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Editorial

3D Imaging and Organoid Bioprinting

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Three decades ago, Charles Hull laid the foundation for 3D bioprinting by initiating a mechanical procedure intended for the fabrication of solid scaffolds. Since then, this field has evolved significantly, transforming into a promising technique for precisely depositing biological substances, living cells, and growth factors to generate bioengineered constructs through computer-assisted deposition and assembly methods [1].

Bioinks for organoid fabrication

Bioinks are printable biomaterials for 3D printing, requiring properties such as biodegradability, bioactivity, and non-toxicity. Natural polymers like agarose, alginate, and collagen are preferred due to their similarity to the Extracellular matrix. Hybrid bioinks, such as matrigel–agarose, support intestinal stem cell growth [2]. Alginate-gelatin blends are common for micro-extrusion printing, combining thermo-sensitive properties and cross-linking. Gelatin and its derivative GelMA form thermoreversible hydrogels. Synthetic polymers like polycaprolactone and polyethyl glycolate offer strong mechanical properties, often combined with natural bioinks [3].

Enhanced bioprinting methods for organoid printing

Crucial for the fabrication of computer-aided designs and scaffolds mirroring native tissues, bioprinters use a range of techniques, including inkjet, micro extrusion, laser-assisted, and stereolithography printers. Inkjet bioprinting, utilizing piezoelectric actuators or thermal microheaters, ejects micrometric droplets of bioink, offering high resolution but limited to low viscosity bioinks due to clogging issues. Extrusion bioprinting deposits cellularised filaments with micrometric resolution, allowing for printing with a multitude of bioink materials and scalable construct sizes. Laser bioprinting, a nozzle-free approach, prints high-viscosity biomaterials and high cell densities with precision and high cell viability. At the same time, stereolithography uses layer-by-layer curing of photosensitive polymers using either ultraviolet or visible light to enable rapid and precise fabrication, enabling directed self-organization and regulated differentiation [4].

In the field of stem cell and organoid bioprinting, two prominent methods have emerged: the direct printing of undifferentiated stem cells and the immediate printing of differentiated cells. Various techniques, spanning from extrusion to laser-based methods, have been used for printing pluripotent cells. Extrusion-based printing, employing polysaccharide-based bio-inks, preserves pluripotency in human iPSCs, while laser-assisted bioprinting ensures precise tissue fabrication with enhanced cell viability [5].

Organoid vascularisation

Efforts to vascularise organoids have encountered significant challenges despite advancements in understanding vascular development. In small-scale cultures, static conditions suffice for adequate nutrient supply. However, large-scale 3D cultures require vascularisation to prevent necrosis, especially in thick tissue arrangements where dynamic nutrient flow is essential. The limitations of organoid cultures, such as ceasing proliferation and developing necrotic cores at certain sizes, emphasize the need for vascularization alongside biofabrication. Extrusion bioprinting has been widely used to print endothelial cells alongside other cells directly [6]. As an example, the fusion of hepatic progenitor cells

(HPC) and liver sinusoidal endothelial cells (LSECs) in a 1:1 proportion has resulted in the formation of hepatobiliary organoids featuring liver-specific vasculature, leading to improved survival and liver functions [7].

For the development of brain organoids, several approaches have been explored. The combination of human embryonic stem cell-derived endothelial cells, neural progenitor cells, microglia, and pericytes in artificial hydrogels has created perineural vascular plexus (PNVP) networks [8]. These networks demonstrate functional attributes, including heightened secretion of neurotrophic factors. Self-organizing human blood vessel organoids, derived from pluripotent stem cells, seamlessly integrate into mouse circulation upon transplantation. At the same time, microfluidic techniques enable the spontaneous vascularization of engineered tissues, facilitating the recreation of tissue- and organ-specific vascular architectures [9].

Applications of bioprinted organoids

The scope of organoids needs to improve regarding structural and physiological relevance, limiting their applicability in functional investigations, pathological emulation, and reparative treatment. Advancements in 3D bioprinting and vascularization tactics offer avenues to enhance their significance. By augmenting complexity and size and providing tissue-specific geometries and architectures, bioprinted organoid structures become valuable tools in developmental modeling, disease simulation, pharmaceutical testing, and tissue repair and rejuvenation. Organoids derived from human stem cells offer unique insights into early human development and disease biology. Unlike conventional 2D cultures, they faithfully recapitulate disease characteristics, making them indispensable for disease modeling and mechanistic investigations. Patient-derived organoids, particularly those derived from induced pluripotent stem cells (iPSCs), are pivotal for studying hereditary diseases and elucidating tumor progression mechanisms [10]. These organoids serve as reliable preclinical models for evaluating pharmacological interventions, potentially mitigating drug development setbacks observed in clinical trials. Organoids hold promise in regenerative therapy. Organoids with integrated vasculature, for instance, can be transplanted *in vivo*, circumventing the need for donor organs [11]. Studies have demonstrated the healing capacity of organoid transplantation in various pathological conditions, such as acute liver failure.

Conclusion and future perspectives

Automated three-dimensional bioprinting technology holds promise for scaling up organoid and tissue construct production. Challenges include enhancing bioprinting resolution, minimizing shear stress-induced cell damage, and developing advanced bioinks for intricate organoid patterning and effective vascularization. Despite these challenges, ongoing refinements in techniques and biomaterials ensure the sustained relevance of bioprinted tissue organoids. In the near future, fully fabricated organs with vascular connections will revolutionize disease modeling and drug testing, offering comprehensive insights and reducing reliance on human trials.

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Review Article

Zeolites: Microporous natural mineral carriers in controlled, targeted (nanocarriers), and gene delivery systems

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Abstract

Ongoing efforts are to attain suitable controlled and targeted drug delivery systems to release the therapeutic agent by designing novel formulations. Numerous delivery systems are being used now a days that have magnificently exhibited a positive outcome in clinical trials. Because of assorted assembly, zeolites have gained substantial attention in pharmaceuticals controlled and targeted drug delivery systems. Due to its microporous, mesoporous, or microporous structure, zeolites can be used as a carrier for the delivery of numerous therapeutic drugs at a target site in a controlled manner. Further, a judicious choice of zeolite nanocarriers will enhance the efficacy of various therapeutic drugs accompanied by reduced dosing and toxicity. Besides, zeolite nanocarriers/ nanocomposites framework carrying the drug substance directly in the living cells might be a novel approach for gene therapy. The current review represents the 21st-century development of zeolite-based controlled and targeted delivery systems to target a specific site with active substance/moiety to lower or prevent the action of diseases. Besides dealing with the controlled/targeted delivery systems, this review provides an updated view of zeolite cytotoxicity, immunostimulatory activity, updated patents, and ongoing clinical trials for using zeolites in drug delivery systems.

Keywords: Clinoptilolite; controlled/targeted delivery systems; cytotoxicity; immune stimulator/modifier; gene therapy; zeolites

Introduction

Conventional delivery systems (tablets and capsules) do not exhibit controlled release of the drug. In most instances, injection as conventional drug delivery provides a swift upsurge in the concentration of the drug, and frequent administration displays toxicity [1]. Controlled-release technology is broadly used in the pharmaceutical domain to deliver active moiety. Controlled and targeted drug release is significant for patients needing medicinal treatment day and night [2]. Numerous diverse controlled and targeted drug delivery systems have been developed, *viz*. liposomes, nanoparticles, and dendrimers, among others [3,4]. In recent decades, there has been a growing interest in using natural and synthetic materials as drug delivery systems, namely carbon materials [5] and inorganic silica [6]. Recently, zeolites have attracted research interest for controlled and targeted drug delivery systems due to their pores' steady and unvarying shape and ion exchange capabilities [7-9]. Beneficial applications of zeolite in the therapy of cancer [10], diabetes [11], HIV [12], Alzheimer's disease [13], the antioxidative effect [14] and other diseases (Figure 1) [15] were examined.

In 1756, Axel Fredrik Cronsted, a Swedish mineralogist and chemist, given the term "zeolites", revealed stilbite [17-18]. Zeolites are microporous crystalline aluminosilicates and the chemical structure of zeolites is composed of SiO4 and AlO4 tetrahedra [19,20]. Zeolites are generally natural minerals which have been broadly used in numerous scientific properes, namely purification for water and air [21], heat transformation applications [22], adsorption refrigeration [23] and as detergents [24]. Natural zeolites appear predominantly in unmetamorphosed sandy rocks, while synthetic zeolite is synthesized by chemical reactions, including breaking and making chemical bonds [25,26]. These

properties are naturally associated with their porous nature [19], high adsorption capacity [27], and their ion-exchange properties [19]. Depending on their pore diameter, the International Union of Pure and Applied Chemistry stated that the porous structures can be arranged into three categories such as microporous structures (pore diameters up to 2 nm), mesoporous materials (containing pores in the range of 2-50 nm), and macroporous solids (pores larger than 50 nm) [20,28,29]. The sizes of these pores and cages are such that drug substances can be encapsulated inside them [30]. Likewise, the drug substances are expected to diffuse out of the channels gradually, consequently controlling the drug release rate [31].

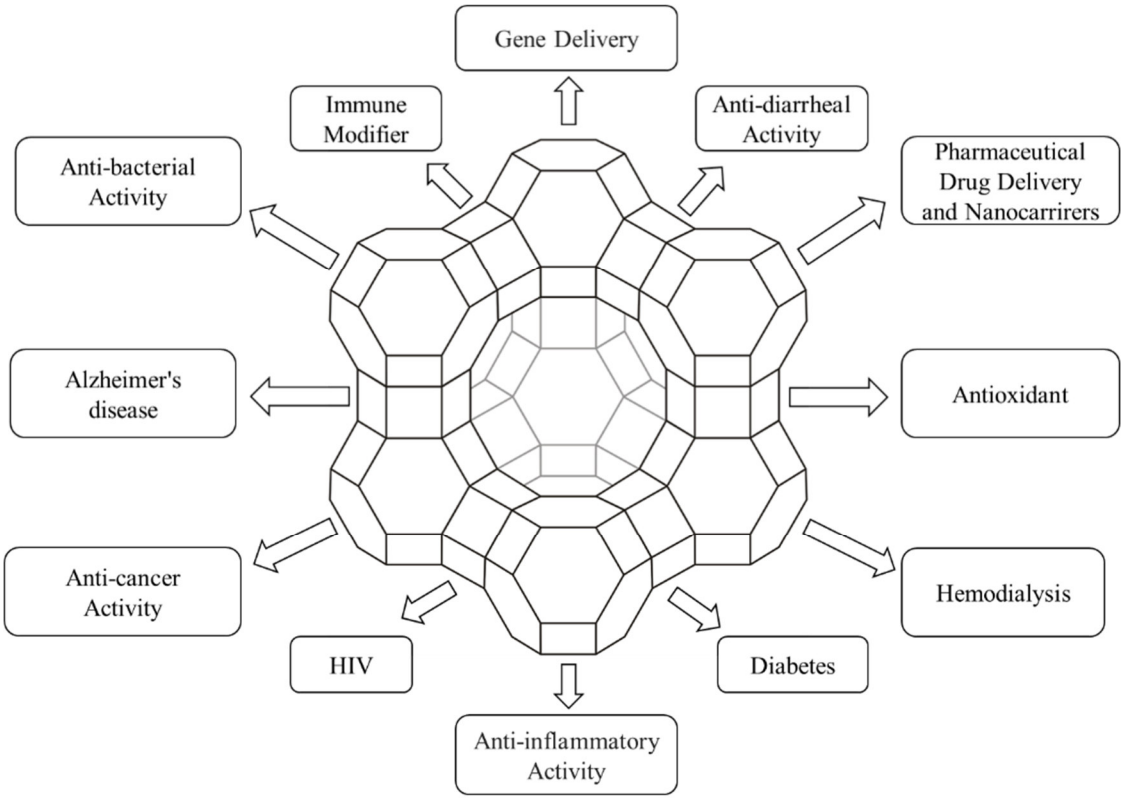

Figure 1. Example of zeolite and its therapeutic applications.
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Numerous drug delivery systems have been suggested, *viz*. nanoparticles, liposomes, microspheres, self-nano emulsifying drug delivery systems, and others [32-34]. Due to their inimitable structural attribute in addition to their biocompatibility and large surface areas, zeolites have been mostly used along with polymeric ingredients/materials in different forms, namely composites [35], blends [36], hydrogels [37] and as a carrier for drug delivery [38,39,8]. Momentous considerations in the biomedical/pharmaceutical domain have attracted the useful characteristics of zeolites with long-lasting biological potency and the capability to regulate the immune system [19]. The Clay minerals such as talc, kaolinite, and others have been used in countless drug delivery formulations as lubricants, disintegrants, diluents, binders, emulsifying, thickening, and anticaking agents [40-43]. Water absorbency variances amongst zeolites and active moieties may limit their charging capacity, even though this can be overwhelmed through surface alteration of the zeolite [44]. Therefore, the zeolite surface can be modified according to the substance/molecule required to be delivered. Numerous zeolites carriers are available according to their use and properties in biomedical as well as pharmaceuticals *viz*. zeolite L [45], mordenite [46], zeolite A [47], ZSM-5 zeolite [35], chabazite [48], zeolite Y [49], zeolite X [50], clinoptilolite [51], zeolite analcime [52] and beta zeolite [53]. Zeolites have gained prodigious attention with more than 15,744 papers published between 1926 and 2024 referenced from PubMed service (Figure 2) [54]. Besides using zeolites in drug delivery, they can be used to carry

DNA to cells because they may be internalized in the cells. Further, cytotoxicity is the biggest challenge to the use of zeolites because some forms are highly carcinogenic [18].

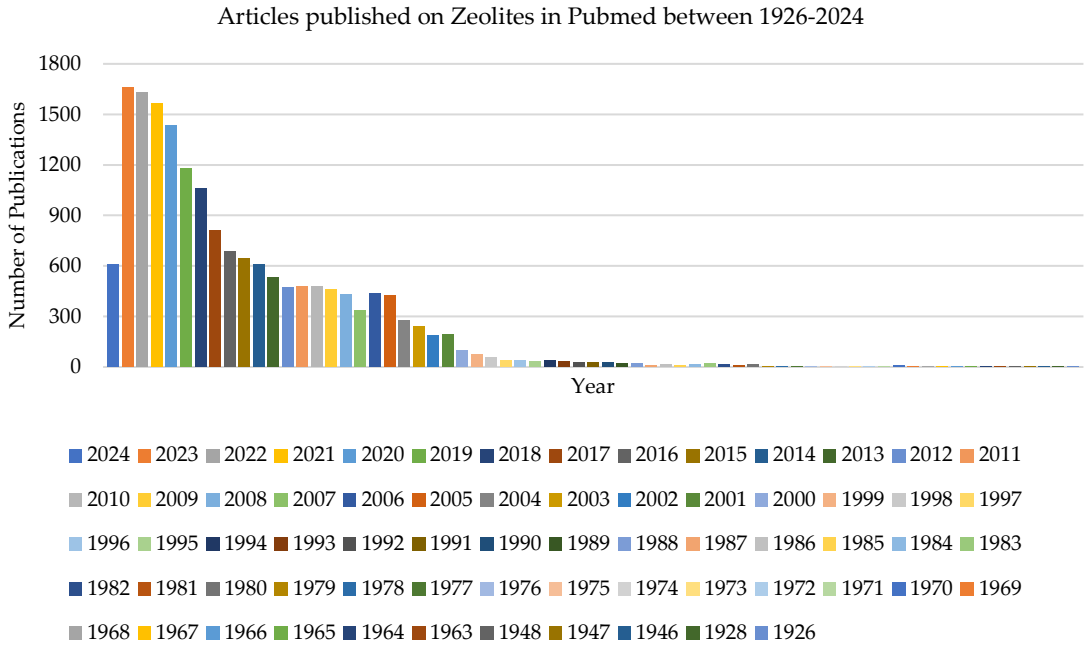

Figure 2. Graphical illustration of publications in PubMed on zeolite over the last 98 years between 1926-2024.

Scope of the review

The current review focuses on zeolite potential in controlled, targeted (nanocarriers) and gene-drug delivery systems in pharmaceuticals. Further, providing a moderately holistic view, this paper thus discusses the cytotoxicity of zeolites, the immunostimulatory/immune modifier activity of zeolites, updated patents, and ongoing clinical trials for zeolites in various diseases.

Zeolites in drug delivery

Primarily, inorganic materials exhibited not plentiful biocompatibility to be used to deliver the drug substance. Nevertheless, the outcomes of fruitful trials on nano zeolites with a more extensive surface area than micron zeolites designate their aptness for an extensive range of drug delivery applications [55,56]. Also, due to its inexpensive and high accessibility, recent research has focused on the use of zeolites for controlled and targeted drug delivery systems. Zeolites exhibit promising delivery of countless drug substances and act as detoxifying, antioxidant, and anti-inflammatory agents [57,9]. Because of conventional delivery system drawbacks and bioavailability issues related to drug substances, current research focus on the formulation and preparation of nanocarriers has expanded [58,20]. The developing arena of nano drug delivery systems encounters the demands for groundbreaking approaches in treating countless diseases. Due to their small size in nanometres and biocompatible and biodegradable, they can target specific sites in the body and reach the systemic circulation *via* Peyer's patches in the ileum region of the small intestine [59]. The essential benefits of nano drug delivery systems are enhanced delivery of insoluble drugs, targeted delivery into a specified cell or tissue and co-delivery and simultaneous delivery of more than one drug with different release rates into one tissue [60].

Controlled drug delivery systems and therapeutic potential of zeolites

After an oral administration, conventional drugs show degradation and excretion while crossing various biological barriers, leaving a minute concentration of the drugs at sites [61]. The goal of controlled and targeted drug delivery systems is to deliver the drugs to the target sites in the body at anticipated rates and time, therefore augmenting the drug absorption and bioavailability. Due to the

inimitable properties/ characteristics of the current controlled and targeted delivery systems, zeolites have offered extraordinary possibilities for controlled drug delivery systems [5]. Recently published data presents the formulation and evaluation of zeolite-controlled and targeted drug delivery systems, as shown in Table 1.

Table 1. Overview of formulation containing different zeolites in drug delivery systems.

Zeolite	Route/Study type	Drug Substance	Study purpose	Reference
CuX zeolite	Oral	Cyclophosphamide	To maintain the cyclophosphamide concentration in blood	[56]
Combination of Zeolite X and zeolite Y	Oral	Indomethacin and ibuprofen	To achieve sustained and controlled release profile and reduced adverse effects	[62]
Combination of Zeolite X and zeolite Y	In vitro	Diclofenac sodium and piroxicam	To obtain a controlled release delivery	[63]
FAU zeolite	In vitro	Doxorubicin	To achieve sustained release of doxorubicin from zeolite-magnetite nanocomposites	[64]
Zeolite Y	In vitro	Ibuprofen	To boost/controlled Ibuprofen delivery rate	[65]
Combination of zeolite X and zeolite A	In vitro	ketoprofen	To achieve modified/controlled release of ketoprofen	[39]
Beta zeolites	-------	ketoprofen, hydrochlorothiazide and atenolol	To confirm the adsorption of drugs by thermogravimetry and X-ray diffraction	[66]
Composites fabricated (Zeolite/polymer chitosan, gelatin and alginate)	In vitro	Cefalexin and Gentamaycin	To achieve prolonged release of the drugs	[67]
Zeolite HY	In vitro	aspirin	To achieve prolonged release of the drugs	[68]
Zeolite-L Nanocrystals	In vitro	Peptide Nucleic Acid	Intracellular Delivery	[45]
Zeolite-L crystals	Cell study	DNA oligonucleotides and organic molecules	Zeolite novel nanoparticles for drug delivery and gene therapy	[69]
Zeolites Y and MOR (mordonite)	Cell line study	temozolomide	To treat glioblastoma brain tumors	[46]
Zeolite/Graphene Oxide Nanocomposite	In vitro and in-vitro toxicity	Doxorubicin	To investigate the biocompatibility used as a drug carrier	[70]
Magnetite-Zeolite	In vitro	5-flourouracil	To obtain sustained release of drug	[71]
Zeolite beta	In vitro	nifedipine	Dissolution enhancement for increase in oral bioavailability	[72]
Zeolite clinoptilolite	In vitro	Diclofenac sodium	To attain oral controlled sustained release	[44]

Uglea et al. [56] synthesized and evaluated the porous physical mixture of CuX zeolite and cyclophosphamide. After oral administration of the physical mixture, the result shows that cyclophosphamide concentration in the blood was maintained. In another study, authors scrutinized the capability of zeolite Y as a slow-release agent for an anthelmintic drug in rats. The outcomes indicated that zeolite Y is an appropriate carrier to slow/control the drug release and enhance its efficacy [73,74]. Clinoptilolite was used as a carrier for pH-controlled oral delivery of aspirin and evaluated for adsorption and desorption of aspirin. The findings revealed that aspirin adsorption and desorption on clinoptilolite depend on particle size and pH [75].

In one study, Microporous zeolites of distinct framework types *viz*. BEA, ZSM, and NaX were used as relevant carriers to evaluate the dissolution pattern of a water-insoluble drug, namely

indomethacin. The possibility of the zeolitic carriers as oral delivery was assessed in Caco-2 cultures. It was observed that intracellular aggregation of the zeolite particles exhibited no cytotoxicity at lesser concentrations and postulated that microporous zeolites can be an appropriate carrier in oral drug delivery [38]. In another study, the zeolitic imidazolate framework was synthesized as the detecting platform for the detection of HIV-1 DNA. These discoveries can guide the synthesis of further metal-organic frameworks with possible applications in the early diagnosis of HIV-1 DNA [12]. Zarkovic et al. analyzed the effect of micronized zeolite clinoptilolite with doxorubicin-induced lipid peroxidation and production of 4-hydroxynonenal. Results suggested that joint therapy with doxorubicin and micronized zeolite clinoptilolite diminished the pulmonary metastasis count and augmented the anticancer effects of doxorubicin [14]. The possible use of zeolite BEA has been assessed as a sustained drug delivery carrier for salbutamol and theophylline. These results indicated that zeolites might act as a probable drug delivery system of active moiety [31]. Zeolite X and zeolite A were investigated and characterized to check their potential to encapsulate and to give controlled release of ketoprofen. The outcomes indicated that zeolitic products release modified/controlled ketoprofen in pH 5 and 6.8 [39].

Serri et al. prepared diclofenac sodium granules by wet granulation for an oral controlled delivery using a surface-modified zeolite as an excipient. Results indicated that surface modified zeolite diclofenac sodium granules gave sustained/modified drug release up to 9 h without any sign of cytotoxicity and can produce an extended anti-inflammatory effect on RAW264.7 cells. Authors concluded that surface-modified natural zeolite could be a potential carrier for sustained release granules [43]. Temozolomide encapsulated into zeolite Y (faujasite) and MOR (mordonite) by liquid-phase adsorption to treat glioblastoma brain tumors and assessed in glioblastoma cell lines. It was observed that mordonite loaded with temozolomide was able to decline 3 fold half-maximal inhibitory concentrations in vitro and in vivo [46]. Neidrauer et al. prepared topical ointment for effective delivery of nitric oxide entrapped in zinc exchanged zeolite A. Findings showed that 5 fold and 3 fold reductions in bacterial and fungal viability, respectively was observed after 8 h to nitric oxide loaded zeolite A ointment against untreated organisms [47]. Controlled oral delivery formulation of indomethacin and ibuprofen was designed using synthetic zeolite X and zeolite Y as carrier/host to evaluate the loading efficiency/capacity followed by in vitro dissolution studies. Authors concluded that loading of drugs in porous structure formulations was able to minimize their release into the stomach followed by the release of drugs in a controlled release pattern up to 3 h in intestinal fluid [62].

Soaking, filtration, and solvent evaporation methods were employed to load diclofenac sodium and piroxicam in zeolites X and Y as polymers/carriers and analyzed for *in vitro* dissolution studies. Results exhibited the controlled release of diclofenac sodium and piroxicam in intestine fluid from zeolite matrixes. It was concluded that zeolites act as a potential polymer to reduce the release of drugs in simulated gastric fluid [62]. In another study, Ibuprofen matrices were prepared using four dealuminated faujasite samples to appraise the adsorption and *in vitro* drug release. It was observed that a diffusion process rules ibuprofen adsorbed in zeolite matrices followed by the release. Results concluded that zeolites (faujasite) play a substantial role in drug delivery systems to sustain/control the drug release [65]. Pasti et al. investigated the adsorption pattern of ketoprofen, hydrochlorothiazide, and atenolol from aqueous solutions of beta zeolites with different SiO2/Al2O3 ratios. It was found that the adsorption volume of beta zeolites was muscularly reliant on both the solution pH and the alumina content of the adsorbent. Findings revealed that atenolol was immediately adsorbed on the less hydrophobic zeolite, whereas hydrophobic interactions mainly determined ketoprofen adsorption. It was clinched that adsorption can be augmented with the upsurge of hydrophobicity [66]. Aspirin was loaded into three zeolites HY carriers/polymers with silica-to-alumina ratios and analyzed. Zeolite HY samples loaded with aspirin showed declined thermogravimetric analysis with augmenting silica-to-alumina ratios. Dissolution data showed that the release rate depends on the zeolite carrier's hydrophobicity; aspirin's release rate depends on silica-to-alumina ratios [68]. The nifedipine dissolution profile was augmented in different ratios after spray drying with zeolite beta (BEA). Results showed that 100% loading efficiency was exposed, followed by a significantly improved *in vitro* dissolution rate in simulated gastric and intestinal fluids against pure drugs. It was further

confirmed and assessed by FTIR, DSC, and XRD, in which the drug displayed substantial amorphization. These findings concluded that zeolitic carriers enhance the dissolution of poorly soluble drugs and upsurge their oral bioavailability [72]. Anticancer drug α-cyano-4- hydroxycinnamic acid was loaded in zeolites (faujasite and Linde type A) to examine their aptness as carriers/hosts for drug delivery systems and investigated. Zeolites alone showed no toxicity to HCT-15 cancer cells, and α-cyano-4- hydroxycinnamic acid loaded in zeolites displayed inhibition of cell viability against the pure drug. Results indicated the possibility of the zeolite drug delivery into cancer cells, which in turn induces cell death [76,77].

Nanocarrier drug delivery systems of zeolites

Recent research shows that applications of nanocarriers containing zeolite in drug delivery systems have been investigated, as depicted in Table 1. In one study, 5-fluorouracil magnetite zeolite nanoparticles were synthesized and evaluated. Sustained release of the drug was obtained from designed nanocomposites without any burst release. Further, 5-fluorouracil magnetite zeolite nanoparticles competently prevent the proliferation of human gastric carcinoma cells *in vitro* and could be an advantageous delivery against cancer [71]. Adhikari et al. formulated zeolitic (ZIF-7 and ZIF-8) imidazole framework nanospheres loaded with doxorubicin and assessed for *in vitro* dissolution studies. Results depicted that ZIF-7 exhibited no drug release delivered up to 10 h when pH alters from physiological to acidic conditions, tuition.

In contrast, ZIF-8 effectively releases the drug in conditions, ion and conditions of the drug obtained up to 3 h. This finding shows a new approach for maximum drug loading and controlled release of the drug to give the maximum therapeutic effect of the drug substance [78]. The outcomes presented nanoparticles with high drug encapsulation efficiency, and the effect of nanoparticles was less cytotoxic against pure mitoxantrone. In addition, it was concluded that zeolite beta nanoparticles could be a fascinating carrier for drug delivery [30]. Guo et al. fabricated and examined ZSM-5 zeolite nanoparticles loaded with gentamicin using a hydrothermal method. Results indicated that ZSM-5 zeolite gentamicin nanoparticles show higher drug encapsulation efficiency followed by sustained drug release, reducing expressively bacterial adhesion and stopping biofilm development in contrast to staphylococcus epidermidis [79]. Sulfadiazine silver nanoparticles were designed using zeolite Y as a carrier and analyzed for topical antibacterial effects. Results concluded that sulfadiazine nanoparticles showed sustained/prolonged drug release using zeolite Y as a carrier and augmented its antibacterial activity [80]. Zeolite magnetite nanocomposites loaded with doxorubicin were prepared by mechanical activation using high-energy milling and evaluated for *in vitro* studies. These findings demonstrated the sustained release of doxorubicin from zeolite magnetite nanoparticles and can be adopted as a potential drug carrier/polymer for delivery systems [69].

Antibiotics-loaded zeolite and biodegradable polymer composite hollow microspheres were developed and fabricated. Results revealed that a prolonged release of the antibiotics from microspheres was observed and concluded as a suitable carrier for sustained and controlled drug delivery [64]. Khatamian et al. prepared and investigated zinc-zeolite (clinoptilolite)/graphene oxide nanocomposites loaded with doxorubicin. Results confirmed high drug loading efficiency of prepared nanocomposites, and slow/controlled release of doxorubicin was obtained [70]. 5-fluorouracil was encapsulated into zeolites (Faujasite) and evaluated for *in vitro* drug release studies. Data exhibited substantial drug loading efficiency followed by controlled drug release, observed in pH 7.4 and fitted to various kinetic models. Moreover, no toxicity was observed, and this finding provided proof of zeolite-cell internalization [77]. Salleh et al. prepared zerumbone-zeolite Y-gelatin nanocomposite by coating technique and were analyzed. Obtained data revealed that coated composite samples demonstrated sustained release of zerumbone from zeolite Y-gelatin nanocomposite up to 24 h. It was concluded that zeolite Y acted as a carrier to control the drug release [81]. Curcumin-loaded ZIF-8 liposomes were fabricated and prepared. Liposomes showed high loading efficiency and good stability, followed by the maximum release of curcumin obtained in an acidic medium against physiological pH. Cytotoxicity data revealed an improved therapeutic potential of ZIF-8 than pure curcumin, indicating an effective

drug carrier for cancer treatment [82]. Nanocomposites of metronidazole were prepared and synthesized using polyethene glycol /NaY zeolite and PEG/MCM-41 to form porous nanocrystals to release the drug and characterized for thermal analysis. Results indicated that prepared nanocomposites could control the release of metronidazole due to hydrogen bonding interactions between the drug and the hydroxyl group on the composite framework [2].

Potential of Zeolites in gene delivery systems

Recently, gene delivery has been shown to offer various advantages and has provided treatment options for diseases that are beyond the reach of traditional approaches. Gene therapy is the transfer/delivery of genetic material to a patient to target tissues or cells to treat a disease [83]. Gene therapy is specifically designed to alter the expression of a gene or to modify the biological properties of cells for therapeutic use. Gene therapy includes the use of nucleic acids (DNA or RNA) for the therapy, heal, or preclusion of human disorders [84]. Recent reports suggested the use of zeolites as a carrier in gene therapy. For example, Bertucci et al. used zeolite-L nanocarriers to deliver peptide nucleic acids and organic molecules into living cells. Particles of zeolite-L were changed by covalently attaching the peptide nucleic acids onto the surface, whereas the channels were loaded with fluorescent molecules. A significant augment of the cellular uptake of peptide nucleic acids /zeolite L hybrid material was observed when entire coated with a thin layer of poly-L-lysine. This finding evidently presented the use of peptide nucleic acids loaded zeolite nanocarriers to target in the living cells might be a novel approach for gene therapy [45]. Zeolite-L crystals were formulated as a versatile nanocarrier to deliver simultaneously DNA oligonucleotides and organic molecules into living cells. Multifunctional zeolite L was formulated by loaded the pore system with a model drug (DAPI), whereas DNA was adsorbed electrostatically on their surface. Results suggested that the release system of DNA and DAPI based on zeolite-L crystals has verified the potential to target in the living cells [69]. In another study, Pearce et al. reported that the efficacy of cell transfection can also be augmented by zeolites. Zeolite silicalite nanoparticles improved polyethylene imine-plasmid DNA induced transfection of HEK-293 cells [85]. Zeolite nanocarriers loading drug molecules, DANN, and other bioactive substances are generally internalized into cells by endocytosis. Thus, zeolite nanocarriers can be used as a novel approach to examine the endocytosing mechanisms and pathways in cells [86].

Brief description of zeolite cytoxicity

Regardless of all the positive effects of zeolites discussed above, some are highly cytotoxic and carcinogenic. The most significant is erionite, a naturally occurring fibrous mineral associated with augmented risks of lung cancer and mesothelioma [87-89]. The cytotoxicity of erionite was examined in *vitro*, where the human monocyte U937 cell line was used to substantiate the toxicity of erionite and offretite asbestiform zeolite fibres. It was observed that erionite fibres were quickly internalized in the membrane and found in the cytosol and the nucleus. Within one day, first erionite fibres rich in sodium and potassium, and then calcium-rich erionite fibres, induced cell necrosis.

Further fibrous zeolites, *like* offretite and skolecite, are also cytotoxic [90]. Another zeolite, zeolite A, also bothered the animals' mineral metabolism and tissue mineral composition. Aluminium retention and augmented calcium concentrations in the liver and muscle were detected in all tissues. In addition, there improved phosphorus concentrations in the aorta but diminished concentrations in plasma; there were amplified magnesium concentrations in the aorta, heart, kidney, liver, and pancreas but reduced concentrations in plasma; and there were lessened iron concentrations in the kidney and liver [91].

Clinical trial of zeolites for medical use

Clinical trials are significant for determining novel disease therapies, including innovative ways to perceive, analyse, and decrease the disease's risk of emerging. Clinical trials can determine how drug substances can show their potential effects on humans, which cannot be learned in the laboratory or animals. Clinical trials are being conducted using zeolites for medical use, as described in Table 2.

Table 2. Clinical trials with zeolites in the site of www.clinicaltrials.gov.

ClinicalTrials.gov identifier	Study phase	Posted year	Disease	Objective of the study	Study status	Reference
NCT04370535	NA	May 1, 2020	Crohn Disease	To evaluate the safety and efficacy of PMA-zeolite in Crohn´s disease patients	Recruiting	[92]
NCT01831492	NA	April 15, 2013	Acidosis Oxidative Stress Inflammation	To investigates the effects of dietary zeolite + dolomite	Completed	[93]
NCT00623675	Phase 4	February 26, 2008	Healthy	To find if urine heavy metal levels are changed in persons who use Mineralox Basic C™ (Mineralox). Mineralox is a zeolite (clinoptilolite) in combination with Vitamin C.	Suspended	[94]
NCT03817645	NA	January 25, 2019	Irritable Bowel Syndrome Microbial Colonization Intestinal Disease	To investigates of possible effects of zeolites on specific indications in human medicine, e.g. irritable bowel syndrome.	Recruiting	[95]
NCT03901989	NA	April 3, 2019	Osteoporosis	To investigates the effect of zeolite on bone mineral metabolism	Completed	[96]

Patents status of zeolites in drug delivery systems

Plentiful patents have been granted for using zeolites in pharmaceutical drug delivery systems, as portrayed in Table 3.

Table 3. A list of patents on the use of zeolite in pharmaceutical drug delivery systems.

Patent number	Purpose	Reference
US7691400B2	Medical device coated with zeolite drug reservoirs for controlled delivery of the therapeutic material	[97]
US9402862B2	Zeolites for delivery of nitric oxide	[98]
US6048830A	Zeolite used as release barrier in delivery system	[99]
US20180169143A1	Zeolite molecular sieves for the removal of toxins	[100]
US9580328B2	Mesoporous framework-modified zeolites	[101]
US8790697B2	Controlled release delivery for bio-active agents	[102]
EP1755569A1	To evaluate the i*n vitro* antimicrobial activity of acrylic resins containing silver and zinc zeolite	[103]
US8273371B2	Crystalline mesoporous oxide-based materials useful for the fixation and controlled release of drugs	[104]
US20040208902A1	Controlled release nano diffusion delivery systems for cosmetic and pharmaceutical	[105]
AU2002351366B2	Encapsulated antimicrobial zeolites for controlled release	[106]
US20060127430A1	Controlled release of cosmetic and pharmaceutical agents via osmotic nano-diffusion from zeolite cage complexes	[107]
US20050058672A1	Controlled release delivery of skin protectant using zeolites	[108]
EP2023971B1	Medical device having coating with zeolite drug reservoirs	[109]
US8524624B2	Method of preparing mesostructured zeolites and degradation catalysts for polymers.	[110]
WO2006122998A1	Method for the controlled release of pharmaceuticals	[111]
US6964781B2	Improved sustained release drug delivery device comprising a drug core, a unitary cup, and a prefabricated permeable plug (zeolite)	[112]
US8440210B2	Zeolites as adsorbent for stabilized pharmaceutical product	[113]
EP0297538B1	Antibiotic zeolite-containing film	[114]
EP1451170A1	Zeolites used as matrices in pharmaceutical	[115]
WO2015100508A1	Subdermal device for the storage and continuous release of an anti-carcinogenic compound for dogs, contained in nano- and micro-particles of natural zeolites	[116]
CA2542968C	Medical use of zeolites for treatment and prevention in humans or animals of deleterious concentrations of ammonia, mercaptans, heavy metals and other toxins by oral administration.	[117]

CA2622022A1	Pharmaceutical composition including clinoptilolite.	[118]
US20050031708A1	Zeolite useful for treating multiple conditions such as, diarrhoea, heartburn, gastrointestinal disease, toxic poisoning, influenza, and the common cold.	[119]
US20090226492A1	Use of an activated zeolite as a pharmaceutical agent for reducing toxic substance	[120]

Zeolites as immune stimulator/modifier

Some published reports suggested that zeolites and other dietary supplements act as immune modifiers [121]. Due to several health benefits, including detoxification, clinoptilolite *in vivo* has augmented vastly [9]. Also, the role of clinoptilolite on the antioxidant mechanisms in the body was perceived in different pathologies and disease models [122]. It was reported that, with prolonged use of the dietary supplement with clinoptilolite, a decrease in the pervasiveness of E. coli carrying some antimicrobial resistance and virulence genes was observed [123]. Ivkovic and colleagues have investigated the effect of supplements with tribomechanically activated zeolite clinoptilolite on the cellular immune system for immunodeficiency disorder. Results showed that expressively enhanced CD4+, CD19+, and HLA-DR+ lymphocyte counts and a significantly decreased CD56+ cell amount, whereas lycopenomin augmented CD3+ cell amount and lessened CD56+ lymphocyte amount [121]. In another study, animals were treated with a chabazitic zeolite supplement: It was observed that chabazitic zeolite supplement may be beneficial to keep a stable intestinal microbial system and to stop stress-related gastrointestinal tract disorders, with a reduction of gut pathogens and an extraordinary upsurge of bifidobacterial [124]. Lastly, other immunomodulatory benefits of zeolites cannot be omitted, but this subject requires further study.

Conclusion and future perspectives

Controlled drug delivery has been widely used to deliver drugs at a controlled rate. Various polymers/carriers have been studied in controlled release systems, such as natural and synthetic. Polymers are the most frequently used ingredients for controlled release. The drug is usually loaded in the polymeric matrix, and release relies on the polymer concentration and active moiety. Zeolites are natural/ synthetic materials well known for a decade and have been demonstrated a controlled release application in pharmaceutical drug delivery systems, mainly regarding the safety and non-toxicity of a few natural zeolites, which were found safe and effective after oral administration. Regardless of the abundant benefits of zeolites as controlled drug delivery systems, the use of zeolites has been linked with challenges. The foremost challenge is controlling the drug release *via* a diffusion mechanism.

Further, the pore size of zeolites is typically bigger than the drug substance, and the drug is released quickly. To overcome this, surface-modified zeolites are used to control the drug release. Moreover, some zeolites are cytotoxic and carcinogenic. Recent literature data presented that clinoptilolite-based materials may be safe for *in vivo* consumption. Because of zeolite surface characteristics and porous structure, research is now focused on nanoparticulate drug delivery systems of zeolites, which can potentially augment drug encapsulating efficiency and control the drug release rate over time. Some studies on zeolite nanocarriers show low toxicity of these frameworks.

Additionally, zeolite gene therapy is beneficial for delivering genetic material to patients to target tissues or cells to treat a disease. It is anticipated that research on the surface alteration of zeolites will be widened to treat various diseases and bring a new opportunity for pharmaceutical drug delivery systems to boost drug efficiency. Besides, altering the size of the zeolites permits them to enter living cells. Moreover, clinoptilolite exhibits immunomodulating properties that human medicine may take advantage of human medicine. Nevertheless, the number of clinical trials on clinoptilolite's immunomodulatory/antioxidant effects is still low, which should be investigated in more detail. As presented, I aimed to deliver an overview of the extensive pharmaceutical applications of zeolites as controlled and targeted (nanocarriers) drug delivery systems in various therapeutic uses followed by gene therapy. When properly designed, this approach makes it conceivable to distinguish the foremost treatment for a specific disease/illness to a patient and in connection with a specific outcome. This review will be helpful for investigators and researchers working on developing zeolite drug delivery systems in pharmaceuticals, and this approach requires further investigation into zeolite use *in vivo*.

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Declaration of interest

The authors declare no conflict of interest.

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Research Article

Assessment of ex-vivo intestinal permeability and lymphatic uptake of curcumin and piperine-loaded nanostructured lipid carriers

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Abstract

Curcumin (CUR) is a naturally occurring compound in food known for its potential pharmacological activity but faces challenges due to its high metabolism. Piperine (PIP) is an effective inhibitor of metabolizing enzymes, enhancing the bioavailability of CUR. This work evaluated the lymphatic absorption and *ex-vivo* intestinal permeability of nanostructured lipid carriers (NLCs) containing PIP and CUR (CP NLCs). The optimized lipid formulation underwent *in-vitro*drug release, *ex-vivo* permeation, and lymphatic uptake studies utilizing chicken intestinal (jejunum) segments. Studies were conducted under different conditions, specifically in the presence and absence of the lymphatic uptake blocker Pluronic-F68 (PF68). PF68 is a non-ionic surfactant commonly used in pharmaceutical research, and it's known for its ability to inhibit lymphatic uptake. *In-vitro* drug release profiles indicated the controlled release of CUR and PIP from NLCs over 24 h. The *ex-vivo* permeability study demonstrated that CP NLCs exhibited higher permeation compared to CUR and PIP Suspension (CP Suspension). Studies on the lymphatic uptake of CP NLCs, conducted with and without the presence of the lymphatic uptake blocker PF68, demonstrated a decrease in drug permeation. However, in the absence of the lymphatic blocker, drug transport via the lymphatic path increased significantly by 4.07-fold for CUR and 6.56-fold for PIP. This means that the NLCs significantly enhanced the lymphatic transport of both CUR and PIP. The results imply that the lipid-based NLC system shows potential as a drug delivery method, improving solubility, and aiding in the lymphatic transport of both CUR and PIP.

Keywords: Curcumin; piperine; nanostructured lipid carriers; lymphatic uptake; payer's patches

Introduction

CUR, a hydrophobic polyphenol with significant therapeutic potential, faces practical challenges that limit its application. These challenges include poor aqueous solubility, swift systemic clearance, metabolism (both intestinal and hepatic), absence of cell targeting, and multidrug resistance despite its proven effectiveness and safety against various cancers [1]. However, when administered together, PIP and CUR co-administration has been reported to increase CUR bioavailability by 200% in human volunteers dramatically. This substantial enhancement is attributed to PIP's role in inhibiting the glucuronidation of CUR, thereby preventing its rapid metabolism and clearance.

Additionally, a similar dosage administered to healthy human volunteers resulted in a remarkable 2000% increase in CUR bioavailability [2,3]. The research started with formulating a combination of CUR and PIP to improve the oral bioavailability of CUR. Various formulation strategies were found during the literature survey, including sodium alginate nanoparticles [4], cubosomes [5], liquid self-nanoemulsifying drug delivery systems [6-8], solid zein-chitosan nanoparticles filled in capsules [9], liposomes [10], immediate-release tablets [11], solid dispersion amorphous powder [12], Solid self-nano emulsifying drug delivery system powder filled in capsules [13], internalizing argineglycine-aspartic

acid liposomes [14], unformulated actives [15], and ethosomes [16]. There was no published literature on CUR and PIP lipid-based matrix systems. Hence, a lipid-based approach was selected for development. Lipid formulations, which include a combination of excipients such as pure triglyceride oils, mixed glycerides, lipophilic surfactants, hydrophilic surfactants, and water-soluble co-solvents, present a promising solution. These formulations aim to improve bioavailability through lymphatic uptake, thereby reducing the need for large doses to achieve suitable serum concentrations. Various mechanisms for NLC disposition inside the body have been proposed via their selective uptake through lacteals or payer's patches. The NLC systems increase absorption from the gastrointestinal tract by accelerating the dissolution process, facilitating the formation of solubilized phases by reduction of particle size to the molecular level, yielding a solid-state solution within the carrier, changing drug uptake, efflux and disposition by altering enterocyte-based transport, and enhancing drug transport to the systemic circulation via the intestinal lymphatic system. During gastrointestinal tract passage, NLCs circumventing the digestion process can be either conveyed to the portal blood via paracellular route bypassing metabolism due to enterocyte enzymes or can be captured by M cells of payer's patches delivering NLCs to the lymphatic system [17-19]. Further, no study has been reported to understand the intestinal permeability and lymphatic uptake of CUR and PIP NLCs. Hence, the main objective of this study was to examine the intestinal permeability and lymphatic uptake of lipid-based nanoparticles to enhance the oral bioavailability of both CUR and PIP. The importance of these objectives cannot be overstated, as they could lead to significant advancements in drug delivery.

Materials and methods

Materials

The CUR gift sample was sourced from VAV Life Sciences, Mumbai, while PIP was procured from Sigma-Aldrich, India. Gift samples of Precirol ATO 5 (PRE), Labrafac Lipophile WL 1349 (LAF), and Gelucire 50/13 (G50/13) were obtained from Gattefosse, India. Additionally, gift samples of Tween 80 (T80), Polaxomer 188 (P188), also known as Pluronic F68 (PF68), and Polaxomer 407 (P407) were acquired from BASF, India. All chemicals used in this study, except for the specified gift samples, were of analytical reagent grade, ensuring their high quality and reliability. They were employed without additional purification, maintaining their integrity. The preparation of all solutions was conducted using Millipore (ultrapure) water, further enhancing the quality of our research.

Nanostructured lipid carriers (NLCs) formulation

The CP NLCs were formulated with the following components: CUR (0.08% w/v), PIP (0.04% w/v), PRE (3% w/v), LAF (2% w/v), T80 (0.125%), G50/13 (0.125%), and purified water. The preparation method utilized a modified hot melt emulsification process. The resulting formulation exhibited a particle size of 248.5 ± 12.8 nm, with a Polydispersity Index of 0.216 ± 0.021. The drug content was 99.70 ± 0.21 % and 100.36 ± 0.12% for CUR and PIP, respectively. The entrapment efficiency of CP NLCs was 99.80 ± 0.21% and 100.05 ± 0.07% for CUR and PIP respectively [20].

In-vitro drug release study

In-vitro drug release studies of CP NLCs were performed employing a systematic dialysis bag method. The dialysis membrane was activated through a 24 h soaking in purified water (hydration). Following this, the CP NLCs and CP Suspensions formulations were loaded into cellulose membrane dialysis bags with a molecular cut-off of 12-14 kDa (Sigma-Aldrich Co., India). Subsequently, the loaded bags were submerged in 100 mL of pH 4.5 acetate buffer containing a 2% sodium lauryl sulfate solution and subjected to magnetic stirring at 100 rpm at 37 °C. Samples were extracted from the vessel at specified time intervals, and equivalent volumes of fresh solvent were introduced. The concentrations of CUR and PIP were assessed spectrometrically using a UV-visible spectrophotometer (Shimadzu 1800, Shimadzu Japan) at wavelengths of 429 nm (for CUR) and 345 nm (for PIP) employing the simultaneous equation method [21].

Ex-vivo intestinal permeation study

An *ex-vivo*intestinal permeability study involving chicken intestinal (jejunum) segments was conducted to assess CP Suspension and CP NLC [22,23]. The small intestine of the chicken was obtained from a slaughterhouse and thoroughly washed with distilled water to eliminate mucous and luminal content. The complete small intestinal segment, spanning from the upper duodenum to the lower ileum, was precisely located and excised. Manual stripping was used to separate the mesentery, and the intestine was thoroughly washed with normal saline (0.9% w/v Sodium chloride). Various segments of the small intestine were delineated, and the chicken intestine was preserved in Tyrode solution. Approximately 10 cm long segments of the jejunum (non-everted tissue) were prepared and filled with a single-unit dose of CP Suspension and CP NLC. The tissue was suspended in 100 ml of phosphate buffer with a pH of 7.4, representing the blood's pH and simulating the lymphatic fluid's pH. The system was agitated at 100 rpm and maintained at 37 °C, with proper aeration provided by O2/ CO2 (95%/ 5%). The study extended over 3 h. At predefined intervals, aliquots were withdrawn, and the pH 7.4 buffer was replenished. The collected aliquots were filtered through a Whatman filter, subsequently diluted with methanol, and analyzed utilizing a UV-visible spectrophotometer (Shimadzu 1800, Shimadzu Japan) at wavelengths of 429 nm (for CUR) and 345 nm (for PIP) employing the simultaneous equation method [21].

Lymphatic uptake study

The lymphatic uptake of NLCs was assessed in a systematic manner. The cleaned tissue segment was first exposed to a 20 µg/mL solution of the lymphatic uptake blocker PF68, ensuring proper aeration. After a systematic incubation period of 1 h, the NLC formulation was introduced to the tissue using the same systematic procedure as previously described. The permeability of the formulations was then compared between untreated tissue and PF68-treated tissue, providing a clear evaluation of the lymphatic uptake of CP NLCs [20,23].

Results and Discussion

In-vitro drug release study

The saturation solubility and solution stability studies were performed as a part of the pre-formulation study. The study revealed that CUR has maximum solubility in phosphate buffer pH 6.8, whereas PIP has maximum solubility in pH 4.5 acetate buffer. CUR and PIP have negligible solubility in alkaline pH buffers (pH 7.4 and 7.5). The pH-dependent stability studies revealed that CUR is unstable at alkaline pH, whereas PIP is stable at all pH levels. Hence, pH 4.5 acetate buffer was evaluated for *in-vitro* drug release studies. The release profiles of CUR from the Suspension and CP NLCs were 57.91 ± 1.24% and 91.55 ± 0.85%, respectively.

Similarly, the release of PIP from the Suspension and CP NLCs was 100.21 ± 0.19% and 98.11 ± 0.78% after 24 h (Figure 1). Due to the poor solubility of CUR, complete release was not achieved, whereas complete release of PIP was observed before 16 h owing to its high solubility. The initial rapid release of both CUR and PIP can be attributed to the presence of actives in liquid lipids, followed by a slower release attributed to the presence of a solid lipid core. The release profile of CP NLCs exhibited a controlled release pattern compared to CP suspension.

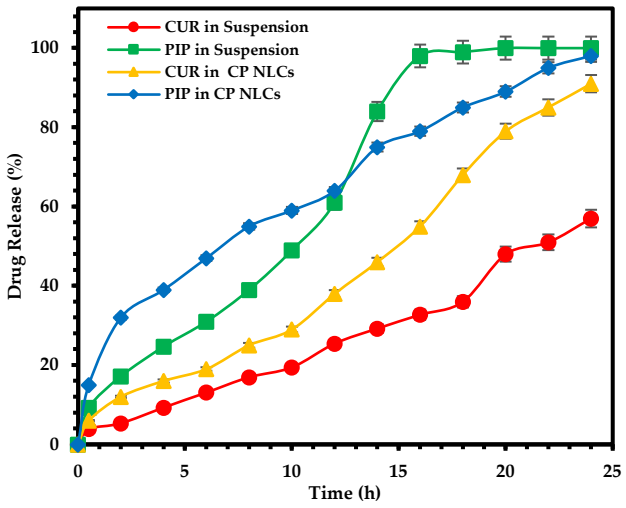

Figure 1. The in-vitro drug release profile of CUR and PIP from both the Suspension and NLC system [Mean ± SD (n=3)].

Ex-vivo intestinal permeation study

The findings of the *ex-vivo* intestinal permeability study, as depicted in Figure 2, are of significant importance. After 3 h of the permeation study, it was observed that the diffusion of CUR and PIP from NLCs was 37.88 ± 2.38% and 37.34 ± 1.54%, respectively. In the case of Suspension, both CUR and PIP diffusion were found to be 26.48 ± 1.97% and 27.08 ± 1.12%, respectively, which is comparatively lower than NLCs. These findings indicate a higher permeation/diffusion occurred with NLCs than with simple Suspension, a crucial discovery in our understanding of drug delivery systems. The enhanced permeation of CUR and PIP from NLCs can be attributed to the presence of nano-sized particles (248.5 ± 12.8 nm) in the formulation and improved permeation facilitated by the surfactant, which reduces the interfacial tension of the formulation [24-26]. The improved permeability may stem from the dissolved state of drugs in lipids and the presence of nano-sized lipid carriers, which increases the surface area. This combination results in a higher drug dissolution and diffusion rate, consequently enhancing permeability [27].

Moreover, it's important to note that NLCs play a crucial role in drug delivery. They can traverse the mucous layer and release the drug directly on the surface of the cell membrane [28]. Existing literature proposes several mechanisms for the enhanced permeability of lipid-based systems following oral administration. These mechanisms include increased membrane fluidity, the opening of tight cellular junctions, inhibition of P-glycoprotein and CYP450 by surfactants, and the stimulation of lipoprotein/chylomicron production by lipids. The latter is particularly significant as lipoproteins/chylomicrons are primarily absorbed by M cells in Peyer's patch [29-31]. The enhanced permeability of CP NLCs may be attributed to the inhibitory action of G50/13 on the P-glycoprotein efflux pump [32,33], resulting in increased absorption after oral administration.

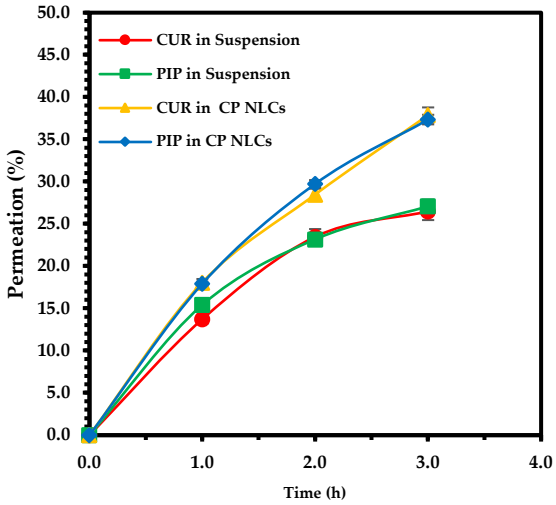

Figure 2. The ex-vivo intestinal permeability of CUR and PIP from both the Suspension and NLC system [Mean ± SD (n=3)].

Lymphatic uptake study

Based on the results of the ex-vivo intestinal permeability study, it was found that the presence of the lymphatic uptake blocker PF68 led to 9.29 ± 1.78% and 5.69 ± 0.59% drug permeation for CUR and PIP, respectively. However, in the absence of PF68, these values increased to 37.88 ± 2.38% and 37.34 ± 1.54% (Figure 3). This indicates a 4.07-fold and 6.56-fold increase in drug permeation for CUR and PIP, respectively, suggesting significant lymphatic transport. The presence of lipids in the NLC formulation primarily contributed to this enhanced lymphatic transport [32,33]. A similar study has addressed the lymphatic uptake of solid lipid nanoparticles in both the presence and absence of P188 [34].

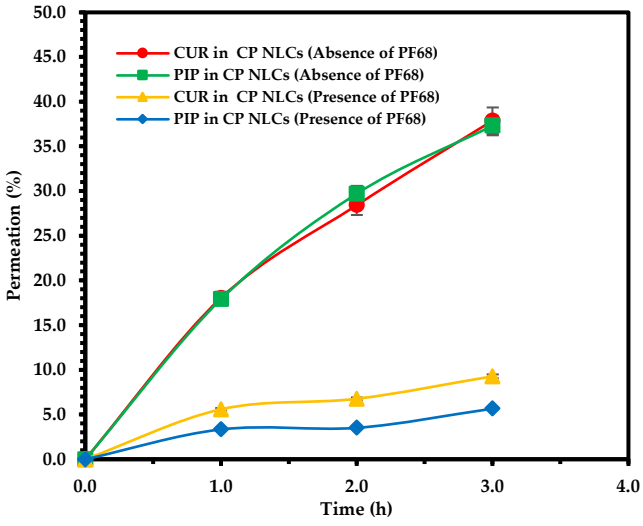

Figure 3. Ex-vivo lymphatic uptake of CUR and PIP from NLC system in presence and absence of lymphatic uptake blocker PF68 [Mean ± SD (n=3)].

Conclusion

The ex-vivo study unequivocally established the critical role of the lymphatic route in significantly enhancing the oral bioavailability of CUR and PIP in the presence and absence of a lymphatic uptake inhibitor. Consequently, CP NLCs unquestionably stand out as a promising drug delivery vehicle for augmenting the oral bioavailability of CUR and PIP. This undoubtedly holds the potential to enhance patient compliance, decrease the required dosage, and ultimately lower the overall cost of therapy.

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Authors contribution

All the authors have contributed equally.

Declaration of interest

The authors declare no conflict of interest.

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