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Chemical Remediation of Aflatoxin B_1 Using Encapsulated Poly-vinylpyrrolidone as an Environmental-friendly Control

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AFLATOXINS (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 *Saccharomycesces cerevisie* cell walls showed the highest AFB_1 adsorption in simulated gastric fluid (SGF) after 10 min, and in simulated intestinal fluid (SIF) after1 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,

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particularly mitochondrial and nuclear nucleic acids and nucleoproteins, resulting in general cytotoxic effects [6]. Due to the extreme concerns about AFB_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 cellulosic 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 cellulosic 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 *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 *Saccharomyces cerevisiae* cell wall has been shown to adsorb selected major mycotoxins *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, β-Glucan particles (GP) extracted from the cell walls of baker's yeast, 1–4μm spherical shells composed primarily of β-1,3/1,6-D-glucan. The hollow cavities of these particles allow adsorption and encapsulation of payload molecules [23]. 1, 3- ß-D-glucan involves both hydrogen and Van der Waals bonding between glucans and AFB_1, whereas 1, 6- ß-D-glucan involves Van der Waals bonding only [15, 24].

Glucan Mannan Lipid Particles are 3-4 μm hollow and porous microspheres derived from *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_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_1 in the gastrointestinal models.

Materials and Methods

Chemicals

Aflatoxin B_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_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_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)

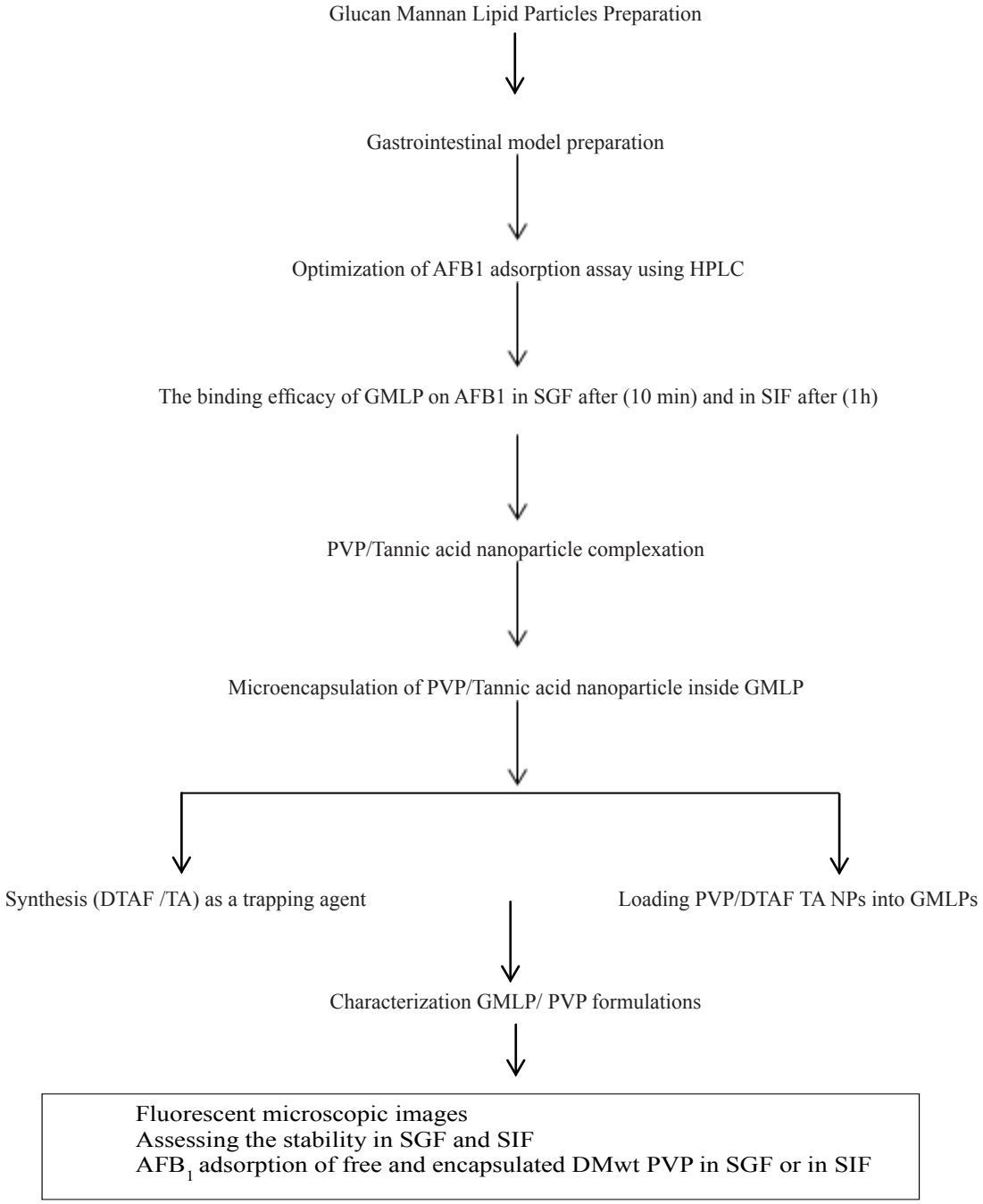

Fig. 1. Flaw chart diagram showing steps undertaken in this study.

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 AFB_1 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_2PO4 (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 and1300 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 1300k Da) 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

assay at 700 nm.

AFB_1 binding capacity of GMLP encapsulated DMwt PVP /TA NPs (GMLP/PVP-TA NPs formulation)

Aflatoxin B_1 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 AFB_1 (µg AFB_1) 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.

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],

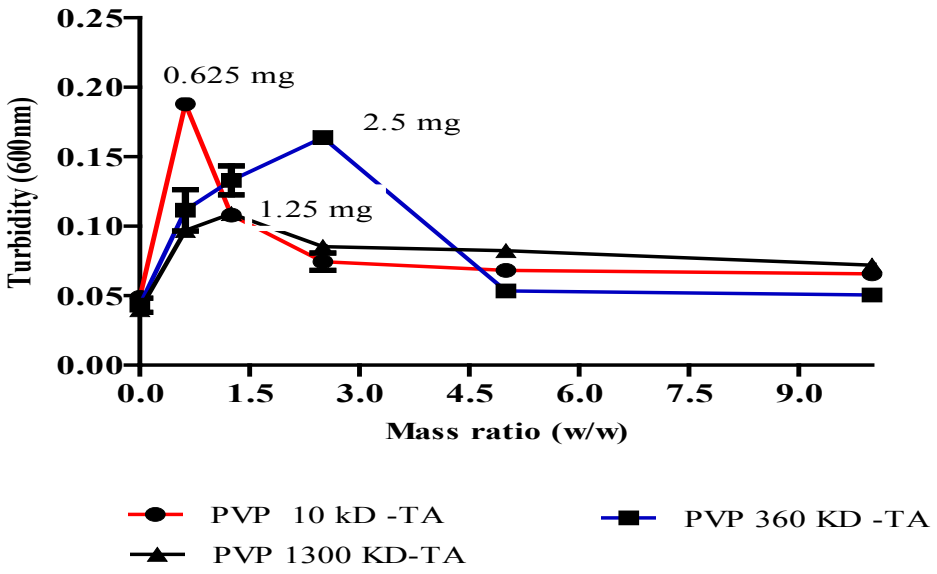

Fig. 2. The mass ratio required to achieve efficient complexation of 1 mg TA using (DMwt of PVP 10, 360 and 1300 kDa).

The optimal mass ratio of PVP/TA as follows

PVP 10 kDa/TA is 0.625 mg

PVP 360kDa /TA is 2.5 mg

PVP 1300kDa /TA is 1.25 mg

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 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.

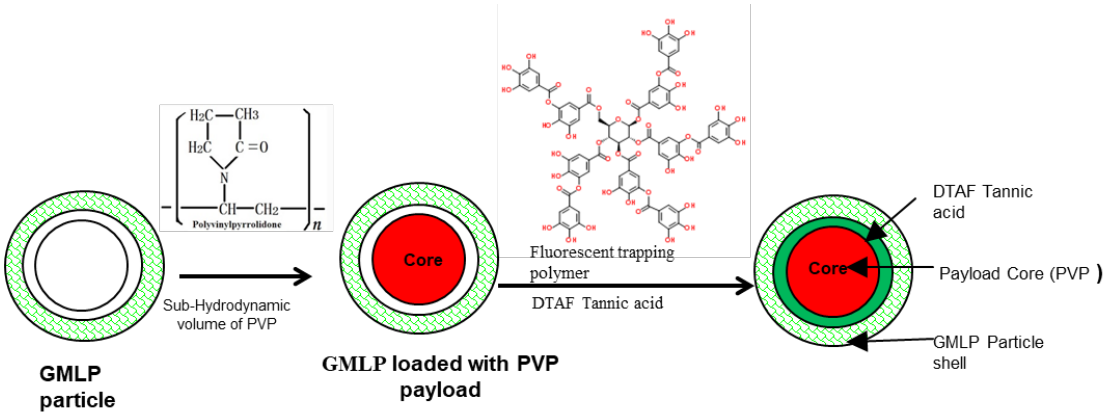

Fig. 3. Schematic representation of PVP (10, 360 and 1300 kDa) as payload core loading into GMLP and trapping reaction to form PVP noncomplex inside GMLP.

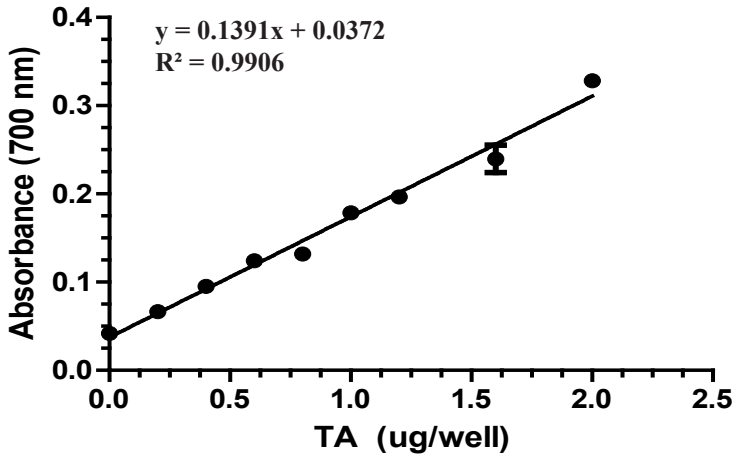

Fig. 4. Folin Ciocalteau tannic acid standard curve.

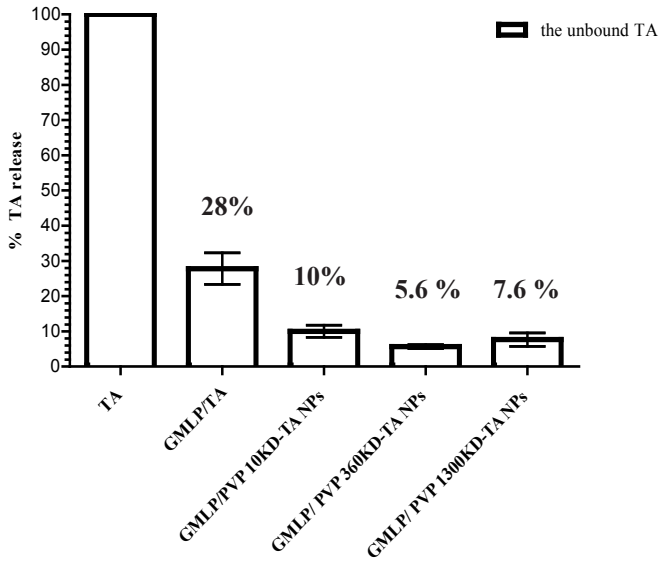

Fig. 5. Stability of GMLP/PVP-NPs. The TA release (%) during the microencapsulation process. Total tannic acid content in the supernatants determined by the Folin-Ciocalteu assay using Spectrophotometric detection at 700 nm. Results are mean ±SD (n=2) (Vertical bars).

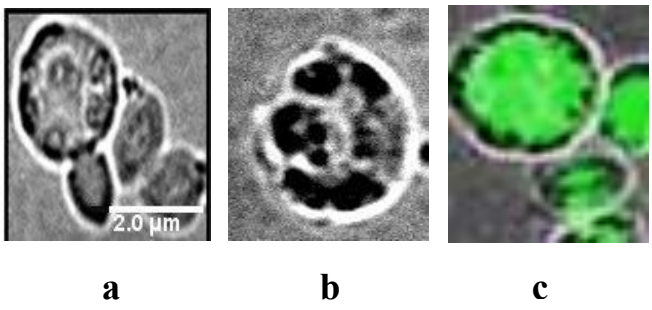

Fig. 6. Fluorescent microscopic images of PVP 10KDa nano-complexes inside GMLP with DTAF TA as a trapping agent
a) GMLP; b) GMLP/PVP 10kDa; c) GMLP/PVP 10 kDa-TA

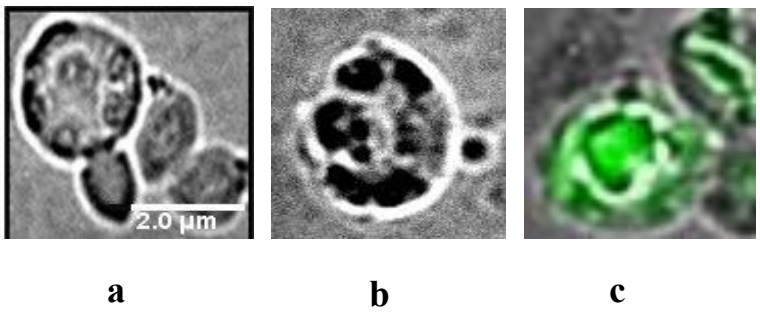

Fig. 7. Fluorescent microscopic images of PVP 360 KDa nanocomplexes inside GMLP with DTAF TA as a trapping agent.
a) GMLP; b) GMLP/PVP 3600 kDa; c) GMLP/PVP360 kDa –TA

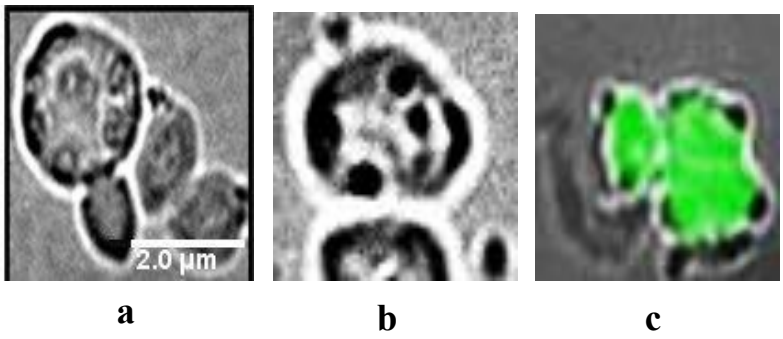

Fig. 8. Fluorescent microscopic images of PVP 1300 KDa nano-complexes inside GMLP with DTAF TA as a trapping agent.
a) GMLP; b) GMLP/PVP 1300 kDa; c) GMLP/PVP -TA

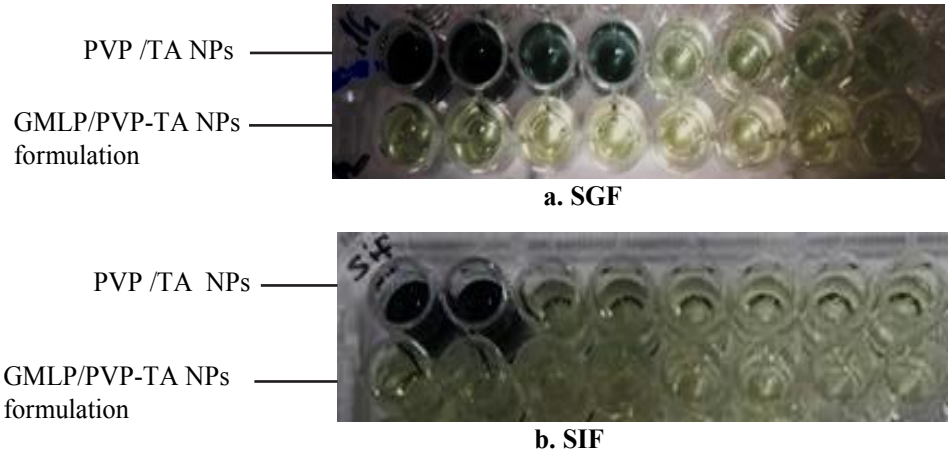

Fig. 9. The release of TA in free and encapsulated PVP/ TA NPs in (a. SGF) followed by (b. SIF) at 37°C after 1h using Folin-Ciocalteu assay.

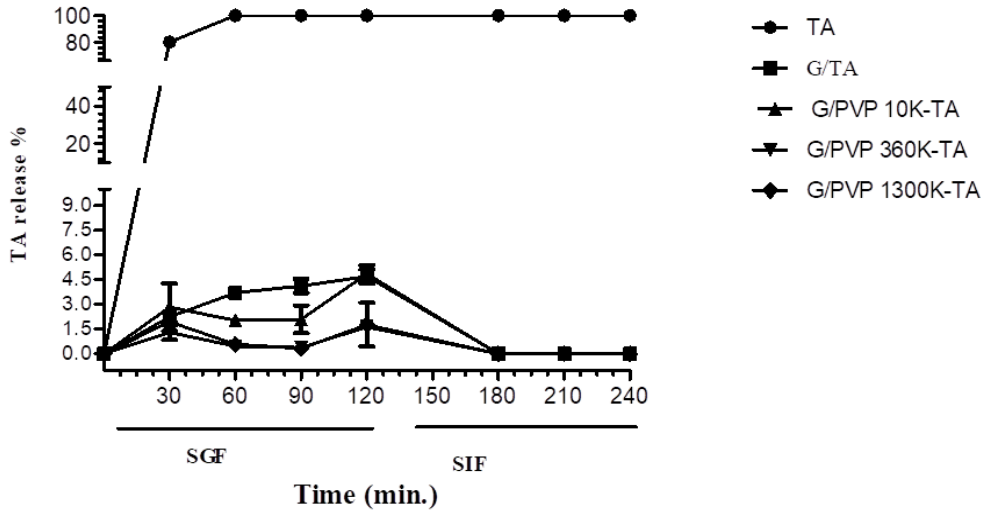

Fig. 10. The stability of DMwt PVP–TA complexed NPs after GMLP encapsulation in SGF followed by SIF at 37°C.
The (%) of TA released in the supernatant at the indicated time points of incubation was calculated using Folin-Ciocalteu assay at 700 nm. Results are mean ± SD (n = 2)

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).

AFB_1-binding properties of free and encapsulated (PVP 10 kDa -TA NPs)

Data in Table 1 shows the difference between the AFB_1 binding capacities of GMLP encapsulated PVP10 kDa NPs before and after formulation in SGF for 10minor in SIF for 1h. Binding capacity of PVP 10 kDa -NPs against 2 µg of AFB_1 was tested using increasing amount of PVP NPs (0.125, 0.375 and 1.25 µg) in SGF and SIF. Based on the remaining AFB_1 in the supernatants, it seems that 0.3175 µg of PVP 10 kDa -NPs was highly efficient to adsorb AFB_1 in SGF and SIF (1.5 and 1.7 µg AFB_1 in SGF and SIF) respectively. On the other hand, the AFB_1 adsorption was similar with the higher quantities of PVP10 kDa–NPs and the interaction of PVP 10 kDa and AFB_1 was not an additive effect.

AFB_1 adsorption by the multi-functional GMLP PVP 10kDa -TA NPs inside GMLP was similar to free PVP-TA that adsorbed (1.4 µg AFB_1 in SGF and 1.6 µg AFB_1 in SIF). After increasing the loaded PVP mass to (1.25 mg) inside GMLP, the AFB_1 adsorption was slightly increased from (1.5 µg AFB_1 for free PVP-TA NPs) to (1.83 µg $AFB_1$for GMLP/PVP –TA NPs) in SGF.

AFB_1-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 AFB_1adsorption. Meanwhile, free PVP 360 KDa-TA recorded the highest AFB_1 adsorption in SGF. After increasing the mass of PVP (5 mg) the AFB_1 adsorption of the encapsulated PVP-TA NPs increased from (1.35 µg AFB_1 for PVP-TA) to (1.55 µg AFB_1 for GMLP/PVP-TA NPs) in SGF (Table 2).

The results showed that the efficient trapping of PVP 360 KDa -TA inside GMLP increased AFB_1 adsorbed mass from (1.07 µg AFB_1 for GMLP/PVP 360 kDa) to (1.55 µg AFB_1 for GMLP/PVP 360 kDa-TA NPs) in SGF after 10 min. In SIF (1.69 µg AFB_1) were bound.

AFB_1-binding properties of free and encapsulated (PVP 1300 KDa-TA NPs)

In the light of the experimental results concerning AFB_1 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 AFB_1 adsorption capacity of free PVP 1300kDa–TA was similar to the AFB_1 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.

The particle composition	PVP 10 kDa (mg)	Adsorbed AFB_1 (μg)	
		SGF	SIF
Before encapsulation		**(μg AFB_1)**	
Free PVP 10 kDa-TA NPs	0.125	1.4±0.02	1.6±0.24
Free PVP 10 kDa-TA NPs	0.375	1.5±0.04	1.7±0.16
Free PVP 10 kDa-TA NPs	1.25	1.5±0.16	1.7±0.02
After encapsulation a. without TA		**(μg AFB_1/mg GMLP)**	
Empty GMLP	0	0.2 ±0.09	0.17 ±0.01
GMLP/PVP 10 kDa	0.125	0.65±0.14	0.2±0.02
GMLP/PVP 10 kDa	0.375	0.83±0.12	0.2±0.02
GMLP/PVP 10 kDa	1.25	0.90±0.11	1.1±0.2
b. with TA			
GMLP/PVP 10 kDa-TA NPs	0.125	1.4±0.09	1.6±0.21
GMLP/PVP 10 kDa-TA NPs	0.375	1.5±0.06	1.74±0.08
GMLP/PVP 10 kDa-TA NPs	1.25	1.83±0.02	1.94±0.02

Results are mean ± SD (*n*=3)

TABLE 2. AFB_1 adsorption by individual components and the multi-functional GMLP PVP 360 KDa-NPs

The particle composition	PVP 360 kDa (mg)	Adsorbed AFB_1 (μg)	
		SGF	SIF
Before encapsulation		**(μg AFB_1)**	
Free PVP 360 kDa-TA NPs	0.5	1.13±0.05	0.80±0.05
Free PVP 360 kDa-TA NPs	1.5	1.31±0.09	1.34±0.02
Free PVP 360 kDa-TA NPs	5	1.35±0.07	1.64±0.03
After encapsulation a. without TA		**(μg AFB_1/mg GMLP)**	
Empty GMLP	0	0.2 ±0.09	0.17 ±0.01
GMLP/PVP 360 kDa	0.5	0.49±0.02	0.20±0.07
GMLP/PVP 360 kDa	1.5	0.62±0.02	0.48±0.06
GMLP/PVP 360 kDa	5	1.07±0.03	1.08±0.02
b. with TA			
GMLP/PVP 360 kDa-TA NPs	0.5	0.91±0.14	0.91±0.02
GMLP/PVP 360 kDa-TA NPs	1.5	1.22±0.02	1.81±0.16
GMLP/PVP 360 kDa-TA NPs	5	1.55±0.06	1.69±0.17

Results are mean ± SD (*n*=3)

TABLE 3. AFB_1 adsorption by individual components and the multi-functional GMLP PVP 1300 kDa-NPs

The particle composition	PVP 1300kDa(mg)	Adsorbed AFB_1	
		SGF	SIF
Before encapsulation		**(µg AFB_1)**	
PVP 1300 kDa-TA NPs	0.25	1.04±0.43	1.18±0.45
PVP 1300 kDa-TA NPs	0.75	1.48 ±0.02	1.73±0.02
PVP 1300 kDa-TA NPs	2.5	1.50±0.02	1.99±0.18
After encapsulation a. without TA		**(µg AFB_1/mg GMLP)**	
Empty GMLP	0	0.20 ±0.09	0.17 ±0.01
GMLP/PVP 1300 kDa	0.25	0.02±0.001	0.03±0.001
GMLP/PVP 1300 kDa	0.75	0.0597±0.20	0.28±0.02
GMLP/PVP 1300 kDa	2.5	0.254±0.06	0.85±0.04
b. with TA		**(µg AFB_1/mg GMLP)**	
GMLP/PVP -TA NPs	0.25	1.28±0.006	1.26±0.01
GMLP/PVP -TA NPs	0.75	1.37±0.08	1.76±0.02
GMLP/PVP -TA NPs	2.5	1.73±0.02	1.89±0.13

Results are mean ± SD (n=3)

predominant role of â-glucans in complexes with AFB_1. β-glucans is also involved in both hydrogen and Van der Waals bonding with AFB_1 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 AFB_1 suggested that these agents strongly bind AFB_1 and decrease its bioavailability in the gastrointestinal tract and consequently reduce its toxicity.

The results of this study give some scientific credence to the AFB_1 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 AFB_1 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 AFB_1 proposed that they have the ability to strongly bind AFB_1 and decrease its bioavailability in the gastrointestinal tract and consequently reduce its toxicity.

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References

1. Kumar, P., Mahato, D.K., Kamle, M., Mohanta, T.K., and Kang, S.G., Aflatoxins: A global concern for food safety, human health and their management. *Frontier Microbiology*, **7**, 1-10 (2017).https://doi.org/10.3389/fmicb.2016.02170

2. Moore, G.G., Mack, B.M., and Beltz, S.B., Genomic sequence of the aflatoxigenic filamentous fungus *Aspergillusnomius*. *BMC Genomics*, **16**, 551 (2015).https://doi.org/10.1186/s12864-015-1719-6

3. Yabe, K., Ozaki, H., Maruyama, T., Hayashi, K., Matto, Y., Ishizaka, M., and Kushiro, M., Improvement of the Culture Medium for the Dichlorvos-Ammonia (DV-AM) Method to Selectively Detect Aflatoxigenic Fungi in Soil. *Toxins*, **10**(12), 519 (2018).https://doi:10.3390/toxins10120519

4. Martins, H.M., Mendes Guerra, M.M., and d'Almeida Bernardo, F.M., Occurrence of aflatoxin B1 in dairy cow's feed over 10 years in Portugal (1995–2004). *Revista Iberoamericana de Micología*, **24**, 69-71 (2007).

5. Ostry, V., Malir, F., Toman, J., and Grosse Y., Mycotoxins as human carcinogens-the IARC Monographs classification. *Mycotoxin Research*, **33**, 65-73 (2017).https://doi.org/10.1007/s12550-016-0265-7

6. Bennett, J.W. and Klich, M., Mycotoxins. *Clinical Microbiology Reviews*, 16, 497-516 (2003).

7. Bures, P., Huang, Y., Oral, E., and Peppas, N., Surface modifications and molecular imprinting of polymers in medical and pharmaceutical applications. Journal of controlled release. *Official Journal of the Controlled Release Society*, **72**, 25-33 (2001). https://doi.org/10.1016/S0168-3659(01)00259-0.

8. Hokkanen, S., Bhatnagar, A., and Sillanpää, M., A review on modification methods to cellulose-based adsorbents to improve adsorption capacity. *Water Research*, **91**, 156-173 (2016). https://doi.org/10.1016/j.watres.2016.01.008

9. Szymczyk, P., Filipkowska, U., Józwiak, T., and Kuczajowska-Zadrozna, M., Phosphate removal from aqueous solutions by chitin and chitosan in flakes. *Progress on Chemistry and Application of Chitin and its Derivatives*, **21**, 192-202 (2016). https://doi.org/10.15259/PCACD.21.21

10. Solís-Cruz, B., Hernández-Patlán, D., Beyssac, E., Latorre, J., Hernandez-Velasco, X., Merino-Guzman, R., Tellez, G. and López-Arellano, R., Evaluation of chitosan and cellulosic polymers as binding adsorbent materials to prevent aflatoxin B_1, fumonisin B_1, ochratoxin, trichothecene, deoxynivalenol, and zearalenone mycotoxicoses through an *in vitro* gastrointestinal model for poultry. *Polymers*, **9**(10), 529 (2017).https://doi.org/10.3390/polym9100529

11. Mahadevappa, Y., Arjumand A., and Ravindra R., Polymer Synthesis and Processing. In: *Natural and Synthetic Biomedical Polymers.* pp. 1-31 (2014). https://doi.org/10.1016/B978-0-12-396983-5.00001-6

12. Alegakis, A., Tsatsakis, A., Shtilman, M., Lysovenko, D. and Vlachonikolis, I., Deactivation of mycotoxins. I. An *in vitro* study of zearalenone adsorption on new polymeric adsorbents. *Journal of Environmental Science and Health Part B,* **34**, 633-644 (1999).https://doi.org/10.1080/03601239909373218

13. Biernasiak J., Piotrowska M., Libudzisz Z., Detoxification of mycotoxins by probiotic preparation for broiler chickens. Institute of Fermentation Technology and Microbiology, Technical University of Lodz, Poland. *Mycotoxin Research,* **22**(4), 230-235 (2006).1007/BF02946747

14. Yiannikouris, A., Andre, G., Poughon, L., Fran¸cois, J., Dussap, C.-G., Jeminet, G., Bertin, G. and Jouany, J.-P., Adsorption of zearalenone by â- D-glucans in the S*accharomyces cerevisiae* cell wall. *Journal of Food Protection,* **67**, 1195-1200 (2004).https://doi.org/10.4315/0362-028X-67.6.1195

15. Shetty, P.H. and Jespersen, L., *Saccharomyces cerevisiae* and lactic acid bacteria as potential mycotoxin decontaminating agents. *Trends in Food Science and Technology,* **17**, 48-55 (2006). https://doi.org/10.1016/j.tifs.2005.10.004

16. Gonçalves, B.L., Gonçalves, J.L., Rosim, R.E., Cappato, L.P., Cruz, A.G., Oliveira, C.A.F., and Corassin, C.H., Effects of different sources of *Saccharomyces cerevisiae* biomass on milk production, composition, and aflatoxin M_1 excretion in milk from dairy cows fed aflatoxin B_1. *Journal of Dairy Science*, **100** (7), 5701-5708 (2017). https://doi.org/10.3168/jds.2016-12215.

17. Diaz, D.E., Hagler, J.R., Blackwelder, W.M., Hopkins, B.A., Anderson, K.L., Jones, F.T. and Whitlow, L.W. Aflatoxin binders II: reduction of aflatoxin M_1 in milk by sequestering agents of cows consuming aflatoxin in feed. *Mycopathologia*, **157**, 233-241 (2004). https://doi.org/10.1023/B:MYCO.0000020587.93872.59

18. Chowdhury, S.R., Smith, T.K., Boermans, H.J. and Woodward, B., Effects of feed-borne Fusarium mycotoxins on hematology and immunology of turkeys. *Poultry Science*, **84**, 1698–1706 (2005). https://doi.org/10.1093/ps/84.11.1698

19. Diaz, D.andSmith, T. Mycotoxin sequestering agents: practical tools for the neutralization of mycotoxins. In: Diaz, D.E. (ed.) T*he mycotoxin Blue Book.* Nottingham University Press, Nottingham, UK, pp. 323-338 (2005).https://digitalcommons.usu.edu/advs_facpub/491/

20. Jouany, J.P., and Diaz, D.E., Effects of mycotoxins in ruminants. *In Mycotoxins Blue Book;* Nottingham University Press: Thrumpton,

Nottingham, UK. pp. 295–321 (2005).https://digitalcommons.usu.edu/advs_facpub/490/

21. Ringot, D., Lerzy, B., Bonhoure, J.P., Auclair, E., Oriol, E. and Lanondelle, Y. Effect of temperature on *in vitro* ochratoxin A bio-sorption onto yeast cell wall derivate. *Process Biochemical*, **40**, 3008-3016 (2005).https://doi.org/10.1016/j.procbio.2005.02.006

22. Upadhyay, T., Fatima, N., Sharma, A., Sharma, D., and Sharma, R., Nano-Rifabutin entrapment within glucan micro-particles enhances protection against intracellular *Mycobacterium tuberculosis*. *Artificial Cells Blood Substitutes and Biotechnology* **47**, 427-435 (2019).https://doi.org/10.1080/21691401.2018.1559180.

23. Yiannikouris, A., Andre, G., Poughon, L., Fran¸cois, J., Dussap, C.-G., Jeminet, G., Bertin, G. and Jouany, J.-P., Chemical and conformational study of the interactions involved in mycotoxin complexation with â-D-glucans. *Biomacromolecules,* **7**:1147-1155 (2006).https://doi.org/10.1021/bm050968t

24. Soto, E.R. and Ostroff, G.R., Characterization of multilayered nanoparticles encapsulated in yeast cell wall particles for DNA delivery. *Bioconjugate Chemistry,* **19,** 840-848 (2008). https://doi.org/10.1021/bc700329p

25. Soto, E., Kim, Y.S., Lee, J., Kornfeld, H. and Ostroff, G., Glucan particle encapsulated rifampicin for targeted delivery to macrophages. *Polymers,* **2**, 681-689 (2010). https://doi.org/10.3390/polym2040681

26. Soto, E.R., Caras, A.C., Kut, L.C., Castle, M.K. and Ostroff, G.R., Glucan particles for macrophage-targeted delivery of nanoparticles. *Journal of Drug Delivery,* (2012). https://doi.org/10.1155/2012/143524

27. Hamza, Z., El-Hashash, M., Aly, S., Hathout, A., Soto, E., Sabry, B. and Ostroff, G. Preparation And Characterization Of Yeast Cell Wall Beta Glucan Encapsulated Humic Acid Nanoparticles As An Enhanced Aflatoxin B_1 Binder. *Carbohydrate Polymers*, **203**, 185-192 (2019). https://doi.org/10.1016/j.carbpol.2018.08.047

28. Blainski, A. Lopes, G.C., Palazzo de Mello, J.C.,.Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from *Limonium Brasiliense* L. *Molecules*, **18** (6), 6852-6865 (2013). https://doi.org/10.3390/molecules18066852

29. Klébert, S, Károly, Z, Késmárki, A, et al. Solvent□ and catalysts□free immobilization of tannic acid and polyvinylpyrrolidone onto PMMA surface by DBD plasma. *Plasma Process and Polymers*, **14** (19), 3-9 (2017) https://doi.org/10.1002/ppap.201600202

30. Loomis, W.D. and Battaile, J., Plant phenolic compounds and the isolation of plant enzymes. *Phytochemistry*, **5**, 423 (1966). https://doi.org/10.1016/S0031-9422(00)82157-3

31. Negm, N.A., El-Farrargy, A.F. and Mohammad, A., Synthesis and Inhibitory Activity of Schiff base Surfactants Derived from Tannic Acid against Bacteria and Fungi. *Egypt. J. Chem.***55** (4), 367-379(2012).https://doi.org/10.1007/s11743-013-1437-5

32. Luo, J., Zhang, N., Lai, J., Liu, R., Liu, X. and Hazard, J., Tannic acid functionalized graphene hydrogel for entrapping gold nanoparticles with high catalytic performance toward dye reduction. *Journal of Hazardous Materials,* **300**, 615-623 (2015). https://doi.org/10.1016/j.jhazmat.2015.07.079

33. Kaal, J., Nierop, K.G. and Verstraten, J.M., Retention of tannic acid and condensed tannin by Fe-oxide-coated quartz sand. *Journal of Colloid and Interface Science*, **287** (1), 72-79 (2005).

34. Lin B., Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. *Environmental Pollution*, **150**, 243–250 (2007). 10.1016/j.jcis.2005.01.104

35. Costa, E., Coelho, M., Ilharco, L.M., Aguiar-Ricardo, A. and Hammond, P.T., Tannic Acid Mediated Suppression of PNIPAAmMicrogels Thermo responsive Behavior. *Macromolecules*, **44** (3), 612–621 (2011).https://doi.org/10.1021/ma1025016

36. Kraal, P., Jansen, B., Nierop, K., and Verstraten, J., Copper complexation by tannic acid in aqueous solution. *Chemosphere*, **65**, 2193-2198 (2006).https://www.doi.org/10.1016/j.chemosphere.2006.05.058.

37. Takemoto, Y., Ajiro, H. and Akashi, M., Hydrogen-Bonded Multilayer Films Based on Poly (*N*-vinylamide) Derivatives and Tannic Acid. *Langmuir*, **31** (24), 6863–6869 (2015). https://doi.org/10.1021/acs.langmuir.5b00767

38. Ren, P.-F., Yang, H.-C., Liang, H., Xu, X.-L., Wan, L.-S.and Xu, Z.-K., Highly Stable, Protein-

Resistant Surfaces via the Layer-by-Layer Assembly of Poly (sulfobetaine methacrylate) and Tannic Acid. *Langmuir*, **31** (21), 5851–5858 (2015).https://doi.org/10.1021/acs.langmuir.5b00920

39. Kolosova, A., and Stroka, J. Evaluation of the effect of mycotoxin binders in animal feed on the analytical performance of standardised methods for the determination of mycotoxins in feed. *Food Additives and Contaminants*: Part A. **29**. 1959-1971 (2012). https://doi.org/10.1080/19440049.2012.720035

40. Avantaggiato, G., Solfrizzo, M. and Visconti, A., Recent advances on the use of adsorbent materials for detoxification of Fusarium mycotoxins. *Food Additives and Contaminants,* **22**, 379-388 (2005). https://doi.org/10.1080/02652030500058312

41. Jard, G., Liboz, T., Mathieu, F., Guyonvarch, A. and Lebrithi, A., Review of mycotoxin reduction in food and feed: from prevention in the field to detoxification by adsorption or transformation. *Food Additives and Contaminant*s, **28**, 1590-1609 (2011). https://doi.org/10.1080/19440049.2011.595377

42. Mezes, M., Balogh, K. and Tóth, K. Preventive and therapeutic methods against the toxic effects of mycotoxins—A review. *Acta Veterinaria Hungarica,* **58**, 1-17(2010).https://doi.org/10.1556/AVet.58.2010.1.1

43. Carrasco-Sánchez, V., Marican, A., Vergara-Jaque, A., Folch-Cano, C. and Comer, J., Polymeric substances for the removal of ochratoxin A from red wine followed by computational modeling of the complexes formed. *Food Chemistry*, (265), 159-164 (2018).https://doi.org/10.1016/j.foodchem.2018.05.089

44. Marican, A., Carrasco-Sánchez, V., John, A., Laurie, V.F., and Santos L.S. The binding of 4-ethylguaiacol with polyaniline-based materials in wines. *Food Chemistry*, (159), 486-492 (2014). https://doi.org/10.1016/j.foodchem.2014.03.053

45. Carrasco-Sánchez, V., John, A., Marican, A., Santos, L., and Laurie, F., Removal of 4-Ethylphenol and 4-Ethylguaiacol with Polyaniline-Based Compounds in Wine-Like Model Solutions and Red Wine. *Molecules*, **20** (8), 14312-14325 (2015).https://doi.org/10.3390/molecules22111890

46. Devegowda, G., Aravind, B. and Morton, M. *Saccharomyces cerevisiae* and mannan oligosaccharides to counteract aflatoxicosis in *broilers. Proceeding of the Australian Poultry Science Symposium,***103**, 106 (1996).

47. Klis, F M., Mol, P., Hellingwerf, K., and Brul. S. Dynamics of cell wall structure in Saccharomyces cerevisiae. *FEMS Microbiology Review,* **26**, 239-256 (2002).https://doi.org/10.1111/j.1574-6976.2002.tb00613.x

48. *Devegowda, G., Arvind, B.I. and* Morton, M.G., *Saccharomyces cerevisiae* and mannan oligosaccharide to counteract aflatoxicosis in broilers. In: *Processing Australian Poultry Science, Sydney, Australia.* **8**, 103-106 (1996). https://doi.org/10.1080/09712119.2004.9706502

التخلص الكيميائي للافلاتوكسين ب1 باستخدام البوليفينيل بيروليدون المكسبل كوسيله صديقه للبيئه

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الأفلاتوكسين هي مشتقات من مركبات الكومارين, تنتج كنواتج أيضيه ثانويه من فطر ينتمي إلي أجناس الأسبراجلس. الأفلاتوكسين بـــ (1) يعد من المواد المسرطنة القويه للكبد لكلا من الإنسان والحيوان وقد صنف كمجموعه أولي ضمن قائمة المواد المسرطنة لانسان. لذا كان الهدف من الدراسه تقدير متراكبات البوليفينيل بيروليدون مع حمض التانيك المكبسله داخل الجدار الخلوي للخميره علي ربط الافلاتوكسين في الوسط المعدي والمعوي. سجلت جزيئات (الجلوكان- المنان- الليبدات)GMLP المفصوله من الجدار الخلوي للخميره أعلي نسبة في امتصاص الافلاتوكسين في الوسط المعدي بعد 10ق وفي الوسط المعوي بعد 60 ق. جزيئات (الجلوكان- المنان- الليبدات) GMLP لها تجويف مسامي داخلي بقطر 4-3 ميكرون مما يجعلها توفر نظاما فعالا لتوليف وتغليف الجزيئات النانومترية التي لها القدره علي ربط الافلاتوكسين. تم تحرير 28% من حمض التانيك اثناء كبسلته داخل جزيئاتGMLP بعد عمليه الغسل ثلاث مرات بالماء, بينما تم تحرير 10 و 5.6 و 7.6 ٪ فقط من إجمالي حمض التانيك عند ارتباطه مع البوليفينيل بيروليدون باوزانه الجزيئة المختلفه (10 PVP و 360 و 1300kDa) المكبسله داخل جزيئات GMLP على التوالي. دعمت الصور المجهرية الفلورية بأن متراكبات البوليفينيل بيروليدون مع حمض التانيك تم كبسلتها بنجاح داخل GMLP وكانت اكثر ثباتا من المتراكبات الغير مكبسله. أظهرت النتائج أيضًا التركيبه GMLP PVP-TA NPs لها القدره علي امتصاص الافلاتوكسين في الوسط المعدي والمعوي مقارنه بجزيئات (GMLP) بمفردها.