Determination of the Age of Gel Pen Inks on Handwritten Documents using Gas Chromatography-Mass Spectrometry and Chemometrics

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Abstract / Overview:
Background: *The accurate determination of age of ink used in the documents related to forensic cases is often submitted to forensic document examiners. Determining the age of ink refers to assessing the most probable time when the ink was used to write on paper. It may become tedious due to the great variety of writing inks available in the market and due to the complexity of chemical processes that occur over time after the deposition of the ink onto the paper. Limited work is available concerning non-ballpoint pen inks. Thus, the study addressed the determination of the age of the writings made using gel pens of 4 different coloured inks by studying their volatile components by employing GC-MS. The effects of the storage conditions and different substrates (paper) on the aging of ink were also assessed.* **Results:** *More than 70% drop in the peak area was observed initially, which increased with time and the fall was more or less stable up to 60 days, after which the peaks disappeared. Slight effect of the type of substrate and storage condition was observed. The results were backed with Karl Pearson's correlation method suggesting comparability between the samples stored in closed and open storage conditions.*
Conclusion: *GC-MS is suitable for studying the solvent components of gel pen inks for the determination of the age of ink. The methodology used in this study provided reliable results for writings stored for up to 75 days.*

Keywords: Documents, questioned documents, determination of the age of the ink, determination of the age of documents, dating of pen inks, dating of documents, gel pen inks, gas chromatography-mass spectroscopy, the effect of substrate, likelihood ratio.

1. Introduction

The constituents of ink are divided into three categories: vehicles, colorants, and additives. A vehicle acts as a carrier and provides a medium for the colorant to flow, and is volatile. Acolorant is mainly dye or pigment, which is used to provide color to the ink. Additives contribute towards viscosity, adhesion, rheological properties, etc. (1). The inks used in pens are broadly classified into ballpoint pen and non-ballpoint pen ink. These inks can also be classified into water-based or oil-based inks. The inks used in ballpoint pens are oil-based, and those in non-ballpoint pens are water-based, which include gel, fountain, pilot, fibre tip pens, etc. (2).

The determination of the age of the ink has been discussed based on the static profile, that is, the evaluation of the properties that do not change with time, and the dynamic profile, that is,the evaluation of the changes that occur in the different components with time (3). Ink has been considered a solution of a volatile solvent and a non-volatile solute present in a vertical container open at one end (for example, a pen cartridge). After the ink gets deposited onto the paper, various physical and chemical changes occur, such as colorant degradation, solvent evaporation,

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S. no.	Reference ID	Reference Solvents	Solubility	Retention Time (minutes)
1.	DEG	Diethylene Glycol, 99%	Soluble	8.27
2.	G	Glycerol, 99+%	Soluble	10.45
3.	PG	Propylene Glycol, 99.5%	Soluble	5.44
4.	TEG	Triethylene Glycol, 99%	Soluble	10.53
5.	EB	Ethyl Benzoate	Soluble	4.03

Table 1: List of reference solvents along with their reference ID, solubility in methanol, and retention time.

and hardening or polymerization of the resins and additives. The solvents evaporate with time, making the solution more concentrated (4).

The assessment of the age of ink has become tedious due to the great variety of writing inks available in the market. The knowledge about the static, relative, and absolute age approaches for determining the age of ink before applying the analytical techniques is important. The static approach deals with the stable components of the ink. The relative age is estimated by comparing the writings made using the same ink, paper, and environmental conditions. The absolute age is estimated based on identification rather than comparison (5-6). Hence, a definitive estimation of the age in the scenario of forensic cases is difficult. However, the boundaries for age determination could be established (7).

Few studies on the dating of iron gallotanate inks were conducted until 1972, as iron gallotanate inks were frequently used to produce documents around that period. Further, most studies related to determining the age of the inks were focused on the inks of ballpoint pens based on their resin, volatile, and dye components. In contrast, work related to non-ballpoint pens and stamp inks is limited (7-8). Thus, the study aimed to determine the age of the writings made using gel pen inks of blue, black, red, and green color by employing gas chromatography-mass spectroscopy (GC-MS) and to study the effects of storage conditions and different papers on the aging of the ink used. Karl Pearson's correlation method was als used to analyze the correlation between different storage conditions (9).

2. Materials and Method

2.1. Sampling

A total of 101 blue, black, red, and green gel pens of different makes and models that were collected from the local market and used to determine the age of gel pen ink writings. Based on the literature, ethyl benzoate (Alpha Aeser) was chosen as the internal standard as it was stable and did not react with other solvents (10-13). Four types of substrates, including white A4-size sheets of 45 GSM, 75 GSM, and 100 GSM, and a sheet of green legal paper of 75 GSM, were collected and used to study the effects of substrate on the ink. Reference solvents and other chemicals of chromatographic grade, including acetic acid (Loba Chemie), acetonitrile (Merck), chloroform (Loba Chemie), distilled water, ethanol (Analytical Reagents), ethyl acetate (Loba Chemie), hexane (Spectrochem), methanol (Merck), and nbutanol (Loba Chemie), were acquired (Table1).

3. Standardization

The sample preparation and analytical methods were standardized before the analysis. The standardization protocol included the extraction

solvents, minimum levels of detection, sample size and type, and the mobile phase used, which are discussed in the subsequent sections and sub-sections.

4. Sample Preparation

For determining the age of gel pen inks, a total of three sets of samples were prepared. In the first set of samples, all the pens were used to write the sentence "Examination of the Document in Question" on all the sheets of paper used in the study. This set was stored in a cupboard using a file without any direct exposure to the light. The second set of samples was prepared in a similar manner. However, this set was kept on the slab of the laboratory exposed to the natural light. The temperature and humidity level of the laboratory were controlled at 27°C and 45% humidity. The third set of samples included the ink taken directly from pen cartridges. All three sets of samples were analyzed at different time intervals.

5. Ink extraction

To extract the components of gel pen ink from ink cartridges and writings made on different types of paper, various solvents, including acetonitrile, ethanol, methanol, and ethanol: water in a ratio of 1:1, were tested individually. In the case of ink cartridges, a 10µl aliquot was taken from the cartridge using a micro-pipette and added to 1 ml of the solvent. In the case o the pen ink strokes made on paper, four micro-punches, each 5 mm in diameter, were made and dissolved in 1 ml of the solvent.

6. Ascertaining the minimum detection levels of the analytical techniques

To determine the minimum detection levels various dilutions of ink and a serial dilution method for ascertaining the aging of the gel pen ink and the writings were performed. The fresh ink extracted from the cartridges, 50µl, 30µl, 20µl, 10µl, 5µl, and 1µl aliquots were added to 1 ml of methanol. A serial dilution of the 50µl aliquot of the ink extracted from the gel pen cartridge was also performed. The writing strokes of 10, 7, 5, 3, 2, and 1 cm using gel pens were made, and the ink was extracted from each using 1 ml of methanol.

7. GC-MS analysis of the inks used in gel pens

7.1. Apparatus

The ink samples were analyzed using a Perkin Elmer Clarus 500 GC-MS with an autosampler. The Turbo Mass 6.1.2 software was utilized for recording the spectra, and a NIST MS 2.2 library was used to identify the separated components.

7.2. Chromatographic Conditions

A Shimadzu SH Rtx-Wax capillary column (30m×0.25 µm ×0.25µm) was used. Helium (He) gas was used as the carrier gas at a 1 ml/min flow rate. The injector temperature was set at 230 °C with a split-less mode. The injection volume of the sample was 0.5 µl. The oven was programmed to heat up to 80 °C, hold for 1 min, increase at a rate of 15 °C/min up to 230 °C and finally hold for 5 mins. A solvent cut-off of 3 minutes was also set. The transfer line temperature was set at 230 °C and the detector source temperature at 200 °C. A mass range of 30 – 450 m/z was evaluated with a scan time of 0.25 seconds and an inter-scan delay of 0.1 seconds.

7.3. Analysis

The extracted ink samples from the gel pens were used for GC-MS analysis. The solution was filtered using a nylon syringe filter of a pore size of 0.2 µm (SimSon Pharma Limited) into aGC-MS glass vial, which was then kept in the autosampler. After updating the data, the software was applied, and the graphs were obtained and saved for data interpretation and further analysis.

8. Interpretation of GC-MS data

The data obtained after analyzing the various gel pen inks using GC-MS at different time intervals were evaluated systematically. The age of the ink writings was determined based on the changes in the peak area of the major solvent components identified in the previous section of this study concerning the time and the solvent loss ratio compared with the internal standard. The writings were studied after 1, 7, 15, 30, 45, and 60 days, and then every 15 days consecutively for up to 1 year to analyze the characteristics related

Group Number	Sample ID	Number of peaks	Retention Time (minutes)
B1	GB07, GB15, GB26, GB29, GB42, GB44, GB56	0	-
B2	GB09, GB51	1	5.44
B3	GB05, GB06, GB10, GB11, GB16, GB18, GB20, GB21, B22, GB23, GB25, GB28, GB31, GB33, GB34, GB36, GB39, B40, GB41, GB45, GB46, GB48, GB49, GB54, GB60	1	5.70
B4	GB30, GB32, GB37, GB43, GB50, GB53, GB58, GB59	1	10.42
B5	GB01, GB02, GB03, GB04, GB08, GB12, GB14, GB17, GB27, GB35, GB38, GB47, GB52, GB55, GB57	2	5.70, 10.42
B6	GB24	2	5.70, 10.55
B7	GB13, GB19	2	10.42, 10.55
K1	GK04, GK17	1	5.44
K2	GK01, GK02, GK03, GK05, GK06, GK07, GK08, GK10, GK11, GK16, GK18, GK23	1	5.70
K3	GK21	1	8.30
K4	GK14	1	10.42
K5	GK09	2	5.44, 10.42
K6	GK12, GK19, GK22	2	5.70, 10.53
K7	GK15, GK20	2	8.30, 10.42
K8	GK13	2	10.87, 11.02
R1	GR11	1	5.44
R2	GR01, GR04, GR08, GR09	1	5.70
R3	GR03, GR05, GR06	1	10.42
R4	GR02, GR07, GR10	2	5.70, 10.42
G1	GG06	1	5.44
G2	GG01, GG02, GG04, GG05, GG07	1	5.70
G3	GG03	2	5.70, 10.42

Table 2: Grouping of blue gel pen ink writings on the basis of the number of major peaks and their retention time obtained using gas chromatography-mass spectroscopy.

to the aging behaviour of the inks.

Samples of the same ink were analyzed in triplicates to determine the repeatability and reproducibility of the method. One pen was randomly selected from each group to calculate the solvent loss ratio, and both the samples stored in open and closed storage conditions were evaluated. An aliquot of 10 µl of ethyl benzoate was added to 1 ml of methanol, and 2 µl of this ethyl benzoate solution was added to each vial before GC-MS analysis.

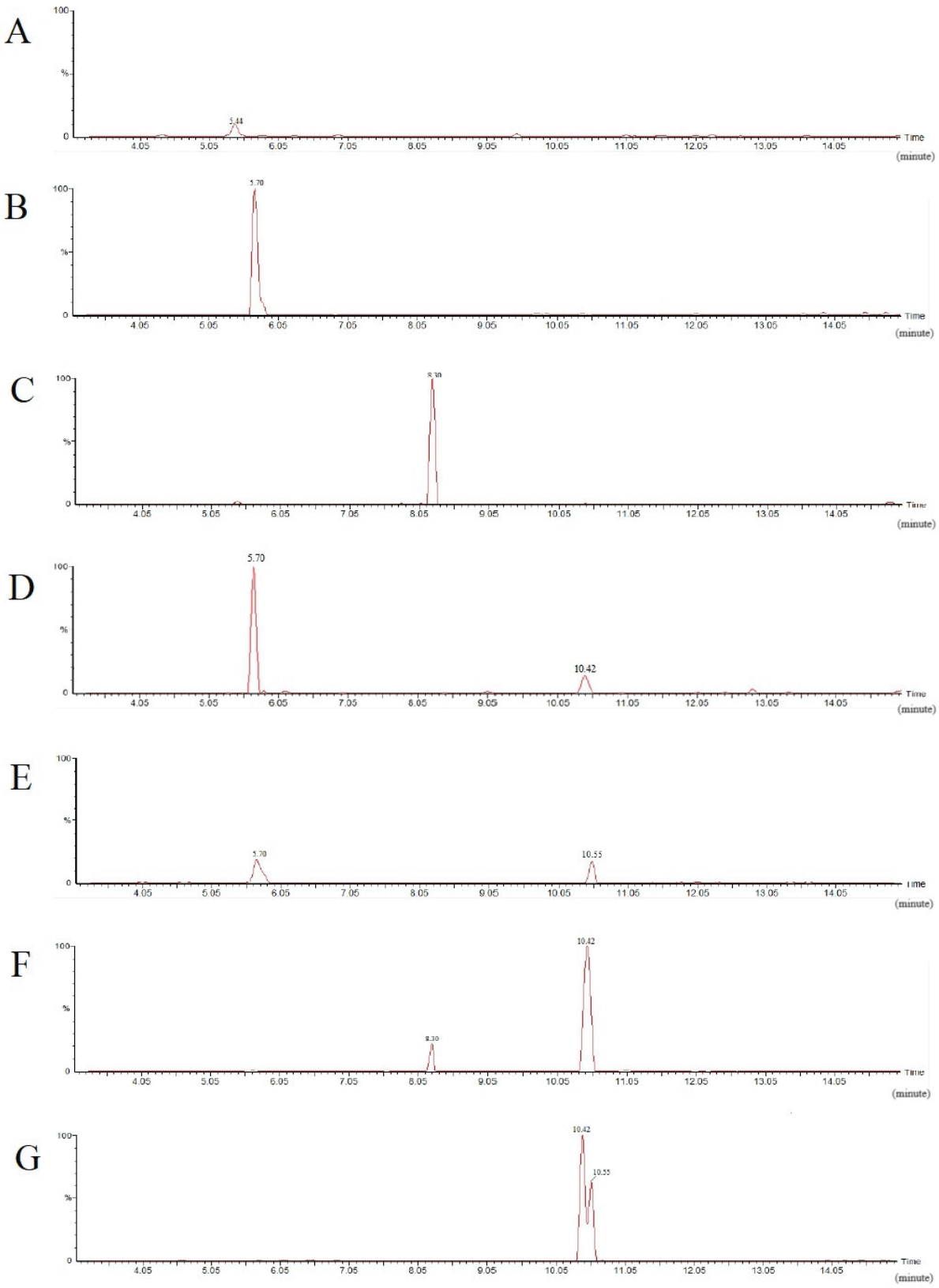

Fig. 1: The graphs obtained for pen ink separated using GC-MS and their retention time in (A) Group B2/K1/R1/G1, (B) Group B3/K2/R2/G2, (C) Group K3, (D) Group B5/R4/G3, (E) Group B6/K6, (F) Group K7, (G) Group B7.

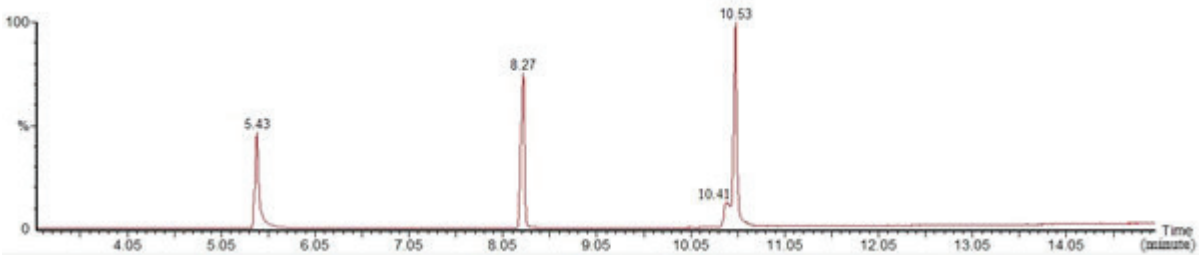

Fig. 2: The graph obtained for the reference solvents using GC-MS along with their retention time.

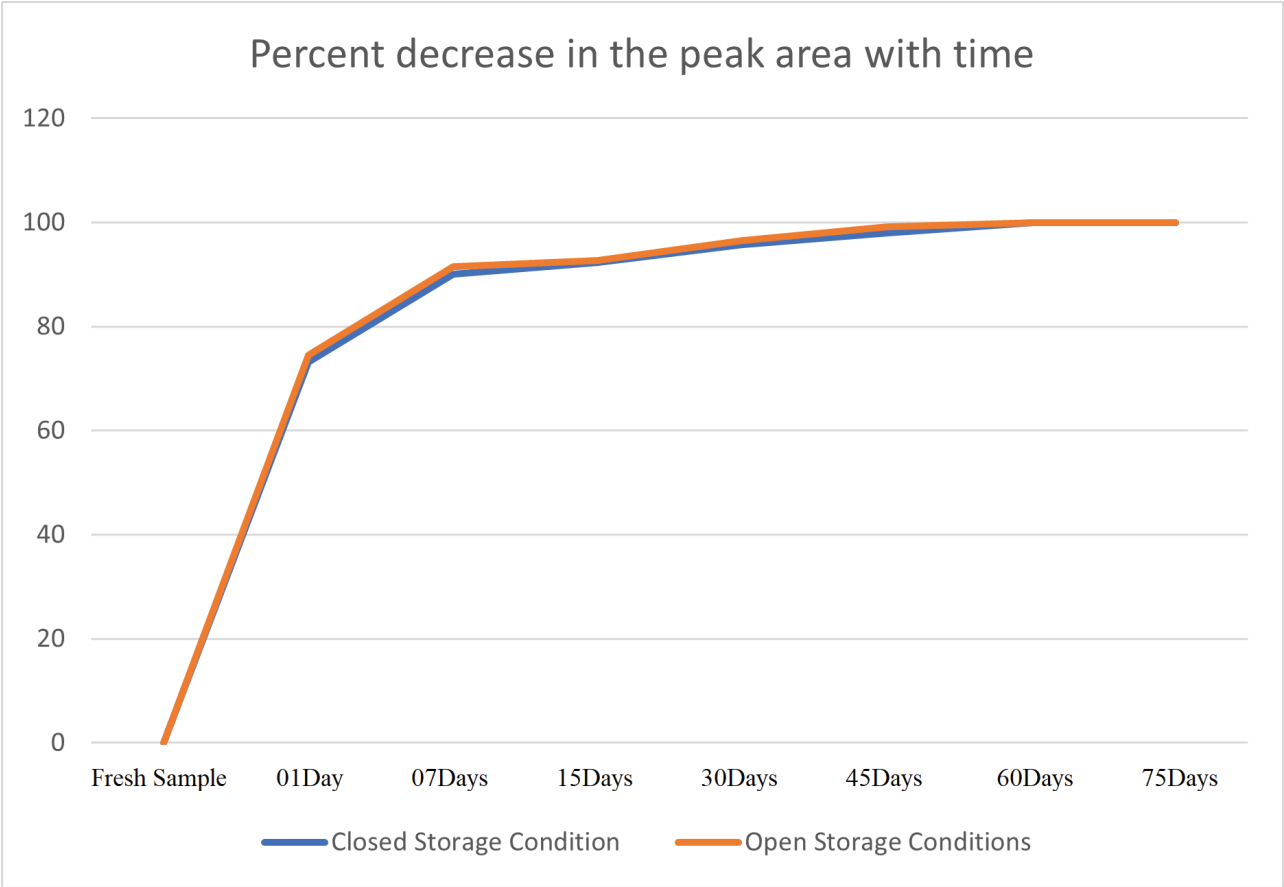

Fig. 3: The percent decrease in the peak area of tetraethylene glycol with respect to time in two different storage conditions.

9. Chemometrics

Karl Pearson's coefficient of correlation (r) was applied to comparatively analyze the writings stored in open and closed conditions. A null hypothesis was proposed, suggesting that they could be compared. However, an alternative hypothesis was also proposed, suggesting the opposite. This method was unaffected by the change in scale and measured the correlation between the two variables. The value of 'r' for the variables X and Y was calculated using the following formula:

$$r = \frac{\text{Covariance}(X,Y)}{\sigma X \,.\, \sigma Y}$$

Where X is the peak area of the solvent of samples stored in open conditions, Y is the peak area of the solvent of samples stored in closed conditions, σ is the standard deviation, and Covariance

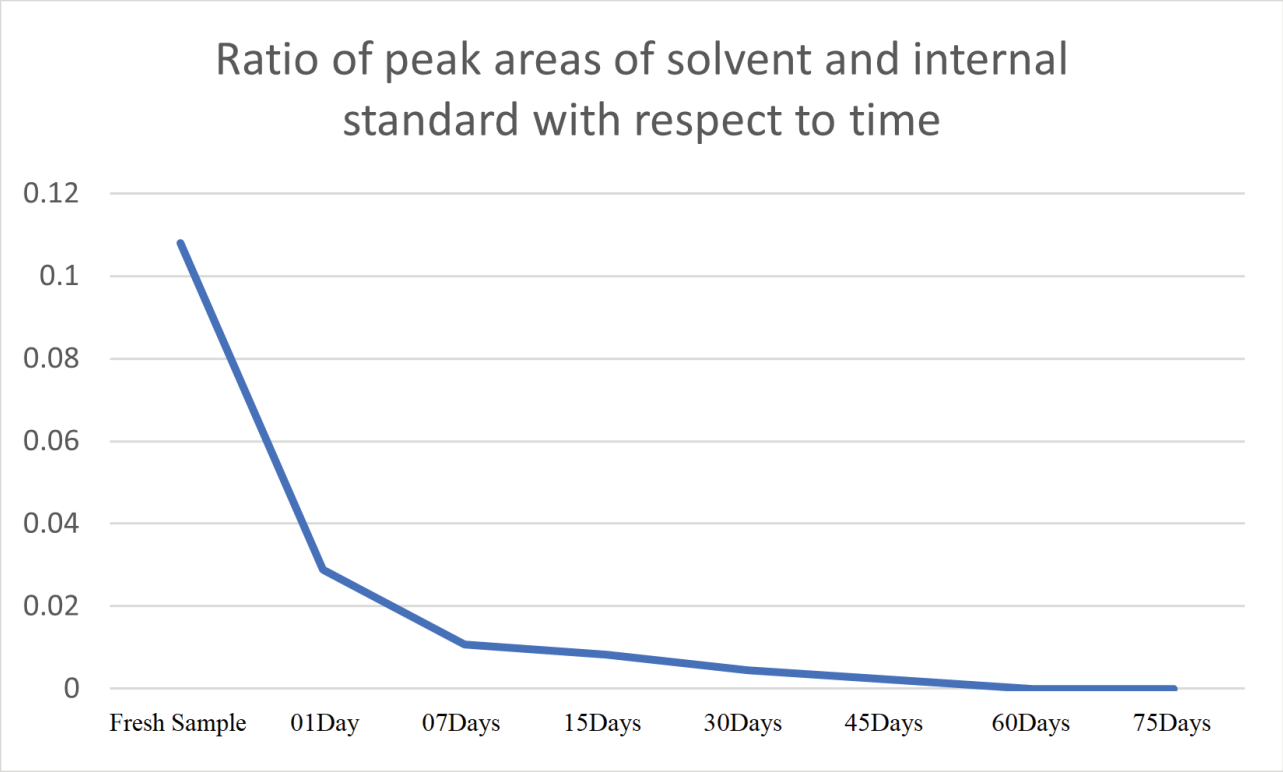

Fig. 4: The ratio of the peak area of tetraethylene glycol and ethyl benzoate (internal standard) with respect to time.

was calculated using the following formula:

$$Covariance(X,Y) = \frac{1}{n}\sum_{i=1}^{n}(Xi - \bar{X})(Yi - \bar{Y})$$

Where n is the total number of samples, *Xi* is the *i*th value of X, is the mean of X, *Yi* is the *i*th value of Y, and is the mean of Y

Karl Pearson's coefficient of correlation always lies between +1 and -1. A value nearer to +1 indicates a positive correlation, while a value nearer to -1 indicates a negative correlation (9). A scatter plot was also prepared to study the correlation between the two.

10. Results

The blue, black, red, and green gel pen ink samples were classified into various groups on the basis of the number of major solvent components separated using GC-MS and their retention time (Table 2 and Fig.1). These pen ink groups were further studied to determine the age of ink writings. The reference solvents were also analyzed using GC-MS, and a graph was obtained for the mixture of the solvents, and their retention time was noted for comparison (Fig. 2).

11. Determination of the age of the gel pen ink writings using GC-MS

11.1. Change in the peak area with time

To study the aging behaviour, the change in the peak areas of different glycols and glycerol was studied, and it varied with the make and model of the pens. A notable drop of more than 70% in the peak area was observed one day after writing, which increased to ~ 90% after seven days. A reduction of 2% – 3% was observed after 7 more days. The fall was more or less stable up to 60 days, with the peaks disappearing after that, except for the writings made with few blue and black pens having a peak at Rt 10.42 minutes, which was detectable for up to 75 days and then disappeared

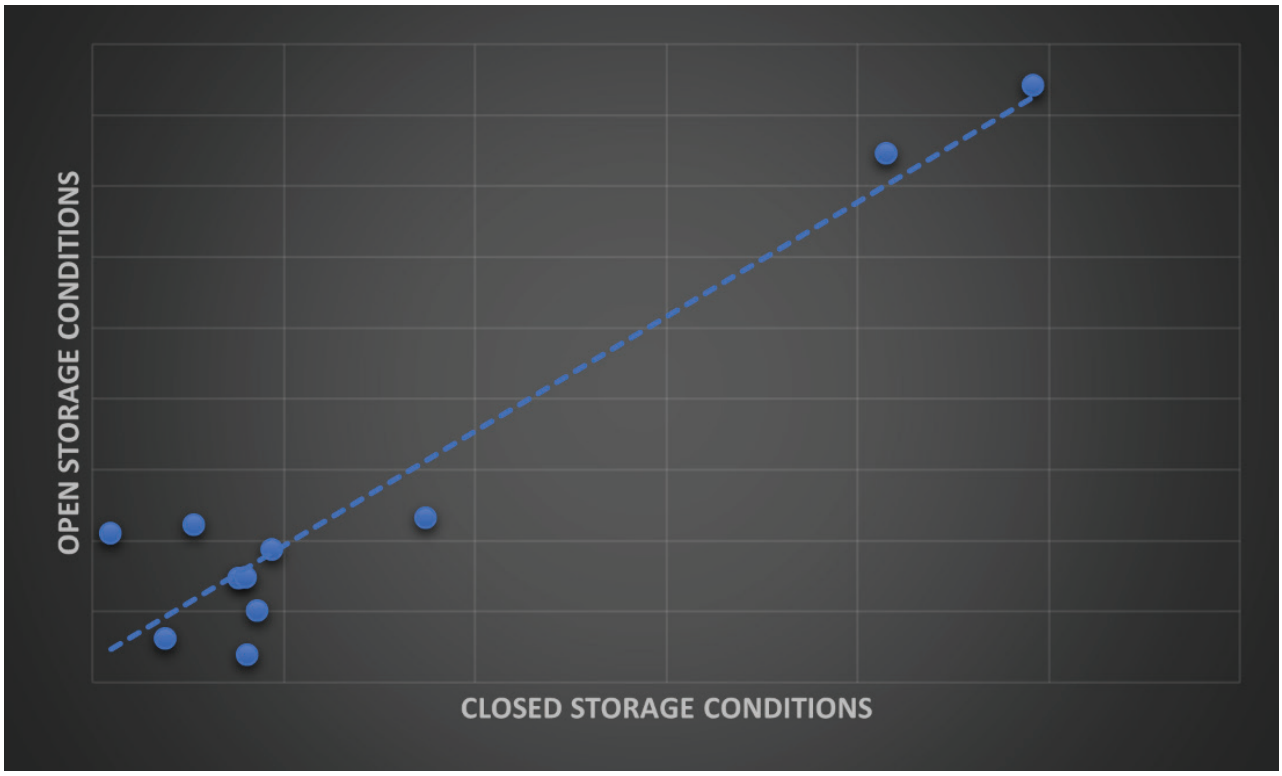

Fig. 5: Scatter plot of peak areas of solvents of samples stored in open and closed storage conditions showing a positive correlation.

(Fig. 3). The method was observed to be repeatable, and the results were reproducible. The reduction was slightly more prominent in the case of the samples stored in the open condition compared to those stored in the closed condition.

11.2. Solvent loss ratio compared to the internal standard

The solvent loss ratio was calculated between the peak area of each major solvent component (different glycols and glycerol) and compared with the internal standard (ethyl benzoate) over time. The ratio decreased with an increase in time which was initially very fast but stabilized with time (Fig. 4).

11.3. Effects of substrate and storage conditions

Gel pen ink writings on all four types of paper were compared with the inks extracted from the pen cartridges to study the effects of paper. A negligible effect of the type of substrate (paper) used on the age of the writings was observed. However, the solvents evaporated relatively faster from the 45 GSM paper than from the 75 and 100 GSM paper. This was a result of the density of the fibers in the sheet of paper. Loose fibers helped in the quick evaporation and migration of the solvents. A slight difference in the peak areas of solvents was observed when the writings were stored under closed and open conditions, suggesting a minute effect of the type of storage on the aging of the ink. The inks aged faster under open storage due to exposure to natural environmental conditions than the writings stored under closed conditions. Open storage conditions made the ink writings potent to heat and air flux, which enhanced the drying of the gel pen ink solvents.

11.4. Statistical analysis

Karl Pearson's coefficient of correlation (r) value of 0.909706 suggested a positive correlation between the open and closed storage conditions. Thus, the null hypothesis was acceptable, suggesting comparability between the samples. The scatter plot also revealed a positive correlation between the two (Fig. 5).

12. Discussion

Glycols were the most common solvents used in gel pen inks, including diethylene glycol, triethylene glycol, propylene glycol, etc. followed by glycerol. Glycols possess extensive intermolecular hydrogen bonding, resulting in higher boiling points, and are polar in nature. The physical properties of these glycols are more or less similar. However, their physiological properties differ, which provide gel pen inks with different characteristics. Li et al. (2014), Sun et al. (2017), and Ni et al. (2020) studied glycol and glycerol, polyethylene glycol polymers, and triethylene glycol solvents, respectively, in black gel pens. However, the sample size was less in all these studies as compared to the present study.

The peak area reduces with time because of oxidation and reduction of different glycols and glycerol and various processes like evaporation, penetration, migration, etc. The results obtained were dependent on the concentration of the ink used for the writing. Li et al. (2014) evaluated the UV induced writing samples and compared these with writings aged under natural conditions. The solvents present in the pen ink decreased with time, and the aging curve was divided into three parts. However, the present study was entirely based on the results of the natural aging of pen ink writings in various storage conditions. Aginsky (2017) studied 2-pyrrolidone in roller ballpoint pen inks, which also decreased with time. The results were similar, thus supporting the findings of this study with a larger sample size.

The results depended on the concentration of the ink used for the writing. The concentration is further dependent on the molarity of the different glycols and glycerol used in the gel pen inks. However, limited work related to the gel pen inks is available. Sun et al. (2017) utilized LC-HRMS to compare the degradation of solvents in artificial and natural aging conditions with standard solvents. The degradation of PEG oligomers was not as significant as the standard; however, the trend was similar. Li et al. (2014) studied UV-induced gel pen ink writing samples, and a correlation between natural and artificial aging conditions was drawn with respect to ethyl benzoate. Ni et al. (2020) utilized GC-MS and GC-FID to study the TEG solvent of carbon-based black gel pen inks. However, these research studies were carried out on smaller sample sizes and majorly focused on black-colored gel pen inks.

13. Conclusions

This study was performed to determine the aging behavior of the solvents in the blue, black, red, and green gel pen inks. Different types of storage conditions and substrate (paper) were used to study their effect on the age of ink. Previous studies for the determination of the age of pen inks were mostly focused on ballpoint pen inks and a few studies on non-ballpoint pen inks were available. Studies on the determination of age of gel pen inks were majorly conducted on black gel pen inks with a small sample size. Thus, the present study was a comprehensive work that analyzed a great number of commercially available gel pens. The peak areas of the solvent components (different glycols and glycerol) were identified by using GC-MS. As the solvent components are volatile, they decrease in concentration with time. The decrease was rapid initially. However, the decrease in the concentration of solvents slowed with time. The solvent concentration decreased due to the solvent flowing through the paper fibers, evaporating, and migrating. Over time, the ratio of solvent loss compared to the internal standard also decreased. Both the analytical techniques used in the study depended on the ink concentration taken for the examination. Hence, the techniques were prestandardized, and their minimum detection abilities were evaluated. A negligible effect of the type of substrate (paper) and storage conditions was observed on the aging process. The results of the analytical examination were supported by the statistical analysis of the samples that were stored under two different conditions. Karl Pearson's coefficient of correlation demonstrated that the samples stored under open and closed conditions could be compared. The aging could be successfully ascertained up to 75 days after the writings were executed. Accordingly, analyzing the changes occurring in solvents, the differentiation of pen inks using GC-MS may only be successful for recently written documents. However, there is still a lot of potential for further studies in this area.

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