



## SEQUENTIAL INJECTION ANALYSIS - LAB AT VALVE (SIA-LAV) FOR CHROMIUM SPECIATION BY COLORIMETRIC METHOD USING H<sub>2</sub>O<sub>2</sub> OXIDIZING AGENT AND 1, 5-DIPHENYLCARBAZIDE COMPLEXING AGENT

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### ABSTRACT

Chromium commonly presence in two different oxidation states, the Cr(III) which is an essential micro-element in living body, and also Cr(VI) which is toxic though in a trace level. Because of its different character for each species, determination of total chromium in water did not provide precise information for its toxicity. Meanwhile, many speciation methods had been developed. In this study, speciation chromium had been done by flow reaction system Sequential Injection Analysis Lab-At-Valve (SIA-LAV) modified by presence of Lab-At-Valve for maximized reaction process. Its determination based on complexed-reaction between 1,5-diphenylcarbazide (DiPC) complexing agent with Cr(VI) that formed Cr(VI)-DiPC complex which can be determined by colorimetric method in 548 nm. Total Chromium level could be achieved by oxidized Cr(III) by H<sub>2</sub>O<sub>2</sub> in alkaline solution, so that Cr(VI) produced by an oxidation process along with Cr(VI) that naturally presence in the artificial samples reacted with DiPC, afterward, the formed Cr(VI)-DiPC determined as total chromium by the same colorimetric method. The optimization of this method was conducted by testing several parameters. Based on the optimization result, the optimum condition obtained: H<sub>2</sub>O<sub>2</sub> concentration 10<sup>-5</sup> M and volume 20 μL, DiPC concentration 2x10<sup>-5</sup> M and volume 50 μL, the sample volume 60 μL and the delay time 10 seconds, flow rate to detector 25 μL s<sup>-1</sup>. The detection limit of Cr(VI) and Cr total were 0.0089 mg/L and 0.0103 mg/L, respectively, and Cr(III) was 0.0043 mg/L. The Chromium speciation by SIA-LAV method outlined above allows the simple, sensitive, rapid and cost-effective analysis for chromium speciation, the Cr(III) and Cr(VI). This result suggests that this method is prospective method to be used for monitoring of chromium species in fresh water.

**Keywords:** SIA-LAV, Chromium speciation.

### INTRODUCTION

Water is the most important component for living. All of the living creatures required water on their entire lives. It is the ultimate necessity for the living processes on earth. However, the rapid industrial growth has been increased the chemical discharge to the environment, which lead to the environmental contamination. Recently, the metal contamination on the environment has become the major issues. Moreover, metals can be accumulated in to food web, and some metals biomagnified along the tropic level. Further, it can be accumulated to the human body through dietary.

Heavy metal pollution arising as industrial development and mining activity continues as well as the anthropogenic activity. Heavy metals contamination has been reported from several points of riverine and coastal area throughout Indonesia, such as the contamination of Hg(II), Cu(II), Pb (II), Cr(VI) and Cr(III), which is come from gold-mining processes, pesticide production and usage, amalgamation, and anthropogenic discharge (Takarina *et al.* 2004); (Edinger *et al.* 2008); (Saputro *et al.* 2014).

Chromium commonly present in two different oxidation state, Cr(III) as cation and Cr(VI) as Oxo-anion. Human body shows different responses for these different species, the Cr(III) is the essential trace metal for metabolism and the Cr(VI) has high toxicity even in trace levels. Thus, the determination of total Cr in environment is unable to provide precise information of Cr toxicity on the samples (Saputro *et al.* 2014).

Chromium can be detected by titration or gravimetric, however, the concentration of the sample possibly lower than 1 ppm or 1 mg/L, as the permitted level of chromium in Indonesia is 0.05 mg/L. Therefore, the determination of chromium using these conventional methods is likely impossible. Moreover, the two different oxidation states of chromium have distinct chemical properties, thus the measurement of total chromium is insufficient to provide information regarding the chromium toxicity level on the water samples. In order to examine the toxicity level of chromium on the water samples, only the level of Cr(VI) should be determined, instead of the total chromium. Therefore, recent studies proposed new methods for each species determination, such as speciation method (Sumida *et al.* 2005) and the



dual auto-membrane extraction for different chromium species determination (Safari *et al.* 2013), chromium speciation using temperature-controlled micro-extraction based on ionic liquid as extraction solvent (Sadeghi and Zeraatkar Moghaddam, 2012) and co-precipitation method (Kongsricharoern and Polprasert 1996). Those methods mostly coupled by different instrumental analysis, such as, Flame Atomic Absorption Spectrometry (FAAS) as reported by (Cespón-Romero *et al.* 1996), X-ray fluorescence spectroscopy (Malherbe and Claverie 2013), high performance liquid chromatography (Safari *et al.* 2013) and Inductively Coupled plasma Mass Spectrometry/ Atomic Emission Spectrometry (ICP-MS/AES) for its determination (Sumida *et al.* 2005); (Mulaudzi *et al.* 2002).

On the other hand, one of the simple methods to determined chromium species is colorimetric method. This method can be carried using simple spectrophotometer. It's provides simple determination technique and easy to be developed on the laboratory. In addition, this method required low-cost equipment that is suitable for routine analysis.

The development of analytical methods for chromium speciation is still ongoing. The simplicity of the sample preparation and measurement, the convenience due to automated sample manipulation, the low-cost equipment with high accuracy are the points which is determining the ultimate applicability of the techniques. These points lead to a technique that has the potential for on-line measurement in routine laboratories, such as Sequential Injection analysis (SIA). This technique is known as second generation of flow injection analysis. The great advantage of this technique is the reduction of sample and reagent volumes for the analysis processes, as the sample and reagent can be manipulated automatically. The SIA operates through the sequential aspiration of a sample and reagent into holding coil, the well-define zone in holding coil attained when the flow is reversed. The forward mode propels the zones through a reaction coil into the detector.

Recently, the SIA has been developed in a new concept, called "Lab-at-Valve" LAV (Burakham R, 2005); Decuir *et al.*, 2007). This technique became prominent because of its economics as an alternative of cost-effective system for online analysis. Various advantages of this SIA modified by LAV system, such as small amount of reagent consumption, automation and integrated instrument. The present of Lab-at-Valve allows the sample and reagent to react completely, thus, rapidly as well as accuracy in analysis are gained in the same time. As reported by (Burakham R 2005), the SIA-LAV has been successfully developed for micro-extraction and determination of anionic surfactant.

Based on the background information above, the speciation of Chromium by on-line system coupled by various determination instruments has been reported. However, it is necessary to developed on-line system

which would provide rapidity, simplicity, accuracy as well as the cost-effective analysis. Thus, in the present work, our group developing a robust online system for determining Cr(III) and Cr(VI) using SIA modified by LAV (SIA-LAV). In order to get the optimum condition of the analysis, we tested several parameters, such as the concentration and volume of the reagent, H<sub>2</sub>O<sub>2</sub> as oxidation agent, DiPC as complexing agent, the delay time on the valve and the flow rate of the analytes to the detector. This proposed method expected to provide fast, accurate and less expensive of chromium speciation with environmental friendly reagent for routine analysis in the laboratory.

## EXPERIMENTAL

### Reagent and solution

The solution was prepared from pro-analysis chemicals. The synthetic samples that contain known concentration of Cr(III) and Cr(VI) were prepared by dissolving Cr(NO<sub>3</sub>)<sub>3</sub>.9H<sub>2</sub>O (Merck, Germany), K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (Merck, Germany) into DI water, the oxidizing agent was prepared by diluting H<sub>2</sub>O<sub>2</sub> 30% (Merck, Germany) into 0.01 %, 1,5-difenilbarbazide (Merck, Germany), and HNO<sub>3</sub> 65% (Merck, Germany), acetone (Merck, Germany), NaOH (SAP Chemica).

### Instrumentation and operating system

The sequential injection apparatus used on this study was assembled from the following component, include *syringe pump* (SP; Hamilton, Reno, Nevada, USA, volume 2.5 mL), eight selection valve (SL; Hamilton, Reno, Nevada, USA), flow cell coupled with RGB detector for the absorbance measurement. Data acquisition and device control were achieved through specific software, the SIA-MPV software interface to control SIA system and FIA-Display software interface for data acquisition and displaying the absorbance peak. The holding coil (HC) and manifold were constructed by Teflon tubing. All the data acquired are the averages of 3 peaks of the samples absorbance.

### The parameter optimization

The parameter optimization included chemical parameters, such as the optimum concentration of DiPC and H<sub>2</sub>O<sub>2</sub>. The optimization of H<sub>2</sub>O<sub>2</sub> concentration was conducted to ensure that all the Cr(III) on the sample was oxidized completely, which was done by varying the

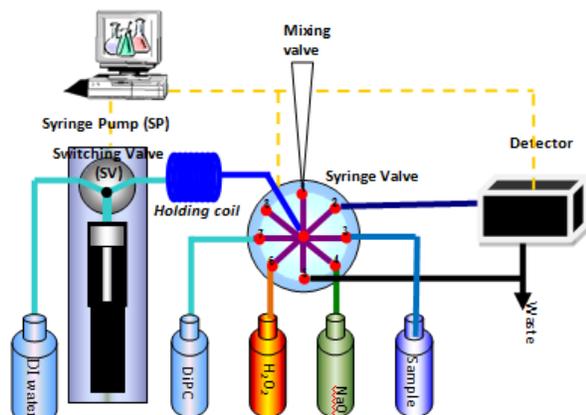


Figure-1. The SIA-LAV instrument scheme.

$\text{H}_2\text{O}_2$   $0.1 \times 10^{-5} - 2 \times 10^{-5}$  M. The concentration of DiPC as the complexing-agent was optimized to ensure the complete formation of Cr(VI)-DiPC complex on the sample, this experiment was conducted by varying the DiPC concentration  $0.5 \times 10^{-5} - 6 \times 10^{-5}$  M. Beside chemical optimization. The physical parameters optimization such as the optimum volume of  $\text{H}_2\text{O}_2$ , DiPC and sample, the delay time on the valve and the flow rate of analyte to the detector was also conducted. The optimum volume of those parameters should be obtained to ensure all the analytes reach to the detector without further dilution by the carrier solution. The range of volume optimization for of  $\text{H}_2\text{O}_2$ , DiPC and sample, respectively, 10 - 80  $\mu\text{L}$ , 20 - 80  $\mu\text{L}$ , and 20 - 100  $\mu\text{L}$ . The delay time optimized in order to get effective time, in a short time yet the complete reaction accomplished, which is addressed for the oxidation of Cr(III) to Cr(VI) by  $\text{H}_2\text{O}_2$  in alkaline solution. The range of the delay time tested 10 - 50 s with interval range 10s. The optimization of flow rate is related to the measurement time or the detection of the formed-complex, the the tested range is 15-35  $\mu\text{L}/\text{s}$ .

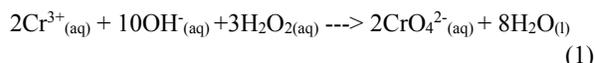
All the quantification was conducted online, directs controlling SIA-LAV system by computer interface and sequentially determined. The scheme of the system depicted in Figure-1.

## RESULTS AND DISCUSSIONS

The methods principle for chromium determination is based on the direct determination of complex compound Cr-diphenylcarbazide (Cr-DiPC) that can be detected colorimetrically at 548nm (Andersen 1998). The proposed system uses a RGB detector that was directly plugged through one of the valve, after being mixed on the Lab-at-Valve, the analytes would be able to undergo direct determination by transporting the product to the flow cell of the RGB detector. This system operates by first measuring the Cr(VI) species followed by the oxidation of Cr(III) to Cr(IV) by  $\text{H}_2\text{O}_2$  and then measured as total chromium. The colorimetric approach that was

chosen based on the specific reaction of Cr(VI) with diphenylcarbazide (DiPC). The reaction occurs reliably in alkaline medium to give an intense violet complex that can be detected colorimetrically at 548 nm. The ligand reacts slightly with other transition metals, thus minimizes the risk of interferences.

The  $\text{H}_2\text{O}_2$  was chosen for the oxidizing agent since it does not absorb at 548nm, thus it would not be the source of the interference. The oxidation of the Cr(III) species in basic solution may be represented by the formulation below:



### The chemical parameters optimization

Although the  $\text{H}_2\text{O}_2$  as the oxidizing agent is free from the interference, it can be slowly decomposed even when it is preserved in basic solution. The decomposition of  $\text{H}_2\text{O}_2$  will be forming bubble of oxygen based on below reaction:



When the oxygen bubble level on the tube is too large, it will result in the higher background level and eventually disturbing the measurement due to unstable or high background level. Therefore, the first chemical parameter optimization is establishing the right amount of the  $\text{H}_2\text{O}_2$  uses for the reactions, afterward determining the effective amount of the complexing agent, DiPC.

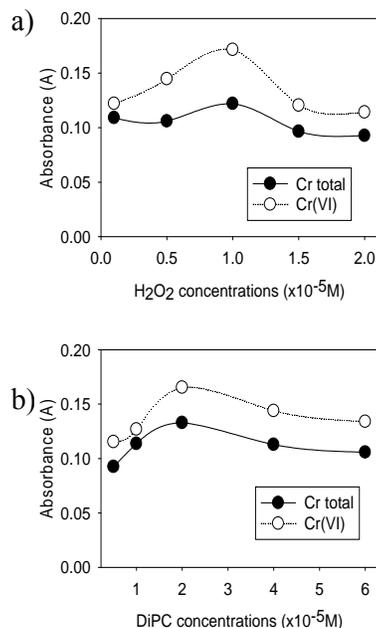
The chemical factors that optimized, the  $\text{H}_2\text{O}_2$  concentration and DiPC concentrations, are plotted against its absorbance, which is consecutively depicted in Figures 2(a) and (b). The absorbance is given by mixture solution of Cr(III) and Cr(VI) as the artificial samples in 0.05 mg/L for each species. The alkaline condition achieved by NaOH solution  $10^{-3}$  M and the complexing agent concentration  $10^{-5}$  M. The volume of  $\text{H}_2\text{O}_2$  and NaOH is 20  $\mu\text{L}$ , and the volume DiPC 40  $\mu\text{L}$ . The delay time on the mixing valve for the oxidation process and the complexing process is 10 seconds, the flow rates of the mixing solution to the detector is 30  $\mu\text{L}/\text{s}$ . These measurement conditions were chosen based on the previous research conducted by (Andersen 1998). However, since the instruments and the parameters included are differ, those parameters then optimized one by one in this research.

Figure-2(a) shows the correlation of  $\text{H}_2\text{O}_2$  concentrations and its absorbance. The Figure-2(a) shows that the maximum absorbance given when the  $\text{H}_2\text{O}_2$  concentration is  $10^{-5}$  M, which is means the highest sensitivity is achieve on its concentration range. The absorbance difference between Cr total and Cr(VI) absorbance was also the highest in this concentration range. When the  $\text{H}_2\text{O}_2$  concentration less than  $10^{-5}$  M, the oxidation process is occurred, however, it cannot be



completed, which means not all the Cr(III) that present on the solution is completely oxidized, thus the amount of Cr(VI) on the sample is not significantly increase. This eventually resulting on the absorbance of Cr total is slightly higher than Cr(VI). When the  $H_2O_2$  concentration higher than  $10^{-5}$  M, the absorbance of Cr total is not significantly higher compare to Cr (VI), this occurrence may cause by the imperfect complexation process, which is not all the Cr(VI) that present on the solution is complexed to the Cr-DiPC complex. When the  $H_2O_2$  concentration about  $0.5 \times 10^{-5}$  M or higher, the oxidation process may occur completely, however, higher  $H_2O_2$  concentration may distract the stability of Cr-DiPC complex compound and the DiPC itself might damage by the acidic condition given by the higher  $H_2O_2$  concentration, as DiPC is susceptible with strong oxidation compound. This occurrence has been reported by (Sumida *et al.* 2005).

Additionally, Figure-2(b) shows the relation of DiPC concentration and its Cr-DiPC complexes absorbance. Based on the data on Figure-2(b), it can be seen that the highest sensitivity is obtained when the DiPC concentration  $2 \times 10^{-5}$  M. This concentration gives the highest absorbance difference between Cr(VI) absorbance and Cr total. This occurrence means that the amount of Cr(III) that oxidized into Cr(VI) is higher than others condition. By the presence of suitable amount of DiPC, the oxidized-Cr(III) along with the Cr(VI) on the artificial samples is complexed completely, on result, the optimum sensitivity and thus the highest absorbance are obtained. The higher concentration of DiPC than  $2 \times 10^{-5}$  M resulting in slightly lower sensitivity, which can be consider into steady states condition, since this is working on trace levels. Its means that there is no need additional DiPC concentration because the formed complex is already reach the optimum state. Thus,  $2 \times 10^{-5}$  M DiPC concentration is chosen as the optimum DiPC concentration. The (Andersen, 1998) was also reported the similar process, which is applied on Flow-Injection Analysis (FIA) system. The FIA system is the first generation of SIA system. Based on the data given in Figure-2, the  $H_2O_2$   $10^{-5}$  M is chosen as the optimum  $H_2O_2$  concentration in this SIA-LAV system.



**Figure-1.** The summary of chemical optimization, included  $H_2O_2$  concentrations (a) and DiPC concentrations (b).

### The physical parameters optimization

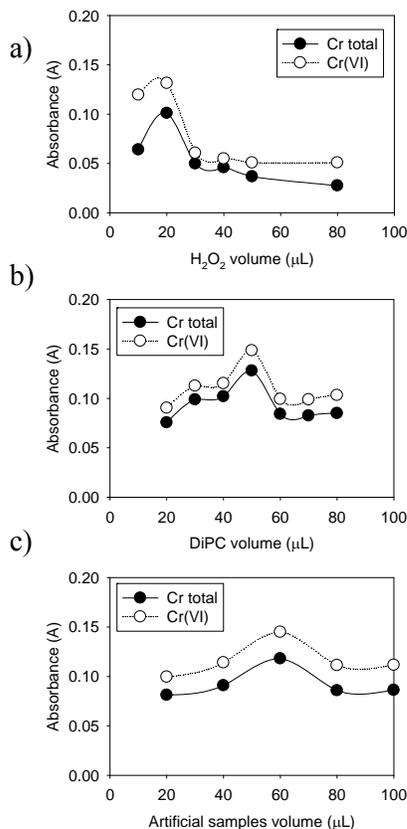
The optimum absorbance value can be obtained by measuring the analyte on its optimum volume, which include the volume of reagents and sample. Therefore, it's also important to determine the optimum volume of  $H_2O_2$ , DiPC and the artificial samples. Figure-3 shows the relation between the absorbance with the reagents and sample volume, which was measured with Cr(III) and Cr(VI) 0.05 ppm and identical condition from the optimum parameter that obtained before, DiPC  $2 \times 10^{-5}$  M and  $H_2O_2$   $10^{-5}$  M.

The greater volume of the oxidizing agent, the higher Cr(III) that oxidized into Cr(VI), which resulting the higher formation of chromium complex and eventually giving the higher sensitivity which giving higher absorbance. However, when the  $H_2O_2$  exceed, there will be dilution effect that affecting the absorbance value, most probably the absorbance value will be decreased. Likely, this dilution effect also occur on the optimization of DiPC and artificial samples on certain volume levels

Similarly, in order to obtain the optimum sensitivity and the highest absorbance value, the DiPC volume should be equal with the amount of chromium on the sample. When the volume of DiPC is lacking, the lower absorbance would be obtained, which is means the incomplete complexation process may occur. However, the exceed DiPC volume would give the dilution effect, and eventually affecting the amount of the analyth on the valve, and thus the absorbance value is decreasing.



The sample volume also needs to be optimized. The sample solution is containing certain amount of Cr(III) and Cr(VI), which is provide the center atom for the complex compound. When the volume is increasing while maintained on its constant concentration, the center atom for the complex compound is also increasing, thus, theoretically the absorbance should be higher. However, the graph on the Figure-3(c) shows that after certain condition the absorbance is getting decrease. This occurrence shows that the reagent, in particular the DiPC, is insufficient to complex all the chromium on the sample, thus the formation of the complex compound is remind constant. Moreover, when dilution effects also take a part on decrement of the complex compound concentration, it will absolutely make the absorbance decreased.



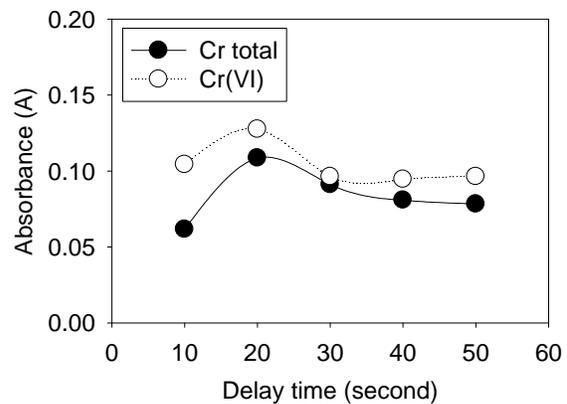
**Figure-2.** The graphs that show the summary of physical parameter optimization, includes the H<sub>2</sub>O<sub>2</sub> volume (a), the DiPC volume (b) and the artificial samples volume (c).

Based on the data above, the optimum volume for H<sub>2</sub>O<sub>2</sub> and DiPC, sequentially, 20 μL 50 μL, and the optimum sample volume is 60 μL. This optimized parameters then use for further optimization steps.

### The determination of the delay time on the valve for the oxidation process

The observation regarding the delay time for the oxidation process had been conducted by the same procedure and condition, in addition the optimized parameters that has been tested above, such as the optimum concentration and volume of the sample and reagents, are used. The relation between the absorbance and the delay time on the valve for the oxidation process is shown in Figure-4. Based in the graph, it can be seen that the absorbance is increasing when the delay time arising from 10 to 20 seconds, and then the decrement of the absorbance is slowly occurred by the increment of the delay time. The decrement of the absorbance is most probably caused by the decomposition of the complex compound. These decompositions might happen when the formed complex compound is loosely bound to each other, the detail interaction of chromium and DiPC had reported by (Willems *et al.* 1977).

Based on this delay time test, the 10 seconds delay time is chosen as the optimum delay time for the oxidation process. In relatively short time, the obtaining absorbance is relatively high. Additionally, inconsideration of shorten the analysis time; the 10 second delay time is the ideal delay time in this SIA-LAV system.



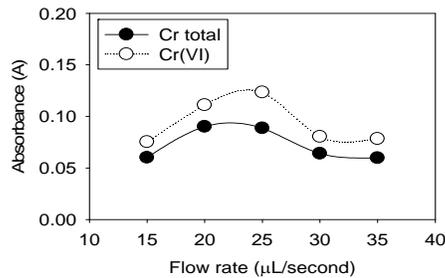
**Figure-3.** The graph shows the absorbance versus the delay time.

### The determination of optimum flow rate

Flow rate affects the reaction time. In case of the flow rate to the detectors, it is affecting the detection process. Based on the Figure-5, the absorbance of the Cr-DiPC complex was increased with the increasing flow rate up to 25 μL/s, and then it was decreased when the flow rate is faster. When the solution flows on the slower rate, the dispersion of the Cr-DiPC complex on the carrier solution may occur that resulting the lower Cr-DiPC complex detected by the detector, and lowering the detected absorbance. While on the faster flow rate, the detector was



not able to detect the amount of the Cr-DiPC complex precisely. The optimum flow rate was chosen at 25  $\mu\text{L/s}$ , as it is giving the highest sensitivity showed by highest absorbance.



**Figure-4.** The tested flow rates versus absorbance.

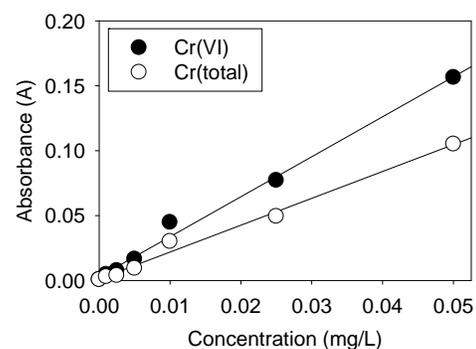
### The optimization summary and linearity measurement

Based on the determination of the optimum parameters that had been conducted, the optimum condition of the tested parameters are obtained and summarized on the Table-1 below:

**Table-1.** The tested parameter for optimization.

Parameters	The tested range	Optimum parameter
H <sub>2</sub> O <sub>2</sub> Conc.	0.1x10 <sup>-5</sup> –2x10 <sup>-5</sup> M (interval 0.5x10 <sup>-5</sup> )	10 <sup>-5</sup> M
DiPC Conc.	0.5x10 <sup>-5</sup> – 6x10 <sup>-5</sup> M (interval 2x10 <sup>-5</sup> )	2x10 <sup>-5</sup> M
Volume H <sub>2</sub> O <sub>2</sub>	10 - 80 $\mu\text{L}$	20 $\mu\text{L}$
Volume DiPC	20 – 80 $\mu\text{L}$	50 $\mu\text{L}$
Volume Sample	20 – 100 $\mu\text{L}$	60 $\mu\text{L}$
Delay time for oxidation	10 – 50 s	10 s
Flow rate	15 – 35 $\mu\text{L/s}$	25 $\mu\text{L}$
Analysis time	$\pm 300$ s (5 mins)	
Analysis rate	12 samples/hr	

Under the optimum condition that summarized in Table-2 the method was found to be linear as seen in Figure-6. The linear equation obtained for Cr(VI)  $Y=2.0711X+0.0012$  with the  $R^2=0.9895$ , and for the Cr(total)  $Y=3.0646X+0.0035$  with the  $R^2=0.9916$ . The Limit Of detection (LOD) measured the minimum analyte that can be detected by the SIA-LAV system. This study determined the LOD by measuring the signal given by the blank up to 10 times ( $S_{LOD}$ ), The detection limit of Cr(VI) and Cr total were 0.0089 mg/L and 0.0103 mg/L, respectively, and Cr(III) was 0,0043 mg/L. The low LOD value obtained from this system shows that the proposed method produces the high sensitivity for chromium speciation, since it's able to detect the low concentration of analyte up to milligram per liter level.



**Figure-5.** The linearity measurement of the synthetic samples on optimum condition.



## CONCLUSIONS

Based on the data above, the optimum condition obtained: H<sub>2</sub>O<sub>2</sub> concentration 10<sup>-5</sup> M and volume 20 µL, DiPC concentration 2x10<sup>-5</sup> M and volume 50 µL, the sample volume 60 µL and the delay time 10 seconds, flow rate to detector 25 µL s<sup>-1</sup>. The Chromium speciation by SIA-LAV method outlined above allows the simple, sensitive and fast analyzing time as well as cost-effective for the determination of each species of chromium, the Cr(III) and Cr(VI). This method is working well on the water sample that contained the chromium species that lower than 0.05 mg/L, as it shows linear range up to this point. This result suggests that this method is prospective method to be used for monitoring of chromium species in environment, such as river. This method is also suitable for determining the toxicity level of chromium, especially in freshwater in Indonesia.

## ACKNOWLEDGEMENTS

The authors are grateful to the University of Brawijaya for the Research facility. The author also acknowledged to Institut Teknologi Adhi Tama Surabaya (ITATS).

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