Research Report

The Theoprax-Method

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Abstract

The **Theoprax-Method**, a new way to use **conductivity measurement** in **analytical chemistry**, allows to **characterize** the **interaction between molecules**. Examples of the interaction between central ion and complexing agent, enzyme and inhibitor as well as antigen and antibody are shown. With the Theoprax-Software it is possible to **calculate** a **pH-spectrum** which shows the interaction between different molecules as a function of the dissociation of their functional groups.

1. Introduction

The **Theoprax-Software**, a new analytical method, makes it possible to calculate pH-spectra which shows the interaction between different molecules as a function of the degree of dissociation of their functional groups. In **complex chemistry,** the pH-spectrum makes it possible to make a statement about the concentration of the complex, **complexing agent and central ion**. Furthermore, it shows the preference of the complexing agent/central ion for an metal ion/complexing agent in the presence of other metal ions/complexing agents. Also you can make a statement about the stoichiometry of the complex in solution. It is no more necessary to isolate it. The pH-spectrum shows the optimum pH-value for complex formation. In **pharmaceutical chemistry** and **biochemistry** for example you can use the Theoprax-Method as a diagnostic tool to speed up your separation technique (affinity chromatography). The pH-spectrum makes it possible to show the **enzyme-inhibitor-complex**, enzyme-substrate-complex, the catabolism of the substrate as well as the pH-activity-curve of the enzyme. It is also possible to show the interaction between **antigen and antibody**.

2. Fundamental principles

The theoretical principles of the Theoprax-Measuring System are based on the following assumptions:

1. The **solutions are ideally diluted**, the concentration of the substances used are in the range of mmol/L.
2. The relaxation effect and the electrophoretic effect can be neglected.
3. The equivalent conductivities at infinite dilution of the ions are additive.

For example, it is possible to find out if there is an interaction between the complexing agent and the metal ion. If there is an interaction, the Kohlrausch Law of independent migration of ions will lose its validity. The **measured specific conductivity** κ, the so-called sample-value, has a deviation of $\Delta\kappa$ from the theoretically calculated value, which is calculated from the specific conductivities of the single components. The specific conductivities of the single components are designated as the single reference value, the value which is calculated from those single reference values is called the total reference value.

The degree of the deviation $\Delta\kappa$ is a measure for the interaction between the ionic molecules. To show **Coulomb interaction**, the tests are carried out in an aqueous solution **without the addition of a foreign electrolyte** and to prove **van der Waals interactions** in an aqueous solution **with the addition of a foreign electrolyte**. If there are **Coulomb interactions**, the deviation $\Delta\kappa$ changes depending on the pH-value of the solution. The pH-spectrum of **the Theoprax-curve will then show peaks**. If there are **van der Waals interactions**, the deviation $\Delta\kappa$ doesn't change with the pH-value of the solution. The pH-spectrum of **the Theoprax-curve will be linear**.

The determination of the deviation $\Delta\kappa$ is shown in an **example** of the Theoprax-curve of the **System (Cu+E)**, for an optional pH-value, see Figure 1. Each value calculated in this way for the deviation $\Delta\kappa$ corresponds to one measuring point in Figure 2, which results in the Theoprax-curve of the System (Cu+E) [1].

Calculation of deviation Δκ

shown by the example of System (Cu+E) for an optional pH-value

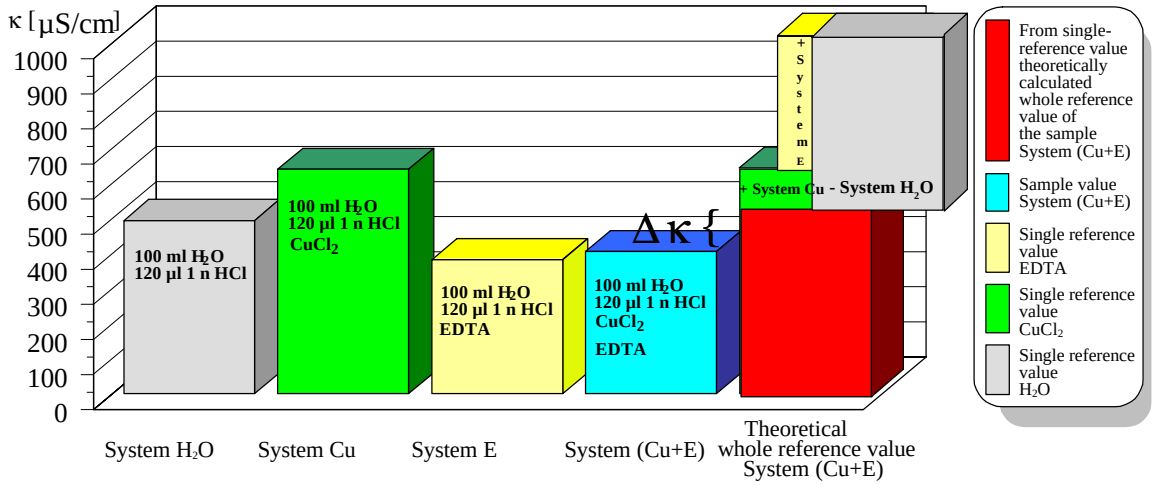

Fig. 1 Calculation of deviation Δκ for the Theoprax-curve from the System (Cu+E)

3. Results and discussion

3.1. Complex chemistry

3.1.1. Preparation of a calibration-curve for the determination of copper concentration, figures 2 to 5

3.1.1.1. The pH-spectrum of Ethylenediaminetetraacetic acid-copper-complex, figure 2

- System Cu02 - (0.2 mmol/L $CuCl_2$ / dist. water)

At pH 12.3 the value for Δκ is - 107 µS/cm, which means that 0.4 mmol/L OH^--ions have been consumed. The copper concentration is 0.2 mmol/L, $Cu(OH)_2$ has been formed. The reason for the continuing decrease of the curve is the forming of $Cu(OH)_3^-$.

- System (Cu02+E) - (0.2 mmol/L $CuCl_2$ + 1.0 mmol/L EDTA / dist. water)

At the beginning of the titration, the pH-value of the System (Cu02+E) is lower than that of System E, figure 6. The forming of the EDTA-copper-complex gives a lower pH-value of 2.7, the value for Δκ is 87 µS/cm. This is equivalent to a conductivity of 0.2 mmol/L H^+-ions. The increase of Δκ means that in comparison with System E, the EDTA-copper-complex inhibits the forming of R-COOH, the value for Δκ of 121 µS/cm is equivalent to the conductivity of 0.3 mmol/L H^+-ions, protonation of the COO^--group is inhibited. Starting from pH 2.7 to a higher pH-value, the variance for Δκ compared to the starting point at pH 2.7 rise up to 69 µS/cm at pH 3.0 where the value for Δκ becomes 156 µS/cm. The variance of 69 µS/cm is equivalent to a conductivity of 0.2 mmol/L OH^--ions. The reasons for the variance of 0.2 mmol/L of free OH^--ions are the additional 0.4 mmol/L of uncomplexed OH^--ions compared to System Cu02 and compared to System E the additional consume of 0.2 mmol/L of OH^--ions in System (Cu02+E) where the forming of the Cu-EDTA complex push the deprotonation of the functional groups. With growing pH the deprotonation of System E becomes equal to System (Cu02+E), the value of Δκ at the starting point becomes zero but the total value for Δκ stay compared to the variance with 68 µS/cm at pH 12.1 nearly constant and doesn't become zero. This indicates that in spite of $Cu(OH)_2$ a Cu(OH)-EDTA-complex is formed.

- System (Cu02+E)`- (0.02 mmol/L $CuCl_2$ + 1.0 mmol/L EDTA + 10 mmol/L KCl / dist. water)

No extra van der Waals interactions could be shown after the addition of KCl.

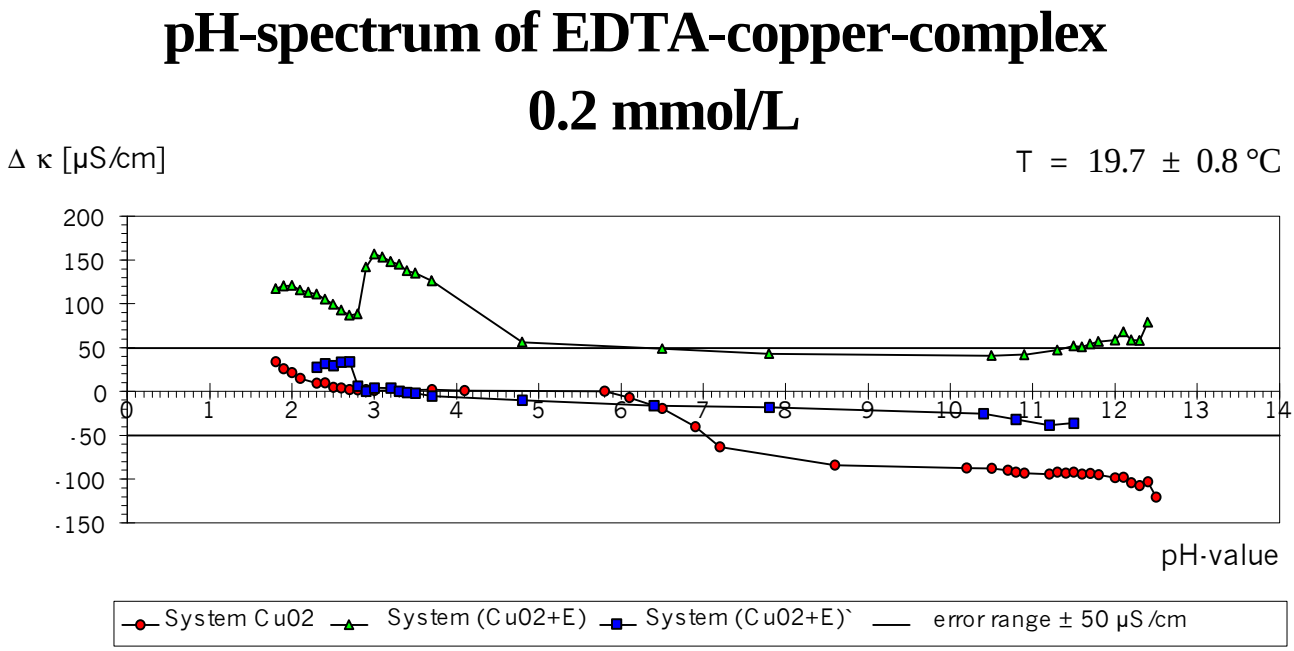

Fig. 2 pH-spectrum of the EDTA-copper-complex System (Cu02+E), pH 3

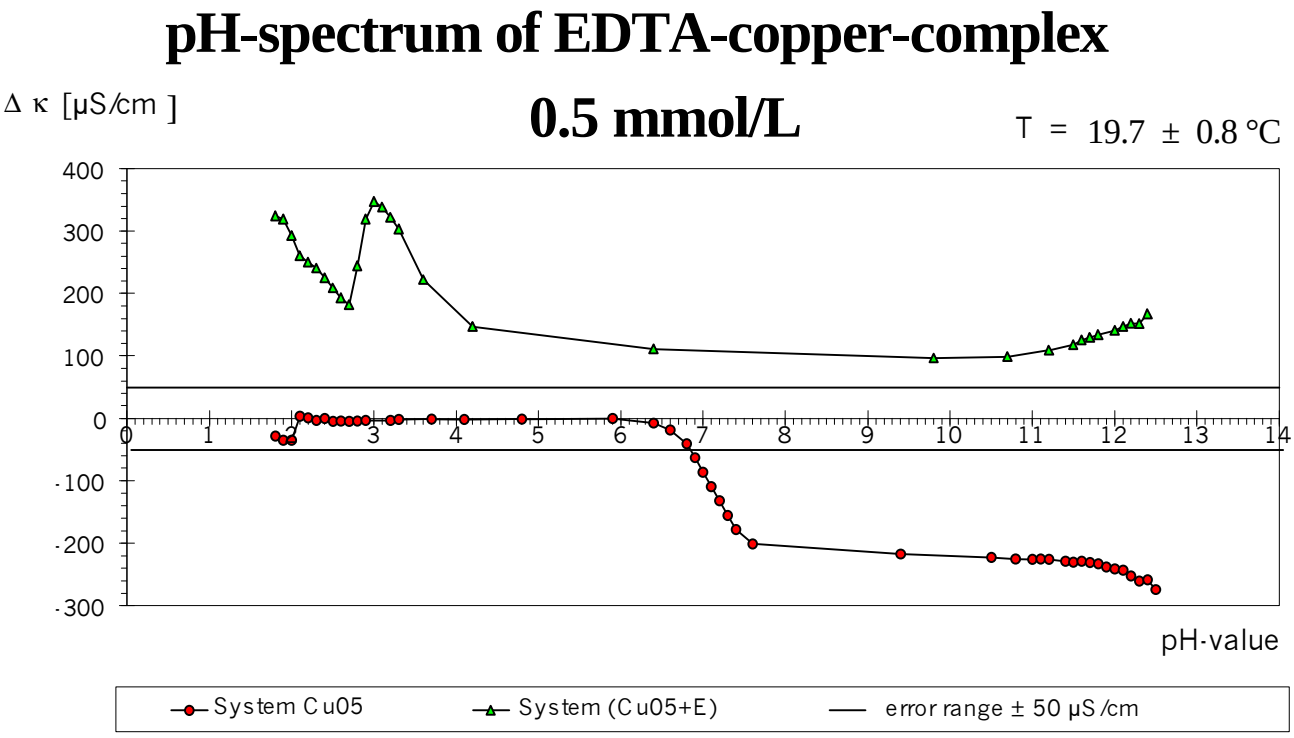

Fig. 3 pH-spectrum of the EDTA-copper-complex System (Cu05+E), pH 3

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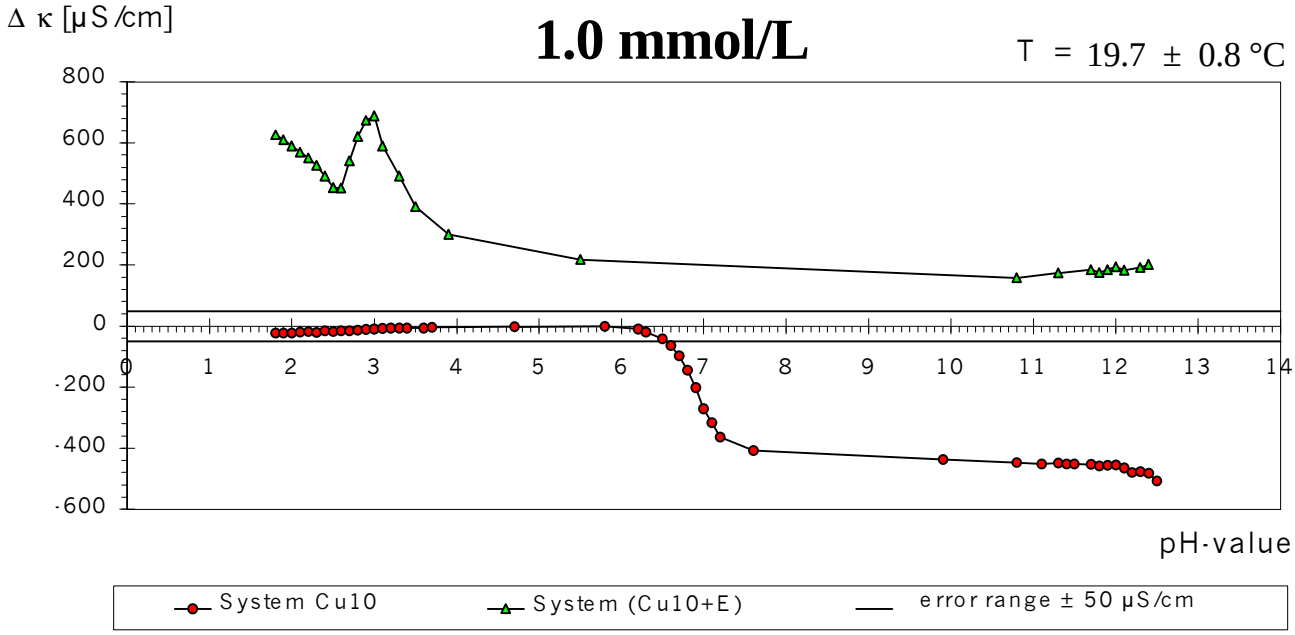

Fig. 4 pH-spectrum of the EDTA-copper-complex System (Cu10+E), pH 3

Peaks	Δκ [µS/cm]	c [mmol/L]
System (Cu02+E)	156	0.2
System (Cu05+E)	347	0.5
System (Cu10+E)	688	1.0

Table 1 Includes the values for the calibration-curve

With figures 2 to 4 it is possible to prepare a calibration-curve to determine the concentration of copper, see figure 5. The Δκ values of the individual peaks (maximum of free OH^--ions) from the Systems (Cu02 / Cu05 / Cu10+E) have been plotted in relation to the copper concentration. There is a linear correlation between Δκ and the EDTA-copper-complex concentration and this also with the concentration of copper. To prepare a calibration-curve it is also possible to plot the Δκ values of the Systems Cu02, Cu05, Cu10 at pH 12 in relation to the copper concentration. With Δκ of -107 µS/cm, -253 µS/cm and -482 µS/cm the concentration of OH^--ions is 0.4 mmol/L, 1.0 mmol/L and 2.0 mmol/L. This corresponds to a copper concentration of 0.2 mmol/L, 0.5 mmol/L and 1.0 mmol/L.

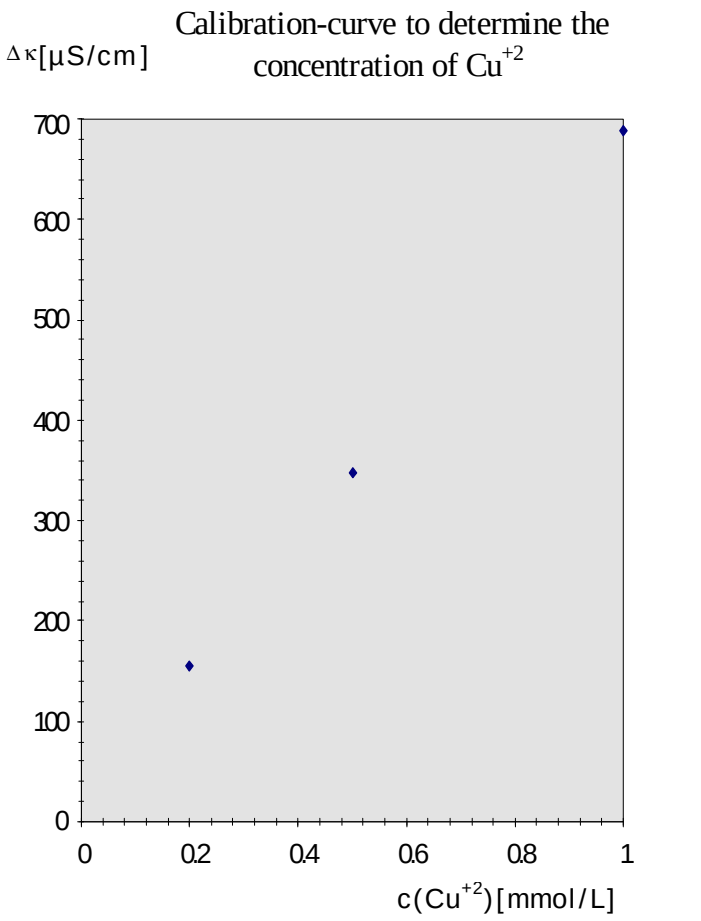

Fig. 5 Calibration-curve to determine the concentration of Cu^{+2}, error range ± 10% of c(Cu^{+2}) [mmol/L]

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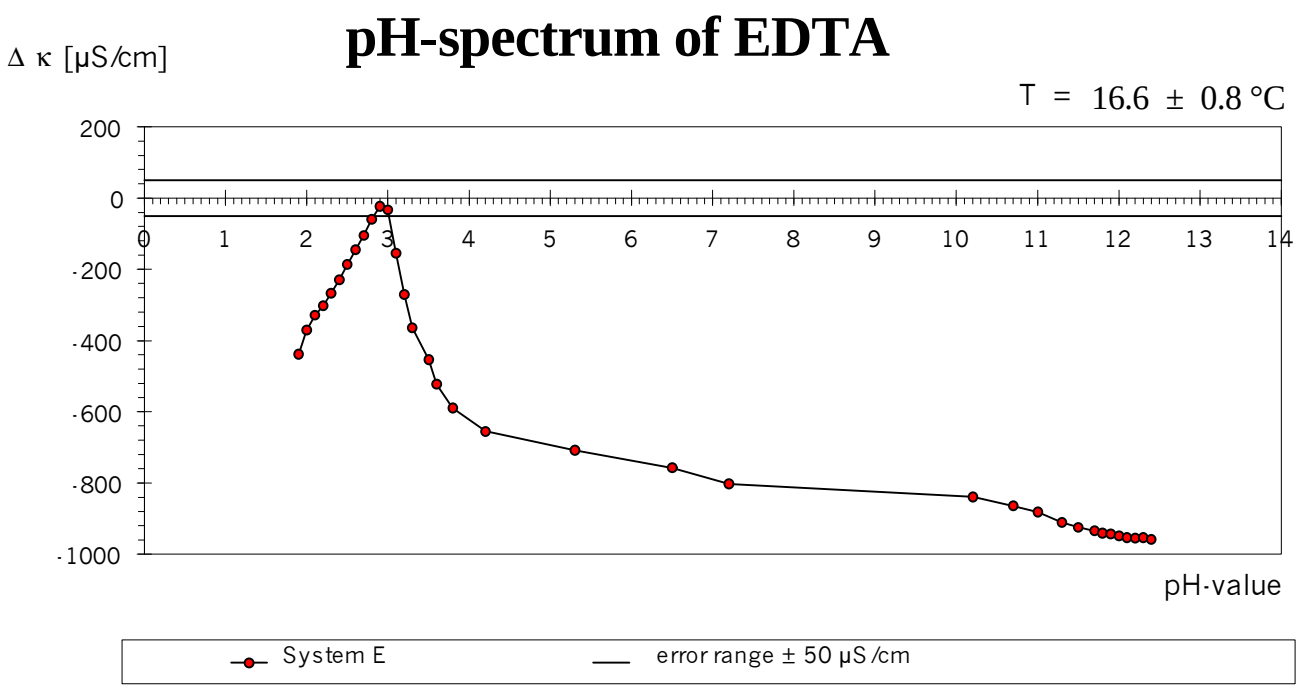

Fig. 6 pH-spectrum of the complexing agent EDTA System E

- System E - (1.0 mmol/L EDTA / dist. water), figure 6

Starting from pH 2.9 to a lower pH-value, $\Delta\kappa$ becomes negative, which indicates that the consumption of H^+-ions is higher than that of the System H_2O. The protonation of the COO^--group from EDTA starts. Starting from pH 2.9 to higher pH-value, $\Delta\kappa$ becomes negative, which indicates that the consumption of OH^--ions is higher than that of the System H_2O. The COOH-group from EDTA starts to deprotonate. At the point of intersection of tangents at pH 3.6, where 53% of the COOH-groups are deprotonated, $\Delta\kappa$ is -523 µS/cm. This is equivalent to a conductivity of 2.1 mmol/L OH^--ions. At pH 12.4, $\Delta\kappa$ is -958 µS/cm, which corresponds to 3.9 mmol/L of OH^--ions, 98% of the COOH-groups are deprotonated, see figure 7.

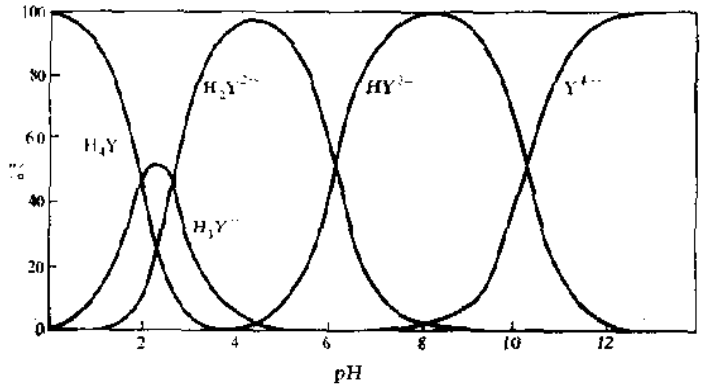

Fig. 7 Dissociation of EDTA as a function of the pH-value [2]

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3.1.2. Determination of the preference of the complexing agent for a central ion, figures 8 to 9

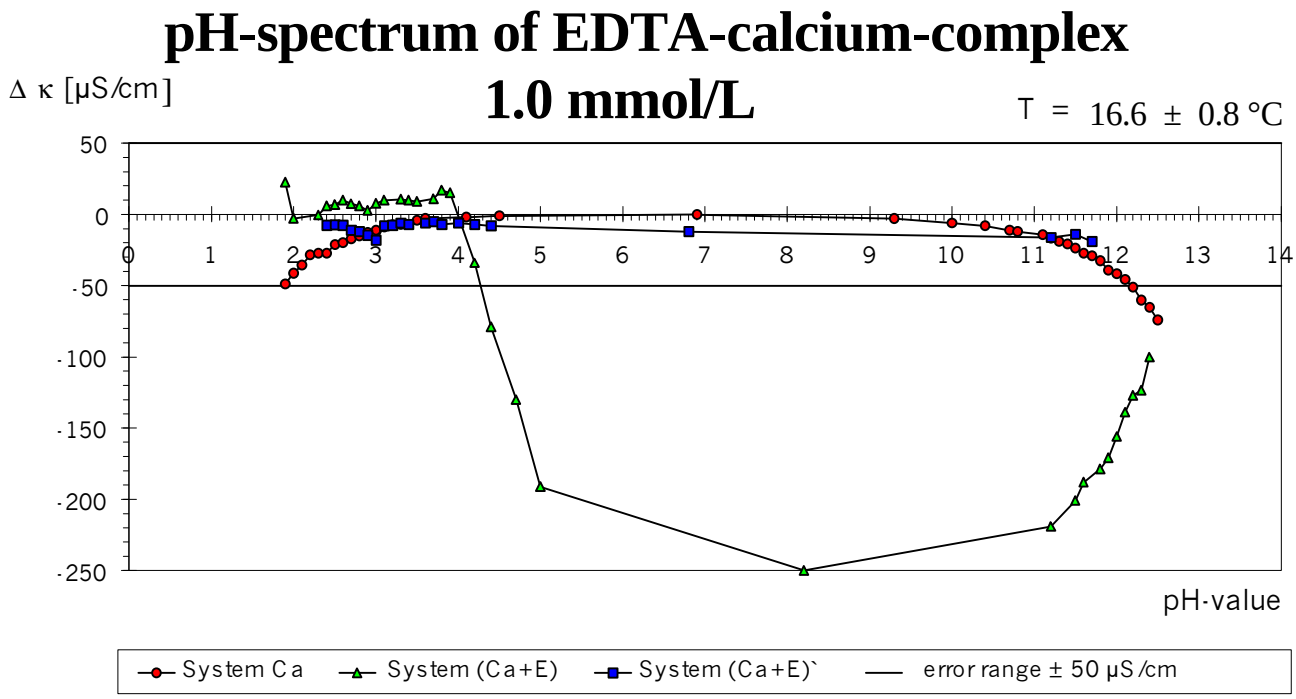

Fig. 8 pH-spectrum of the EDTA-calcium-complex System (Ca+E), pH 8.2

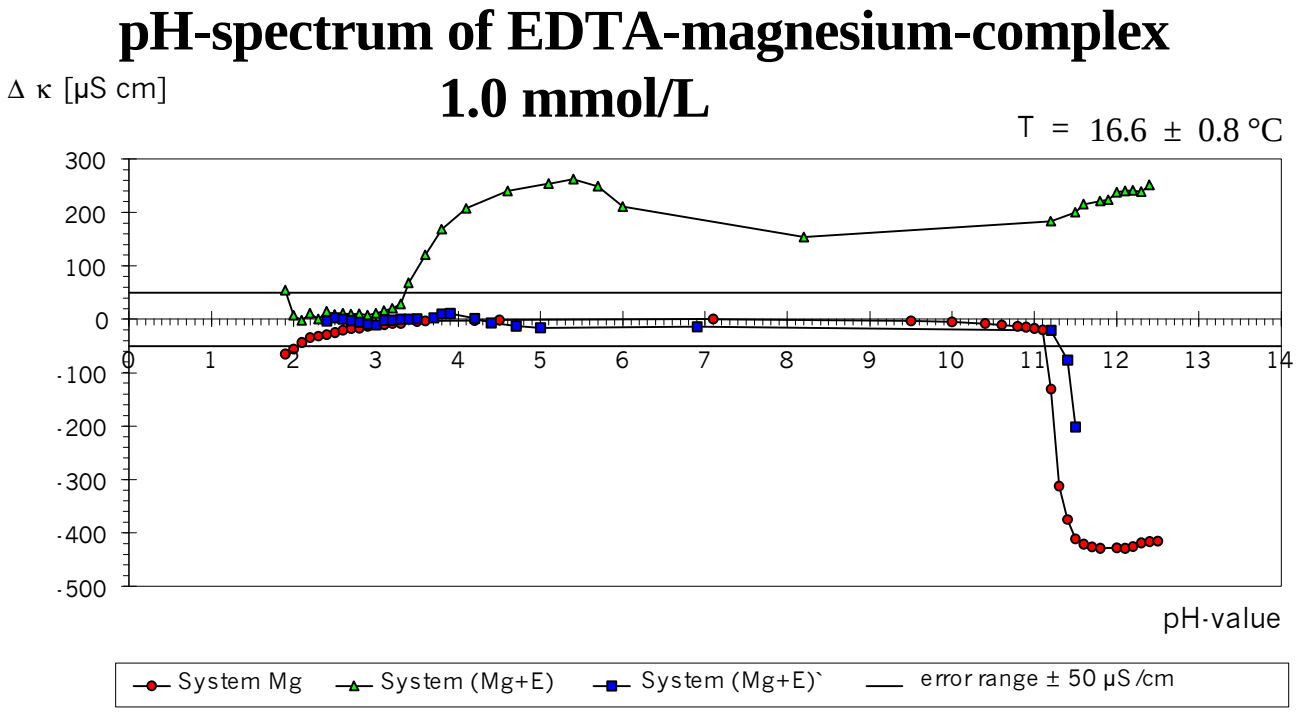

Fig. 9 pH-spectrum of the EDTA-magnesium-complex System (Mg+E), pH 5.4

3.1.2.1 The pH-spectrum of EDTA-calcium-complex, figure 8

- System Ca - (1.0 mmol/L $CaCl_2$ / dist. water)

Since the value of $\Delta\kappa$ is within the maximum error range of ± 50 µS/cm, one can deduct that there will be no formation of a Ca-OH-complex in the pH range from 2 to 12.

- System (Ca+E) - (1.0 mmol/L $CaCl_2$ + 1.0 mmol/L EDTA / dist. water)

At pH 8.2, the value of $\Delta\kappa$ is -250 µS/cm, which corresponds to a conductivity of 1.0 mmol/L OH^--ions, a EDTA-Ca-complex is formed. The forming of the EDTA-Ca-complex push the deprotonation of the functional groups of EDTA so that at pH 8.2 in relation to System E the consume of OH^--ions is higher. With growing pH the deprotonation of System E becomes equal to System (Ca+E), the value for $\Delta\kappa$ becomes lower.

- System (Ca+E)`- (2.0 mmol/L $CaCl_2$ + 1.0 mmol/L EDTA / dist. water)

No additional peak appears, because the competing ions are also Ca^{2+}-ions.

3.1.2.2 The pH-spectrum of EDTA-magnesium-complex, figure 9

- System Mg - (1.0 mmol/L $MgCl_2$ / dist. water)

At pH 12, the value of $\Delta\kappa$ is -430 µS/cm, which corresponds to a consumption of 1.7 mmol/L OH^--ions, $Mg(OH)_2$ is formed, but not completely.

- System (Mg+E)- (1.0 mmol/L $MgCl_2$ + 1.0 mmol/L EDTA / dist. water)

The forming of the EDTA-Mg-complex in System (Mg+E) push the deprotonation of the functional groups of EDTA so that at pH 5.4 the value for $\Delta\kappa$ is 262 µS/cm where in relation to System E the consume of OH^--ions is higher but lower than in System Mg, where $Mg(OH)_2$ is formed. With growing pH the deprotonation of System E becomes equal to System (Mg+E), the value for $\Delta\kappa$ becomes lower. At pH 12.4 the value for $\Delta\kappa$ is 251 µS/cm which means that a total of 1.0 mmol/L of OH^--ions remain uncomple1xed and a EDTA-Mg-OH-complex is formed.

- System (Mg+E)`- (1.0 mmol/L $MgCl_2$ + 1.0 mmol/L $CaCl_2$ + 1.0 mmol/L EDTA / dist. water)

According to table 2, the common logarithm for the formation constant of EDTA-complex for Mg^{2+} is 8.69 and for Ca^{2+} it is 10.96 there will be a ionic exchange, which can be seen in the decrease of the Theoprax-curve at pH 11.4. Due to the greater log K_{MY} of Ca^{2+}, Mg^{2+}-ions will be set free and begin to complex with the OH^--ions, $\Delta\kappa$ becomes negative. The preference of a complexing agent for a metal ion can be demonstrated in this way.

Cation	Complex	log K_{MY}
Ca^{2+}	CaY^{3-}	10.96
Cu^{2+}	CuY^{2-}	18.80
*Mg^{2+}	MgY^{3-}	8.69

Table 2 Common logarithm of the dissociation and stability constant of EDTA for different cations, measured at 20° C (pK_1 = 2.0; pK_2 = 2.76; pK_3 = 6.16; pK_4 = 10.26) [2]
* 0.1 M KCl

3.1.3. Determination of the preference of the central ion for a complexing agent / ligand, figure 10

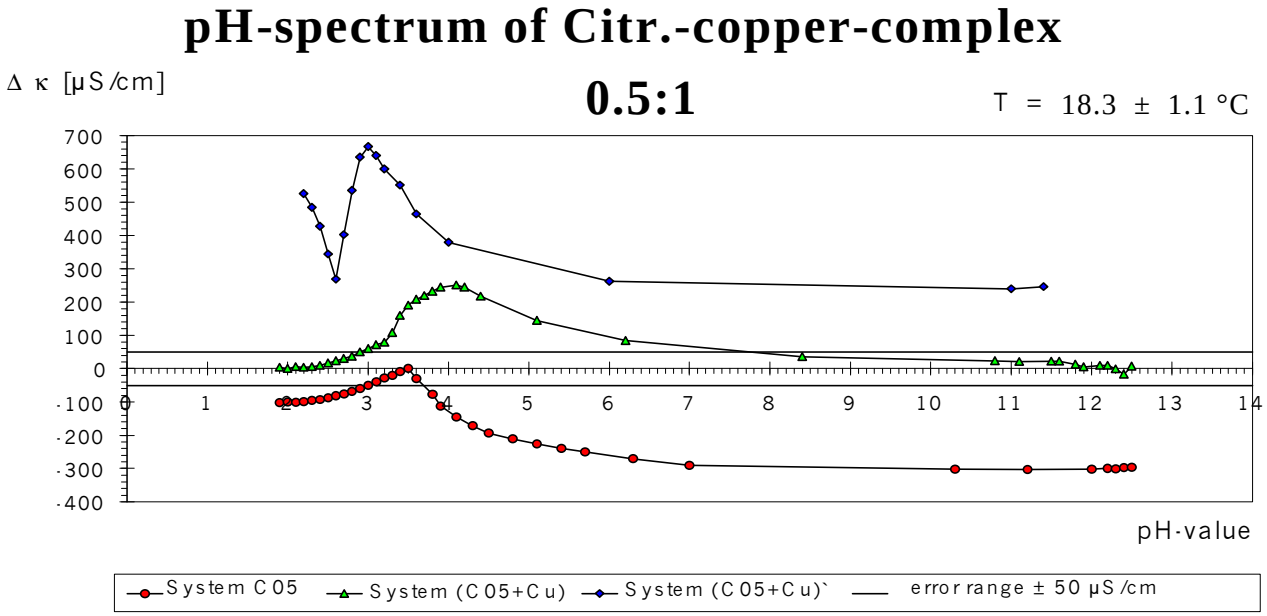

Fig. 10 pH-spectrum of the Citr.-copper-complex System (C05+Cu), pH 4

3.1.3.1 The pH-spectrum for citric acid, figure 10

- System C05 - (0.5 mmol/L citric acid / dist. water)

Starting from pH 3.5 to lower pH-values, $\Delta\kappa$ becomes negative, that means the consumption of H^+-ions is higher than that of System H_2O. The COO^--group of the citric acid begins to protonate. The participation of the citric acid in the conductivity of the solution no longer exists. For low pH-values, the value of $\Delta\kappa$ approximates the absolute κ-value for citric acid of 117 µS/cm at pH 3.5, which has been measured before starting the titration. If the substrate begins to decompose on low pH or to form cationic groups, the value of $\Delta\kappa$ gets higher than the absolute κ-value. Starting from pH 3.5 to higher pH-value, $\Delta\kappa$ becomes negative because of the higher consumption of OH^--ions compared to System H_2O. The COOH-group of the citric acid begins to deprotonate. At the point of intersection of tangents at pH 4.5 and at pH 12, where 50% and 78% of the COOH-groups are deprotonated the value for $\Delta\kappa$ is -193/ -300 µS/cm. This correspond to a conductivity of 0.75/1.17 mmol/L OH^--ions.

- System (C05+Cu) - (1.0 mmol/L $CuCl_2$ + 0.5 mmol/L citric acid / dist. water)

The lower pH-value of 3.2 of System (C05+Cu) at the beginning of the titration compared to System C05 indicates that a Citr.-Cu-complex has been formed, the protonation of the COO^--group in System (C05+Cu) was not inhibited by the Citr.-Cu-complex (see also EDTA-Cu-complex, figures 2 to 4). Starting from pH 2.8 to higher pH-values, $\Delta\kappa$ becomes positive because at the beginning of the titration with 1 N KOH the consumption of OH^--ions is in System (C05+Cu) lower compared to System C05 and System Cu. At pH 4.1 the value of $\Delta\kappa$ is 251 µS/cm, which is equivalent to a conductivity of 1.0 mmol/L OH^--ions what means that the forming of the Citr-Cu-complex push the deprotonation of the second COOH-groups. The beginning decrease of the curve indicate that with a growing concentration of OH^--ions, the exchange of Citr.-ligands with OH^--ligands begins, the forming of $Cu(OH)_2$ is privileged. The value of $\Delta\kappa$ approximates zero at pH 12.5, which means that there are no more interactions between citric acid and the copper ions, as if the ions would be in two different beakers.

- System (C05+Cu)`- (1.0 mmol/L $CuCl_2$ + 0.5 mmol/L Citr. + 1.0 mmol/L EDTA / dist. water)

With the addition of 1.0 mmol/L EDTA to the System (C05+Cu), a curve will appear which is similar to the curve of System (Cu10+E) in figure 4. For the Cu^{+2}-ion the preferred complexing agent is EDTA, it forms a stabler copper-complex than citric acid. The generation of the Theoprax-curve of the System (Cu10+E) could be mathematically deducted in the following simplified way.

- Sample value: System (C05+Cu)` (1)

= Conductivity of 0.5 mmol/L Citr. + 1.0 mmol/L EDTA-Cu-complex + 2.0 mmol/L uncompl. OH^--ions

- Single reference value: System (C05+Cu) + System E - System H_2O (2)

= Conductivity of 0.5 mmol/L Citr.-Cu-complex + 0.5 mmol/L $Cu(OH)_2$ + 1.0 mmol/L uncompl. OH^--ions + 1.0 mmol/L EDTA

From (1) - (2) follows (3)

= Conductivity of 0.5 mmol/L Citr. + 1.0 mmol/L EDTA-Cu-complex + 1.0 mmol/L uncompl. OH^--ions - 0.5 mmol/L Citr.-Cu-complex - 0.5 mmol/L $Cu(OH)_2$ - 1.0 mmol/L EDTA (3)

For a pH-value with lim conc. - Citr.-Cu-complex towards 0 results from (3):

= 0.5 mmol/L Citr. + 1.0 mmol/L EDTA-Cu-complex + 1.0 mmol/L uncompl. OH^--ions - 0.5 mmol/L Citr. - 0.5 mmol/L $Cu(OH)_2$ - 0.5 mmol/L $Cu(OH)_2$ - 1.0 mmol/L EDTA - 1.0 mmol/L compl. OH^--ions (4)

This is equivalent to the Theoprax-curve of the System (Cu10+E)

= 1.0 mmol/L EDTA-Cu-complex + 2.0 mmol/L uncompl. OH^--ions - 1.0 mmol/L $Cu(OH)_2$ - 1.0 mmol/L EDTA (5)

3.1.4. Determination of the stoichiometry of the complex in solution, figure 11

3.1.4.1 The pH-spectrum for citric - copper - complex, figure 11

- System Cu - (1.0 mmol/L $CuCl_2$ x 2 H_2O / 100 ml dist. water), figure 11

In comparison with System H_2O, the System Cu has a higher consumption of OH^--ions, therefore the curve of the System Cu has a strong decrease at a pH-value above 6.5. The forming of $Cu(OH)_2$ can be shown by the consumption of OH^-- ions in the pH-area of 6.5 to 11.6, a $\Delta\kappa$ value of - 440 µS at pH 11.6 shows the consumption of 2.0 mmol/L of OH^--ions, the solution is turbid (blue flocs).

- System CS05 - (0.5 mmol/L sodium citrate dihydrate / 100 ml dist. water), figure 11

In comparison with System H_2O, the System CS05 has a higher consumption of H^+-ions, therefore the curve of the System CS05 has a strong decrease at a pH-value below 6.4. The protonation of the three COO^--groups can be shown by the consumption of H^+- ions in the pH-area of 6.4 to 2.0, a $\Delta\kappa$ value of - 524 µS at pH 2.0 shows the consumption of 1.4 mmol/L of H^+-ions.

- System (CS05/1/2+Cu) - (0.5/1.0/2.0 mmol/L sodium citr. / 1.0 mmol/L $CuCl_2$ / 100 ml H_2O), fig. 11

Before titration the pH of the solution drift from pH 7.2/7.6/7.7 of pure sodium citrate down to 4.2/4.7/5.7 by the addition of 1.0 mmol/L $CuCl_2$ x 2 H_2O. The solution is clear and blue, the value for $\Delta\kappa$ is - 117/-212/-237 µS/cm, which indicates that there is an interaction between sodium citrate and copper ions before the beginning of the titration. On the basis of Coulomb interactions, there is a citrate-copper complex in the pH area of 3.9/3.8/3.7 to 12.4, $\Delta\kappa$ is at pH 11.5/11.7/11.8 -92/-135/ -155 µS/cm. In the direction of lower pH-values the protonation of the COO^--groups in System (CS05/1/2+Cu) was not inhibited by the Citr.-Cu-complex.
In the direction of higher pH the variance from the basis line at pH 4.2/4.7/5.7 is 209/347/392 µS/cm at pH 11.5/11.7/11.8, what means that 0.9/1.6/1.8 mmol/L OH^--ions are free in compare to System Cu so that only 1.1/0.4/0.2 mmol/L of OH^--ions have been consumed, explainable by a citrate-copper complex, 0.5 mmol/L of HO-Cu-Citr.-Cu-OH/0.5 mmol/L of Citr.-Cu-Citr.-Cu-OH/ 0.75 mmol/L Citr.-Cu-Citr. and 0.25 mmol/L Citr.-Cu-OH at pH 11.5/11.7/11.8.

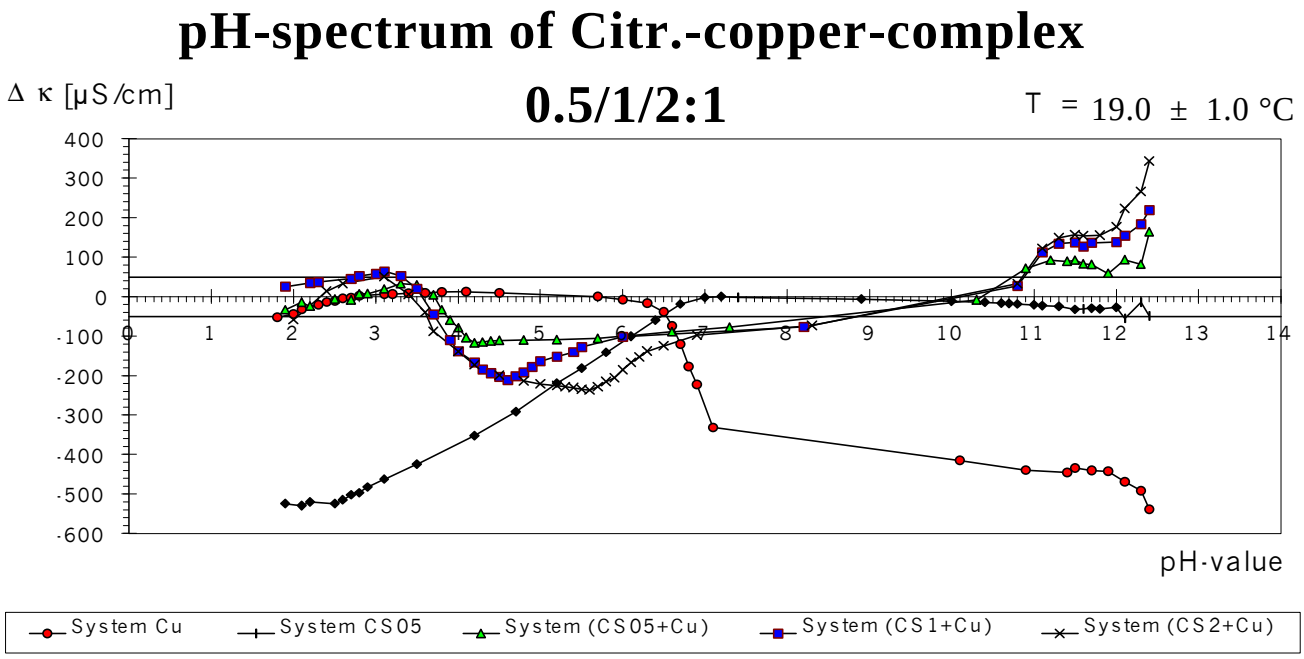

Fig. 11 pH-spectrum of the Citr.-copper-complex System (CS05/1/2+Cu), pH 3.9/3.8/3.7 - 12.4

Citr. : Cu/* conz. OH^-	$Cu(OH)_2$/Citr.-Cu [3]	Citr.-Cu/ Citr.-Cu-OH	Citr.-Cu-OH/ Citr.-Cu-Citr.	Citr.-Cu-Citr.
0.5 mmol/L : 1.0 mmol/L	*209 µS/cm = 1 mmol/L	at 0.5 mmol/L	not possible	not possible
1.0 mmol/L : 1.0 mmol/L	at 2 mmol/L	*347 µS/cm = 1.6 mmol/L	not possible	not possible
2.0 mmol/L : 1.0 mmol/L	at 2 mmol/L	at 1 mmol/L	*392 µS/cm = 1.8 mmol/L	at 2 mmol/L

Table 3 * Concentration of uncomplexed free OH^--ions calculated according to figure 11

Citr. : Cu	Complex	Number of surplus of free OH^--ions
0.5 mmol/L : 1.0 mmol/L	0.5 mmol/L HO-Cu-Citr.-Cu-OH	0.9 mmol OH^--ions
1.0 mmol/L : 1.0 mmol/L	0.5 mmol/L Citr.-Cu-Citr.-Cu-OH	1.6 mmol OH^--ions
2.0 mmol/L : 1.0 mmol/L	0.75 mmol/L Citr.-Cu-Citr. + 0.25 mmol/L Citr.-Cu-OH	1.8 mmol OH^--ions

Table 4 Postulated complexes with the surplus of free OH^--ions

3.2. Pharmaceutical Chemistry
3.2.1. Investigation of the enzyme-inhibitor-complex
3.2.1.1 The pH-spectrum for the complex between the digestive enzyme Trypsin and the inhibitor Soybean / Chicken Egg White, figures 12 to 13

For the **recording** of the **pH-spectrum** of a enzyme-inhibitor-complex, **System (IS+T), eight measurements** are necessary. The quantity of **0.5 g of Trypsin** (Boehringer Mannheim, 10000 BAEE) and an **equivalent quantity** of **Soybean inhibitor** (Sigma Aldrich, 1 mg inhibit 1.7 mg Trypsin 10000 BAEE) are necessary. To investigate the interaction of Trypsin with an **another inhibitor 250 mg of Trypsin**, the equivalent quantity of inhibitor and **four additional measurements** will be necessary. For **every run** it is possible to investigate the **interaction of three inhibitors with Trypsin.**

- System IS - (Soybean inhibitor / dist. water), figure 12

In comparison with System H_2O, the System IS has a higher consumption of H^+/OH^--ions; therefore the Theoprax-curve of the System IS has a strong decrease at a pH-value below 4 and above 12 (the point of intersection of tangents lies around pH 3.6 and 11.6). The decomposition of the Soybean inhibitor begins.

- System (IS+T) - (Soybean inhibitor + Trypsin / dist. water)

At pH 5.1 the solution is turbid at the beginning of the titration, the value for $\Delta\kappa$ is - 61 µS/cm. On the basis of Coulomb interactions, there is an enzyme-inhibitor-complex in the pH area of 3.5 to 11.8. This is nearly equivalent to the enzyme-inhibitor-complex between Trypsin and Kunitz Soybean inhibitor, which is in the pH area of 3.5 to 7.5 [4]. After the addition of HCl / KOH, the solution becomes clear, when the pH gets lower or higher. The decomposition of the complex begins. In the direction of lower pH-values, the value of $\Delta\kappa$ gets less and becomes positive, because the protonation of the enzyme and the inhibitor is inhibited by the enzyme-inhibitor-complex. The same is true for a higher pH-value, where the deprotonation and the hydrolyse are also inhibit by the complex, the peak is next to pH 11.8.

- System (IS+T)` - (Soybean inhibitor + Trypsin + KCl / dist. water)

With the addition of KCl, the van der Waals interaction becomes stronger the value of $\Delta\kappa$ is –200 µS/cm. The share of the complex in the specific conductivity κ of the solution becomes zero, K^+-ions are also complexed because the specific conductivities κ of the solution gets below that of the pure KCl solution. The decrease of the specific conductivity is very strong and can also be shown by the addition of the inhibitor to the KCl solution. The inhibitor is a strong complexing agent which complexes the enzyme as well as the K^+-ions. With the addition of the enzyme to the KCl solution, the specific conductivity of the pure enzyme solution does not raise the value of the specific conductivity κ of the pure KCl solution, it stays almost constant. This means that the van der Waals forces make the interaction between the enzyme molecules stronger and the migration more difficult.

- System IC - (Chicken Egg White inhibitor / dist. water), figure 13

The higher consumption of H^+/OH^--ions, compared to System H_2O, makes the Theoprax-curve of the System IC decrease. At a pH below 3 and above 11 (the point of intersection of tangents is next to pH 2.5 and 11.8) the decomposition of the Chicken Egg White inhibitor begins.

- System (IC+T) - (Chicken Egg White inhibitor + Trypsin / dist. water)

After the start of the titration with KOH the solution begins to become turbid, the value of $\Delta\kappa$ is about 100 µS/cm at the pH 5.7 to pH 10.6. On the basis of Coulomb interactions an enzyme inhibitor complex is formed. The deprotonation and hydrolyse of the enzyme and inhibitor are inhibited, the peak is next to pH 11.8.

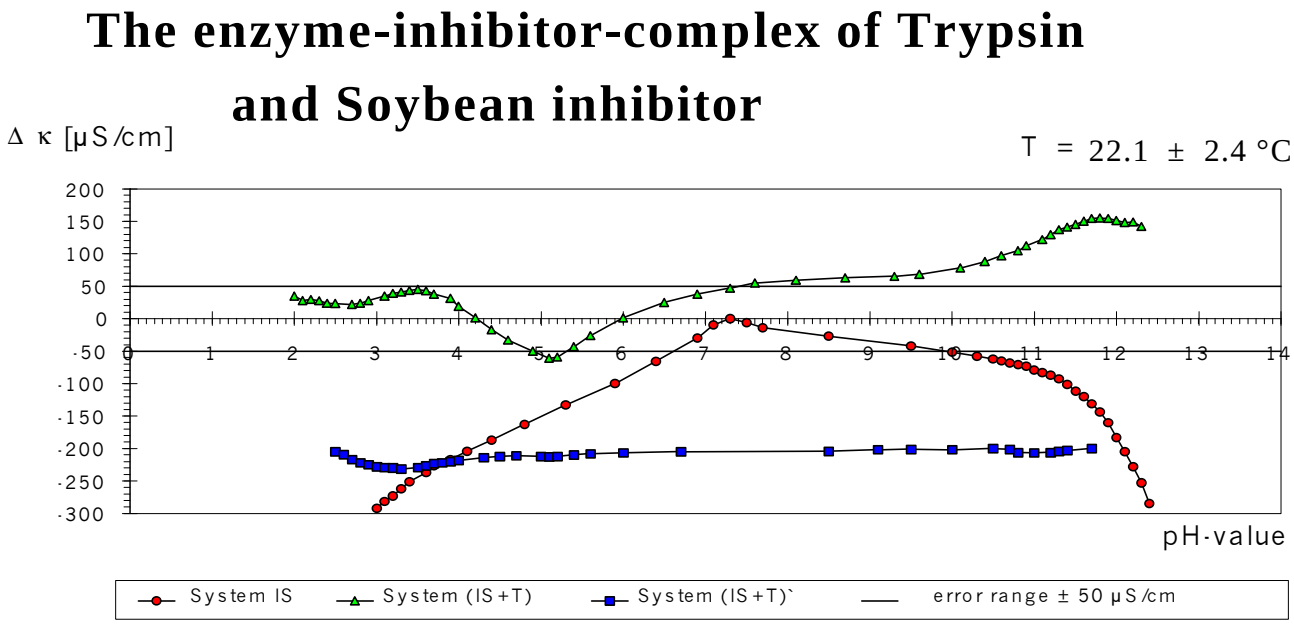

Fig. 12 pH-spectrum of the enzyme-inhibitor-complex (System (IS+T), pH 3.5 - pH 11.8) between Trypsin (120 mg, Boehringer Mannheim, 10000 BAEE) and Soybean inhibitor (72 mg, Sigma Aldrich, 1 mg inhibit 1.7 mg Trypsin 10000 BAEE)

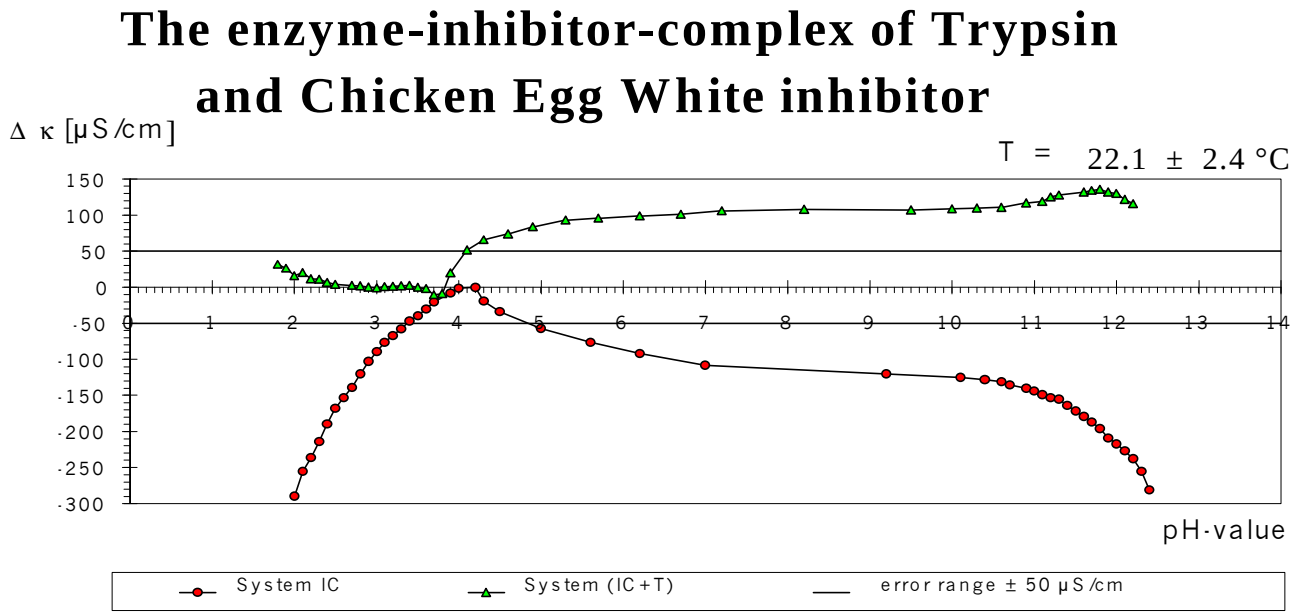

Fig. 13 pH-spectrum of the enzyme-inhibitor-complex (System (IC+T), pH 4.3 - pH 11.8) between Trypsin (120 mg, Boehringer Mannheim, 10000 BAEE) and Chicken Egg White inhibitor (120 mg, Sigma Aldrich, 1 mg inhibit 1 mg Trypsin 10000 BAEE)

3.2.2 Investigation of the catabolism of Gelatine by Trypsin, figures 14 to 15

In figure 14 the investigation has been made with the lowest possible quantity of Trypsin (4 mg Trypsin / 0.1% solution of Gelatine). Figure 14 shows that, although the small values of $\Delta\kappa$ are still in the error range, a tendency statement of the expected results can be made. With this pH-spectrum, the necessary quantity of Trypsin for figure 15 can be calculated. Here five times the quantity is necessary to record figure 15 (20 mg Trypsin / 0.2% solution of Gelatine). Before the recording of the pH-spectrums begins, the solution had to rest a certain time and then after the addition of HCl respective KOH the pH-spectrum was recorded. The enzyme Trypsin separates specifically the peptide bond at the carboxy-groups from Lysin and Arginin [5].

- System (G+T) - (Gelatine + Trypsin / dist. water)

Due to the catabolism of Gelatine by Trypsin in System (G+T), compared to System G, 0.2/0.4 mmol/L H^+-ions have been consumed at pH 2, the value of $\Delta\kappa$ is -61/ -165 µS/cm. The same happened at pH 12, where 0.3/0.6 mmol/L of OH^--ions have been consumed, $\Delta\kappa$ is -65 µS/ -172 µS/cm. The use of twice the quantity of Gelatine in figure 15 results in the double quantity of consumption of H^+/OH^--ions. The enzyme Trypsin separates the peptide bond at the carboxy-groups of Lysin and Arginin at a preferable pH area of 7.5 to 9 [4].

- System (I0.5+T)` - (Gelatine + 50% Soybean inhibitor + Trypsin / dist. water)

The inhibition of the enzyme by the Soybean inhibitor, figures 14/15 at pH 2.5 with $\Delta\kappa$ -33/ -89 µS/cm corresponds to a consumption of 0.1/0.2 mmol/L H^+-ions. This correspond to 50% of the consumption of System (G+T). At pH 11.5 with $\Delta\kappa$ -44/ -147 µS/cm, 0.2/0.5 mmol/L OH^--ions are being consumed. The reason for the reduced inhibition is that the reaction rate is higher, so that the end of the reaction is reached very early. The reaction time is long enough to catabolize 80% of the Gelatine with an inhibit enzyme. For a correct evaluation of the results, the waiting time before the beginning of the titration must be calculated in such a way that the reaction time is so long that Trypsin can catabolize Gelatine to 100%. Refer to the results for the acid, where a correct waiting time had been chosen.

- System (I1+T)` - (Gelatine + 100% Soybean inhibitor + Trypsin / dist. water)

In comparison with System G, no additional H^+/OH^--ions have been consumed. The value of $\Delta\kappa$ is in the error range, the inhibition of the enzyme is 100%.

3.2.3 Investigation of the interaction between the enzyme and Gelatine, figures 14 to 15

Figures 14/15 show the interaction between Gelatine and Trypsin at pH 4.5 to 9. At pH 8.4 the value for $\Delta\kappa$ is 17/74 µS/cm, in figure 14 it is 0.05 mmol/L and in figure 15 it is 0.26 mmol/L. The surplus of OH^--ions like the quantity of Trypsin increases five-fold. In the acid pH-area at pH 5, where $\Delta\kappa$ of the System (I0.5+T)` is lower than $\Delta\kappa$ of the System (G+T), the inhibitor reduces the interaction between Trypsin and Gelatine. At pH 9 and higher, where the consumption of $OH^-$-ions at the System (I0.5+T)` begins, in comparison with System (G+T), at a higher pH-value, the decomposition of the enzyme-substrate-complex begins later. At the isoelectric point at pH 7.5 until 9.3, the molecules of Gelatine are spherical, the Carboxyl-groups are inside and the protonated positive amine-groups are outside of the spherical at the end of the side chain [6, 7]. The enzyme molecules have the same character as the gelatine molecules so that in the acid, the inhibitor with his protonated functional groups, raises the positive charge of the enzyme by forming a complex and the repulsion between the enzyme and the

substrate gelatine becomes stronger. In the basic, the repulsion between the positive charged amine-groups of the enzyme becomes weaker, the interaction between enzyme and Gelatine grows. Since the interaction of the inhibitor with the enzyme increases the positive charge of the enzyme, the Theoprax-curve of the System (I1+T)` reaches its maximum at a higher pH-value. At the maximum, the value of $\Delta\kappa$ is due to sterical reason lower than $\Delta\kappa$ of the System (G+T)`. On the formation of the enzyme-substrate-complex, like the key and lock principle, the positive charge of the functional groups outside of the enzyme, which have to pass the positive functional groups of gelatine, determine the level of the activation energy. The level of the activation energy can be changed by the pH-value, in example the percentage of protonated functional groups and their distance to each other, this means by the length of the side chain of the amino acids.

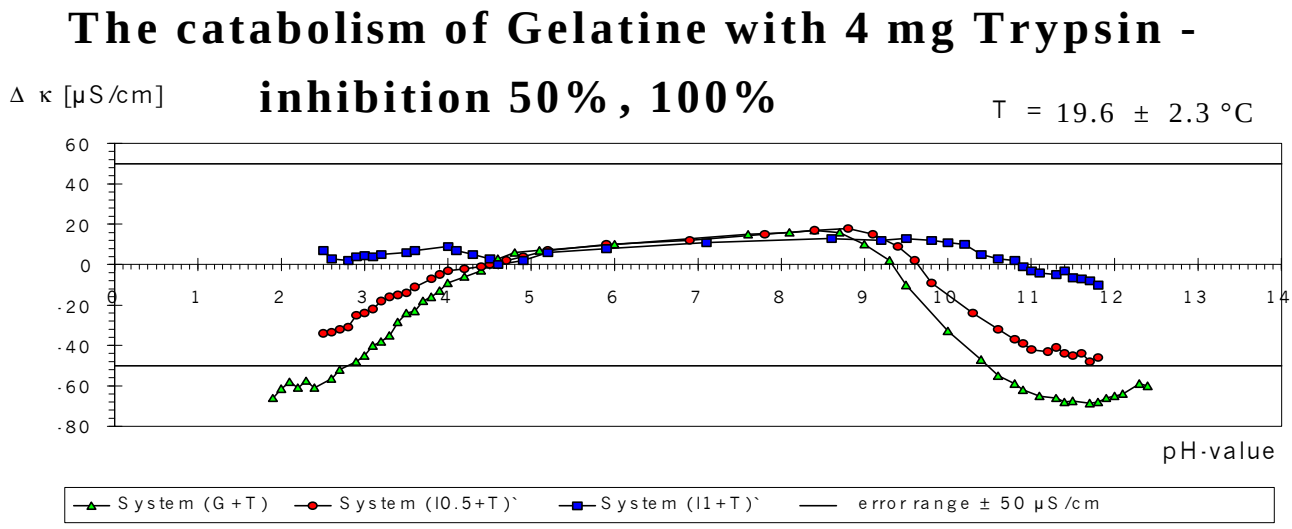

Fig. 14 The catabolism of Gelatine by the enzyme Trypsin (4 mg Trypsin / 0.1% solution of Gelatine) inhibition of the enzyme of 50% respectively 100% by the addition of inhibitor (1.2 mg / 2.4 mg Soybean inhibitor)

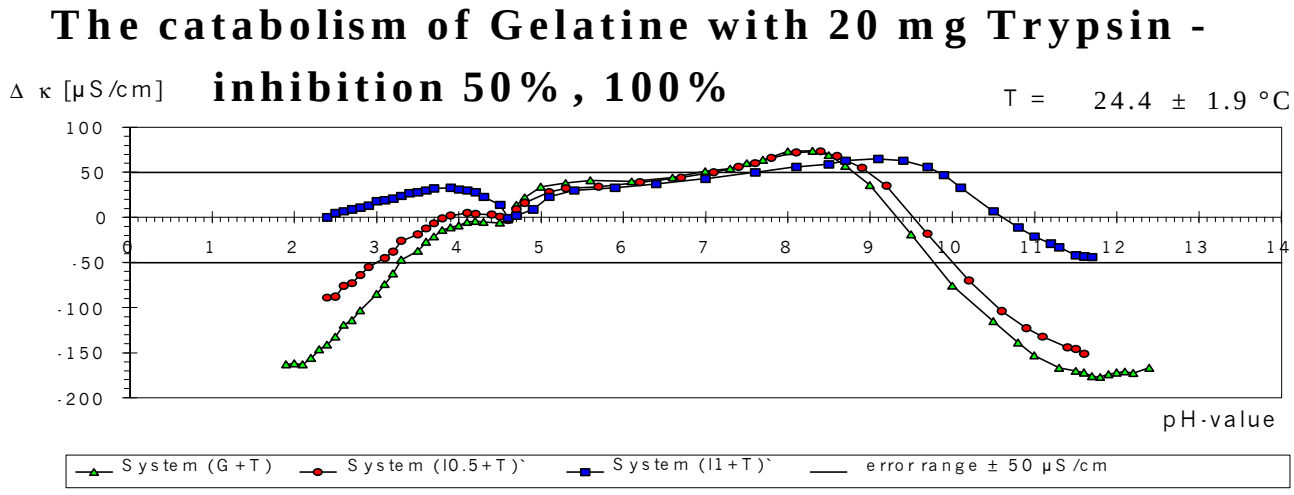

Fig. 15 The catabolism of Gelatine by the enzyme Trypsin (20 mg Trypsin / 0.2% solution of Gelatine) inhibition of the enzyme of 50% respectively 100% by the addition of inhibitor (6.0 mg / 18.0 mg Soybean inhibitor)

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3.2.4 Determination of the pH-activity-curve of Trypsin, figure 16

- System G/T - (Gelatine / dist. water) / (Trypsin / dist. water)

In comparison with System H_2O, the System Gelatine/Trypsin consumes more H^+/OH^--ions.

- System (G+T) - (Gelatine + Trypsin / dist. water)

Through the catabolism of Gelatine with Trypsin, an additional 0.4/0.6 mmol/L of H^+/OH^--ions are consumed, compared to System G.

- System (G+T)` - (Gelatine + Trypsin / dist. water)

Both peaks at pH 4/8.3 are equivalent to the activity-area of the enzyme Trypsin. The value of $\Delta\kappa$ is 148/392 µS/cm which means that the surplus of H^+/OH^--ions is 0.4/1.45 mmol/L.

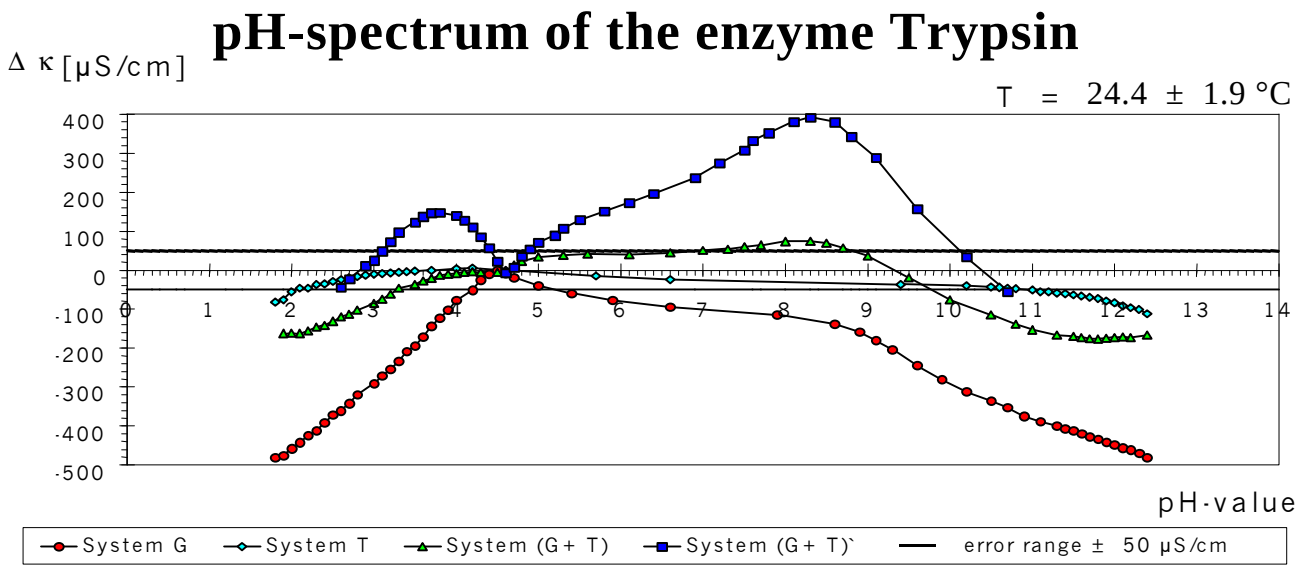

Fig. 16 The pH-spectrum shows with the Theoprax-curve System (G+T)`the pH-activity-curve of Trypsin (20 mg Trypsin / 0.4% solution of Gelatine)

3.3. Biochemistry

3.3.1 Investigation of the interaction between antigen and antibody

3.3.1.1 The pH-spectrum for the antigen-antibody-complex between the antigens Sheep/Bovine Albumin and the antibody to Sheep whole Serum, figure 17 to 18

For the **recording** of the **pH-spectrum** of a antigen-antibody-complex, **System (S+AS) eight measurements** are necessary. The quantity of **1 ml antibody to Sheep whole Serum** (Liquid, Host Animal Rabbit, Sigma Aldrich) and an **equivalent quantity** of **antigen Sheep Albumin** (Fraction V Powder, Sigma Aldrich) are necessary. To investigate the interaction of the antibody with an **another antigen. 0.6 ml antibody**, the equivalent quantity of antigen and **four additional measurements** are necessary. For **every run**, it is possible to investigate the **interaction of three antigens** with the antibody.

- System S - (Sheep Albumin / dist. water), figure 17

The reason of the sharp decrease of the Theoprax-curve of System S, at a pH below 4 and above 11 (the point of intersection of tangents is next to pH 3.7 and 11.0) is the higher consumption of H^+/OH^--ions compared to the System H_2O, the decomposition of Sheep Albumin begins.

- System (S+AS) - (Sheep Albumin + antibody to Sheep whole Serum / dist. water)

The antigen-antibody-complex forms in the acid pH range below 7, because in the basic, the RNH_3^+-group is deprotonated [8, 9]. The maximum is at pH 5.1 with a $\Delta\kappa$ value of 76 µS/cm.

- System B - (Bovine Albumin / dist. water), figure 18

In comparison with System H_2O, the consumption of H^+/OH^--ions is higher, so that the Theoprax-curve for the System B has a strong decrease. The decomposition of Bovine Albumin begins at a pH below 4 and above 11 (the point of intersection of tangents is next to pH 3.6 and 11.1)

- System AS - (antibody to Sheep whole Serum / dist. water)

At the System AS, the higher consumption of H^+/OH^--ions begins at a pH below 3 and above 10.

- System (B+AS) - (Bovine Albumin + antibody to Sheep whole Serum / dist. water)

The antibody reacts with the antigen protein in the acid, because in the basic, the RNH_3^+-group is deprotonated [8, 9]. The antigen-antibody-complex forms below the pH of 5.3. The maximum is at pH 4.4 with a $\Delta\kappa$ value of 70 µS/cm. The peak of the System (B+AS) is in comparison with the System (S+AS) lower because the antigen is characteristic to the species [8].

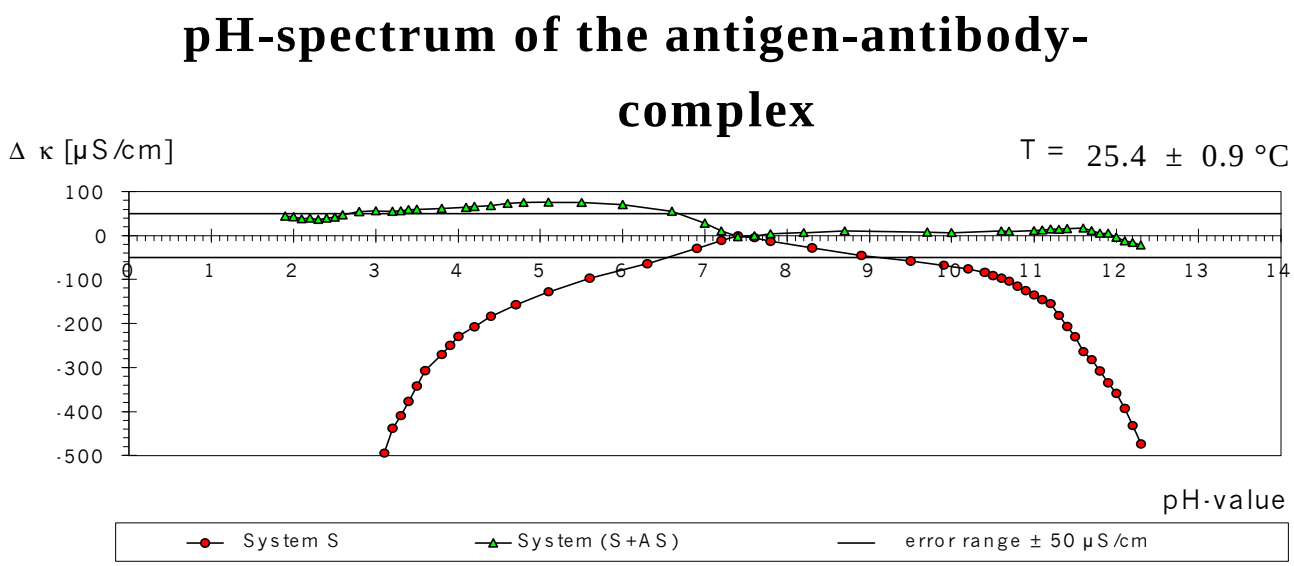

Fig. 17 The pH-spectrum of the antigen-antibody-complex (System (S+AS), pH 5.1) between Sheep Albumin (231 mg, Fraction V Powder, Sigma Aldrich) and the antibody to Sheep whole Serum (0.3 ml, Liquid, Host Animal Rabbit, Sigma Aldrich)

pH-spectrum of the antigen-antibody-complex

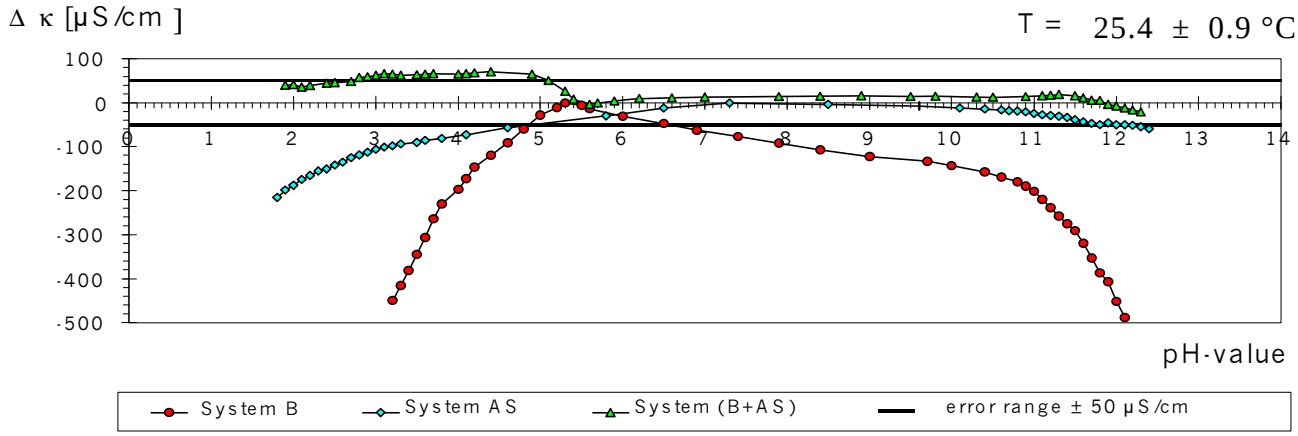

Fig. 18 The pH-spectrum of the antigen-antibody-complex (System (B+AS), pH 4.4) between Bovine Albumin (231 mg, Fraction V Powder, Sigma Aldrich) and the antibody to Sheep whole Serum (0.3 ml, Liquid, Host Animal Rabbit, Sigma Aldrich)

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