Study the Properties of Color Image of Different Chromatography Plates to Estimate the Type, Thickness, and Integrity of Plates

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Abstract: *The study concern is an analysis of the properties of color images at different wavelengths belongs to the HPTLC, TLC, and preparative TLC silica gel 60 F254 glass plates. Where the silica plates differ from each other depending on the manufacturing method and the thickness of silica on the plates, where the thin layer chromatography is usually done using a thin, uniform layer of silica gel or alumina coated onto a piece of glass, metal or rigid plastic. The silica gel (or the alumina) is the stationary phase often contains a substance which fluoresces in UV light. So the aim of the work is to estimate the thickness of silica depending on the properties of the color image of the chromatography plate, in addition to edge detection using Sobel operator on the image plate to ensure that the chromatography plate is clean and free from any damage. The results indicate that use of color properties analysis is simple and efficient method for differentiation among types of plates in addition to estimation of thickness and integrity of plates*

Keywords: Image digitalization, Chromatography, Sobel

1. Introduction

The thin layer chromatography (TLC) silica plates are different from HPTLC plates in three respects, which explain the different chemical properties. The glass silica gel is slightly thicker, 0.25 mm, compared with 0.20 mm in HPTLC plates. TLC plates are made of silica granules varying from 5 to 20μm, the average being between 10 and 12 μm. The granule size of the HPTLC plates vary from 4 to 8 μm and the average is between 5 and 6 μm [1]. Although the TLC –mass spectrometry (TLC–MS) is a readily implemented technique in its simplest form, puts few demands on either chromatography or spectrometry, nevertheless, compared to the situation with high performance liquid chromatography, it is less developed. Currently, the bulk of the practical applications of TLC–MS are directed towards the use of fast atom, or ion bombardment [2]. Among the commonly used preparative-scale separation techniques, centrifugal chromatography has so far played only a minor role, although radial TLC became commercially available, in this system, the TLC plate (rotor) is not horizontal but inclined and thus allows more efficient collection of the elute[3].

The image processing on the digital computers has gained high acceptance in recent years. Early techniques in image processing concentrated on procedures that were carried out in the frequency domain, which was a natural extension of one-dimensional linear signal processing theory. It became well known computing that a two-dimensional transform for large data array is a very active by time-consuming especially with fast transform techniques on large computers. Hence, frequency domain procedures for real-time processing of images wereimplanted [4].

The sharpness of measure or blurriness of edges in the TLC plate image can be useful for a number of applications in image processing, such as checking the focus of a camera lens, also helping to identify shadows (whose edges are often less sharp than object edges), the separation of variations in illumination from the reflectance of the objects in the TLC plate image (known as intrinsic image extraction), and in-focus areas (or foreground)[5]. So the edge detection is used for image data segmentation extraction in areas such as image [6]. In addition to detecting continuity is so difficult, often single technique is insufficient for unambiguous determination of the blend morphology [7].

Digital image which processed by computer is composed of a finite number of elements, where x and y are spatial coordinates, and the amplitude off any pair of coordinates (x, y) is called the intensity or gray level of the image at that point, when x, y and the amplitude values of all finite discrete quantities, thus the image can be known as digital image [8]. The Sobel operator algorithm relates the change in image brightness at any point to the brightness pattern; images may be sampled at intervals on a fixed grid of points. Also, Sobel has advantages in case of tessellations other than the obvious one and can test differences in levels of additive Gaussian noise [9, 10].

Mostly edges created by a change in brightness, as indicated by gray scale level value, can be examined. This involves probably 90% of edges of interest in real problems, but not all of them. It turns out that changes in color, or hue, are not always detected by the edge detectors described so far. If edges are the boundaries of objects, then boundaries that are marked by color alone should be detectable, and because most images involve color, it is important to consider it when looking for edges [11].

For simple yet lucid extension of black and white density slicing is to use colors to highlight and brightness value ranges, rather than simple gray levels. This is known as color density slicing. Provided the colors are chosen suitably, it can allow fine detail to made immediately apparent [12].

2. Methods

1. Glass plate (HPTLC, TLC, preparative TLC plate) silica gel 60 F254 were prepared, and pictures were obtained using TLC visualizer (CAMAG, Swiss) at different wave length (366 & 254 nm) and 10x10 cm (X, Y) part from the obtained images was taken. The properties whole image (width 1800 PXL, height 1543 PXL) and engrave annotations (width 15.4 cm, height 13.1cm), resolution 300 DPI as shown in figure (1).
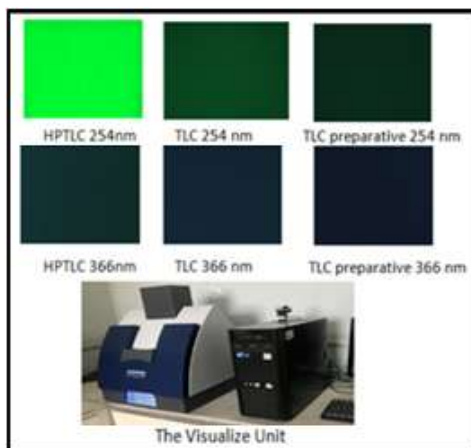

Figure 1: Different chromatographic plate images obtained by CAMAG visualizer unit

2. The histograms of chromatograph plate images at two wave lengths (254, 366) nm were recorded. So the properties of image analyzed by calculation the histogram of red, green, and blue bands as shown in figure (2).
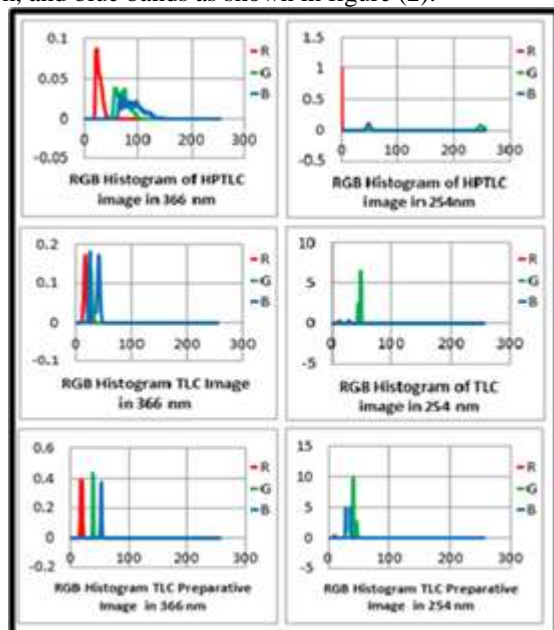

Figure 2: The RGB histogram analysis of TLC, HPTLC, and preparative TLC plates at different wave lengths

3. Values of the minimum and maximum reading of red, green, and blue bands for all images were calculated as shown in the table (1).

Table 1: The minimum and maximum values of RGB in chromatograph images

Type of plate	HPTLC			TLC			Preparative TLC		
Wave length	254 nm			254 nm			254 nm		
Type of band	R	G	B	R	G	B	R	G	B
Minimum value	0	229	0	5	42	24	5	40	22
Maximum value	18	255	70	15	49	34	15	47	34
Wave length	366 nm			366 nm			366 nm		
Type of band	R	G	B	R	G	B	R	G	B
Minimum value	17	51	58	14	35	47	9	19	33
Maximum value	53	115	155	22	42	58	28	37	53

4. The Sobel operator was applied on one region zoon 100x100 pixels from the image as shown in figure (3). And three different thresholds (15, 30, and 45 pixels) in Sobel operator was applied, the point detects the edges and cracks in the picture of the chromatogram as shown in the table (2).
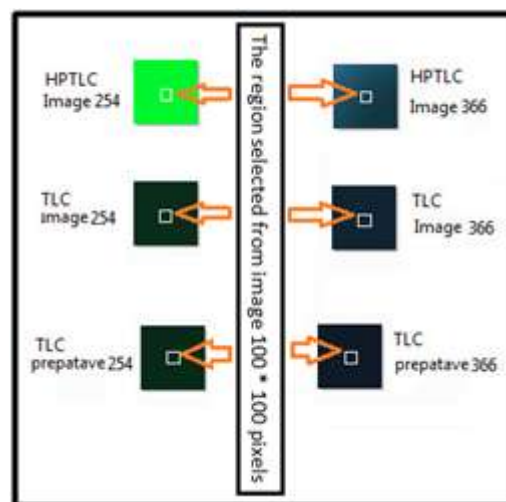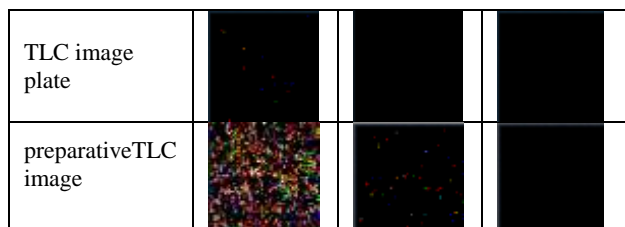

Figure 3: The selected region for Sobel operator application for all images

Table 2: Chromatograph images with Sobel operator application

	254nm 15 pixel	254 nm 30 pixel	254 nm 45 pixel
HPTLC image plate			
TLC image plate			
preparativeTLC image			
	366 nm 15 pixel	366 nm 30 pixel	366 nm 45 pixel
HPTLC image plate			

| TLC image plate | |
| preparativeTLC image | |

3. Results and Discussion

- The results indicate that the differences in intensity of histogram curve were clear observed as shown in figure (2). So in the histogram for HPTLC plate image at 366 nm, the peak is less than 0.1 while in TLC is higher and preparative TLC is the highest .
- At 254 nm similar observation was noticed since the peak histogram for HPTLC plate image is approximately 1, while in TLC is higher and preparative TLC is the highest .

The peak reading in histogram curve is proportional to the thickness of the chromatograph plate. Thus the thickness can be estimated.

- The results in table (1) indicate that the red band is too low while the blue and green is higher, the explanation for this observation may be related to the wave length that used in this experiment which is 254 and 366 nm
- The results of Sobel Operator (table 2) application on the chromatograph picture image at 254 nm indicate that the there are many cracks in the HPTLC plate while no cracks or damages in the TLC & preparative TLC plates.
- •The results of Sobel Operator (table 2) application on the chromatograph picture image at 366 nm indicate that the there are many cracks in the HPTLC & preparative TLC plates while no cracks or damages in the TLC plates.
- Thus it can be concluded from overall results that the analysis using Sobel operator at 366 nm is more precise since it obeys the results by objective test to find the cracks and damages in the chromatograph plates

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