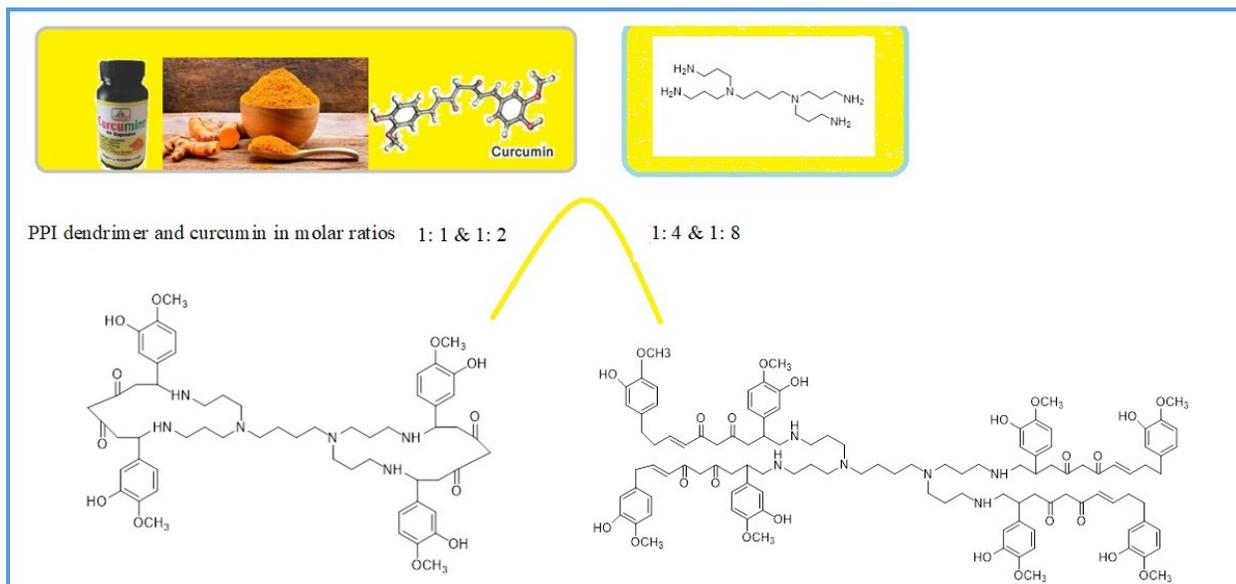
Cancer cell

ABSTRACT

The dendrimers are the macromolecules that branch out from the same nuclear and they finally reach the same central nucleus. The solubility of the dendrimer is strongly affected by the surface groups. These compounds are the ideal carriers for biomedical applications. One of the oldest identified dendrimer is poly (propylene imine) dendrimer (denoted as PPI dendrimer). The present study consists of two sections. In the first section, the interaction between the (PPI) dendrimer with different molar ratios of curcumin was investigated at the presence or absence of the ultrasonic waves. In the second section, the anti-cancer effects of this compound on BRC-9 cell line (of breast cancer cells) were investigated with the help of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay.

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Graphical Abstract



Introduction

There is no doubt that nano-based therapy is one of the main fields addressed in nanotechnology studies in order to cure some diseases such as cancer or atherosclerosis. In spite of the recent advances in medicine, cancer is still considered one of the main reasons for early mortality along with the cardiovascular diseases. Therefore, the development and progress in modern treatment of cancer have changed into a priority for the researchers [1]. Many studies have been conducted using a wide range of nanotechnology to cure numerous cancers including breast cancer [2, 3]. A large number of researches have been conducted using the principles of nanotechnology for drug and therapeutic delivery [4]. Because of distinct structure and some specific features, these systems have concentrated greatly attention for their application in several fields. However, concerning the treatment of the major cancers, the final goal is to increase the drug absorption and permeability as well as the site-control feature and drug diffusion [5]. Nano-carriers

can specifically penetrate the cancerous tissues through inactive and/or active transmission system to this end, different structures have been developed for the drug releasing systems [6]. Dendrimers are a class of spherical polymers having the structural control of the competition among the traditional molecules. They were introduced in the mid-1980s, and they are now known as kinetic proteins [7]. Dendrimers are the large nano macromolecules that have some highly flexible points. They have also some three-dimensional and steady dimensions (1 nm to 100 nm). Also, they consist of a central nucleus with some surface groups and the units derived like the tree branches [8–11]. Several varieties of dendrimers are poly (propylene imine) dendrimer that are available commercially, and they were first synthesized and introduced by Voegtle [11, 12]. Dendrimer poly (propylene imine) is applied to define dendrimers that include the central nucleus of diaminobutane of tertiary propylene amine part and the amine terminus [13]. In recent years, the researchers have used the dendritic structures especially dendrimer in medical

sciences, pharmacy, biology of pharmaceutical systems, growth of cellula praecursoria, and treatment of different types of tumors, improvement in medical disorders, detection of the cancer cells, antiviral and antibacterial applications [14–19]. The curcumin is one of the polyphenolic compounds and it is the main active turmeric factor involved in the diet lacking water solution. The curcumin has a wide range of applications like anti-inflammatory, antioxidant, anti-diabetic, anti-microbial, anti-cancer effects and also the treatment of many liver diseases, dementia, heart diseases, hypoglycemia, digestive disorders, and osteoporosis [20]. The curcumin is an efficient molecule in the phenomena of multidrug resistance and also in developing tumor cells sensitivity for chemotherapy and radiotherapy. The clinical development of curcumin as a drug depends on the creation of a nanocarrier for efficient body transmission. In addition, the curcumin non-formulation improves the cell absorption in the cancer models which increases the probability of a positive therapeutic result [21]. A wide variety of research has been performed in the field of curcumin derivatization and confirming anticancer effects of this herbal material. Nabati synthesized silylated derivatives of curcumin and suggested that bulky silylated substituents result in more stable compounds [22]. Basil *et al.* [23] synthesized two curcumin derivatives containing bis-dimethoxy curcumin (bDMC) and diacetyl curcumin (DAC) to improve the activity and stability of curcumin representing proper stability of derivatives relative to pure curcumin. Ding *et al.* [24] synthesized three groups of curcumin derivatives including phosphorylated, etherified, and esterified products to examine their anti-tumor activity against breast cancer (MCF-7), bone cancer cells (Hep-G2), cervical cancer cells (HeLa). Lal *et al.*

[25] evaluated one of curcumin derivatives namely 3, 4-dihydropyrimidinone/thione.

Experimental

Materials and methods

Curcumin and methanol were purchased from the Merck, Germany. DMEM and trypsin culture media were provided by Cytomethin Gene, BRC-9 cell from the Pasteur Institute of Iran, Gibco bovine serum, and Kit MTT from the Sigma Aldrich USA. The BRUKER 400 (MHz) Nuclear Magnetic Resonance (NMR) device was used to identify the structure of the products, the number and types of carbon and hydrogen. PerkinElmer RX I model by KBr tablet was used to identify the product functional groups. The purity of the products and the reaction progress were measured using the thin-layer chromatography (TLC) and UV spectrophotometer. To carry out this research, the BRC-9 breast cancer cell line was purchased from the Pasteur Institute, Iran. Cells were cultured in RPMI (Roswell Park Memorial Institute) medium (USA, Gibco) containing 10% Fetal bovine serum (FBS) (USA, ATCC), 2 mM L-glutamine solution and penicillin-streptomycin (Gibco, Germany). The cells were plated in 25 cm² flasks and incubated at 37 °C with atmosphere of 5% CO₂. Two days after culture initiation, the first medium replacement was performed and then medium was changed two times per week till the bottom of the flask was covered with the cells (till confluency). The cells were trypsinized (trypsin-EDTA, Gibco, Germany) and passed to another culture flask as the first passage and then the cultures were expanded through two additional subcultures which were used for further investigation.

General

In the present study, different products were synthesized through changing the molar ratio of the curcumin with PPI dendrimer. Also, a comparison was made between the change in the conditions of reactions at the presence and absence of the ultrasonic waves.

Curcumin-dendrimer interaction

The poly (propylene imine) dendrimer was added to the solution after the complete dissolution of curcumin in methanol whenever

a certain amount of curcumin was dissolved in the least amount of methanol. Then, it underwent the conditions of the methods 1 and 2 to be converted to the intended product (Table 1 and 2). Then, it was dried for 3 days at room temperature. Thin-layer chromatography was used to determine the purity of the products (Hexane: Acetone, 40:60). The final structure of the product was formed with the help of FT-IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ spectra (Table 3).

Table 1. Curcumin-dendrimer interaction (the molar ratio of curcumin with PPI dendrimer 1:1 and 1:2)

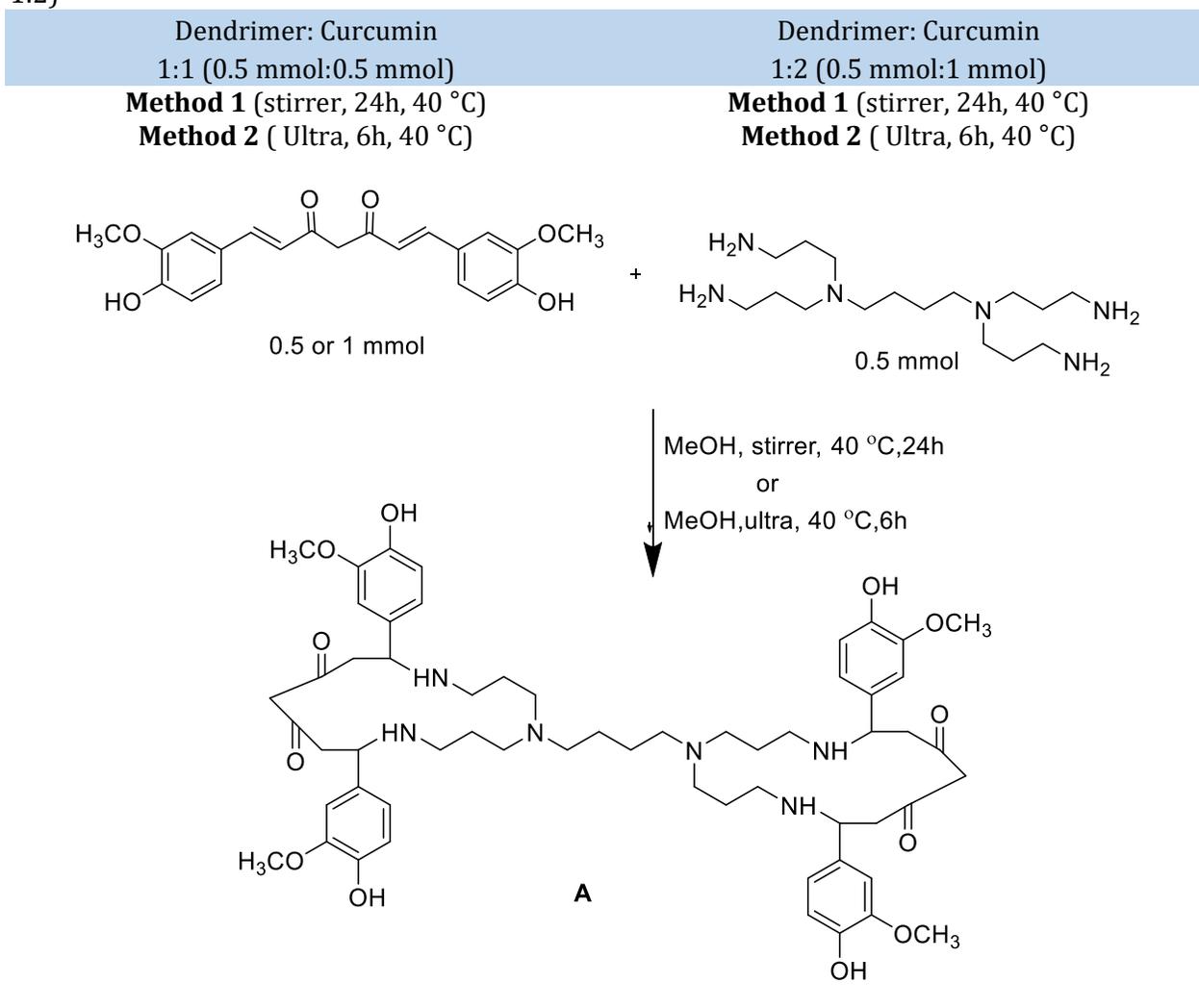
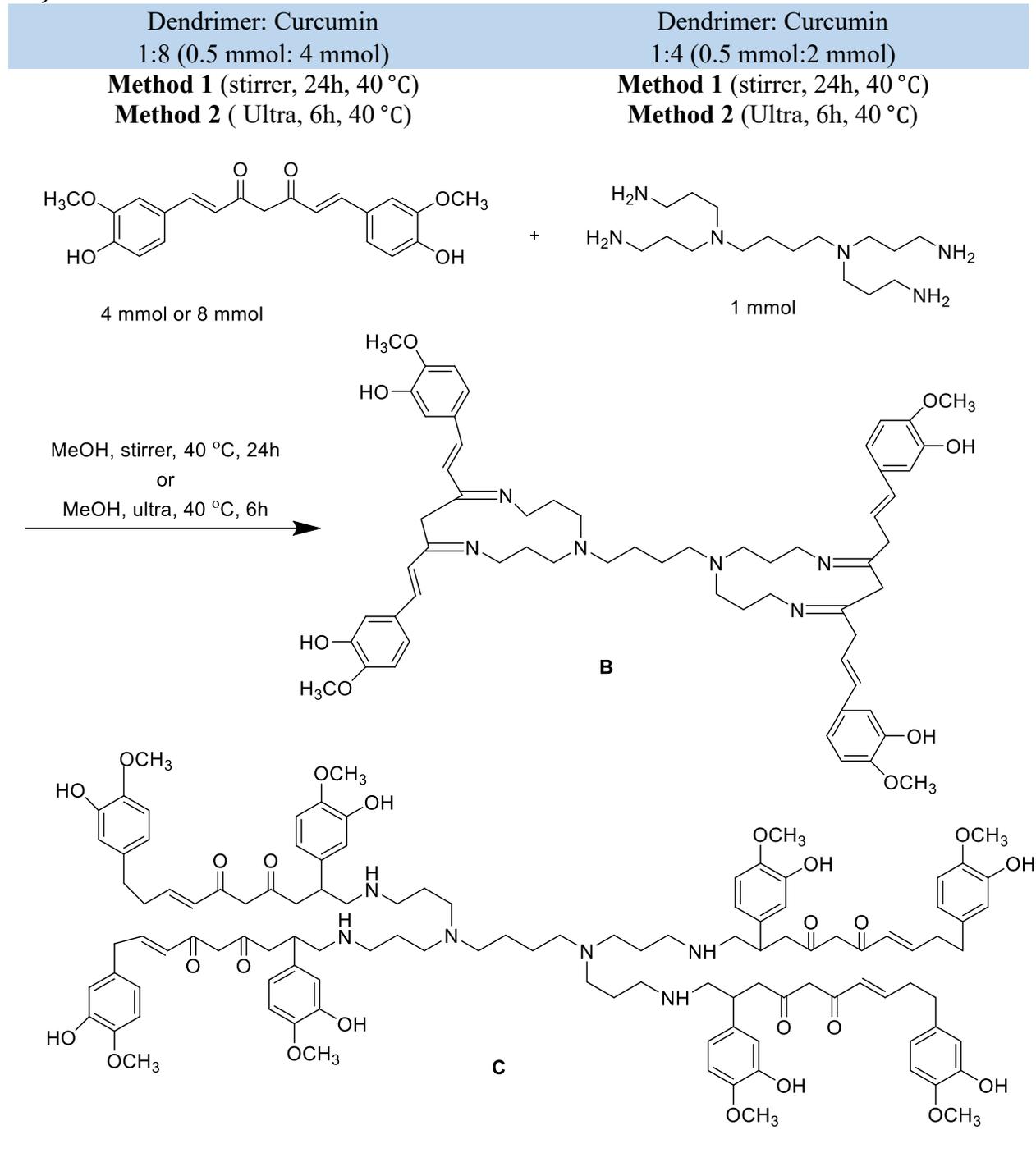


Table 2. Curcumin-dendrimer interaction (the molar ratio of curcumin with PPI dendrimer 1:4 and 1:8)**Table 3.** The results of IR,¹H NMR, and ¹³C NMR, spectra of title compound

| | IR | HNMR | CNMR |
|-------|---------------------------------|----------------------------------|---|
| (1:1) | C=O (cm ⁻¹ 1581/65) | DMSO(solvent) 2.509, phenolic | Acetic acid (solvent)(18.36- 19.53ppm) |

| | | | |
|-------------------------|---|--|--|
| Without ultra. | Stre. OH, NH, CH respectively (2937.11,3058.82,3221.82) | H(6.833ppm), Aromatic ring H(6.719), Methoxy H(3.727ppm) DMSO(solvent) | C=O(176.99ppm) C(4)(172.08ppm), C of methoxy(55.35ppm), CH(a) and CH2(b) 37.07 and 47.05ppm. |
| (1:1) ultra. | C=O (1647.55 cm ⁻¹) Stre. CH, NH, OH respectively (2917.15,3098.02,3210.08 cm ⁻¹) | 1.23-2.509, phenolic H(6.322ppm), Aromatic ring H(6.715), Methoxy H(3.167ppm) DMSO(solvent) | Acetic acid (solvent)(18.36- 19.53ppm) C=O(177.23ppm) C(4)(172.11ppm), C of methoxy(55.35ppm), CH(a) and CH2(b) 37.09 and 45.05ppm. |
| 1:2 Without ultra | C=O (1581.47 cm ⁻¹) Stre. CH, NH, OH respectively (2937.11,3058.82,3182.07 cm ⁻¹) | 1.23-2.51, phenolic H(6.322ppm), Aromatic ring H(6.715), Methoxy H(3.167ppm) DMSO(solvent) | Acetic acid (solvent)(18.36- 19.54ppm) C=O(177.29ppm) C(4)(172.02ppm), C of methoxy(55.35ppm), CH(a) and CH2(b) 37.07 and 40.29ppm. |
| 1:2 Ultra | C=O (1541.25cm ⁻¹) Stre. CH, NH, OH respectively (2934,3014.00, 3131.65cm ⁻¹) | 1.23-2.55, phenolic H(7.33ppm), Aromatic ring H(6.84), Methoxy H(3.73ppm) | Acetic acid (solvent)(18.36- 19.53ppm) C=O(177.23ppm) C(4)(172.11ppm), C of methoxy(55.35ppm), CH(a) and CH2(b) 37.09 and 45.05ppm. |

Treatment of cells with different derivatives of curcumin-PPI

In order to use these samples (curcumin-PPI derivatives), it is necessary to obtain the effective dose of curcumin in the cells. For that matter, Concentrations of 10, 20 and 40 µm

Cell viability assays

The viability test on control and treated cells was carried out in a 96 well-plate using MTT (4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide), where after 4 h of incubation, the mitochondrial succinate dehydrogenase in the living cells reduces the yellow color tetrazolium into purple formazan. Then, 100 µL of DMSO was added to each well of the plate and formazan crystals were dissolved

were prepared from 7 different samples and added to the culture medium of the cells. For 24 h, cells were exposed to different doses of curcumin and PPI-dendrimer derivatives. The MTT assay (methylthiazole tetrazolium) was then performed to evaluate cell proliferation.

at room temperature. The absorbance of solutions was measured on an automated microplate reader (SCO diagnostic, Germany) at 505 nm.

Statistical analysis

Statistical evaluation of the data was performed using the one-way analysis of variance (ANOVA) Tukey,s test, with the help of SPSS. Results were expressed as mean± S.D and

$P < 0.05$ was accepted as the minimum level of significance.

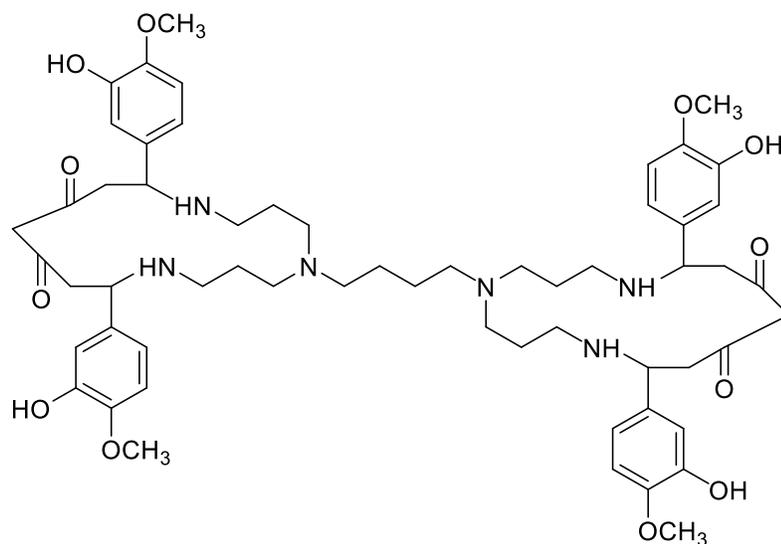
Result and Discussion

Chemical result

In this paper, the interaction of PPI dendrimer to curcumin with different molar ratios of 1:1, 1:2, 1:4, 1:8 respectively in the presence and absence of the ultrasonic waves was studied. The results of the IR, ^1H NMR, ^{13}C NMR, SEM, and FESEM spectra showed that a new product, that is called A, is formed through the interaction between PPI dendrimer and curcumin in molar ratios of 1:1 and 1:2 (Scheme 1, Table 3). However, the products (1:1 and 1:2 in the presence or absence of ultrasonic waves) were powder-shaped and decomposed at a temperature higher than 200 °C. Also, in these four structures, the ring structure was formed because of the existence of a carbonyl group in their structures and considering the molar ratios by adding the given amount of curcumin and presence of NH. In this regard, structure A

is the intended structure for all 4 derivatives obtained from the results of the research. Also, the studies on the SEM images in both derivatives 1:2 (presence or absence of ultrasonic waves) show the confirmation of structure A (Figure 1 and 2).

Concerning the derivatives 1:4 and 1:8 (in the presence and absence of ultrasonic waves), the products are provided in powder and the melting points of the products were between 162 °C to 185 °C. The results showed that the number of lateral branches increased with increasing in curcumin where the molecule got larger and the melting points decreased. This is the case because the steric hindrance increases along with the increase in dendrimer bands. Also, the IR studies (Table 3) on these derivatives showed that the presence of two types of carbonyl and considering the large amount of curcumin, each curcumin mole interacts with each NH that shows that the confirmation of the C structure and linear structure of the derivatives (Scheme 2). Also, the studies on the SEM images illustrated the confirmation of structure C (Figure 3).



Scheme 1. A structure is the recommended Structure for Products (1:1 & 1:2) (in the presence and absence of Ultrasonic waves)

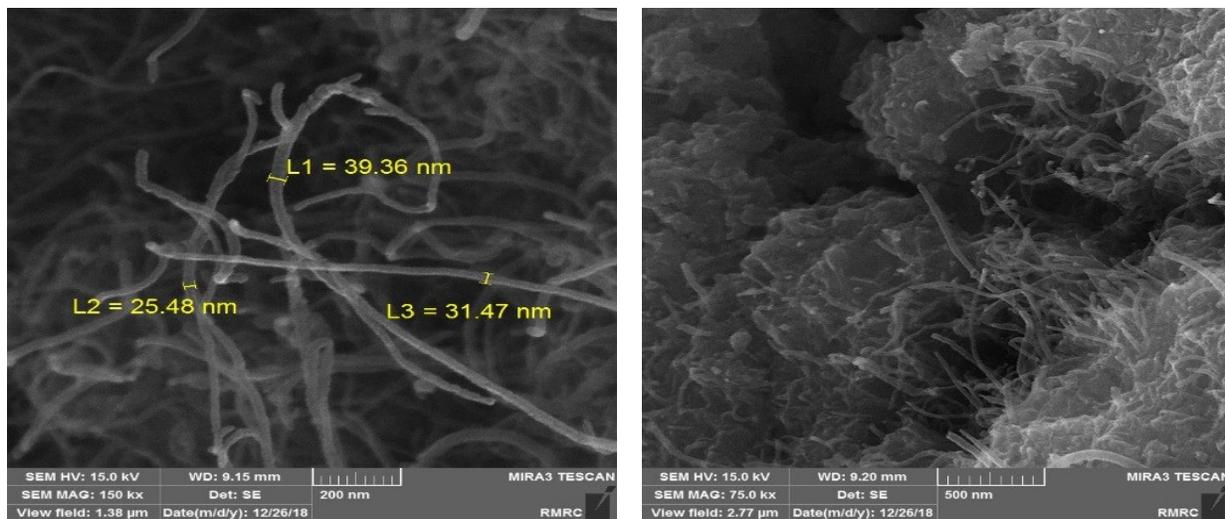


Figure 1. FE-SEM images of 1:2 product (In the absence of ultrasonic waves), SEM growth images show the length of the arms

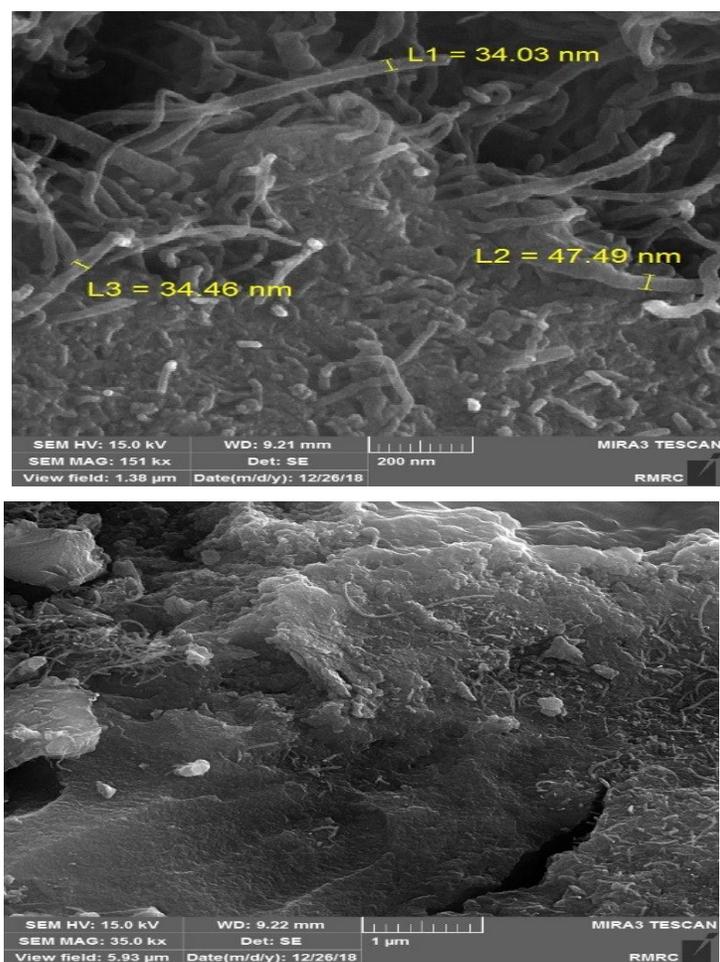
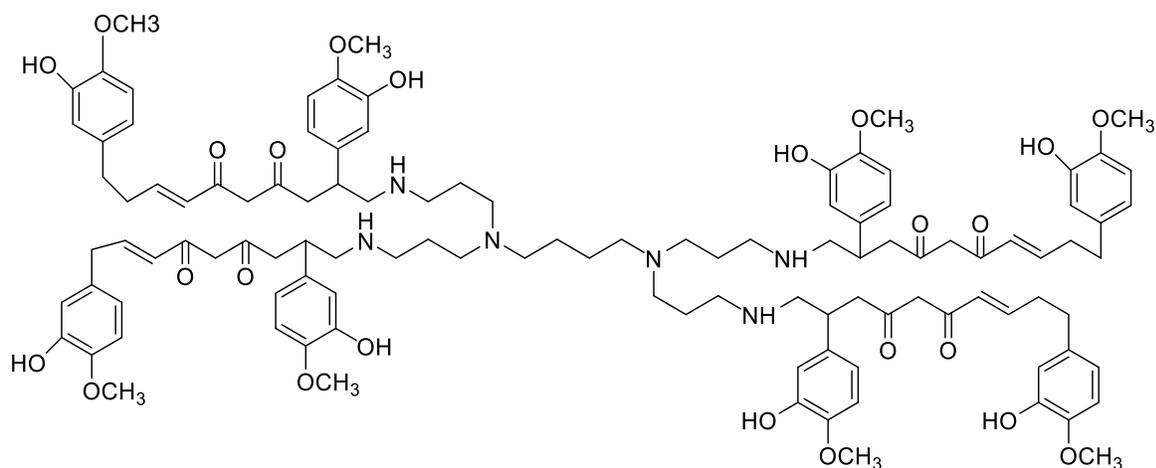


Figure 2. FE-SEM images of 1:2 product (in the presence of ultrasonic waves), SEM growth images show the length of the arms



Scheme 2. C structure is the recommended Structure for Products (1:4 & 1:8) (in the presence and absence of Ultrasonic waves)

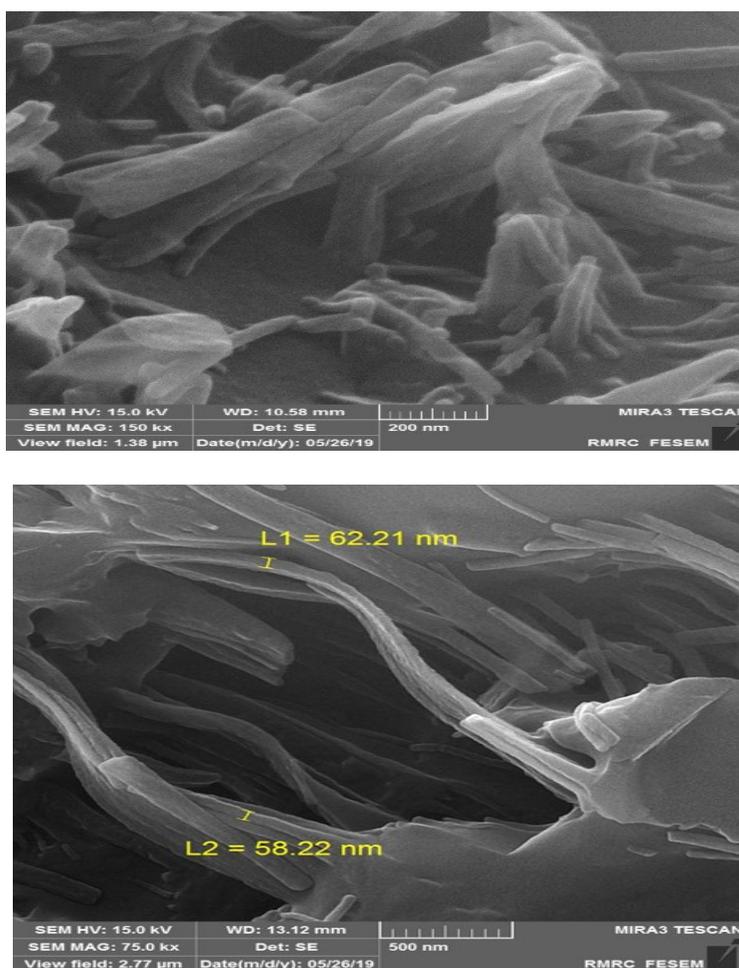


Figure 3. FE-SEM images of 1: 8 product (in the presence of ultrasonic waves), SEM growth images show the length of the arms

Clinical results

The results obtained from the analytical studies conducted by SPSS showed that no significant difference was made in the number of cells in dose of 0 (control group) and 10 micromolar of all samples over 24 h. However, a significant decrease was observed in the number of cells after the treatment at a dose of 20 micromolar in all samples. This is the case while the number of cancer cells decreased by a

smaller degree in sample curcumin (without dendrimer) in relation to other samples (Table 4). Also, the treatment of cancer cells at a dose of 40 micromolar in different samples led to the death of about 50% of the cells after 24 h, and the number of cancer cells has decreased by 50% in relation to those of the control group and other samples. This means that dendrimer is effective in greater penetration of curcumin to the cancer cells and applying the fatal properties of curcumin.

Table 4. The comparison between the number of live cells after the treatment with different doses of 8 substances after 24 h was made using MTT assay for the amounts as means±sd. The a, b c,d means with the different code letters mean different things (one-way ANOVA, p<0.05)

| | 40 | 20 | 10 | 0 |
|-----------------------|-----------------------|------------------------|-------------------------|------------------------|
| 421 ^d ±2.5 | 976 ^a ±7.3 | 1987 ^b ±2.5 | 2021 ^b ±7.5 | A,1 |
| 511 ^d ±6.5 | 765 ^a ±4.1 | 2234 ^b ±2.1 | 1934 ^b ±6.1 | A,2 |
| 603 ^d ±2.7 | 879 ^a ±3.2 | 2322 ^b ±1.2 | 2032 ^b ±2.20 | A,4 |
| 552 ^d ±1.3 | 863 ^a ±9.4 | 2192 ^b ±3.3 | 1992 ^b ±9.3 | A,8 |
| 493 ^d ±4.4 | 789 ^a ±0.9 | 1911 ^b ±2.4 | 2111 ^b ±0.4 | B,1 |
| 499 ^d ±0.8 | 811 ^a ±4.1 | 2087 ^b ±3.1 | 1987±0.1 | B,2 |
| 589 ^d ±2.7 | 747 ^a ±5.4 | 1977 ^b ±2.6 | 2002 ^b ±2.4 | B,4 |
| 535 ^d ±3.8 | 749 ^a ±2.4 | 1969 ^b ±7.4 | 1991 ^b ±3.4 | B,8 |
| 712 ^a ±7.4 | 934 ^c ±0.9 | 1964 ^b ±1.4 | 1988 ^b ±0.4 | Curcumin(no dendrimer) |

Conclusions

The solubility and permeability of curcumin to the cancer cells can be increased through combining the curcumin with other dendrimer. The dendrimer are a new class of macromolecules that have a three-dimensional and spherical structure with a great thickness. The structures of these substances have a great effect on their physical and chemical properties. These specific features, especially in the medical and chemical behaviors and nanotechnology, have made them more interesting. Also, they are used in a wide range of medical and industrial applications for the specific behavior of dendrimer. The dendrimer solved in water can connect to the hydrophobic molecules with the antifungal and antibacterial

properties. The importance of dendrimer lies in the fact that the therapeutic effect of each drug depends on its good solubility in the aqueous solution. Therefore, these complexes are considered as the systems delivering drugs. In this study, the connection of dendrimer poly (propylene imine) to curcumin as a drug deliverer and its anti-cancer effects on the BRC-9 line of the breast cancer cells were evaluated. Our studies demonstrated that the treatment of cancer cells at a dose of 40 micromolar in different samples led to the death of about 50% of the cells after 24 h, and the number of cancer cells decreased by 50% in relation to those of the control group and other samples. This means that the dendrimer is effective in greater penetration of curcumin into the cancer cells and applying the fatal properties of curcumin.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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