FLATOXINS (AFs) are difuranocoumarin derivatives produced as secondary metabolites by fungi belonging to several *Aspergillus* species. Aflatoxin B$_1$ (AFB$_1$) is one of the most potent naturally occurring hepatic carcinogens to both human and animals and is classified as a group 1 (human carcinogen). Therefore, the aim of this study is to assess the effect of encapsulating polyvinylpyrrolidone (PVP 10, 360 and 1300 kDa)-Tannic acid complexed nanoparticles (PVP-TA NPs) inside yeast cell walls (YCW) to remediate AFB$_1$ in the gastrointestinal models. Glucan Mannan Lipid Particles (GMLPs) from *Saccharomyces cerevisiae* cell walls showed the highest AFB$_1$ adsorption in simulated gastric fluid (SGF) after 10 min, and in simulated intestinal fluid (SIF) after 1 h. Glucan Mannan Lipid Particles are hollow 3–4-micron porous microspheres that provide an efficient system for the synthesis and encapsulation of AFB$_1$-absorbing nanoparticles (NPs). Although tannic acid (28%) was released from GMLP particles after three water washes, only 10, 5.6 and 7.6% of the total loaded TA was released when complexed with optimal ratios of PVP 10, 360 and 1300 kDa; respectively. Fluorescence microscopic images supported the conclusion that PVP TA complexed NP cores were successfully synthesized inside the GMLPs. Encapsulation of PVP TA NPs inside GMLPs significantly increased the stability of the GMLP encapsulated PVP TA NPs formulation. Data also showed that AFB$_1$ adsorption by the multi-functional GMLP PVP-TA NPs was enhanced synergistically in SGF and in SIF binding compared to individual GMLP.

**Keywords:** Chemical, Remediation, Polyvinylpyrrolidone, Aflatoxins B$_1$, Encapsulation.

**Introduction**

Aflatoxins (AFs) are highly toxic, carcinogenic, and teratogenic secondary metabolites produced by fungi [1]. They are produced mainly by certain strains of *Aspergillus flavus* and *Aspergillus parasiticus*, as well as several other species such as *A. nomius* [2], and more. The contamination of agricultural commodities with AFs is not only a serious health hazard to humans and animals, but also a cause of huge economic losses worldwide [3].

Fungi can invade, colonize, and produce mycotoxins either during preharvest or postharvest and may grow on foods during storage under favorable conditions (temperature, moisture, water activity, relative humidity) [4]. AFB$_1$ is classified by the International Agency of Research on Cancer (IARC) as a Group 1 carcinogen, with high risks for hepatocellular carcinoma (HCC) in individuals exposed to aflatoxins [5]. The toxic effects of AFB$_1$ are principally due to the binding of bioactivated AFB$_1$-8,9-epoxide to cellular macromolecules,
particularly mitochondrial and nuclear nucleic acids and nucleoproteins, resulting in general cytotoxic effects [6]. Due to the extreme concerns about AFB\textsubscript{1} in food and feed and their negative public health and economic impacts, therefore, there is a great need to increase the safety of food and feed for human and animal consumption by using detoxification methods.

Recently, the use of polymeric adsorbents has gained increased interest given that their structures can be synthetically modified to achieve molecules with more specificity in their trapping capabilities due to its bio-inert characteristic and its ease of tailoring their physical and chemical properties for a given purpose [7]. Several studies have demonstrated that cellulose materials have adsorption capacities for pollutants [8]. Similarly, some researchers evaluated the binding activity of chitosan against several mycotoxins [9, 10]. Recently, Solís-Cruz et al., [11] suggested that cellulose polymers have the highest adsorption capability for all mycotoxins.

Meanwhile, polyvinylpyrrolidone commonly called polyvidone or povidone is widely used in medical products, hair care products and cosmetics. Povidone iodine is a compound of polyvinylpyrrolidone and iodine, which is commonly used as an antibacterial agent and antiseptic [12]. Polyvinylpyrrolidone is a water-soluble polymer adsorbent has the capacity to adsorb zearalenone and aflatoxin concentrations \textit{in vitro} [13].

On the other hand, the use of organic materials such as yeast cell walls is considered a successful strategy for the management of multi-mycotoxin contamination of feedstuffs [14]. A preparation of chemically modified \textit{Saccharomyces cerevisiae} cell wall has been shown to adsorb selected major mycotoxins \textit{in vitro} [15-17] and alleviate the effect of dietary mycotoxin exposure in various animal species [18-20]. Yeast cell wall are composed mainly of polysaccharides, proteins, and lipids which offer numerous functional groups for the interaction, such as carboxyl, hydroxyl, phosphate and amine groups, as well as hydrophobic adsorption sites, such as aliphatic chains and aromatic carbon rings [21, 22].

Lately, \(\beta\)-Glucan particles (GP) extracted from the cell walls of baker’s yeast, 1–4\(\mu\)m spherical shells composed primarily of \(\beta\)-1,3/1,6-D-glucan. The hollow cavities of these particles allow adsorption and encapsulation of payload molecules [23]. 1, 3- \(\beta\)-D-glucan involves both hydrogen and Van der Waals bonding between glucans and AFB\textsubscript{1}, whereas 1, 6- \(\beta\)-D-glucan involves Van der Waals bonding only [15, 24].

Glucan Mannan Lipid Particles are 3-4 \(\mu\)m hollow and porous microspheres derived from \textit{Saccharomyces cerevisiae} that provide an efficient system for encapsulation, transport, delivery, and release of a wide range of molecules [25] such as water-soluble macromolecules and insoluble preformed nanoparticles (NPs) of less than 30 nm in diameter as cores inside GMLP (GMLP-NP) or onto the surface of GMLPs [26,27].

The present work objectives to develop a new innovative technology for controlling mycotoxins using microparticulate delivery system by yeast cell wall encapsulated nanoparticulate mycotoxin binders as polyvinylpyrrolidone using a defined nanomaterials engineering approach and hypothesized that the combination, termed GMLP bio-hybrid NPs would show enhanced AFB\textsubscript{1} detoxification properties. Therefore, the aim of this study is to assess the effect of encapsulating polyvinylpyrrolidone (PVP 10, 360 and 1300 kDa)-Tannic acid complexed nanoparticles (PVP-TA NPs) inside yeast cell walls (YCW) to remediate AFB\textsubscript{1}, in the gastrointestinal models.

**Materials and Methods**

**Chemicals**

Aflatoxin B\textsubscript{1} (Cayman Chemical Company, USA), Polyvinylpyrrolidone (PVP), Tannic acid (TA), and Folin-Ciocalteu (FC) reagent were purchased from Sigma Aldrich (St. Louis, MO, USA). GMLPs and the other particle types were prepared in the Ostroff laboratory by varying the chemical extraction treatments (acid/base hydrolysis, organic solvent extraction) using Baker’s yeast obtained from Biospringer, Juno, WI.

In our previous work [28] we observed that AFB\textsubscript{1} was stable in SIF during the incubation period, and was degraded in SGF, therefore in this study we used a 60 min incubation period for SIF binding studies, and 10 min incubation period for SGF binding studies. Also we noticed that glucan mannan lipid particles showed the highest AFB\textsubscript{1} adsorption efficacy of eight different types of the yeast cell wall-derived materials, thus in this study glucan mannan lipid particles were used. A flaw chart diagram displaying steps undertaken in this study was shown in Fig. 1.

**Preparation and characterization of Glucan Mannan Lipid Particles (GMLPs)**

Glucan Mannan Lipid Particles Preparation

Gastrointestinal model preparation

Optimization of AFB1 adsorption assay using HPLC

The binding efficacy of GMLP on AFB1 in SGF after (10 min) and in SIF after (1h)

PVP/Tannic acid nanoparticle complexation

Microencapsulation of PVP/Tannic acid nanoparticle inside GMLP

Synthesis (DTAF/TA) as a trapping agent

Loading PVP/DTAF TA NPs into GMLPs

Characterization GMLP/ PVP formulations

Fluorescent microscopic images
Assessing the stability in SGF and SIF
AFB1 adsorption of free and encapsulated DMwt PVP in SGF or in SIF

Fig. 1. Flow chart diagram showing steps undertaken in this study.

Glucan Mannan Lipid Particles were prepared from Baker’s yeast as previously described by Hamza et al., [28]. The dry particles were milled, and one mg/mL (w/v) of the extracted GMLP suspension was prepared in 0.9% saline and sonicated to single particles. The particles were evaluated under the microscope for intact yeast cell wall ghosts. Particle numbers/mg was quantified using a hemocytometer.

Development of an in vitro method to measure AFB1 binding

Gastrointestinal model preparation

The simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to European Pharmacopeia (07/2010:51701 Recommendations on Dissolution Testing) for the in vitro digestion test. Briefly, the simulated gastric fluid was prepared by dissolving 3.2 g pepsin powder (derived from porcine stomach

mucosa with an activity of 800 to 2500 units per mg of protein) in 1L HCl solution (pH = 1.6 containing 2 g/L sodium chloride) at room temperature. The simulated intestinal fluid was prepared by dissolving monobasic KH$_2$PO$_4$ (13.6 g) into 1L of water and 20 g of pancreatin was added; pH of the resultant solution was adjusted with either 0.2N NaOH or 0.2N HCl solution to 6.8 ± 0.1 at room temperature.

GMLP microencapsulation of mycotoxin binding materials to enhance AFB$_1$ binding

PVP/Tannic acid nanoparticle complexation as a nanoparticulate payload (PVP-TA NPs).

A stock solution of different molecular weight (DMwt) of PVP (10, 360 and 1300 kDa) was prepared at concentrations 25, 100 and 50 mg/mL in water. To determine the maximum complexation capacity of TA for DMwt PVP, a fixed concentration of tannic acid was placed at a concentration of (1 mg/mL) in micro-tubes with different concentrations of PVP ranging from (0.1–10 mg/mL). The interaction of TA and DMwt PVP (10, 360 and 1300 kDa) was based on the turbidity values at 600 nm.

In vitro assessment of PVP/TA complexed NPs as aflatoxin binders.

The binding capacity of AFB$_1$, by unencapsulated PVP (10, 360 and 1300 kDa) TA complexed NPs was tested in SGF for 10 min and SIF for 1h using variable PVP (10, 360, 1300 kDa) weights. The bound AFB$_1$ (µg) was calculated from the amount of unbound AFB$_1$ remaining in the supernatants using HPLC (Beckman Coulter, Inc.) by measuring the peak area and interpolating concentration using a calibration curve obtained with an AFB$_1$ standard.

Synthesis of PVP--NPs inside GMLPs (GMLP PVP-NP formulation).

Synthesis of fluorescent TA by using 5-(4,6-dichlorotriazinyl) aminofluorescein (DTAF) as a trapping agent.

Tannic acid (100 mg) was dissolved in 10 mL of carbonate buffer pH 9; 5 mg of DTAF was dissolved in DMSO (1mL), with stirring. The two solutions were mixed together at a room temperature, and the incubation was done at a room temperature in the dark overnight (24 h) to allow the labeling reaction to complete. 1mL of 1M Tris buffer pH 8 was added and incubated for 30 min at room temperature and the labeled tannic acid was purified by the precipitation method.

Loading PVP/DTAF TA NPs into GMLPs

Soluble PVP (payload) was absorbed into GMLPs by swelling a dry GMLP pellet (5 mg) with a sub-hydrodynamic volume of DMwt PVP (10, 360 and 1300 KD at concentrations (25, 100 and 50 mg/mL, respectively) (5 µL/mg GMLPs). GMLP samples containing DMwt PVP were incubated at room temperature for 30 min to allow for passive PVP diffusion into the GMLPs by capillary action. The sample was then frozen and lyophilized (lyophilizer, Virtis Company, Gardiner, NY). A series of water hydration steps with 2.5 µL water/mg GMLPs were carried out twice to increase PVP encapsulation efficiency. After lyophilization, the loaded GMLP PVP was then treated by swelling the pellets in a subhydrodynamic volume of the previously prepared DTAF TA (40 mg/mL) for 1X cycle of the formulation and 120 mg/mL of DTAF TA to 3x cycle and 400 mg/mL DTAF TA to 10x cycle of the formulation as a trapping agent to produce insoluble PVP-NPs inside GMLPs. The GMLP PVP-NPs were then washed three times with water to remove uncomplexed PVP-TA to synthesize the GMLP PVP-TA NPs formulation. The GMLP DMwt PVP formulation was prepared as above without DTAF TA complexation. The percentage of unbounded TA (three water washes) in the formulation was determined by the Folin-Ciocalteu assay using spectrophotometric detection at 700 nm (Safire Tecan 2 plate reader). Folin-Ciocalteu assay was prepared according to Blainski et al. [29].

Characterization of GMLP/ PVP and GMLP/ PVP-TANPs formulations.

Microscopic images of GMLP PVP formulations.

To visualize the location of the PVP-TA NPs in GMLP formulations, the particles were imaged by fluorescence microscopy (Zeiss Axiovert 200 microscope equipped with a Zeiss Axio Cam HR CCD camera with 1300x1030 pixel resolution) to demonstrate the synthesis of PVP-TA complexes inside GMLPs.

Stability of GMLP PVP-NPs through simulated gastrointestinal conditions.

The stability of GMLP DMwt PVP formulations was assessed by quantifying the concentration of TA released in the supernatant after each time point in SGF (30, 60, 90 and 120 min.) followed by sequential transfer to SIF (150, 180, 210 and 240 min.) at 37°C. The released TA was measured spectrophotometrically using the Folin-Ciocalteu
CHEMICAL CHARACTERIZATION OF CONSTITUENTS ISOLATED...

**Afloatin B<sub>1</sub> binding capacity of GMLP encapsulated DMwt PVP /TA NPs (GMLP/PVP-TA NPs formulation)**

Afloatin B<sub>1</sub> binding capacity of GMLP encapsulated PVP/TA NPs (0.125 mg PVP 10 kDa/ mg GMLP; 0.5 mg PVP 360 kDa /mg GMLP and 0.25 mg PVP 1300 kDa /mg GMLP) was tested in SGF for 10 min or SIF for 1h at 37°C. The bound AF<sub>B1</sub> (µg AF<sub>B1</sub>) was calculated from the amount of unbound AF<sub>B1</sub> remaining in the supernatants using HPLC (Beckman Coulter, Inc.) by measuring the peak area and interpolating concentration using a calibration curve obtained with an AF<sub>B1</sub> standard.

**Statistical analysis**

The experiments were expressed in replicates, except where indicated. The statistical significance of the differences in the means of experimental groups was determined by t-test and ANOVA analysis using Graph Pad Prism 5.0a Software.

**Results and Discussion**

**PVP/TA NPs as a nanoparticulate payload (PVP-TA NPs)**

The binding of PVP (Molecular weight; 10, 360 and 1300 kDa) to TA was measured by the turbidity developments, whereas the turbidity values increased with the increase of molecular weight of PVP. The complete TA complexation was found to occur at mass ratios of 0.625:1 PVP 10 kDa: TA; 2.5:1 PVP 360 kDa: TA and 1.25:1 PVP 1300 kDa: TA w/w (Fig. 2).

Tannic acid is a specific form of tannin, a type of polyphenol, and its weak acidity is due to the numerous phenol groups in the structure. As all phenols, tannic acid can establish H-bonds with N-substituted amide and this bond is one of the strongest types of H-bond [30]. Tannic acid was discovered from both soluble and insoluble PVP that form stable insoluble complexes with tannins [31]. The tannins form H-bond with the peptide linkages, probably through the peptide oxygen, and with the tannins furnishing the hydrogen. Recently, tannic acid compound has received considerable attention due to its functional properties such as antioxidant, antimutagenic, anti-inflammatory, and antitumor activity, as well as antifungal and antibacterial activity [32]. Tannic acid complexing agents find application in various biological processes. It is among the most intriguing building blocks in nanotechnology due to the unique chemical properties of this material, which allow interactions with various metals [33].
minerals [34], metal oxides, carbon nanotubes [35] and graphene [33]. The chemical structure of TA includes multiple galloyl groups, which promote electrostatic, hydrogen bonding, and hydrophobic interactions [36]. The galloyl groups of TA provide binding sites for the formation of chelates with different metals [37]. Moreover, TA showed interesting complexation behavior with various macromolecules [38,39], including carbohydrates, proteins, enzymes, and synthetic polymers.

**GMLP encapsulated PVP/TA NPs as a nanoparticulate payload (GMLP/PVP-TA NPs).**

The development and optimization of GMLP/PVP- NPs synthesis process (Fig. 3) is required a method to measure unencapsulated TA in the supernatant fractions. Folin-Ciocalteu assay was used to measure the total TA in the supernatant fractions during the microencapsulation process inside GMLP using Folin Ciocalteau tannic acid standard curve at 700 nm (Fig. 4).

Most of tannic acid (28%) was released from GMLP particles after three water washes. In contrast, only 10, 5.6 and 7.6% of the total loaded TA was released when complexed with optimal ratios of PVP 10, 360 and 1300 kDa; respectively (Fig. 5).

In Fig. 6, 7 and 8 fluorescence microscopic images captured by Axiovert 200 M from Image Xpress Microshow the location of the encapsulated PVP TA NPs complexes, as visualized by DTAF TA inside the GMLPs; thus supporting the conclusion that PVP TA complexed NP cores were successfully synthesized inside the GMLPs.

**Fig. 3. Schematic representation of PVP (10, 360 and 1300 kDa) as payload core loading into GMLP and trapping reaction to form PVP noncomplex inside GMLP.**
Fig. 5. Stability of GMLP/PVP-NPs. The TA release (%) during the microencapsulation process. Total tannic acid content in the supernatants determined by the Folin-Ciocalteu assay using Spectrophotometric detection at 700 nm. Results are mean ±SD (n=2) (Vertical bars).

Fig. 6. Fluorescent microscopic images of PVP 10KDa nano-complexes inside GMLP with DTAF TA as a trapping agent.
   a) GMLP; b) GMLP/PVP 10kDa; c) GMLP/PVP 10 kDa-TA

Fig. 7. Fluorescent microscopic images of PVP 360 KDa nanocomplexes inside GMLP with DTAF TA as a trapping agent.
   a) GMLP; b) GMLP/PVP 3600 kDa; c) GMLP/PVP360 kDa –TA
Fig. 8. Fluorescent microscopic images of PVP 1300 KDa nano-complexes inside GMLP with DTAF TA as a trapping agent.

a) GMLP; b) GMLP/PVP 1300 kDa; c) GMLP/PVP-TA

Fig. 9. The release of TA in free and encapsulated PVP/TA NPs in (a. SGF) followed by (b. SIF) at 37°C after 1h using Folin-Ciocalteu assay.

Fig. 10. The stability of DMwt PVP–TA complexed NPs after GMLP encapsulation in SGF followed by SIF at 37°C.

The (%) of TA released in the supernatant at the indicated time points of incubation was calculated using Folin-Ciocalteu assay at 700 nm. Results are mean ± SD (n = 2)

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To assess the stability of the free PVP TA NPs and GMLP PVP NPs materials through a simulated digestion system, TA release was measured following sequential incubation in SGF followed by SIF at 37 °C. GMLP/PVP TA NP formulations were stable during the incubation in SIF. Encapsulation of PVP TA NPs inside hollow GMLPs significantly reduced TA solubilization in simulated gastric fluid and increased the stability of the GMLP encapsulated PVP TA NPs formulation (Fig. 9, 10).

AFB₁-binding properties of free and encapsulated (PVP 10 kDa -TA NPs)

Data in Table 1 shows the difference between the AFB₁ binding capacities of GMLP encapsulated PVP10 kDa NPs before and after formulation in SGF for 10min in SIF for 1h. Binding capacity of PVP 10 kDa-NPs against 2 µg of AFB₁ was tested using increasing amount of PVP NPs (0.125, 0.375 and 1.25 µg) in SGF and SIF. Based on the remaining AFB₁ in the supernatants, it seems that 0.3175 µg of PVP 10 kDa-NPs was highly efficient to adsorb AFB₁ in SGF and SIF (1.5 and 1.7 µg AFB₁, in SGF and SIF) respectively. On the other hand, the AFB₁ adsorption was similar with the higher quantities of PVP10 kDa-NPs and the interaction of PVP 10 kDa and AFB₁ was not an additive effect.

AFB₁ adsorption by the multi-functional GMLP PVP 10kDa -TA NPs inside GMLP was similar to free PVP-TA that adsorbed (1.4 µg AFB₁ in SGF and 1.6 µg AFB₁ in SIF). After increasing the loaded PVP mass to (1.25 mg) inside GMLP, the AFB₁ adsorption was slightly increased from (1.5 µg AFB₁ for free PVP-TA NPs) to (1.83 µg AFB₁ for GMLP/PVP –TA NPs) in SGF.

AFB₁-binding properties of free and encapsulated (PVP 360 KDa -TA NPs)

In the present investigation, it appears that the loading (0.5 and 1.5 mg) of PVP 360 KDa inside GMLP did not enhance the AFB₁ adsorption. Meanwhile, free PVP 360 KDa-TA recorded the highest AFB₁ adsorption in SGF. After increasing the mass of PVP (5 mg) the AFB₁ adsorption of the encapsulated PVP-TA NPs increased from (1.35 µg AFB₁ for PVP-TA) to (1.55 µg AFB₁ for GMLP/PVP-TA NPs) in SGF (Table 2).

The results showed that the efficient trapping of PVP 360 KDa -TA inside GMLP increased AFB₁ adsorbed mass from (1.07 µg AFB₁ for GMLP/PVP 360 kDa) to (1.55 µg AFB₁ for GMLP/PVP 360 kDa-TA NPs) in SGF after 10 min. In SIF (1.69 µg AFB₁) were bound.

AFB₁-binding properties of free and encapsulated (PVP 1300 KDa-TA NPs)

In the light of the experimental results concerning AFB₁ adsorption by individual components and the multi-functional (GMLP/PVP 1300kDa-NPs), the results revealed that the loaded mass (0.25 and 0.75 mg) of PVP 1300 kDa inside GMLP showed the same effect as the previous results in PVP (10 and 360kDa), whereas, the binding capacity was not enhanced in SGF or SIF.

In SIF, the AFB₁ adsorption capacity of free PVP 1300KDa–TA was similar to the AFB₁ adsorption capacity of encapsulated PVP 1300 KDa-TA. The data showed that the binding capacity of PVP 1300 KDa in SGF and SIF was lost after loading PVP 1300 KDa inside GMLP without trapping agent (TA) and did not show any effect as most of PVP 1300 KDa was released during the washing step (Table 3).

Mycotoxin binders, which are considered promising for the prevention of hazardous effects of mycotoxins, may be reversible during passage through the digestive tract. As a result binding reactions that occur in the acid pH of the stomach may decompose further down the digestive tract (e.g., more neutral pH); thus releasing the mycotoxin and leading to toxicity. Therefore, it is important to ensure the stability of the mycotoxin binder through an in vitro system that mimics the changes in physiological conditions along the gastrointestinal tract.

Strategies for the detoxification of mycotoxin-contaminated food and feed stuff in a cost-effective way are still under developed. The greatest promising approaches for the elimination of mycotoxin problem in feed stuff are the addition of non-nutritive adsorptive materials [40].

A search of the literature has shown that polyvinylpyrrolidone (a highly polar amphoteric polymer) have been demonstrated to bind mycotoxins in vitro and in vivo [41-43]. Carrasco-Sánchez et al. [44] evaluated the capacity of the PVP for the removal of other type of mycotoxin (Ochratoxin) from acidic model solutions and red wine. The ability of various polymers to remove undesired substances in wine was also studied [45,46].

Moreover, mannan from the yeast cell wall was reported to play a role in aflatoxin binding [47]. Additionally, Yiannikouris et al. [24] found a
TABLE 1. AFB$_1$ adsorption by individual components and the multi-functional GMLP PVP 10kDa -NPs.

<table>
<thead>
<tr>
<th>The particle composition</th>
<th>PVP 10 kDa (mg)</th>
<th>Adsorbed AFB$_1$ (µg)</th>
<th>SIF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SGF</td>
<td>SIF</td>
</tr>
<tr>
<td><strong>Before encapsulation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free PVP 10 kDa-TA NPs</td>
<td>0.125</td>
<td>1.4±0.02</td>
<td>1.6±0.24</td>
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<tr>
<td>Free PVP 10 kDa-TA NPs</td>
<td>0.375</td>
<td>1.5±0.04</td>
<td>1.7±0.16</td>
</tr>
<tr>
<td>Free PVP 10 kDa-TA NPs</td>
<td>1.25</td>
<td>1.5±0.16</td>
<td>1.7±0.02</td>
</tr>
<tr>
<td><strong>After encapsulation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>a. without TA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Empty GMLP</td>
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<td>0.2±0.09</td>
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<tr>
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<tr>
<td>GMLP/PVP 10 kDa-TA NPs</td>
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<td>Results are mean ± SD (n=3)</td>
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TABLE 2. AFB$_1$ adsorption by individual components and the multi-functional GMLP PVP 360 KDa-NPs

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<tr>
<th>The particle composition</th>
<th>PVP 360 kDa (mg)</th>
<th>Adsorbed AFB$_1$ (µg)</th>
<th>SIF</th>
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<td>SGF</td>
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<tr>
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<td><strong>a. without TA</strong></td>
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<tr>
<td>Empty GMLP</td>
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<td>0.2±0.09</td>
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<td>GMLP/PVP 360 kDa-TA NPs</td>
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<tr>
<td>Results are mean ± SD (n=3)</td>
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</table>

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The predominant role of α-glucans in complexes with AFB₁, β-glucans is also involved in both hydrogen and Van der Waals bonding with AFB₁, although (1,6)-β-glucan is involved in only Van der Waals bonding. The (1,3)-β-D-glucan chains form triple helix three-dimensional structures with spring-like mechanical properties and are responsible for the strength of yeast cell walls (1,3) and their ability to bind toxins [48, 49]. Taken together, the ability of PVP-TA NPs and glucans to bind AFB₁ suggested that these agents strongly bind AFB₁ and decrease its bioavailability in the gastrointestinal tract and consequently reduce its toxicity.

The results of this study give some scientific credence to the AFB₁ adsorption capacity increased by using free PVP–TA NPs and did not enhance by loading PVP-TA NPs inside GMLP. While the incorporation of PVP as an AFB₁ binding material inside GMLP enhanced the binding capacity of GMLP.

**Conclusion**

A micro-particulate delivery system using GMLP encapsulated PVP-TA NPs as a multi-functional mycotoxin binding material was developed. The mycotoxin binding capacity of the developed materials were enhanced by polyvinylpyrrolidone inside GMLP. The ability of PVP-TA NPs and glucans to bind AFB₁ proposed that they have the ability to strongly bind AFB₁ and decrease its bioavailability in the gastrointestinal tract and consequently reduce its toxicity.

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**References**


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