EFFECTS OF HOMOGENISATION AND ULTRASONICATION ON CURCUMIN-CHITOSAN NANOPARTICLES

L.H. Chuah[1], N. Billa[1]*, M. Sivakumar[2]

[1]School of Pharmacy, University of Nottingham Malaysia Campus
Jalan Broga, 43500 Semenyih, Selangor, Malaysia.

[2]School of Chemical and Environmental Engineering
University of Nottingham Malaysia Campus, Jalan Broga
43500 Semenyih, Selangor, Malaysia.

***(Nashiru.Billa@nottingham.edu.my)**

ABSTRACT : Homogenisation and ultrasonication are widely used techniques to produce nanoparticles. In the present study, we compared the physical characteristics of curcumin-chitosan nanoparticles produced by these two processes and the combination of both. Parameters studied included homogenisation and ultrasonication speed and amplitude respectively, as well as duration in each instance. The decrease in mean particle size from homogenisation alone to the combination of both methods were calculated to be 29.6 % and 18.6 % for 5 minutes homogenisation and 10 minutes homogenisation respectively. On the other hand, the decrease in mean particle size from ultrasonication alone to the combination method were 30.47 % and 31.40 % for 5 minutes homogenisation and 10 minutes homogenisation respectively. Increase in zeta potential was also observed with the combination method. Hence it may be concluded that the combination method of homogenisation and ultrasonication produces better curcumin-chitosan nanoparticles with superior physical characteristics in terms of size and zeta potential compared to either method alone.

KEYWORDS : Curcumin, nanoparticles, ultrasonication, homogenisation

INTRODUCTION

"Nano" formulation has become an attractive means of delivery due to the several attributes associated with nanoparticles which include higher drug load capacity, high stability, ability to incorporate both hydrophilic and hydrophobic drug entities, feasibility for various different routes of administration, as well as formulation for controlled drug release. In order to exploit these attributes however, the formulator must choose between the right balance of formulation and processing variables. In the present study, processing variables related to homogenisation and ultrasonication techniques were studied in the production of curcumin-containing nanoparticles made from chitosan.

Curcumin (diferuloylmethane) is a hydrophobic polyphenol derived from the rhizome of the traditional Indian herb known as turmeric *(Curcuma longa)*. It has been shown to possess a wide spectrum of therapeutic actions including anti-inflammatory, antioxidant, antifungal, antiviral, antidiabetic as well as anticancer activities (Aggarwal *et al.*, 2007), and in this regard, its use in colon cancer treatment is of particular interest. However, the oral bioavailability of curcumin is so low that even oral ingestion of 8 g curcumin resulted in approximately 0.5-2 μM of curcumin peak levels in blood plasma (Cheng *et al.*, 2001). Hence, various formulations of curcumin have emerged in an attempt to improve its oral bioavailability, including poly(butyl)cyanoacrylate nanoparticles (Mulik *et al.*, 2009), PLGA nanoparticles (Shaik *et al.*, 2009), alginate-curcumin-pluronic composite nanoparticles (Das *et al.*, 2007), and curcumin-phospholipid complex (Maiti *et al.*, 2007). We hypothesise that by incorporating curcumin into colon-specific mucoadhesive nanoparticulate drug delivery systems, it may be possible to limit the activity of the latter to the colon for localised effects. This strategy is expected not only to minimize side-effects arising from unrestrained activity from curcumin, but is also likely to improve the efficacy of the drug due to possible mucoadhesion within the colon.

Chitosan is used in the present study due to its biocompatibility, biodegradability, non-toxicity as well as mucoadhesive properties (Kumar *et al.*, 2000). Moreover, it can be formulated as controlled release matrix. It has been widely used as carrier in drug delivery, either on its own or in combination with other polymers. Some applications of chitosan in drug delivery including chitosan – polyethylene glycol (PEG) nanocapsules (Prego *et al.*, 2006), chitosan-polyethylene oxide (PEO) nanoparticles (Calvo *et al.*, 1997) and polymeric chitosan based vesicle encapsulated in liposome (McPhail *et al.*, 2000).

The physical characteristics of nanoparticles are influenced by the physicochemical properties of the drug as well as processing variables. Saether *et al.*, (2008) investigated the effects of several parameters on polyelectrolyte complex formation between alginate and chitosan. The various parameters studied include homogenisation speed, diameter of the dispersing element, charge ratio of alginate to chitosan, mixing order of the two compounds

as well as some molecular parameters. They reported that smaller particle sizes were obtained with increased diameter of dispersing element as well as homogenising speed (Saether *et al.*, 2008). On the other hand, Mulik *et al.*, (2009) studied the effects of stirring speed and stirring time. They reported that increased stirring time decreases the particle size initially, but increases the size after a longer time due to aggregation of the particles (Mulik *et al.*, 2009).

In this study, we have investigated the influences of homogenisation speed and time along with ultrasonication on the physical properties of the nanoparticles produced. These parameters will in turn be used for optimisation of the formulation and processing parameters.

MATERIALS AND METHODS

Materials

High purity curcumin and low molecular weight chitosan were purchased from Sigma Aldrich, USA and used as such. Sodium hydroxide was from Merck chemicals, Germany while glacial acetic acid was from Fisher Scientific, UK.

Methods

Preparation of curcumin-chitosan nanoparticles

Curcumin dissolved in 0.1 M NaOH was added dropwise into chitosan which was dissolved in 2 % (v/v) acetic acid in a ratio of 1 to 5 respectively, under stirring for 10 minutes. The resultant mixture was subjected to either homogenisation, ultrasonication or the combination of both processes. Homogenisation was conducted using Ultraturrax homogeniser (IKA, Germany) with a T25 dispersing element; whilst ultrasonication was carried out using Sonics Vibra Cell (Sonics & Materials Inc., USA). The mixtures containing formed nanoparticles were incubated for 2 hours at room temperature prior to further investigation. Table 1 shows the parameters studied during the production of the nanoparticles.

Table 1. Parameters of homogenisation and ultrasonication

Sample	Homogenisation Time (min)	Homogenisation Speed (rpm)	Ultrasonication Time (min)	Ultrasonication Amplitude (%)
S1	5	9,500	-	-
S2	5	17,500	-	-
S3	5	24,000	-	-
S4	2.5	17,500	-	-
S5	10	17,500	-	-
U1	-	-	1	20
U2	-	-	1	30
U3	-	-	1	40
C1	5	17,500	1	40
C2	10	17,500	1	40

Size and zeta potential measurement

Zeta Sizer Nano series (Malvern instruments, UK) was used to measure the size and zeta potential of the nanoparticles. The nanoparticle suspension was centrifuged at 14800 rpm for 30 minutes. The supernatant was discarded and the nanoparticles were collected from the bottom of the centrifuge tube and then redispersed in purified water for size and zeta potential measurement. A replica of three assessments was taken to characterise each reading.

RESULTS AND DISCUSSION

The mean particle size and PdI values of the samples were presented in Table 2

Table 2. Effects of homogenising speed on size and PdI of nanoparticles

Sample	Homogenisation Speed for 5 min (rpm)	Size (nm)	Pdi value
S1	9,500	652.4	0.617
S2	17,500	578.9	0.237
S3	24,000	717.6	0.357

Homogenisation speed of 17,500 rpm produced particles with superior characteristics in terms of size and PdI values when compared to those produced at 9,500 and 24,000 rpm (Table 2). During the formation of the nanoparticles, high shear forces are required for the formation of smaller dimension particles. This would invariably also result in the production of lower PdI, which explains the difference between size and PdI values of particles produced at 9,500 and 17,500 rpm. However, at much higher homogenisation speed, a diminishing effect is experienced, possibly attributable to aggregation of particles from smaller size dimensions. A similar phenomenon has been reported by Mulik *et al.*, (2009) who had shown that longer stirring time led to inter-particle aggregation hence increasing the particle size (Mulik *et al.*, 2009). Since aggregation is a random phenomenon, the size range produced is normally wide and therefore higher PdI values were obtained.

Table 3. Effects of homogenizing time on size and PdI nanoparticles

Sample	Homogenisation time at 17,500 rpm (min)	Size (nm)	PdI value
S4	2.5	820.2	0.335
S2	5	578.9	0.237
S5	10	493.8	0.199

Homogenisation time has a profound effect on the particles produced (Table 3). Shorter homogenisation times produce insufficient shear forces to reduce the size of particles, hence producing large particles. S5 which was subjected to 10 minutes homogenisation produced smaller particles than that of S2, which underwent homogenisation for only 5 minutes. Furthermore, there was also a reduction in the PdI values after a longer duration of homogenisation, which corresponded to the homogenising purpose to produce better size distribution. Therefore it appears that higher homogenisation speed and duration are both crucial for the production of nanoparticles with superior physical characteristics. It must be added however that longer duration of homogenisation at moderate speed is more favourable than higher homogenisation speeds.

Table 4. Effects of ultrasonication amplitude on size and PdI of nanoparticles

Sample	Ultrasonication amplitude for 1 min (%)	Size	PdI value
U1	20	512.3	0.892
U2	30	548.2	0.883
U3	40	586	0.788

Ultrasonication at lower amplitude produces smaller particle size with higher PdI values (Table 4). Although higher amplitude creates more cavitation which is required to break down the particle size, it can also dampen the efficiency of energy transmission and

reduce the ultrasonic effect (Mason, 1999), as observed in the present study. This appears to mirror the observed increase in size arising from higher homogenisation speeds discussed earlier. All three samples that underwent ultrasonication had high PdI values, suggesting that the samples were polydispersed, even at 40 % amplitude.

Table 5. Effects of homogenisation and ultrasonication on size and PdI of nanoparticles

Sample	Homogenisation time at 17,500 rpm (min)	Ultrasonication amplitude for 1 min (%)	Size (nm)	PdI value
C1	5	40	407.7	0.327
C2	10	40	402.0	0.258

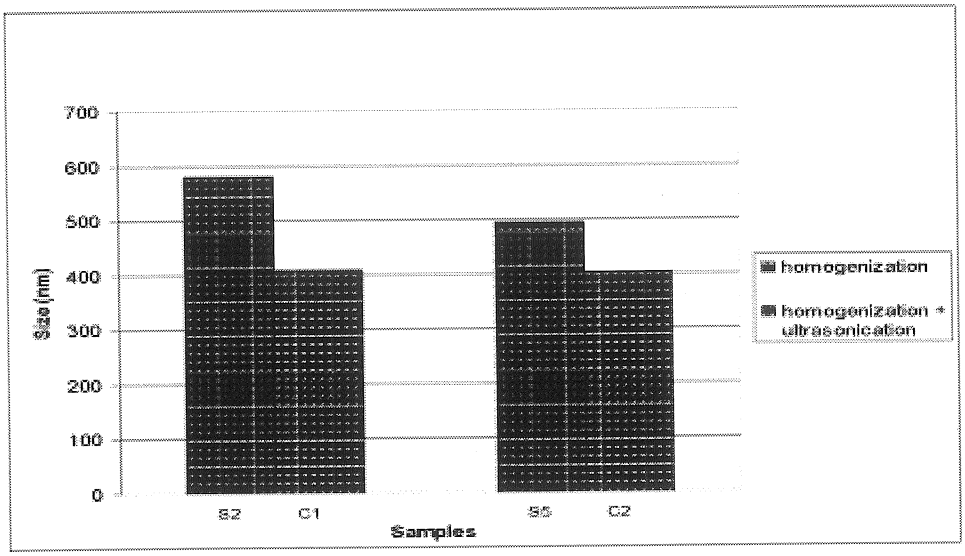

Figure 1. *S2 & C1 (5 minutes homogenisation). S5 & C2: (10 minutes homogenisation). Homogenisation at 17,500 rpm. Ultrasonication at 40 % amplitude for 1 minute.*

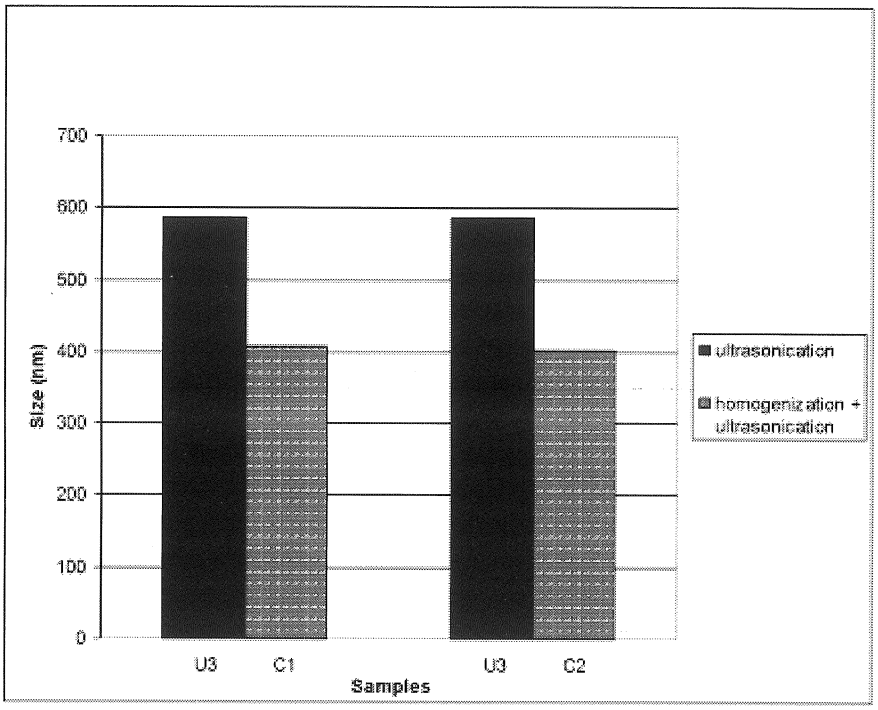

Figure 2. *C1: (5 minutes homogenisation). C2: (10 minutes homogenisation). Homogenisation at 17,500 rpm and ultrasonications at 40 % amplitude for 1 minute.*

An obvious size reduction can be seen when the particles were subjected to homogenisation and ultrasonication (Table 5). The decrease in size from S2 (5 minutes homogenisation) to C1 (5 minutes homogenisation and 1 minute ultrasonication) was calculated to be 29.6 %; whereas the decrease in size from S5 (10 minutes homogenisation) to C2 (10 minutes homogenisation and 1 minute ultrasonication) was 18.6 % (Figure 1). On the other hand, there was a 30.47 % size reduction from U3 (1 minute ultrasonication) to C1 (5 minutes homogenisation and 1 minute ultrasonication) and 31.40 % size reduction from U3 (1 minute ultrasonication) to C2 (10 minutes homogenisation and 1 minute ultrasonication) (Figure 2). Although homogenisation is comparatively superior in production of smaller sized nanoparticles with lower PdI, the combination of both methods is more effective not only in reducing the size but also in producing a good size distribution of the particles. This combination allows the two methods to compromise with each other, making it more superior than when using either method alone.

Table 6. Zeta potential determination

Sample	Homogenisation time (min)	Ultrasonication time (min)	Zeta Potential (mV)
S2	5	-	+48.4
S5	10	-	+42.7
U3	-	1	+43.2
C1	5	1	+54.5
C2	10	1	+44.4

The zeta potentials were higher for nanoparticles produced by the combination method suggesting higher stability (Table 6). Comparing the zeta potentials of the particles treated for 5 minutes homogenisation and 10 minutes homogenisation, the latter had slightly lower zeta potentials. However the particles were still considered very stable having zeta potentials of more than 40 mV. Ultrasonication alone produced particles with the lowest zeta potential among all. On the whole, all particles were stable in term of zeta potential.

CONCLUSION

Moderate speed and duration of homogenisation produce better physical characteristics of curcumin-chitosan nanoparticles. Homogenisation and ultrasonication have their own limits in nanoparticle production. However, the combination makes use of the advantages of both methods hence producing better nanoparticles.

ACKNOWLEDGEMENT

The authors would like to acknowledge the School of Pharmacy of University of Nottingham Malaysia Campus for supporting this work with the Faculty Research Grant.

REFERENCES

Aggarwal, B.B., Sundaram, C., Malani, N. (2007). Curcumin: The Indian solid gold. In: *The molecular targets and therapeutic uses of curcumin in health and disease,* ed. Aggarwal, B.B., Surh, Y.J., Shishodia, S., Springer Science + Business Media LLC, New York, pp 1-75.

Calvo, P., Remuñán-López C., Vila-Jato, J.L., Alonso, M.J. (1997). Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. *J. Appl. Polym.* Sci. **63**: pp 125-132.

Cheng, A.L., Hsu, C.H., Lin J.K., Hsu, M.M., Ho, Y.F., Shen, T.S., Ki, J.Y., Lin, J.T., Lin, B.R., Wu, M.S., Yu, H.S., Jee, H.S., Chen, G., Chen, C.A., Chen, T.M., Lai, M.K., Pu, Y.S., Pan. M.H., Wang, Y.J., Tsai, C.C., Hsieh C.Y. (2001). Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or premalignant lesions. *Anticancer Res.* **21**: pp 2895-2900.

Das, R.K., Kasoju, N., Bora, U. Encapsulation of curcumin in alginate-chitosan-pluronic composite nanoparticles for delivery to cancer cells. Nanomedicine: NMB. xx: 1-8. doi:10. 1016/j.nano.2009. 05 .009

Kumar, M.N.V.R. (2000). A review of chitin and chitosan applications. *Reactive and Functional Polymers* **46**: pp 1-27.

Maiti, K., Mukherjee, K., Gantait, A., Saha, B.P., Mukherjee, P.K. (2007).Curcumin – phospholipid complex: Preparation, *therapeutic evaluation and pharmacokinetic study in rats. Int. J. Pharm.* **330**: pp 155-163.

Mason, T.J.(1999). Laboratory equipment and usage considerations. In: Crum, L.A., Mason, T.J., Reisse, J.L., Suslick, K.S. (Eds.), Sonochemistry and Sonoluminescence NATO ASI Series. Kluwer Academic Publishers, Netherlands, pp. 245–258.

McPhail, D., Tetley L., Dufes, C., Uchegbu, I.F.(2000). Liposomes encapsulating polymeric chitosan based vesicles – a vesicle in vesicle system for drug delivery. *Int. J. Pharm.* **200**: pp 73-86.

Mulik, R., Mahadik, K., Paradkar, A.(2009). Development of curcuminoids loaded poly(butyl)cyanoacrylate nanoparticles: Physicochemical characterization and stability study. *Eur. J. Pharm. Sci.* **37**: pp 395-404.

Prego, C., Torres, D., Fernandez-Mergia, E., Novoa-Carballal, R., Quiñoá, E., Alonso, M.J. (2006). Chitosan-PEG as new carriers for oral peptide delivery: Effect of chitosan pegylation degree. *J. Control. Rel.* **111**: pp 299-308.

Sæther, H.V., Holme, H.K., Maurstad, G., Smidsrød, O., Stokke, B.T. (2008). Polyelectrolyte complex formation using alginate and chitosan. *Carbohydrate Polymers.* **74**: pp 813-821.

Shaikh, J., Ankola, D.D., Beniwal, V., Singh, D., Ravi Kumar, M.N.V. (2009). Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. *Eur. J. Pharm. Sci.* **37**: pp 223-230.