CHALMERS

Synthesis and studies of cancer active substances

Bachelor Science Thesis

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CHALMERS UNIVERSITY OF TECHNOLOGY
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ABSTRACT

The objectives of this thesis and its experiments was to see if the aggregations of hyaluronic acid and cyanine dyes could be formed, and as a consequence, get fluorescent properties with high emissions.

Hyaluronic acid is present in high concentrations close to some cancer tumors and could possible therefor be used as a way to signal where the cancer is in the body. If the hyaluronic acid can bind to the cyanine dyes forming high fluorescent aggregates at the locations of cancer, treatment could be sent to the infected parts of the body minimizing collateral damage.

Older theses and literature have results of cyanine dyes forming aggregates with DNA. Because of the structure of hyaluronic acid having similar charge as DNA and there are some literature reports of successfully increasing the emission when aggregation between dye and hyaluronic acid is formed the thesis was continued on this premise in the beginning.

Different in-house dyes where experimented on together with dyes synthesized for this thesis. These dyes where analyzed on their fluorescent properties (absorption and emission) through UV/Vis spectrometer and fluorescence spectrometer.

Due to low intensity emission and some changes in absorption the results were hard to use when making a conclusion. The results on emission did not enter the levels of practical use and sometimes the emission even was lowered with addition of hyaluronic acid. Due to these results the formation of the hyaluronic cyanine dye aggregates cannot be confirmed, but there are trends indicating that the changes in absorption and intensity are more than just dilutions and may be aggregates. Because of the low emission values there couldn't be any conclusions drawn about the ability to use cyanine dyes as a signal substance for cancer tumors.

Low intensity emissions instead of the high intensity in literature could be due to the many environmental parameters that effect aggregation such as structure of dye, what kind of hyaluronic acid, temperature, pH and solvent.

Keywords: Cyanine dyes, aggregation, DNA, spectroscopy, emission.

SAMMANFATTNING

Målen med denna avhandling och dess experiment var att se om aggregeringarna av hyaluronsyra och cyaninfärgämnen skulle kunna bildas och följaktligen få fluorescerande egenskaper med höga ljusutsläpp.

Hyaluronsyra är närvarande i höga koncentrationer nära vissa cancertumörer och kan därför användas som ett sätt att signalera var cancer föreligger i kroppen. Om hyaluronsyran kan binda till cyaninfärgämnena och bilda fluorescerande aggregat vid cancern, då skulle cancerbehandling kunna sändas till de smittade delarna av kroppen och då minimera indirekta skador till närliggande vävnad.

Äldre avhandlingar och litteratur har resultat där cyaninfärgämnen bildar aggregat med DNA. På grund av att strukturen av hyaluronsyra är något liknande DNA samt att några litteraturrapporter har framgångsrikt fått en ökning av emission när aggregeringen mellan färgämne och hyaluronsyra bildas sker. På denna premiss fortsatte avhandlingen.

Olika in-house färgämnen experimenterades på tillsammans med färgämnen som syntetiserades fram för denna avhandling. Dessa färgämnen analyserades på deras fluorescerande egenskaper (absorption och emission) genom UV / Vis-spektrometer och fluorescensspektrometer.

På grund av låg emission och vissa förändringar i absorption var det svårt att dra en slutsats. Resultaten av emission gick inte upp i nivåerna av praktisk användning och ibland sänktes ljusutsläppen med tillsats av hyaluronsyra vilket är motsatsen till önskad effekt. På grund av dessa resultat kan bildandet av hyaluronsyra och cyaninfärgämnes aggregaten inte bekräftas, men det finns trender som indikerar att förändringarna i absorption och intensitet är mer än bara utspädningar vilket skulle kunna vara aggregat. En följd av den låga emissionen är att användningen av cyaninfärgämnen som signalsubstans för cancertumörer ej kunde bekräftas.

Låg emission istället för den höga emissionen i litteraturen kan bero på de många parametrar som påverkar aggregeringen som cyaninfärgämnets struktur, vilken typ av hyaluronsyra, temperatur, pH och lösningsmedel.

CONTENTS

1 INTRODUCTION

1.1 Background:

Cancer is one of the diseases causing major health concern and due to its mortality rate and is therefore a huge area that needs improvement with new treatments and to improve the old treatments [1]. The problem with the current treatments is that the tumour cells either become drug resistant or it causes damage to healthy cells and the body in severe ways. One way to solve this problem is making the drugs specifically target infected cancer tumours and for them to ignore healthy cells [2].

Cyanine dyes are one compound that show promising properties, where one of them is inhibition of cancer cells [3]. Cyanine dyes are cationic and are built up of heterocyclic ring systems with nitrogen. One example of a cyanine dye that shows inhibition of cancer cells is heptamethine cyanine. Heptamethine cyanine releases cytotoxicity-free radicals in photodynamic therapy. These radicals can either kill tumour cells directly or damage their growth potential [4]. One more suitable property for cyanine dyes are that they can form aggregates with other materials which leads to suitable optical properties like spectra shifting and visualization of DNA [5]. The aggregation of cyanine dyes occurs naturally and the reason for the formation is thought to be due to van der Waals forces, hydrogen bonding with solvent and coordination with metal ions. The spectra of the aggregated dyes are different to the monomers where J-aggregates form longer wavelength with intense excitonic absorption while H-aggregates have lower wavelength. The formation of the aggregate depends on the structure of the dye but also environment, polarity of solvent and pH. The definite structures of aggregates are not yet determined but is a task for the future and right now the structures are merely speculations.

One way to make the treatments target infected cells is by using Hyaluronic acid (HA) which according to literature increases the fluorescence of cyanine dyes. HA is a natural polysaccharide that is presented in for example skin, cartilage and other connective tissues. The HA can bind to a receptor called CD44, this receptor is very common in different tumour cells and therefore the HA is likely to be found in cancer infected tumour complexes. These properties make HA an excellent shuttle or target for the drugs [6][7].

1.2 Objectives

The main aim of the bachelor thesis is to further work on the research made in the older theses to use their results on cyanine dyes to synthesize a new cyanine dye with all the desired properties. Former theses have made the cyanine dyes emit light when forming aggregates with DNA using crystalline nanocellulose/hemicellulose as a shuttle to DNA. This thesis will try to form aggregates using HA with different cyanine dyes to retrieve similar spectral properties as when aggregating with DNA. After finding some promising results a mix of the promising cyanine dyes will be synthesized trying to take the good qualities of them and merging them together.

2 THEORY

2.1 Cyanine Dyes

The interest of cyanine dyes in later years has increased a lot due to its versatile usage in engineering, pharmacology and medicine. It has been researched to the point where it is used as spectral sensitizers in photographic industries, as an acid-base indicator, as an anti-cancer agent and for detection of nucleic acids and proteins and this is just some of its applications [8]. A cyanine dye is normally built up of two heterocyclic rings and with two nitrogen atoms where one of the nitrogen atoms is positively charged. The rings block the formation of two conjugated double bonds. These dyes can absorb light, the absorption mainly comes from the methine groups which are either in E or Z configuration [5]. The strong colours that are associated with cyanine dyes is due to delocalized electrons within the dye [9].

The dye can in some situations, as mentioned, work as an anti-cancer agent [4]. One example is the compound heptamethine cyanine, IR-808, that when aggregated starts to produce cytotoxicity-free radicals. These radicals can terminate cancer cells directly or just inhibit the cancer from spreading by blocking the formation of new blood vessels nurturing the cancer.

2.1.1 Aggregation of Cyanine Dyes

Cyanine dyes as mentioned absorbs a lot of light but do not emit that much on their own. One way for the dyes to have high fluorescent properties is by forming aggregates [5] [10]. There are some common known aggregates formed by cyanine dye monomers which are dimer aggregates, J-aggregates and H-aggregates which can be viewed in figure 1 [9]. Aggregation for dyes often occur when in a solution with polyelectrolytes or biological macromolecules, both forming reversible interactions [5]. The forming of the aggregations has been debated and the different explanations for them are van der Waal forces, hydrogen bonding with solvent or when binding with the metals, the coordination with ions in the metals. When the aggregations have been formed, the dyes are no longer applicable to the lambert-beer law since they may change in extinction. The structure of the dye aggregations has never been definitely proven.

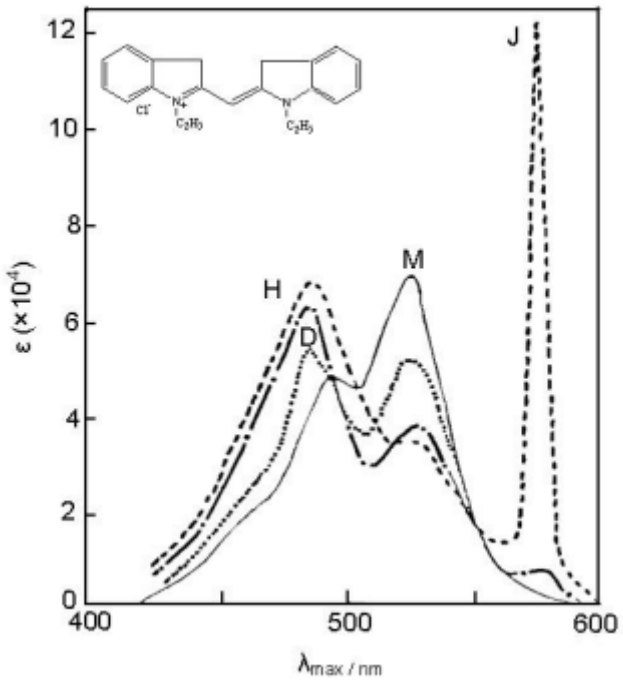

Figure 1. *The absorption spectra for the monomer (M) and the dye aggregates dimer (D), H-aggregates (H) and J-aggregates (J) of the dye 1,1'-diethyl-2,2'-cyanine chloride at room temperature [5].*

The most common and easiest aggregate to form is the dimer aggregate which is formed in high concentrations of cyanine dyes and can visually be seen in figure 2. This aggregation tends to be at lower wavelength than the monomer. [14] [15]

H-aggregates is when the molecules of the dye start to face itself in a vertical stack, can be seen in figure 2. This form of aggregates has their absorption band at a high energy level greater than that of the monomer leading to a higher intensity in the fluorescent emissions (and lower wavelength). How much of an increase this intensity is depends on the amount of cyanine monomers that form the H-aggregate.

J-aggregate differs from the H-aggregates in a way that they do not form vertical stacks but instead they form slanted stacks, which can be seen in figure 2, which in turn gives the aggregates a longer wavelength (lower energy) that still can have strong intensity emissions.

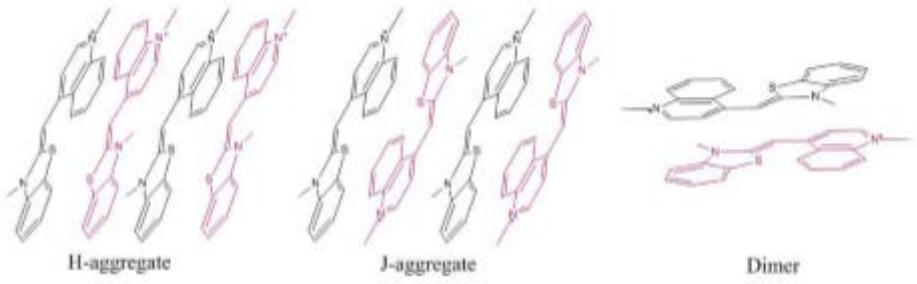

Figure 2. *The different forms of aggregates for the common cyanine dye thiazole (TO) orange can form with itself using its monomers. [3]*

For a dye to aggregate there are different properties that control the aggregation, foremost the structure of the dye. Then there are also properties like temperature, which when lowered often promote aggregation, polarity and pH of the solvent. Water is a good solvent for the cyanine dyes to form aggregates due to its dielectric constant and its ability to disrupt water-like hydrogen-bond structures. The dielectric constant makes the repulsive forces within the cyanine dye structure to diminish. The dielectric constant can be both increase and lowered with inorganic salts respectively alcohol solvents.

2.1.2 Cyanine dyes and DNA

Cyanine dyes are a future use in anti-tumour drugs and this is because of the interactions the dyes have with molecules similar to DNA [11]. When binding to a molecule like DNA, nucleic acids, the otherwise pretty much non-fluorescent dye becomes intensely fluorescent. The binding to the nucleic acid is called intercalation, intercalation is when a molecule, like cyanine dyes, binds into a nucleic acid between its base pairs into the minor and major groove [12]. The great shift in emission intensity is believed to have its basis in the fact that when the two molecules bind, the torsion of the cyanine dye is hindered between the two heterocyclic rings [11]. This torsion hindering then absorbs a lot of energy and stores it when it would normally be released as movement. To remove all this excess energy the molecule complex instead starts to emit light with a high intensity.

2.2 Hyaluronic acid

Hyaluronic acid is a carbonated polymer consisting of a flexible disaccharide, where the saccharides are uronic acid and amino sugar units [13], the structure of the HA used in this thesis can be seen in figure 3.

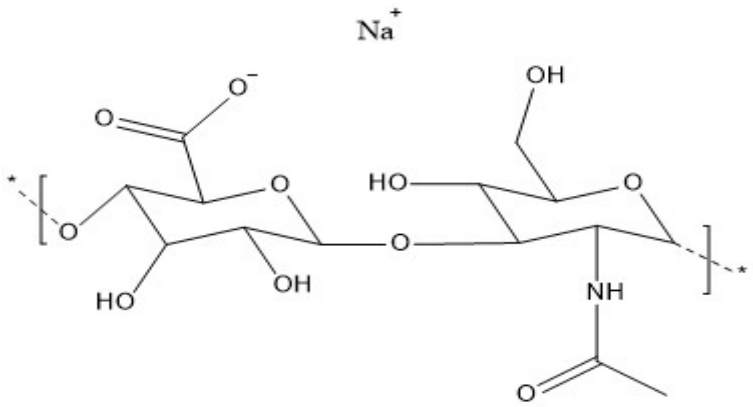

Figure 3. *The structure of hyaluronic acid monomer used in the experiments in this thesis.*

In the human body HA is produced within the cells and is used as a membrane for the cells. HA can control the level of the water balance in cells and tissues, this skill gives HA treated molecules the ability to change location during growth of the cells. This is also the downfall of the acid since this ability enables cancer tumour cells, with HA as a membrane, to migrate through the body and affect other healthy parts of the body with the specific cancer, this is called metastasis.

The reason why HA is forming the membrane on cancer cells is due to the CD44 receptors that there is a high concentration of in cancer cells [6]. HA can bind to these receptors and therefore aiding cancer to spread through the body. When HA is present in the tumours some of it starts to degenerate through enzymes called hyaluronidases, these enzymes have a higher concentration in different type of cancers. These enzymes deteriorate the HA in two steps which makes the product of these steps very small in size and perfect as a shuttle for drugs to enter tumours at an intracellular level.

The cyanine dyes will form a bond with the HA at the left part of the structure at COO^- just like in DNA it will be bonding by intercalation.

3 EXPERIMENTAL

3.1 materials

3.1.1 Chemicals

Dyes:
Thiazole orange (TO)
1-Methyl-4-[(3-methyl-1,3-benzothiazol-2(3H)-yliden)methyl]pyridinium (BOXTO)
TO-3-propyl-Br (SB1)
2-TO-p-acetofenon (LB2)
2-TO-m-Nitrobensen (LB3)
Thiazole-dimer (SL)
Quinoline-dimer (VL)
2-hydroxy Nile red (LE7)
3-hydroxyl Nile red (OH-3)
Bo-neu (VK1)
Other chemicals:
Methylthiazole tosylat
4-metyl quinolinium tosylat
Hyaluronic acid
Hydrochloric acid, sigma aldrich
MQ-water
Dimethyl sulfoxide (DMSO), sigma aldrich
Acetone, sigma aldrich
Dichloric methane, sigma aldrich
Methanol, sigma aldrich
Triethylamine, sigma aldrich
Sodium dihydrogenphosphate, sigma aldrich

They structure of the dyes can be seen in appendix (appendix 13-22)

3.1.2 Apparatus

Nuclear magnetic resonance (NMR)
UV-Vis, Cary 50 Bio from Varian Australia Fluorescence
Fluorescence, Cary Eclipse from Varian Australia

3.2 METHOD

3.2.1 Preparation of spectroscopy samples

All mixtures of the different samples were made in a 1x1cm quartz cuvette. All cuvettes where filled with three millilitres of MQ-water that was 5% DMSO. The sample in the cuvettes was mixed by pipetting the liquid up and down to get a homogenous mixture. This method was used instead of shaking the cuvettes to lower the chance of bubbles disturbing the results. All samples were prepared and performed in room temperature.

3.2.2 Synthesis of cyanine dye VL and SL

The materials 4-metyl quinolinium tosylat and methylthiazole tosylat (0,3295g and 0,3355g) where added to 3ml respectively 4ml of acetic anhydride in two different glass jars able to withstand higher temperature without braking. This was then stirred with a magnetic pill, heated to 90°C and 0,4416g of triethyl orthoformate was added to both jars (around 0,4ml). The reaction with Methylthiazole tosylat after 15 minutes became very purple conforming the reaction according to literature. These mixtures were then allowed to be stirred for a total of one hour at 90°C. After one hour were taken off the heating plate and they cooled down to room temperature for 24 hours to form crystals. These crystals where then washed with around 10 ml diethyl ether per gram product (crystals) using filtration. NMR was used to confirm product. Another synthesis was made but due to the product disappearing after three steps of separation the continuation of the synthesis was stopped. All syntheses of cyanine dyes where analyzed with NMR to confirm the product.

4 RESULTS AND DISCUSSION

4.1 Fluorescence spectra

In this section the results of some cyanine dyes will be shown and discussed. Some extra experimental results on different dyes can be viewed from Appendix 8.1 to Appendix 8.6. Theses dyes are not discussed because the lack of new results and continuation of low intensity emissions that are going to be examined in this part of the thesis. Also in the appendix there are the concentrations of each cyanine dye and HA used in all the different spectra.

4.1.1 TO spectra

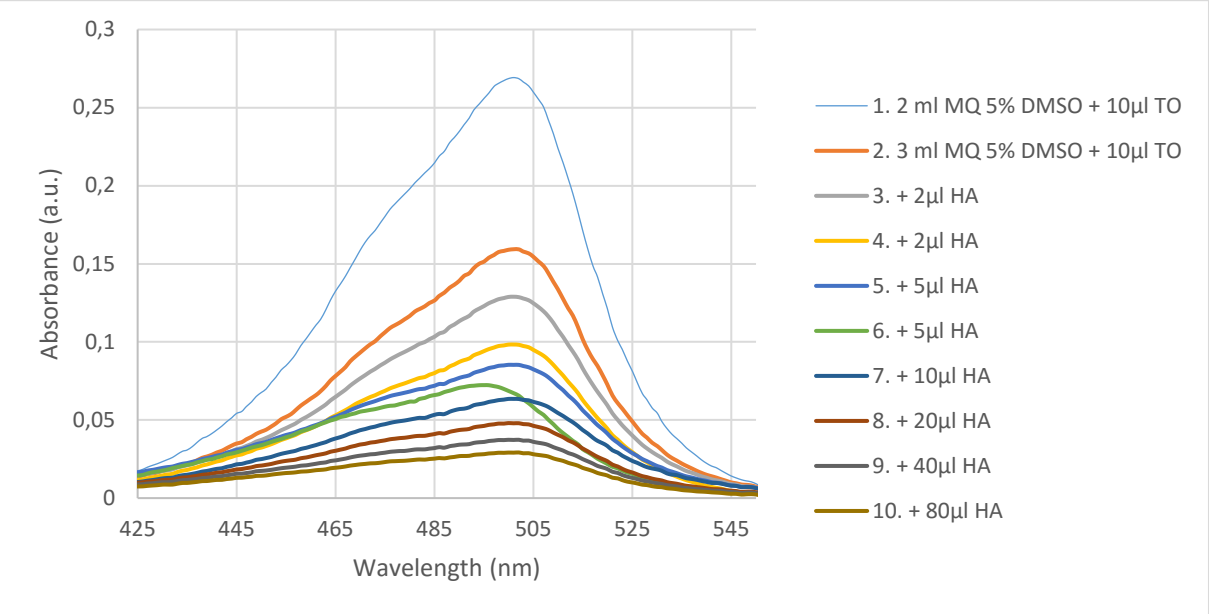

Figure 4. *Absorption spectra of different concentrations of HA and 10µl TO. All samples after sample 1 mixed with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide. Each step is a titration, meaning every step down the HA is added to the old sample. Total added HA is 164µl.*

In figure 4 you can see that the absorbance is decreasing the more HA is added, the most reasonable explanation is probably dilution. The large amount of the decrease could be as mentioned due to the HA diluting the sample but also there could be a lack of aggregation formation. Suppose aggregation was being formed the absorbance spectra should start to level out. It can be seen though that at the later parts of the titration the increased HA doesn't give increase absorbance reduction, meaning there could be some form of aggregation making the decrease in absorbance less than if it was not there. The mechanism behind this cyanine dye and all the other cyanine dyes are that just like in DNA the cyanine dye will intercalate with the HA and by dying so get hindered movement in the form of aggregation, this hindered movement produces light.

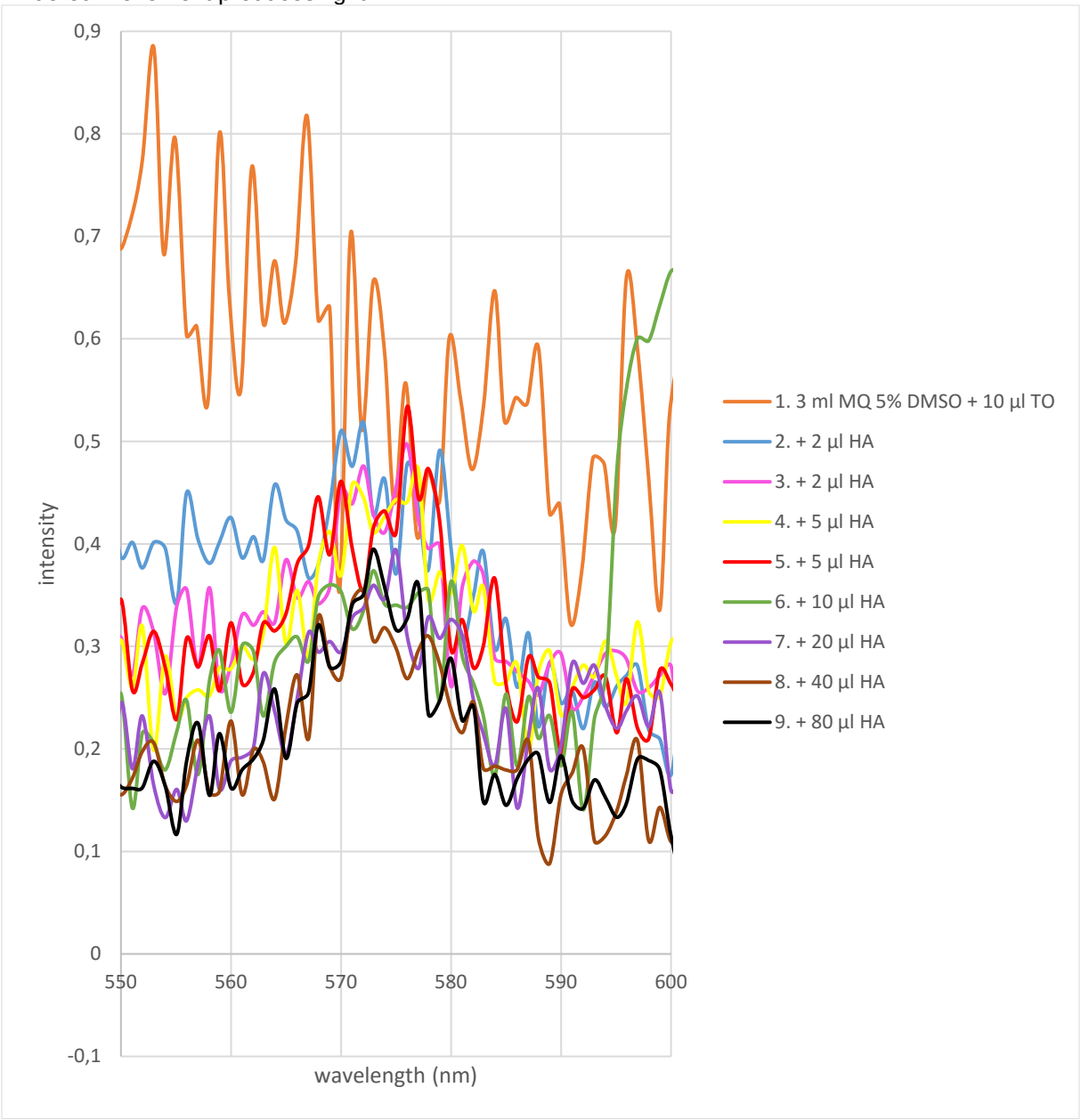

Figure 5. *Emission spectra of different concentrations of HA and 10µl TO. All samples contained 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide. Each step is a titration, meaning every step down the HA is added to the old sample. Total added HA is 164µl.*

When looking at the emission spectrum of TO, figure 5, the shift when adding HA is so little it is considered just to be disturbance. When not considering the result to just be disturbance the very minor changes are still going in the wrong direction, the intensity is decreasing. For comparison the increase in intensity that was desired was supposed to increase, at least within the chosen concentration spans, up to everything between 1 000-1 000 000.

4.1.2 BOXTO spectra

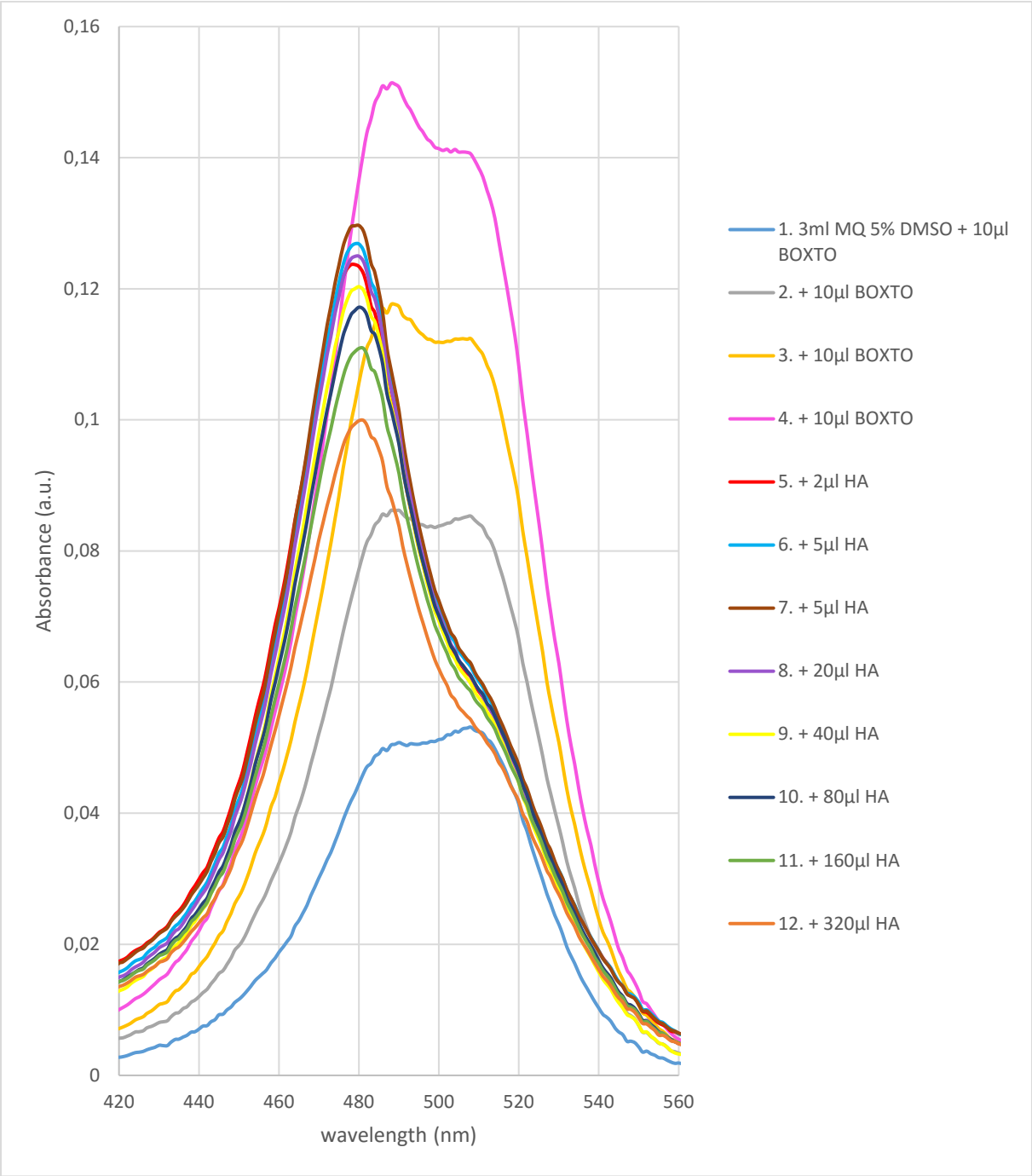

Figure 6. *Absorption spectra of different concentrations of HA and titration of HA starts at a total of 40µl BOXTO. All samples contained 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide. Each step is a titration, meaning every step down the BOXTO/HA is added to the old sample. Total titrated HA is 632µl.*

In this spectrum, figure 6, there was addition of extra BOXTO to get better curves that would show better results. In all these four first steps where it is just BOXTO we can see there are two peeks every addition meaning there is some form of self-aggregation and with the two tops that might be a dimer aggregate with itself. When then HA was added to the sample the possible dimer structure broken and became one single peak with a tiny dent where it used to be another peak. This could be that the HA is forming an aggregation with the BOXTO making it lose its main structure in its pure condition and entering perhaps a H- or J-aggregate.

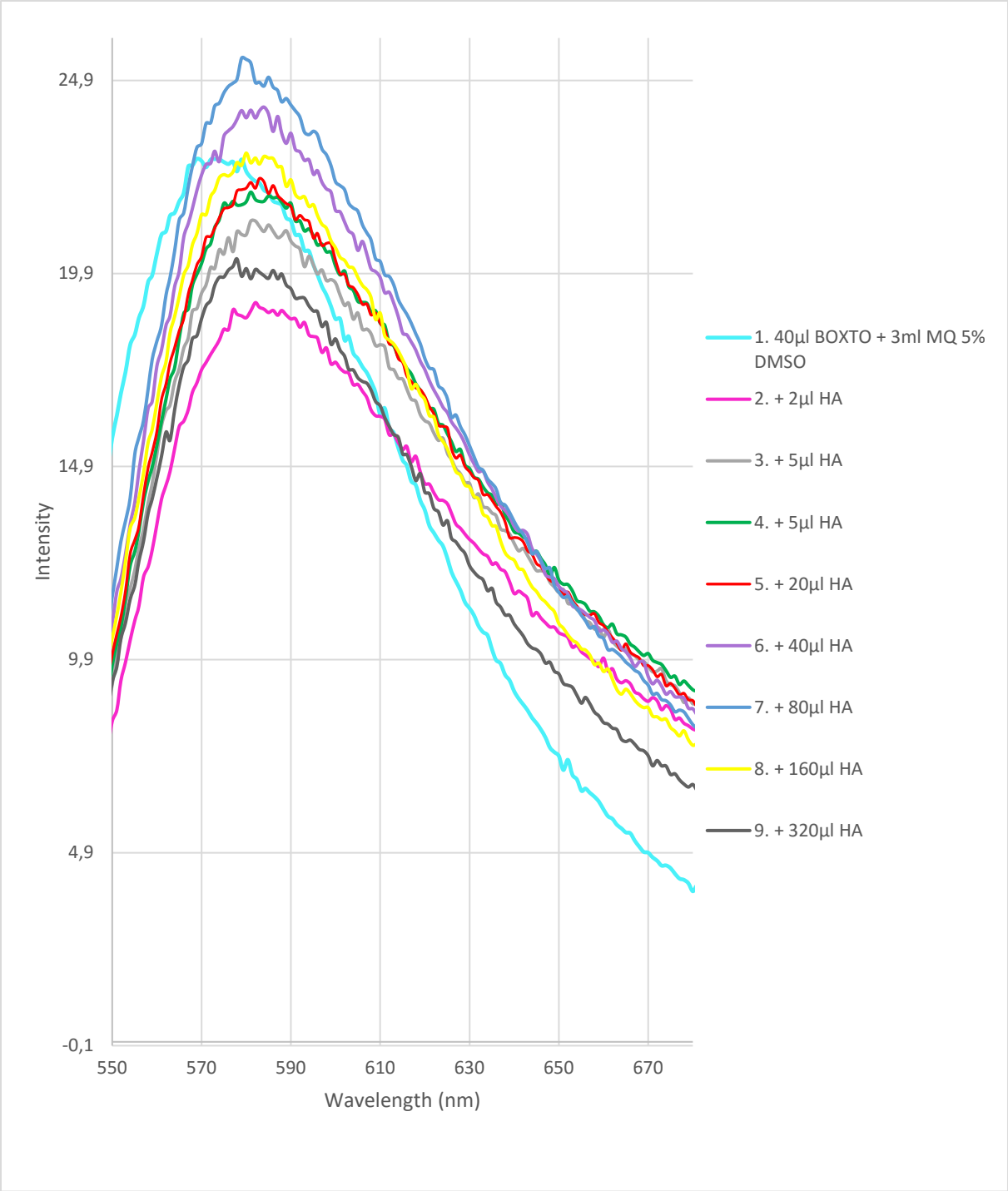

Figure 7. *Emission spectra of different concentrations of HA and a titration of HA starts at 40µl BOXTO. All samples contained 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide. Each step is a titration, meaning every step down the HA is added to the old sample. Total added HA is 632µl.*

In figure 7 we can see that is shift in intensity, this is still too low to be considered anything else than disturbance. If you look at the graph without considering the disturbance there is change in intensity and it is going partly in the right direction, for example sample 6 to 7 is increasing. Then again, there are samples going the wrong direction like sample 7 to 8. So, there is something happening, some form of aggregation, but due to the low intensity shifts the results are not conclusive and what can be seen can't for certain be correct.

4.1.3 BOXTO reference spectra

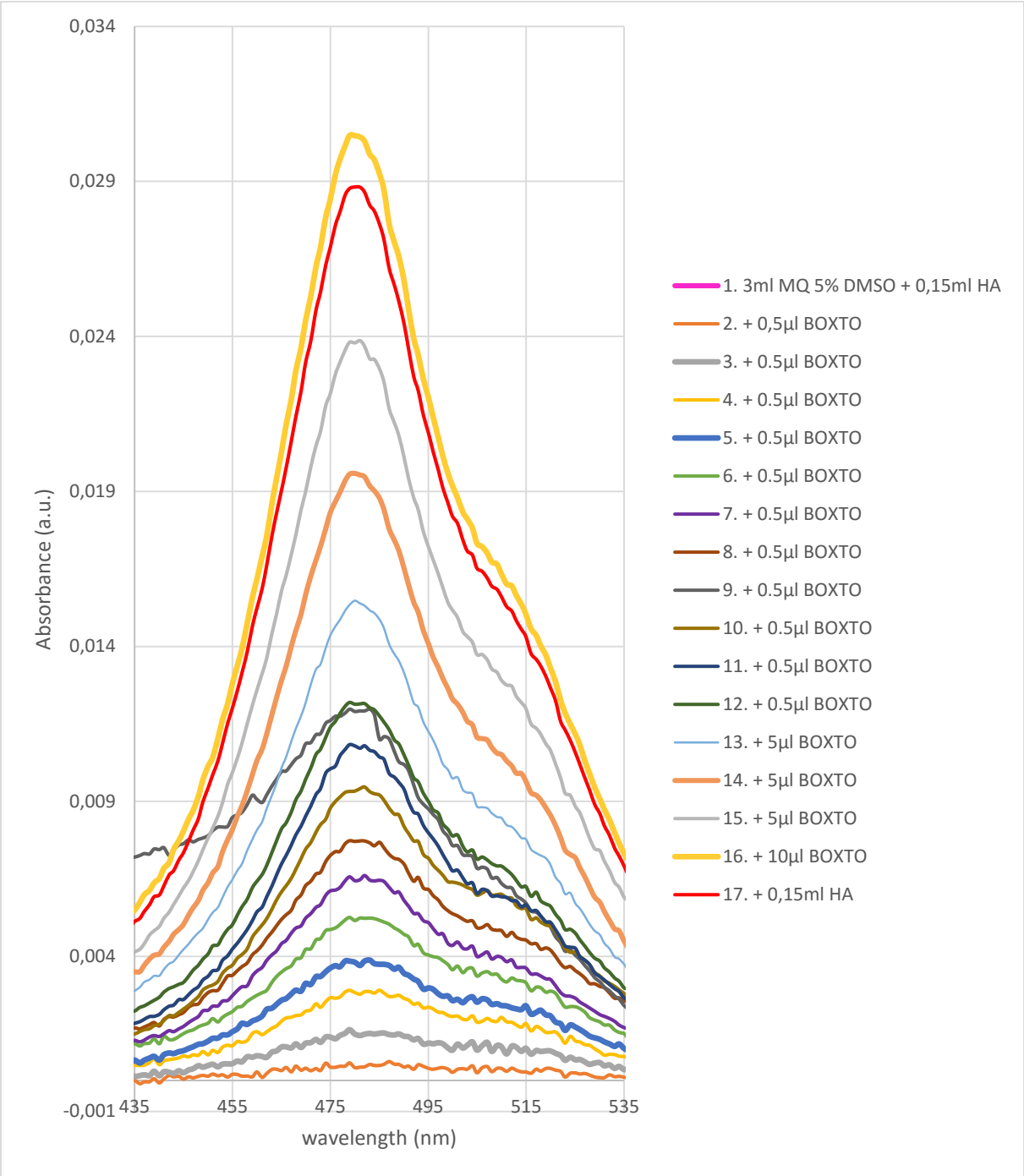

Figure 8. *Absorption spectra of different concentrations of BOXTO and a titration of BOXTO starts with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide and 0,15ml HA. Each step is a titration, meaning every step down the BOXTO/HA is added to the old sample. Last step an extra 0,15 ml HA was added. Total BOXTO added was 30,5µl.*

In figure 8 there are mixed results, but the usual trend can still be seen, that when you add more cyanine dye the absorption increases, that is basically the result here and the deviations are just that, deviations. In this BOXTO trial the addition of BOXTO was used for every sample instead of adding more HA because of trouble with dissolving the BOXTO properly. To know that the sample is fully diluted and not over-saturated the BOXTO was added to the bigger solution. This setup was also used to look at a promising dye from another direction. There was some data malfunction on the first curve, so it is not visible at the desired location, but it was formed under the second sample following the usual trend.

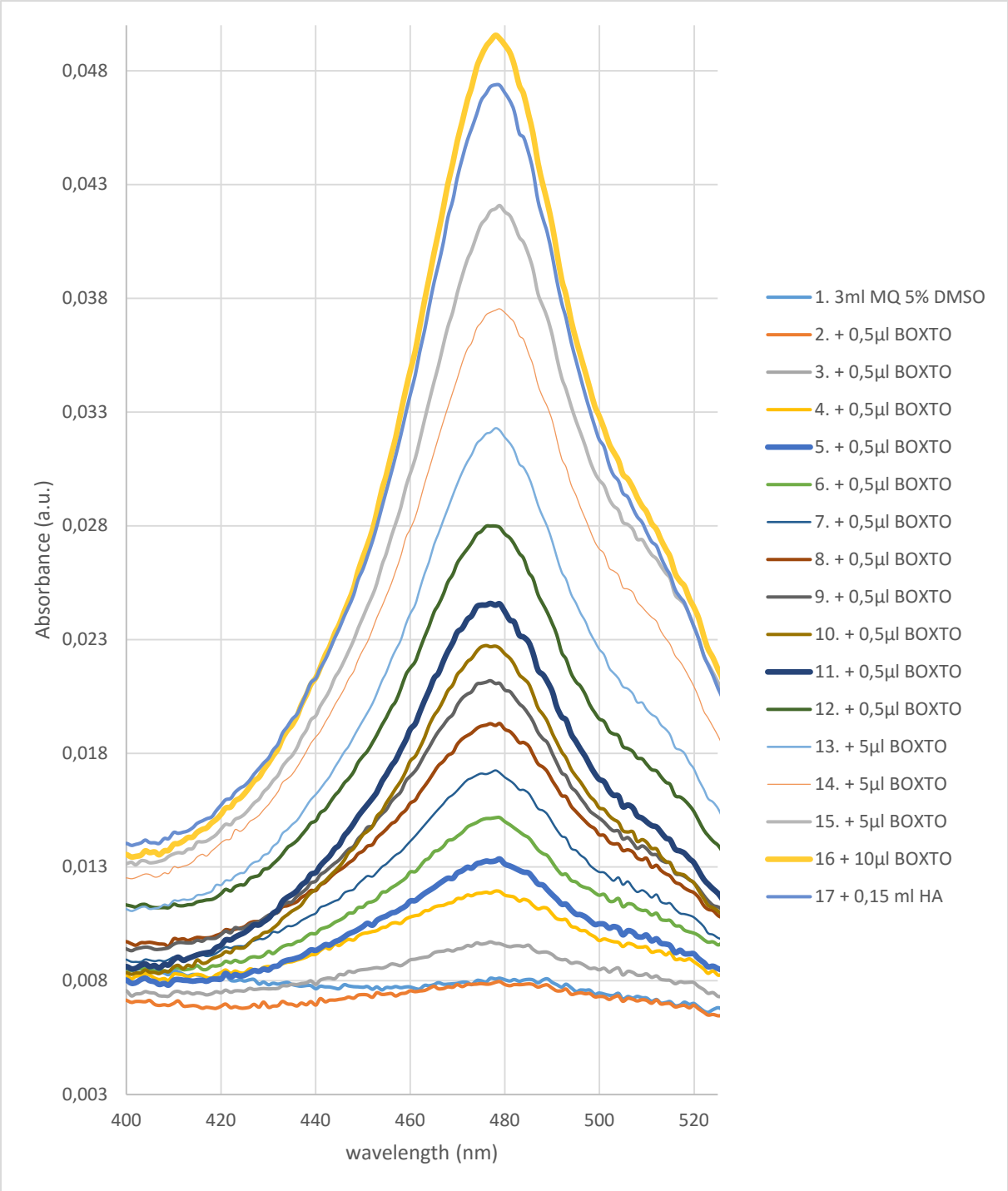

Figure 9. *Absorption spectra of a reference sample to BOXTO (figure 8.) Titration of BOXTO starts with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide and with no HA. Each step is a titration, meaning every step down the BOXTO/HA is added to the old sample. Last step an extra 0,15 ml HA was added. Total BOXTO added was 30,5µl.*

A reference sample was used to look at the differences if not any HA was added in figure 9. In the final sample there was addition of HA just as an experiment. The same results were given where the more dye you add the more absorbance there was. When the last sample was made by adding some HA in the end of the titration the trend was seen, again, of HA not aiding the absorbance but instead working as a dilution.

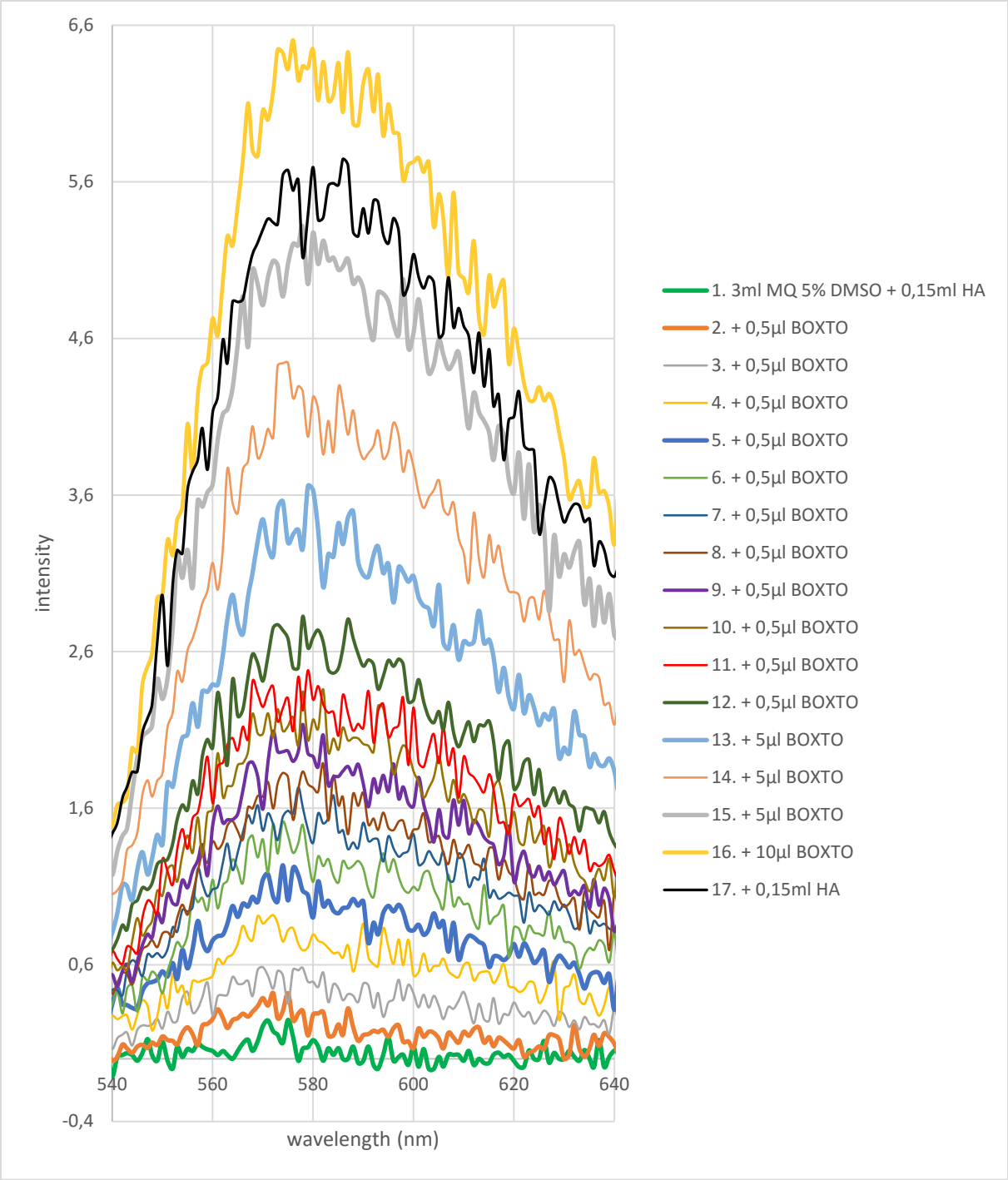

Figure 10. *Emission spectra of different concentrations of BOXTO and a titration of BOXTO starts at 15ml BOXTO and with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide. Each step is a titration, meaning every step down the BOXTO/HA is added to the old sample. Last step an extra 0,15 ml HA was added. Total BOXTO added was 30,5µl.*

In this first emission spectra, figure 10, that is connected to figure 8 we can see that the fluorescence of the dye is increasing the more dye you use. When then the final sample, sample 17, was added the trend of HA decreasing the fluorescence did not change. Just like figure 7 and figure 5, the intensity differences are very low and could be considered disturbance. This disturbance however is following a pattern so the conclusion that HA decreasing the intensity of BOXTO at these concentrations are still a valid conclusion.

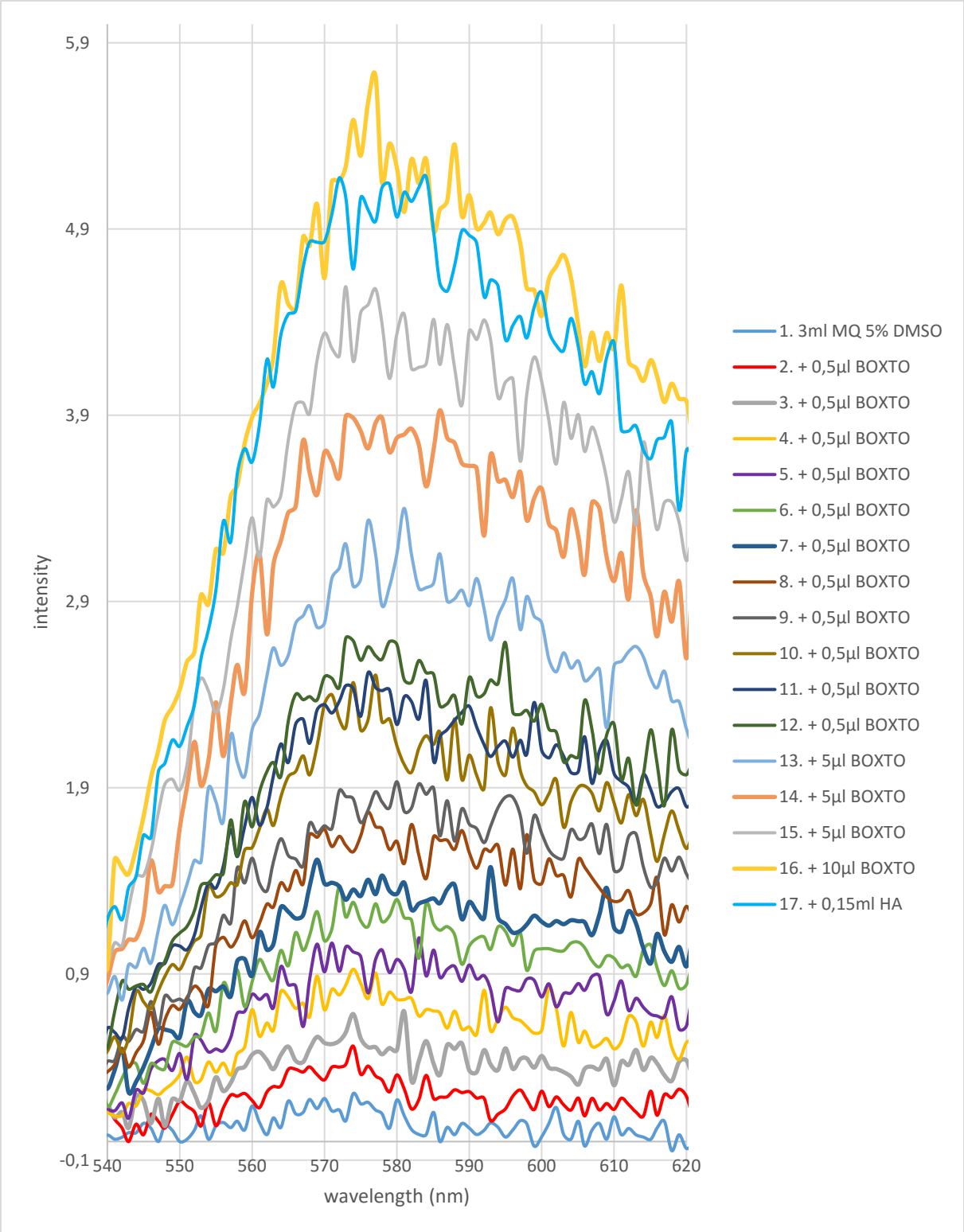

Figure 11. *Emission spectra of a reference sample to BOXTO (figure 7.). Titration of BOXTO starts at 0ml HA and with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide. Each step is a titration, meaning every step down the BOXTO/HA is added to the old sample. Last step an extra 0,15 ml HA was added. Total BOXTO added was 30,5µl.*

Figure 11 is following the same trend as figure 10 but with the difference of maximal intensity where actually in figure 10 there is a higher maximum intensity even though HA is added. There is a trend in all this even though the intensity is low, so some conclusions can be drawn but this difference in intensity maximum is hard to really consider anything other than just disturbance.

4.1.4 LB2 spectra

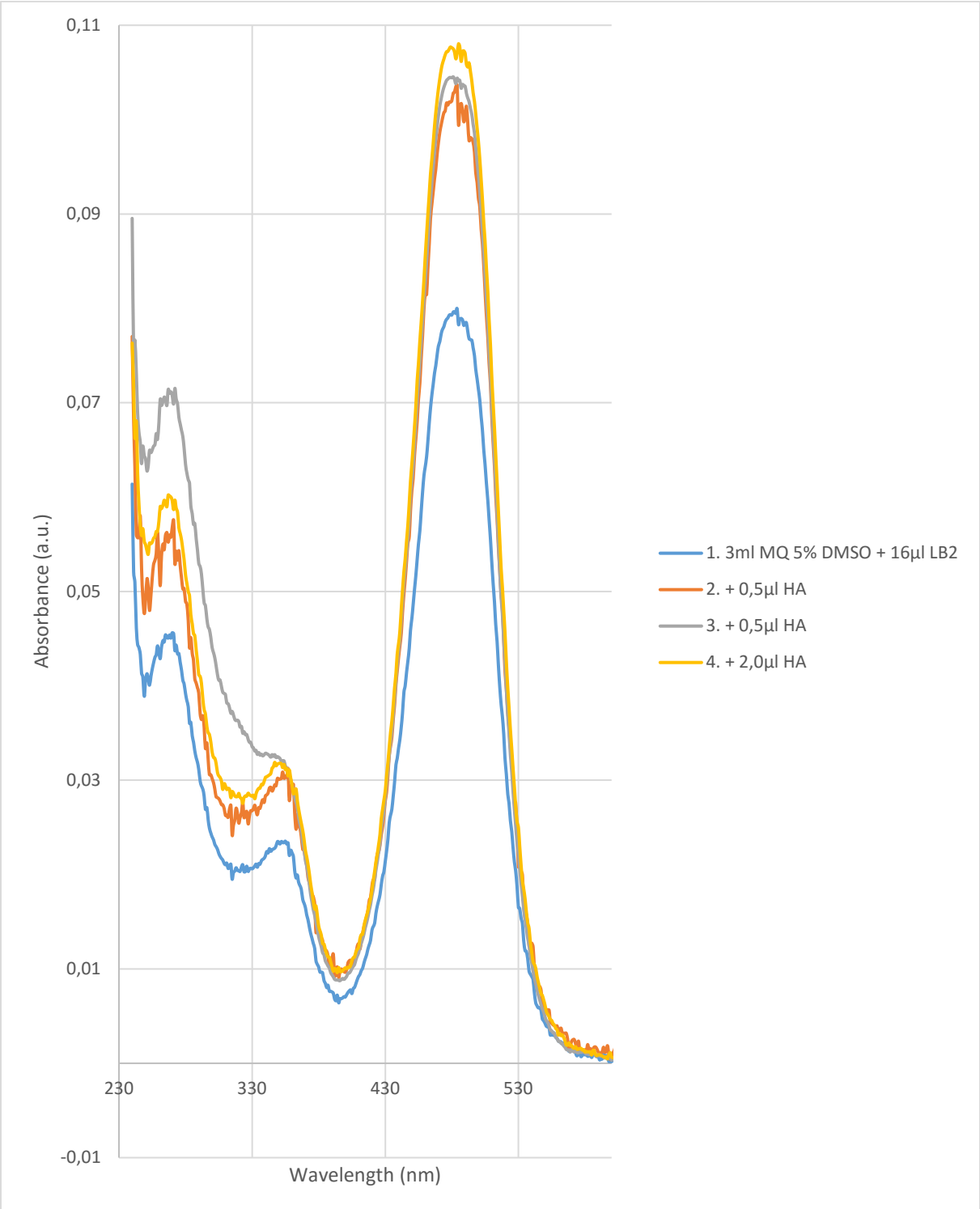

Figure 12. *Absorption spectra of different concentrations of HA and the titration of HA starts with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide and 16µl LB2. Each step is a titration, meaning every step down the HA is added to the old sample. Total HA added was 3µl.*

Here in figure 12 it is possible to see a different trend of the absorption, increasing with addition of HA. This is more of a positive trend, making this an interesting dye that could be used for further testing.

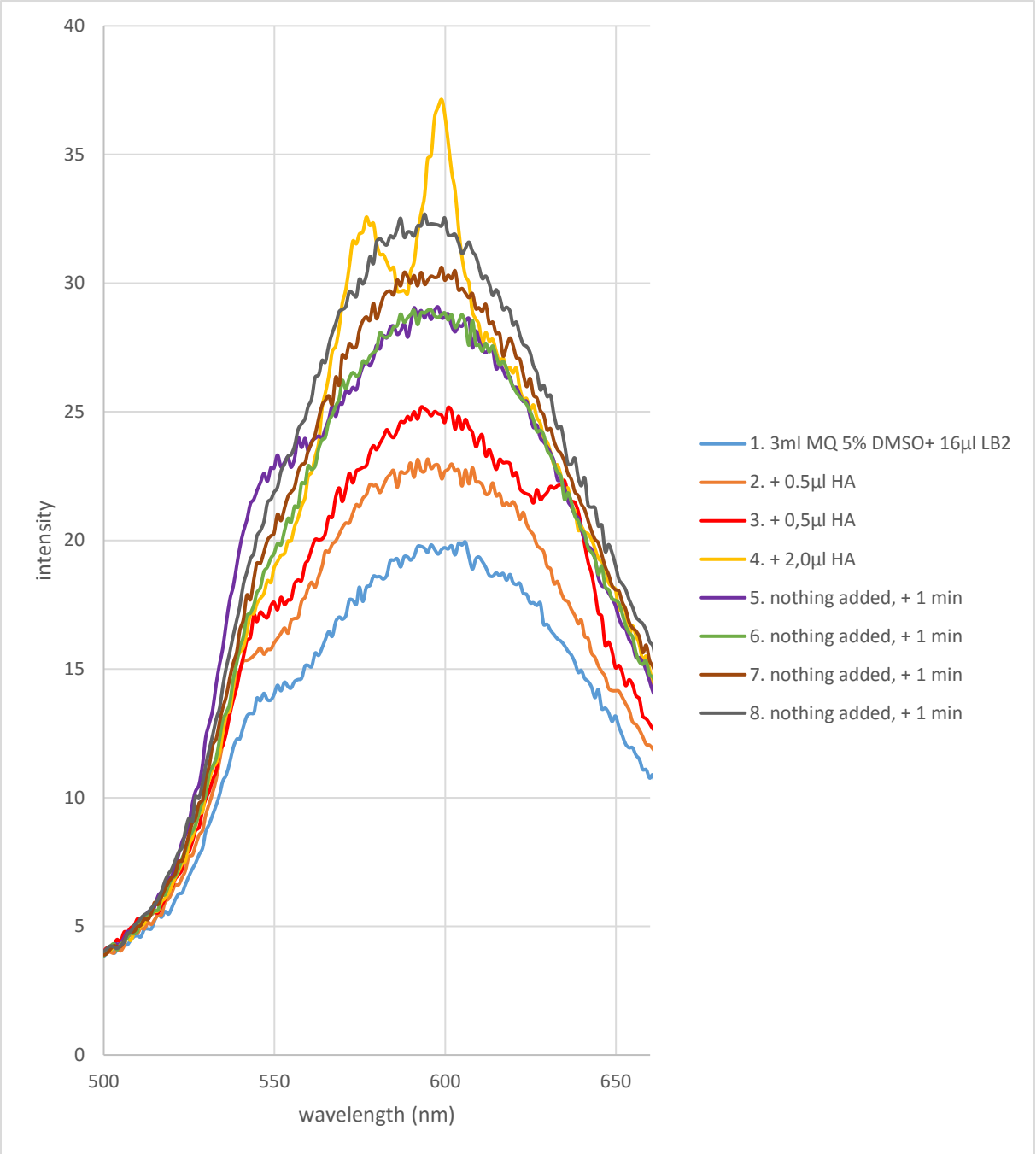

Figure 13. *Emission spectra of different concentrations of HA and the titration of HA starts with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide and 16µl LB2. Each step is a titration, meaning every step down the HA is added to the old sample. Last four steps nothing was added but the different measurements were taken one minute apart. Total HA added was 3µl.*

The emission spectrum of figure 13 is interesting and promising from the aim of increasing the fluorescence by adding HA. By adding more HA, the fluorescence increased with some weird dents forming in sample 3 and 4, this led to the possibility of the dye-HA-aggregation being time dependent. After the samples, samples 5 to 8 were taken, these are just taken at separate times, it was confirmed that the aggregates are time dependent. This makes it hard to monitor any real effect since there is already an unknown of how much concentration needed to make a substantial change in intensity but now there is the element of time. The current setup of the titration could not handle that effect. The results are promising and if the titration setup could be formed so that the time dependency would not affect the result this could be very interesting to further look at.

4.1.5 VK-1 spectra

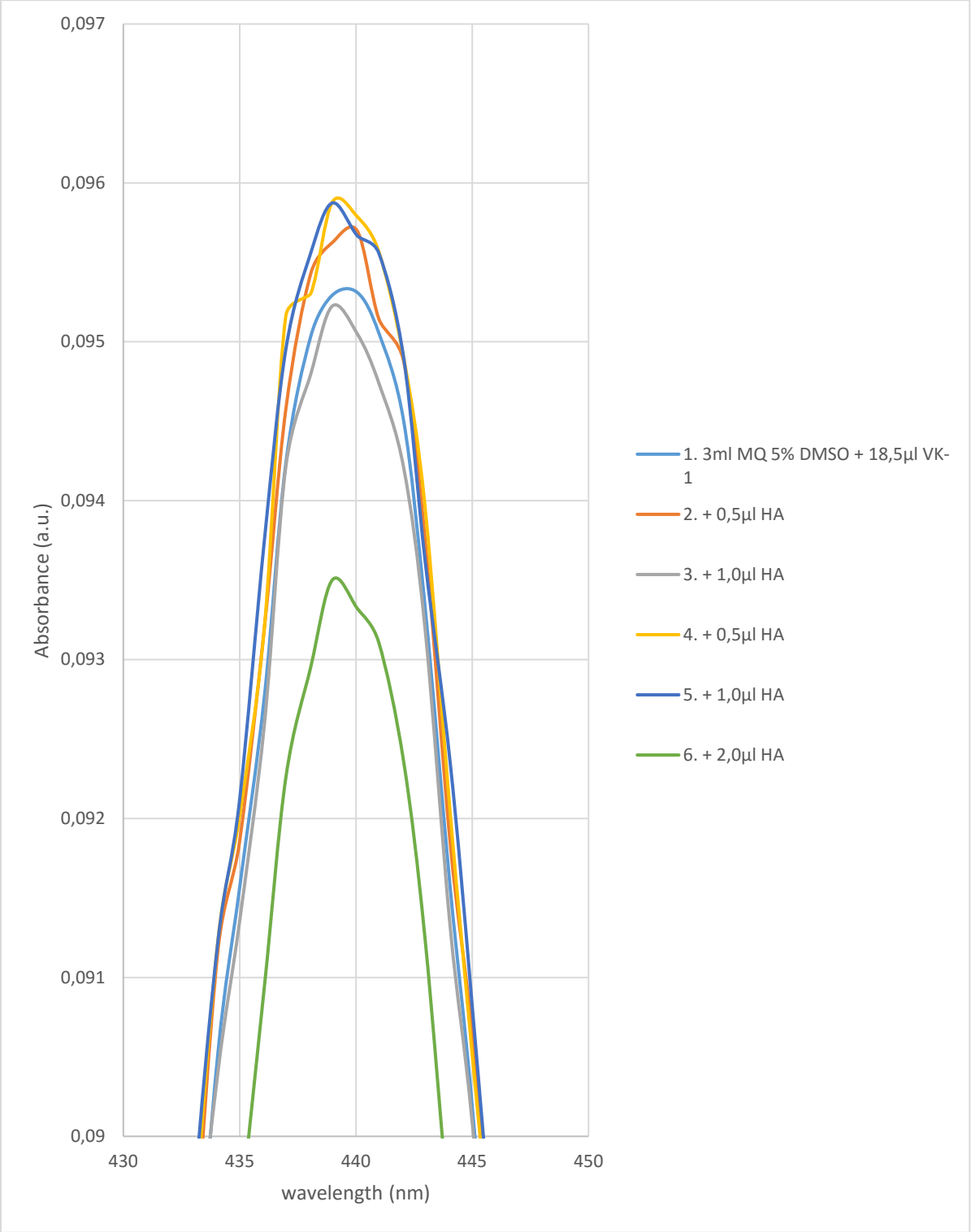

Figure 14. *Absorption spectra of different concentrations of HA and the titration of HA starts with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide and 18,5µl VK1. Each step is a titration, meaning every step down the HA is added to the old sample. Total HA added was 5µl.*

There is a typical decline in adsorption in figure 14 that could be due to dilution like in some of the previous figures. The last sample, sample 6, did however have a major decrease which could indicate some form of aggregation happening that lowers absorption or it could be a plateau.

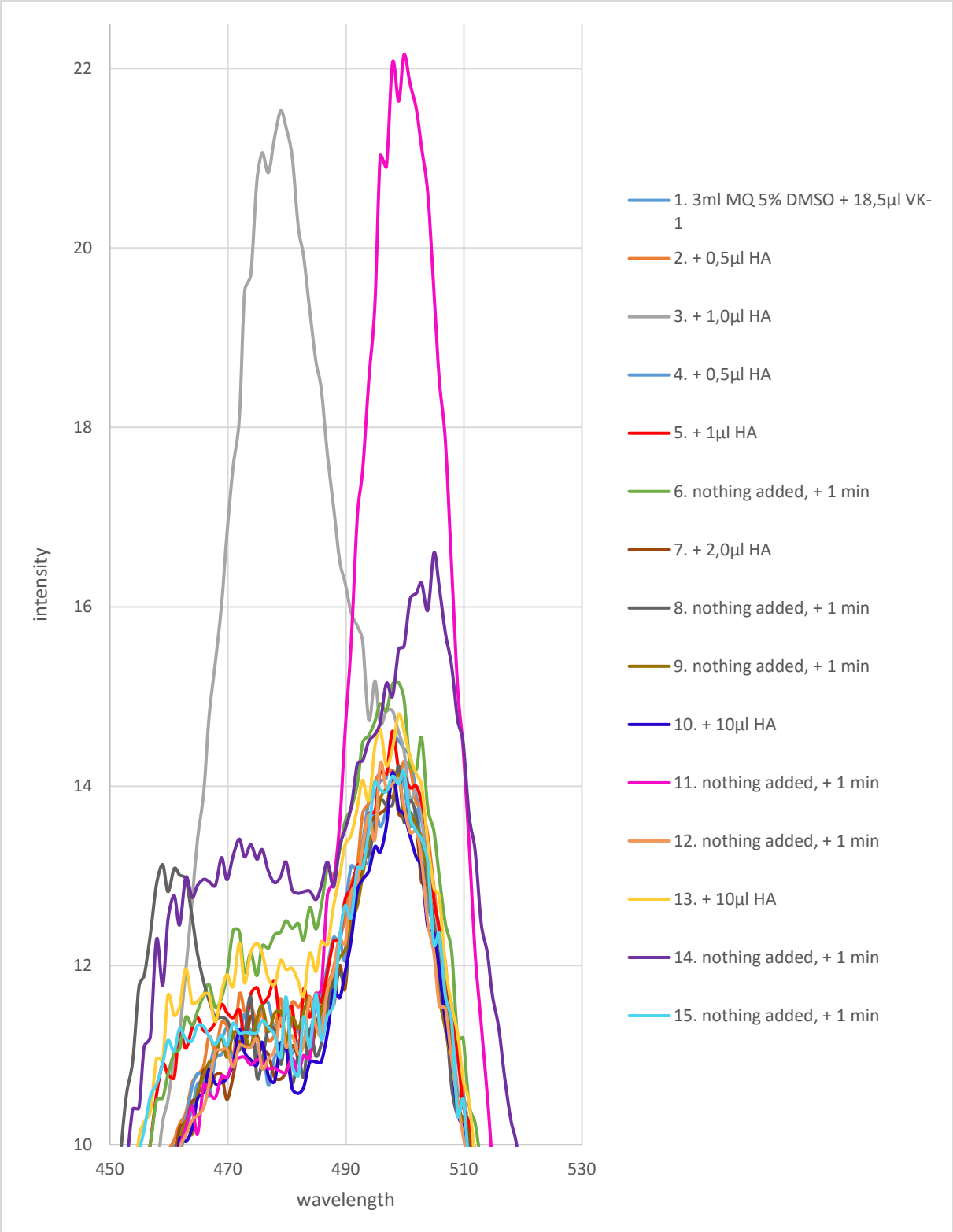

Figure 15. *Emission spectra of different concentrations of HA and the titration of HA starts with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide and 18,5µl VK-1. Each step is a titration, meaning every step down the HA is added to the old sample. Last two steps nothing was added but the different measurements were taken one minute apart. Total HA added was 25µl.*

In figure 15 there are both highs and lows where the intensity is very increased by the addition of HA but also decrease with the addition of HA. This particular dye as can be seen in sample 3, 11 and 14

there is a spike of intensity. If the steps of preparing the sample was done quick this kind of peak appeared leading to the conclusion that there is a very rapid change in aggregation with time. But as the other results the changes are very low and could still only be considered disturbance. Then again to see the same kind of peaks three times with three different concentrations would argue to say there is a positive result that it is time dependent and if the initial binding could be prolonged there would be a good result. The last steps 10 and 13 where not analyzed in figure 14, with respect to absorbance, to make the procedure faster to see the increased peaks and how the sample intensity varied with time in some form.

5 CONCLUSION

Firstly, after having a hard time finding high emission on any of the dyes used in this study the aim, merging the different good dyes to have all the good optical properties, was revoked. Instead following the instructions to reproduce the results from literature and synthesizing dye SL and VL so that high emission peaks might appear.

To summarize all the results, we can see that there are positives and negatives. The results are not close to the amount of intensity that is need for it to be called a conclusive result in that part of the experiments. But if we instead look at the trends between every result and the trends reoccurring in some results it is possible to conclude that the aggregation of the cyanine dyes with the HA is working in some situations. For example, in Figure 18 we can see there rather enlarged peaks if the procedure was done quick meaning it is working but the formation of the aggregates stops and reverse with time.

An exact explanation

6 FUTURE WORK

For future work it could be interesting to try the dyes with promising results, namely BOXTO, LB2 and VK-1 with other concentrations and with different buffer solutions. Not only that but also LB2 and VK-1 could be tested with a different method to eliminate the element of time dependency to get more conclusive results showing if they are suitable dyes to be used in the inhibition and localization of cancer. There are a lot of possibilities for the future since there are many parameters that can decide the results, therefor some of the dyes, like TO, is a dye that should have continued work on because of the positive results found in literature.

7 ACKNOWLEDGEMENTS

I would like to thank the following people and departments who have helped me with this project:

Gunnar Westman, for his advice and guidance throughout the project giving ideas and help with solving problems.

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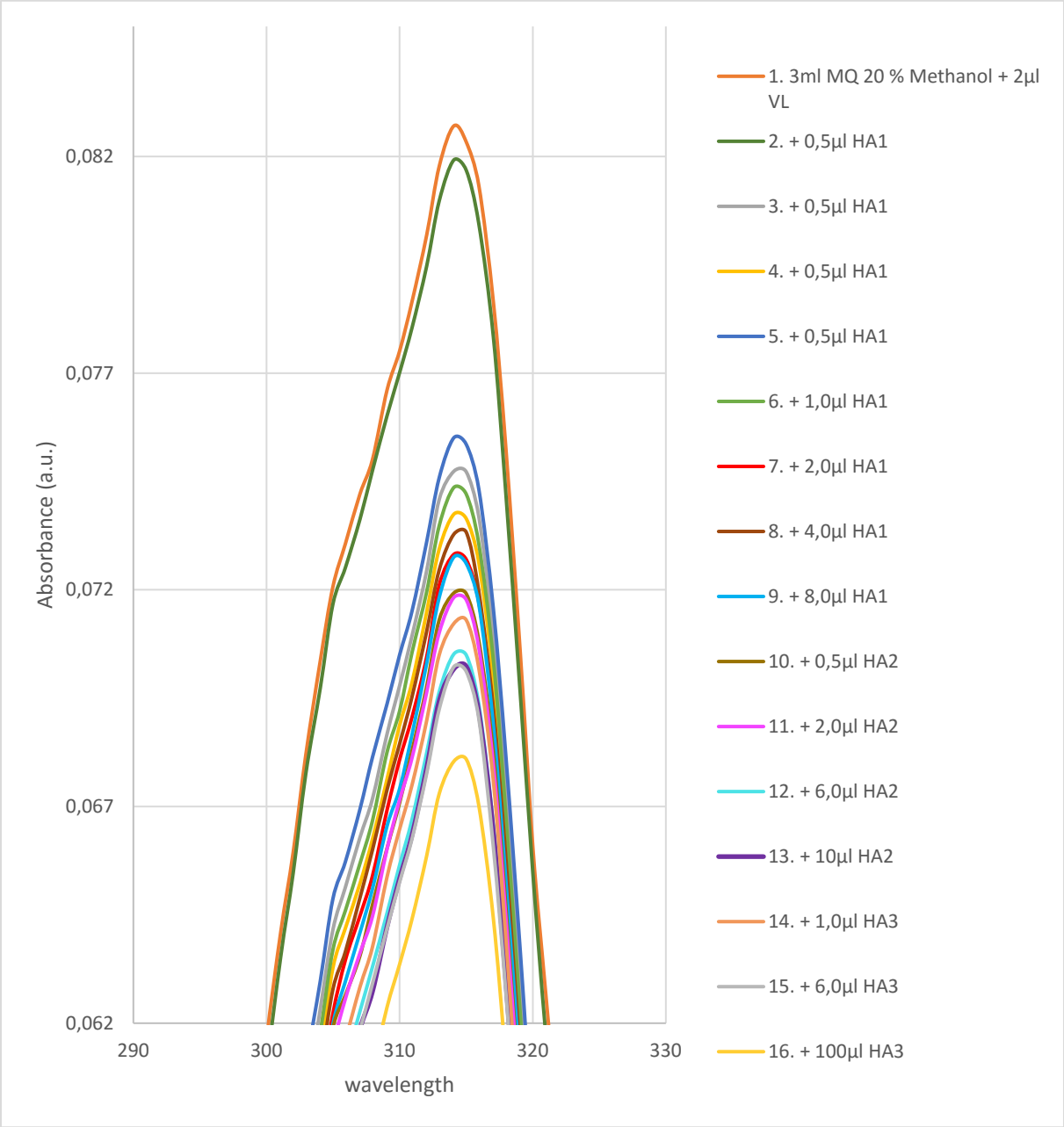
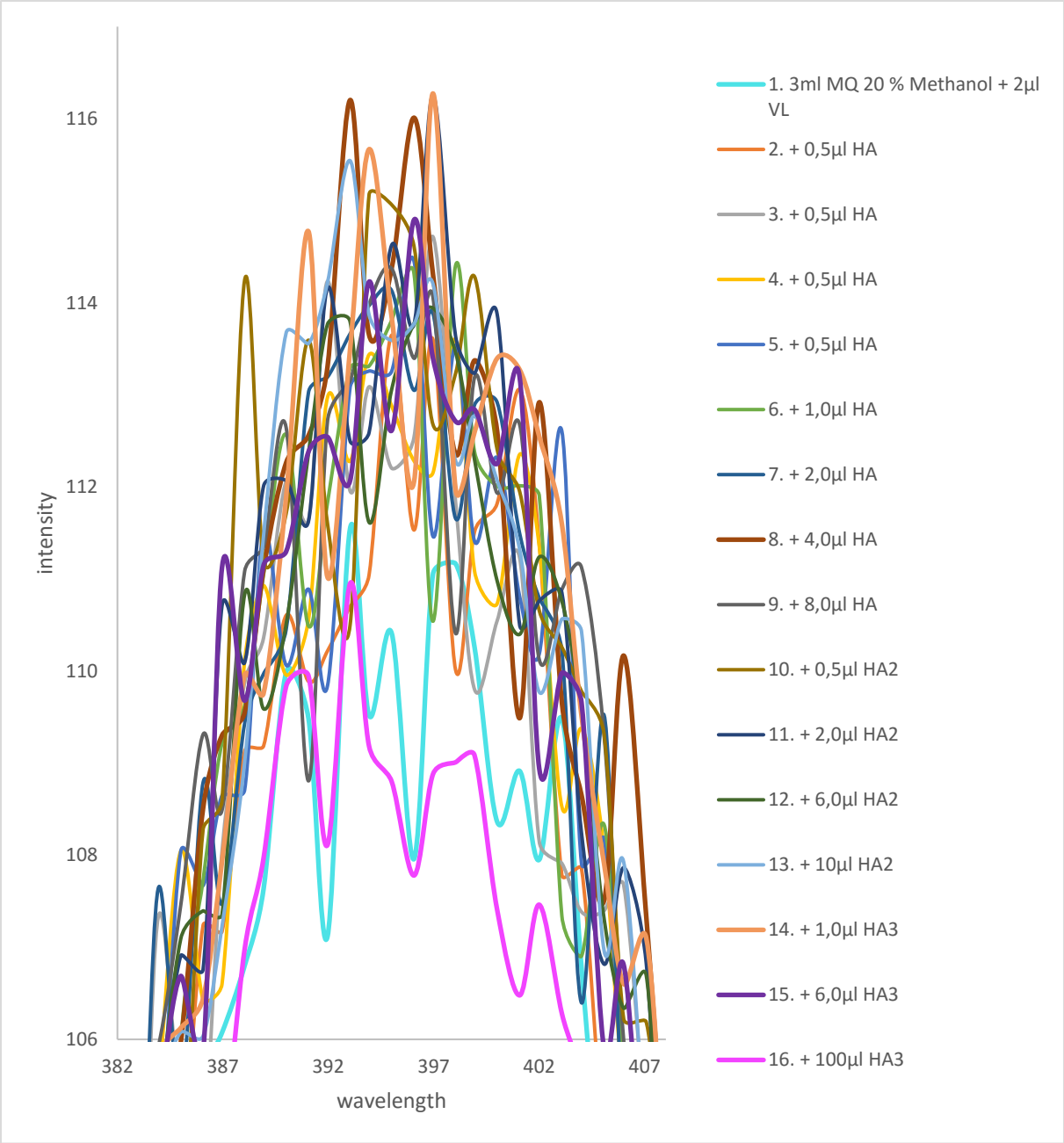
9 APPENDIX

9.1 Extra fluorescent spectra

9.1.1 VL spectra

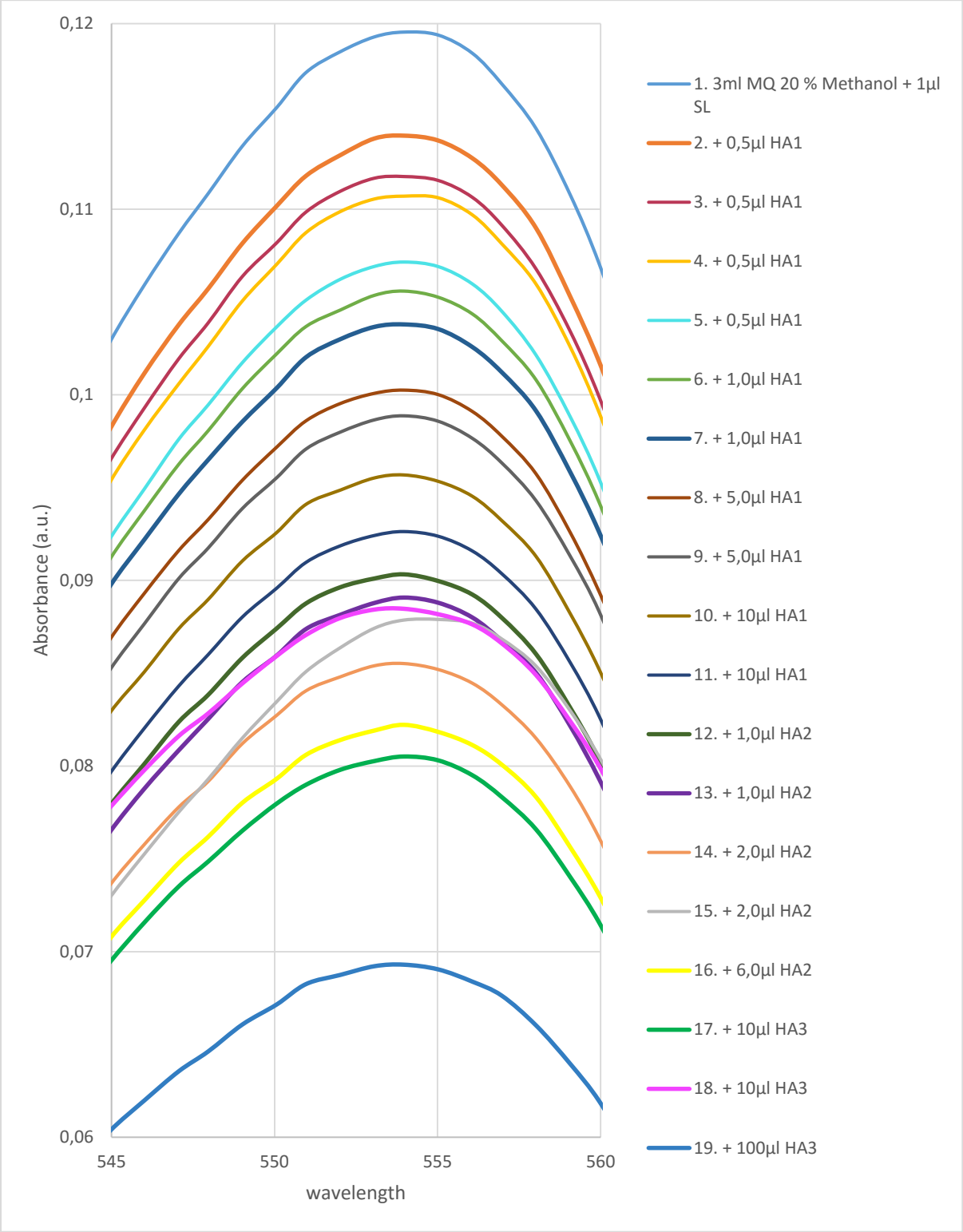


Appendix 1. *Absorption spectra of different concentrations of HA and the titration of HA starts with 3 ml of 80% MilliQ-water + 20% methanol and 2µl VL. Each step is a titration, meaning every step down the HA is added to the old sample. Three different concentrations of HA were used. HA1 was 6,5*10^{-11}M, HA2 was 5*10^{-6}M, HA3 was 3,8*10^{-4}. Total HA1 added was 17µl, total HA2 added was 18,5µl, total HA3 added was 107µl.*

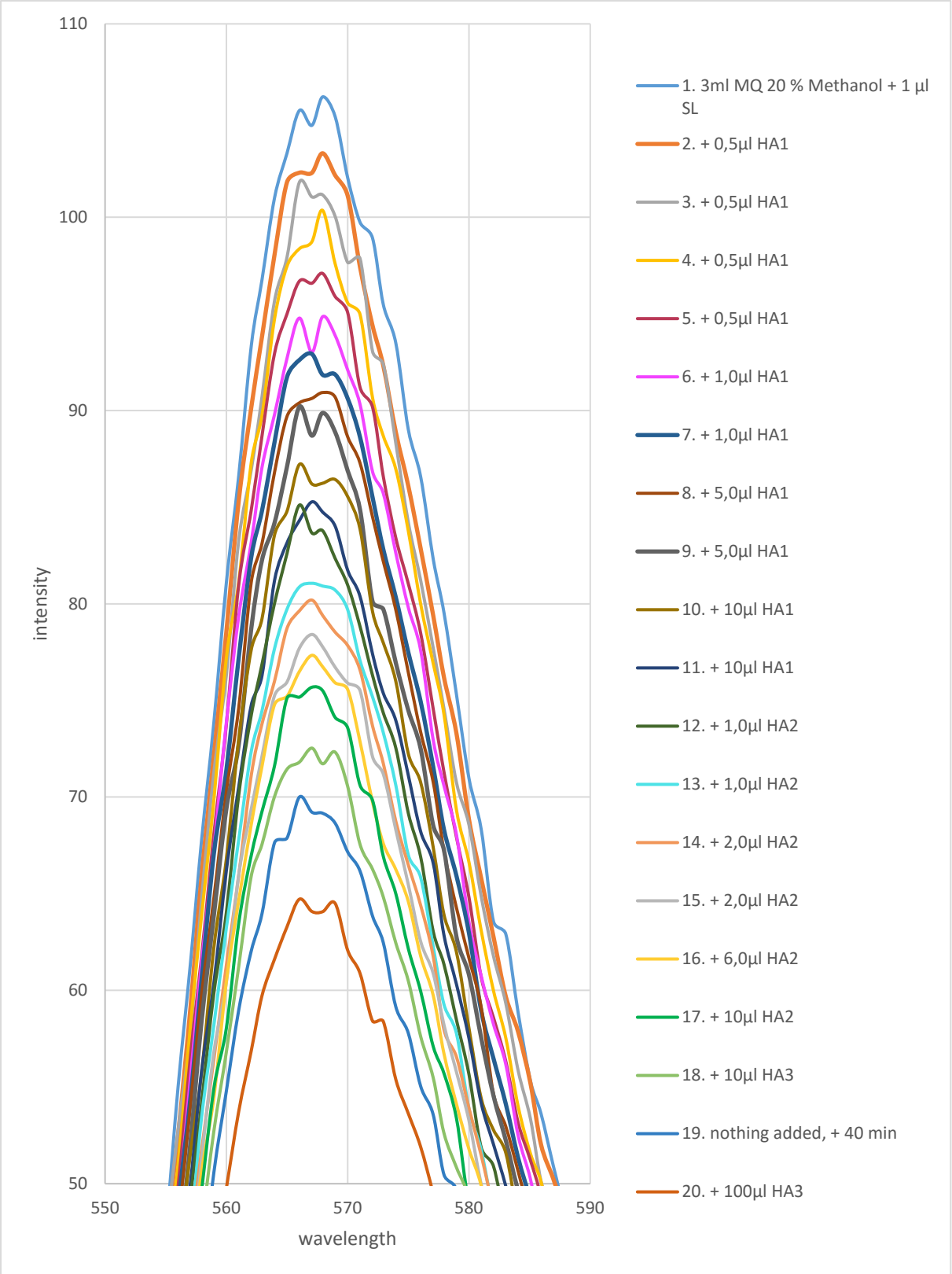


Appendix 2. Emission *spectra of different concentrations of HA and the titration of HA starts with 3 ml of 80% MilliQ-water + 20% methanol and 2µl VL. Each step is a titration, meaning every step down the HA is added to the old sample. Three different concentrations of HA were used. HA1 was 6,5*10^{-11}M, HA2 was 5*10^{-6}M, HA3 was 3,8*10^{-4}. Total HA1 added was 17µl, total HA2 added was 18,5µl, total HA3 added was 107µl.*

9.1.2 SL spectra

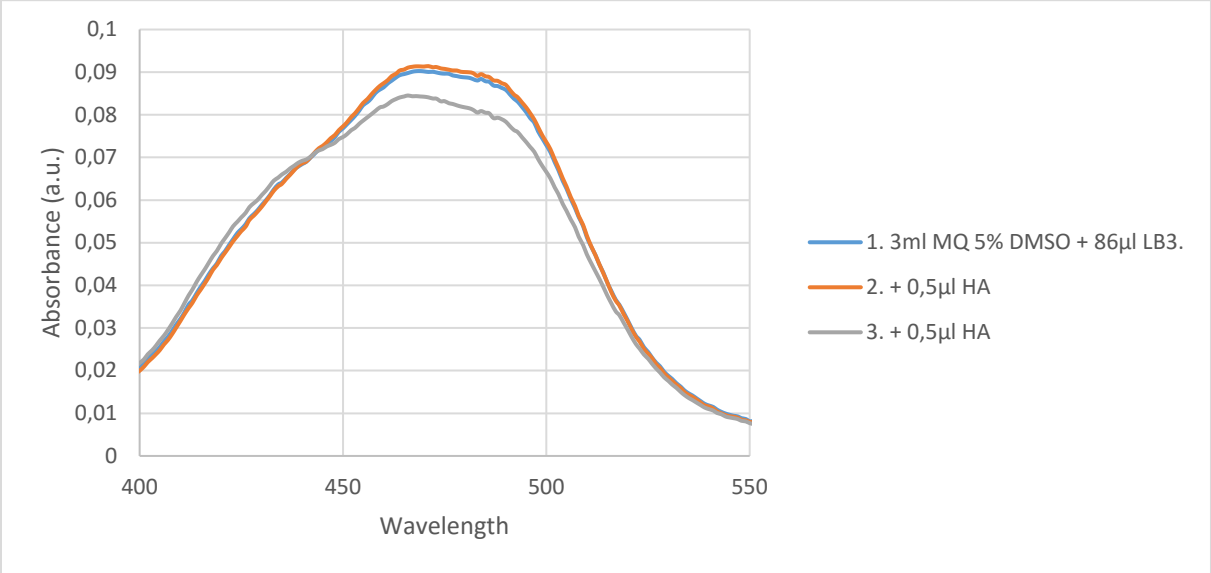


Appendix 3. *Absorption spectra of different concentrations of HA and the titration of HA starts with 3 ml of 80% MilliQ-water + 20% methanol and 1µl VL. Each step is a titration, meaning every step down the HA is added to the old sample. Three different concentrations of HA were used. HA1 was 6,5*10^{-11}M, HA2 was 5*10^{-6}M, HA3 was 3,8*10^{-4}. Total HA1 added was 34µl, total HA2 added was 12µl, total HA3 added was 120µl.*

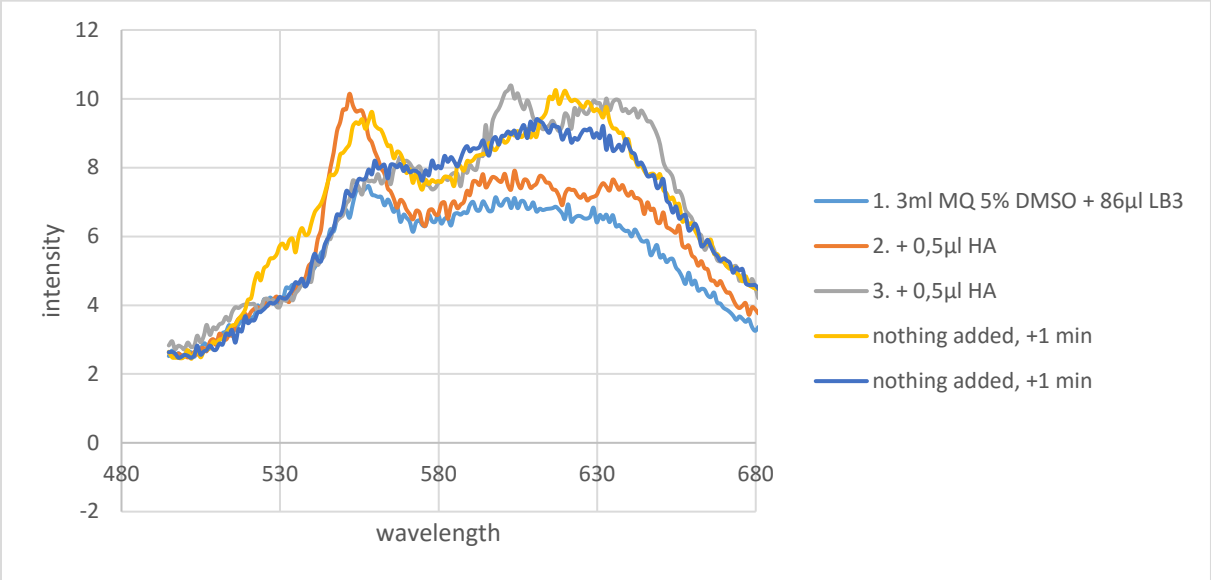


Appendix 4. *Emission spectra of different concentrations of HA and the titration of HA starts with 3 ml of 80% MilliQ-water + 20% methanol and 1µl SL. Each step is a titration, meaning every step down the HA is added to the old sample. Three different concentrations of HA were used. HA1 was 6,5*10^{-11}M, HA2 was 5*10^{-6}M, HA3 was 3,8*10^{-4}. Total HA1 added was 34µl, total HA2 added was 12µl, total HA3 added was 120µl.*

9.1.3 LB3 spectra

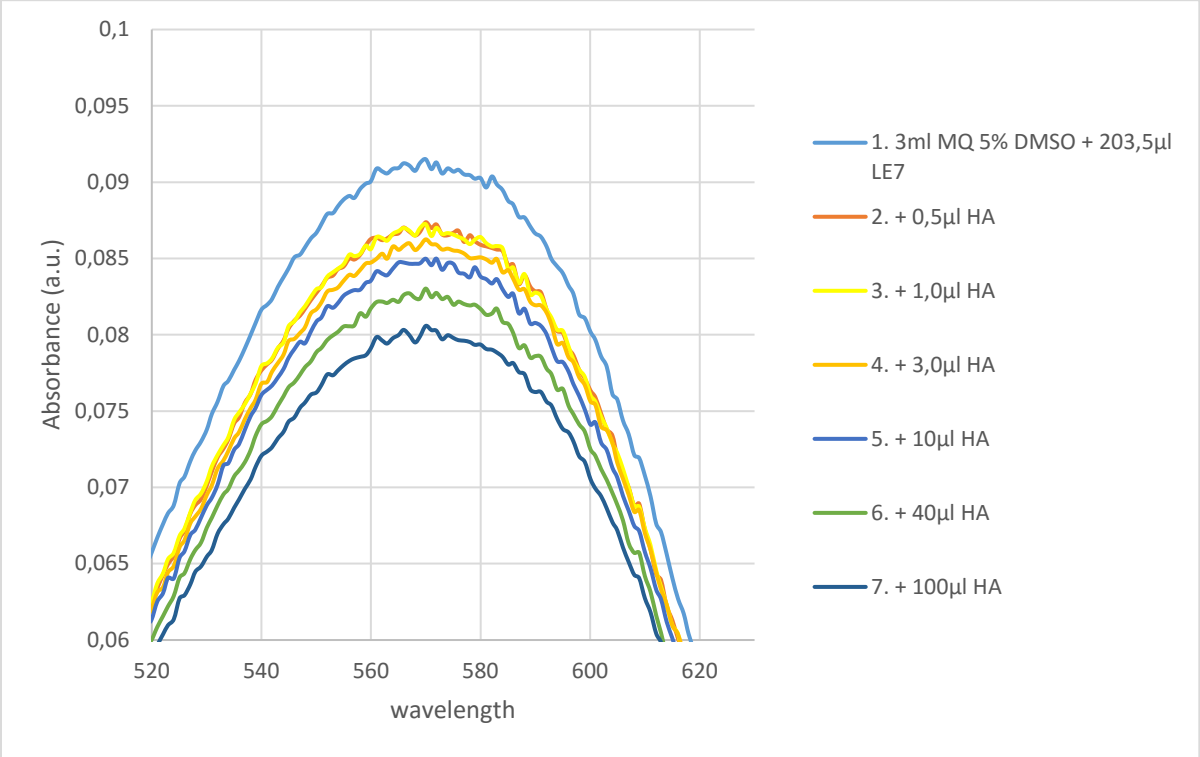


Appendix 5. *Absorption spectra of different concentrations of HA and the titration of HA starts with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide and 86µl LB3. Each step is a titration, meaning every step down the HA is added to the old sample. Total HA added was 1µl.*

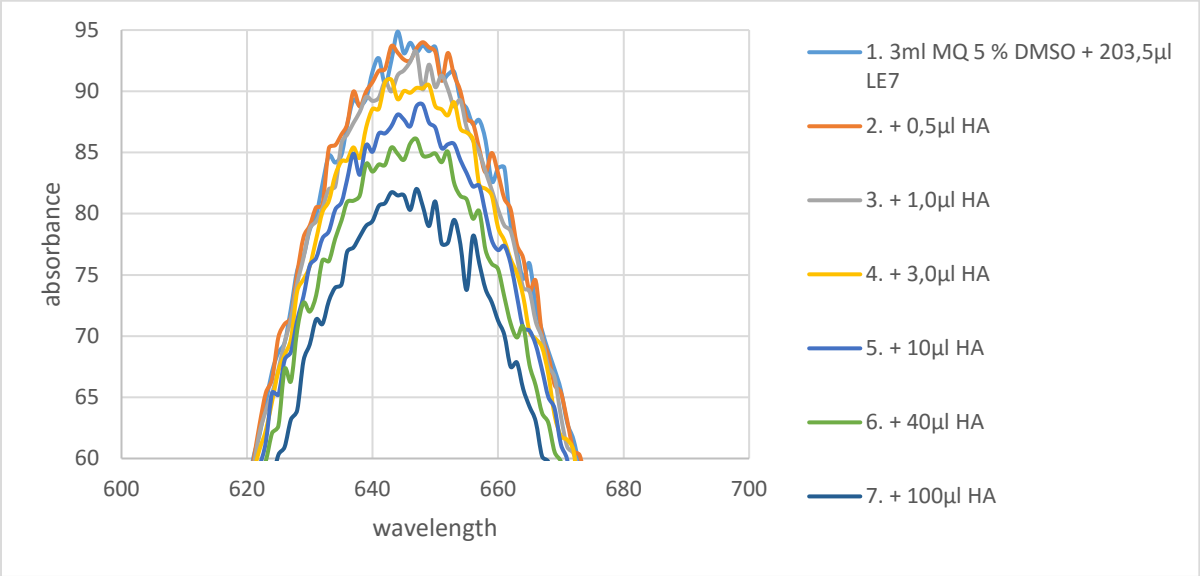


Appendix 6. *Emission spectra of different concentrations of HA and the titration of HA starts with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide and 86µl LB3. Each step is a titration, meaning every step down the HA is added to the old sample. Last two steps nothing was added but the different measurements were taken one minute apart. Total HA added was 1µl.*

9.1.4 LE7 spectra

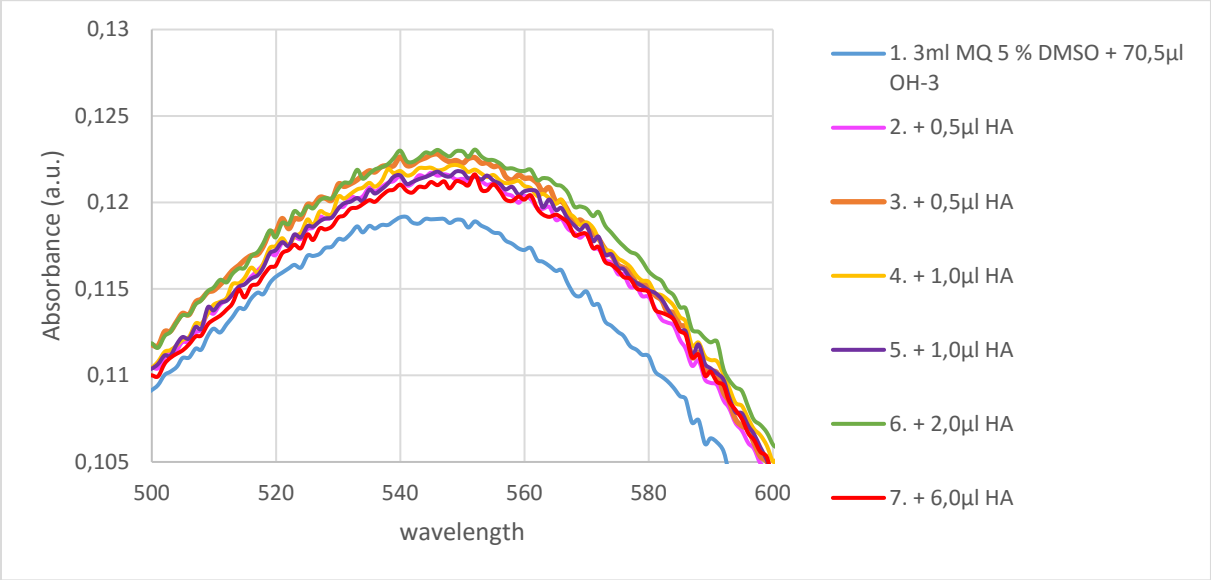


Appendix 7. *Absorption spectra of different concentrations of HA and the titration of HA starts with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide and 203,5µl LE7. Each step is a titration, meaning every step down the HA is added to the old sample. Total HA added was 154,5µl.*

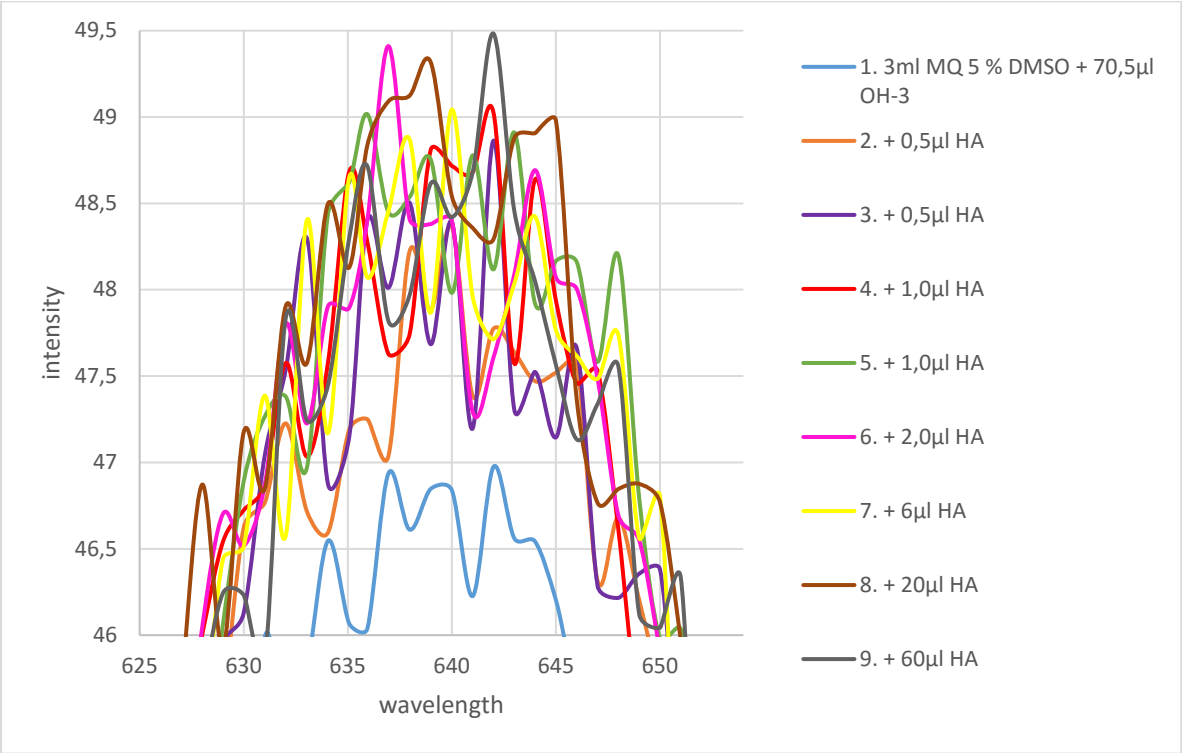


Appendix 8. *Emission spectra of different concentrations of HA and the titration of HA starts with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide and 203,5µl LE7. Each step is a titration, meaning every step down the HA is added to the old sample. Last two steps nothing was added but the different measurements were taken one minute apart. Total HA added was 154,5µl.*

9.1.5 OH-3 spectra

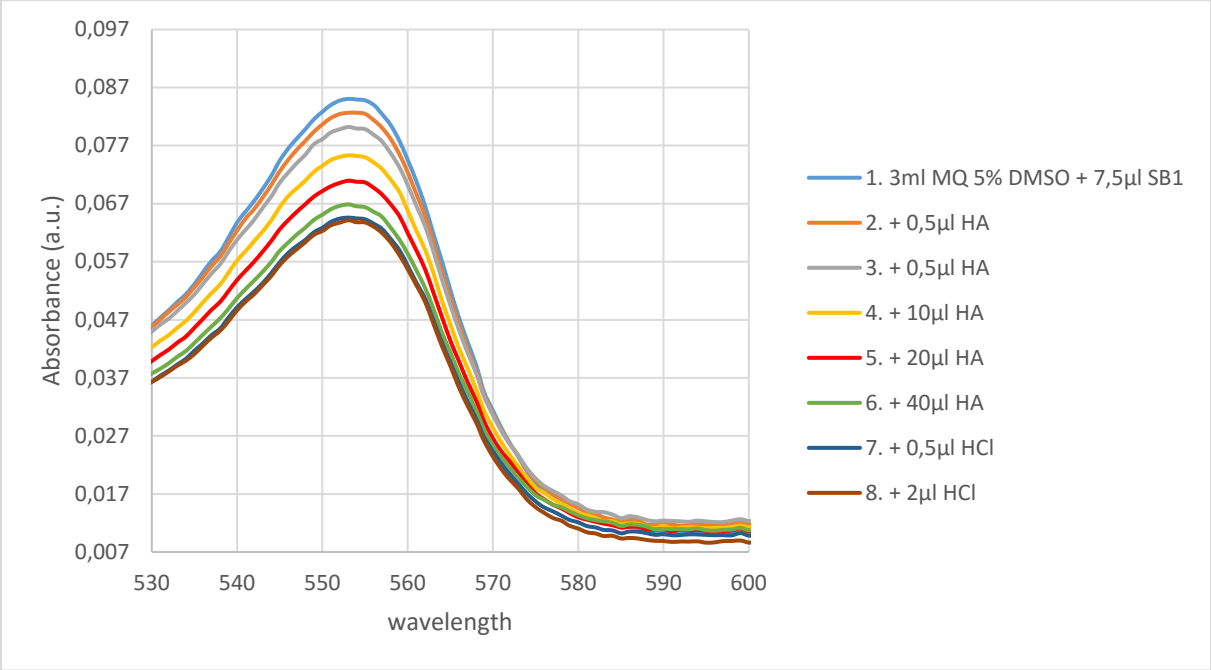


Appendix 9. *Absorption spectra of different concentrations of HA and the titration of HA starts with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide and 70,5µl OH-3. Each step is a titration, meaning every step down the HA is added to the old sample. Total HA added was 11µl.*

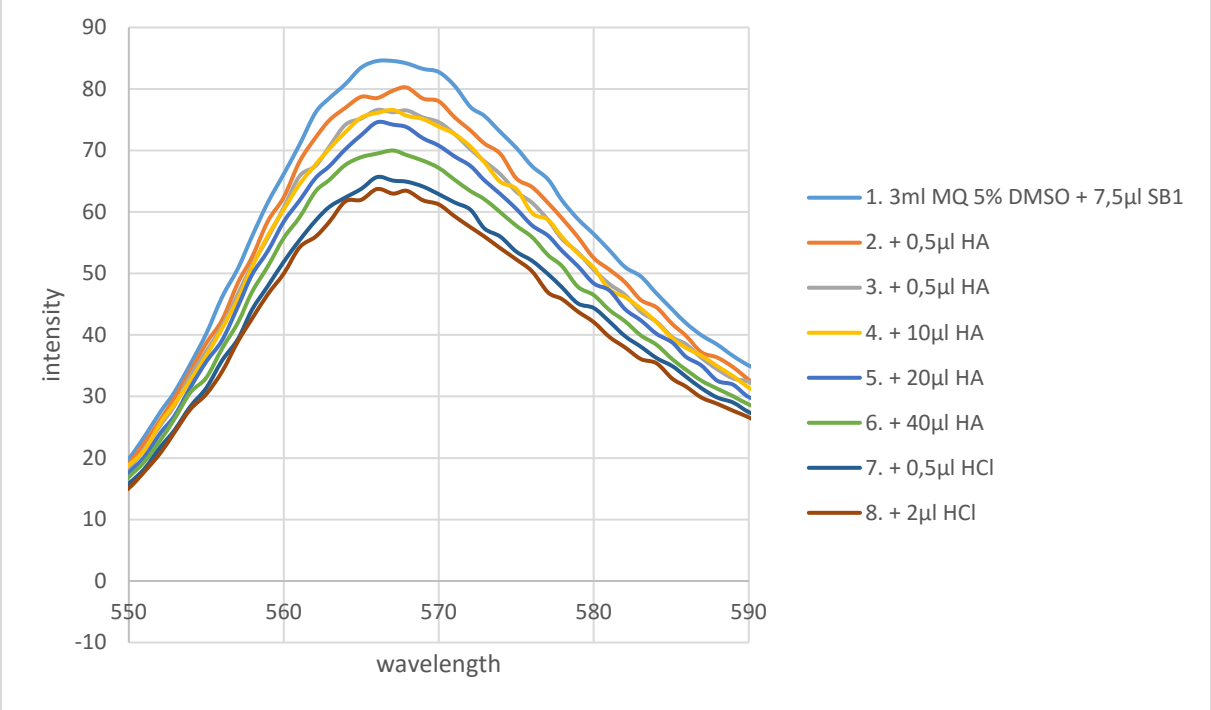


Appendix 10. *Emission spectra of different concentrations of HA and the titration of HA starts with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide and 70,5µl OH-3. Each step is a titration, meaning every step down the HA is added to the old sample. Last two steps nothing was added but the different measurements were taken one minute apart. Total HA added was 91µl. The last 2 samples where not observed in figure 15 due to technical difficulties but the trend was the same.*

9.1.6 SB1 spectra

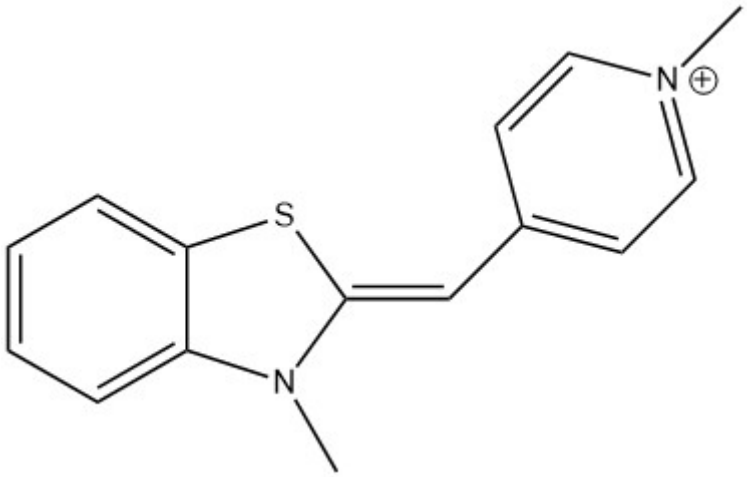


Appendix 11. *Absorption spectra of different concentrations of HA and the titration of HA starts with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide and 7,5µl SB1. Each step is a titration, meaning every step down the HA is added to the old sample. Last two steps extra HCl was added. Total HA added was 71µl.*

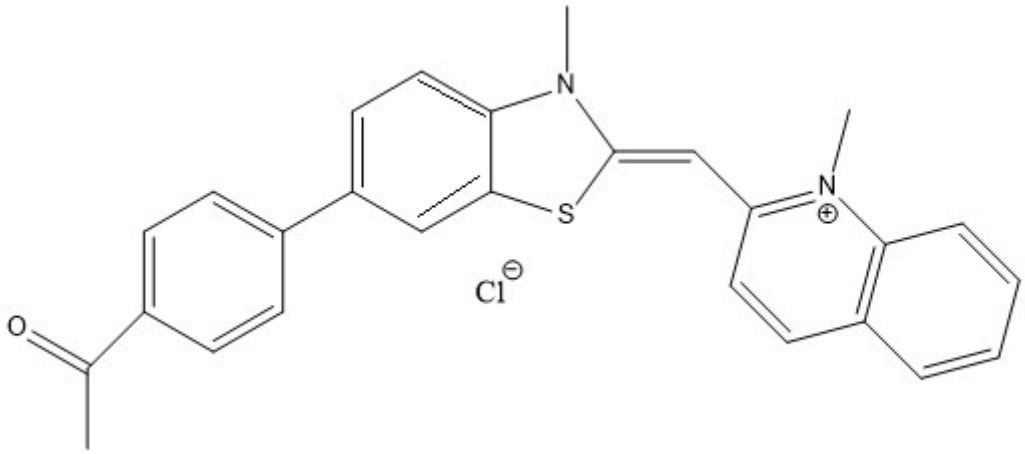


Appendix 12. *Emission spectra of different concentrations of HA and the titration of HA starts with 3 ml of 95% MilliQ-water + 5% Dimethyl sulfoxide and 7,5µl SB1. Each step is a titration, meaning every step down the HA is added to the old sample. Last two steps extra HCl was added. Total HA added was 71µl.*

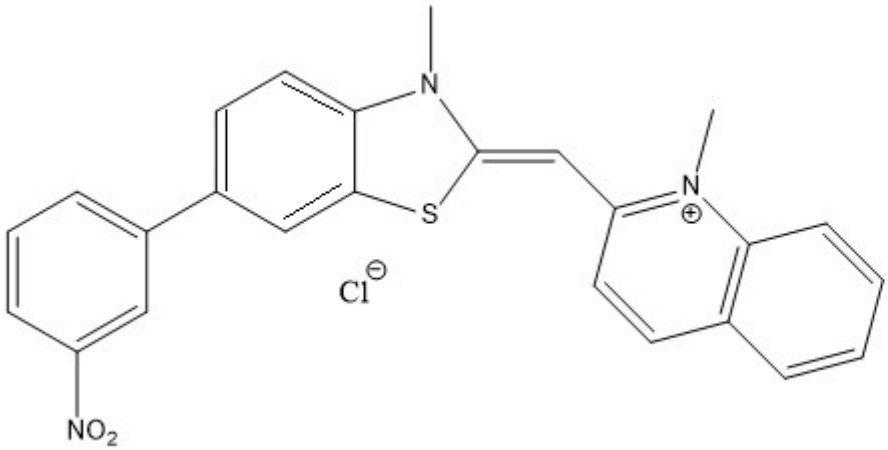
9.2 Structure of dyes investigated



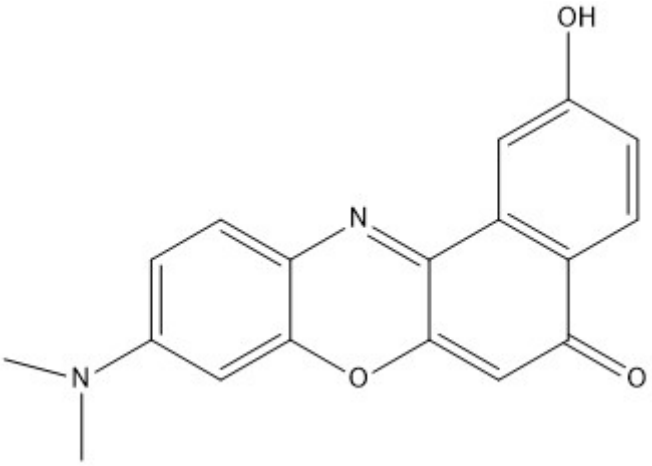
Appendix 13. *Structure of BOXTO*



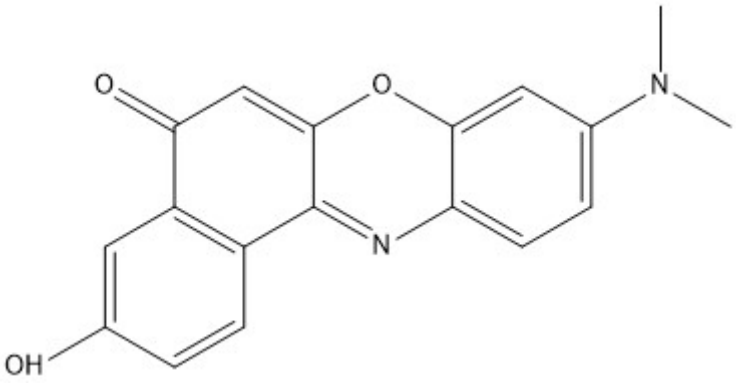
Appendix 14. *Structure of LB2*



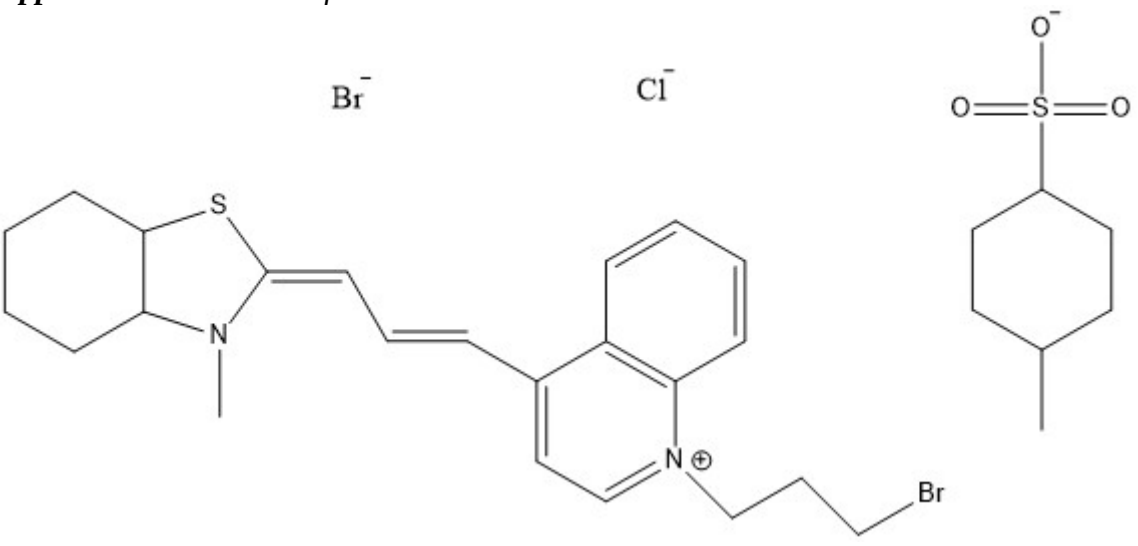
Appendix 15. *Structure of LB3*



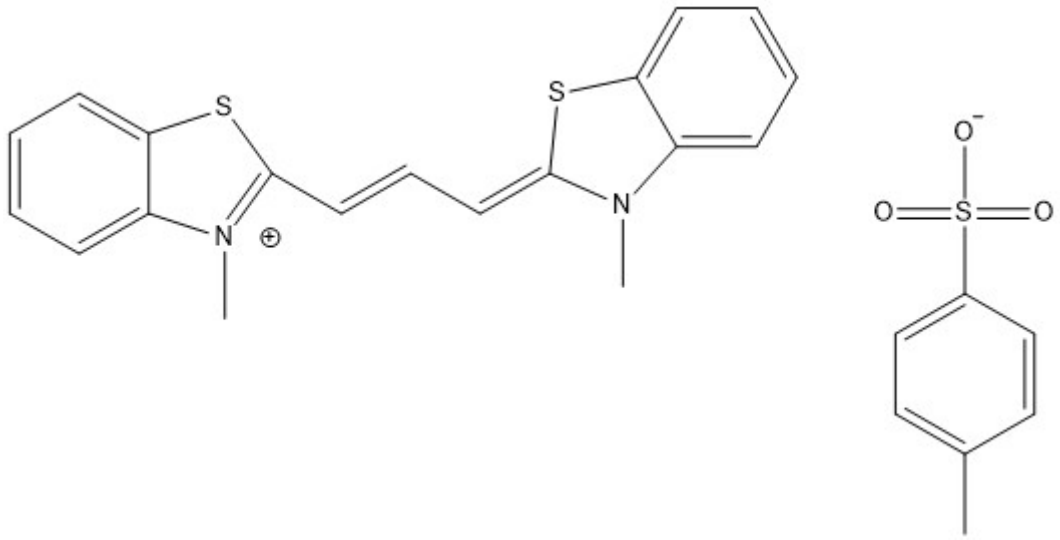
Appendix 16. *Structure of LE7*



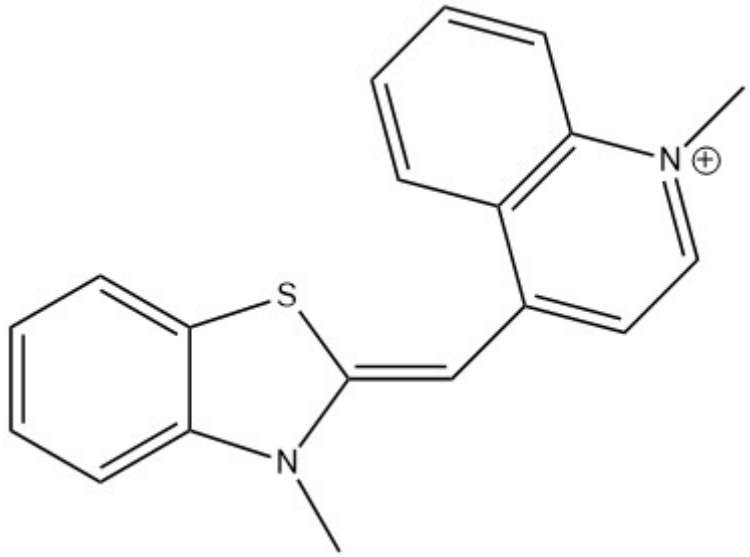
Appendix 17. *Structure of OH-3*



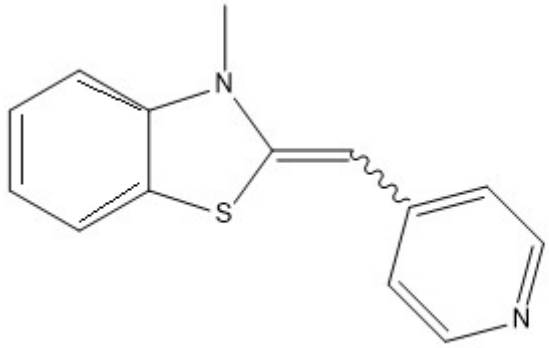
Appendix 18. *Structure of SB1*



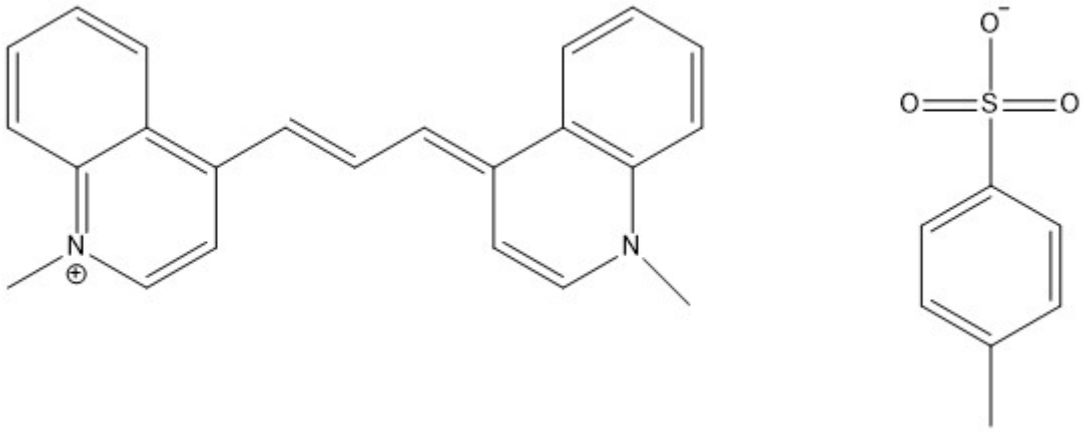
Appendix 19. *Structure of SL1*



Appendix 20. *Structure of TO*



Appendix 21. *Structure of VK-1*



Appendix 22. *Structure of VL1*

9.3 Concentrations and molar mass for cyanine dyes and solvents in the experiments

HA was calculated with the molar mass 776,651g/mole

4.1.1: TO: 0,001M and 476,61g/mole; HA: 0,6mM

4.1.2: BOXTO: 1mM and 426,55g/mole; HA: 0,6mM

4.1.3: BOXTO: 15,6mM and 426,55 g/mole; HA 1mM

4.1.4: LB2: 2,76mM and 458,518g/mole; HA 1mM

4.1.5: VK-1: 4,374mM and 240,065g/mole; HA 1mM

9.1.1: VL: 5µM and 495g/mole; HA1:6,5*10^{11}M, HA2: 5µM, HA3: 0,38mM

9.1.2: SL: 5µM and 388g/mole; HA1:6,5*10^{11}M, HA2: 5µM, HA3: 0,38mM

9.1.3: LB3: 7,22mM and 461,518g/mole; HA: 1mM

9.1.4: LE7: 1,38mM and 306g/mole; HA: 1mM

9.1.5. OH-3: 3,676mM and 306g/mole; HA: 1mM

9.1.6. SB1: 0,6mM and 515g/mole; HA: 1mM