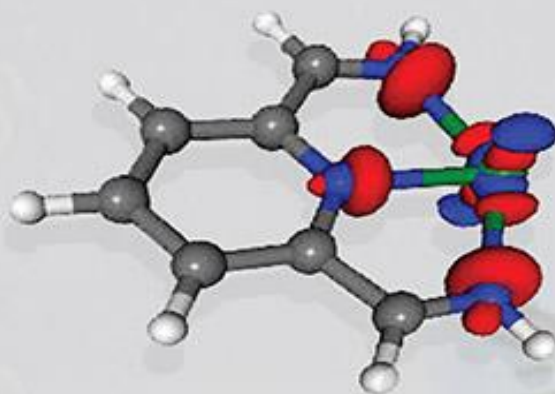




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📞 01111273794



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✉ ef.ewies@nrc.sci.eg

📞 01009957717



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📍 *National Research Center*

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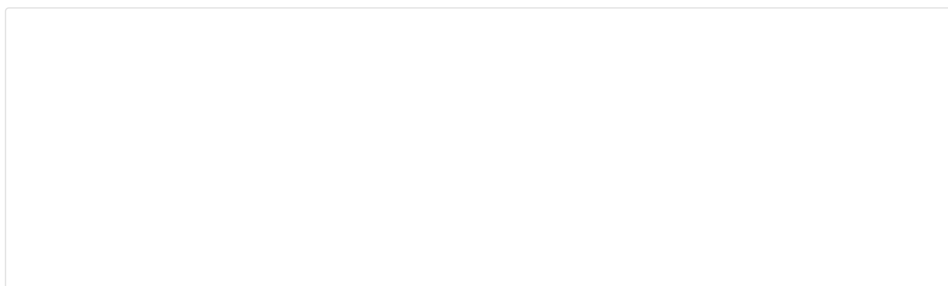


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✉️ shaymaaabdallaismail@gmail.com





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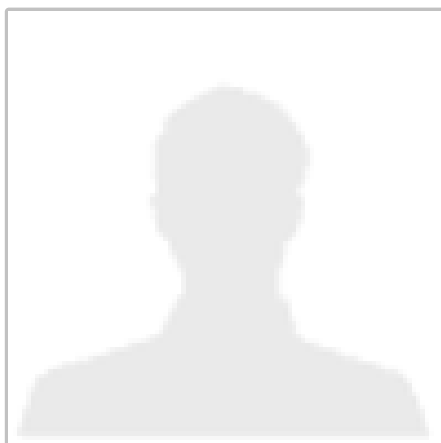


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✉️ drhanansibrahim@gmail.com



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Environmental Chemistry

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✉️ gnawwar@yahoo.com



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Inorganic Chemistry

📍 *National Distinguished Expert (China)*

Distinguished Fellow IETI (Hong Kong)

Ghent University (Belgium)

Wuhan University of Technology (China)

✉ francis.verpoort@ghent.ac.kr

🆔 0000-0002-5184-5500



Ammar Labib

Inorganic Chemistry

📍 *National Research Center*

✉ ammara133@gmail.com



Peter Hessemann

Ionic Liquids and catalysts

📍 *Institut Charles Gerhardt Montpellier*

UMR 5253 CNRS-UM-ENSCM

*Université de Montpellier, Place E. Bataillon, Bât 17, cc
1701*

34095 Montpellier cedex 5 - France

<http://Scopus Author ID: 6603113289>

✉ peter.hessemann@univ-montp2.fr

🆔 0000-0001-5266-979



👤 Mohamed Othman

Catalysis

📍 *NormandieUniv, UNILEHAVRE, CNRS 3038, URCOM,
76600 Le Havre, France*

✉️ *mohamed.othman@univ-lehavre.fr*



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Catalysis

📍 *Ain-Shams University*

✉️ *ahm512@sci.asu.edu.eg*



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Material Science

📍 *Nuclear Materials Authority*

✉️ *a.mahrous@nma.org.eg*



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📍 *National Research Center*

✉️ hishamayosef@yahoo.com



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Organic Chemistry

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✉️ sm_yakout@hotmail.com



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Organic Chemistry

📍 *National Research Center*

✉️ hatem_741@yahoo.com



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Organic Chemistry

National Research Center

✉ mrw_elhussieny@yahoo.com



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Food Chemistry

National Research Center

✉ hazahran23@gmail.com



Samir Kamel

Cellulose

National Research Centre

✉ samirki@yahoo.com



Amgad Al Bohy

Pharmaceutical Chemistry

British University in Egypt

albohy@ualberta.ca



Sudip Kumar Mandal

Pharmaceutical chemistry (Medicinal chemistry)

Dr. B. C. Roy College of Pharmacy & AHS

Durgapur-713206, W.B., India

gotosudip79@gmail.com



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Physical Chemistry

National Research Center

omniashehata@yahoo.com



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Applied Physical Chemistry

📍 *Surface Chemistry and Catalysis Laboratory
Physical Chemistry Department,
National Research Center,
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✉ *fathyna.77@hotmail.com*



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Physical Chemistry

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Laboratory of Separation and Reaction Engineering -
Laboratory of Catalysis and Materials
Department of Chemical and Biological Technology
Polytechnic Institute of Bragança
Campus Santa Apolónia*

5300-253 Bragança, Portugal

🌐 *CIMO: cimo.ipb.pt LSRE-LCM: lsre-
lcm.fe.up.pt*

✉ *htgomes@ipb.pt*

📞 *0000-0001-6898-2408*



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Polymer Chemistry

📍 *National Research Center*

✉ *salwa_elsabbagh@yahoo.com*



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Textile

📍 *National Research Centre*

✉️ aga.hassabo@hotmail.com



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Chemical Engineering

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✉️ shahir.sadek@bue.edu.eg



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Chemical Engineering

📍 *National Research Centre*

✉️ monaamin46@gmail.com



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Biochemistry

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✉ msaleh@sci.cu.edu.eg



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✉ shimarb@uomisan.edu.iq

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









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Dry Powder Inhalation Microparticles (Alginate, Carrageenan, Chitosan, and Combination Polymers): A Review on Characteristics and In Vivo Activity

Iqlima Ayu Prestisya^a, Andang Miatmoko^a, Mahardian Rahmadi^b, Dewi Melani Hariyadi^{1*}

^aDepartment of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Campus C Mulyorejo, Surabaya 60115, Indonesia

^bDepartment of Pharmacy Practice, Faculty of Pharmacy, Universitas Airlangga, Campus C Jl. Mulyorejo 60115, Surabaya, Indonesia



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Abstract

Dry Powder Inhaler (DPI) delivers one or more drug substances to the site of action through the inhalation route. It is used to treat respiratory diseases characterized by airflow obstruction and shortness of breath, including asthma and chronic obstructive pulmonary disease (COPD), respiratory infections, and cystic fibrosis. The inhalation route offers further potential for systemic drug delivery. DPI products consist of a drug formulation (the drug constituent part) and a container closure system. A DPI drug formulation contains the drug substance and excipients, including a drug carrier. Drug formulation plays an essential role in producing an effective inhalable medication. Formulating dry powders for inhalation involves micronization with various methods using various excipients, such as lipids, lactose, and polymers. Each one offers its unique advantages and disadvantages, depending on the therapeutic agent being formulated. This review will be highlighting the use of biodegradable polymers, such as alginate, chitosan, carrageenan, and combination polymers, in inhalation drug delivery systems. Particularly polymers microparticles, known as microspheres, received much attention because of their sustained and prolonged release properties and their application for targeting respiratory diseases. Moreover, this review will also summarize the in vivo drug deposition, lung localization, and histopathological study of microparticles.

Keywords: microparticles; dry powder inhaler (DPI); carrageenan; alginate; chitosan; in-vivo

1. Introduction

A dry powder inhaler is a device that has been widely used in various respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) and respiratory infections, and cystic fibrosis. It delivers active pharmaceutical ingredients (API) substances by inhalation to the target site. It is hoped that the delivered drugs can reach the target and treat the target site appropriately. Dry powder inhalers (DPIs) are currently considered a pulmonary drug administration device with the most significant potential for improved and new therapies.

The use of DPI in this therapy transports various active pharmaceutical ingredients that must-have characteristics such as less than 5-micron size, mucoadhesive material, and different other properties and characterization parameters which will be discussed further in the chapter 2. This

review aims to summarize the DPI mechanism, the use of DPI with various types of biodegradable polymers; such as alginate, chitosan, carrageenan, and combination polymers, in inhalation drug delivery system particularly polymers microparticles as known as microspheres which received much attention because of their sustained and prolonged release properties, and also their application for targeting of respiratory diseases. This review also explains both in vitro tests of DPI microparticles and in vivo test drug deposition, lung localization, histopathological study, and macrophages uptake.

2- Dry Powder Inhaler (DPI) Drug-Loaded Polymeric Microparticles for Inhalation Delivery

Dry powder inhaler (DPI) is one of the inhaler delivery devices commonly and ideally used to generate aerosols that have advantages over nebulizers, such as the convenience of a compact,

*Corresponding author e-mail: dewi-m-h@ff.unair.ac.id

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portable device and rapid medication delivery in the pulmonary route [1]. It is a breath-actuated device developed to overcome the difficulty of achieving the proper hand-breath coordination required to actuate a pMDI device for effective drug delivery in the lungs. Moreover, some DPI devices emit inspiratory-flow signals that promote suitable techniques and patient adherence. A DPI device consists of a medication reservoir, air inlet, deagglomeration compartment, and mouthpiece. Drug formulations for DPIs are micronized drug particles either in a pure form or bound to an inert, larger carrier molecule to form loose agglomerates. Rapid patient inspiration passes the drug formulation through a screen of spinning surfaces or generates turbulent airflow that disaggregates drug particles into a respirable dose [2].

Medication delivery depends on the peak inspiratory flow rate (PIFR) that a patient can generate through the device. This threshold peak inspiratory flow is, in turn, dependent on the internal resistance of the inhaler and is thus device-specific. A patient-generated PIFR of greater than 60 L/min is considered ideal for the use of most DPI devices. Conversely, a PIFR of less than 30 L/min may be insufficient for optimal pulmonary deposition, while 30–60 L/min rates may still provide sufficient therapeutic effects [2].

Sham et al. (2004) developed a platform for aerosol delivery of nanoparticles by preparing carbohydrate (e.g., lactose) carrier particles containing nanoparticles using a spray-drying technique. Carrier particles can be fabricated with an appropriate MMAD to optimize lung deposition. Dispersion of the lactose carrier containing either gelatin or poly butyl cyanoacrylate nanoparticles by a DPI showed a fine particle fraction (FPF) of about 40% [3]. Upon reaching the deep lung and igniting with the aqueous lining fluid of the lung epithelium, the carrier particles dissolved and released the drug of nanoparticles. A novel type of effervescent carrier particle-containing nanoparticles with an MMAD suitable for deep lung delivery was reported by Ely et al. (2007). Effervescent technology incorporation into the carrier particles added an active release mechanism for nanoparticles after pulmonary administration using DPI [4].

Available DPIs are single and multiple-dose devices. DPI has to be loaded before each inhalation in single-dose products, with a capsule containing powder. After loaded, the capsule is perforated within the device, and the powder is inhaled. Loading, piercing and discarding the capsule require manual dexterity and strength, which can be a problem for the elderly and patients with severe shortness of breath. Even there have been reports of patients swallowing the capsules instead of inhaling the contents. In addition, patients may need to take two or more breaths to inhale the therapeutic dose from the device caused

by high internal resistance. As a precaution, patients should be instructed to check the capsule in the device after the first inhalation and repeat the inhalation process if the capsule is not empty. Labeling one single-dose device to deliver formoterol fumarate stipulates refrigeration of the medication capsules before dispensing. The need for such additional steps and instructional measures may contribute to an already cumbersome process and lead to poor acceptance by patients. Multiple-dose DPI devices either deliver an individual dose of powder from a reservoir (e.g., Turbuhaler) or deliver premeasured individual doses from blisters, disks, or strips (e.g., Diskhaler). Patients tend to favor multiple-dose devices due to their ease and quick use, generally lower costs, and integral dose counters that allow them to view the remaining medication level.

2.1 Inhalation Delivery

It is well known that drugs administered by this route of pulmonary delivery are readily absorbed through the alveolar region directly into the blood circulation. Pulmonary drug delivery systems offer many advantages, such as an absorption area of up to 100 m² with a thin absorption membrane (0.1–0.2 μm) and a low blood supply. The required dose is lower than the oral dose so that side effects can be minimized, the onset of action is speedy, degradation of the drug by the liver is also unavoidable. In addition to these advantages, the delivery of drugs in the lungs also has many influencing factors.

2.1.1 Factors Influence Inhalation Delivery

The physiological factors affect the therapeutic effectiveness of inhalation drug delivery, including aerosol particle size, airway geometry, lung clearance mechanisms, and lung disease [5].

Aerosol particle size

One of the essential variables in determining the deposited dose and the distribution of drug aerosol in the lungs is aerosol particle size. Fine aerosols are distributed on the peripheral airways however store less drug per unit surface area than larger particle aerosols but on the larger particle more central airways. Most therapeutic aerosols are almost always heterodisperse, consist of varying particle sizes and are described by a log-normal distribution with the log of the particle diameters plotted against the number of particles, surface area, or volume (mass) on a linear or probability scale and expressed as absolute values or cumulative percentages. The particle size is determined from this distribution by several parameters. First is the mass median diameter of an aerosol. The aerodynamic diameter relates to a particle of a spherical diameter with a unit density that has the same depositional velocity as the desired particle regardless of its shape or density.

Second, the geometric standard deviation (GSD) measures the particle diameter variability in the aerosol and is calculated from the particle diameter ratio at the 84.1% point on the cumulative distribution curve to the MMAD. The GSD is the same for a log-normal distribution sum, surface area, or mass distribution. GSD's value is 1 indicates monodispersed aerosol, while GSD's more than 1.2 indicates heterodispersion aerosol. Most particles, more than 10 μm , are deposited in the oropharyngeal region with large amounts impacting the larynx, especially when the drug is inhaled from devices requiring a high inspiratory flow rate (DPIs). The large particles are subsequently swallowed and contribute minimally, if at all, to the therapeutic response. Particles 1–5 μm in diameter are deposited in the small airways and alveoli with >50% of the 3 μm diameter particles deposited in the alveolar region. In the case of pulmonary drug delivery for systemic absorption, aerosols with a small particle size would be required to ensure peripheral penetration of the drug. Particles lower than 3 μm have an approximately 80% chance of reaching the lower airways, with 50–60% deposit in the alveoli. However, the most effective particle size for treating systemic diseases has not been determined.

Airway geometry

Branching and narrowing of the airways encourage the impaction of particles. The larger the particle size, the greater the velocity of the incoming air, and the smaller the airway radius, the greater the probability of deposition by impaction. The lung has a relative humidity of approximately 99.5%. The addition and removal of water can significantly affect the particle size of a hygroscopic aerosol and thus deposition [5]. Drug particles are known to be hygroscopic and grow or shrink in size in high humidity, such as in the lung. A hygroscopic aerosol delivered at relatively low temperature and humidity into one of high humidity and temperature would be expected to increase in size when inhaled into the lung. The increase in particle size above the initial size should affect the amount of drug deposited, particularly the distribution of the aerosolized drug within the lung. Ferron et al. have predicted that for initial sizes between 0.7 μm and 10 μm , total deposition of hygroscopic aerosols increases by a factor of 2. However, for NaCl particles with an initial size of 0.1 μm , the distribution pattern in the airways was similar to that for non-hygroscopic particles of the same size with diffusion remaining the primary mechanism of deposition. The total deposited dose may decrease, but the resolution of current imaging techniques is not significant enough to distinguish the shifts in generations in this peripheral lung region. For particles with an initial size of 1 μm , we predict changes in the distribution pattern due to particle growth. The calculations showed a shift from

sedimentation to primary impaction on more central airways [5].

Lung clearance mechanisms

Once the inhaled drug is deposited in the lungs, either cleared from the lungs, absorbed into the systemic circulation, or degraded via drug metabolism, particles of drugs deposited in the conducting airways are mainly removed through mucociliary clearance and, to a lesser extent, are absorbed through the airway epithelium into the blood or lymphatic system. Ciliated epithelium extends from the trachea to the terminal bronchioles. Insoluble particles are trapped in the gel layer of mucus. They are moved toward the pharynx (and finally to the gastrointestinal tract) by the upward movement of mucus produced by the beating of metachronous cilia. In the normal lungs, the rate of mucus movement varies according to the airway area and is determined by the number of ciliated cells and their beat frequency. Movement is faster in the trachea than in the small airways and is affected by ciliary functioning and mucus quantity and quality [6]. For average mucociliary clearance to occur, airway epithelial cells must be intact, ciliary structure and activity normal, the depth and chemical composition of the sol layer optimal, and the rheology of the mucus within the physiological range. Mucociliary clearance is impaired in lung diseases such as immotile cilia syndrome, bronchiectasis, CF, and asthma. In CF, the ciliary structure and function are normal, but the copious amounts of thick, tenacious mucus present in the airways impair their ability to clear the mucus effectively.

Lung disease

Lung diseases (i.e., cystic fibrosis/CF, chronic obstructive pulmonary disease/COPD, asthma, and bronchiectasis) alter the architecture of the lung through changes in bifurcation angles and airway obstruction due to mucus accumulation modifying aerosol deposition and distribution patterns. A decrease in the cross-sectional area of the lung caused by obstruction increases air velocity and turbulence in areas where the airflow is usually laminar. Airway obstruction diverts inspired air to unobstructed airways. Thus, the minimal drug is deposited in the blocked sites, and often it needs to be reached to achieve an optimal therapeutic effect of the drug. In an obstructed lung, the aerosolized drug will be deposited more centrally in the lungs by inertial impaction compared with the uniform distribution achieved in the normal lung [7].

2.2 DPI Polymeric Microparticles

2.2.1 Alginate Microparticles

The use of alginate polymers in microparticles for inhalation drug delivery has begun to be widely studied. Alginate is a polymer polysaccharide, alginate as a matrix due to its biodegradable nature, derived from nature; therefore, it has nontoxic

properties or no subacute/chronic toxicity or carcinogenicity reactions, no risk of immunosuppression, is expected to achieve the ideal local release effect: better biocompatibility

and good stability to avoid second operative resection; as well as low cost and good economic benefits. The following table summarizes various studies that examine the use of alginate.

Table 1: The use of Alginate Microparticles for inhalation

No	Drug	Method	Results	References
1	Paclitaxel	Emulsification/gelation	<ul style="list-style-type: none"> - It is a continuous and direct pathway for lungs chemotherapy. - In endotracheal delivery, PTX-ALG-MPs diminish the drug loss during application. - Encapsulation of alginate microparticles resulted in a better lung tissue AUC with a longer residence time. 	[8]
2	Bovine Serum Albumin (BSA)	Spray-Drying	<ul style="list-style-type: none"> - Particles size suitable for deep lung administration. - The addition of zinc causes a more collapsed geometry. - Protein release depended on the (i) alginate: ZnSO₄ ratio, (ii) BSA content, (iii) type of release medium (rising release rate with rising phosphate concentration). 	[9]
3	Hyaluronic acid	The internal setting, ionotropic gelation with subsequent drying using supercritical CO ₂	<ul style="list-style-type: none"> - The Association of HA with Alginate showed a positive effect in lessening the particles' agglomeration and improving biodegradation. - Physicochemical and aerodynamic properties suitable as a drug carrier for the pulmonary tract. 	[10]
4	Salbutamol sulfate (SBS)	Melting, blending, freezing, powdering, and sieving	<ul style="list-style-type: none"> - Hydrophilic matrix prolongs hydrophilic drug diffusion path. - In vitro dissolution study indicates that when the ratio of the hydrophilic polymer is equated, the drug release will take a long time. - The amount of alginate aid in H-bonding increases the miscibility of alginate and PEG6000 for the former and electrostatic interactions between SBS and alginate for the latter. 	[11]
5	Ciprofloxacin HCl	Ionotropic gelation	<ul style="list-style-type: none"> - The polymer concentration affected the drug release pattern of microspheres but did not affect the particle size. - All formulas produced small size particles which fulfilled the lung region. - Drug release from ciprofloxacin HCl-alginate microspheres followed the Matrix-Higuchi model (diffusion-controlled drug release mechanism). - The highest concentration of alginate polymer in the formula (more than 0.75%) 	[12]

			<p>had the best flow property and demonstrated suitable free flow property.</p> <ul style="list-style-type: none"> - The selected best ciprofloxacin HCl-alginate microspheres issued significant inhibition of microbial against <i>S. aureus</i> ATCC 25923 and <i>P. aeruginosa</i> ATCC 27853. 	
6	Ropinirole hydrochloride (RH)	Spray-drying	<ul style="list-style-type: none"> - The inlet temperature had a remarkable effect on the morphology and yield of the spray-dried microparticles. - On the microparticles' morphology, size, and size distribution, and alginate to drug ratio of 90:10 (w/w) was considered the best formulation. - X-ray diffraction studies showed the spray-dried microparticles were stable for at least two months. - The release rate of RH was significantly affected by polymer concentration in the formulation. 	[13]
7	Ciprofloxacin HCl	Dropping, emulsification	<ul style="list-style-type: none"> - Solvent engineering during the ionic gelation process enables a high degree of molecular encapsulation from low to high molecular weight. - Co-immobilization of an antibiotic, CIP, an enzyme, and AL (Alginate Lyase), was achieved without losing their activities. - The blend microspheres appeared a controlled release profile of active CIP and AL molecules in a simulated gastrointestinal environment. - The incorporation of HMP into the matrix allowed the release of both charge molecules and provided a protective mechanism to negligibly prevent AL inactivation under acid conditions (stomach). - The inclusion of AL in the microsphere biomatrix formulation does not influence CIP antimicrobial activity. However, it improves its release profile at simulated intestinal conditions. 	[14]
8	Salbutamol Sulphate	Prilling, inkjet printing	<ul style="list-style-type: none"> - Particles produced by thermal inkjet printing and aerogel technology are unique nanostructures (highly porous and spherical alginate-based microspheres). - Process development unveiled a feasible printability region limited by ALG concentration in the printable aqueous fluid. - The optimized biopolymer aerogel particle 	[15]

			<p>formulation has excellent and homogenous textural properties falling in the nanoporous range with narrow particle size distribution ($23.8 \pm 4.5 \mu\text{m}$).</p> <ul style="list-style-type: none"> - The processing technique is compatible with incorporating a bioactive compound (salbutamol sulfate) in the aerogel carrier for concealed sustained release. 	
9	Roflumilast	Emulsified spray-drying	<ul style="list-style-type: none"> - The particle size and SPAN values for the crosslinked spray-dried alginate particles were more extensive than microparticles composed without a crosslinking agent. - The formulation prepared using β-cyclodextrin as a carrier (CD formulation) had an enormous particle size value and a more controlled drug release pattern than those made using lactose, mannitol, and maltodextrin as carriers. - The CD formulation with spherical-shaped microparticles gradually bloats to reach its maximum size within three hours. - Analysis of the aerodynamic data for the selected formulation revealed its efficient aerosolization compared to a pure drug for deposition in the alveolar region. - The selected CD formulation had a strong inhibitory effect on the growth of A549 cells compared to the pure drug. Furthermore, they had a marked inhibitory effect on pro-inflammatory cytokines (TNF-α, IL-6, and IL-10) in A549 cells. 	[16]

2.2.2 Chitosan Microparticles

Table 2: The use of Chitosan Microparticles for inhalation

No	Drug	Method	Results	References
1	Fluticasone and (FLU) Salmeterol xinafoate (SX)	Ionotropic gelation	<ul style="list-style-type: none"> - SEM images proved the spherical shape, the FLU and SX loading process in these formulations showed an increase in drug encapsulation within the MPs' networks with increasing drugs concentration. - FLU microencapsulated in an amorphous form in the acrylic derivatives, whereas in CS, CS-tAcon, and CS-Succ, the encapsulation was induced mainly in crystalline form. - In vitro release studies revealed a substantial increase in the dissolution of both drugs from all CS derivatives. The release rate is much higher for SX drugs due 	[17]

			<p>to their complete amorphization, following a similar release rate pattern across all derivatives.</p> <ul style="list-style-type: none"> - CS-g-PHEA and CS-g-PAA derivatives are more promising due to their advanced FLU release profile. 	
2	Isoniazid and Rifabutin	Spray-drying	<ul style="list-style-type: none"> - Inhalable CS has a drug association efficiency of 93% (INH) and 99% (RFB). - The microparticles developed display MMAD around 4 μm and FPF of approximately 45%, suitable for deep lung delivery. - Cytotoxicity assays demonstrated that the formulation is well tolerated by alveolar epithelial cells. - A marginal decrease in cell viability of macrophage-like cells (to 60%) was observed at the highest microparticle concentration tested (1.0 mg/mL) after 24 h exposure. However, this dose is feasibly overestimated compared to actual conditions in vivo. 	[18]
3	Prothionamide (PTH)	Ionic gelation	<ul style="list-style-type: none"> - The prepared PTH nanoparticles were spherical with a particle size of 314.37 ± 3.68 nm. - Optimized PTH nanoparticles have an aerodynamic particle size of 1.76μm and signify their suitability for effective delivery for pulmonary administration. - In-vitro release study indicated the release occurred due to a combination of erosion and diffusion mechanism followed by the Korsmeyer-Peppas kinetic model. - Particle sizes were changed in a narrow range during storage time, but it did not significantly affect the release of PTH from Chitosan nanoparticles. - Prepared DPI prolonged PTH concentration above the MIC for more than 12h after single-dose administration and can raise the treatment's effectiveness by increasing PTH concentration in the lungs tissue with a reduced dose. 	[19]
4	Bovine serum albumin (BSA)	Physically crosslinked hydrogel, graft copolymerization	<ul style="list-style-type: none"> - The hydrogel microparticles were designed in such a fashion to offer good aerodynamic properties (10.01 ± 0.45 and 13.73 ± 0.07 μm) that can confer sustained release of drug once deposited in the lung. 	[20]
5	Clindamycin HCl	Spray-drying	<ul style="list-style-type: none"> - The formaldehyde (crosslinking agent) concentration affected prepared 	[21]

			<p>microspheres' particle size and drug release behavior.</p> <ul style="list-style-type: none"> - Spray drying technology has remained as individual entities with less aggregation. - The characterization of microspheres in terms of size and shape showed that the spray drying technique is handy for manufacturing inhaled powders. 	
6	Levofloxacin	Spray-drying	<ul style="list-style-type: none"> - Spray-dried microparticles containing octanoyl chitosan proclaimed a greater dispersibility and higher FPF when compared to non-modified chitosan. - Morphological investigation showed that the engineered particles have a suitable aerodynamic particle size and relatively low physical contact due to the layers on their surface, leading to a decreased density and hence high dispersibility. - Spray-dried formulations containing octanoyl chitosan were comparable with those containing L-leucine, showing effectiveness as a dispersibility enhancer. - Chitosan hydrophobically modified has dispersibility enhancement property compared to the model hydrophobic amino acid L-leucine due to its antibacterial and mucoadhesive properties. - Mucoadhesion will increase the residence time of the formulations in the lungs, thereby increasing the efficiency of antibiotics to treat infections. 	[22]
7	Rifampicin (RIF) and Rifabutin (RFB)	Ionotropic gelation, spray-drying	<ul style="list-style-type: none"> - Have excellent aerodynamic characteristics, as evidenced by their deposition in the later stages of the ACL. - Chitosan-based microparticles containing antitubercular drugs are nontoxic to the lung tissues. However, repeated-dose inhalation toxicology studies of these formulations will be needed to assess their durable safety better. - The microparticles are also taken up by alveolar macrophages, thus enabling targeting of the <i>Mycobacterium tuberculosis</i> occupying within the macrophages. - Antitubercular drug microparticles, RIF and RFB, are excellent for direct delivery to the lungs when formulated as dry powder for inhalation (DPI). 	[23]
8	Rifampicin	Ionic gelation probe sonication method	<ul style="list-style-type: none"> - The size range and entrapment efficiency of prepared nanoparticles were estimated from 	[24]

			<p>124.1±0.2 to 402.3±2.8 nm and 72.00±0.1%.</p> <ul style="list-style-type: none"> - The nanoparticle formulation was used to conduct in vitro lung deposition studies via Andersen cascade impactor (ACI). - The cumulative in vitro drug release studies with developed nanoparticle formulation demonstrated sustained release for up to 24 hours. - Pharmacokinetic and toxicity studies carried out with prepared NPs DPI formulations compared with conventional DPI marketed formulation showed RFM release for an extended period. 	
9	Insulin	Supercritical fluid assisted atomization	<ul style="list-style-type: none"> - Well-defined spherical TMC microparticles with maintained structure and thermal stability could be obtained. - SAA-HCM proved to be a promising method without using any organic solvents. - The MMAD of the amorphous composite microparticles is within 1-5 µm, which is advantageous for inhalation therapy with rapid dissolution. - In rats, intratracheal administration of TMC/insulin formulations enhanced insulin absorption with relatively higher bioavailability. 	[25]

2.2.3 Carrageenan Microparticles

Table 3: The use of Carrageenan Microparticles for inhalation

No	Drug	Method	Results	References
1	Isoniazid	Emulsification	<ul style="list-style-type: none"> - Various concentrations of surfactant and polymer controlled the sizes of the microspheres. - The absorption of isoniazid into the microspheres depends on the concentration of isoniazid solution. The higher the concentration of isoniazid solution, the higher the percent encapsulation. - The stability of the microsphere depends on crosslinking and the concentration of isoniazid in the solution. The higher the concentration of isoniazid solution, the lower the stability. 	[26]
2	Ciprofloxacin HCl	Ionotropic gelation	<ul style="list-style-type: none"> - Ciprofloxacin HCl-Carrageenan microspheres resulted round spherical shape with a smooth surface. - Increasing the concentration of carrageenan polymers (0.5 to 1.0%) and KCl crosslinker (0.2 to 0.6%) increased particle size, yield, 	[27]

entrapment efficiency, and drug loading.

3	Isoniazid and Rifabutin	Spray-drying	<ul style="list-style-type: none"> - CRG/INH/RFB microparticles were efficiently associated with the model drugs, which antibacterial effect was not affected antibacterial effect. [28] - A common toxic effect was observed in alveolar epithelial cells and macrophages, but further testing is needed. - CRG microparticles demonstrated some ability to interact with macrophages and induced moderate activation of these cells.
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2.2.4 Combination Polymer Microparticles

Table 4: The use of Combination Polymers Microparticles

No	Drug	Polymer	Method	Results	References
1	Tobramycin	Alginate-Chitosan	Cationic crosslinking	<ul style="list-style-type: none"> - The formulations showing mucoadhesive properties and the conjugation of SLPI strengthen it. [29] - The particles reported herein could deliver a potent antimicrobial (tobramycin) to <i>P. aeruginosa</i> and dormant increase drug delivery efficacy over prolonged periods. 	
2	Ethionamide	Carrageenan stabilized, Alginate-Chitosan	Ionotropic gelation	<ul style="list-style-type: none"> - Carbohydrate-based polymers are due to their biodegradability and safety, and good physical characteristics. [30] - The physical properties of the nanoparticles, including particles size, shape, and zeta potential, are promising for further researches. - Abilities to reach a reasonable entrapment, a controlled drug release, and a comparable antimycobacterial activity such as <i>Tuberculosis</i>. 	
3	Tobramycin	Alginate-chitosan	Isothermal titration calorimetry	<ul style="list-style-type: none"> - The flexibility of tobramycin NPs by designing a dual-modality NPS incorporating DNase exhibits the effective penetration and anti-pseudomonal activity in the sputum of CF patients. [31] - Tobramycin polymeric NPs have high antibiotic loading, stability, and mucus penetration abilities. 	
4	Losartan	Chitosan-Dextran Sulfate	Spray-drying	<ul style="list-style-type: none"> - LS-MC-DPI exhibited complete burst release during the first 2 h (>95% within 30 min), and LS-MC- 	[31]

				<p>DPI was revealed as a continuous release, accounting for 61.47% throughout 24 h.</p> <ul style="list-style-type: none"> - It showed favorable PS and morphologic structure, reasonable flowability, swelling, mucoadhesive potentials, and sustained drug release. - Superior in vitro lung deposition and tolerability were attained.
5	Budesonide	Alginate-chitosan	Cation induced gelification	<p>- The DPI formulation results were primarily influenced by calcium chloride and chitosan. [32]</p> <ul style="list-style-type: none"> - Main correlations between the flowability, surface charges, and physical properties compared to particle size for particle dynamics in the respiratory tract. - With effective fluidization, particle trajectories, and morphological properties, there was a higher probing effect of surface charge than the formulated DPI and acts as a standard evaluation of lung deposition.
6	Vancomycin	Alginate-chitosan	Emulsion crosslinking	<p>- Vancomycin-loaded alginate-chitosan microspheres having mean particle size and drug loading were $25.3 \pm 5.4 \mu\text{m}$ and $18.5 \pm 2.3\%$. [33]</p> <ul style="list-style-type: none"> - The kinetic profile of vancomycin microspheres indicated a sustained release of the drug. - The higher AUC and the encapsulated half-life of vancomycin propose a longer duration of action than free vancomycin.
7	Resveratrol and Curcumin	Alginate-CMC	Spray-drying	<p>- Improved encapsulation efficiency with 82.91% for resveratrol (in the core) and 59.64% for curcumin (in the shell of the microcapsules) were obtained. [35]</p> <ul style="list-style-type: none"> - In vitro release profiles under simulated gastric and intestinal conditions confirmed controlled release of resveratrol encapsulated in core (or interior surface) of the microcapsules resulting from the 3FN process compared to resveratrol from the microparticle matrix generated from the 2FN spray drying

				<p>process.</p> <ul style="list-style-type: none"> - Curcumin from both formulations showed a slower cumulative release compared to resveratrol. - Both bioactive fit a Korsmeyer-Peppas release kinetic model with a pseudo-Fickian diffusion mechanism for resveratrol and anomalous diffusion for curcumin. - Co-encapsulation did not affect the release profiles of individual bioactive molecules. 	
8	Doxorubicin	BSA, Alginate and Chitosan	Co-precipitation	<ul style="list-style-type: none"> - Owing to the charge variability of BSA with changing pH, the pH-controlled loading effect and release behavior were observed. - Real-time biodistribution of DOX showed the metabolism of DOX-loaded BSA-gel-capsules(MPs) over 48 h post-injection. 	[36]
9	Acetylsalicylic acid (aspirin)	Alginate-pectin	Internal gelation	<ul style="list-style-type: none"> - Drug release is influenced by the medium condition and the properties of the polymer and drug. - More drug release was obtained for an acidic pH environment, and also, the increase in pectin soared the percentage of drug release. The drug release process was affected by the physical and mechanical properties of the gel barrier created around the capsules. - Enhancement of pectin led to poor gel barrier and increased the drug release percentage. - The addition of pectin amounts in the microcapsule increased the particle size and size distribution broader. - Having porous microstructure morphology of alginate pectin microcapsules and also the presence of drug crystals. - Controlled drug release was achieved for up to 350 min by the moderate increase from time to time for the alginate pectin combinations and three pH levels. 	[37]

2. In Vivo Drug Deposition, Lung Localization, Histopathological Study

Table 5: Drug Deposition of Microparticles

No	Drug	Polymer	Method	Main Parameter	References
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1	Paclitaxel	The sodium salt of alginic acid (Na-Alg) and hydroxypropyl methylcellulose (HPMC)	Aerodynamic assessment of fine particles using a Model DP-4 dry powder insufflator for a rat.	<ul style="list-style-type: none"> - SEM of alginate microparticles has a diameter of less than 5 μm. Maximum drug loading and encapsulation efficiency was 61%. In vitro cytotoxicity of paclitaxel against tumor cell lines agrees with the mechanism of action of paclitaxel. In more extended incubation periods, a more significant number of cells enter the G2 and M cell cycle phases, during which paclitaxel is more active. Fine particle fractions (FPF) (<5 m) were found to be $13.9 \pm 0.57\%$. - Mass median aerodynamic diameters (MMAD) and geometric standard deviation (GSD) were 5.9 ± 0.33 and $1.84 \pm 0.14 \mu\text{m}$. - The percent emitted fraction was 92% 	[8], [38]
2	Bovine serum albumin (BSA)	Sodium alginate	In vitro using a Multi (5)-stage Liquid Impinger.	<ul style="list-style-type: none"> - A decrease in the alginate: ZnSO_4 ratio led to an increase in particle size from 2.9 (± 2.1) μm (10:1 ratio) to 5.0 (± 2.2) μm (1:1 ratio) with spherical shape. - In vitro aerosolization and aerodynamic flow behavior: The addition of $\text{Zn}(\text{NH}_3)_4\text{SO}_4$ to the spray-drying solution during microparticle preparation resulted in a remarkable increase in the FPF (to 32.8% at alginate: ZnSO_4 ratio of 10:1, and to 40.4% at alginate: ZnSO_4 ratio of 2:1). 	[9]
3	Rifampicin-loaded liposomes	Chitosan and k-carrageenan	Powder aerosol performances using a next-generation impactor and a Turbospin as the inhaler device.	<ul style="list-style-type: none"> - The acceptable particle dose and the fine particle fraction were ~ 1.5 mg and 50%, respectively, denoting positive aerosol performances for both coated formulations. - Both coated formulations' mass median aerodynamic diameter was much lower ($\sim 2 \mu\text{m}$) than uncoated liposomes ($\sim 9 \mu\text{m}$). - Drug dispersion showed substantial toxicity ($\sim 40\%$) after 2 h incubation and increased to $\sim 65\%$ after 48 h. - Rifampicin-loaded uncoated- and coated-liposomes did not cause significant cytotoxic activity during the first 4 h incubation (less than 15% mortality), and it slowly 	[39]

				increased up to ~30% after 48 h.
4	Bovine serum albumin (BSA) and fetal bovine serum (FBS)	Chitosan, pentasodium tripolyphosphate and κ-carrageenan	Aerodynamic characterization using a TSI Aerosizer LD equipped with an Aerodisperser and assuming actual density as the density parameter to determine the aerodynamic diameter.	<p>- The protein-loaded nanoparticles resulted in a dry powder with suitable properties for lung delivery.</p> <p>- The pulmonary administration of nanoparticles is severely hindered by their low inertia, which makes alveolar deposition practically impossible, mainly resulting in the exhalation of the carriers.</p> <p>- The spray-drying of CS-based nanoparticles can reach the deep lung.</p> <p>- The nanoparticle carriers were determined to have a Feret diameter of $2.3 \pm 0.6 \mu\text{m}$, an absolute density of $1.44 \pm 0.01 \text{ g/cm}^3$ and a tap density of $0.42 \pm 0.04 \text{ g/cm}^3$. The aerodynamic diameter was determined to be $1.80 \pm 0.11 \mu\text{m}$.</p>
5	Fraction V Bovine Serum Albumin (BSA)	Chitosan, Alginate, PLGA, Gelatin, HPC-L, Ovalbumin, Sodium hyaluronate	Aerosolization efficiency aerosolized at 60 l/min through a DPI using an Andersen cascade impactor.	<p>- The fine particle fraction (FPF) was highest in the case of HPC-L particles (26.1%) and lowest with ovalbumin particles (11.9%). The other polymers produced FPF values between 14 and 21%.</p> <p>- The MMAD values were more prominent than the theoretical; between 2.9 and 4.7 m, possibly due to particle aggregation.</p> <p>- Emitted dose uniformity for all formulations as tested using DUSA were in the acceptable range (80.9–91%). The values were $82.4 \pm 7.5\%$ (chitosan), $84.4 \pm 6.1\%$ (alginate), $85.5 \pm 8.7\%$ (PLGA), $80.9 \pm 2.3\%$ (gelatin), $91.1 \pm 4.5\%$ (HPC), $81.3 \pm 3.2\%$ (ovalbumin), and $80.2 \pm 2.6\%$ (sodium hyaluronate).</p>
6	Hyaluronic acid (HA)	Sodium Alginate (Alg)	Aerodynamic properties were estimated by the d_A , while dv of the aerogel microspheres was calculated from the particle size distribution data measured with a Camsizer XT.	<p>- The mean particle diameter of the Alg microspheres decreased from 51.4 ± 12.6–$22.5 \pm 0.7 \mu\text{m}$, and the Alg-HA microspheres from 40.0 ± 0.8–$33.0 \pm 8.8 \mu\text{m}$, caused by the stirrer rate were increased from 850 to 1200 rpm. The experimental d_A values for the Alg microspheres were well estimated, except for sample 1% Alg₁₂₀₀, which has the smallest dv of $22.5 \pm 0.7 \mu\text{m}$ that</p>

				might lead to aggregation of the solid microspheres because of the high surface energy, which increases the experimental d_A values.	
7	Budesonide	Sodium alginate-chitosan	In vitro deposition study uses an eight-stage, non-viable cascade impactor. In water, the impaction plates were precoated with a 1.5% (w/v) of HPMC (4000 Hz) gel. In vivo lung deposition fraction: Healthy male Wistar albino rats weighing between 250 and 350 g were used for lung deposition fraction study. They were maintained in cages with a preserved 12: 12 h dark/light cycle and free access to standard food and tap water.	- The MMAD of obtained budesonide and commercial DPI were of 1.16 ± 0.01 and $5.04 \pm 0.03 \mu\text{m}$, which was an acceptable range (0.5 to $5 \mu\text{m}$) for the lung deposition. - The fine particle fraction reached the lower seven stages of the impactor (corresponding to aerodynamic diameters $<5.8 \mu\text{m}$) or the lower five stages (corresponding to aerodynamic diameters $<3.3 \mu\text{m}$). The lower MMAD was also reflected in fine particle fraction, $56.18 \pm 0.01\%$ for obtained DPI and $22.83 \pm 0.02\%$ for commercial DPI, respectively. - The improved deposition fraction in the tracheobronchial area of formulated budesonide loaded biopolymer DPI attributes better aerodynamic behavior such as less MMAD ($1.16 \pm 0.01 \mu\text{m}$), deaggregation, and better flowability.	[33]
8	Roflumilast	Alginic acid sodium	The MKII ACI consists of an induction port, pre-separator, stage-0 to stage 6 and filter. The selected formulation (10 mg) and the pure drug were filled separately into size-3 hard gelatin capsules. DPI was loaded at a flow rate of 60 L/min with a flow duration of 4 sec.	- The microparticles had the highest deposition in stage 3 (particles between $3.4\text{-}4.6 \mu\text{m}$) and stage 4 (particles between $2.1\text{-}3.2 \mu\text{m}$) of the ACI, most relevant for deep lung penetration. - Conversely, drug powder showed deposition from stage 0 (particles more than $9.0 \mu\text{m}$) to stage 3 (particles between $3.4\text{-}4.6 \mu\text{m}$), which is relevant for deposition in the bronchial and laryngeal areas of the pulmonary tract.	[16]
9	Salbutamol sulfate	Alginic acid sodium salt from brown algae	In vitro aerodynamic test uses a next-generation impactor and a medium resistance single-	- Due to the high porosity of the aerogels, the aerodynamic diameter obtained for the particles was ca. 6-fold lower than the aerogel particle size and in the respirable range. - The emitted dose as the powder was	[15]

			<p>dose DPI. The capsules size 3 were manually filled with 8.0 ± 0.2 mg of powder, and the vacuum pump was activated at a flow of 60 L min^{-1} for 4.0 s. Before use, the seven collection stages of the impactor were coated with a 1 % (w/v) solution of glycerin in methanol and then allowed to dry.</p>	<p>close to 100%, indicating the aerogel particles' good flowability with reduced particle cohesion forces.</p> <ul style="list-style-type: none"> - The FPF values of the aerogels were close to 50 %, which means better performance than other inkjet-printed (5-23 %) particles and some SS-commercial formulations. - The SS deposition profile showed in the first stage of the impactor (36.7 %), and the deposited drug contents gradually decreased from stage 1 to 7.
10	Budesonide	Chitosan-gelatin	<p>Using a twin impinger. The 25-mg formulation loaded into an HPMC stick-free capsule was then installed in a Rotahaler® device containing 7 and 30 ml of collecting solvent (acetonitrile/ buffer) in stages 1 and 2, respectively. The system was vacuumed to produce air streams of 60 l/min for 5 s. The liquid in stages 1 and 2 was collected, diluted to 100 mL, and measured by UV spectrophotometry at 244 nm. Formulations were also subjected to Anderson cascade impactor (ACI) to determine the MMAD and GSD.</p>	<p>Pulmonary scintigraphy is a [44] noninvasive method for visualizing deposition patterns and quantifying the amount of drug deposited and the concept of proof of bioavailability. BUD was radiolabeled with ^{99m}Tc by physical adsorption of radioactivity, then blended with inhalable lactose and filled in a size 3 capsule.</p> <ul style="list-style-type: none"> - Scintigraphic measurement after DPI inhalation—The radioactivity was obtained from RMC and was measured that the maximum respirable fraction obtained from radiolabeled BUD formulation was 22.16%, with a standard deviation of 4.31.
11	Rifampicin (RIF) and Rifabutin	Chitosan	<p>In vitro lung deposition of drug-loaded</p>	<ul style="list-style-type: none"> - A significant portion of the emitted dose was found deposited on the pre-separator stage (oral cavity). It is due [23]

(RFB)	<p>microparticles, plain drug, and microparticles with lactose blends was evaluated on an Andersen Cascade Impactor (ACI) at a flow rate of 28.3 L/min using a Lupihaler device. Acceptable particle dose (FPD), fine particle fraction (FPF), mass median aerodynamic diameter (MMAD), and geometric standard deviation (GSD) were calculated as per USP using the CITDAS software application.</p>	<p>to the large particle size of the drug crystals, rod-shaped morphology of RIF, and agglomerated nature of RFB as observed in SEM images.</p> <p>- No significant differences were observed among the two batches of RIF-loaded microparticles concerning MMAD, GSD, and FPF. However, the values of MMAD and GSD near 5 μm and 1.2, respectively, indicate a monodisperse DPI system suitable for deep lung delivery.</p> <p>- RFB-loaded microparticles were similar to RIF-loaded microparticles. However, RFB-loaded microparticles showed better deposition in the later stages of the ACI, indicating a greater FPF.</p> <p>- RFB microparticles appear smooth and have a corrugated surface compared to RIF microparticles, which exhibited a rough surface.</p>
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Table 6: Lung Localization of Microparticles

No	Drug	Polymer	Method	Main Parameter	References
1	Paclitaxel	The sodium salt of alginic acid (Na-Alg) and hydroxypropyl methylcellulose (HPMC)	Anzatax, Free-PTX, and PTX-ALG-MPs were administered i.v. and endotracheally, and paclitaxel amount in lung tissue was determined at 0.5, 6, and 24 h after administration. Rats were killed, and the lungs were rapidly excised, weighed, frozen, and stored at $-70\text{ }^{\circ}\text{C}$ until analyzed.	- The amount of paclitaxel per gram of lung tissue after 0.5, 6 and 24 h was 19.1 ± 2.4 , 2.7 ± 0.55 and $0.35 \pm 0.13\text{ }\mu\text{g}$ for Free-PTX and 28.3 ± 0.4 , 3.8 ± 0.5 and $0.35 \pm 0.08\text{ }\mu\text{g}$ for PTX-ALGMPs. Lung tissue AUC for Free-PTX and PTX-ALG-MPs was 87.2 ± 2.4 and $124.8 \pm 15.5\text{ }\mu\text{g.h/ml}$.	[8]
2	Isoniazid (INH)	Sodium alginate, Ispaghula husk and $^{99\text{m}}$ -Technetium ($^{99\text{m}}\text{Tc}$).	Using three Wistar rats weighing about 300–350 g. The microparticles were administered orally, a dose of 37 mBq, after overnight fasting for 8–10 h. The animals were	- The gamma image was recorded by injecting the $^{99\text{m}}\text{Tc}$ via i.v. Route. High levels of $^{99\text{m}}\text{Tc}$ were detected in the lung, liver, and spleen immediately after injection.	[45]
				- During the gamma scintigraphy study, the presence of microparticles could be marked in	

			<p>carried out normal activities but were not allowed to take any food or water until the formulation had emptied the stomach. The scintigraphic examination was done at 1, 4, and 12 h to assess the mobilization of the microparticles in the GIT. Images were recorded for a preset time of 5 min/view with a 15% window centered on including the 140 keV photopeak of ^{99m}Tc.</p>	<p>the intestinal lumen 1 h after the oral administration. Microparticles could also be detected in the intestine after 12 h. However, the percent radioactivity had significantly decreased ($t_{1/2}$ of ^{99m}Tc $\frac{1}{4}$ 4–5 h). Due to negligible radioactivity, microparticles in the GIT could not be assessed after 12 h of administration.</p> <ul style="list-style-type: none"> - The optimized microparticles based on design software exhibited 83.43% drug entrapment and 51.53 μm particle size with 97.80% and 96.37% validity, respectively. 	
3	Lipopolysaccharide (LPS) from <i>K. pneumonia</i>	Sodium alginate	<p>The in vivo efficacy evaluation of free LPS and encapsulated LPS was carried out in Swiss albino mice. Groups of six mice were administered 100 μg free LPS, and LPS encapsulated microparticles (2 mg microparticles equivalent to 100 mg LPS) via intramuscular, intratracheal, and intranasal routes.</p>	<ul style="list-style-type: none"> - The control group mice did not show any antibody titer against <i>K. pneumonia</i> LPS. In the case of the mice immunized with free and encapsulated LPS, variable PHA titers were observed, thus indicating that the LPS antigen is presently capable of inducing an immunological response. - Comparison of PHA titers of mice immunized with free LPS with those immunized with LPS encapsulated microparticles revealed a better antibody response irrespective of the route of administration. Maximum antibody response was observed in mice immunized with LPS encapsulated microparticles by intra-tracheal, intranasal, and intramuscular routes. The free LPS vaccine, on the contrary, has evoked very low PHA titers. 	[46]
4	Isoniazid	Sodium alginate	<p>Nine Wistar Rats (300–350 g; 15–20 weeks old) were used, each treated and untreated animal group. The microspheres were administered 2 mg/ml after overnight fasting for 8–10 h. Animals were given free access</p>	<ul style="list-style-type: none"> - The i.v.-route administers the passage of ^{99m}Tc. Radioactivity was recorded in the thoracic and upper abdominal region and the tail region. High levels of ^{99m}Tc were detected in the lung, liver, and spleen immediately after injection. - Microspheres could be marked in the intestinal lumen 4 h post-oral administration. It showed the 	[47]

			<p>to water, but the food was restored 1–2 h after dosing. The animals' examination was done at 4 and 24 h to assess the mobilization of the microspheres in the GIT. Images were recorded for a preset 5 min/view, including the 140 keV photopeak of ^{99m}Tc.</p>	<p>contamination of the windpipe in one of the animals during oral administration. Microspheres could also be detected in the intestine after 24 h although the percent radioactivity had significantly decreased ($t_{1/2}$ of ^{99m}Tc = 4–5 h).</p> <ul style="list-style-type: none"> - The presence of microspheres in the GIT could not be assessed after 24 h of administration due to negligible radioactivity.
5	Rifampicin (RIF) and Isoniazid (INH)	Chitosan-Guar Gum	Spray-drying, ionotropic gelation	<ul style="list-style-type: none"> - All optimized formulations showed controlled and sustained drug release for a more extended period. - The optimized formulations also showed lower cytotoxicity and enhanced lung uptake of drugs. - Formulations also resulted in an almost 5-fold reduction in the number of bacilli in the lungs compared to free drugs.
6	Isoniazid (INH) and Rifabutin (RFB)	Carrageenan	<p>HPMC size 3 capsules were filled with 30 mg of CRG/ INH/RFB microparticles. The content of capsules loaded using the high resistance RS01® inhaler. The device was connected to the Andersen cascade impactor (ACI), operating at 60 L/min. It was activated for 4 s using the appropriate adaptor kit for the 60 L/min air flow rate test. Cut-offs of the stages (-1 to 6) at the airflow rates adopted. The impactor plates were coated with a solution of Tween 20® in ethanol 1% (w/v). The drugs were recovered with a water/ acetonitrile mixture (50/50, v/v) and quantified by HPLC. The recovery ranged</p>	<ul style="list-style-type: none"> - CRG/INH/RFB microparticles efficiently associated the model drugs, which antibacterial effect was not affected antibacterial effect. - The powder aerosolization performance was efficient, with emitted doses of 91% and MMAD of 3.3–3.9 μm, for RFB and INH, indicating a strong possibility of co-deposition of drugs in the deep lung region.

between 77 and 91% in all the experiments.

Table 7: Histopathological of Microparticles

No	Drug	Polymer	Method	Main Parameter	References
1	Ropinirole hydrochloride	Low viscosity sodium alginate	Using an isolated nasal sheep mucosa	<ul style="list-style-type: none"> - The size of microparticles had a narrow range (between 2.35 ± 0.10 and $2.58 \pm 0.06 \mu\text{m}$). - Histopathological of the microparticles (formulation A2) and drug-free microparticles did not show remarkable effects on the overall appearance of the animal's nasal mucosa, which is contrary to the positive control. No necrosis was detected. As shown, goblet cells, sero-mucinous glands, and ciliated cells were intact, with detection of only slight focal sloughing of the cells. 	[13]
2	Rifampicin (RIF) and isoniazid (INH)	Chitosan-based Nano embedded microparticles (CNPs), Mannan based Nano embedded microparticles (MNPs) and Guar gum-based Nano embedded microparticles (GNPs)	The isolated lung tissue was fixed in 5 mL of 10% neutral buffered formalin on female mice. Lungs were embedded, sectioned horizontally, stained with hematoxylin and eosin.	<ul style="list-style-type: none"> - Mannan batches show relatively higher pathological consequences evident by inflammatory cells and parenchyma degeneration. It could be related to the high burst release of ATDs, resulting in increased local drug concentration causing accidental cell death. - More substantial evidence of toxicity in mannan formulation is further related to preferential accumulation of mannan carrier in phagocytic cells due to the presence of mannose receptors predominantly found on their cell surface. - Other drug-loaded formulations show a thin layer of connective tissue and numerous capillaries lined with simple squamous epithelium without necrotizing granuloma. 	[48]
3	Budesonide	Sodium alginate-chitosa	The lung tissue of rats was fixed in 10% formalin for 24 h. The samples were then embedded in paraffin, cut into a 5 mm section,	<ul style="list-style-type: none"> - The obtained budesonide and commercial DPI particle sizes were 3.059 ± 0.03 and $1.521 \pm 0.04 \mu\text{m}$, respectively. - The formulated DPI showed higher entrapment efficiency 	[43]

			and stained with hematoxylin and eosin.	(87.16 ± 0.09%). - The MMAD of obtained budesonide and commercial DPI was 1.16 ± 0.01 and 5.04 ± 0.03 µm. - No signs of inflammation were observed in both samples treated animals. However, some basal membrane congestion was rarely observed in formulated DPI due to the installation procedure, which can be reversed by the anionic nature of the engineered particles.
4	Doxorubicin	Chitosan, BSA (fraction V, 99%), and Sodium alginate	Histology studies: lung specimens were fixed with formalin for at least 24 h, embedded in paraffin, sectioned, stained with hematoxylin and eosin.	- The H&E-stained tissue sections also showed that only a few small nodules existed in the lungs of mice treated with DOX-loaded capsules; in contrast, the mice treated with saline showed many melanomas, and metastasis nodules occupied a significant number of pulmonary parenchyma. [36]
5	Lovastatin (LS)	Sodium alginate (AG) powder and chitosan (CS) powder	On the 29th day, 40 adult Swiss mice were randomly divided into 4 groups, with 10 animals per group were injected with ketamine at a dose of 30mg/kg, and their kidneys and livers were excised, weighed, and examined macroscopically. Therefore, the livers and kidneys were preserved in 10% buffered formalin for histopathological study.	- The ACL nanoparticles containing a high LS content during the initial phase of the test period released less LS when compared with the ACL nanoparticles having a low LS concentration, where the release of LS from the ACL particles was higher. This drug release pattern occurred at all tested pH values, including 7.5, 6.5, 4.5, and 2. - Image of H&E-stained sections showing the glomerular and renal vein with edema exudate and a clear Bowman cavity with a normal kidney basement membrane. The vascularity of the cells was normal with relatively healthy tubular epithelial cells. The scale bar is 100 µm. [49]
6	Vancomycin hydrochloride	Sodium alginate and chitosan	Histological analysis was prepared in 4% phosphate-buffered paraformaldehyde and then washed overnight in water, dehydrated	- For more than 14 days, microspheres formulations showed no severe signs such as epithelial necrosis. The sloughing of epithelial cells was detected in histological studies. [34]

			through alcohols, cleared in xylene, and embedded in paraffin wax. The cut sections (5 μm in thickness) were stained with hematoxylin and eosin (H&E) and observed by microscope.	
7	Lipopolysaccharide (LPS) from <i>K. pneumoniae</i>	Sodium alginate	For histopathological evaluation, lung tissue was preserved in a 10% v/v aqueous formaldehyde solution. The excised lung tissues were dehydrated in ascending series of ethanol (70-100 %), embedded in paraffin wax, sectioned, and stained with hematoxylin-eosin. The severity of the pathological lesions was assessed on a semi-quantitative scale of 0-4, and a total score indicative of the overall severity was determined by adding the individual scores.	- Vaccinated animals showed significantly low severity scores (P-value < 0.05) compared to control animals, except for the animals that received encapsulated vaccines, which showed comparatively high lesion scores. - There is no edema or congestion in these animals, as seen in the untreated control group. Such infiltration of cells may be attributed to the particulate nature of the microparticles, which has caused a mild reaction and is a commonly reported observation on the administration of particulate delivery systems. - The LPS encapsulated microparticles exhibit greater efficacy when administered by the intra-tracheal route than the free LPS vaccine. The mice immunized with LPS encapsulated microparticles showed more significant PHA titers and revealed complete elimination of <i>K. pneumoniae</i> from lungs. [46]
8	Budesonide	Chitosan	The right lobes of mice and rats (which were not lavaged) were excised, fixed with 10 % formalin for 48 h, embedded in paraffin after alcohol gradient treatment, and then sliced into 5 μm thin sections for H&E staining. The inflammatory cell infiltrate was analyzed	- No statistical difference in the histology, inflammation cells, and IL-4 mRNA expression was found, indicating the chitosan microparticle itself did not affect asthma mice. - The significantly enhanced sustained therapeutic effect of chitosan swellable microparticles was demonstrated in allergic asthma models in mice and rats. After single treatment with SM50 and SM200, a time-dependent [50]

by light microscopy at a magnification of 100x. Briefly, the inflammation score was designated as none (score, 0), mild (score, 1-2), moderate (score, 3-4), or severe (score, 5-6). The final score was calculated by the addition of both peribronchial and perivascular inflammation.

therapeutic impact was investigated to test whether prolonged therapeutic efficacy can be achieved as a reflection of in vitro sustained release. - Both inflammatory cells and IL-4 and IL-5 levels in BALF showed delivery of SM200 and SM50, which had a sustained drug release for 12 h, had a longer therapeutic effect than budesonide physical mixture alone. - SM200 showed significant therapeutic efficacy up to 18 h.

3. Conclusions

This paper provides a comprehensive review of the current researches of various polymers in drug and protein delivery, particularly for inhalation in DPI dosage forms. Applications of polymers in these researches have a promising future. The characterization and testing of the polymer include stability, drug deposition to its histopathological. This review highlights the recent advances in polymers because of their convenience, biodegradability, and nontoxicity, and it is applied to various drug-delivery technologies. Thus, researchers need to update the advances in polymer-based drug delivery systems, and this review is a source of guidance for future research.

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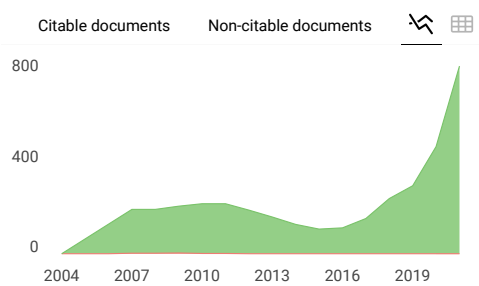
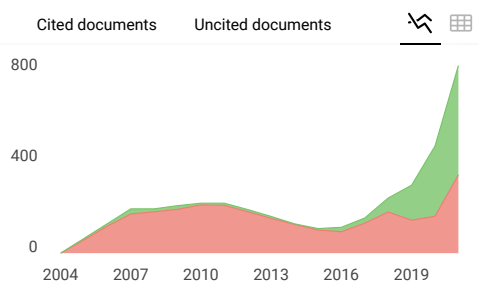
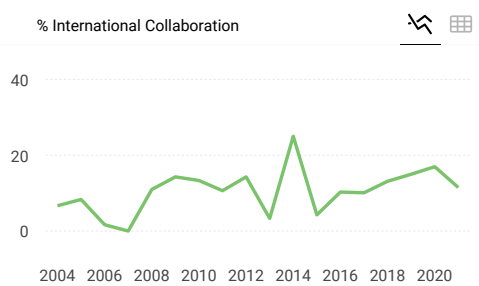
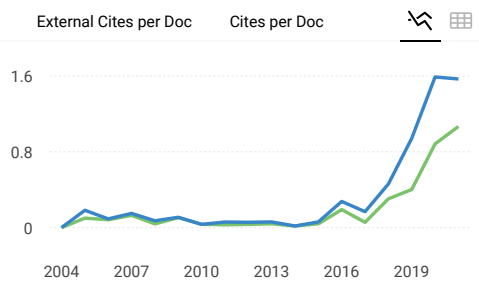
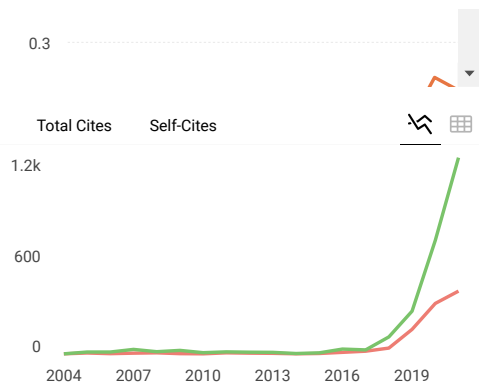


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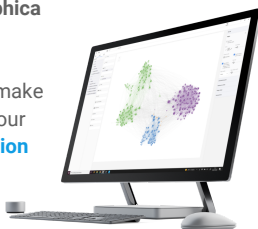
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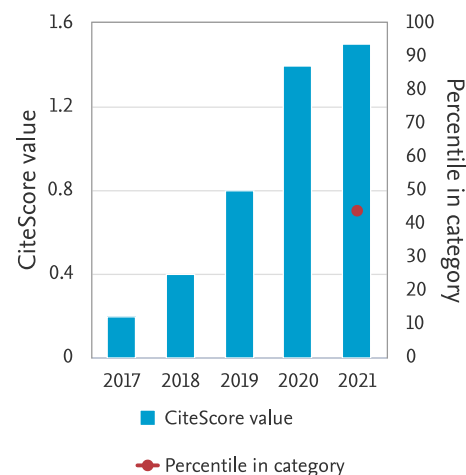
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