Chapter-4

Nano-RNA aptamer based biosensing for analysis of vitamin $B_{12}$
4.1. INTRODUCTION

Aptamers (DNA or RNA) have attracted an increasing amount of interest in development of sensors for drugs, proteins, amino acids, organic, inorganic molecules and even supramolecular complexes such as viruses or cells (Luzi et al., 2003; You et al., 2003). Aptamers have potential application as recognition element in analytical and diagnostic assays, as aptamers can be easily screened, designed and evolved in vitro selection process known as systematic evolution of ligands by exponential enrichment (SELEX) (Ellington and Szostak, 1992; Herman and Patel, 2000). Relative to antibodies, aptamers have several advantageous properties, including stability, low cost, and ease of synthesis (Sumedha et al., 1999).

Natural RNA or DNA is nuclease sensitive, therefore, nuclease resistant oligonucleotides are generated for wide applications in biosensor. To generate nuclease-resistant oligonucleotide libraries, we used modified RNA in which the 2'-hydroxy (-OH) group in the pyrimidine nucleotides was substituted with the fluoro (F') functionality. This modification is known to impart a substantial degree of mechanism-based protection against the majority of endo and ribonucleases (Pieken et al., 1991). Importantly, the 2'-fluoro substitution is compatible with the enzymatic steps of SELEX. Thus, SELEX experiments with libraries carrying such modifications can lead to nuclease resistant ligands. 2' fluoro modified RNA aptamers potentially have more rigid structures than 2' amino aptamers because of their stronger intramolecular helices than 2' amino RNA leading to thermodynamically stable secondary structure and higher affinities.

We have chosen to focus on the vitamin B\textsubscript{12} specific aptamer isolated by Lorsch and Szostak (1994) as a model system for biosensor development. Adenosylcobalamin (the biologically active form of vitamin B\textsubscript{12}) is believed to be one of the most evolutionarily ancient enzyme cofactors and has been postulated to have played a key role in the transition from RNA-based biology to modern DNA and protein-dominated biology (Benner et al., 1989). Using in vitro selection from a pool of $10^{15}$ random-sequence molecules, Lorsch and Szostak were able to isolate one RNA molecules with 35-nucleotide...
pseudoknot that specifically recognize vitamin B$_{12}$ only. The aptamer binds cyanocobalamin with relatively high affinity (Kd approx. 90nM), suggesting a large number of specific interactions stabilize the RNA ligand complex. The aptamer requires 1 M lithium chloride for proper folding and binding because lithium ions form tight complexes with phosphates leads to covalent binding than ionic pairing (Cotton and Wilkinson, 1988). Further lithium ions helps in neutralization of charge backbone of RNA enabling RNA to fold into a more compact and stabilized structure.

Followed by its evolutionary importance, vitamin B$_{12}$ is naturally gifted biomolecule with carbon-metal bond in foods and medicines. Natural and commercially known cyanocobalamin is a stable form of vitamin B$_{12}$. The conventional methods are time consuming, nonspecific, low sensitive and expensive. To overcome these issues, aptamers offer themselves as ideal candidates for use in biosensor applications. The potential use of aptamers in biosensor applications is further enhanced upon their conjugation to nanoparticles.

Colorimetric aptasensor using gold nanoparticles (GNPs) have been considered as an on-site detection method with high specificity and sensitivity, because of its easy preparation, simple operation, and detection of its colorimetric signal with naked eye (Cho et al., 2008). GNPs were extensively used as important colorimetric materials because they have strong distance-dependent optical properties that are used for aptamer based biosensors. Zhao and co-workers (Zhao et al., 2007) and Wang and co-workers (Wang et al., 2006) have detected adenosine and potassium ions separately based on a non-crosslinking mechanism of GNPs aggregation followed by Lu’s group (Liu and Lu, 2004; Liu and Lu, 2006; Liu et al., 2006) that worked on detection of adenosine and cocaine based on a crosslinking mechanism of GNPs by hybridizing aptamers with complementary sequences. Similarly, Pavlov and co-workers (Pavlov et al., 2004) have used aptamer- functionalized GNPs as a catalytic label for amplified detection of thrombin in solution and on surface.
Recently, Song and co-workers focused on colorimetric detection of kanamycin using GNPs and aptamers (Song et al., 2011). There are several reports on successful DNA aptamer-based sensors using GNPs. In contrast, only one RNA aptamer-based sensors for theophylline has been developed which suffers from low stability (Elena et al., 2008) and further improvement in the stability of RNA aptamer is done in the present report by 2’-fluoro modifications for vitamin B\(_{12}\) detection. RNA aptamer for vitamin B\(_{12}\) is known and hence it provided starting point for our studies. In general, RNA forms diverse 3D structures formation with high affinity and specificity and makes screening procedure easier for isolation of RNA aptamers from RNA library (Gopinath, 2007). In the proposed colorimetric aptasensor, there is a target-specific aggregation of GNPs due to interaction of aptamers to analyte after physical adsorption of aptamers on GNPs (Rothberg, 2004). We have successfully conducted the target vitamin B\(_{12}\) specific aggregation of GNPs as a result of aptamer–vitamin B\(_{12}\) interaction.

### 4.2. MATERIALS AND METHODS.

#### 4.2.1. Reagents

Vitamin B\(_{12}\) RNA aptamer sequence (5’ GGA ACC GGU GCG CAU AAC CAC CUC AGU GCG AGC AA 3’) was adapted from previous report (Lorsch and Szostak, 1994). Further, all pyrimidines are 2’-flouro modified and obtained from Trilink Biotechnologies, San Diego, CA. Vitamin B\(_{12}\), diethylpyrocarbonate (DEPC), gold (III) chloride, tri sodium citrate, and silver nitrate were procured from Sigma Aldrich chemicals, USA. All stock solutions were prepared in DEPC treated water and further diluted accordingly.
4.2.2. Apparatus and principle of detection of vitamin B$_{12}$ using proposed method.

Spectral analysis of GNPs was taken in the range from 300 to 700 nm using spectrophotometer UV-1601 (Shimadzu, Japan). Fluorimetric analysis of aptamer was taken in the emission range from 250-500 nm at an excitation of 260 nm using spectrofluorometer RF-5301 PC (Shimadzu, Japan).

We demonstrate that color changes of GNPs which are visible with the naked eye can be used as probe for the detection of vitamin B$_{12}$. Solutions of GNPs are red colored due to their specific and size-dependent surface plasmon resonance (SPR) absorption at 520 nm. Addition of salt screened electrostatic repulsion between negatively charged GNPs, and resulted in aggregation of GNPs that led to red-to-purple color change and an additional absorption was observed above 600 nm. We then treated GNPs with the vitamin B$_{12}$ RNA aptamer for 15 min, in the presence and the absence of vitamin B$_{12}$. Upon the addition of salt, the former solution showed a color change from red to purple, while the latter retained its original red color as shown in scheme-4. Aggregation of GNPs was specifically induced by desorption of the vitamin B$_{12}$ binding RNA aptamer from the surface of GNPs as a result of the aptamer target interaction, leading to the color change from red to purple.
Scheme 4. Scheme of the vitamin B$_{12}$ RNA aptamer and colorimetric detection of vitamin B$_{12}$-induced structural variation.

4.2.3. Solution preparation

A stock solution of vitamin B$_{12}$ was prepared by dissolving 1 mg of pure crystalline B$_{12}$ in 1 ml of B$_{12}$-binding buffer (Na-HEPES, pH 7.4). A stock solution of 1mM RNA aptamer was prepared by dissolving 11 mg of desalted RNA aptamer in 1 ml of DEPC water. Further dilutions of this stock solution were prepared in HEPES buffer to obtain 5 µM to 100 µM aptamer concentrations. An aqueous solution of monodisperse quasi-spherical GNP's was prepared by modified Turkevitch et al., method as described in previous chapter section 3.2.4. A stock solution of 1M NaCl was prepared by dissolving 40 mg in 1 ml of DEPC water and accordingly diluted for optimization studies. The effect of incubation on binding and stability was checked using fluorimeter during the analysis. All samples and standard solutions was prepared and stored in a brown calibrated flask at 4°C.
4.2.4. Analysis of vitamin B$_{12}$ in pharmaceutical samples

A mixture of total volume 300µl consisting of optimized concentrations of GNPs and aptamer were shaken mildly for 10 min at room temperature and incubated for 10 min by adding 100µl of different concentrations of vitamin B$_{12}$ (0.1 µg/ml, 0.5 µg/ml, 1 µg/ml, 5 µg/ml and 10 µg/ml). After slow addition of optimized concentration of NaCl into the incubated sample, color and spectra changes were observed by UV/vis spectrophotometer. Vitamin B$_{12}$ injection ampoules labelled a concentration of 0.334 mg/ml were diluted to 100 ml with water, making a stock solution of 10 µg/ml. At least 6 numbers of each of capsules and tablets containing 15 µg vitamin B$_{12}$ were taken, which is equivalent to 90 µg of vitamin B$_{12}$, and diluted to 9 ml, giving a stock of 10 µg/ml, which was suspended in DDW and directly used for the proposed and spectrophotometric method. For reference, pharmaceutical preparations were made to approximate 10 µg/ml solutions, which were then determined at 273, 361 and 551 nm using UV-vis spectroscopy respectively.

4.3. RESULTS AND DISCUSSION

4.3.1. Preparation and spectrophotometric characterization of GNPs

Details of the preparation and spectroscopic characterization of GNPs is described in section 3.3.1 to 3.3.2.

4.3.2. Optimization of aptamer molar ratio to GNPs

It has already been reported that uncoiled ssDNA can be adsorbed on GNPs due their bases facing the GNPs which lead to electrostatic interaction between the bases of ssDNA and GNPs. GNPs are normally stable due to the adsorbed ssDNA aptamers even if salt is added (Rothberg, 2004). But, as expected, the targets specific to the aptamers induced the adsorbed aptamers to detach from GNPs and resulted in the subsequent aggregation of GNPs, leading to the color change from red to purple. In this study, the 35-mer size of vitamin B$_{12}$ binding 2'-flouro modified RNA aptamer was used for the stabilization of GNPs. The stability of GNPs can be maintained by the aptamers.
adsorbed on them preventing the salt induced aggregation. Assay was done to optimize the minimum amount of aptamer needed to stabilize GNPs as shown in Fig. 4.1. The resultant graph showed that, the most of the GNPs get aggregated at aptamer concentration less than 25µM. This indicates that lower concentration of aptamers are not sufficient to shield the GNPs, leaving more free GNPs for salt induced aggregation whereas, at aptamer concentration higher than 25µM, GNPs do not get aggregated indicating that aptamers are completely shielding the GNPs. Thus, 25µM of aptamer concentration was selected as optimum because visually and spectrophotometrically distinct color change was observed. The color change of the GNPs is mainly due to varying interparticle plasmon coupling during aggregation and resulting in plasmon band shift.

**Fig. 4.1.** Absorbance spectra for an optimization of aptamer concentration (10µM, 25µM, and 50µM) to GNPs in a wavelength range 400-700nm.

### 4.3.3. Optimization of GNPs to RNA aptamer concentration

In order to increase the sensitivity of vitamin B₁₂, an assay was performed to optimize the minimum amount of GNPs needed to adsorb on 25µM concentration of aptamer with observable spectra and colorimetric
changes. As shown in Fig. 4.2, most of the GNPs get aggregated at amount higher than 300µl of 4.19 x 10⁻⁹ M concentration which indicates that the aptamer concentration is not sufficient to shield the complete GNPs leaving more free GNPs that leads to salt induced aggregation. Whereas, at lower amount of GNPs, the aptamer completely shield the GNPs and do not allow salt induced aggregation. Thus, 300µl of GNPs was selected as optimum because visually and graphically distinct color change was observed.

![Optimization of GNP to Aptamer concentration](image)

**Fig. 4.2.** Absorbance spectra for an optimization of GNPs concentration (200µl, 300µl, 400µl and 500µl) to aptamer in a wavelength range 400-700nm.

### 4.3.4. Optimization of salt concentration

An assay was performed to optimize the optimum concentration of salt required for the aggregation of GNPs and desired colorimetric changes observed as salt increases the ionic strength and decreases the interparticle distance among the GNPs that leads to change in color from red to purple due to aggregation. From the results of spectra observed at different concentration of NaCl as shown in Fig. 4.3, it was found that, below 0.5M NaCl concentration GNPs are not aggregated effectively. Whereas, above 0.5M NaCl concentration effective aggregation of GNPs was observed. Thus, among all
the concentration of salt 0.5M NaCl was selected as optimum because visually and spectrophotometrically distinct color change was observed. The salt induced aggregation of GNPs is mainly due to Van der Waals forces of attraction against repulsion between the GNPs. Therefore, the addition of sufficient amount of salt screens the repulsion between the negatively charged GNPs and lead to the aggregation which is responsible for the color change.

Fig. 4.3. Absorbance spectra for an optimization of salt concentration (0.1M, 0.25M, 0.5M and 1M) in a wavelength range 400-700nm

4.3.5. Incubation studies

Incubation studies were done to optimize the time required to bind the aptamer to vitamin B_{12} from GNPs. From the results of spectra observed at different incubation time as shown in Fig. 4.4, it was found that at 0\textsuperscript{th} min. there was no difference in spectra after addition of salt immediately, as sufficient reaction time was not given to the aptamers to dissociate from GNPs and bind to vitamin B_{12}. Therefore, visually the color observed was red. Whereas, after 15 min. of incubation, the aptamers bound to GNPs get dissociated and bind with vitamin B_{12}, as aptamers are highly specific for the target, leaving GNPs
for salt induced aggregation and the color changed to purple. Thus, among all the incubation studies, 15 minutes was selected as optimum time.

![Incubation studies](image)

**Fig. 4.4.** UV-vis absorbance spectra of 25µM aptamer, 0.1 µg/ml vitamin B\(_{12}\) and 300µl of GNPs after different incubation time periods (0min., 15min., 30min. and 60min.)

### 4.3.6. Binding studies of RNA aptamer to vitamin B\(_{12}\) using Fluorimeter

Binding of RNA aptamer to vitamin B\(_{12}\) was confirmed using the emission intensity of RNA aptamer with different concentrations of vitamin B\(_{12}\) as shown in Fig. 4.5. The resultant graph showed that, the control, containing only aptamer had higher emission intensity as compared to aptamer with different vitamin B\(_{12}\) concentrations. At higher concentrations of vitamin B\(_{12}\) there is less emission intensity and at lower concentrations higher emission intensity was observed. This shows that emission intensity is indirectly proportional to vitamin B\(_{12}\) concentrations. The emission of aptamer is mainly due to its bases. When aptamer and vitamin B\(_{12}\) get bound their emission gets decreased due to conformational changes in the aptamer and reduced exposure of bases on its surface. It is a well known phenomenon that native RNA or DNA has less emission properties due to their week fluorophore
nitrogen bases (Sidney and Perola, 1962) but in our studies we have observed distinguishable emission intensities due to incorporation of 2'-fluoro moiety in all pyrimidines. It is proved that aromatic substitution with fluorine generally leads to increased fluorescent properties, partly due to the greater absorption of these compounds in the ultraviolet region as compared to the unsubstituted molecules (Gordon and Michael, 1962).

![Fluorimetric assay of binding of Aptamer to Vitamin B₁₂](image)

**Fig. 4.5.** Fluorimetric emission spectra of aptamer with different concentration of vitamin B₁₂ (100 ng/ml, 1000 ng/ml and 10,000 ng/ml).

### 4.3.7. Stability of RNA aptamer at different incubation time using Fluorimeter

Incubation studies were done to determine the stability of RNA aptamer after incubating at different time period at room temperature as shown in Fig. 4.6. The results obtained showed that, at 0th hr. aptamer showed optimal fluorescent intensity. As time increased there was a decrease in fluorescent signals because the aptamers were exposed to nuclease attack due to working in an environment without any major RNA safety precautionary measures. Therefore, there may be a degradation of aptamers which resulted in less emission intensity. It is a well-known phenomenon that native RNA is not stable for even seconds but 2'-flouro modified RNA are stable for more than 15 hours.
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(Nikos et al., 1997; Seong wook and Bruce, 1997). In our studies, the overall time of analysis is only 15 min for which 2'-fluoro modified aptamers are better than native RNA aptamers without losing its activity. The same aptamer would be carried for biosensor application which requires stringent and different environmental conditions therefore 2'-fluoro modified RNA aptamers may be well suited for such analytical applications.

![Fluorimetric assay of Aptamer stability at room temperature](image)

**Fig. 4.6.** Fluorimetric detection of aptamer stability after particular time interval (0hr, 3hrs, 6hrs, 9hrs, 12hrs and 24hrs).

### 4.3.8. Performance of proposed method for vitamin B\textsubscript{12} determination in pharmaceutical preparations

Based on the understanding on the interaction between aptamer and GNPs, the distinct color was appeared as the results of GNPs aggregation at the different concentrations of vitamin B\textsubscript{12} as shown in Fig. 4.7. The resultant graph, showed that, vitamin B\textsubscript{12} at the highest concentration of 10 µg/ml leads to more binding of aptamer, as aptamers are highly specific and have high affinity towards the target leaving GNPs for salt induced aggregation due to
which color turns to purple. In case of 1 µg/ml of vitamin B$_{12}$ concentration, few aptamers get bound with vitamin B$_{12}$ and remaining with GNPs, leaving free GNPs to get aggregated partially, so color turned to a lighter purple. The control sample with no vitamin B$_{12}$ retains red color due to physical adsorption of aptamer to GNPs and so a sharp absorbance peak was obtained at wavelength 520 nm. UV-vis studies provided quantitative results, which clearly showed that adsorption at 520 nm gradually decreased while adsorption at 640 nm increased. This blue shift in the SPR absorption suggested the formation of large-sized aggregates of GNPs. Significantly, color change is visible at as low as 0.1 µg/ml of vitamin B$_{12}$, suggesting that GNPs are sensitive probes for aptamer structures. As can be seen in Fig. 4.7, the spectral and color changes of GNPs solution were measured up to 0.1 µg/ml of vitamin B$_{12}$ with spectrophotometry and visually respectively. For the convenient analysis of the spectral and color changes, the absorbance ratios at A$_{520}$/A$_{640}$ were plotted, and found to be well matched with the spectra and visual observation. The data points in Fig. 4.7 inset show the A$_{520}$/A$_{640}$ ratio versus the vitamin B$_{12}$ concentration (from 10 to 0.1 µg/ml) of the solution consisting of 25 µM of the vitamin B$_{12}$ RNA aptamer, 300 µl of 4.19 x 10$^{-9}$ M of GNPs concentration, and 0.5 M NaCl. The higher the vitamin B$_{12}$ concentration, the greater the change in color occurred and the higher the A$_{520}$/A$_{640}$ ratio was. In this experiment, vitamin B$_{12}$ was easily detected at 0.1 µg/ml, but it was not possible to detect it below this value. In addition, the degree of GNPs aggregation did not change in the presence of more than 10 µg/ml vitamin B$_{12}$. Furthermore, the well-fitted curve from A$_{520}$/A$_{640}$ ratio data shows that the designed method is a reliable system for vitamin B$_{12}$ detection with 10 µg/ml as upper limit and 0.1 µg/ml as lower limit.

The method was applied to the analysis of three preparations of vitamin B$_{12}$, including injections, tablets, and capsules, which were purchased from the local market. To check the accuracy of the proposed method, UV-vis spectroscopy method was used to measure the content of vitamin B$_{12}$ in all samples in triplicate. The results are given in Table 4.1. The two methods show good agreement. To check the accuracy of the results, a recovery study was carried out by comparing the concentrations found in three pharmaceutical samples spiked with known amounts of vitamin B$_{12}$. The overall recovery was
92–95% with a relative standard deviation in the range of 2.08–8.27 % as shown in Table 4.2. The results obtained by the current method agreed well with the labeled values for vitamin B₁₂ injection, tablets, and capsules. To assess the possible analytical applications of the proposed method described, the effect of various water soluble vitamins and metal ions contaminants were tested by analyzing a standard solution of vitamin B₁₂ (1 µg/ml) to which increasing amounts of interfering compounds were added. A standard solution of 1 µg/ml of vitamin B₁₂ was used to analyze non-specific aggregation. A₀ and A are the absorbance ratio (A₅₂₀/A₆₄₀) of proposed method in absence and presence of interfering compounds as shown in Table 4.3. Tolerable concentrations, defined as the concentrations of foreign compounds causing less than ± 3% relative error ΔA/A (%), were examined. The results showed that the tolerable concentration ratios of coexisting substances to 1.0 µg/ml vitamin B₁₂ was over 1000-fold for Al³⁺, Zn²⁺, Pb²⁺, Hg²⁺, Ca²⁺, Ag⁺; Mn²⁺, Fe²⁺, Mg²⁺, Cd²⁺, Co²⁺, Cu²⁺, Fe³⁺, Li²⁺ and vitamin B₁₂ analogs; over 3000 for water soluble vitamins. It can be seen that some impurities in pharmaceutical preparations have no effect on the determination of low concentrations of vitamin B₁₂. However, metal ions are severe interferers for the determination. Because almost no commercial pharmaceutical injections, capsules and tablet contain the above interference level, therefore, the method can be applied for the direct determination of vitamin B₁₂ in pharmaceuticals. Thus the proposed method showed good selectivity and indicates that prior to evaluation samples with excess metal ions should be taken care of by chelating with EDTA (Youngjin et al., 2001; Nan Dinga et al., 2012).
Fig. 4.7. UV-vis absorption spectra for limit of detection of different concentration of Vitamin B_{12} (100 ng/ml, 500 ng/ml, 1000 ng/ml, 5000 ng/ml and 10,000 ng/ml) using RNA aptamer and GNPs. Inset, Plot of absorption ratio (A_{520}/A_{640}) vs. vitamin B_{12} concentration. Coloured photographs of 300 µL of 15 nm GNPs stabilized by the aptamer (100 µL 25µM) after the addition of salt (50µL 0.5 M NaCl) in presence of different concentrations of vitamin B_{12} (from left to right).

Table 4.1. Analysis of vitamin B_{12} in pharmaceutical samples using proposed method in comparison with UV-vis spectroscopy. The average of five measurements (± SD). Amount of vitamin B_{12} labelled in multivitamin injection is 0.334 mg/ml, a multivitamin tablet is 15 µg/tablet, and in multivitamin capsule is 15 µg/tablet.
Pharmaceutical Samples | Amount labelled | Amount found | Proposed Method | UV-Vis Spectrophotometric method
--- | --- | --- | --- | ---
Multivitamin Injection | 0.334 | 0.32 (± 0.04) | 0.33 (± 0.06)
Multivitamin Tablet | 15 | 14.06 (± 0.35) | 14.24 (± 0.46)
Multivitamin Capsule | 15 | 14.44 (± 0.50) | 14.6 (± 0.35)

**Table 4.2.** Recovery of externally added (1 µg/ml) vitamin B₁₂ in pharmaceutical samples was analysed using proposed method. Data were derived from triplicate assays (n=3).

<table>
<thead>
<tr>
<th>Pharmaceutical samples</th>
<th>Added (µg/ml)</th>
<th>Found (µg/ml)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injections</td>
<td>0</td>
<td>0.926</td>
<td>38.6</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.84</td>
<td>5.22</td>
<td></td>
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<tr>
<td>Tablets</td>
<td>0</td>
<td>0.898</td>
<td>8.27</td>
<td>94.3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.92</td>
<td>3.08</td>
<td></td>
</tr>
<tr>
<td>Capsules</td>
<td>0</td>
<td>0.92</td>
<td>4.861</td>
<td>95.3</td>
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<td></td>
<td>1</td>
<td>1.906</td>
<td>2.08</td>
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</table>

**Table 4.3.** Tolerable concentration of coexisting substances on the absorbance ratio ($A_{520}/A_{640}$) of proposed method with respect to 1 µg/ml vitamin B₁₂. Relative error $\Delta A = A_0 - A$, where $A_0$ and $A$ are the absorbance ratio in absence and presence of interfering species.

<table>
<thead>
<tr>
<th>Interfering substances</th>
<th>Tolerable concentration (µg/ml)</th>
<th>Relative Error $\Delta A/A$ (%)</th>
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<tbody>
<tr>
<td>Al³⁺</td>
<td>1300</td>
<td>0.60</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>1500</td>
<td>-0.30</td>
</tr>
<tr>
<td>Pb²⁺</td>
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<td>0.73</td>
</tr>
<tr>
<td>Hg²⁺</td>
<td>1300</td>
<td>0.50</td>
</tr>
<tr>
<td>Ca²⁺</td>
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<td>-0.30</td>
</tr>
<tr>
<td>Ag⁺</td>
<td>1250</td>
<td>-1.70</td>
</tr>
<tr>
<td>Mn²⁺</td>
<td>1150</td>
<td>-3.00</td>
</tr>
<tr>
<td>Fe²⁺</td>
<td>1000</td>
<td>0.50</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>1450</td>
<td>-0.28</td>
</tr>
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</table>
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<table>
<thead>
<tr>
<th></th>
<th>Conc. (µM)</th>
<th>Slope (M⁻¹)</th>
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<tbody>
<tr>
<td>Cd²⁺</td>
<td>1300</td>
<td>-1.30</td>
</tr>
<tr>
<td>Co²⁺</td>
<td>1150</td>
<td>0.56</td>
</tr>
<tr>
<td>Cu²⁺</td>
<td>1400</td>
<td>-1.23</td>
</tr>
<tr>
<td>Fe³⁺</td>
<td>1500</td>
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</tr>
<tr>
<td>Li²⁺</td>
<td>1000</td>
<td>0.03</td>
</tr>
<tr>
<td>Vitamin B₁₂ Analogs</td>
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</tr>
<tr>
<td>Water soluble vitamins</td>
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</tbody>
</table>

4.4. CONCLUSIONS.

In conclusion, vitamin B₁₂ was analysed up to a minimum concentration of 0.1 µg/ml using 25µM of RNA aptamer, 300µl of 4.19 x 10⁻⁹ M GNPs and 0.5M NaCl concentration. Modified RNA aptamer was stable up to 2 hr, a feature not explored previously. Compared with conventional analytical techniques, such as HPLC, AAS, inductively coupled plasma mass spectrometry, radioisotope assay, microbiological assay, and fluorimetric detection, the current proposed method was sensitive, rapid, economical, required no expensive instrumentation and can be used for onsite analysis. The level of detection obtained for vitamin B₁₂, which was 0.1 µg/ml in terms of spectral change or colorimetric change, is still higher than the RDA value (2-3 µg/100g in food) regulation. Therefore, the following optimization was conducted to achieve the lower level of detection than the regulation. While this work is in its preliminary stage, it could be further improved by introducing several existing technologies, which might eventually lead to a cost-effective biosensor platform. Since the detection is homogeneous, it is easily adaptable for high throughput assays in microwell-based plates and even automated analysis. More importantly, this strategy is not limited to detection of vitamin B₁₂. In fact, RNA aptamers can be stabilized by 2'-fluoro modifications for general biosensor development. Therefore the principle described in this work can be conveniently generalized to other aptamer systems. Eventually, we expect that it will be possible to visually detect a large number of important analytes with unmodified GNPs and aptamers generated from SELEX.
REFERENCES


