Stability of B-TRAXIM®2C towards acid conditions

KEY FINDINGS

The complexes issued from B-TRAXIM®2C in aqueous solution

- ***Have a relatively good stability in very acid conditions.***
- ***Were all detected in the 2 to 7 pH range.***
- ***Have a variable stability in acid condition depending on the size of the complex, larger complexes were less affected than smaller ones.***

INTRODUCTION AND OBJECTIVE

B- Traxim 2C are crystalline chelates of glycine that are more bioavailable than metal sulphates.
When set in aqueous solution at a neutral pH, the metal remains bound to glycine (TB n°322). The formula is $[M(Gly)(SO_4)]_x(H_2O)_n$.

When absorbed by an animal, B- Traxim 2C faces different conditions of acidity, ranging from 2 in the stomach to a neutral pH in the intestines. The objective of the study is to determine the stability of B- Traxim 2C in this pH range.

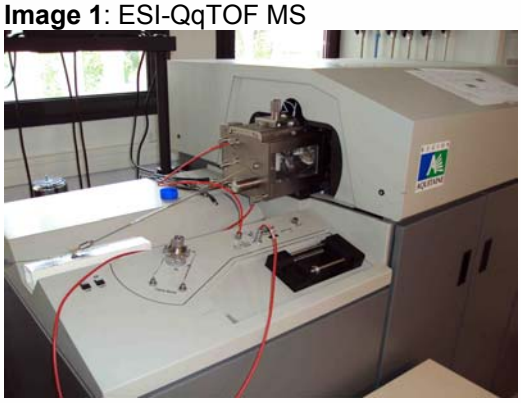
MATERIALS AND METHOD

The study was conducted at the Laboratory of Bio-Inorganic and Environmental Analytical Chemistry of the CNRS in Pau (France).

The measurements were performed by high resolution mass spectrometry.

Apparatus:
Electrospray QqTOF mass spectrometer (ESI-QqTOF MS) QSTAR XL from Applied Biosystems (iamge 1)

Image 1: ESI-QqTOF MS



Reagents and solvents:
All the solvents and reagents were of analytical grade.

Samples:

- B-TRAXIM 2C Zn $[Zn(C_2H_5NO_2)(H_2O)_2(SO_4)]_n$
- B-TRAXIM 2C Cu $Cu(C_2H_5NO_2)(H_2O)_2(SO_4)]_n$
- B-TRAXIM 2C Mn $[Mn(SO_4)(C_2H_5NO_2)]_n$
- B-TRAXIM 2C Fe $\{[Fe(C_2H_5NO_2)(H_2O)_2(SO_4)_2][Fe(C_2H_5NO_2)(H_2O)_4]\}_n$

Method of analysis
The glycinates were dissolved at 100mg/L in an optimised buffer (50% methanol, 50% of a 10 mM ammonium acetate solution) and the pH was changed between 2 and 7 by steps of 1. For each pH and each complex, the mass spectrum was recorded. If the complex was dissociated, glycine would be freed. Therefore the evolution of the glycine peak detected at 76amu (atomic mass unit) was also recorded.

The mass accuracy of the mass spectrometer was calibrated with a reserpin standard ($C_{33}H_{40}N_2O_9$): $[M+H]^+$ 609.28121amu.

The optimum settings were:

- ion spray voltage: 4850 V,
- curtain gaz: 25 V;
- GS1: 17 V;
- GS2: 0 V;
- collision gas : 15 to 30eV with N_2 depending on the compound.

The mass spectra were recorded across the range m/z 70-2000amu.

To prevent oxidation of Fe(II) in Fe(III), all the solutions containing B-TRAXIM 2C Fe were degassed by nitrogen bubbling just before analysis. B-Traxim 2C Fe was dissolved in the degassed solution just before analysis.

Technical Bulletin

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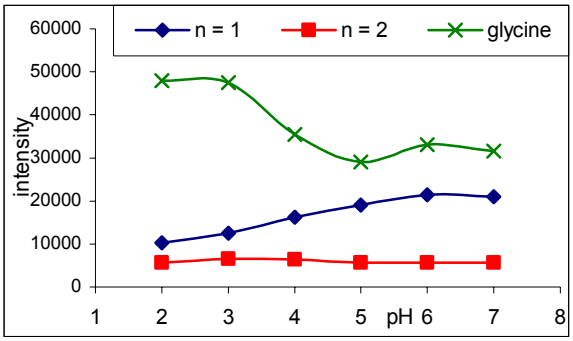
RESULTS AND CONCLUSION

B-Traxim 2C Zn

For B- Traxim 2C Zn, the ions at 254 and 473amu were used as markers for the monomer and dimer complexes. Their intensity depending on the pH is illustrated in figure 1.

Figure 1: pH Stability of B- Traxim 2C Zn

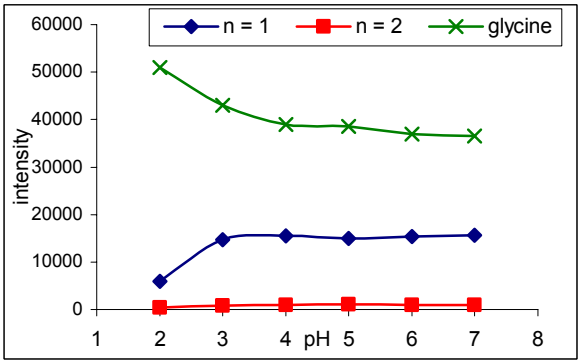


The zinc complexes were detected in the whole pH range. An acid pH partly degraded the monomer complex, as the intensity of its signal decreased while the signal of glycine increased. The dimer complex was not affected by the pH.

B-Traxim 2C Fe

In the case of B- Traxim Fe (figure 2), the 2 iron complexes were also observed at every pH tested.

Figure 2: pH Stability of B- Traxim 2C Fe.



The complexes were partly degraded in acid conditions, as the signal of glycine slightly increased and the signal of the complexes decreased at pH 2.

B-Traxim 2C Cu and Mn

All the complexes were also identified from pH 7 to pH 2 for B- Traxim Cu (figure 3) and B- Traxim Mn (figure 4).

Figure 3: pH Stability of B- Traxim 2C Cu

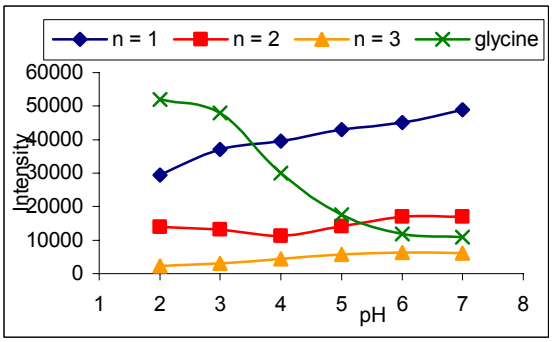
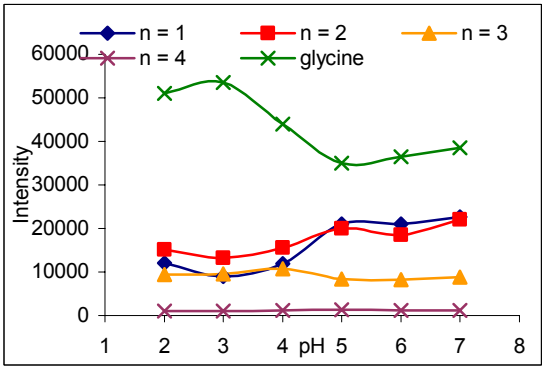


Figure 4: pH Stability of B- Traxim 2C Mn



The monomer complexes were partly degraded as observed with zinc and iron, as their signal decreased and the glycine increased with a lower pH value. The larger complexes were less (or even not) affected by an acid pH. This was particularly the case of the copper dimer and the manganese trimer and tetramer.

The glycine complexes identified in neutral pH conditions were detected at pH 2 for all B-Traxim 2C (Zn, Fe, Cu and Mn),
Glycine was apparently generally slightly more abundant at acid pH, while the peak intensity of the complexes seemed to decrease when the solution was acidified. This indicated that an acid pH induced a small degradation of the complexes. This degradation remained limited, as even at pH 2 all the complexes were still detected.
Moreover the bigger the complex, the less it was affected by the pH.

In conclusion B-Traxim 2C has a relatively good pH stability, as even at pH 2 an important part of the metal remained bound to the glycine ligand.