ELCTROSPUN NANOFIBRES FOR CO–ENCAPSULATION OF CURCUMIN AND PIPERINE

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Curcumin is the major chemical compound found in turmeric (Curcuma longa). It shows anti-cancer, antioxidant, anti-inflammatory and antibacterial effects. However, it suffers drawbacks such as low solubility, poor bioavailability and rapid metabolism to tetrahydro curcumin and phase II glucuronidation in the liver. Studies show that piperine improves the bioavailability of curcumin. Hence, the right combination of curcumin and piperine will resolve the said issues associated with curcumin. This study aims to develop curcumin and piperine-loaded PVA (polyvinyl alcohol) nanofibers using the electrospinning technique in which curcumin is loaded to the core and piperine to the sheath of the nanofibre. Curcumin was extracted into ethanol using the Soxhlet extraction method from dry turmeric rhizomes and dried ground black pepper seed powder was refluxed with dichloromethane to isolate piperine and purified by recrystallization. The presence of piperine and curcumin was confirmed with UV-vis and FT-IR spectroscopies. Fabrications of nanofibres were carried out under different electrospinning and experimental conditions to counter the transition phase from coaxial electrospray to coaxial electrospinning. The fibre was developed by electrospinning technique with various formulation parameters like concentration of PVA, curcumin, piperine, dichloromethane and isopropyl alcohol. Also, preparation parameters like applied voltage, needle tip to collector distance and flow rate of the solutions. The developed fibres were morphologically characterized using SEM. Polyvinyl alcohol-based curcumin in the core and polyvinyl alcohol-based piperine in the outer layer fibres showed better distribution in fibre-diameter in the range of 0.0 to 0.5 μm.

Keywords: curcumin, piperine, polyvinyl alcohol, co-encapsulation, electrospinning, coaxial electrospinning

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PREPARATION AND CHARACTERIZATION OF CURCUMIN AND PIPERINE CO – ENCAPSULATED ELCTROSPUN NANOFIBRES

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INTRODUCTION

Curcumin is the major chemical compound found in turmeric (*Curcuma longa*) (Lal, n.d.). It shows anti-cancer, antioxidant, anti-inflammatory and antibacterial effects (Nisar et al., 2015). However, it suffers from low solubility, poor bioavailability and rapid metabolism to tetrahydro curcumin and phase II glucuronidation in the liver (Hoehle et al., 2007). Studies show that piperine improves the bioavailability of curcumin; hence, the right combination of curcumin and piperine will resolve the said issues associated with curcumin (Farooqui & Farooqui, 2019). This study aims to develop curcumin and piperine-loaded PVA (polyvinyl alcohol) nanofibres using the electrospinning technique. In this work, curcumin and piperine-loaded PVA (polyvinyl alcohol) (Lee et al., 2004) nanofibres were made using an electrospinning technique under different electrospinning and experimental conditions with single and coaxial needles (Subbiah et al., 2005).

METHODOLOGY

MATERIALS

Extraction solvents: -

Dichloromethane, Diethyl ether, Acetone, Hexane, Ethanol

Materials used for electrospinning: -

Polyvinyl alcohol (PVA) (Degree of hydrolysis = 4000), 2-Propanol (Isopropyl alcohol), dichloromethane and distilled water

METHODS

Extraction of curcumin from turmeric rhizomes

The turmeric rhizomes were dried under ambient conditions until a constant weight was obtained. The dried turmeric rhizomes were ground (20 g) and extracted for 8 hours with 200 ml of distilled ethanol. The extract was then concentrated using a rotary evaporator at 90 °C. Residue was placed in a desiccator to remove the residual ethanol. The crude curcumin extract was weighed and the yield was recorded (Nabati et al., 2014; Sahne et al., 2016).

Extraction, isolation and recrystallization of piperine from black pepper seed

10.0 g of ground black pepper was refluxed in 20 ml of dichloromethane for 30 minutes. The mixture was then allowed to cool down to room temperature followed by filtering with a Büchner funnel. The extract was then concentrated using a rotary evaporator. The resulting olive-brown, thick oil was cooled in an ice bath before adding 3 – 4 ml of cold ether to the oil with gentle stirring. The resulting oil was cooled again in an ice bath, and 3 ml of cold ether was added with gentle stirring. The straw-yellow crystals obtained were washed with cold ether. Next, the crude piperine was dissolved in a small volume of heated 3:2 acetone: hexane

solution to recrystalize. After allowing the mixture to remain at room temperature for 15 minutes, rod-like, yellow crystals of piperine began to appear. The solution was cooled for 30 minutes in an ice bath. The crystals were placed in a desiccator and then the weight was obtained (Epstein & seide, 1993; Shingate et al., 2013).

Electrospinning solutions

Weighed proportions of PVA, curcumin, piperine, 2-Propanol, dichloromethane and Distilled water were used in the preparation of core and sheath solutions as given in the Table 01. Solutions were prepared at 60 °C.

Table 01: Weight parentages (W/W) % electrospinning of solutions

	Core				Sheath			
Sample no:	PVA (w/w) %	Curcumin (w/w) %	Iso-propanol (w/w) %	Water (w/w) %	PVA (w/w) %	Piperine (w/w) %	Dichloro methane (w/w) %	Water (w/w) %
1	8	2	10	80	10	0	0	90
2	8	2	10	80	10	0	0	90
3	8	2	10	80	10	0	0	90
4	8	2	10	80	10	0	0	90
5	8	2	10	80	10	0	0	90
6	8	2	10	80	10	1	9	80

Nanofibre preparation

The prepared solutions were fed to the electrospinning system using a syringe pump. Needle to collector distances (TCD), needle size, collector drum speed, sliding speed and the relevant flow rate were adjusted according to Table 02. A voltage was adjusted across a collector aluminium foil attached to the drum from the spinneret tip. Electrospinning was performed at room temperature for 2 hours. to obtain sufficiently smooth nanofibre membranes.

Table 02: Electrospinning parameters

Electrospin sample no:	Spinning voltage (kv)	Flow rates (ml/h) core	Flow rates (ml/h) sheath	TCD (cm)	Drum speed (rpm)	Sliding speed (mm/s)	Needle size
1	10.07	0.1	0.1	8	50	20	22G/16G
2	11	0.1	0.2	8	50	20	22G/16G
3	7.72	1	1	8	50	20	22G/16G
4	7.75	1	5	8	50	20	22G/16G
5	7.93	1	1	10	50	20	22G/16G
6	9.08	1	1	8	50	20	22G/16G

RESULTS AND DISCUSSION

Percentage yield of curcumin and piperine

70 g of turmeric powder was used for the extraction of curcumin and the percentage yield was 19%. Also, 80 g of black pepper seeds powder was used to extraction and percentage yield was 2.5%.

FT-IR spectroscopy conformation of curcumin and piperine

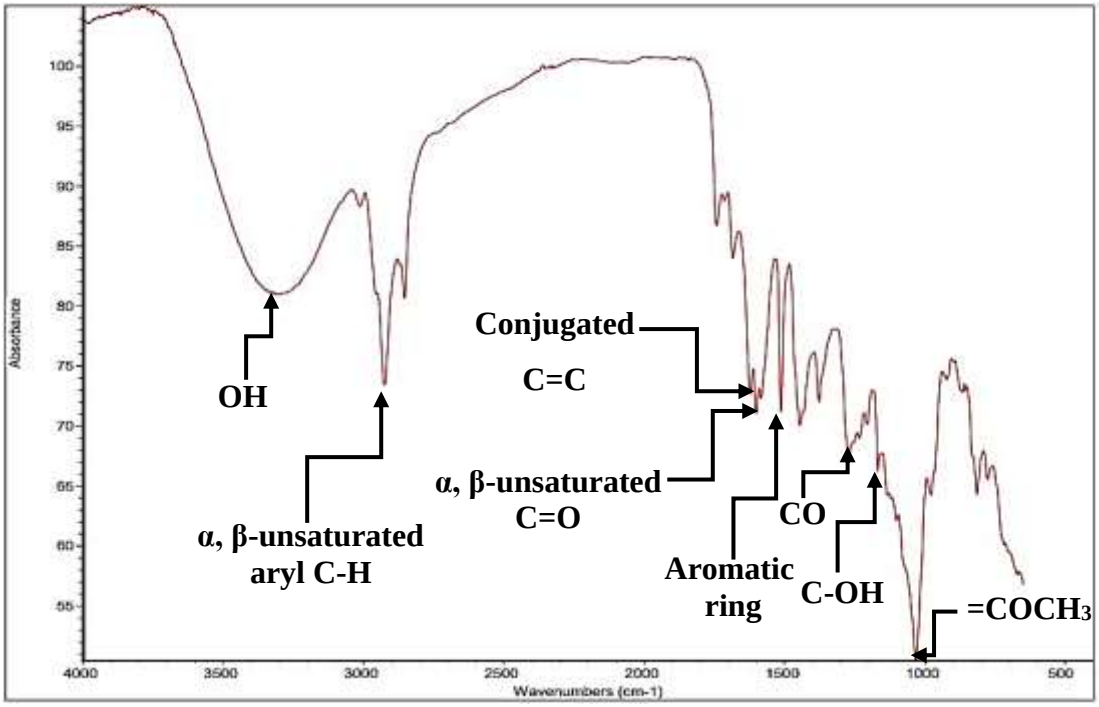

Figure 01: FTIR spectroscopy of crude curcumin

Table 03: Characteristic peaks of curcumin (Chearwae et al., 2006)

FTIR (wave number cm^{-1})	Functional group
1050	=C-O-CH_3
1140	C-OH
1287	CO
1510	Aromatic ring
1609	α, β-unsaturated C=O
1624	Conjugated C=C
2950–3000	α, β-unsaturated aryl C-H
3328	OH

Characteristic peaks of curcumin are given in Table 03. Characteristic peaks of curcumin are as follows: 3300 cm^{-1} (-OH), 2920 cm^{-1} (α, β-unsaturated aryl C-H), 1670 cm^{-1} (Conjugated C=C) and 1595 cm^{-1} (α, β-unsaturated C=O), 1510 cm^{-1} (Aromatic ring), 1285 cm^{-1} (CO), 1140 cm^{-1} (C-OH), 1040 cm^{-1} (=C-O-CH_3) which are consistent with the literature (Chearwae et al., 2006).

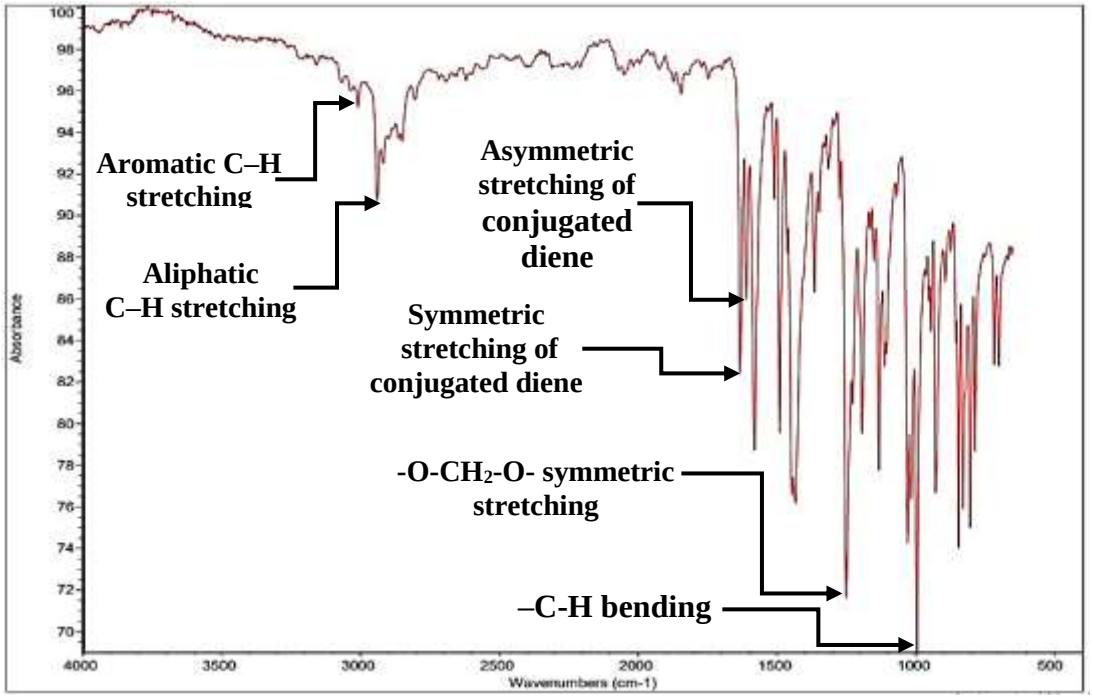

Figure 02: FTIR spectroscopy of piperine

Table 4**:** Characteristic peaks of piperine (Shingate et al 2013)

FTIR (wave number cm^{-1})	Functional group
above 3000	Aromatic C–H stretching
2925	Aliphatic C–H stretching
1633	Symmetric stretching of conjugated diene
1610	asymmetric stretching of conjugated diene
1230	-O-CH_2-O- symmetric stretching
1000	–C-H bending

Characteristic peaks of piperine are given in Table 4: 3000 cm^{-1} (Aromatic C–H stretching), 2930 cm^{-1}(Aliphatic C–H stretching),1630 cm^{-1} (Symmetric stretching of conjugated diene) and 1610 cm^{-1} (asymmetric stretching of conjugated diene), 1235 cm^{-1} (-O-CH_2-O- symmetric stretching), 995 cm^{-1} (–C-H bending), which is consistent with the literature (Shingate et al 2013).

Morphological characterization of nanofibres using SEM

Morphology of the electrospun nanofibres was studied using SEM.

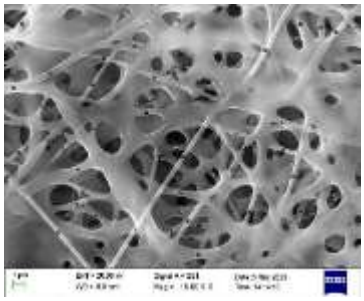

Figure 03: Sample 1 core flow rate 0.1 ml/h sheath flow rate 0.1 ml/h TCD 8 cm (mag.10 K)

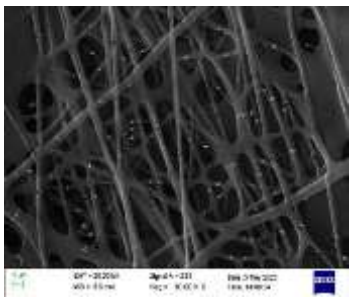

Figure 04: Sample 2 core flow rate 0.1 ml/h sheath flow rate 0.2 ml/h TCD 8 cm (mag.10 K)

Figure 05: Sample 3 core flow rate 1 ml/h sheath flow rate 1 ml/h TCD 8 cm (mag.10 K)

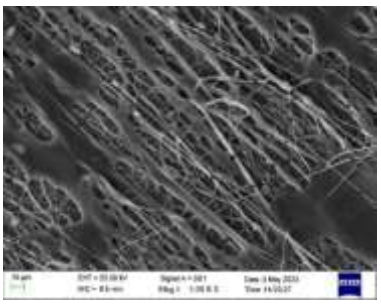

Figure 06: Sample 4 core flow rate 1 ml/h sheath flow rate 5 ml/h TCD 8 cm (mag.1 K)

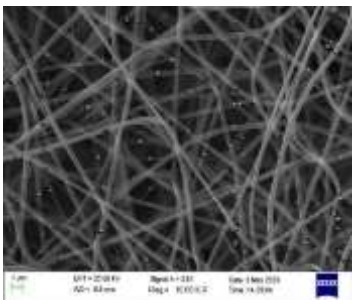

Figure 07: Sample 5 core flow rate 1 ml/h sheath flow rate 1 ml/h TCD 10 cm (mag.10 K)

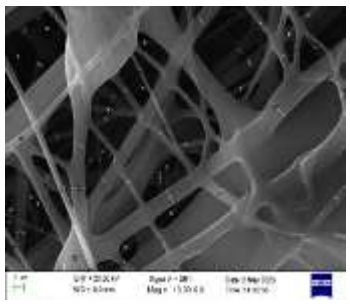

Figure 08: Sample 6 core flow rate 1 ml/h sheath flow rate 1 ml/h TCD 8 cm curcumin core and piperine sheath nanofiber sample (mag.10 K)

The 80 fibres were measured from sample 01 (Figure 03) using ImageJ software and the analysis as given in Figure 07 and the fibre diameter ranged from 0.2 μm to 1.0 μm and highest relative frequency percentage of fibre diameter was observed in the range of 0.3 μm to 0.4 μm. 100 fibres were measured from sample 02 and the analysis given in Figure 10 and the fibre diameter ranged from 0.0 to 1.3 μm and highest relative frequency of fibre diameter was observed in the range of 0.3 μm to 0.4 μm. With increasing flow rate, ratio distribution of diameters is more acceptable.

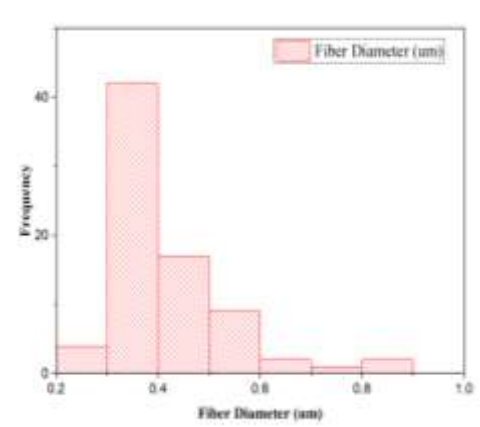

Figure 09: Sample 1 fibre diameter distribution

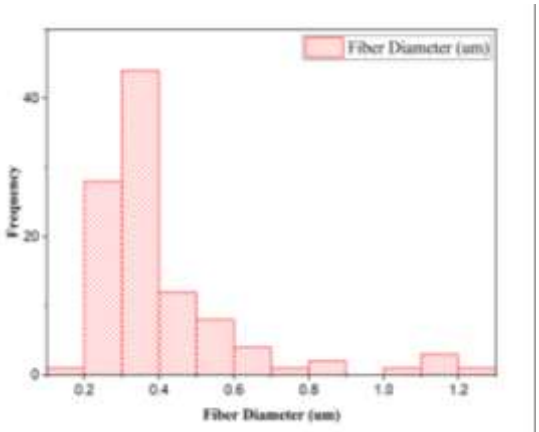

Figure 10: Sample 2 fibre diameter distribution

In sample 03, the tip of the needle to collector distance was kept at 8 cm; other electrospinning parameters and weight percentages of component are similar to that of sample 05. The only difference was the tip of the needle to collector distance. 150 fibres form sample 03 (Figure 05) were measured and the analysis is given in Figure 11. Fibre diameter ranged from 0 to 10 μm and the highest relative frequency percentage of fibres was observed in the range of 1 μm to 2 μm. 120 fibres were measured from sample 05 and the analysis is given in Figure 12. Fibre diameter ranged from 0.2 μm to 1.0 μm and the highest relative frequency was observed in the range of 0.3 μm to 0.4 μm. When Figures 11 and 12 are compared, the increasing tip of needle to collector distance has decreased the fibre diameter.

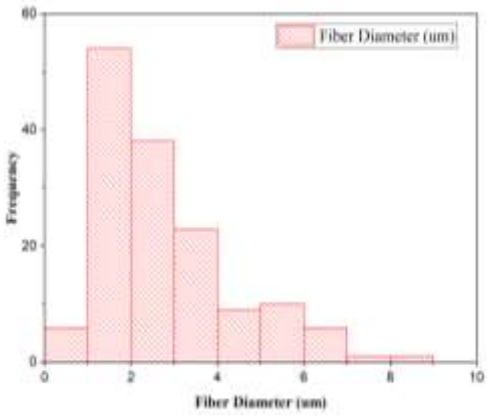

Figure 11: Sample 3 fibre diameter distribution

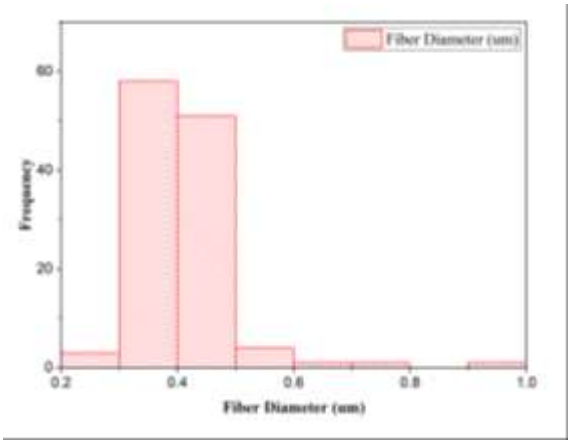

Figure 12: Sample 5 fibre diameter distribution

From Figure 08, sample 06 considering 70 particles through ImageJ software analysis as depicted by figure 13 fibre diameter ranges from 0.0 to 3.0 μm and a higher relative frequency of fibre diameter was observed in the range of 0.0 to 0.5 μm.

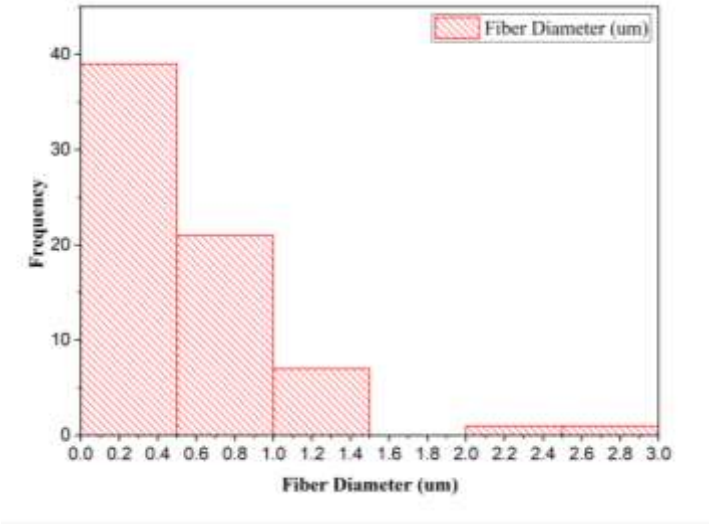

Figure 13: Sample 5 fibre diameter distribution

CONCLUSIONS

Curcumin exhibits very important health benefits to the human body. Some of them are antioxidant activity, antimicrobial activity, ant-cancer properties, neuroprotective and anti-inflammatory activity. However, due to low solubility and hepatic metabolism in the liver, its benefits are limited. This study aimed to develop a piperine-curcumin composite to evade the hepatic metabolism of curcumin. As piperine can inhibit the curcumin metabolism. Hence, having piperine in the outer layer can potentially deactivate the hepatic enzymes responsible for curcumin metabolism. Nanofibres were successfully fabricated under different conditions. Further analyses are under way such as TEM to confirm the multilayer structure of the nano fibres.

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