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Review

Integrating VOC-Based Signatures and *in Silico* Approaches: A Conceptual Framework for the Management of Respiratory Tract Infections

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Abstract

Volatile organic compound (VOC)-based signatures and *in silico* approaches offer promising strategies for early disease diagnosis and improved nasal drug delivery in the management of respiratory disorders. VOCs are of both natural and anthropogenic origin. They are low-molecular-weight (MW) metabolic compounds characterised by high vapour pressure and low boiling points. Emerging evidence shows that specific microbial VOC profiles are associated with distinct disease states, as observed in patient-derived samples. These compounds can serve as biomarkers for pathogen identification and disease characterisation when analysed using advanced analytical techniques and computational models. *In silico* stimulation may further enable the optimisation of nasal drug delivery systems by enhancing drug bioavailability and circumventing first-pass metabolism. Chronic exposure to anthropogenic VOCs through one's occupation can impair respiratory health, increasing susceptibility to infections such as respiratory syncytial virus (RSV), severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), and influenza, while compromising mucosal integrity, which is critical for both immune defence and effective intranasal drug absorption. This study explores the potential of VOC-based signatures (biomarkers) and computational modelling as integrated tools to improve nasal drug delivery strategies for managing respiratory diseases.

Keywords

Volatile organic compounds, Nasal cavity, Respiratory infection, *In silico*, Screening tools

Article History

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1. Introduction

The quest for solutions amid the increasing health challenges in our society is inexhaustible, as humans are daily exposed to various chemicals that act as agents inducing many health disorders [1]. These health disorders are sometimes byproducts of long-term exposure to chemical compounds, such as Volatile organic compounds (VOCs), in the form of workplace fumes, or of exposure to microorganisms. VOCs are carbon-based molecules, naturally or artificially emitted, that include ketones, xylene, alcohols, toluene, sulfides, aldehydes, amines, hydrocarbons, terpenes, formaldehyde, and isocyanates [2]. They are emitted by some pathogens and various chemical compounds, such as paints and pharmaceutical products, and are characterised by high volatility, high saturation pressure, and low boiling points. VOCs are also classified into three major groups: VOCs (50-100 to 240-260 °C), Very Volatile Organic Compounds (VVOCs) (0 to 50-100 °C), and semi-volatile organic compounds (SVOCs) (240-260 to 380-400 °C) based on the degree of boiling point [2,3]. Humans are affected by VOCs through inhalation, dermal contact, and ingestion of man-made or pathogen-borne solid or liquid chemical substances [2]. Production of VOCs from pathogens, lung injury, pathiophysiological factors or due to exposure to environmental pollutants brings about ROS imbalance leading to oxidative stress with implication of DNA damage, protein oxidation and lipid peroxidation resulting to increased risk of respiratory diseases such as bronchial asthma, pulmonary fibrosis, chronic obstructive pulmonary disease, acute lung injury and lung cancer [4], as shown in Figure 1 (Pathological sources and effects of VOCs). This study adopted a systematic search of electronic databases, including PubMed, Scopus, Web of Science, Google Scholar and ScienceDirect. The search included studies published between 2000 and 2025.

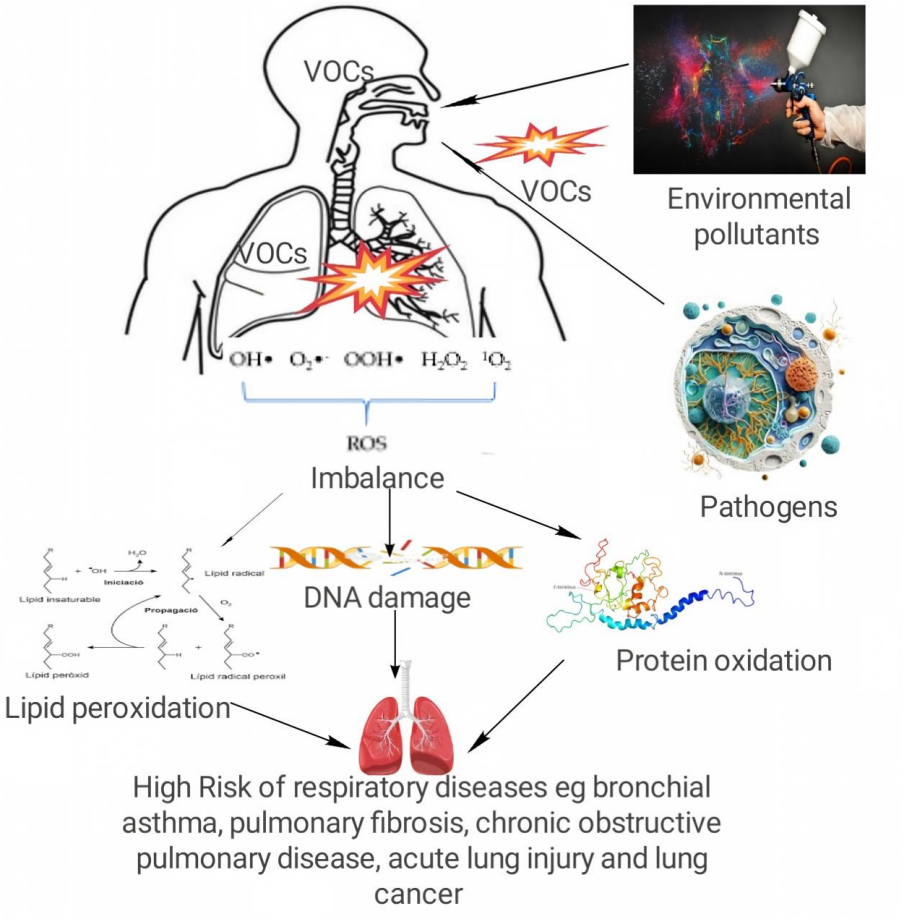

Figure 1. Pathological sources and effects of VOCs.

Notwithstanding the negative effects of VOCs, microbial-based VOCs are currently employed by health specialists in diagnosing and managing diseases using computer-based approaches, because microbial organisms, such as bacteria, emit a myriad of VOCs associated with various health disorders, including respiratory diseases [4]. Microbial-produced VOCs are byproducts of the primary and secondary metabolism of many microbes. Microbes produce many types of VOCs during the metabolic processes of carbohydrates, lipids, amino acids, and other molecular compounds containing aromatic compounds, sulphur, and nitrogen [5]. The comprehensive set of these microbial-produced VOCs, known as the volatilome, can be analysed using pure cultures grown under various laboratory conditions. Microbial cultures can also be used to classify

bacteria by their lipid and fatty acid profiles using computer-assisted techniques. This method of scientific disease diagnosis is currently gaining attention as a major pathway towards the use of microbial VOCs as viable infectious biomarkers for respiratory disorders such as tuberculosis (TB) and pneumonia [6]. Some microbial species, such as Pseudomonas aeruginosa, Aspergillus fumigatus, and Mycobacterium TB, are associated with lung infection. Alcohol and ketones are VOC metabolites associated with fermentation, whereas hydrogen cyanide is a major biomarker for identifying Pseudomonas aeruginosa [7,8].

Diagnosis is becoming easier with the advent of computer-based approaches. The application of computational tools is currently the most widely accepted scientific approach in disease diagnosis, discovery, and the design of new therapeutic compounds [9]. *In silico* approaches are scientific methods that leverage computational tools to analyse and model biological processes, diagnose disease, and provide therapeutic solutions. It is a method of experimental stimulation using various computer techniques, including artificial intelligence, machine learning, deep learning, imaging, statistical analysis, and data-driven methodologies. *In silico* approaches in disease analysis help to accelerate and scale up diagnoses with the aid of pattern recognition techniques in digital image analysis to improve clinical output, while in deriving therapeutic solutions, they allow rapid screening of large libraries of diseases to identify a potential drug that is suitable for the disease conditions [9].

For rapid diagnosis and treatment of respiratory tract infections, a proper understanding of the anatomy and physiology of the nasal cavity, along with the application of novel drug formulations and improved clinical devices such as computer-based stimulation, can make nasal drug delivery more efficient and an excellent therapeutic method [10]. Nasal drug delivery systems are in the limelight compared to the conventional routes of drug administration for the treatment of respiratory diseases because of their ability to bypass hepatic first-pass metabolism (physiological barriers) in the treatment of disease conditions. The mechanism of nasal drug delivery is based on the complex network of the nasal cavity (Figure 2) that makes it possible to break the blood-brain barrier (BBB) for drugs to enter the brain tissue or cerebrospinal fluid through the nose (olfactory neurons), giving it a great advantage over the conventional, widely used oral method of drug delivery [11].

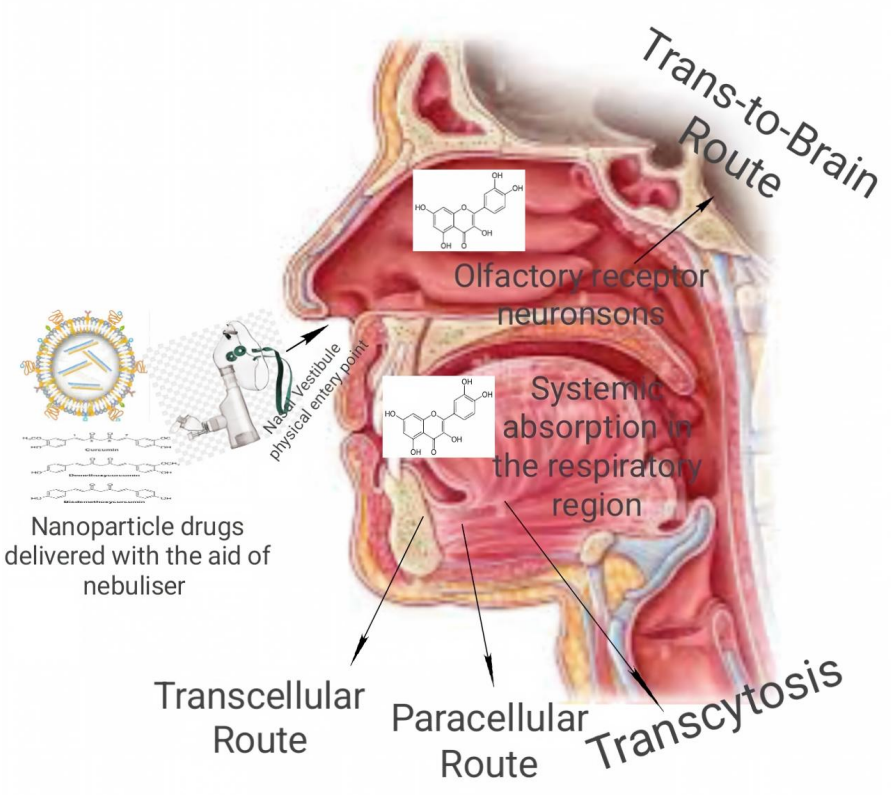

Figure 2. Anatomy of the nasal system and routes of drug delivery.

Respiratory tract infections are diseases that affect the lungs, airways, and related structures. They include asthma, TB, chronic obstructive pulmonary disorder (COPD), and lung cancer [12]. The primary causes of respiratory tract infections are viruses such as coronaviruses (SARS-CoV-2), respiratory syncytial virus (RSV), influenza viruses, rhinovirus, parainfluenza viruses, and human metapneumovirus. Respiratory tract infections can also be caused by bacteria such as Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis. But not frequently like viruses [13]. The major route of attack for respiratory tract infections is the upper respiratory tract, thereby creating a clinical need for efficient delivery tools to enhance drug delivery through the nasal cavity [14]. In respiratory infection, there are also cases of co-infection. Respiratory viruses

can be co-infected with other respiratory Viruses, bacteria, and fungi, causing detrimental effects on the host. Patients with respiratory co-infections have been observed to have more complications, severe disease, and a higher rate of intensive care unit admissions [15]. The diagnosis and treatment of various respiratory disorders can be effectively achieved through *in silico* simulation of active compounds to enhance disease identification and drug discovery and development, and to reduce the need for preclinical studies [16].

Over the years, the world has consistently highlighted the negative impact of VOCs, but recently, medical scientists have begun to demonstrate that VOC-based diagnostics have great potential as next-generation medical screening tools for pathogen identification and characterisation, enabling improved medical intervention against infectious diseases. Although this practice has been around for the last two decades, the premise of using VOCs as biomarkers for disease identification and rapid diagnosis dates back to ancient times. This was at the time when the ancient Greek physician Hippocrates poured human sputum over hot coals to remove the distinct odours indicative of TB during the period 460-370 BC [17]. The medical application of VOCs faces challenges because VOC identification requires sophisticated analytical techniques.

Currently, there is growing evidence that certain VOCs, or their profiles, are unique to various disease states. Scientific research has shown that different pathogenic species produce VOC profiles with characteristics unique to them during metabolism (Table 1). Certain bacteria and plants produce a specific type of VOCs. For instance, Escherichia coli produces indole, Actinomycete bacteria produce geosmin, and Pseudomonas aeruginosa, a good breath biomarker for identifying respiratory infections, produces 2-aminoacetophenone. Plant VOCs include terpenoids, compounds with aromatic rings, amino acids, and fatty acid derivatives [18]. This was also seen from the metabolite profile of the patient's sample, analysed for non-invasive pathogenic identification to enhance early diagnosis and treatment [19]. Also, pathogens can produce various VOCs; however, some metabolites are produced specifically by a single bacterial species, as shown in Table 1 below [20].

Table 1. Pathogens and their specific VOC types, along with the associated infectious diseases.

Pathogen	The Biomarker VOCs for Disease	Infectious Disease	Sources of Infections	References
Mycobacterium TB	Methyl nicotinate, 1-methyl-naphthalene and 1,4-dimethyl-cyclohexane	TB	Airborne droplets (cough/sneeze)	[18,21,22]
Klebsiella pneumoniae	Octyl acetate, butyraldehyde, butanoic acid, tridecanol, and dodecanal	Pneumonia, Bronchitis	Normal microbiota of the intestine (health care facilities)	[23]
Pseudomonas aeruginosa	1-vinylaziridine, 1-undecene, 2-nonanone, 2-aminoacetophenone, methyl thiocyanate and pyrrole	Pneumonia and Sinusitis	Medical equipment's	[24]
Staphylococcus aureus	Acetic acid, acetoin, acetaldehyde, hydroxyacetone, 2-propanol, 3-methyl-1-butanol and isovaleric acid	Pneumonia and Sinusitis	Nasal passages (contaminated surfaces and objects	
Haemophilus influenzae	Benzaldehyde, benzyl alcohol, acetic acid, Indole			
Vibrio cholera	Dimethyl disulfide, p-menth-1-en-8-ol	Cholera	Contaminated water and food through infected faeces	
Burkholderia cepacian				
Campylobacter jejuni	Butanoic acid, 1-butoxy-2-propanol and 1-octen-3-ol,	Ulcerative colitis, diarrhea	Animal feces	
Moraxellacatarrhalis	Benzaldehyde, benzyl alcohol, and 2-phenylethanol			
Clostridioides difficile (clostridium difficile)	Ethanol, Butanol, Isopropanol	Ulcerative colitis, diarrhea	Hardy spores	
Streptococcus neumoniae	Acetic acid, Benzyl alcohol, benzaldehyde, ethanol, aminoacetophenone, acetaldehyde, 2-phenylethanol, indole, methyl mercaptan, dimethyl sulfide, hexanal and trimethylamine			

2. VOC Biomarkers in Respiratory Infections

Worldwide, there is a public need for a rapid means or methods of identification of pathogens, determination of antibody resistance or susceptibility for effective treatment of disease conditions because most of the conventional diagnostic methods

are faced with limited sensitivity and specificity, are very expensive and time-consuming, as they require several days for cell culture and low-throughput microscopy assays. The use of VOCs as medical diagnostic tools is made possible as a result of normal metabolic activities of the body which produces different kinds of VOCs that are emitted through exhaled breath, saliva, skin secretions, blood, faces and urine likely derived from the activities of commensal microbes detectable in culture sample grown *in vitro* can be properly identified (Table 2) and monitored through computational tools (Figure 3) [25]. These microbes produced a characteristic VOC profile due to their distinct metabolisms [26].

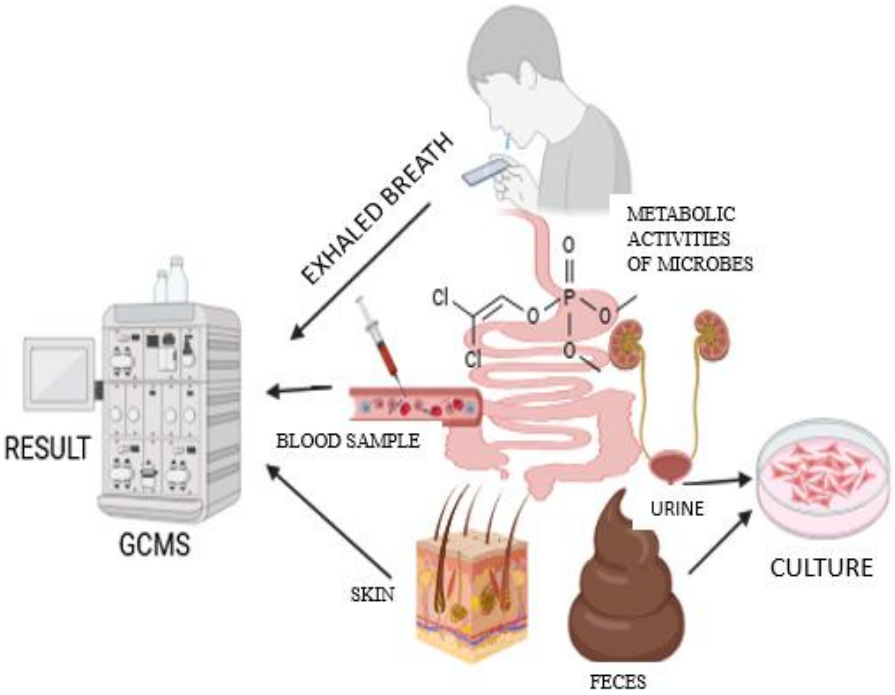

Figure 3. Computer-based study of human sample used for VOCs medical analysis.

Table 2. Specimens used in VOCs analysis.

Specimen	Target Infections	VOCs	Method	Instruments	References
Exhaled breath	Respiratory tract infection	Sulfur-containing VOCs	Membrane extraction with a sorbent interface, chemical trapping, sorbent trapping, cold trapping, or condensate trapping, thermal desorption and Solid-phase micro-extraction (SPME),	GC-Mass spectrometer	[21,25,27,28]
Saliva (spatum)	Respiratory infection, e.g., TB	Alcohols, aldehydes, ketones, carboxylic acids, esters, amines, amides, lactones, and hydrocarbons	Culture-based	GCMS	[28,29,30]
Skin	Commensal skin bacteria		Wiping and collecting the VOCs directly from the affected skin with organic solvent (acetone) onto an absorbent SPME fibre		
Feces	Gastrointestinal and liver diseases		Stool culturing		[31,32]
Blood	Diseases of the internal body	Hydrocarbons (pentane and isoprene), ketones (acetone), halogenated compounds (isoflurane), and thioethers (dimethyl sulfide)	SPME	Gas chromatography-mass spectrometry (GC-MS)	[33,34]
Urine	Urinary tract infection	ketone, alcohol, furan, pyrrole and sulfide	Culturing	Gas-liquid partition chromatography	[35,36]

2.1 Breath Analysis and Diagnostic Technologies

Breath analysis is a grey area of medical diagnostic testing for managing respiratory disorders. It is a VOC biomarker for diagnosing respiratory diseases that is easy to use in patients. Exhaled breath contains a myriad of VOCs that can be traced to a particular disease. Breathing VOCs for diagnostic purposes is achieved using chemical, sorbent, cold, or condensate trapping, followed by SPME, which adsorbs VOCs onto a coated microfiber and delivers them to a mass spectrometer [28]. Disease identification and treatment via the nasal cavity can also be improved with an electronic device known as the e-nose (electronic nose), which mimics the human olfactory system. It consists of a micro-array gas sensor combined with pattern recognition software to adsorb exhaled breath and produce output. It has the capacity of differentiating non-identical samples without the need to separate the mixture into its individual components; however, before an e-nose can be effectively applied for practical diagnosis of VOCs biomarkers, it will need an improvement in the area of accuracy and sensitivity to enhance reliable recognition of a large number of VOCs profiles as a specific compound [30].

Currently, GC-MS remains the gold standard for separation, detection, identification and quantification of VOCs [30]. However, there are several other analytical tools that work in synergy with GC to achieve desired results, such as GC ion mobility spectrometry (GC-IMS), GC flame ionisation detection (GC-FID) (widely used for breath analysis) [37]. Optical spectroscopic and non-optical direct injection methods are used in VOC measurement. The optical spectroscopic methods include laser absorption spectrometry, while the non-optical direct injection methods are ion-molecule reaction mass spectrometry (IMR-MS), selected ion flow tube mass spectrometry (SIFT-MS), and proton transfer reaction mass spectrometry (PTR-MS). SIFT-MS stands out in clinical analysis for its ability to provide real-time, absolute quantification of multiple VOCs simultaneously [38,39,40]. Treatment of Respiratory Tract Infection has been a common challenge over the years, with the common cold being the most frequent diagnosis, as patients present with symptoms consistent with it [41]. Laboratory Investigations such as sputum culture and also with the help of the patient's past history and self-examination, conclusions are made to proffer solutions to the Infection 'common cold' by giving broad spectrum antibiotics through the nasal route with the aid of inhaler for a long duration due to the fact that the suspect bacteria agent cannot be confirmed because of the minimal time required to cultivate the bacteria in the Laboratory media [42,43,44].

With an ever-growing population and advancing technology, rapid point-of-care diagnosis to detect the specific bacterial agent is needed to enable direct treatment. Breath testing has been found to diagnose specific bacterial agents based on volatile compounds these agents produce, the host, or the result of their interaction with the host [45]. A study conducted by Rosario C. et al. demonstrated the effectiveness of a breath analyser, Aeonose TM, in differentiating healthy individuals from TB patients. The analyser was used to monitor the patient's treatment response. Many diagnoses have been made using VOCs; they serve as strong biological markers [46]. Exhaled Nitric Oxide (NO) levels have been used in the diagnosis of Asthma [46]. Octane, Acetaldehyde, and 2,3-methylheptane were identified as VOC biomarkers for acute respiratory distress syndrome [47]. Exhaled hexanal was also identified as a biomarker for Asthma exacerbations in childhood [46].

Ventilator-associated pneumonia (VAP), which is a common hospital-acquired infection seen in patients in the Intensive Care Unit [48], has a high mortality rate due to its severe complications. In a more recent study, the common diagnostic modality for VAP, Bronchoalveolar lavage (BAL), was compared with an exhaled breath analyser, which provided immediate results within an hour, unlike the normal 48 hours seen with BAL [48]. The exhaled breath analyser detected VOCs associated with the infection and those not associated with it. Exhaled breath clearly has an edge over BAL analysis, as it can be analysed quickly, is very easy to perform, and is non-invasive [48]. This rapid diagnostic tool will go a long way toward identifying the best medication and preventing antibiotic resistance [49].

2.2 Application of VOCs in Nasal Drug Delivery System

The nasal cavity is multifunctional, serving as a gateway for gaseous filtration (breathing), humidification, pathogen entry, and therapeutic drug administration. Nasal drug delivery is preferred over systemic delivery because of its ability to bypass the BBB [50]. The nasal cavity extends from the nostrils towards the nasopharynx and is separated into the olfactory, respiratory, and vestibular regions. The nasal cavity is a specialised network of structures for effective drug delivery while bypassing the BBB [51]. The olfactory region is the first point of entry for respiratory infection because of its primary exposure to the environment [52]. Viruses use the receptor-mediated endocytosis mechanism to enter the olfactory epithelium; they bind to glycans with terminal sialic acid that is linked to galactose in the nasal epithelium, and those such as herpesvirus use heparan sulphate and nectin-1 on the cell surface [53]. Also, studies have shown that SARS-CoV-2 enters the human system through the nasal and olfactory epithelia. They make use of obligatory receptor, with the aid of an enzyme angiotensin 11 and the priming protease [54,55].

There is growing evidence supporting the efficacy of VOC analysis in identifying a wide range of respiratory diseases, thereby improving treatment through *in silico* simulation [55]. Pathogens are capable of producing a metabolite that are exclusively to their species, particularly in polymicrobial respiratory infections such as seen in RSV, SARS-CoV-2 and Influenza and the most efficient way of drug delivery for effective therapeutic response, is through the use of inhaler devices like nebulizers,

metered-dose inhalers, and dry powder inhalers which is continuously improved through virtual laboratory experiment (*In silico* experimentation) [12]. Selecting a drug through in silico experimentation before clinical testing for nasal cavity drug delivery is important, as it plays a key role in drug design and selection. VOC signatures for disease diagnosis enhance sample collection from all forms of patients, such as children, the aged, and those who are very sick. The breakthrough in analysing the various VOC biomarkers is possible through various scientific methods (chemical trapping, sorbent trapping, condensate trapping, thermal desorption, the use of SPME) and analytical tools (gas chromatography mass spectrometer [21].

2.3 Challenges of Nasal Drug Delivery

The challenges of nasal drug delivery include the following.

2.3.1 Physiological Barriers

The physiological characteristics of the nasal cavity, such as mucociliary clearance and the limited nasal mucosal surface area, dramatically affect nasal drug administration in the treatment of respiratory infections. Mucociliary clearance is an integral natural defence mechanism that prevents the entry of noxious materials (bacteria, viruses, allergens, or toxins) into the lungs [55]. The cilia lining the nasal mucosal surface sway at an estimated speed of 5-6 mm/min to move mucus to the pharynx and remove substances adhered to the nasal mucosa [56]. When noxious substances dissolve in or adhere to the mucus lining in the nasal cavity, they will be transported to the nasopharynx for upward discharge into the gastrointestinal tract. Although this is a protective mechanism, the rapid removal of substances from the nasal cavity by ciliated cells is a primary constraint on nasal drug absorption [56]. Mucociliary clearance shortens the time a drug remains on the nasal mucosa, thereby affecting absorption, as the drug remains in the nose for 20 to 30 minutes after administration. This ultimately reduces the drug's bioavailability in the nasal cavity [57].

2.3.2 Physicochemical Properties of the Drug

The molecular weight (MW), solubility, degree of ionisation, viscosity, and the balance of lipophilic and hydrophilic substances in drugs significantly affect their absorption in the nose. High-molecular-weight drugs may have difficulty traversing the nasal mucosa and entering the respiratory system, leading to low drug absorption efficiency [55]. For lipid-soluble drugs, absorption rates are nearly 100% across the nasal mucosa; however, absorption decreases significantly when a drug's MW exceeds 1 kDa. Also, the concentration of water-soluble macromolecular medicines entering the CSF negatively correlates with MW [56]. Moreover, the permeation efficiency of polar drugs is highly dependent on the MW, with compounds having MW $\geq$ 300 Da demonstrating low permeation [58]. According to Barros et al. [59], such as desmopressin acetate, cyanocobalamin, nafarelin acetate, and salmon calcitonin with an MW of 1000-3400 Da have a low nasal bioavailability of approximately 10%. Ideally, the nasal route may not be ideal for administering compounds with high MW to the respiratory tract. In addition, drug absorption via the nasal mucosa depends on the drug's ability to dissolve in nasal secretions.

Furthermore, drug solubility in water and lipid affinity affect its potential to traverse the nasal epithelium [55]. However, nasal secretions comprise approximately 90% water, with small amounts of proteins, mucin, lipids, and inorganic salts. Notably, the degree of ionisation (pKa), which connotes the pH of the un-ionised and ionised form existing in equal concentrations, is a crucial physiological property that controls drug absorption across the epithelial membrane [59]. As only the non-ionised fraction of drugs can diffuse across the epithelial membrane, drug bioavailability is influenced by the pH at the absorption site and the water solubility. Considering the pH of the nasal cavity, only weak base salts having a pKa above 7.5 will usually be ionised, while weak acid salts with a pKa value below 5 will predominantly remain unionised in the nasal cavity [59]. Additionally, drug stability in the nasal cavity is an essential factor in its efficacy. For instance, some drugs may degrade under acidic pH or by nasal enzymes [55]. Studies have also shown that drugs with higher viscosity have greater contact time with the nasal mucosa, thereby enhancing permeation time and absorption efficacy (Moinuddin et al. 2019). However, highly viscous drugs interfere with nasal physiological functions, such as ciliary beating and mucociliary clearance, thereby altering the permeability of the target drug [59].

2.3.3 Patient Factors

Comprehensive knowledge of human nasal anatomy and physiology is crucial, as variables such as mucosal permeability, surface area, and vascular blood supply can influence the distribution and absorption of drugs administered through the nasal route to the respiratory tract. So, when formulas are evaluated, they need to account for differences in anatomy between species. These differences include nasal pH, mucociliary clearance, blood flow, dosage volume, and enzyme activity. Hence, variability in nasal anatomy and conditions (e.g., nasal congestion) across species poses a major challenge for nasal drug formulation. In recent decades, animal models, including rats, mice, rabbits, dogs, monkeys, and sheep, have been widely used in research on intranasal drug administration [60]. Caution is necessary when extrapolating findings to humans due to anatomical and physiological differences. For instance, the nasal structure of dogs, which depends on their olfactory sense, is

intricate, featuring branched turbinates, in contrast to the single scroll found in humans. The olfactory area in rats constitutes over 50% of the nasal cavity, a fivefold greater proportion than in humans [60]. Consequently, a crucial challenge in implementing pulmonary drug delivery is the reliance on animal models for *in vivo* studies, which require active drug administration because they cannot perform the required inhalation manoeuvres. Achieving adequate dispersion and deposition profiles with commonly used *in vivo* delivery devices for pulmonary drug delivery in humans is challenging. Consequently, *in vivo* investigations may not precisely represent a patient's state. Given the respiratory tract morphology and immunological responses to pathogens, vaccine candidates must be evaluated in animals with respiratory tract structures and immune responses analogous to those in humans [11]. It's also possible that differences in nasal architecture, such as differences in thickness, airway flow, the properties of the mucosal lining, and how well people can breathe, can greatly affect how well nasal drugs (NDs) are retained, absorbed, and distributed in the respiratory tracts.

3. *In Silico* Approaches as Strategies to Overcome Challenges

In silico therapeutic approaches in disease modelling create a detailed model of diseases, aiding a broad understanding of disease mechanisms, identification of biomarkers, and testing of potential treatments in a virtual environment using a series of computer-based techniques, thereby reducing the need for extensive *in vivo* studies, as it provides a platform to combine both *in vivo* and *in vitro* experimentation [16]. Strictly adhering to and applying various *in silico* disease diagnostic and drug development models for effective respiratory disease treatment will help reduce the excessive sacrifice of animals for experimentation, costs, and the long time spent on disease diagnosis and effective treatment [61]. *In silico* stimulation can be used to study the activity of a drug during drug delivery to improve the design of drug delivery to reach the target organs using the following drug delivery tools.

3.1 The Use of Absorption Enhancers

Absorption enhancers (AEs) are formulations used to improve drug absorption across biological barriers. They enhance the efficacy of proteins, peptides, and other pharmacologically active agents that have poor permeability across biological barriers [62]. *In silico* stimulation can be used enhance the absorption of NDs to the respiratory tracts via multiple mechanisms, such as improving the mucus solubility and stability of the drug, increasing the residence time the drug stays on the mucosa, modifying drug physicochemical properties, and improving the permeation of substances across the epithelial barrier through paracellular or intracellular mechanisms through the combination of *in vitro* and *in vivo* platform provided by *in silico* approaches [58,60]. This could be achieved by adding excipients, using a drug delivery system, or adjusting the pH of the mucosa solution. Maggio and Pillion [63] reported that adding AEs can raise epithelial permeability up to 30-fold, allowing larger molecules up to 30 kDa to pass. The commonly used AEs for nasal drug delivery systems are surfactants, cyclodextrins (CDs), protease inhibitors, tight junction modulators, and cationic polymers [60]. Surfactants used as AE are classified as bile salts, phospholipids, salts of fatty acids, non-ionic surfactants, and alkyl glycosides [55]. Opening of tight junctions, leaching of membrane proteins by perturbing the cell membrane, or inhibition of enzymatic degradation of NDs are the proposed mechanisms of action of surfactants [56]. The CPEX Pharmaceuticals Serenity has developed two surfactants: testosterone (Testim) and Nocturia for nasal and pulmonary formulations. CDs are chemical reagents comprising six, seven, or eight glucose units linked in a cyclized ring with a central cavity that can accommodate other substances, especially lipophilic agents [56]. Their outer hydrophilic surface, in contact with the external aqueous environment, makes the complex soluble in water [64]. CD polar cavities incorporate NDs with hydrophobic moieties to prevent potential modification after administration. CD promotes drug absorption directly or indirectly by acting as an absorption enhancer, stabiliser, or solubiliser. Specifically, modified CDs such as dimethyl-β-cyclodextrin, hydroxypropyl-β-cyclodextrin, and methylated-β-cyclodextrin are being studied as nasal drug enhancers. Interestingly, Kleptose® HPB (hydroxypropyl-cyclodextrin), a commercial CD product, has been proposed as an appealing excipient for nasal and pulmonary drug administration because of its ability to solubilise drugs, improve drug absorption, and have a low toxicity profile [62]. Overall, the Food and Drug Administration has listed both natural and modified CD as accepted excipients for pharmaceutical products [64].

Nucleases and proteases in the mucus and liquid on the airway surface can degrade drugs before they reach the absorptive membrane. Enzyme inhibitors are an effective strategy for preventing enzymatic degradation of proteins, peptides, and nucleic acids in the respiratory and nasal regions. Over the last decade, enzyme inhibitors such as aprotinin, nafamostat, bacitracin, mesilate, soybean trypsin inhibitor, bestatin, eupeptin, and phosphoramidon have been studied as AE in nasal/pulmonary drug administration [58]. Additionally, fusidic acid derivatives and salts are enzyme inhibitors that increase the bioavailability and absorption of NDs [58]. Emerging research indicates that the use of neutrophil elastase inhibitors, such as engineered protease inhibitors like human neutrophil elastase 4 (EPI-HNE-4), attenuates poor absorption of NDs during chronic inflammation due to excessive accumulation of neutrophil elastase in tissue and pulmonary fluid in patients with chronic lung infection. Moreover, several protease inhibitors have registered research interest in enhancing NDs' stability and pulmonary absorption in respiratory diseases. Remarkably, enhancement of nasal drug delivery can be achieved using cationic polymers such as chitosans, cationized gelatin, poly-L-arginine, cationized pullulan, and polyethylenamine, as they interact with mucosal

barriers to modify tight junctions, thereby improving the absorption of hydrophilic macromolecules. For example, the cations in the amino acid residues of chitosan usually bind to mucosal anions, increasing epithelial cell membrane permeability and opening tight junctions to improve drug absorption [56]. Studies have shown that chitosan's desirable qualities, such as being biodegradable, biocompatible, and low in toxicity, make it a great choice for use as a pharmaceutical excipient. Intranasal apomorphine, intranasal fentanyl citrate (NasalFent), and intranasal diazepam, produced by Archimedes Pharma Ltd., are chitosan-based absorption enhancers undergoing 2- and 3-phase developmental stages for nasal drug formulation. Additionally, Archimedes Pharma Ltd. has employed polyethylenamine (PEI) as an excipient for nasal drug administration. Interestingly, the degree of positive charge was directly related to how well PEI absorbed things. Collectively, several absorption-enhancing products in stages of development for nasal and respiratory formulations are not limited to nocturia (surfactant).

3.2 Development of Nanoparticles for Enhanced Drug Delivery

Nanoparticles offer a flexible approach to overcoming the obstacles of nasal drug delivery by improving drug stability, regulating release, and increasing absorption. This is achieved by their ability to regulate the drug-release rate, which ensures that the optimal therapeutic concentration is maintained, thereby enhancing treatment [55]. Their biocompatibility, lack of toxicity, and increased uptake make them safe for nasal drug delivery to the respiratory system [55]. In recent years, many nanosized drug delivery systems, including polymeric nanoparticles, solid lipid nanoparticles (SLN), micelles, dendrimers, nanoemulsions, and liposomes, have been used as platforms for enclosing NDs [55]. Polymeric nanoparticles comprise water-insoluble polymers in which drugs are entrapped, dissolved, attached, or encapsulated. Polymeric carriers are tailored to adjust cargo loading, surface charge, surface decoration, and cargo-release capability with specific chemical entities that help drugs reach targeted therapeutic targets [64]. The controlled drug release capacity and surface hydrophobicity of polymeric nanoparticles make them suitable for prolonging the therapeutic effect of nasal formulations [64]. The SLN is a lipid-based nanocarrier with a single lipid coat outside and a hydrophobic core inside. They are prepared by ultrasonication, microemulsion, high-pressure homogenization, emulsification, solvent evaporation, and double emulsion, which replace liquid lipids with solid lipids, with hydrophobic lipid chains embedded to improve drug dispersion [65]. The advantages of SLNs in drug delivery include their high physicochemical stability, low biotoxicity, and slow, controlled drug release [65]. Studies have shown that SLNs increase nasal retention primarily due to mucous membrane adhesion and occlusive effects [64]. However, the main drawbacks of SLNs are their nonuniform particle size distribution and limited shape options. To overcome these challenges, nanostructured lipid carriers (NLCs) composed of liquid and solid lipids, using stable, less toxic physiological lipids such as fatty acids, triglycerides, and waxes, have been developed [65]. Notably, NLCs achieve greater encapsulation efficiency relative to SLNs since the solubility of hydrophobic molecules is higher in liquid lipids compared to solid lipids. According to Pires et al. [64], NLCs have high drug loading and allow rapid uptake with long-term stability. Collectively, their increased stability, controlled drug release, low toxicity, and high loading make them stand out as promising delivery systems.

Liposomes, produced from phospholipids and cholesterol, are spherical, nanosized vesicles mimicking the biological membranes [64]. They are biocompatible, biodegradable, and highly safe, making them ideal carriers for drug delivery routes [57,65]. Liposomes enhance drug absorption, protect against enzymatic degradation of drugs, and also increase drug stability [57]. Encapsulating the drug in liposomes can reduce direct contact between the drug and the nasal mucosa. At the same time, the lipid bilayer can regulate drug release and uptake to achieve the desired response [65]. The unique structure of liposomes makes them suitable for loading both hydrophobic molecules and hydrophilic molecules. Although liposomes can be loaded into gels, decoration of liposomes with mucoadhesive polymers such as chitosan, alginates, gelatin, starch, carboxymethylcellulose, methylcellulose, hyaluronate, and polyacrylates prolongs residence time while reducing mucociliary clearance [57]. Moreover, functionalized liposomes with cell-penetrating peptides enhance their transport across absorption barriers. In a study by Qiang et al. [66], fexofenadine was loaded into chitosan-coated liposomes to enhance systemic exposure and extend drug release. They realised that in-coated liposomes had higher plasma concentration and bioavailability than uncoated liposomes. Additionally, the drug exposure time was sustained in the blood for 12 hours after the coating of the liposome with chitosan. Chitosan and other mucoadhesive polymers can interact with mucus via van der Waals interactions, hydrogen bonding, and electrostatic attraction, thereby significantly prolonging residence time and reducing mucociliary clearance [57]. By and large, the components, viscosities, and pH values of nanosized drug formulations must be compatible with the epithelium to prevent irritation, destruction, and inflammation of the nasal lining [57].

3.3 pH and Osmolarity Adjustments for Mucosal Compatibility

The nasal mucosa has a slightly acidic pH of 5-6.5 to facilitate normal ciliary function and good permeation, reduce drug losses via leaching, and prevent nasal irritation [59]. Thus, any significant deviation from this pH elicits nasal irritation and causes poor drug absorption. Hence, only drugs that can be solubilised at this pH are ideal for intranasal drug delivery. Moreover, fluctuations in the nasal cavity pH, from mildly acidic to neutral, can affect the stability of nasal drug formulations

that are susceptible to pH alterations [56]. Drugs solubilised at extreme pH or below 4 exhibit reduced tolerance, thereby impeding drug permeation. To overcome nasal irritation and low permeation, the pH of the nasal formulation needs to be adjusted to 4.5-6.5 to activate the lysozyme responsible for destroying bacteria in nasal secretion [58]. This is mostly achieved by including a buffer in the nasal formulation to prevent drastic changes in pH and maintain isotonicity, as physiological pH changes may be better tolerated [59]. Ideally, lysozyme works optimally at acidic pH and is inactivated at alkaline pH, making the nasal cavity susceptible to infection. Thus, adjustments of ND pH prevent bacterial growth, enhance drug permeation, and prevent irritation [59]. Furthermore, studies in rat models have shown that adjusting the dosage form's osmolarity improves nasal drug absorption. The highest absorption level was observed at a sodium chloride concentration of 0.462 M in a study examining the effect of formulation sodium chloride concentration on nasal absorption. Also, a higher concentration of sodium chloride increased bioavailability but elicited toxicity in the nasal epithelium [58].

3.4 Integration of VOC Diagnostics and Computational Modelling

To improve the management of respiratory diseases such as asthma and chronic obstructive pulmonary disease, exhaled breath VOCs from metabolic and inflammatory processes are used as non-invasive biomarkers [66]. Early detection, personalised therapy and therapeutic monitoring of respiratory tract infection with the combination of *in silico* disease modelling and drug delivery simulations [67]. The application of in silico therapeutic approaches in the management of respiratory tract infection provides computer-based platforms capable of simulating disease mechanisms, predicting biomarker behaviour and evaluating drug interactions before *in vivo* experimental testing [68]. Integrating VOC diagnostic mechanisms with a computer-based system provides a framework for modelling the associations between specific VOC signatures and airway inflammation, oxidative stress, microbial infection, or tissue degeneration. It enhances understanding of disease mechanisms and methods of disease progression while reducing the use of animals in experiments, lowering experimental costs, and shortening diagnostic and therapeutic development timelines [69]. Respiratory VOC diagnosis using *in silico* modelling improves the optimisation of targeted nasal and pulmonary drug delivery systems. Patient breath VOC biomarkers can guide computational simulations that predict how therapeutic agents should be formulated and delivered to target affected tissues [70]. In silico simulations can evaluate how absorption enhancers such as surfactants, CDs, protease inhibitors, and cationic polymers enhance mucosal permeability, drug stability, and epithelial penetration under disease-specific VOC conditions, thereby enabling the design of personalised formulations that respond to the biochemical environment associated with patients' respiratory conditions [71].

4. Future Opportunities for Improving Nasal Drug Delivery

The future of nasal drug administration remains promising because of the abundant blood vessels in the nasal mucosa, which enhance drug absorption and bypass the need for intravenous injection. This position has been reiterated by the conference on European Academic Research on buccal and nasal administration as an alternative to intravenous delivery. The nasal route of drug delivery has been the predominant route for treating systemic diseases compared with other routes because it can cross the BBB [71]. This opportunity of nasal drug delivery can also be improved through the following, in addition to what has been discussed above:

Emerging technology: There should be an application of emerging technologies that is simple and easy to use.

Formulation of inhalable drugs. Drugs in aerosol or dry powder formulations can be formulated to contain anti-inflammatory agents or bronchodilators to treat conditions aggravated by VOCs, such as asthma or allergic rhinitis, in which certain VOCs trigger airway inflammation and bronchoconstriction.

Targets on VOCs as biomarkers for disease diagnosis. Some VOCs act as biomarkers for conditions such as lung cancer, diabetes, asthma, and bacterial infections. VOC-specific drug formation can be designed to target these compounds for early detection or therapy.

Production of breath-based inhaler: Breath-based diagnostics: inhalers or nasal sprays that release drugs or diagnostic agents in response to VOC associated with certain diseases can be produced. Drug delivery based on VOC profile: medication that can be activated or delivered based on the presence of certain VOCs found in the breath or bodily fluids of individuals, such as using VOC biomarkers in lung cancer for precise treatment

Application of VOC-triggered Drug Release (Prodrugs): A prodrug is an inactive compound that becomes active only when it encounters a specific biomarker or environmental trigger. VOC-triggered prodrugs would be formulated to release their active drug components when exposed to certain VOCs.

VOC-specific nanomedicine: Nanoparticles or nano-carriers could be designed to target or respond to specific VOCs, either as therapeutic agents or diagnostic tools. These systems can improve drug delivery by enhancing solubility, targeting specific tissues, or controlling the release of active ingredients. VOC-responsive nanoparticles could be engineered to release drugs

upon detection of specific VOCs, such as in the lungs for individuals exposed to VOCs from industrial pollution. These nanoparticles can be designed to target the respiratory system, delivering drugs directly to the site of action while minimising systemic side effects.

Personalised Medicine Based on VOC Sensitivity: VOC sensitivity, particularly toxic Chemicals such as formaldehyde and toluene, can cause asthma or chemical sensitivity. Drug formulation could be personalised based on an individual's sensitivity to specific VOCs, providing more effective treatment and reducing side effects. Personalised nasal sprays or inhalers tailored to an individual's VOC triggers, helping control inflammation and symptoms during VOC exposure.

5. Conclusion

Volatile organic compound (VOC)-based signatures and *in silico* approaches offer promising strategies for early disease screening and diagnosis, as well as for improved nasal drug delivery, in the management of respiratory disorders. It provides a significant opportunity to advance precision medicine and improve nasal drug delivery strategies for the management of respiratory viral disorders. VOC signatures, such as low molecular weight, high volatility, and disease-specific metabolic origins, can provide valuable insights into pathological investigations and facilitate early identification and characterisation of respiratory diseases. The integration of advanced analytical technologies with VOC profiling will further enhance the accuracy and reliability of disease detection, paving the way for personal therapeutic interventions.

Data Availability Statement

Not applicable.

Author Contributions

JOO conceptualisation and writing of the original manuscript, review/editing. CGO writing of original manuscript, review/editing, IFC writing of original manuscript, review/editing, CO writing of original manuscript, review/editing, SIE writing of original manuscript, review/editing. SIO, writing of original manuscript, review/editing, CPA writing of original manuscript, review/editing and CO writing of original manuscript, review/editing.

Conflicts of Interest

The authors declare no conflicts of interest.

Generative AI Statement

The authors declare that no generative artificial intelligence technologies were used when preparing this manuscript.

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