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Research Article

QUERCETIN NANOPARTICLES: PREPARATION AND CHARACTERIZATION

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ABSTRACT

Purpose –Quercetin (QU) is a polyphenolic flavonoid and shows several biological effects such as antioxidant, antitumor, antibacterial and antiproliferative effects both in-vitro and in-vivo. Major limitation of QU in clinical application is administration of high dose due to its poor bioavailability. Also, its water soluble derivative has been synthesized but its bioavailability was only 20%. The purpose of this paper is to prepare Quercetin nanoparticles (QUENPs) with the improved solubility in water and bioavailability.

Design/methodology/approach - This paper is based on nanoparticipation technique for preparation of QUENPs by adding ethanol to water volume ratio (1:35), fixed flow rate (8 and 10 ml/min). Physicochemical characterization was studied by using Dynamic Light Scattering (DLS) usingZetasizer Nano ZS, SEM, FTIR, X-ray diffraction and BET surface area.

Findings - The data suggest that when resuspend QUENPs in water appeared to be soluble compared with original QU. Decrease in particle size of QUENPs than original QU. In addition, QUENPs are more crystanility and absence of larger particles compared with original QU.

Research limitations/implications - The source of preparation technique was previously researched. The Physicochemical characterization was study in University of Dammam.

Practical implications -Positive outcomes can be used to improve the bioavailability of QU antioxidant, antitumor, antibacterial and antiproliferative effects.

Social implications - The outcomes of this research would be used in healthcare and medical field, in areas, such as, faster diagnosis, drug delivery and tissue regeneration.

Originality/ value -This paper used a new solvent/ anti-solvent (S/AS) ratio and new flow rate into the anti-solvent.

KEY WORDS: Quercetin nanoparticles, SEM, FTIR, X-ray, BET, solvent/ anti-solvent, solubility in water.

INTRODUCTION

Nanotechnology deals with structures sized 100 nanometers or smaller in at least one dimension, and includes developing materials within that size. Nanotechnology, ranging from extensions of conventional device physics to completely new approaches based upon molecular self-assembly, from developing new materials with dimensions on the nanoscale. Rajendran, et. al [1] and Dubey, et. al [2] suggested that on the future nanotechnology has the potential to create many new materials with a vast range of applications, such as in medicine, biomaterials and energy production. The rapid development of nanotechnology worldwide is accompanied by massive generation of nanoparticles (NPs) even though the potential health impacts of these materials are largely unknown. The increased industrial use of NPs can result in frequent exposure through inhalation, ingestion or dermal contact during manufacture, use and disposal, therefore, studies are needed to understand the

potential biological effects of exposure to NPs. Engineered NPs possess greater surface-to-volume ratio and functionalities on their surfaces which could result in greater biological activity if these are taken into the body, making them a potential health concern. Gao, et. al [3]and Nazem, et. al [4] reported that the application of nanotechnology to healthcare holds great promise in medical field in areas, e.g imaging, faster diagnosis, drug delivery and tissue regeneration. Also, they said nanotechnology is approach in biochemical applications with a focus on the synthesis of NPs with the improved antioxidants activities against degenerative diseases like Cancer and Alzheimer's. Ferry, et.al.[5] and Fiorani, et.al.[6] indicated that natural products and dietary components have been evaluated as potential chemo protective agents. Flavonoids are known as phytophenolic compounds with strong antioxidant properties. This bioflavonoid is a potent oxygen free radical scavenger and a metal chelator, capable of inhibiting lipid peroxidation. In addition, it is particularly known for its ion- chelating and iron stabilizing action, therefore, helps in inhibition of lipid peroxidation [7]. Quercetin (3,5,7,3',4'-pentahydroxyflavone) (QU) (Fig. 1)is one of the most abundant flavonoids in the human diet, present in edible fruits, vegetables[8], herbs, oils and tea[9][10] or related products, e.g. apples, onions[11], Ginkgo biloba[12] and red wine[13], respectively. Even though pharmacokinetic and bioavailability information on QU is scarce and contradictory, QUhave many beneficial effects such as antioxidant, antitumor and antibacterial activities, hence, it has attracted a lot of attention [14]. QU shows several biological effects such as a strong inhibitory effect on the growth of several animal and humancancer cell lines [15], cardioprotection, anticancer, antiulcer, antiallergic, antinflammatory, antiviral, antiproliferative activities [16] and enhances the antiproliferative effect of cisplatinin-vitro and in-vivo [17]. Moreover, QU exhibits its antioxidant property and is effective against neurodegenerative diseases [18] [19]. Nuengchamnong, et. al. [20] reported that the antioxidant activity of QU is higher than well-known antioxidant molecules trolox, ascorbyl and rutin. This is due to the number and position of the free hydroxyl groups in the QU molecule [21].Therefore, QU has been extensively investigated for its pharmacological effects that include anti-tumor [22] and effectively heal the wounds [23].

Mulholland, et. al. [24] found that water soluble derivative of QU has been synthesized but its bioavailability was only 20%and major limitation of QU in clinical application is administration of high dose (50 mg/kg) due to its poor bioavailability. Therefore, various techniques have been used to increase the solubility of QU including thecomplexation with cyclodextrinand liposome [25][26]. But the use of cyclodextrin is associated with a risk of nephrotoxicity [27] and employing liposome might incur stability problems during storage [28]. Hence, it is necessary that a stable, safe, and efficient delivery method in increasing the solubility of QU. Alternative QU formulation strategies like liposomes and nanocapsules have been introduced to reduce the dose and to improve the therapeutic efficacy of the compound [29].Therefore, our investigation aimed to prepare quercetin nanoparticles and study its physicochemical characterization by using Fourier transform infrared spectroscopy (FTIR), powder X-ray diffraction (XRD),Dynamic Light Scattering (DLS) using Zetasizer Nano ZS, Scanning electron microscopy (SEM) and Brunauer, Emmett and Teller (BET) surface area.

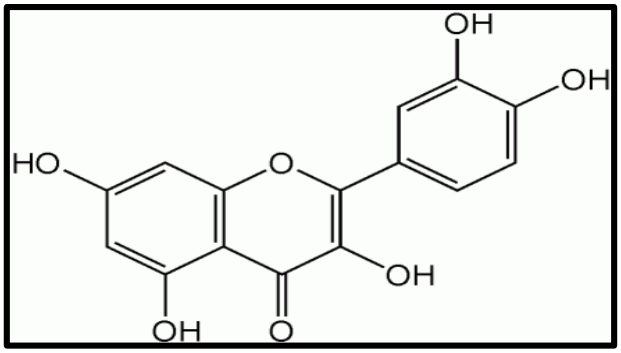


Fig. (1): Chemical structureofQuercetin (3, 5, 7, 3', 4'-pentahydroxyflavone)

MATERIALS AND METHODS

Materials

Quercetin was purchased from Sigma-Aldrich, Singapore and used as received. All reagents used were of technical grade. The absolute ethanol (99.5–99.8%) wasobtainedfrom J.T. Baker (Avantor Performance materials, Phillipsburg, NJ).

Preparation of quercetin nanoparticles

Quercetin nanoparticles were prepared adding ethanol to water volume ratio (1:35), fixed flow rate (8 and 10 ml/min) under magnetic stirring (1000 rpm) by the nano participation technique [30]**.** Ethanol at predetermined concentration of 5mg/ml was used as a solvent to dissolve commercial quercetin. The prepared solution was filled and secured onto a syringe pump. At a fixed flow rate the drug solution was quickly injected in to the anti-solvent (deionized water) of definite volume under magnetic stirring. The nanoparticles of quercetin were filtered and vacuum dried.

Characterization of quercetin nanoparticles

Particle size analysis

Quercetin nanoparticle were diluted with ethanolto ensure that the signal intensity was suitable for the instrument. Particle size was determined by dynamic

light scattering (DLS) using Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK). Nanoparticles were diluted with ethanol (viscosity (cp) 0.1000) and intensity scattered lightwasdetectedatascatteringangleof173°to anincidentbeamatatemperatureof25°C.The polydispersity in dexrange was comprised between 0and1 and the measurements were done in triplicate.

Morphology of the particles

The morphology of samples was observed using a SEM (Quanta 3D FEG/ FEI)with 20 kV, a collector bias of 300 V. The powder samples were spread on a SEM stub and sputtered with gold before the SEM observations.

FTIR spectroscopy analysis

The structure was analyzed by FTIR spectra (FTIR-Nicolet 6700**)**. Samples were ground and mixed with Potassium bromide (KBr) to make pellets. It was compressed under high pressure to prepare pellets of 10.0 mm and 1–2 mm thick. The pellets were scanned over a range of 4000 cm^{-1} to 400 cm^{-1}. FTIR spectra in the transmission mode were recorded using a Nicolet Nexus, connected to a PC, in which the number of scans was 128 and the resolution was 4.

X-ray diffraction analysis

The crystalline state of the samples was estimated by an X-ray diffractometer (D/max r-B, Rigaku, Japan). The experiments were performed in symmetrical reflection mode with a Cobalt (Co)line as thesource of radiation. Standard runs using a 40 kV voltage, a 40 mA current and a scanning rate of 0.02°min^{-1} over a 2 range of 5–40° were used.

Brunauer, Emmet and Teller (BET) surface area

The specific surface area per mass unit (m^2g^{-1}) was determined by means of Brunauer, Emmet and Teller (BET) analysis (adsorption of nitrogen in cryogenic condition) using a Micromeritics Gemini V instrument. Nitrogen adsorption measurements were performed at five different partial pressures (p/p_0 0.10–0.25), temperature of 77.350°C, cross section in 16.200A^2, Liquid density was 0.808 g/cc and sample density was 3.9 g/cc, with a standard deviation between replicate measurements of <1%, as previously described.

RESULT AND DISCUSSION

Preparation and characterization of QU and QUENPs

The preparation based on nano participation technique[30] involved dissolving QU in the solvent (ethanol) at predetermined concentration of 5 mg/ml and injected with syringe pump at fixed flow rate into the anti-solvent (deionized water) under magnetic stirring and then filtered and vacuum dried (Fig. 2 and 3). When resuspended in water, the lyophilized powder formed a very fine dispersion and appeared to be soluble.Unlike original QU, which is completely insoluble in water (Fig. 3). The saturation solubility and dissolution rate of a drug can be increased by reducing the particle size to increase the particle surface area [31]. Therefore, quercetin nanoparticles made by syringe pump with the increased dissolution rate could translate into an enhanced bioavailability upon oral administration[30][32].Previous studies have shown that, for solids dispersed in a liquid medium under agitation, a decrease in particle size results in a thinner hydrodynamic layer around particles and an increase of the surface specific dissolution rate[33].Other study have found a hyperbolic relation between the particle size and the surface specific dissolution rate corrected for solubility[34]. This phenomenon is especially pronounced for materials which have a mean particle size less than 2 mm. At a particle size of 1 mm the intrinsic dissolution rate is very fast, and further decrease in size will lead to no practical advantage in the case of e.g. oral adsorption [35].

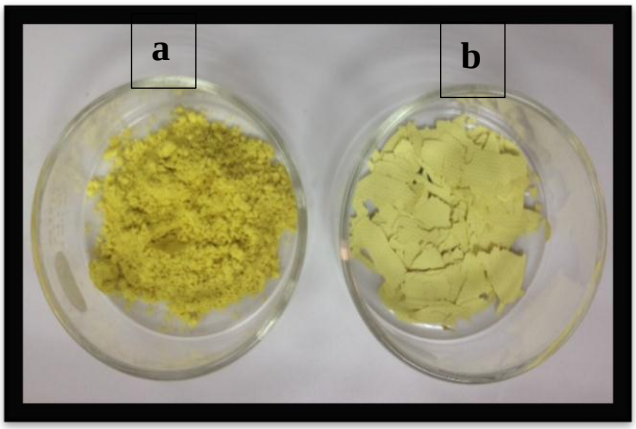


Fig. (2): Image of(a)original Quercetin and (b) Quercetin nanoparticles powder

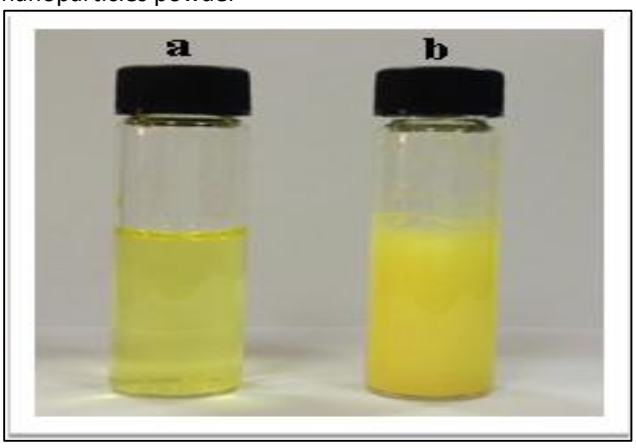


Fig. (3): Solubility of (a)Quercetin nanoparticles and (b) original Quercetin in waterParticle size analysis

The particle size analysis was performed by DLS (Fig. 4 and 5). DLS of the ethanolic dispersion of QUENPs revealed the formation of nanoparticles with an average hydrodynamic diameter of 17.35 and 16.13 nm at flow rate 8 and 10 ml/min, respectively.

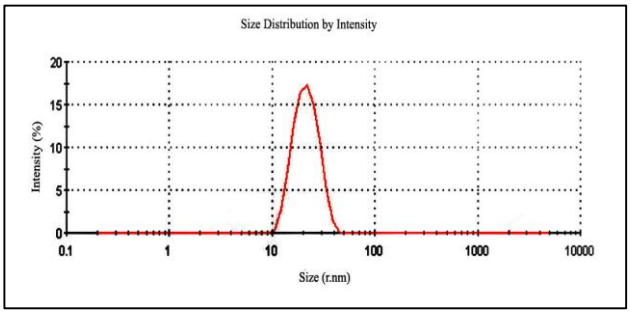


Fig. (4): Size characterization of Quercetin nanoparticles at flow rate (8 ml/min)

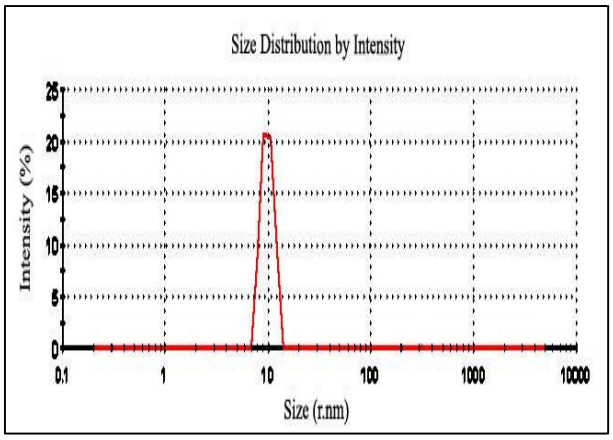


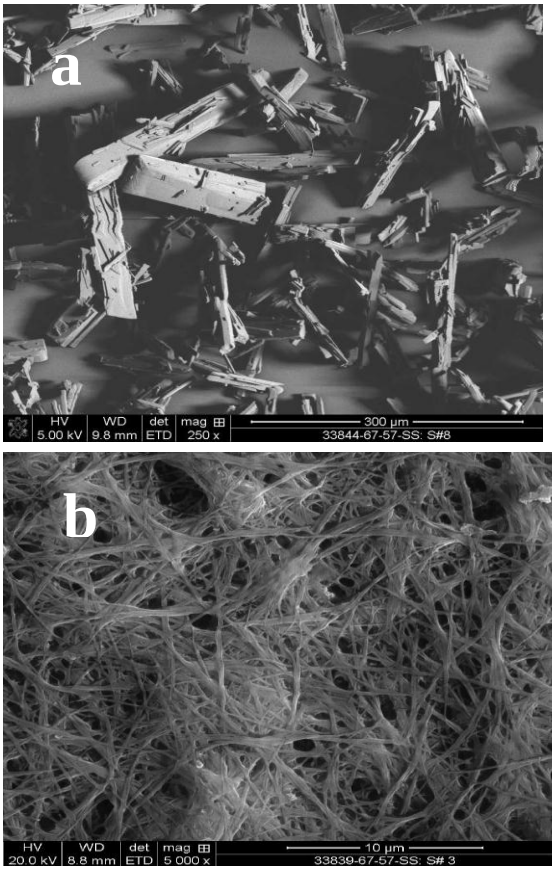
Fig. (5): Size characterization of Quercetin nanoparticles at flow rate (10 ml/min)

SEM analysis

The effects of the quercetin concentration, solvent/anti-solvent ratio (S/SA), stirring speed, and flow rate on the particle diameter and morphology were examined using the SEM (Fig. 6). The description of all the samples discussed is provided in table 1. The original QU powder (Fig. 6 a) exhibited particles lacking uniformity in size was relatively much larger than the QUENPs. On the other hand, QUENPs prepared by syringe pump exhibited particles uniformity in size, less crystanility and absence of larger particles (Fig. 6b,c). The particle diameter of the QUENPs at flow rate 8 ml/ min, S/SA volume ratio 1:35, stirring speed 1000 rpm and drug concentration 5mg/ml was 17.35nm. From table 1, it was observed that the particle diameter decreases from 17.35 nm to 16.13 nm as the flow rate increased from 8 ml/min to 10 ml/min (Fig b and c), respectively. From these results, we conclude that the particle size of the quercetinmade by syringe pump was significantly smaller and more uniform than that of the commercial quercetin, which was more evidenced in the case of sample prepared at lower drug concentration (5 mg/ml), higher stirring speed (1000), higher S/AS volume ratio (1:35) and higher flow rate (10 ml/min). Solvent to anti-solvent ratio, stirring speed and flow rate are important parameters affecting the particle size and increasing the values of these factors reduces the particle size of quercetin to 16.13 nm. The yield of QUENPs at flow rate (8 and 10 ml/min) are presented in table 1. Table 1. Shows that the yield of QUENPs at flow rate (8 and 10 ml/min) were 0.064 and 0.065 mg, respectively. This results agree with the other resultwhich indicated that decreasing the drug concentration, increasing the stirring speed, flow rate and the S/AS volume ratio favored the reduction in the particle diameter.It was investigated that at avolume ratio of 1:25, the quercetin particles diameter was 220 nm[30]. Also, as the flow rate increased from 2 ml/min to 8 ml/min, the particle size decreased from 220 nm to 170 nm. Other study showed that the S/AS volume ratio was decreased to 1:10, the particle diameter was increased to about 486 nm[36].

sample	Quercetin (mg/ml)	S/SA ratio	Stirring speed (rpm)	Flow rate (ml/min)	Particle diameter (nm)	Yield
1	5	1:35	1000	8	17.35	0.064 mg
2	5	1:35	1000	10	16.13	0.065 mg

Table.1: Fabrication conditions of the various quercetin samples prepared by using the syringe pump.



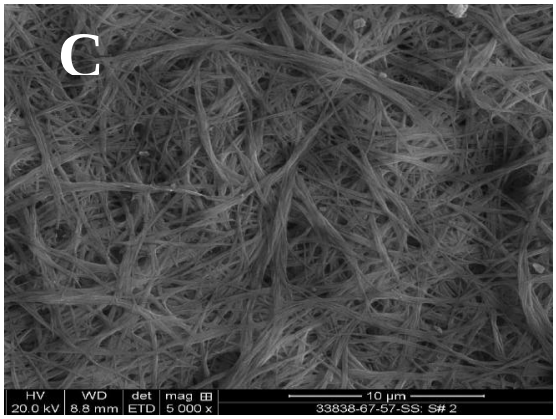
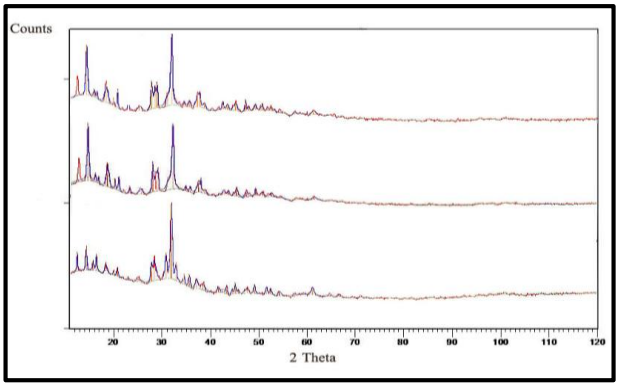


Fig. (6): SEM photographs of(a) original Quercetin, (b) Quercetin nanoparticles at flow rate (8ml/ min) and (c) Quercetin nanoparticles at flow rate (10ml/ min)

(FT-IR) spectroscopy

FTIR analysis is one of the important tools for the quickand efficient identification of encapsulated chemical molecules. Both spectra of QU and QUENPs have the same characteristic peaks but with lower intensity (conc) of sample peaks (Fig 7 a, b, c). This observation indicated that no intermolecular interaction occurred. QU presenting the characteristic intensities of O-H stretch at 3400 cm-1, =C-H stretch at 2930 cm-1, conj C=O stretch at 1680 cm-1, aromatic C=C stretch at 1510 cm-1 and 1610 cm-1and aromatic C-O stretch at 1220 cm-1. The spectra from the QUENPs showed that O-H stretch of QU was disappeared (Fig 7 b, c). These results suggested that intermolecular hydrogen bonding occurred in the QUENPs. Furthermore, this also indicated that the formation of hydrogen bonding in the QUENPs system correlated with the less crystalline compared toQU.There are several studies that have Fig. demonstrated that hydrogen bonding can affect the transformation of drug crystal to amorphous state[37][38][39][40].



(7): FTIR spectra of (a) original Quercetin, (b) Quercetin nanoparticles at flow rate (8ml/ min) and (c) Quercetin nanoparticles at flow rate (10ml/ min)

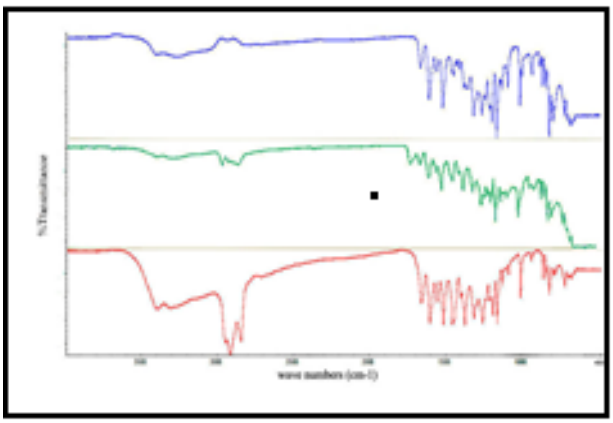


Fig. (8): X-ray diffraction pattern of (a) original Quercetin, (b) Quercetin nanoparticles at flow rate (8ml/ min) and (c) Quercetin nanoparticles at flow rate (10ml/ min)

X-ray diffraction analysis

The representative X-ray diffraction patterns of the original QU and QUENPs at flow rate (8 and 10 ml/min) are shown in Fig 8 a, b and c. The figures indicated the changes in the QU crystal structure. The X-ray patterns of the original QU powder displayed the presence of the numerous distinct peaks at 2θ(12.4662°, 14.4166°, 15.7372°, 16.4365°, 18.4199°, 19.9994°, 20.8063°, 25.0563°, 27.8273°, 28.4001° and 30.8483°) which suggested that the drug was of a high crystalline form. The QUENPs prepared also showed the similar peaks but with slightly different peaks intensities. The QUENPs at flow rate (8 and 10 ml/min) showed peaks at 2θ(12.6803°, 14.6068°, 16.0944°, 16.6714°, 18.6049°, 20.1795°, 20.9740°, 23.2009°, 25.5010°, 27.9646° and 28.9808°) and (12.6593°, 14.5891°, 16.0467°, 16.7004°,18.5869°, 20.1514°, 20.9422°, 21.9380°, 23.1811°, 25.4628° and 27.9501°), respectively. This result indicates that QUENPs prepared withnano participation technique appeared no changes in the crystal structure of the material.The X-ray pattern of the quercetin powder in other study showed the presence of numerous distinct peaks at 2θ of 5.54°, 10.22°, 11.82°,14.18°, 17.24°, 22.16°and 27.48°, which suggested that the drug was of a high crystalline form. The quercetin nanoparticles prepared also showed the similar peaks but with slightly different peak intensities. The quercetin nanoparticles showed peaks at 5.2°, 10.06°, 11.76°, 13.52°, 26.38°, and 27.38°. Some of the peaks were absent. This result indicates that quercetin nanoparticlesmade by syringe pump possessed lower crystallinity than the raw quercetin powder. Besides that, no significant effect of the quercetin concentration, solvent/anti-solvent ratio, stirring speed, and flow rate on the crystallinity of the samples produced by the syringe pump[30].Similar findings were also observed by other studies[31][41]. Also, It has been

reported thatif water is used as an anti-solvent rather than any organic solvent, the product is more crystalline in nature [42]. This is because of the fact that the amorphous form is easily converted to its crystalline state in the presence of water.Other study showed that the characteristic peaksof QU exhibited at a diffraction angle of 2θ, 10.73°, 12.33°, 15.87°, 24.41°, 26.50°, and 27.40°, can be inferred to traits of a high crystalline structure. The crystalline structure ofthe drug still persisted. Moreover, there were no characteristic peaks appearing on the patterns of all lyophilized QUENPs du to different nanoprecipitation technique for preparation[39]. Bunauer, Emmet and Teller (BET). The surface area of original QU and QUENPs were performed by BET. Original QU surface area was 1.591 m2/g. On the other hand, surface area of QUENPs at flow rate (8 and 10 ml/min) was 74.611m2/g and 76.060m2/g, respectively. The increased in surface area of QUENPs might be due to the decreased in particle size of QUENPs.Similar findings were also observed by other studies [31][40] .

CONCLUSION

This study demonstrated that theLiquid solvent/anti-solvent precipitation method is able to produce quercetin nanoparticles with the significantly smaller particle size by using a new solvent/ anti-solvent (S/AS) ratio (1:35) and thus, the higher percent dissolution in water as compared to the original QUpowder. The original idea of making very small drug particles in order to improve the bioavailability and make drugs more effective. We suggest that the QUENPs may be applied in clinical setting and warrant further studies.

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