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Mn^{2+} - Negatively Charged Pegylated Dendrimer G2-Tryptophan: Novel Nano Magnetic Resonance Imaging Agent

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Abstract: Due to the new proclamation of the harmful symptoms of Gd (III) based MRI differentiate operators in the patients with harmed renal capacities, there is a solid consideration on emerging substitute differentiate specialists for MRI. A polymer with core of PEG and citrus extract as a shell was created for double modular imaging. After amalgamation of the polymer, ALGD-Trp Conjugate and after that Mn^{2+} stacked on the dendrimer. Differentiate upgraded MRI of the focused on double imaging operator was assessed by AFM magnifying lens. Growth is one of driving reason for death in everywhere throughout the world. Early determination of tumor known as a decent solution. Achieving a capable and non-obtrusive contrast agent with high specificity, sensitivity and capacity to target cancer cells are essential. Among of different malignancies distinguishing strategies, Magnetic Resonance Imaging is an important technique, however its burdens including low resolution, In this study, Tryptophan was appended on the Anionic direct globular dendrimer with polyethylene glycol center and citrus extract bunches around it. Tryptophan was utilized to enhance intra-cell take-up and gathering in human bosom malignancy cells lastly differentiate operator (Mn^{2+}) stacked in orchestrated dendrimers. The contrast agent was characterized with different techniques such as FT-IR, FTIR, Zeta sizer, UV-spectroscopy. ICP-AES was used to assess the intra-cell take-up of Mn^{2+} iones. Cytotoxicity studies and evaluation of cell passing mechanism were performed in bosom tumor MCF-7 cell line. Then, impact of various grouping of new difference specialist on T1 and T2 assessed in-vitro utilizing MR imaging. In light of the outcomes exhibited that Mn^{2+} - Anionic straight globular dendrimer G2-Tryptophancontrastagentdid not demonstrate any significant toxicity (cancer and ordinary cells), The outcomes demonstrated the new focused on nano differentiate specialist fitting accumulation in the tumor cells and solid T1intense flag on compared to Magnevist as a standard CA. This novel complexity specialist can be presented as a decent CA applicant in early identification of tumor.

Keywords: Synthesis, Biological Activity, Mn^{2+} Dendrimer G_2-Tryptophan, MRI

1. Introduction

Nano materials are a favorable theranostic authority with sales for tumor imaging and picked development calm transport [1]. New techniques in nanoparticle plans exploit synergism of different imaging modalities to move development disclosure, cure and to screen response to treatment [2]. Two such relating systems are optical imaging and alluring resonation imaging (MRI) [3]. X-beam gives highlight significant tissue multifaceted nature and three-dimensional assurance; nonetheless, it is not a quantitative procedure and it is exorbitant. Optical imaging is simplicity, delicate and gives tremendous spatial and short lived assurance, yet infiltration is kept to two or three millimeters under the tissue [4]. By arranging nano construction formulations with twofold imaging uses, the benefits of MRI as an identify instrument can be jointed with optical imaging to quantitatively track and control the bio spreading of nanomaterial's in vivo [5]. MRI as a non-meddling expository system that can give viable high-assurance anatomical pictures of fragile tissues [6, 7]. Nano-sized appealing resonation imaging is one of the intense imaging modalities for imagining. In any case, because of MRI low affectability, it's not feasible for sub-nuclear imaging. Presently, commonly little sub-nuclear Gd (III) chelates are open that improve picture separate vial imitation of the loosening up times of the abutting water protons [8]. The administrators are generally not specific extracellular multifaceted nature masters and no fitting for target-specific nuclear imaging in both preclinical and clinical wards [9].There have been relentless attempts in the advance of centered distinction administrators for more precise symptomatic imaging [10]. Gadolinium-based chelates are the most ordinarily used multifaceted nature authorities for clinical MRI. Magnesium salts are second groups of separate administrators can production of free Mn^{2+} particles [11]. Though, as an outcome of metabolic process can be assembled in a couple of organs, for instance, liver, pancreatic and heart, subsequently can be compelled their application [12]. Dendrimer-based contrast administrator's proposal an agreeable number of limiting regions to which many imaging and concentrating on moieties can be conjugated. Because of bio degradable core, dendrimers are hurtful [13]. The G1Dendrimer with more flexibility, lower negative charge and more multi-dispersity in size against the G2showedfurther toxic quality than to G2. The G2polyamidoamine-NH2 (PAMAM-NH2) dendrimer has a fairly broad assortment of safe use for potential healing sales. The G2 conjugate may permit less difficult ID and section to the telephone without requiring further imperativeness. In this way, the more negative charge of the G2makesan increase the amount of confining regions prepared to interface with the unequivocally charged substrate of the phone layer in contrast to the G1. This may come to fruition on account of the declaration that the aftereffect of the extra commonsense social occasions in the G2 can be executed by its more related steric revultion in against with the G1 [14-16]. In like manner, the purpose of the present study, Synthesis and natural development appraisal of Mn^{2+} Anionic direct globular Dendrimer G2-Tryptophan as another nano-separate administrator for upgrading of MRI.

2. Material and Method

2.1. Chemical and Materials

Polyethylene glycol (PEG), citric corrosive anhydrous, sodium bicarbonate and methanol were bought from the Merck Co., Germany. Mn, Tryptophan and Cephadex were acquired from Sigma Aldrich compound operator were utilized as a part of the study (2, 3-Bis (2-methoxy-4-nitro-5-sulfophenyl)- 2H-tetrazolium-5-carboxanilide) (XTT) pack was obtained from Sigma-Aldrich, (Sigma-Aldrich Inc, and St. Louis, MO, USA). MCF7 Cell line (Breast malignancy Cell line) was bought from The Pasteur Institute (Tehran, IR Iran), and Annexin-PI pack was gotten from (Roche Company, AnnexinV-FLUOS®, Basel, Switzerland).

2.2. Instrumental Analysis

FTIR (Nicolet), Lyophilizator (Wisco), ELISA peruser (Bio-Tek), Zetasizer, UV-spectroscopy, AFM, ICP-AES (Varian AX150 torbo) were utilized as a part of the study.

2.3. Cell Lines

In vitro studies were performed utilizing human bosom growth cell lines (MCF-7). The cell lines were given from National Cell Bank of Pasteur Institute of Iran (Pasteur, IR Iran). The Cell culture was done in the DMEM media (Gibco, New York, NY, USA) supplemented with 10% fetal ox-like serum (FBS) and a blend of penicillin–streptomycin (100unit/ml) and brooded at 37°C in a 5% CO_2 atmosphere. Trypsin compounds were utilized for cell treatment and isolated with centrifugation at 2000 rpm for 5 min.

2.4. Nano-Contrast Specialist Synthesis

The method of Nano difference specialist blend comprises of three primary strides: (1) combination of the Anionic Linear Globular G2Dendrimer (2) Conjugation of the tryptophan amino corrosive to the ALGD (3) Manganese stacking on the incorporated Nano atom.

2.5. Anionic Linear Globular Dendrimer (G2) Combination

Poly ethylene glycol (PEG) and citrus extract framed center and shell of the dendrimer, respectively. 5.65 mmole PEG engineered (PEG600) were added to dried Dimethyl Sulfoxide (DMSO) and Cacl2 and blended for 10 minutes. At

that point, 2 mmole N, N'- Dicyclohexylcarbodiimide (DCC) were included for 15 minutes. In the following stride, 0.5 gr citrus extract was added to the response compartment and mixed on 400 RPM for 1h. Synthesize of G2was finished by adding of 3 mmole DCC to 5.2 mmol citric corrosive and mixed on 400 rpm for 7 days. The shading change to the dim chestnut shading were checked the combination of the G2dendrimer. Decontamination strategy was performed by filter paper strip chromatography [17].

2.6. Anionic Linear Globular Dendrimer-G2 (ALGDG2) Purification Methods

Channel paper chromatography utilized for sanitization of cephadex. Dicyclohexylurea (DCU) and dicyclohexylcarbodiimide (DCC) were removed by a channel paper chromatography technique and lyophilized, for DMSO evacuation. Along these lines, FTIR, TLC, Zetasizer and molecule measure analyzer were utilized for assurance of the structure, filtration, charge and size, individually.

2.7. Conjugation of the Tryptophan Amino Corrosive on ALGD G2

The G2 dendrimer lyophilized and DCC were blended for 15 min. For conjugation Tryptophan with the ALGDG2 utilitarian groups, DCC was utilized. At that point Tryptophan with a 1:1 molar proportion was added to enact ALGD G2 and centrifuged at 300 RPM for 72h. Conjugation was done between the NH2 gatherings of folic acid with the carboxyl group of the dendrimer Figure 1.

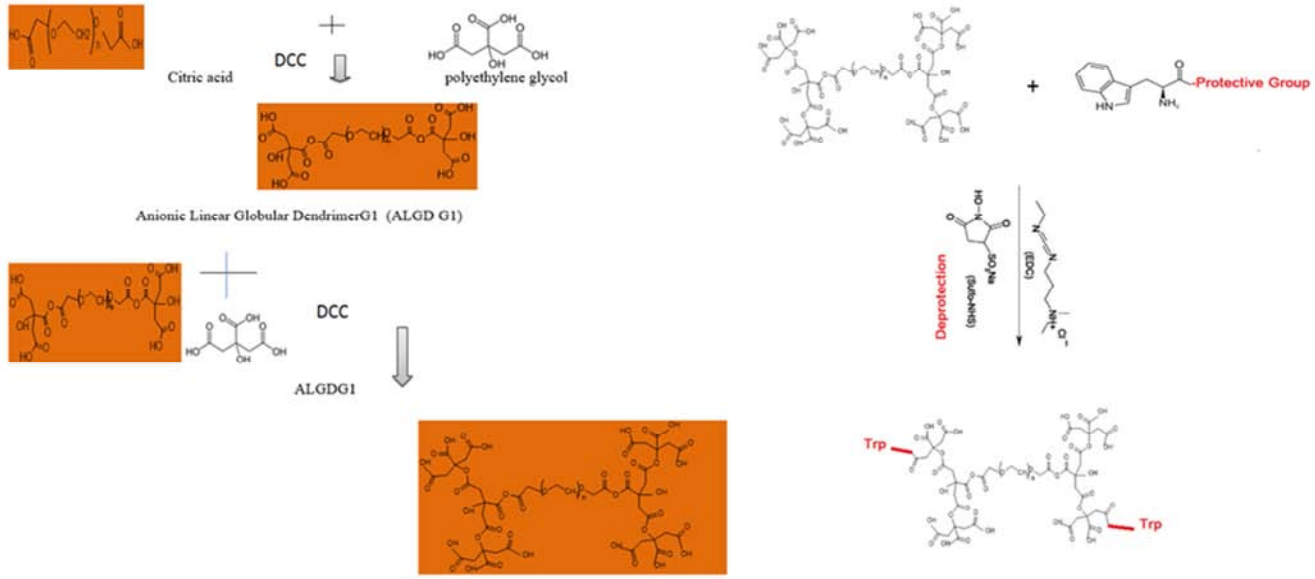


Figure 1. *Depiction of the synthesis procedure of Trp-Anionic Linear Globular Dendrimer nano-conjugate.*

2.8. Purification and Verification of Tryptophan-ALGD G2

Chromatography (section with Cefadex G75 gel) was utilized for cleansing of the Tryptophan-ALGDG2molecule. TLC and FTIR were done for verifying the Tryptophan-ALGDG2 structure. FTIR was utilized as an approval test for Tryptophan conjugation on surface of Mn^{2+} [18]. A solution of $Mn^{2+}$was arranged in chloroform dissolvable and exchanged to the sheet plates.

2.9. Mn^{2+}Loading on the Tryptophan-ALGD G2

Tryptophan-dendrimer conjugate (2.01mg) was broken down in refined water. Sodium bicarbonate was included request to make of basic specimen. Gadolinium stacking was performed by an attractive radiator stirrer (IKA-Werke, Staufen, Germany) for10min at 100°C and blended at 300 rpm for 6days. Mn^{2+} authoritative to Tryptophan-AL G2 affirmed by the inductively coupled plasma

Atomic emission spectrometry (ICP-AES) and UV spectroscopy. Therefore, the ratio of manganese in Nano contrast agents was determined by ICP-AES.

3. *In Vitro* MRI Imaging

MRI was performed using a 1.5 Tesla General Electric (US) in the Rajaei Hospital Tehran, Iran. Mn^{2+}-ALGD G_2-Tryptophan was used in various concentrations (0.1 M, 0.25 M, 0.5 M, 0.75 M and 1M). Sterile distilled water and Magnevist were used as a blank and standard sample, respectively. T_1 and T_2 weighted MRI at 1.5 T intensity were performed on Phantom axial slices. AFM microscopy imaging was performed by in two forms; the compound complex with/without manganese.

3.1. Intracellular Uptake Study

Intra cellular uptake of Mn^{2+}Dendrimer G_2-Tryptophan was determined by two methods;(I) concentration of 2×10^5 cells per well were passaged and incubated in 37°C and 5% CO_2 for 24 h. Negative and positive controls were included; first well counting 400µg/ml Mn^{2+}, 800µg/ml Mn^{2+}in a

second well and 200µl Magnevist drug to 200µl Mn^{2+}-Dend rimer G_2- Tryptophan in the third well, respectively. After incubation cells at 37°C with 5% CO_2 for2 h, washing was done twice with 500µl of phosphate buffer saline (PBS) and centrifuged at 1500 rpm for 15 minthen reconstruction in 2 ml of cell culture media. (II) Quantitative determination of cellular uptake of Mn^{2+} - Dendrimer G_2- Tryptophan was completed by ICP-AES. These measurements were performed in triplicate and mean and standard deviation were calculated by SPSS software (ver. 19) ($P<0.05$).

3.2. In Vitro Toxicity Assay

XTT examine is one of the proper strategy for estimation of cell reasonability. In the initial step, the cells (MCF7) were brooded with different concentrations of the Mn^{2+}-Anionic Globular Dendrimer G2-Tryptophan (1, 0.1, 0.01, 0.001 µgr/ml (Magnevist (as a positive control) and DMSO (as a negative control) for 24h. 50 µl XTT arrangements were added to every well, and after the cells hatching for an extra 4 hours. The plates were kept in a dim place to be prepared for spectrophotometric assurance. The ingestion rate in every well was computed by a computerized microplate peruser at 450 nm. The outcomes were isolated into two gatherings (1) the ingestion of the untreated control culture and (2) communicated as rate of practical cells. Living cells delivered a larger amounts off or mazande hydrogenase. The red shading change demonstrated the nearness of reasonable cells.

3.3. In Vitro MRI Measurement

The MCF-7 cells line (1×10^5) were incubated with different doses of synthesized CA and Magnevist (as a standard) (0.1, 0.25, 0.5, 0.75, 1M) for 1h. MRI imaging was done with 3T MRI scanners. Untreated cells were used as a control group. The calculations were performed based on the following protocols: Standard Spin Echo, # of Echoes =32

TE=13/26/39/52/66/79/92/105/118/145/158/171/184/198/211/237/250/264/277/290/303/330/343/356/369/382/396/422 ms, TR=20/50/100/200, /400/2000/3000ms, Matrix=256*256, Slice Thickness=1/5mm, FOV=18*18cm, NEX=Non

For quantitative analysis, the MRI images were analyzed by DICOM software version 1.3. 0.5. (MEDAV GmbH Company) Microdicom, MATLAB and Microsoft office Excel 2007 software.

3.4. Statically Analysis

Statistical data analysis and T_1 and T_2 were done using Dicomwork, Matlab and excel software.

4. Results and Discussion

4.1. Nano-Contrast Agent Synthesis

Carboxyl group of G2 dendrimers forms amide bonds with a peripheral amine group of the Tryptophan which loaded with Mn^{2+} composing Mn^{2+} -G2 dendrimer-Tryptophan structure (Figure 1). The structure was confirmed by FTIR spectroscopy (Figure 2). Fourier-transform infrared (FTIR) spectroscopy was used to confirm the existence of the Mn^{2+}-ALGD G2-Tryptophan conjugate in. The peak observed in the wave of number 1232 cm-1wavelengthis related to CO groups in the esterifies bond which confirms the synthesis of G2 dendrimers and esterifies bond formation between terminal citric acid groups of G1 dendrimers and new citric acid groups. Alsothe peak 1726cm-1 is related to C = O groups of terminal citric acids of G2. The peaks in of 2500-3430cm-1wavelength are related to acidic OH groups of citric acid and the peak 1500-1600 cm -1exclusively depicts amid bond formation. (Figure 2).

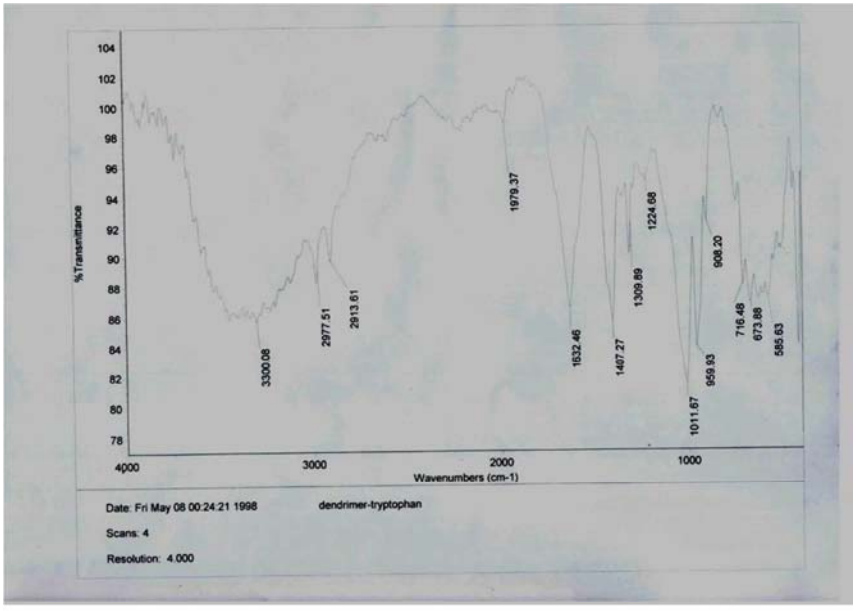
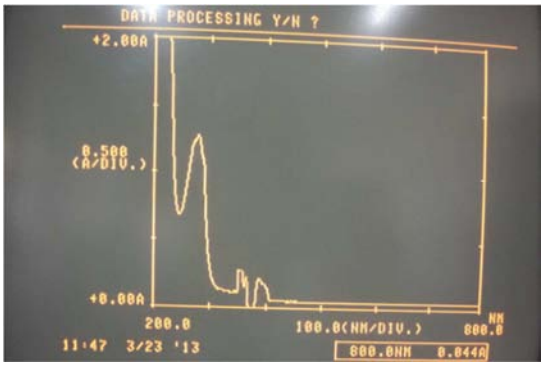


Figure 2. *Dendrimer-Tryptophan FT-IR spectrum.*

4.2. UV Spectrometry Result

Based on Absorption peak at ? nm in the UV spectra in and Mn^{2+}-ALGD G2-Tryptophan formation and without such a peak in G2 dendrimer –Tryptophan demonstrating the conjugation. (Figure 3).

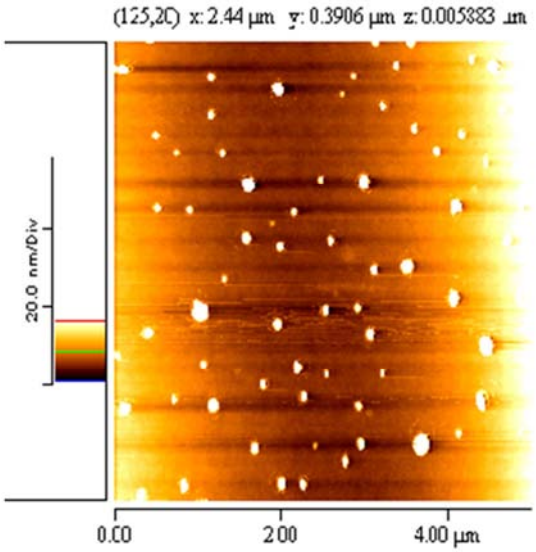
A



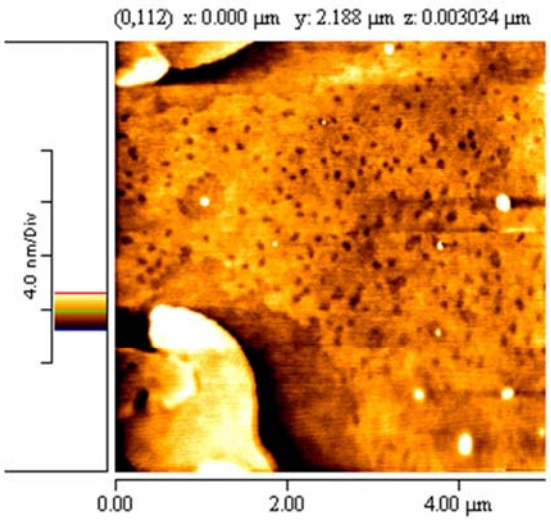
B

Figure 3. *The UV spectrum of G2dendrimer-Tryptophan (A) and Mn-ALGD G2-Tryptophan (B).*

4.3. Determination of Conjugation Morphology

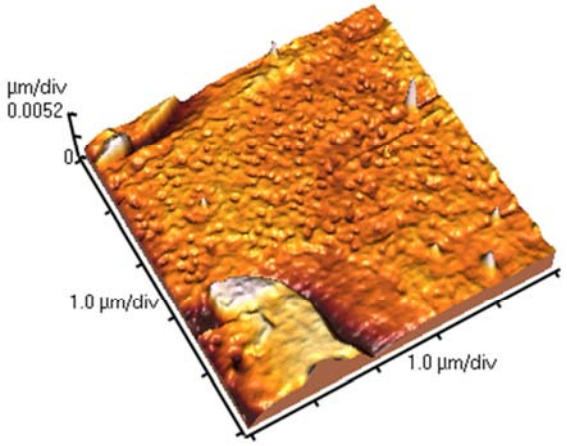


A

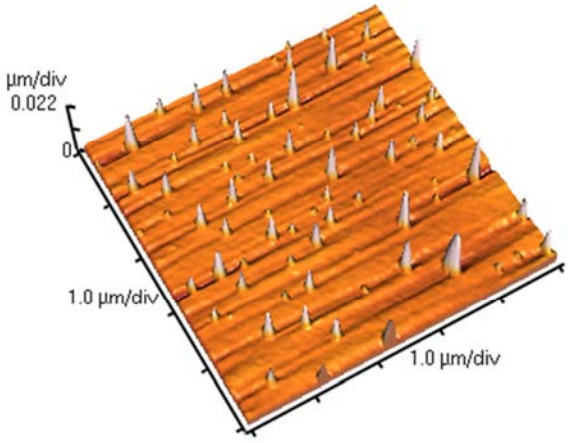


B

Figure 4. *2D (A) and 3D (B) AFM image of ALGD G2-Tryptophan.*



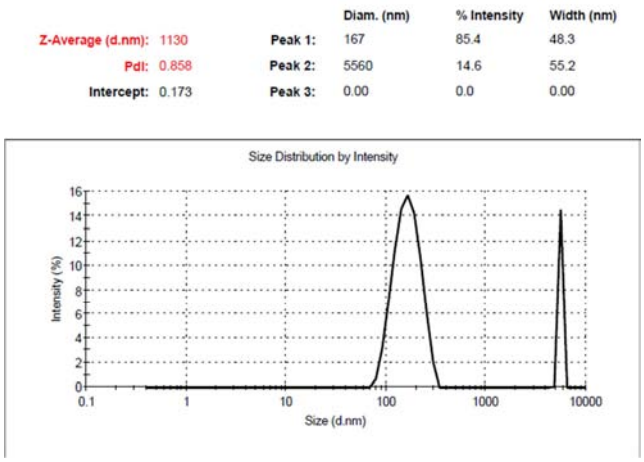
A



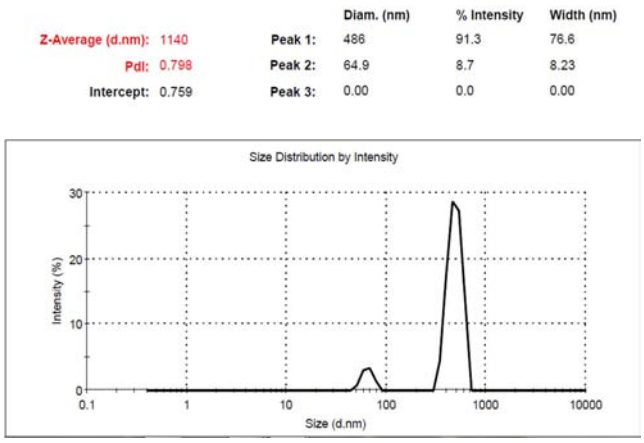
B

Figure 5. *2D (A) and3-D (B) AFM image of Mn^{2+}-ALGD G2-Tryptophan.*

Size and charge of the nanoparticles molecule after conjugation to the ligand i.e Mn^{2+} experienced significant changes (Figure 6, 7). The same confirming results were emerged by atomic force microscopy (AFM) (Figure 4, 5).

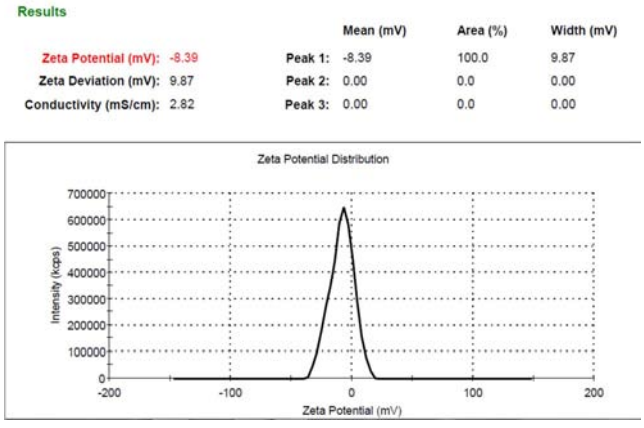


A

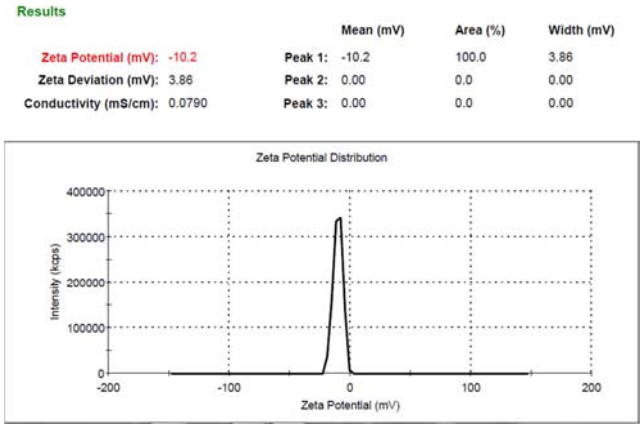


B

Figure 6. *Particle size of the ALGD G2-Tryptophan and Mn^{2+}-Anionic Globular Dendrimer G2- Tryptophan (respectively A, B).*



A



B

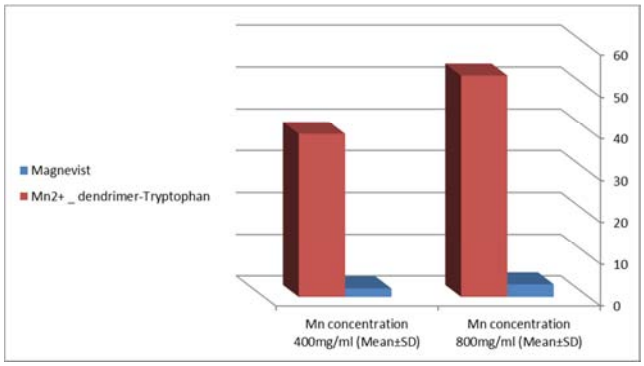
Figure7. *Article charge of the ALGD G2-Tryptophan and Mn^{2+}-ALGD G2-Tryptophan (respectively A, B).*

4.4. Cellular Uptake Result

Loading of Mn^{2+} in ALGD G2 Dendrimer can increase the accumulation of drugs enter the MCF7 cell line in voth applied different doses and made much better pharmacokinetics compared to Magnevist.(equation 1) (table 1).

Table 1. *Comparison ofcellular uptake between Mn^{2+}-ALGD G2-Tryptophan andMagnevist into thecellattwo different doses.*

	Mn concentration (Mean±SD)	**Mn^{2+}concentration (Mean±SD)**
	400mg/ml	**800mg/ml**
Magnevist	2.1±0.333	3.02±0.456
Mn2+ - dendrimer-Tryptophan	39.2±2.1	53.34±4.445



Equation 1. *Cellular uptake assay.*

4.5. XTT Assay Results

Biocompatibility of Mn^{2+}-Anionic Globular Dendrimer G2 - Tryptophan conjugate has been evaluated in vitro using XTT assay at different concentrations (1, 0.1, 0.01, 0.001µg) for 24 hours. The results have been shown no specific toxicity (table 2).

Table 2. *Results of Mn^{2+}-Anionic Globular Dendrimer G2- Trypophan XTT assay on MCF7 cellas a percentage cell viability at different concentrations of nano new contrast agent.*

Test group	Elisa OD at(450nm)	*Mean OD*	*Cell Viability %*
Untreated MCF7 cell line (1µlit DMSO)	1.25, 1.42	1.33	100%
ALGD G2-Tryptophan (1µg)	0.48, 0.43	0.45	33%
ALGD G2-Tryptophan (0.1µg)	0.95, 0.87	0.91	68%
ALGD G2-Tryptophan (0.01 g)	0.98, 1.03	1.00	75%
ALGD G2-Tryptophan (0.001µg)	1.01, 1.05	1.03	77%
Mn^{2+}-ALGD G2-Tryptophan (1µg)	0.18, 0.17	0.175	12%
Mn^{2+}-ALGD G2-Tryptophan (0.1µg)	0.75, 0.79	0.77	57%
Mn^{2+}-ALGD G2-Tryptophan (0.01µg)	0.87, 0.76	0.81	60%
Mn^{2+}-ALGD G2-Tryptophan (0.001µg)	1.07, 0.82	0.94	70%

5. Relaxation Time Evaluations (*in Vitro* MRI Imaging)

Graph and related to the T2 relaxation time in various TE and the effect of different concentrations of the Mn^{2+}-ALGD G2-Tryptophan on T1 relaxation time in comparison with water

Figure 7 shows T1relaxation time decay. Results indicate that Mn^{2+}_ALGD G2-Tryptophan shows a better relaxation time in comparison with water and standard Magnevist (figure 8) Table shows the signals obtained from different concentrations and TR times. T1 was determined by using the linear equation and compared with Magnevist and water.

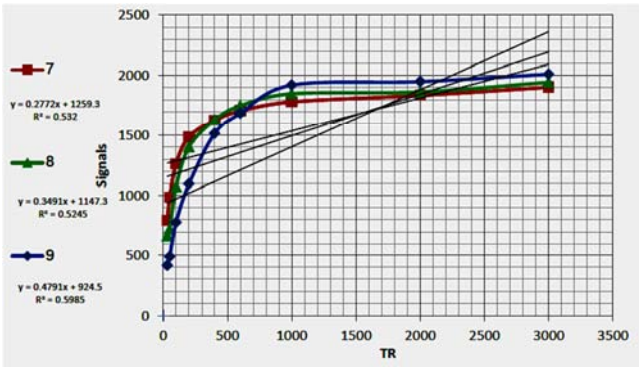


Figure 8. *Signal intensity in different concentration and TR time showing good relaxivity parameter for suggested nanoconjugate-Mn^{2+}.*

6. Conclusion

Most normal little molecule iodinated many-sided quality administrators are low nuclear weigh. In this study we created thought to decide this issue. The productive progression of dendrimer added to their fascinating physico chemical features including controlled release systems, evaluate, shape, degradation, surface science and concentrating on. The wealth of valuable social events on the outer shell of dendrimers and their water dissolvability incorporate serves as a Contrast masters modified by expanding imaging times. In this study, for the first time, the Mn^{2+}-Anionic Globular Dendrimer G2-Tryptophan, as a novel targeted molecular separate master for application in imaging of active tumort issue was exhibited. Conjugated Nanoparticles' physicochemical properties were examined in this study. also cell death instrument used to assess the threat of in vitro. Anionic straight globular Dendrimer G2 made out of polyethylene glycol-citrate which is absolutely biocompatible and non-noxious separating to non-unsafe presenting in each and every living being so is absolutely biodegradable[18-30]. Another favored angle of this separate pro is targeting ability. In late years, there is an example to use methods for metabolic imaging of pharmaco dynamic biomarkers. Tumor cells have an extended enthusiasm for glucose and amino acids to support, Therefore there are a couple considers drove on the usage of amino acids to target infection cells starting late [31-51]. Concentrated taking drugs movement is relied upon to decrease the side effects of meds. Late studies have shown that Tryptophan is the specific concentration for cancer-causing cells. Conjugation Tryptophan on the surface of the new CA can be given fitting affectability and specificity in MR imaging besides upgrade the phone take-up of CA in view of the specific social occasion of tryptophan on the surface of CA. By novel physical and invention characteristics of this new many-sided quality expert which can be coordinated adequately and quickly obliging almost no exertion additionally biocompatibility; it would be as a sensible confident passing on an imaging contrast administrator of danger cells.

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