Cazacu M. et al./**Scientific Papers: Animal Science and Biotechnologies, 2014, 47 (2)**

Synthesis and Preliminary Characterization of a New Hexadentate Sequestrant in the Ethylenediaminetetrapropionic Acid Class

Mihaela Cazacu*, Teodor Traşcă, Adrian Riviş, Corina Costescu, Ducu Ştef, Călin Jianu

Faculty of Food Processing Technology, Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" at Timişoara, Calea Aradului 119, RO-300645 Timişoara, Romania

Abstract

Ethylenediaminetetraacetic acid (EDTA) (E-385) as such and/or derivatized (salified) occupies a separate chapter, of wide interest in food sciences, because of its coordinating (sequestering) competences towards various organic and/or inorganic structures (especially metal cations) with small geometric dimensions. This paper aims to diversify the structural range of these hexadentate ligands, by accessing a synthetic protocol different from the classic one, the cyanoethylation of primary amine functional groups of ethylenediamine (ED). In this paper are considered the main dependences of the yields of nucleophilic addition of the amino groups to the activated double bond in acrylonitrile (AN) monomer, on the operating parameters (temperature, processing time, molar ratio of reactants), and finally the preliminary data on the coordinating (sequestering) competence of ethylenediaminetetrapropionic acid (EDTP). The conclusions drawn support further research to extend the area of hexadentate sequestering structures.

Keywords: hexadentate ligands, complexone II, ethylenediaminetetraacetic acid, Trilon B, *N*,*N'*-bis-(2-hydroxyethyl)-ethylenediamine-*N*,*N'*-diacetic acid.

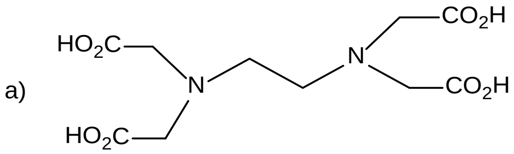
1. Introduction

Metal chelates (m.c) are coordinative compounds which are characterized by the presence within their coordination sphere of ligands grafted to the metal ion through more than one donor atom, so that they form with it a "heterocycle" (chelate). These molecular architectures or ions are also known as chelating agents (chelators). The process of formation of a metal chelate is called by this term, having been proposed in 1920 by Morgan and Drew from the Greek χηλή, *chēlē* = "claw" (crawfish pincer) [1,2]**.** Chelators (chelating agents) are chemical compounds able to form stable cyclic structures (chelate rings). These structures of 5 or 6 ring atoms, less frequently of 4 or more than 6 carbon atoms, are also called "internal complexes", because both active groups that generate the ring belong to the same molecule. This cyclic architecture may be formed in two ways, by the coordination of a hydrogen atom bound to an electronegative element to an atom with lone-pair electrons or engaged in a multiple bond or in an "aromatic cloud", or the coordination of the unshared electrons of an (electronegative) element to an electron-deficient metal (Al, Be, etc.) or one having vacant orbitals (Mn, Fe, Co, Ni, Cu, etc.). The number and variety of structures for which chelating competences have been confirmed has increased considerably. The most general criterion of their classification is denticity, which refers to the number of atoms through which the ligand can graft to a given metal ion. Based on this criterion, chelating agents can be classified into di-, tri-, tetra-, penta-, or hexadentate, respectively. At the formation of a metal chelate, a multidentate agent may not access all its donor atoms (a potentially

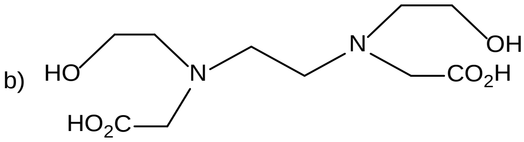
---

* Corresponding author: Cazacu Mihaela, Tel 0724316959, Email cazmih@yahoo.com

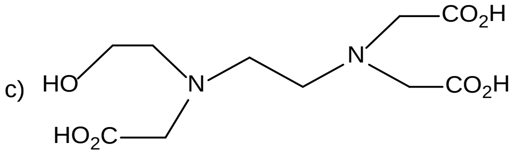
hexadentate agent can act as a pentadentate one, behavior observed for a large number of ligands of this type) [1,2]. In this work the authors have aimed to diversify the structural range of hexadentate ligands, by accessing a classical reaction scheme adapted to the synthesis of ethylenediaminetetrapropionic acid (EDTP) (Figure 1, 2) [3,4].



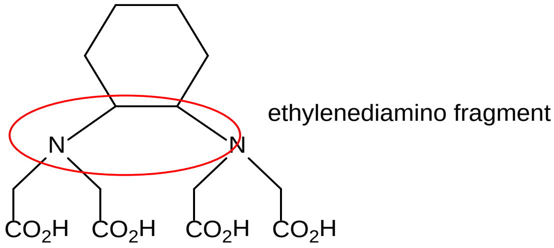
ethylenediaminotetraacetic acid

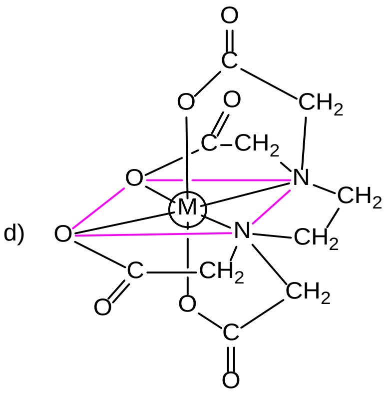


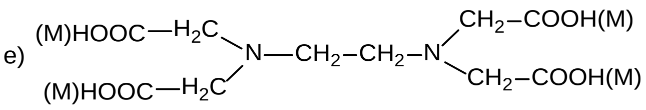
N,N′-bis-(2-hydroxyethyl)-ethylenediamino-*N,N′*-diacetic acid



N′-(2-hydroxyethyl)-ethylenediamino-*N,N,N′*-triacetic acid







EDTA (E-385)
M - earth alkaline metal cation (Ca, Mg)

Figure 1. Structures of certain hexadentate complexones, derivatives of ethylenediamine

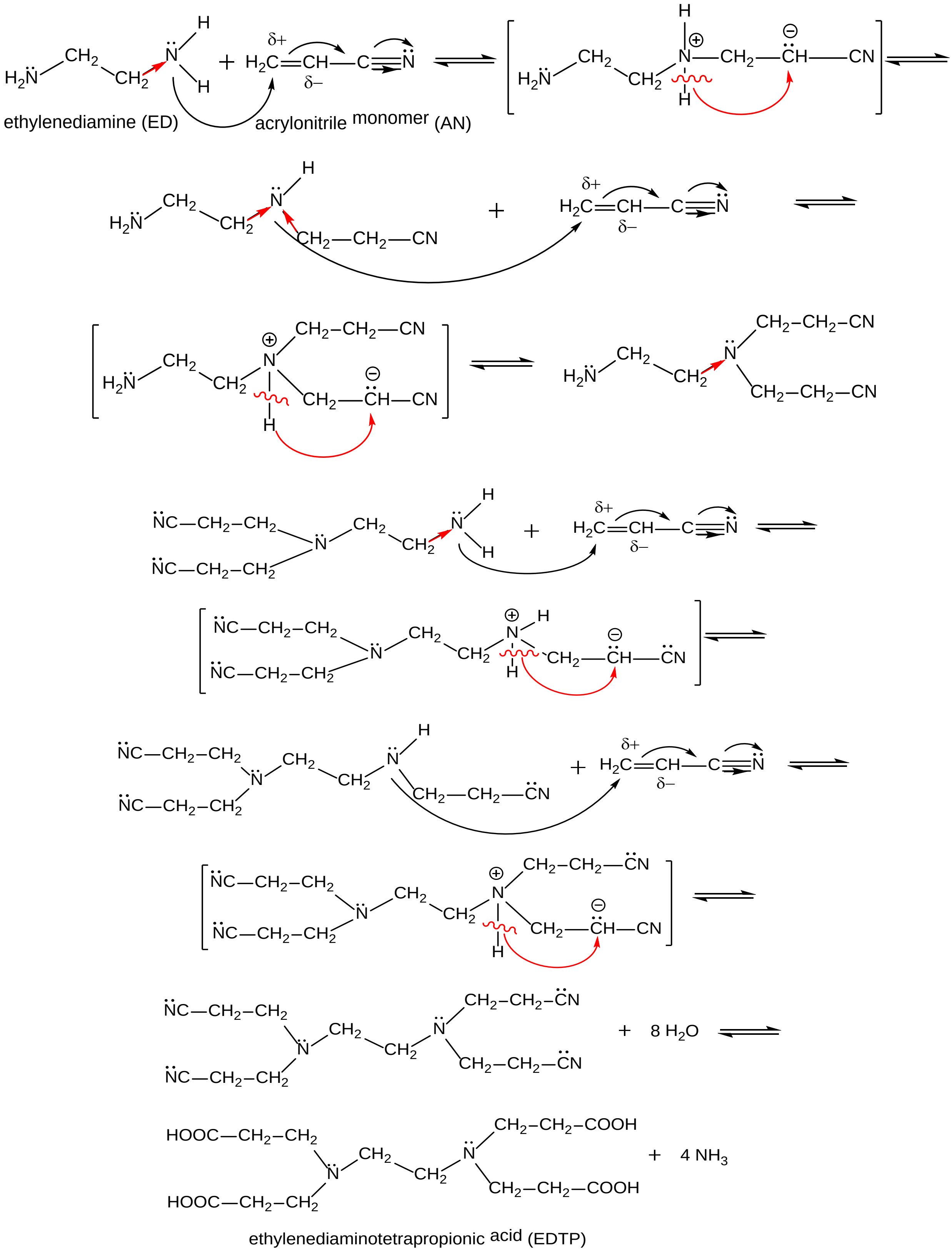


Figure 2. Processing scheme of ethylenediaminetetrapropionic acid

There are other ways for the classification of chelating agents, of which can be mentioned those based on the nature of the functional groups or on the structural features of the molecule. Ligands with two basic groups coordinate as neutral species. The chelate formed has the same charge as the metal ion. In that category of chelating agents should be mentioned primarily aliphatic diamines (ethylenediamine) or heterocyclic ones (dipyridyl and phenanthroline). Ethylenediamine is the most accessed chelating agent (Figure 1).

Ethylenediaminetetraacetic acid (EDTA) (E-385) at doses of ca. 75mg/kg is well tolerated by the human consumer, sequesters primarily alkaline earth cations (Ca, Mg), generating a stable, water-soluble complex, without any physiological role as such, only occasionally with the risk of decalcification (hypocalcemic tetany) over the long term. As such or salified (di-, tri-, tetrasodium salt), anhydrous or hydrated (Figure 1), it is commonly used in food processing as sequestering (complexing) agent of many metal cations (Table 1), to stabilize vitamins from fruit juices, wines, vinegar etc., to prevent the non-enzymatic browning of fruits and vegetables, in the processing of aquatic and marine fauna, of classic dairy and meat products (E-385). In the last decades the interest in these structures has also been steadily increasing in the food sciences [5-8].

Table 1. Structure and physical characteristics of certain EDTA derivatives

Compound	Molecular weight	EDTA content (%)	Solubility (g/100mL HCl)	pH
EDTA (H)	292	99	0.02	2.6
Disodium salt EDTA $(Na_2)\cdot 2H_2O$	372	78.5	11	5 – 6.5
Trisodium salt EDTA $(Na_3)\cdot 2H_2O$	376	77.5	57	8 – 9.0
Tetrasodium salt EDTA $(Na_4)\cdot 2H_2O$	416	70	105	10.5 – 11.5

Organisms able to accumulate high concentrations of heavy metals (with no noticeable symptoms) are often referred to in literature as metallophilic or hiperaccumulators [3,4]. The concept of heavy metal flora/fauna refers to species that normally grow in natural and anthropogenic environments enriched with heavy metals. Organisms have different capacities to store heavy metals [3]. The absorption intensity and “transport” of heavy metals does not depend solely on the type of metal cation, but also on its action, particularly in biochemical reactions. The mobility and distribution of the cations of heavy metals in plants depends mainly on their ability to form complexes, chelates with the xylem components [3,4].

2. Materials and Methods

- Ethylenediamine (Sigma Aldrich)
- Acrylonitrile (stabilized monomer) (Sigma Aldrich)

Methods [10-14**]**

Preparation of exhaustively cyanoethylated ethylenediamine

In a reaction vessel equipped with an effective cooling system, mechanical stirring, thermometer, dropping funnel, under inert atmosphere, at 20-30°C 0.1 moles ethylenediamine (ED) are introduced over 30-45 minutes and then 0.0025 moles finely divided anhydrous $FeSO_4$ are added. 5.83 g (0.11 moles) acrylonitrile monomer (AN) are added cautiously, so that the temperature of the reaction mixture does not exceed the recommended range. If necessary the reaction vessel is cooled. It is allowed a further 2 hours under the same conditions to complete the reaction, and the precipitated salts are filtered hot. The yellowish-colored fluid residue is purified if necessary from traces of acrylonitrile oligomers by eluting on a silica gel column. Yields against ED are virtually quantitative.

Preparation of ethylenediaminetetrapropionic acid (EDTP)

The acids were obtained by total acid hydrolysis with hydrochloric acid of the propionitriles previously prepared. In a reaction vessel equipped with effective mechanical stirring, thermometer, reflux cooler and dropping funnel, 0.1 moles nitriles are introduced at 60-70°C to avoid abundant foaming or the reaction mass overflow, respectively, 0.4 moles (14.5g) hydrochloric acid (40-42 mL concentrated hydrochloric acid) are added over 30-45 minutes. The reaction is perfected at 90-100°C for another hour, then the mixture is neutralized with ca. 16 g divided NaOH (54 mL NaOH 30% aqueous solution). The precipitated inorganic salts are filtered and the water in the reaction product is removed under vacuum (10-20 mm Hg). The fluid residue obtained after concentration is then purified by repeated extractions in the ethyl ether/water system, followed by elution on a column packed with ion exchangers. The purity of EDTP is checked by thin-layer chromatographic separation. Yields against nitriles starting materials are between 95-98%.

Preparation of EDTP alkaline salts

In a reaction vessel equipped with effective mechanical stirring, thermometer and dropping funnel 0.1 moles EDTP are introduced, then the neutralizing agent is added cautiously at 60-70°C under continuous stirring, in a suitable form and amount. After ca. 1 hour the pH of the mixture is adjusted to a 7.5-8.5 value, the traces of water are removed under vacuum (10-20 mm Hg), and the residue is purified by repeated alcoholic extractions. Purity was determined by the titration method.

3. Results and discussion

Preliminary tests of ethylenediamine cyanoethylation [3] have revealed that the oligomerization processes of the acrylic monomer, especially at temperatures above 30°C, have a significant relevance. The yields of nucleophilic addition decrease considerably, and the reaction tends to proceed vehemently, strongly exothermically, with the formation of aggregated reddish-brown resins, soluble in polar solvents (water, acetone, etc.) or dispersed yellow solid products soluble in solvents with medium, low polarity. Initially we worked without any oligomerization inhibitor of the acrylic monomer in order to follow the evolution of the cyanoethylation and oligomerization yields (Table 2).

Subsequently, under the conditions of the optimal parameters set, anhydrous $FeSO_4$ was introduced as oligomerization inhibitor. Under equimolar conditions, by increasing the temperature in the range of 25-35°C, the yields decrease. Under considerable excess of acrylic monomer, the evolution of the yield follows a similar trend. In the 25-40°C range, the amount of oligomers formed is below 1%, independently of the excess of monomer used, while between 40-60°C it increases markedly [9].

Table 2. Dependence of the ethylenediamine cyanoethylation (ED)/oligomers yields (%) on the AN/ED molar ratio and temperature, processing time 60 minute, without polymerization inhibitor

Temperature (°C)	**Molar ratio AN/ED**						
	1.0/1	**1.1/1**	**1.3/1**	**1.5/1**	**1.7/1**	**1.9/1**	**2.0/1**
25	48.61	64.42	70.84	74.43	76.17	80.74	83.52
	-	-	0.12	0.13	0.13	0.25	0.28
30	43.38	66.50	73.46	75.52	78.44	82.54	88.08
	0.14	0.14	0.15	0.16	0.27	0.27	0.31
35	50.62	67.78	74.22	76.34	80.04	87.73	96.18
	0.47	0.48	0.50	0.52	0.54	0.59	0.61
40	40.50	63.28	71.80	69.34	75.03	78.58	8.21
	0.89	0.95	1.08	1.13	1.35	1.48	1.62
50	43.68	46.07	66.48	68.16	71.84	74.79	70.77
	2.43	2.95	2.88	3.02	3.39	3.87	4.12
60	31.47	36.53	42.51	57.48	53.51	50.82	45.56
	4.20	4.62	4.92	5.25	5.71	5.99	6.32

Under equimolar conditions or monomer excess the addition yields increase in the 30-40°C range.

At the same time the content of oligomers increases with temperature and excess monomer. These behaviors suggest that the cyanoethylation reaction of ED is reversible. In the process of ED cyanoethylation the reaction time favors the formation of propionitriles up to 180 minutes, and the oligomerization of the monomer over the entire duration of the process. After this period the cyanoethylation yields decrease, further suggesting the reversible character under prolonged contact between reactants. The maximum yield value is achieved at shorter reaction times. On the whole process, increasing the reaction time up to ca. 180 minutes favors all the reaction processes, including the formation of AN oligomers. After approximately 180 minutes the content of propionitriles decreases due to prolonged contact with the basic environment of the reactants. In this work the formation of AN oligomers was avoided by the introduction of anhydrous ferrous sulfate ($FeSO_4$) as polymerization inhibitor of the acrylic monomer (Table 3).

Table 3. Dependence of the ethylenediamine (ED) cyanoethylation yield on the polymerization inhibitor (anhydrous $FeSO_4$) content, molar ratio AN/ED 1/1, temperature 30°C, processing time 2 hours

Crt. No.	Polymerization inhibitor (%)	Yield (%)
1	0.5	82.25
2	0.6	86.31
3	0.7	89.42
4	0.8	93.77
5	0.9	96.82
6	1.0	98.90

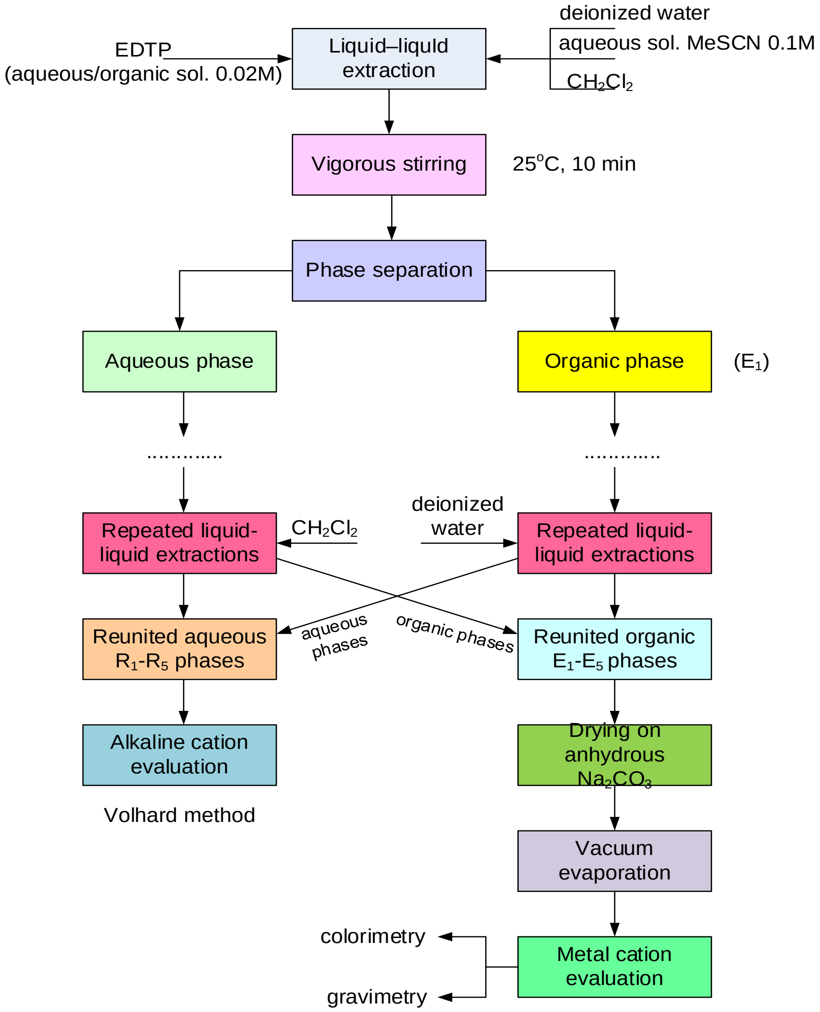


Figure 3. The block diagram of operations for the preliminary evaluation of the sequestering capacity of some metal cations with EDTP

The nature of the acid catalyst (HA) favorably influences the hydrolysis yields through the acid's strength: if in the presence of concentrated sulfuric acid at low temperatures propionamides are mainly obtained, in the presence of hydrochloric acid above 80°C substituted propionic acids are formed. Increasing the temperature and the HA/nitrile molar ratio favors the total hydrolysis yields. The amount of acid selectively influences the hydrolysis to propionic acids, along with their esters, while an excess of *n*-dodecylbenzenesulfonic acid (catalyst) favors more the formation of propionamides and less that of propionic acids, without the formation of the corresponding esters. In all cases studied, above 80°C the propionitriles content decreases markedly regardless of the acid catalyst used, and the presence of propionic esters is observed. Above 110°C in the presence of hydrochloric acid increasing amounts of ED are noticed due to the cleavage of ether bridges. Increasing the reaction time favors the total acid hydrolysis. At higher temperatures (over 80°C) in the presence of hydrochloric acid, after ca. 60 minutes acrylonitrile disappears from the reaction mixture, and after 90 de minutes so does the propionamide formed intermediately. From the interpretation of the preliminary values of the distribution balance of the sequestrating matrix between the two phases, a convincing distribution of them is noticed (Figure 3).

4. Conclusions

From the analysis of the experimental data of the ethylenediamine cyanoethylation process, the reaction proceeds by a specific mechanism in which the rate-determining step is the proper nucleophilic addition. Its pre- and post-stages are protolytic equilibria (acid–base) in which the hydroxylic nucleophile characteristic to the substrate is formed, and the carbanionic structure resulting from the addition is stabilized, respectively. Because in the formation of the activated complex participate two polarized molecular species, the acrylic monomer and ethylenediamine, the reaction is strongly influenced by effects of solvation (coordination), due both to the polarity of the reaction medium and the nature of the cation. Increasing the ionic character of the nucleophile–cation bond favors the coordination effects specific to the conformation of the processing intermediates. The distribution balance of metal cations between the two phases of the sequestration process is encouraging and suggests further research in the EDTP class.

Acknowledgements

The authors express their entire gratitude to Prof. Univ. Emeritus Ionel Jianu for the suggestions put forward during the development of this work.

References

1. C. Liteanu, E. Hopîrtean, Chimie analitice cantitativă. Volumetria, Ediția 6. Editura Didactică și Pedagogică București, 1972.
2. *** Enciclopedia de chimie, volumul 3. Editura Academiei, București, 1972.
3. Jianu C. Research concerning the of some metal ion complexing additives to improve food value in horticultural raw. PhD thesis. Banat's University of Agricultural Sciences and Veterinary Medicine, 2006,. Jianu I. Aminoethers surfaceactive agents. Cationic surfaceactive agents. PhD thesis. Polytehnic University, 1984.
4. G. Schwarzenbach, Die Komplexometrische Titration, II Anfl., Enke – Verlag, Stuttgart, 1985.
5. G. Marcu, Chimia compușilor coordinativi. Editura Academiei R.S. România, București, 1987.
6. C. Drăgulescu, E. Petrovici, Chimie structurală modernă. Chimia coordinației 8. Editura Academiei, București, 1977.
7. Haiduc, Chimia compușilor metaloorganici. Editura Științifică București, 1972.
8. Jianu C. Ethylene Oxide Homogeneous Heterobifunctional Acyclic Oligomers Chapter 4 in Oligomerization of Chemical and Biological Compounds, edited by Claire Lesieur, Intech 2014.
9. Hoey M, Gadberry J, Polyoxyethylene Alkylamines. In Nonionic Surfactants, Organic Chemistry, Surfactant Science Series. Volume 72. Edited by van Os NM. Marcel Dekker, Inc., New York, USA, 1998:163-175.
10. Bujancă G, Jianu I: Synthesis of salifed β-alkyl ($C_{12}H_{25}/C_{18}H_{37}$) polyethyleneoxy (n=3-20) propionamides, potential components in food sanitation(I). J Food Agric Environ 2012, 10:182-188.
11. Jianu C. Colloidal competences of some food cleaning agents based on alkaline and ammonium nonionic soaps. J. Food Agric Envirom 2012, 10:10-15.
12. Jianu C, Trașcă T, Riviș A, Mișcă C, Chiș M, Jianu I. Hydrolysis of β-alkyl ($C_{12}H_{25}/C_{18}H_{37}$) polyethyleneoxy (n=3-20) propionitriles in phase

transfer catalysis conditions (II). J Agroalim Proc Technol 2009, 15:79-87.
13. Jianu C. Synthesis of nonionic-anionic colloidal systems based on alkaline and ammonium beta-nonylphenol polyethyleneoxy (n=3-20) propionates/dodecylbenzenesulfonates with prospects for food hygiene. Chemistry Central Journal 2012, 6:95, doi:10.1186/1752-153X-6-95.
14. Jianu C, Jianu I. Colloidal competences of new tailor-made lipids. J Food Agric Environ 2010, 8:148-155.