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To examine the feasibility of using thin layer chromatography to analyze tattoo ink.

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ABSTRACT :

The best secondary proof for identification, both for the living and the deceased, is a tattoo. From the discovery of mummified remains to the various chemical processes utilized in contemporary tattoo inks, tattoo techniques have evolved over time. To differentiate authentic genuine products from counterfeits, modern tattoo ink are evaluated chemically and their ratio frequency values is calculated to distinguish genuine goods from fakes. A survey questionnaire was created to find out which tattoo ink was most frequently used in the study area (Salem taluk, Tamil Nadu, India). Thin layer chromatography instrumentation methods were used to examine these tattoo ink mixtures. Thin layer chromatography serves an efficient and dependable instrument to ascertain whether tattoo inks RF (retardation factor) values can be analyzed using this instrumentation method. This article's goal to analyses the movement of tattoo ink from the stationary phase in thin layer chromatography using different types of solvents (mobile phase). The aim to identify whether tattoo ink can be analyzed using TLC and components of tattoo ink, evaluating the effectiveness of various solvents in separating these components.

Keywords: Tattoo inks, Thin layer chromatography, RF (Retardation Factor), polarity, silica gel, mobile and stationary phase.

Introduction

Tattoos are further classified into five groups according to American board of dermatology [ABD], namely amateur tattoos, professional tattoos, cosmetic tattoos, medical tattoos, traumatic tattoos. Tattoos and tattoo ink play a very critical role in aspects of forensic investigation. Tattoos are most useful as identification for both the living and deceased. In forensic aspects tattoos are considered as secondary identification markers which can be used for positive identification.

Tattooing Is A Process Of Marking Almost Indelible Designs On The Human Body By Inserting Pigments Into The Skin (1).During The Procedure Of Tattooing Into The Skin Using Rotating Needles Tattoo Machines A Larger Amount Of Ink Is Injected About 0.4-14.36 Mg Of Pigments Per 1cm² (2).Tattoos Refer To Various Skin Markings Done By Impregnating The Skin's Epidermis With Varied Pigments Like Tattoo Ink Remains In Dermis Level Macrophages, Fibro-blasts. Permanent Tattoos Inks Are Pigments Which Holds Indigo, Cobalt, Carbon, China Ink, Cinnabar, Vermilion Prussian Blue Etc (3).

Nowadays you can see tattoos on any person regardless of sex, age race, occupation etc. Tattoos have become more common in recent years in younger individual so excepted to be found with increasing incidence at time of forensic autopsy examinations (4).Tattoos are always visible to some extent because of their chemical composition and way of making. Permanence of marks depends on both the type of the dye used and the depth of the skin involved. Deeper layers of the skin last longer. Tattoo marks are observed by decomposition/mummified they can be visualized by treating area with 3% h202 [hydrogen peroxide] which temporarily removes the product's of decomposition. Tattoo can be treated by surgical method skin grafting, laser beams used for removing Tattoo Marks without Permanent Marks. Tattoos Do Not Disappear Even When The Body Is Decomposed Or Deeply Burnt. This Makes Tattoos Extremely Reliant In Cases Where The Body Is Decomposed Or Is Burnt (5).

1.1. Thin Layer Chromatography

Thin layer chromatography (TLC) is one of the most useful tools for monitoring the progress of organic chemical reactions and for assessing the purity of organic compounds in photochemistry and Biotechnology. TLC takes advantage of the different affinities of the analyses with the mobile and stationary phases to achieve the separation of complex mixtures of organic molecules. TLC plate consists of glass, metal or plastic in which a thin layer of a solid adsorbent is coated with. The TLC plate is then placed at solvent (developing chamber) to ensure the bottom of the plate is immersed in the liquid (6).

1.2. Why thin layer chromatography

Tattoo inks are commonly composed of complex mixtures of pigments, solvents and various additives. Understanding the chemical composition of these inks is crucial for assessing their safety and potential health risks. Thin layer chromatography (TLC) is simple, rapid, and cost- effective method for

analyzing the components of tattoo inks. If we find the Rf (Retardation Factor) value of a Particular brand tattoo ink we easily able to identify the counterfeit products that may be introduced in the market.

1.3. Importance of Thin layer chromatography

Thin Layer Chromatography (TLC) plays a significant role in forensic science due to its simplicity, efficiency, and ability to analyze a wide variety of substances. It can be used to in Ink and Dye Analysis allowing for comparisons inks from documents (e.g., checks, letters) or dyes from fibers, helping forensic experts in cases of forgery, fraud, or document alteration. It is a Cost-effective and Fast method, providing affordable and quick preliminary results, allowing forensic investigators to screen substances before moving to more advanced and expensive techniques such as GC-MS (Gas Chromatography-Mass Spectrometry). A Non-destructive Nature preserves a significant portion of the sample, which is crucial in forensic investigations where sample amounts are limited and evidence preservation is critical.

Materials And Methods

1.4. Materials required

1. Survey questionnaire
2. Thin layer chromatography (TLC)
3. Solvent system (mobile phase)
4. Tattoo ink

QUESTIONNAIRE

Each tattoo shop located in Salem taluk, Salem district, tamilnadu has been probed using survey questionnaire. The Predominantly Used Tattoo Ink brand in Salem taulk Tamilnadu, India has been identified. The Most Common Tattoo Ink Brand Used Is - Dynamic tattoo ink -TBK.

1.5. Method

STATIONARY PHASE (TLC SHEETS)

This research focuses on the principle of the stationary phase, utilizing Silica Gel plates for solvent analysis. The most commonly used stationary phase is employed for adsorption mechanism. Silica gel is polar making it ideal for separating compounds in tattoo ink based on their polarity. The stationary phase enables the study of various solvents with different compositions, making it ideal for comprehensive ink analysis throughout the research.

MOBILE PHASE (SOLVENT SYSTEM)

Different pigments have varying polarities. It is necessary to select an appropriate solvent system to carry out the separation process. The reason behind affecting the travel of pigment can be poor solvent system.

THIN LAYER CHROMATOGRAPHY PROCEDURE

- To perform Thin Layer Chromatography (TLC), start by handling a silica gel plate carefully to avoid contamination.
- With the help of a pencil mark a line 2-3mm above the bottom of the plate. And apply the sample or ink on the TLC plate by using capillary tube. Repeat this process 3-5 times for better results with dilute samples.
- Then place the TLC plate in the developing chamber.tlc plate should immersed in the solvent system (mobile phase) should not exceed the marked line. Cover the chamber and allow the solvent vapour equilibration to be established.
- Let the solvent rise up the plate until separations occur, typically reaching 80% of the stationary phase. Afterwards Record the observations.
- To calculate the Rf value, with the help of a ruler measure the (distance travelled by the compound) and (the distance travelled by solvent front). Different ink components may have multiple Rf values, so organize them in a table. Some inks may exhibit fluorescence, which can be observed with a UV light source. This process helps analyze and characterize different ink components. Rf = dist. Travelled by sample / dist. Travelled by solvent.

Table 1 - Solvent system used.

S.no	Solvent system (with ratio)	Justification
01	Ethyl acetate : hexane (3:7)	To separate non-polar compounds to adequately polar compounds. Ethyl acetate adjusts polarity whereas Hexane provides non-polar base.
02	Acetone : water (7:3)	Acetone helps dissolve pigments water increases polarity. Mostly used during food dye analysis.
03	Toluene : ethyl acetate (4:1)	Toluene dissolves hydrophobic pigments, ethyl acetate used to separate slightly polarity compounds. Used for aromatic and poly aromatic hydrocarbons.
04	Hexane : acetone (8:2)	Low polarity system useful for separating hydrophobic pigments. Acetone slightly increases polarity to enable better migration.
05	Cyclohexane : ethyl acetate (7:3)	Cyclohexane offers slightly different elution strength. Preferable for pigments and oils.
06	Toluene : acetone (7:3)	Common system for separating lipophilic dyes. Toluene solubilises pigments, acetone controls elution.
07	Hexane : isopropanol (9:1)	Hexane is highly non-polar, and isopropanol helps to elute slightly polar components.
08	Ethanol : water (9:1)	The mixture is often used for polar compounds. Ethanol is a polar solvent and the small amount of water increases the polarity.
09	Ethanol : acetone (1:1)	Balances polarity well. Acetone increases volatility and can separate compounds with intermediate polarity. Used in pharmaceutical TLC.
10	Ethyl acetate : acetone (4:1)	Both solvents are mid-polarity suitable for separating aromatic esters, certain pigments. Ethyl acetate slows elution, acetone speeds it up.

11	**Isopropanol : acetone (7:3)**	**This system provides moderate polarity and is commonly used in cosmetic and ink analysis. Less aggressive than ethanol-based systems.**
12	**Chloroform : ethanol (9:1)**	**Chloroform dissolves hydrophobic compounds well; ethanol helps with polar functionality. Frequently used for separating pigments and resins.**
13	**Ethanol : propan-1-ol (1:1)**	**Both are polar solvents with different salvation properties used to explore migration behaviour of amphiphilic or complex molecules**
14	**Ethanol (100%)**	**Organic dyes and small molecules. This tests direct mobility in a highly polar but volatile environment.**
15	**Hexane : toluene : glacial acetic acid (14:9:0.4)**	**Offers a unique balance of polarity. Acetic acid modifies silica surface slightly, improving resolution for acidic/basic dyes Used in separating natural pigments.**

SOLUBILIZATION PROCESS:

An Appropriate solvent system has been chosen and the ink was dissolved by placing a small amount of tattoo ink (milligrams) in a clean vial. And the mixture was shaken and stirred thoroughly to ensure the ink is fully dissolved. Water and ethanol (commonly used).and kept at room temperature with drying time for each solvent on approximately of 2-3 minutes.

Results

Table 2 - Results seen on TLC sheets.

S. No.	Solvent System Used	Ratio	TLC Plate Image	Result (Separation)	Notes
1	**Ethyl acetate : Hexane**	**3:7**		**Negative**	**No distinct spots observed**
2	**Acetone : Water**	**7:3**		**Negative**	**Smearing seen, no clear Rf values**

3	**Toluene : Ethyl acetate**	**4:1**		**Negative**	**Baseline retention**
4	**Hexane : Acetone**	**8:2**		**Negative**	**No movement from baseline**
5	**Cyclohexane : Ethyl acetate**	**7:3**		**Negative**	**No visible separation**
6	**Toluene : Acetone**	**7:3**		**Negative**	**No Rf values recorded**
7	**Hexane : Isopropanol**	**9:1**		**Negative**	**No pigment migration**

8	**Ethanol : Water**	**9:1**		**Negative**	**Minimal diffusion, no spots**
9	**Ethanol : Acetone**	**1:1**		**Negative**	**Faint trail, no separation**
10	**Ethyl acetate : Acetone**	**4:1**		**Negative**	**Overlapping streaks**
11	**Isopropanol : Acetone**	**7:3**		**Negative**	**No spot development**
12	**Chloroform : Ethanol**	**9:1**		**Negative**	**No migration**

13	**Ethanol : Propan-1-ol**	**1:1**		**Negative**	**Strong baseline retention**
14	**Ethanol (100%)**	**10 ml**		**Negative**	**No pigment mobility**
15	**Hexane : Toluene : Acetic Acid**	**14:9:0.4**		**Negative**	**Slight streak, no separation**

4. Discussion

The study aimed to determine whether Thin Layer Chromatography (TLC) could be used to separate and analyze the components of tattoo ink. Where fifteen different solvent systems (ranging from non-polar to polar) were tested.There are several reasons why thin layer chromatography (TLC) might not be effective for analyzing tattoo ink. The solvent system might not be suitable for the solute, suggesting that the solute is not soluble in the solvent or the solvent is too polar/non-polar for the solute.

- The tattoo ink must be too strongly adsorbed to the TLC sheet.
- The concentration of the solute might be too low to be detected.

Outcome of the experiment noted:

1. No separation of tattoo ink components was observed on any TLC sheets.
2. Pigments remained at the baseline and the solvent system did not effectively move the solute
3. No Rf values (measurements of how far a compound travels) were recorded.

Reasons for negative outcomes:

1. Tattoo inks are not fully soluble – they consist of large, suspended particles (such as carbon black or titanium dioxide), not dissolved molecules.
2. Strong adhesion to silica gel – due to pigment polarity or charge, they stick to the TLC plate and did not travel.
3. Silica gel may not be appropriate stationary phase for such materials.TLC is not suitable for analyzing tattoo inks in their raw form.

To achieve Better results we can use alternative techniques such as

- High-Performance Liquid Chromatography HPLC
- Ultraviolet-visible spectroscopy
- Fourier transform infrared spectroscopy FTIR
- Mass Spectrometry

In conclusion, although no successful separations were achieved in this TLC-based study, the work provides valuable negative data to the field and underscores the challenges of analyzing complex particulate mixtures like tattoo ink using conventional chromatographic techniques.

5. Conclusion

This study was conducted to determine whether Thin Layer Chromatography (TLC) could be used to separate the components and analyze the tattoo ink to find out the Rf value. Fifteen different solvent systems were tested, but none were successful no clear separations or spots appeared on the TLC plates. This is likely because tattoo inks contain insoluble pigments that do not move well on TLC, especially on silica gel.

Even though the results were negative, they are still very important in demonstrating that TLC is not suitable for analyzing tattoo inks in their raw form. Future research should explore other methods like High-Performance Liquid Chromatography (HPLC), as well as incorporate pre-treatment steps to filter or dissolve parts of the ink, and combine chromatography with techniques like UV or IR spectroscopy to better identify what is present inside the ink. This study provides valuable information and assists in guiding better methods for analyzing tattoo inks in the future**.**

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