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FORMULATION AND EVALUATION OF A DRY POWDER INHALER OF CURCUMIN FOR LUNG CANCER TREATMENT

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ABSTRACT

Lung Cancer is most devastating tumor destroying human life in recent times. The lethal nature of this aggressive disease is shown by the fact that incidence rate and mortality rate are almost equivalent to each other. In the present work, we developed dry powder formulation of the curcumin by spray drying technique. In this review we have to look at glance on various kind of method of Preparation of Dry Powder Inhaler and its Evaluation for various parameters i.e. Mean Particle size and Polydispersity index, Zeta potential, Drug content, XRD, SEM and Density etc.

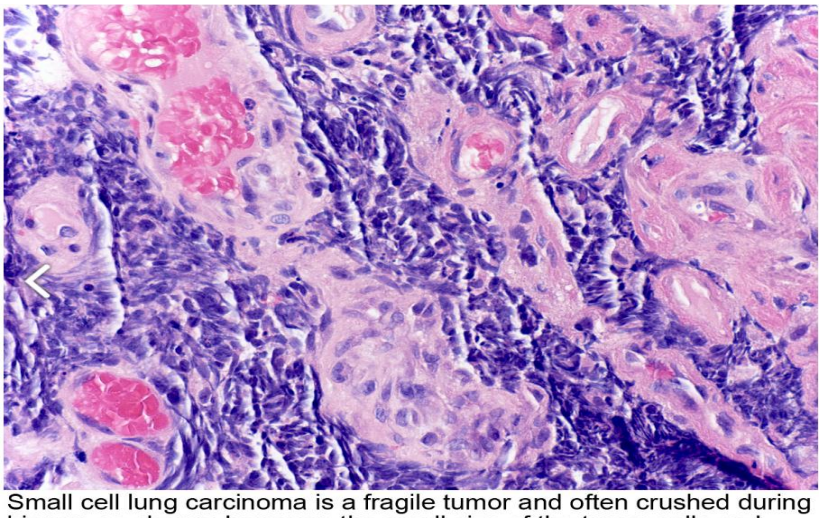
KEYWORDS: Lung Cancer, NSCLC, SCLC, Curcumin, Dry Powder Inhaler, Evaluation.

INTRODUCTION

Cancer is one of the human severe diseases and causes increasing morbidity and mortality every year in the world. As a leading cancer type, pulmonary carcinoma is account for 14% of new cancer cases and is associated with more than 25% of the total cancer-induced deaths in United States of America. Tumor growth and systemic metastasis are highly dependent on angiogenesis. Angiogenesis promotes tumor growth by supplying nutrients and oxygen and removing waste products, meanwhile, facilitating tumor invasion and metastasis. Angiogenesis is essential for tumor growth and metastasis, therefore antiangio- genesis has been proposed as a promising therapeutic strategy for clinical therapy of tumors. Anti angiogenic therapy aims to prevent the formation of new vessels around tumors and to frustrate the existing abnormal capillary network that supports the tumor. Cancer takes place by uncontrolled growth and spreading of abnormal cells. It is of two types: benign and

malignant. The cells of malignant tumors have the ability to attack neighbouring tissues and organs, also the possibility of cancerous cells to rupture from the tumor and enter into the blood stream, thus spreading of the disease to other organs occur. This process of spreading is called metastasis.

Lung cancer (also known as carcinoma of the lung) is a malignant lung tumor characterized by uncontrolled cell growth in tissues of the lung. If left untreated, this growth can spread beyond the lung by process of metastasis into nearby tissue or other parts of the body. Mostcancers that start in the lung, known as primary lung cancers, are carcinomas that derive from epithelial cells. The main primary types are small-cell lung cancer (SCLC), also called oat cell cancer, and non-small-cell lung cancer (NSCLC). The most common symptoms are coughing (including coughing up blood), weight loss, shortness of breath, and chest pains. The most common cause is long-term exposure to tobacco smoke, which causes 80–90% of lung cancers. Nonsmokers account for 10–15% of lung cancer cases, and these cases are often attributed to a combination of genetic factors, and exposure to; radon gas, asbestos, and air pollution including second-hand smoke.



Small cell lung carcinoma is a fragile tumor and often crushed during biopsy, as shown; however, the small size of the tumor cells and lack of nucleoli can still be appreciated; the high nuclear to cytoplasm ratio is also demonstrated

Curcumin possesses wide-ranging anti-inflammatory and anticancer properties. Many of these biological activities can be attributed to its potent antioxidant capacity at neutral and acidic pH, its inhibition of cell signalling pathways at multiple levels, its diverse effects on cellular enzymes, and its effects on cell adhesion and angiogenesis. In particular, curcumin's ability to alter gene transcription and induce apoptosis in preclinical models advocates its potential utility in cancer chemoprevention and chemotherapy. With regard to considerable

public and scientific interest in the use of phytochemicals derived from dietary components to combat or prevent human diseases, curcumin is currently a leading agent. Curcumin have revealed poor absorption and rapid metabolism that severely curtails its bioavailability. Curcumin absorption is very low only 40% of drug absorbed that will go extensive metabolism in guts. The mean oral bioavailability of curcumin is 2% due mainly to hepatic first pass metabolism. Curcumin is given by oral, intravenous, intraperitoneal, suppositories, topical route.

The dry powder inhaler device is one of the types of delivery systems that enable generation and delivery of aerosolised drug particles into the respiratory tract. The principle of this therapy is to transfer powdered medication into a clinical effect. However, the respiratory tract is a strongly branched system, which works excellently as filtering system to avoid penetration of particles into the lungs. To enable penetration into the lungs, the aerodynamic particle size of the inhaled medication has to be small, preferably below 5 to 10 μm. The particle size distribution of the drug is one of the major determinants in drug delivery to the respiratory tract. Special techniques, such as micronisation of drugs in dry powder systems, are required to produce particles (or droplets) in this size range. Because the handling of micronized particles is very difficult, and the amounts of drugs that have to be metered in inhalation therapy are often very low (6 mg to 500 mg), special formulations and devices have to be applied to obtain the required clinical effect from the administered dose.

Dry powder formulations for inhalation consist of fine drug particles and coarse carrier particles like lactose. The fine drug particles adhere to the carrier surface to form ordered mixtures. The carrier particles are used to improve the flow of the drug particles which are usually present in a low concentration, with a usual drug-carrier ratio of 1:67.5 (w/w) (Zeng *et al.,* 2000, Timsina *et al.,* 1994). The improvement in the flowability of the powders helps in the reproducible dose metering. The carrier particles also help to reduce the high cohesive forces among micron sized drug particles which prevents the aggregation of the particles. Interactions between the drug and carrier particles are mainly dependent on the physicochemical characteristics such as particle size, shape, surface morphology, contact area and hygroscopicity (Berard *et al.,* 2002, Ferron, 1994). The adhesion between carrier and drug must be sufficient for the drug-carrier blend to be stable. Simultaneously, the adhesion between the drug-carrier has to be weak enough to enable the detachment of drug from carrier during patient inspiration. If the above two criteria are fulfilled then the drug will be

able to reach the lungs efficiently.The carrier widely used in DPI formulations is lactose monohydrate. Lactose in solid form can be crystalline or amorphous. Crystalline lactose can exist in one of two distinct forms: β-lactose and α-lactose monohydrate (Larhrib *et al.*, 1999). Crystals of α-lactose monohydrate have a characteristic to mahawk-like shape. Crystals of pure β-lactose have a characteristic kite-like form. They do not contain crystal water and often referred to as anhydrous lactose. The advantages of lactose as a carrier are its ease of availability, low price, its well-investigated toxicity profile and well-established stability profile (Telko *et al.*, 2005 and Saint-Lorant *et al.*, 2007).

METHOD OF PREPARATION

A. Method of preparation of dry powder inhaler (DPI)

Two separate steps were performed to produce the formulation. First Curcumin dispersion was prepared in 50 mL of water. The curcumin dispersion was transferred in surfactants solution i.e. DPPC and Pluronic F-127.Thesuspended solution was ultarasonicated for 30 min and homogenized at 10000 rpm for 12 min. The homogenized solution was passed through high pressure homogenizer at pressure of 600 bars and 8 cycles to produce nanoparticles. The solvent evaporation was done by lyophilization and/or vacuum drying by rotary evaporation. The DPI was prepared by using different concentration of drug and surfactant. Curcumin containing DPI was prepared using DPPC and Pluronic F-127 as surfactant to formulated batch.

B. Lyophilized Nanoparticles

Aqueous dispersion of the curcumin surfactant was frozen with 6% inhalac 400 (Inhalation grade lactose as cryoprotectant) in deep freezer at -70 ˚C for 12 hrs. Then the sample was lyophilized using a freeze-drying was carried out at -75 ˚C for 24 hrs. The role of cryoprotectant is to decrease nanoparticle aggregation during the process of freeze drying. The vacuum was maintained at 76 mTorr (Baheti *et. al.*, 2010).

C. Vacuum drying of Nanoparticles by Rotary Evaporation

Inhalac-400 (Inhalation grade lactose as cryoprotectant) 6% was added in aqueous dispersion of the curcumin and surfactant. Then sample was placed in rotary evaporator at 30 ˚C, 22 bar pressure, 50 rpm for 1:30 hrs. Dried formulation was kept under vacuum condition for 12 hrs.

EVALUATION

1. Mean Particle size (MPS) and Polydispersity index (PDI)

The MPS and PDI were determined by PCS with a Malvern Zetasizer (Nano ZS 90, Malvern ltd., UK). The measurement using PCS is based on the light scattering phenomena in which the statistical intensity fluctuations of the scattered light from the particles in the measuring cell are measured. Prior to the measurements, all samples were diluted with double distilled water to produce a suitable scattering intensity. The z-average and PDI values were obtained at an angle of 90° using disposable polystyrene cells having 10 mm diameter cells at 25°C, which were equilibrating for 120 seconds. All measurements were performed in triplicate at 25°C.

2. Zeta potential

The zeta potential (ZP), reflecting the electric potential on the particle surface and indicating the physical stability of colloidal systems, was measured by determining the electrophoretic mobility using the Malvern Zetasizer (Nano ZS 90, Malvern ltd., UK). The measurements performed with diluting in double-distilled water. It was measured using Dip cell with applying field strength 20 V/cm and the average of the zeta potential was given from 30 runs.

3. Determination of production yield of DPI

The production yield of DPI of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and surfactant used for preparation of DPI and percent production yields were calculated as per the formulae mentioned below equation.

$$Production\ Yield = \frac{amount\ of\ freeze\ dryer\ or\ rotary\ evaporator\ powder}{amount\ of\ curcumin\ and\ surfactant\ in\ feed} \times 100$$

4. **Determination of Drug content:** Curcumin containing DPI formulation was weighed 10 mg and dissolved in 100 ml of methanol. Absorbance of the above solution was taken at λ max 420 nm.

Angle of repose: (Lachman *et al.*, 1990)

The angle of repose was determined by the funnel method. The accurately weighed powder was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of the powder. The powder was allowed to flow

through the funnel freely onto the surface. The diameter of the powder cone was measured. The angle of repose was calculated by using the following equation.

$$tan\theta = \frac{h}{r}$$

Where 'h' and 'r' are the height and radius respectively of the powder cone.

6. Determination of Density

A. Determination of bulk density: (USP 2002)

Accurately weighed 25 g of formulation was taken, previously passed through 20 no. sieve and transfer in 100 ml graduated cylinder. The powder was carefully leveled and unsettled.

Apparent volume (V0) was noted, then the apparent bulk density in g/ml was calculated by the following formula.

$$Bulk\ density = \frac{weight\ of\ powder}{volume(\mathrm{V0})}$$

B. Determination of tapped bulk density: (USP 2002)

Accurately weighed 10 gm of formulation was taken, and transferred in 100 ml graduated cylinder. Mechanically tapped the cylinder containing the sample by raising the cylinder and allowing it to drop under its own weight using mechanically tapped density tester that provides a fixed drop of 14±2 mm at a nominal rate of 300 drops per minute. Tapped the cylinder for 100 times initially and measured the tapped volume (V1) to the nearest graduated units, repeat the tapping an additional 750 times and measured the tapped volume (V2) to the nearest graduated units.

$$Tap\ density = \frac{weight\ of\ powder}{tapped\ volume}$$

C. Compressibility index: The compressibility index of all ingredients was determined by following equation.

$$Car`s\ index = \frac{tap\ density - bulk\ density}{tap\ density}$$

D. Hausner's ratio: Hausner's ratio was determined by following equation.

$$\mathbf{Hausner's\ ratio} = \frac{\mathbf{tap\ density}}{\mathbf{bulk\ density}}$$

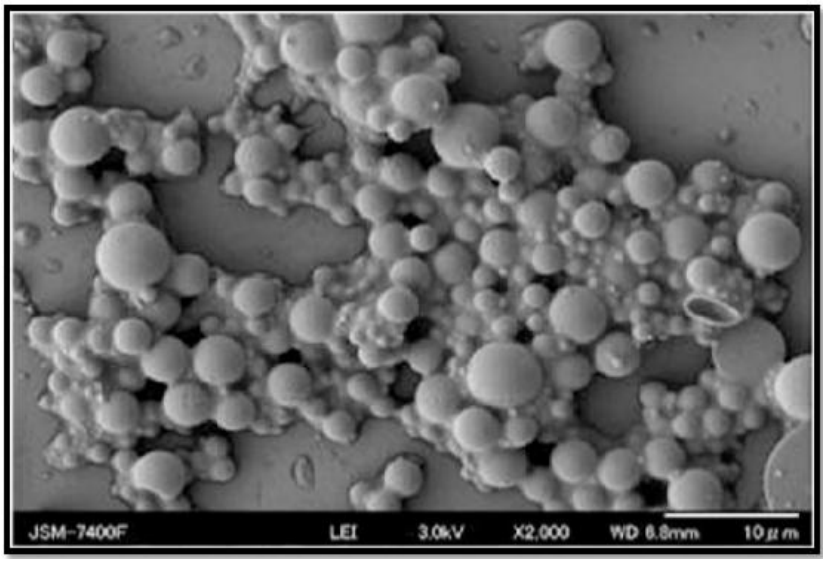
7. X-ray diffraction study (XRD)

The XRD measurements curcumin and formulation were carried out using Bruker D8 Advance X-ray diffractometer. The x-rays were produced using a sealed tube and the wavelength of x-ray was 0.154 nm (Cu K-alpha). The measurements were performed at a voltage of 40 kV and 80 mA. The scanned angle was set from 20˚- 60˚ and the scan rate was 1˚/min-1.The x-rays were detected using a fast counting detector (Bruker Lynx Eye detector) based on Silicon strip technology.

The X-ray powder diffraction study of pure drug (curcumin) was performed X-ray diffractometer. The 2θ values were recorded for samples. In the diffractogram of curcumin 2θ values percents are 8.89˚, 14.48˚, 17.22˚, 18.18˚, 23.33˚, 24.60˚ and 25.52˚. These recorded 2θ values of drug curcumin matches with reported values of curcumin, it means the curcumin was present in crystalline form (Yen and Wu *et al.*, 2010).

8. Scanning electron microscopy

The morphology of optimized formulation was examined by scanning electron microscopy. samples of optimized surfactant NPs dusted onto double sided tape on an aluminum stub and coated with gold using a cold sputter coater to a thickness of 400˚ A, and then imaged using a 20 kv electron beam.



9. *In-vitro* Diffusion studies

The *in vitro* diffusion study of prepared curcumin containing DPI was carried out by using Franz diffusion cell having 2.0 cm diameter which having 12 mL capacity of medium is used.

Dialysis membrane having molecular weight cut off range 12000 – 14000 kDa was used as diffusion membrane. Pieces of dialysis membrane were soaked in distilled water solution (pH 7.4). Diffusion cell was filled with simulated lung fluid solution (pH 7.4) then dialysis membrane was mounted on cell. The temperature was maintained at 37 ± 0.5 °C. After a pre-incubation time about 20 min, 10 mg formulations was placed in the donor chamber. At predetermined time points, 0.5 mL samples were withdrawn from the receiver compartment, replacing the sampled volume with simulated lung fluid solution (pH 7.4) after each sampling, for a period of 30 minute. The samples withdrawn were filtered using membrane filter (0.45µm) and used for analysis. The amount of permeated drug was determined using a UV spectrophotometer at 420 nm (Tagami *et al*., 2015).

10. Stabilities study

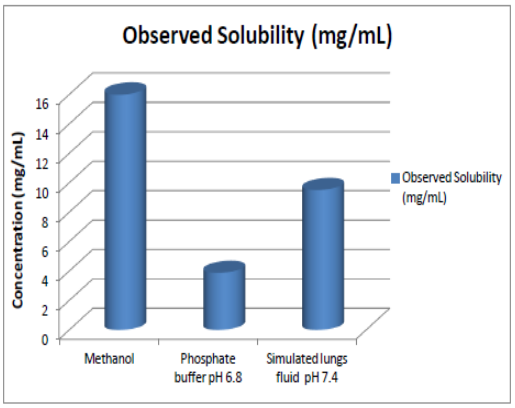
An accelerated stability study was carried out for optimized lyophilized and vacuum dried Curcumin containing DPI formulation. The stability study was performed at 25 ± 2˚C and 60 ± 5% RH in an environmental stability chamber over a period of 90 days to assess the stability of curcumin containing DPI. The dried powder was transferred to amber-colored glass vials, which were plugged, sealed and kept in the stability chamber. These dried powder samples were determined drug content were measured up to three months.

11. Solubility Determination

The solubility of curcumin was assessed in different solvent system viz., Methanol, Phosphate buffer pH 6.8 at 37 ± 0.5°C and Simulated Lung Fluid pH 7.4 at 37 ± 0.5°C. The solubility data is shown in (Table 1) and (Figure 2).

Table 1: Solubility of Curcumin in Different Solvent System.

Sr. No.	Solvent	Observed Solubility (mg/mL)
1	Methanol	16±1.13
2	Phosphate buffer pH 6.8	3.88±1.05
3	Simulated lungs fluid pH 7.4	9.512±1.42



12. Melting Point Determination

a. Melting point study by Glass Capillary Method

Melting point of curcumin was found by glass capillary method. The observed melting point of curcumin was confirmed with the standard melting point of curcumin i.e. 183˚C (I.P. 2014).

b. Melting point study by DSC

The melting point of curcumin was confirmed by Differential Scanning Calorimetry which was performed at the scanning rate of 10˚C/Min with 40 mL/min of Nitrogen splurging. The thermogram exhibited melting endothermic peak at 178.81˚C.

CONCLUSION

Lung cancer (also known as carcinoma of the lung) is a malignant lung tumor characterized by uncontrolled cell growth in tissues of the lung. The main primary types are small-cell lung cancer (SCLC), also called oat cell cancer, and non-small-cell lung cancer (NSCLC). Curcumin possesses wide-ranging anti-inflammatory and anticancer properties. Curcumin absorption is very low only 40% of drug absorbed that will go extensive metabolism in guts. The mean oral bioavailability of curcumin is 2% due mainly to hepatic first pass metabolism. Curcumin is given by oral, intravenous, intraperitoneal, suppositories, topical route.

The dry powder inhaler device is one of the types of delivery systems that enable generation and delivery of aerosolised drug particles into the respiratory tract. Dry powder formulations for inhalation consist of fine drug particles and coarse carrier particles like lactose. The fine drug particles adhere to the carrier surface to form ordered mixtures. Curcumin formulation procced by dry powder inhaler (DPI) method by using DPPC and Pluronic F-127. The formulation of curcumin DPI procced for different evaluation/physiochemical characterisation method.

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