Investigating the Most Effective Solvent for Isolating Different Components of a Black Marker using Paper Chromatography

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<u>Abstract</u>

The processes of chromatography have become prevalent in society with each passing day. This idea of chromatography has been incorporated into this investigation, which aims to test several varying solvents to determine which is most effective for separating the different components that make up a black marker (Artline 210). The technique manipulated is chromatography, a method of analysis used to enable the viewer to visually comprehend the various elements present in a mixture, which in this case is the marker. The investigation was conducted in two major parts: the first was the execution of the experiment and the second which were the calculations necessary to draw adequate conclusions. During procedure one, the investigator set up the trial, using 10.0 ± 0.5 mL of each solvent and measured the distance travelled by the separated components and the solvent when given five minutes to separate. Part two revolved around appropriate calculations regarding the obtained results. From this, the investigator observed that different solvents could only separate specific components to a disparate degree. Overall, tap water was best at separating the individual components present in the black marker, evident by the large distances travelled by each component as well as the solvent.

Introduction

Chromatography is a scientific technique of separating mixtures into the components they are made from in order to analyse, identify and quantify the mixture or components. There are many different types of chromatography: liquid chromatography, gas chromatography, and paper chromatography; however, they all act on the same basic principles of attraction. Chromatography utilises the differential affinities of the components for a gas or liquid medium (mobile phase) and the stationary medium (stationary phase). Due to their varying adsorption and desorption rates, they will move at different speeds, in turn separating the distinct constituents. The separation of a mixture into its disparate components is a physical process; that is, because the components of the mixture are not chemically bound, they can be separated by physical means (TeachEngineering, 2010). This property of mixtures sanctions an application of chromatography in the police department. When solving crime, chromatography can be used to determine the presence of cocaine in urine, alcohol in blood, PCBs in fish and lead in water (Soinc, n,d). The results of such an investigation can be visually presented on a graph with a calibration curve, hence allowing detectives to calculate the concentration of a particular substance in the sample.

Using paper chromatography, this investigation aims to determine which solvent is most effective for the separation of the different components of a black marker. Ozlem Coskun's research demonstrated the significance of chromatography and suggested methods to perform trials, which served as the

2 | Page

basis for the subsequent study (NIH, 2016). It was hypothesised that as the interaction between the solvent and the sample increased, then the rate of separation of components will increase proportionately, displaying more considerable distances between the disparate constituents. The results and data collected from conducting the investigation would then be used to graph the function of distance travelled by the separate component (cm) over the distance travelled by the solvent (cm) in order to deduce the most effective solvent for separating the elements. This report will summarise the findings and information gathered throughout the study to determine the optimum solvent for separating the components found in the black marker. The report will also cover the method's validity and reliability as well as the precision and accuracy of the data produced and if these factors had an improper impact on the final conclusions drawn.

Methodology

Task 1 – The Practical Execution of the Experiment

Task 1 began by measuring 10 ± 0.5 mL of five different solvents (1% sodium chloride solution, cloudy ammonia, methylated spirits, tap water and kerosene) into five test tubes in a test tube rack. Five pieces of chromatography paper were then cut out into 16 cm x 2 cm dimensions, with a 1 cm fold at the top to enable easy removal of the strips. We then created our line of origin on the chromatography strips 3 cm up the strip on the side that does not have the fold. This was done to ensure that the level at which the solvent sat was always under the line of origin, allowing the solvent to travel up the strip and separate the components. This process was repeated on each strip until they were all the same, ensuring that the line of origin was straight. All five chromatography strips were then simultaneously placed into each of the five test tubes housing the five different solvents, whilst synchronically a stopwatch had started. After 5 minutes, all of the chromatography strips were, in unison, taken out of the test tubes and placed on a paper towel, allowing them to rest and dry out naturally. Each test tube was then thoroughly rinsed with water, and each solution was disposed of in the waste container provided before the test tubes were refilled, ensuring no residual solvent was present in the test tube to impact subsequent trials. This whole process was repeated another two times. After the third trial, another trial was executed. This time, however, we extended the time the solvent had to travel up the chromatography strip from 5 minutes to 10 minutes. This acted as a comparability result, aimed to view any correlations between increased time and rate of separation of components. After leaving the chromatography strips to dry overnight, we measured the distance each component and the solvent travelled from the origin line using a ruler, with an uncertainty mark of \pm 0.05 cm. These results were then noted in a textbook. In an attempt to minimise external factors that would skew findings, the experiment was conducted over a double period to ensure consistency in environmental implications.

Task 2 – The Calculations

Task 2 focused on calculations that would aid in drawing a pertinent conclusion and was based on procedures from earlier studies relating to Amber Hess' enquiry on the isolation of various mixtures (sciencebuddies, 2022). They involved the results obtained from Task 1 and offered a numerical value representing the ratio between the distance travelled by each component and the solvent from the starting point (line of origin). This ratio can then be used to deduce which solvent was best at separating the components present in the black marker, as the higher the value, the better the separation of the solvent. This value is known as the retardation factor (R_f value) and is calculated by dividing the distance travelled by the sample component by the overall distance travelled by the solvent. These calculations were then repeated for every element discerned in each trial for each solvent, ensuring the values obtained were always lesser than one. The average R_f value for each component in each solvent was then calculated, excluding the measurements from the 10-minute trial. Finally, each of these calculations was computed twice to account for accidentally misinterpreted values and resulted in outcomes of an uncertainty mark of \pm 0.05, followed through from Task 1.

Results



 $Graph 1: A \ column \ graph \ that \ displays \ the \ average \ R_f \ values \ obtained \ from \ the \ separation \ of \ the \ three \ components \ of \ the \ black \ marker$

Investigating the Relationship Between Distance Travelled by Separate Components and Solvent in 2 minutes						
	Solvent (10 mL)	R _f Values				
		Trial 1				
Blue	1% Sodium Chloride	N/A				
Yellow	Solution	N/A				
Pink		N/A				
Blue	Cloudy Ammonia	N/A				
Yellow		N/A				
Pink		N/A				
Blue	Methylated Spirits	N/A				
Yellow		N/A				
Pink		0.57 ± 0.05				
Blue	Tap Water	N/A				
Yellow		N/A				
Pink		N/A				
Blue	Kerosene	0.14 ± 0.05				
Yellow		N/A				
Pink		0.29 ± 0.05				

Table 1: A table of preliminary results recording the trial conducted with a Sharpie Marker (marker used before changing to Artline 210)

Solvent in 5 minutes						
	Solvent (10 mL)		R _f Values	Average R _f Value		
		Trial 1	Trial 2	Trial 3		
Blue	1% Sodium Chloride	0.66 [°] ±	0.76 ±	0.77 [•] ±	0.73 ± 0.05	
	Solution	0.05	0.05	0.05		
Yellow		0.42 ±	0.46 ±	0.44 [·] ±	0.44 ± 0.05	
		0.05	0.05	0.05		
Pink		0.19 ±	0.20 ±	0.22 [·] ±	0.21 ± 0.05	
		0.05	0.05	0.05		
Blue	Cloudy Ammonia	0.56 ±	0.71 ±	0.62 [·] ±	0.63 ± 0.05	
		0.05	0.05	0.05		
Yellow		N/A	N/A	N/A	N/A	
Pink		0.19 ±	0.29 ±	0.24 [•] ±	0.24 ± 0.05	
		0.05	0.05	0.05		
Blue	Methylated Spirits	0.40 ±	0.46 [°] ±	0.35 ±	0.41 ± 0.05	
		0.05	0.05	0.05		
Yellow		N/A	N/A	N/A	N/A	
Pink		N/A	N/A	N/A	N/A	
Blue	Tap Water	0.82 ±	0.80 ±	0.77 [·] ±	0.81 ± 0.05	
		0.05	0.05	0.05		
Yellow		0.68 ±	0.62 [·] ±	0.60 ±	0.63 ± 0.05	
		0.05	0.05	0.05		
Pink		0.31 ±	0.31 ±	0.31 ±	0.31 ± 0.05	
		0.05	0.05	0.05		
Blue	Kerosene	N/A	N/A	0.03 ±	0.03 ± 0.05	
				0.05		
Yellow		N/A	N/A	N/A	N/A	
Pink		N/A	N/A	N/A	N/A	

Investigating the Relationship Between Distance Travelled by Separate Components and

Table 2: Results showing the trials conducted with the marker Artline 210. It also shows the Rf values produced as well as the average $R_{\rm f}$ value induced by the predominant three components separated



Graph 2: A pictorial representation of the single trial conducted with the Artline 210, allowing the components 10 minutes to separate, and demonstrating the R_f values produced thereafter

After combining the procedures conducted in both Tasks 1 and 2, along with extra routines, the results above are acquired. In particular, Graph 1 and Table 2 are intertwined, representing the same information differently. Upon analysis, when allowing the solvents to separate the marker for five minutes, tap water gave average R_f values of 0.81 ± 0.05, 0.63 ± 0.05 and 0.31 ± 0.05 respectively, for the components blue, yellow, and pink, whilst the 1% sodium chloride solution, the next most comparable solvent, produces average R_f values of 0.73 ± 0.05, 0.44 ± 0.05 and 0.21 ± 0.05 respectively for the same colours. The next solvent with similar results is cloudy ammonia, reproducing results of 0.63 ± 0.05 , N/A and 0.24 ± 0.05 for blue, yellow, and pink, respectively. Having not separated the component of the colour yellow, cloudy ammonia is the only polar solvent used in the investigation not isolating all the constituents. Methylated spirits was next, generating average R_f values of 0.41 ± 0.05, N/A and N/A for the colours blue, yellow, and pink, respectively and were most comparable with kerosene, exhibiting the least separation of the predominant components of the black marker with results of 0.03 \pm 0.05, N/A and N/A respectively. From these findings, the investigator was able to draw adequate conclusions regarding the ability of each solvent to separate, coming to the deduction that tap water, and to some extent, the 1% sodium chloride solution, were undoubtedly most fit for isolating the components of the black marker within the five minutes.

Graph 2 represents the data collected from the trial in which the solvent was allowed 10 minutes to separate the components instead of five in an attempt to realise if more time enabled better isolation.

Judging from overall findings, this was not the case. However, in the extra five minutes, the methylated spirits' ability to separate the marker components spiked, separating all the hidden constituents with significantly larger R_f values.

Table 1 is a body of results that recounts the preliminary trials. It was initiated by the investigator to familiarise themselves with the investigation and acted as a means to roughly understand the ability of each solvent to separate the components. It was because of the preliminary trials that the investigator changed the time length that allowed for the solvent to separate the components within the marker as well as change the marker as a whole.

Discussion

The investigation's purpose of determining the optimal solvent for isolating the various components that make up the black marker is fulfilled by patterns and trends visible by analysis of the data described above. Upon examination, it is evident that different solvents separate the distinct elements within a black marker at different rates, some of which do not exhibit any distinguishment whatsoever. From Graph 1, the obvious pattern apparent is how tap water, along with the 1% sodium chloride solution, was best at separating all of the predominant components of the marker (blue, yellow and pink). This level of isolation is only possible due to the components of the marker readily dissolving in the solvent. As a result of their desorption, their attraction to the solvent increases and their affinity to the chromatography paper is weakened, resulting in the sample components travelling up the strip at varying rates depending on their level of interaction with each phase, hence separating the constituents.

The overall trend revealed the efficiency with which tap water and the 1% sodium chloride solution were able to separate the distinct components, giving R_f values of 0.8 ± 0.05, 0.63 ± 0.05, and 0.31 ± 0.05 respectively for tap water and the values 0.73 ± 0.05, 0.44 ± 0.05, and 0.21 ± 0.05 respectively for the 1% sodium chloride solution, with variation most likely due error or unwarranted influence of external factors. A particular outlier to this trend occurred on the first trial of the cloudy ammonia solution when the R_f value calculated for the discerned colour blue was 0.56 ± 0.05. Whilst this is a slight difference from the average R_f value for blue in cloudy ammonia of 0.63 ± 0.05, there should be no inconsistent values unless an accidental variation in method or incorrect measurement occurs. This is presumably the outcome of random error imposed on by poor method, causing disparity to control variables. Whilst the abolition of all random errors is not possible, to reduce their influence, one can conduct more trials, using a larger sample to mask the effect of the hindered result. However, since we sought to minimise environmental impacts, there was no time for further experiments since we had to complete all of our trials within the double period. This limitation allowed for outliers such as

the one outlined above to arise. Other potential limitations include the lack of solvents available, limiting the polarities of solvents being tested. As outlined by Fiona Middleton's research, limitations such as these could be easily overcome by completing more repeat trials and involving a wide range of polar and non-polar solvents in an attempt to draw stronger conclusions, as direct correlations between varying polarities on a constant sample would become apparent (Scribblr, 2019).

It was hypothesised that as the interaction of the solvent with the sample increased, then the pace of component separation would follow suit, resulting in greater distances between the different constituents. As displayed in Table 2, tap water, 1% sodium chloride solution and cloudy ammonia interacted most with the solvents and hence separated the best, wherein the cloudy ammonia was inferior to the other two. This effectively relates back to the aim of the investigation, which was to deduce which solvent was most suitable for separating the individual components that make up the black marker. Hence, it can be concluded that these solvents were the optimal apparatus for this investigation. However, this conjecture is not entirely correct due to the cloudy ammonia being unable to separate the colour yellow, as demonstrated in Graph 1. This suggests the presence of flaws in the method as the produced result does not reflect the actual, true value that should be imitated. Furthermore, it is known that the trials were likely hurried with subpar execution owing to time and resource constraints. This discrepancy is thus likely the result of improper measurement of the R_f values and solvent quantity. As a result, this conclusion may be partially incorrect and needs to be supported by further scientific investigation.

The method used in this study allowed for the efficient collection of data that, in essence, supported the findings of Amber Hess' research, but eventually resulted in inaccurate conclusions as a result of cloudy ammonia being unable to distinguish all the different parts of the black marker (sciencebuddies, 2022). The approach taken for this experiment may be considered reasonably valid, given the method's ability to provide data that tests the predetermined hypothesis and aim of the investigation. This validity is further reinforced by the competence of the method to carefully change the independent variable by varying the solvents, whilst measuring the dependent variable of the distance each component and solvent travelled up the chromatography strip with a precision of \pm 0.05 cm, and controlling externally influential factors such as the environmental conditions and time allowed for the solvent to separate components. The preciseness of this investigation can be attested by the closeness of the results obtained. For example, the R_f values they reproduced from the three trials for tap water concerning the colour blue were 0.82 \pm 0.05, 0.8 \pm 0.05 and 0.77' \pm 0.05, respectively, which appear quite precise. However, not all the R_f values replicated precise results, evident by outliers. When looking at the accuracy of the results, the investigator can acknowledge they are unreliable, in that the results attained do not reflect the anticipated outcome. To increase

the reliability of the results, the investigator could complete more trials. Successive repetition of experimental trials greatly decreases large statistical and random errors from having a substantial impact on the results, thus increasing the precision and accuracy of the conclusions drawn. The majority of random errors that may have impacted the method root from group dynamics and carelessness of the investigators regarding simple procedures, wherein certain members may conduct certain tasks to their plea and not the quality at which they should be executed. An example of this would be parallax error, in which the angle of perceiving the quantity of solvent poured out may vary among group members at each trial. This error could be easily avoided by observing the amount of solvent poured out at eye level, ensuring the reading is consistently taken from the top or bottom of the meniscus. Further repeated trials may minimise other human errors, such as inaccurately calculating the duration of response, which also influenced the data acquired. Systematic errors that may have influenced the results is the transference of solvent from the measuring cylinder to the test tube, wherein not all of the solvent has been changed over. In this case, the true volume of the solvent is always less than the quantity used. This error could be avoided by using test tubes that incrementally mark 10 mL to ensure all the solvent is used in the trial. Regarding the amount of solvent, the investigator used an uncertainty mark of \pm 0.5 mL and a \pm 0.05 cm mark when measuring the distance each component and solvent had travelled on the chromatography strip, as quantification of measurement is always subject to variations in accuracy and precision. Further alterations to the method to improve it include using different forms of chromatography, and in particular, thin-layer chromatography (TLC). This chromatography technique is based on adsorption principles and is frequently used to isolate non-volatile mixtures. The investigator's preference for using paper chromatography over TLC is centralised around the hassle-free experience associated with paper chromatography; it is easy to set up, simple to operate and does not require too many parts. The disadvantage of paper chromatography, however, is the amount of time it takes for ascending analysis and the qualitative form of data it offers, allowing for subjectivity among individuals to arise. Paper chromatography cannot be used to separate volatile substances and is incompatible with larger or complex samples. This extended amount of time necessary for the trial influences the investigation as it limits the number of trials the investigator could complete, ultimately lessening the preciseness and accuracy of the results obtained. If more trials were conducted, it might affect the outcome to largely different results to those obtained in this investigation. Additionally, more time would reduce potential bias as the investigator would not be inclined to change the marker based on preliminary trials. According to the preliminary trials from Table 1, only methylated spirits and kerosene could distinguish between any of the components in the experiment, with methylated spirits only being able to isolate one colour. If more time had been provided, the investigator would have conducted more

trials to see if the results obtained were accurate and precise. Being influenced by bias provoked change as the expected trend was not being met and enabled bias to sway the overall results obtained.

Processing and Evaluating Results

Upon analysis of the obtained results, it was concluded that tap water was the best solvent for separating the components of the black marker, producing R_f values of 0.81 ± 0.05, 0.63 ± 0.05 and 0.31 ± 0.05 respectively, for the colours blue, yellow and pink. This effectively relates back to the purpose of the investigation, which was to deduce which solvent was most suitable for separating the components, which in this case is tap water. Throughout the investigation, three main-coloured components were separated frequently: blue, yellow and pink. Upon examination of Graph 1, it was revealed that blue was readily dissolved in the mobile phase, producing the highest R_f values when compared to the other components. This implies that the polarity of the blue component is closest to that of the mobile phases, being attracted to it more than the stationary phase. These large amounts of interaction with the mobile phase indicate that the component forms stronger intermolecular bonds with the solvent (mobile phase) than that of the paper (stationary phase), allowing it to be carried up the chromatography strip via desorption and thus exhibiting the highest binding position on the chromatography paper. The opposite can be said about the yellow and pink components. Retrospectively speaking, yellow had more interactions with the mobile phase than pink, evident by its intermediate positioning on the stationary phase between blue and pink. It can be inferred from this data that yellow had, to some extent, experienced equal forces of attraction to both the chromatography strip and the solvent, regularly dissolving into the solvent to travel up the paper whilst also being adsorbed quite strongly to the paper itself. Furthermore, this expounds on how yellow was equally competed for, highlighting its median polarity relative to the stationary and mobile phases. The last of the components is the component pink. As discernible by its low R_f values, pink was generally bound strongest to the stationary phase as its binding position to the chromatography paper was typically the lowest. In addition, it can also be deduced that the components of the colour pink do not dissolve easily into the solvents, reinforced by the apparent physical separation, which indicates their stronger attraction intermolecularly to the stationary phase and how it was competed for more by the chromatography paper. This eventuates in its interactions with the chromatography paper being significantly more prominent than with the solvents, clarifying the similar polarity of the stationary phase with the component pink. Hence, the trend extracted is how blue was the most soluble component, followed by yellow and then pink; however, this trend is not constant for the cloudy ammonia solution as the pink exhibited larger R_f values than the yellow. In this case, the pink dissolved more regularly in the mobile phase than the solvent.

Conclusion

This investigation aimed to determine which solvent was most effective for separating the coloured components present in a black marker. It was hypothesised that as the interaction between the solvent and sample increased, then the degree of isolation of the constituents would follow suit. To some extent, this hypothesis was supported as the most interactive solvents demonstrated the highest level of separation. From the findings, tap water produced R_f values of 0.81 ± 0.05, 0.63 ± 0.05 and 0.31 ± 0.05 , the 1% sodium chloride solution generating the values 0.73 ± 0.05 , 0.44 ± 0.05 and 0.21 ± 0.05 whilst the cloudy ammonia reproduced values of 0.63 ± 0.05 , N/A and 0.24 ± 0.05 for the colours blue, yellow and pink respectively. Being the three most effective solvents for the task, it can thus be concluded that tap water is the optimum solvent for separating the individual components present in the black marker. This pattern can be explained by the general idea of chromatography, according to which the components that were strongly adsorbed to the stationary phase, and therefore moved the least, and the components that were most easily dissolved into the mobile phase, and therefore had the highest R_f value, have similar polarities to the phases. The separation of the constituents is caused by the intermolecular interactions between the phases and the sample components, only possible by the unique affinities of the constituents to the various phases. Further study is required to demonstrate the accuracy of this outcome since it has not been verified by the research. Furthermore, due to the influence of bias, mistakes, and inaccurate results acquired, these results may be confirmed or refuted by subsequent testing of other substances. This might lead to more findings suggesting a different solvent best for separating the various parts of a black marker.

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