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Colorimetric and chemiluminescence based enzyme linked apta-sorbent assay (ELASA) for ochratoxin A detection

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ABSTRACT

Ochratoxin A (OTA) is one of the most widespread mycotoxin found to contaminate various food products such as cereals, spices, groundnuts, coffee, wine, beer etc. It is also carried over from contaminated feed and fodder to milk, blood, meat, kidney and liver of animals consuming it. Enzyme-linked to biorecognition molecules like antibodies or aptamers are very popular due to their ability to be used as labels or tags in biosensing formats. In this work, OTA aptamer based colorimetric and chemiluminescence biosensing formats were evaluated for the detection of OTA. The colorimetric enzyme linked apta-sorbent assay (Co-ELASA) and chemiluminescence enzyme linked apta-sorbent assay (Cl-ELASA) showed a linear detection range from 1 pg/mL to 1 μg/mL with a limit of detection (LOD) of 0.84 pg/mL for Co-ELASA (limit of quantification (LOQ) = 2.54 pg/mL) and 1.29 pg/mL for Cl-ELASA (LOQ = 3.94 pg/mL) under optimized buffer conditions. Comparison of ELASA methods with sandwich ELISA indicated that the developed techniques had sensitivity similar to the conventional technique which indicated a LOD of 1.13 pg/mL and LOQ of 3.41 pg/mL. Studies in simulated contaminated food samples by spiking OTA in groundnut and coffee bean at concentrations of 0.1, 1 and 10 ppb, indicated recoveries in the range of 50.21 to 113.27% for Co-ELASA, 90.47 to 107.72% for Cl-ELASA and 76.23 to 141.49% for ELISA. Results of the study indicate that Co-ELASA and Cl-ELASA assays could be an alternate approach for ultrasensitive detection of OTA in food samples, which can also be adapted for biosensor development.

1. Introduction

Ochratoxins are a group of polyketide derived secondary toxic metabolite produced by various fungi of genera *Aspergillus*, *Penicillium* and *Fusarium*. Among all the ochratoxins, ochratoxin A (OTA) produced by *Penicillium verrucosum* and *Aspergillus ochraceus* is the most predominant [1]. OTA is found as a common contaminant in cereals, pulses, groundnuts, coffee, beer, wine and can also be carried over to blood, kidney, liver, meat and milk of animals fed with contaminated feed [2]. Due to its toxicological effects like neurotoxicity [3], nephrotoxicity [4], hepatotoxicity [5], teratogenicity [6] and immunotoxicity [7], OTA has been classified as a potential carcinogen (group 2B) for humans by the International Agency for Research on Cancer [8]. A maximum level for OTA of 15 μg/kg in spices and 20 μg/kg in liquorice was set by European Union [9]. The Codex Alimentarius commission has set a maximum level for OTA of 20 μg/kg in all spices [10]. Conventional analytical techniques like thin layer chromatography (TLC) [11], high-performance liquid chromatography (HPLC) [12], gas chromatography (GC) [13] and liquid chromatography-mass spectrometry (LC-MS) [14] are routinely used for the detection of OTA in real samples. Although these techniques are accurate and sensitive, they suffer from drawbacks like costly instrumentation, requirement of skilled personnel for handling and elaborate sample preparation steps including analyte derivatization and use of immunoaffinity columns for sample extraction and purification prior to HPLC. Molecular methods like polymerase chain reaction and immunoassays available for OTA detection, suffer from cross-reactivity, stability and cost of analysis [15,16].

Biosensors which use biological elements such as enzymes, antibodies, aptamers, whole cells, affimers etc. offer several diagnostic advantages like high sensitivity, selectivity, no (or less) sample clean-up and preparation steps, minimal matrix interference, etc. These biological molecules can easily be integrated to develop various biosensing formats such as optical platforms like colorimetric, fluorescence, luminescence based assays, electrochemical platforms such as amperometric, potentiometric assays, piezoelectric based assays, etc. [17]. Various technologies which use combination of biological molecules have been described for sensitive detection of numerous analytes. These include potentiometric immunoassay [18], microarray immunoassay

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[19], microfluidic immunoassay [20], magnetic immunoassay [21] etc. Combination of these technologies results in increase in sensitivity as well as selectivity of the developed assay.

Aptamers are short single-stranded oligonucleotide or peptide sequences that have high affinity, specificity and selectivity for the analyte against which it is generated. They are developed by an in vitro selection procedure known as systematic evolution of ligand by exponential enrichment (SELEX). Aptamers can conform into specific three dimensional structures, where target binding occurs through structural compatibility, stacking of aromatic rings, electrostatic interactions, Van der Waal forces, hydrogen bond formation etc. [17].

This study reports the development of two (aptamer-based) enzyme-linked apta-sorbent (ELASA) assays – colorimetric (Co) and chemiluminescence (Cl) for the detection of OTA, where OTA specific aptamer served as a capture agent in Co-ELASA and detection agent in Cl-ELASA. Both the Co-ELASA and Cl-ELASA were compared to sandwich ELISA for its sensitivity and recovery in food samples like coffee beans and groundnut. The main objective of the work was to compare aptamer based ELASA with antibody based ELISA and its potential to replace antibodies in typical immunoassay formats, either as capture agents or detection agents, without compromising on the sensitivity.

2. Experimental

2.1. Chemicals and reagents

OTA aptamer (5′GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGA CA3′) [22] and 5′ biotin modified OTA aptamer was synthesized by Sigma Chemicals, Bengaluru, India. Standards of OTA and aflatoxin B_1 (AFB_1) were procured from Himedia Laboratories Ltd. (Mumbai, India). Poly L-lysine, bovine serum albumin (BSA), luminol, urea-hydrogen peroxide, streptavidin-horse radish peroxide (HRP) conjugate, anti-rabbit goat secondary antibody alkaline phosphatase (ALP) conjugate, aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) standards were purchased from Sigma-Aldrich (Bangalore, India). Para nitrophenyl phosphate (*p*-NPP) was procured from SRL (Mumbai, India). Anti-OTA rabbit polyclonal antibodies were obtained from Abcam (Kolkata, India). Microtitre plate (96 well) were purchased from NUNC, Roskilde, Denmark. Other analytical grade chemicals were procured from Merck, Himedia Laboratories (Mumbai, India) and SRL (Mumbai, India).

2.2. Colorimetric ELASA assay (Co-ELASA)

Poly-L-lysine was coated on 96 well microtiter plates for overnight at 4 °C. The plates were then washed with Tris hydrochloride-tween 20 (Tris-T) buffer, followed by addition and incubation of OTA aptamer at 37 °C for 1 h. To reduce the background interference, blocking agent was added to wells and incubated for another hour. After washing with Tris-T, analyte OTA was added and incubated at 37 °C for 1 h. Anti-OTA IgG rabbit antibodies were then added and incubation was proceeded for next 1 h. After a Tris-T wash, anti-rabbit goat secondary antibody conjugated with ALP was added and incubated for 37 °C for 1 h. Colour was developed using *p*-NPP as substrate and 3 M NaOH as stop solution. The colored product was measured at 405 nm using Infinite Pro 200 ELISA Reader. For the optimization studies of the Co-ELASA assay, absorbance values were plotted after subtracting the absorbance readings of control wells without target. All the experiments were performed in duplicates.

2.3. Chemiluminescence ELASA assay (Cl-ELASA)

96 well microtiter plate was coated with anti-OTA IgG antibodies for overnight at 4 °C. The plates were then washed with phosphate buffer-tween 20 (PB-T). To reduce the background interference, blocking agent was added to wells and incubated at 37 °C for 1 h. After PB-T wash, analyte OTA was added and incubated for another hour. Analyte addition was followed by the addition of biotinylated OTA aptamer which was incubated at 37 °C for 1 h. After washing with PB-T, streptavidin-HRP conjugate was added and incubation was proceeded at 37 °C for 1 h. Luminescence was observed after the addition of substrate luminol along with urea-hydrogen peroxide. Luminol was dissolved in dimethyl sulfoxide whereas urea-hydrogen peroxide was dissolved in buffer. To 70 μL of buffer, 20 μL of 5 mM luminol and 10 μL of 1 mM urea-hydrogen peroxide was added before luminescence measurement. The assay was performed in duplicates and the absence of analyte to particular wells served as a negative control. The relative luminescence unit (RLU) was measured using the Varioskan Flash microplate reader.

2.4. Sandwich ELISA

96 well microtiter plate was immobilized with anti-OTA IgG capture antibody for overnight at 4 °C. The capture antibody was removed and the plate was washed with Tris-T wash buffer. The vacant sites were then blocked with 0.2% BSA for 1 h. After Tris-T wash, analyte OTA was added and incubated at 37 °C for 1 h. Anti-OTA IgG detection antibody was added and incubation was carried out for another hour. After washing with Tris-T, anti-rabbit goat secondary antibody conjugated with ALP was added and incubated at 37 °C for 1 h. Colour was developed using *p*-NPP as substrate and 3 M NaOH as stop solution. The colored product was measured at 405 nm. The assay was performed in duplicates.

2.5. Calibration curve

The calibration curve for Co-ELASA and sandwich ELISA assays were plotted by considering OTA concentrations (1 pg -1 μg) on the x-axis against percent inhibition on the y-axis. Percent inhibition was calculated by the following formula.

$$\text{Percent Colour} = \frac{100 \times \text{OD sample}}{\text{OD control}}$$

$$\text{Percent Inhibition} = 100 - \text{Percent colour}$$

The calibration curve for Cl-ELASA assay was plotted with OTA concentrations (1 pg – 1 μg) on the x-axis against relative luminescence units (RLU) on the y-axis. LOD and LOQ was calculated using S/N ratio of 3 [23].

2.6. Food sample preparation

Groundnut and coffee samples in this study were prepared according to our previous reported protocol [24]. Briefly, the samples were extracted using 80% methanol. The final concentrated sample for Co-ELASA as well as sandwich ELISA was suspended in Tris buffer (pH -9), while samples for Cl-ELASA was taken in phosphate buffer (pH -7.2). All the samples were 0.45 μm filtered, prior to further analysis.

2.7. Cross-reactivity

Specificity studies were carried out with toxins from the aflatoxin group namely AFB_1, AFB_2, AFG_1 and AFG_2. All toxin standards were prepared at concentrations of 100 ng/mL. Toxin dilutions were prepared in Tris buffer (pH -9) for Co-ELASA and phosphate buffer (pH -7.2) for Cl-ELASA.

3. Results and discussion

3.1. Detection principle

The Co-ELASA assay was based on the principle of target capture by aptamer where, OTA specific aptamer was used for detection of toxin.

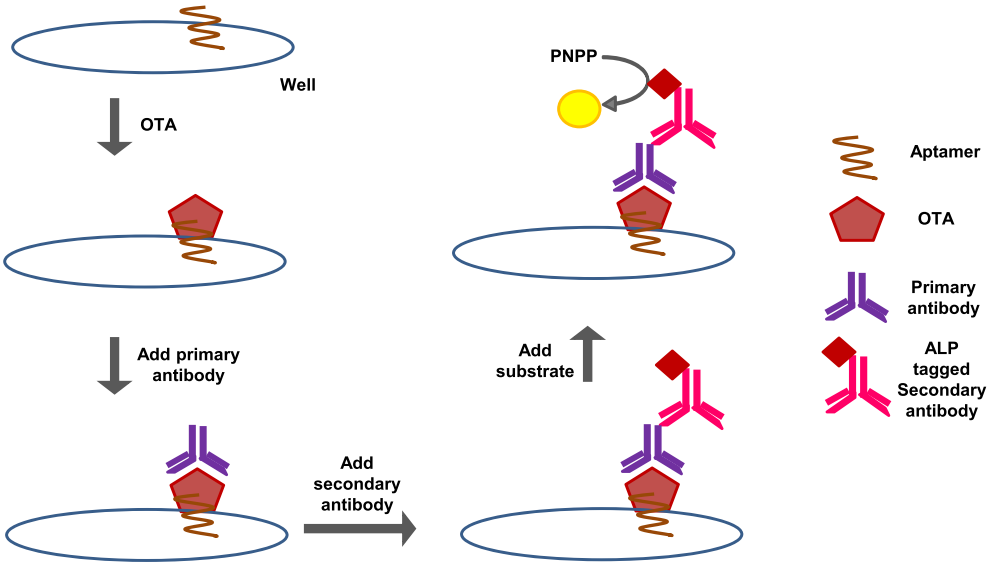

Fig. 1. Schematic representation of the colorimetric ELASA assay.

This was followed by the addition of anti-OTA IgG primary antibody and anti-rabbit ALP tagged secondary antibody, which served as detection agents. The colour development was aided by the addition of *p*-NPP as substrate, which reacted to form a yellow colour product, the absorbance of which was measured at 405 nm (Fig. 1). The Cl-ELASA assay is based on the principle of antibody based target capture where, anti-OTA IgG rabbit antibody was used to capture OTA toxin. OTA capture was followed by the addition of biotin labelled OTA aptamer and streptavidin-HRP, which served as a detection agent. Luminescence was observed after the addition of luminol and urea-hydrogen peroxide (Fig. 2).

3.2. Optimization of assay conditions

3.2.1. Aptamer concentration

Polycationic nature of poly L-lysine makes it an attractive molecule for biomolecule attachment. The positively charged amine groups in poly L-lysine electrostatically bind to the negatively charged DNA backbone. This principle was adopted for binding of OTA aptamers to microtiter plates [25]. Aptamer in Co-ELASA determines the effective capture of analyte, whereas in Cl-ELASA, biotin labelled OTA aptamer served as a detection agent, thus concentrations of OTA aptamer was optimized separately for both these methods. Concentrations of aptamers in the range of 0.2–1 μM were varied, keeping all other variables like blocking agent, primary antibody, secondary antibody and streptavidin-HRP constant. As indicated in Fig. 3A, aptamer concentration of 1 μM resulted in maximum absorbance values in Co-ELASA assay. The higher concentrations of aptamer for the assay may be attributed to requirement to completely cover the surface of the plate for effective capture. Concentrations below 1 μM showed lesser absorbance values apparently due to inefficient capture. According to Fig. 3B, a concentration of 0.4 μM was sufficient to bind to OTA and resulted in maximum luminescence in Cl-ELASA assay. Concentration <0.4 μM resulted in insufficient

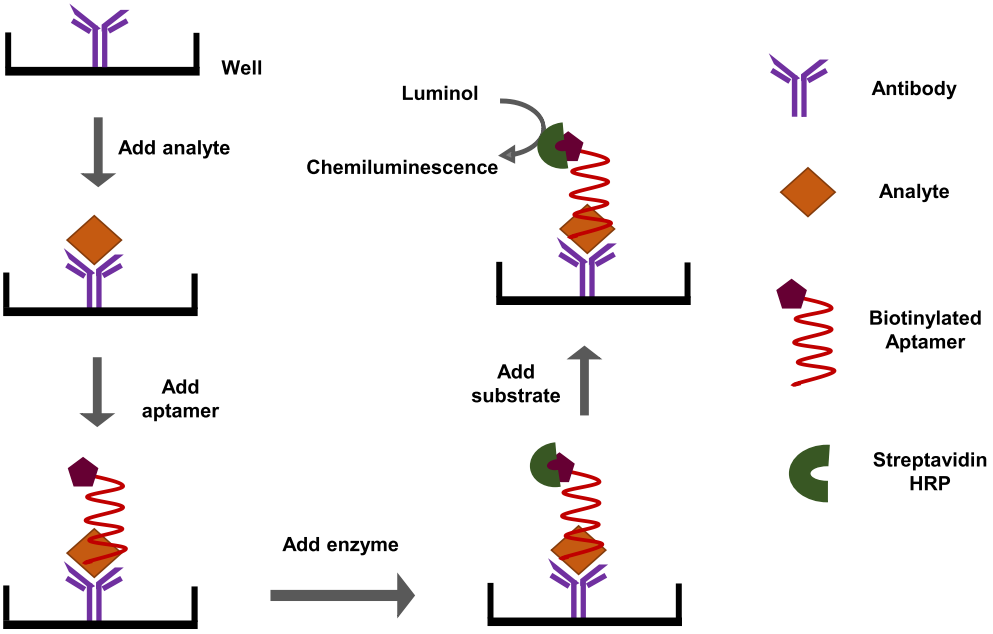

Fig. 2. Schematic representation of the chemiluminescence ELASA assay.

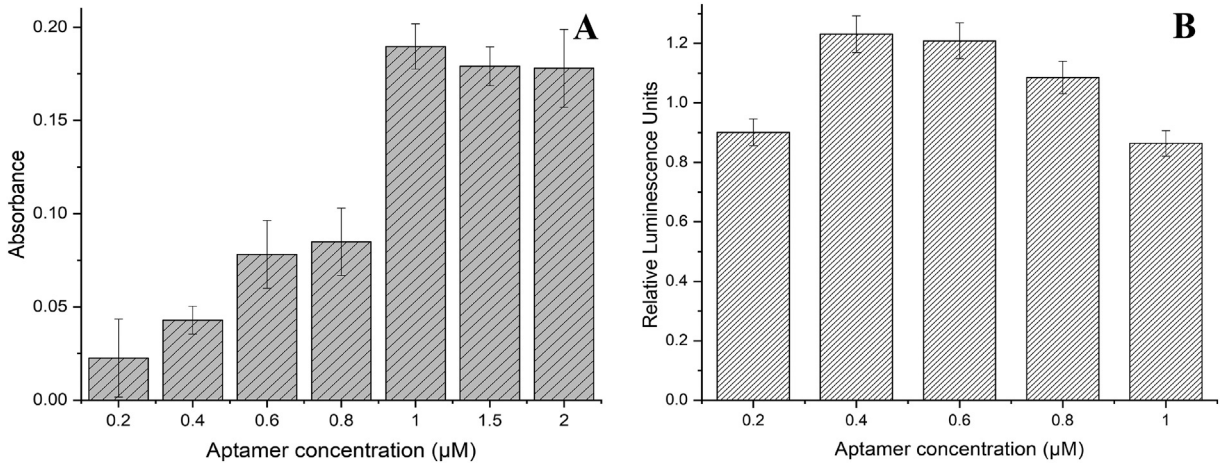

Fig. 3. (A) Absorbance values for optimization of aptamer concentration (0.2, 0.4, 0.6, 0.8, 1, 1.5 and 2 μM) for Co-ELASA assay. (B) Relative luminescence values for optimization of aptamer concentration (0.2, 0.4, 0.6, 0.8 and 1 μM) for Cl-ELASA assay.

binding of aptamer to OTA while concentrations above 0.4 μM effected the OTA-aptamer complex formation resulting in decrease in luminescence value. Therefore, 1 μM of OTA aptamer and 0.4 μM biotin OTA aptamer were used for further experiments on Co-ELASA and Cl-ELASA assays, respectively.

3.2.2. Blocking agent

Plate assays often include protein antibodies or targets immobilized to plastic surfaces through non-specific binding. Binding of non-specific protein compounds appearing during the consequent steps may lead to decline in sensitivity, specificity and can also give false positive results

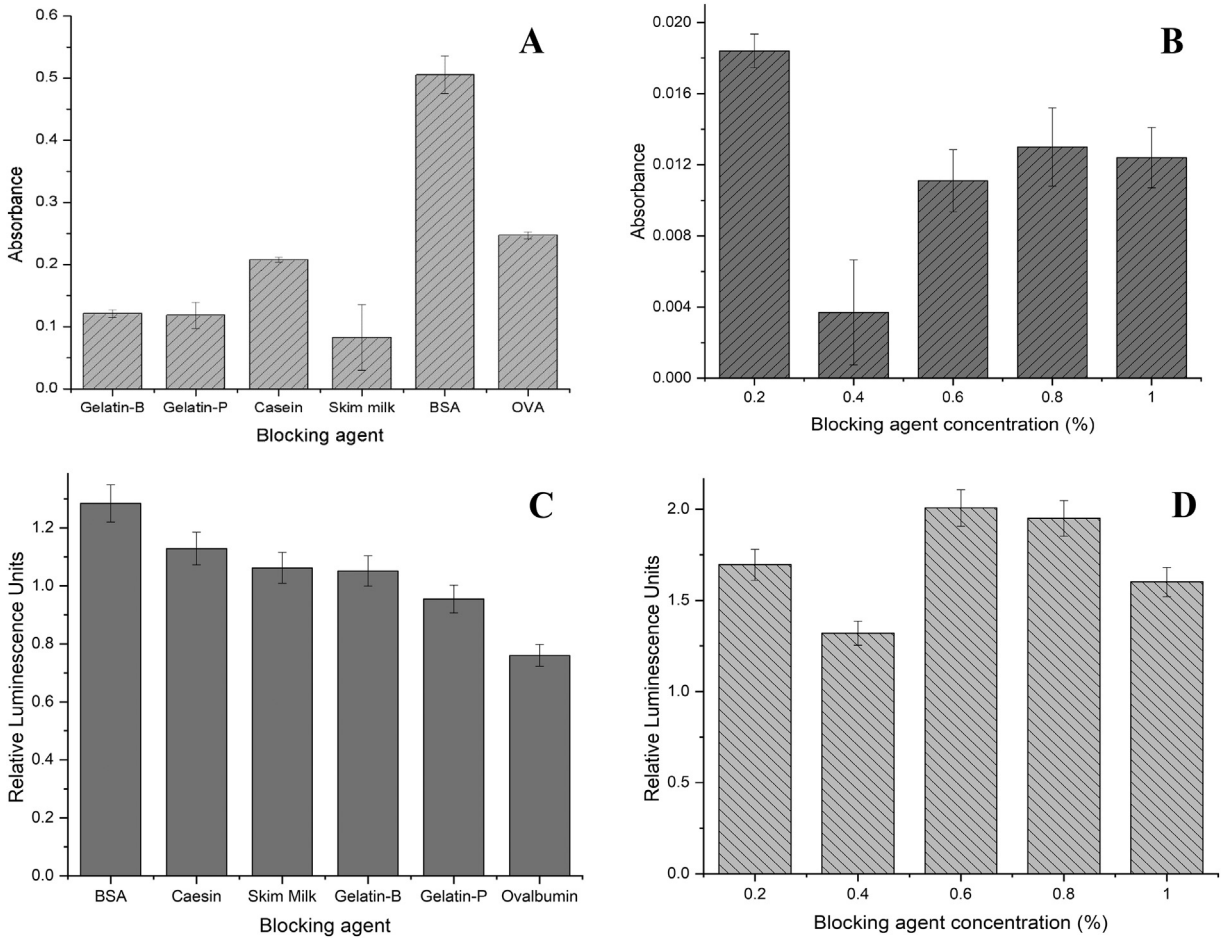

Fig. 4. (A) Absorbance values for optimization of different blocking agents (gelatin bacteriology (gelatin-B), gelatin from porcine skin (gelatin-P), skim milk, bovine serum albumin (BSA) and ovalbumin A (OVA)) for Co-ELASA assay. (B) Optimization of blocking agent BSA at various concentrations (0.2, 0.4, 0.6, 0.8 and 1%) for Co-ELASA assay. (C) Relative luminescence values for optimization of different blocking agents (gelatin-B, gelatin-P, skim milk, BSA and OVA) for Cl-ELASA assay. (D) Optimization of blocking agent BSA at various concentrations (0.2, 0.4, 0.6, 0.8 and 1%) for Cl-ELASA assay.

[26]. Therefore, to avoid this undesirable binding to vacant sites, the remaining adsorptive surface of the plastic wells were treated with blocking proteins. In this study, common blocking proteins used in plate assays like gelatin bacteriology grade (gelatin-B), gelatin from porcine skin (gelatin-P), skim milk, bovine serum albumin (BSA) and ovalbumin A (OVA) were used. In Co-ELASA and Cl-ELASA assays, among all the blocking agents, BSA showed optimum results by effectively blocking the vacant sites on microtiter plates (Fig. 4A & C). Further, the concentration of BSA was also tested in the range of 0.2–1% where, a concentration of 0.2% for Co-ELASA and 0.6% for Cl-ELASA was adequate to saturate vacant sites (Fig. 4B & D). BSA concentration above 0.2% in Co-ELASA resulted in lower absorbance value probably due to blocking of specific aptamer-target interaction [27]. Thus, all further experiments were carried out using BSA at 0.2% for Co-ELASA and 0.6% for Cl-ELASA.

3.2.3. Buffer pH

pH plays an important role in aptamer-target interactions where, it effects the aptamer binding site and its consequent binding to the target molecule [28]. pH also effects antibody-target interactions, where it influences protein-protein interaction, thermal unfolding transition and colloidal stability of the complex [29]. In this work, buffers of different pH ranging from 5.6 to 11 were tested. Acetic acid/sodium acetate (pH 5.6), phosphate buffer (PB) (pH 7.2), HEPES (pH 7.4), tris-HCl (pH 9), and carbonate/bicarbonate (pH 11) buffers were used in this study. According to Fig. 5A, tris-HCl buffer (pH 9) gave the maximum absorbance compared to control samples in Co-ELASA assay, whereas as shown in Fig. 5C, PB buffer (pH 7.2) showed the maximum luminescence value for Cl-ELASA assay. This behaviour can be corroborated to the fact that optimum amount of aptamer-OTA and IgG-OTA complexes were formed at pH 9 and 7.2, which resulted in higher absorbance/luminescence values. As shown in Fig. 5B & D, tris-HCl and PB buffer concentrations were also optimized in the range of 5–100 mM where, 10 mM tris-HCl (pH 9) and 75 mM PB buffer (pH 7.2) were found to be more effective. Concentrations of 10 mM and 75 mM resulted in maximum absorbance/luminescence and concentrations below or above this might not be effective in stabilizing the aptamer-OTA or IgG-OTA complex. Therefore, 10 mM tris-HCl (pH 9) and 75 mM PB (pH 7.2) buffer were chosen as optimal buffer for further experiments.

3.2.4. Antibody

Antibodies in Co-ELASA assay act as the detection molecule for OTA. Different concentrations of anti-OTA IgG rabbit antibody and anti-rabbit ALP tagged secondary antibody were optimized. As shown in Fig. 6A, anti-OTA IgG antibody was optimized in the range of 1:1000–1:10000 dilution (stock solution of 5 mg/mL) where, 1:2500 dilution showed the best result. This dilution of primary antibody was sufficient enough to detect OTA and dilutions below or above this could not efficiently bind to OTA, resulting in lower absorbance values. Similarly, anti-rabbit ALP tagged secondary

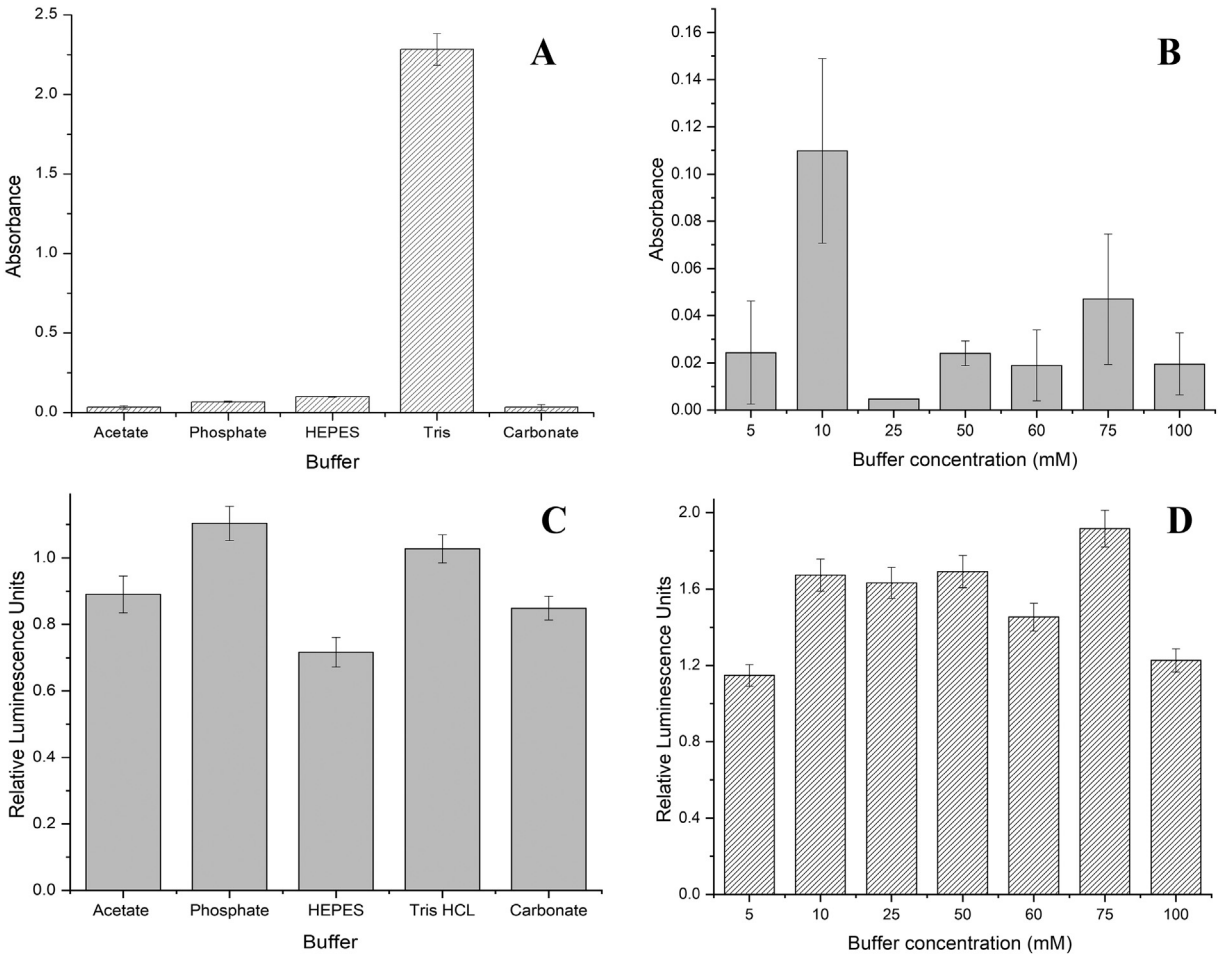

Fig. 5. (A) Absorbance values for optimization of different buffers (acetate (pH 5.6), phosphate (pH 7.2), HEPES (pH 7.4), tris-HCl (pH 9) and carbonate (pH 11)) for Co-ELASA assay. (B) Optimization of tris-HCl buffer at various concentrations (5, 10, 25, 50, 60, 75 and 100 mM) for Co-ELASA assay. (C) Relative luminescence values for optimization of different buffers (acetate (pH 5.6), phosphate (pH 7.2), HEPES (pH 7.4), tris-HCl (pH 9) and carbonate (pH 11)) for Cl-ELASA assay. (D) Optimization of phosphate buffer at various concentrations (5, 10, 25, 50, 60, 75 and 100 mM) for Cl-ELASA assay.

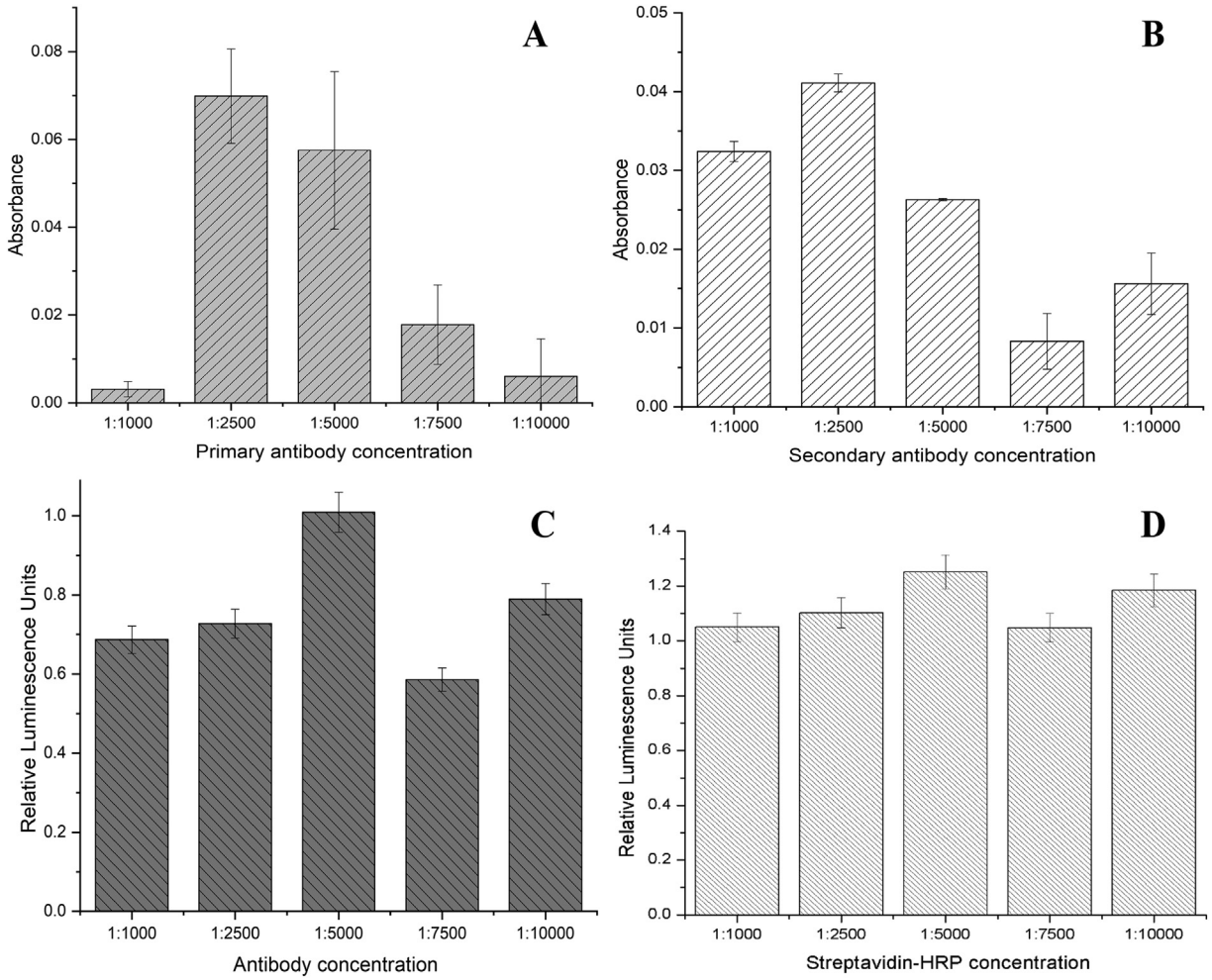

Fig. 6. (A) Absorbance values for optimization of anti-OTA rabbit antibody concentrations (1:1000, 1:2500, 1:5000, 1:7500 and 1:10000) for Co-ELASA assay. (B) Absorbance values for optimization of anti-rabbit ALP tagged secondary antibody concentrations (1:1000, 1:2500, 1:5000, 1:7500 and 1:10000) for Co-ELASA assay. (C) Relative luminescence values for optimization of anti-OTA rabbit antibody concentrations (1:1000, 1:2500, 1:5000, 1:7500 and 1:10000) for Cl-ELASA assay. (D) Relative luminescence values for optimization of streptavidin-HRP conjugate concentrations (1:1000, 1:2500, 1:5000, 1:7500 and 1:10000) for Cl-ELASA assay.

antibody was also optimized in the range of 1:1000–1:10000 dilution. According to Fig. 6B, 1:2500 dilution of secondary antibody was adequate to detect anti-OTA primary antibody. Hence, all further experiments of Co-ELASA were performed using 1:2500 dilution of anti-OTA IgG primary antibody and anti-rabbit ALP tagged secondary antibody. In Cl-ELASA assay, anti-OTA IgG rabbit antibody served as capture molecule, thus, different concentrations of anti-OTA primary antibody was optimized. From Fig. 6C, out of all dilutions, 1:5000 dilution resulted in maximum luminescence, probably due to optimum capture of analyte as well as maximum well coverage. Therefore, 1:5000 dilution was selected for further Cl-ELASA experiments.

3.2.5. Streptavidin-horse radish peroxidase

Streptavidin-HRP conjugate was used as a signal generator, where HRP acts as a catalyst for the reaction between hydrogen donor (luminol) and hydrogen acceptor (hydrogen peroxide) [30]. This reaction results in the formation of a product 3-aminopthalate, which gives off a bright blue-green luminescence [31]. Various dilutions of streptavidin-HRP were tested in the range of 1:1000–1:10000. According to Fig. 6D, 1: 5000 dilution exhibited the maximum luminescence, as this concentration may be optimal for the oxidation of luminol to 3-aminopthalic acid. Hence, 1: 5000 dilution of streptavidin-HRP was used for further experiments of Cl-ELASA.

3.3. Calibration curve

The efficiency of the Co-ELASA and Cl-ELASA to detect OTA was studied under optimized conditions in the range of 1 pg to 1 μg (per mL) of analyte concentration. The LOD of OTA for Co-ELASA assay was determined to be 0.84 pg/mL with a LOQ of 2.54 pg/mL. In the Cl-ELASA assay, the LOD was found to be 1.29 pg/mL, and no considerable change in RLU was observed below this concentration. The LOQ of Cl-ELASA was found to be 3.94 pg/mL. Results indicate that both the assays showed linearity over a wide range (1 pg/mL to 1 μg/mL) with appreciable regression coefficient of 0.9757 ($n = 6$), 0.977 ($n = 6$) for Co-ELASA and Cl-ELASA assay, respectively (Fig. 7A, B & C).

The performance and sensitivity of the Co-ELASA and Cl-ELASA assay was compared to conventional sandwich ELISA method. The comparison study was carried out using OTA standards in the range of 1 pg to 100 ng (per mL) made in 10 mM tris-HCl buffer. The LOD for ELISA was found to be 1.13 pg/mL with LOQ of 3.41 pg/mL. The standard curve showed linearity in the range of 10 pg/mL to 100 ng/mL with a regression coefficient of 0.9517 ($n = 6$) (Fig. 7D). Comparing all the three methods, we found out that our Co-ELASA and Cl-ELASA assay had broader detection range and comparable sensitivity to sandwich ELISA method. The presence of dual biorecognition molecules i.e. aptamer and antibody, can be associated with the improvement in sensitivity of both the ELASA assays. Detection studies of several analytes also

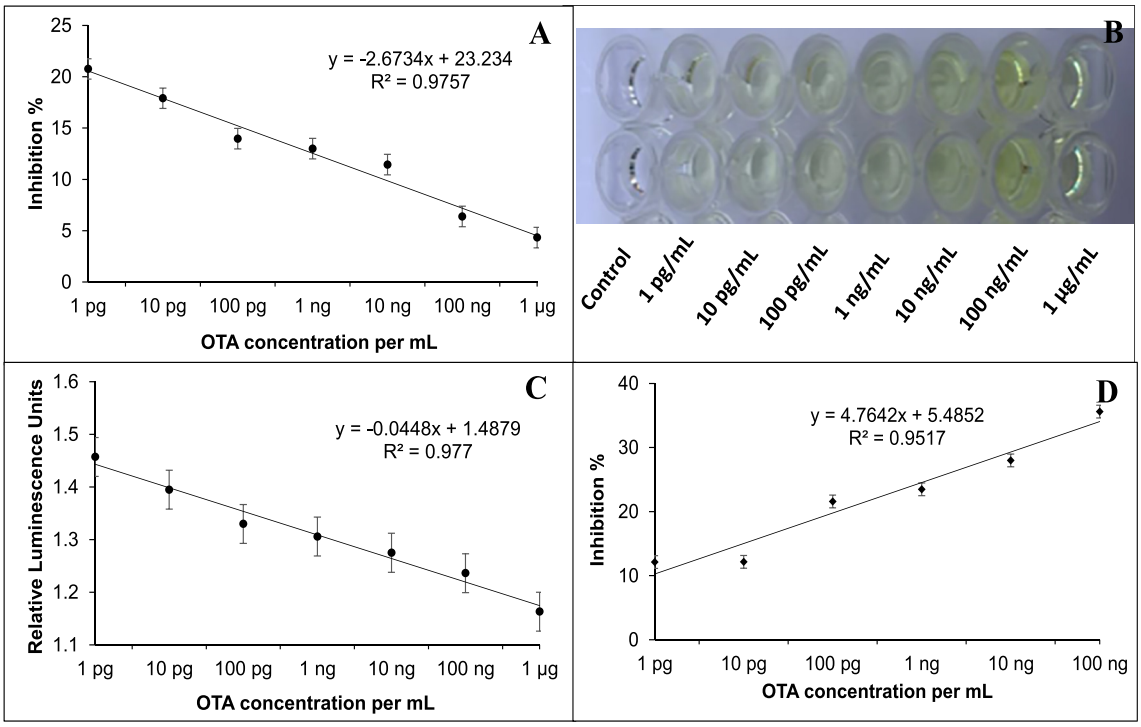

Fig. 7. (A) Calibration curve of Co-ELASA assay for OTA (1 pg to 1 µg) spiked in 10 mM tris-HCl (pH 9) buffer. (B) Microplate wells with different concentration of OTA in Co-ELASA assay. (C) Calibration curve of Cl-ELASA assay for OTA (1 pg to 1 µg) spiked in 75 mM phosphate (pH 7.2) buffer. (D) Calibration curve of sandwich ELISA assay for OTA (1 pg to 100 ng) spiked in 10 mM tris-HCl (pH 9) buffer.

reveal that aptasensor offer better sensitivity and lower detection limits and can be good alternative technique to ELISA [32,33].

3.4. Recovery studies

Food commodities like groundnuts and coffee beans often contain OTA as a major contaminant [34,35], therefore, these food samples were preferred for our recovery study. Groundnut and coffee bean samples were spiked at concentrations of 0.1 ppb, 1 ppb and 10 ppb for this study. The recovery study was carried out under optimized condition and was also compared with conventional sandwich ELISA for its performance. According to Table 1, for Co-ELASA assay, recovery of OTA in spiked samples varied from 90.80 to 107.85% in groundnut and 50.21 to 113.37% in coffee bean samples. For Cl-ELASA assay, recovery varied in the range of 94.85 to 107.72% in groundnut and 90.47 to 107.60% in coffee bean samples. In comparison, the conventional ELISA method showed a recovery of 83.37 to 141.49% in groundnut and 76.23 to 90.88% in coffee bean samples. Out of all the three assays, Cl-ELASA assay showed the best recovery results, where recovery of 90.47 to 107.72% was achieved for both the samples. At lower concentration of 0.1 ppb of groundnut, both Co-ELASA and Cl-ELASA showed better recovery than ELISA. Lower recovery of 50.21% was only achieved at 0.1 ppb of coffee bean (Co-ELASA), which may be due to matrix interference. All other sample concentrations of Co-ELASA showed comparable recovery to that of ELISA method.

3.5. Cross reactivity studies

Specificity of the Co-ELASA and Cl-ELASA assay was tested with other commonly occurring mycotoxins such as the aflatoxin group. Cross-reactivity was analysed with AFB_1, AFB_2, AFG_1 and AFG_2 and was measured in terms of percent inhibition of activity. According to Fig. 8A & B, the OTA aptamer showed less than 40% cross-reactivity with aflatoxins for Co-ELASA assay, whereas the biotin OTA aptamer showed less than 35% cross-reactivity with aflatoxins for Cl-ELASA assay. This indicated the specificity of the Co-ELASA and Cl-ELASA assays for selective detection of OTA.

4. Conclusion

OTA, which is one of the most commonly occurring mycotoxin in food and feed, requires early detection in order to avoid health hazards. Herein, we developed two assays i.e. Co-ELASA and Cl-ELASA for the detection of OTA using OTA specific aptamers as capture and detection agents, respectively. Although both these methods use antibodies, the use of aptamer as capture or detection agent replaces the use of hapten

Table 1
Recovery of spiked OTA (0.1 ppb, 1 ppb and 10 ppb) in food samples (groundnut and coffee beans) using Co-ELASA, Cl-ELASA and sandwich ELISA assays.

Sample	Spiked (ppb)	Co-ELASA (ppb)	% recovery	Cl-ELASA (ppb)	% recovery	ELISA (ppb)	% recovery
Groundnut	10	10.79 ± 0.43	107.85	10.44 ± 2.08	104.38	8.73 ± 2.69	87.3
	1	0.91 ± 0.39	90.80	0.95 ± 0.62	94.85	0.83 ± 0.24	83.37
	0.1	0.09 ± 0.03	90.83	0.11 ± 0.03	107.72	0.14 ± 0.09	141.49
Coffee beans	10	8.96 ± 1.53	89.63	9.75 ± 3.19	97.46	8.97 ± 2.15	89.73
	1	1.13 ± 0.74	113.37	0.90 ± 0.12	90.47	0.76 ± 0.2	76.23
	0.1	0.05 ± 0.007	50.21	0.11 ± 0.01	107.60	0.09 ± 0.03	90.88

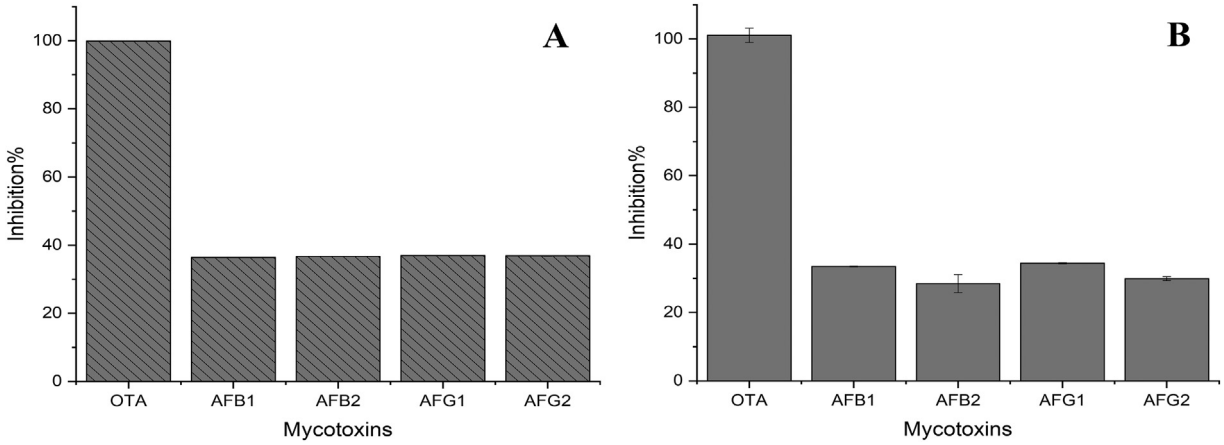

Fig. 8. Cross reactivity of (A) Co-ELASA assay (B) Cl-ELASA assay with aflatoxins like AFB_1, AFB_2, AFG_1 and AFG_2.

or antibody in conventional immunoassays where immunoreceptor generation is tedious as well as costly. Both the assays showed a wide detection range from lower picogram to microgram levels. The Co-ELASA and Cl-ELASA assays showed comparable sensitivity to conventional ELISA. Recovery studies in groundnut and coffee bean showed comparable results between Co-ELASA and ELISA, whereas Cl-ELASA had better recovery than ELISA. Therefore, both the Co-ELASA and Cl-ELASA assays can be used as a replacement for ELISA in screening OTA contaminated food samples. The principle of detection used for both the assays can also be applied to detect other analytes at ultrasensitive level.

CRediT authorship contribution statement

Monali Mukherjee: conceptualization, visualization, investigation, data curation, formal analysis, methodology and manuscript original draft, **C. Nandhini:** investigation, formal analysis and methodology, **Praveena Bhatt:** conceptualization, supervision, resources, project administration, validation, reviewing and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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