IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 3 Ver. III (May - Jun.2016), PP 120-127
www.iosrjournals.org

Tailor Made Phospholipid Based Curcumin Phytosomes: Fabrication, Characterization and Ex-Vivo Permeation

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Abstract:*Curcumin (CUR), a highly lipophilic molecule has wide range of pharmacological activities. Nevertheless its limited aqueous solubility and extensive pre-systemic metabolism confine its bioavailability. CUR phytosomes were prepared by simple method and were evaluated in comparison to commercial patent product by DSC, FTIR, TEM, SEM as well as in vitro drug release profile in different dissolution media. Furthermore, a new ex-vivo experimental design utilizing non everted sac model was held out to study the permeability behavior of prepared phytosomes. Results revealed that prepared phytosomes were similar to commercial product in all physicochemical properties. They were spherical; self-closednano-sized structure with good physical stability. DSC and FTIR studies showed a strong complex formation between CUR and phospholipid. Change in surfactant concentration had no effect on the dissolution profile for both CUR-phytosomal powders. Ex-vivo permeation and mucosal uptake studies across rat intestine revealed a good similarity. Complexation of CUR with phospholipid leading to 4 times enhancement in permeation compared with drug suspension. Lipophilic nature of CUR could be the reason for drug entrapment inside the intestinal mucosal cells with limited permeation.The prepared phytosomes could be successfully tailored as a promising and effective formulation for CUR delivery.*

Keywords:*Curcumin; Meriva; Phytosomes; Bioavailability; Ex-vivo; Permeation*

I. Introduction

Many obstacles stand against medicinal use of herbal derived drugsparticularly those containing polyphenolic rings in their chemical structure owing to their poor oral bioavailability. Some of those reasons are low aqueous solubility, high molecular weight/size, poor membrane permeability and low stability in the presence of gastric fluids. One of the methods to respond to latter challenges is the application of phytosomal technology [1].

Phytosomes were developed at Indena (Milan. Italy) in herbal formulations that provide better pharmacokinetic and pharmacodynamic behavior than conventional botanical extracts. This technique utilized complex formation between phospholipid molecules and herbal extracts or its constituents producing a lipid compatible molecular complex soluble in both water and lipid environment [2]. Water soluble drugs as well as poorly water soluble candidates can be incorporated in phospholipid complex to enhance their bioavailability either by increasing penetration through the plasma membrane or improving their solubility in gastric fluids [3].

CURhas been reported to have a diversity ofbiological and pharmacological activities [4]. Extremely low aqueous solubility or extensive pre-systemic metabolism may be responsible for the unfavorable pharmacokinetics of this molecule[5]. Various products are marketed worldwide containing CUR as principle powder ingredient.Keeping in mind, the importance of CUR as a therapeutically active agent with its poor oral absorption problem and the need to develop new formulationsthat can increase oral absorption and enhance therapeutic activity, CUR phytosomes were patently developed by Indena (Milan. Italy) and Meriva® is the only patent commercial product for formulated CURphospholipid complex[6]. Compared bioavailabilities of pure CUR and Meriva® has been evaluated in rats, showing that Meriva® is five times more bioavailable than CUR[7].

CUR-SPC complex in a semisolid form were examined by many authors [8-10], nevertheless, no studies evaluated their formulations in comparison to commercial patent product in order to get a similar formula with a simple low cost method.

The main aim of our study is to prepare CURphospholipid complex in a dry powder form resemble to patently Indena product (Meriva®) using simple and low cost industrially applicable method, different physicochemical characterization for the prepared complex were assessed in comparison to commercial product. Furthermore, the permeability of CUR across the intestinal mucosa in addition to mucosal uptake for both products utilizing non everted sac model were also studied.

II. Materials And Methods

Curcumin powder was purchased from Shenzhen chemrider, China; the soy phosphatidylcholinephospholipid (SPC-Lipoid S-100) was kindly supplied by Lipoid Co., Ludwigshafen, Germany; Curcumin - phospholipid S 100 complex(Meriva®) was a gift from IndenaSpA, Italy. Polyoxyl 35 castor oil (CRM EL) by Basf, USA; Collidal silicon dioxide (Aerosil 200), Sodium lauryl sulphate (SLS) by Evonik Degussa, UK; All other reagents and chemicals were of analytical grade.

2.1. Preparation of curcumin phytosomes (CUR-PHY)

CUR and SPC S 100 in molar ratio of 1:1 were dissolved in 20 mL of dichloromethane andthe mixture was refluxed for 2 h at room temperature and poured into colloidal silicon dioxide powder with the same weight of SPC. The solvent was evaporated in a controlled temperature water bath with application of a mixer (IKA T25, Germany) at 40 - 45°for 4 hours. The prepared flakes were grinded into fine powder.

2.2. Characterization of the prepared CUR-PHYpowder

2.2.1. Transmission electron microscope (TEM)

Morphological examination of phyto-vesicles for CUR-PHY and Meriva® were carried out using TEM (model JEM-100S microscope, Jeol, Japan). Samples were diluted and sonicated for 10 mins. A drop of the resultant phospholipids complex dispersions was placed onto a carbon-coated copper grid, leaving a thin liquid film. The film was dried by air then viewed. The mean particle size for vesicles was measured and photographed using TEM.

2.2.2. Scanning electron microscope (SEM)

SEM photographs were taken to study the difference in morphology among CUR powder, CUR-PHY complex and Meriva®. Where the dried sample was fixed on aluminum stubs using double-sided adhesive tape and coated with gold. A scanning electron microscope (model JSM-6360LV, Jeol, Japan) with a secondary electron detector was used to obtain digital images of the samples at an accelerating voltage of 10 kV.

2.2.3. Differential scanning Calorimetry (DSC)

Thermal behavior of CUR-SPC complex was assessed using DSC analysis (Perkin Elmer, USA). The samples were sealed in the aluminum crimp cell and heated at the speed of 10℃/min from 30 to 300℃ in nitrogen atmosphere (60 mL/min). Thermograms of CUR, SPC, CUR-PHY andMeriva® were examined.

2.2.4. Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR spectra were obtained using a FTIR spectrometer (Perkin Elmer Life and Analytical Sciences, Shelton, CT, USA). Samples were mixed with dry crystalline KBr in a ratio 1:100 and tablets were compressed for measurements. A spectrum was collected for each sample within the wave number region 4,000 – 500 cm^{-1}. Samples assessed encompassed CUR, SPC, CUR-PHY and Meriva®.

2.2.5. In-vitro dissolution studies

According to dissolution test for CUR at USP 32[11]; 900 mL 1% SLS were used as a dissolution medium using apparatus II (Hanson research, USA) at 100 rpm.Different dissolution media (0.25%, 0.5% and 1%SLS & 0.5% Tween 80[12] were used to study the dissolution pattern for CUR-PHY for 4 hours at the same pharmacopeial conditions. CUR powder, CUR-PHY and Meriva® were weighed to 40 mg (in terms of CUR powder) for the dissolution test. 10 mL sample was withdrawn at intervals; 60, 120, 180, 240 min and replaced by fresh medium. Samples were filtered and diluted to a proper concentration with the corresponding medium and analyzed spectrophotometrically at wave length 420 nm.

2.2.6. Ex vivo intestinal permeation studies

2.2.6.1. Animals' protocol

Ex vivo permeation studies for CUR pure powder, CUR-PHY and Meriva® were carried out using non-everted gut sac technique[13-15]. A total number of 15 male wistar rats (weighing 200-250 g) were obtained from animal house of faculty of pharmacy (Alexandria, Egypt). Experiments were performed in accordance with the European community guidelines for the use of experimental animals and were approved by the institutional ethics committee. The rats were housed in a temperature and humidity controlled room (23℃, 55%) with free access to water and standard rat chow. The rats were acclimated for at least 5 days and fasted overnight but supplied with water and libitum before the experiment. Animals were sacrificed by spinal dislocation and small intestine was immediately removed after sacrifice by cutting across the upper end of the duodenum and the lower end of the ileum and manually stripping the mesentery. The small intestine was washed out carefully with

cold normal oxygenated saline solution (0.9% w/v, NaCl) using a syringe equipped with blunt end. The clean intestinal tract was prepared into 12 ± 0.5 cm long sacs having a diameter of 3 ± 0.5 mm.

2.2.6.2. Permeation experimental study

Each sac was filled via a blunt needle with 1 mL of test sample (equivalent 1 mg CUR) dispersed in a solution compromising from (0.9% w/v, NaCl), 100 µm itraconazole and 2% Tween 80 . Each sac was tied tightly with a thread and placed in a conical flask containing 50 mL of ethanolic saline solution with ratio (98:2) containing 10% CRM EL. The entire system was maintained at 37℃ in a shaking water bath (Bunsen, India) operated at 100 rpm and aerated with 5% CO_2 and 95% O_2 (10-15 bubble/min) using laboratory aerator. Samples were withdrawn from outside of the sac and replaced by a fresh medium for 3 hours[16] at time intervals 30, 60, 120 and 180 mins. Samples were analyzed spectrophotometrically at wave length 420 nm. At the end of permeation test, each sac was withdrawn from its solution and cut from its two ends to wash the inner part of sac with a saline solution. The intestinal sac was opened longitudinally to isolate the mucosal layer by scratching by a spatula and 1 ml of acetone was mixed with mucous samples. The sample was centrifuged at 4000 rpm for 15 min prior to be analyzed by HPLC. Studies were performed in triplicates and all data were statistically analyzed by student t-test $P< 0.05$ (GraphPad Prism version 3.02, GraphPad Software, SanDiego, CA).

2.2.6.3. High-performance liquid chromatography (HPLC)

A validated HPLC method of analysis for CUR was adapted by Marchzylo et al[7]. The HPLC instrument (Agilant, USA) was equipped with C18 column (4.6 x150 mm, 3µm) with a guard (4.6 x 20 mm, 3 µm) kept at 35℃ and UV detector adjusted at 426 nm. Mobile phase was composed 10 mM of ammonium acetate buffer and Acetonitrile at flow rate 1.5 mL per minute. It was initially adjusted at 95% of a progressing to 55% at 20 mins until reaching 5% at 33 mins

III. Results And Discussion

3.1. Preparation of CUR-PHY

In the preparation of CUR-PHY, method expressed byGupta et al[9] and Maiti et al[10]was developed with some modification. A very low density powder (Aerosil 200) was selected among different fillers to be the most proper diluent used as a carrier to produce CUR-PHY powder. The formed complex flakes were mechanically grinded to produce fine and flowable powder.

3.2. Transmission electron microscopy (TEM)

Figure 1 indicated that CUR PSC was spherical or ellipsoidal; self-closed structure for both prepared CUR-PHYand Meriva® with mean PS 24.2 ± 1.66 nm and 37.2 ± 7.07 nm respectively, which demonstrated that the complex formation did not change the amphiphilic properties and water dispersion associated with phospholipids [12].

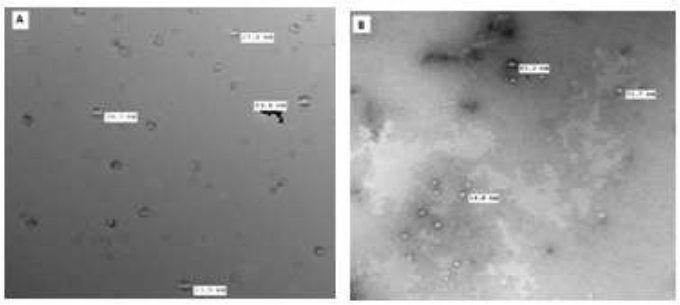

Fig. 1:Transmission electron microscopic photograph of CUR-SPC phytosome (A) and Meriva (B) with 20 folds dilution in distilled water (magnification 10,000 X)

3.3. Scanning electron microscope (SEM)

Figure 2(a) showed flat broken needles with different size, with well-developed edges of CUR powder which lines with observation by paradkar et al[17], conversely, figure 2 (b, c) showed an irregular, round shaped and smooth surface for both Meriva® and CUR-PHY which could be attributed to change in internal crystalline structure due to complex formation.

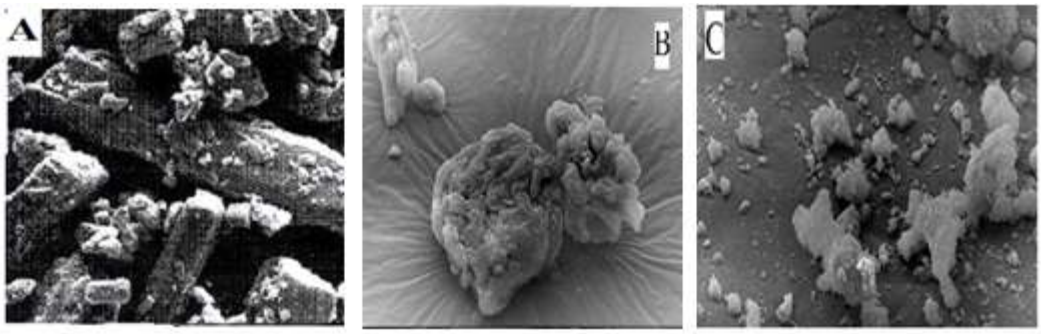

Fig. 2: Scanning electron microscope photograph of (A) CUR, (B) Meriva and (C) prepared complex

3.4. Differential Scanning Calorimetry

DSC is a fast and a crucial tool to screen drug-excipient compatibility and possible interactions at solid state of matter. An interaction is concluded by elimination of endothermic peak(s), appearance of new peak(s), change in peak shape and its onset, peak temperature/melting point and relative peak area or enthalpy. Consequently, DSC thermograms of CUR (A), SPC (B), Meriva® (C) andCUR-PHY (D) were presented at figure 3. The thermogram of CUR showed a melting range from 177.638℃ to 185.268℃ with one single peak.Thermogram of SPC showed two different melting ranges; the first one is mildfrom154.306℃to 160.029℃andthe second one is broadfrom232.577℃ to 295.66℃, that may be owing to the movement of phospholipids polar head group and phase transition from gel to liquid crystalline state, respectively as reported in many literatures [9, 10, 12, 18]. A melting range from 134.7℃to 171℃ appeared in the thermogram of CUR-PHY, which was different from each individual components of the complex. This could be due to complete and strong complex formation of CUR with SPC during the preparation process throughhydrophilic/ hydrophobic interaction. The –OH groups of the phenol rings of curcumin are involved in hydrogen bonding whereas the aromatic rings could be involved in hydrophobic interaction[10]. For Meriva®, a similar melting range to that of locally prepared phytosomes wasobserved from 134.6℃ to 164.200 ℃ with small minor peaks which could be attributed to uncomplexed SPC or incomplete evaporation of residual solvent during the preparation process.

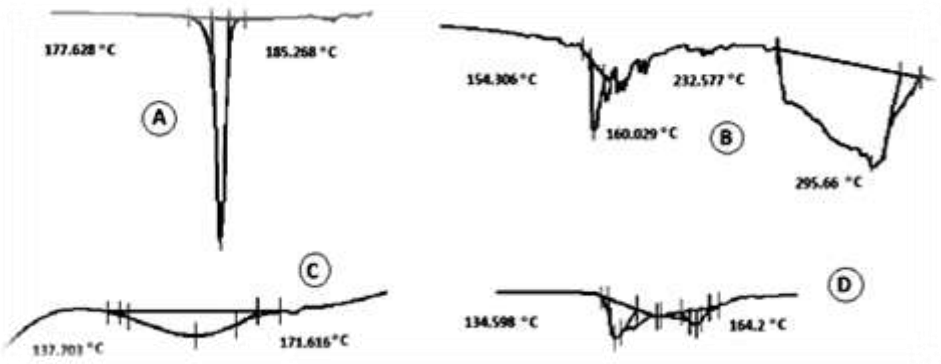

Fig. 3: DSC thermograms exhibiting the peak transition of pure CUR (A), SPC (B), prepared complex (C) and Meriva®(D)

3.5. Fourier Transform Infrared Spectroscopy (FT-IR)

Further investigation for complex formation was carried out by using infrared spectra spectroscopy. As shown in figure 4, the band at 3501 cm^{-1} was attributed to the stretching vibration of the phenolic –OH group in natural CUR (A), additionally two bands were observed at 1625 cm^{-1} and 1275 cm^{-1} which attributed for C = O and C – O respectively[19]. For SPC (B) strong peaks at 1736 cm^{-1} and 1238 cm^{-1} were due to C = O absorption and P = O absorption respectively, strong peaks at 2925 cm^{-1} and 2855 cm-1 and weak peak at 1376 cm^{-1} due to stretching and deformation of methyl groups. The peak at 1465 cm^{-1} observed in SPC could be due to bending vibration of CH2[12].

A shift from 3500 cm^{-1} to broad peak at 3345 cm^{-1} and 3403 cm-1 for Meriva® (C), CUR-PHY (D) respectively compared with natural CUR and a shift from 1238 cm^{-1} to 1249 cm^{-1} compared with SPC were excited which indicated that a complex is formed by hydrogen bonding between the OH group of the phenol rings of curcumin and the P = O group of the phospholipids. For CUR-PHY (D), a strong broad sharp peak was

observed at 1096 cm^{-1} which may be attributed to SiO2 group of colloidal silicon dioxide used in the preparation of CUR-SPC complex. This observed peak may mask a peak of P = O of SPC at 1267 cm^{-1}.

3.6. In-vitro dissolution studies

Due to the absence of a specified dissolution medium for CUR-PHY, different dissolution media (0.25%, 0.5% & 1%) SLS and 0.5% Tween 80[10] were used to study the dissolution pattern for CUR-PHY for 4 hours at the same pharmacopeial conditions for CUR powder. By using the above mentioned dissolution systems, a comparative in-vitro dissolution study were carried out between Meriva® and our prepared CUR-PHY.

As shown in figure 5A, dissolution profile for CUR powder couldn't be measured in 0.25% SLS medium due to limited solubility of CUR in 0.25% SLS . On increasing the SLS concentration, the dissolution profile for CUR in its complexed powder form was enhanced due to better wettability for the complex powder resulting in better dissolution profile[20, 21].

CUR-PHYshowed better dissolution profile than Meriva®at 0.25%, 0.5% and 1% SLS, this may be attributable to better wettability of free flowing prepared complex powder than Meriva® powder. On the other hand, dissolution profile of CUR-PHYpowder is very similar to Meriva® in 0.5% Tween 80 (Figure 5D).

A similar dissolution profile for both CUR-SPC complex powder on using 0.5% SLS and 0.5% Tween 80 was appeared in Figure 5B, 5D. These indicated that change in surfactant concentration, either ionic or non-ionichad no effect on the dissolution profile for CUR-SPC complex powder.

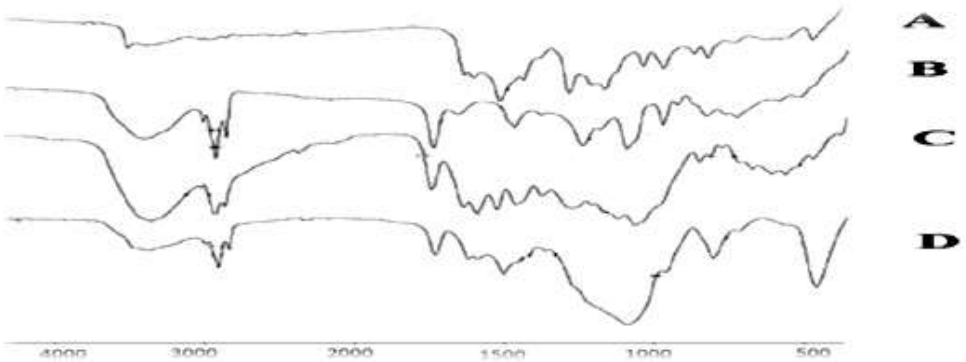

Fig. 4. FTIR spectra of (A) curcumin, (B) phospholipids, (C) Meriva® and (D) prepared phytosomes.

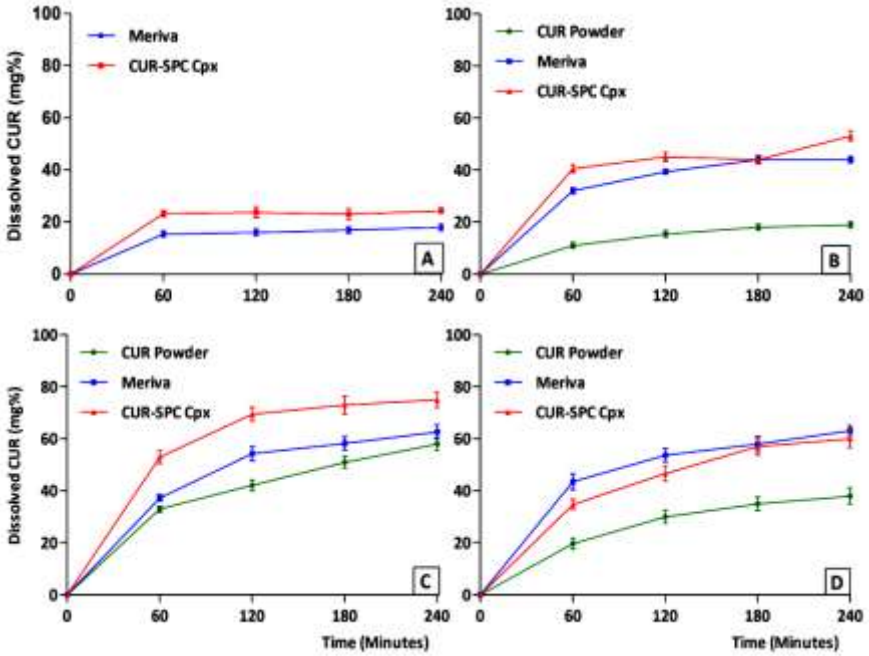

Fig. 5. Comparative dissolution profile between Meriva®, CUR SPC Cpx and CUR powder in aqueous 0.25% SLS (A), 0.5% SLS (B), 1% SLS (C) and 0.5% Tween 80 (D)

3.7. Ex vivo intestinal permeation studies

In vitro absorption models as non- everted intestinal sac was commonly used to study drug absorption[22] through the intestine which reflects the in-vivo drug absorption in humans with less labor and experimental costs to be compared with in vivo studies. For passive transported drugs with norequired energy for permeation, Genty et al[23] reported the permeability of passive absorption drug remained the same whether the sacs were everted or not. These results support the use of non-everted sac model to study the permeation of passively diffused CUR[24]. Several advantages to utilize non everted sac model over everted sac model including simplicity, need for less amount of test sample and ability to collect successive of serosal samples with less morphological changes[25].

3.7.1. Preparation of receiver solution and test samples

Although Gupta et al[9] utilized everted intestinal sac model to study the permeation difference between CUR-SPC complex and CUR powder, the design of the used system could be criticized due to the use of phosphate buffered saline with pH 7.4, knowing the reported instability of CUR powder in alkaline medium[26], in addition to, Long period of the experiment time up to 6 hours with an absence for any data guarantee the intestine viability for a long period of time.

Conversely, Shishu et al[14] utilized non-everted intestinal sac model to show effect of different vehicles on the permeation of CUR from various sources where the receiving solution compromised from Ringer phosphate buffered saline at pH 7.4 and isopropyl alcohol in ratio (7:3), but the design of the experiment could be also criticized due to the same above reasons, in addition toabsence of an evidence data on using a strong dehydrating agent as isopropyl alcohol which may affect on intestine viability leading to false results.

From previous criticized experiments, a new experiment design was held out to study the permeability behavior of our prepared CUR-PHY compared to Meriva®. Depending on data published by Hamid et al [27] about the effect of different solubilizing agents on the intestine viability of rat, various solubilizing agents were tested according to their ability to solubilize CUR including 10% PEG 400, 10% Ethanol and 10% Cremophor. From this viewpoint, 10% CRM EL showed the highest solubilizing effect for CUR to be mixed with saline ethanolic solution in ratio (98:2) to form the receiver solution for ex-vivo permeation study.

3.7.2. Preparation of test samples

Being a class IV drug[24], CUR possess poor solubility and poor permeability. The poor permeability was encountered to intestinal metabolism by CYP 3A4 enzymes resulting in formation of curcumin glucouronide and curcumin sulphate metabolites[28]. To avoid any obstacle that may interfere with CUR permeability through non everted rat intestine, 100 µm itraconzaole which was reported to be CYP 3A4 inhibitor[29, 30] was added to dispersion solution for test samples before the injection process. Also, 2% Tween 80, which has no reported effect on intestinal viability of rats[27], was added as a wetting agent for Meriva® and CUR-PHY powder to enhance the dispersion of the powder in the prepared solution.

3.7.3. Permeationand Mucosal uptake

Intestinal permeation profiles for Meriva® and prepared phytosomes in comparison to CUR suspension were depicted inFigure 6. A good similarity between the amounts of CUR permeated across rat intestine for Meriva® and CUR-PHY complex, the maximum permeated amount in the receiver compartment was 45 µg after 60 mins and no further increase till the end of experiment. The lipophilic nature of CUR could be the main reason for drug entrapment inside the intestinal mucosal cells leading to limited permeation across rat intestine. On the other hand, permeability results of CUR suspension was 11 µg after 3 hours, complexation of CUR with phospholipid leading to 4 times enhancementin permeation compared with corresponding drug suspension. Phospholipids can act as an effective emulsifier which could provide dramatically enhanced permeation for lipid soluble highly lipophilic drugs[31].A similar work was reported by Freag et al[16] to study the permeability difference between diosmin phytosomes and diosmin powder suspension utilizing non everted sac model, a relative much lower similar results was obtained in our study which could be related to strong complex formation between CUR and phospholipid in contrary to diosmin-phospholipid complex which showed a weak complex form.

Results obtained from the analysis of the amount of CUR retained inside the mucosal cells were graphically illustrated in Figure 6. A similarity between Meriva® and the prepared CUR-PHYwas also revealed. In contrary, mucosal uptake for CUR suspension was much higher than those obtained from application of CUR in its phytosomal structure. This may be also come across high lipophilicity and low aqueous solubility of CUR powder leading to attachment of CUR to the outside part of the mucosal cells resulting in low permeation of CUR across the rat intestine. Phytosomal structure of curcumin either for Meriva or prepared CUR-PHY increased intestinal permeation of curcumin compared to CUR suspension which subsequently decreases CUR appendance inside the mucosal cells.

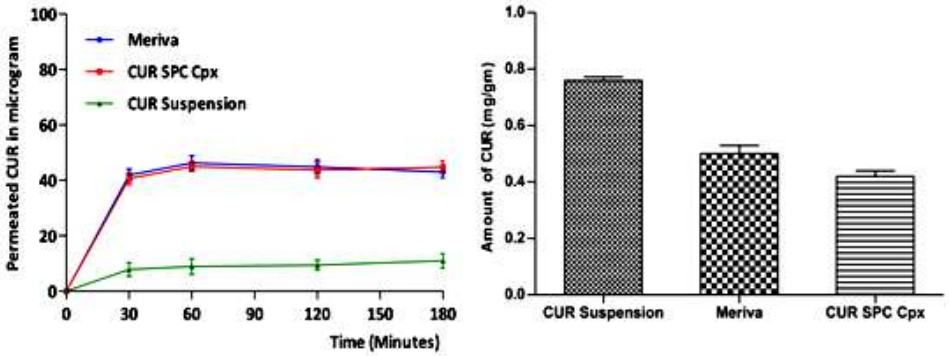

Fig. 6. Comparative permeation results and mucosal uptakefor Meriva®, prepared CUR-SPC complex and CUR suspension.

IV. Conclusion

In our study, we successfully developed a dry powder form of CUR-PHY by a simple method. Different in-vitro characterization revealedgood similarity between prepared CUR-PHY andMeriva®. Complexation of CUR with phospholipid bring about 4 times enhancement in permeation compared with corresponding drug suspension. This approach opens much more chances to produce similar products with low cost price. Formulation of our product in a final dosage form utilizing the advantages of softgel technology using lipid based excipients with reported bioactive effect on CURmetabolism is under investigation.

References

[1]. ZTeng, CYuan, FZhang, MHuan, WCao, KLi, JYang, DCao, SZhou and Q Mei. Intestinal Absorption and first-pass metabolism of polyphenol compounds in rat and their transport dynamics in Caco-2 cells. Plos One, 7, 2012, 1-9.
[2]. P Shivanand and P Kinjal. Phytosomes: Technical revolution in phytomedicine. Int J Pharmatech Res, 2, 2010. 627-631.
[3]. G Fricker, TKromp, AWendel, ABlume and A Zirkel. Phospholipids and lipid based formulations in oral drug delivery. PharmRes, 27, 2010, 1469-1486.
[4]. RKMaheshwari, AKSingh, JGaddipati and RC Srimal. Multiple biological activities of curcumin: a short review.Life Sci,78, 2006, 2081–2087.
[5]. S Oetari, M Sudibyo, NM Commandeur, R Samhoedi and PEVermeulen. Effects of curcumin on cytochrome P450 and glutathione S-transferase activities in rat liver.Biochem Pharmacol, 51, 1996, 39-45.
[6]. http://www.phytosomes.info/public/meriva.asp
[7]. THMarczylo, RDVerschoyle, DNCooke, P Morazzoni, WPSteward and AJ Gescher. Comparison of systemic availability of curcumin with that of curcumin formulated with phosphatidylcholine. Cancer Chemother Pharmacol,60, 2007, 171-177.
[8]. F Zhang, GY Koh, DP Jeansonne, J Hollingsworth, PS Russo, G Vicente, RW Stout and Z Liu. A novel solubility-enhanced curcumin formulation showing stability and maintenance of anticancer activity. J Pharm Sci, 100, 2011, 2778-2789.
[9]. NK Gupta , VKDixit. Bioavailability enhancement of curcumin by complexation with phosphatidyl choline. J Pharm Sci, 100, 2011, 1987-1995.
[10]. KMaiti, KMukherjee, AGantait, BSaha andPK Mukherjee. Curcumin-phospholipid complex:Preparation, theraputic evaluation and pharmacokinetic study in rats. Int J Pharm, 330, 2007,155-163.
[11]. United States pharmacopeia, Thirty second edition. National Formulary, twenty seventh edition, 2011.
[12]. JZhang, QTang, XXu andNLi. Development and evaluation of a novel phytosome-loaded chitosan microsphere system for curcumin delivery. Int J Pharm, 448, 2013, 168-174.
[13]. LPRuan, SChen, BYYu, DN Zhu, GA Cordell andSX Qiu. Prediction of human absorption of natural compounds by the non-everted rat intestinal sac model. Eur J Med Chem, ,41,2006, 605-610.
[14]. Shishu, Kamalpreet and M Maheshwari. Development and evaluation of novel microemulsion based oral formulations of 5-fluorouracil using non-everted rat intestine sac model. Drug Dev Ind Pharm, 38,2012, 294-300.
[15]. C Bothiraja, APPawar, GYDama, PPJoshi and KSShaikh. Novel solvent-free gelucire extract of Plumbago zeylanica using non-everted rat intestinal sac method for improved therapeutic efficacy of plumbagin. J Pharmacol Toxicol Methods,66, 2012, 35-42.
[16]. MSFreag, YSElnaggar andOYAbdallah. Lyophilized phytosomal nanocarriers as platforms for enhanced diosmin delivery: optimization and ex vivo permeation. Int J Nanomedicine, 8, 2013 ,97-85.
[17]. AParadkar, AAAmbike, BKJadhav, KRMahadik. Characterization of curcumin-PVP solid dispersion obtained by spray drying. Int J Pharm, 271, 2004, 281-286.
[18]. XYanyu, SYunmei, CZhipeng and PQineng. The preparation of silybin-phospholipid complex and the study on its pharmacokinetics in rats. Int J Pharm, 307, 2006, 77-82.
[19]. MKModasiya and VM Patel. Studies on solubility of curcumin. Int J Pharmacy life sci, 3, 2012, 1490-1497.
[20]. JCui, BYu, YZhao, W Zhu, HLi and H Lou. Enhancement of oral absorption of curcumin by self-microemulsifying drug delivery systems. Int J Pharm, 371, 2009, 148-155.
[21]. RShelma and CPSharma. Invitro and invivo evaluation of curcumin loaded lauryolsulphated chitosan for enhancing oral bioavailability. Carbohydrate polymers, 95,2013, 441-448.
[22]. PSharma, HPChawlaa andRPanchagnula. TLC determination of cephalosporins in in vitro rat intestinal sac absorption model. J Pharm Biomed Anal, 27, 2002, 39-50.
[23]. M Genty, G Gonzalez, C Clere, V Desangle-Gouty and JY Legendre. Determination of the passive absorption through the rat intestine using chromatographic indices and molar volume. Eur J Pharm Sci,12, 2001, 223-229.

[24]. BWahlang, YBPawar and AK Bansal. Identification of permeability-related hurdles in oral delivery of curcumin using the caco-2 cell model. Eur J Pharm Biopharm. 77, 2011, 275-282.
[25]. PDixit, KM Jain and J Dumbwani. Standardizationof an ex vivo method for determination of intestinal permeability of drugs using everted rat intestine apparatus. J Pharmacol Toxicol Methods, 65, 2012, 13-17.
[26]. YJWang, MPan, ACheng, LLin, YHo, CHsieh and J Lin. Stability of curcumin in buffer solutions and characterization of its degradation products. J Pharm Biomed Analysis, 15, 1997, 1867-1876.
[27]. KAHamid, HKatsumi, TSakane and A Yamamoto. The effects of common solubilizing agents on the intestinal membrane barrier functions and membrane toxicity in rats. Int J Pharm, 379, 2009, 100-108.
[28]. SOetari, MSudibyo, NMCommandeur, RSamhoedi and PE Vermeulen. Effects of curcumin on cytochrome P450 and glutathione S-transferase activities in rat liver'. Biochem Pharmacol, 51, 1996, 39-45.
[29]. XRen, XMao, L Cao, K Xue, L SI and J Qiu. Nonionic surfactants are strong inhibitors of cytochrome P450 3A biotransformation activity in vitro and in vivo. Eur J Pharm Biopharm, 36, 2009, 401-411
[30]. XRen, XMao, LSi, LCao andHXiong. Pharmaceutical excipients inhibit cytochrome P450 activity in cell free systems and after systemic administration. Eur J Pharm Biopharm, 70, 2008, 279-288.
[31]. KRVinod, S Sandhya, JChandrashekar, BDavid and S Anbuazaghan. A review on genesis and characterization of phytosomes. Int J Pharm Sci Rev Res, 4, 2010, 69-75