Application of microwave plasma atomic emission spectrometry in bioanalytical chemistry of bioactive rhenium compounds

Mariano Soba[a,b,c], Gonzalo Scalese[a], Leticia Pérez-Díaz[d], Dinorah Gambino[a], Ignacio Machado[b*]

[a] *Área Química Inorgánica, Facultad de Química, Universidad de la República, Montevideo, Uruguay.*

[b] *Área Química Analítica, Facultad de Química, Universidad de la República, Montevideo, Uruguay.*

[c] *Programa de Posgrado en Química, Facultad de Química, Universidad de la República, Montevideo, Uruguay.*

[d] *Laboratorio de Interacciones Moleculares, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.*

* *Corresponding author: Ignacio Machado. e-mail address: imachado@fq.edu.uy*

ABSTRACT

Five newly synthetized *fac*-Re(I) tricarbonyl compounds were explored as prospective antitrypanosomal agents. The biological activity of the whole series was evaluated preliminarily against the epimastigote form of *Trypanosoma cruzi*. All compounds showed activity against epimastigotes with IC_{50} values in the low micromolar range. The most active compound [*fac*-Re(I)$(CO)_3$(tmp)(CTZ)](PF_6), with CTZ = clotrimazole and tmp = 3,4,7,8-tetramethyl-1,10-phenantroline, showed good selectivity towards the parasites and thus was selected to carry out further metallomic studies. For this task, a newly bioanalytical method based on microwave plasma atomic emission spectrometry (MP-AES) was developed and validated. The accuracy of the method was ensured by testing a certified reference material. Results of rhenium elemental analysis by MP-AES agreed with the proposed formula of the studied compounds, contributing to the overall validation of the method, which was then applied to evaluate the percentage of rhenium uptaken by the parasites and the association of the compounds with parasite biomacromolecules. Metallomics results showed low total rhenium percentage uptaken by parasites (~1.2%) and preferential accumulation in the soluble proteins fraction (~82.8%). Thus, the method based on MP-AES turned out to be an economical and green alternative for metallomics studies involving potential rhenium metallodrugs. Moreover, a comparison against rhenium determination by electrothermal atomic absorption spectrometry (ET-AAS) was included.

Keywords: Rhenium(I) compounds; *Trypanosoma cruzi*; Metallomics; Microwave plasma atomic emission spectrometry (MP-AES).

1. Introduction

Parasitic diseases produced by trypanosomatid protozoa, like American Trypanosomiasis (Chagas disease), constitute an overwhelming health issue in poor regions, particularly in Latin America. Chagas disease is considered by the World Health Organization (WHO) as a neglected tropical disease, which is endemic in Latin America, but has also spread in recent decades to non-endemic regions owing to the migration of unaware infected people that transmit the disease through blood transfusions, organ transplants and from mother to fetus. Its etiological agent is the homoflagellated protozoan parasite *Trypanosoma cruzi*, that is transmitted to mammalian host mainly by blood-sucking triatomine infected bugs. The current chemotherapy is based on two old drugs, Nifurtimox and Benznidazole, that show several therapeutic disadvantages such as severe toxic effects and poor efficacy in the chronic phase of the disease. In this regard, more efficient and non-toxic drugs are urgently needed [1-7].

In this framework, our research group has contributed to demonstrating that the strategy of hybridization in a single molecule, of bioactive moieties "metal ion or organometallic core + bioactive organic ligand", leads in many cases to antiparasitic metal-based compounds bearing improved biological properties. In particular, we have expanded our research on the effect of metal coordination of bioactive ligands by including the *fac*-Re(I) tricarbonyl organometallic core, since *fac*-$\{Re(CO)_3\}^+$ compounds have been described in the last years as a new class of promising antiproliferative compounds [8-9]. Although rhenium compounds have been poorly explored as antitrypanosomal agents, our group counts with previous experience with promising *fac*-Re(I) tricarbonyl complexes with salicylaldehyde semicarbazone and

thiosemicarbazone ligands [10-11]. Currently, we propose to explore the potentiality as prospective antitrypanosomal agents of new *fac*-Re(I) tricarbonyl compounds that include in the same molecule two ligands bearing antiparasitic activity: a bidentate 1,10-phenanthroline derivative and a monodentate azole, namely, clotrimazole [12-13]. At a first stage, the biological activity of the whole series of rhenium compounds was evaluated against the epimastigote form of *Trypanosoma cruzi*. In addition, to study the metallomics of the most active compound of the series in *Trypanosoma cruzi*, the percentage of rhenium uptaken by the parasite and the preferred association of the compound with parasite biomacromolecules were also determined. For this task, a newly bioanalytical method based on microwave plasma atomic emission spectrometry (MP-AES) was developed and applied.

In general terms, trace inorganic elements can be determined in many matrixes using several methods consisting of microwave-assisted digestion with mineral acids and subsequent measurement using atomic spectrometry techniques. The trend in analytical chemistry is to avoid drastic treatments and to look for efficient extraction procedures under mild conditions, in accordance with green analytical chemistry principles [14]. In the case of rhenium, it can be determined by flame atomic absorption (FAAS) in a nitrous oxide-acetylene flame with a characteristic concentration of around 14 mg L^{-1} at 346.0 nm. However, due to its extremely high melting point of 3186 °C and its boiling point of around 5900 °C, it cannot be practically determined by electrothermal atomic absorption spectrometry (ET-AAS) since an adequate vapor pressure cannot be attained at the prevailing temperatures [15]. Recently, MP-AES has reemerged with several improvements, and it can be considered as a strategy for the determination of highly refractory elements such as rhenium. This technique employs the concept of "running on air" with a nitrogen plasma that operates at around 5000 K, which results in cost savings

and the removal of expensive and flammable gases from the laboratory [16-17]. This high plasma temperature provides a higher sample matrix tolerance, lower detection limits and an expanded working concentration range, when compared to FAAS. However, it is not the first choice for trace elements determination since detection limits are not as low as those achieved with other techniques such as ET-AAS or inductively coupled plasma – mass spectrometry (ICP-MS) [18-20]. However, in this work it was demonstrated for the first time that MP-AES can be used as a tool to perform rhenium elemental analysis as well as to probe the fate of potential rhenium-based drugs for metallomic studies with excellent performance. MP-AES technique had not been used for metallomic studies in the past, hence, this study assumes importance also from the technique's point of view. Moreover, an attempt to perform rhenium determination by ET-AAS according to the only publication found on the scientific literature [21], was also discussed and compared. The results obtained in this work provide new knowledge and deeper insights to the field of bioanalytical chemistry applied to medicinal inorganic chemistry.

2. Materials and methods

2.1. *Reagents*

Calibration curves were prepared by serial dilution of a rhenium 1000 mg L^{-1} solution prepared from ammonium perrhenate (NH_4ReO_4) (Sigma Aldrich, St. Louis, MO, USA) in 0.01 mol L^{-1} nitric acid (HNO_3) prepared from concentrated HNO_3 (Merck, Darmstadt, Germany).

A NIST 1643e (Gaithersburg, MD, USA) standard reference material (SRM), consisting of trace elements in water, was used for trueness and precision evaluation of the analytical method.

Ultrapure water of 18.2 MΩcm resistivity was obtained from a Millipore Direct Q3 UV water purification system (Bedford, MA, USA). All glassware was soaked overnight in 1.4 mol L^{-1} HNO_3 and then rinsed exhaustively with ultrapure water for decontamination.

2.2. *Studied rhenium compounds*

A new series of five [*fac*-Re(I)$(CO)_3$(NN)(CTZ)](PF_6) recently synthetized compounds was used, where CTZ is clotrimazole and NN are bidentate 1,10-phenanthroline derivatives, namely: 1,10-phenantroline (phen); 5-amino-1,10-phenantroline (aminophen); 4,4′-dimethyl bipyridine (dmb); 3,4,7,8-tetramethyl-1,10-phenantroline (tmp) and 2,2′-bipyridine (bipy) (Fig. 1).

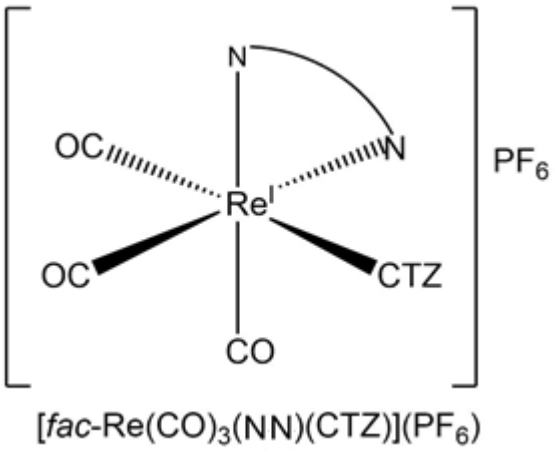


Fig. 1. General structure of the new Re(I) tricarbonyls. CTZ is clotrimazole and NN are five different bidentate 1,10-phenanthroline derivatives.

2.3. *Total digestion of rhenium compounds for elemental analysis of the metal*

A microwave-assisted acid digestion of rhenium compounds was carried out using a CEM Mars 6 microwave digestor (Matthews, NC, USA) provided with 12 Easy Prep Plus® vessels. For sample preparation 5.0 mg of sample were accurately weighted into

each vessel and 10.0 mL of 3.5 mol L^{-1} HNO_3 were added. The program consisted of a 15-minute ramp time until 200 °C and then holding for 10 min, with power varying between 400 and 1800 W, and a maximum pressure of 3.4 MPa. The obtained solutions were used for analytical determinations after an appropriate dilution with ultrapure water. Samples were run in triplicate. Reagent blanks were also run.

2.4. Rhenium uptake by parasites

The rhenium compound, [*fac*-Re(I)$(CO)_3$(tmp)(CTZ)], uptake percentage was performed as previously described [7]. Briefly, epimastigotes of CL Brener strain in a density of 3×10^6 parasites mL^{-1} were cultured in brain-heart infusion (BHI) medium at 28°C and incubated with concentrations of the rhenium compound corresponding to 1 × and 10 × IC_{50} previously determined on epimastigotes of *Trypanosoma cruzi* (3.43 µmol L^{-1} and 34.3 µmol L^{-1} respectively). Parasites were collected at 4 h and 24 h after incubation with the most active compound. Each sample (3×10^6 parasites) was centrifuged at 1000 *g* for 10 min. The supernatant containing uncaptured compound was separated from the pellet of parasites. The parasites in the pellet fraction were washed with 1 × phosphate buffered saline (PBS) and resuspended in 500 µL of 0.1 mol L^{-1} HNO_3 and subjected to ultrasonication for 5 min in a Cole Parmer 8893 (Vernon Hills, IL, USA) ultrasonic bath at 47-kHz for optimum solubilization. The supernatant was appropriately diluted with 0.1 mol L^{-1} HNO_3. Both fractions (pellet and supernatant) were analyzed by MP-AES. The uptake percentage of rhenium in the parasites was determined according to the following equation: % entry = P/(P + S), where "P" corresponds to total µg of rhenium in the parasites (pellet), "S" corresponds to µg of rhenium in the supernatant and "P + S" is total µg of the metal incorporated in the experiment (supernatant + pellet).

154 Three independent experiments were performed for each concentration at each analyzed
155 time point.
156
157 *2.5. Rhenium association with parasite biomacromolecules*
158 Rhenium association with different biomacromolecules (DNA, RNA, soluble
159 proteins, and insoluble fraction) was studied for [*fac*-Re(I)$(CO)_3$(tmp)(CTZ)](PF_6) as
160 previously described [7]. Insoluble fraction mainly contained insoluble proteins and
161 membrane lipids among other insoluble molecules. Mid exponential phase parasites were
162 incubated with 1 × and 10 × IC_{50} (3.43 µmol L^{-1} and 34.3 µmol L^{-1} respectively). After 4
163 h of incubation, biomacromolecules were isolated for further analysis. For DNA isolation,
164 2×10^6 parasites were collected using Monarch Genomic DNA Purification Kit provided
165 by New England Biolabs (Ipswich, MA, USA). For protein isolation, 2×10^6 parasites
166 were resuspended in 1 mL of parasite lysis buffer containing Tris-HCl 10 mmol L^{-1} pH
167 7.5, EDTA 1 mmol L^{-1}, CHAPS 1%, glycerol 10%, Triton 0.5%, and Complete™
168 Protease Inhibitor Cocktail provided by Roche (Manheim, Germany). After stirring on
169 ice for 30 min, the lysate was centrifugated at 20,000 *g* for 1 h at 4 °C. The soluble proteins
170 were isolated from the supernatant and the insoluble ones from the pellet, which were
171 resuspended in 200 µL of PBS. Total RNA was isolated using Trizol™ reagent provided
172 by Life Technologies (Gaithersburg, MD, USA) starting from 2×10^6 parasites. All
173 fractions were diluted to 500 µL with 0.1 mol L^{-1} HNO_3 and subjected to ultrasonication
174 for 5 min in the ultrasonic bath at 47 kHz for optimum solubilization. Three independent
175 experiments were performed for each fraction at each concentration.
176 Samples were analyzed by MP-AES. Associated percentages were calculated as
177 follows: [µg of rhenium in fraction *x*/total µg of rhenium] × 100, where "*x*" was a given

biomacromolecule fraction, and "total μg of rhenium" was the sum of rhenium in RNA fraction + DNA fraction + soluble protein fraction + insoluble fraction.

2.6. Rhenium analytical determinations

Total rhenium determinations were performed by MP-AES using an Agilent 4210 spectrometer (Santa Clara, CA, USA) equipped with an inert One Neb nebulizer with a double-pass glass cyclonic spray chamber system, and a standard torch. The spectrometer used an online nitrogen generator Agilent 4107 (Santa Clara, CA, USA), which takes in air from the environment through a Dürr Technik KK70 TA-200K compressor (Bietigheim-Bissingen, Germany). The plasma gas flow was fixed at 20 L min^{-1} and the auxiliary gas flow at 1.5 L min^{-1}. The following operational settings were applied: uptake time of 70 s, plasma stabilization time with sample aspiration of 15 s, read time of 3 s (in triplicate), wash time of 20 s, wavelength 346.046 nm, viewing position -20, nebulizer flow 0.8 L min^{-1}. Automatic background correction was used.

For comparison reasons, total rhenium determinations were also performed by electrothermal atomic absorption spectrometry (ET-AAS) using a Thermo Scientific iCE 3500 spectrometer (Cambridge, United Kingdom) equipped with auto-sampler and employing Zeeman correction. The spectrometer was connected to a transversely heated graphite tube furnace module from Thermo Fisher Scientific (Cambridge, United Kingdom). A rhenium hollow cathode lamp from Green Scientific (Beaconsfield, Australia) was used. The analytical line employed was 346.0 nm and the signal used for quantification was integrated absorbance (peak-area). Omega platform pyrolytically coated graphite tubes from Thermo Scientific (Cambridge, United Kingdom) were used. Argon 99.998% of purity provided by Linde (Montevideo, Uruguay) was used as purge

202 and protective gas. The graphite furnace heating program used is shown in Table 1. No
203 matrix modifier was used.

Table 1. Temperature program for the determination of rhenium by ET-AAS.

Stage	Temperature (ºC)	Ramp rate (ºC s^{-1})	Hold time (s)	Argon flow (L min^{-1})
Drying	100	10	40	0.2
Pyrolysis	1500	150	20	0.2
Atomization	2800	0	3	-
Cleaning	2850	0	3	0.2

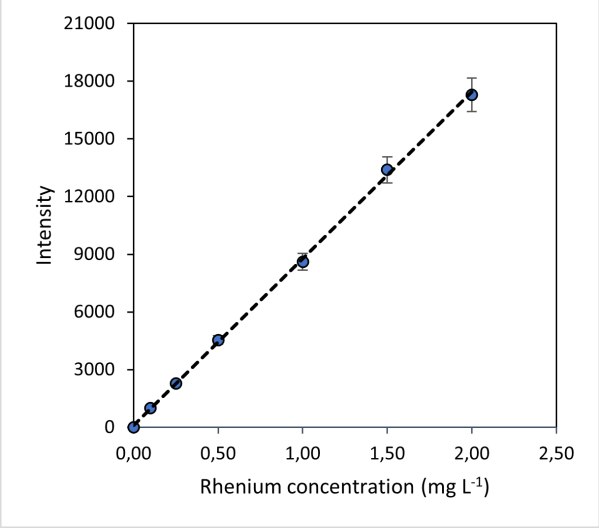
204

205 ## 3. Results and discussion

206 *3.1. MP-AES analytical method validation*

207 The analytical method was validated for rhenium based on the Eurachem guide
208 recommendations [22]. Validation was performed using direct calibration, at five
209 concentration levels, in the range presented in Table 2.

210 Linearity was verified in a suitable range for this application, up to 2.0 mg L^{-1}, using
211 the lack-of-fit test [23]. For all calibration functions, the determination coefficient value
212 (R^2) was greater than 0.999. The resulting calibration curve is shown in Fig. 2, being the
213 equation as follows: Intensity = (8672.3 ± 104.0)*Concentration (mg L^{-1}) + (101.9 ± 1.5)



214

215 **Fig. 2.** Calibration curve for rhenium determination.

For trueness evaluation, a student's *t*-test was performed to compare the obtained values with the certified values of the SRM. All experimental *t*-values were below the theoretical *t* (0.05, 5) of 2.57 indicating that, at the 95% confidence level, the concentrations did not differ significantly from the informed value [23]. Average recovery was 98%. Precision estimated as repeatability and expressed as percent relative standard deviation (%RSD) for the analysis of the SRM (n = 6) was better than 5%. These results for precision were adequate, considering that the analytical determinations were performed at trace levels. So, the accuracy of the method was ensured [22].

To estimate the limits of detection and quantification (LOD and LOQ), the criteria established by the Eurachem guide was used, namely, 3s and 10s, being "s" the standard deviation of the blank [22]. These limits are summarized in Table 2, which proved to be suitable for the objectives of this work, being the LOQ at least five times below the lower value obtained in the samples, enough to be able to quantify rhenium with an adequate confidence level. It should be noted that this LOQ obtained by MP-AES is slightly lower than that reported by Koide *et al*. [21] using ET-AAS.

In sum, this method can be considered as a reliable alternative to perform rhenium metallomic studies. Furthermore, the MP-AES technique pose several advantages over other widely used atomic spectrometry techniques. For instance, with MP-AES, the use of expensive gases such as high-purity argon and acetylene can be avoided.

Table 2. Main analytical figures of merit obtained after rhenium validation by MP-AES.

Parameter	LOD (mg L^{-1}) (3*s*; n=10)	LOQ (mg L^{-1}) (10*s*; n=10)	Linearity (mg L^{-1})	Precision (%RSD; n=6) *	Trueness (%Recovery; n=6) *
Re	0.005	0.015	0.015 – 2.0	4.5	98.0

*SRM: NIST 1643e.

238 *3.2. Comparison with ET-AAS analytical method*

239 The optimization of pyrolysis and atomization temperatures was carried out in an
240 exhaustive way, by performing the corresponding pyrolysis-atomization curves from
241 1000 to 3000 °C at 200 °C intervals. Once the optimum temperature ranges were found,
242 the fine adjustment was made by using 50 °C intervals, obtaining the heating program
243 presented in Table 1. As reported by Koide *et al*. [21] strongly broadened peaks and a
244 sensitivity highly dependent on the atomization temperature were observed in this work.
245 Temperatures below 2800 °C did not result in complete atomization. Also, to avoid
246 memory effects several heat-out cycles at 2850 °C were necessary to obtain base-line
247 readings which was very unfavorable for the lifetime of the graphite tubes, which are
248 quite expensive laboratory supplies. All these drawbacks, led to a low precision of the
249 method, which turned out to be 25% expressed as %RSD. Under these conditions, an
250 instrumental LOD of 0.027 mg L^{-1} was attained, employing the 3s criteria as
251 recommended by Eurachem guide, in accordance with that obtained by Koide *et al*. [21]
252 of 0.020 mg L^{-1}. All the disadvantages presented during rhenium determination by ET-
253 AAS promoted the development and validation of the MP-AES method.

254

255 *3.3. Elemental analysis of rhenium compounds*

256 The sample preparation procedure for rhenium compounds using dilute HNO_3 acid
257 resulted very efficient with the advantage of reducing the use of dangerous residues and
258 lowering costs. The solution obtained after microwave-assisted digestion was limpid and
259 no further procedures were required, except for the dilution with ultrapure water prior to
260 instrumental analysis by MP-AES.

261 Rhenium elemental analysis results agree with the proposed formula for the five new
262 [*fac*-Re(I)$(CO)_3$(NN)(CTZ)](PF_6) complexes as shown in Table 3, being the percentage

recoveries between the experimental and the theoretical value in the range 97 – 101 %. These results contribute to the overall validation of the newly developed method based on MP-AES, demonstrating good selectivity towards rhenium in real samples.

Table 3. Elemental analysis of rhenium on studied compounds.

Compound	Theoretical %Re	Experimental %Re	%Recovery
[*fac*-Re(I)(CO)$_3$(phen)(CTZ)](PF$_6$)	19.8	19.5	99
[*fac*-Re(I)(CO)$_3$(aminophen)(CTZ)](PF$_6$)	19.5	19.0	97
[*fac*-Re(I)(CO)$_3$(dmb)(CTZ)](PF$_6$)	19.7	19.3	98
[*fac*-Re(I)(CO)$_3$(bipy)(CTZ)](PF$_6$)	20.3	20.5	101
[*fac*-Re(I)(CO)$_3$(tmp)(CTZ)](PF$_6$)	18.7	18.3	98

3.4. Rhenium uptake of the most active compound on Trypanosoma cruzi

The five new [*fac*-Re(I)(CO)$_3$(NN)(CTZ)](PF$_6$) complexes were previously evaluated *in vitro* for their anti-*Trypanosoma cruzi* activities against epimastigotes of CL Brener strain. To determine the IC_{50} value (50% growth inhibitory concentration) a method adapted by our research group was employed [7]. The newly synthesized compounds showed IC_{50} values in the low micromolar range (3.43–10.2 μmol L^{-1}), the same order as those of the reference drug Nifurtimox (2.86 μmol L^{-1}), being [*fac*-Re(I)(CO)$_3$(tmp)(CTZ)](PF$_6$) the most active compound.

Thus, for metallomics studies, epimastigotes were incubated with the most promising compound, [*fac*-Re(I)(CO)$_3$(tmp)(CTZ)](PF$_6$), for 4 and 24 h at concentrations of 1 × and 10 × the IC_{50} value (3.43 μmol L^{-1}). Rhenium uptaken by the parasites or strongly bound (not removable by washing), and rhenium remaining in the culture medium, were determined by MP-AES to estimate the amount of compound into epimastigote form of the parasites. The average amount of rhenium determined for three independent experiments is shown in Table 4. To evaluate the accuracy of this assay, the

corresponding mass balance was calculated to verify that the mass of rhenium in the pellet + the mass of rhenium in the culture, was statistically equivalent to the total mass of rhenium used for incubation. A Student's *t*-test ($p < 0.05$) was performed for this task, showing experimental *t* values below the theoretical ones. Thus, it was concluded that the sum of experimental masses obtained by MP-AES were statistically equal to the theoretical masses used for incubation.

As presented in Table 4 the metal incorporation percentages do not change significantly between 4 and 24 h. The uptake percentages are quite low when compared with those previously reported in epimastigotes for promising vanadium compounds synthesized by our research group [24-25]. This differential uptake could arise from the different chemical nature of the compounds and the different metallic center.

Table 4. Calculated percentages of rhenium uptaken by the parasites after 4 h and 24 h incubation with 1 × and 10 × the calculated IC_{50} value for [*fac*-$Re(I)(CO)_3(tmp)(CTZ)](PF_6)$ on epimastigotes of *Trypanosoma cruzi*.

Compound concentration (μM)	%Entry ± SD	
	4 h	24 h
1 × IC_{50}	1.24 ± 0.03	1.16 ± 0.04
10 × IC_{50}	1.28 ± 0.03	1.25 ± 0.02

%Entry corresponds to % of rhenium uptaken by the parasite (pellet) relative to the total rhenium in the parasite culture. The average of three independent experiments and standard deviation (SD) are presented for each point.

3.5. Rhenium distribution in the parasite of the most active compound on Trypanosoma cruzi

Rhenium association with biomacromolecules was studied in order to evaluate the subcellular distribution of the compound [*fac*-$Re(I)(CO)_3(tmp)(CTZ)](PF_6)$ inside the parasites. For this task, epimastigotes from *Trypanosoma cruzi* were incubated for 4 h with the chosen compound. Afterwards, different biomacromolecules (DNA, RNA, and

306 proteins) were isolated, being total rhenium associated with each fraction determined by
307 MP-AES. Results are shown in Table 5.

308 A similar pattern of rhenium distribution was observed in epimastigotes after 4 h of
309 treatment with 1 × and 10 × the IC_{50} value (3.43 μmol L^{-1}) previously determined on cell-
310 derived epimastigotes. As a matter of fact, no statistically significative differences were
311 found between incubation with different compound concentrations according to by a one-
312 way analysis of variance (ANOVA) followed by Student's *t*-test ($p < 0.05$).

313 As can be observed in Table 5, less than 1% was found associated to nucleic acids
314 fractions (DNA and RNA) at both concentration levels. A preferential association to the
315 soluble proteins fraction was observed with an average value of 82.8%. Similar
316 preferential association with soluble proteins was previously reported by our research
317 group for heteroleptic oxidovanadium(V) complexes [7, 24-25].

318

319 **Table 5.** Calculated percentage of rhenium associated with the different isolated
320 biomacromolecules after 4 h of treatment with 1 × and 10 × the calculated IC_{50} value of
321 [*fac*-Re(I)$(CO)_3$(tmp)(CTZ)](PF_6) on epimastigotes of *Trypanosoma cruzi*.

Compound concentration (μM)	%Association ± SD			
	SP	IF	ADN	ARN
1 × IC_{50}	82.1 ± 0.8	16.3 ± 0.7	0.85 ± 0.05	0.76 ± 0.07
10 × IC_{50}	83.5 ± 0.3	15.0 ± 0.2	0.81 ± 0.03	0.69 ± 0.06

322 %Association corresponds to % of rhenium associated with the fraction relative to the total
323 rhenium uptaken by the parasite. The average of three independent experiments and standard
324 deviation (SD) are presented for each fraction: DNA, RNA, soluble proteins (SP) and insoluble
325 fraction (IF).

326

327 **4. Conclusions**

328 The validated analytical method based on MP-AES technique, which was used in this
329 study for rhenium determination, can be considered as a reliable, economical, and green

alternative for metallomic studies, constituting an efficient strategy for bioanalytical evaluation of bioactive rhenium compounds.

The use of this method allowed to get relevant biological parameters for the rhenium agent [*fac*-Re(I)$(CO)_3$(tmp)(CTZ)](PF_6). Metallomics studies of this most promising compound, performed on the epimastigote form *Trypanosoma cruzi*, showed a low uptake of rhenium by the parasites and a preferential accumulation in the soluble proteins fraction. A negligible localization of the compound in the DNA and RNA fractions, which allows to discard these biomolecules as the main targets.

This work highlights once again the importance of providing validated methods before its application to the performance of different sort of bioanalysis, such as metallomics studies, to obtain accurate results. Also, the incorporation of MP-AES technique is worth to mention, considering all the many advantages previously enumerated. In sum, the work promotes the key role of bioanalytical chemistry in supporting medicinal inorganic chemistry in the development of newly metallic drugs and in the study of their mechanism of action.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgements

This research was funded by Agencia Nacional de Investigación e Innovación (ANII, Uruguay), grant FCE_1_2019_1_155971. M. Soba acknowledges the support of the Agencia Nacional de Investigación e Innovación (ANII, Uruguay) through the grant POS_FCE_2020_1_1009195 and the support of PEDECIBA-Química.

References

[1] World Health Organization 2022, Chagas disease (American trypanosomiasis). http://www.who.int/chagas/en/. (Accessed 9 March 2022).

[2] J. Brindha, M. Balamurali, K. Chanda, An overview on the therapeutics of neglected infectious diseases. Leishmaniasis and Chagas diseases, Front. Chem. 9 (2021) 1–19. https://doi.org/10.3389/fchem.2021.622286

[3] E. Chatelain, J.R. Ioset, Phenotypic screening approaches for Chagas disease drug discovery, Expert. Opin. Drug. Discov. 13 (2017) 141–153. https://doi.org/10.1080/17460441.2018.1417380

[4] O.T. Kayode, C.K. Lele, A.A.A. Kayode, Trypanosomiasis: recent advances in strategies for control, Glob. J. Infect. Dis. Clin. Res. 6 (2021) 037–041. http://doi.org/10.17352/2455-5363.000033

[5] R. Paucar, E. Moreno-Viguri, S. Pérez-Silanes, Challenges in Chagas disease drug discovery: a review. Curr. Med. Chem. 23 (2016) 3154–3170. https://doi.org/10.2174/0929867323999160625124424

[6] A.J. Pérez-Molina, I. Molina, Chagas disease, Lancet. 391 (2018) 82–94. https://doi.org/10.1016/s0140-6736(17)31612-4

[7] G. Scalese, I. Machado, G. Salinas, L. Pérez-Díaz, D. Gambino, Heteroleptic oxidovanadium(V) complexes with activity against infective and non-infective stages of *Trypanosoma cruzi*, Molecules. 26 (2021) 5375. https://dx.doi.org/10.3390%2Fmolecules26175375

[8] G. Gasser, N. Ott, J. Metzler-Nolte, Organometallic anticancer compounds, Med. Chem. 54 (2011) 3–25. https://doi.org/10.1021/jm100020w

[9] A.V. Shtemenko, P. Collery, N.I. Shtemenko, K.V. Domasevitch, E.D. Zabitskaya, AA. Golichenko, Synthesis, characterization, *in vivo* antitumor properties of the

cluster rhenium compound with GABA ligands and its synergism with cisplatin, Dalton Trans. 26 (2009) 5132–5136. https://doi.org/10.1039/B821041A

[10] I. Machado, S. Fernández, L. Becco, B. Garat, J.S. Gancheff, A. Rey, D. Gambino, New *fac*-tricarbonyl rhenium(I) semicarbazone complexes: synthesis, characterization, and biological evaluation, J. Coord. Chem. 67(10) (2014) 1835–1850. https://doi.org/10.1080/00958972.2014.926008

[11] E. Rodríguez Arce, I. Machado, B. Rodríguez, M. Lapier, M.C. Zúñiga, J.D. Maya, C. Olea Azar, L. Otero, D. Gambino, Rhenium(I) tricarbonyl compounds of bioactive thiosemicarbazones: synthesis, characterization, and activity against *Trypanosoma cruzi*, J. Inorg. Biochem. 170 (2017) 125–133. https://doi.org/10.1016/j.jinorgbio.2017.01.011

[12] M. Fernández, J. Varela, I. Correia, E. Birriel, J. Castiglioni, V. Moreno, J.C. Pessoa, H. Cerecetto, M. González, D. Gambino, A new series of heteroleptic oxidovanadium(iv) compounds with phenanthroline-derived co-ligands: selective *Trypanosoma cruzi* growth inhibitors, Dalton Trans. 42 (2013) 11900–11911. https://doi.org/10.1039/C3DT50512J

[13] D. Gambino, L. Otero, Design of prospective antiparasitic metal-based compounds including selected organometallic cores, Inorg. Chim. Acta. 472 (2018) 58–75. http://dx.doi.org/10.1016/j.ica.2017.07.068

[14] I. Machado, I. Dol, E. Rodríguez-Arce, M.V. Cesio, M. Pistón, Comparison of different sample treatments for the determination of As, Cd, Cu, Ni, Pb and Zn in globe artichoke (*Cynara cardunculus* L. subsp. *Cardunculus*), Microchem. J. 128 (2016) 128–133. http://dx.doi.org/10.1016/j.microc.2016.04.016

[15] B. Welz, M. Sperling, Atomic absorption spectrometry, third ed., Wiley-VCH Verlag GmbH, Weinheim, 1999.

405 [16] L. São Bernardo Carvalho, C. Santos Silva, J. Araújo Nóbrega, E. Santos Boa Morte,
406 D.C. Muniz Batista Santos, M.G Andrade Korn, Microwave induced plasma optical
407 emission spectrometry for multielement determination in instant soups, J. Food
408 Compos. Anal. 86 (2020) 103376. https://doi.org/10.1016/j.jfca.2019.103376
409 [17] C.B. Williams, R.S. Amais, C.M. Fontour, B.T. Jones, J. Araujo Nóbreg, G.L.
410 Donati, Recent developments in microwave-induced plasma optical emission
411 spectrometry and applications of a commercial Hammer-cavity instrument, Trends
412 Anal. Chem. 116 (2019) 151–157. https://doi.org/10.1016/j.trac.2019.05.007
413 [18] M. Pistón, A. Suárez, V. Bühl, F. Tissot, J. Silva, L. Panizzolo, Influence of cooking
414 processes on Cu, Fe, Mn, Ni, and Zn levels in beef cuts, J. Food Compos. Anal. 94
415 (2020) 103624. http://dx.doi.org/10.1016/j.jfca.2020.103624
416 [19] A.R. Corrales Escobosa, K. Wrobel, E. Yanez Barrientos, S. Jaramillo Ortiz, A.S.
417 Ramírez Segovia, K. Wrobel, Effect of different glycation agents on Cu(II) binding
418 to human serum albumin, studied by liquid chromatography, nitrogen microwave-
419 plasma atomic-emission spectrometry, inductively-coupled-plasma mass
420 spectrometry, and high-resolution molecular-mass spectrometry, Anal. Bioanal.
421 Chem. 407 (2015) 1149–1157. http://dx.doi.org/10.1007/s00216-014-8335-1
422 [20] V. Balaram, Microwave plasma atomic emission spectrometry (MP-AES) and its
423 applications. A critical review, Microchem. J. 159 (2020) 105483.
424 https://doi.org/10.1016/j.microc.2020.105483
425 [21] M. Koide, V. Hodge, J.S. Yang, E.D. Goldberg, Determination of rhenium in marine
426 waters and sediments by graphite furnace atomic absorption spectrometry, Anal.
427 Chem. 59(14) (1987) 1802–1805. https://doi.org/10.1021/ac00141a014

[22] Eurachem 2014, Eurachem guide: the fitness for purpose of analytical methods – A laboratory guide to method validation and related topics. http://www.eurachem.org. (Accessed 9 March 2022).

[23] J.N. Miller, J.C. Miller, Statistics and chemometrics for analytical chemistry, sixth ed., Ashford Colour Press Ltd., Gosport, 2010.

[24] G. Scalese, I. Machado, C. Fontana, G. Risi, G. Salinas, L. Pérez-Díaz, D. Gambino, New heteroleptic oxidovanadium(V) complexes: synthesis, characterization, and biological evaluation as potential agents against *Trypanosoma cruzi*, J. Biol. Inorg. Chem. 23 (2018) 1265–1281. https://doi.org/10.1007/s00775-018-1613-1

[25] G. Scalese, I. Machado, I. Correia, J.C. Pessoa, L. Bilbao, L. Pérez-Diaz, D. Gambino, Exploring oxidovanadium(iv) homoleptic complexes with 8-hydroxyquinoline derivatives as prospective antitrypanosomal agents, New J. Chem. 43 (2019) 17756–17773. https://doi.org/10.1039/C9NJ02589H

441 **Captions to tables**

442

443 **Table 1.** Temperature program for the determination of rhenium by ET-AAS.

444 **Table 2.** Main analytical figures of merit obtained after rhenium validation by MP-AES.

Table 3. Elemental analysis of rhenium on studied compounds.

445 **Table 4.** Calculated percentages of rhenium uptaken by the parasites after 4h and 24 h
446 incubation with 1 × and 10 × the calculated IC_{50} value for [*fac*-
447 Re(I)$(CO)_3$(tmp)(CTZ)](PF_6) on epimastigotes of *Trypanosoma cruzi*.

448 **Table 5.** Calculated percentage of rhenium associated with the different isolated
449 biomacromolecules after 4 h of treatment with 1 × and 10 × the calculated IC_{50} value of
450 [*fac*-Re(I)$(CO)_3$(tmp)(CTZ)](PF_6) on epimastigotes of *Trypanosoma cruzi*.

451

452 **Captions to figures**

453

454 **Fig. 1.** General structure of the new Re(I) tricarbonyls. CTZ is clotrimazole and NN are
455 five different bidentate 1,10-phenanthroline derivatives.

456 **Fig. 2.** Calibration curve for rhenium determination.