Design of Indicator Strip Using Polystyrene (PS) and Polymethylmethacrylate (PMMA) for Detection of Diclofenac Sodium in Traditional Pain Relief Herbal Medicines

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ABSTRACT

Diclofenac sodium is one of analgesic which added to herbal medicines to enhance the effects. One of a qualitative method that is easy, efficient and simple is an indicator strip test. Indicator strip based polystyrene (PS) and polymethylmethacrylate (PMMA) was made to detect of diclofenac sodium which misused in traditional pain relief herbal medicine. Indicator strip was made by reagent blending method with specific reagents, copper sulfate ($CuSO_4$), ferric chloride ($FeCl_3$) and vanillin sulfate. The principle of the indicator strip in detecting diclofenac sodium is with the occurrence of a color reaction. The PS and PMMA were made in the concentration of 5, 7.5 and 10% and the mixture of PS and PMMA 1:5 and 1:6 respectively with a ratio of solvent and reactant 6:4; 7:3 and 8:2. The best indicator strip is PMMA-$CuSO_4$ 5% (7:3), PMMA-$FeCl_3$ 5% (7:3) and PMMA-Vanillin sulphate 7.5% (7:3). The detection limit of the PMMA-$CuSO_4$ 5% (7:3) was at 50 ppm, PMMA-$FeCl_3$ 5% (7:3) showed detection limit of 12,500 ppm and PMMA-vanillin sulfate 7.5% (7:3) showed detection limit of 500 ppm. All indicator strips stable up to 29 weeks. The indicator strip can be used as an alternative method to detect diclofenac sodium in herbal medicine.

***Keywords**: diclofenac sodium; PS; PMMA; indicator strip*

ABSTRAK

Natrium diklofenak adalah salah satu analgesik yang ditambahkan ke obat-obatan herbal untuk meningkatkan efek. Salah satu metode kualitatif yang mudah, efisien dan sederhana adalah strip indikator. Strip indikator polystyrene (PS) dan polymethylmethacrylate (PMMA) dibuat untuk mendeteksi natrium diklofenak yang disalahgunakan dalam jamu pegal linu. Strip indikator dibuat dengan metode reagen blending dengan reagen tembaga sulfat ($CuSO_4$), besi klorida ($FeCl_3$) dan vanillin sulfat. Prinsip strip indikator dalam mendeteksi natrium diklofenak adalah dengan terjadinya reaksi warna spesifik. PS dan PMMA dibuat konsentrasi 5, 7,5 dan 10% dan campuran PS: PMMA 1:5 dan 1:6 dengan perbandingan pelarut dan reaktan 6:4; 7:3 dan 8:2. Strip indikator terbaik adalah PMMA-$CuSO_4$ 5% (7:3), PMMA-$FeCl_3$ 5% (7:3) dan PMMA-vanilin sulfat 7,5% (7:3). Batas deteksi PMMA-$CuSO_4$ 5% (7:3) adalah pada 50 ppm, PMMA-$FeCl_3$ 5% (7:3) menunjukkan batas deteksi 12.500 ppm dan PMMA-vanillin sulfat 7,5% (7:3) batas deteksi 500 ppm. Semua strip indikator stabil sampai dengan 29 minggu. Strip indikator dapat digunakan sebagai metode alternatif untuk mendeteksi natrium diklofenak dalam pengobatan herbal.

***Kata Kunci**: natrium diklofenak; PS; PMMA; strip indikator*

INTRODUCTION

Diclofenac sodium is a non-steroidal anti-inflammatory drug (NSAID) with an indication of anti-inflammatory, analgesic and antipyretic comparable or superior to other NSAIDs. Diclofenac showed preferential inhibition of cyclooxygenase 2 (COX-2). Diclofenac sodium is mainly indicated in the treatment of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis [1-2,4]. Diclofenac sodium also has anti-bacterial action shown by inhibition of DNA synthesis. As with other NSAIDs, diclofenac sodium is known to increase the risk of gastrointestinal bleeding and side effects cardiovascular [1-2]. Diclofenac was associated with severe gastrointestinal toxicity and few adverse effects on the lungs, liver and renal peripheral tissue [3,5-6].

In the current information on the security aspects of drugs containing diclofenac (systemic formulation), has been done a comprehensive study in which the systemic formulation of diclofenac in improving cardiovascular risk in a consistent and comparable risk with rofecoxib that has toxicity to the heart. In safety alert issued by Badan Pengawasan Obat dan Makanan (BPOM) on July 13th, 2015 related to the regulation of repair and restrictions on dosages and contraindications product, diclofenac associated with cardiovascular risk, among others: ischemic heart

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disease, peripheral arterial disease, cerebrovascular disease and congestive heart failure *(class II-IV)*.

Based on the Indonesian Ministry of Health number 246/Menkes/Per/V/1990 (regarding traditional medicine industry business license and registration of traditional medicine) as well as the decision of the Head of POM HK numbers 00.05.41.1384 2005, traditional herbal medicine should not contain synthetic chemicals or medicinal isolation results, as well as material belonging to hard drugs or narcotics or psychotropic substances, and animal or plant to be protected. Consumers usually do not aware on the hazards of traditional medicines being consumed, especially to the contraindications or drug interactions that can occur when traditional medicine contain chemicals being taken together with other medicine [7].

The method commonly used to determine diclofenac sodium is using the HPLC method, UV spectrophotometer, and fluorimetry [8-12]. The method is less effective and efficient, especially in the field. People need an alternative method to help them to analyze chemicals in herbal medicine without taking it to the lab. Thus, in this research, a qualitative analysis technique which is simpler, easier and faster was embodied in the indicator strip. In the method, the indicator strip was made of polystyrene (PS), polymethylmethacrylate (PMMA) and a mixture of PS and PMMA containing a specific reagent that will react with the analyte and produced specific color belong to that analyte. The design of the indicator strip will be conducted using reagent blending method followed by the characterization of the resulting indicator strips includes a test of accuracy, sensitivity, stability and robustness.

EXPERIMENTAL SECTION

Materials

Acetosal, mefenamic acid, methampyron, paracetamol, ethanol 96%, ethyl acetate and methanol 96% from Brataco Chemistry. Copper sulfate, ibuprofen, ferric chloride, sulphuric acid and Vanillin from Merck. Phenylbutazone, polymethylmethacrylate (PMMA) and polystyrene (PS) from Sigma-Aldrich. Diclofenac sodium from Promed, extract of herbs simplisia and sample of traditional pain relief herbal medicine.

Instrumentation

A magnetic stirrer (Cimarec), micropipette (Socorex), Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX, Hitachi TM 3000), ultrasonic (Neyo) and UV spectrophotometer (Analytik Jena Specord 200).

Procedure

Design of indicator strip from polymers by specific chemical reagent using reagent blending method

Polymers (PS, PMMA, as well as a mixture of PS and PMMA) were made in three varying concentrations of 5, 7.5, and 10%. Each concentration was made in ethyl acetate as solvent and mixed with a specific chemical reagent of diclofenac sodium (copper sulfate, vanillin sulfate, and ferric chloride) with a variation of solvent and reagent 6:4, 7:3 and 8:2 respectively. Polymer blends of PS and PMMA are made with the mass ratio between the PS:PMMA of 1:5 and 1:6.

Polymers 5, 7.5, and 10% were made by mixing 0.5, 0.75, and 1.0 g polymers, respectively, in 10 mL of solvent-reagent mixture. Each polymer solution was stirred on a magnetic stirrer until all the polymer is dissolved homogeneouskly. The polymer solution was coated on a glass plate, allowed to stand until dry. The polymer membranes are ready to use and test.

Test of indicator strip performance

The performance of indicator strip including sensitivity, stability, and robustness test was tested at each optimum conditions.

Sensitivity Test. Indicator strips tested to various concentrations of diclofenac sodium. The results of the color change of indicator strips were observed and the reaction time was measured. The concentration of diclofenac sodium where strips indicator no longer able to detect (no color change) is the limit of detection of the strips.

Stability Test. Indicator strips were tested in a standard solution of diclofenac sodium at a concentration of 50,000 ppm is then observed color change is given within a certain timeframe after the indicator strip is made. The stability of the indicator strip is marked on the day that the indicator strip is not yielded positive results.

Robustness Test. Indicator strips were tested against a variety of chemicals that are often used in herbal medicine. Substances that are tested include the active substance antalgin class of NSAIDs, acetosal, ibuprofen, paracetamol, mefenamic acid, and phenylbutazone each dissolved in a suitable solvent with a concentration of 50,000 ppm and three types of ethanol extract of herbs simplisia were made in a concentration of 15,000 ppm.

Confirmation of sample with ultraviolet spectro photometer and indicator strip using spiked-placebo recovery method

A total of 400 mg of traditional pain relief herbal medicine that has been powdered fine, extracted with methanol 96% in 10 mL, sonicated for 30 min and filtered. The filtrate was evaporated and dissolved in distilled water in 10 mL. Then 3 mL of diclofenac sodium 50,000 ppm in 2 mL samples was added. Spiked sample was tested then using indicator strip and confirm the result using the Ultraviolet Spectrophotometer in 20 ppm dilution. The spiked sample solution is analyzed by UV spectrophotometer to scan mode 200-350 nm and the spectrum was overlayed with diclofenac sodium standard.

Characterization indicator strips by using Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX)

Indicator strips were characterized by using Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX) with a magnification of 1000x to determine whether the surface topography and the reagents have been mixed well with the polymer.

RESULT AND DISCUSSION

Indicator strips were made of PS and PMMA for the examination of diclofenac sodium were misused as medicinal chemicals in traditional herbal medicine. This indicator strip is a qualitative analysis method that is simpler, easier and faster to detect the presence of the chemical that contained in the sample. Indicator strip was made with reagent blending method with specific reagents. The principle of the indicator strip in detecting the presence of diclofenac sodium sample is with the color reaction. The color reaction is marked by the change in color due to the reaction between the specific chemical reagents to a particular functional group in a substance.

Design of Indicator Strip from Polymers by Specific Chemical Reagent Using Reagent Blending Method

The solubility of PS and PMMA in various solvents can be predicted based on the value of Hildebrand solubility parameter. Hildebrand solubility parameter was used to select a solvent and additives in the formulation and mixing polymer [13]. The closer Hildebrand solubility parameter of a polymer with a value of Hildebrand solubility of a solvent, the greater the solubility of the polymer in the solvent [14]. The value of Hildebrand solubility of PMMA and PS are 9.3 $cal^{1/2}$ and 9.1 $cal^{1/2}$ $cm^{-3/2}$. Hildebrand solubility values approaching solvent PS and PMMA is ethyl acetate (9.1 $cal^{1/2}$ $cm^{-3/2}$) [15-18]. Solubility process is controlled by the solubility of the polymer chain tangling or by diffusion through the boundary layer adjacent to the polymer-solvent interface [19].

Phase inversion technique also called by blending reagent. Phase inversion is the transformation of the polymer from a liquid phase to a solid phase. Compaction process of the polymer begins with the formation of a liquid phase of the two liquid phases are intermingled. One of the liquid phases is a polymer-rich phase. This phase will condense during the phase inversion process so as to form a solid matrix [20].

Indicator strips are made from polymers with three variations of concentrations of 5, 7.5, and 10%. PMMA polymer concentrations less than 5% produces a very fragile membrane, whereas concentrations above 10% causing a pore of size polymer sheet smaller and the water permeability decreases, make it difficult to adsorb reagents. The ratio of the solvent and the reagent used is 6:4; 7:3; and 8:2, so as ethyl acetate-reagent mixture can be mixed well with the polymer. The amount of solvent is too much beyond this comparison on the final result causes the resulting polymer is too brittle, resulting in a non-homogenous membrane indicator strip. While too much amount of reagent in the mix causes the polymer can not be dissolved well because there are too many non-solvent phases in the mixture.

Performance of Polymers

The indicator strip of polymer PS can not be formed. When the reagent blending process is done, PS polymer solidification occurs directly without mixing homogeneously with the reagent. This is because the PS polymer is non-polar, while solvent-reagent mixture more polar than the PS [23-24]. So that PS can not be mixed better with each specific water-based reagent compared to PMMA. PS elections due to be rigid and resistant to corrosive substances but readily soluble in aromatic hydrocarbons and chlorine. Polystyrene is resistant to alkaline substances, acid halide and an oxidation-reduction agent [21-22].

PMMA is a polymer that has high resistance to the weather, quite strong, impact resistant and bending, a good insulator, give a stable color to various chemicals, and optically most transparent of all kinds of plastic [25]. Results of testing the indicator strip based on PMMA to a standard solution of diclofenac sodium to 50,000 ppm, shown in Table 1. Based on Table 1, it showed that the indicator strip of PMMA can be formed and give better membrane than the indicator strip of PS. This is because PMMA is more polar than PS so that PMMA can be mixed better with each specific water-based reagent than PS [20]. With that, the reagent

Table 1. Result Test of PMMA Indicator Strips with 50,000 ppm Standard Solution of Diclofenac Sodium

Reagent	% PMMA	EA:Reagent	Polymer Condition	Result	Time of Reaction
$CuSO_4$	5	6:4	Homogenous	+	2”
		7:3	Homogenous	+	2”
		8:2	Homogenous	+	2”
	7.5	6:4	Homogenous	+	7”
		7:3	Homogenous	+	7”
		8:2	Homogenous	+	8”
	10	6:4	Homogenous	+	40’
		7:3	Homogenous	+	1’ 56”
		8:2	Homogenous	+	1’ 40”
$FeCl_3$	5	6:4	Homogenous	+	2”
		7:3	Homogenous	+	2”
		8:2	Homogenous	+	2”
	7.5	6:4	Homogenous	+	28”
		7:3	Homogenous	+	12”
		8:2	Homogenous	+	9”
	10	6:4	Homogenous	+	4”
		7:3	Homogenous	+	6”
		8:2	Homogenous	+	2”
Vanillin Sulfate	5	6:4	Non-Homogenous	-	-
		7:3	Non-Homogenous	-	-
		8:2	Non-Homogenous	-	-
	7.5	6:4	Non-Homogenous	-	-
		7:3	Homogenous	+	6”
		8:2	Homogenous	+	26”
	10	6:4	Non-Homogenous	-	-
		7:3	Homogenous	+	43”
		8:2	Homogenous	+	32”

Table 2. Result test of PS:PMMA Indicator Strip with 50,000 ppm Standard Solution of Diclofenac Sodium

Reagent	% PS:PMMA	EA:Reagent	Polymer Condition	Result	Time of Reaction
$CuSO_4$	**1:5 (5%)**	6:4	Homogenous	+	11”
		8:2	Homogenous	+	3”
	1:6 (5%)	6:4	Homogenous	+	2”
		8:2	Homogenous	+	4”
$FeCl_3$	**1:5 (5%)**	6:4	Homogenous	+	3”
		8:2	Homogenous	+	4”
	1:6 (5%)	6:4	Homogenous	+	9”
		8:2	Homogenous	+	1”
Vanillin Sulfate	**1:5 (5%)**	6:4	Non-Homogenous	-	-
		8:2	Non-Homogenous	-	-
	1:6 (5%)	6:4	Non-Homogenous	-	-
		8:2	Non-Homogenous	-	-

Information: EA: Ethyl acetate; (+): Positive color change; (‘): min; (“): sec

blending process of PMMA with a reagent to deliver results homogeneous so that the polymer membrane well formed.

Indicator strip was also made from a mixture of PS and PMMA with a ratio of 1:5 and 1:6, respectively. The purpose of the variation of PS and PMMA polymer blends are to provide a better indicator strip membrane results than the indicator strip comprising only a single polymer. Results of testing the indicator strip polymer mixture against a standard solution of diclofenac sodium 50,000 ppm, shown in Table 2.

According to the Table 1 and 2, it showed the differences time reaction at each concentration of polymer and ratio of solvent and reagent, The reaction time of the indicator strip is affected by the pores formed in the indicator strip. The pore size of the indicator strip is affected by the concentration of the polymer and the concentration of water. High polymer

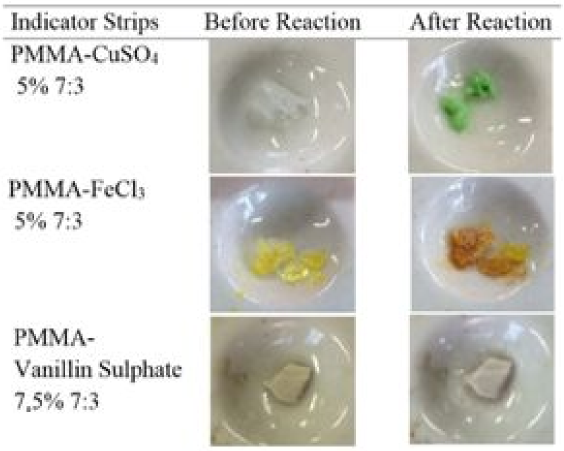

Fig 1. Color test result between indicator strips (before reaction) and after reaction with 50,000 ppm standard solution of diclofenac sodium

concentrations lead to pore formed more tightly [26]. Increasing concentrations of polymers will make polymer intermolecular pores become smaller and be selective [27]. The smaller the pore size of the polymer, causes the indicator strip impenetrable by diclofenac sodium solution that takes a long time to react with a reagent of diclofenac sodium in the indicator strip. In addition, the mixing ratio of solvent and reagent also affects the homogeneity of the indicators strip. Homogeneity of reagent can affect the performance indicator for piling reagent strip in a certain area. Less homogenous areas caused by the phase difference between the polarity of solvents and reagents [28]. At some concentration of polymer and also the ratio of solvent and vanillin sulfate was given that indicator strip was not homogeneous. Non-homogenous indicator strip makes it can not be coated and can not do testing reaction time. This is caused by using of vanillin sulfate as a reagent with 15 N sulfuric acid, making the process of solidification the polymer was not homogeneously. This happens because PMMA is irresistance to inorganic reagents, including strong alkali and acid liquids [29]. The concentration of vanillin sulfate as a reagent can not be lower than 15 N because as vanillin sulfate will not provide a significant color change.

Test of Indicator Strip Performance

The strip was tested to know sensitivity, stability, and robustness. Indicator strip that showed the optimum condition are PMMA-$CuSO_4$ 5% (7:3), PMMA-$FeCl_3$ 5% (7:3) and PMMA-vanillin sulphate 7.5% (7:3). Selection of optimal reagent strip-indicator is based on the significant color change, time observation of the color change and the efficient use of polymers and ratio polymer solvent and reagent. The color test result of indicator strip and with adding 50,000 ppm standard solution of diclofenac sodium shown in Fig. 1. PMMA-$CuSO_4$ indicator strip gave a color change from white to green. The reagent of copper sulfate reacts with chloride ions to form complexes of *tetrachlorocuprate* (II). PMMA-$FeCl_3$ indicator strip gave a color change from yellow to orange-brown. Ferric chloride reagents react with acetate ions form a precipitate of alkaline iron (II) acetate. PMMA-vanillin sulfate gave a color changes from yellow to purple as it reacts with a secondary amine functional groups.

Sensitivity and stability test

PMMA polymer-$CuSO_4$ to 5% (7:3) can detect the concentration of diclofenac sodium up to 750 ppm with a reaction time of 2 min 30 sec. PMMA-$FeCl_3$ 5% (7: 3) can detect the concentration of diclofenac sodium up to 12,500 ppm with a reaction time of 1 min 30 sec. PMMA-vanillin sulfate 7.5% (7:3) can detect diclofenac sodium at a concentration of 500 ppm with a reaction time of 10 sec.

The test of the stability of indicator strip was done every week until the indicator strip did not provide the color changes according to the results of the optimization. The entire indicator strip shows the stability in the period up to 29 weeks.

Robustness test

The robustness test indicator strips do against other drugs that are often added to traditional pain relief herbal medicines i.e. antalgin, acetosal, ibuprofen, paracetamol, mefenamic acid, and phenylbutazone. Beside chemical NSAID addition, the strip was also tested with three types of extracts which are all of the three extracts have been tested for secondary metabolite with phytochemical qualitative analysis. The extract 1 is the ethanol extract of jawer kotok leaves *(Plectranthus scutellarioides* L.) contains polyphenols, flavonoids, saponins and quinone as the composition of secondary metabolites. The extract 2 is ethanol extract of rambutan binjai rind *(Nephelium lappaceum* L.) contain polyphenols, flavonoids, tannins, monoterpenes, sesquiterpenes and saponin as the composition of secondary metabolites and the extract 3 is ethanol extract of the fruit of Malacca *(Phyllanthus emblica* L.) contain polyphenols, flavonoids, tannins, monoterpenes, sesquiterpenes, quinone and saponin as the composition of secondary metabolites. The content of a variety of secondary metabolites in the extract can represent a variety of other substances contained in the sample which can possibly give false positive results on the indicator strip when tested with a sample of traditional pain relief herbal medicines. The result of robustness test showed in Table 3 and 4. Based on the results of the robustness test can be concluded the indicator strips have good selectivity.

Table 3. The Robustness Test Results of the Indicator Strip with NSAID

NSAID	Color Change Indicator Strip		
	$CuSO_4$-PMMA 5% 7:3	$FeCl_3$-PMMA 5% 7:3	Vanillin Sulfate-PMMA 7.5% 7:3
Antalgin	-	-	-
Acetosal	-	-	-
Ibuprofen	-	-	-
Paracetamol	-	-	-
Mefenamic Acid	-	-	-
Phenylbutazone	-	-	-

Table 4. The Robustness Test Results of the Indicators Strip with Extracts of Herbs

Indicator Strip	Extract 1	Extract 2	Extract 3
	Color Change	Color Change	Color Change
$CuSO_4$-PMMA 5% 7:3	-	-	-
$FeCl_3$-PMMA 5% 7:3	-	-	-
Vanillin Sulfate -PMMA 7.5% 7:3	-	-	-

Information: (-): No color change reaction

Table 5. Percentage of Elements Mass in Indicator Strips

Indicator Strips	Type of Elements	% Mass
PMMA-$CuSO_4$ 5% 7:3	Carbon	47.007
	Oxygen	35.550
	Sulfur	4.466
	Copper	12.997
PMMA-$FeCl_3$ 5% 7:3	Carbon	53.822
	Oxygen	14.495
	Chlorine	18.960
	Iron	12.723
PMMA-Vanillin Sulfate 7.5% 7:3	Carbon	38.335
	Oxygen	50.489
	Aluminum	0.349
	Sulfur	10.828

Confirmation of Sample with Ultraviolet Spectro photometer and Indicator Strips Using Spiked-Placebo Recovery Method

Measurements result on samples of traditional pain relief herbal medicine showed that diclofenac sodium was not found in the sample. Simulations of herbal medicine samples were tested through the drugs substances-placebo spiked recovery method by adding 3 mL of 50,000 ppm of diclofenac sodium into 2 mL sample extract. For the analysis by spectrophotometer UV, spiked samples were diluted to a concentration of 20 ppm. With the accuracy of test results on the spiked sample, strip showed that three indicator strips provide the color changes according to the results of the optimization. PMMA-$CuSO_4$ to 5% (7:3) to react for 2 min and 40 sec. PMMA-$FeCl_3$ 5% (7:3) react for 1 min 28 sec. PMMA-vanillin sulfate 7.5% (7:3) to react for 25 sec. Overlay of spectrum between the samples of the spiked sample with diclofenac sodium 18 ppm shown in Fig. 2.

Characterization of Indicator Strip by Using Scanning Electron Microscope-Energy Dispersive X-Ray (SEM-EDX)

SEM characterization of the indicator strip at 1000x magnification to observe the surface topography and microstructure of the indicator strip are presented in Fig. 3. Analysis using Energy Dispersive X-ray (EDX) produced a distinctive spectrum which shows the content of the elements of reagent in the indicator strip. The concentration of elements that contained in the indicator strip is shown in Table 5. The color change in strips was based on the reaction between the elements in the reagent with the functional groups of diclofenac sodium. In order to confirm the presence of the elements inside the strips, we have done EDX analysis. From the EDX analysis, we have concluded that all elements of the reagents were a presence on the strip and can form a chemical reaction with functional groups of diclofenac sodium. SEM was used to determine the homogeneity of the strip. The reaction time of the reagent inside the indicator strip with

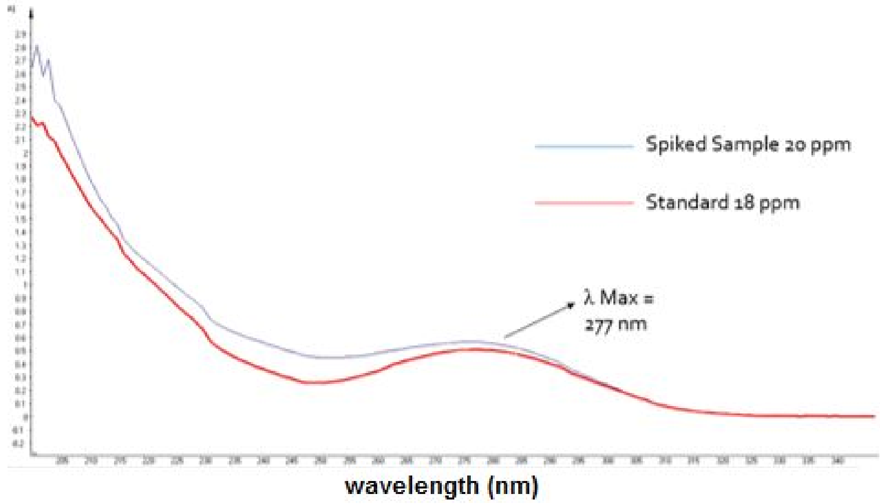

Fig 2. Result of Overlay Spiked Sample with Standard of Diclofenac Sodium

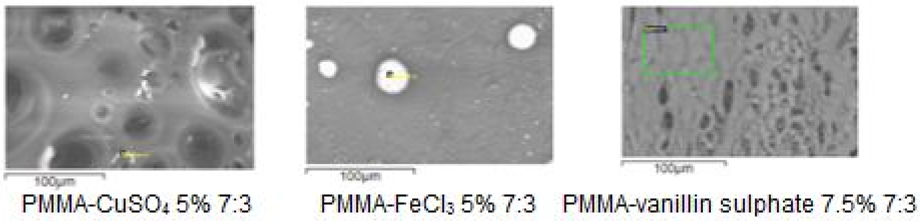

Fig 3. Surface Topography and Microstructure of Indicator Strips

diclofenac sodium is affected by the pores that formed in the strip. The *homogenous* microstructure can lead to reproducibility and fasten reaction. From SEM result, we can found out that all strips had a good homogeneity.

CONCLUSION

Indicator strip based on polymethylmethacrylate-specific reagent can be used to detect diclofenac sodium in herbal medicine with a good performance and accuracy.

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