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Effect of nanotization on therapeutic activities of biomolecules: Synthesis and Characterization

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ABSTRACT

The bioactive organic nanoparticles were synthesized by precipitation-dialysis method from their corresponding bulk forms. The morphology and average particle size of the synthesized nanoparticles were characterized by scanning electron microscope (SEM) and dynamic light scattering (DLS) techniques. The nanotization decreases the particles size by 2-20 times with a narrow range of distribution and also considerable change in the shape. Further, the nanoparticles (**1-10)** and their bulk forms (**1B-10B**) were evaluated for *in vitro* antioxidant, anti-inflammatory and antimicrobial activities. The biological assay data revealed that new organic nanoparticles showed enhanced biological activity compared to their corresponding bulk forms. This may be due to increase in the surface area and decrease in the particles size after nanotization.

Keywords: Nanoparticles; Dialysis; Quinazolinone; Dipeptides; Bioactivity.

INTRODUCTION

The application of nanomaterials ranging from 1 to 1000 nanometers (nm) is an emerging area of nanoscience and nanotechnology. The problem associated in the area of catalysis, medicines, solar energy conversion and water treatment were successfully solved by use of nanomaterials (Dahl et al . 2007; Hutchison 2008). Compare to their macro-scaled counterparts nanomaterials shows considerable changes in their physical, chemical and biological properties (Li et al. 2001). Synthesis and use of nanoparticles of noble metal in the field of electronics, optics, environment and biotechnology is a constant area of interest for researcher (Hussain et al. 2003; Burleson et al. 2004; Cheng 2004; Obare and Meyer 2004; Yuan 2004; Masciangioli and Zhang 2003; Albrecht et al. 2006). In the field of photography, biological labeling, photonics and optoelectronics the stable and dispersed nanoparticles of gold, silver and copper were used (Smith et al. 2006; Kearns et al. 2006).

Inclusion of nanotechnology and nanoscience with medicine presents an outstanding opportunity for developing novel materials that can significantly improve treatment and diagnosis of diseases (Alshehri et al. 2015). The nanoparticles which provide large surface area become ideal candidates for high efficacy in both diagnostics and therapeutics. Further, it is categorized into nanocarrier, for carrying conventional drugs and, nanodrugs with direct curing of target disease (Yang et al. 2014). There are numerous methods were reported in the literature for the synthesis of nanoparticles among them nanoprecipitation and solvent evaporation were most widely accepted methods, but these methods associated with several problem including removal of non-chlorinated solvent and surfactants. To overcome these difficulties, researcher developed dialysis method to prepare the nanoparticles.

We are the research group working on conjugation of amino acids/peptides to small bioactive molecules which is now considered as a promising and successful approach for developing new lead candidates with enhanced potency. These have shown diverse biological applications including antimicrobials, anti - inflammatory, antiglycation, antiurease, antioxidants and H^+/K^+-ATPase inhibitors (Suhas et al. 2012a; Suhas et al. 2012b; Shantharam et al. 2013; Vardhan et al. 2013; Kumara and Gowda 2016; Sharma et al. 2013). In continuation of this project, we have now directed our systematic effort towards development of bioactive organic nanoparticles from their corresponding bulk forms. So the present study involves the synthesis, characterization and biological studies of organic nanoparticles followed by correlating the biological activity to size of nanoparticles.

MATERIALS AND METHODS

Dialysis membrane with a molecular weight cutoff 1.0 kDa was purchased from Bio Design Inc. (New York). The reagents used for biological assays were procured from Sigma Aldrich (India). All the reagents and solvents used for the synthesis were of analytical grade.

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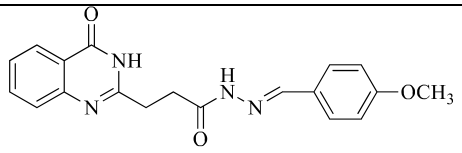
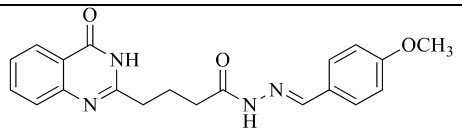
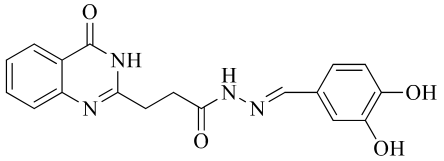
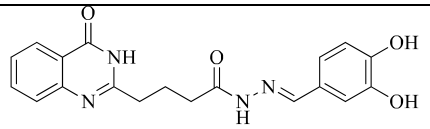
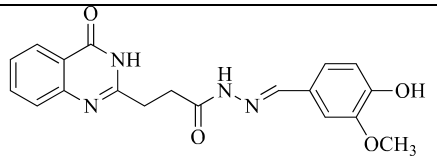
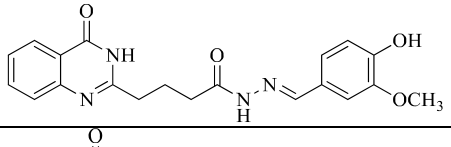
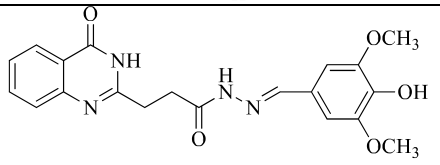
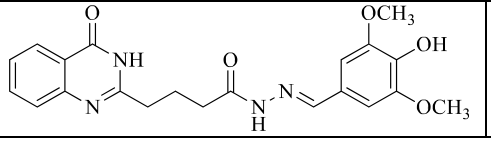
Table 1: Physical and analytical data of synthesized nanoparticles and their bulk forms

Sl. No.	Structure	R_f values		m.p. °C	Theoretical Mol. Wt.	Actual MS values (M^+)
		R_f^a	R_f^b			
1	Boc-Lys(2-ClZ)-Asp(OBzl)-OBzl	0.65 (0.63)	0.83 (0.81)	97-99 (95-97)	710.2130 (710.2130)	710.3890 (710.3890)
2	Boc-Lys(2-ClZ)-Trp-OMe	0.63 (0.62)	0.78 (0.78)	96-98 (95-97)	615.1170 (615.1170)	615.3478 (615.3478)
3		0.56 (0.54)	0.62 (0.61)	195-197 (194-196)	350.1365 (350.1365)	350.2687 (350.2687)
4		0.55 (0.52)	0.63 (0.62)	234-236 (235-238)	364.2104 (364.2104)	364.2456 (364.2456)
5		0.49 (0.50)	0.56 (0.58)	210-212 (209-211)	352.1245 (352.1245)	352.6591 (352.6591)
6		0.52 (0.53)	0.59 (0.60)	220-222 (218-220)	366.8452 (366.8452)	366.9615 (366.9615)
7		0.52 (0.54)	0.57 (0.59)	222-224 (222-224)	366.3812 (366.3812)	366.9615 (366.9615)
8		0.54 (0.54)	0.59 (0.60)	238-240 (238-241)	380.2145 (380.2145)	380.2456 (380.2456)
9		0.59 (0.56)	0.68 (0.69)	204-206 (204-205)	396.4062 (396.4062)	396.4529 (396.4529)
10		0.57 (0.56)	0.64 (0.65)	224-226 (222-225)	410.3654 (410.3654)	410.4513 (410.4513)

[a]The values in parentheses corresponds to their bulk form

The solvent system comprising chloroform/methanol/acetic acid in the ratio 98:02:03 (R_f^a) and 95:05:03 (R_f^b) was used to determine the R_f value. Melting points were determined on a Superfit melting point apparatus (India) and are uncorrected. The morphology of nanoparticles was examined by SEM, Hitachi (Japan) and the mean particle size was determined by DLS, Microtrac Bluewave (USA).

Synthesis of nanoparticles

The bulk forms of dipeptides (1B and 2B) and hydrazones of quinazolinone (3B-10B) were synthesized and characterized by following our earlier reports (Kumara and Gowda 2016; Rakesh et al. 2015). The organic nanoparticles (1-10) were prepared from their bulk forms by literature reported dialysis method (Fessi et al. 1989; Jeong et al. 2001; Kostog et al. 2010; Jeon et al. 2000) with slight modification. The bulk forms of samples (0.5 mmol) were dissolved separately in 100 mL of dimethyl sulfoxide (DMSO). The resulting solution was taken in a 1000 molecular weight cut-off dialysis tubing and was immersed into the external aqueous phase of nanopure water. The external aqueous phase was allowed to constant stirring to aid the diffusion and

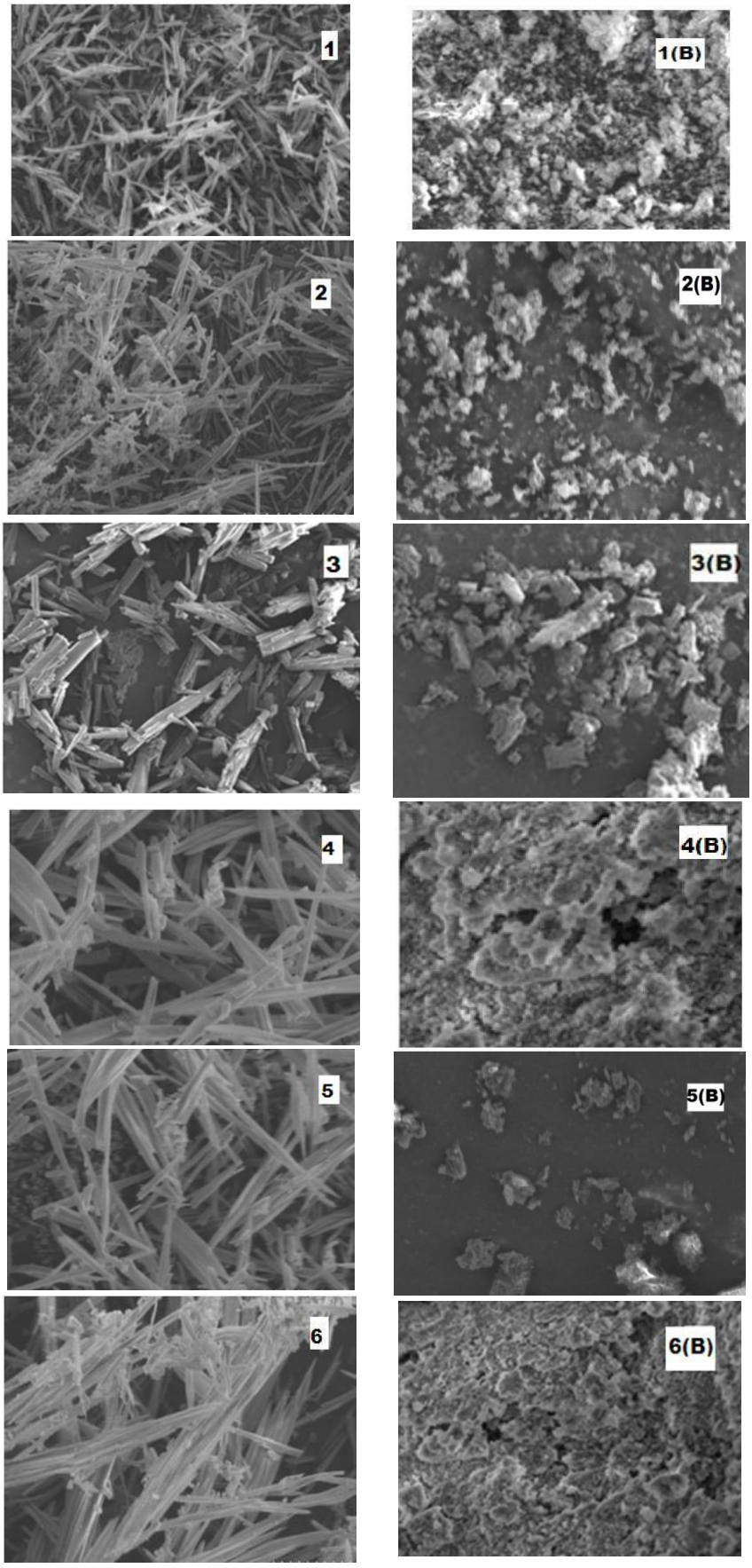
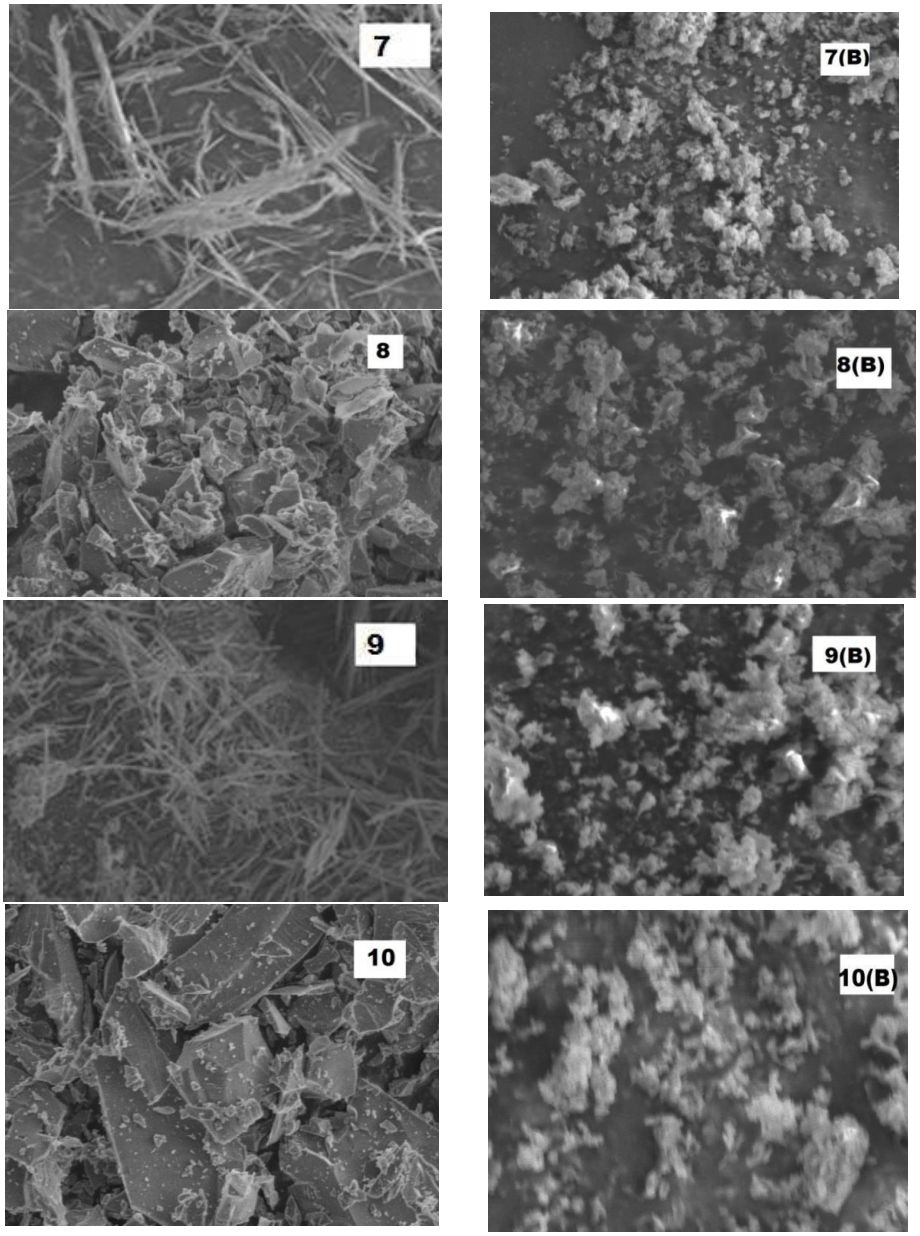


Figure 1: SEM images of nanoparticles and their corresponding bulk forms. Right panel shows the nanotized compounds. Left panel indicates the corresponding bulk forms



B= their corresponding bulk forms

Figure 1: SEM images of nanoparticles and their corresponding bulk forms. Right panel shows the corresponding bulk forms. Left panel indicates the nanotized compounds

replaced at an interval of 3 to 4 h for a period of 24 h. The organic solvent diffused out of the membrane and water diffused inside during the dialysis process, leads to precipitation of organic molecules which in turn self-assembled to form nanoparticles. Then, the sample in the dialysis membrane was collected and centrifuged at 11500 rpm for 30 minutes in Kemi Centrifuge shrc-1 (India). The nanoparticales were obtained as pellet and further freeze-dried to obtain 1-10.

Scanning electron microscope

The scanning electron microscopy (SEM) observation was performed with EVO LS 15 Hitachi (Japan) equipped with an energy dispersive X-ray spectrometer. The sample placed on the aluminum holder stub using a double sticky carbon tape and it's coated with gold and electrically grounded. Then the sample was dried at 60 °C for overnight in the drying oven. Acquire the image electronically and save.

Dynamic Light Scattering

The particle diameter of sample was measured by dynamic light scattering (DLS) performed on a Microtrac Bluewave (USA). The sample was prepared by taking 1 mg of the sample in 10 mL of distilled water. For analysis, a minimum of 2 mL of the prior prepared suspension has to be transferred into a 4.5 mL disposable plastic cuvette, placed in the analysis device and subsequently analyzed for size analysis. For each sample, five scans were collected and the averages were calculated.

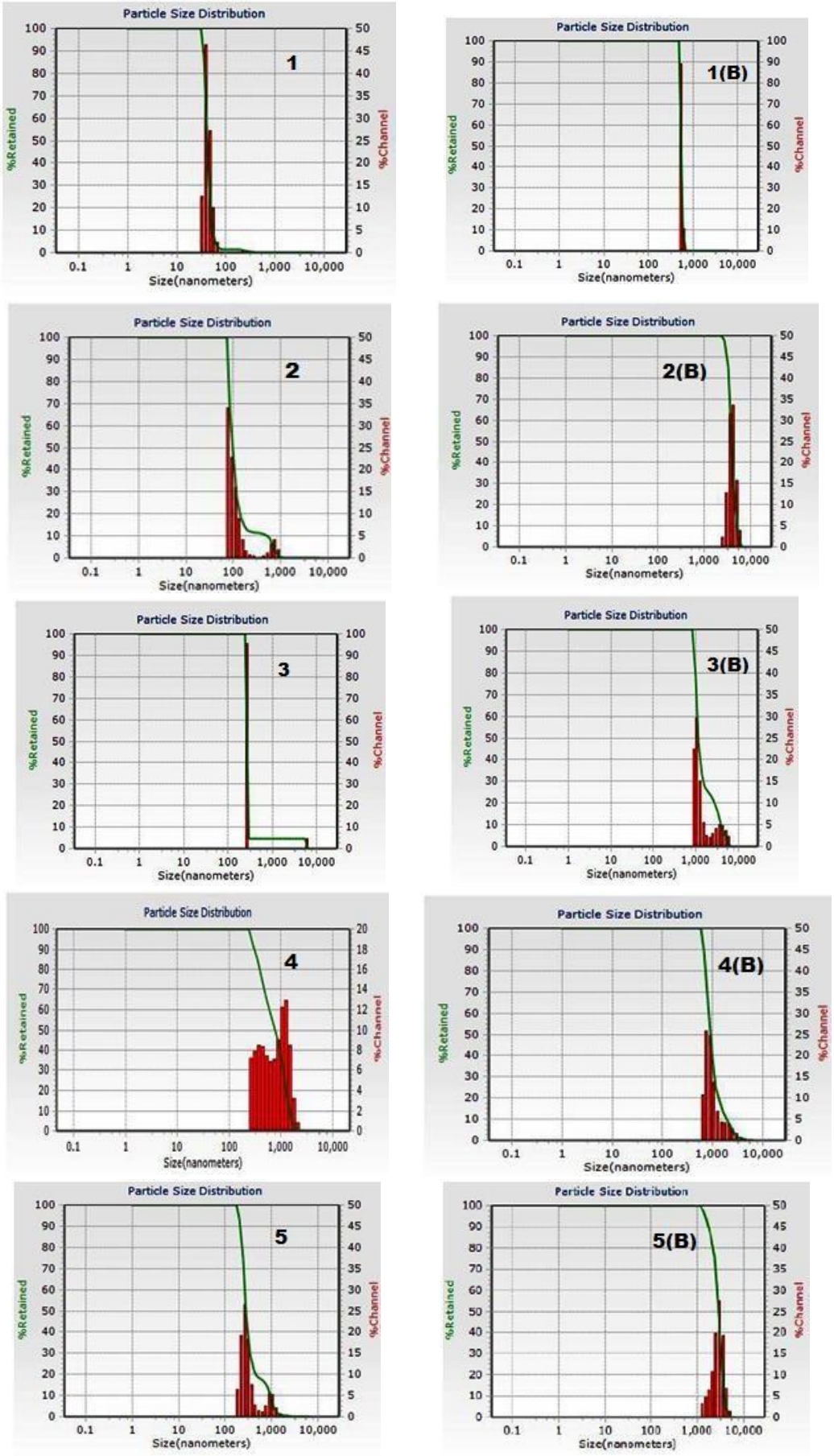


Figure 2: Average particle size distribution of nanoparticles and their bulk forms. Right panel shows the corresponding bulk forms. Left panel indicates the nanotized compounds

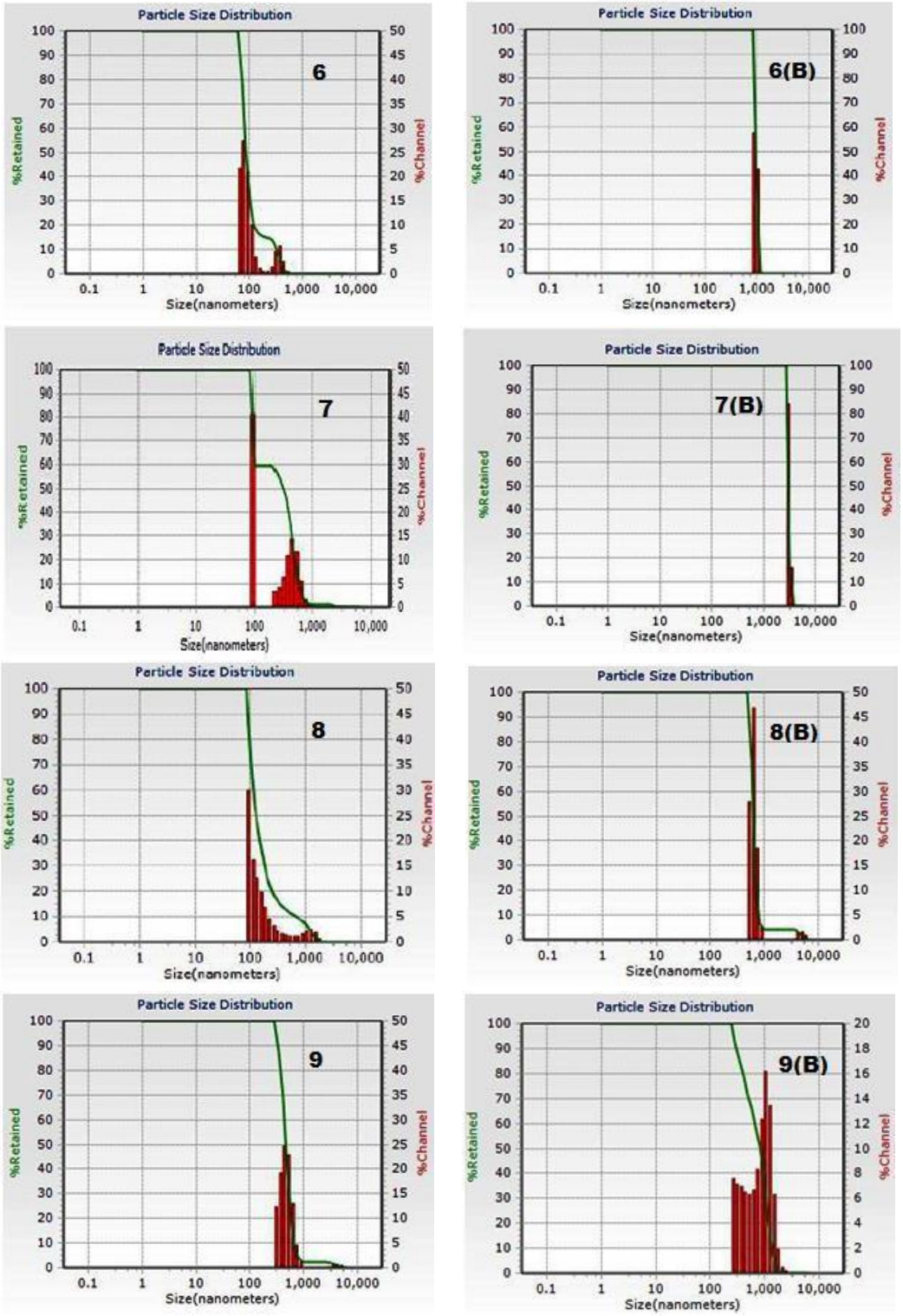


Figure 3: Average particle size distribution of nanoparticles and their bulk forms. Right panel shows the corresponding bulk forms. Left panel indicates the nanotized compounds

RESULTS AND DISCUSSION

The bulk form and nano form of dipeptides and hydrazones of quinazolinone were synthesized and characterized by R_f values, m.p. and spectroscopic techniques like IR, NMR and mass spectrometry. Both the forms have the same physical and spectroscopic data indicating there is no change in structures upon nanotization. The R_f values, m.p. and mass spectral data of both the forms are tabulated in **Table 1**. The ^1H NMR and ^{13}C NMR values are in complete agreement with articles (Kumara and Gowda 2016; Rakesh et al. 2015). To the best of our knowledge, this is the first report on synthesis of bioorganic nanoparticles wherein the biological activities has been compared with their corresponding bulk forms.

Table 2: Antioxidant and anti-inflammatory activities of the synthesized nanoparticles and their corresponding bulk forms[a]

Entry	Antioxidant activities IC_{50} (µg/mL)[b]			Anti-inflammatory activity IC_{50} (µg/mL)[b]
	DPPH	DMPD	ABTS	
1	130±1.65 (250±2.56)	60±1.35(240±2.65)	85±1.35 (200±2.45)	145±0.98 (240±1.20)
2	50±0.96 (115±2.09)	75±1.35(140±2.56)	45±2.06 (125±1.24)	65±1.35 (150±1.54)
3	60±0.54 (75±0.62)	60±2.98(95±1.26)	45±0.94 (75±1.35)	235±2.30 (300±1.85)
4	55±2.35 (80±1.23)	65±0.98(80±2.13)	50±1.80 (65±2.65)	225±1.85 (300±2.65)
5	30±1.65 (45±0.95)	25±1.23(35±1.64)	25±1.28 (40±1.65)	100±1.65 (160±1.45)
6	35±1.85 (40±1.54)	40±1.65(45±1.74)	40±0.64 (45±0.68)	125±1.36 (180±0.98)
7	40±0.65 (45±1.26)	45±0.67(50±1.34)	45±1.35 (60±1.75)	200±1.98 (240±1.65)
8	45±1.20 (60±1.25)	50±1.65(65±0.94)	40±0.65 (55±1.65)	100±1.20 (160±1.28)
9	35±0.89 (40±0.92)	60±1.25(80±1.23)	25±0.46 (40±1.85)	100±1.84 (175±1.69)
10	35±1.10 (50±2.56)	35±2.31(60±1.85)	40±1.65 (45±1.25)	75±1.35 (100±1.58)
AA	**50±1.26**	**65±1.65**	**55±0.69**	-
GA	**50±1.85**	**55±1.67**	**60±0.98**	-
Indomethacin	-	-	-	**60±1.65**
Ibuprofen	-	-	-	**65±1.98**

[a] The values in parentheses corresponds to their bulk forms

[b]Values are mean of three determination, the ranges of which are >5% of the mean in all cases

Table 3: Antimicrobial activity of the synthesized nanoparticles and their corresponding bulk forms[a]

Entry	Antibacterial activity (Zone of Inhibition in mm)[b]		Antifungal activity (Zone of Inhibition in mm)[b]	
	E. coli	*S. aureus*	*A. niger*	*F. oxysporum*
1	9±0.13 (5±0.07)	14±0.20 (9±0.10)	10±0.21 (6±0.08)	13±0.32 (9±0.12)
2	8±0.11 (6±0.05)	15±0.18 (10±0.25)	7±0.15 (4±0.02)	13±0.19 (8±0.09)
3	7±0.11 (3±0.05)	6±0.02 (4±0.06)	9±0.12 (5±0.09)	7±0.21 (3±0.05)
4	5±0.04 (2±0.07)	7±0.11 (3±0.02)	6±0.23 (4±0.05)	9±0.19 (5±0.05)
5	13±0.24 (10±0.14)	14±0.21 (12±0.15)	14±0.16 (9±0.22)	15±0.24 (11±0.25)
6	6±0.11 (5±0.12)	9±0.09 (7±0.13)	7±0.13 (2±0.06)	8±0.14 (5±0.15)
7	14±0.09(10±0.26)	12±0.16 (9±0.18)	15±0.08 (12±0.21)	13±0.24 (10±0.28)
8	13±0.16 (8±0.13)	10±0.05 (7±0.12)	11±0.15 (6±0.10)	12±0.11 (8±0.32)
9	9±0.15 (5±0.06)	9±0.20 (6±0.13)	8±0.25 (4±0.13)	10±0.30 (5±0.12)
10	8±0.09 (6±0.01)	10±0.11 (8±0.05)	9±0.21 (7±0.11)	13±0.09 (9±0.16)
Streptomycin	**12±0.15**	**13±0.16**	-	-
Bavistin	-	-	**13±0.21**	**14±0.19**

[a] The values in parentheses corresponds to their bulk forms

[b]Values are mean of three determination, the ranges of which are >5% of the mean in all cases

SEM analysis

The surface morphology of the synthesized nanoparticles (1-10) and their corresponding bulk forms (1B-10B) are shown in Figure 1. The SEM images of samples obtained before the dialysis shows the aggregate foam like particles with structural morphology very ambiguous and incomplete (right panel, Fig 1). The morphology of particles obtained after dialysis consist of elongated tubular structure and morphology is very well defined (left panel, Fig 1).

Figure 1: SEM images of nanoparticles and their corresponding bulk forms. Right panel shows the corresponding bulk forms. Left panel indicates the nanotized compounds.

DLS analysis

The average particle size of bioorganic molecules before and after dialysis was measured and presented in **Figure 2.** The average particle size of bulk forms were found to be around 500-10000 nm which decreases to 40-500 nm after dialysis. It indicates 2-20 times decrease in particle size after dialysis. This clearly indicates the conversion of bulk form of particles into nanoform.

Biological activities

The *in vitro* antioxidant activity, anti-inflammatory activity and antimicrobial activity of the synthesized nanoparticles (1-10) and their bulk forms (1B-10B) were evaluated by following literature methods (Perez et al.1990; Singh and Singh 2000; Shinde et al. 1999; Blois 1958; Gulcin 2010; Re et al. 1999). The IC_{50} values (µg/mL) for antioxidant and anti-inflammatory activities and zone of inhibition in mm for antimicrobial activities are tabulated in Table 2 and Table 3 respectively.

In all the three antioxidant assays there is a considerable difference in the IC_{50} values of nanoparticles and their corresponding bulk forms and the values were found to be significantly lower for former compared to later in all the three assays. All the synthesized nanoparticles showed good anti-inflammatory activity compared to their corresponding bulk forms. The antimicrobial activity results revealed that all nanoparticles showed enhanced zone of inhibition compared to their bulk forms. This enhanced biological activity of nanoparticles may be due to small size, shape and high surface area (i.e. surface area to mass ratios). These physically small sized nanoparticles create a strong possibility of their interaction with biological system compared to micro sized molecules (Tiwari et al. 2013; Aggarwal et al. 2009; Desai et al. 1996).

CONCLUSION

The development of new therapeutic agents is time consuming process and hence nanotechnology provides opportunity to develop new diagnostic tools from already existing drug candidates. From this tool we can synthesize finest small sized molecules with improved biological activity and also enhanced bioavailability. These hitherto primary investigations of nanotized molecules have proven to be effective therapeutic agents. The studies focus the preliminary stage and our research group is presently working on more applications on these nano based structures. Hence these could be considered as new lead structures in the drug discovery.

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REFERENCES

Aggarwal P, Hall JB, McLeland CB, Dobrovolskaia MA, McNeil SE (2009) Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. Advanced Drug Delivery Reviews 61:428–437

Albrecht MA, Evans CW, Raston CL (2006) Green chemistry and the health implications of nanoparticles. Green Chem 8:417−432

Alshehri R, Ilyas AM, Hasan A, Arnaout A, Ahmed F, Mamic A (2016) Carbon nanotubes in biomedical applications: factors, mechanisms, and remedies of toxicity. J Med Chem 59:8149–8167

Blois MS (1958) Antioxidant determinations by the use of a stable free radical. Nature 181:1199–1200

Burleson DJ, Driessen MD, Penn RL (2004) On the characterization of environmental nanoparticles. J Environ Sci Health A 39:2707−2753

Cheng M-D (2004) Effects of nanophase materials (≤20 nm) on biological responses. J Environ Sci Health A 39:2691−2705

Dahl JA, Maddux BLS, Hutchison JE (2007) Toward greener nanosynthesis. Chem Rev 107:2228−2269

Desai MP, Labhasetwar V, Amidon GL, Levy RJ (1996) Gastrointestinal uptake of biodegradable microparticles: effect of particle size. Pharm Res 13:1838–1845

Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S (1989) Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm 55:R1–R4

Gulcin I (2010) Antioxidant properties of resveratrol: a structure – activity insight. Innov food sci & emerg technol 11:210–218

Hussain I, Brust M, Papworth AJ, Cooper AI (2003) Preparation of acrylate-stabilized gold and silver hydrosols and gold-polymer composite films. Langmuir 19:4831−4835

Hutchison JE (2008) Greener nanoscience: a proactive approach to advancing applications and reducing implications of nanotechnology. ACSNano 2:395−402

Jeon HJ, Jeong YI, Jang MK, Park YH, Nah JW (2000) Effect of solvent on the preparation of surfactant-free poly(dl-lactide-co-glycolide) nanoparticles and norfloxacin release characteristics. Int J Pharm 207:99–108

Jeong YI, Cho CS, Kim SH, Ko KS, Kim SI, Shim YH, Nah JW (2001) Preparation of poly(dl-lactide-co-glycolide) nanoparticles without surfactant. J Appl Polym Sci 80:2228–2236

Kearns GJ, Foster EW, Hutchison JE (2006) Substrates for direct imaging of chemically functionalized SiO_2 surfaces by transmission electron microscopy. Anal Chem 78:298−303

Kostog M, Kohler S, Liebert T, Heinze T (2010) Pure cellulose nanoparticles from trimethylsilyl cellulose. Macromol Symp 294:96–106

Kumara HK, Gowda DC (2016) Synthesis and SAR studies of bisthiourea derivatives of dipeptides Lys/lys - Asp, Lys/lys-Trp conjugated benzo[d]isoxazole as promising antioxidants. Int J Pept Res Ther doi: 10.1007/s10989-016-9557-1

Li L, Hu J, Alivistos AP (2001) Band gap variation of size and Shape-Controlled Colloidal CdSe Quantum Rods. NanoLett 1:349−351

Masciangioli T, Zhang W-X (2003) Environmental technologies at the nanoscale. Environ Sci Technol 37:102A-8A

Obare SO, Meyer GJ (2004) Nanostructured materials for environmental remediation of organic contaminants in water. J Environ Sci Health A 39:2549−2582

Perez C, Pauli M, Bazerque P (1990) An antibiotic assay by agar-well diffusion method. Acta Biol Med Exp 15:113–115

Rakesh KP, Manukumar HM, Gowda DC (2015) Schiff's bases of quinazolinone derivatives: synthesis and SAR studies of a novel series of potential anti - inflammatory and antioxidants. Bioorg Med Chem Lett 25:1072-1077

Rao JP, Geckeler KE (2011) Polymer nanoparticles: preparation techniques and size-control parameters. Prog Polym Sci 36:887–913

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26:1231–1237

Shantharam CS, Vardhan DMS, Suhas R, Sridhara MB, Gowda DC (2013) Inhibition of protein glycation by urea and thiourea derivatives of glycine/proline conjugated benzisoxazole analogue – synthesis and structure-activity studies. Eur J Med Chem 60:325–332

Sharma A, Suhas R, Chandan KV, Banu SH, Gowda DC (2013) tert-Butyl 1,5-bis(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)-1,5-dioxopentan-2-ylcarbamate urea/thiourea derivatives as potent H+/K+-ATPase inhibitors. Bioorg Med Chem Lett 23:4096–4098

Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN (1999) Membrane stabilizing activity – a possible mechanism of action for the anti-inflammatory activity of Cedrus deodara wood oil. Fitoterapia 70:251–257

Singh I, Singh VP (2000) Antifungal properties of aqueous and organic solution extracts of seed plants against Aspergillus flavus and A. Niger. Phytomorphology 50:151–157

Smith AM, Duan H, Rhyner MN, Ruan G, Nie S (2006) A systematic examination of surface coatings on the optical and chemical properties of semiconductor quantum dots. Phys Chem Chem Phys 8:3895−3903

Suhas R, Chandrashekar S, Gowda DC (2012) Synthesis of uriedo and thiouriedo derivatives of peptide conjugated heterocycles – a new class of promising antimicrobials. Eur J Med Chem 48:179-191

Suhas R, Gowda DC (2012) Design and synthesis of tryptophan containing peptides as potential analgesic and anti-inflammatory agents. J Pept Sci 18:535–540

Tiwari MN, Agarwal S, Bhatnagar P, Singhal NK, Tiwari SK, Kumar P, Chauhan LKS, Patel DK, Chaturvedi RK, Singh MP, Gupta KC (2013) Nicotine-encapsulated poly(lactic-co-glycolic) acidnanoparticles improve neuroprotective efficacy against MPTP-induced parkinsonism. Free Radical Biology and Medicine 65:704–718

Vardhan DMS, Shantharam CS, Suhas R, Sridhara MB, Gowda DC (2013) Synthesis and urease inhibition studies of ureas and thioureas derived from amino acids conjugated heterocycle. Int J Chem Pharm Sci 4:65–69

Yang Z, Kang S, Zhou R (2014) Nanomedicine: de novo design of nanodrugs. Nanoscale 6: 663−677

Yuan G (2004) Environmental nanomaterials: occurrence, syntheses, characterization, health effect and potential applications. J Environ Sci Health A 39:2545−2548