International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2017; 5(3): 45-48
Received: 08-03-2017
Accepted: 09-04-2017

DS Aniesrani Delfiya
Scientist, ICAR-Central Institute of Fisheries Technology, Cochin - 682029, Kerala, India

K Thangavel
Department of Food and Agricultural Process Engineering, Tamil Nadu Agricultural University, Coimbatore – 641 003, India

Correspondence
DS Aniesrani Delfiya
Scientist, ICAR-Central Institute of Fisheries Technology, Cochin - 682029, Kerala, India

In vitro release kinetics of spray dried curcumin-loaded egg albumin microparticles

DS Aniesrani Delfiya and K Thangavel

Abstract
Curcumin-loaded egg albumin microparticles were prepared by spray drying and the *in vitro* release profile was analyzed for 72 h. Results showed that the curcumin release rate was controlled by egg albumin concentration. The initial fast release and further controlled release of curcumin was observed in all formulations. Release data was fitted in mathematical models and found that curcumin release from all the formulations were best explained by Hixson-Crowell model followed by first order. Best fit of Hixson-Crowell model (R^2>0.99) indicated a change in surface area and diameter of the microparticles with the progressive dissolution of egg albumin as a function of time. Release exponent (n) values of less than 0.4 indicated the Fickian diffusion controlled release of curcumin from microparticles. Spray drying can be suggested as a suitable method to prepare microparticles with controlled delivery of curcumin

Keywords: Spray drying, curcumin, egg albumin, *in vitro* release, kinetics

1. Introduction
Curcumin is a low molecular weight polyphenol with flexible medicinal properties and it is obtained from the rhizome of turmeric (*Curcuma longa* L.) Interest on curcumin increased remarkably because of its potent antioxidant, anti-inflammatory, antimicrobial *etc*. activities [1]. Even though curcumin possess various therapeutic effects its application is less due to the hydrophobic nature, poor aqueous solubility, rapid metabolism and poor bioavailability because of physicochemical and biological instability. The solubility of curcumin was reported to be 11 ng/mL (in plain aqueous buffer pH 5.0) and rapid degradation at physiological pH [2]. To use curcumin as an efficient drug the problems like poor solubility, low bioavailability and stability should be solved.
Albumin based carrier systems could be used for drug encapsulation, because different drug binding sites are present in the albumin molecules [3]. The method of spray drying is advantageous for the preparation of albumin-based drug delivery systems because it is able to make compact particles with reproducible size, surface morphology, and release characteristics [4]. Egg albumin has been used for the microencapsulation of drugs [5]. The use of egg albumin in formulations is due to the fact that it is a naturally obtained polymer and is biodegradable in nature with good aqueous solubility. Egg albumin also has a property of protein binding and physical entrapment and supports passive as well as facilitated release of various types of incorporated drugs from the polymer matrix [6]. The objective of this study was to prepare the curcumin-loaded egg albumin microparticles by spray drying method and to evaluate the effect of egg albumin concentration on *in vitro* release kinetics of microparticles.

2. Materials and Methods
Egg albumin powder (minimum 80.5% protein) was obtained as a gift from M/s SKM egg products, Erode, Tamil Nadu. Curcumin (minimum 95%) was obtained from M/s Synthite Industries Ltd, Kolenchery, Kerala. Glutaraldehyde (25% w/v solution) was purchased from M/s Astron Chemicals (India), Ahmedabad. All other reagents used in the experiments were of AR grade.
Based on literature review, egg albumin concentration, curcumin concentration and air inlet temperature were considered as the most important factors affecting the quality characteristics of microparticles. Egg albumin concentration of 2.5 to 7.5% (w/v), curcumin concentration of 0.25 to 0.75% (w/v) and air inlet temperature of 110 to 130^{0}C were selected for spray drying and experimental runs were obtained by a three-factor, five-level CCD, with egg albumin solution concentration (EAC, A), curcumin concentration (CC, B) and inlet air temperature (DT, C) as independent variables (factors). The design included 20 experiments as shown in Table 1.

2.1 Preparation of emulsion

Curcumin-egg albumin emulsion was prepared by dissolving egg albumin (%w/v based on the total solution) in distilled water and subsequent addition of curcumin (%w/v based on total solution) into it according to the experimental design (Table 1). Prepared emulsion was stirred at 700 rpm for 10 min using magnetic stirrer.

2.2 Spray drying of emulsion

Spray drying of prepared curcumin-egg albumin emulsion was carried out in a lab model spray dryer (Spray Mate, M/s JISL, Mumbai, India) with a spray nozzle of 0.7 mm. Emulsion was fed into the spray drier equipped with the two fluid nozzle atomizer using a peristaltic pump at the rate of 2 ml min^{-1}. The pressure of compressed air for the flow of the spray was adjusted to 2 bar. The vacuum pressure was maintained at -80 mm of water column. An air outlet temperature was maintained at 50 – 60 °C. Inlet temperature of drying air was maintained according to the experimental design (Table 1). Microencapsulated powders obtained from spray dryer were filled in airtight, self-sealable polyethylene pouches. Then these pouches were packed in aluminium foil pouches and sealed using the hand sealer (Make: Sevana, India) to protect it from light. Sealed bags were stored at refrigeration temperature (4±2 ºC) until further studies.

2.3 *In vitro* release studies of microparticles

The *in vitro* drug release profiles of curcumin-loaded egg albumin microparticles were determined by the method suggested by Jithan *et al*. [7] as follows. These experiments were conducted in dark conditions as curcumin extensively degrades in the presence of light. The release medium was phosphate-buffered saline (PBS 0.1M, pH 7.4), which contained 1% ascorbic acid and 0.1 butylated hydroxytoluene to prevent further degradation of curcumin. One hundred milligrams of microparticles were re-dispersed in 200 ml of the release medium (PBS). The entire system was kept at 37±1°C under stirring at100 rpm. At the desired time intervals of 0, 2,4, 6, 8, 24, 48 and 72 h, 20 ml of release medium was removed and replaced with the same volume of fresh medium. The samples withdrawn were filtered using 0.2 µ sterile filter and the amount of curcumin in release medium was determined by UV-VIS spectrophotometer at 425nm. All measurements were performed in triplicate. The release was quantified as follows:

$$Release\ (\%) = \frac{Released\ curcumin}{Total\ curcumin}\ x\ 100 \quad \ldots\ldots\ldots\ldots (1)$$

2.4 Release kinetics

To study the release mechanism of curcumin from spray dried microparticles, data obtained from *in vitro* release studies were fitted to zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas kinetics models and the models are as follows:

Zero Order Model:

$$Q_t = Q_0 + K_o t \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where Q_t is the amount of curcumin released in time t, Q_0 is the initial amount of curcumin in the solution and K_0 is the zero order release constant. Data obtained from *in vitro* drug release studies were plotted as cumulative amount of drug released versus time.

First Order Model [8].

$$ln\ M_t = ln\ M_0 + K_1 t \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where M_t is the cumulative amount of curcumin released at any specified time point and M_o is the initial amount of curcumin in the prepared particles. The data obtained are plotted as log cumulative percentage of drug remaining vs. time.

Higuchi Model [9].

$$Q_t = K_H t_{1/2} \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where Q_t is the amount of curcumin released in time t, K_H is the Higuchi dissolution constant. The data obtained were plotted as cumulative percentage drug release versus square root of time.

Hixson-Crowell Model [10].

$$Q_0{}^{1/3} - Q_t{}^{1/3} = K_{HC} t \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

Where, Q_t is the amount of curcumin released in time t, Q_0 is the initial amount of curcumin in prepared particles and K_{HC} is the rate constant for Hixson-Crowell rate equation. Data obtained from *in vitro* drug release studies were plotted as cube root of drug percentage remaining in matrix versus time.

Korsmeyer-Peppas Model [11].

$$M_t \Big/ M_{\infty} = K_K\, t^n \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Where Mt / M∞ is a fraction of curcumin released at time t, K_K is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices. Data obtained from *in vitro* drug release studies were plotted as log cumulative percentage drug release versus log time.

Plotted release data were fitted using a linear equation using Microsoft Excel and the correlation coefficient (R^2) was obtained for each graph. Selection of model for the release data was based on the comparison of correlation coefficients [12].

3. Results

In vitro release of curcumin from curcumin-loaded egg albumin microparticles prepared using various combinations of egg albumin concentration and pH of solution is presented in Table 1. Microparticles prepared using lowest egg albumin concentration resulted in maximum curcumin release of 85.64% within 72 h. The lowest release of 45.2% was observed in the microparticles prepared using egg albumin concentration of 9.2 (%w/v) in the solution.

To describe the dissolution profile of curcumin from formulations the *in vitro* release data was evaluated using various kinetic models such as zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas models. The best fit of these models investigated based on their correlation coefficient values for each model. Correlation coefficients, release rate constants and release exponent (n) values for each model is presented in Table 2. *In vitro* release pattern from all the formulations were best explained by Hixson-Crowell model (R^2>0.99) followed by first order (R^2>0.99).

Table 1: *In vitro* release data of curcumin loaded egg albumin microparticles prepared by spray drying method

Factors (EAC/CC/DT)	Cumulative curcumin release (%)							
	0 h	2 h	4 h	6 h	8 h	24 h	48 h	72 h
0.8/0.5/120	7.25±0.22	18.47±0.66	33.28±1.00	54.16±2.48	66.59±1.99	74.13±2.22	80.58±2.90	85.64±2.26
2.5/0.25/110	6.52±0.17	16.3±0.59	26.53±0.70	38.83±1.16	49.9±1.32	58.44±1.55	64.67±2.96	69.21±2.49
2.5/0.25/130	6.17±0.22	14.7±0.67	23.83±0.86	34.81±1.04	44.82±1.62	54.14±1.95	61.31±1.84	65.68±2.37
2.5/0.75/110	7.08±0.25	17.01±0.51	28.33±1.02	40.47±0.81	51.03±1.53	59.86±2.16	67.51±2.02	73.63±3.37
2.5/0.75/130	6.87±0.31	15.76±0.47	26.57±1.22	37.58±1.13	47.3±1.25	56.19±2.57	63.42±1.27	70.27±2.11
5/0.08/120	4.77±0.14	11.92±0.36	20.65±0.62	30.49±0.81	39.21±1.41	46.33±1.39	51.94±1.56	56.83±1.70
5/0.5/103	5.14±0.15	12.96±0.26	21.83±0.65	31.77±1.14	40.6±1.46	48.16±1.44	54.05±1.43	59.03±1.18
5/0.5/120	5.21±0.10	12.84±0.38	21.63±0.43	31.72±0.95	40.67±1.86	48.13±0.96	54.14±1.95	59.31±1.78
5/0.5/120	5.34±0.16	12.49±0.33	21.46±0.64	32.03±0.85	41.06±1.23	48.94±1.46	54.89±1.65	59.93±1.58
5/0.5/120	5.45±0.14	13.01±0.47	22.05±0.58	32.87±1.18	42.04±1.26	49.63±1.31	55.52±1.47	60.89±2.19
5/0.5/120	5.38±0.19	12.81±0.38	21.79±0.78	32.36±1.17	41.43±1.10	48.92±1.76	54.77±1.97	60.1±1.80
5/0.5/120	5.27±0.16	12.56±0.33	21.24±0.64	31.47±1.44	40.67±1.47	47.99±1.44	53.66±1.93	59.3±1.57
5/0.5/120	5.43±0.14	13.32±0.48	21.75±0.57	32.03±0.96	41±1.48	48.61±1.29	54.6±2.50	59.55±2.15
5/0.5/136	4.96±0.18	11.68±0.42	20.24±0.73	30.37±0.91	38.38±1.76	45.31±1.63	50.93±1.53	55.38±1.99
5/0.92/120	5.55±0.20	13.11±0.60	22.59±0.81	34.04±0.90	43.82±1.31	52.67±1.90	59.41±1.78	64.45±2.95
7.5/0.25/110	3.75±0.11	8.98±0.27	17.21±0.52	26.82±0.97	35.52±1.06	42.42±1.27	47.84±1.72	53.01±1.59
7.5/0.25/130	3.58±0.11	9.64±0.25	16.74±0.50	25.45±1.17	33.6±0.67	39.88±1.20	46.04±1.66	51.02±1.35
7.5/0.75/110	3.83±0.10	9.52±0.34	18.45±0.49	28.5±0.85	37.08±1.11	44.66±1.18	51.41±2.35	56.3±2.03
7.5/0.75/130	3.62±0.13	9.31±0.33	17.29±0.62	26.97±0.81	35.5±0.94	42.85±1.54	49.38±1.48	54.06±1.95
9.2/0.5/120	2.47±0.09	7.04±0.32	13.27±0.48	21.5±0.37	28.28±1.02	34.06±1.23	40.22±1.21	45.2±2.07

EAC – egg albumin solution concentration (%w/v); CC- curcumin concentration (%w/v); DT – Inlet air temperature (^{0}C); mean±standard deviation value

Table 2: Release kinetics parameters obtained from model fitting of *in vitro* release data of curcumin loaded egg albumin microparticles prepared by spray drying

Factors (EAC/CC/DT)	Zero order		First order		Higuchi		Hixson-Crowell		Korsmeyer- peppas		
	R^2	K_o	R^2	K_1	R^2	K_H	R^2	K_{HC}	R^2	K_K	n
0.8/0.5/120	0.943	10.42	0.991	-0.278	0.960	11.84	0.991	-0.316	0.828	9.55	0.324
2.5/0.25/110	0.960	8.07	0.995	-0.167	0.984	9.37	0.995	-0.211	0.859	8.19	0.310
2.5/0.25/130	0.958	7.49	0.992	-0.151	0.991	8.93	0.995	-0.195	0.88	7.40	0.314
2.5/0.75/110	0.964	8.38	0.993	-0.184	0.991	9.8	0.998	-0.228	0.867	8.72	0.307
2.5/0.75/130	0.964	7.85	0.992	-0.165	0.994	9.29	0.998	-0.209	0.878	8.22	0.306
5/0.08/120	0.960	6.53	0.997	-0.117	0.99	7.74	0.997	-0.157	0.866	5.93	0.326
5/0.5/103	0.962	6.77	0.997	-0.124	0.991	7.98	0.997	-0.165	0.865	6.42	0.320
5/0.5/120	0.961	6.77	0.997	-0.125	0.991	8.02	0.997	-0.166	0.870	6.42	0.320
5/0.5/120	0.957	6.83	0.995	-0.128	0.990	8.16	0.996	-0.170	0.876	6.40	0.322
5/0.5/120	0.959	6.94	0.996	-0.131	0.99	8.24	0.997	-0.173	0.872	6.62	0.319
5/0.5/120	0.959	6.85	0.996	-0.128	0.990	8.13	0.997	-0.169	0.872	6.53	0.319
5/0.5/120	0.959	6.72	0.996	-0.125	0.991	8.01	0.997	-0.166	0.875	6.37	0.320
5/0.5/120	0.961	6.79	0.996	-0.126	0.991	8.03	0.997	-0.167	0.872	6.65	0.315
5/0.5/136	0.958	6.35	0.996	-0.113	0.989	7.53	0.996	-0.152	0.870	6.01	0.320
5/0.92/120	0.955	7.36	0.993	-0.146	0.991	8.85	0.996	-0.190	0.878	6.63	0.327
7.5/0.25/110	0.949	6.06	0.994	-0.107	0.991	7.42	0.995	-0.146	0.876	4.48	0.356
7.5/0.25/130	0.956	5.80	0.996	-0.100	0.995	7.04	0.997	-0.137	0.872	4.48	0.349
7.5/0.75/110	0.951	6.46	0.994	-0.118	0.993	7.90	0.996	-0.159	0.873	4.66	0.359
7.5/0.75/130	0.950	6.20	0.994	-0.111	0.993	7.60	0.996	-0.151	0.875	4.43	0.360
9.2/0.5/120	0.948	5.11	0.994	-0.085	0.996	6.36	0.996	-0.119	0.870	6.42	0.320

EAC – egg albumin solution concentration (%w/v); CC- curcumin concentration (%w/v); DT – Inlet air temperature (^{0}C)

Korsmeyer-Peppas model was further used to verify the mechanism of curcumin release from formulations and the R^2 values were found to be greater than 0.82. In Korsmeyer-Peppas model release exponent (n) is the indicative of mechanism of drug release. All the microparticles prepared by spray drying obtained the 'n' values of less than 0.36.

4. Discussion

From all the formulations initial burst release and further sustained release was observed in 72 h. The fast initial release was mainly due to the dissolution of egg albumin and diffusion of curcumin nearer to the surface of microparticles. Further slower release might be due to the diffusion of encapsulated curcumin from inner part of microparticles. It was observed that curcumin release rate could be controlled by egg albumin concentration. An increase in the egg albumin concentration decreased the curcumin release rate in PBS release medium. This might be due to the formation of high density gel like viscous layer when egg albumin was used at high concentrations. This layer might have resisted the faster diffusion of curcumin and resulted in the controlled release of curcumin from formulations. But in case of lower egg albumin concentration curcumin release rate was found to be high due to the faster diffusion of curcumin from formulations.

Best fit of Hixson-Crowell model to the release data indicated a change in surface area and diameter of the microparticles with the progressive dissolution of egg albumin as a function of time. First order model described the concentration dependent release of curcumin from microparticles. Higuchi model with the correlation coefficient of greater than 0.96 explained the diffusion controlled release pattern.

Release exponent (n) values of spray dried particles indicated the Fickian diffusion controlled release of curcumin from microparticles [11]. Fickian diffusional release occurs by the usual molecular diffusion of the drug due to a chemical potential gradient. Jain [13] prepared the norethisterone loaded egg albumin microspheres and studied the *in vitro* diffusion of norethisterone from microspheres and found that drug release decreased from 95 – 60%. Drug was released by following swelling control release and followed Higuchi diffusion controlled model.

5. Conclusion

The release profile of curcumin from spray dried microparticles was assessed and found that the release rate was controlled by egg albumin concentration and increase in egg albumin concentration decreased the release rate in PBS. Various release models were applied to curcumin release data in order to evaluate release mechanisms and kinetics. *In vitro* release pattern from all the formulations were best explained by Hixson-Crowell model followed by first order. Korsmeyer-Peppas model release exponent (n) showed the mechanism of drug release is only through diffusion. Prepared curcumin microparticles could be used as an effective drug delivery system for curcumin with controlled delivery characteristics. Hence curcumin can be encapsulated by spray drying method using egg albumin to improve its bioavailability in physiological pH conditions.

6. Acknowledgement

This work has been carried out during the doctoral studies of author D.S. Aniesrani Delfiya, in Tamil Nadu Agricultural University, Coimbatore. The authors would like to acknowledge Department of Science and Technology, Govt. of India for supporting this study through INSPIRE Fellowship 2013.

7. References

1. Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Research. 2003; 23(1A):363-98.
2. Yallapu MM, Jaggi M, Chauhan SC. beta-Cyclodextrin-curcumin self-assembly enhances curcumin delivery in prostate cancer cells. Colloids Surf B Biointerfaces. 2010; 79(1):113-125.
3. Patil GV. Biopolymer albumin for diagnosis and in drug delivery. Drug Development Research.2003; 58(3):219-247.
4. Elzoghby AO, Samy WM, Elgindy NA. Albumin-based nanoparticles as potential controlled release drug delivery systems. Journal of Controlled Release. 2012; 157(2):168-182.
5. Torrado JJ, Illum L, Cadorniga R, Davis SS. Egg albumin microspheres containing paracetamol for oral administration. I. *In vitro* characterization. Journal of Microencapsulation. 1990; 7(4):463-470.
6. Shailesh TP, Vipul PD, Girishbhai JK, Manish CJ. Preparation and *in-vitro* evaluation of ethyl cellulose coated egg albumin microspheres of Diltiazem Hydrochloride. Journal of young pharmacists. 2010; 2(1):27-34.
7. Jithan AV, Madhavi K, Madhavi M, Prabhakar K. Preparation and characterization of albumin nanoparticles encapsulating curcumin intended for the treatment of breast cancer. International Journal of Pharmaceutical Investigation. 2011; 1(2):119-125.
8. Khatun M, Islam SMA, Akter P, Quadir MA, Reza MS. Controlled release of Naproxen sodium from Eudragit RS100 Transdermal Film Search Results. Dhaka University Journal of Pharmaceutical Sciences. 2004; 3(1-2):1-10.
9. Higuchi T. Mechanism of sustained action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. Journal of pharmaceutical sciences.1963; 52(12):1145-1149.
10. Hixson AW, Crowell JH. Dependence of reaction velocity upon surface and agitation: I-theoretical consideration. Industrial and Engineering Chemistry. 1931; 23(10):1160-1168.
11. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. 1983. Mechanisms of solute release from porous hydrophilic polymers. International Journal of Pharmaceutics. 1983; 15(1):25-35.
12. Ranjan AP, MukerjeeA. Scale up, optimization and stability analysis of curcumin C3 complex-loade nanoparticles for cancer therapy. Journal of Nanobiotechnology. 2012; 10(38):20.
13. Jain BK. Preparation and *in vitro* characterization of muco adhesive Norethisterone - egg albumin microspheres for nasal administration. Asian Journal of Biomedical and Pharmaceutical Sciences. 2012; 2(15):49-57.