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Original Article

DESIGN OF HYDROQUINONE INDICATOR STRIP BASED ON POLYMETHYLMETHACRYLATE (PMMA) FOR IDENTIFICATION OF HYDROQUINONE IN WHITENING COSMETICS: CASE STUDY IN BANDUNG INDONESIA

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ABSTRACT

Objective: The objective of this research was to developing an indicator strips made from polymer polymethylmethacrylate (PMMA) to detect hydroquinone in whitening cosmetics.

Methods: The strips were prepared by reagent blending method with a polymer PMMA that mixed with a chemical reagent for hydroquinone based on chemical structure: ferric chloride ($FeCl_3$), Benedict, and ammoniacal silver nitrate. PMMA were varied with concentrations of 5%, 7.5%, and 10%. The ratios of ethyl acetate and the reagents were off 6:4; 7:3; and 8:2.

Results: The results showed that the following concentration was an optimum mixture of chemical reagent-polymer and ethyl acetate $FeCl_3$-PMMA 7.5% 7:3; benedict-PMMA 5% 6:4; and ammoniacal silver nitrate-PMMA 5% 7:3. 22 samples of whitening cream were not contained hydroquinone based on strip indicator analysis. These results are appropriate with a confirmatory test using UV spectrophotometry and thin layer chromatography. The indicator strips showed a positive result in detect the samples that had been spiked by hydroquinone but has a limitation when applied to cream dosage forms. The limit of detection (LOD) and stability of the indicator strip $FeCl_3$-PMMA 7.5% 7:3 was 20.05 mg/l and stable up to 157 d, Benedict-PMMA 5% 6: 4 was 0177 mg/l and stable up to 123 d, and ammoniacal silver nitrate-PMMA 5% 7:3 was 0.025 mg/l and stable up to 174 d. The selectivity test results showed that indicator strip did not give false positive results.

Conclusion: Based on the tests, the indicator strip has limitations to detect hydroquinone in whitening cream cosmetics that required further modifications to the detection process.

Keywords: Hydroquinone, UV-Vis spectrophotometry, Thin Layer Chromatography (TLC), Indicator strip, Polymethylmethacrylate (PMMA)

INTRODUCTION

Beauty skin whitening cosmetics is considered important for women's beauty [1]. This is because of an assumption that beauty means having a smooth and white skin [2]. In 2013, Nielsen Indonesia conducted a survey of cosmetic using in Indonesian women, that concluded the women's use of cosmetics in Indonesia has increased [3]. The large market share of the cosmetic product makes some manufacturers produce the product with an addition of hazardous ingredients and without the distribution license. One of the frequently used hazardous ingredients for whitening cosmetics was hydroquinone [4]. Hydroquinone is a substance that can be used as the depigmenting agent [5]. Hydroquinone uses as skin lightening agent through the mechanism of inhibition of the enzyme tyrosine in melanin formation. Hydroquinone competes with tyrosine as a substrate for tyrosinase; then hydroquinone is oxidized by tyrosinase and produce benzoquinone which is toxic to melanocytes [6]. FDA proposes that whitening cosmetics contain hydroquinone should not be available as over the counter (OTC) drugs and should be restricted use with doctor's prescription only. The patient that use hydroquinone product should be monitored by a medical supervisor [4].

The analytical methods for hydroquinone analysis that has been studied were using a visible spectrophotometer, Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC) [7-9]. The instrumental analytical method needs a long time for analysis, not simply to do especially in the field, and should be done by trained person. The instrumental analytical method is not practical and not effective to be applied to common people. To facilitate public for detection hydroquinone in whitening cosmetics, this study designed a method for detection hydroquinone in whitening cosmetics using indicator strip that already contain a specific reagent for hydroquinone. Indicator strip is polymer based material. The polymer is selected as a raw material in the manufacture of the indicator strip because the polymer form pores that the specific reagent of hydroquinone can penetrate and enter.

To ensure the quality of indicator strip, it was tested to some whitening cosmetic samples that were marketed in Bandung, West Java, Indonesia. The results were compared with the identification method using an ultraviolet-visible spectrophotometer and TLC).

MATERIALS AND METHODS

Materials

This study used some materials, such as concentrated ammonia (Merck), aqua dest, acetone (CV. Rachmat Putra), Cera alba (Bratacho Chemistry), cera flava (Bratacho Chemistry), ethanol 96% (Bratacho Chemistry), ethyl acetate (Bratacho Chemistry), ferric chloride hexahydrate (Merck), hydroquinone (CV. Dwi Putra Utama), lanoline (CV. Rachmat Putra), sodium carbonate anhydride (Merck), sodium citric (Merck), nipagin (Merck), nipasol (Merck), copper sulfate (Merck), n-hexane (Bratacho Chemistry), silver nitrate (Merck), polymethylmethacrylate (PMMA) (Aldrich Chemistry), spermaceti (Bratacho Chemistry), triethanolamine (Bratacho Chemistry), resorsinol (Merck), and samples whitening cream with or without registration number.

Instruments

The instruments that used for this study were UV254 lamp (Camag UV Betrachter), magnetic stirrer (Cimarec), micropipette (Socorex), oven (Memmert), silica gel 60 F254(Merck), scanning electron microscope-energy dispersive X-ray (SEM-EDX) (Hitachi TM300), ultraviolet-visible spectrophotometer (Analytic Jena Specord 200), ultrasonic (Neyo), and glass tools that commonly used in analytical chemistry laboratory.

Searching specific reagent for hydroquinone identification

Specific reagents for detection of hydroquinone refers to Moffat et. al., were ferric chloride ($FeCl_3$), benedict, and ammoniacal silver nitrate [10]. The specific reagent was tested to hydroquinone solution to check the color changes. Specific reagents that give different color change from literature was not used in the design of indicator strips. Optimization of hydroquinone concentration was carried out by reacting the specific reagent with 100 mg/l, 1000 mg/l, 10.000 mg/l, and 100.000 mg/l hydroquinone solution in ethanol. The concentration that gives contrast color change then used for further usage.

Design the indicator strips based on PMMA polymerizing reagent blending methods

Indicator strips based of PMMA was made using reagent blending methods with a various concentration that are 5%, 7.5%, and 10%. The PMMA was solved in ethyl acetate; then the specific reagent adds into it. The ratio of ethyl acetate and specific reagents were 6:4; 7:3; and 8:2. The Indicator strips were tested toward standard hydroquinone solution. The indicator strips that give the fastest color change were used to next test.

Collecting whitening cosmetics that marketed in Bandung for samples

The samples were collected randomly refers to previous research [7]. 22 whitening cream were collected from Bandung.

Identification of hydroquinone in sample using ultraviolet-visible spectrophotometer and TLC

Identification of hydroquinone in a sample using a ultraviolet-visible spectrophotometer and TLC was started with sample preparation. 1 g sample of skin whitening creams was weighed and extracted using 25 ml of 96% ethanol. The mixture was homogenized in a water bath at 60 °C for±10 min and cool it down to separate from the fatty phase. The mixture was filtered. The filtrate was used for analysis of TLC and ultraviolet spectrophotometer [7].

For analysis using an ultraviolet spectrophotometer, the filtrate was measured at the maximum wavelength in the range 200–400 nm. The maximum wavelength of the sample was compared to the maximum wavelength standard.

Identification using TLC was carried out using silica gel GF254 as the stationary phase. Based on research conducted by Siddique *et al.*, (2012) the mobile phase used was n-hexane: acetone (3:2). The spot was detected on UV 254 nm, with spray reagent silver nitrate and ammonia concentrated. The Rf of the spot then compared with Rf of hydroquinone standard [7].

Examination the indicator strips toward hydroquinone standard solution and samples

Indicator strips examination consist of sensitivity test, selectivity test, and stability test. The sensitivity test of indicator strips carried out by reacting the indicator strip with various concentration of hydroquinone solution to obtain the minimum concentration that is still producing same color changes as literature.

The stability of indicator strip was carried out by reacting the indicator strips with standard hydroquinone solution then observed the color change in a certain interval time after the indicator strip was made. The stability of the indicator strip was marked on the day when the indicator was already not given positive results. Selectivity test of indicator strips was carried out by tested the indicator strips against a variety of chemicals substances that are often added to skin whitening preparations either excipient or active ingredient and have similar structure and activity with hydroquinone.

Indicator strip was tested toward whitening cream. 10 g samples were extracted with ethanol±20 ml, then homogenized then incubated±30 min and applied to the indicator strip.

Tests on samples include testing by UV and TLC using spiked-placebo recovery method. 1 g sample of skin bleaching cream hydroquinone added 1 ml of 100.000 mg/ml hydroquinone solution, homogenized and then extracted with 25 ml of 96% ethanol. The mixture was homogenized in a water bath at 60 °C for±10 min and cooled it down then filtered. The filtrate was used for analysis of TLC and ultraviolet spectrophotometer [7].

Characterization of indicator strip using SEM-EDX

Indicator strip was characterized to ensure the specific reagent was blended with the polymer. The indicator strip was characterized using SEM-EDX with a magnification of 5000x to view the microstructure and composition of the indicator strip.

RESULTS

The specific reagents from hydroquinone are ferric chloride, benedicts, and ammoniacal silver nitrate. The result of these reagents optimization showed that the three reagents are able to give appropriate colors which can be used for a strip indicator.

The optimization in Table1 showed that the higher hydroquinone concentrate in a liquid used means the stronger color is changing. It could be seen when the 100.000 mg/l of hydroquinone concentrate used; three reagents can produce significant colors in order to make the design easier.

Indicator strips based on PMMA was made using reagent blending methods with a various concentration that is 5%, 7.5%, and 10%. The ratio of solvent and specific reagents were 6:4; 7:3; and 8:2. The indicator strips were tested toward standard hydroquinone solution. Then, the best strip indicator was chosen by evaluating the condition and fastest time reaction for each reagent, which are; $FeCl_3$-PMMA 7.5% 7:3; Benedict-PMMA 5% 6:4; and ammoniacal silver nitrate-PMMA 5% 7:3 (table 2).

The result of identification hydroquinone in samples using UV-Visible spectrophotometer and TLC showed in table 3. Based on that identification, 22 samples did not contain hydroquinone.

The result of sensitivity and stability test on the indicator strips showed in table 4. The result of indicator strips selectivity test showed in table 5.

Table 1: The results of hydroquinone liquid tested by various concentrate to ferric chloride, benedicts, and ammoniacal silver nitrate reagents

Hydroquinone liquide concentrate	The result of reagents tested		
	$FeCl_3$	benedict	Ammoniacal silver nitrate
100 mg/l	-	+	+
1.000 mg/l	-	+	+
10.000 mg/l	+	+	++
100.000 mg/l	++	++	++

Description: (-): no color changing, (+): weak color changing, +: intense color changing, ++: more intense color changing.

The strip indicator testing toward 22 samples showed that all samples did not contain hydroquinone (table 6). This result was appropriate with the result of identification using an ultraviolet spectrophotometer and TLC (table 3).

Table 2: The result of strip indicator with PMMA concentrate variation testing and ethyl acetate reagent comparing with 100.000 mg/l hydroquinone

Reagents	PMMA concentrate	EA: reagents	Polymers conditions	Result	Reaction time
$FeCl_3$	5%	6:4	inhomogeneous	+	2' 14"
		7:3	inhomogeneous	+	3' 43"
		8:2	Inhomogeneous, fragile	+	3' 56"
	7.5%	6:4	Homogeneous, fragile	+	3' 38"
		7:3	homogeneous	+	3' 38"
		8:2	homogeneous	+	5' 58"
	10%	6:4	inhomogeneous	+	5'
		7:3	homogeneous	+	6' 4"
		8:2	inhomogeneous	+	2'
Benedict	5%	6:4	homogeneous	+	1'12"
		7:3	inhomogeneous	+	5"
		8:2	inhomogeneous	+	5' 50"
	7.5%	6:4	homogeneous	+	2' 30"
		7:3	Homogeneous	+	4' 10"
		8:2	inhomogeneous	+	2' 10"
	10%	6:4	homogeneous	+	42"
		7:3	homogeneous	+	1' 7"
		8:2	homogeneous	+	3' 30"
Ammoniacal Silver Nitrate	5%	6:4	homogeneous	+	5"
		7:3	homogeneous	+	1"
		8:2	homogeneous	+	1"
	7.5%	6:4	homogeneous	+	2"
		7:3	homogeneous	+	1"
		8:2	inhomogeneous	+	1"
	10%	6:4	homogeneous	+	48"
		7:3	homogeneous	+	15"
		8:2	homogeneous	+	1"

Table 3: The result of ultraviolet spectral photometer maximum wavelength and TLC in whitening skin cream samples

S. No.	Sample	Wavelength (nm)	TLC	No	Sample	TLC
1	Sample A	299	-	12	Sample L	-
2	Sample B	259	-	13	Sample M	-
3	Sample C	-	-	14	Sample N	-
4	Sample D	299	-	15	Sample O	-
5	Sample E	231	-	16	Sample P	-
6	Sample F	284	-	17	Sample Q	-
7	Sample G	276	-	18	Sample R	-
8	Sample H	-	-	19	Sample S	-
9	Sample I	219	-	20	Sample T	-
10	Sample J	224	-	21	Sample U	-
11	Sample K	238	-	22	Sample V	-

Description: (-): contain hydroquinone, (+): no hydroquinone

Table 4: The result of minimum detection parameter test and balance strip indicator to 100.000 mg/l hydroquinone

Reagents	Indicator strip ingredients	Minimum concentration limits	Time stability
$FeCl_3$	PMMA 7.5% 7:3	20050 mg/l	157 D
Benedict	PMMA 5% 6:4	177 mg/l	123 D
Ammoniacal Silver Nitrate	PMMA 5% 7:3	25 mg/l	174 D

Table 5: The result of selectivity to an excipients

Indicator strip	$FeCl_3$-PMMA 7.5% 7:3		Benedict-PMMA 5% 6:4		Ammoniacal silver nitrate-PMMA 5% 7:3	
	Color change	Reaction time	Color change	Reaction time	Color change	Reaction time
Nipagin	Grey	1"	-	-	-	-
Nipasol	Grey	1"	-	-	-	-
Resorcinol	Grey	1"	-	-	dark brown	2' 30"
Cera Alba	-	-	-	-	-	-
Cera Flava	-	-	-	-	-	-
Lanolin	-	-	-	-	-	-
TEA	-	-	-	-	-	-
Spermaceti	-	-	-	-	-	-

The result of the skin whitening cream sample testing provides that the sample does not contain the maximum absorption on wavelength 296 nm and the sample does not give the same Rf on the TLC plate.

The strip indicator characteristic can be seen from fig. 1 by using scanning electron microscope in 5000x magnifications. It shows that the strip indicator $FeCl_3$-PMMA 7.5% 7:3; Benedict-PMMA 5% 6:4; and ammoniacal silver nitrate-PMMA 5% 7:3 has not had homogeny microstructure pores. The EDX analysis result shows that all the strip indicators are containing carbon and oxygen element which is part of PMMA polymer composer. The other elements are part of specific reagents that showed that it already entered the pores of the strips.

Table 6: The result of strip indicator testing to skin whitening cream samples

Sample	Indicator strips			Details information
	$Fecl_3$	Benedict	Ammoniacal silver nitrate	
Sample A	Yellow	Blue	White	-
Sample B	Yellow	Blue	White	-
Sample C	Yellow	Blue	Grey	-
Sample D	Yellow	Blue	Reddish brown	-
Sample E	Yellow	Blue	Twany	-
Sample F	Yellow	Blue	White	-
Sample G	Brown	Blue	White	-
Sample H	Black	Blue	Brown	-
Sample I	Black	Blue	Grey	-
Sample J	Yellow	Blue	White	-
Sample K	Black	Blue	White	-
Sample L	Yellow	Blue	White	-
Sample M	Yellow	Blue	White	-
Sample N	Yellow	Blue	Brown	-
Sample O	Black	Blue	Grey	-
Sample P	Yellow	Blue	Grey	-
Sample Q	Black	Blue	White	-
Sample R	Yellow	Blue	Grey	-
Sample S	Yellow	Blue	Grey	-
Sample T	Black	Blue	Grey	-
Sample S	Yellow	Blue	Grey	-
Sample U	Reddish yellow	Blue	Black	-
Sample V	Yellow-black	Blue	Grey	-

Description: (-) = Consider does not contain a hydroquinone, (+)= Consider contain a hydroquinone

Table 7: The result of strip indicator to skin whitening cream with placebo spike method

Sample	Indicator strips			Details information
	$FeCl_3$	Benedict	Ammoniacal silver nitrate	
Sample A	Yellow	Red	Greyish brown	-
Sample B	Yellow	Red	Greyish brown	-
Sample C	Yellow	Red	Greyish brown	-
Sample D	needle-shaped green	Red	Greyish brown	+
Sample E	needle-shaped green	Red	Greyish brown	+
Sample F	Reddish brown	Red	Greyish brown	-
Sample G	needle-shaped green	Red	Grey	+
Sample H	Reddish brown	Red	Greyish brown	-
Sample I	Reddish brown	Red	Greyish brown	-
Sample J	Yellow	Red	Greyish brown	-
Sample K	Reddish brown	Blue	Greyish brown	-
Sample L	needle-shaped green	Red	Greyish brown	+
Sample M	Yellow	Red	Greyish brown	-
Sample N	needle-shaped green	Red	Greyish brown	+
Sample O	Reddish brown	Red	Greyish brown	-
Sample P	needle-shaped green	Red	Greyish brown	+
Sample Q	Reddish brown	Red	Greyish brown	-
Sample R	needle-shaped green	Red	Greyish brown	+
Sample S	needle-shaped green	Red	Greyish brown	+
Sample T	Reddish brown	Red	Greyish brown	-
Sample S	needle-shaped green	Red	Greyish brown	+
Sample U	Reddish brown	blue	Greyish brown	-

Description: (-) = No reaction, (+)= reaction

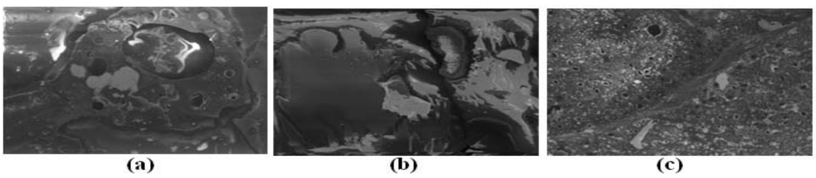

Fig. 1: The characteristic result of strip indicator of PMMA reagent blending method using SEM in 5000x magnification, (a) microstructure strip indicator of $FeCl_3$-PMMA 7.5% 7:3, (b) microstructure strip indicator of benedict-PMMA 5% 6:4 and (c) microstructure strip indicator of ammoniacal silver nitrate PMMA 5% 7:3

DISCUSSION

The specific reagents from hydroquinone are ferric chloride, benedicts, and ammoniacal silver nitrate. The result of these reagents optimization showed that the three reagents are able to give appropriate colors which can be used as for strip indicator.

The liquid hydroquinone concentrates need to be optimized in order to reach a significant color changing. The optimization in table 1 showed that the higher hydroquinone concentrate in a liquid used means the stronger color change. It could be seen when the 100.000 mg/l of hydroquinone concentrate used; three reagents can produce significant colors in order to make the manufacture easier. Therefore, the test of the next strip indicator will be used a hydroquinone liquid with 100.000 mg/l concentrate.

The reagent blending method in manufacturing strip indicator is a phase inversion precipitation with solvent evaporation. An inversion phase is a polymer transformation process from the liquid phase into the solid phase. Then, one of the liquid phases which reached by the polymer is a PMMA liquid in ethyl acetate. This phase is condensing as the phase inversion process happen to shape solid matrix. PMMA phase are on 5%, 7.5%, and 10%. The PMMAliquid in an acetate ethyl and specific reagent was mixed with various ethyl acetate: reagent 8:2; 7:3; and 6:4. An improvement of polymer concentrate made pores between the PMMAmolecule smaller and selective [11] so, it took a long time to make hydroquinone reacted with the reagent in the strip indicator. The made strip indicator was tested with 100.000 mg/l hydroquinone liquid. Then, the best strip indicator was chosen by evaluating the condition and fastest time reaction for each reagent, which are; $FeCl_3$-PMMA 7.5% 7:3; Benedict-PMMA 5% 6:4; and ammoniacal silver nitrate-PMMA 5% 7:3 (table 2). The nonpolymer phase liquid is a hydroquinone specific reagent (ferric chloride, benedicts, and ammoniacal silver nitrate) these two phases is combined and evaporated. The nonpolymer liquid hydroquinone (as a specific reagent) will be absorbed with the manufacturing solid matrix PMMA. The strip indicator is made by the three polymethylmethacrylate concentrate variations.

The determination of maximum wavelength in the hydroquinone liquid was showed 296 nm as a maximum wavelength. There was a friction about 1 nm from the maximum wavelength based on the 295 nm as a determination if it was reconstituted by ethanol [10]. Based on table3 sample, all the samples were considered not containing hydroquinone. In table 3 there is no spot on the TLC plate which is observed by UV 254 nm light. On the other methods by using spray reagent ammoniacal silver nitrate, there is no spot in the plate. This situation identifies there is no hydroquinone in the sample. The test which is done on the performance of strip indicator is including sensitivity, balance, and rigidity test (table 2). The result of sensitivity competency strip indicator (table 4) shows that the ammoniacal silver nitrate strip indicator has the highest sensitivity since it reaches the lowest detection limit. The smaller hydroquinone concentrate detected, also shows a smaller reaction hydroquinone with the reagent.

The result of lifetime test on table 4 provides that the strip indicator with the polymer basis has a particular lifetime. This condition is happened by PMMA which bears with environment influences [12]. In the design of strip indicator the reagent is combined with the polymer, so in a span long time the strip indicator still on a stable condition [13].

The selectivity test is done by reacting strip indicator with another component which can be added into skin whitening cream and has a similar structure with the hydroquinone. The $FeCl_3$ strip indicator gives a positive result to nipagin, nipasol, and resorcinol. It could happen because that three component contains a phenol group. The benedict strip indicator gives a negative result to nipagin, nipasol, and resorcinol. The benedict reagent causes it is only able to react to the phenol group which is containing at least 4 hydroxyl group at the aliphatic chain. The ammoniacal silver nitrate strip indicator shows a negative effect to nipagin and nipasol. However, it provides a positive result to resorcinol. It could happen since the ammoniacal silver nitrate can be reacted with a compound which is containing reduction power [10]. Resorcinol is a reductor [14,15]. Therefore, if it reacts with the ammoniacal silver nitrate it will show brownies color. The result of strip indicator to cera alba, cera flava,lanolin, triethanolamine, and spermaceti do not give any reaction. Based on the selectivity test, no component reacted positively with all the three reagents; it is showed the selectivity of the strip.

The result of the skin whitening cream sample testing provides that the sample does not contain hydroquinone. Therefore, the spiked-recovery method is done for a further study and to ensure the quality of the strip. The testing result after adding the hydroquinone provides that all the samples contains hydroquinone based on the maximum absorption on UV and Rf on TLC.

The strip indicator testing result to the sample on table 7 shows that there are strip indicator which gives a positive results (sample D, E, G, L, N, P, R, S, and U) and negative results (sample A, B, C, F, H, I,J, K, M, O,Q,T, and V) which are supposed to give positive result because of standard spiked that already done. This situation is contradicted with the TLC and UVtesting result. This result was because the matrix contained and showed the limitation of the strip on some samples.

The strip indicator characteristic can be seen from fig. 1 by using SEMin5000x magnifications. It shows that the strip indicator $FeCl_3$-PMMA 7.5% 7:3; Benedict-PMMA 5% 6:4; and ammoniacal silver nitrate-PMMA 5% 7:3 has not had homogeny microstructure pores. It is caused by a difference polarity between the polymer and reagent resulted reagent and polymer are not able to mix well.

The EDX analysis result shows that all the strip indicators are containing carbon and oxygen element which is part of PMMA polymer composer. However, the other elements are part of specific reagents. Overall, the reagent is able to enter the polymer pores because of the elements contain in the reagent is detected by EDX.

CONCLUSION

The strip indicator is created by using reagent blending method which is combining PMMA liquid in the specific hydroquinone ethyl acetate reagent; such as $FeCl_3$, benedict, and ammoniacal silver nitrate. The results showed that the strip can be use as an alternative method for early detection of hydroquinone content in cosmetic samples even it still have a limitation due to matrix interference.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest

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