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# REVIEW ARTICLE

# PULMONARY DELIVERY OF ESSENTIAL OIL

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## ABSTRACT

The study embroils development of pulmonary drug delivery system based on dry powder inhalers containing microencapsulated essential oil and characterization of the developed formulation. Microparticles of eucalyptus oil to be incorporated for pulmonary delivery was formulated using technique of microencapsulation of inclusion complex by spray drying. Encapsulating and complexing materials used were Gum acacia, maltodextrin & betacyclodextrin. This dry powder was then blended with Respitose ML006 & transfer into Aphaler DPI device. To determine the eucalyptol content, a gas chromatography method was developed using the solvent ethanol, nitrogen as the carrier gas and 15% OV17 column; and validated for various parameters like linearity (R2of 0.9998). The encapsulation efficiency of the formulation procedure determined by GC analytical method was 93.95 % w/w. Formulated powder of eucalyptus oil exhibited excellent flow properties. The SEM studies confirmed the encapsulation of essential oil. In vitro pulmonary deposition studies were carried out using twin stage impinger apparatus Cascade impact apparatus. From the current study it could be concluded that the technique of microencapsulation of inclusion complex by spray drying enhanced the availability and stability of eucalyptus oil. The developed DPI formulations containing natural oil hold promising future due to reduction in problems associated with inhalation of eucalyptus oil and have potential for improving patient compliance & showed high potential for successful pulmonary delivery.

**Key words:** Dry powder inhaler, eucalyptus oil, micro encapsulation, spray drying, gas chromatography, twin stage impinge

## INTRODUCTION

## PULMONARY DRUG DELIVERY SYSTEMS

Inhalation of medicated aerosols for delivery of drugs to the systemic circulation through lungs has developed into one of the most promising alternatives to oral or other invasive routes of administration. It is a needle free delivery system capable of administering a variety of therapeutic substances. Large protein molecules which degrade in the harsh gastrointestinal conditions and are eliminated by the first-pass metabolism in the liver, may be delivered now via the pulmonary route by depositing in the respiratory zone of the lungs. The drugs delivered by

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pulmonary route are readily absorbed through the alveolar region directly into blood circulation. Increasing prevalence of pulmonary diseases with high mortality and morbidity such as chronic obstructive pulmonary disease (COPD), asthma, cystic fibrosis, infectious diseases like tuberculosis and lung cancer, makes pulmonary drug delivery as a non-invasive and attractive approach for local drug administration. In treatment of these pathologies by use of pulmonary delivery, lower dosages than by the oral route can be used with comparable effectiveness which will reduce unwanted side effects. Particles with aerodynamic diameters between 2 and 6 μm are expected to efficiently deposit in the lung periphery and release the drugs for regional aerosolization.[1]

The lung also provides a non-invasive route of delivery for the systemic circulation, due to its unique characteristics such as large surface area, thin epithelial barrier and high blood flow. Lack of first pass metabolism and less enzymatic activity make pulmonary delivery as an ideal administration route for extensively degraded drugs following oral delivery and for macromolecules, such as proteins and peptides.[2]

The broncho-constriction is caused due to narrowing of the airways in the lungs and tightening of surrounding smooth muscles. Bronchial inflammation also causes narrowing due to edema and swelling caused by an immune response to allergens. People with asthma may also have asthmatic bronchitis, inflammation of the lining of the bronchial tubes.

Chronic pulmonary diseases are characterized by irreversible airflow limitation that is usually progressive in majority of patients and is associated with abnormal inflammatory response by lungs to noxious inhalants.[3]

**DRY POWDER INHALERS**

Dry powder inhalers (DPIs) have attained considerable attention due to their propellant-free formulations and the patient's inherent coordination with actuation. Dry powders for inhalation are formulated either as loose agglomerates of micronised drug particles with aerodynamic particle sizes of less than 5μm or as carrier-based interactive mixtures with micronised drug particles adhered onto the surface of large lactose carriers.[4] The powder formulation is aerosolized through a DPI device under the influence of inspiratory flow, where the drug particles are detached from the carrier surface (from drug–carrier mixtures) or deagglomerated, and the dose is deposited into the patient's deep lungs. The individual patient's skill to inhale vigorously and deeply is the limiting factor for the optimum performance of the DPI and MDI. Aerodynamic diameter is the diameter of a sphere of unit density that has the same terminal settling velocity as the particle under consideration and it is required to study the deposition mechanisms & flow properties. Anderson cascade impactor (Copley Scientific, UK) & Twin Impinger are used to determine particle size distribution, to estimate respirable fraction and for the aerosolization and deposition properties in vitro.[5]

ESSENTIAL OILS

Essential oils are complex mixtures, constituted by terpenoid hydrocarbons, oxygenated terpenes and sesquiterpenes. They originate from the plant secondary metabolism and are responsible for their characteristic aroma.Aromatic plants and oils have been used for thousands of years, as incense, perfumes and cosmetics and for their medical and culinary applications. Their ritual use constituted an integral part of the tradition in most early cultures, where their religious and therapeutic roles became inextricably intertwined. The Vedic literature of India dating from around 2000 BC, lists over 700 substances including cinnamon, peppermint, spikenard, ginger,

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myrrh, eucalyptus and sandalwood etc. Throughout the Renaissance period, aromatic materials filled the pharmacopoeias which for many centuries remained the main protection against epidemics. Over the next few centuries the medicinal properties and applications of increasing numbers of new essential oils were analysed and recorded by the pharmacists. The list included both well-established aromatics such as cedar, cinnamon, frankincense, juniper, rose, rosemary, peppermint, lavender, eucalyptus and sage, but also essences like artemisia, cajeput, Chervil, orange flower, valerian and pine.[6]

The various applications of essential oils account for the great interest in their study. Such applications may be found in the cosmetic industry, as ingredients of fragrances, decorative cosmetic, fine fragrances and flavoring, in the food industry, as aromas and flavours, in the pharmaceutical industry, as active components of medicines and as antibacterials/antimicrobials, and in aromatherapy.[7] Also, It is important to recognize that essential oils have three distinct modes of action with regard to how they inter-relate with the human body: pharmacological, physiological and psychological. The pharmacological effect is concerned with the chemical changes which take place when an essential oil enters the bloodstream and reacts with the hormones and enzymes etc; the physiological mode is concerned with the way in which an essential oil affects the systems of the body, whether they are sedated or stimulated, etc; the psychological effect takes place when an essence is inhaled, and an individual responds to its odour. With relation to the first two points, aromatherapy has a great deal in common with the tradition of medical herbalism or phytotherapy – in other words, it is not simply the aroma which is important but also the chemical interaction between the oils and the body, and the physical changes which are brought about. Although most plants which yield essential oils are also used in medical herbalism, it is important to distinguish the therapeutic qualities of a particular oil from those of the herb taken as a whole or prepared in another manner. German chamomile, for example, is used extensively in the form of a herbal preparation such as an infusion, tincture or decoction, apart from being utilized for its volatile oil. For the treatment of respiratory conditions, nervous conditions, insomnia and dermal irritation or disease, the essential oil is both useful and effective. The volatile oil is, of course, less concentrated in the form of an infusion, tincture or decoction, the potency of the oil is reduced (and inherently the safety margin increased), thus making the herbal preparation more suited to internal use. Similarly with peppermint whilst the oil is eminently suited to the treatment of respiratory conditions as an inhalant. [8]

But volatile oils suffer oxidation and volatilisation or react with other formulation component that may cause skin irritation. However, some of researcher reported that encapsulation is a feasible alternative way to increase the stability of this compound. Besides that, the physical form of essential oil is liquid and sticky make it difficult for storage and transportation, so it will increase in production cost. Essential oils also have limited usage because of its low water solubility. [9]

A formulation that allows protecting the essentials oils from high temperatures, oxidation and UV light, live must be found. The microencapsulation process provides several benefits to essential oils, such as the protection and stability of released volatiles and storage. This study significantly endeavors in microencapsulating of Essential oils. It can be useful, especially in food industry and any other field including pharmaceutical and medical areas. Besides, this study can be used as a model study for future research on Microencapsulation of any plant materials. [10]

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## MICROENCAPSULATION OF ESSENTIAL OILS

Following the first commercial use of microencapsulation in 1954 to create a carbonless copy paper (Dziezak et.al.,1988), different encapsulation techniques were developed and accepted within the pharmaceutical, chemical, cosmetic, and food industries (Gibbs etal., 2006). Microencapsulation is the process by which active ingredients (core materials) such as food oils and flavours are packaged within a secondary (wall) material.The main advantage of microencapsulation is the formation of a barrier between the compound and the environment. This barrier can protect against oxygen, water and light and can prevent contact with other ingredients in a prepared meal or, for example, in a controlled diffusion of the encapsulated compound. The food industry applies microencapsulation process for a variety of reasons: (1) Encapsulation= entrapment can protect the core material from degradation by reducing its reactivity to its outside environment (e.g., heat, moisture, air, and light), (2) evaporation or transfer rate of the core material to the outside environment is decreased=retarded, (3) the physical characteristics of the original material can be modified and made easier to handle, (4) the product can be tailor to either release slowly over time or at a certain point (i.e., to control the release of the core material to achieve the property delay until the right stimulus), (5) the flavor of the core material can be masked, (6) the core material can be diluted when only very small amounts are required, yet still achieve a uniform dispersion in the host material, and (7) it can be employed to separate components within a mixture that would otherwise react with one another.[11]

## THERAPEUTIC POTENTIAL OF ESSENTIAL OILS IN THE TREATMENT OF RESPIRATORY CONDITIONS

Nose, throat and lung infections are conditions which respond very well to treatment with essential oils. Inhalation is a very effective way of utilizing their properties, for ‘although after arriving in the bronchi the main part will be exhaled directly by the lungs, they cause an increased bronchial secretion (a protective reaction) which is beneficial for many respiratory ailments’. By inhalation they are absorbed into the blood circulation even faster than by oral application. In addition, most essential oils which are absorbed from the stomach are then excreted via the lungs, only a small part in the urine. The categories include;

- Expectorants for catarrh, sinusitis, coughs, bronchitis, etc: Eucalyptus, pine, thyme, myrrh, sandalwood, fennel.
- Antispasmodics for colic, asthma, dry cough, whooping cough, etc: Eucalyptus Hyssop, cypress, Atlas cedarwood, bergamot, chamomile, cajeput.
- Antiseptics for ’flu, colds, sore throat, tonsillitis, gingivitis, etc: Thyme, sage, eucalyptus, hyssop, pine, cajeput, tea tree, Borneol.

## INTRODUCTION TO EUCALYPTUS OIL

Eucalyptus oil is obtained by steam distillation and rectification from the fresh leaves or fresh terminal branchlets of various species of Eucalyptus rich in 1,8-Cineole also called as **Eucalyptol**. The species mainly used is **Eucalyptus globulus- Labill**. Commercial cineole-based eucalyptus oils are produced from several species of Eucalyptus such as Eucalyptus cneorifolia, Eucalyptus dives, Eucalyptus dumosa, Eucalyptus globulus, goniocalyx, horistes,

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kochii, leucoxylon, oleosa, polybractea, radiata, Eucalyptus sideroxylon, smithii, tereticornis. Eucalyptus oil has a history of wide application, as a pharmaceutical, antiseptic, repellent, flavouring, fragrance and industrial uses. Eucalyptus oils in the trade are categorized into three broad types according to their composition and main end-use: medicinal, perfumery and industrial. The most prevalent is the standard cineole-based "oil of eucalyptus", a colourless mobile liquid (yellow with age) with a penetrating, camphoraceous, woody-sweet scent, **(Ammon, D.G*et al*., 1996)**.

Australian Aboriginals use eucalyptus leaf infusions (which contain eucalyptus oil) as a traditional medicine for treating body pains, sinus congestion, fever, and colds. Dennis Considen and John White, surgeons on the First Fleet, distilled eucalyptus oil from Eucalyptus piperita found growing on the shores of Port Jackson in 1788 to treat convicts and marines. Eucalyptus oil was subsequently extracted by early colonists, but was not commercially exploited for some time **(Brophy JJ*et al*., 1995)**.

Eucalyptus oil has antibacterial effects on pathogenic bacteria in the respiratory tract and therefore eases breathing difficulties in people with croup, asthma and bronchitis. Inhaled eucalyptus oil vapor is a decongestant and treatment for bronchitis. Eucalyptol controls airway mucus hypersecretion and asthma via anti inflammatory cytokine inhibition. Eucalyptus oil also stimulates immune system response by effects on the phagocytic ability of human monocyte derived macrophages. Hence attempts were made to develop dry powder inhalers containing microencapsulated Eucalyptus oil for pulmonary delivery.[12]

**PROBLEMS ASSOCIATED WITH THE USE OF ESSENTIAL OILS**

The major challenges associated with essential oils include the following:

- Volatility
- Poor water solubility
- Aptitude for oxidation
- Loss of flavors during storage
- Incompatibility with other components.
- Difficult for storage
- Transportation
- Increase in production cost.

**7.7 REMEDIES**

Volatile oils suffer oxidation and volatilization or react with other formulation components that may cause skin irritation. However, some of researchers have reported that encapsulation is a feasible alternative way to increase the stability of such compounds. Besides that, the physical form of essential oil is liquid and sticky make it difficult for storage and transportation, so it may

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result in increase in production costs. Essential oils also have limited usage because of their low water solubility. A formulation that allows protecting the essentials oils from high temperatures, oxidation and UV light, live must be found. The microencapsulation process provides several benefits to essential oils, such as the protection and stability of released volatiles and storage. Such studies significantly endeavor in microencapsulating of Essential oils **(Leimann F V *et al*., 2009)**.

Curiosity in essential oils has revitalized in recent times with the fame of aromatherapy, a division of substitute medicine that asserts that essential oils and other related aromatic compounds have healing effects. Oils are diluted in carrier oil and employed in massage, dispersed in the air by the nebulizer, heated by candle flame and burned as incense **(Shiga H *et al*., 2001)**.

### 7.8 ENCAPSULATION OF ESSENTIAL OILS

Following the first commercial use of microencapsulation in 1954 to create a carbonless copy paper **(Kim YD *et al*., 1996)**.Different encapsulation techniques were developed and accepted within the pharmaceutical, chemical, cosmetic, and food industries **(Bhandari BR*et al*., 1999)**. Microencapsulation is the process by which active ingredients (core materials) such as food oils and flavors are packaged within a secondary (wall) material **(Desai KGH*et al*., 2005)**. The main advantage of microencapsulation is the formation of a barrier between the compound and the environment. This barrier can protect against oxygen, water and light and can prevent contact with other ingredients in a prepared meal or, for example, in a controlled diffusion of the encapsulated compound. The efficiency of controlled release or protection depends mainly on the composition and structure of the established wall and on the process conditions (temperature, pH, pressure and moisture) during the production and use of such particles. The barrier is generally formed by components that create a network through the hydrophilic or hydrophobic properties **(Fuchs M*et al*., 2005)**.

In this endeavour following encapsulation and delivery techniques have been reported in literature for the stabilization and delivery of Eucalyptus & other essential oils in food, cosmetics and pharmaceutical industries, most of them being based on the soft colloidal systems.

- Spray drying
- Inclusion complexation
- Alginate beads formation
- Oil-in-water emulsions

### MICROENCAPSULATION BY SPRAY DRYING:

Spray-drying process has been used for decades to encapsulate food ingredients such as flavors, lipids, and carotenoids. It is of great importance because solid or liquid microencapsulated food flavors using such technique exhibit a good chemical stability and controlled release. In addition, spray-drying is generally known to produce flavor powders in a short duration of time. The

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process of spray drying is economical and flexible, uses equipment that is readily available, and produces Powder particles of good quality **(Campanile F*et al*., 2007)**.

## METHODOLOGY

Spray-drying is a unit operation by which a liquid product is atomized in a hot gas current to instantaneously obtain a powder. The gas generally used is air or more rarely an inert gas as nitrogen. The initial liquid feeding the sprayer can be a solution, an emulsion or a suspension. Spray-drying produces, depending on the starting feed material and operating conditions, a very fine powder (10–50µm) or large size particles (2–3 mm). **Shahidi and Han (1993)** suggested that the microencapsulation by spray-drying involves four stages:

- Preparation of the dispersion or emulsion
- Homogenization of the dispersion
- Atomization of the infeed emulsion, and
- Dehydration of the atomized particles

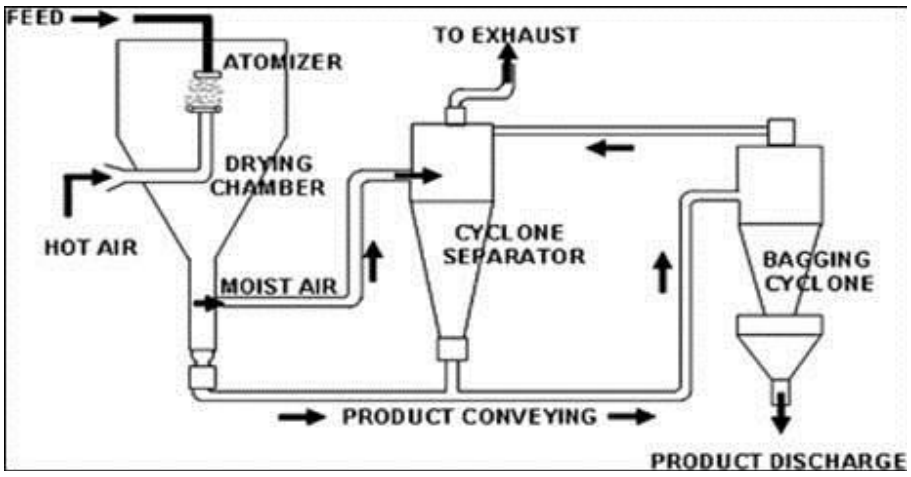


**Figure 1: Process of Spray Drying**

The first stage is the formation of a fine and stable emulsion of the core material in the wall solution. The mixture to be atomized is prepared by dispersing the core material, which is usually of hydrophobic nature, into a solution of the coating agent with which it is immiscible. The dispersion must be heated and homogenized, with or without the addition of an emulsifier depending on the emulsifying properties of the coating materials because some of them have themselves interfacial activities **(Jafari SM*et al*., 2009)**.

The obtained oil-in-water emulsion is then atomized into a heated air stream supplied to the drying chamber and the evaporation of the solvent, usually water, consequently leads to the formation of microcapsules. As the sprayed particles fall through the gaseous medium, they

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assume a spherical shape with the oil encased in the aqueous phase [20]. The short time exposition and the rapid evaporation of water keep the core temperature below 40°C, in spite of the high temperatures generally used in the process **(Nylson G*et al*., 1996)**.

**Operating conditions**

The main factors in spray-drying that must be optimized are feed temperature, air inlet temperature, and air outlet temperature **(Weißbrodt J*et al*., 2005)**.

When the feed temperature is increased, viscosity and droplets size should be decreased but high temperatures can cause volatilization or degradation of some heat-sensitive ingredients. The rate of feed delivered to the atomizer is adjusted to ensure that each sprayed droplet reaches the desired drying level before it comes in contact with the surface of the drying chamber. Moreover, appropriate adjustment of the air inlet temperature and flow rate is important **(Chemistry F*et al*., 2002)**.

The air inlet temperature is usually determined by two factors: The temperature which can safely be used without damaging the product or creating operating hazards and the comparative cost of heat sources **(Templeton AC *et al*., 2005)**. The temperature at the end of the drying zone, also called in literature exhaust temperature or air outlet temperature, obtained under given conditions can be considered as the control index of the dryer. It is quite difficult to predict this outlet temperature in advance for a given product, since it depends on the drying characteristics of the material. Contrary to the air inlet temperature, the air outlet one cannot be directly controlled since it depends on the air inlet temperature, and the ideal air outlet temperature for the microencapsulation of food ingredients such as flavors has been reported to be 50–80°C. The best spray-drying conditions are a compromise between high air temperature, high solid concentration of the solution, and easy pulverization and drying without expansion and cracks of final particles **(Moretti MDL*et al*., 2002)**.

**Most commonly used wall materials**

The chemical functionality, the solubility and the diffusion through the forming matrix determine the retention degree of core compounds during the preparation of microcapsules by spray-drying. Therefore, microencapsulation efficiency and microcapsules stability during storage are largely dependent on wall material composition. Carbohydrates such as starches, maltodextrins and corn syrup solids are usually used in microencapsulation of food ingredients. However, wall materials that are based on these compounds have poor interfacial properties and must be chemically modified in order to improve their surface activity. In contrast, proteins have an amphiphilic character that offer physicochemical and functional properties required to encapsulate hydrophobic core materials. Moreover, protein compounds such as sodium caseinate, soy protein isolate, and whey protein concentrates and isolates, could also be expected to have good microencapsulating properties **(Jafari SM *et al*., 2008).**

**INCLUSION COMPLEXATION**

This method involves delivering of active ingredients in aqueous foods by physically complexing them with other molecules, so that a better solubilization and/or an increase in the chemical stability of the complexed bioactive material of interest can be achieved. In this context a molecular complex refers to the physical association between a host and a guest (active ingredient) molecule. The most studied host molecules are the cyclodextrins **(Marques HMC*et al*., 2010)**.

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Inclusion complexes are formed by the insertion of the molecule or the non polar region of one molecule (known as guest) into the cavity of another molecule or group of molecules (known as host). The major structural requirement for inclusion complexation is snug fit of the guest into the cavity of host molecule. The cavity of host must be large enough to eliminate water, so that the total contact between the water and the nonpolar regions of the host water and the guest is reduced **(Menezes PPHM C*et al*., 2012)**.

Cyclodextrins (CDs) are cyclic carbohydrates derived from starch. The parent CDs contain six, seven and eight glucopyranose units and are referred as α-, β- and γ-CD, respectively. The most important property of the CDs is the ability to establish specific interactions – molecular encapsulation – with various types of molecules through the formation of non-covalently bonded entities, either in the solid phase or in aqueous solution.

These nano-encapsulating agents may form inclusion complexes with essential oils and volatiles, or their components, in order to improve their characteristics, such as transformation of liquid compounds into crystalline form; masking unpleasant smells and tastes of some compounds; improving the physical and/or chemical stability; and stabilizing volatile compounds by reducing or eliminating any losses through evaporation. Complexation has been used to avoid the destruction of certain flavors by processing or, on storage, allowing the use of minor amounts of flavors. The guest molecule is released in the warm moisture of the mouth. There are several methods for the preparation of inclusion complexes; kneading, co-precipitation, freeze-drying and spray-drying the most commonly used **(Szente L*et al*., 2003)**.

**FORMATION OF INCLUSION COMPLEX WITH CD:** It involves many steps;

- Approach of the guest or substrate molecule to CD molecule.
- Loss of the water structure within the cavity with removal of same water molecule.
- Breakdown of water structure around the portion at the substrate that will be included and transport of some water molecules in solution.
- Interaction of the substituent groups of substrate with groups on the rim or inside the CD ring.
- Possible formation of bonds between the CD and the substrate.
- Re-establishment of water structure around the external part of substrate after the inclusion has occurred.

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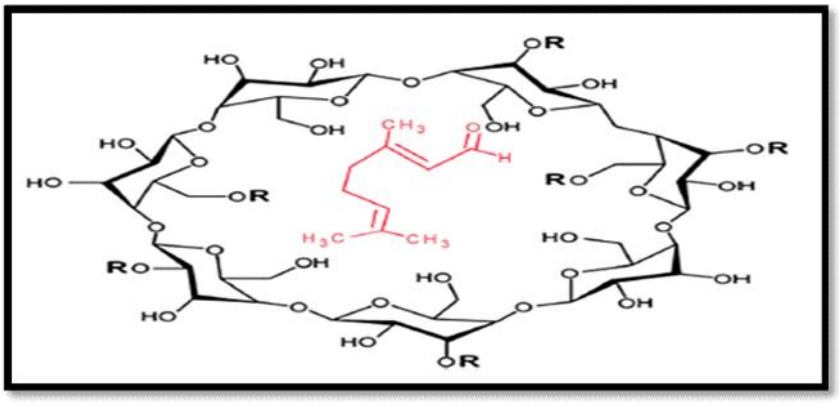

**Fig 2. Entrapment of molecule in the cavity of Betacyclodextrin**

In aqueous solution the slightly a polar CD cavity is occupied by water molecule which are energetically unfavored and so can be readily substituted by guest molecule which are less polar than water.The dissolved CD is the 'Host' molecule and the driving force of the complex formation is the substitution of the high enthalpy water molecule by appropriate 'Guest' molecule. CD complexes are relatively stable their water solubility compared to pure CD is strongly reduced so they rapidly separated from the solution in crystalline form. Evidence for a guest inclusion into the apolar CD cavity may be obtained by various analytical techniques, including NMR spectroscopy, UV–visible absorption spectroscopy, optical rotatory dispersion and circular dichroism, fluorescence, infrared/FT-IR spectroscopy, thermo-analysis, TLC, mass spectromety, and powder X-ray diffractometry **(Ciobanu a*et al*., 2011)**.

## DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD

Analytical Method Development & Validation part was considered as initial part of preformulation studies, because this developed and validated analytical method was used in formulation development, for drug content & stability studies.

## PREPARATION OF STANDARD CURVE OF EUCALYPTUS OIL

Analytical method for eucalyptus oil was developed based on Gas Chromatography (Perkin Elmer) and was found to be linear in concentration range of 50- 400 µl/ml. The areas under curve of solutions were determined in triplicate using flame ionization detector. Temperature of the column (15 % OV-17) was programmed from 50 to 300°C, rise rate 4°C/min, injector temperature 320°C, detector temperature 310°C, nitrogen flow rate 1.2 ml/min. The content of components was determined by the inner normalization method. The developed method was then validated for linearity, precision, accuracy, limit of detection and limit of quantification. The Gas Chromatographic calibration curve obtained is depicted in fig 3;

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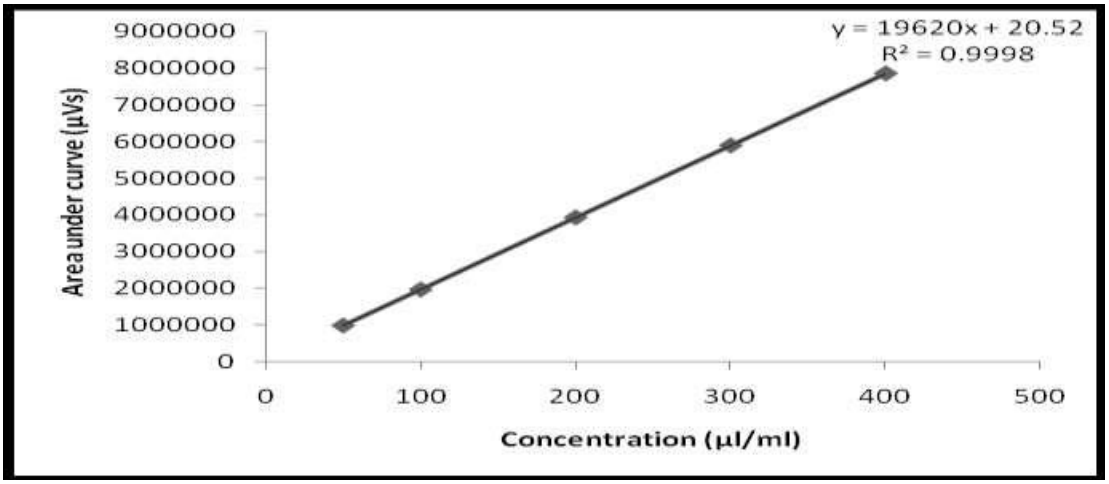


Fig 3. Gas Chromatographic calibration curve of eucalyptus oil

**FORMULATION DEVELOPMENT**

The aim of this study was to develop dry powder inhalers of Eucalyptus oil for pulmonary delivery, initially the oil was encapsulated by microencapsulate formation & then spray dried.

**MICROENCAPSULATION OF INCLUSION COMPLEX BY SPRAY DRYING**

Microparticle formulations were developed by inclusion complexation method using Betacyclodextrin as complexing agent. This process was carried out by dissolving both the calculated amount of guest and CD in a common solvent and then sprays drying the solution into particles with the complex formation by a spray dryer. βCD powder was dissolved in water :ethanol mixture by heating at temperature 60˚C- 65˚C and cooled at room temperature. Aqueous gum arabic (GA) and maltodextrin (MD) solution of appropriate concentrations were prepared by magnetic stirring for 3-4 hrs. For preparation of the inclusion complex slurry of βCD with the Oil, essential oil was added to βCD solution. Then aqueous GA solution and MD solution were blended with βCD solution containing included Oil. Thereafter, the mixture was homogenized using High Pressure Homogenizer make at 10-50 MPa & then it was spray dried in a spray-dryer (Labultima L222). The operational conditions of the spray-drying were Inlet temperature of air: 150˚C, Outlet temperature of air: 80˚C, Feed rate: 3-4 ml/mi, Aspirator Flow Rate: 65-80 Nm3/hr.

**FORMULATION DEVELOPMENT OF DPI CONTAINING MICROENCAPSULATED EUCALYPTUS OIL**

DPI formulations were developed by blending spray dried powder of Eucalyptus oil with inhalable lactose such as Respitose ML006. This blend was then filled with 25± 2mg of the

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powder into Size 3 Empty Hard Gelatin capsules & was transferred into Aphaler DPI device.

**Fig 4.Dry Powder Inhaler Device "Aphaler"**

**CHARACTERIZATION OF DEVELOPED MICROPARTICLE OF EUCALYPTUS OIL**

**APPEARANCE**

The resulting spray dried powder was observed for colour, presence or absence of an odour, surface texture & degree of fineness.

**FLOW PROPERTIES**

The density of microspheres was determined using a Tap densitometer apparatus. To measure the bulk density, a 5 gm of powder blend was added to a 100 ml graduated measuring cylinder and mounted on tap densitometer apparatus, the cylinder was dropped from the height of 1 inch resultant volume was measured. The tap volume occupied by a mass of powder of about 5 gm, placed into a 100ml graduated measuring cylinder, determined after 100 tappings. Bulk and tapped density values helped in determination of Hausner's ratio and Carr's index. Hausner's ratio is a measurement of flow ability of powder and was calculated using following equations;

**Hausner's ratio = $\frac{\text{Tapped Density}}{\text{Bulk Density}}$** **Equation 1**

**Carr's Index = $\frac{\text{Tapped density- Bulk density}}{\text{Tapped density}}$** **Equation 2**

**Angle of repose (θ) is one of the simple fast and popular method of predicting powder flow characteristics**

Fixed height funnel method was used to determine angle of repose in which funnel was adjusted such that the stem of the funnel lies 2 cm above the horizontal surface. The drug powder was allowed to flow from the funnel under the gravitational force till the tip of the pile just touched the tip of the funnel. The diameter of the pile was determined by drawing a boundary along the circumference of the pile. Experiment was performed in triplicate to calculate average diameter. Values of height and radius were substituted in the equation 3;

Equation 3

$$\theta = \tan^{-1} h/r$$

Where h = Height of pile

r = Radius of pile

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**OIL CONTENT**

**(i) TOTAL VOLATILE OIL (TVO)**

Total oil content of the spray dried powder was determined using Clevenger distillation. During hydrodistillation the essential oil components form an azeotropic mixture with water. Approximately 10 g of spray dried powder was dissolved in 200 mL of distilled water in a 250-ml round-bottom flask. Then, the Clevenger trap was connected to the flask with a water cooled condenser on top. The distillation was carried out under constant stirring for 3 h, and the volume of distilled oil was read directly from the collection arm. The volatile oil retention (overall aroma retention) during drying was calculated as follows:

Volatile retention %w/w = (measured oil content / theoretical oil content)*100 Equation 4

**(ii) SURFACE OIL CONTENT (SOC)**

It is determined by phase separation miscibility. Surface oil content of the powders was determined by washing 4 g of powder with n-Hexane (10 ml) with magnetic stirring for 5 min. The filtrate was then collected and n-Hexane was evaporated. The amount of residual oil was measured to obtain surface oil content.

**(iii) MICROENCAPSULATION EFFICIENCY (ME)**

Microencapsulation Efficiency (ME) was assessed by determining the total oil content of the powders and surface oil on the powders using the methods described previously. The ME was calculated as follows:

ME %w/w = {(TVO-SOC)/TVO}*100 Equation 5

Where TVO is total volatile oil and SOC is surface oil content, both on a dry basis.

**(IV) EUCALYPTOL CONTENT**

A sample of 400 µl of extracted oil from the developed formulation by hydrodistillation method was added to 1 ml of ethanol and analysed by Gas Chromatography using the developed calibrated method for determination of eucalyptol content.

**FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)**

IR spectra were recorded with FTIR spectrometer (Perkin Elmer RX1). The samples were prepared by KBr mixture method IR spectrum was recorded. The spectra of oil & formulation were recorded in the range of 4000-400 cm-1.

**SCANNING ELECTRON MICROSCOPIC STUDIES**

A JSM 820 model JEOL (Akishima, Tokyo, Japan) scanning electron microscope was used to investigate the microstructural properties of spray-dried microencapsulated products. Microencapsulated specimens were loaded onto a specimen stub with two-sided adhesive tape (Ted Pella, Redding, CA). Specimens were coated with 60% gold and 40% palladium with a sputter coater, Model Desk II (Denton Vacuum Inc., Cherry Hill, NJ). The conditions used to operate the electron microscope were as follows: objective aperture, 10 µm; sample distance, 18-23 mm; accelerating voltage, 20 kV; and tilt angle, 0°. Examinations were made at 700×, 1200×, and 2200× magnifications.

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**IN-VITRO AERODYNAMIC PARTICLE SIZE DISTRIBUTION OF DEVELOPED FORMULATIONS:**

1. **Twin Stage Impinger Snalysis:** Developed DPI formulations were subjected to investigate % respirable fraction using Twin Stage Impinger (Copleys) apparatus.

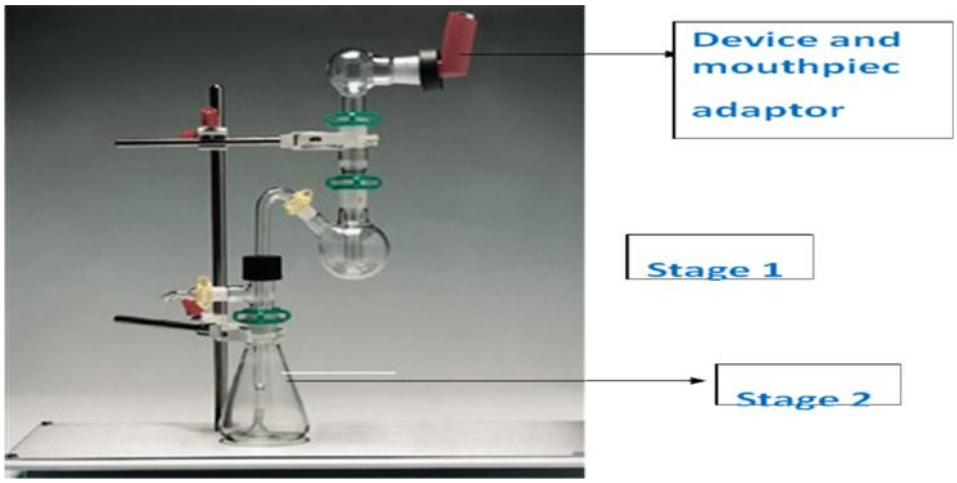


**Fig 3. Twin Stage Impinger (Copleys) apparatus**

**PROCEDURE**

25 milligram Spray dried DPI formulation, loaded in size 3 hard gelatin capsule (Associated Capsules Pvt. Ltd., India), was installed into the device. The device was attached to the impinger apparatus mouth containing, 7ml of solvent (ethanol) was dispensed into the upper impingement chamber (stage 1) and 30ml to lower impingement (stage 2). The set assembly was ensured to be vertical and adequately supported. The jet-spacer peg of the lower jet assembly was ensured to just touch the bottom of lower impingement chamber (S2). Capsules contents were released by puncturing the capsules from both the sides by the needles of the device. The system was subjected to vacuum to produce air flow rate of 60±2L/min. The vacuum pump was switched off and the empty capsule was removed. The discharge sequence was repeated for 9 capsules and then the assembly was dismantled. The inner surface of the inlet tube to the lower impingement chamber and its outer surface that projects into the chamber were washed with the solvent and washings were collected in lower impingement chamber (S2). The liquid in stages S1 and S2 was collected and diluted to 50ml with the solvent. The device (D) and the mouthpiece were washed with the solvent and volume was made up to 50ml with the same. The content of the active substance Eucalyptol was determined by developed GC method of analysis.

**QUANTIFICATION OF AEROSOL DISPERSION OF DPI FORMULATIONS BY TIA**

Fine particle dose (FPD) is denoted as the quantity of drug per capsule that is deposited in the lower stage of TIA i.e. S2

Recovered dose was taken as the total quantity of the drug recovered per capsule after each actuation i.e.

RD=D+S1+S2 Equation 6

Emitted dose is that emitted from inhaler device i.e.

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ED= S1+ S2 Equation 7

Percent emission was calculated as the percentage of emitted dose to recovered dose.

Fine particle fraction (FPF) is the ratio of FPD to RD.

**% Fine particle fraction = Fine particle dose (S2) X 100 Equation 8**
**Recovered dose**

The dispersibility was calculated as percentage of Fine particle dose to emitted dose.

**% Dispersibility = Fine particle dose (S2) X 100 Equation 9**
**Emitted dose**

2. **Cascade Impactor Analysis:** Cascade impactors operate on the principle of inertial impaction. Each stage of the impactor comprises a series of nozzles or jets through which the sample laden air is drawn, directing any airborne towards the surface of the collection plate for that particular stage. Whether a particular particle impacts on that stage is dependent on its aerodynamic diameter. Particles having sufficient inertia will impact on that particular stage collection plate, whilst smaller droplets will remain entrained in the air stream and pass to the next stage where the process is repeated. The stages are normally assembled in a stack or row in order of decreasing particle size. As the jets get smaller, the air velocity increases such that smaller particles are collected. At the end of the test, the particle mass relating to each stage is recovered using a suitable solvent and then analysed usually using HPLC to determine the amount of drug actually present.

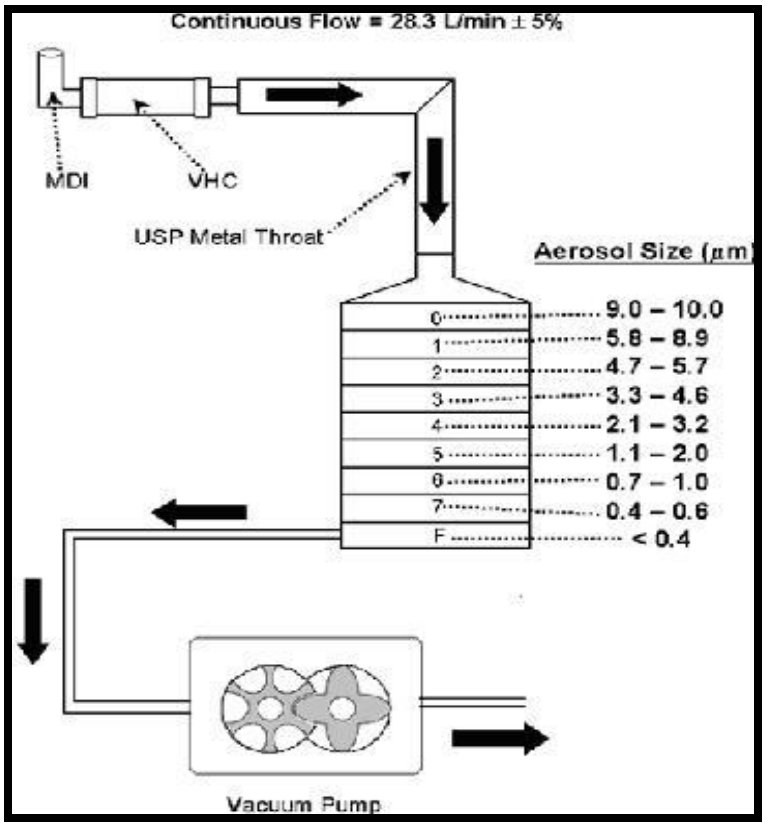


**Fig 5. Andersen Cascade Impactor Apparatus (Schematic view)**

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**Flow Rate:** 28.3 liters/min

**Procedure:** Aphaler device was attached with cascade impactor simultaneously and the flow rate was28.3 l/min. All the stages were rinsed using mobile phase of developed analytical method. Collected samples were analyzed by developed HPLC analytical method. The total dose of particles with aerodynamic diameters smaller than 5.0 µm was calculated by interpolation from the cumulative mass against cut-off diameter of the respective stages and considered as the fine particle dose (FPD) or fine particle fraction (FPF), expressed as a percentage of the emitted dose (ED). The ED was determined as the percentage of total dose. The mass median aerodynamic diameter (MMAD) of the particle was defined from the same plot as the particle size at which the line crosses the 50% mark.

## STABILITY STUDIES OF DEVELOPED FORMULATIONS

Stability is an essential factor of quality, safety and efficacy of a drug product. A drug product which is not of a sufficient stability can result in changes in physical as well as in chemical characteristics (formation of high risk decomposition substances). Microbiological instability of a sterile drug product could also be hazardous. The stability study consists of a series of tests in order to obtain an assurance of stability of a drug product, namely maintenance of the specifications of the drug product packed in its specified packaging material and stored in the established storage conditions within the determined time period **(Asian guideline on stability study, 2005).** Stability testing, as a function of time against a variety of environmental factors such as temperature, humidity, light and combination of these parameters, is critical for establishment of recommended storage conditions, retest periods, expiry dates and shelf lives of pharmaceutical products **(FDA, cGMP Guidelines, 2011).**

The selected DPI formulations of Glycopyrronium Bromide and Formoterol Fumarate were subjected to stability studies. The formulations were filled in size 3 stick free hard gelatin capsule in vials and stored at following conditions for a period of two months.

1. $25^0C \pm 2^0C$ / 60% RH ± 5%RH
2. $40^0C \pm 2^0C$ / 75% RH ± 5% RH
3. $5^0C \pm 3^0C$

The samples were withdrawn periodically at the interval of 1 month and analyzed for Drug content by HPLC.

## CONCLUSION

Eucalyptus oil microparticles were successfully formulated using Betacyclodextrin, as inclusion complex forming agent, maltodextrin as an emulsifier & carrier lipid and gum arabic as stabilizer using the technique of microencapsulation by spray drying of inclusion complex. The process parameters such as feed temperature, air inlet temperature, and air outlet temperature for spray drying were optimized and the selected batch showed promising results as the % Microencapsulation efficiency was up to 93.95 % w/w indicating good entrapment & retention of essential oil. The particle size obtained in the range 2 µm - 6 µm indicating particles could efficiently be deposited in the lung periphery and could release the contents after regional deposition. The developed DPI formulations of eucalyptus oil showed high respirable fraction

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(S2) in the range 36 % - 40 % indicationg the drug can be delivered directly to the alveoli. The developed formulations hold promising future due to reduction in problems associated with inhalation of eucalyptus oil and have potential for improving patient compliance.

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