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DRY POWDER INHALATION FORMULATION CONTAINING SPRAY DRIED ESSENTIAL OIL FOR PULMONARY DELIVERY

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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 inti 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 ($R^2$of 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 & it showed high respirable fraction up to 38 %. Formulated powder of eucalyptus oil was found to be stable over a period of 2 months. 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 inhalers, Eucalyptus oil, Microencapsulation, Spray drying, Gas chromatography, Twin stage impinger.

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 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, 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]

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.

Among these essential oils 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]

MATERIALS AND METHODS

Eucalyptus oil was procured from Enovate Biolife sciences, Mumbai. Eucalyptol reference standard was procured from Total Herb Solution (THS), Mumbai. Excipients such as Betacyclodextrin and Maltodextrin provided by Signet, Mumbai and Gum arabic was obtained from Drytech India, Mumbai as gift sample. All other chemicals such as ethanol, n-hexane etcwere procured locally from S.D. Fine Chemicals. Inhalable lactose such as Respitose ML006 was obtained as a gift sample from Macleods Pharmaceuticals & the DPI device Aphaler was received from Ajanta Pharma, Mumbai.

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 1;

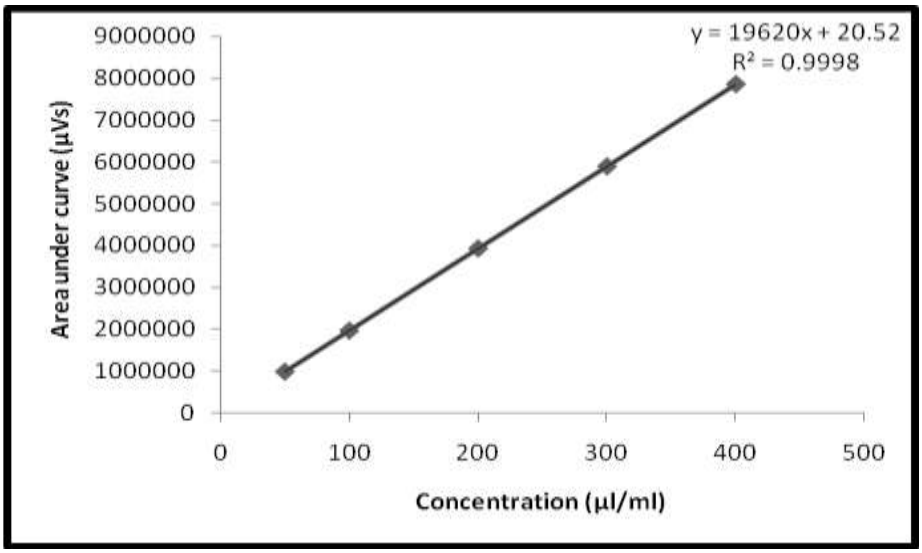

Fig 1: Calibration curve of Eucalyptus oil in Ethanol

FORMULATION DEVELOPMENT

The aim of this study was to develop dry powder inhalers of Eucalyptus oil for pulmonary delivery, here initially the oil was encapsulated by microencapsulate formation & 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 Nm^3/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 powder into Size 3 Empty Hard Gelatin capsules & was transferred into Aphaler DPI device.

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 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 flowability of powder and was calculated using following equations;

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

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

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;

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

Where h = Height of pile
r = Radius of pile

Equation 3

The Eucalyptol content in the developed formulation was analysed after the extraction of Eucalyptol from the developed spray dried powder. For the extraction of Eucalyptol, firstly, the Eucalyptus oil has been extracted by Hydro distillation method using Clevenger apparatus.

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.

IN-VITRO AERODYNAMIC PARTICLE SIZE DISTRIBUTION OF DEVELOPED FORMULATIONS: Developed DPI formulations were subjecyed to investigate % respirable fraction using Twin Stage Impinger (Copleys) apparatus.

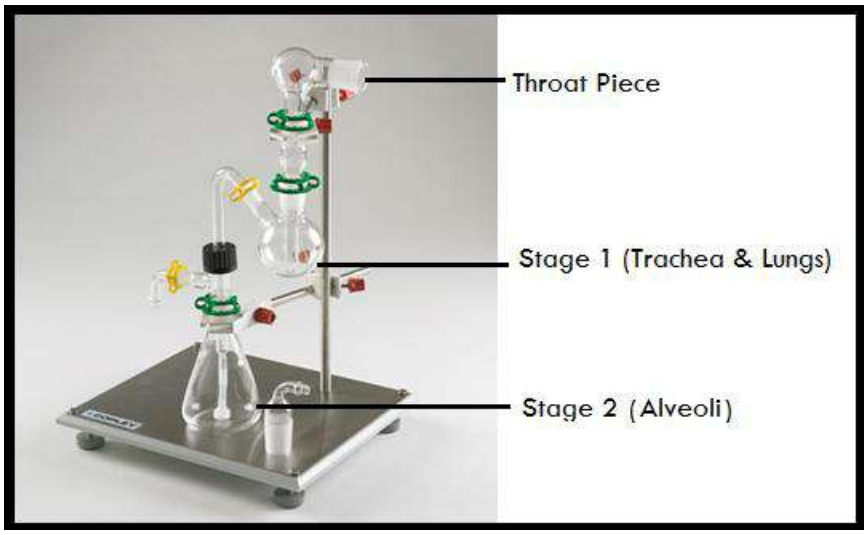

Fig 2. Twin Impinger Apparatus (Copleys apparatus)

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 (S_2). 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 S_1 and S_2 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. S_2

Recovered dose was taken as the total quantity of the drug recovered per capsule after each actuation i.e. RD= $D+S_1+ S_2$ Equation 6

- Emitted dose is that emitted from inhaler device i.e. ED= $S_1+ S_2$ 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.

$$\% \text{ Fine particle fraction} = \frac{\text{Fine particle dose } (S_2)*100}{\text{Recovered dose}} \quad \text{Equation 8}$$

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

$$\% \text{ Dispersibility} = \frac{\text{Fine particle dose } (S_2)*100}{\text{Emitted dose}} \quad \text{Equation 9}$$

RESULTS AND DISCUSSION

The optimized formulation of dry powder of essential oil developed by microencapsulation of inclusion complex by spray drying was evaluated for the following parameters.

APPEARANCE

The resulting spray dried powder of eucalyptus oil was white free flowing in appearance with strong cineolic odour.

FLOW PROPERTIES

Following parameters were determined to assess the flowing ability of the spray dried powder. Good flowability of powder tends to achieve weight uniformity and content uniformity of final formulation. The flow properties of spray dried powder are described in Table 1;

Table 1: Flow properties of spray dried eucalyptus oil

PARAMETERS	Spray dried microparticles of eucalyptus oil	DPI containing microencapsulated eucalyptus oil
Bulk Density (gm/ml)	0.261	0.176
Tapped Density (gm/ml)	0.304	0.193
Hausner's ratio	1.15	1.09
Carr's index (%)	14.1	8.81%
Angle of repose (˚)	32.21	28.5˚

The above result indicates that the developed microparticles of Eucalyptus oil showed Good flow properties whereas DPI formulation containing microencapsulated Eucalyptus oil showed excellent flow properties of formulation of Eucalyptus essential oil when compared with the standard values of scale on flowability. From this it can be concluded that after addition of fine grade lactose such as Respitose ML006, the flow properties were found to be enhanced.

OIL CONTENT

The oil content was analysed by hydrodistillation method. Results of the same are depicted in the table 2 below& discussed in following sections.

Table 2: Oil content of spray dried eucalyptus oil

PARAMETERS	RESULTS
Total Volatile Oil (TVO)	92.2% w/w
Surface Oil Content (SOC)	3.75 % w/w
Microencapsulation Efficiency (ME)	93.95 % w/w
Eucalyptol Content	84.4 %

The TVO of the developed formulations of spray dried Eucalyptus oil was obtained to be 92.2% w/w indicating good entrapment & retention of essential oil. The TVO of 92.2 % indicates 92.2 % entrapment of essential oil in the developed formulations. The determination of Surface oil content has played important role in analysis of extracted oil. It has indicated the percentage of Eucalyptus oil on the surface of the microparticles. It thus denotes the efficiency of microencapsulation process. Microencapsulation Efficiency has played important role in determining the capability of entrapment tendency of the spray drying process. Thus, its measurement gives the indication of inclusion of essential oil content in the developed microparticles.

CHARACTERIZATION OF DEVELOPED MICROPARTICLE FORMULATION OF EUCALYPTUS OIL BY FOURIER TRANSFORM INFRARED SPECTROSCOPY

FTIR is a prevailing means of identifying types of chemical bonds in molecule by producing an infrared absorption spectrum that is like a molecular fingerprint. It is the most powerful tool of identifying types of functional groups present in the molecule.

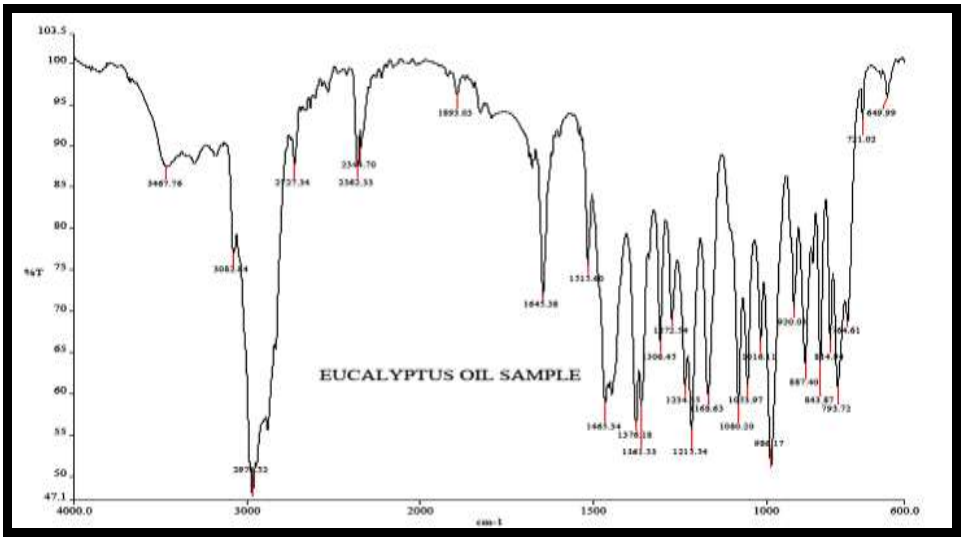

Fig 3: FTIR Spectrum of Eucalyptus oil

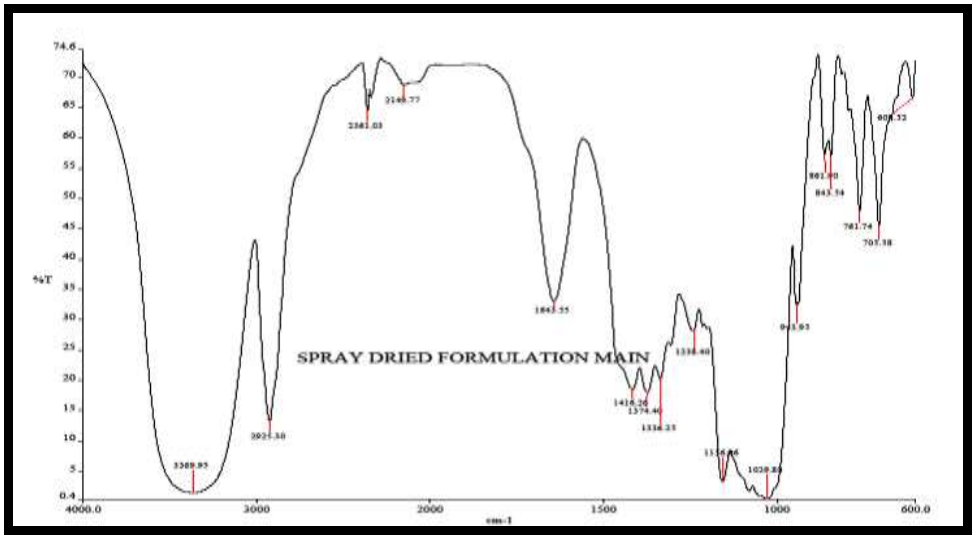

Fig 4: FTIR Spectrum of Spray dried formulation

The IR spectrum of procured Eucalyptus oil showed the diagnostic peaks at 2923 cm-1, 1739 cm-1,1054 cm-1, 1029 cm-1 are due to (–CH2) asymmetric stretching, C=O stretching (ketone carbonyl), C-O stretching, O-H in plane bending respectively. All the identical peaks were also present in FTIR spectrum of Spray dried formulation **(Fig 4)**, hence confirming their compatibility.

CHARACTERIZATION OF DEVELOPED MICROPARTICLE FORMULATION BY SCANNING ELECTRON MICROSCOPIC STUDIES

Scanning electron microscopic studies were investigated to depict the microstructural properties of spray-dried microencapsulated products & to confirm encapsulation of oil in the polymer matrix. SEM results in Figure 5 depict quite dramatically the SEM Image of Spray dried Formulation & of oil encapsulated in the polymer matrix.

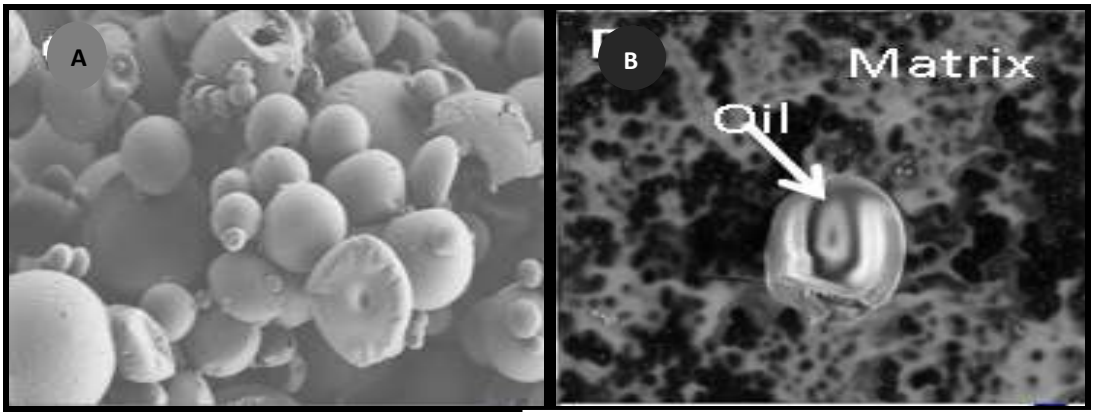

Figure 5A: SEM Image of Spray Dried Formulation

Figure 5B: SEM Image of oil encapsulated in polymer matrix

Figure 5: Scanning Electron Microscopic Studies images

SEM results for microencapsulated Eucalyptus oil particles exhibited spherically shaped smoothed surfaced particles that were uniform in size and were free of visible cracks and pores. On the other hand, Figure 7B revealed the oil encapsulation in microparticles.

IN-VITRO AERODYNAMIC PARTICLE SIZE DISTRIBUTION OF DEVELOPED FORMULATIONS USING TWIN STAGE IMPINGER:

The aerodynamic particle size distribution of DPI formulation containing microencapsulated eucalyptus oil was determined by Twin stage impinger apparatus. Drug Deposition was studied in three stages device, stage1 and stage2 each representing the drug retained in the device, oropharynx and pulmonary region. Content of the drug at each stage was calculated by GC method. The results are as given in **Table 3.**

Table 3: Deposition Pattern of DPI formulation containing microencapsulated eucalyptus oil using TIA

PARAMETERS	RESULTS
Device Fraction (%)	4.8 %
Stage 1 (% S1)	38.5 %
Stage 2 (% S2)	24.6 %
% Recovered dose	64.9 %
% Emitted dose	60.1 %
Fine particle fraction (%FPF)	37.90 %
% Dispersibility	35.00 %

It can be concluded from above table that DPI formulation delivered suitable portion of drugs to lungs. Figures of above table showed that around 25 % drug can be delivered directly to the alveoli. Around 39 % was found to distribute to stage-1 (the lungs), which might slowly diffuse towards the alveoli and then enters to the blood stream. Fine particle fraction of DPI formulations containing microencapsulated eucalyptus oil was found to be 38 %. These results confirm that both the particle size of the spray dried powder of microencapsulated oil and added fine lactose play an important role in determining the in vitro pulmonary deposition pattern.

CONCLUSION

The project was aimed at development of dry powder inhaler formulations containing microencapsulated eucalytpus oil. 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 (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. Thus, the developed DPI formulations containing natural oil showed high potential for successful pulmonary delivery.

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