

Studying the Effect of Functional Group and Size of Silica Nanoparticles Loaded with Quercetin on their *in vitro* Characteristics

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ABSTRACT

Silica nanoparticles (SNs) possess unique properties making them ideal carriers for many agents. Both the size and the surface chemistry are important features that impact the *in vitro* characteristics of their loaded agents. In this study, different surface functionalization of SNs with a particle size of 200 nm (propyl thiol, propyl carboxylic acid, and propyl amine) and two different sizes of propyl amine SNs (200 and less than 100 nm) were investigated. The nanoparticles (NPs) parameters were characterized using Dynamic Light Scattering (DLS) and their Encapsulation Efficiency (EE) and Loading Capacity (LC) with quercetin were measured using UV Spectrophotometer. Quercetin cumulative release was studied in phosphate buffer saline (PBS) (pH 7.4, 37°C) and its *in vitro* cytotoxicity toward HeLa cells was evaluated using an MTT assay. Our results showed that the mean particle size of all samples increased after drug loading and the polydispersity (PD) values were all within the acceptable range (0.2-0.5). All SNs exhibited negative values of zeta potential with the highest value for propyl-carboxylated NPs. The EE and LC percentages of quercetin in SNs depend on the type of surface functional group where the aminated SNs showed higher percentages compared to the other groups. A direct relation was observed between the drug release rate and the cytotoxicity where the highest and smallest values were exhibited by thiolated and aminated SNs, respectively. Surface modifications have thus a more pronounced effect on the *in vitro* properties of our studied SNs compared to the size.

Keywords: Silica nanoparticles; quercetin; surface functionalization; size of SNs; DLS analysis; cumulative release; MTT assay.

1. INTRODUCTION

Nanotechnology has drawn a great attention in the pharmaceutical and medical fields by playing an important role and acting as a driving force for their evolution¹. It is also a part of drug delivery field that not only enhances the efficacy of already available drugs but also provides a chance for the discovery of new therapies¹. This technology utilizes particles within the size range of 1-1000 nm referred to as nanoparticles (NPs) into which many therapeutic agents can be either entrapped,

encapsulated or attached to their surfaces². Numerous medications, including hydrophilic and hydrophobic tiny medications, vaccinations, and biological macromolecules, can be given using nanoparticles³. It is well known that particle size and size distribution are the most important features to impact particles' *in vivo* distribution as well as targeting ability². Therefore, their small size allows their entry into the cells more efficiently than larger molecules⁴. It had been reported that the cellular uptake of nanoparticles by Caco – 2 cells (human epithelial colorectal adenocarcinoma) are up to 2.5 fold higher than 1 µm micro-particles⁵. Therefore, they are used as carriers for different therapeutic agents to be delivered to their sites of action inside the human body².

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Targeted drug delivery can also be achieved by such delivery systems as they transport the therapeutic agents to their required site of action without affecting other tissues which in turn will reduce the undesirable adverse effects⁴. Such targeted delivery can be achieved by either attaching a specific molecule on NPs' surface such as antibodies or by manipulating physical stimuli such as pH⁴. Additionally, these systems are known to increase the bioavailability and the absorption of medications that are poorly soluble in water, such as hydrophobic pharmaceuticals⁶. In addition, such systems can protect the therapeutic agents from undesired metabolism, degradations and clearance which in turn will lower the required dose used, improve patient compliance and lower the cost⁴. Moreover, they can be utilized in different routes of administration including oral, parenteral, nasal and other². Among all NPs found Silica nanoparticles (SNs) are recently of great interest in the field of drug delivery systems. They are inorganic-based solid particles with a honeycomb – like porous structure into which many drugs can be incorporated². They have superior properties including: simple synthesis techniques, relatively low cost, good thermal and chemical stability and biocompatibility at adequate concentration^{7,8}. More importantly, they have a large surface area and pore volume allowing for high drug concentration to be loaded, if needed⁸. Simply, by manipulating the synthesis parameters such as pH, temperature and reaction time, different particles sizes and shapes can be obtained⁹. The control of both the shape and the particle size are highly essential to assure efficient cellular internalization of the delivery systems. In addition, NPs' morphology and electrostatic interaction between their surface groups and cells also impact their cellular uptake^{10,11}. The outer surface of the particles can also be easily modified by adding different functional groups⁸. It has been found that both the size and the surface functional groups affect many of the particles' characteristics such as their liver metabolism, uptake by human lymphocyte cells and many others¹². For instance, it was revealed that the

cellular uptake of aminopropyl vinyl modified particles is more efficient compared to the vinyl modified and unmodified ones¹². NPs' size, on the other hand, has a great significance in developing a successful delivery system by influencing many important points such as the NPs' cellular uptake, tumor penetration and blood circulation half-life¹³. As drug delivery systems, SNs can be used to encapsulate different therapeutic agents¹⁴ and some of NPs' properties such as their size and surface functional groups would affect the in vitro characteristics of the drugs when encapsulated into these NPs, such as the drugs' loading, release rate and cytotoxicity profiles. The effect of certain simple functional groups on the encapsulation efficiency, loading capacity, physical stability, drug cumulative release and cell toxicity has not been thoroughly studied on many therapeutic agents such as quercetin.

Quercetin(3, 3', 4', 5', 7-pentahydroxyflavone)(Figure 1)¹⁵, is the most abundant natural flavonoids that present in many fruits and vegetables such as grapes, onions and many others¹⁶. The stability of quercetin can be drastically reduced as a result of either degradation or oxidation during processing and storage¹⁷. However, many factors are found to affect its stability such as temperature, pH, metal ions and others¹⁸⁻²⁰. This promising potent flavonoid has numerous health beneficial effects²¹. It displays a wide variety of pharmacological impacts including anti-inflammatory, anti-diabetic, hepato-protective and anti-obesity^{22,23}. It is considered a powerful antioxidant along with its capability to eliminate free radicals²⁴. Its antioxidant ability is due to the presence of antioxidant pharmacophore such as the catechol group in the ring B and the hydroxyl group (OH) at the position 3 of ring AC²⁵. Based on previous studies, quercetin is considered the most powerful scavenger of (ROS) such as peroxides and nitrate radicals which makes quercetin a good inhibitor of lipid peroxidation²⁴. This property along with its ability to increase glutathione levels help in preventing atherosclerotic plaques formation, thus reduce the risk of

cardiovascular and neurodegenerative diseases^{24,26}. It was reported by previous studies that quercetin is able to block the action of several enzymes such as xanthine oxidase, phosphate oxidase and NADP that are responsible for the formation of oxidative species²². In addition, quercetin has an anti-inflammatory effect by preventing Ca⁺²-dependent cell death²⁴. Most important, quercetin has a cancer preventive effect by different mechanisms including the anti-oxidant effect, enzymes inhibition which involve in carcinogens activation and modifications in signal transduction pathways²⁷. In addition, it has a synergistic effect when combined with other anti-cancer agents such as cisplatin²⁸. However, quercetin exhibit variable characteristics regarding its aqueous solubility which will eventually impact its activity²⁹. Being a lipophilic, it has a moderate solubility in organic solvent such as ethanol with solubility of about 4 mg/ml at 37 °C³⁰ and highly soluble in DMSO of about 150 mg/ml at 25 °C²⁰. It had been reported by previous studies that the aqueous solubility of quercetin range from about 2.63 ug/ml at 25 °C to 1490 ug/ml at 140 °C²⁹. Therefore, its low hydrophilicity and poor solubility result in very minimal gastro – intestinal absorption and subsequent low oral bioavailability up to only 17 % in rats and 1 % in man^{31,32}. In addition to solubility challenges, physiological instability is another issue that limited quercetin clinical applications as well as its poor absorption, fast metabolism and short half - life which all restrict severely its bioavailability³³. In order to overcome these disadvantages, it is highly recommended to get advantages of different applicable delivery systems

such as NPs²⁷. Such systems are able to improve the poor solubility by reducing the drug aggregation due to their favoring greater surface area, thus, better dispersion for drug loaded nanoparticles will be achieved³⁴. Quercetin was already included in different types of delivery systems including nano-materials such as cyclodextrins or methoxyoly (ethylene glycol)-Poly (lactide), chitosan NPs, micelles and liposomes to evaluate the best delivery vehicle for such flavonoids^{35,36}. It has been reported by previous studies that using NPs with many modifications such as folic acid incorporation or PEG successively implement better anti-proliferative effect toward different types of tumors with better stability of released quercetin in tumor cells with limited distribution in normal cells as well as enhanced neuro protection against neurodegenerative diseases^{22,37,38}.

One of the most obstacles associated with NPs is their high surface energy resulting in their aggregation³⁹. In order to eliminate such difficulties and to gain the maximum drug bioavailability, different functional groups had been used as a surface coating to NPs such as cyclodextrin, PEG and many others^{37,39}. Our goal here was to study the effect of three specific functional groups on the *in vitro* characteristics of 200nm SNs (EE, LC, stability, release rate and cell toxicity) when loaded with quercetin. In addition, we will investigate the NPs' size effect on the same characteristics. Furthermore, the three different functional groups will be compared with unmodified SNs which will be treated as a control sample.

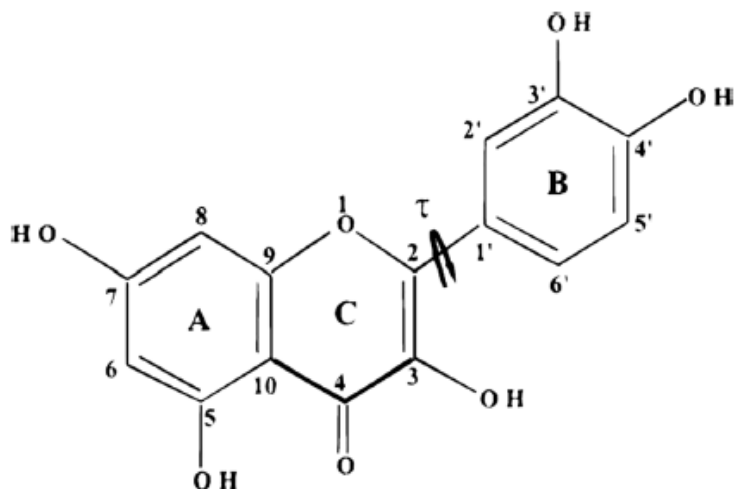


Figure 1: Chemical structure of quercetin

2. Materials and Methods

2.1. Materials

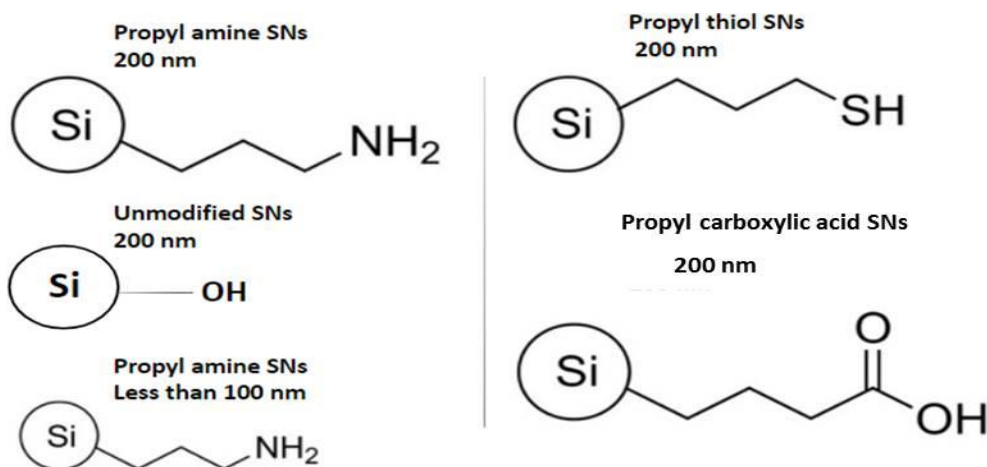
Silica NPs (200 nm), propyl thiol silica NPs (200 nm), propyl carboxylic acid silica NPs (200 nm), 3- aminopropyl silica NPs (of less than 100 nm), quercetin hydrate and phosphate buffer saline were all purchased from Sigma Aldrich. Propyl amine silica NPs (200 nm) were purchased from Nanoshell company UK. Cell culture components including the tissue culture media was obtained from Caisson Laboratories, fetal calf serum and Trypsin – EDTA from Biowest, gentamicin sulfate and Penicillin-Streptomycin were both obtained from EuroClone Italy.

2.2. Methods

2.2.1. Preparation of quercetin stock solution and quercetin loaded silica NPs

In order to obtain a standard solution having a

concentration of (100 ug/ml) of quercetin, an accurately weighed 10 mg of quercetin powder was transferred to 100ml volumetric flask, dissolved well with methanol and diluted to the mark. Then 1ml from the standard solution was transferred to a 10 ml volumetric flask and diluted to the mark by adding methanol to obtain a solution with a quercetin concentration of (10ug/ml) which in turn will be the stock solution. For SNs' loading, 4 ml of this solution was mixed with 0.01 g of SNs (Propyl thiol SNs 200 nm, Propyl carboxylic acid SNs 200 nm, Propyl amine SNs 200 nm, Unmodified SNs 200 nm and 3-Aminopropyl modified SNs of less than 100 nm as shown in scheme 1). This mixture was then stirred for 24 hrs and the resulting SNs were centrifuged for at least 25mins at 14000 rpm. After centrifugation step, they were washed well with deionized water then dried³⁸.



Scheme 1: Unmodified and modified SNs with different functional groups (Propyl amine, propyl thiol and Propyl carboxylic acid) and sizes (200 nm and less than 100 nm) which investigated in this study

2.2.2. Quercetin calibration curve construction in methanol and PBS

From the stock solution (10 ug/ml), different concentrations (2, 4, 6 and 8 ug/ml) were prepared by drawing each time 1 ml of the stock solution and complete the volume with either pure methanol or BPS according to our required concentration and calibration curve, by applying the following equation:

$$\text{Concentration 1} * \text{volume 1} = \text{Concentration 2} * \text{volume 2}$$

The absorbance of each solution was measured at $\lambda = 372$ nm (λ_{max} for quercetin⁴⁰). Two different calibration curves (in methanol and PBS) were then plotted at this specific wavelength and the line equation was generated.

2.2.3. DLS analysis

The mean particle size of all modified SNs was measured using the DLS technique (Zetasizer nano series, Malvern U.K). The particle size was measured using dilute suspensions of SNs (5×10^{-4} mg/ml) before and after quercetin loading in PBS with a refractive index of 1.326 for PBS and 1.48 for SNs and viscosity = 1.2 cp. All suspensions were vortexed for at least 5 mins in order to separate the large aggregates, if exist. The measurements were performed in triplicate with 10 runs for each and the average was then taken. The polydispersity (PD) and the

zeta potential averages were also measured simultaneously before and after quercetin loading.

2.2.4. Evaluation of encapsulation efficiency and loading capacity of the NPs

A precise amount (0.01 g) of five different samples of SNs were mixed separately with 4 ml of quercetin stock solution (10 $\mu\text{g/ml}$) and stirred for 24 hrs. The NPs were then separated by centrifugation at 14000 rpm for 25 mins. The supernatant absorbance was then measured using UV spectrophotometer at $\lambda = 372$ (λ_{max} for quercetin). By applying the line equation of our set calibration curve in methanol, the amount of free (un trapped) quercetin in the supernatant was measured and then the LC and EE were calculated using the following equations:

$$\text{EE}\% = (\text{weight of initially added drug} - \text{weight of free drug in supernatant}) / \text{weight of initially added drug} \times 100 \%^{41}.$$

$$\text{LC}\% = (\text{weight of initially added drug} - \text{weight of free drug in supernatant}) / \text{weight of NPs} \times 100 \%^{42}.$$

2.2.5. Assessment of *in vitro* drug release

A precise quantity of modified SNs loaded with quercetin (0.015 g) were first mixed with 2 ml PBS (pH =7.4) in a dialysis bag (SnakeSkin Dialysis Tubing, 22mm \times 35 feet dry diameter, 3.5K MWCO), and then added to a clean beaker containing 23ml PBS and shaken inside a

water bath at 37°C. At various times (1, 2, 4, 6, 24, 26, 28 and 30 hrs.), a sample of 1ml was drawn and replaced with 1ml of fresh PBS. The amount of quercetin released was measured using UV spectrophotometer based on calibration curve in BPS.

2.2.6. Antiproliferative (cell viability) assay

Frozen HeLa cells were thawed carefully in water bath at 37 °C, re-suspended in cell culture media and allowed to grow in cell culture flasks inside the incubator for 24 hrs. Under aseptic conditions, grown cells were de – attached with trypsin and counted. Into 96- well tissue culture, HeLa cells (100 µl/well) were dispensed in tissue culture medium which consist of DMEM media, 10 % fetal bovine serum, 1 % L – glutamine, 1 % penicillin streptomycin and 0.1 % gentamicin solution at an optimized concentration of 1000 cells/well. After 24 hrs, the media was completely removed and 200 µl of 0.15 mg/ml of different quercetin loaded SNs suspensions were added to the cells and incubated for 48 hrs. Then, cell viability was determined using MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide)

assay. From each well, 100 µl of culture media was removed and 10 µl of thiazolyl blue tetrazolium solution was added to the wells and left in CO2 incubator for at least 3 hrs. Finally, MTT solubilization solution (100 µl/well) was added in order to stop the reaction, mixed well and incubated for 1 hr. Absorbance was measured using microplate reader at 550 nm and cell viability was calculated to determine the cell toxicity percentage.

3. Results and Discussion

3.1. DLS analysis

Favorable characteristics of SNs such as their high surface area and diverse surface functionalization, allow such NPs to be suitable for loading of many drugs and their involvement in other biomedical applications⁴. However, among the most important properties that need to be controlled are the size and surface charge. The mean particle size and the polydispersity (PD), which provides an indication of the heterogeneity of particle size in a mixture⁴³, of all samples of SNs were measured in PBS by DLS technique using (Zetasizer nano series, Malvern U.K), as shown in Table 1.

Table 1. DLS analysis of SNs in PBS (pH=7.4) before and after quercetin loading, data were shown as mean ±SD (n=3).

Unloaded SNs	Mean particle size±SD	PD±SD	Zeta potential (mV)±SD	Loaded SNs	Mean particle size±SD	PD±SD	Zeta potential (mV)±SD
Unmodified SNs 200 nm	204.8±13.28	0.378±0.038	-16.9 ±2.92	Unmodified SNs 200 nm	295.8±50.64	0.46±0.18	-20.7±1.83
Propyl thiol SNs 200 nm	236.3±15.64	0.223±0.156	-13.6±0.40	Propyl thiol SNs 200 nm	249.4±19.41	0.42±0.06	-13.9±0.55
Propyl carboxylic acid SNs 200 nm	204.1±3.37	0.33±0.237	-18.98±1.99	Propyl carboxylic acid SNs 200 nm	290.4±8.16	0.44±0.14	-19.95±0.94
Propyl amine SNs 200 nm	203.3±19.29	0.34±0.039	-11±0.5	Propyl amine SNs 200 nm	214.7±16.85	0.46±0.09	-14.1±0.4
Aminopropyl SNs of less than 100nm	128.2 ±25.9	0.49±0.03	-7.5±2.67	Aminopropyl SNs of less than 100nm	161.9±4.19	0.333±0.037	-14.4±0.45

The experimentally measured particle size for the unloaded samples was close to their original sizes (200 nm and less than 100 nm). The PD of unloaded samples was measured simultaneously and found to be within the acceptable range (0.2- 0.5)⁴⁴. The average particle size for all loaded samples was mainly higher than the unloaded samples suggesting a successful quercetin loading and/or adsorption, as shown in Table 1. The adsorption arises from the interaction that occurs between the different functional groups on the surface of SNs and the quercetin groups via, most likely, the hydrogen bonds resulting from quercetin B ring that can rotate freely along the 1'-2 connecting bond as well as the 3-2-1'-2' angle plus the non-aromatic hexagonal ring C which gives the molecule more flexibility to be adsorbed on SNs' surfaces¹⁵, as shown in Figure 1 which represents the chemical structure of quercetin.

The PD values were also slightly increased after loading except for the aminopropyl SNs of less than 100nm. This can be explained by the fact that after drug loading, the particle size of the aminopropyl SNs (less than 100 nm) increased and thus reducing the surface area and the tendency for aggregation. In addition, for SNs of less than 100 nm, the presence of quercetin after loading appeared to have much less effect on their stability as compared to those of 200 nm.

Simultaneously, zeta potential was measured in PBS for SNs before and after quercetin loading (Table 1). Zeta potential is the charge that arises between the solid surface of the NPs and its liquid media at the interface⁴⁵. Although it is of great importance in evaluating the NPs' stability but it is not the absolute measure to describe stability⁴⁵. This measurement describes the degree to which charged particles repel⁴⁶. Therefore, higher zeta potential values implies higher degree of repulsion among particles and thus higher stability and lower chance for aggregation⁴⁷. It is also known that the magnitude of the charge on the nanoparticle surface depends on the solution pH. The zeta potential for the unloaded and unmodified SNs was found to be -16.9 mV. The unmodified SNs have only silanol groups on their surfaces. The pKa value for the silanol

group is 5.6⁴⁸. At neutral pH, since their pKa is lower than pH, silanol groups will be deprotonated resulting in an overall negative surface charge. The pKa of propyl thiol and propyl amine SNs are 10.2 and 10.56, respectively. They will both be protonated at neutral pH. Since the percentage of modification of our NPs is less than 25%^{6,38}, the zeta potential values were -13.6, -11 mV and -7.5 mV for SH-SNs, NH₂-SNs 200nm and NH₂-SNs 100 nm, respectively. Even though all possess negative charge, they were generally less than the unmodified ones due, most likely, to the effect exhibited by the surface protonated groups. The pKa of propyl carboxylic acid is 4.81⁴⁹ which will be de-protonated at neutral pH and thus had the highest negative value. After quercetin loading, the net zeta potential increased for all samples. According to previous studies, the pKa value of quercetin at neutral pH is about 6⁵⁰. Therefore, at neutral pH, quercetin will be negatively charged as a result of its hydroxyl groups causing an elevation in the net negative charge of the loaded SNs. In addition, the increased values of zeta potential after drug loading indicates the presence of drug molecules adsorbed on NPs' surface to a certain extent.

3.2. Measuring the encapsulation efficiency and loading capacity of the NPs

Encapsulation efficiency refers to the capability of the therapeutic agent to be entrapped into NPs while loading capacity gives an idea about the ability of NPs to entrap drugs. Both measurements are important parameters to evaluate the performance of a drug delivery system. Particularly when designing a delivery system for cancer therapy, it is beneficial to ensure that the system provides a high drug loading so that increasing the amount of therapeutic agent delivered to the cancer cells⁵¹. Generally, the surface modifications affect the interaction between NPs and the therapeutic agents. The values of both LC and EE were close for unmodified, propyl thiolated and propyl carboxylated SNs. On the other hand, these values were the highest for propyl aminated SNs (both 200 and less than 100 nm), as shown in Table 2. These findings are most

likely attributed to the preferable interaction between propyl amine functional groups and quercetin through hydrogen bond formation. The strength of the intermolecular hydrogen bond between quercetin and

propyl thiol, propyl carboxylic acid and silanol groups seems to be weaker than propyl amine, therefore, all have smaller values of EE and LC.

Table 2. EE (average %) and LC (average %) for SNs loaded with quercetin, data wear shown as mean \pm SD (n=3).

SNs	EE% \pm SD	LC% \pm SD
Unmodified SNs 200 nm	79.29% \pm 1.52	0.312 \pm 0.002
Propyl thiol SNs 200 nm	81.12% \pm 2.22	0.321 \pm 0.007
Propyl carboxylic acid SNs 200 nm	76.31% \pm 3.81	0.301 \pm 0.015
Propyl amine SNs 200 nm	98.4% \pm 0.18	0.393 \pm 0.0007
Amino propyl SNs of less than 100 nm	99.21% \pm 0.13	0.396 \pm 0.0005

3.3. *In vitro* drug release study

Surface functionalization affects drug loading as well as the rate of drug release. Both surface charge and hydrophobicity of SNs are significant parameters that modulate drug release⁵². Quercetin release was studied at pH 7.4 using warm PBS (37 °C) as the release medium to simulate physiologic conditions. The release patterns of quercetin from SNs are plotted in Figure 2 as cumulative release percentage at different time intervals (1, 2, 3, 4, 6,

24, 26, 28 and 30 hrs). Release results were obtained based on calibration curve in PBS (Figure 2).

Results showed that the free quercetin dissolved faster than quercetin loaded SNs where the difference was statistically significant at all time intervals. Within the first 6 hrs, about 42% of drug was released then increased after 24 hrs to reach a maximum of about 90% at 30 hrs. On the other hand, quercetin loaded SNs samples exhibited a much slower release pattern at the exact time intervals.

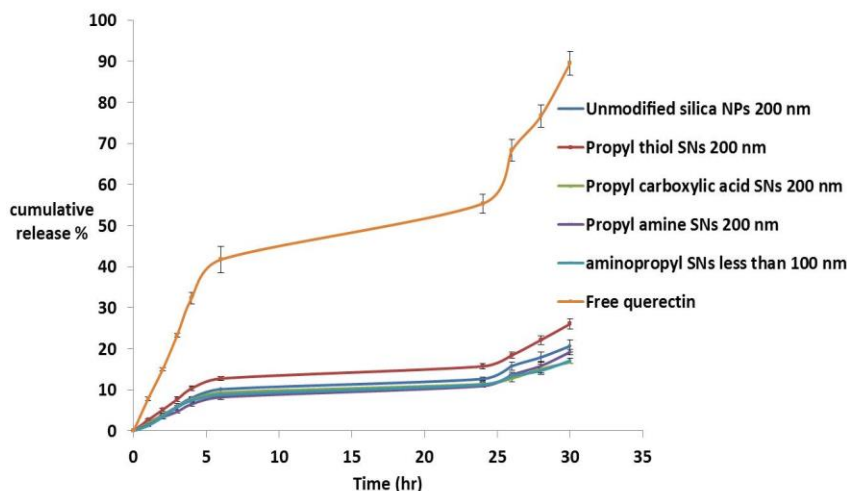


Figure 2: Cumulative release percentage of free quercetin and quercetin loaded SNs, data wear shown as mean \pm SD (n=3). One Way Anova Calculator was used to assess the differences, when free quercetin was excluded; P value <0.0001 after 6 hrs, P<0.001 at 1, 3 and 4 hrs and P<0.01at 2 hrs, when quercetin was included; P value <0.00001, at all-time intervals.

For quercetin loaded SNs, sustained drug release was generally observed with all samples with a statically significant difference among them even though the difference was more statically significant when free quercetin was included. Also, the difference was more significant between SNs after 6 hrs compared to the first four hrs, as shown from the calculated P values (Figure 2). Within the first 6 hrs, quercetin release from SNs was found to be only between 9 – 13 %. The release percentages were then increased over time to reach their maximum values at 30 hrs. At this time interval, propyl thiol SNs exhibited the highest percentage compared to other NPs. As also shown in Figure 2, both sizes of aminated NPs exhibited similar pattern at the first 6 hrs with a slight difference after 30 hrs. Both aminated as well as carboxylated modified SNs were associated with the slowest cumulative release that might be related the favorable interaction of these groups and quercetin that eventually restrict its release.

3.4. In vitro cell viability assay

In vitro cytotoxicity is highly dependent on cell – type as well as the characteristics of NPs’ drug carrier. For cell viability assay, a suspension of quercetin loaded SNs with a concentration of 0.15 mg / ml as well as free drug was incubated with HeLa cells for 48 hrs under the same conditions. Then, MTT assay was conducted to evaluate the cell toxicity and P values were calculated between two different individual groups as well as between all groups

simultaneously (Figure 3). All P values were less than 0.05, indicating a statistically significant difference, except the one between propyl thiol and unmodified SNs. As shown in Figure 3, the highest cell toxicity percentage of about 60% was associated with free quercetin. For quercetin loaded SNs, the highest cell toxicity was shown with propyl thiol SNs which is in agreement with its cumulative release as propyl thiol SNs showed the fastest quercetin release compared to other loaded SNs. The lower cell toxicity associated with propyl carboxylic acid compared to both unmodified and propyl thiol SNs might be explained by their lower drug content as was shown from their EE values (Table 2). The minimal in vitro cytotoxicity was observed with both quercetin loaded aminated SNs. This is in consistent with their slowest release rates behavior which in turn might be related to the favorable interaction between quercetin hydroxyl groups and amine group on SNs’ surfaces.

Indeed, quercetin loaded aminated SNs of less than 100 nm showed a higher percentage of toxicity compared to the bigger aminated NPs (200 nm) with a P value of 0.005. This result is in agreement with the fact that the NPs’ size is one of the key factors influencing the biological uptake and the internalization kinetics of NPs⁵³. In general, smaller NPs are more easily up-taken by cells compared to larger ones⁵³.

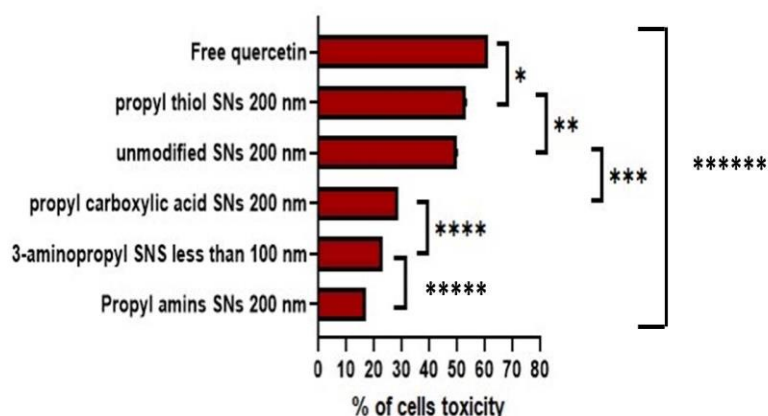


Figure 3: The results of MTT cell viability assay used to get the percentages of cell toxicity for HeLa cells after incubation with 0.15 mg/ml of quercetin loaded SNs as well as free quercetin for 48 hrs. Data were shown as ± SD (n = 3).

Unpaired t test was used to assess the differences between the two groups while the One Way Anova Calculator used to assess the difference between all groups: P*= 0.025, P ** = 0.52, P ***= 0.0008, P****= 0.001, P*****= 0.005, P ***** <0.05

Despite the fact that free quercetin exhibited a higher cytotoxicity compared to the loaded SNs, diverse advantages can be achieved from these NPs. The slower release rate observed would reduce the side effects especially toward normal cells in the case of loading with anticancer agents. In addition, all the characteristics of SNs can be exploited to enhance the drug delivery such as their high EE and the capability of further surface modification with other groups to target specific cells. Keeping in mind that *in vitro* studies are different than *in vivo* behavior, such systems can protect the therapeutic agents from undesired metabolism, degradations and clearance which in turn will lower the required dose used, improve patient compliance and lower the cost⁴. Moreover, cellular targeting can be achieved by systems such as NPs. In cancer therapy, targeting is crucial so that normal cells are protected and all therapeutic doses reach their site of actions in order to get the maximum benefits with the minimal adverse effects⁴.

The reason of the overall low cytotoxicity of our SNs might not due only to their slow cumulative release behavior but also to the effect of their surface charge. In general, positively charged NPs have higher internalization than neutral and negatively charged NPs⁵⁴. For instance, it was found that positively charged silica, silica-titania hollow and gold NPs are more cytotoxic than negative variants of similar sizes in non-phagocytic cells⁵⁵. The type of functional group itself also might determine the extent of NPs' internalization. Thus, to better evaluate the extent of our SNs' internalization, and to better assess the relation between their release rate, cell toxicity and their uptake, a further research is needed in the near future.

4. Conclusion

Silica NPs are found to be ideal carriers for many therapeutic agents due to their special properties. Our objective here was to study the effect of three specific functional groups (propyl thiol, propyl carboxylic acid and propyl amine SNs, 200 nm) on their encapsulation

efficiency, loading capacity, physical stability, drug release rate and cell toxicity when loaded with quercetin. In addition, we aimed to investigate the NPs' size effect on the same characteristics using two different sizes of propyl amine SNs (200 and less than 100 nm). The physical stability of the NPs was characterized using DLS analysis. The results showed an increase in the size, PD and zeta potentials after drug loading suggesting a successful quercetin loading. EE and LC were also investigated where the highest percentages were observed with aminated SNs due to favorable drug – carrier interaction as explained before. The *in vitro* quercetin release study was performed in PBS at 37°C and the subsequent cytotoxicity against HeLa cells was evaluated by MTT assay. Quercetin cumulative release from SNs and the cytotoxicity results were in agreement as the highest release and percentage of cytotoxicity were observed with propyl thiolated SNs and the lowest with aminated ones. Moreover, both sizes of aminated NPs exhibited nearly similar results in terms of their EE, LC and cumulative release rate and a slightly higher cytotoxicity of the smaller ones. The surface functional group has thus a more pronounced effect on studied *in vitro* characteristics compared to the size effect and it can hence serve a basic tool for controlling the loading, cumulative release and cytotoxicity of variety of therapeutic agents and their subsequent clinical efficacy.

Author contributions

Areen M. Khattabi conceived and designed the experiments and revised the manuscript; The Lina M. Ibraheem wrote the manuscript and conducted the experiments.

Conflicts of interest

The authors declare no conflict of interest.

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دراسة تأثير المجموعة الوظيفية وحجم جسيمات السيلكا النانوية المحملة بالكيرستين على خصائصها المخبرية

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ملخص

تمتلك جزيئات السيلكا النانوية (SNs) خصائص فريدة تجعلها حاملة مثالية للعديد من العوامل. يعتبر كل من الحجم وكيمياء السطح من السمات المهمة التي تؤثر على الخصائص المخبرية للمواد المحملة. في هذه الدراسة، تم فحص وظائف سطحية مختلفة لجزيئات السيلكا النانوية بحجم جسيم 200 نانومتر (بروبيلثيول، بروبيلكربوكسيلك، و بروبيلا أمين و حجمين مختلفين من بروبيلا أمين (200 و أقل من 100 نانومتر). تم تمييز معلمات الجسيمات النانوية باستخدام تشتت الضوء الديناميكي DLS و تم قياس كفاءة تحميل دواء الكيورستين EE و سعة التحميل LC باستخدام مقياس الطيف الضوئي للأشعة فوق البنفسجية. تمت دراسة الإطلاق التراكمي للكيورستين المحمل على جسيمات السيلكا النانوية في محلول الفوسفات الملحي PBS (درجة الحموضة 7.4، 37 درجة مئوية) و تم تقييم سمية الخلايا في المختبر تجاه خلايا هيللا باستخدام اختبار ال MTT. أظهرت نتائجنا أن متوسط حجم الجسيمات لجميع العينات زاد بعد تحميل الدواء وأن قيم التشتت المتعدد PD كانت كلها ضمن النطاق المقبول (0.2-0.5). أظهرت جميع جسيمات السيلكا النانوية قيمة سلبية لشحنة الزيتا مع أعلى قيمة لبروبيلكاربوكسيلكسيلكا. يعتمد تحميل الدواء وسعة التحميل بشكل كبير على نوع المجموعة الوظيفية السطحية حيث أظهرت كل من الجسيمات النانوية التي تحتوي على مجموعة البروبيل أمين على سطحها أعلى نسب مئوية متشابهة. ولوحظ وجود علاقة مباشرة بين الإطلاق التراكمي للدواء والسمية الخلوية حيث أظهرت الجسيمات النانوية التي تحتوي على مجموعة بربيل ثايول أعلى قيم الإطلاق التراكمي للدواء والسمية الخلوية تجاه خلايا هيللا بينما أظهر كلا الحجمين للجسيمات التي تحتوي على مجموعة البروبيل أمين القيم الأصغر. وبالتالي، فإن تعديلات السطح لها تأثير أكثر وضوحاً على الخصائص المخبرية لل SNs مقارنة بالحجم.

الكلمات الدالة: جزيئات السيلكا النانوية، كيورستين، تشغيل سطح الجسيمات النانوية، حجم الجسيمات النانوية، تشتت الضوء الديناميكي DLS، الإطلاق التراكمي، اختبار سمية الخلايا MTT.

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