A Novel Micellar Medium Using Triblock Copolymer for Cobalt Determination

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This work proposes a new method for Co(II) determination based on the use of the triblock copolymer as micellar medium instead of chloroform. The proposed strategy is environmental friendly because the copolymer is biodegradable and nontoxic. The method is based on the formation of a cobalt-1-nitroso-2-naphthol complex, in the micellar triblock copolymer compound solution constituted by polyethylene oxide (PEO) and polypropylene oxide (PPO). Experimental conditions such as pH, the molecular weight and the PEO/PPO ratio of triblock copolymer were optimized. Results obtained for cobalt determination in vitamins with this novel method showed excellent agreement with those obtained using atomic absorption spectrometry. The relative standard deviation for a solution containing 0.125 mg L^{-1} Co was 1.0% (n = 5); the relative error varied from 2.8 to 4.4%; the detection limit was 13.3 μg L^{-1} and the quantification limit was 44.3 μg L^{-1}.

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Recently, researchers have started to pay attention to the applications of micellar systems in analytical chemistry.[1] Micelles have been accepted as a microscopic medium, which provides a new basis for the development of separation techniques.[2-6] Micellar media are convenient to use because they are readily available, stable and can provide sensitive measurements in some tailored applications.[7]

The investigations described so far have utilized auto-organized systems (micelles) formed by classical low molecular weight surfactants; the surfactants used included the Triton X series,[3,6] Tween series,[2] sodium dodecylsulfate,[5,6] and others.[8-13] However, those surfactants generally present absorbance values in the ultraviolet region, which could cause interferences. The triblock copolymer systems proposed in this work have the advantage of presenting a negligible absorbance in the ultraviolet (UV) region. In fact, with respect to the surfactants used in early investigations, triblock copolymers have, in general, a very low critical micellar concentration (c.m.c.) and a low diffusion coefficient. This is beneficial in micellar systems where the concentration of individual molecules in equilibrium with the micelles has to be kept to a minimum.[14]

Water-soluble triblock copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO), often denoted PEO-PPO-PEO or $(EO)_n(PO)_m(EO)_n$, are commercially available. These nonionic linear macromolecules are surface-active agents with well-defined compositions, molecular weights, and structures. They are usually referred to by their trade names, the most common being: Pluronics, Poloxamers and Synperonics. The nomenclature for the Pluronic triblock copolymers starts with the letters L (for liquid), P (for paste), or F (for flakes). The first one or two numbers are indicative of the molecular weight of the PPO block, and the last number indicates the weight fraction of the PEO block. Additionally, they are low cost, biocompatible, and environmentally safe compounds. The advantage of these macromolecular systems, when compared with other non-polymeric surfactants is an increase of the size of internal micellar cavity, which leads to an increase of the hydrophobic character of the copolymer system. This in turn, allows for the presence of a greater solute content inside the cavity.[15]

These properties and characteristics demonstrated the possible advantages of using macromolecular micellar systems in the development of new spectrophotometric methods applied to the micro-determinations of metal ions, biological compounds, drugs and pesticides.

In this work, cobalt was chosen as the analyte because it is involved in human biochemical metabolism and it is a constituent of the essential vitamin B_{12}. This vitamin is involved in a wide variety of metabolic processes, which include factors affecting growth, hemopoiesis, and the maintenance of the integrity of nerve cells.[16-18] It is also well known that some metals that are essential nutrients can also exert a toxic action, depending on their concentrations. For some of these metals, there is only a narrow concentration difference between the toxic and the essential levels.[6] As a result, knowledge of the metal content in various matrices, together with the development of reliable analytical methods for cobalt determination is mandatory.[19] Hence the need for a precise and accurate cobalt determination *via* an inexpensive method, for instance, using chromophore agents and UV-Vis spectrophotometry.

The reagent 1-nitroso-2-naphthol (1N2N) forms highly stable complexes with several metal ions including Co(II), Cu(II), Fe(II), Fe(III), V(V), Pd(II) and U(VI). Many of these complexes are strongly colored species.[2] This chelating agent has been used to determine the concentration of several metal ions by UV-Vis spectrophotometry. These complexes are not soluble in aqueous medium, so it is necessary to perform a solvent extraction. It is well known that this process is time-

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consuming, but also is an efficient way for preconcentration of metals. In most studies involving these complexes, the extraction was carried out with chloroform,[20-23] which is a carcinogenic agent.[24]

The work here described proposes a new method for Co(II) determination in vitamins based on the use of the triblock copolymer as micellar medium instead of chloroform. This strategy is compatible with the green chemistry approach, because the copolymer is biodegradable and nontoxic.

Experimental

Apparatus

Spectrophotometric measurements were performed with an UV-Vis spectrophotometer (Shimadzu UV-1601PC, Japan) utilizing a quartz cell (1.00 × 1.00 × 3 cm). The slit aperture was kept at 2.0 nm.

The pH measurements were performed with a combined glass electrode using a digital pH meter (DIGIMED, Model DM-20, Brazil).

A Carl Zeiss JENA (Model AAS-3, Germany) atomic absorption spectrophotometer was utilized for the comparative studies to check accuracy. The operating conditions were: wavelength 304.4 nm, resolution 0.5 nm, air–acetylene flame (air and acetylene flow rates 13.5 and 1.97 $\text{dm}^3\ \text{min}^{-1}$, respectively) and, burner height 13.5 mm.

A Milli-Q II water deionizer (Millipore, USA) was utilized for the final purification of distilled water.

Reagents

Three poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) copolymers with the basic backbone $(EO)_n(PO)_m(EO)_n$ were used. They are L35: $(EO)_{11}(PO)_{16}(EO)_{11}$ with average molecular weight (M_w) 1900 g mol^{-1} and 50% ethylene glycol; P103: $(EO)_{20}(PO)_{69}(EO)_{20}$ with M_w 5800 g mol^{-1} and 30% ethylene glycol and F68: $(EO)_{76}(PO)_{28}(EO)_{76}$ with M_w 8400 g mol^{-1} and 80% ethylene glycol; these were acquired from Aldrich Company, USA. The concentration of the copolymer aqueous solutions, 2.5% (m/v), was utilized for all analyses.

A 19.0 mg L^{-1} solution of 1-nitroso-2-naphthol (Merck, Darmstadt, Germany) was prepared in 2.5% (m/v) triblock copolymers (L35, P103 or F68) aqueous solution.

Reference solutions of Co(II) were diluted from 100 μg mL^{-1} stock solution prepared from $CoCl_2\cdot 6H_2O$ (Carlo Erba, Italy).

To evaluate the pH effect, we prepared the following solutions:[25] HCl/KCl (pH 1.0), tartaric acid/NaOH buffer (pH 2.0 and 3.0), CH_3COONa/CH_3COOH buffer (pH 4.0, 5.0, and 6.0) and $Na_2B_4O_7\cdot 10H_2O/HCl$ buffer (pH 8.0). These reagents were obtained from Merck, Germany.

All the reagents were analytical-grade; deionized water ($R \geq$ 18 MΩ cm^{-1} was used throughout all the experiments.

Procedure

An appropriate volume of the cobalt stock solution was mixed with a 1N2N solution in 2.5% (m/v) triblock copolymers (L35, P103 or F68) aqueous solution to give a final concentration of 1.56 mg L^{-1} (pH 1 to 8). The 1.56 mg L^{-1} cobalt solution was diluted with 1N2N solution in 2.5% (m/v) triblock copolymers (L35, P103 or F68) aqueous solution to give a final concentration in the range of 0.0625 to 1.56 mg L^{-1}, in each pH. UV-Vis spectrophotometric measurements were carried out at 318 nm.

The correlation coefficient of the analytical curve was estimated. The detection limit (LD), the quantification limit (LQ), and concentration of cobalt in the samples were obtained.

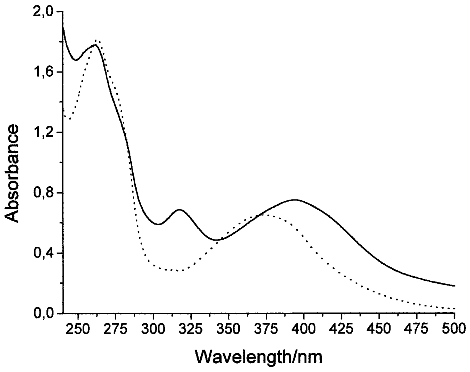

Fig. 1 UV-Visible spectra of 1-nitroso-2-naphthol (dotted line) and cobalt-1-nitroso-2-naphthol complex (solid line) both in 2.5% (m/v) P103 aqueous solution.

Samples

Aliquots of 500 μL of ampoules of vitamin B_{12} in three pharmaceutical preparations: hydroxicobalamin (Bristol-Myers Squibb Brasil®), cyanocobalamin (Merck®) and cyanocobalamin (Bunker®) were transferred to a porcelain crucible. After 2.5 mL potassium hydrogen sulfate 12.5% (m/v) was added, the contents were heated for 4 h at 500°C. The powered material obtained was then dissolved in 10.00 mL of acetate buffer (pH = 4). An aliquot of 180 μL of these solutions was taken and the volume was adjusted to 5.0 mL with 1-nitroso-2-naphthol solution in 2.5% (m/v) P103. Cobalt was determined by both UV-Visible spectrophotometry (318 nm) and atomic absorption spectrometry (AAS).

Results and Discussion

Figure 1 shows typical UV-Vis spectra obtained for 1-nitroso-2-naphthol and cobalt-1-nitroso-2-naphthol (Co-1N2N) complex both dissolved in a 2.5% (m/v) P103 aqueous solution. The solid curve corresponds to the electronic transitions associated with cobalt-1-nitroso-2-naphthol complex dissolved inside the hydrophobic micelles core with absorbance maxima at 262, 318, and 394 nm. The dotted line corresponds to the molecular spectrum of 1-nitroso-2-naphthol dissolved in the hydrophobic core of the aggregate formed by copolymer molecules, in the absence of cobalt. The spectrum of the ligand alone presents two peaks with maximum absorbances at 263 and 373 nm. It is interesting to note the similarities between our data and the spectrum obtained in chloroform solvent.[26] A diluted macromolecular solution was used for this analysis in order to avoid any light scattering. Initial measurements were performed for two wavelengths, 318 and 394 nm. However, the absorbance maximum at 318 nm was chosen as the analysis wavelength, because the highest signal-to-blank ratio was obtained at this value.

Triblock copolymer solutions 0.5, 1.0, 1.5, 2.0, 2.5, 3.5, and 5.0% (m/v) were tested in the present work. We have noticed that the solubility property of the 1N2N was very low below 2.5% (m/v), which is probably a result of the small numerical density of polymolecular aggregate present in the macromolecular solution. The opposite happens above 2.5% (m/v), which is related to an increase in the number of micelles

Table 1 Influence of the variation of the average molecular weight and of the ratio PEO/PPO of the triblocks copolymer in the cobalt determination

[Co(II)]/ mg L^{-1}	Absorbance		
	P103[a] (2.88×10^4)[d] (0.9997)[e]	L35[b] (2.64×10^4)[d] (0.9999)[e]	F68[c] (1.75×10^4)[d] (0.9984)[e]
0.249	0.130	0.111	0.0737
0.374	0.185	0.166	0.119
0.499	0.250	0.223	0.150
0.624	0.309	0.281	0.192
0.811	0.405	0.362	0.242

a. $(EO)_{20}(PO)_{69}(EO)_{20}$ with M_w 5800 g mol^{-1} and 30% EO.
b. $(EO)_{11}(PO)_{16}(EO)_{11}$ with M_w 1900 g mol^{-1} and 50% EO.
c. $(EO)_{76}(PO)_{28}(EO)_{76}$ with M_w 8400 g mol^{-1} and 80% EO.
d. Molar absorptivity (L mol^{-1} cm^{-1}; λ_{max} = 318 nm).
e. Correlation coefficient.

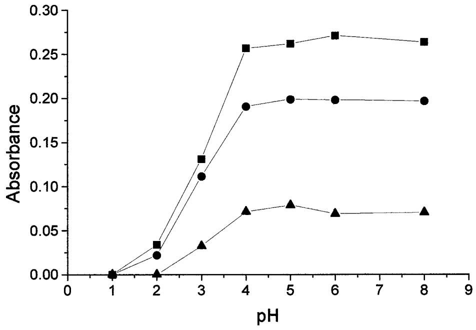

Fig. 2 Influence of pH on the complexation between cobalt and 1-nitroso-2-naphthol in 2.5% (m/v) of P103 copolymer solution. Here concentration cobalt was: ▲, 0.125 mg L^{-1}; ●, 0.374 mg L^{-1}; ■, 0.499 mg L^{-1}.

promoting an enlargement in the amount of substance of 1N2N, dissolved in the system, capable of carrying out the cobalt complex formation in the concentration range studied. However, since the 1N2N solubility did not change significantly at higher concentration, we have chosen 2.5% (m/v) as reference throughout our study.

Selection of the triblock copolymer

Aqueous solutions of three different triblock copolymers: L35, P103 and F68, with a macromolecular concentration of 2.5% (m/v), were tested. The absorbance data for solutions containing different Co(II) concentrations are shown in Table 1, for the range 0.249 to 0.811 mg L^{-1}, pH 4. It can be seen that all copolymers can be successfully applied, but the best absorbance signals were obtained for P103 copolymer. The higher absorbance signal observed for P103 aqueous solution could be attributed to its comparatively better ability to enhance the aqueous solubility of 1-nitroso-2-naphthol, which, otherwise, is only sparingly soluble in water. This increase in the solubility arises from the fact that the micellar cores for block copolymer micelles can serve as compatible microenvironments for water-insoluble molecules.

The differences among the three copolymers include their average molecular weights and the PEO/PPO ratios, which led to different behaviors in solution. Experimental data obtained using dynamic light scattering and small-angle neutron scattering has been used to show the phase behavior of PEO-PPO-PEO triblock copolymers dissolved in water.[27-29] The molecular processes occurring in solution depend on both the polymer concentration and temperature. At low temperatures ($T \leq 15^\circ C$) and low polymer concentrations, the macromolecules were fully dissolved in Gaussian chains with a gyroscopic radius (R_g) of approximatly 1.7 nm.[14] When the temperatures were increased, the hydrophobic nature of the PPO group caused aggregation of the macromolecules into spherical micelles with core sizes on the order of 5 – 10 nm, consisting of only the PPO block and a corona region consisting of the water and PEO blocks. The characteristics of the micelles formed are somewhat temperature and PPO size dependent. It is apparent from these experimental studies that PEO-PPO-PEO copolymers, which are relatively less hydrophobic, either due to a high PEO content or a low molecular weight, form micelles at larger c.m.c., with aggregates formed with a small hydrophobic core size. Tuzar and Kratochvil[30] pointed out that the partition coefficient of the solute is mainly controlled by the Flory–Huggins parameter that characterizes the interaction between the solute and the core-forming block copolymer. This thermodynamic parameter is similar for the three copolymers used in this work. However, the amount of substance of 1-nitroso-2-naphthol incorporated inside of micelles depends on the size of hydrophobic core and on the number of aggregates formed. Surface tension measurements[31] and dye solubilization[32] techniques associated with dynamic light scattering experiments[33] on Pluronics aqueous solutions revealed that Pluronic P103 has a lower c.m.c. and a higher micellar core radius than the other two copolymers, F68 and L35. Based on these results we have chosen P103 as the micellar medium.

Influence of pH

An appropriate volume of the Co(II) stock solution (pH 1 to 8) was mixed with 1N2N solution in 2.5% (m/v) P103 aqueous solution (pH 1 to 8) to give a final concentration of 1.56 mg L^{-1}. The 1.56 mg L^{-1} cobalt solution was diluted with 1N2N solution in 2.5% (m/v) P103 aqueous solution (pH 1 to 8) to give final concentrations of 0.125, 0.374 or 0.499 mg L^{-1}. The optimum pH value was determined by measuring the absorbance of the solutions conditioned at different pH values. The pH influence on the metal complex absorbance in 2.5% (m/v) P103 solution is presented in Fig. 2.

This cobalt complex had high absorbance in the pH range 4 – 8. The pH = 4 was chosen because preliminary tests showed that the interference of other metal ions could be minimized at this pH. The results shown in Fig. 2 can be attributed to two factors: one of them is the pH dependence of the partition coefficient of the cobalt-1-nitroso-2-naphthol complex from water to micelle core and the other is the influence of hydrogen ion concentrations on the thermodynamic stability constant of the complex formed. The former influence should be expected to produce a proportional absorbance signal for the three different Co(II) concentrations, and this behavior was not observed. The relationship between the pH and the partition coefficient should be insignificant, since the free energy of the transference process is strongly influenced by the hydrophobic interactions between solute molecules and the PPO core of the micelles.[30] The second factor is the influence of hydrogen ion concentrations on the thermodynamic stability constant of the complex formed. It is well known that the stability constant of the complex is dependent on pH.[26,34] These experiments are supported by Tun *et al.* and Sandell's comments[26,34] that cobalt-

Table 2 Influence of pH on cobalt-1-nitroso-2-naphthol complex stability

Co(II) concentration/ mg L^{-1}	Absorbance pH 4	Absorbance pH 1
0.0490	0.0241	0.0236
0.293	0.150	0.145
0.440	0.228	0.226
0.635	0.326	0.328
0.880	0.433	0.449

Table 3 Tolerance ratio of foreign ions on the determination of 0.0625 mg L^{-1} cobalt

Ion	Tolerance ratio [foreign ion]/[cobalt]	Tolerance ratio after adjusting the solution pH to 1
Ni(II)	100	1000
Cu(II)	10	500
Fe(II)	1	100[a]
Fe(III)	1	100[a]

a. Adding NaF as masking agent.

1-nitroso-2-naphthol complex requires a weakly acidic solution for quantitative formation.

The influence of pH on complex stability

To apply the proposed method to real samples it is necessary to verify its capacity to eliminate interferences. It is well known that Ni(II) and Cu(II) form stable complexes with 1N2N, turning them into powerful interferers. A very useful approach to eliminate the Ni(II) and Cu(II) interferences is to form naphtholate complexes of Ni(II), Cu(II) and Co(II) at pH 4, and after that, decreasing the pH to 1, to destroy all complexes formed except the Co-1N2N. However, in order to apply this pH change approach to eliminate interference, one must investigate the general aspects of kinetic dissociation of cobalt-1-nitroso-2-naphthol at pH 1. Initially the Co-1N2N complex was formed at pH 4; after that, the pH was adjusted to 1. This was done for solutions containing different cobalt concentrations. The absorbances were measured for pH 4 and pH 1 and the values were compared. The results are shown in Table 2. Because the Co-1N2N complex has a very low rate of dissociation, during a relatively long time it is metastable at pH 1. It is evident from these results that, once formed, the complex is not significantly affected by solutions containing elevated concentrations of acids, but an acidic solution with low concentration of H_3O^+ ions is required for its quantitative formation.[34] The reason for the stability of the complex in acidic medium is the oxidation of the Co(II)-1N2N to Co(III)-1N2N and this complex is known to be quite stable toward acids.[35]

Interference study

The interferences of co-existing ions in the determination of 0.0625 mg L^{-1} cobalt by the proposed method were evaluated. The relative error of less than 5% was considered within the range of experimental error. The results obtained are summarized in Table 3. The foreign ions Ni(II), Cu(II), Fe(II) and Fe(III) were studied because they form complexes with 1N2N. Iron(II), Fe(III) and Cu(II) interfered significantly; however, Ni(II) only interfered when its concentration is 100 times larger than the cobalt concentration. A volume of 50 µL HCl conc. was added to reduce the interferences caused by Ni(II) and Cu(II) to acceptable levels. The naphtholate complexes of Ni(II) and Cu(II) dissociate in strongly acidic medium, while the naphtholate complex of cobalt is marginally affected. The interferences caused by Fe(II) and Fe(III) were corrected by adding NaF as a masking agent.

Table 4 Cobalt determination in B_{12} vitamin samples (mean ± standard deviation, $n = 4$)

Sample	$[Co]_{AA}$/ mg L^{-1}	$[Co]_{PM}$/ mg L^{-1}	Relative error
Cyanocobalamin[a]	0.366 ± 0.009	0.373 ± 0.011	3.32
Cyanocobalamin[b]	0.436 ± 0.002	0.448 ± 0.004	2.75
Hydroxicobalamin[c]	1.13 ± 0.03	1.08 ± 0.02	4.42

$[Co]_{AA}$: concentration of cobalt obtained by AAS.
$[Co]_{PM}$: concentration of cobalt by the proposed method.
a, Bunker®; b, Merck®; c, BMSB®.

Optimum analytical conditions for Co(II) determination

The optimum conditions for the proposed method were: 19.0 mg L^{-1} solution of 1-nitroso-2-naphthol, 2.5% (m/v) P103 triblock copolymer and pH 4. After the method was optimized, the relative standard deviation (RSD%) for 5 replicates at a concentration of 0.125 mg L^{-1} for cobalt was 1.0%. The linear calibration range is from 0.0625 to 1.56 mg L^{-1}, the correlation coefficient being 0.9999. The equation obtained by linear regression is: absorbance = 0.489[Co(II)] + 0.00435; where n = 9, with a molar absorptivity of 2.88×10^4 L mol^{-1} cm^{-1} at λ_{max} = 318 nm. The detection and quantification limits[36] were 13.3 µg L^{-1} and 44.3 µg L^{-1}, respectively.

Determination of cobalt in B_{12} vitamin samples

The method was applied for the determination of Co(II) in three pharmaceutical preparations, in the form of vitamin ampoules: hydroxicobalamin (Bristol-Myers Squibb Brasil®), cyanocobalamin (Merck®) and cyanocobalamin (Bunker®). Cobalt was determined with the proposed method and compared with the results obtained by atomic absorption spectrometry. The pretreatment of vitamin samples was done for both methods, proposed method and AAS method. Results in Table 4 indicate that the proposed method can be successfully applied to the determination of Co(II) in vitamin B_{12} formulations. A *t*-test (95% confidence level) was applied and did not show significant differences between the two methods. Additionally, the proposed method presented good accuracy (relative error varied from 2.75 to 4.42%).

The proposed method is environmentally friendly, low in cost, simple, and easily applicable to any ordinarily equipped laboratory. A comparative evaluation between the developed method and others reported in the literature for cobalt determination (Table 5) put forward advantages of the former in terms of broader determination range, greater correlation coefficient, and lower reagent consumption in terms of surfactant amount. In addition, the method presented herein has shown a high molar absorptivity. In comparison to those in Table 5 it can therefore be classified as the second highest.

Conclusion

The green chemistry method developed in this work demonstrated the suitability of triblock copolymer as an

Table 5 Comparison of analytical characteristics of the cobalt determination by the proposed method and by other micellar methods

Photometric reagent	1N2N	1N2N	CDBA[c]	PAR[d]	1N2N
Medium and solvent	Aqueous Triton X-100[a]	Aqueous Tween 80[b]	Aqueous Tween 80[b]	Aqueous Triton X-100[a]	Aqueous P103
$\lambda_{\max}$ (nm)	420	445	555	620	318
ε (L mol^{-1} cm^{-1})	3.18×10^4	1.86×10^4	1.64×10^4	1.9×10^4	2.88×10^4
Linear range (mg L^{-1})	0.205 – 3.00 (0.9997)[e]	0.300 – 1.50 (0.9986)[e]	0.100 – 3.52	0.40 – 3.2	0.0625 – 1.56 (0.9999)[e]
LD (μg L^{-1})	6.16	16	4.0	—	13.3
LQ (μg L^{-1})	20.5	53	—	—	44.3
Reference	6	2	37	38	Present work

a. Polyoxyethylene *p*-*ter*-octylphenol. b. Polyoxyethylene sorbitan monooleate. c. 5-(*o*-Carboxyphenylazo)-2,4-dihydroxybenzonic acid. d. 1-(2-Pyridylazo)naphthol. e. Correlation coefficient.

extracting agent for cobalt determination by liquid–liquid extraction. The three macromolecular compounds used showed a good performance, but the best sensitivity was obtained with P103, probably due to large hydrophobic cores existing in its aggregates. In this work, it was demonstrated that pH variation is an effective way to eliminate the interference of other metals. The better selectivity of 1N2N makes it feasible in the context of the described methodology for the determination of cobalt in other samples. A precision of 1% and a relative error lower than 4.5% were obtained, and the method demonstrated that it is possible to determine cobalt with reagents that are environmentally friendly. The application of this strategy is being presently extended to Ni(II), Cu(II), and Fe(III) and promising preliminary results have been obtained.

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