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THE REACTION OF NINHYDRIN AND 1,2- INDANEDIONE WITH GLYCINE ON WHATMAN'S FILTER PAPER

By

Mark Steven Bliss

A THESIS

Submitted to
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2003

ABSTRACT

THE REACTION OF NINHYDRIN AND 1,2- INDANEDIONE WITH GLYCINE ON WHATMAN FILTER PAPER

By

Mark Steven Bliss

Using sequential dilutions of the amino acid glycine, and of either ninhydrin or 1,2-indanedione as the chemical developer, the stoichiometry of the reactants will be investigated based on the color produced on filter paper. The characteristic evaluated in ninhydrin will be its Ruhemann's Purple color using visual observation, a UV-Vis Spectrophotometer, and a Xerox photocopier. For 1,2-indanedione, fluorescence will be evaluated by a fluorescence spectrophotometer, and visually by an alternate light source.

Also in this study the dilutions of the amino acid glycine and ninhydrin or 1,2-indanedione will each be mixed into one beaker and allowed to react in solution. Each of the eleven beakers will contain a known ratio of ninhydrin or 1,2-indanedione to glycine. The product being formed will then be evaluated by the same methods used above on the sequential solutions on paper.

DEDICATION

To the one who kept me determined and patient, thank you Gail Bliss.

ACKNOWLEDGEMENTS

A sincere thanks to my professor and advisor, Dr. Jay Siegel, for his professionalism and commitment to broadening my knowledge of forensic science. His encouragement, insight, and support helped make this research project one that I will remember forever.

I would also like to express my gratefulness to Dr. Antonio Cantu and Robert Ramotowski M.S., both forensic scientists from the United States Secret Service, who proposed the project and also provided the materials to carry it out. They were invaluable to me when I had questions about the project and often pointed me in the right direction to find a solution. Without them, this research would not have been possible.

Lastly, to Cory Painter and Lori Wash, two good friends of mine. They listened to my ideas and provided me with support when it was short at hand. Thanks!

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LIST OF ABBREVIATIONS

AFIS - Automated Fingerprint Identification System

ALS - Alternate Light Source

mM/L - $1x10^{-3}$mole/liter

INTRODUCTION

Arguably, there is nothing more probative than a suspect's fingerprint at a crime scene. Since Bertillion's initial proposal for human classification, which ended in failure, to the present day with automated fingerprint identification system (AFIS) technology, man has searched for ways to identify individuals. The promise of a computer linked fingerprint database that can search through millions of fingerprints in seconds has always been one of the central focuses of police science. When a surface is touched, fingerprint residue is usually transferred to that surface forming a latent print. To be useful the latent print has to be visualized or developed.

Fingerprint residue is composed of many different components that can be utilized to develop a latent print. One group of components that were examined was water-soluble. In one study it showed that chloride, lactic acid, calcium, sulfur, urea, amino acids, phenol, sodium, potassium, and ammonia could be found in varied amounts [10]. The United Kingdom Home Office did another study of the water-insoluble component of fingerprints. The results showed that squalene, cholesterol, free fatty acids,

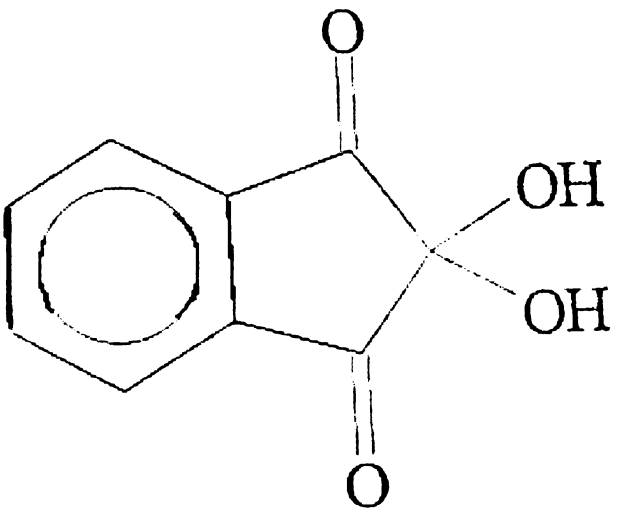


Figure 1 - Molecular Structure of Ninhydrin

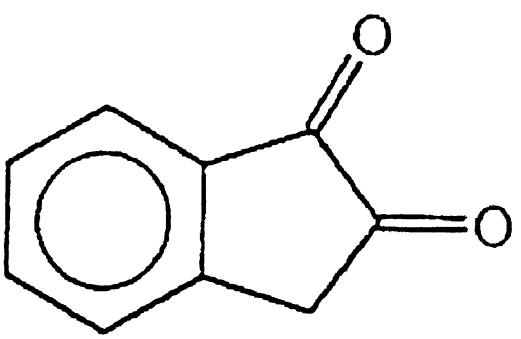


Figure 2 - Molecular Structure of
1,2-Indanedione

wax esters, and triglycerides make up the composition of water-insoluble molecules [10]. Mixtures of these make up the latent fingerprint and are reacted with the developers to visualize that print.

One of the earliest and most used fingerprint developers is Ninhydrin, which was first composed by Siegfried Ruhemann [17]. Ninhydrin (figure 1) reacts with the amino acids in a fingerprint, resulting in a complex, Ruhemann's purple, which has a faint pink to a dark purple color [14]. Development with ninhydrin is usually performed on paper and other, similar porous materials.

There are a number of ninhydrin analogues that have been synthesized to try and improve the sensitivity of the original reaction, and to enhance its properties by adding elements like fluorescence, which ninhydrin alone does not possess [5,8,12,15]. One such analogue is 1,2-indanedione, which, when developed, also produces a faint pink color for latent fingerprints. This compound, unlike ninhydrin, will fluoresce when excited with light at a wavelength of 555nm.

The current literature suggests that Ruhemann's purple consists of ninhydrin and an amino acid (e.g. glycine) in a 2:1 ratio. This theory was developed in experiments that dealt with Ruhemann's purple being formed and tested in solution with glycine. Because latent fingerprints are

primarily developed on a substrate or porous surface it is important to know if the product being formed has the same or different reactant ratio. There also is currently no research examining the ratio of 1,2-indanedione and glycine when developed on a porous surface.

By understanding the stoichiometric ratio of the product of the reactants on a substrate as opposed to solution, better reagents can be developed to enhance the formation of the developed product with improved clarity pertaining to the fingerprint. Also, better development techniques can be probed based on the reaction chemistry resulting in the known final product.

In this study, using sequential dilutions of the amino acid glycine, which is commonly found in fingerprints, and of either ninhydrin or 1,2-indanedione as the chemical developer, the stoichiometry of the reactants will be investigated based on the color produced on filter paper. The correct ratio of the amino acid and chemical developer will produce the darkest color on paper, which is the most desired (greatest sensitivity). The characteristic evaluated in ninhydrin will be its Ruhemann's purple color using visual observation, a UV-Vis Spectrophotometer, and a Xerox photocopier. For 1,2-indanedione, fluorescence will

be evaluated by a fluorescence spectrophotometer, and visually by an alternate light source.

Also in this study the dilutions of the amino acid glycine and ninhydrin or 1,2-indanedione will each be mixed into a beaker and allowed to react in solution. Each of the eleven beakers will contain a known ratio of chemical developer to glycine. The first solution in the initial beaker will start with a 100% chemical developer and 0% glycine concentration, and then the chemical developer solution will be diluted by 10% for each beaker while the glycine solution will be increased by 10%. The final solution in beaker number eleven would then contain a concentration of 100% glycine. A strip of filter paper will then be dipped in each solution and allowed to develop. The product being formed will then be evaluated by the same methods used above on the sequential solutions. This is to examine if the product being formed in solution will have the same ratio of reactants when developed on paper after it is initially allowed to react in solution.

Ultraviolet-Visible Reflectance Spectrophotometry

Ultraviolet-Visible Spectrophotometry [UV-Vis] is an analytical technique that records the absorption of far ultraviolet to near infrared radiation by a molecule [20].

The radiation produced in the spectrophotometer causes the molecule being analyzed to go into a transition to a higher energy level. For the visible spectrum, the outer electrons go through the transitions, while in the ultraviolet range the middle or outer electrons perform the transition depending if the light affects the near or far ultraviolet range [20].

For many solutions over a narrow range of concentrations, absorption of light follows the Beer-Lambert law, which states that absorbance and concentration have a linear relationship. The radiation used in the wavelength range of 390-780 nanometers [nm] is the visible spectrum; the ultraviolet spectrum has a range of 180-390nm. In the visible spectrum, the color of the sample will be absorbed at certain wavelengths [20]. (TABLE 1)

The basic parts of a common UV-Vis Spectrophotometer are its radiation source, wavelength selector, sample chamber, detector, and the output device [20]. The typical radiation source or lamp, can be either incandescent with either a glass or iodine envelope, deuterium, or xenon. The device that selects the wavelengths is a monochromator. This provides the mechanism for the spectrophotometer to select certain wavelengths of light, which is essential if the analyte is to follow the Beer-Lambert law.

The sample can either be a solid or liquid. If it is a liquid, a sample cuvette is used, and if it is a solid, it has a holder to keep it in place. The sample is then exposed to the light and the light that is not absorbed is, in the case of a solid sample, reflected to the detector. The detector then records which wavelengths are reflected back. Finally the output device plots a spectrum, which at the x-axis shows wavelength and the y-axis plots the percent that was reflected.

Fluorescence Spectrophotometry

Molecular Fluorescence Spectrophotometry is a technique that is used to measure the emission of light from molecules that have become excited by absorbing radiation from either the visible or ultraviolet spectrum [19]. Fluorescence is a type of photoluminescence, in which a molecule or species emits radiant energy from an excited state [18]. Fluorescence occurs rapidly, about 10^{-6}-10^{-9} seconds, and it is not possible for the human eye to perceive it once the source of radiation is removed [18].

Wavelength (nm)	Color Absorbed	Color Observed
390-420	Violet	Green-Yellow
420-440	Violet-Blue	Yellow
440-470	Blue	Orange
470-500	Blue-Green	Red
500-520	Green	Purple
520-550	Yellow-Green	Violet
550-580	Yellow	Violet-Blue
580-620	Orange	Blue
620-680	Red	Blue-Green
680-780	Purple	Green

Table 1 - The Visible Spectrum.

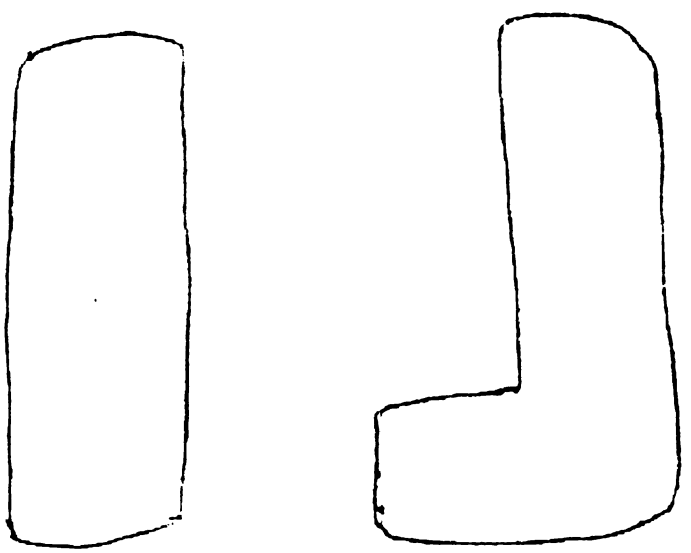


Figure 3 - Whatman Filter Paper Strips.

Fluorescence is usually seen in molecules that are aromatic and conjugated and have easily excited delocalized π electrons [9]. Fluorescence is caused by the molecule starting off in a low energy state (S_0), and then being excited into a higher energy state (S_1)[13]. Once at this higher state the molecule will give off this excess energy at a different wavelength than the original as fluorescence, and return to its ground state. The energy that is given off will be the difference between the initial state and the excited state. Like Uv-vis Spectrophotometry, for solutions in a certain concentration range this analytical technique also follows the Beer-Lambert Law.

Most fluorescence spectrophotometers consist of these core parts: a source or lamp that gives the excitation light, a device that selects the excitation and emission wavelength, the sample chamber, detector, and a program that will determine where the fluorescence is most intense [19]. The excitation wavelength is used to excite the molecule to a higher energy level. Once at this wavelength, the instrument will then scan at the emission wavelength, this is where the molecule will emit the energy and fluoresce.

Using the fluorescence spectrophotometer's software, a scan is taken first of a blank strip of filter paper. This is excited with the same wavelength that will be used on the filter paper containing the amino acid and chemical developer. The instrument is also set to look at the same emission wavelength that the chemical developer is set to fluoresce at. The initial blank scan is then used by the software and subtracted from the spectrum with the chemical developer and amino acid. The resulting spectrum printed out is the fluorescence of the molecule containing the chemical developer and amino acid.

Alternate Light Source

An alternate light source [ALS] is an intense light that can be used to visualize latent fingerprints [10]. Most common ALS possess a source for creating the light and then a light pipe that permits easy placement of the light. Attached to the end of the pipe is a wavelength selector, which moves different filters over the light. These filters then allow the operator to direct a specific wavelength of light onto a piece of evidence that was initially treated with a chemical developer, which fluoresces at a certain wavelength.

Photocopier

The Xerox photocopier was used as a secondary source to test the results of the visual examination of the filter paper strips. The photocopier's scanner, like the human eye, observes and distinguishes between the strips in the visible spectrum. By manipulating the photocopier's darkness and lightness controls while making copies of the filter paper strips, the machine uses its own scanning eye and determines which strip is the darkest or lightest out of the set. The copy produced, which has all the filter paper strips in a row, would show what strip(s) is the darkest when the machine is set to produce a copy at the lightest setting. The darkest strip at that setting would be the one that is easily discernable from the rest.

In a photocopier, the basic premise of image copying is due to xerography. An image, in this case the filter paper that has been developed, is transferred to a photoconductive surface. A positive charge is applied to the surface areas where the image is present. The areas where no image is recognized remain uncharged. Toner, which has a negative charge, is released over the surface of the photoconductive surface. The negatively charged toner will adhere to the positively charged surface due to electrostatic attraction. A piece of paper is then given a

positive charge and placed over the surface. The toner will be transferred to the piece of paper. Heat is then applied to the surface of the paper. This will melt and seal the toner to the paper.

REVIEW OF THE LITERATURE

Ninhydrin

There have been numerous research papers written on the ninhydrin reaction forming a purple complex commonly known as Ruhemann's Purple, and one result of these findings is that there are many different reaction pathways that occur in solution [6,8]. There has not been any research on the reaction chemistry of ninhydrin when developed on a paper substrate containing the stoichiometry of the product formed on the substrate.

Since the discovery that ninhydrin can detect latent fingerprints on paper and other similar material, the forensic science community has been trying to perfect the reaction to maximize latent print yields [1,12]. In 1993 Cantu *et al* [4] used a paper substrate for development when examining several fingerprint reagents with the amino acid Glycine. Although the purpose of the research was to evaluate the reagents for color development, fluorescence, fluorescence after being treated with a secondary developer of zinc chloride, and background interference, the paper emphasized the importance of considering a paper substrate when evaluating fingerprint developers.

Lennard *et al* examined the structure of Ruhemann's Purple when it complexes with Group IIb Metal Salts [11]. The paper used spectroscopic data to deduce the structure of Ruhemann's purple and a metal complex. The chemical developer used in the paper was ninhydrin, and the amino acid was glycine. In the study spectroscopic data was collected at 485 and 580nm based on absorbance. The results showed that a 1:1 ratio of the Ruhemann's Purple and the three Group IIb metals was formed in solution.

Another similar experiment was done by Davies *et al*, in which zinc(II) and cadmium(II) complexes were produced with ninhydrin developed prints and Group 12 metal salts [5]. Davies *et al* investigated the stoichiometry 1:1 metal:ligand ratio so that better enhancement procedures could be replicated. In one of the experiments the complex was synthesized on filter paper with a concentrations ranging from 1% to 0.05%. The procedure detailed that one-drop of each ninhydrin solution was coupled with one-drop of a glycine solution on the filter paper to show various Ruhemann's Purple intensities.

1,2-Indanedione

One analog of ninhydrin that has shown promise for use as a chemical developer is 1,2-indanedione. Ramotowski *et*

al evaluated the effectiveness of several indanedione compounds at visualizing spots containing glycine [15]. The experiment involved stoichiometricly spotting grade 2 Whatman's filter paper with Glycine and then with 1,2-indanedione. The filter paper was developed in a number of different ways that were assessed over a period of seven days based on the product's fluorescence. Fluorescence measurements were recorded by a Hitachi model F-4500 fluorescence spectrophotometer in the same excitation (450-600nm) and emission (525-725nm) wavelengths. All the indanedione compounds were then compared to each other for their ability to visualize the amino acid glycine on filter paper. In the study it was noted that the product formed and analyzed had not been determined thus far.

Other preliminary results of the 1,2-indanediones were released by Hauze et al. in 1998, stating that this was a new reagent for visualizing amino acid latent prints [7]. Again, filter paper was the substrate used for the development of the 1,2-indanedione product. The filter paper was first saturated with a 0.25% solution of glycine and water, and then allowed to dry for a number of days before 1,2-indanedione was added. Different drying conditions were then evaluated to determine what would be

the optimal conditions for developing the product's maximum fluorescence.

The study done by Hauze *et al* also compared the color of 1,2-indanedione developed prints to that of ninhydrin prints. The color of 1,2-indanedione was found to be relatively poor compared to the intense color change of ninhydrin, but the fluorescence of the product was considerably greater than other latent fingerprint developers currently being used.

Almog *et al* then tested 1,2-indanedione's fluorescence on common photocopy paper [2]. In the experiment prints were placed on white A4/80 gram photocopy paper and then cut, the paper was then sprayed with a 0.025% (w/vol) concentration of 1,2-indanedione and viewed under a Rofin Polilight at 530nm, with a cutoff filter at 549nm. Almog et al. compared these developed prints for fluorescence, background fluorescence, and color. There were also other reagent concentrations of 0.05%, 0.01%, and 0.0004% formulated, these were then judged by the above standards. Unlike previous research done on 1,2-indanedione, in this experiment the researchers used an individual's fingerprint instead of using a solution containing an amino acid for reactants.

1,2-indanedione was also tested using different carrier solutions and developing conditions to evaluate its effectiveness on porous surfaces [16]. The preliminary evaluation exposed 1,2-indanedione's limits of detection on porous surfaces by using decreasing dilutions of glycine in water on filter paper. The test sheets containing glycine were spotted and allowed to air-dry overnight before being developed with 1,2-indanedione. The developed sheets were then viewed for fluorescence with a Polilight PL10 filtered light source at an excitation wavelength of 555nm, and an emission wavelength of 610nm. The results were compared against other types of development sequences and treatments to find which gave the optimal development.

Wilkinson studied 1,2-indanedione and various solutions [21]. She found that when 1,2-indanedione is dissolved in methanol the reactivity of the solution is reduced due to hemiketal formation. When ethyl acetate is used in place of methanol in solutions there is improved sensitivity to amino acids. Wilkinson's research indicates that alcohol formulations should not be used when making a 1,2-indanedione solution.

Materials and Methods

A. Materials

1. Whatman Filter Paper - Grade 2
2. Ninhydrin Crystals (Sirchie)
3. Methanol - Reagent Grade (Aldrich)
4. Deionized Water
5. Ethyl Acetate - ACS Reagent Grade (CCI)
6. Glacial Acetic Acid
7. Glycine (Tyron)
8. 1,2-Indanedione Crystals - Obtained from Dr. Cantu and Robert Ramotowski from the United States Secret Service.

B. Equipment

A Mettler model B303 balance was for all weighings. The UV-Vis Spectrophotometer used was a Hitachi U-4001 with an RSA-HI-40S Reflectance Spectroscopy accessory. The conditions for the UV-Vis instrument included a scan speed of 120(300) nm/min, auto-sampling interval, zero delay time, 2.00nm fixed slit, PMT voltage was on auto gain, and Pbs gain was set at two.

For measuring the fluorescence a Hitachi F-4500 fluorescence spectrophotometer was used with a solid sample holder. A cut off filter up to 555nm was used for viewing

fluorescence. The instrument parameters included an excitation slit of 5.0nm, emission slit of 5.0nm, auto response, PMT Voltage was auto, response was set to auto, and the shutter control and corrected spectra categories were both turned off. The alternate light source used was a MCS-400 with a filter of 555nm. A Xerox machine, model 5365, was used to obtain black and white copies.

C. Concentrations of the Solutions

Ninhydrin solutions in methanol were prepared in multiple concentrations of 10,40,90, and 100 mM/L ($1x10^{-3}$mole/liter). The 1,2-indanedione solutions consisted of ethyl acetate and 1,2-indanedione at concentrations of 1,3,5,10,40, and 60mM/L ($1x10^{-3}$mole/liter) acidified with 1% glacial acetic acid. The glycine solution was prepared in deionized water.

Ninhydrin Experiment Method

Ninhydrin crystals were weighed out and dissolved in methanol. Glycine was measured and then dissolved in deionized water. Also, a carrier solution of methanol was made for the ninhydrin solution. Another carrier solution of just distilled water was also prepared for the glycine solution.

Eleven beakers were set up for the glycine and distilled water carrier solutions. Eleven dilutions of glycine were prepared according to table 1, and the corresponding solution number went with the same beaker number. Once completed, the glycine carrier solution of distilled water was then added to each of the eleven beakers to make each beaker have a total volume of ten milliliters. The beakers were then mixed to ensure that the solution was homogenous throughout.

Filter paper was then cut into eleven strips and numbered one to eleven (Figure 3). Filter paper number one was then dipped in the glycine/carrier beaker one. The paper strip was held in the solution for five seconds and then paper-clipped to a string suspended in the air so the strip could air dry. Each of the remaining ten filter paper strips were dipped in the corresponding beaker and allowed to air dry for one hour.

Another eleven beakers (table 3) were then prepared for the ninhydrin dilutions and a methanol carrier solution, was used on above. The glycine-laden strip number one was then dipped in the ninhydrin/methanol beaker eleven. Each glycine-laden strip was dipped in

SOLUTION	GLYCINE (mL)		Carrier Solution (mL)	Total Volume in Beaker (mL)
Solution 1	0	+	10	10
Solution 2	1	+	9	10
Solution 3	2	+	8	10
Solution 4	3	+	7	10
Solution 5	4	+	6	10
Solution 6	5	+	5	10
Solution 7	6	+	4	10
Solution 8	7	+	3	10
Solution 9	8	+	2	10
Solution 10	9	+	1	10
Solution 11	10	+	0	10

Table 2 - Amino Acid Glycine Beaker Solutions.

SOLUTION	Ninhydrin or 1,2-Indanedione (mL)		Carrier Solution (mL)	Total Volume in Beaker (mL)
Solution 1	0	+	10	10
Solution 2	1	+	9	10
Solution 3	2	+	8	10
Solution 4	3	+	7	10
Solution 5	4	+	6	10
Solution 6	5	+	5	10
Solution 7	6	+	4	10
Solution 8	7	+	3	10
Solution 9	8	+	2	10
Solution 10	9	+	1	10
Solution 11	10	+	0	10

Table 3 - Chemical Developer Beaker Solutions.

the ninhydrin solution in the following way; strip two with beaker ten, strip three with beaker nine, and so on. The filter paper strips were then allowed to air dry for thirty minutes.

After all excess solution was dry on the filter paper the eleven strips were then placed on a paper towel and put in an oven. The strips were developed in the oven at 100 degrees for twenty minutes.

Once the strips were developed they were then placed in order on a brown cardboard backing placed out to be viewed visually in the artificial light of the laboratory. When viewed, each strip that was not the darkest was removed from the cardboard backing until the strips, which were the darkest, could be viewed together. The darkest strip(s) were then recorded.

The next analysis done on the filter paper strips was measuring the amount of reflectance using the UV-Vis spectrophotometer. Before any tests were attempted using the instrument, a filter paper strip that was not dipped in either of the solutions was scanned as a blank. This was used as a background for the reacted strips. Once the background scan was acquired each strip was scanned to generate a reflectance spectrum.

Each spectrum was taken and evaluated to find the lowest point of reflectance, which was around 550nm. The distance from zero (the x-axis) up to the peak was measured in millimeters using a standard ruler. The shortest distance between those two points confirmed the darkest strip from the eleven test samples.

The last analysis run on the ninhydrin test strips involved the use of a Xerox copy machine. All eleven filter paper strips were first laid out in order on the copy machine. The machine's contrast was set at zero and copies of the strips were made one at a time while adjusting the contrast from the lightest setting to the darkest before each separate copy. The copies were then evaluated by observing the darkest strip compared to the others while viewing each setting.

1,2-indanedione

Solutions of 1,2-indanedione and glycine were prepared in the same manner as the ninhydrin except that the carrier solution was ethyl acetate instead of methanol. The filter paper was also cut into eleven different strips (Figure 4) and they were dipped and dried using the same process that was done with the ninhydrin and glycine strips. The 1,2-

indanedione strips where developed in the oven at 100 degrees Celsius for twenty minutes.

Once the strips were developed they were first evaluated for the darkest color in artificial laboratory light. Next the strips were taped, in order, to a brown cardboard backing and tested for florescence. Using the alternate light source at 555nm the strips were excited and through observation, the most florescent strip was picked from a distance of four feet up to ten feet away from the filter paper strips. Each strip was then placed in the fluorescence spectrophotometer. Each strip was analyzed using an excitation wavelength of 555nm and viewed from 575nm to 750nm. The emission wavelength of 1,2-indanedione is 610nm. The spectrum from the scan was then printed out and compared to the other strips for the highest peak.

The Mixed Beakers

Using tables two and three as an outline, the eleven beakers of ninhydrin were combined with the eleven glycine containing solutions. Glycine beaker number one was combined with solution number eleven of ninhydrin. The remaining beakers were combined in this way. The eleven 1,2-indanedione beakers were then mixed with the same eleven beakers containing the same concentrations of

glycine as above. The mixed solutions were allowed to react in vitro for ten minutes.

A strip of filter paper was then dipped in each of the beakers and allowed to air dry by being hung on a string. All of the strips were then placed in an oven at 100 degrees Celsius for twenty minutes to develop. The strips were then taken out and evaluated using the same criteria depending on if the strips contained ninhydrin or 1,2-indanedione. This was done for each of the concentrations listed above in the solutions section. The observations were then recorded.

RESULTS AND DISCUSSION

Because most fingerprint comparisons are done using the human eye, the chief mode of evaluation of the experimental test strips was done visually with artificial light. The results reported by the analytical equipment were regarded as secondary to what the human eye saw. The strips were categorized based on the highest intensity of the color change or, in the case of 1,2-indanedione, based on the highest degree of fluorescence.

It was expected that the results form the instruments used in the research would correspond to what the human eye discerned. In some instances, the spectrophotometer picked a strip other than the one picked visually. This is because the instruments are more sensitive to slight differences in color intensity or fluorescence than the human eye.

Ninhydrin and 1,2-indanedione, the two compounds evaluated in this study, had decisively different methods of data collection. For ninhydrin, the concentrations ranged from 10mM to 100mM. At 100mM, the color change was too intense; thereby rendering the technology unable to determine which strip was the darkest. Because there has not been any previous work done in this area a procedure

for strips being too light or too dark had to first be set. Starting with a low concentration, the next concentration was determined by increasing the concentration incrementally. Although they were not prepared in this order, the results will be summarized in order from lowest to highest concentrations.

For 1,2-indanedione, fluorescence was the primary characteristic that determined the most desirable strip. The concentrations were relatively small compared to ninhydrin, owing to the fact that quenching effects on the fluorescence was a concern. The quenching characteristic occurs when the fluorescent product's concentration overpowers and acts as an "inner filter" that blocks the emitting fluorescence. This can result in an improper determination of the most fluorescing strip. By reducing the concentration of the fluorescing product the fluorescence given off is not inhibited by the concentration and has no quenching effect.

Results

Ninhydrin Sequential Strip Experiment

For the 10mM concentration (table 4) the visual examination was unable to single out one strip. Strips four, five, and six were counted as the darkest, while the

other strips were excluded because they were too light. The Xerox and UV-Vis examinations picked strip number six as the darkest. Using the concentration ratios, according to the Xerox and UV-Vis, the ratio of the Ninhydrin molecule to Glycine is one to one.

Because the ratio could not be determined visually, the next step was to increase the concentration to 40mM. All three tests, visual examination, UV-Vis, and Xerox chose strip number six as the darkest. This also confirmed that the product formed on the strips has a one to one ratio. The 40mM concentration was repeated. In this instance, the visual and spectroscopic evaluations again chose strip six. The Xerox machine, however, was unable to discern between strips number six and seven. Possible explanations could be due to lack of sensitivity to small contrasts with the copier, or an inadequate amount of toner was used on the printout.

Concentration (mM)	Visual Evaluation	Xerox Evaluation	Spectrophotometer
10	4,5,6	6	6
40	6	6	6
40 (2^{nd} Try)	6	6,7	6
90	6	6	6
90 (2^{nd} Try)	5,6	5,6	5
100	Too Dark	Too Dark	Inaccurate Reading

Table 4 - Ninhydrin Sequential Strip Experiment Results.

Concentration (mM)	Visual Evaluation	Xerox Evaluation	Spectrophotometer
10	7	7	7
40	6,7	6	6
40 (2^{nd} Try)	5,6	5,6	6
90	7	7	7
90 (2^{nd} Try)	6,7	6,7	6,7
100	Too Dark	Too Dark	6

Table 5 - Ninhydrin Mixed Strip Experiment Results.

Concentration (mM)	Visual Evaluation	Xerox Evaluation	Fluorescence Spectrophotometer	Alternate Light Source
1	No Color	No Color	3,4,5,6	3,4,5,6
3	3,4	3	6	6
5	3,4	4	6	6
10	4,5	3	8	5,6,7,8
40	4,5	3,4,5	8	5,6,7,8
60	4,5	3,4	8	5,6,7,8

Table 6 - 1,2-Indanedione Sequential Strip Experiment Results.

Concentration (mM)	Visual Evaluation	Xerox Evaluation	Fluorescence Spectrophotometer	Alternate Light Source
1	No Color	No Color	None	None
3	4,5	2,3	7	5,6
5	2,3	4	3,4,5,6	3,4,5,6
10	3,4,5	4	4	3,4,5
40	6,7	3	6,7	6,7
60	2,3	2,3	5	4,5

Table 7 - 1,2-Indanedione Mixed Strip Experiment Results.

Next, the concentration was raised to 90mM. This resulted in the formation of very dark strips that showed strip number six being the darkest in all three of the evaluations. The concentration was then prepared again and under artificial light strips five and six were the darkest. The Xerox machine also showed strips five and six being equal in contrast, and the UV-Vis selected strip number five.

The final concentration prepared for ninhydrin was 100mM. The strips were too dark to visually separate out the darkest one. The Xerox and the UV-Vis also could not differentiate between the other strips. This batch of strips was discarded, and thus the upper limit of concentration using ninhydrin was found to be 100mM.

Ninhydrin Mixed Strip Experiment

When evaluating the mixed strips the first observation was that the strips-even when compared with sequential strips with the same concentration-were noticeably lighter. Using table 5, with the concentrations 10mM and 90mM (1^{st} try) the strip that was the darkest was number seven. This meant that the product being formed in solution had a ratio of 6:4 of glycine to ninhydrin. When the concentration was changed to 40mM, the first batch of strips showed upon

visual examination to be between strips six and seven, whereas the Xerox and the UV-Vis selected strip number six. This suggests that the complex being formed in solution could possibly be more than one species of the ninhydrin and glycine molecule.

Strips were developed using a 40mM concentration again showed somewhat different results. The visual examination and Xerox had strips five and six being the darkest while the UV-Vis picked strip six. This again suggests that there are other ratios of ninhydrin to glycine complexes that are formed in solution.

Like the ninhydrin sequential strips, when 100mM solutions were mixed and developed they were also too dark to be visually evaluated. When the strips were put in the Xerox machine there was no distinction between any of the strips.

1,2-Indanedione Sequential Strip Experiment

The 1,2-indanedione solution was first evaluated at a 1mM concentration (see table 6). Visual and Xerox examinations were not able to detect any color change whatsoever. When the strips were viewed under an alternate light source, strips three, four, five, and six showed very low fluorescence. When analyzed with a UV

spectrophotometer, strips three four, five and six again showed low level of fluorescence. There was some variance in the amount of fluorescence recorded, but the values were so low they were below the instruments level of detection. Therefore, these values were not considered accurate.

When the concentration of 1,2-indanedione was increased to 3mM for the solutions. There was some initial color to the strips compared to the previous concentration. Strips three and four looked the darkest upon initial visual evaluation. The Xerox machine though showed that strip number three was the darkest. The alternate light source and the fluorescence spectrophotometer both showed strip six as the most fluorescent strip.

With a concentration of 5mM the visual examination was split between strips three and four, while the Xerox machine showed strip number four for the darkest. According to the spectrophotometer the sixth strip was the most fluorescent, and this was confirmed with the alternate light source.

For the solutions containing 10, 40, and 60mM concentrations, strips four and five were the darkest when visually examined. The Xerox machine selected strip number three at a concentration of 10mM. At 40mM, the Xerox then had strips three, four, and five being equal in their color

contrast. Using a 60mM concentration, the Xerox again showed strips three and four as the darkest, omitting strip number five.

With the last three concentrations (10, 40, and 60) the fluorescence spectrophotometer resulted in finding strip number eight as the most fluorescent. The alternate light source then could not differentiate between strips five, six, seven, or eight. Those four strips all were equal in fluorescence.

1,2-Indanedione Mixed Strip Experiment

The results for the 1,2-indanedione developer did not show any one strip or strips as being the darkest or most fluorescent. Each time the concentration was increased the strips did not show any pattern of concentration quenching or any significant development on the filter paper strip (table 7). The complex that was formed in solution seemed to not want to react with the filter paper strips. This problem lead to mixed results, which do not give any clear ratio for what was being formed in the beakers.

Discussion

Ninhydrin Sequential Experiments

The initial concentration of 10mM was far too light to get any accurate results when the strips were viewed visually after their development. For this reason the concentration was increased even though the Xerox and UV-Vis spectrophotometer both showed that the product on the strips had a 1:1 ratio of ninhydrin and Glycine (table 8). Because of the lightness of the strips the concentration was increased significantly to see how dark the strips could be made in order to positively pick out the one right strip under artificial light. Since the visual examination was the most important test it was a priority to clearly see the darkest strip.

A solution of 90mM was tested and the strips were all dark enough to see that each of the evaluations selected strip six, which stated that the product again had a 1:1 ratio with ninhydrin and glycine. When the reflectance spectrum was compared on each of the strips numbers five, six, and seven were extremely close according to the UV-Vis.

The concentrations then were decreased to 40mM to see if the ninhydrin and glycine concentration had any effect on the product being formed on the filter paper strips. In

this test only strips number five, six and seven were developed because they were the darkest strips based on the previous experiments. This narrowed the search for the darkest strips, the others were discarded because they were not dark enough compared to the previous three strips.

A concentration of 40mM was chosen because it would be significantly lighter than the 90mM solution but would also be darker than the initial 10mM solution. Visually and with the analytical machines, strip six was the darkest. The product being developed on filter paper was then confirmed to have a 1:1 ratio of ninhydrin and glycine.

Another solution of 40mM was prepared to replicate the previous results. The results showed strip six as the darkest visually and also with the UV-Vis spectrophotometer. The Xerox machine could not differentiate between strip six or strip seven this time. A 90mM concentration was prepared and tested. The visual examination showed that strip five and six were the darkest, as did the Xerox machine. The UV-Vis then selected strip number five as the darkest of the strips. A 100mM concentration was also developed but the strips were too dark to give an accurate reading so the strips were discarded.

Based on these tests, the product being formed does not have a 2:1 ninhydrin to glycine ratio as was previously thought. The product being formed on the filter paper strips is possibly an intermediate of the ninhydrin and glycine reaction. The intermediate could be formed because the reaction between ninhydrin and glycine has not gone to completion. It is feasible that the development of the 2:1 ratio of Ruhemann's purple is inhibited by the filter paper causing it to stay in the intermediate form.

1,2-Indanedione Sequential Experiments

The characteristic tested in these experiments was the fluorescence of the chemical developer 1,2-indanedione with glycine. Using a concentration of 1mM the fluorescence was too weak, meaning that strips three through six all fluoresced evenly. The fluorescence spectrophotometer and alternate light source all showed the same strips as the most fluorescent. Since there was no difference between the filter paper strips the concentration was increased in hope that the strips would separate out more with their fluorescence.

When the concentration was increased to 3mM and then to 5mM the results showed that strip number six- that was a 1:1 ratio (table 9) of 1,2-indanedione and glycine-

fluoresced the most according to the alternate light source, as well as by the fluorescence spectrophotometer. The concentration of the solutions was then again increased to evaluate if the compound formed was dependent on the reactants concentration.

Using the concentrations of 10,40, and finally 60mM there was concentration quenching. One sign that is occurring is that strips five through eight all looked like they fluoresced the same under the alternate light source. The fluorescence spectrophotometer selected strip number eight as the most fluorescent, but it is important to note that starting from strip five through eight all showed very similar excitation peaks that were difficult to separate.

As shown by the tests, the product being formed on paper between 1,2-indanedione and glycine has a 1:1 ratio. A concentration of 1mM or less for the reactants causes the fluorescence of the product to be too weak, and then the strip cannot be selected according to the product's concentration. It has been shown that by raising the concentration to 10mM and above the product formed on the strips cannot be properly evaluated because of concentration quenching of the strips.

Strip Number	Percent Ninhydrin	Percent Glycine
1	100%	0%
2	90%	10%
3	80%	20%
4	70%	30%
5	60%	40%
6	50%	50%
7	40%	60%
8	30%	70%
9	20%	80%
10	10%	90%
11	0%	100%

Table 8 - Percent of Ninhydrin and Glycine ratios for the filter paper strips.

Strip Number	Percent 1,2-Indanedione	Percent Glycine
1	100%	0%
2	90%	10%
3	80%	20%
4	70%	30%
5	60%	40%
6	50%	50%
7	40%	60%
8	30%	70%
9	20%	80%
10	10%	90%
11	0%	100%

Table 9 - Percent of 1,2-Indanedione and Glycine ratios for the filter paper strips.

Ninhydrin and 1,2-Indanedione Mixed Strip Experiments

In essence the mixed strip experiment was originally performed to show a difference between developing fingerprints on a solid sample versus in solution. Like previous research done on the Ruhemann's Purple complex, the ratio of ninhydrin to amino acid was 2:1, since the experiments were done in solution there was a question about what would the ratio be on paper.

The sequential strip experiments done have shown that there is evidence that the product being formed on paper is a 1:1 ratio of ninhydrin and glycine compared to the 2:1 ratio previously thought. It was expected that the mixed strip experiments would show that the ratio of the product in solution was still 2:1 when they were allowed to react in solution and then forced to react on the filter paper instead of the reactants being reacted and developed solely on the filter paper.

In the case of ninhydrin, the darkest strips started at strip number five and continued to strip number seven. As shown in table 5, each evaluation had different results. Only with a 10mM concentration did all three evaluations share a single strip. These results could be caused by a number of reasons, (1) the solutions - when allowed to react in solution before being put on paper - allowed a

number of intermediates to be formed and each had a different affinity to the filter paper, (2) the original Ruhemann's Purple product, once formed in solution, does not react well with the filter paper, and the product reacted randomly on the strips, and (3) the reactants did not reach their equilibrium with the formation of the product, this could mean that the reactants were then transferred from the solution and developed on the paper. This would lead to the product being formed on the filter paper having a 1:1 ratio. In all these cases the signs of this would be that the color on the filter paper strips would be lighter than usual and the development on the surface of the filter paper would not be consistent.

The mixed strips containing ninhydrin and glycine each showed the above signs of an incomplete reaction or that the product being formed on the strip was an intermediate.

When 1,2-indanedione was used as a chemical developer in place of ninhydrin the results were identical. The solutions that were mixed together were also allowed to react for a period of time like the ninhydrin, but unlike the ninhydrin, the product formed in solution did not want to react with the paper. The excitation peaks were also significantly lower when they were compared against the fluorescence of the sequential strips. The strips varied

with concentration and also with the evaluation method being used. In numerous occasions the alternate light source would single out a number of strips but the fluorescence spectrophotometer would select different ones. This could be due to the product being formed in solution not having a high affinity for the paper. The solution did not want to react with the filter paper and this caused some strips to react compared to the others but no clear results could be acquired because the results were random.

CONCLUSION

This study suggests that the product being formed when reacted and developed on filter paper with ninhydrin and glycine has a 1:1 stoichiometric ratio compared to the proposed 2:1 ratio that is commonly believed. There are also preliminary results indicating that an analogue to ninhydrin - 1,2-indanedione - also could have a 1:1 stoichiometric ratio of the chemical developer and glycine when reacted and developed on filter paper.

The study also indicates that when ninhydrin or 1,2-indanedione are reacted with glycine in solution-and then is allowed to react with filter paper - there will be competing reactions causing the color and development of the product to diminish or the development of the product to be nonexistent altogether.

The stoichiometric ratios found in this study are extremely beneficial to the forensic science community. Because latent fingerprints are primarily developed on a substrate or on a porous surface it is important to know what product is being formed, compared to what the forensic science community currently believes, to increase the chance of optimal development of the print. By understanding the stoichiometric ratio, one can better

understand what reaction could be occurring between the chemical developer and the fingerprint versus a reaction examined in a test tube. Also better development techniques can be pursued because the forensic scientist knows the reaction pathway to the final product and what the developed product consists of.

At the present time numerous proposals of reaction pathways have been put forth to explain ninhydrin's reaction with an amino acid, but none of them have conclusively shown to be correct. For 1,2-indanedione, there has not been any reaction pathway presented in the literature at this time. This research investigated the reaction of 1,2-indanedione and what product is formed when reacted on a paper substrate, which in this case was filter paper.

In order to continue progress in forensic science increased research is necessary. Ideally, more research needs to be done on identifying what product is being formed on various substrates using different chemical developers and development techniques. Another area that can be explored is the reaction of ninhydrin and 1,2-indanedione with proteins instead of single amino acids. This research centered on looking at ninhydrin and 1,2-indanedione, there are many other common fingerprint

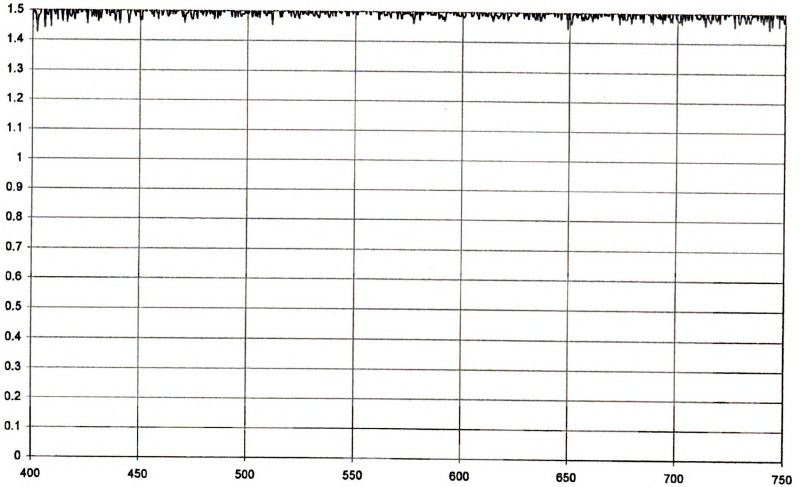
developers used to visualize a latent fingerprint using a variety of techniques that can be explored.

APPENDICES

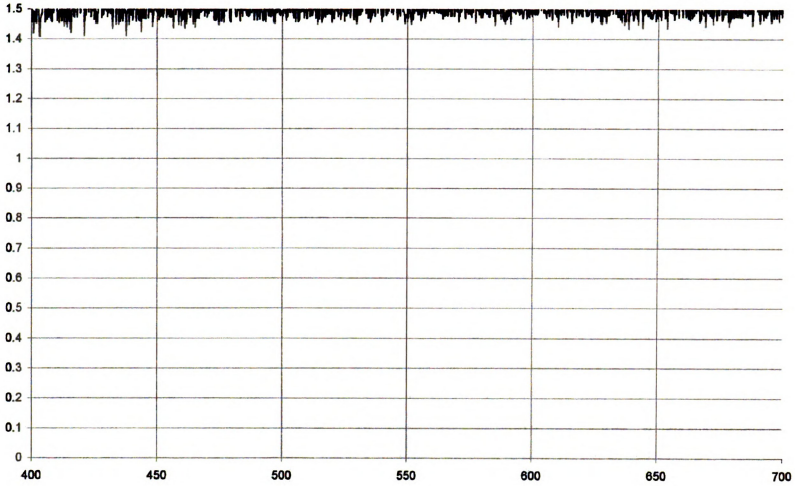
APPENDIX A

UV-VIS Spectra of Ninhydrin Tested Sequential Whatman Filter Paper Strips

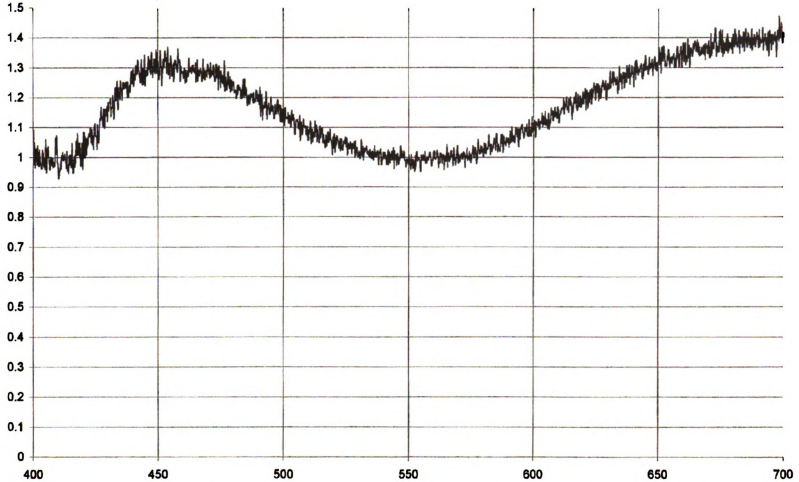
Ninhydrin Blank Strip

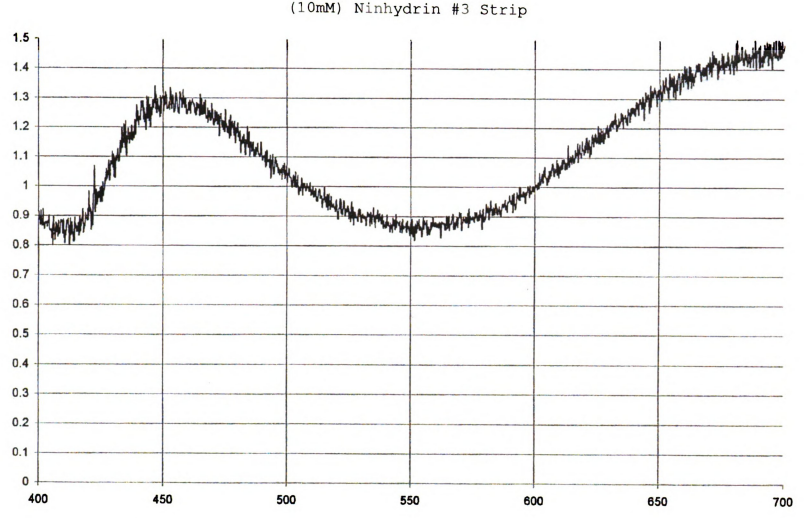


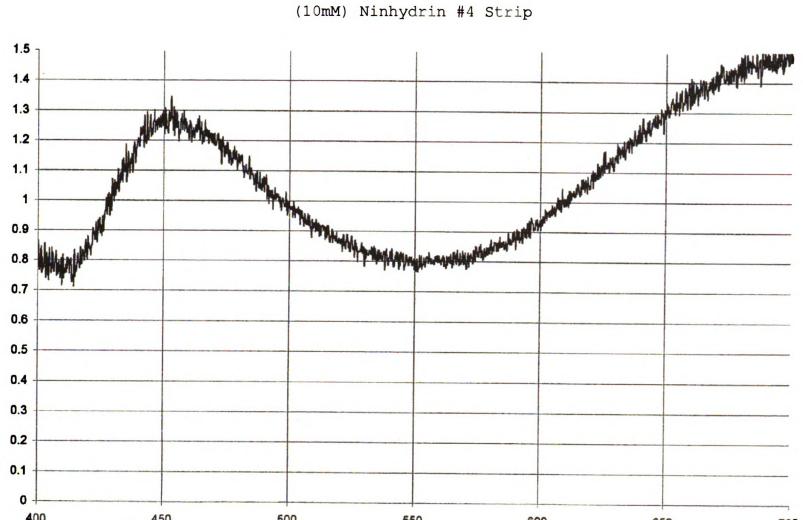
(10mM) Ninhydrin #1 Blank Strip

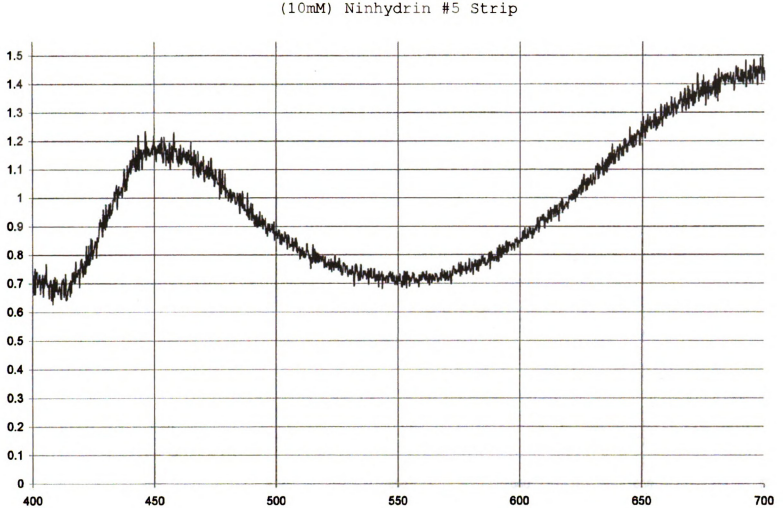


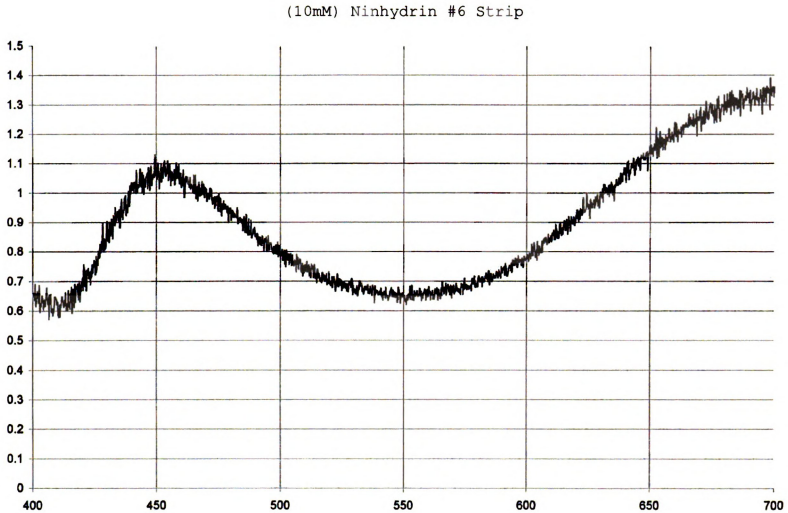
(10mM) Ninhydrin #2 Strip

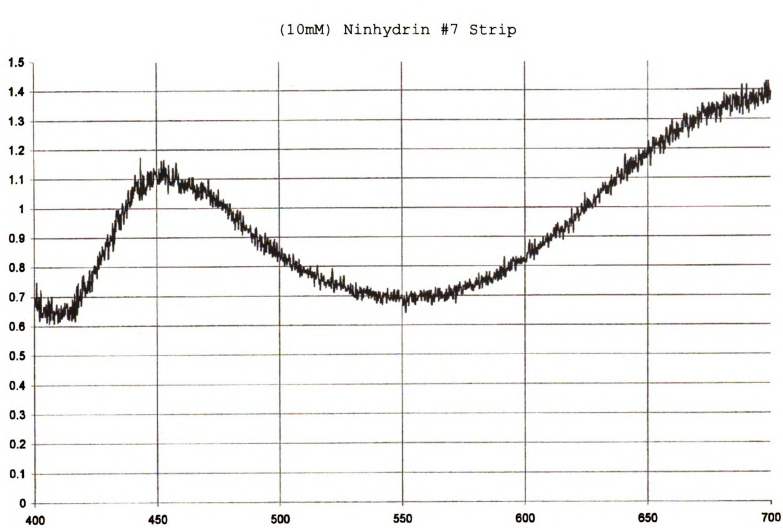


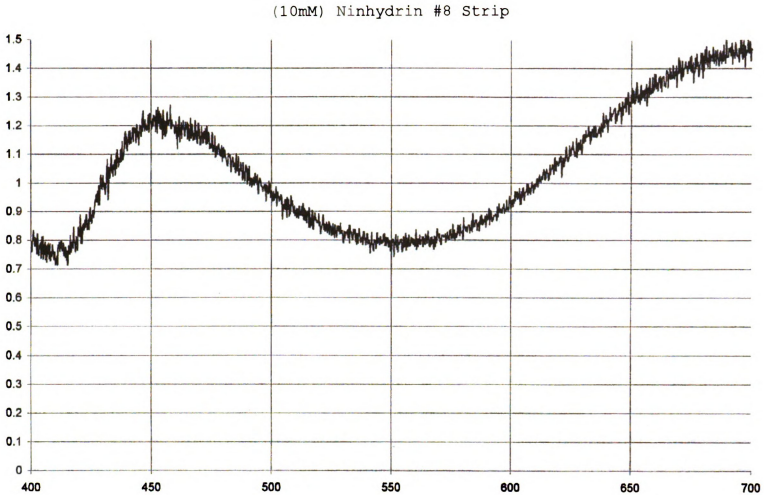

(10mM) Ninhydrin #3 Strip
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700

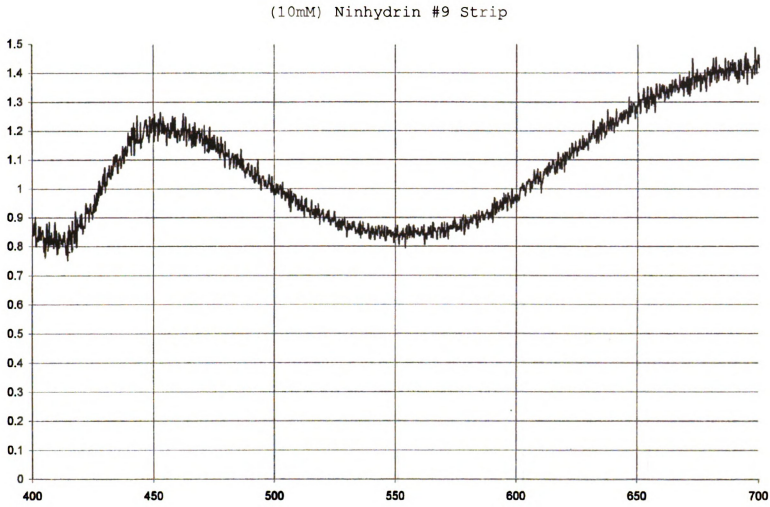

(10mM) Ninhydrin #4 Strip
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700

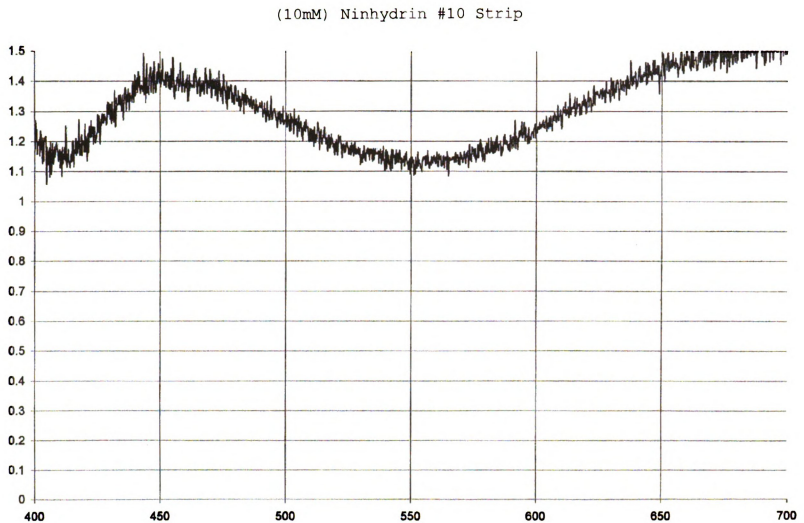

(10mM) Ninhydrin #5 Strip
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700


(10mM) Ninhydrin #6 Strip
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700

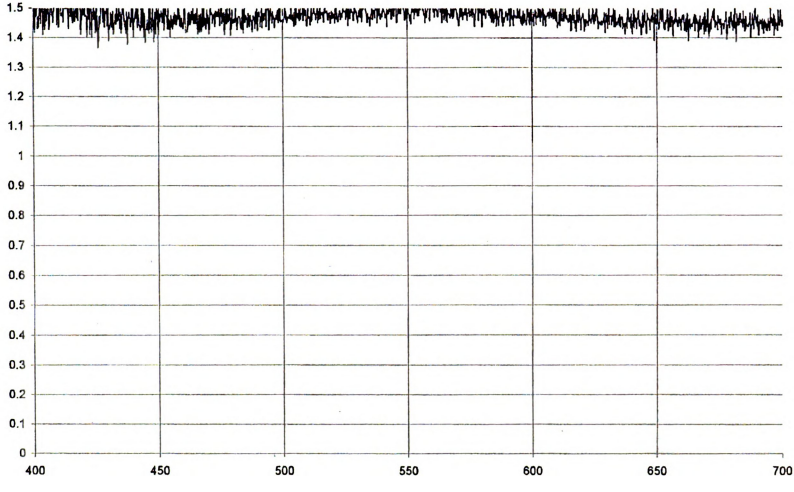

(10mM) Ninhydrin #7 Strip
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700


(10mM) Ninhydrin #8 Strip
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700

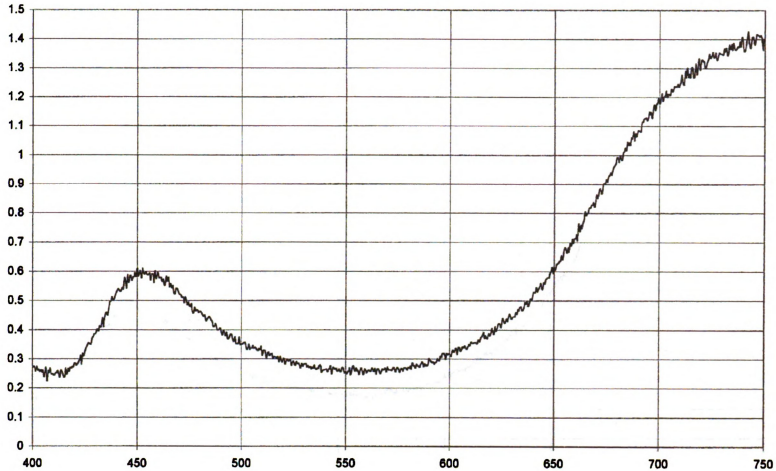

(10mM) Ninhydrin #9 Strip
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700


(10mM) Ninhydrin #10 Strip
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700

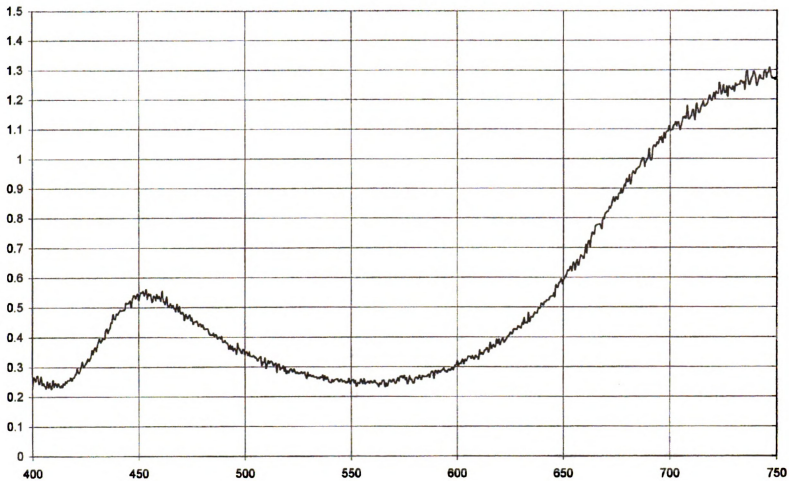
(10mM) Ninhydrin #11 Strip



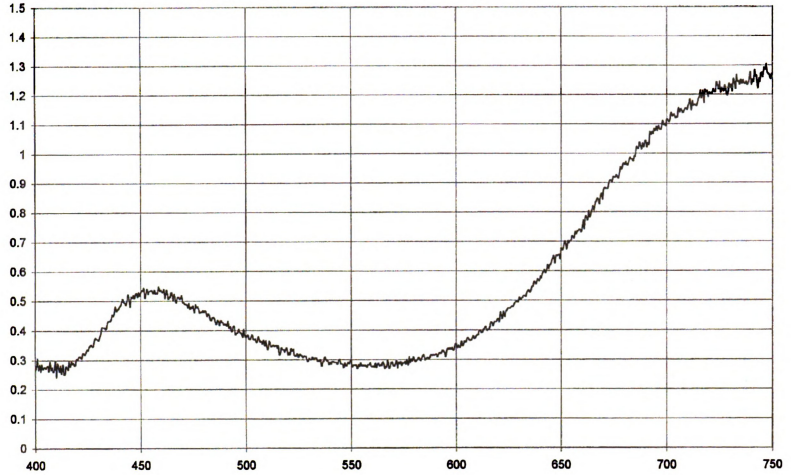
(40mM) Ninhydrin #5 Strip



(40mM) Ninhydrin #6 Strip



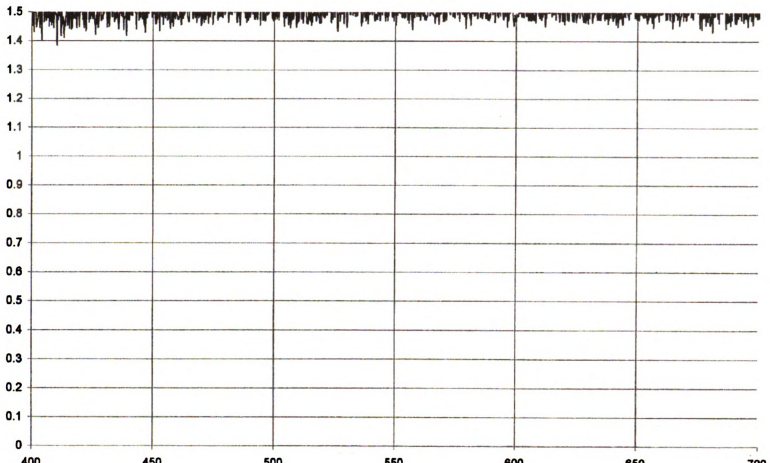
(40mM) Ninhydrin #7 Strip

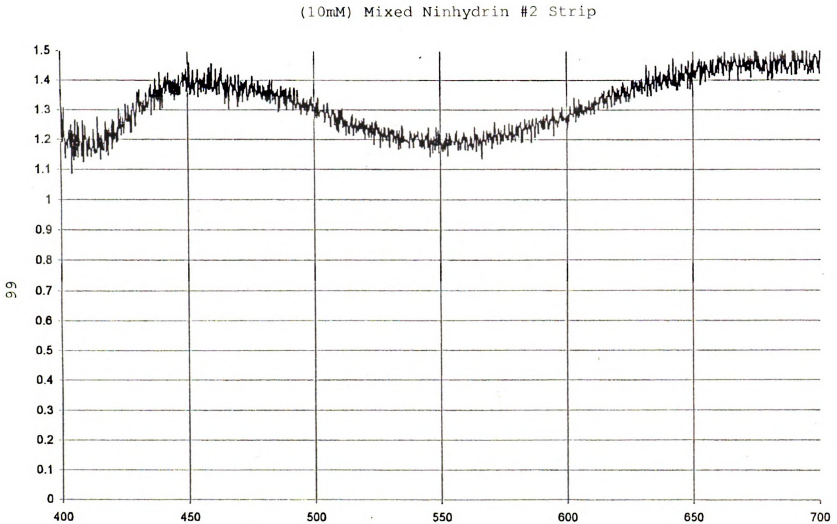


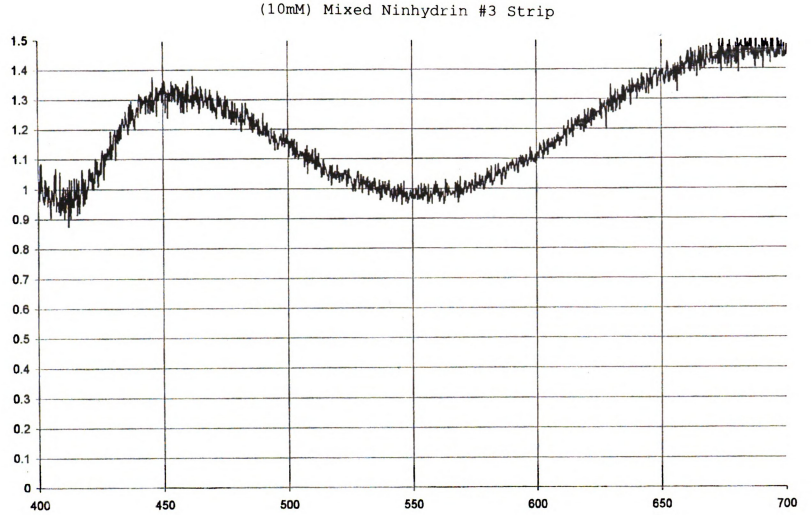
APPENDIX B

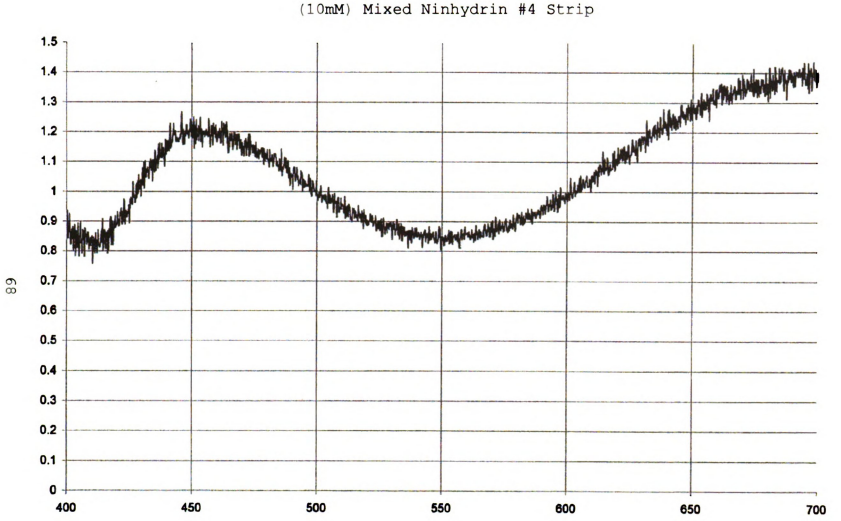
UV-VIS Spectra Of Ninhydrin Tested Mixed Whatman Filter Paper Strips

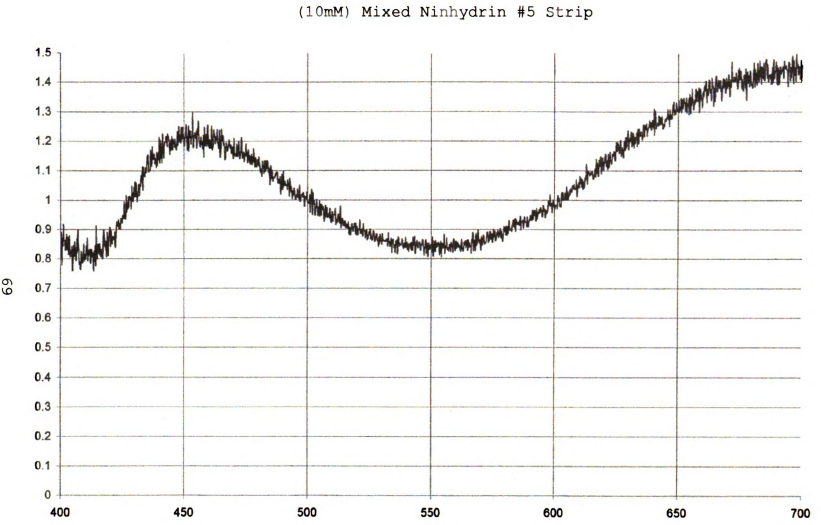
(10mM) Mixed Ninhydrin #1 Strip

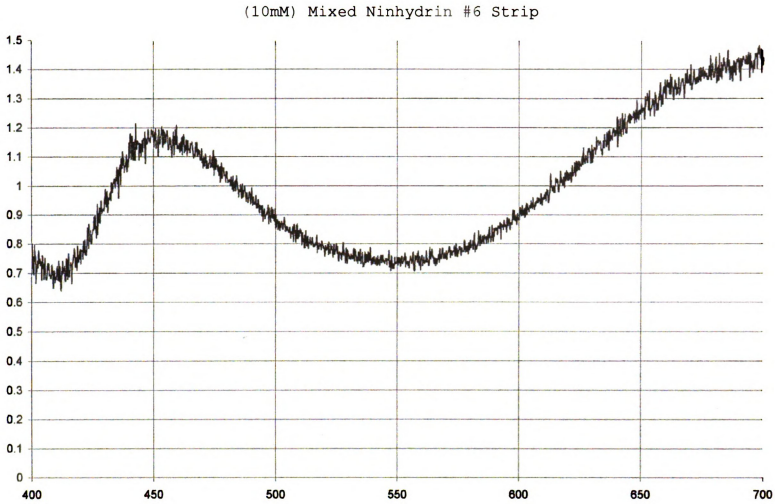


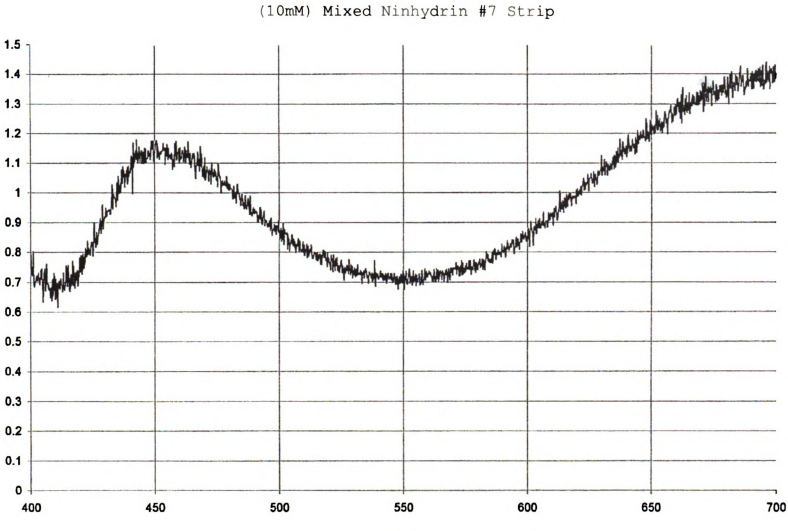

(10mM) Mixed Ninhydrin #2 Strip
99
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700


(10mM) Mixed Ninhydrin #3 Strip
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700

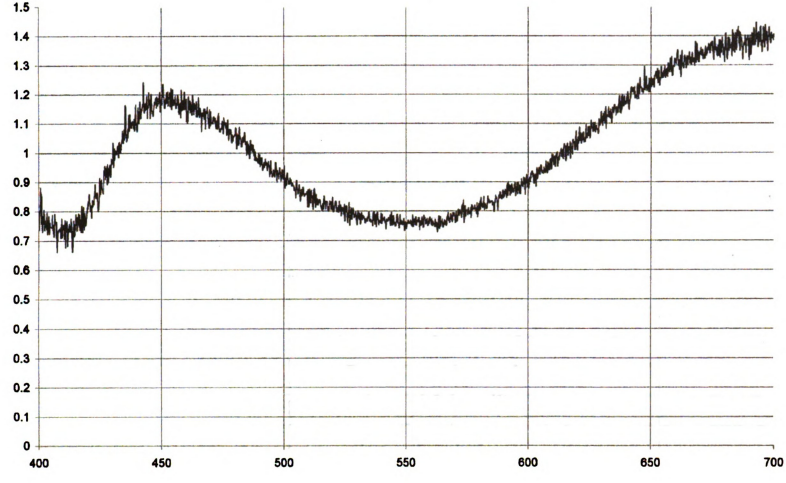

(10mM) Mixed Ninhydrin #4 Strip
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700

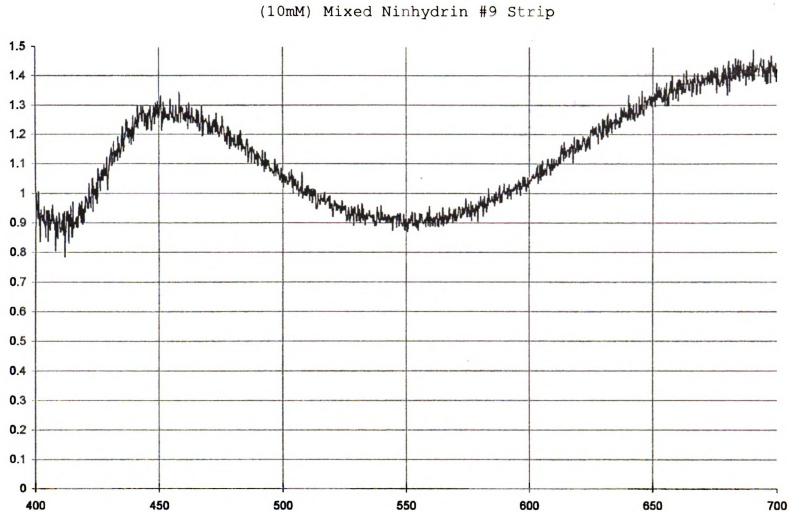

(10mM) Mixed Ninhydrin #5 Strip
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700


(10mM) Mixed Ninhydrin #6 Strip
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700

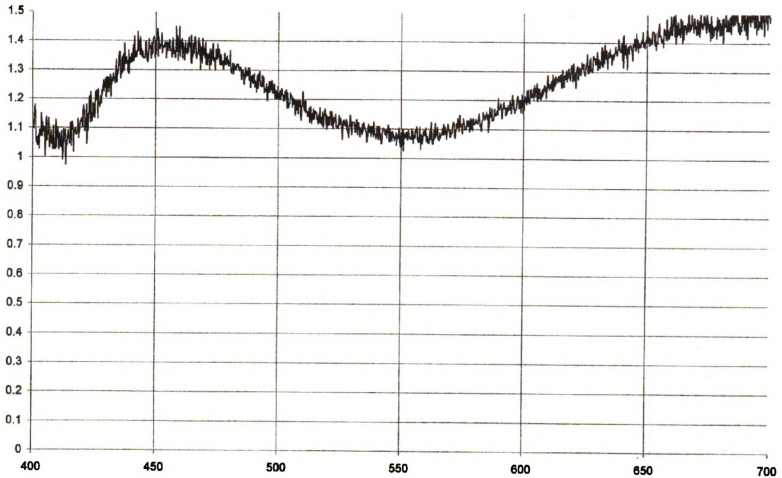

(10mM) Mixed Ninhydrin #7 Strip
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700

(10mM) Mixed Ninhydrin #8 Strip

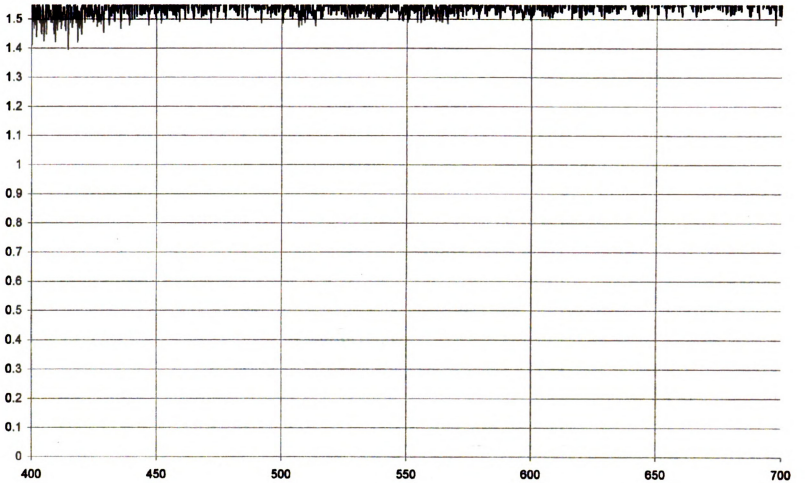



(10mM) Mixed Ninhydrin #9 Strip
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700

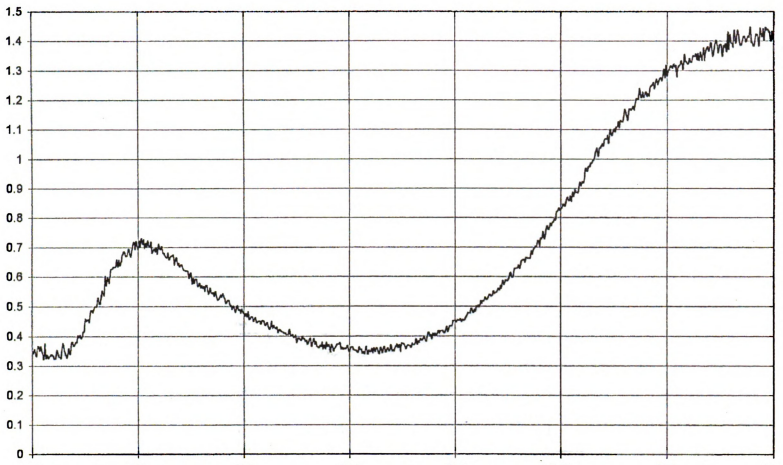
(10mM) Mixed Ninhydrin #10 Strip



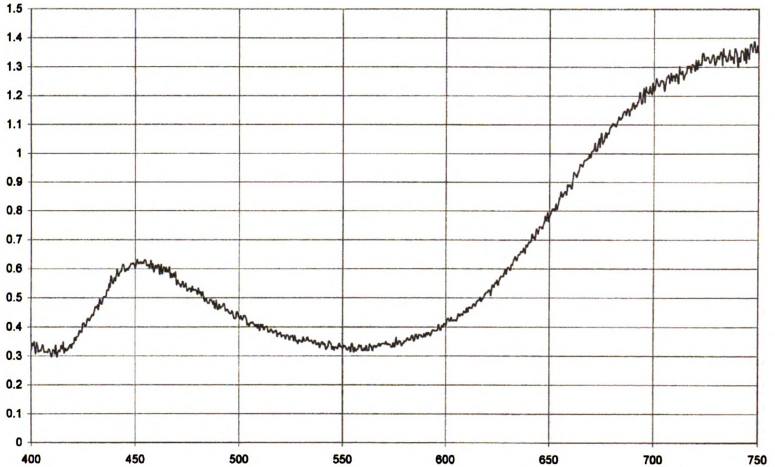
(10mM) Mixed Ninhydrin #11 Strip



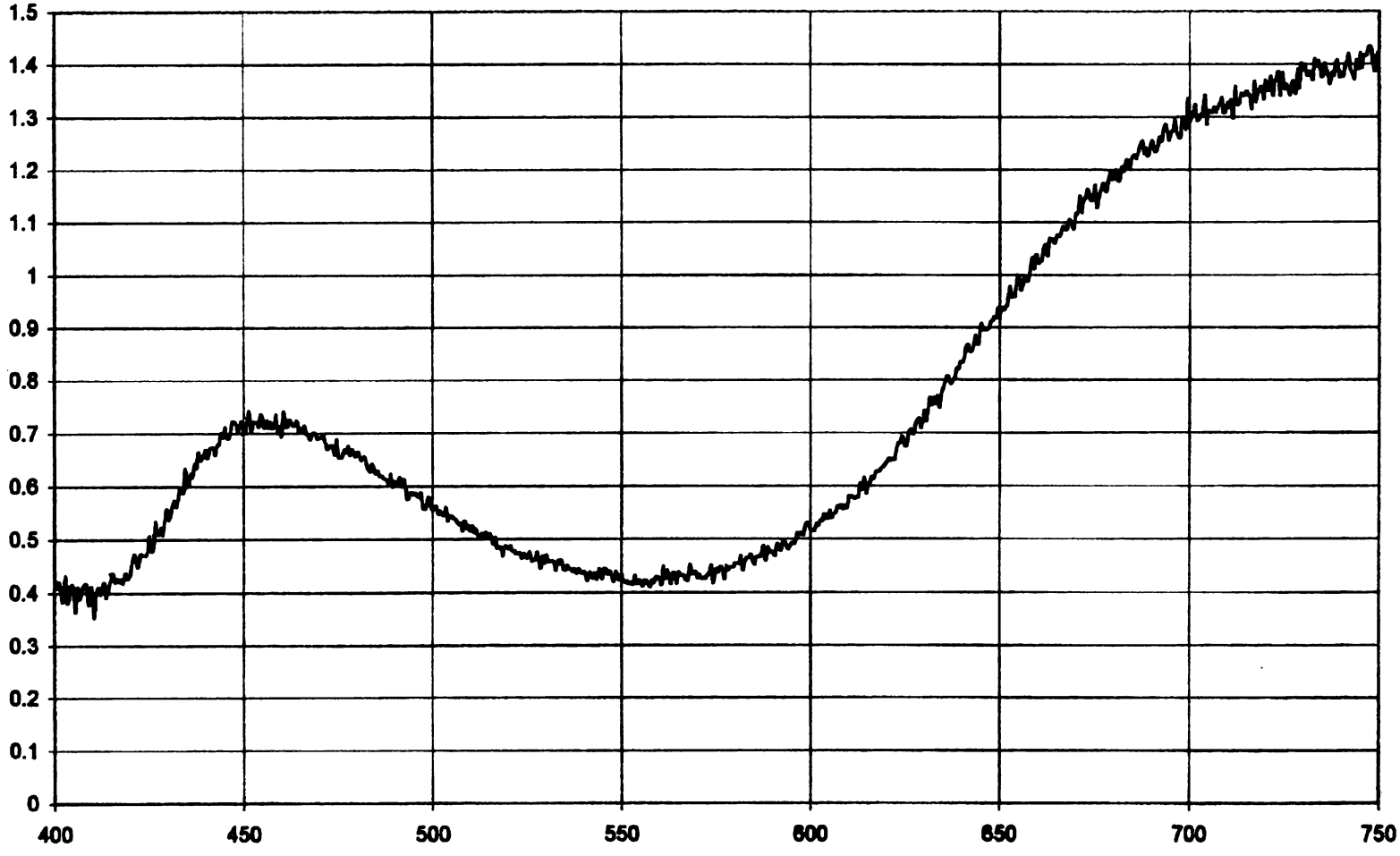
(40mM) Mixed Ninhydrin #5 Strip



(40mM) Mixed Ninhydrin #6 Strip



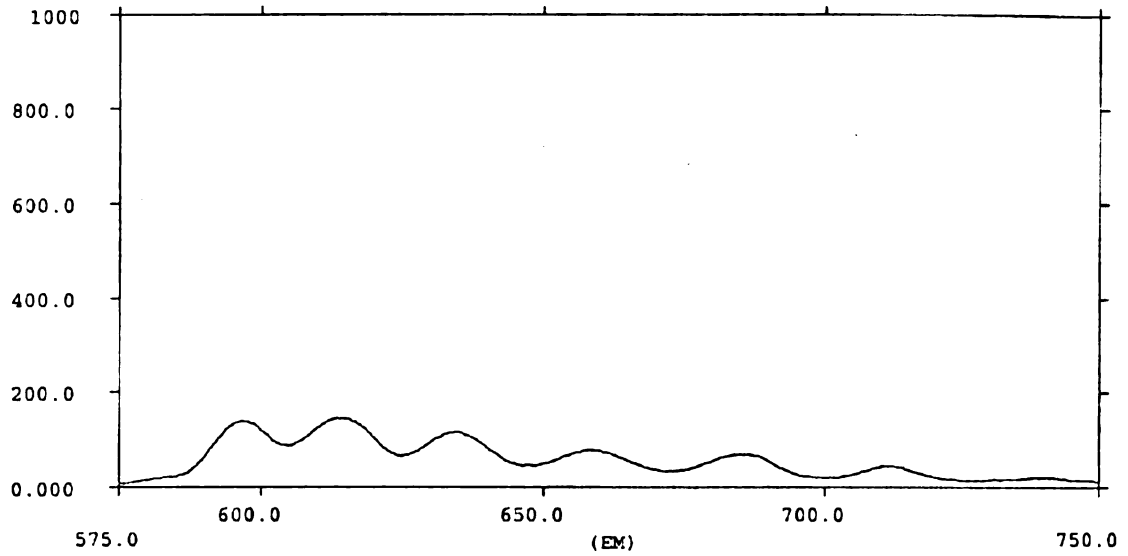
(40mM) Mixed Ninhydrin #7 Strip



APPENDIX C

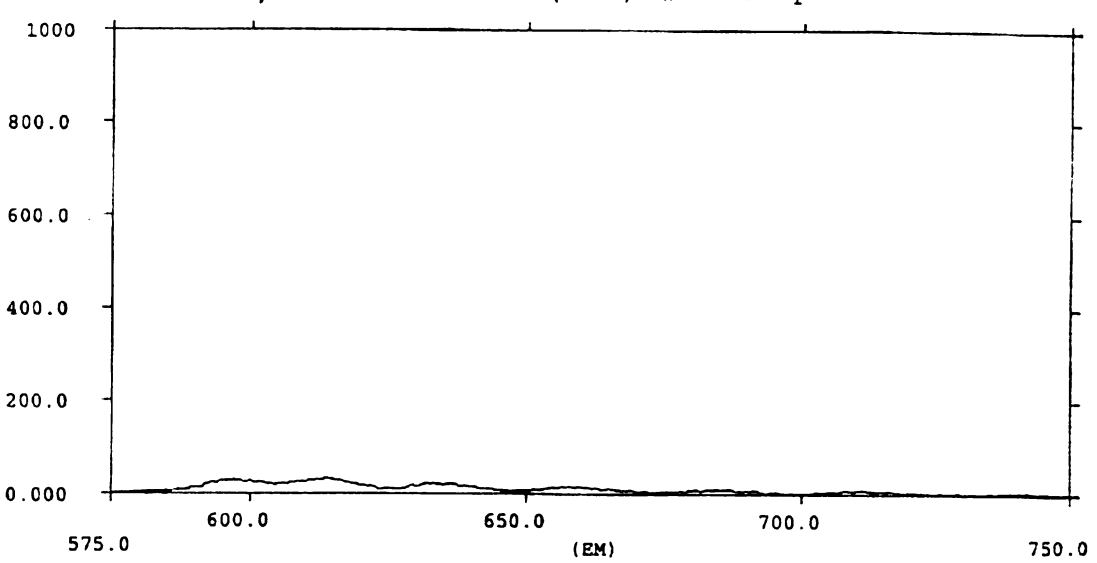
Fluorescence Spectra of 1,2-Indanedione Tested Sequential Whatman Filter Paper Strips

1,2-Indanedione (1mM) Blank Strip



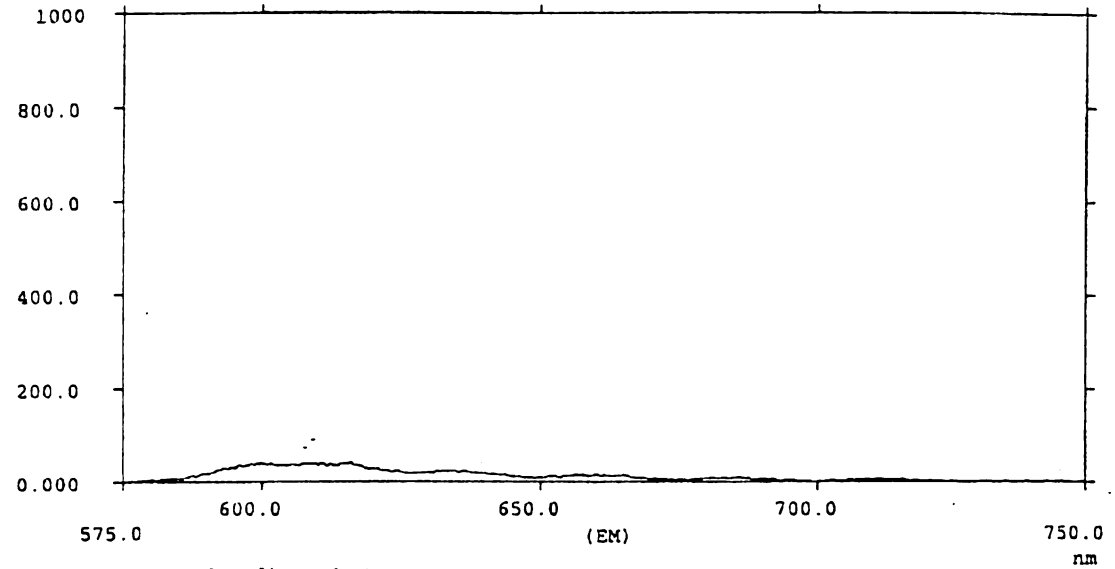
Sample : Indanedione 1mM
Comment : Blank Strip
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

1,2-Indanedione (1mM) #1 Strip



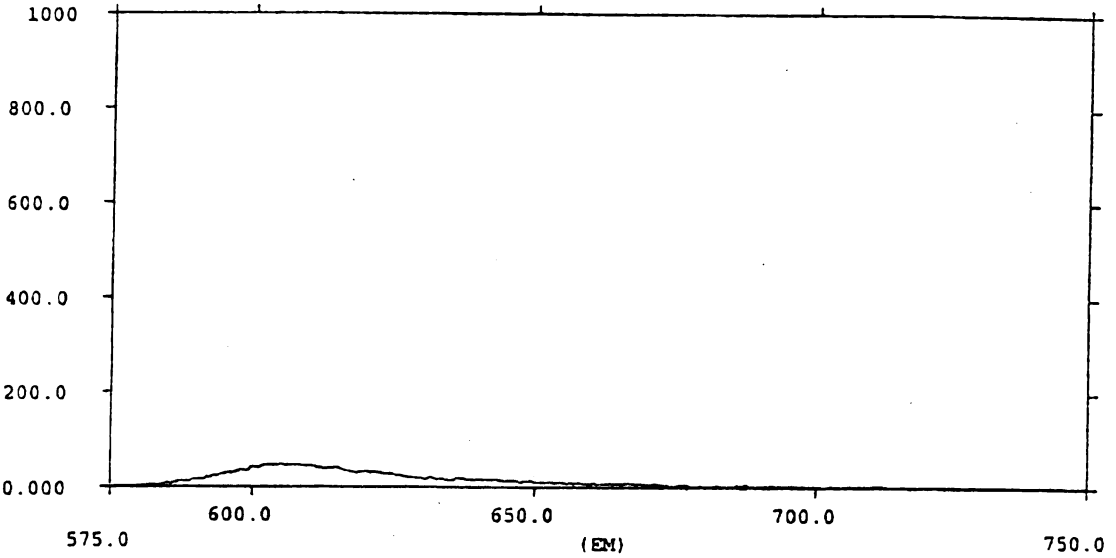
Sample : Indanedione 1mM
Comment : Strip #1
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

1,2-Indanedione (1mM) #2 Strip



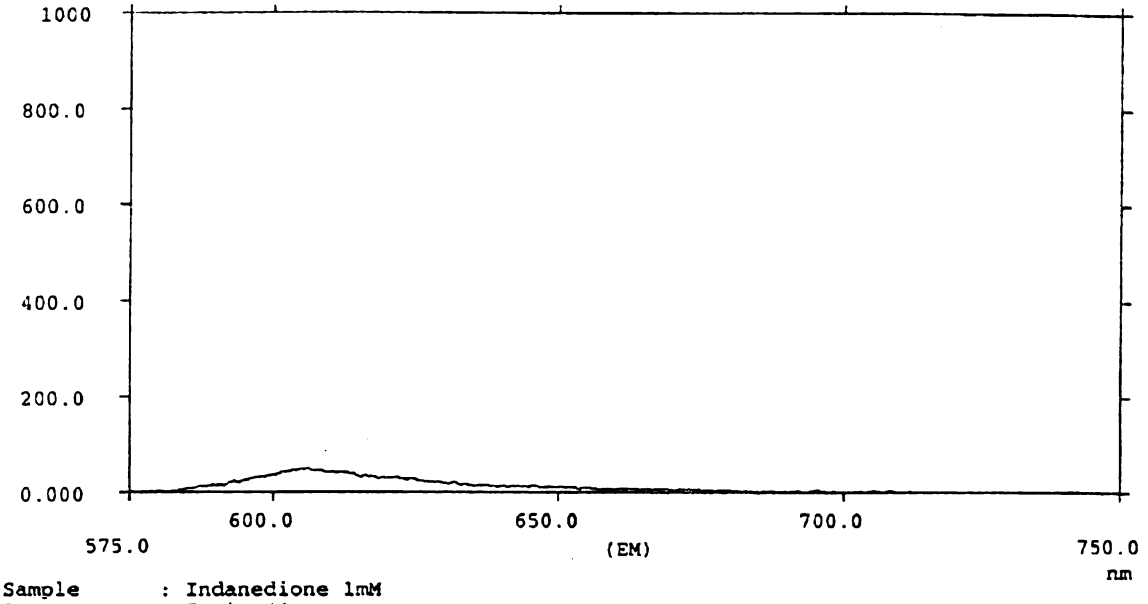
Sample : Indanedione 1mM
Comment : Strip #2
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

1,2-Indanedione (1mM) #3 Strip



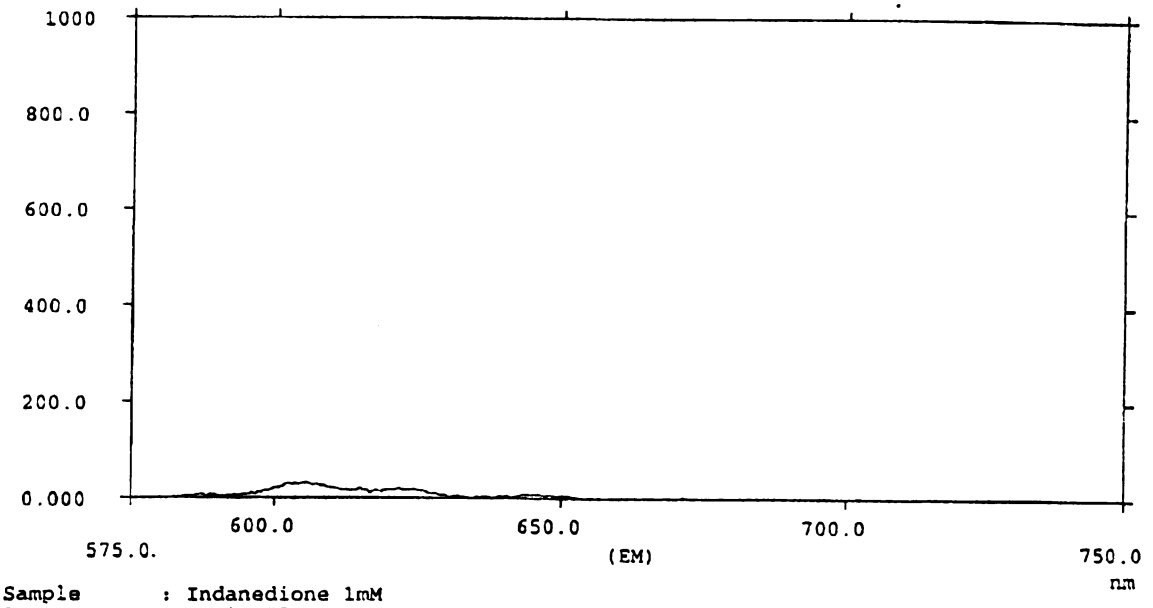
Sample : Indanedione 1mM
Comment : Strip #3
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

1,2-Indanedione (1mM) #4 Strip



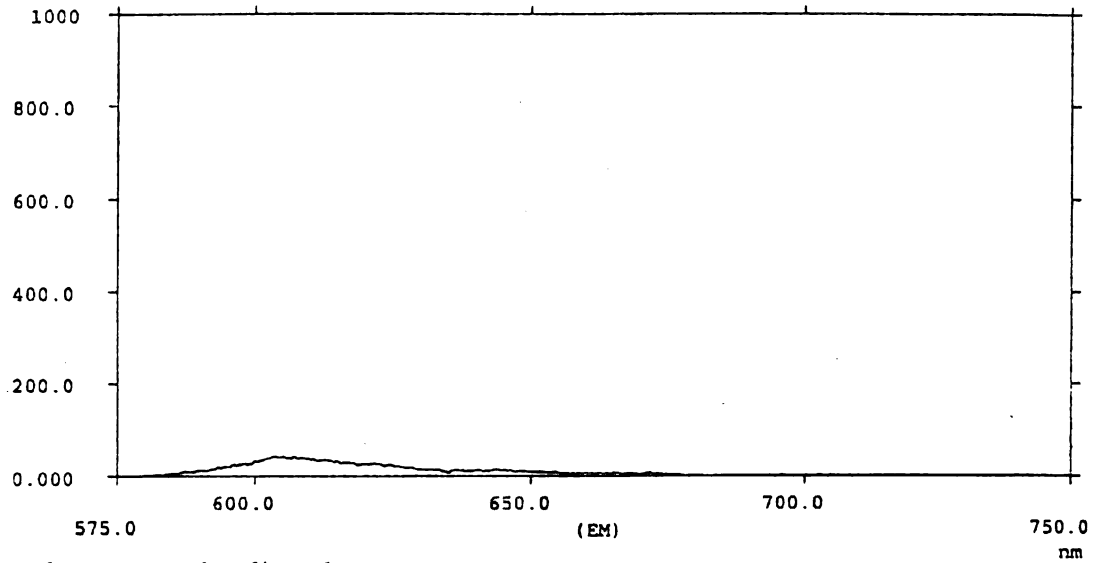
Sample : Indanedione 1mM
Comment : Strip #4
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

1,2-Indanedione (1mM) #5 Strip



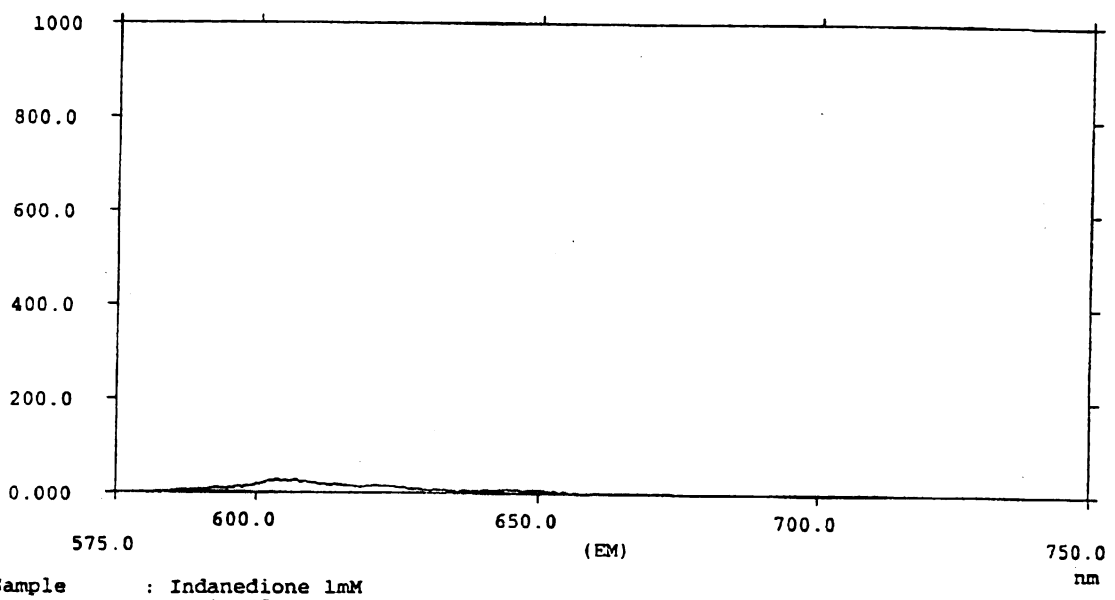
Sample : Indanedione 1mM
Comment : Strip #5
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

1,2-Indanedione (1mM) #6 Strip

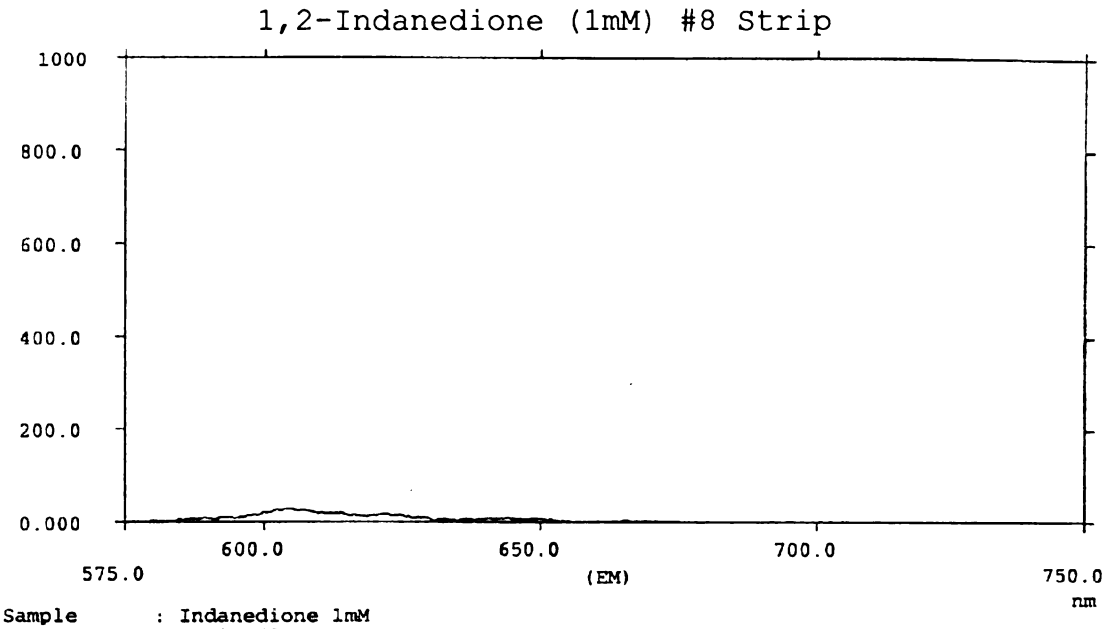


Sample : Indanedione 1mM
Comment : Strip #6
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

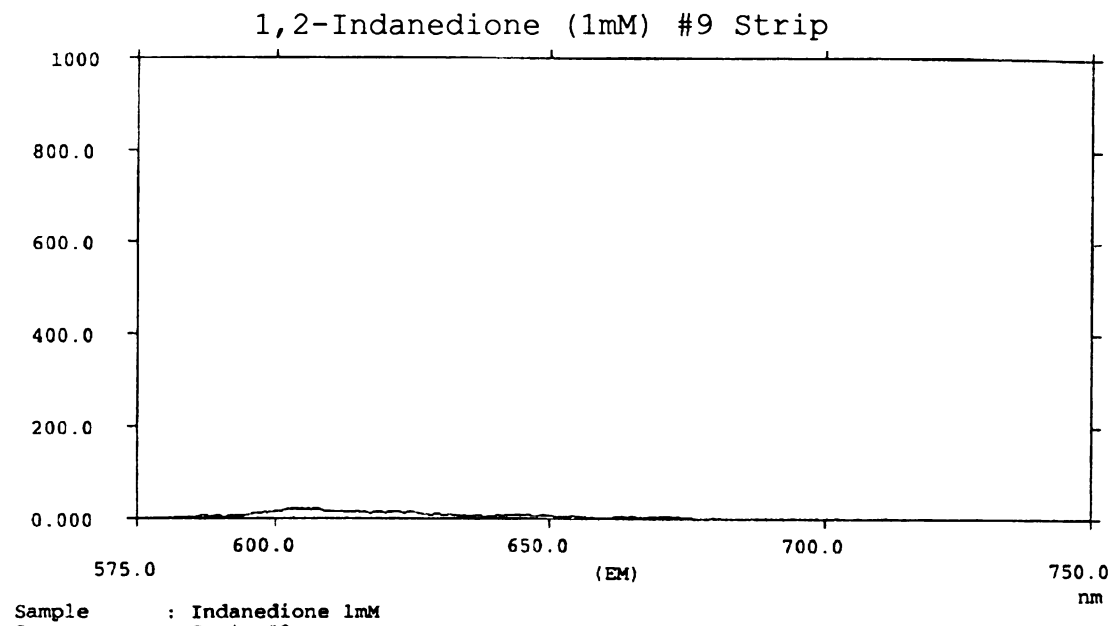
1,2-Indanedione (1mM) #7 Strip



Sample : Indanedione 1mM
Comment : Strip #7
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

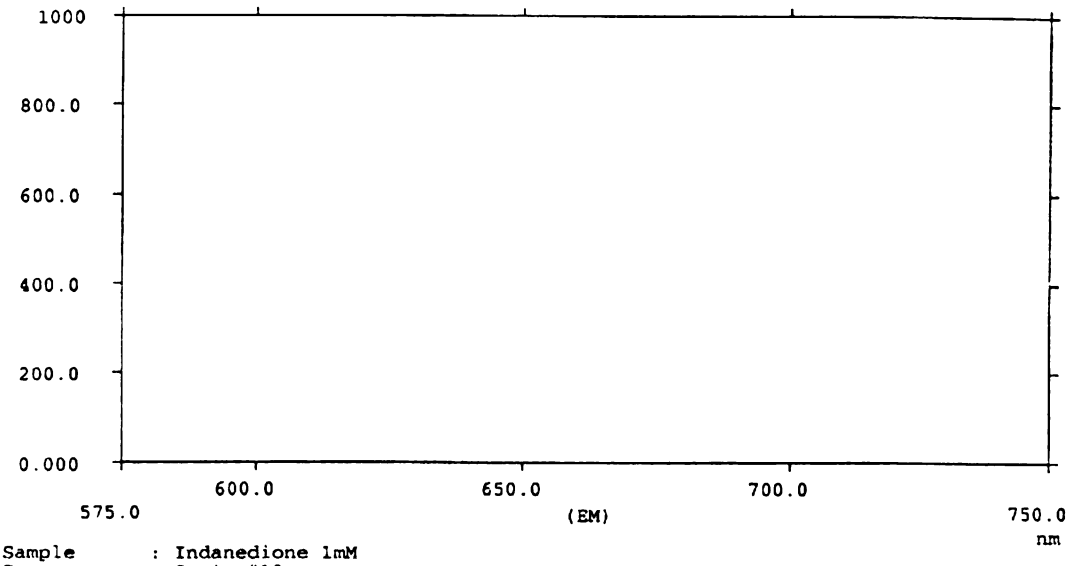


Sample : Indanedione 1mM
Comment : Strip #8
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto



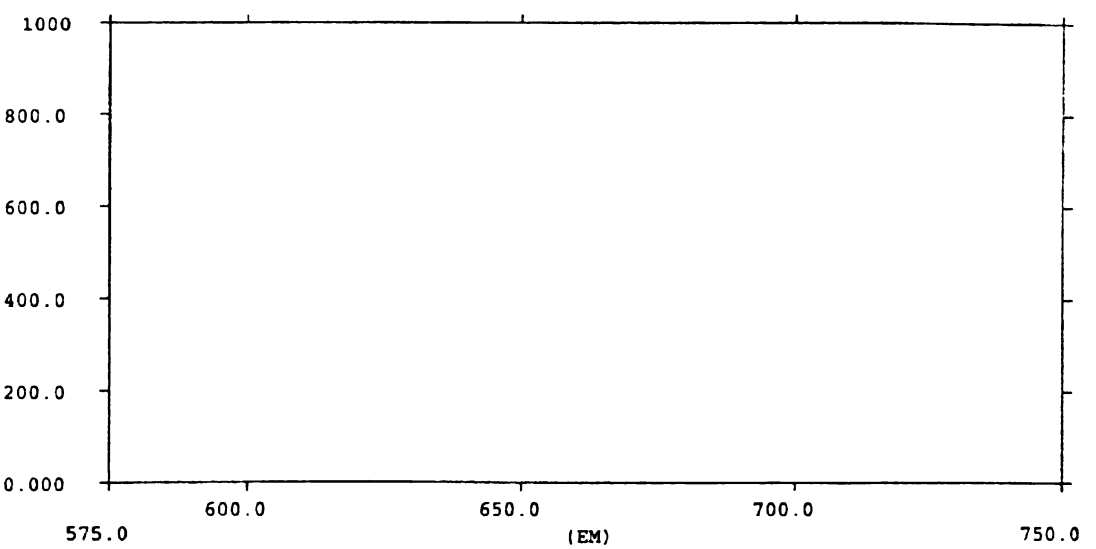
Sample : Indanedione 1mM
Comment : Strip #9
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

1,2-Indanedione (1mM) #10 Strip



Sample : Indanedione 1mM
Comment : Strip #10
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

1,2-Indanedione (1mM) #11 Strip

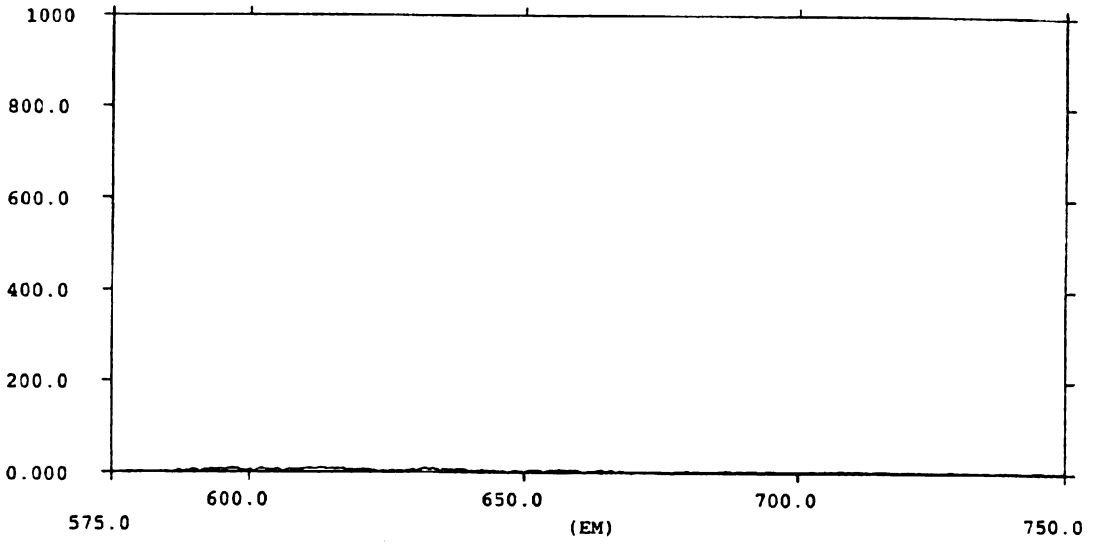


Sample : Indanedione 1mM
Comment : Strip #11
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

APPENDIX D

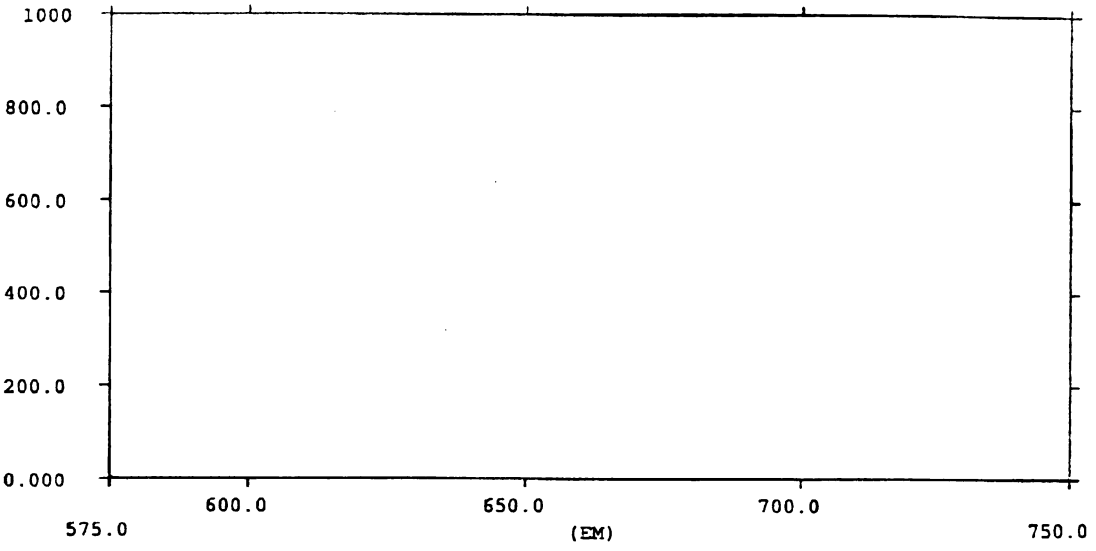
Fluorescence Spectra of 1,2-Indanedione Tested Mixed Whatman Filter Paper Strips

1,2-Indanedione (1mM) Mixed #1 Strip



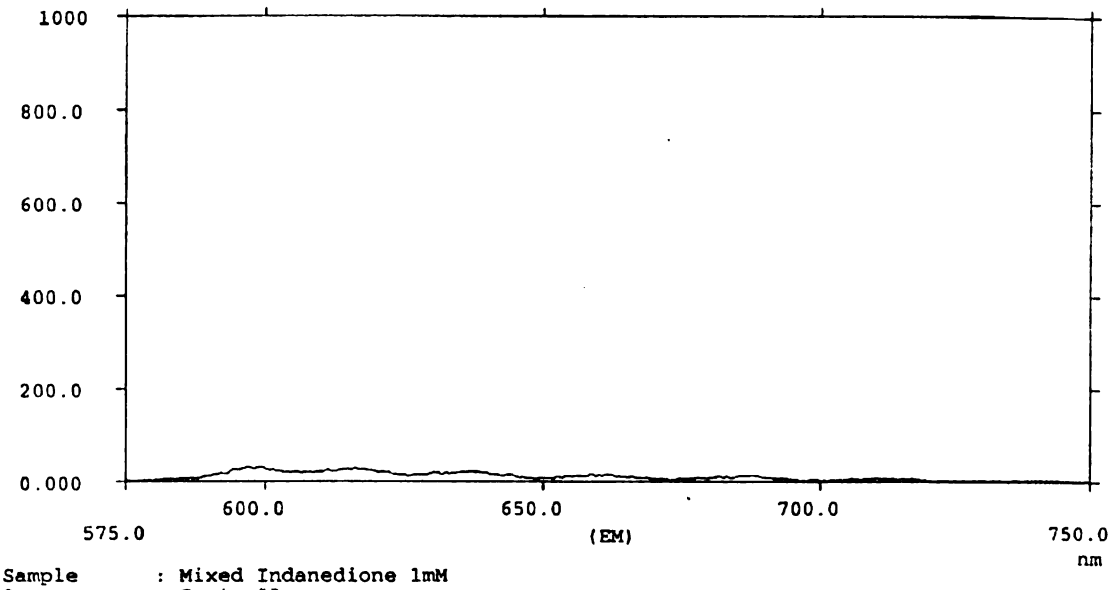
Sample : Mixed Indanedione 1mM
Comment : Strip #1
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

1,2-Indanedione (1mM) Mixed #2 Strip



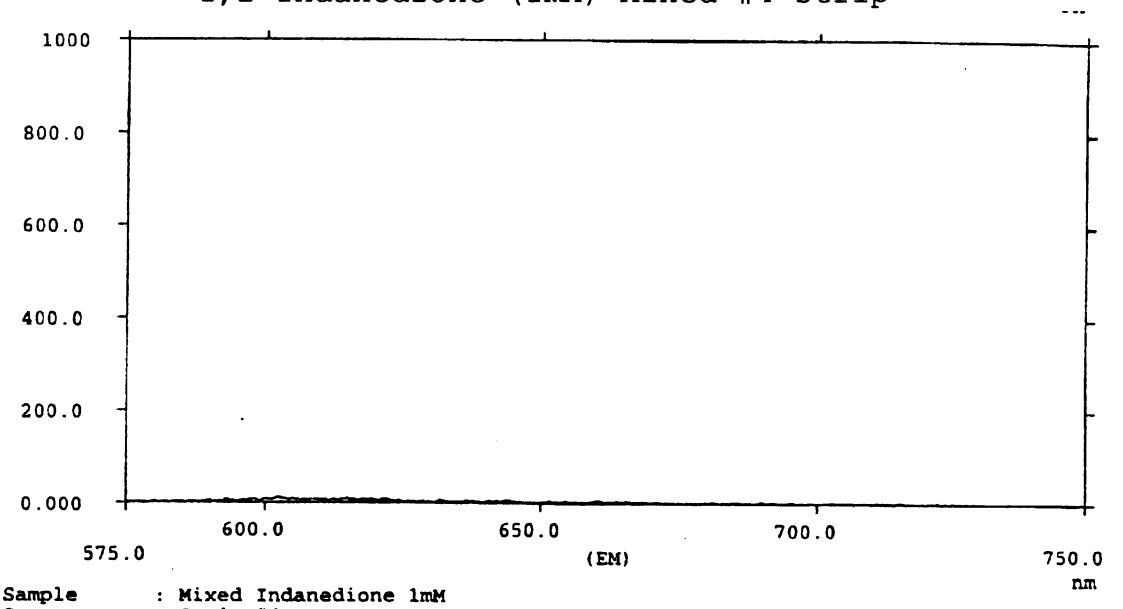
Sample : Mixed Indanedione 1mM
Comment : Strip #2
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

1,2-Indanedione (1mM) Mixed #3 Strip



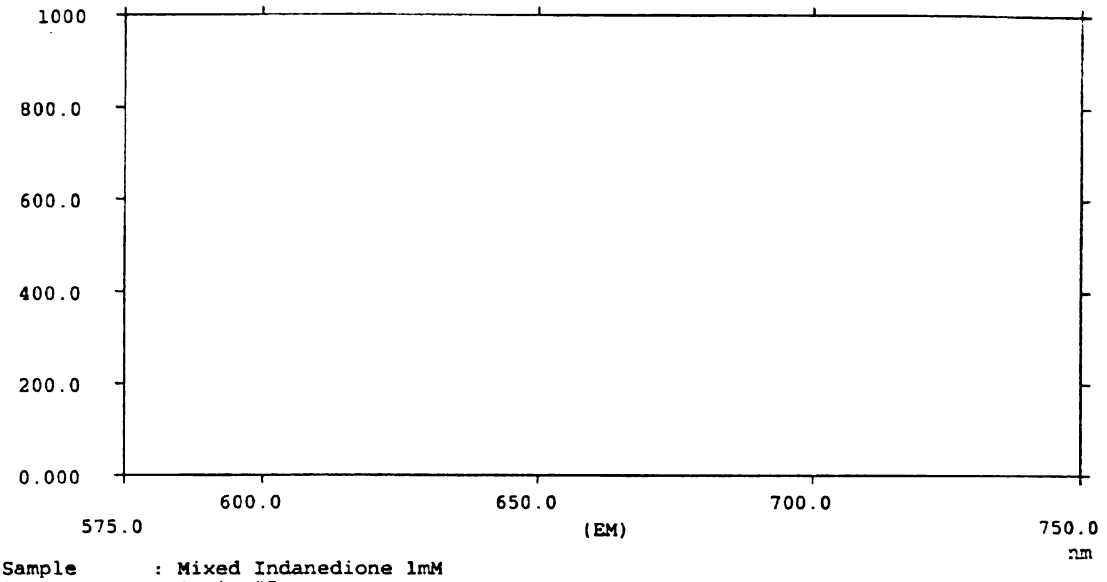
Sample : Mixed Indanedione 1mM
Comment : Strip #3
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

1,2-Indanedione (1mM) Mixed #4 Strip



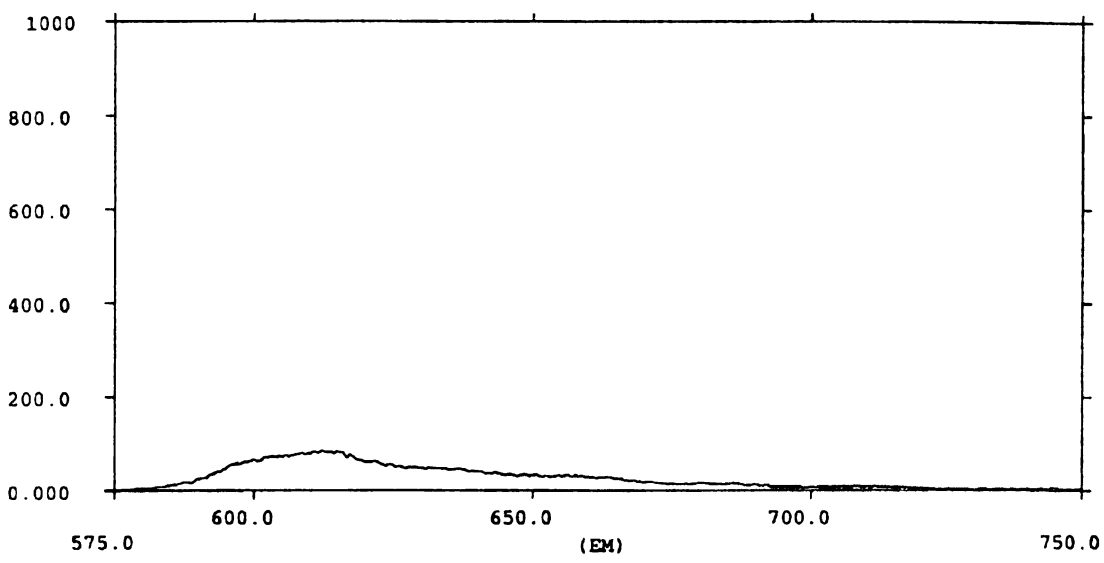
Sample : Mixed Indanedione 1mM
Comment : Strip #4
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

1,2-Indanedione (1mM) Mixed #5 Strip



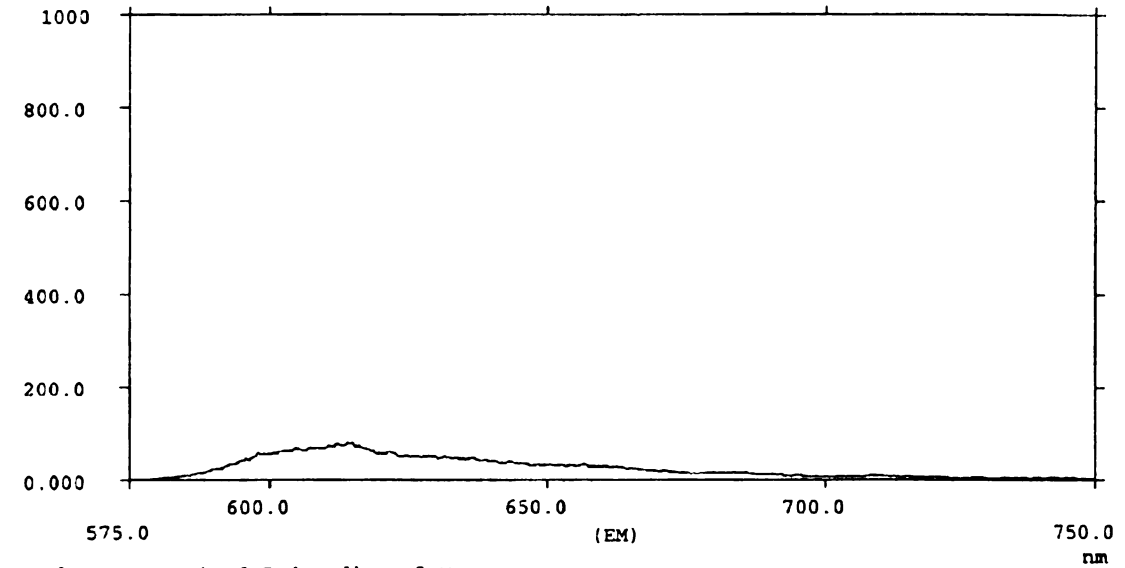
Sample : Mixed Indanedione 1mM
Comment : Strip #5
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

1,2-Indanedione (5mM) Mixed #3 Strip



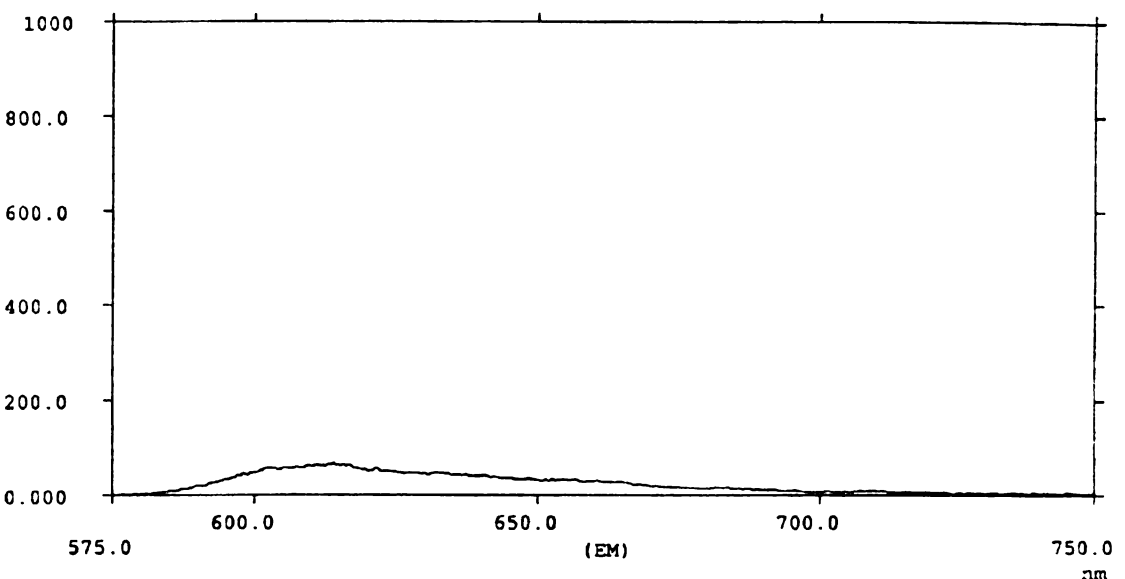
Sample : Mixed Indanedione 5mM
Comment : Strip #3
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

1,2-Indanedione (5mM) Mixed #4 Strip



Sample : Mixed Indanedione 5mM
Comment : Strip #4
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

1,2-Indanedione (5mM) Mixed #5 Strip



Sample : Mixed Indanedione 5mM
Comment : Strip #5
EX : 555.0 nm
Data Mode : Fluorescence
Scan Speed : 240 nm/min Slit (EX/EM) : 5.0 nm / 5.0 nm
PMT Voltage : 700 V Response : Auto

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