The influence of certain additives on the stability of all-trans astaxanthin dissolved in methylene chloride and chloroform

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1. Introduction

Astaxanthin (AXT) is a lipophilic pigment [Bri95b]. The substance occurs naturally, generated by a diversity of plants, microorganisms and algae [Gup07], but can also be chemically synthesized [Loc06].

The molecule contains a long chain of conjugated double bonds, which can be isomerized easily, especially, in solution [Bri95b]. This structural feature is a chromophore and because of the length of the double bond chain the AXT molecule absorbs light in the visible region [Bri95b]. AXT being a natural pigment with the exact features it inhibits, it is perfectly suitable for feed supplementation. However, the chromophore also brings an unfortunate feature with it, the high susceptibility of the molecule. The aftermath being isomerization and degradation of the chromophore and the molecule as a whole [Bri95b]. This causes an immense loss of material. This work`s endeavor is to discover countermeasure to approach this circumstance.

The main isomers transformed out of all-trans AXT are 9-cis and 13-cis AXT [Yua01]. Their incorporation is not selectively into the muscle tissue of the fish [Yua01]. For instance, in case of *Salmo salar* and *Salvelinus alpinus* 13-cis AXT was mostly found in the liver [Lia08].

Animals, incapable of producing AXT, access the substance via digestion. The xanthophyll gets absorbed into the plasma and transported to potential binding sites [Hen87, Hen89]. For sockeye salmon (O. nerka) it is understood, that all-trans AXT is connected to the muscle tissue fiber (actomyosin) by a weak hydrophobic bond [Sto92]. Also lobster shell contains the all-trans isomer of AXT. In the shell the isomer is bound to the protein crustacyanin [Cia02]. A reduced absorption of AXT in vivo has been observed and ascertained by several scientific studies concerning intake and retention of AXT [Ytr06, Sto92, Sah06]. Though the number of bonding sites can be considered restricted, it is not yet proven to be the actual reason for the diminished deposition [Sah06, Mat06]. Therefore a distinct amount of all-trans AXT is sufficient to achieve an appealing color result [Øst99, Sto92, Tor89].

It is of importance that the amount of all-trans AXT embedded into the feed remains in the all-trans configuration for as long as possible, providing a high quality and long lasting product.

AXT can be either extracted from biomass or synthesized chemically. On either way the molecule gets in contact with solvents, which endanger its structural stability and subsequently the quality of the feed product. The idea is to test and apply additives, beneficial to maintain the all-trans configuration, once the molecule is dissolved and structurally most vulnerable [Yua99, Per11]. The application of additives is ubiquitous and the fields of their application widely dispersed. Therefore the chances to discover suitable substances seem rewarding.

In their studies [Hen87, Fos84, Tor96, Tor89, Bje00, Ytr06] were able to reveal the outstanding role (all-trans) AXT occupies, over other carotenoids, by coloring the salmonid muscle tissue. AXT, synthesized by microorganisms in nature, is part of the food chain of many animals. The main demand of fish is met by fish farms, however, the appealing flesh color can`t be achieved naturally in fish farms. Feed must be supplemented with carotenoids for a satisfying coloration.

The factors to which AXT is susceptible to are light, oxygen, acid, base and oxidant [Sch95, Che07]. For the experiments chlorinated solvents were employed. When there are traces of water in chlorinated solvents, the formation of hydrochloric acid is a given [Che03, Moh10]. The source material was exposed to unfavorable conditions, seeking to ensure acceleration of unintentionally occurring processes decreasing the all-trans AXT concentration.

2. Fundamentals

2.1 Carotenoids

The term “carotenoid” encompasses a class of unsaturated hydrocarbons and the corresponding oxygenated derivates [Jee94]. The structure of carotenoid molecules includes a segment of conjugated double bonds (polyene chain) located in the center of the molecule. This segment is essentially involved in the establishment of the physical and chemical properties, due to its delocalization of π-electrons [Bri95c]. Carotenoids are known for their intense and characteristic color, which they occupy by absorbing light of the visible region of the spectrum [Fra08].

The reason for the susceptibility of carotenoids towards oxidizing agents, light, metal ions, high temperature, acid and peroxides, is up to the polyene chain and results in isomerization and degradation of the molecule [Koh95, Rod99, Sch95].

Carotenoids without polar moieties are very hydrophobic and therefore either not soluble in water at all or merely to a small extent. However, is a polar moiety part of the carotenoid molecule, the polarity is changed towards a more hydrophilic character. There are different configurations possible for the chain of conjugated double bonds to transform into and thus changing the configuration of the entire molecule. The distinction is made between all-trans and cis isomers [Bri95a].

2.2 Isomerization of Carotenoids

Carotenoids, either formed naturally or assembled synthetically, do not necessarily occur in their most stable conformation [Cha06, Ern02]. Even though in nature isomerization of molecules can be and most certainly is a part of complex mechanisms in every living organism, like photosynthesis and vision [Bri08]. The process of isomerization is not a desirable event concerning the production of specific isomers for drugs or supplements for utilization in food or feed products (e.g. [Lia08]). Thermal isomerization can be applied to reconfigure cis isomers into the desired all-trans molecules [Ern02].

2.3 Application of additives

Additives are ubiquitous in a plethora of industrial branches, for example pharmaceutical industry, petroleum industry and food industry. Additives in the petroleum industry improve properties of fuels and oils. In emulsions additives can increase the stability of a dispersion [Pet13]. The application of additives in crystallization can alter the crystal growth rate, nucleation kinetics and the particle size distribution (e.g. [Nýv95]). The application of additives can lead to the nucleation of the desired meta-stable form of crystals by suppressing the effect of kinetic factors. Additives that affect the nucleation rate similarly influence the growth rate. Tailor-made additives are an option to modify certain characteristics that could not be influenced by none tailor-made additives so far [San07, Sch13]. When the effect provided by an additive is ascertained for a specific process its time constant can be influenced selectively by the additive. The procedure to achieve this kind of knowledge includes the following three steps: Screening, measurement series and data evaluation. Additives provide a certain level of manipulation to process controlling by stocking up the choices of operating parameters like temperature and stirring speed [Str04]. In literature an optimum concentration for the additive application was stated to range from 1 – 1000 ppm [Rau00]. It was also stated that the range depends strongly on the system and that often the concentration of the additive affects the effect provided by the additive. Therefore the same substance can initiate very different progresses in a process, merely by the variation of the concentration [Rau00].

2.4 HPLC

As a physical separation method, the high performance liquid chromatography (HPLC) has become a very important tool in the modern analytical laboratory [Kaz07, McM07]. The separation is highly versatile and facilitates the isolation of substance mixtures that are very alike. For instance, in isomer analysis, the geometrical isomers of the same carotenoid can be isolated by HPLC. A simple construction scheme of an HPLC includes the mobile phase, a pump, an injection valve and sample loop, a degasser, a control unit, the column (additional precolumn), a detector, an evaluation unit and a waste container. The inside of the column is filled with the solid phase material and through the entire system the mobile phase is pumped. Samples that are to be investigated by HPLC will be transferred to a liquid stage, e.g. solution, and injected into the valve. A precisely defined volume stays in the

sample loop and, by turning the valve, enters the system. The mobile phase transports the sample through the column. As soon as the sample enters the column and gets into contact with the solid phase physical interactions between the sample molecules and the solid phase material occur. The sample molecules get adsorbed differently to the solid phase and depending on the intensity of the adsorption the different molecules remain on the column for distinct periods of time. The mobile phase transports the sample molecules onto the column and contributes to rinse them off the column again. For separation of polar substances a normal phase system is used, consisting of a polar stationary phase and a nonpolar mobile phase. When a mix of nonpolar substances is supposed to be separated a reversed phase system will be applied. The nonpolar substances will adsorb to the nonpolar solid phase and get rinsed off by a polar mobile phase. By the time the separated substances elute from the column the respective detector will measure the signals caused by the substances. The information will be recorded by the evaluation unit. The correct separation of the individual peaks is fundamental, for analytically and quantitatively accurate work. For each separation problem the best possible conditions should be chosen to ensure optimal resolution and separation efficiency [Mey06].

3. Aim of the work

A known principle in crystallization is directed to maintain the required metastable form of a crystalline substance for a prolonged amount of time, by the application of additives. The aim of this work was to adapt this fact to dissolved material and to achieve the preservation of the desired metastable form.

It was found by Torrisen *et al*. [Tor89] as well as by Storebakken and No [Sto92] that the utilization of AXT is most efficient for pigmentation of salmonids. All-trans AXT is the isomer accumulating selectively into muscle tissue of salmonid fish [Koh95]. To achieve an appealing look for farm-raised salmonids the fish feed is supplemented with all-trans AXT. During synthesis and processing, AXT, susceptible as it already is, gets in contact with substances and factors which can initiate isomerization and degradation of the molecule.

The aim is to achieve a significantly reduced loss of all-trans AXT, dissolved either in chloroform (also known as trichloromethane, here exclusively referred to as chloroform and abbreviated as TCM) or methylene chloride (also known as dichloromethane, here exclusively referred to as methylene chloride and abbreviated as DCM), by the application of additives. Therefore a selection of substances should be tested with the focus on their improvement for the structural stability of all-trans AXT, in solution, by monitoring the concentration changes of the main isomers (all-trans, 9-cis and 13-cis) via HPLC. If this approach is successful the results could be presented in diagrams, showing the concentration of the main isomers with time. A concept of the reduction of an all-trans AXT loss could then be presented and proven.

4. Materials and Methods

Due to the absorption properties of carotenoids the concentration detection is realizable via HPLC, to detect the individual isomers, and UV/VIS photometer to detect the combination (dissimilar to sum) of all carotenoid isomers present in solution. The water content of the utilized solvents was distinguished via Karl-Fischer titration.

4.1 Karl-Fischer Titration

Even in so called “purified solvents” traces of other substances or elements can be found, including water. Among other things the water content in the solvent is crucial for the stability of the dissolved AXT molecules. The distinction of the water content present in both pure and water-saturated solvents was determined by titration according to the method of Karl-Fischer. The water content is determined in accordance to the following principle and reaction. Iodine (methanolic iodine solution) reacts with SO_2 (dissolved in pyridine) under the condition of water being present. As long as water is still available in the solution the chemical reaction shown in equation (4.1) will continue to take place.

$$SO_2 + I_2 + 2\,H_2O \rightarrow 4\,H^+ + 2\,I^- + SO_4^{2-} \qquad \textbf{(4.1)}$$

This is visualized by the continuous degeneration of the brown iodine color of the solution which is added. At the very moment the water is used up completely, by iodine and SO_2, the brown iodine color stays visible and signals the end of the titration [Kun02].

4.2 HPLC

The source material (crystalline all-trans AXT powder) was provided by DSM Nutritional Products. It was kept under argon atmosphere, at a temperature of 4 °C and light exclusion. The solvents methylene chloride (HPLC grade, not stabilized) and chloroform (HPLC grade), were purchased from Carl Roth GmbH & Co. KG, Germany. Deionized water (pH = 6.4) was used to prepare water-saturated methylene chloride and water-saturated chloroform. The water content was measured via Karl-Fischer titration in both, water-saturated and pure, solvents. Further solvents: n-heptane (HPLC grade), acetone (HPLC grade), methanol (HPLC grade) and ortho-phosphoric acid (85 %, extra pure) were purchased likewise from Carl Roth GmbH & Co. KG, Germany.

A variety of additives was tested and a selection was taken into the further experimental approach. For the selection practical and economical reasons were taken into account.

The initial assortment of additives was: potassium chloride (KCl), potassium nitrate KNO_3, potassium sulfate (K_2SO_4), sodium carbonate (Na_2CO_3), calcium chloride ($CaCl_2$), magnesium sulfate hexahydrate ($MgSO_4*6H_2O$), magnesium acetate tetrahydrate ($(CH_3COO)_2Mg*4H_2O$) potassium hydroxide (KOH), dipotassium phosphate (K_2HPO_4), potassium acetate (CH_3COOK (≥ 99 %, p.a.)) and potassium carbonate (K_2CO_3 (≥ 99 %, p.a., ACS, ISO)). The prior screening lead to the following selection of substances: KOH, K_2HPO_4, CH_3COOK and K_2CO_3. For the experiments an amount of approximately 5 mg was weighed and added to each solution. The sample solutions were prepared by adding all-trans AXT powder to either pure/water-saturated chloroform or pure/water-saturated methylene chloride to establish an AXT level of 0.2 g/L.

A reference solution consisting of AXT and the solvent in question was prepared in all 4 cases. Illustrating the actual situation in AXT synthesis and processing, without application of additives, revealing processes taking action unhindered. Measurements were taken by HPLC and also photometer to determine concentration changes. The HPLC system consisted of the system controller (Shimadzu SCL-10A VP), the Shimadzu UV/VIS detector (SPD-10A) with a deuterium lamp as light source. Furthermore, a pump control unit (Shimadzu LC-10AD VP) and a low pressure gradient unit (Shimadzu FCV-10AL VP) complete the HPLC system. Part of the instrument was also a degasser (Shimadzu DGU-14A) and a rheodyne injection valve with a 20 µL sample loop. The column specifications were a particle diameter of 5 µm and a length of 125 mm. The inner diameter was 4 mm and a pre-column with the same specifications except a length of only 20 mm was located directly in front of the main column. The pre-column and the main column were packed with LiChrosorb®Si60 as stationary material. A modification of the stationary phase was performed with methanolic phosphoric acid solution to avoid tailing of the peaks. The eluents n-heptane and acetone were used in a ratio of v/v 86/14.

Two different solvents were used to distinguish the effects caused by a variety of additives. For each solvent and each additive three experiments were performed under the same environmental conditions. The same applies to reference experiments with additive-free solutions of each solvent. The experiments were carried out to distinguish the imprint of the chosen additive into the system and molecular stability of all-trans AXT. The experimental conditions were as follows: light exclusion, a set temperature of 30 °C for methylene chloride

and 40 °C for chloroform and a stirring speed of 300 rpm. Double walled beaker with screw lids were used to safeguard an exact temperature and prevent loss of solvent by evaporation.

4.3 UV/VIS Photometer

To photometrically detected organic substances in the UV/VIS region these substances have to have an unsaturated character, like a system of conjugated double bonds inside the molecule. The measuring technique uses ultraviolet light and the light of the visible region to put valence electrons inside of molecules into an excited state. The electrons absorb the light and the diminishing of the light intensity is measured. Molecules show specific absorption peak(s) at certain wave lengths in which the intensity of the light absorption is much higher [Ott06]. Ethyl acetate (purchased from Carl Roth GmbH & Co. KG, Germany) was used to dilute the samples with. For the sample preparation 100 µL of the sample solution was added to 900 µL of ethyl acetate. The solution was thoroughly mixed. 100 µL of the freshly prepared mixture was added to 900 µL of ethyl acetate and mixed well. The second mixture was transferred into a quartz glass cuvette closed with a stopper and placed into the UV/VIS photometer for the measurement. Before the actual samples were measured a reference solution (without AXT and without additives) was used previously to exclude the absorption caused by the solvent and set the device to zero. The additives K_2CO_3, KOH, CH_3COOK and K_2HPO_4 were tested upon their possible UV activity, however, none was found. The detection of the signal of the sample solutions was carried out at a wavelength of 478 nm and an integration time of 2.0 s. The device used was an analytikjena SPECORD 40 UV/VIS photometer (version: 3.2.3.0; no.: 232E158). The evaluation of the detected signals was performed with the software WIN ASPECT.

5. Results

According to the experiment descriptions, specified in chapter 4, the results are presented in the following and in the same sequence in which they were described previously. After due consideration of the screening experiments, the experimental running times were planned to be 5 days. Occasionally adjustments and changes in the schedule had to be made.

5.1 Karl-Fischer titration

The measurements conducted with a Karl-Fischer device show a distinct difference in water contents between the pure solvents taken from the reservoir bottle and the previously water-saturated solvents. The water content determination was of interest as potential influencing factor on the isomerization and degradation processes all-trans AXT can undergo.

The water content of the pure and water saturated solvents was determined via Karl-Fischer titration. Chloroform, fresh from the reservoir bottle had a water content of about 68 ppm. Water-saturated chloroform, whereby the saturation took place over more than 24 hours in advance to the experiments, contained 1145 ppm. This is an increase of 1684 %. Methylene chloride, taken from the supply bottle had a residue of 96 ppm water. And likewise to the water-saturated chloroform, the water content in the water-saturated methylene chloride increased, to 1847 ppm.

5.2 Screenings

5.2.1 Screenings by HPLC

A variety of organic and inorganic salts were chosen to be tested regarding their stabilizing qualities for dissolved all-trans AXT in chloroform. Desirable would be a slower decrease of all-trans AXT and the reduction of the loss that is faced in untreated solution. The surveyed substances were potassium carbonate (K_2CO_3), potassium acetate (CH_3COOK), sodium carbonate (Na_2CO_3), magnesium acetat tetrahydrate ($(CH_3COO)_2Mg*4H_2O$), magnesium sulfate hexahydrate ($MgSO_4*6H_2O$), calcium chloride ($CaCl_2*2H_2O$) and molecular sieve (3 Å). One solution for each additive was prepared and tested (onefold measurements for screening purposes) by HPLC and UV/VIS photometer. The results obtained from the HPLC measurements are presented in Fig. 1. The information was only used as an orientation and

therefore the area-% values were plotted against the elapsed time of the respective experiments, which vary slightly. The very starting point at the time zero was assumed to be 100% of the measured area, which represents that the initial material only contained the all-trans isomer of AXT. A rather steep descent right from the beginning applies equally to all curves. The area percentage value of the all-trans peak diminishes the most in the first section, which ends at about 30 hours. In the second section the area decrease is significantly lower.

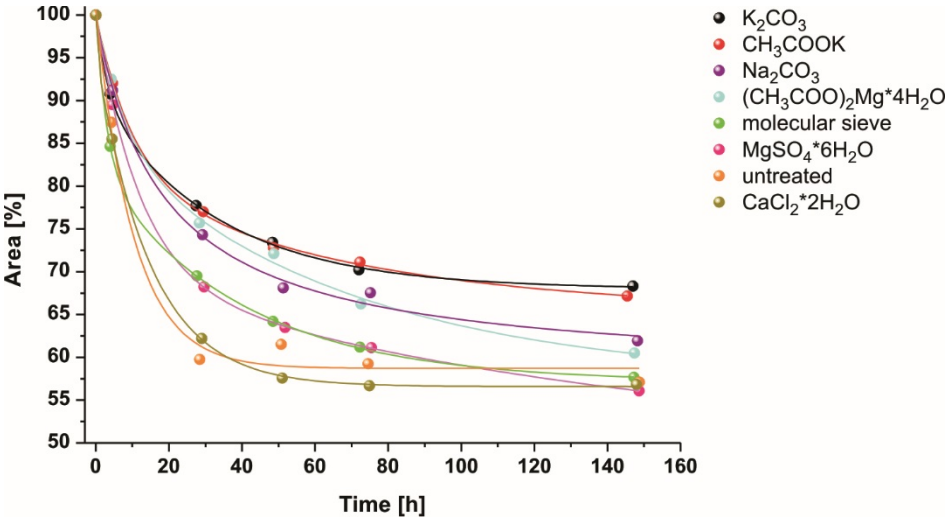

Fig. 1: Additive screening in TCM, plotted is area [%] vs. time [h] indicating concentration losses at different rates, provided by the utilized additives, at a temperature of 40 °C.

After 150 hours all all-trans peak areas were diminished to a percentage value of below 70 %. Two of the tested substances were close to only 60 %, and measured values of four of the experiments were even below 60 %. The experiments that ended below 60 % were the solutions which were equipped with $CaCl_2*2H_2O$, $MgSO_4*6H_2O$, the untreated solution and the solution which was pretreated with a molecular sieve. Close to 60% but above were the values measured in the solutions equipped with $(CH_3COO)_2Mg*4H_2O$ and Na_2CO_3. The two highest values were measured in the solutions equipped with potassium salts (K_2CO_3 and CH_3COOK) the values stand out from the rest with a certain clearance in the space between the graphs. This data lead to further investigations on the potassium salts, because a link between the potassium and the increased stability of all-trans AXT was suspected.

A potassium salt screening followed and brought interesting results which are described in the following. The information extracted from the onefold executed experiments was not as expected. The results were broadly diversified, basically just like in the previously performed

additive screening. The longest duration of experiments in this screening was 93 hours. Four of the potassium salts show such a good stabilizing effect that the area-% values remained between 70 and 80 %. Those salts were CH_3COOK, dried K_2CO_3, KOH and K_2HPO_4. The deviations in the "K_2CO_3"-graph could be a result of measuring errors of the onefold executed experiment. Once again, the values are only meant for guidance. The measurements were performed onefold and hence the results will have an uncertainty, because the screenings only serve the purpose of a survey.

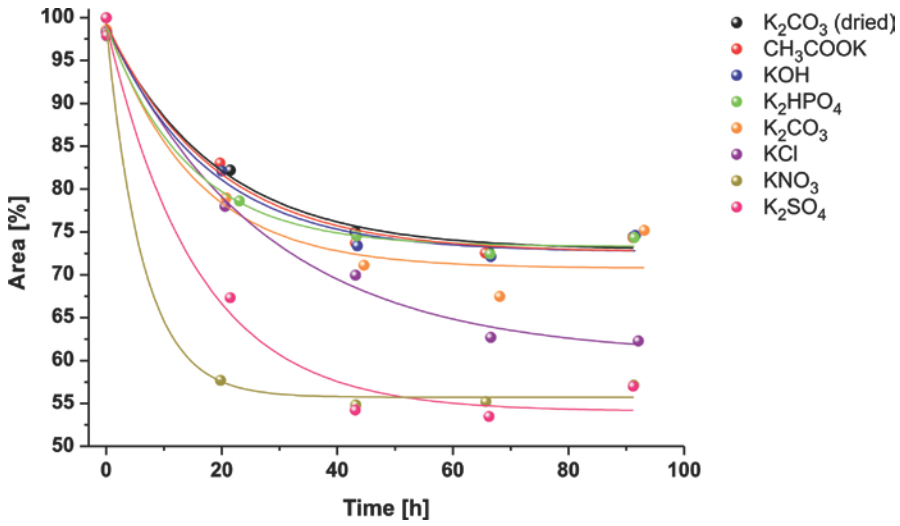

Fig. 2: Potassium salt screening in TCM, plotted is area [%] vs. time [h] indicating concentration losses at different rates, provided by the utilized additives containing potassium, at a temperature of 40 °C.

Subsequently, the following substances were selected for further measurements, in chloroform, in order to acquire precise data: K_2CO_3, CH_3COOK, KOH and K_2HPO_4. Concerning the second solvent, methylene chloride, two other screenings were performed likewise. At first an additive screening was executed to achieve a broader view over the subject matter. Fig. 2 shows the obtained data from the onefold executed experiments. Besides the usage of a different solvent (methylene chloride instead of chloroform) also the temperature was adjusted to 30 °C. The negative slope of all curves is extensively lower than expected and almost all curves descend in a nearly straight manner. The addition of four of the salts into the sample solutions brought remarkable results concerning the all-trans AXT stabilization. Na_2CO_3, K_2CO_3, CH_3COOK and $(CH_3COO)_2Mg*4H_2O$ provided a protection for the structure of the all-trans isomer thus far, that the area of the all-trans peaks diminished no further than 10 % after 65 hours elapsed. It must be remembered that the temperature setting to 30 °C plays a big part for the diminished loss of substance. It is an acknowledged

principle in science, a rule of thumb of chemical kinetics, so to say, that a temperature increase by 10 K increases the rate of chemical reactions by twice to four times (see e.g. [Hol95]). In this regard, a decrease in temperature of 10 K would decrease the rate of a chemical reaction twice to four times as well. The graphs of the untreated solution and the solution which was pretreated with molecular sieve run close to each other and are centrally located between the two other groups of graphs clustered together.

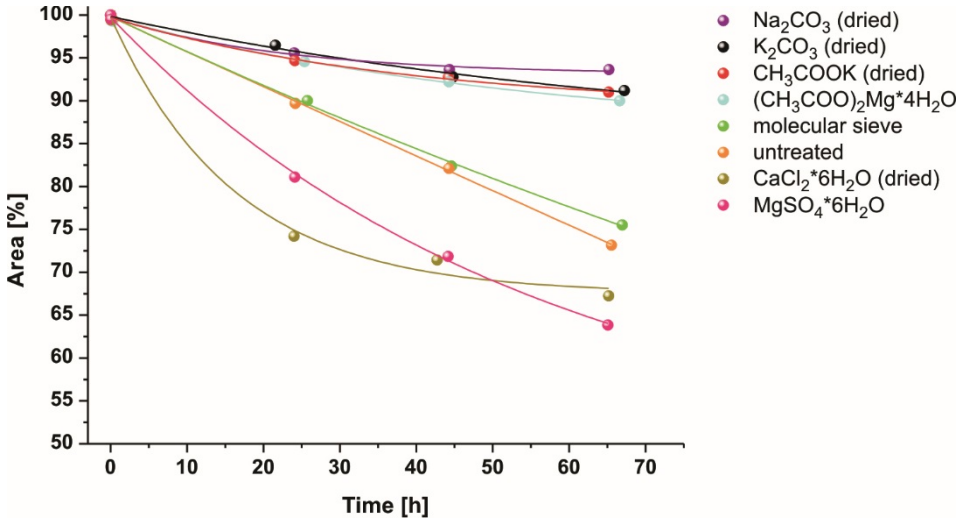

Fig. 3: Additive screening in DCM, plotted is area [%] vs. time [h] indicating concentration losses at different rates, provided by the utilized additives, at a temperature of 30 °C.

These two graphs embody the progress that is strived to be improved by application of additives. According to this guidance (Fig. 3), the pretreatment of the solvent (DCM) by molecular sieve is not worth the effort. The salts $CaCl_2*6H_2O$ and $MgSO_4*6H_2O$ favor the very opposite of the intended purpose of this work and enhance the destruction processes, diminishing the all-trans AXT concentration. Since again both potassium salts are among the group of substances showing the best results, a potassium salt screening was performed subsequently.

The data measured in the potassium salt screening are shown in Fig. 4. The data were acquired in two sets of experiments. After the first set was evaluated the run time for the second set of experiments was reduced to about 70 hours instead of 170 hours. The reason was the steadiness of most of the curves. The majority of the tested potassium salts run in close proximity to one another. They appear to have almost the same rate throughout the entire time. Only two of the tested salts (KCl and K_2SO_4) showed a strong deviation with high decrease of the area of the all-trans peak. A very much unexpected result obtained was the

location of the graph of the untreated solution, which is actually among the cluster of graphs, unlike the data of the untreated solution shown in Fig. 3. Also for the screening of potassium salts in methylene chloride all experiments were performed onefold for orientation purposes.

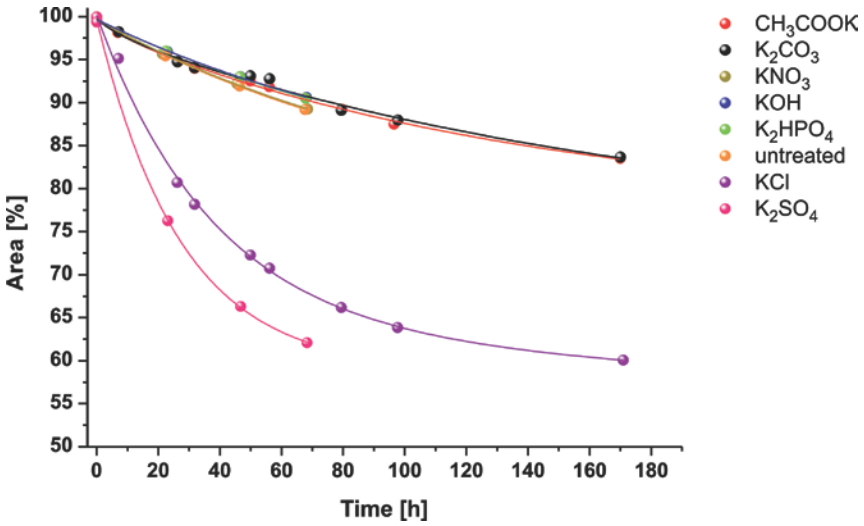

Fig. 4: Potassium salt screening in DCM, plotted is area [%] vs. time [h] indicating concentration losses at different rates, provided by the utilized additives containing potassium, at a temperature of 30 °C.

Since not just potassium salts (CH_3COOK, K_2CO_3, KNO_3, KOH and K_2HPO_4) showed good results, but also the untreated solution a repetition was performed to make sure that no aberration has happened unnoticed. For the repetition six runs were performed. Two untreated solutions and solution treated with K_2CO_3/dried K_2CO_3 and CH_3COOK/dried CH_3COOK were measured. The data in the diagram (see Fig. 5) made it very clear that no error influenced the previous results (see Fig. 4) and the location of the graph of the untreated solution is not questionable. After about 67 hours the area decrease for the majority of the salts was located at about 90 %, including the untreated solution. The curves of the experimental data of KCl were below 70 %, and K_2SO_4 was above 60 %. Both salts (KCl and K_2SO_4) are not suitable for stabilization of dissolved all-trans AXT in methylene chloride. After the lapse of 71 hours in the repetition set of experiments the area had decreased to about 79 %, which is 10 % less than in the actual potassium salt screening in methylene chloride. This is the case for all tested solutions in the repetition. The reason is unidentified so far, but it has to be considered that these experiments were measured onefold, only to provide an orientation.

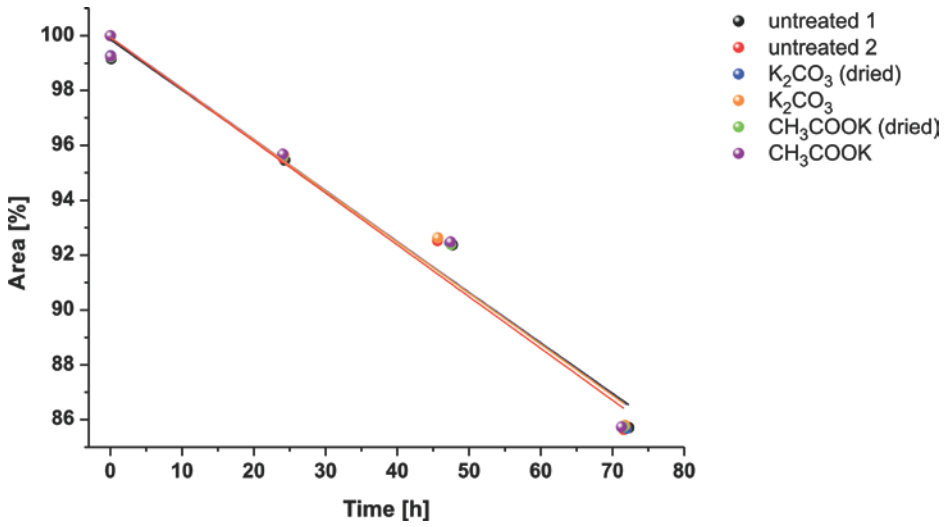

Fig. 5: Repetition of a part of the potassium salt screening in DCM, plotted is area [%] vs. time [h] indicating concentration losses at nearly equal rates, for the untreated solutions as well as for the solutions treated with additive, at a temperature of 30 °C.

5.2.2 Screenings by UV/VIS photometer

For every sample solution tested by HPLC an additional sample of that solution was tested by photometer. The sample solutions were measured onefold and therefore show large variations and as a consequence were excluded from the data that were used for the additive selection. Only the area-% data obtained from the HPLC measurements were taken into account for the selection choices of additives for the data acquisition.

5.3 Measurements

5.3.1 Measurements by HPLC

Three assumptions have been made and mentioned before in [Kre14]. First, at the time zero the concentration is assumed to be 0.2 g/L for all-trans AXT and consequently the total isomer concentration. Second, in each diagram the graph showing the total isomer concentration has been totalized out of the concentration values of all-trans, 9-cis and 13-cis AXT. The area between this graph and the all-trans graph embodies the amount of measured cis AXT; therefore, it has been assumed that this area stands for the amount of initial material lost due to isomerization. The third assumption has been made accordingly. Presuming the formation of other cis isomers, despite 9-cis and 13-cis AXT is negligible, the area of each diagram between the initial concentration and the total isomer concentration is

assigned to concentration losses due to degradation of the initial material and, as the case may be, cis isomers.

Measurements were taken threefold.

The experimental results are depicted in diagrams outlining the changes of the concentrations of the measured isomers of AXT. In each solvent, alongside an additive-free experiment, four different additives were applied to a combination of the starting material (which is assumed to be 100% pure all-trans AXT) and the representative solvent. Each diagram includes 6 graphs. Starting at high concentration, successively, the graphs are “initial concentration” (all initial concentrations were normalized to 0.2 g/L AXT in the used solvent), “total isomer concentration” (totalized out of the three measured isomers), “all-trans” (concentration of the all-trans isomer), “total cis concentration” (totalized out of the two measured cis isomers) and “9-cis” and “13-cis” (each showing the concentration changes of one of the cis isomers).

The data obtained from the untreated solution is considered the “worst case scenario”. Though there are substances which increase the isomerization and degradation rate of all-trans AXT (as demonstrated in the prior additive survey) the untreated solution is usually the current procedure in production and processing.

5.3.1.1 HPLC results in pure chloroform

The water content in the “pure solvent” was 68 ppm. The calibration was performed using chloroform as solvent. Equal volumes of sample solution and n-heptane were mixed together and injected via syringe into a 20 µL sample loop. That means only 10 µL chloroform got transported with the mobile phase through the column. A flow rate of 1.2 mL/min was applied. Under these premises it was acceptable to use the same calibration equation for methylene chloride and chloroform.

Additive-free solution

The solvent was taken from the supply bottle, without prior treatment. No additive was applied to the solutions. AXT was dissolved almost instantaneously. The data obtained by the UV/VIS detector are shown in Fig. 6. A rapid decrease of all-trans AXT takes place in the first 5 hours of the experiment, as well as a quick formation of cis isomers. Both cis isomers reach a threshold level and then a slow degradation process of the cis isomers can be

observed. As soon as the threshold level of the cis-isomers is reached a distinct deceleration of the decrease of all-trans AXT occurs. There is still a continuing loss of all-trans AXT yet at a more moderate pace. 25 % of the initial material is condemned within the first three hours. Over the entire course of the experiment the largest loss can be attributed to the degradation of the all-trans isomer. Another suggestive hint for a degradation of the cis isomers is the reduction in the distance between the total isomer graph and the all-trans graph. If there was no degradation the gap would maintain the same size exactly like at the point with the greatest space between the lines. 50 % of the all-trans isomer is lost after 14 hours and a total of 75 % became unusable after 45 hours had elapsed. The last value measured for all-trans AXT was 0.0224 g/L.

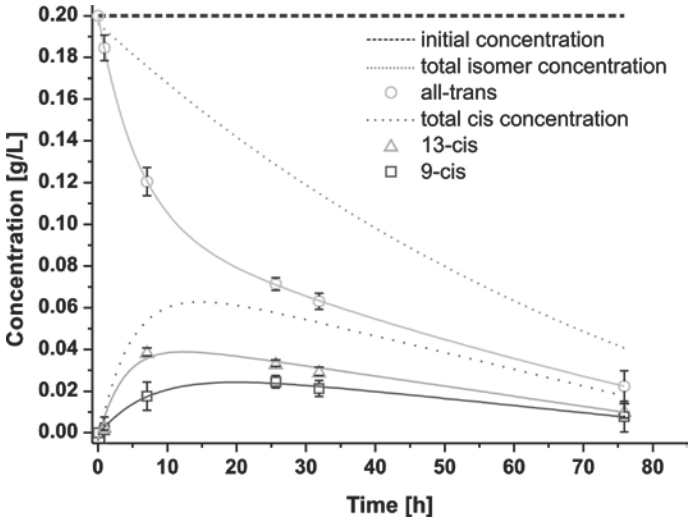

Fig. 6: Concentration changes of all-trans AXT in additive-free, pure chloroform, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

In untreated chloroform the formation of 13-cis AXT is higher than the formation of 9-cis AXT. In the present system both formation processes can be considered unhindered. Roughly 30 % of the initial substance got isomerized after approximately 10 hours. The end values of the cis isomers were 0.0106 g/L for 13-cis AXT and 0.0077 g/L for 9-cis AXT [Kre14].

Solution containing the additive K_2HPO_4

The sample solution was prepared as described in chapter 4. A mean value of 70 ppm of the additive was applied to each sample solutions. The effect of K_2HPO_4 is rather minor

compared to the data obtained from the additive-free solution. The decrease, described by the all-trans graph, is likewise dramatic in the first few hours. After about 7 hours 25 % all-trans isomer have been partially isomerized and/or degraded. K_2HPO_4 increased the stability of the all-trans isomer by a factor of 3.3 for the end value measurable. The formation rate and ratio of 9-cis and 13-cis AXT influenced by the additive differ slightly from the data obtained from the additive-free solutions. In this case the formation of 13-cis is also higher than of the 9-cis isomer. The initial amount 9-cis AXT formed is smaller than in the additive-free solution. The same applied to the 13-cis isomer formation. If there is a degradation taking place decreasing the value of the cis isomers, it is too small to be of any importance. The indication is that the formation and the presence of the cis isomers undergo a stabilization provided by the additive. This is also visible in the constant distance between the two graphs, all-trans and total isomer concentration, in Fig. 7. After approximately 24 hours 50 % of the all-trans material was condemned. At this point the formation of the cis isomers reached equilibrium and stayed at a nearly constant concentration level. By reaching this just described equalization a change of course happens to the all-trans graph. Further loss of all-trans concentration proceeds less steep compared to the first part of the graph. The end value measured was 0.0741 g/L for the all-trans isomer. The last concentration determined for 13-cis was 0.0340 g/L and 0.0172 g/L for the 9-cis isomer.

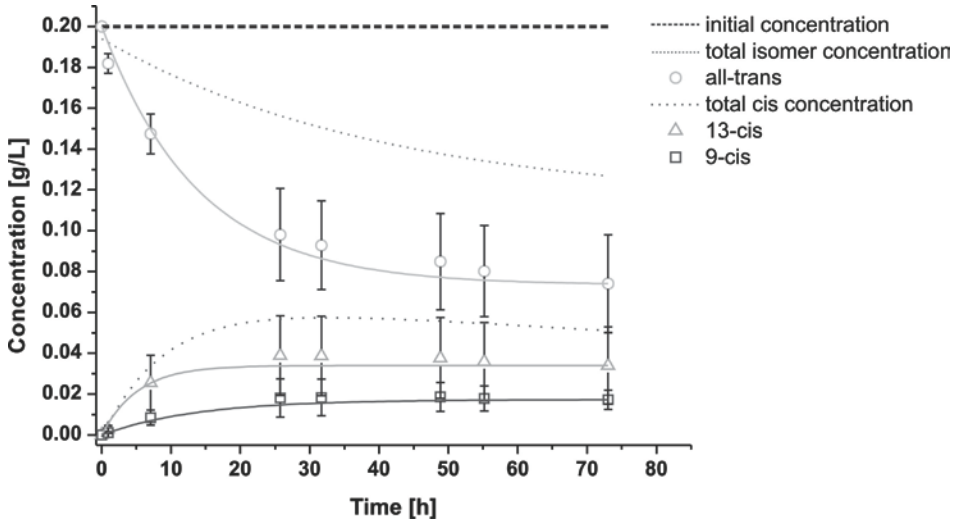

Fig. 7: Concentration changes of all-trans AXT in pure chloroform in presence of K_2HPO_4, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

The error bars are large. Nonetheless the improvement on the all-trans isomer stability appears to be insufficient for industrial application of K_2HPO_4 as stabilizing agent for all-trans AXT.

Solution containing the additive KOH

The application of KOH is a significant improvement over the additive-free solution (see Fig. 8). Each test solution contained a mean value of 567 ppm KOH. Even in the critical early stage the reduction of initial material decelerated prior to the deceleration that took place in the additive-free solution. 25 % of the initially employed all-trans isomer was lost after almost 7 hours, which is likewise to the case of K_2HPO_4 and equally an improvement to the additive-free solution. However, the destructive processes are decelerated by the additive to such an extent that a loss of 50 % of the initial substance is reached at about 65 hours, which is remarkable. The all-trans AXT concentration at the last measurement was 0.0989 g/L. Concerning the formation of cis isomers KOH appears to be nearly inhibiting the formation of 9-cis AXT. The highest measured value was 0.0024 g/L. The amount formed of 13-cis AXT increased with time, rather slow in the beginning, but steady over the entire duration. In comparison to the additive-free solution almost the same amount of 13-cis isomer is formed (0.0432 g/L), but in the case of KOH the amount is reached at a posterior point (at about 71 hours). Unlike to the untreated solution no degradation of cis isomers occurred.

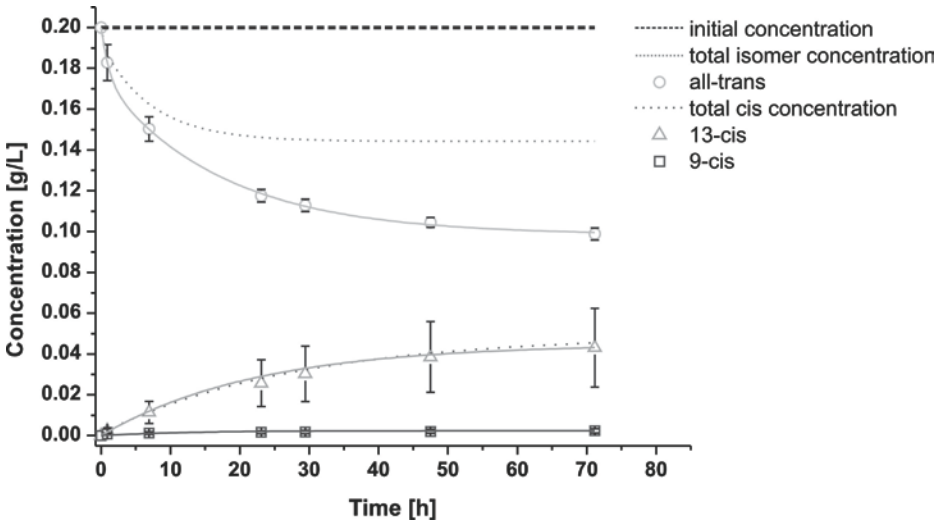

Fig. 8: Concentration changes of all-trans AXT in pure chloroform in presence of KOH, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

In fact by the end of the experiment it was obvious, that 13-cis hadn`t reached a maximum value, since the graph continued to rise. KOH is an excellent candidate for additive application considering the provided inhibition of the 9-cis AXT formation in combination with the deceleration of the formation of 13-cis AXT.

Solution containing the additive CH_3COOK

For the test solutions in this experiment a mean value of 140 ppm of additive was added. The data obtained from solutions equipped with CH_3COOK provided the information presented in Fig. 9. CH_3COOK appears to influence the isomerization towards both, 9-cis and 13-cis AXT, wherein one is hindered to a larger extent (9-cis isomer) than the other (13-cis AXT). The additive emerges to have the same effect on the formation of 9-cis AXT as does KOH. The detected content of the 9-cis isomer is almost negligible (0.0029 g/L). Furthermore, the increase of the 13-cis AXT content was decelerated from the very start and rose slowly over duration, to a point where hardly any growth was detectable anymore. In neither one of the two cases (9- and 13-cis) occur decreases of concentration. So either the cis formation reaches equilibrium and causes the graph to proceed more even towards the end or there is actually a degradation taking place, but at such a small percentage that the graph is not forced to decrease, but rather to proceed more evenly.

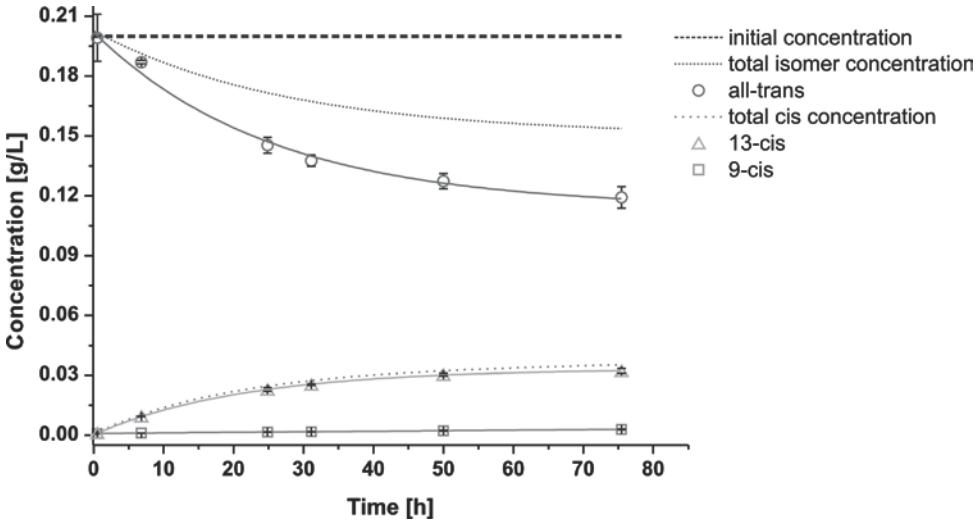

Fig. 9: Concentration changes of all-trans AXT in pure chloroform in presence of CH_3COOK, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

With regard to the concentration changes of all-trans AXT the application of CH_3COOK provided an even slightly better improvement than the application of KOH provided. A loss of 25 % initial substance took place after the elapse of 26 hours. That is a remarkable enhancement. By the end of the experiment the concentration of all-trans AXT reached a value of 0.1192 g/L which is a total loss of about 40 % ([Kre14] gives a value of 0.1192 g/L which corrected here). This data represents the best result triggered by an additive so far. The amount of all-trans AXT which was detected by the end of the experiment was 10 % higher than the amount detected in the solution to which KOH was applied.

Solution containing the additive K_2CO_3

The solutions prepared for this experiment contained a mean value of 32 ppm of K_2CO_3. The course of the graph representing the total isomer concentration is quite remarkable. After an initial drop the graph proceeds in a horizontal manner for the entire duration. The implication behind this is that despite a small amount of approximately 94 % no further all-trans AXT got degraded throughout the entire experiment. The formation of the 9-cis isomer is again negligibly minor; the consequence being that the main decrease of the all-trans molecule is due to isomerization into 13-cis (see Fig. 10).

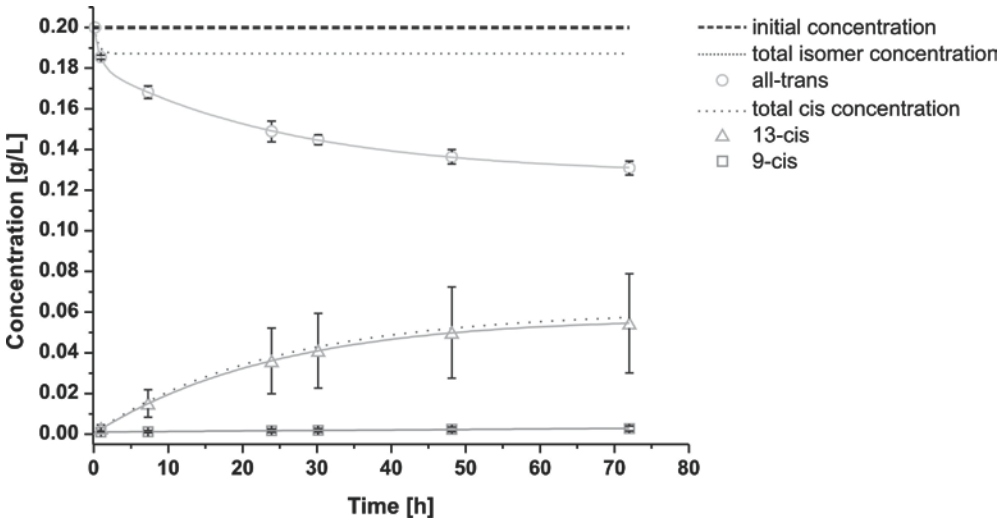

Fig. 10: Concentration changes of all-trans AXT in pure chloroform in presence of K_2CO_3, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

The two curves are almost an identical reflection of one another. In all prior experiments the degree of degradation of AXT increased throughout the entire process. None of the previously utilized additives influenced this process to a consistent level, like K_2CO_3 did in this case. Under the application of this substance the loss of 25 % starting material is reached at about 23 hours. And by the end of experiment the total loss of all-trans AXT lists approximately 35 % with a measureable concentration of 0.1310 g/L. The end value of 13-cis AXT was 0.0546 g/L and of 9-cis AXT it was 0.0028 g/L [Kre14].

5.3.1.2 HPLC results in water-saturated chloroform

The water-saturation of the solvents was performed to elucidate the effect of a high amount of water present in the solution and the consequences towards the AXT stabilization. In view of the fact that in industrial processes, whether synthesis of AXT or processing of the carotenoid, an increased proportion of water may be present in the solvent, due to recycling.

Additive-free solution

The reference data, to distinguish the additive effects, were obtained from an additive-free solution of AXT in water-saturated chloroform.

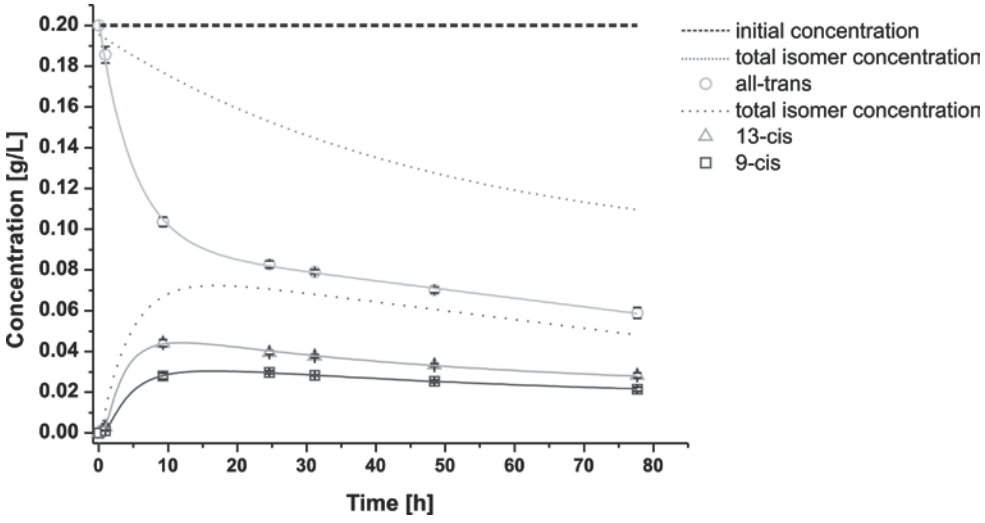

Fig. 11: Concentration changes of all-trans AXT in additive-free, water-saturated chloroform, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

The initial decrease of the all-trans graph (see Fig. 11) is immense (48 % in about 9 hours). The cis formation took place almost as rapid. Likewise to the processes occurring in pure chloroform the concentration increase of 9- and 13-cis AXT proceeds unhindered, up to a certain level (9-cis (0.0281 g/L), 13-cis (0.0442 g/L)). At this point the concentration value experiences no further increment but rather a decrease, stronger pronounced in case of 13-cis than 9-cis AXT. Therefore, in additive-free, water-saturated chloroform degradation affects not only the initial isomer (all-trans), but also the newly transformed cis isomers. A classification of the all-trans graph into two sections is just as appropriate as the corresponding classification of the 9- and 13-cis graphs. The first section is heavily influenced by isomerization processes and the second section obtained its appearance primarily from degradation processes. The isomerization section descends rapidly, suggesting a high rate with which all-trans AXT is transformed into its 9- and 13-cis isomers. The reason for the sudden change is unknown so far. A presumption is the potential achievement of an equilibrium state when a certain level of cis isomers has been formed, inhibiting an additional formation of cis isomers. This indicates that under these conditions isomerization is the limiting factor for the process itself. By the last measurement the concentration levels were determined to be 0.0216 g/L for the 9-cis isomer, 0.0284 g/L for the 13-cis isomer and 0.0590 g/L for the all-trans isomer.

In the following the degradation is more prominent and comes to the fore after taking place almost imperceptibly in the first section. This interpretation is reinforced by the steadiness and slower course of the total isomer concentration graph. Degradation took place all along but has been overshadowed by the superiority of isomerization.

Solution containing the additive K_2HPO_4

The amount of additive added to the solutions in this experiment was a mean value of 78 ppm. In comparison with other additives K_2HPO_4 is lacking a sufficient feature of suppressing the formation of 9-cis AXT under the set conditions. However, K_2HPO_4 appears to diminish both cis formations to a certain extent. The velocity of the 9-cis isomer formation was faster in the first half of the experiment, which also applies to the velocity of the 13-cis isomer formation. By the end of the first half, at about 35 hours, both cis isomers reached a content, which probably corresponds to an equilibrium state, under the given conditions. In the second half both courses of the cis graphs are characterized by their constant content. The final values were 0.0149 g/L of 9-cis and 0.0248 g/L of 13-cis AXT. By the end of the

experiment approximately 20 % of the initially deployed all-trans AXT was transformed to either 9-cis or 13-cis AXT. Fig. 12 shows the progression of the decrease of the all-trans isomer. For a considerable amount of time the concentration decreased at a relatively high speed, due to the occurrence of both, isomerization and degradation processes. However, according to the obtained data the content barely changed anymore over the last 20 hours.

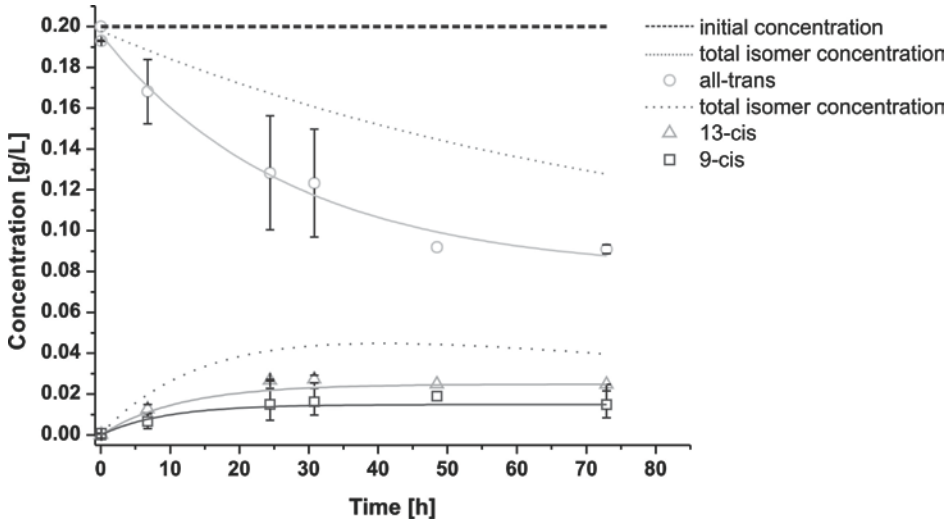

Fig. 12: Concentration changes of all-trans AXT in water-saturated chloroform in presence of K_2HPO_4, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

The final concentration measured for all-trans AXT was 0.0910 g/L. The loss of material due to degradation is nearly linear over the entire period, as visualized by the graph of the total isomer concentration. More than 50 % of the initial amount was condemned by the end of the experiment, 20 % due to isomerization and roughly 35 % due to degradation.

Solution containing the additive KOH

In this experiment (see Fig. 13) occurred massive disturbances, causing significant deviations. Closest to reality are probably only the last measured values of the respective isomers (all-trans AXT (0.1148 g/L), 9-cis AXT (0.0029 g/L), 13-cis AXT (0.0302 g/L)). Intrinsically there occurred no obvious abnormalities or malfunctions during the execution of the measurements for this experiment. Also based on the unobtrusive photometer data (see Fig. 33) it was not apparent that such a deviation occurred for the all-trans AXT values detected by HPLC. This leads to the conclusion that the reason was either a

pipetting/handling error which remained unnoticed in the process of sample preparation or a detection problem that occurred during the HPLC measurement. Due to consideration of physical plausibility the first three actual measurements of the all-trans concentration and the first three calculated dots for the total isomer concentration are not included into the creation of the fits. In this case the dots indicating the total isomer concentration are illustrated by curved brackets. And the data points showing the all-trans AXT values are encompassed in square brackets. A mean value of 93 ppm KOH was added to the test solutions. In the context of all experiments performed in water-saturated TCM only the end values may be considered as usable. Hence, all other values have been discarded.

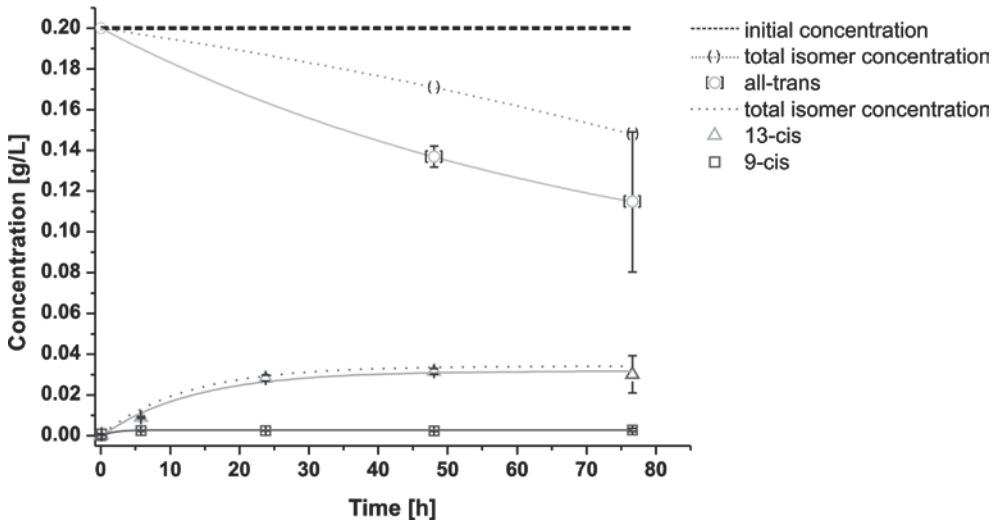

Fig. 13: Concentration changes of all-trans AXT in water-saturated chloroform in presence of KOH, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved. Not all points of the total isomer concentration and the all-trans concentration are included into the respective fit. The dots indicating the total isomer concentration are illustrated by curved brackets. The data points showing the all-trans AXT values are encompassed in square brackets.

Solution containing the additive CH_3COOK

With solutions containing CH_3COOK the data shown in Fig. 14 was generated. About 145 ppm of additive was added to the test solutions at the beginning of the experiment. The 13-cis formation took place at a reasonable pace until a certain concentration level was approached in the liquid stage. The closer the content got towards that level the slower the rate of further 13-cis formation. The final amount was measured to be 0.0332 g/L.

Throughout the entire experiment the formed 9-cis content didn`t exceed 0.0029 g/L. In water-saturated TCM CH_3COOK provided an almost complete inhibition on the 9-cis formation and a diminution on the 13-cis formation. The highest value of the total cis isomer concentration was 0.0361 g/L. This is conform to 18 % of initial substance being transformed into the 13-cis isomer. The all-trans AXT concentration decreased more quickly in the first segment due to the isomerization of a larger share of all-trans AXT into cis isomers. The decrease was also due to degradation. In the second segment, starting from approximately 40 hours, the course of the curve changed to a slower pace. This deceleration is the result of the retarded formation of the cis isomers.

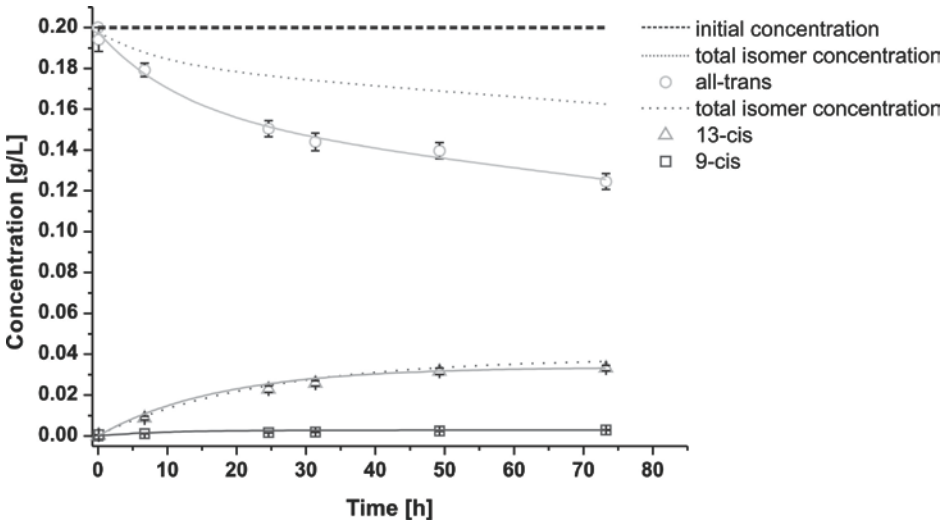

Fig. 14: Concentration changes of all-trans AXT in water-saturated chloroform in presence of CH_3COOK, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

Starting from approximately 30 hours also the degradation processes were diminished in their extent. After the termination of the experiment a total all-trans isomer content of 0.1245 g/L was still measurable. By the end of the experiment approximately 20 % of the initial material was transformed into cis isomers and 62 % remained in the all-trans form. The remaining portion was degraded.

Solution containing the additive K_2CO_3

Fig. 15 shows data obtained from experimental runs in which AXT was dissolved in water-saturated chloroform and all occurring processes were influenced by the addition of K_2CO_3.

In this case a mean value of 23 ppm of the additive was added to each of the test solutions. The 9-cis isomer was hindered in its formation. The end value was determined to be 0.0034 g/L. The concentration of 13-cis AXT increased slowly with time and was still rising by the end of the experiment. By this time the content of the 13-cis isomer was 0.0529 g/L. Neither one of the cis isomers reached a visibly observable maximum concentration (graphs continue to rise). The course of the graph of the cis isomer concentration is a close reflection of the trend of the all-trans concentration change. The isomerization is hindered to a certain extent but not inhibited throughout the entire time frame. The high water content of the used chloroform in this case appears to have a minor impact on the degradation.

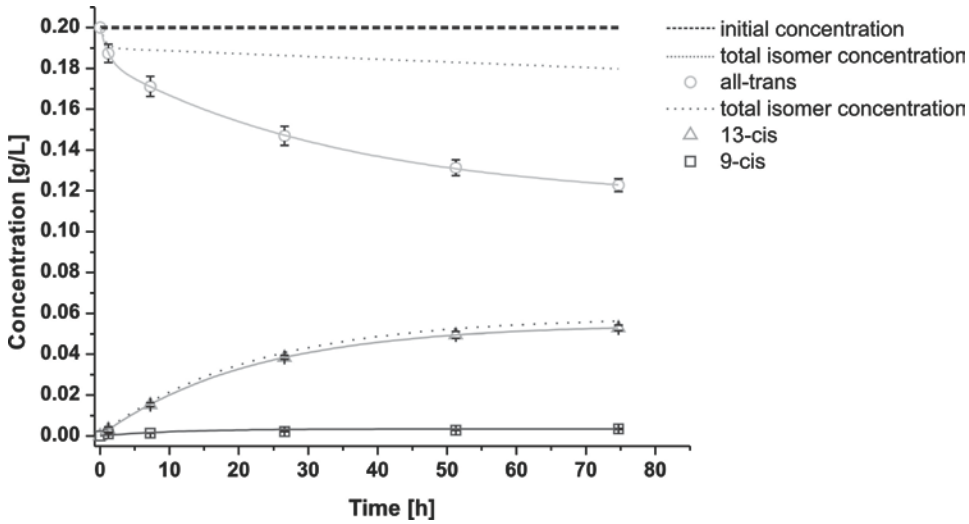

Fig. 15: Concentration changes of all-trans AXT in water-saturated chloroform in presence of K_2CO_3, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

The degradation process is not blocked completely like it had been in the case of pure chloroform equipped with K_2CO_3. The graph, showing the total isomer concentration, decreases slightly towards the end. By the elapse of about 24 hours the content of initial isomer was reduced to 25 %. The end content of all-trans AXT was determined to be 0.1228 g/L, which is equivalent to 61 % of the initially applied concentration.

5.3.1.3 HPLC results in pure methylene chloride

Additive-free solution

The sample solution was prepared with pure methylene chloride fresh from the supply bottle. A malfunction appeared at the end of the measurements. The data were only recorded for a period of about 48 hours. The remaining data show all important processes in Fig. 16. For instance, the decrease of all-trans AXT proceeds much slower than in pure, additive-free chloroform. At this point it should be mentioned once again that the temperature for the experiments in methylene chloride was set to 30 °C instead of 40 °C, which was applied in all experiments employing chloroform. This temperature alteration for methylene chloride was necessary to ensure more accurate sample taking. A lower temperature influences both isomerization and degradation of the starting material, resulting in more moderate concentration changes. 25 % of all-trans AXT were isomerized/degraded by the elapse of roughly 7 hours. 40 hours later 50 % became undetectable. The end value of the all-trans isomer measured at 48 hours was 0.0944 g/L. The formation of both cis isomers occurred to more equal proportions than in any other case, where chloroform was used as solvent. It can be presumed that in additive-free methylene chloride the isomerization process towards 9-cis formation is higher, caused by the chemical methylene chloride.

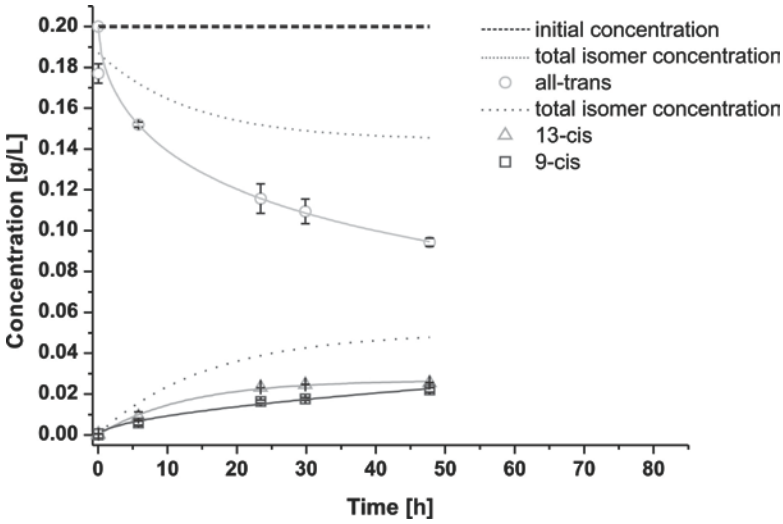

Fig. 16: Concentration changes of all-trans AXT in additive-free, pure methylene chloride, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

Further investigation is needed on whether the formation of 13-cis is unaffected or slightly hindered by the applied solvent. While the 9-cis concentration increases further towards the end, the 13-cis concentration appears to have reached a nearly stagnant state. If further data was available it might show an intersection of the two cis isomer graphs, but at this point this is merely a speculation. Another possibility would be a behavior likewise to both additive-free experiments performed in chloroform, where a maximum is reached and subsequently further isomerization might be inhibited. This would result in two-section graphs for all curves except the total isomer concentration, which would continue to diminish.

Solution containing the additive K_2HPO_4

A mean value of 132 ppm K_2HPO_4 was added to the test solutions. In this experiment (see Fig. 17) occurred relevant disturbances, causing significant deviations. Intrinsically there occurred no obvious abnormalities or malfunctions during the execution of the measurements for this experiment. Also based on the unobtrusive photometer data (see Fig. 37) it was not apparent that such a deviation occurred for the all-trans AXT values detected by HPLC.

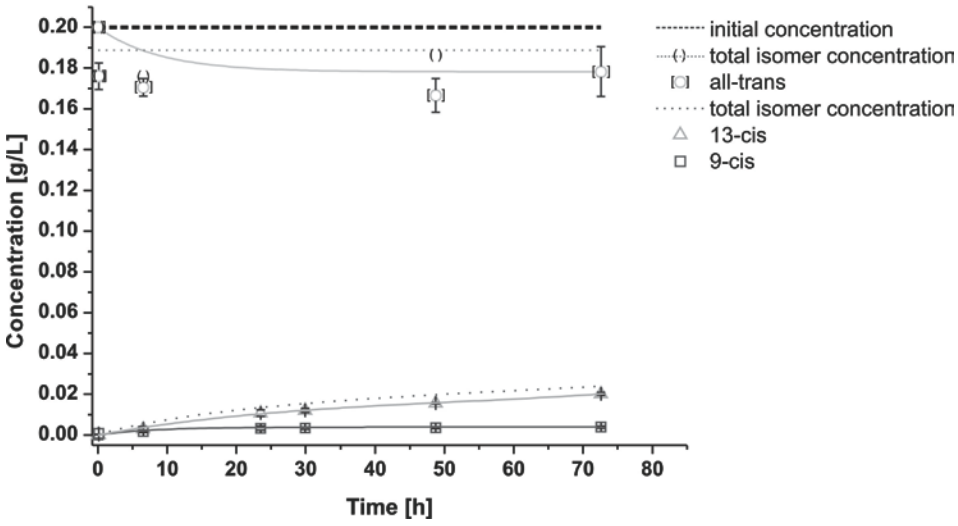

Fig. 17: Concentration changes of all-trans AXT in pure methylene chloride in presence of K_2HPO_4, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved. Not all points of the total isomer concentration and the all-trans concentration are included into the respective fit. The dots indicating the total isomer concentration are illustrated by curved brackets. The data points showing the all-trans AXT values are encompassed in square brackets.

This leads to the conclusion that the reason was either a pipetting/handling error which remained unnoticed in the process of sample preparation or a detection problem that occurred during the HPLC measurement. Closest to reality are probably only the first measured values of the respective isomers. In the context of all experiments performed in pure DCM only the first measured values may be considered as possibly usable, but nonetheless inconclusive, since the course of the data as a whole can`t be ranged in with the other results. Hence, all values have been discarded. For the creation of the fit of the total isomer concentration two of the calculated values were excluded, due to consideration of physical plausibility. Those two values are the ones located above the initial concentration of 0.2 g/L. In this case the dots indicating the total isomer concentration have been assigned brackets to illustrate their deviation more clearly. The same applies to the creation of the fit for the all-trans concentration points, the data located above the initial concentration is excluded. And the data points showing the all-trans AXT values are encompassed in square brackets.

Solution containing the additive KOH

The transformation towards 9-cis, shown in Fig. 18, is hindered, when KOH is present in solution. Approximately 2640 ppm (one pellet) of the additive was brought into the solution of methylene chloride and all-trans AXT.

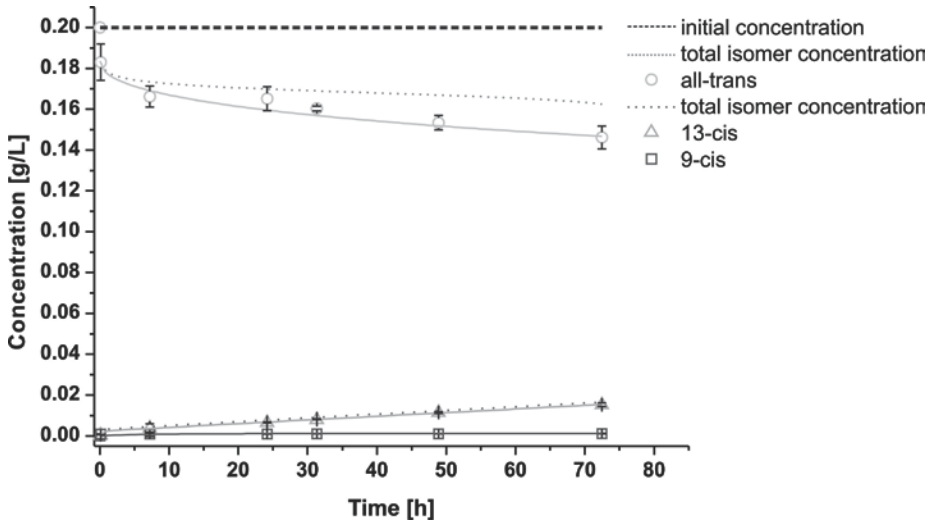

Fig. 18: Concentration changes of all-trans AXT in pure methylene chloride in presence of KOH, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

The highest measured content of 9-cis AXT is 0.0011 g/L. The corresponding graph proceeds in a horizontal line. The formation of 13-cis AXT rose linearly. The increase of the isomer was restricted by the additive contained in the solution. The value of 13-cis AXT formed in this experiment amounted to 0.0153 g/L. In total an amount of about 8 % of the starting material isomerized. The end value of all-trans AXT (0.1462 g/L) is equivalent to 73 % of the initial substance. Accordingly the total amount of degraded substance is equivalent to 0.0374 g/L. A loss of more than 25 % all-trans isomer was only slightly exceeded by the time the last measurement was taken.

Solution containing the additive CH_3COOK

By the application of CH_3COOK (approximately 267 ppm per test solution) the extent of isomer formation, shown in Fig. 19, was narrowed and mostly composed of 13-cis AXT.

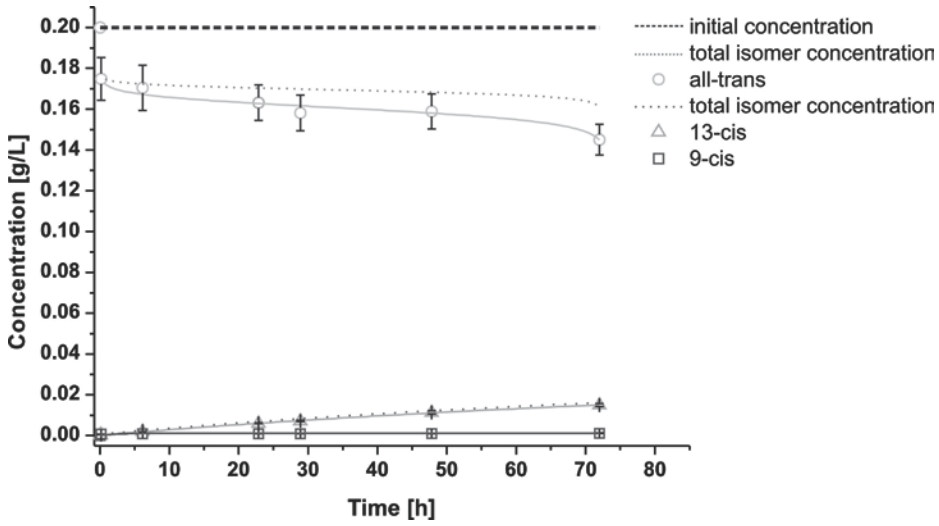

Fig. 19: Concentration changes of all-trans AXT in pure methylene chloride in presence of CH_3COOK, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

After 72 hours an amount of 0.0150 g/L 13-cis was measured. Merely traces of the 9-cis isomer were formed (0.0011 g/L). A share of approximately 8 % of the initial substance has been altered by isomerization processes. By the end of the experiment the amount of all-trans AXT had decreased to 0.1450 g/L. According to the diagram the material loss caused by degradation was approximately 19 %.

Solution containing the additive K_2CO_3

The obtained results are shown in Fig. 20. Any transformation of initial substance towards the 9-cis isomer was largely hindered by utilization of K_2CO_3. An amount of about 180 ppm was applied to each test solution. The presence of the additive also led to a slightly diminished formation of 13-cis AXT. The highest content measured of 9-cis AXT was 0.0013 g/L and 0.0187 g/L of 13-cis AXT. By the end of the experiment the total cis concentration was 10 % of the initial all-trans AXT concentration. The deviations which occurred in the measurements of the all-trans isomer also affected the total isomer concentration.

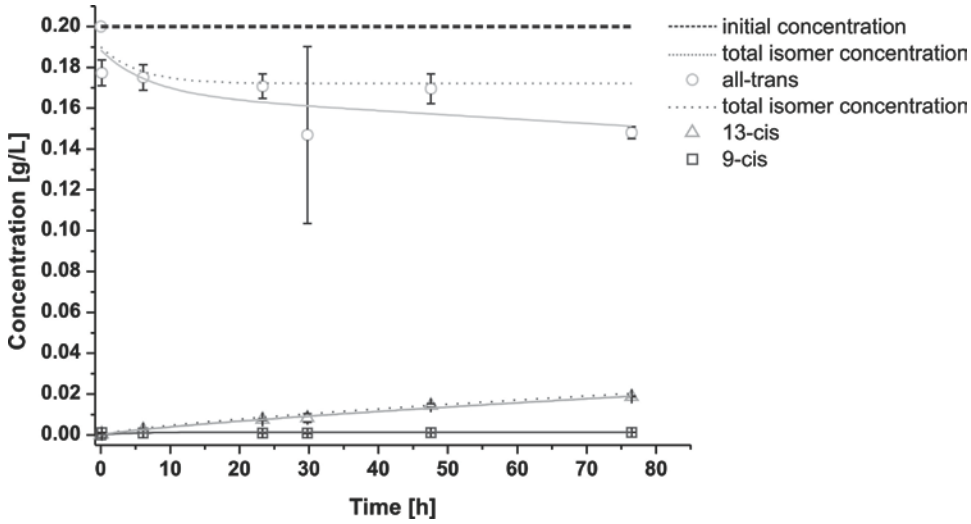

Fig. 20: Concentration changes of all-trans AXT in pure methylene chloride in presence of K_2CO_3, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

According to the trend line of the all-trans decrease a loss of 25 % all-trans AXT isn`t reached. However, with the last measurement a concentration of 0.1481 g/L was found which is conform with a decrease of 26 % of the initial substance.

5.3.1.4 HPLC results in water-saturated methylene chloride

Additive-free solution

Measurements taken in water-saturated methylene chloride, without any additive application, provided the data shown in Fig. 21. As suspected there was a visible increase of the 9-cis AXT concentration, since there was no additive interaction throughout the duration of the

experiment. The concentration level of both cis isomers proceeded closely in the beginning and again towards the end. During the middle section more 13-cis AXT was formed. Both graphs nearly converge in the end. The 9-cis graph was still increasing with time when the experiment was stopped. The posterior two measurements, concerning the 13-cis isomer, showed hardly any concentration difference. By the end the 13-cis concentration was 0.0257 g/L and the value of 9-cis AXT reached 0.0245 g/L. The last measurement revealed a total cis isomer formation of 25 %. More than 50 % of the initial amount of all-trans AXT has been isomerized and degraded after approximately 75 hours. According to the obtained data 0.1021 g/L all-trans AXT was lost by that time. The last measurement revealed a remaining all-trans AXT concentration of 0.0980 g/L.

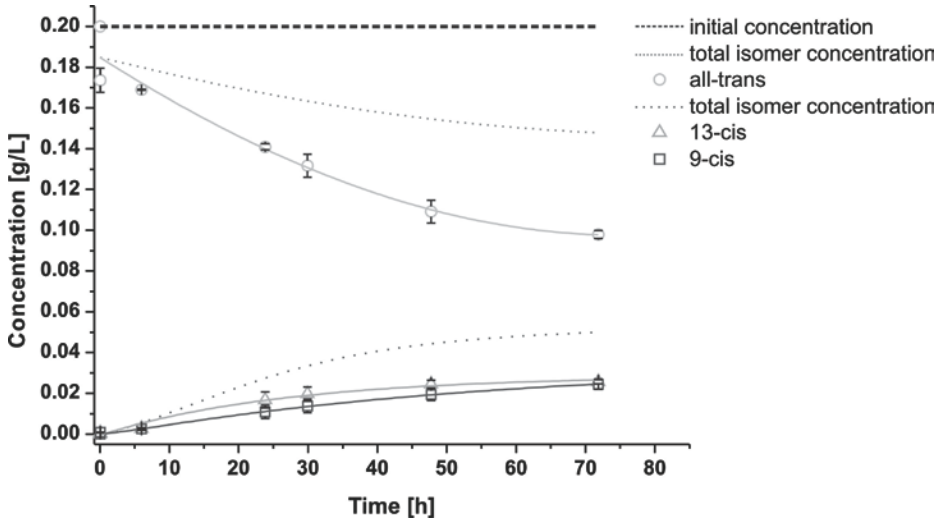

Fig. 21: Concentration changes of all-trans AXT in additive-free, water-saturated methylene chloride, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

Other than the initial decrease, the subsequent part of the all-trans graph descended slowly in the first 6 hours and then more steeply in the middle part until the descend slowed again towards the end. There is a mild consistency in the data sequence of the 13-cis isomer.

Solution containing the additive K_2HPO_4

The additive amount applied to each test solution was a mean value of 49 ppm. The data in this trial, where K_2HPO_4 was used to influence the stability of all-trans AXT in water-saturated methylene chloride, took a surprising twist. So far K_2HPO_4 was considered to be less

effective in stabilizing the all-trans isomer (see Fig. 22). It is remarkable that in this case no intense decrease occurred in the very beginning, as it did in all other cases (even with those additives, which so far proved to be more effective in stabilizing all-trans AXT). The 9-cis formation is hindered by the presence of K_2HPO_4 in solution. The increase of the 13-cis concentration proceeds slowly and steady in a linear way. The end values of both cis isomers are 0.0177 g/L for 13-cis and 0.0016 g/L for 9-cis AXT. The added up result is a value of about 10 %. The diminution of all-trans AXT proceeds quite slowly for a considerable amount of time. A slightly enhanced decrease occurred in the timeframe from 30 to 48 hours.

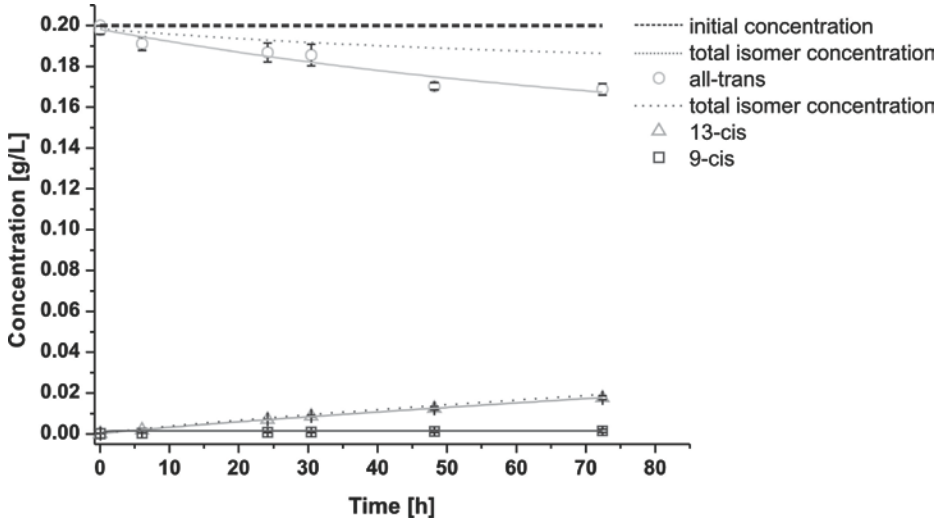

Fig. 22: Concentration changes of all-trans AXT in water-saturated methylene chloride in presence of K_2HPO_4, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

The posterior segment displayed hardly any further concentration change, regarding the data points. The all-trans content measured last was 0.1688 g/L which is about 84 % of the initial value. The “degradation area” between the total isomer concentration and the initial concentration increased slowly, as did the isomerization area. The individual data points are slightly different from the course of the respective trend line. The amount of substance lost due to degradation is smaller than the value of isomerized material (degraded material: 6%; isomerized material: 10 %).

Solution containing the additive KOH

For the investigation approximately 105 ppm of KOH was added to the respective test solutions. An initial decrease of the all-trans AXT concentration occurred. The results are plotted in Fig. 23. There was a hindrance towards the formation of the 9-cis isomer but less effective than in prior cases, e.g. 5.3.1.1 (K_2CO_3 and CH_3COOK in pure TCM) and 5.3.1.3. (K_2CO_3 and KOH in pure DCM). The yield of the 9-cis isomer turned out to be 0.0034 g/L by the end of the experiment. The slowly increasing level of 13-cis AXT was smaller than in prior cases where KOH was utilized, for instance in 5.3.1.1 (pure TCM) and 5.3.1.3 (pure DCM). The final concentration of 13-cis AXT was 0.0141 g/L. The second half of the curve of the total isomer concentration proceeded nearly horizontal. The implication is that no further degradation of any of the present isomers takes place in that time frame. In contrast the all-trans curve descended continuously to a final yield of 0.1579 g/L. The reduction of all-trans AXT came up to 21 % of the initial concentration.

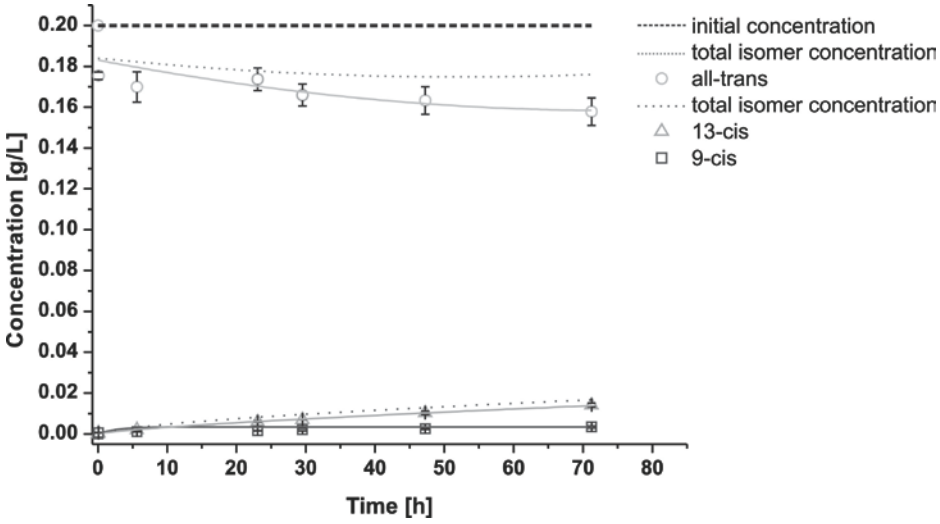

Fig. 23: Concentration changes of all-trans AXT in water-saturated methylene chloride in presence of KOH, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

Solution containing the additive CH_3COOK

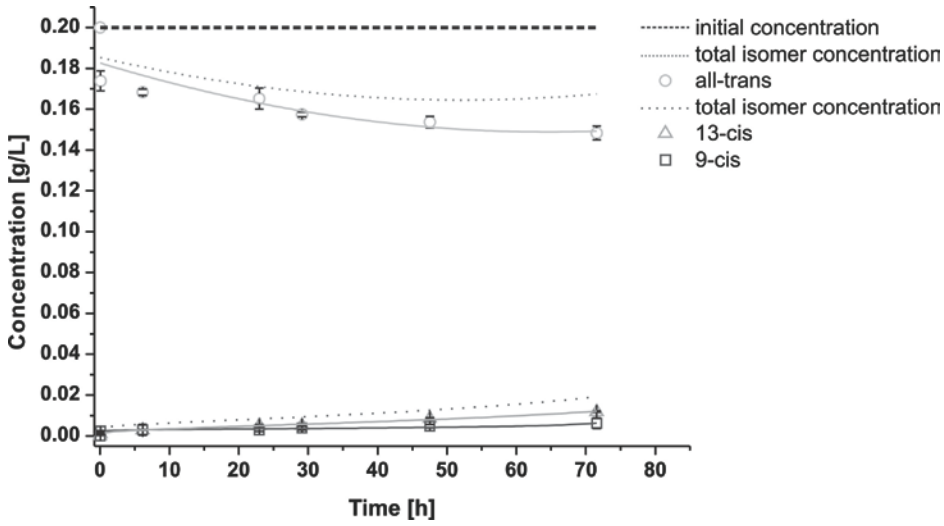

Fig. 24: Concentration changes of all-trans AXT in water-saturated methylene chloride in presence of CH_3COOK, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

Both cis isomer formations were influenced by the applied additive CH_3COOK (see Fig. 24). The additive amount utilized in this investigation was approximately 93 ppm for each respective test solution. A yield of 0.0062 g/L was reached for 9-cis AXT and the final concentration of 13-cis AXT was 0.0119 g/L. Approximately 9 % of the initial substance were isomerized in the course of the experiment. The concentration of the all-trans isomer experienced a total reduction of about 26 % to a concentration of 0.1484 g/L. The more extensive part (about 17 %) of the main isomer was consequently degraded. Considering the initial drop of the all-trans concentration is caused by deviations due to different measuring techniques and normalization of the all-trans data, then the degradation of all-trans AXT would be just a fraction amount of the value provided by the diagram.

Solution containing the additive K_2CO_3

The all-trans AXT graph started out with an initial decrease, implying an abrupt diminution of concentration. The total isomer concentration descended with a smaller negative slope than the all-trans graph.

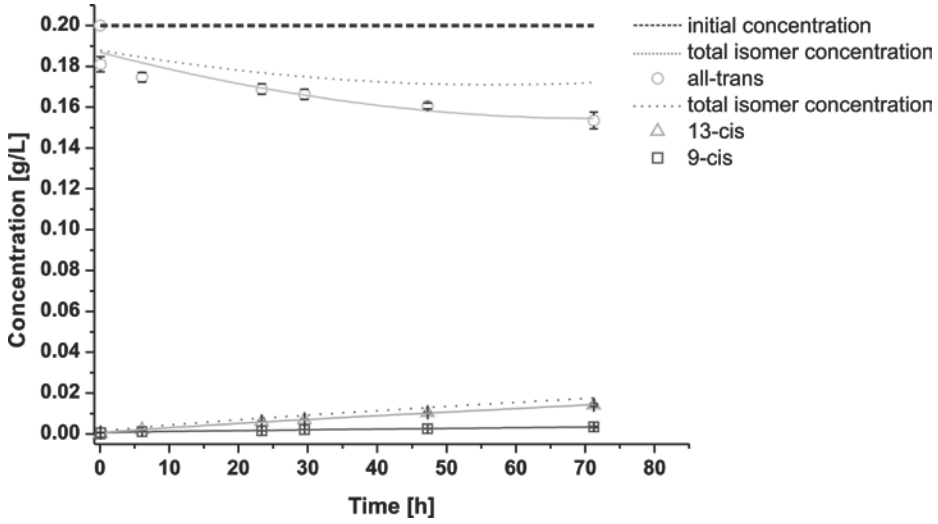

Fig. 25: Concentration changes of all-trans AXT in water-saturated methylene chloride in presence of K_2CO_3, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid lines represent actual measured values, dotted lines represent calculated values of the actual measurements to assess the extent of processes (isomerization, degradation) involved.

Both processes (degradation and isomerization) influenced the concentration diminution of all-trans AXT, but only degradation caused the reduction shown by the total isomer concentration graph. By the end of the experiment the all-trans concentration was reduced to 0.1536 g/L. Almost 77 % of the initial material remained intact. In this investigation the applied amount of additive valued 78 ppm in each respective test solution. The data presented in Fig. 25 shows that in a solution, consisting of water-saturated methylene chloride and AXT, to which the additive K_2CO_3 was applied the formation of both cis isomers, is hindered to a certain extent. The increase in both graphs was linear. More 13-cis than 9-cis AXT was formed. The final values were 0.0034 g/L of 9-cis isomer and 0.0141 g/L 13-cis isomer.

5.3.2 Measurements by UV/VIS photometer

The solution measured by photometer was the same as the solution measured by HPLC. As mentioned before it was assumed that the all-trans AXT was the only present AXT isomer in solution at the time zero. According to that there were 100% all-trans AXT present and 0% cis isomers. In the course of time isomerization occurred and cis isomers were formed. As soon as a mixture of AXT isomers was present in solution the determination of the absorption of only one isomer, insignificant whether a cis or the trans isomer, was no longer possible. Yuan and Chen [Yua99] published absorption spectra of the three most commonly occurring

AXT isomers. The absorption maximum of each isomer was in close proximity to the others. The superposition of the absorption spectra did not permit a concentration determination. The data obtained could only serve as an indication as to what was happening in the sample solution in the course of time. The concentration calculated from the photometer data represents a somehow combined concentration of the AXT isomers present in solution. The absorption spectra of the isomers overlay and so are the calculable concentrations. However, the overlay is not resulting in an equally intensified signal. Therefore a exact content couldn`t be determined.

5.3.2.1 UV/VIS photometer results in pure chloroform

The measurements were taken at a wavelength of 478 nm.

Additive-free solution

The untreated solution showed a fast decrease of the concentration which was presumed to be total AXT and not a specific isomer. The concentration values have been normalized to a starting value of 0.2 g/L. The solutions were prepared by mixing all-trans AXT powder into pure chloroform. No additive was applied. All isomerization and degradation processes took place unhindered.

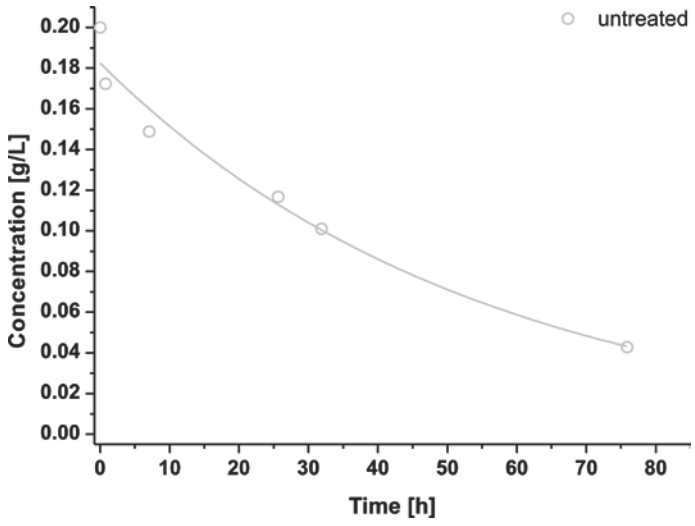

Fig. 26: Concentration changes of AXT in additive-free, pure chloroform, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

The rapid decrease of concentration between the first two data points was a result of different measurement techniques that have been used to obtain the data and additionally manual pipetting. In accordance with the HPLC data also the data obtained from the UV/VIS photometer showed that the concentration of AXT diminished, when dissolved in pure chloroform. After 7 hours the initial concentration was decreased by 25 %. 30 hours after the initiation only 50 % of the material was lost. After 75 hours the experiment was terminated and the end value calculated from the measured data was 0.0429 g/L for AXT. The information obtained from the experiment is shown in Fig. 26.

Solution containing the additive K_2HPO_4

The photometer data demonstrate that the utilization of K_2HPO_4 increased the stability of AXT in the solution. There was an initial steep decrease between the first and second data point. The subsequent data points demonstrated a decrease with a velocity slightly too slow for the graph to run linear. A hindrance towards isomerization and degradation of AXT was clearly readable from the slope. A loss of 25 % was reached after 3 hours. The final concentration amounted to 0.1093 g/L which represents 55 % of the initial material. According to the data shown in Fig. 27 less than 50 % of the initial amount of AXT got either isomerized and/or degraded.

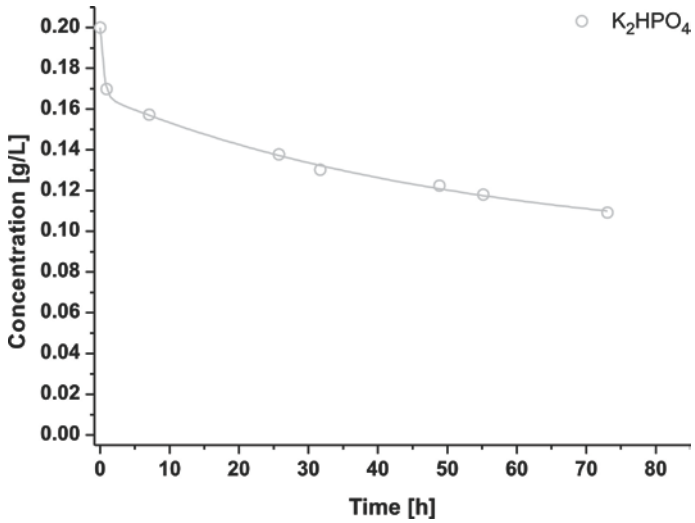

Fig. 27: Concentration changes of AXT in pure chloroform in presence of K_2HPO_4, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

Solution containing the additive KOH

In Fig. 28 the measurement results are shown, obtained from the solutions of pure chloroform and AXT which was supplemented with KOH. After the initial concentration decrease the presence of KOH appeared to have a stabilizing influence on the system, which led closely towards an equilibrium state, concerning the posterior three data points.

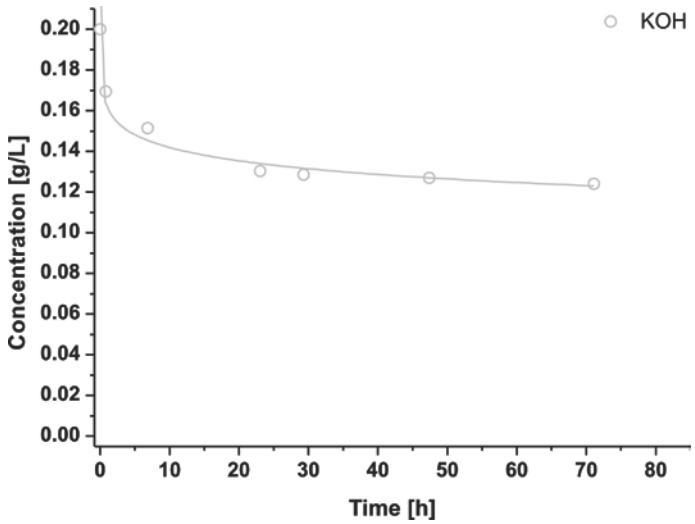

Fig. 28: Concentration changes of AXT in pure chloroform in presence of KOH, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

The suitability of the additive KOH, for the chosen system and the applied conditions, is represented by the inclination of the graph. At about 7 hours 25 % of the initial concentration was lost, though throughout the entire duration the concentration level never sinks below 0.1240 g/L. This means 62 % of photometrically measureable AXT isomers stayed intact.

Solution containing the additive CH_3COOK

The decrease of the concentration (see Fig. 29) was affected and clearly slowed due to the presence of CH_3COOK, but no equilibrium state was reached in this case. After the initial steep decrease the course of the curve was nearly linear, which means the rate with which the concentration decreased was virtually constant. However, it took about 25 hours to decrease the measurable concentration by 25 %. The end concentration was 0.1334 g/L AXT.

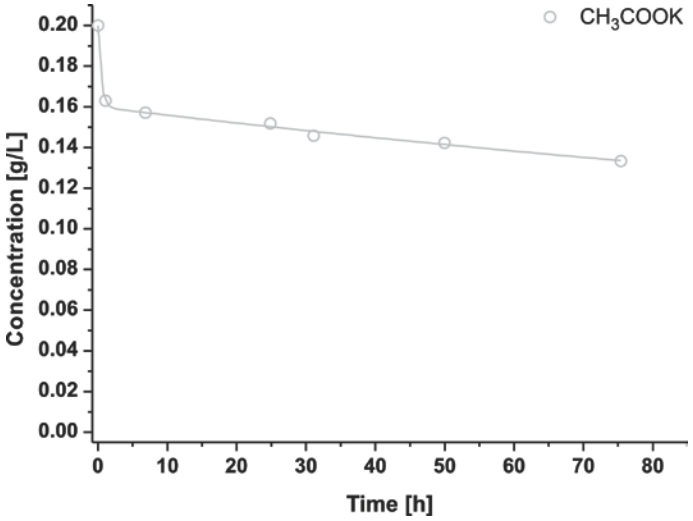

Fig. 29: Concentration changes of AXT in pure chloroform in presence of CH_3COOK, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

Solution containing the additive K_2CO_3

K_2CO_3 was remarkable also, considering the UV/VIS photometer data shown in Fig. 30. After the initial decrease the concentration change could only be described as minimal.

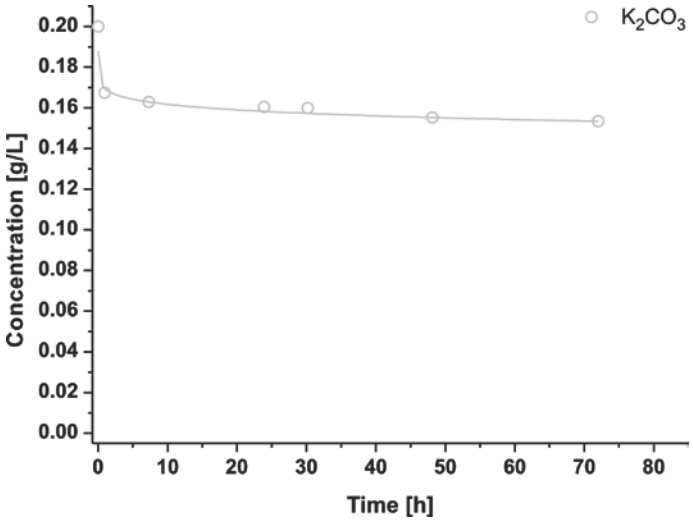

Fig. 30: Concentration changes of AXT in pure chloroform in presence of K_2CO_3, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

The achievement of an equilibrium state appeared to be very close. The suitability of K_2CO_3 to stabilize AXT in pure chloroform was clearly stated by the obtained data. By the end of the

experiment the entire loss didn´t reach the 25 % mark. The experiment was terminated at an AXT concentration of 0.1535 g/L.

5.3.2.2 UV/VIS photometer results in water-saturated chloroform

Additive-free solution

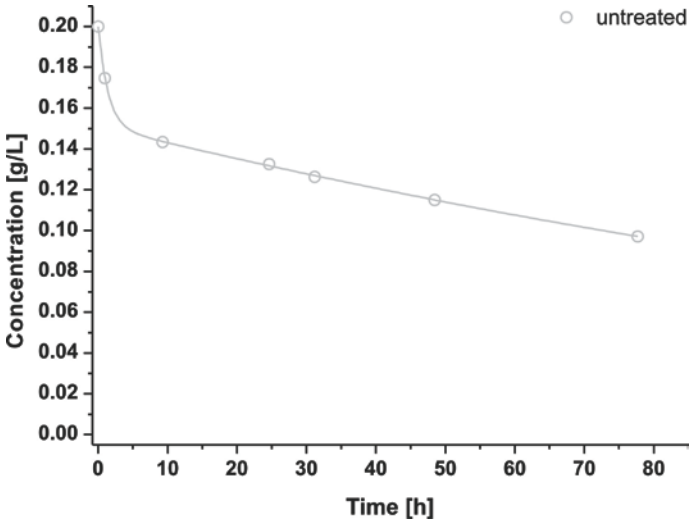

Fig. 31: Concentration changes of AXT in additive-free, water-saturated chloroform, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

There were two sections described by the graph in Fig. 31. The first part extends a little further than the distance from the first to the second data point, which was basically the initial concentration decrease. Starting from about 9 hours the second part extends to the end. The first part of the curve had higher errors due to different measuring techniques. In the second part the data points ran closely linear (with a deviation from linearity of 0.07 % - 1.38 %). In the untreated solution the concentration decreased by 25 % at approximately 8 hours. At about 77 hours the last measurement was taken (0.0972 g/L AXT) and revealed a total loss of about 51 % over the time.

Solution containing the additive K_2HPO_4

The application of K_2HPO_4 in water-saturated chloroform led to the data plotted in Fig. 32. Throughout the experiment the formation of an equilibrium state didn`t manifest itself. The concentration decrease proceeded quite steady. The system experienced 25 % loss at about 17 hours after the initiation. By the usage of K_2HPO_4 the concentration loss could be

diminished, however the continued decrease of AXT showed that K_2HPO_4 is not a suitable additive for industrial application. The percentage of loss didn`t exceed 64 %. The last measurement yielded to 0.1279 g/L.

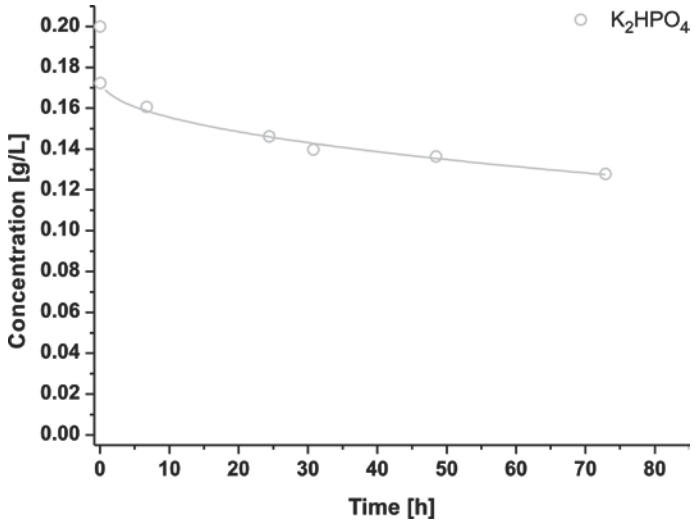

Fig. 32: Concentration changes of AXT in water-saturated chloroform in presence of K_2HPO_4, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

Solution containing the additive KOH

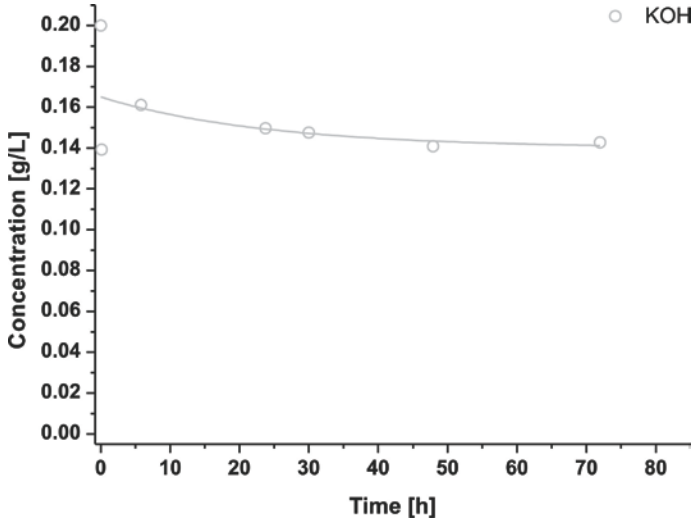

Fig. 33: Concentration changes of AXT in water-saturated chloroform in presence of KOH, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

The presence of KOH led closely toward an equilibrium, after roughly 30 hours elapsed.

But despite the formation of the equilibrium state a total loss of about 29 % couldn`t be prohibited by the application of KOH. Immediately after the initial steep concentration decrease (from 0.2 g/L to 0.1393 g/L), the concentration rose to 0.1612 g/L AXT. This type of event is highly implausible and was only observed in this particular case (see Fig. 33). During the preparation of the first sample a measuring error of some sort must have occurred unnoticed. The last measurement yielded to 0.1429 g/L.

Solution containing the additive CH_3COOK

In the experiment in which CH_3COOK was used as an additive, an extensive loss of concentration was measured.

It took only 7 hours to decrease the concentration by 25 % and 32 hours to reach a loss of 50 % of AXT. By the end of the experimental trial merely a concentration value of 0.0429 g/L was measureable, which was conform with a total loss of almost 80 % (see Fig. 34).

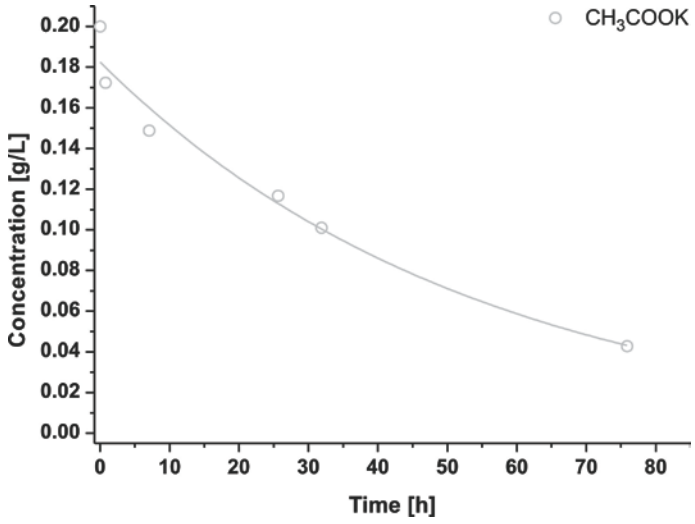

Fig. 34: Concentration changes of AXT in water-saturated chloroform in presence of CH_3COOK, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

Solution containing the additive K_2CO_3

After the initial decrease of concentration the further occurring loss of AXT was rather small in this experiment (see Fig. 35). All in all the amount of starting material decreased by approximately 26 % over the entire duration.

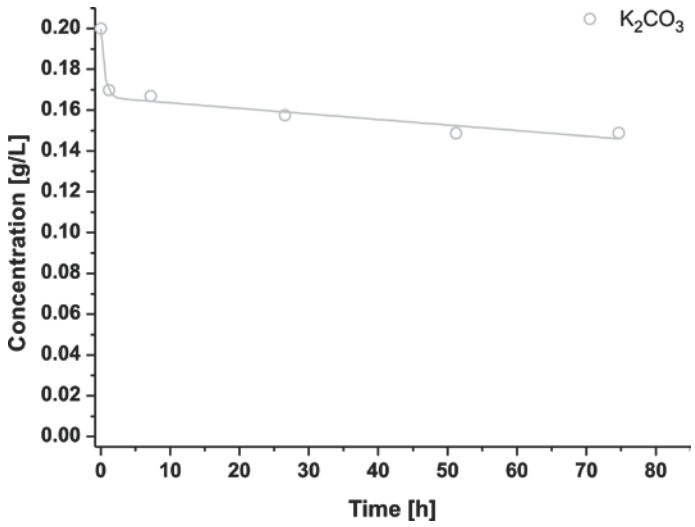

Fig. 35: Concentration changes of AXT in water-saturated chloroform in presence of K_2CO_3, at 40 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

The concentration difference between the first photometrical measurement at about one hour and the last one at 72 hours amounted to 0.0211 g/L. This concentration matches about 11 % of the initially added substance. The total loss yielded to 0.0512 g/L.

5.3.2.3 UV/VIS photometer results in pure methylene chloride

Additive-free solution

In pure methylene chloride, and with no additive application, the photometrical measurements revealed a remarkable course of events, for this particular experiment (see Fig. 36). The system reached an equilibrium state, quite promptly, all by itself. Although, almost 25 % of the initially applied substance disintegrated, according to the first two data points, the subsequent decrease turned out to be substantially minor. The highest concentration difference among the data points after the initial drop amounted to 0.0226 g/L, which equals 11.3 % of the initially added amount of AXT. The lowest concentration measured was 0.1312 g/L AXT.

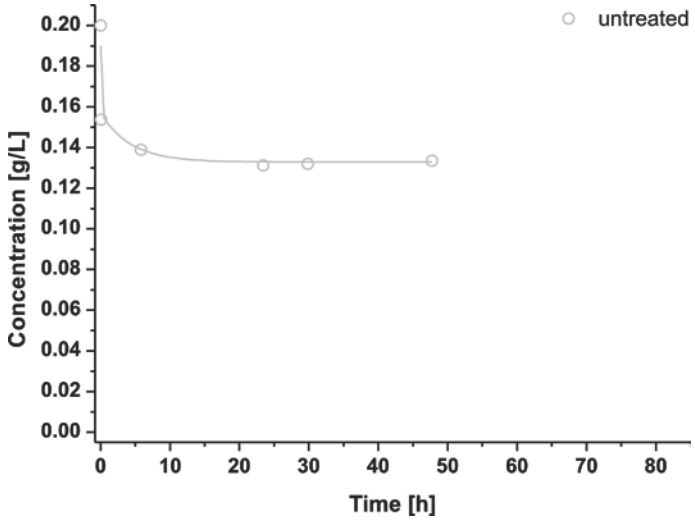

Fig. 36: Concentration changes of AXT in additive-free, pure methylene chloride, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

Solution containing the additive K_2HPO_4

By application of K_2HPO_4 to the solution of pure methylene chloride and all-trans AXT the data shown in Fig. 37 was obtained. Between the first two data points exists a concentration difference of 25 %.

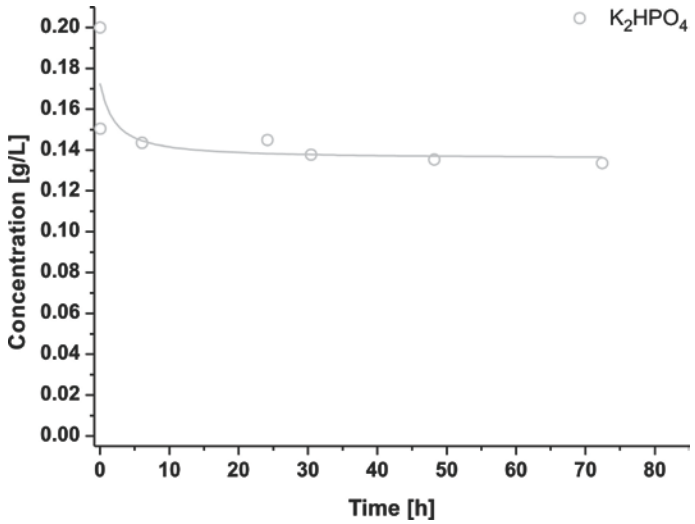

Fig. 37: Concentration changes of AXT in pure methylene chloride in presence of K_2HPO_4, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

The end concentration yielded to 0.1337 g/L which was equivalent to 67 % of measureable AXT. Though the concentration was continuously decreasing with time the course of the curve turned out to be close to being parallel, since the decrease was rather minor.

Solution containing the additive KOH

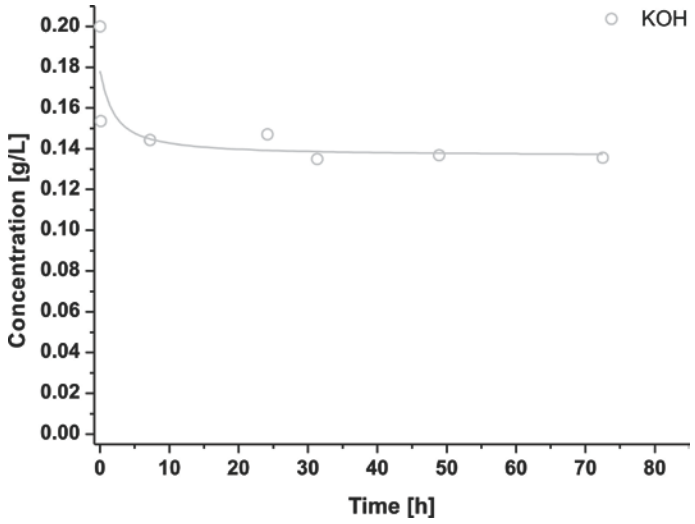

Fig. 38: Concentration changes of AXT in pure methylene chloride in presence of KOH, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

The initial steep decrease of concentration showed an immediate loss of almost 25 %. All photometrically obtained data points (see Fig. 38) stayed in a range of 9.3 % to one another over time. The lowest measured concentration was 0.1350 g/L, which is equivalent to 67.5 % of the initial concentration. The location of the data points also indicated that the system was close to attaining equilibrium.

Solution containing the additive CH_3COOK

Throughout the entire experiment the concentration level stayed almost constant, except for the concentration decrease between the first and second data point. Between the second and the last measurement (see Fig.39) the concentration diminished only by 7 %. The final value yielded to 0.1385 g/L. By the end of the trial there were still 69 % of the AXT measureable.

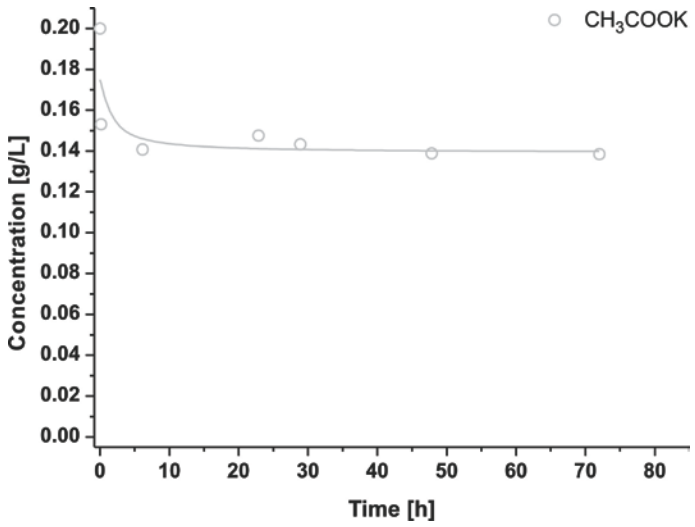

Fig. 39: Concentration changes of AXT in pure methylene chloride in presence of CH_3COOK, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

Solution containing the additive K_2CO_3

The data obtained from this experiment is shown in Fig. 40. Starting from the second data point the values fluctuated slightly. The fluctuation describes a range of almost 6 %. Despite the fluctuations the course of the graph was close to equilibrium state. The end value was measured to be 0.1461 g/L which is equivalent to 72.8 % of starting material.

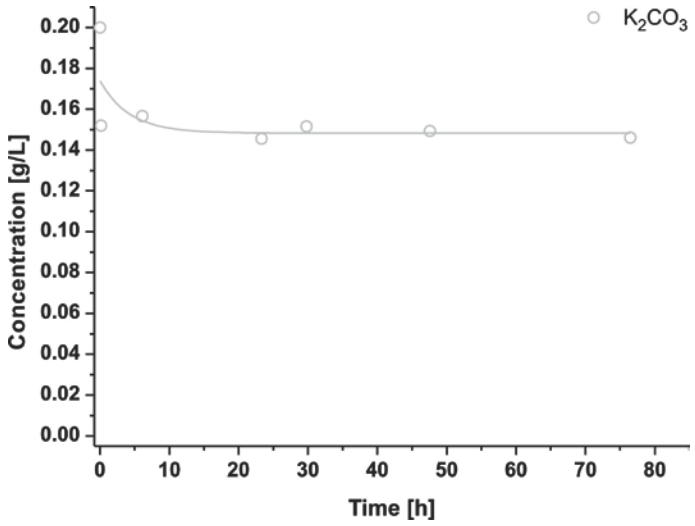

Fig. 40: Concentration changes of AXT in pure methylene chloride in presence of K_2CO_3, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

5.3.2.4 UV/VIS photometer results in water-saturated methylene chloride

Additive-free Solution

The final concentration, shown in Fig. 41, yielded to 0.1289 g/L (64 %) of measurable AXT.

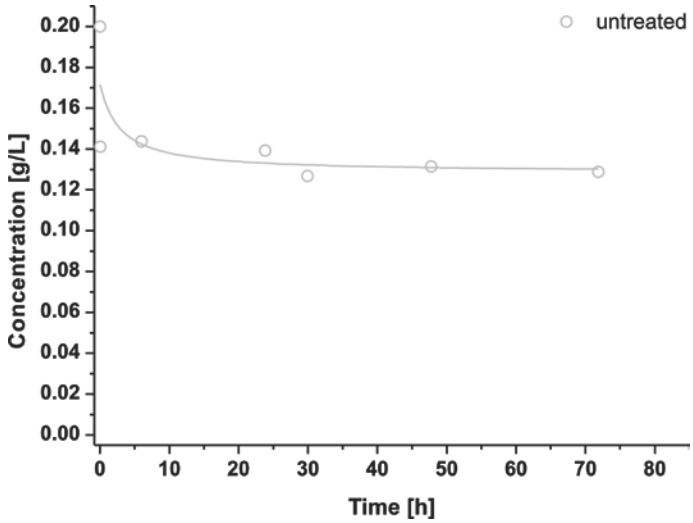

Fig. 41: Concentration changes of AXT in additive-free, water-saturated methylene chloride, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

From the second to the last data point the course of the curve strived for a horizontal alignment. In this experiment the concentration decrease between the first data point and the first data point obtained photometrically amounted to 0.0588 g/L, which was a loss of initial material of about 29 %.

Solution containing the additive K_2HPO_4

The obtained data from the experiment of K_2HPO_4 as additive in a solution of water-saturated methylene chloride and AXT is presented in Fig. 42. The concentration measureable after the immense concentration decrease, which happens at the very beginning, was 0.1504 g/L. The total loss was 25 % of the initially applied substance. There were slight fluctuations among the photometrical measurements in a range of about 5 %. The lowest measured concentration was 0.1398 g/L, which is equivalent to 70 % of the initially measureable AXT concentration.

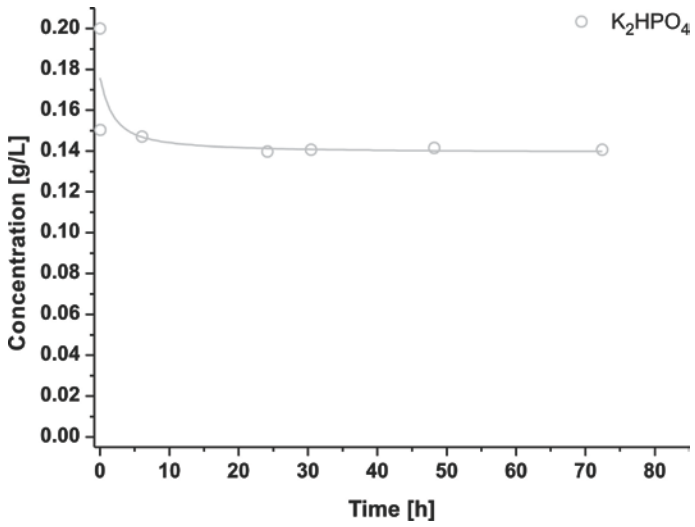

Fig. 42: Concentration changes of AXT in water-saturated methylene chloride in presence of K_2HPO_4, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

Solution containing the additive KOH

The usage of KOH as additive led to the data presented in Fig. 43. The calculated concentrations, obtained from the photometrical measurements, showed a slight decrease throughout the duration of the experiment. The lowest concentration measured is 0.1395 g/L. This equals about 70 % of the initially utilized concentration.

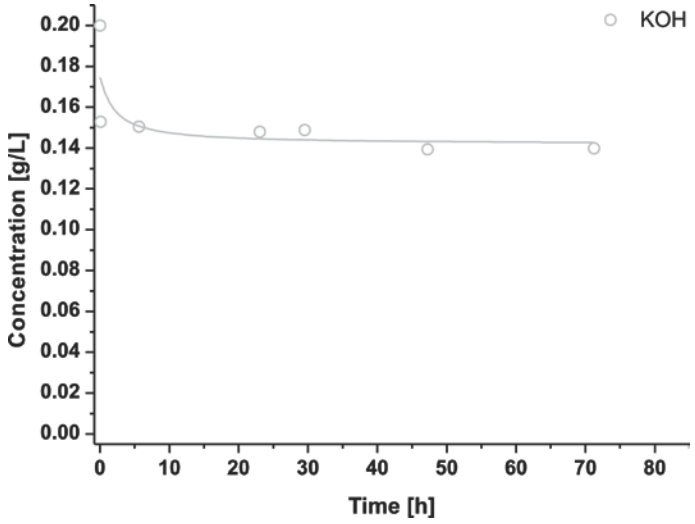

Fig. 43: Concentration changes of AXT in water-saturated methylene chloride in presence of KOH, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

Between the second and the last data point was a concentration range of about 6 %. Despite the initial decrease the concentration diminution that took place in the first 20 hours only yielded to 0.4 %.

Solution containing the additive CH_3COOK

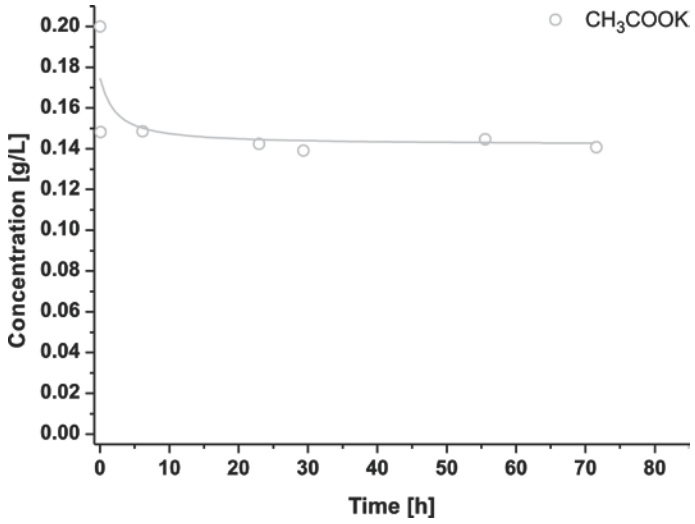

Fig. 44: Concentration changes of AXT in water-saturated methylene chloride in presence of CH_3COOK, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

In this experiment CH_3COOK was added to the solution of water-saturated methylene chloride and AXT. The information obtained from the experiment is presented in Fig. 44. After the initial concentration decrease the course of the curve proceeded closely below the concentration of 0.15 g/L. The end value measured was 0.1408 g/L. A total of 70.4 % AXT was therefore still measurable.

Solution containing the additive K_2CO_3

Fig. 45 shows the results of the experiment, in which K_2CO_3 was applied as additive. The amount of substance lost between the first and second data point covered a range of 23.2 % AXT. The first photometrically obtained concentration value was 0.1536 g/L. The data points were located quite closely to a concentration level of 0.15 g/L. That means under the influence of K_2CO_3 the entire loss was not higher than 28 %. The end value of AXT amounted to 0.1442 g/L.

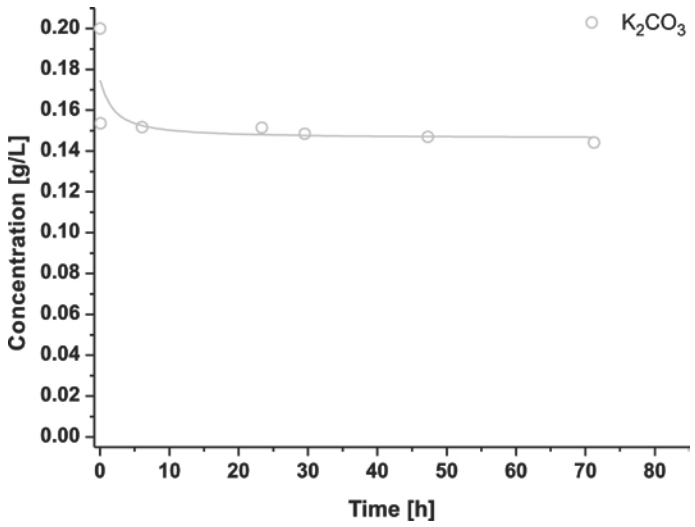

Fig. 45: Concentration changes of AXT in water-saturated methylene chloride in presence of K_2CO_3, at 30 °C. Plotted is concentration [g/L] vs. time [h]. Solid line represents the decrease rate of the AXT concentration due to degradation and isomerization.

In this case the total loss detected for the all-trans AXT by HPLC was 5 % lower than the data obtained via photometer. However, having regard to all AXT isomers present in solution and their signal superposition a difference of 5 % is in good agreement of both measuring techniques.

6. Discussion

Certain additives appear to have an accelerating effect on the formation of the equilibrium state of AXT in a given solvent. The equilibrium state of all-trans AXT in either chloroform or methylene chloride can be affected by application of additives to different extents. In regard to the HPLC data all experiment were terminated before reaching the equilibrium state. However, several of the negative slopes of the graphs visualizing the concentration decrease of the all-trans isomer approach zero, for instance see Figs. 7 and 8. Under these circumstances it was not possible to determine a value for all-trans AXT which could be defined as the equilibrium state. However, by acceleration of the equilibration and influencing the position of the equilibrium, the initial substance (all-trans AXT) could be kept intact. In untreated solutions the establishment of an equilibrium state takes a lot longer, than for the additive containing solutions to approach that state. However, the duration time of the experiments (measurements performed by HPLC) was too short either way. Therefore only a graph, that descended was obtained from the data of the untreated solutions. In the process of establishing an equilibrium state in the untreated solutions copious amounts of initial substance degraded. If there exists one specific substance that would increase the acceleration of the establishment of the equilibrium state to the highest possible acceleration and/or would shift the equilibrium so all-trans AXT is favored greatly, there wouldn´t be a need for other “stabilizing” agents to safeguard the molecule structure of all-trans AXT in solution. Simply by accelerating the process of equilibrium establishment, all-trans AXT could be protected. The formation of the equilibrium state needs to be understood in order to find the best working substance, in regard to optimization of synthesis and processing of all-trans AXT.

The HPLC results for the concentration changes of all-trans AXT in pure chloroform are plotted together in Fig. 46. End values of each graph are given in chapter 5.3.1.1.

The comparison of the additive treated solutions with the untreated solution in Fig. 46 shows that the application of different additives produces a gradation in the stabilization of all-trans AXT. The intensity of the additive provided stability differs. In pure chloroform the order of ascending priority of additives is K_2HPO_4, KOH, CH_3COOK and K_2CO_3. All tested additives increased the stability of the all-trans isomer. However, the solution equipped with K_2CO_3 delivered the best result in pure chloroform at 40 °C. As assumed the application of additives

increased the all-trans AXT stability. So far degradation could be stopped only in case of application of K_2CO_3 to a solution of pure chloroform and AXT.

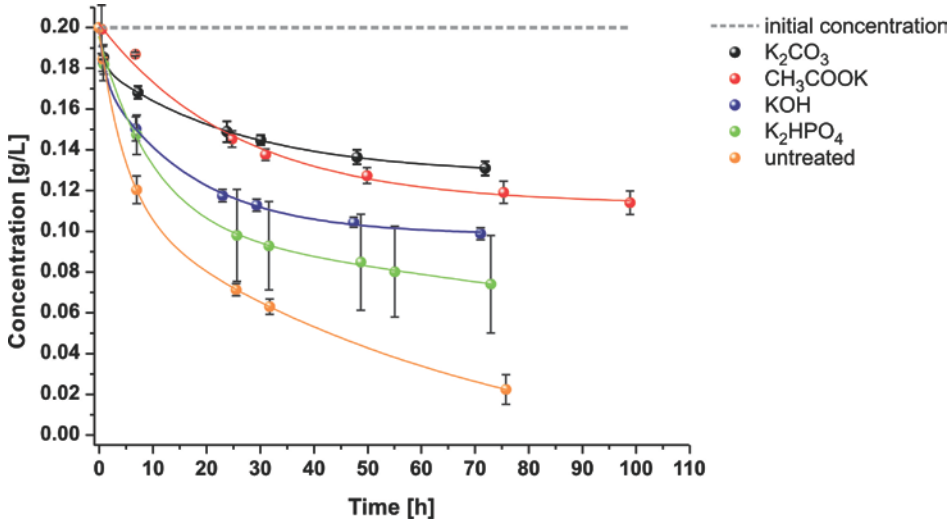

Fig. 46: HPLC results. Comparison of all-trans AXT concentration decreases in pure TCM, at 40 °C. Plotted is concentration [g/L] vs. time [h]. The data points represent the measured all-trans AXT concentration of each experiment. The solid lines represent the decrease rate of the all-trans AXT concentration due to degradation and isomerization.

The HPLC results for the concentration changes of all-trans AXT in water-saturated chloroform are plotted together in Fig. 47. The increased water content was assumed to increase the destructive processes, isomerization and degradation of AXT. The results of the solution containing KOH are to be neglected like established in chapter 5.3.1.2 (KOH in water-saturated TCM). The concentration loss measured in the untreated solution is highest among all solutions tested in water-saturated chloroform at 40 °C (see Fig. 47). However, the decrease of all-trans AXT in water-saturated chloroform was assumed to be higher than the loss in the pure chloroform. The experiments showed, however, an end value of 0.0224 g/L for the untreated solution in pure chloroform and the last measurement of the untreated solution in water-saturated chloroform produced a value of 0.0589 g/L all-trans AXT. In water-saturated chloroform there is a distinct similarity between the slope of the graphs of the sample solutions containing K_2CO_3 and CH_3COOK, this applies also to those solutions containing K_2CO_3 and CH_3COOK in pure methylene chloride. Suggesting that under the prevailing conditions the effects triggered by the two additives are very similar. Though the measured values differ between the two diagrams, those graphs run almost identical in their respective diagram. For water-saturated chloroform K_2CO_3 and CH_3COOK are found to be suitable additives in order to diminish the decrease of all-trans AXT.

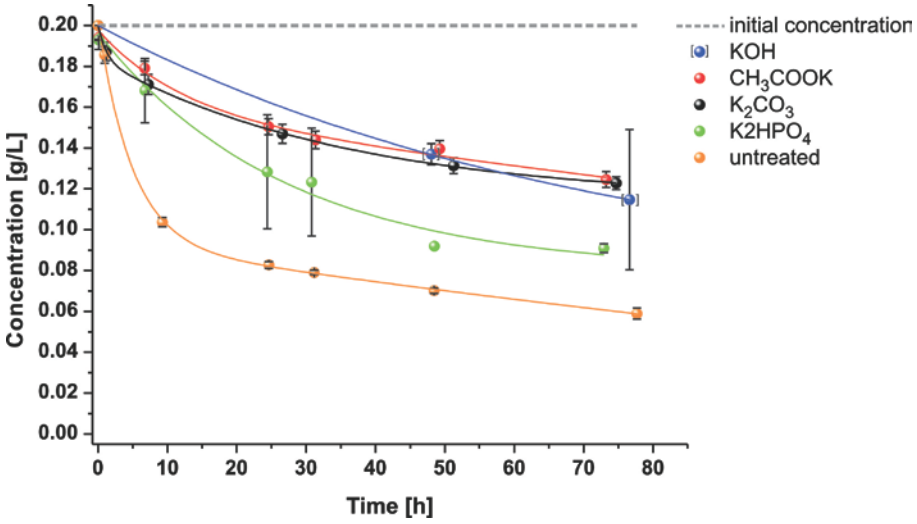

Fig. 47: HPLC results. Comparison of all-trans AXT concentration decreases in water-saturated TCM, at 40 °C. Plotted is concentration [g/L] vs. time [h]. The data points represent the measured all-trans AXT concentration of each experiment. The solid lines represent the decrease rate of the all-trans AXT concentration due to degradation and isomerization. The fit of the solution containing KOH (data points in square brackets) was created under exclusion of all values with a higher concentration than 0.2 g/L.

Though the results for K_2HPO_4 display a higher concentration of all-trans AXT than the results found for the untreated solution, the application of K_2HPO_4 should be replaced by either K_2CO_3 or CH_3COOK in order to maintain a higher all-trans concentration.

The concentration changes of all-trans AXT in pure methylene chloride measured by HPLC are plotted together in Fig. 48. Considering the results of the pure methylene chloride group there is really not much difference between the graphs. The graph of the untreated solutions already looks like an additive was applied to the solution. This most likely depends on the lower temperature of 30 °C. The results of K_2HPO_4 are to be neglected as stated previously under 5.3.1.3 (K_2HPO_4 in pure DCM). The graphs of all other additive equipped solutions run fairly close to each other in the diagram. In pure methylene chloride and at 30 °C a solution containing all-trans AXT should also include either K_2CO_3, KOH or CH_3COOK. Due to the inconclusive results found for K_2HPO_4, described in its particulars in 5.3.1.3, there is no statement possible. The application of an additive was assumed to increase the stability of all-trans AXT in methylene chloride and has been proven correct. The decrease of all-trans AXT in pure methylene chloride at 30 °C is considerably lower than in pure chloroform at 40 °C.

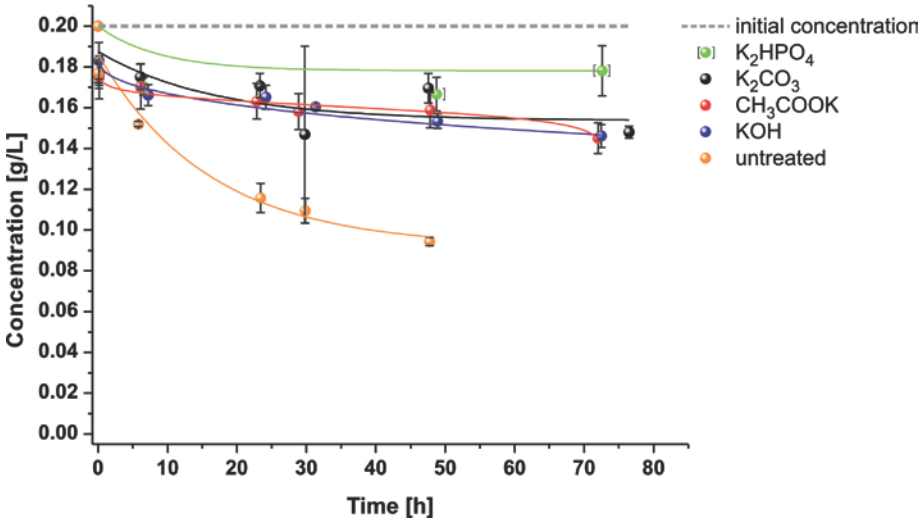

Fig. 48: HPLC results. Comparison of all-trans AXT concentration decreases in pure DCM, at 30 °C. Plotted is concentration [g/L] vs. time [h]. The data points represent the measured all-trans AXT concentration of each experiment. The solid lines represent the decrease rate of the all-trans AXT concentration due to degradation and isomerization. The fit of the solution containing K_2HPO_4 (data points in square brackets) was created under exclusion of all values with a higher concentration than 0.2 g/L.

The HPLC results for the concentration changes of all-trans AXT in water-saturated methylene chloride are plotted together in Fig. 49. In case of an increased water content the measured values, especially, for K_2HPO_4, are higher, if only slightly, than in the “pure methylene chloride”. The lowest values were measured in the untreated solution. The graphs of the additive containing solutions run close to each other in the upper quarter of the concentration scale. Solely in the additive-free solvents (pure + water-saturated TCM) it is possible to distinguish two sections of all graphs except for the total isomer concentration. This is only possible because in additive-free solutions isomerization can flourish freely. This is expressed by reaching of a maximum value of both 9- and 13-cis isomers in a quite short period of time. It is possible that this maximum value represents the equilibrium state for the system under the present conditions. The result, which is met by reaching the maximum value, is a probably complete hindrance of any further isomerization of all-trans AXT towards its cis isomers. This is represented by an abrupt change of course of the graphs. At this point the extent of degradation becomes clearly visible. The degradation proceeds always slower than isomerization (this applies only to the first graph section) in additive-free solvents. Inhibition of the degradation processes did not occur throughout the entire duration of the experiments, unlike apparently for isomerization.

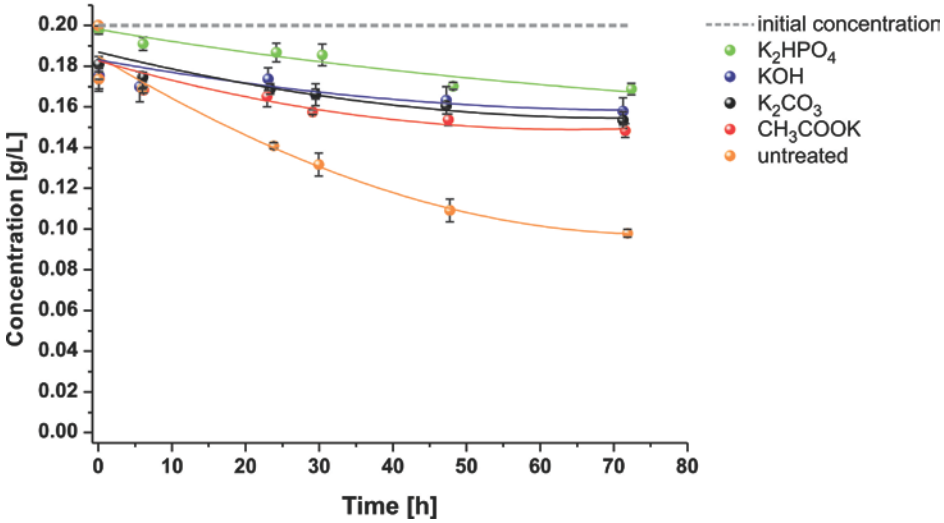

Fig. 49: HPLC results. Comparison of all-trans AXT concentration decreases in water-saturated DCM, at 30 °C. Plotted is concentration [g/L] vs. time [h]. The data points represent the measured all-trans AXT concentration of each experiment. The solid lines represent the decrease rate of the all-trans AXT concentration due to degradation and isomerization.

The HPLC results of the solutions with pure and water-saturated chloroform show that the saturation of chloroform with water leads to an improved stability of all-trans AXT. This applies to the untreated solution, the solution containing CH_3COOK and KOH. The solution containing K_2HPO_4 has to be exempted from this point of discussion due to the course of its data. The solution containing K_2CO_3 is the exception. For the solutions in which K_2CO_3 was used as additive a lower value of all-trans AXT was measured for the water-saturated solution.

The comparison of the HPLC data of the experiments performed in pure and water-saturated methylene chloride shows that the results of the solutions with higher water content were measured with a higher concentration value than the respective solutions which were prepared with pure methylene chloride. This statement does not include the experiments in which K_2HPO_4 was used as additive due to its discontinuous course (see Fig. 48).

A couple of questions need to be discussed in this context: Has the content of water an impact upon the rates of isomerization and degradation? It was shown for the case of the untreated solution of all-trans AXT in water-saturated chloroform (compare 5.3.1.1 (additive-free solution of pure TCM) and 5.3.1.2 (additive-free solution of water-saturated TCM)) that the increased water content provided a distinct improvement on the stability of the

all-trans isomer. The decrease of both cis isomers is mitigated as well by the higher water concentration. The last measurements of the two experiments revealed an all-trans AXT concentration of 0.0224 g/L in case of the pure solvent and 0.0590 g/L for the water-saturated solution. The same applies to the experiments in which K_2HPO_4 is utilized as additive in pure and water-saturated chloroform. The all-trans isomer concentration in the water-saturated solution is higher (0.0910 g/L) than in the pure solvent (0.0741 g/L). In the case of KOH no comparative statement can be made because no data were obtained from the water-saturated solution. However, in this context it is noteworthy that the final concentration of the all-trans isomer in the pure solution (0.0989 g/L) is slightly higher than the all-trans concentration in water-saturated chloroform in the presence of K_2HPO_4. Concerning the application of CH_3COOK the data states a 3 % higher concentration of all-trans AXT in water-saturated chloroform (0.1245 g/L) than in the pure solution (0.1192 g/L). In the K_2CO_3 containing solution prepared with water-saturated chloroform, the concentration of all-trans AXT (0.1228 g/L) is 4 % lower than measured in the pure solvent (0.1310 g/L).

The influence of the water concentration in the methylene chloride based experiments is in all cases (except K_2HPO_4, due to lack of comparative values) a positive improvement over the results achieved in pure solution. The determined difference in the untreated solutions is rather minor: 0.0944 g/L all-trans AXT in the pure solution and 0.0980 g/L in the water-saturated methylene chloride. For the application of KOH a clear difference in the all-trans isomer values was measured. The last value measured in the pure solution showed a remaining concentration of 0.1462 g/L, while the value measured in the water-saturated solution was 0.1579 g/L. For the solutions containing CH_3COOK the difference, in all-trans AXT concentrations, attributed to the different water contents was again rather minor. The pure solution contained 0.1450 g/L all-trans isomer and the amount determined in the water-saturated solution was 0.1484 g/L. More prominent was the distinction shown in the pure solution (0.1481 g/L) containing K_2CO_3 and the water-saturated solution (0.1536 g/L) containing the same additive. In summary it can be said that in most cases the increased content of water present in solution had a positive impact on the stabilization of the desired all-trans isomer. However, in regard to the cases of CH_3COOK and K_2CO_3 in chloroform, in which the concentration of all-trans AXT was distinctly lower, raises the question if it is possible that the positive effect of a higher water content only noticeably emerges when the all-trans concentration is not well enough stabilized already by the applied additive? For clarification further investigation will be necessary.

Is there a link between the presence of potassium and the stability increase of all-trans AXT? The way in which an additive influences a complex system is difficult to explore and determine [Str04]. However, the information obtained from the screenings (see section 5.2) does suggest that a connection between an increased stability of all-trans AXT and the presence of potassium exists.

Will a combination of several additives have an even more stabilizing effect than only one? In the realization of this project the investigation of the impacts of additive combinations would have exceeded the set time frame. Nonetheless, further investigations on the subject matter may prove the application of an additive combination to be worthwhile.

For all experimental data obtained from experiments in which pure chloroform was used as solvent it was found that the values measured by photometer were higher than the values measured by HPLC. This is due to the fact that via photometer only the combined signal of all AXT isomers present in solution is measurable the values obtained by photometer are higher than the respective values obtained by HPLC. This also applies to all experiments in which water-saturated chloroform was used as solvent. However, in all experiments in which either pure methylene chloride or water-saturated methylene chloride was used as solvent the values obtained from HPLC measurements were higher than the respective values measured by photometer. With one exception; the photometrically measured values for the untreated solutions in both, pure and water-saturated methylene chloride, were distinctly higher than the respective values measured by HPLC.

In pure chloroform the application of additives shows diverse outcomes (see Fig. 50). The end results vary between 0.1535 g/L for the solution containing K_2CO_3 and 0.0429 g/L for the untreated solution. This is a range of 55 % of AXT.

The values measured via photometer are of higher concentration than the values of the same solutions measured by HPLC, due to the missing distinctiveness for multi-component solutions, of the photometrical measuring technique. Nonetheless, the order of the additives is the same as it is from the HPLC results. In this case the photometer results are supporting the HPLC results.

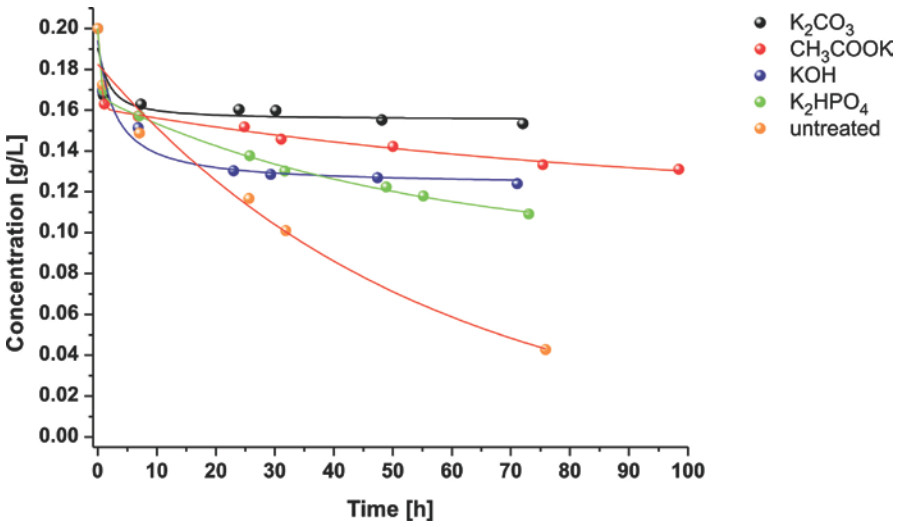

Fig. 50: Comparison of UV/VIS photometer results of AXT concentration decreases in pure TCM, at 40 °C. Plotted is concentration [g/L] vs. time [h]. The data points represent the measured AXT concentration of each experiment. The solid lines represent the decrease rate of the AXT concentration due to degradation and isomerization.

The comparison of the results, acquired from all experiments in water-saturated chloroform, is presented in Fig. 51.

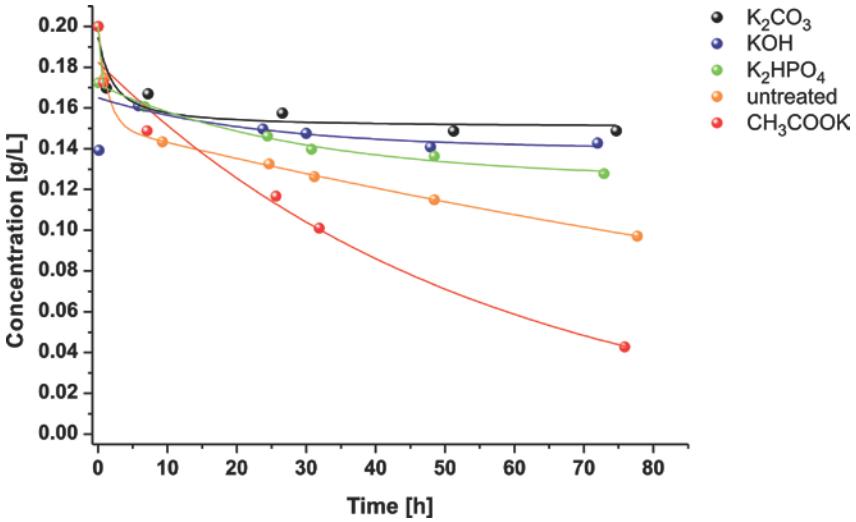

Fig. 51: Comparison of UV/VIS photometer results of AXT concentration decreases in water-saturated TCM, at 40 °C. Plotted is concentration [g/L] vs. time [h]. The data points represent the measured AXT concentration of each experiment. The solid lines represent the decrease rate of the AXT concentration due to degradation and isomerization.

Very peculiar is the course of the graph indicating the location of the results measured in the solution containing CH_3COOK. According to the photometer results the decrease in this solution was very high, even higher than the decrease of AXT in the untreated solution. This isn`t conform to the HPLC results for the same sample solutions. The HPLC results (see Fig. 47) show that CH_3COOK influenced the AXT decrease almost identical to K_2CO_3, which led to a loss of only 0.0603 g/L (all-trans AXT) in case of the CH_3COOK containing solution. The value of the loss based on the photometrical measurement was 0.1571 g/L (AXT). The loss measured by photometer is 260% higher than the loss measured via HPLC, though the loss measured via HPLC only refers to the all-trans AXT isomer. The loss measured by photometer refers to all measurable AXT isomers.

Though the photometrically acquired graph appears to be ordinary and rather smooth, without any discordant values, in accordance to all previously obtained results for solutions containing CH_3COOK it is assumed that the photometer data is not conform with reality.

The photometrical measurements show, that concerning the influence of the water content of chloroform it is obvious, that in case of the untreated solution the high water amount was highly beneficial. The opposite applied to the solution treated with CH_3COOK. The increased water content mitigated the stabilizing effect provided by the additive. The stabilizing attributes of K_2CO_3, KOH and K_2HPO_4 appear to be unaffected by an increased amount of water present in the solvent.

The application of additives in pure methylene chloride in order to increase the stability of AXT led to the data plotted in Fig. 52. The initial decrease of all tested solutions was rather high, however, in the following the loss of AXT wasn`t as extensive as at the very start. The order in which the graphs descend is the solutions containing K_2CO_3, CH_3COOK, KOH, K_2HPO_4 and the untreated solution. The location of the data points obtained via photometer for the solution containing K_2HPO_4 appears to be rather reasonable. However, due to an error, the HPLC values for this solution couldn`t be adduced for interpretation, and therefore also not for comparison with the photometrical results, of the effect provided by the presence of K_2HPO_4.

The sample solutions prepared with pure and water-saturated methylene chloride turned out to deliver quite similar results concerning their photometer values. The photometer data obtained from all experiments performed in pure methylene chloride are plotted in Fig. 52.

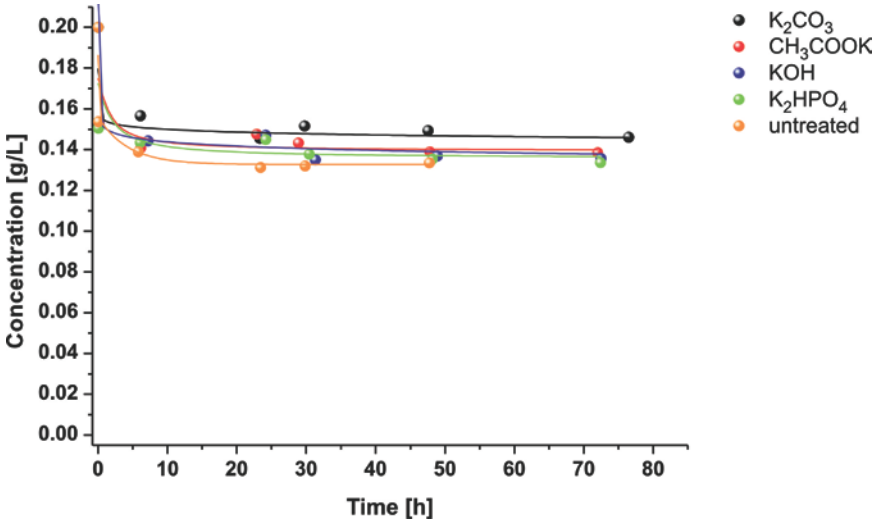

Fig. 52: Comparison of UV/VIS photometer results of AXT concentration decreases in pure DCM, at 30 °C. Plotted is concentration [g/L] vs. time [h]. The data points represent the measured AXT concentration of each experiment. The solid lines represent the decrease rate of the AXT concentration due to degradation and isomerization.

The initial concentration decrease is quite similar in all five cases, with about 23 % to 25 %. In the following the loss of AXT rose only by 2 % to 9 %. The course of the graphs is so similar that an order in their sequence couldn`t be established clearly. The concentration difference between the photometer data of all sample solutions prepared with pure methylene chloride and those prepared with water-saturated methylene chloride is rather indistinct. The high water content in the water-saturated solution is the only factor that differs between those two sets of experiments but there is no obvious improvement on the AXT stability. The same has been shown by the respective HPLC results (for KOH, K_2CO_3 and CH_3COOK) in pure and water-saturated methylene chloride.

That the graphs in the diagrams (see Figs. 50 and 51) are drifting further apart in pure and water-saturated chloroform, compared to the data shown in Figs. 52 and 53 is mostly due to the lower temperature that was applied for the experiments in pure and water-saturated methylene chloride.

The data shown in Fig. 53 is giving the information of all photometer experiments performed in water-saturated methylene chloride. In comparison the graphs run fairly close to one another. Throughout the duration time, starting from the first photometrical measurement, the concentration difference of the points furthest apart from each other was 0.0245 g/L which is similar to a fluctuation of only about 13 %. The application of additives provided an

improvement on the stability of AXT, even if it wasn`t remarkably large. The initial concentration decrease valued between 23 % and 29 % of the initially applied amount of AXT.

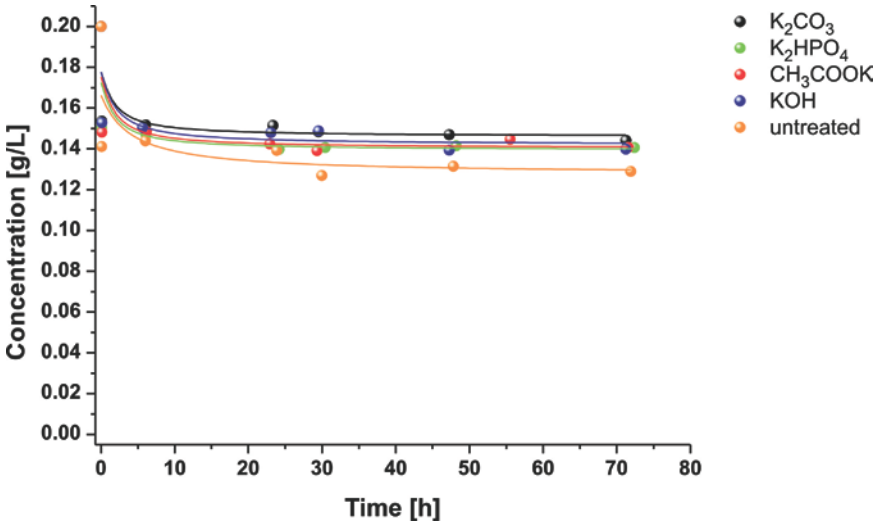

Fig. 53: Comparison of UV/VIS photometer results of AXT concentration decreases in water-saturated DCM, at 30 °C. Plotted is concentration [g/L] vs. time [h]. The data points represent the measured AXT concentration of each experiment. The solid lines represent the decrease rate of the AXT concentration due to degradation and isomerization.

The photometrical measurements reveal no significant advantageous or disadvantageous influence of an increased water content of the used solvent.

6.1 Result overview

All tested additives (KOH, K_2CO_3, K_2HPO_4, CH_3COOK) increased the stability (to varying degrees) of the dissolved all-trans AXT in all tested solvents (screening results are excluded from this statement) in comparison to the respective additive-free solutions.

In “pure chloroform” the highest remaining all-trans isomer concentration was achieved by application of K_2CO_3. Because of the inconclusiveness of the KOH experiments in water-saturated chloroform the conclusively best result and therefore highest remaining concentration of all-trans AXT was achieved by usage of CH_3COOK. In methylene chloride were the results concerning the application of K_2HPO_4 inconclusive and therefore the demonstrably highest remaining concentration of the all-trans AXT isomer was achieved by application of K_2CO_3, as similar to chloroform. The highest concentration of all-trans AXT at

the end of the experiments in water-saturated methylene chloride was achieved by application of K_2HPO_4.

In most cases (encircling both solvents) the impact of the high water content is reflected in an improvement concerning the remaining concentration of all-trans AXT in solution. Most significant in the additive-free solution of water-saturated chloroform compared to the results in “pure chloroform”. In one case, the increased water content led to a lower all-trans isomer concentration, this applies to K_2CO_3 comparing both sets of chloroform experiments.

The data collected in this project suggests that a connection between the stabilization of all-trans AXT and the presence of potassium in solution exists.

A combination of additives, present in solution of all-trans AXT, in either chloroform or methylene chloride may preserve an even higher amount of the sought isomer than provided by only one additive.

The photometer results showed differences in comparison to the HPLC results concerning the amount detected of AXT. All photometer results, in “pure chloroform” and water-saturated chloroform, reflected higher concentrations because with the photometer device the combined signal of all AXT isomers in solution was detected. In “pure methylene chloride” and in water-saturated methylene chloride the concentrations of all-trans AXT measured by HPLC were higher than the AXT concentrations obtained from the photometer measurements, except for both additive-free solutions.

The photometer results support the HPLC results concerning the order of additives arranged in Fig. 50 and Fig. 46 in “pure chloroform” and also in pure DCM (except K_2HPO_4) in Figs. 48 and 52.

The UV/VIS photometer results state that K_2CO_3 is the best stabilization agent in all tested cases.

6.2 Evaluation of results

Application of additives is generally recommended to improve the yield which can be recovered of all-trans AXT from solution (of chloroform or methylene chloride). K_2CO_3, CH_3COOK, KOH and K_2HPO_4 can be suggested for industrial application to stabilize all-trans AXT in either one of these solvents. In general the adaption from lab scale to industrial scale is associated with obstacles. The order in which the applied additives stabilized the

remaining all-trans AXT concentration can be different when it comes to industrial conduction. It is advisable to go from lab scale to pilot plant to industrial scale.

7. Summary

The stabilization of the chemically reactive structure of all-trans AXT, which is susceptible to a plethora of factors and substances, is a challenging endeavor. Especially so, when the isomer is transferred into a dissolved state in order to process it into a delivery system meant to enhance the quality and effectiveness of a feed supplement in its field of application. In this regard high bioavailability is essential, a major characteristic and has to be guaranteed.

By acceleration of isomerization processes it was possible to demonstrate positive impacts on the stability of the molecule, provided by several additives. It was also proven by HPLC that under defined conditions different additives provide different levels of efficiency. The best result in stabilizing all-trans AXT in pure methylene chloride was achieved by application of K_2CO_3. In water-saturated methylene chloride the smallest loss of all-trans isomer occurred in the presence of K_2HPO_4. In case of pure and water-saturated chloroform the most effective stabilization was provided by K_2CO_3 and CH_3COOK.

In industry the chemicals used as solvents are most likely to be recycled and therefore an increased water content is a valid presumption, even though there are of course measures to remove water from recycled solvents (under additional expenditure). An elevated water content inside the used chlorinated solvent was found to be a better precondition for the cases in which untreated sample solutions were measured and for several cases in which additives were applied. Most pronounced was this in the untreated solutions in pure and water-saturated chloroform. Also in case of K_2HPO_4 was the all-trans AXT concentration higher in the water-saturated solution than in the pure chloroform. However, a lower all-trans AXT concentration occurred for the experiment in chloroform containing K_2CO_3, comparing the water-saturated solution and the pure solvent.

In accordance with that the altered water-content of methylene chloride created results that showed the highest stabilizing effect was supported by KOH, followed by K_2CO_3, the untreated solution and CH_3COOK. All experimental results (except K_2HPO_4) in methylene chloride showed that the concentration of all-trans AXT was higher in the water-saturated solvent.

The obtained data suggests that a connection exists between the presence of potassium in solution and an increased stability of the dissolved all-trans isomer in either methylene chloride or chloroform.

The photometer results showed differences in comparison to the HPLC results concerning the amount detected of AXT. All photometer results, in “pure chloroform” and water-saturated chloroform, reflected higher concentrations because with the photometer device the combined signal of all AXT isomers in solution was detected. In “pure methylene chloride” and in water-saturated methylene chloride the concentrations of all-trans AXT measured by HPLC were higher than the AXT concentrations obtained from the photometer measurements, except for both additive-free solutions.

The photometer results support the HPLC results concerning the order of additives arranged in Fig. 50 and Fig. 46 in “pure chloroform” and also in pure DCM (except K_2HPO_4) in Figs. 48 and 52.

The UV/VIS photometer results state that K_2CO_3 is the best stabilization agent in all tested cases which is only partially conform with the HPLC results. According to HPLC K_2CO_3 was the best stabilizing agent in “pure chloroform” and “pure methylene chloride”. The respective additive in water-saturated chloroform was CH_3COOK closely followed by K_2CO_3 and for water-saturated methylene chloride K_2HPO_4 was the best stabilizing agent. Application of additives is generally recommended to improve the yield which can be recovered of all-trans AXT from solution (of chloroform or methylene chloride). K_2CO_3, CH_3COOK, KOH and K_2HPO_4 can be suggested for industrial application to stabilize all-trans AXT in either one of these solvents.

8. Zusammenfassung

Die Stabilisierung eines relativ unbeständigen und anfälligen Moleküls, wie dem all-trans Isomer des Carotinoids AXT, ist ein anspruchsvolles Unterfangen. Ganz besonders dann, wenn das Isomer in einen gelösten Zustand überführt wird, um es zu einer Verabreichungsform zu verarbeiten, die dazu gedacht ist die Qualität und die Effektivität eines Futterzusatzes in dem vorgesehenen Einsatzfeld zu erhöhen. Eine hohe Bioverfügbarkeit muss gewährleistet sein, da auf diesen Faktor ein Hauptaugenmerk fällt.

Mittels experimenteller Versuche, in denen die Isomerisierungsprozesse beschleunigt abliefen, war es möglich den positiven Einfluss auf die Stabilisierung des Isomers durch die Additiv-Anwendung zu demonstrieren. Darüber hinaus war es ebenfalls möglich per HPLC nachzuweisen, dass unter den fest definierten Versuchsbedingungen der Einsatz unterschiedlicher Additive unterschiedliche Resultate liefert. Das beste Resultat bezüglich der Stabilisierung von all-trans AXT in reinem Dichlormethan lieferte der Einsatz von K_2CO_3. In wassergesättigtem Dichlormethan war der Verlust an all-trans Isomer am geringsten in der Anwesenheit von K_2HPO_4 in der Lösung. CH_3COOK und K_2CO_3 lieferten jeweils in reinem und wassergesättigtem Chloroform die effektivste Stabilisierung.

Industriell verwendete Lösungsmittel werden zur Kostenersparnis recycelt. Dadurch kommt es zur Zunahme an Verunreinigungen in den Chemikalien und es ist eine berechtigte Annahme, dass durch die Wiederverwendung ebenfalls der Wassergehalt erhöht ist. Der höhere Wasseranteil der verwendeten chlorierten Lösungsmittel konnte als verbesserte Grundvoraussetzung nachgewiesen werden. Dies gilt für die Additiv-freien Lösungen und ebenso für einige der Lösungen, in denen Additive zum Einsatz kamen. Am deutlichsten war der positive Einfluss des hohen Wassergehalts in der Additiv-freien Lösung zu sehen, die mit reinem und wassergesättigtem Chloroform hergestellt wurde. Der all-trans AXT Gehalt war ebenfalls in der wassergesättigten Lösung, die K_2HPO_4 enthielt, höher als in der Vergleichslösungen, die mit reinem Chloroform hergestellt wurden. Jedoch zeigten die weiteren Ergebnisse (CH_3COOK, K_2CO_3) auch, dass im Vergleich die all-trans Isomer Konzentration durch den erhöhten Wassergehalt in der wassergesättigten Lösung niedriger ausfallen kann, als in der Lösung, die mit reinem Lösungsmittel hergestellt wurde.

Bezug nehmend auf das Lösungsmittel Dichlormethan brachte der Vergleich der zuletzt gemessenen all-trans Werte die Erkenntnis, dass sämtliche im wassergesättigten

Lösungsmittel durchgeführten Experimente (ausgenommen K_2HPO_4) eine höhere all-trans Konzentration des Carotinoids verbuchen konnten. Der veränderte Wassergehalt im Dichlormethan beeinflusste maßgeblich die Resultate, der größte Unterschied zwischen den Lösungen aus reinem und denen aus wassergesättigtem Lösungsmittel wurde bei dem Additiv KOH gemessen. Darauf folgten K_2CO_3, die Additiv-freie Lösung und CH_3COOK.

Die experimentell gesammelten Daten deuten auf eine bestehende Verbindung zwischen dem Vorhandensein von Kalium in der Lösung und der erhöhten Stabilität des gelösten all-trans AXT in beiden Lösungsmitteln.

Die Resultate der photometrischen Experimente zeigten, bezüglich des detektierten Betrags an AXT, Unterschiede im Vergleich zu den Resultaten, die mit der HPLC gewonnen wurden. Höhere Gehalte wurden bei den Experimenten in „reinem“ Chloroform und in wassergesättigtem Chloroform gemessen, da mit dem Photometer sämtliche, in der Lösung vorhandenen, AXT isomere detektiert werden. Die all-trans AXT Konzentrationen, die in „reinem“ und in wassergesättigtem Dichlormethan mittels HPLC bestimmt wurden waren allesamt höher, als die AXT Konzentrationen, die mittels Photometer gemessen wurden (ausgenommen der Additiv-freien Lösungen).

Die Photometerdaten bekräftigen die die Resultate, die mit der HPLC erlangt wurden bezüglich der Reihenfolge in der die Additive die Stabilisierung fördern. So z. B. beim Vergleich der Abbildungen 46 und 50 in „reinem“ Chloroform.

Die UV/VIS Photometer Resultate sagen aus, dass K_2CO_3 in allen durchgeführten Versuchen den größten stabilisierenden Effekt aufwies. Dies stimmt nur teilweise mit den HPLC Resultaten überein. In den „reinen“ Lösungsmitteln brachte K_2CO_3 die beste Stabilisierung des Isomers. Für wassergesättigtes Chloroform war das CH_3COOK, dicht gefolgt von K_2CO_3 und in wassergesättigtem Dichlormethan wurde die höchste Stabilisierung durch die Verwendung von K_2HPO_4 erreicht. Die Verwendung von Additiven zur Verbesserung der Ausbeute an all-trans AXT, welches aus Lösungen mit Chloroform oder Dichlormethan zurückgewonnen werden soll wird im Allgemeinen empfohlen. K_2CO_3, CH_3COOK, KOH und K_2HPO_4 werden für den industriellen Einsatz, zum stabilisieren des all-trans Isomers in einem der angeführten Lösungsmittel, vorgeschlagen.

9. List of abbreviation

Abbreviations

AXT	Astaxanthin
DCM	Methylene chloride
TCM	Chloroform
ppm	Parts per million
g/L	Gramm per liter
%	Percent
SO_2	Sulfur dioxide
I_2	Iodine
H_2O	Water
H^+	Hydron
I^-	Iodide ion
SO_4^{2-}	Sulfate ion

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Paper

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