The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

# Equilibrium characteristic and biological activity of aqueous cobalt(II) complexes with reduced Schiff base *N*-(2-hydroxybenzyl)phenylalanine

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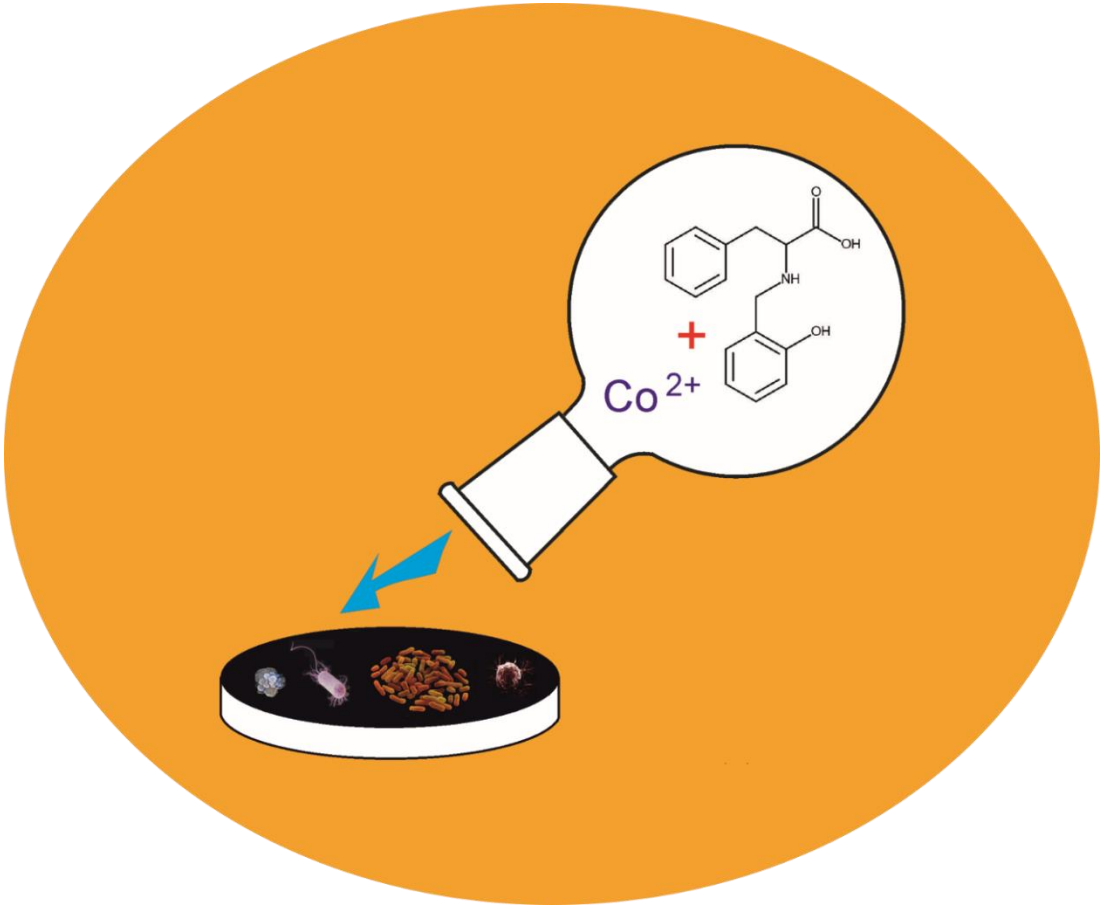
# Equilibrium characteristic and biological activity of aqueous cobalt(II) complexes with reduced Schiff base *N*-(2-hydroxybenzyl)phenylalanine



**Abstract:**
The present study describes the coordination properties of a reduced Schiff base, *N*-(2-hydroxybenzyl)phenylalanine, towards cobalt(II) using potentiometric method. The speciation model was confirmed by UV–Vis studies. The appearance of a precipitate in the pH range 2-7.3 meant that measurements in aqueous solution were only possible in an alkaline medium. The results show the formation of four mononuclear complexes in aqueous solution with the different degree of ligand deprotonation. At physiological pH, the antimicrobial properties of *N*-(2-hydroxybenzyl)phenylalanine and of the Co(II)–ligand complexes were determined against Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Helicobacter pylori*), Gram-positive bacteria (*Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*), and a fungal strain (*Candida*). The results indicate that both the complexes and the ligand alone exhibit anti-cellular toxicity against L929 mouse fibroblasts and gastric cancer cells. This double activity of the tested preparations prompts further study on the development of a composition to combat *H. pylori* infection and eliminate gastric epirthelial cells with altered phenotype.

**Keywords:** biological activity; cobalt(II) complexes; coordination modes; reduced Schiff base; stability constant

# Introduction

Reduced forms of the Schiff bases, as good chelating agents, can form transition metal complexes with important applications such as antimicrobial and antifungal activity, cytotoxicity, and DNA-binding. Schiff bases are a combination of two structural fragments derived from amino acids and carbonyl compounds. The formation of a reduced Schiff base structure increases its conformational flexibility, making the molecule more stable and able to be used as a model in biological reactions.

The present study examines the complexation equilibria in the aqueous system of cobalt(II) with the reduced Schiff base *N*-(2-hydroxybenzyl)phenylalanine, PhAlaSal. Increasing drug resistance requires continued development of new therapeutic compounds. Therefore, an important objective of this study is to evaluate the antimicrobial activity of the ligand and its cobalt(II) complexes in conjunction with biocompatibility to eukaryotic cells and potentially cytotoxicity towards gastric cancer cells.

## Results and discussion

The protonation constants of PhAlaSal (Fig.1), determined in aqueous solution, indicate the dissociation of three functional groups. The first dissociation constant, equal to 2.90, is assigned to the carboxyl group. In turn, p$K_{a2}$ = 8.19 and p$K_{a3}$ = 9.95 relate to the amine and phenolic groups, respectively.

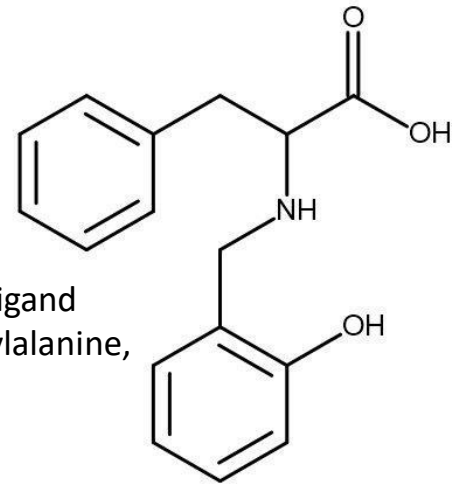



**Figure 1.** Structure of the ligand *N*-(2-hydroxybenzyl) phenylalanine, PhAlaSal [$LH_2$].

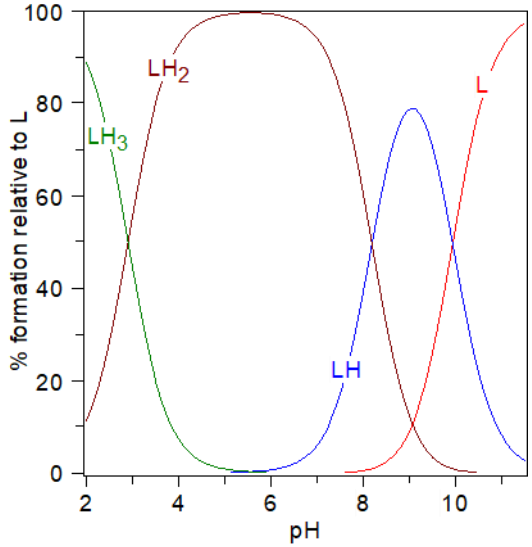



**Figure 2.** Species distribution curves of the PhAlaSal; $c_{PhAlaSal}$ = 1.0x10$^{-3}$ M.

This order of deprotonation is confirmed by previous studies involving a hydroxybenzyl derivative. The pH–dependent species distribution diagram shows the individual ionic forms of the ligand (Fig. 2).

# Results and discussion

The pH-metric results confirm the formation of four complexes in the aqueous Co(II)–PhAlaSal system. The overall stability constants of these species are presented in Table 1.

Table 1. Decimal logarithms of overall formation constants in the Co(II)–PhAlaSal system, $\beta_{mlh} = [M_mL_lH_h]/[M]^m[L]^l[H]^h$ at 25.0 x 0.1 °C, I = 0.1 ($KNO_3$). Standard deviations in parentheses after overall protonation and stability constants refer to random errors only.

| Species | $\log_{10}\beta_{mlh}$ | Stepwise stability constants |
|---|---|---|
| [CoL] | 6.41(13) | 6.41 |
| $[CoL_2]^{2-}$ | 11.13(5) | 4.72[b] |
| $[CoL_3]^{4-}$ | 15.27(8) | 4.14[c] |
| $[CoL_2H]^-$ | 19.61(9) | |
| σ ; n [a] | 2.92; 156 | |

[a] $\sigma$ – the value of the normalized sum of squared residuals; *n* – number of titration points

[b] $\log_{10} K^{CoL}_{CoL_2} = \log_{10}\beta_{CoL_2} - \log_{10}\beta_{CoL} = 11.13 - 6.41 = 4.72$

[c] $\log_{10} K^{CoL_2}_{CoL_3} = \log_{10}\beta_{CoL_3} - \log_{10}\beta_{CoL_2} = 15.27 - 11.13 = 4.14$

# Results and discussion

A representative species distribution graph is presented for PhAlaSal-to-Co(II) molar ratio 2:1 (Fig. 3).

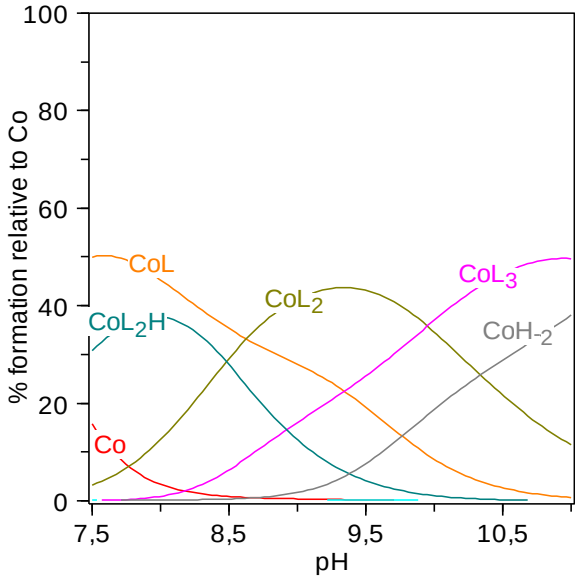



**Figure 3.** Species distribution curves for the complexes formed in the Co(II) – PhAlaSal system as a function of pH relative to Co(II); $C_{PhAlaSal}$ = 5.0x10$^{-3}$ M.

PhAlaSal, as a deprotonated tridentate ligand, most likely occupies only equatorial positions in the [CoL] complex, binding to the central ion via amine, phenolic and carboxyl groups and forming two chelate rings.
This coordination mode has been previously indicated for analogous systems of the reduced Schiff base, N-(2-hydroxybenzyl)alanine, with transition metal ions, both in the solid state and in aqueous solution.

## Results and discussion

In turn, in the $[CoL_2]^{2-}$ complex, as reported for analogous X-ray crystal forms, one PhAlaSal molecule most likely coordinates equatorially in the $\{O^-_{phenolic}, N, O^-_{carboxyl}\}$ chelation mode.
The nitrogen atom of the second molecule is also likely bound at this position, while the oxygen donors are weakly coordinated at the axial sites.

An excess of donor groups relative to the coordination sites is observed in $[CoL_3]^{4-}$. Presumably, as in the case of the tri-ligand complex in the Co(II)–AlaSal system, coordination of the metal ion takes place by three amine nitrogens and one phenolic oxygen in the equatorial positions. The remaining phenolic groups of two ligand molecules occupy the axial sites.

## Results and discussion

For only one protonated complex, $[CoL_2H]^-$, a stability constant was determined in the Co(II)–PhAlaSal system. The deprotonation step occurs according to the equation:

$[CoL_2H]^- = [CoL_2]^{2-} + H^+$

hence

$$pK_{CoL_2}^{CoL_2H} = \log_{10}\beta_{CoL_2H} - \log_{10}\beta_{CoL_2} = 19.61 - 11.13 = 8.48$$

reaches a lower value than the corresponding free ligand deprotonation constant for the phenolic proton.

# Results and discussion

The electronic absorption spectra in the presence of the metal ion indicate a blue shift of the d-d bands relative to the $Co(H_2O)_6^{2+}$ aqua-ion (Fig. 4), 515 nm ($\varepsilon$ = 4.6).

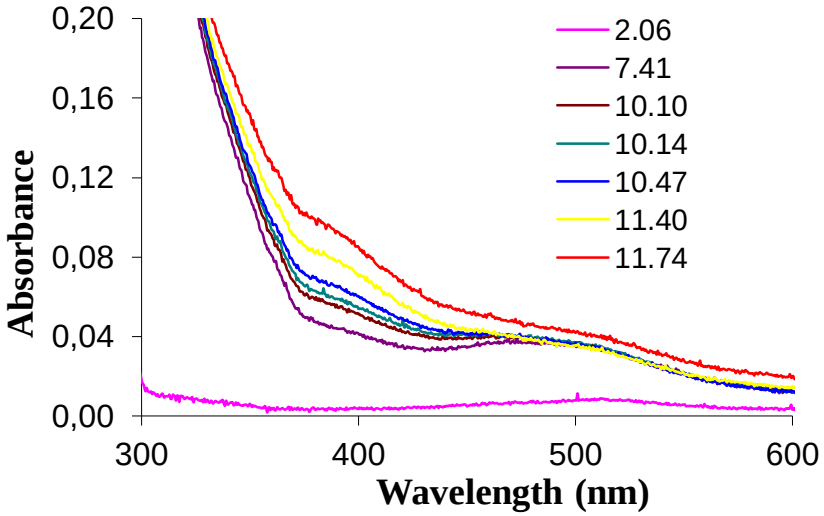



**Figure 4.** The spectra of the complexes in the Co(II)–PhAlaSal system at ligand-to-metal molar ratio 2:1; $C_{Co(II)}$ = 1x10$^{-3}$ M.

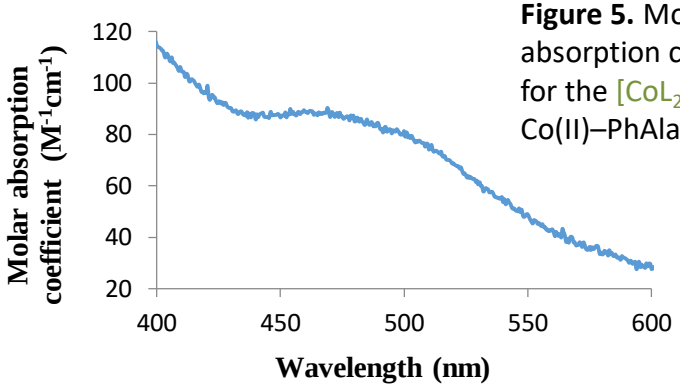



**Figure 5.** Molar absorption coefficients ($\varepsilon$) for the $[CoL_2]^{2-}$ complex in Co(II)–PhAlaSal system.

HypSpec deconvolution (part of Hyperquad 2008 suite, Protonic Software) confirmed the formation of one octahedral complex $[CoL_2]^{2-}$ (Fig.5). Presence of only this species probably indicates the dominance of $[CoL_2]^{2-}$ form within the studied pH range. The maximum molar absorption coefficient for the complex is equal to 90 $M^{-1}cm^{-1}$ at 469 nm.

# Results and discussion

PhAlaSal alone and the complexes in the Co(II)–PhAlaSal system presented antibacterial and antifungal activity. The Co(II)–PhAlaSal complexes exhibited the highest bacteriostatic and bactericidal activity against two ***Helicobacter pylori*** strains (Table 2), for which minimal inhibitory concentration (MIC), and minimal bactericidal concentration (MBC) were 1.82 mM, while for all other tested microorganisms MIC/MBC/minimal fungicidal concentration (MFC) values were >1.82.

**Table 2**

Antimicrobial activity against Gram-negative bacteria of tested compounds, prepared directly and stored for two weeks; MIC - minimal inhibitory concentration and MBC - minimal bactericidal concentration.

| Microorganism | MIC/MBC (mM) | | | |
|---|---|---|---|---|
| | Co(II)–PhAlaSal complexes | | PhAlaSal | |
| | MIC | MBC | MIC | MBC |
| | Gram-negative bacteria | | | |
| Pseudomonas aeruginosa ATCC 27853 | >1.82 | >1.82 | 7.30 | >7.30 |
| Escherichia coli ATCC 25922 | >1.82 | >1.82 | 7.30 | >7.30 |
| Helicobacter pylori CCUC 17874 | 1.82 | 1.82 | 3.65 | >7.30 |
| Helicobacter pylori ATCC 700392 | 1.82 | 1.82 | 3.65 | >7.30 |

# Results and discussion

**Table 3**
Antimicrobial activity against Gram-positive bacteria and fungi of tested compounds, prepared directly and stored for two weeks; MIC - minimal inhibitory concentration and MBC - minimal bactericidal concentration or MFC - minimal fungicidal concentration.

| Microorganism | MIC/MBC/MFC (mM) | | | |
|---|---|---|---|---|
| | Co(II)–PhAlaSal complexes | | PhAlaSal | |
| | MIC | MBC/MFC | MIC | MBC/MFC |
| Gram-positive bacteria | | | | |
| Enterococcus faecalis ATCC 29212 | >1.82 | >1.82 | 7.30 | >7.30 |
| Staphylococcus aureus ATCC 29213 | >1.82 | >1.82 | 3.65 | >7.30 |
| Staphylococcus aureus ATCC 6538 | >1.82 | >1.82 | 3.65 | >7.30 |
| Staphylococcus epidermidis ATCC 12228 | >1.82 | >1.82 | 3.65 | >7.30 |
| Fungi | | | | |
| Candida albicans ATTC 10231 | >1.82 | >1.82 | 3.65 | >7.30 |
| Candida glabrata ATCC 2001 | >1.82 | >1.82 | 3.65 | >7.30 |
| Candida parapsilosis ATCC 22019 | >1.82 | >1.82 | 3.65 | >7.30 |

In turn, the PhAlaSal alone showed the MIC values for ***H. pylori*** and ***S. aureus***, ***S. epidermidis*** as well as fungi equal to 3.65 mM, while for ***P. aeruginosa***, ***E. coli*** and ***E. faecalis*** the MIC value was equal to 7.30. For all tested microorganisms MBC/MFC values were > 7.30 (Tables 2, 3).

# Results and discussion

PhAlaSal ligand statistically significantly diminished the ability of L929 mouse fibroblasts to reduce within the range 1.82– 3.56 mM, whereas ligand solution stored for two weeks have a lower inhibitory effect than freshly prepared (Fig. 6). In turn, Co–(II) PhAlaSal complexes show significantly cytotoxic effect towards L929 cells (48%-89% of dead cells) within the range 0.06-0.91mM (freshly prepared), and within the range 0.03-0.91 mM (stored for two weeks).

Only PhAlaSal stored for two weeks inhibits metabolic activity of human AGS gastric adenocarcinoma epithelial cells within the range of 1.82– 3.56 mM (58-84% of dead cells), whereas Co(II)–PhAlaSal complexes caused dead 44-63% cells (concentration range 0.23-0.91 mM); Fig. 6.

PhAlaSal alone freshly prepared statistically significantly diminished the ability of human HeLa cervix adenocarcinoma epithelial cells to reduce MTT (38%-50% of dead cells) within the range 0.91–3.56 mM. Co(II)–PhAlaSal complexes shows significantly cytotoxic effect towards HeLa cells (45%-81% of dead cells) within the range 0.11-0.91mM (freshly prepared) and within the range 0.46-0.91 mM (stored for two weeks); Fig. 6.

# Results and discussion

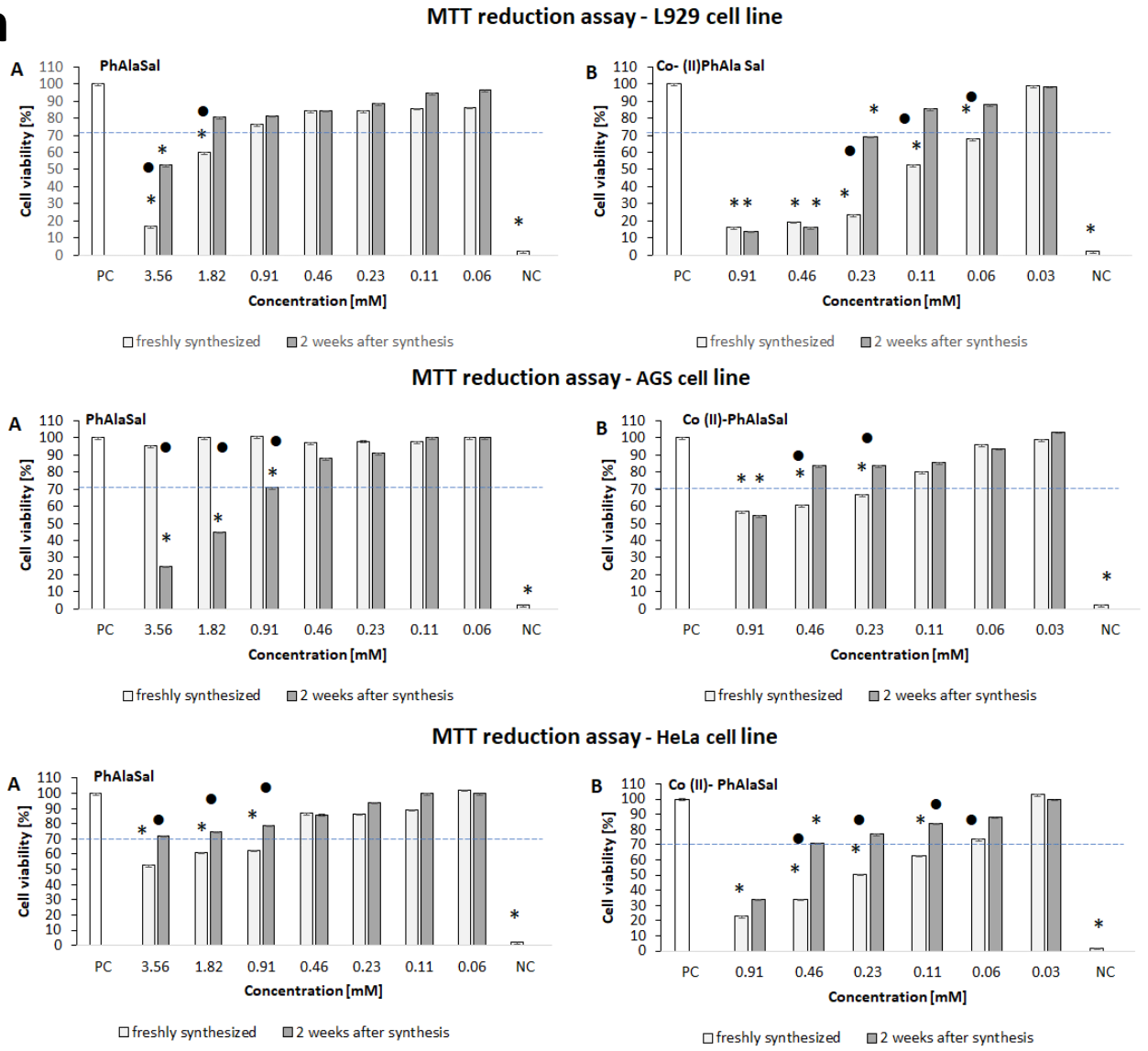



**Figure 6.** Cytotoxic effect of the studied ligand and complex forms: **(A)** PhAlaSal, **(B)** Co(II)–PhAlaSal towards L929, AGS and HeLa cells.
The cytotoxicity was assessed by MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] reduction assay.
Complete RMPI-1640 medium (cRPMI) was used as a positive control (C+) of cell viability (100% viable cells) and 0.03% $H_2O_2$ as a negative control (C−) of cell viability (100% dead inactive cells).

Statistical significance: *● $p < 0.05$;
* untreated cells vs. cells treated with tested solution (solution prepared directly or solution stored for two weeks); ● solution prepared directly vs. solution stored.
The blue line indicates the minimal percentage of viable cells (70%) required to confirm the compound as non-cytotoxic at the *in vitro* level.

## Conclusions

As shown by potentiometric measurements, four complexes were formed in the Co(II)–PhAlaSal aqueous system and the overall stability constants of all species were calculated. The Co(II)–PhAlaSal complexes exhibit equatorial coordination in the $\{O^-_{phenolic}, N, O^-_{carboxyl}\}$ chelation mode. The $[CoL_2]^{2-}$ complex, as dominant in the studied pH range, was confirmed by UV/Vis studies. Under physiological pH conditions, cobalt(II) complexes revealed the highest bacteriostatic and bactericidal activity against two *Helicobacter pylori* strains. Furthermore, the ligand alone and complexes showed different levels of anti-cellular toxicity towards L929 mouse fibroblasts, human HeLa cervix adenocarcinoma epithelial cells and human AGS gastric adenocarcinoma epithelial cells. These compounds may therefore be the basis for the development of new formulations combining cytotoxic activity towards gastric carcinoma cells with antibacterial activity against antibiotic resistant ***H. pylori*** strains, which may have applications in control of gastric diseases induced by these bacteria that belong to class I carcinogens.

## Acknowledgments

This work was supported by the Medical University of Lodz (Statute Fund No. 503/3-014-02/503-31-001) and by the Fundação para a Ciência e a Tecnologia (FCT, project UIBD/00100/2020 of the Centro de Química Estrutural), Portugal. M. S. acknowledges the FCT and IST for a working contract "DL/57/2017" (Contract no. IST-ID/102/2018).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