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# Analysis of Rhodamin B on Lipstick, Blush On and Eye Shadow in Pekalongan Regency With UV-Vis Spectrophotometer

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**Abstract:** Rhodamine B is a synthetic dye that is often misused in cosmetic products. These dyes can cause irritation to the respiratory tract, are carcinogenic and cause liver damage in high concentrations. This study aims to determine the content of Rhodamine B in lipstick, blush on and eye shadow in the Pekalongan Regency. The analysis used in this research is qualitative and quantitative analysis. Qualitative study using thin layer chromatography (TLC) with silica gel F254 as stationary phase and mobile phase n-butanol, ethyl acetate, ammonia (5.5 : 2 : 2.5) v/v and then detected on UV light at 254 nm and 366 nm. Quantitative analysis using UV-Vis spectrophotometer at a maximum wavelength of 557 nm. The results of the qualitative analysis showed that three samples of lipstick (LA, LD dan LE), two samples of blush on (BB and BC) and three samples of eye shadow (EA, EB and EE) contained Rhodamine B. The TLC results showed that the samples LA, LD, LE, EA, EB, EE, BB, and BC had a pink color and had the same Rf value between the sample and the standard. Rhodamine B levels in the sample LA 0.00244%, LD 0.00052%, LE 0.00065%, EA 0.00057%, EB 0.00140%, EE 0.00047%, BB 0.00239%, and BC 0.01091%. It is hoped that the public will pay more attention to the quality of the Lipstick, Blush On, and Eye Shadow products that are used without being affected by the price and striking colors.

**Keywords:** Analysis, Cosmetics, Rhodamin B, UV-Vis Spectrophotometer.

## 1. Introduction

Cosmetics, according to the Regulation of the Minister of Health of the Republic of Indonesia Number 1176/MENKES/PER/VIII/2010 concerning cosmetic notifications, are materials or preparations intended to be used on the external parts of the human body (epidermis, hair, nails, lips and external genital organs) or teeth and the oral mucosa mainly to clean, perfume, change appearance and improve body odor or protect or maintain the body in good condition (Dominika & Hasyim, 2019). Cosmetics often used by women today are lipstick, blush on and eye shadow.

Lipstick is a lip cosmetic, basically a dispersion of a coloring agent in a base consisting of a suitable mixture of oils, fats and waxes. Lipstick is used to give color, making the lips look more attractive (Nanda & Darayani, 2018). Blush on is a cosmetic preparation that is used to color the cheeks in various red colors and in various dosage forms so that it can improve the aesthetics of facial makeup and is in great demand because it creates a new and beautiful effect *(Andayani et*

*al.,* 2018). Eye shadow is a cosmetic preparation used to color the eyelids, which makes the eyes look more prominent and more attractive (Komarudin *et al.,* 2019). The three types of cosmetics are often added with dyes. The dye added in the cosmetic preparation is used to increase the consumer's attractiveness to the product. However, many irresponsible people add harmful dyes to cosmetic preparations such as Rhodamine B.

Rhodamine B is often used as a dye because the price is relatively lower, the color produced is more attractive and the level of color stability is better than natural dyes. Through the Minister of Health Regulation No. 239/Menkes/Per/VI/1985, the Indonesian Government stipulates more than 30 hazardous dyes, one of which is Rhodamine B (Mamoto & Citraningtyas, 2013). Rhodamine B is a synthetic dye that is often misused in cosmetic products. Rhodamine B is a synthetic dye in the form of a crystalline powder, odorless, purplish-red in color, and a bright red, fluorescing solution. This dye can cause irritation to the respiratory tract and is carcinogenic; besides, Rhodamine B in high concentrations can cause liver damage (Nanda & Darayani, 2018).

Identification of Rhodamine B content in lipstick, blush on and eye shadow preparations can be made by qualitative methods using thin layer chromatography (TLC) and quantitative methods using UV-Vis Spectrophotometry. Thin Layer Chromatography is a qualitative analysis to detect a sample by separating the sample components based on their polarity (Nanda & Darayani, 2018). UV-Vis spectrophotometry is a simple type of quantitative analysis; the analysis is fast and can analyze solutions with very small concentrations (Asmawati *et al.,* 2019). In addition, the UV-Vis spectrophotometry method has a good level of accuracy (Fauziah *et al.,* 2020).

In Pekalongan regency, there are still cosmetic sellers who sell fake cosmetics or cosmetics without registration number. It can be usually seen in night or traditional market such as pasar tiban. Examples of this cosmetics that sold are lipstick, eye shadow and blush on. Therefore, it is necessary to identify whether there are harmful ingredients in the cosmetics being sold. The purpose of this study was to identify the content of Rhodamine B in lipstick, blush on and eye shadow circulating in the Pekalongan regency using the UV-Vis spectrophotometry method. The introduction highlights the place or location of the study and the main problems that it is important.

## 2. Materials and Methods

### Materials

The materials used in this research are chemicals with pro-analytical quality from Merck's production include ammonia, ethanol 96%, diethyether, ethyl acetate, HCl 37%, silica Gel F254, NaOH, n-butanol and Rhodamine B. A total of 15 samples of lipstick, blush on and eye shadows in this research without having a registration number with 5 samples each other.

### Tools

The tools used in this research include analytical balance (Ohaus), UV-Vis spectrophotometer (Shimadzu, 1280), oven (Mammert), micropipette (Scilogex), water bath and a set of glassware (Pyrex).

## Methods

### 2.1. Extraction of Rhodamine B

Five (5) grams of the sample was weighed and then put into an erlenmeyer. Samples were extracted using 50 mL of 2% ammonia in 70% ethanol, then allowed to stand for 24 hours, and then filtered. The result of the extraction is evaporated over a water bath at a temperature of 65°C until the solution is concentrated; this concentrated solution is dissolved with 15 ml of distilled water while stirring. Then 3 mL of 10% NaOH was added. Then the solution was extracted with 15 ml of diethylether, then shaken and allowed to stand to form 2 layers. The ether extract was washed with 2.5 ml of 0.5% NaOH, then shaken and allowed to stand until two layers were formed. The ether extract was extracted three times with 5 ml of 0.1 N HCl each time until the ether layer was clear. The bottom layer is taken and put into a measuring cup, where this layer contains Rhodamine B (Patimah *et al.*, 2020).

### 2.2. Preparation of the standard solution

Ten (10) mg of Rhodamine B were carefully weighed and put in a beaker, added with 0.1 N HCl until dissolved; then the solution was put in a 10 mL volumetric flask and then added with HCl to the mark, a solution of Rhodamine B with a concentration of 1000 µg/mL was obtained. Prepared five of 10 mL volumetric flasks, from 1000 g/mL standar solution diluted to concentrations of 2, 4, 6, 8 and 10 µg/mL with 0.1 N HCl (Patimah *et al.*, 2020).

### 2.3. Sample Identification

TLC plate measuring 20 × 10 cm was activated by heating in an oven for 30 minutes at 105°C. The TLC plate to be used is lined with a pencil at a distance of 1 cm from the top edge and 1 cm from the bottom edge. The spotting distance for each test sample is 1 cm. The plate is left for some time to dry. The TLC plate containing the sample was inserted into a previously saturated chamber with the mobile phase n-butanol: ethyl acetate: ammonia (5.5: 2: 2.5) v/v. The plate was left completely eluted, then removed and dried, and the color of the spots was observed visually and under UV light at 254 and 366 nm (Yuniarto & Maryam, 2019)

### 2.4. Determination of Rhodamin B

Samples that have been extracted using standard procedures were measured for absorbance using a UV-Vis spectrophotometer, using 0.1 N HCl as a blank, at a maximum wavelength of 557 nm. The concentration of Rhodamine B in samples that detected Rhodamine B was calculated using a calibration curve with the regression equation $y = ax \pm b$ (Rembet *et al.*, 2017).

## 3. Results and Discussion

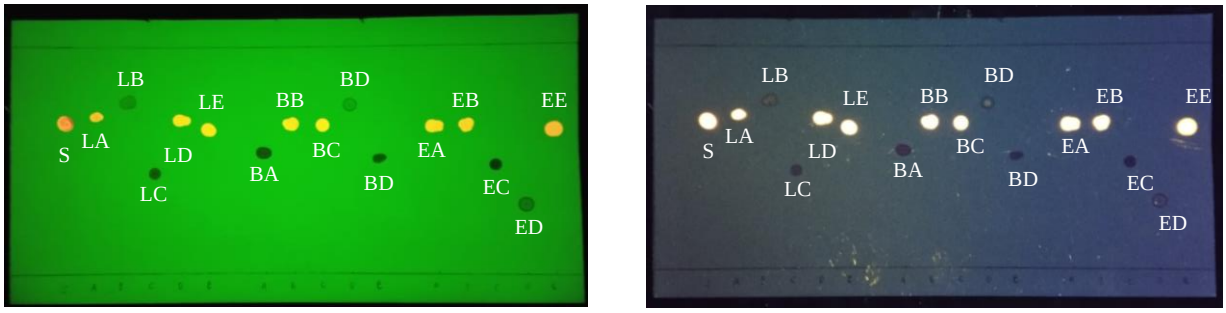
The sample that will be used for testing is first extracted to attract Rhodamine B substances that may be contained in cosmetic preparations. Samples were extracted using 2% ammonia solvent in 70% ethanol. Ammonia is a binder and bleach solution for Rhodamine B dye, and

ethanol is a solvent for Rhodamine B so that Rhodamine B will be extracted perfectly (Patimah *et al.*, 2020). After that, the solution is filtered to separate the dye contained in the sample to be analyzed from impurities that can interfere with the analysis. The filtered filtrate was evaporated at a temperature of 65°C until it became concentrated. Evaporation process at a temperature of 65°C to avoid sample damage (Samosir et al., 2018).

The resulting concentrated extract was then added with distilled water to rinse the remaining extract in the evaporating dish. This solution is then put into a separating funnel, and then 10% NaOH is added, which aims to reduce the solubility of Rhodamine B in distilled water. Rhodamine B is a class of basic dyes; when in a non-ionized state or at high pH, alkaline compounds tend to dissolve in non-polar solvents, so that when Rhodamine B is added with diethyl ether solvent, Rhodamine B will be extracted in the ether phase. The washing of the ether phase using 0.5% NaOH aims to remove residues or impurities, where these residues will dissolve in the aqueous phase and then be removed through a separating funnel. The addition of 0.1N HCl at the final stage of extraction aims to create an acidic atmosphere, causing Rhodamine B to be dissolved into the aqueous phase (Patimah *et al.*, 2020).

The qualitative analysis of this study used the TLC method to determine the presence or absence of Rhodamine B in the samples studied before quantitative analysis was carried out. In the TLC method, the separation of compounds based on adsorption and partition occurs; polar solvents will bind to polar compounds and vice versa. In the analysis of Rhodamine B in TLC using n-butanol: ethyl acetate: ammonia in a ratio of 5.5 : 2 : 2.5 v/v as the mobile phase and silica gel F 254 as the stationary phase. The mobile phase used is polar, the same as Rhodamine B, which is also polar. polar, so that the eluent can elute Rhodamine B well (Samosir *et al.*, 2018).

The examination was carried out by spotting the concentrated sample on a TLC plate and then eluting it using n-butanol: ethyl acetate: ammonia in a ratio of 5.5 : 2 : 2.5 v/v. The mobile phase is expected to produce an Rf value that meets a good Rf value range of 0.2-0.8 and produces circular spots that are neither wide nor tailed (Ananda et al., 2014). These spots from 15 samples in TLC can be seen visually under UV light at 254 nm (**Fig.1.a.**) and 366 nm (**Fig.1.b.**), respectively.




(a) (b)

**Fig.1.** Thin Layer Chromatoghraphy profiles of Rhodamine B qualitative test on samples under UV light at 254 (a) and 366 (b)

From the TLC profile above, nine spots. One spot is for standard and eight spots are samples where Rhodamine B is detected. After the stain is visible, the Rf value can be calculated. This value is used for the relative comparison between samples (**Table 1**).

**Table 1**. Rf value of Rhodamine B in samples lipstick, blush on and eye shadow

| No | Sample | Lipstick | Blush On | Eye Shadow |
|---|---|---|---|---|
| 1 | A | 0.69 | 0.53 | 0.64 |
| 2 | B | 0.75 | 0.67 | 0.65 |
| 3 | C | 0.44 | 0.66 | 0.48 |
| 4 | D | 0.68 | 0.74 | 0.31 |
| 5 | E | 0.64 | 0.50 | 0.63 |

*Rhodamine B standard Rf value is 0.66

Table 1 shows the results of the TLC test on five lipstick samples, five blush on samples and five eyeshadow samples. The resulting Rf value indicates that a good Rf value of Rhodamine B is 0.2 - 0.8 (Elfasyari *et al.,* 2020). The standard Rf value in this study is 0.66, and the sample Rf value can be seen in the table. The result of the difference in Rf values is declared positive if 0.05 and negative if the difference in Rf is >0.05 (Agustin *et al.,* 2021). From the 15 samples, eight samples were obtained that were suspected of containing Rhodamine B by looking at their Rf values where the Rf values of the samples were the same or close to the standard values of Rhodamine B. The samples were lipstick samples, namely LA, LD, and LE, and blush on samples, namely BB and BC and eye shadow, namely EA, EB and EE, in the sample. The results of the qualitative test it was continued for quantitative testing, which aims to measure the levels of Rhodamine B contained in each of these samples.

Samples suspected of containing Rhodamine B were then analyzed for levels using UV-Vis spectrophotometry. The UV-Vis spectrophotometry method was used because the Rhodamine B compound has a chromophore group, which is a group of organic compounds capable of absorbing ultraviolet light and UV light such as carboxyl groups, aromatic compounds and also has an auxochrome group, which is a group that has a lone pair of electrons such as NR2 (Fauziah *et al.,* 2020). The chromophore group in Rhodamine B is a quinoid and the auxochrome group is dimethyl amine. Before calculating the levels of each sample, it is best to determine the maximum wavelength of the Rhodamine b standard using UV-Vis spectrophotometry. Determination of the maximum wavelength of the Rhodamine B standard solution was carried out by measuring the maximum absorbance of the standard solution of 10 µg/mL. The maximum wavelength absorbance measurement was carried out in the wavelength range of 400-800 nm. The maximum absorbance measurement results are at a maximum wavelength of 557 nm with an absorbance value of 0,495. This is in accordance with previous research, which stated that the maximum wavelength of Rhodamine B was 557 nm (Riyanti et al., 2018). The maximum wavelength of Rhodamine B can be seen in **Fig.2.**

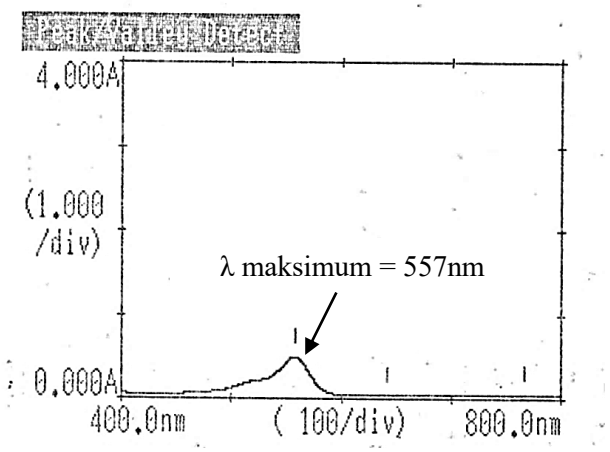



**Fig.1.** Maximum wavelength of Rhodamine B standard solution

The calibration curve data was obtained from the absorbance readings of the Rhodamine B concentration series solutions with concentrations of 2, 4, 6, 8, and 10 µg/mL at a wavelength of 557 nm using UV-Vis spectrophotometry. The absorbance results from the five concentration series solutions were then used as a standard curve **(Fig.3.)**. From the relationship between absorbance and concentration, the resulting linear regression equation is y = 0.0822x + 0.0005 with the value of $R^2$ (relational coefficient) is 0.9997. The value of the relation coefficient is close to 1, indicating that there is a linear relationship between the concentration and absorbance of the reference standard solution. The correlation between concentration and absorption is positive, meaning that with increasing concentration, the absorbance will also increase. The concentration of Rhodamine B and the sample is calculated by entering the absorbance data into the "y" value of the linear regression equation (Nanda & Darayani, 2018).

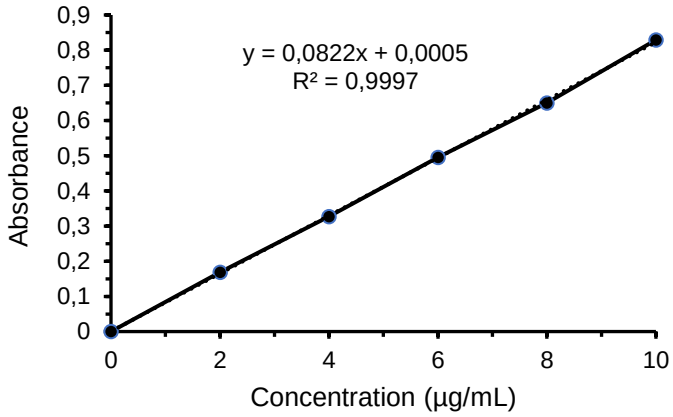



**Fig.2.** Standard Curve of Rhodamine B

Determination of Rhodamine B levels is done by measuring the absorbance of the sample. The concentration of Rhodamine B in the sample was obtained by substituting the absorbance value of the sample solution obtained into the linear regression equation of the solution y =

0.0822x + 0.0005. The results of measuring levels of Rhodamine B in the samples lipstick, blush on and eye shadow are shown in **Table 2.**

**Table 2.** Rhodamine B Levels in Samples Lipstick, Blush On and Eye Shadow

| Sample | Replication | Rhodamine B Levels (%) | Average Rhodamine B Levels (%) ± SD |
|---|---|---|---|
| LA | 1 | 0.00244 | 0.00244 ± 0.000018 |
| | 2 | 0.00245 | |
| | 3 | 0.00241 | |
| LD | 1 | 0.00052 | 0.00052 ± 0 |
| | 2 | 0.00052 | |
| | 3 | 0.00052 | |
| LE | 1 | 0.00065 | 0.00065 ± 0.000007 |
| | 2 | 0.00066 | |
| | 3 | 0.00065 | |
| EA | 1 | 0.00057 | 0.00057 ± 0.000014 |
| | 2 | 0.00057 | |
| | 3 | 0.00059 | |
| EB | 1 | 0.00141 | 0.00140 ± 0.000007 |
| | 2 | 0.00139 | |
| | 3 | 0.00139 | |
| EE | 1 | 0.00047 | 0.00047 ± 0 |
| | 2 | 0.00047 | |
| | 3 | 0.00047 | |
| BB | 1 | 0.00239 | 0.00239 ± 0 |
| | 2 | 0.00239 | |
| | 3 | 0.00239 | |
| BC | 1 | 0.01091 | 0.01091 ± 0 |
| | 2 | 0.01091 | |
| | 3 | 0.01091 | |

Based on the results above, it was stated that eight samples contained Rhodamine B. The levels of each sample were LA 0.00244%, LD 0.00052% and LE 0.00065%. The sample of BB was 0.00239%, and BC was 0.01091%. EA 0.00057%, EB 0.00140%, EE 0.00047%. Based on the results obtained, 8 out of 15 samples have violated the Minister of Health RI No. 239/MenKes/Per/V/85, which prohibits the addition of harmful dyes into cosmetics. The results of this research corroborate the previous research by Yuniarto & Maryam (2019) nine lipstick samples tested, six samples containing Rhodamine B. Likewise, Fauziah *et al*., (2020) research on five eye shadow samples found that two positive samples had Rhodamine B. This is similar to

the reaserch by Satiyarti *et al.*, (2021) the results showed that from three samples blush on, two samples were contained rhodamin B. Significant levels of Rhodamine B can be dangerous because the more significant the levels of Rhodamine B, the greater the toxic effect that will be caused. Rhodamine B, in high frequency, will accumulate in the body. The impact can be in the form of irritation to the skin and respiratory tract and can cause impaired function of the day or liver cancer (Elfasyari *et al.*, 2020)

## 4. Conclusion

The fifteen samples of eye shadow, lipstick and blush on circulation in the Pekalongan regency tested included three eye shadow samples, three lipstick samples, two blush samples that were positive for Rhodamine B, and seven samples were negative for Rhodamine B. The TLC results showed that the samples LA, LD, LE, EA, EB, EE, BB, and BC had a pink color and had the same Rf value between the sample and the standard. Rhodamine B levels in the sample LA 0.00244%, LD 0.00052%, LE 0.00065%, EA 0.00057%, EB 0.00140%, EE 0.00047%, BB 0.00239%, and BC 0.01091%. It is hoped that the public will pay more attention to the quality of the Lipstick, Blush On, and Eye Shadow products that are used without being affected by the price and striking colors.

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