

Bioenhancement of Curcumin by Combined Approaches of Adjuvants and Liposomal Fabrication

Amit Roy, Suman Saha*, Ananta Choudhury, Sanjib Bahadur

Department of Pharmaceutics, Columbia Institute of Pharmacy, Raipur, Chhattisgarh, India

Abstract

Aim: To improve oral bioavailability of curcumin by a combination of approaches. **Materials and Methods:** Liposomes were prepared by thin film hydration method. Prepared liposomes were evaluated for its physical characteristics such as particle size, zeta potential, and encapsulation efficiency. Release study and bioavailability was also determined on rat. Blood samples were collected at specific intervals, and plasma was separated by ultracentrifugation. Plasma was analyzed by high-performance liquid chromatography at 420 nm taking acetic acid:acetonitrile (9:1) ratio as a mobile phase at a flow rate of 0.5 ml/min using C18 column. **Result and Discussion:** Increase in zeta potential indicates more uniform size distribution. From *in-vivo* study, it is clear that liposomal curcumin and piperine increases C_{max} , area under the curve, and mean residence time significantly as compared to other approaches. **Conclusion:** Oral bioavailability of curcumin can be significantly increased by combining two approaches. The single approach may not be sufficient to overcome the hindrance of curcumin.

Key words: Bioenhancement, curcumin, liposome, piperine

INTRODUCTION

Curcumin is a dynamic ingredient of a much-known spice, turmeric (*Curcuma longa*), used in cuisine in whole India and additionally other areas of Asia, which belongs to ginger family (Zingiberaceae) having variety of pharmacological properties together with anti-inflammatory, antioxidant, antiproliferative, and antiangiogenic activities. Commercially, curcumin consists of about 77% diferuloylmethane, 17% demethoxycurcumin, and 6% bisdemethoxycurcumin.^[1-7]

Regardless of that, the efficiency and safety of curcumin has not been yet authorized as a therapeutic agent, and the comparative bioavailability of curcumin has been tinted as a major problem for this. The reasons for low bioavailability of any compound within the body are little inherent solubility, reduced absorption, and elevated rate of biotransformation and/or quick elimination and clearance from the body.^[8-10] Concerning the distribution of curcumin, it showed its accumulation within the intestine, colon, and liver, that is one of the key reasons of giving most promising *in-vivo* activity in gastrointestinal ailments when compared with different organ structures. The liver and intestinal

mucosa is the fundamental organs liable for metabolism of curcumin. On absorption, curcumin usually transformed into metabolite form, i.e. glucuronides and sulfates or it is reduced to hexahydro curcumin.^[11,12] How to enhance the bioavailability of curcumin is a provocative question. The activity of adjuvant that may block metabolic pathways of curcumin is one of the major means utilized to get better bioavailability.^[13,14] Nanoparticles, liposome, micelles, and phospholipids complexes are other hopeful new formulations, which seem to offer better bioavailability of curcumin. The main purpose of any drug delivery system is to attain required concentration of the moiety in blood or tissue, which is therapeutically useful and nontoxic for a prolonged time.^[15]

MATERIALS AND METHODS

Soy lecithin was purchased from HiMedia Laboratories Pvt. Ltd.; cholesterol from Loba Chemie Pvt. Ltd.; curcumin

Address for Correspondence:

Suman Saha, Department of Pharmaceutics, Columbia Institute of Pharmacy, Raipur, Chhattisgarh, India.
E-mail: suman_hpi@yahoo.com

Received: 27-09-2016

Revised: 07-10-2016

Accepted: 15-10-2016

from the Natural remedies Bangalore; piperine from the Alfa Aesar, U.K., and all other chemicals are of AR grade and high-performance liquid chromatography (HPLC) grade and used as received.

Preparation of liposome

Liposomal curcumin has been prepared as per thin film hydration method of Bangham *et al.*^[4,16,17] In this process, the curcumin, piperine, soya lecithin, and cholesterol have been dissolved in 10 ml methanol and chloroform blend (9:1 ratio), and the solution was taken in a round bottom flask, as mentioned in table 1. The flask was kept in a rotary shaker in low speed so that solvent can be evaporated and a thin film was produced. The flask was kept overnight for complete evaporation of organic solvents. Then to shape the vesicles, the dried layer was hydrated with 30 ml of standard saline solution and vortexed for 1 hour (higher than the gel - liquid transition temperature of soya lecithin). After hydration, a milky suspension was produced. To trim down the vesicle dimension, the liposomal suspension was irradiated to ultrasonic irradiation with an output of 50 watt for three cycles of 1 min each.

Mean particle size and zeta potential measurement

Mean vesicle size and zeta potential of prepared liposomes were estimated using Zetasizer 300HSA (Malvern instrument, Malvern, UK).

Drug entrapment studies

The percentage drug entrapped (PDE) was estimated by ultracentrifugation. The liposomal formulations were exposed to ultracentrifugation (ultracentrifuge, Remi Laboratories,

Mumbai, India) at 11000 rpm for 30 min in an ultracentrifuge to separate the confined drug from the free drug. Then, the apparent supernatant was separated and analyzed for drug content following suitable dilution by ultraviolet-visible (UV-VIS) spectrophotometer. This indicates the quantity of free drug. The liposome was redispersed in methanol and analyzed for drug content after proper dilution using UV-VIS spectrophotometer.^[18-20] This indicates amount of drug entrapped. The entrapment efficiency of liposomes was calculated as follows.

$$PDE = \left[\frac{(T-C)}{T} \right] \times 100$$

Where, T is the total amount of drug that is detected both in the supernatant and sediment, and C is the amount of drug detected only in the supernatant.

In-vitro drug release studies

In-vitro release studies were performed by dialysis membrane method. Membrane was drenched in lukewarm water at 45°C for 30 min before using it for release study. This membrane was then cautiously clamped to one end of the hollow glass cylinder and considered as the donor section. The dissolution medium, i.e., phosphate buffer solution pH 7.4 (200 ml) was taken into the receiver compartment. The donor section was immersed into the receiver compartment in such a way that the rim just touched the receiver compartment. Before the release test, 0.5 ml of curcumin formulation was diluted to 3 ml in release medium and placed into the donor compartment at 37°C at 100 rpm using magnetic stirrer and bead. Samples (5 ml) were collected from the receptor section at predetermined intervals and replaced with fresh medium immediately. The samples were analyzed by UV-VIS spectrophotometer at 420 nm. Drug release was monitored for 12 h.^[20-22]

Pharmacokinetic study for determination of curcumin

In-vivo study was investigated in Sprague-Dawley rat. All the animal experiments were conducted according to the rules and guidelines of the committee for the purpose of control and supervisions of experiments on animals. The Institutional animal ethical committee approved the study.

Construction of curcumin standard graph

A standard graph of curcumin was developed for the assessment of pharmacokinetics of the curcumin formulations

Table 1: Composition of liposome

Compositions of liposomal formulation (mg)	Formulation code	
	F1	F2
Curcumin	250	
Curcumin+piperine (10:1)		250
Soya lecithin	900	900
Cholesterol	200	200
Methanol: chloroform (v/v)	9:1	9:1
Normal saline solution (ml)	30	30
Method used	Thin film hydration	Thin film hydration

Table 2: Evaluation parameters of prepared liposome

Formulation code	Zeta potential (mV)	Mean particle size (nm)	Percentage encapsulation efficiency
F1	-26.15±0.231	198.26±3.56	66.51±1.28
F2	-26.30±0.243	235.56±4.69	68.35±1.95

in rat by the following method. 100 mg of pure curcumin was dissolved in 100 ml of methanol and sonicated for 10 min to prepare stock solution. 10 ml stock solution was taken and diluted with the same solvent, i.e., methanol up to 100 ml and again sonicated to form standard solution. From standard solution, further dilution was prepared, *viz.*, 0.5 ml was transferred from the standard solution into test tubes and diluted to 10 ml with methanol to form 5 µg/ml. The solutions so prepared (5, 10, 15, 20, and 25 µg/ml). The samples were analyzed in HPLC at 420 nm taking acetic acid:acetonitrile (9:1) ratio as a mobile phase at a flow rate of 0.5 ml/min using C18 column. The retention time and peak area were noted and data obtained through HPLC analysis was further used to interpolate, the experimental peak area values to get the corresponding concentration of curcumin in plasma.

Animals were divided into four groups; each group contains six rats.

Group 1 received: Liposomal curcumin

Group 2 received: Liposomal curcumin+pure piperine

Group 3 received: Liposomal curcumin: Piperine formulation

Group 4 (standard) received: Pure curcumin.

Blood samples (0.5 ml) from the experimental rats were collected by retro-orbital plexus technique into a sequence of microcentrifuge tubes containing 0.3 ml of sodium citrate solution. Blood samples were taken at different time intervals such as 30 min, 1, 2, 3, 6, 12, 18, and 24 h. The collected blood samples were centrifuged at a speed of 5000 rpm for 10 min, and plasma was separated into an additional microcentrifuge tube using micropipette and stored in deep freeze until analysis. The drug was extracted out from plasma by adding in a methanolic solvent, and it was centrifuged for 10 min from which the organic layer of drug comes out other than sediment. The organic layer of drug was then injected into the HPLC system for the further processing of determination of plasma drug concentration and other parameters, i.e., area under the curve (AUC), C_{max} , etc.^[16-18,19]

RESULT AND DISCUSSION

Surface charge

From the data obtained from particle size analysis and surface charge (as per table 2), it seems that, as zeta potential increases, the repulsive interactions will be larger, leading to the formation of more stable particles with a more uniform size distribution.^[14,17]

The *in-vitro* drug release of curcumin from liposome was carried out using dialysis membrane in 7.4 pH phosphate buffer for 12 h. The *in-vitro* release profile obtained from formulation, are shown in Table 3 and Figure 1, respectively. It reflects slow and steady release of curcumin from the liposome. It may be due to the improved stability of curcumin inside liposome and release at a steady fashion.

In-vivo study

Standard graph of curcumin by HPLC

Data obtained for calibration curve are given in Table 4, and a graph was plotted [Figure 2] by taking area on y-axis and concentration on x-axis. R^2 value was found to be 0.998. The equation of linearity was also calculated using MS Excel worksheet which shall be used further.

It is very much clear from the *in-vivo* study (Table 4) that there are definite scopes to improve the bioavailability

Table 3: *In-vitro* release profile of liposome formulation F1 and F2

Time (h)	Cumulative percentage drug release	
	F1	F2
0.5	2.87	2.52
1	4.35	4.54
1.5	7.93	8.06
2	10.34	10.58
3	16.74	18.08
4	25.51	26.74
6	37.38	35.16
8	46.13	48.67
10	59.391	62.32
12	74.752	76.86

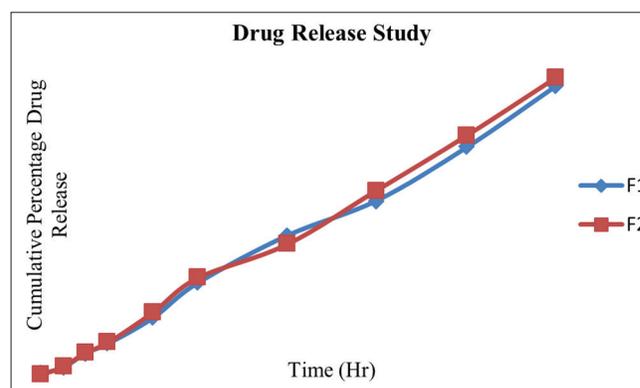


Figure 1: Drug release profile

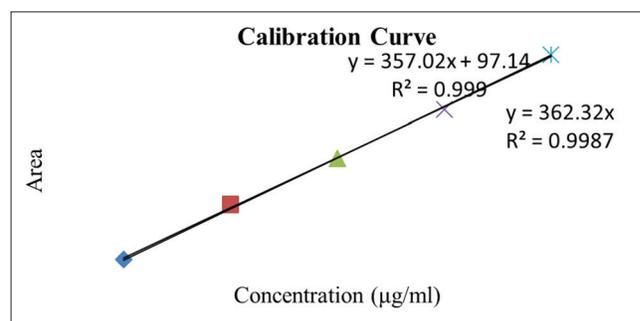


Figure 2: Standard graph of curcumin for pharmacokinetic evaluation

Table 4: Standard graph of curcumin

Concentration ($\mu\text{g/ml}$)	Area
5	1837.65
10	3786.3
15	5412.85
20	7138.4
25	9087.15

Table 5: *In-vivo* pharmacokinetic parameters

Parameters	Curcumin liposome formulation	Curcumin liposome+pure piperine	Curcumin and piperine liposome	Pure curcumin
C_{\max} ($\mu\text{g/ml}$)	3.362	14.724	20.17	2.501
T_{\max} (h)	6	6	6.7	3
$AUC_{0-\infty}$ ($\mu\text{g/ml/h}$)	51.3	223.373	419.409	47.724
MRT	13.813	15.22	20.52	13.261
K_{el}	0.0723	0.0657	0.04873	0.0754

Values are average value of 6 animals, MRT: Mean residence time, AUC: Area under the curve

of curcumin. In contrast with pure curcumin, liposomal curcumin reflects superior bioavailability. These demonstrate that novel drug delivery system has the potential to get better bioavailability. When adjuvant was added with it, then also AUC value amplified. In next step, piperine was also trapped in liposome along with curcumin and shows enhanced bioavailability and improved C_{\max} value. Which may be a sign of slow liposomal drugs release, thus metabolic pathway is also clogged up for longer period; thus, circulation time for curcumin was increased.

CONCLUSION

The present work deals with the development of liposomal drug delivery system for curcumin, an isolate from *C. longa*. Liposomes were fabricated using phospholipid as carrier. From all the formulations, F2 was selected as best formulation due to its ideal particle size high entrapment efficiency and desirable drug release. Further, there is a need to improve the dissolution rate of the curcumin liposomes. When it used with adjuvant, it shows better *in-vivo* parameters. One such ideal approach is to use effective bioenhancers in the formulation. Hence, in future, further work has to be carried out by applying multiple concepts like application of novel drug delivery system and aid of adjuvant in curcumin formulation.

REFERENCES

- Gupta SC, Kim JH, Prasad S, Aggarwal BB. Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. *Cancer Metastasis Rev* 2010;29:405-34.
- Chouhan M, Saha S, Roy A. Curcumin: A review. *J Appl Pharm Res* 2014;11:18-28.
- Saha S, Roy A, Bahadur S, Chandrakar S, Choudhury A, Das S. Phytochemicals as adjuvant in neoplasia. *Asian J Res Chem* 2014;7:765-70.
- Goel A, Aggarwal BB. Curcumin, the golden spice from Indian saffron, is a chemosensitizer and radiosensitizer for tumors and chemoprotector and radioprotector for normal organs. *Nutr Cancer* 2010;62:919-30.
- Gupta SC, Kismali G, Aggarwal BB. Curcumin, a component of turmeric: From farm to pharmacy. *Biofactors* 2013;39:2-13.
- Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: Problems and promises. *Mol Pharm* 2007;4:807-18.
- Du WZ, Feng Y, Wang XF, Piao XY, Cui YQ, Chen LC, *et al.* Curcumin suppresses malignant glioma cells growth and induces apoptosis by inhibition of SHH/GLI1 signaling pathway *in vitro* and *in vivo*. *CNS Neurosci Ther* 2013;19:926-36.
- Aggarwal S, Ndinguri MW, Solipuram R, Wakamatsu N, Hammer RP, Ingram D, *et al.* [DLys(6)]-luteinizing hormone releasing hormone-curcumin conjugate inhibits pancreatic cancer cell growth *in vitro* and *in vivo*. *Int J Cancer* 2011;129:1611-23.
- Moorthi C, Kumar CS, Kathiresan K. Synergistic anti-cancer activity of curcumin and bio-enhancers combination against various cancer cell lines. *Int J Pharm Pharm Sci.* 2014;6:901-3.
- Wahlström B, Blennow G. A study on the fate of curcumin in the rat. *Acta Pharmacol Toxicol (Copenh)* 1978;43:86-92.
- Li H, Zhang N, Hao Y, Wang Y, Jia S, Zhang H, *et al.* Formulation of curcumin delivery with functionalized single-walled carbon nanotubes: Characteristics and

- anticancer effects *in vitro*. *Drug Deliv* 2014;21:379-87.
12. Li C, Zhang Y, Su T, Feng L, Long Y, Chen Z. Silica-coated flexible liposomes as a nanohybrid delivery system for enhanced oral bioavailability of curcumin. *Int J Nanomedicine* 2012;7:5995-6002.
 13. Isacchi B, Bergonzi MC, Grazioso M, Righeschi C, Pietretti A, Severini C, *et al.* Artemisinin and artemisinin plus curcumin liposomal formulations: Enhanced antimalarial efficacy against *Plasmodium berghei*-infected mice. *Eur J Pharm Biopharm* 2012;80:528-34.
 14. Agarwal NB, Jain S, Nagpal D, Agarwal NK, Mediratta PK, Sharma KK. Liposomal formulation of curcumin attenuates seizures in different experimental models of epilepsy in mice. *Fundam Clin Pharmacol* 2013;27:169-72.
 15. Zhang L, Lu CT, Li WF, Cheng JG, Tian XQ, Zhao YZ, *et al.* Physical characterization and cellular uptake of propylene glycol liposomes *in vitro*. *Drug Dev Ind Pharm* 2012;38:365-71.
 16. Pawar YB, Purohit H, Valicherla GR, Munjal B, Lale SV, Patel SB, *et al.* Novel lipid based oral formulation of curcumin: Development and optimization by design of experiments approach. *Int J Pharm* 2012;436:617-23.
 17. Saengkrit N, Saesoo S, Srinuanchai W, Phunpee S, Ruktanonchai UR. Influence of curcumin-loaded cationic liposome on anticancer activity for cervical cancer therapy. *Colloids Surf B Biointerfaces* 2014;114:349-56.
 18. Mukerjee A, Vishwanatha JK. Formulation, characterization and evaluation of curcumin-loaded PLGA nanospheres for cancer therapy. *Anticancer Res* 2009;29:3867-75.
 19. Pandelidou M, Dimas K, Georgopoulos A, Hatziantoniou S, Demetzos C. Preparation and characterization of lyophilised egg PC liposomes incorporating curcumin and evaluation of its activity against colorectal cancer cell lines. *J Nanosci Nanotechnol* 2011;11:1259-66.
 20. Chang MT, Tsai TR, Lee CY, Wei YS, Chen YJ, Chen CR, *et al.* Elevating bioavailability of curcumin via encapsulation with a novel formulation of artificial oil bodies. *J Agric Food Chem* 2013;61:9666-71.
 21. Sharma RA, Euden SA, Platton SL, Cooke DN, Shafayat A, Hewitt HR, *et al.* Phase I clinical trial of oral curcumin: Biomarkers of systemic activity and compliance. *Clin Cancer Res* 2004;10:6847-54.
 22. Sasaki H, Sunagawa Y, Takahashi K, Imaizumi A, Fukuda H, Hashimoto T, *et al.* Innovative preparation of curcumin for improved oral bioavailability. *Biol Pharm Bull* 2011;34:660-5.

Source of Support: Nil. **Conflict of Interest:** None declared.