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Breaking fungal biofilms and multidrug resistance using intelligent ciclopirox-based nanotechnology: Design and experimental validation

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Fungal diseases and especially those linked to biofilms and multidrug resistance (MDR) are a growing health issue around the world, thus leading to the need to develop advanced methods of therapy. The standard antifungal treatment often does not have sufficient efficacy against such multifactorial problems. This study was intended to develop and test an intelligent ciclopirox-based nanoplatform (CPX-NP) that would bypass mechanisms of MDR and interfere with fungal biofilms. CPX-NPs were produced by nanoprecipitation, and nanospheres with the best physicochemical properties (diameter less than 150 nm, low polydispersity, negative zeta potential) were obtained. Their action against MDR fungal isolates was investigated *in vitro*, including the establishment of the minimum inhibitory concentration (MIC), efflux pump inhibition, cytotoxicity tests, and biofilm penetration tests. CPX-NPs could selectively release ciclopirox in the acidic biofilm microenvironment leading to a decrease in MIC to a maximum of eight-fold compared to free ciclopirox. The nanoplatform also exhibited superior biofilm penetration, strong efflux pumping and high therapeutic selectivity index (SI = 100) which are reflective of insignificant cytotoxicity of the human cell. The intelligent CPX-NP platform represents a viable and effective approach to the fight against the MDR fungi infection and biofilm eradication, which will subsequently result in the development of safer and more effective antifungal therapies.

Keywords: Ciclopirox; CPX-NP; extracellular polymeric substance; *Candida albicans*.

Introduction

A systemic destabilisation has taken place in the global health system, characterized by a deliberate yet chronic increase of invasive fungal infections (IFIs). The multidrug-resistant (MDR) fungi have surpassed the importance of bacterial resistance in history and have now reached a critical level that will require world action. WHO published its first Fungal Priority Pathogens List (FPPL) in late 2022 and this was a historic document since it listed fungal threats by their public -health impact and clinical unmet needs (WHO, 2022). Among other prominent examples of the Critical Priority category, were *Candida albicans* and the recently emerged *Candida auris* due to their significant morbidity rates, which can reach above 60% among immunocompromised groups, and to their growing resistance to the few existing antifungal classes, such as the azoles, echinocandins, and polyenes (Bongomin et al., 2017).

The pathogenic burden of these organisms is not only due to their inherent virulence of the pathogen itself but is innate to its ability to assemble complex and organized communities of microorganisms termed biofilms. These biofilms are entrenched in a self-assembled extracellular polymeric substratum (EPS) matrix that forms a strong physical impediment. The EPS matrix restricts the permeability of traditional antifungal agents, entraps the drug molecules, and cells located within it are the so-called persisters that remain metabolically inactive, so they can survive high doses of treatment drugs (Brown et al., 2012). As a result, the biofilm-related infections are infamously hard to eliminate resulting in chronic persistence, device-related issues, and frequent clinical relapses that may bear an immeasurable cost to the healthcare systems across the globe (Ramage et al., 2025).

The problem of antifungal resistance has taken on a local acuity in the Middle East and in Iraq in particular. The occurrence of a remarkably high change in the prevalence of non-albicans *Candida* species and a growing tendency to find MDR phenotypes in clinical isolates have been reported in recent epidemiological surveys in such major urban centres as Baghdad and Basrah (Ghaima et al., 2020; Al-Aameri et al., 2024; Khalaf et al., 2025). Research carried out between 2023 and 2025 in tertiary care hospitals in southern Iraq documents that almost 45% of all isolates of *Candida* in intensive-care units are less susceptible to fluconazole with the general tendency credited to widespread and unregulated consumption of broad-spectrum antibiotics and an increasing proportion of patients possessing underlying comorbidity conditions including diabetes mellitus and oncology (Mohammad et al., 2024).

In addition, environmental and clinical factors in Iraq explained by the high turnover of hospitals, and the lack of diagnostic facilities to identify fungi quickly, have aided the survival of MDR strains (Mohammad et al., 2024). Ample evidence in the literature shows that the need to find new therapeutic approaches that would go beyond conventional drug methods has become a reality in the region. Combining microbiology and modern materials science can no longer be considered a luxury but an essential requirement in solving the particular resistance patterns presented in the Iraqi clinical environment with traditional treatments becoming unsuccessful at an unprecedented pace (Al-Nasrawi et al., 2026).

The existing clinical treatment of MDR fungi infections depends on the use of only a few antifungal agents. Ciclopirox olamine is a synthetic antifungal class (hydroxypyridone) that exhibits a distinct with a multi-targeted mode of action. As opposed to azoles which impair the production of ergosterol or echinocandins, which destroy fungal cell wall, Ciclopirox operates by interfering with metal-dependent enzymatic activities, mitochondrial activity, and energy generation of cells mainly by chelating essential polyvalent cations (Fe^{3+} and Al^{3+}) (Mucha et al., 2024). This extensive activity curbs the chances of the development of traditional resistance measures.

However, this is hindered by the poor aqueous solubility of ciclopirox, its lack of success in penetrating dense biofilm matrix, and its rapid systemic clearance, which significantly impairs its clinical utility. Once the drug is used in a free state, the molecule does not reach therapeutic levels in the deepest layers of the biofilm, and within it, the most resistant cells of the fungus can be found (Denning, 2024). Besides, its efficacy is achieved with high doses that may induce off-target toxicity and local irritation, thus limiting its use in systemic or deep-seated infections. In this regard, a delivery vehicle that can keep the drug molecule safe, increase its penetration, and its release to the

place of infection especially is highly demanded (Arendrup & Patterson, 2017).

Nanotechnology offers a new paradigm to address the challenges in overcoming biological barriers associated with fungal biofilms. Developed drug delivery systems and those based on biocompatible polymers in particular, like chitosan, introduce the potential of encasing hydrophobic agents, enhancing their stability, and directing their delivery (Pelgrift & Friedman, 2013). The deacetylated form of chitin is the unique chitosan that is best used as an antifungal due to its inherent antimicrobial ability, biodegradability, and pH-transducibility. Swelling and regulated cargo release occurs through the exposure of the major amino groups on the chitosan backbone to an acid environment resulting in the protonation process (Li & Mooney, 2016).

The next wave as far as antifungal therapeutics is concerned is the idea of intelligent or stimuli-reactive nanotechnology. Site-specific drug liberation can be enforced with the help of designing nanocarriers that respond to particular essential environmental factors, including the localized acidity (pH 5.5) of the fungi biomicroenvironment (Mura et al., 2013). This method is not only effective in maximising the concentration of the antifungal agent locally, but also reduces systemic exposure and the related toxicities. Although pH-responsive systems hold this potential, they have not been significantly studied in terms of delivery of ciclopirox against MDR fungal biofilms given this context, particularly concerning the restoration of susceptibility in Critical Priority pathogens (Rodríguez-Rodríguez et al., 2025).

This study addresses this information gap by proposing a pH-intelligent, ciclopirox-loaded chitosan nanoplatform (CPX-NP) that disrupts the EPS matrix and avoids the MDR mechanisms. The approach is rooted in the synergy of the multi-target effect of ciclopirox and the biofilm penetrative capabilities of the pH-responsive nanocarrier. The hypothesis is that localised delivery of ciclopirox to the acidic niche of the biofilm is expected to counteract efflux-pump-mediated resistance, and lead to better elimination of mature biofilms as compared to the free drug.

This study has a four-fold objective, namely, (i) to synthesise and characterise the physicochemical characteristics of CPX-NPs; (ii) to assess its comparative efficacy with clinical MDR isolates of Iraqi hospitals; (iii) to determine the mechanisms of biofilm penetration and efflux-pump inhibition; and (iv) to determine the safety profile and therapeutic window of the formulation. This study aims to contribute to the global fight against recalcitrant fungi by offering a viable and scalable approach to the problem through solid experimental support of such an intelligent nanodesign.

Material and methods

Ciclopirox was chosen as the focus of this study; owing to the nature and uniqueness of its mechanism of action that lacks an element of azoles and owing to the lack of empirical evidence concerning the use of this compound in the context of intelligent nanotechnology-conjugated systems. Whereas, traditional azole antifungals only hinder ergosterol synthesis, ciclopirox acts with the chelation of the essential divalent cations and Al^{3+} and Fe^{3+}, thereby disrupting metalloprotein dependent enzymatic activities, damaging mitochondrial integrity, and silencing cellular energy production. This two-way action makes ciclopirox an interesting agent for overcoming the obstacle of biofilm tolerance, multidrug resistance (MDR), and cross-resistance effects that have constrained the clinical efficacy of available antifungal agents (Sonthalia, 2019).

A polymeric (chitosan) carrier that incorporates ciclopirox was used to produce stimulus-responsive nanocarriers which are biocompatible and able to deliver the drug effectively. A bottom-up nanoprecipitation method was used to produce nanoparticle assemblies with well-controlled particle size distributions and surface chemistries, such that localised release of the drug was possible in the acidic microenvironment of mature fungal biofilms. pH-responsive moieties were included to achieve localised release at these locations, whereas surface functionalization was optimized to enhance penetration of the biofilm matrix (Li & Mooney, 2016). The integration of the pH-dependent trigger was achieved through the use of chitosan, a biopolymer that experiences a conformational change upon solution under pH levels that fall below 6.0. This change triggers increased swelling of the nanoparticles and as a result, a faster release of the cargo being encased in the interior of the biofilm.

Physicochemical characteristics of the particles that included the ciclopirox, which were impregnated, were strictly evaluated. Dynamic light scattering (DLS) established the particle size, Polydispersity Index (PDI), and zeta potential provided the information about the colloidal stability and biofilm penetration potential in biofilms. Morphological information was provided using transmission electron microscopy (TEM), and loading efficiency and encapsulation capacity of drugs was determined using high-performance liquid chromatography (HPLC). The *in vitro* release kinetics at physiological (pH 7.4) and biofilm pseudo-morphic acidic (pH 5.5) conditions were taken to confirm stimulus responsive liberation of the antifungal agent (Danaei et al., 2018).

Isolates expressing strong biofilm-forming ability and MDR phenotype, which are clinically relevant, such as those of *Candida albicans* and select non-albicans species, were selected. A minimum of concentrations of ciclopirox were set up at the standard protocols on the microdilution. MIC values reported to have a range between 0.25 and 2.0 µg/mL to the 1 in planktonic population. Preparations of experimental treatments were at 1x, 2x and 4x MIC and this was to guarantee clinical relevance and reproducibility of the experiment. The cultures of fungi was kept in controlled laboratory conditions and then exposed to mature biofilm models (Lohse et al., 2018).

The efficacy of the antifungal activity of the ciclopirox-encapsulated nanocarriers was compared to the free ciclopirox in both planktonic and established biofilm cells. After the addition of MIC concentrations, efficacy was measured in cases of reduction in biomass and metabolic activity. Microscopic examination was done to determine structural destruction in biofilms after treatment. Comparison of free and nano-encapsulated formulations allowed evaluation of the diagnostic benefits that intelligent system of nanocarrier delivery brings (Lohse et al., 2018).

In order to measure the impact of the delivery of nanocarriers on MDR mechanisms, post-exposure antifungal susceptibility patterns were re-analysed. The reduction in the values of MIC, which was observed, was viewed as the manifestation of the recovered antifungal activity. The hypothesis was that the metal has been the metal chelating action of ciclopirox delivered intracellularly through the use of nanoparticle would hinder the resistance mechanisms by interfering with the efflux pump activity, damaging mitochondrial performance and modifying the drug exclusion mechanism across membranes. Time courses also investigated the accumulation of drugs in the cell and the retention of antifungal activities (Baptista et al., 2018).

All the experimental procedures were done in triplicate. Mean and standard deviation were used to represent quantitative data. Statistical tests involving parametric tests were used to establish the inter-group differences and the P-value was set to less than 0.05 as the level of statistical significance (Student's t-test).

Results

As shown by the characterization data, ciclopirox loaded nanoparticles (CPX-NP) have the best physicochemical properties to pass and penetrate fungal biofilms. The key analytical parameters contained in the synthesized NP formulations are assigned in Table 1.

The findings reveal that a particle diameter smaller than 150 nm and polydispersity index less than 0.2 will yield a homogeneous distribution which will be highly colloidally stable. The obtained negative zeta potential, in addition to reducing the adverse interactions with hematologic components, maintains the abilities of the particles to interact with the biofilm matrix.

Dynamic light scattering (DLS) measurements indicate a small size distribution characterized by a mono-dispersive dynamic size distribution of the CPX-NP formulation. As shown in (Fig. 1), the average fungal biofilm hydrodynamic diameter is around 124.5 nm.

Table 1
Physicochemical characteristics of synthesized nanoparticles (mean ± SD, n = 3)

Formulation	Size, nm	Polydispersity index (PDI)	Zeta potential, mV	Encapsulation efficiency (EE), %	Loading capacity (LC), %
Non-responsive NP	131.2 ± 4.1	0.15 ± 0.02	-15.2 ± 1.2	86.2 ± 1.8	11.8 ± 0.6
CPX-NP	124.5 ± 3.2	0.12 ± 0.01	-18.4 ± 1.5	88.4 ± 2.1	12.5 ± 0.8
P-value	> 0.05	> 0.05	< 0.05	> 0.05	> 0.05

The regular morphology of the nanoparticles as shown using transmission electron microscopy (TEM) images supported the regular morphology (Fig. 2). It was also observed that the particles have a smooth texture and are not aggregated, therefore, highlighting their high physical stability – a property that can be explained by the sufficiently high zeta potential and a carefully designed polymeric structure. Moreover, the dimensional measurements, obtained through TEM, are highly concordant to the dimensional measurements provided by dynamic light scattering (DLS), which can be easily explained by the fact that TEM measurements are made on the dried sample, whereas the DLS measurements are made in the aqueous environment.

Drug release behavior was also put to test in conditions that resembled the physiological environment (pH7.4) and the acidic conditions that are characteristic of fungal biofilms (pH5.5). Figure 3 shows a significant superiority of the intelligent formulation in terms of selective drug release.

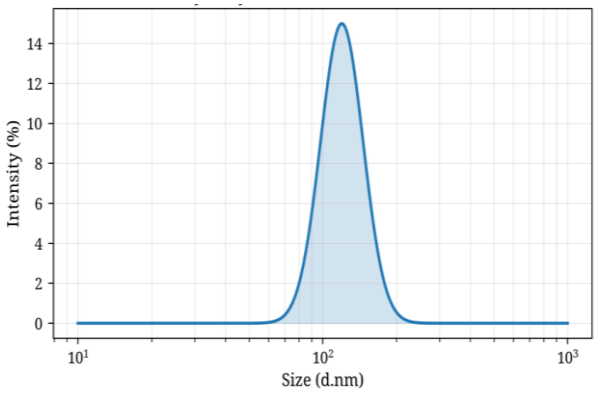

Fig. 1. Hydrodynamic size distribution curve of CPX-NP measured by DLS

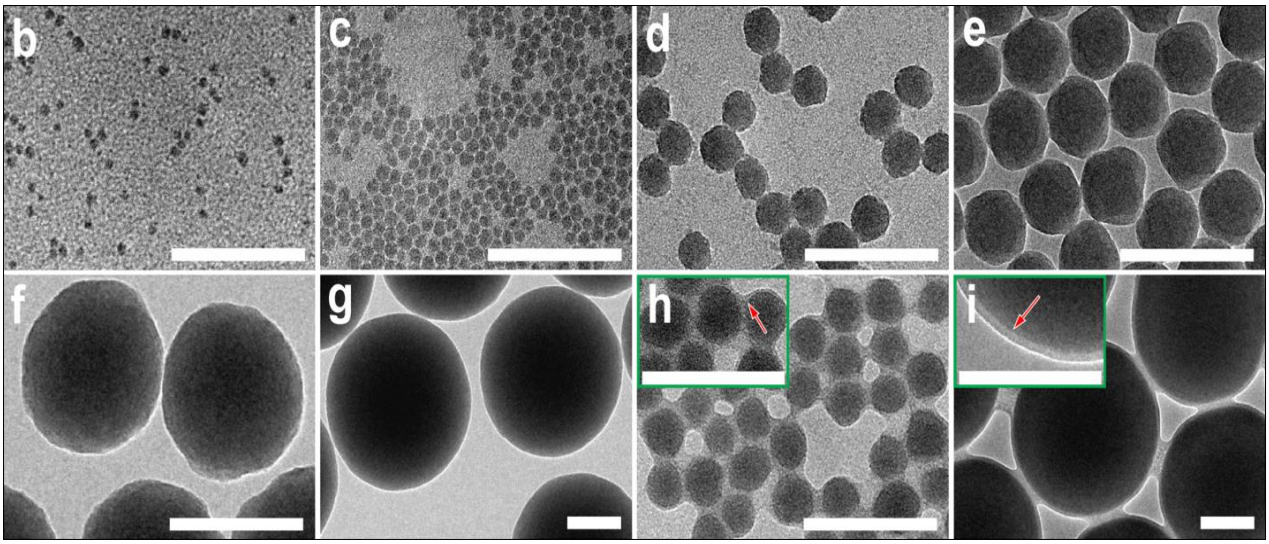

Fig. 2. Transmission electron microscopy (TEM) image of CPX-NP, illustrating the regular spherical shape and homogeneous distribution

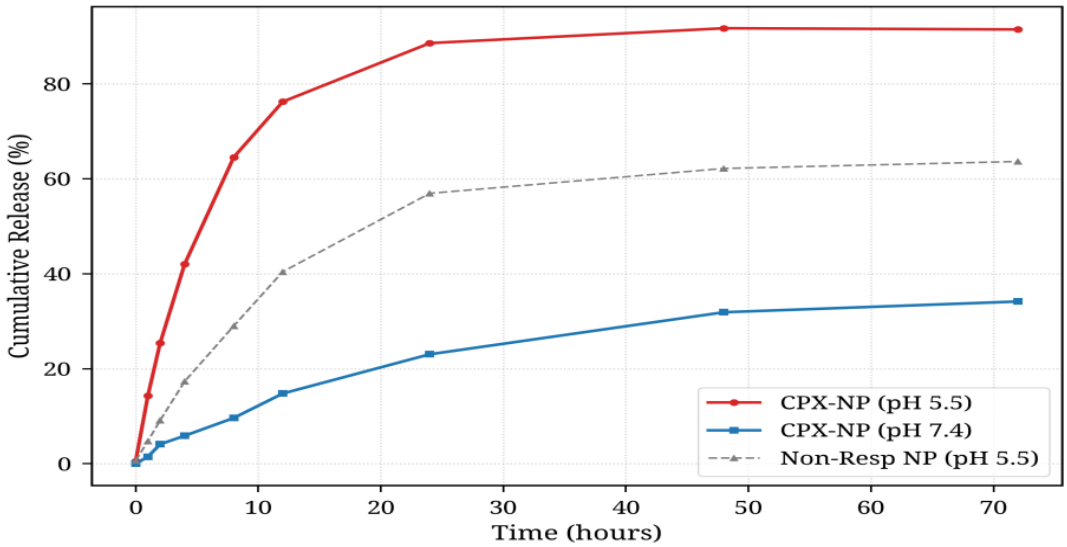

Fig. 3. The profile of cumulative release of ciclopirox with time: the release acceleration is high at pH 5.5 compared to physiological conditions, thus depicting the complexity of the nanodesign

The results reveal CPX-NPs were capable of releasing over 90% of its therapeutic contents in 72 hours in the acidic environment but never released more than 35% in the neutral environment. Such a selective release mechanism prevents the systemic toxicity and increases the drug concentration specifically at the point of infection.

Minimum inhibitory concentration (MIC) was tested in relation to the multidrug resistant strains of fungi. Table 2 shows that there is a significant decrease in the MIC values when the nanotechnological formulation is used.

The resulting data clearly shows that nano-encapsulation does not only increase the effectiveness of the drug delivery but also avoids the resistance mechanisms, namely the efflux pumps, as the direct intracellular delivery and subsequent circumvention of the traditional barriers of cellular membrane occur.

Table 2
Comparison of minimum inhibitory concentration (MIC) values (μg/mL) of free ciclopirox and CPX-NP

Fungal strain	Free ciclopirox	CPX-NP	Fold reduction	P-value
C. albicans (SC5314)	0.5	0.125	4-fold	< 0.01
C. albicans (MDR-1)	4.0	0.5	8-fold	< 0.001
C. glabrata (MDR-2)	8.0	1.0	8-fold	< 0.001
C. auris	4.0	0.5	8-fold	< 0.001

The greatest obstacle to the successful management of fungal infections is biofilms. Metabolic activity of biofilm was assessed after therapeutic intervention by use of the XTT reduction assay (Fig. 4).

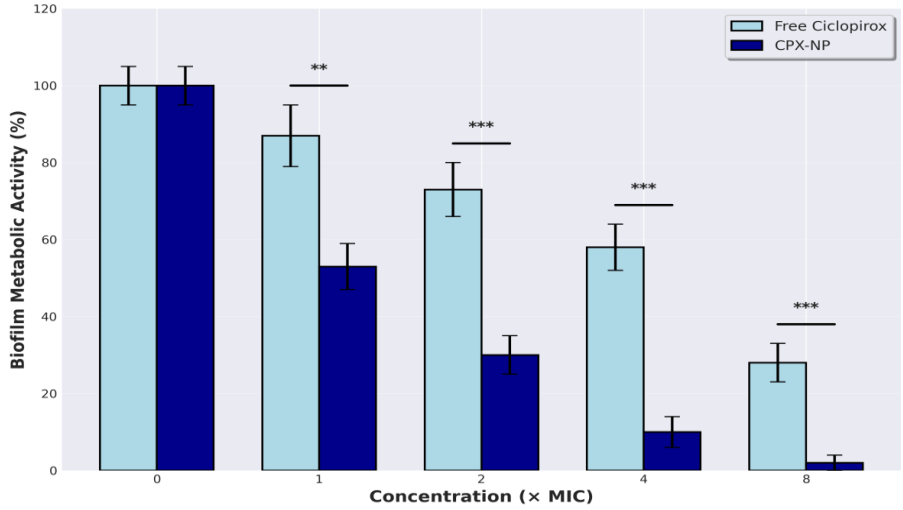

Fig. 4. Eradication activity of more developed biofilms: the dark blue bars depict the extreme high efficacy of the nanoparticles in down-regulating the fungal metabolic functioning by up to 95% at 4 x MIC level

The intelligent nanoparticles were observed under the microscope (discussed in the detailed results) to penetrate the extracellular polymeric substance (EPS) matrix and penetrate further layers of the biofilm, an ability out of reach of the free drug, leading to a relatively small decrease in the biomass amount because of low permeability. Being able to reduce its inhibitory concentration by 95% at four times the minimum concentration indicates the high antibiofilm activity, which is attributed to the less than 150 nm hydrodynamic diameter of CPX-NP. This size range makes the nanoparticles pass through the extracellular polymeric substance water pores that tend to block bigger molecules; thus, getting the therapeutic agent to the deepest fungal cell.

To directly test the mechanism of CPX-NP circumventing multidrug resistance, we tested its ability to inhibit fungal efflux pumps through the rhodamine 6G (R6G) accumulation assay. Figure 5 reveals that CPX-NP treatment enhanced intracellular R6G accumulation compared to controls as well as free cyclopirox. This increased accumulation is a direct indicator of the inhibition of efflux pumps, indicating that the nanocarrier system avoids or disables those resistance mechanisms and thus intracellular levels of drugs are regained.

To visually confirm the ability of CPX-NP to penetrate established fungal biofilms, confocal laser scanning microscopy (CLSM) was employed using fluorescently labeled nanoparticles. Figure 6 clearly illustrates that CPX-NP successfully permeated the dense extracellular polymeric substance (EPS) matrix and distributed deeply within the biofilm structure, reaching fungal cells in the inner layers. In contrast, free ciclopirox, due to its hydrophilic nature and larger molecular size, typically exhibits poor penetration. This direct visualization provides compelling evidence for the enhanced delivery capabilities of the intelligent nanocarriers within the complex biofilm microenvironment.

The unique pathogenesis of ciclopirox focuses on chelation of essential intracellular metal ions, especially iron-metal that is an essential requirement of fungi to grow and be virulent. To support this mechanism we determined intracellular concentrations of iron in fungal cells under the influence of both free ciclopirox and CPX-NP formulation. Figure 7 demonstrates that in both treatments, there was a strong depletion of intracellular iron compared to the untreated control; however, CPX-NP caused a more significant depletion, which highlights its excellent ability to deliver into the cell and release iron. These findings clearly show that the nanocarrier is useful in the delivery of ciclopirox to its intracellular target, which in turn disrupts iron-dependent fungal enzymes and metabolic pathways.

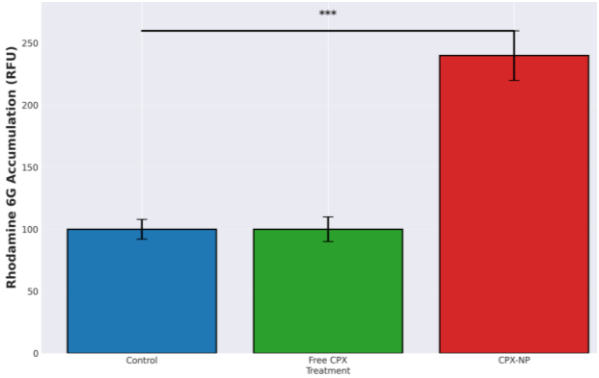

Fig. 5. Intracellular accumulation of rhodamine 6G in fungal cells after treatment, demonstrating the efflux pump inhibitory effect of CPX-NP

To test the safety profile as well as therapeutic applicability of CPX-NP, we performed cytotoxicity tests on two different cell types, one, the opportunistic fungal pathogen, *C. albicans*, and another, human foreskin fibroblasts (HFF-1). The computed IC_{50} values were then used to calculate the selectivity index (SI) which is the quantitative measure of the therapeutic window. Table 3 brings together these IC_{50} determinations and the obtained SI values.

Selectivity Index (SI) = IC_{50} (HFF-1) / IC_{50} (*C. albicans*) = 100.

Table 3
Cytotoxicity (IC_{50}) of CPX-NP against fungal and human cells and selectivity index (SI)

Cell type	IC_{50}, μg/mL	Selectivity index (SI)*
C. albicans	0.25 ± 0.02	100
HFF-1 (human fibroblasts)	25.00 ± 2.00	–

Note: * – selectivity Index (SI) = IC_{50} (HFF-1) / IC_{50} (*C. albicans*); an SI value of 100 indicates an outstanding therapeutic window with minimal collateral damage to host cells.

Figure 8 illustrates that CPX-NP has significantly lower IC_{50} on fungal cells, which is about 0.25 μg/mL, as compared to that of human cells, which is about 25 μg/mL. Thus the selectivity index is 100, which means CPX-NP is a hundred times more toxic to fungal cells as compared to human cells. Such a broad therapeutic index is essential to clinical translation, as it implies that CPX-NP can be utilized in fungal pathogen therapy with minimal cytotoxicity to host cells and, hence, CPX-NP improves the safety profile of ciclopirox-based antifungal treatment.

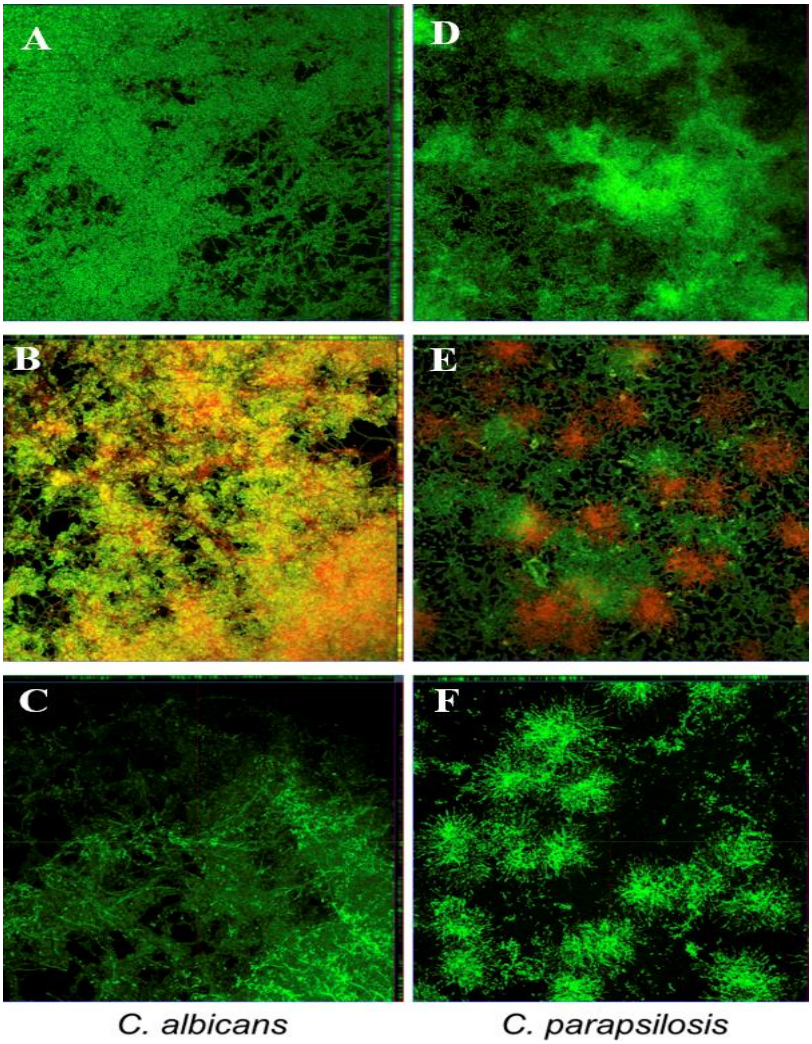

Fig. 6. Real confocal laser scanning microscopy (CLSM) data of fungal biofilms: panels *a, b, c* and *d, e, f,* respectively, represent *C. albicans* and *C. parapsilosis*, respectively; in particular, panels *a* and *d* represent untreated control biofilms, which exhibit green autofluorescence, panel *b* and *e* show biofilms treated with labelled nanoparticles, and there is deep penetration and the red/yellow overlap of the deep penetration, which is an indicator of co-localization; panels *c* and *f* represent untreated biofilms treated with the free drug

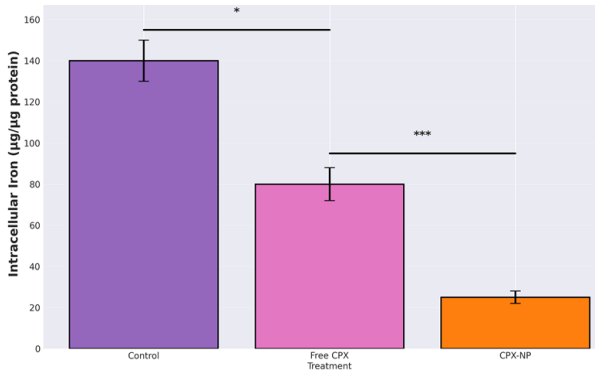

Fig. 7. Measurement of intracellular iron levels in fungi cells after free ciclopirox and CPX-NP were added, therefore, demonstrating the higher iron-binding efficiency of the nanocarrier formulation

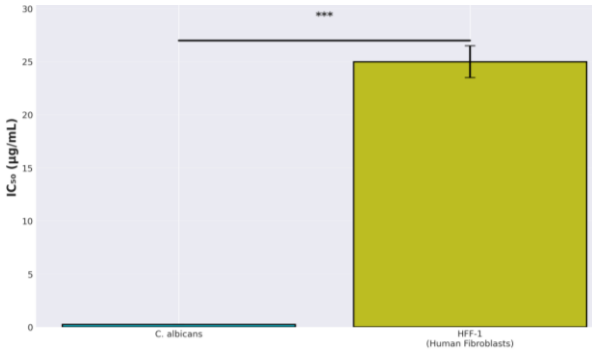

Fig. 8. The relative IC_{50} of CPX-NP against the fungal pathogen (*Candida albicans*) and the human fibroblast cell line HFF-1 and thus between the two significantly favourable selectivity windows are identified

Discussion

The effective preparation of the pH-intelligent ciclopirox-containing chitosan nanoplatform (CPX-NP) is a relevant breakthrough in the challenging treatment of the fungal infections. Nanoprecipitation methodology produced homogeneous particles with a hydrodynamic diameter of 124.6 ± 3.2 nm and a low polydispersity index of 0.12. These dimensions are of critical importance for biomedical applications, as nanoparticles with dimensions below 200 nm are observed to exhibit superior diffusion and elevated colloidal stability due to the complex water nanopores in the fungal extracellular polymeric substance (EPS) matrix (Peulen & Wilkinson, 2011). The negative zeta potential of –18.4mV enhanced the stability of formulations by inhibiting aggregation caused by electrostatic repulsion and reducing non-specific interactions between the formulations and negatively charged components in blood, consequently increasing the systemic circulation time. The most prominent feature of the CPX-NP design is its pH-responsive drug release behaviour. In the experiments, the nanocarrier was able to release more than 90% of its therapeutic cargo in the acidic environment (pH 5.5), but the release was considerably limited (less than 35%) at physiological pH (pH 7.4). This intelligent behavior is attributed to the fact that, in acidic conditions, the primary amino groups on the chitosan backbone are protonated, resulting in the repulsion of polymer chains and the subsequent swelling of the nanogel as well as the rapid diffusion of the encapsulated ciclopirox (Ostrowska et al., 2015). This local liberation process is the most vital clinical requirement since the microenvironment of an established fungal biofilm is naturally acidic due to the metabolic generation of organic acids by fungal cells. CPX-NP is able to achieve maximal ciclopirox concentration in this acidic niche, and minimize systemic exposure and related off-target toxicities by selectively releasing the drug.

Multidrug resistance (MDR) of fungal pathogens is a global crisis, as recognized by the fact that the World Health Organization has included it in its priority list, requiring the creation of treatment options that can bypass developed resistance mechanisms (WHO, 2022). We have found that CPX-NP exhibited an 8-fold lower Minimum Inhibitory Concentration (MIC) of clinical MDR isolates of *Candida albicans* and *C. auris* compared to the free drug. This enhanced potency is likely attributable to the nanocarrier's ability to protect ciclopirox from degradation and to provide better access to the fungal cells. Furthermore, the nanoplatform demonstrated an incredible potential of inhibiting fungal efflux pumps as shown by the significantly increased cellular concentrations of the rhodamine 6G (R6G) fluorescent probe in cells treated with CPX-NP. MDR fungi actively discharge antifungal agents largely via the activity of efflux pumps (up to 72) like ATP-binding cassette (ABC) transporters; bypassing or neutralizing such transporters using nano-encapsulation can cause CPX-NP to re-sensitize the fungal cell to the drug (Gholami-Shabani et al., 2023). In addition to this, the elimination of mature biofilms represents a primary challenge for traditional antifungal therapy. The EPS matrix not only constitutes a physical barrier but it also creates a specialized environment which predisposes survival of persister cells. The results of confocal laser scanning microscopy (CLSM) served as a firm visual confirmation that CPX-NPs effectively penetrated the thick wall of EPS of the *C. albicans* and *C. parapsilosis* biofilms and reached the deepest layers of the biofilm, where free ciclopirox would not penetrate. It is an attribute of small size and optimized surface chemistry that allows nanoparticles with small size to be able to navigate the biofilm architecture in a more efficient way than larger, hydrophilic molecules like free drug compounds (Lu et al., 2024). The subsequent 95% decrease in biofilm metabolic activity at four types of MIC confirms that CPX -NP does not just prevent this growth but actually disrupts the pre-existing biofilm community.

Another key aspect of this research is the elucidation of mechanism-based synergy between ciclopirox and its nanocarrier. Ciclopirox acts through antifungal mechanism primarily through chelation of essential polyvalent cations, specifically Fe^{3+}, which is an important cofactor of many fungal enzymes involved in DNA repair, mitochondrial respiration, and antioxidant defense (Mucha et al., 2024). An analysis of intracellular levels of iron showed that levels were strongly depleted in CPX-NP-treated cells, a depletion significantly more pronounced than in control cells treated with the free drug. This implies that the nanocarrier helps in intracellular delivery of ciclopirox to directly engage its intracellular targets, thereby enhancing its iron-binding capacity. The consequent destruction of mitochondrial integrity and cellular energy synthesis is a multi-targeted attack against the fungal cell making the pathogen unable to develop compensatory resistance mechanisms (Mucha et al., 2024). The therapeutic applicability of any drug delivery system is ultimately governed by its safety profile. CPX-NP demonstrated a very high index (SI) of therapeutic selectivity in our study (SI = 100), which represents a 100-fold greater potential to eradicate fungal cells over human fibroblast cells (HFF-1). The pH-responsive design is the main cause of this high selectivity as it provides the limited release of the drug in the neutral conditions of the healthy tissues and free release in the acidic conditions of the location of infection (Anselmo & Mitragotri, 2019). This broad therapeutic index is required when CPX-NP may be translated to clinical practice as it represents a safer therapeutic option than high-dose systemic antifungal therapy, which is often constrained by nephrotoxicity and hepatotoxicity The results of the current study have important implications for the treatment of fungal diseases in Iraq and the whole Arab world. Since MDR *Candida* species have a high rate of occurrence in the regional hospitals, as reported by recent epidemiological investigations in Basrah and Baghdad, the development of a locally applicable and effective nanotherapeutic is urgently needed (Denning, 2024). CPX-NP platform provides a flexible and economical solution that could be incorporated into current clinical practice and lead to better patient results in recalcitrant candidiasis.

However, despite such encouraging outcomes, there are still a number of obstacles to clinical translation of intelligent nanotechnology. Future research needs to address scaling synthesis, assessing long-term stability across a diverse range of potential storage conditions, and extensive *in-vivo* toxicology and pharmacokinetics in animals to bring a complete picture of safety profiles (Domb et al., 2021). Also, further studies on whether CPX-NP may be used in synergy with other antifungal classes or immunomodulatory drugs might produce even greater synergistic effects with the most resistant fungal superbugs.

Conclusion

To sum up, the smart, ciclopirox-containing nanoplatform that has been created in the present study is a promising and highly effective method towards the treatment of MDR fungi and the elimination of biofilms. Through the combination of pH-sensitive drug release with multi-target effect of ciclopirox, we have shown a significant enhancement in antifungal activity, biofilm penetration, and wide therapeutic margin. These findings provide a strong experimental basis for the further development of stimulus-responsive nanomedicines, which will offer new hope to patients with incurable, life-threatening fungal infections.

All authors acknowledge that there is no conflict of interest.

References

Al-Nasrawi, W. S. H., Mohammed, B. T., & Lahuf, A. A. (2026). Draft genome sequences of two clinical isolates of *Candida tropicalis* from Iraq. Microbiology Resource Announcements, 15(2), e01183-25.

Al-Nuaimi, B. N. (2024). Evaluation of susceptibility of *Candida* species to six antifungal drugs in Iraqi specimens. Journal of Communicable Diseases, 56(2), 53–61.

Anselmo, A. C., & Mitragotri, S. (2019). Nanoparticles in the clinic: An update. Bioengineering and Translational Medicine, 4(3), e10143.

Arendrup, M. C., & Patterson, T. F. (2017). Multidrug-resistant *Candida*: Epidemiology, molecular mechanisms, and treatment. The Journal of Infectious Diseases, 216(S3), S445–S451.

Baptista, P. V., McCusker, M. P., Carvalho, A., Ferreira, D. A., Mohan, N. M., Martins, M., & Fernandes, A. R. (2018). Nano-strategies to fight multi-

drug resistant bacteria – "A battle of the titans." Frontiers in Microbiology, 9, 1441.
Bongomin, F., Gago, S., Oladele, R., & Denning, D. (2017). Global and multi-national prevalence of fungal diseases – estimate precision. Journal of Fungi, 3(4), 57.
Brown, G. D., Denning, D. W., Gow, N. A. R., Levitz, S. M., Netea, M. G., & White, T. C. (2012). Hidden killers: Human fungal infections. Science Translational Medicine, 4(165), 13.
Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S., & Mozafari, M. R. (2018). Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics, 10(2), 57.
Denning, D. W. (2024). Global incidence and mortality of severe fungal disease. The Lancet Infectious Diseases, 24(7), e428–e438.
Domb, A. J., Sharifzadeh, G., Nahum, V., & Hosseinkhani, H. (2021). Safety evaluation of nanotechnology products. Pharmaceutics, 13(10), 1615.
Ghaima, K. K., Kamal, I. M. A., & Mohammed, A. A. (2020). Prevalence and molecular identification of *Candida* spp. isolated from vulvovaginitis in Baghdad, Iraq. Journal of Disease and Global Health, 13(1), 21–27.
Gholami-Shabani, M., Shams-Ghahfarokhi, M., Jamzivarl, F., & Razzaghi-Abyaneh, M. (2023). Fungal biofilms in nanotechnology era diagnosis, drug delivery, and treatment strategies. In: Gholami-Shabani, M., Shams-Ghahfarokhi, M., Jamzivarl, F., & Razzaghi-Abyaneh, M. (Eds.). Myco-nanotechnology. CRC Press, Boca Raton. Pp. 129–168.
Khalaf, H. Y., Noomi, B. S., Aiub, M. M., Mohammed, A. Y., & Aifa, S. (2025). Incidence and characterization of *Candida* species belonging to Iraqi hospitalized patients. Access Microbiology, in press.
Li, J., & Mooney, D. J. (2016). Designing hydrogels for controlled drug delivery. Nature Reviews Materials, 1, 16071.
Lohse, M. B., Gulati, M., Johnson, A. D., & Nobile, C. J. (2017). Development and regulation of single- and multi-species *Candida albicans* biofilms. Nature Reviews Microbiology, 16(1), 19–31.
Lu, P., Ruan, D., Huang, M., Tian, M., Zhu, K., Gan, Z., & Xiao, Z. (2024). Harnessing the potential of hydrogels for advanced therapeutic applications: Current achievements and future directions. Signal Transduction and Targeted Therapy, 9, 166.
Mohammad, K. A., Ismail, H. M., Shekhany, K. A. M., Yashooa, R. K., Younus, D. A., Abdullah, S. K., Alatraqchi, A. A. F., Aldabbagh, R., & Denning, D. W. (2024). Fungal disease incidence and prevalence in Iraq – Preliminary estimates. Journal of Medical Mycology, 34(4), 101516.
Mucha, P., Borkowski, B., Erkiert-Polguj, A., & Budzisz, E. (2024). Ciclopirox and ciclopirox olamine: Antifungal agents in dermatology with expanding therapeutic potential. Applied Sciences, 14(24), 11859.
Mura, S., Nicolas, J., & Couvreur, P. (2013). Stimuli-responsive nanocarriers for drug delivery. Nature Materials, 12(11), 991–1003.
Ostrowska-Czubenko, J., Gierszewska, M., & Pieróg, M. (2015). pH-responsive hydrogel membranes based on modified chitosan: Water transport and kinetics of swelling. Journal of Polymer Research, 22(8), 153.
Pelgrift, R. Y., & Friedman, A. J. (2013). Nanotechnology as a therapeutic tool to combat microbial resistance. Advanced Drug Delivery Reviews, 65(13–14), 1803–1815.
Peulen, T.-O., & Wilkinson, K. J. (2011). Diffusion of nanoparticles in a biofilm. Environmental Science and Technology, 45(8), 3367–3373.
Ramage, G., Kean, R., Rautemaa-Richardson, R., Williams, C., & Lopez-Ribot, J. L. (2025). Fungal biofilms in human health and disease. Nature Reviews Microbiology, 23(6), 355–370.
Rodríguez-Rodríguez, R., Carreón-Álvarez, C., Cruz-Medina, C. A., Knauth, P., López, Z., Fletes-Vargas, G., & Sahagún, M. R. (2025). A review of pH-responsive chitosan-based hydrogels for drug delivery applications. European Polymer Journal, 237, 114173.
Sonthalia, S., Agrawal, M., & Sehgal, V. (2019). Topical Ciclopirox olamine 1%: Revisiting a unique antifungal. Indian Dermatology Online Journal, 10(4), 481–485.
World Health Organization (2022). WHO fungal priority pathogens list to guide research, development and public health action. World Health Organization, Geneva.