

JIMMA UNIVERSITY
SCHOOL OF POST GRADUATE STUDIES
COLLEGE OF NATURAL SCIENCE
DEPARTMENT OF CHEMISTRY



MSc THESIS ON
SALTING-OUT ASSISTED LIQUID-LIQUID EXTRACTION OF
Cr (VI) IN WATER PRIOR TO ITS DETERMINATION BY UV-
VIS SPECTROPHOTOMETRY

JOTE DAME

FEBRUARY, 2021
JIMMA, ETHIOPIA

**SALTING-OUT ASSISTED LIQUID-LIQUID EXTRACTION OF
Cr (VI) IN WATER PRIOR TO ITS DETERMINATION BY UV-
VIS SPECTROPHOTOMETRY**

JOTE DAME

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MSc THESIS APPROVAL SHEET

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ABBREVIATIONS

WHO	World health organization
USEPA	United states Environmental Protection Agency
SALLE	Salting out assisted liquid-liquid extraction
SPE	Solid phase extraction
LLE	Liquid-liquid extraction
DLLME	Dispersive liquid-liquid microextraction
LDR	Linear dynamic range
EPA	Environmental protection agency
LOQ	Limit of quantification
LOD	Limit of detection
DPC	Diphenyl carbazide
RSD	Relative standard deviation
RR	Relative recovery
FAAS	Flame atomic absorption spectrophotometry
GFAAS	Graphite furnace atomic absorption spectrophotometry
IL	Ionic liquid

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ABSTRACT

In this study salting out-induced liquid-liquid extraction was developed for UV-Vis spectrophotometric determination of Cr (VI) in water samples. Prior to salting out-induced liquid-liquid extraction, Cr (VI) has been reacted with a chelating agent, 1, 5-diphenyl carbazide (DPC), in an acidic medium. Then, the Cr (VI)-DPC complex formed has been extracted by the proposed method and analyzed at 540 nm monitoring wavelength. Various parameters influencing the complexation and extraction efficiency of the method were evaluated and the optimal conditions were established. Under optimum conditions, the calibration curve constructed at seven concentration points exhibited satisfactory linearity range from 0.25–2 mg/L, with coefficient of determination (R^2) of 0.9973. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.004 mg/L and 0.015 mg/L, respectively. The LOQ of the method was below the maximum tolerable limit (MTL) of Cr (VI) in drinking water, which is 0.05 mg/L. The method also demonstrated satisfactory enrichment factor (EF), which was 5.8. The intra-day and inter-day precision studies at concentration levels (Level 1; 0.75 mg/L and level 2: 1.25 mg/L) showed that proposed method has satisfactory precision (i.e., $RSD < 9.0$). The recovery studies, which were performed by spiking tap, well and river water samples with the above two concentration level, showed recovery values $88.0 \pm 2.5\%$ - $97.3 \pm 0.1\%$, which are in acceptable range. The interference studies also demonstrated Fe, Cu, V, and Cd the proposed method have negligible interferences ($< 5\%$) up to 500 mg/L. Generally, the proposed method is simple and selective, and to be used as alternative method for analysis of Cr (VI) from water and other related matrices.

Keywords: *Salting-out assisted liquid-liquid extraction; Chromium (VI); 1, 5-Diphenyl carbazide UV-Vis spectrophotometry; Water samples.*

1. INTRODUCTION

1.1 Background of the study

Starting from the industrial revolution to today, environmental pollutions due to heavy metals and toxic organic compounds are causing enormous problems worldwide [1]. The pollution of rivers and streams with chemical contaminants has become one of the most critical environmental problems every nation. Wastewaters which are released from industries contain various heavy metals, which can persistently exist in the environment [2].

Chromium is one of the metals which can be released to the environment from various industries. Chromium usually exists in two oxidation states as Cr (III) and Cr (VI). Cr (VI) is more toxic than Cr (III). It is soluble in almost the whole pH range and has greater mobility than Cr (III) [3]. Due its toxicity, United State Environmental Protection Agency (USEPA) set the maximum tolerable level of Cr (VI) in surface water to be below 0.05 mg/L and for total Cr: which include Cr(VI), Cr(III) and other forms) 2.0 mg/L [4]. Similarly, World Health Organization (WHO) set the maximum tolerable limit of total chromium 0.05 mg/L [4].

Due to its toxicity, the maximum tolerable level of Cr (VI) in water sample is very small. On the other hand, in the real samples, the presence of interfering substances could also complicate its analysis. Thus, the desired analyte, in this case Cr (VI), should be selectively extracted and/or pre-concentrated prior to its determination by various analytical techniques. A range of extraction and/or preconcentration procedures have been used for analysis of Cr (VI) from water and other samples. These methods include liquid-liquid-extraction (LLE), solid phase extraction, dispersive liquid-liquid microextraction (DLLME), and so on have been reported for extraction of Cr (VI) from various sample matrices [5- 8]. Although, these methods have several advantages, they use toxic organic solvents. Thus, replacing another attractive and alternative sample preparation procedure that uses less toxic and easily accessible solvent is crucial. In this regard, optimizing a salt-out induced LLE, a method which has recently taken great attention as a simple and rapid environmentally benign method is important to use alternative sample preparation procedure for monitoring Cr (VI) in water samples. To the best of the researcher knowledge, there is no report on salt-out assisted liquid-liquid extraction (SALLE) for

determination of Cr (VI) from any sample prior to its UV-Vis determination. Therefore, in this study, SALLE has been proposed for first time for extraction of Cr (VI) after chelating with DPC for its quantitative determination by UV-Vis spectrophotometer.

1.2. Statement of the problem

Because of its toxicity, monitoring of the level of Cr (VI) in environmental water or industrial effluent is so crucial [3, 4]. Thus, numerous sample preparation methods such as LLE, SPE, liquid phase microextraction and so on have been employed to extract Cr (VI) from water prior to instrumental analysis. These methods usually use toxic organic solvents during extraction [5 - 8]. Hence looking for a simple, fast, cheap and environmentally friendly sample preparation method for analysis of Cr (VI) is crucial. Recently, SALLE has gained particular recognition for extraction and or pre-concentration of different organic and inorganic compounds. SALLE uses water-miscible organic solvents such as acetone, acetonitrile, ethyl acetate and isopropanol as extraction solvent [9-11]. Addition of appropriate amount of salts such as magnesium sulfate (MgSO_4), ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), sodium chloride (NaCl), calcium chloride (CaCl_2), potassium carbonate (K_2CO_3) and calcium sulfate (CaSO_4) reduces the mutual miscibility of the aqueous sample and water-miscible organic solvent, causing formation of a two-phase system with the simultaneous extraction of the target analytes into the organic phase [11]. Therefore, developing SALLE for extraction and/or preconcentration of Cr (VI) prior to its determination by UV-Vis spectrophotometry is important, since the method is simple, fast and relatively environmentally safe than the several existing sample preparation methods.

1.3. Objectives of the study

1.3.1. General objective

- To develop salting-out assisted liquid-liquid extraction technique that enable selective extraction of Cr(VI) form water sample prior to its determination by UV-Vis spectrophotometry

1.3.2. Specific objectives

- To optimize parameters affecting the extraction efficiency of SALLE method such as extraction solvent type and its volume, volume of the chelating agent, type and amount of salt, sample pH, extraction time, interfering ions, and stability of the complex for the analysis of Cr (VI).
- To validate the proposed method in terms of the working linear range, limit of detection, limit of quantification, repeatability, intermediate precision, and recovery studies.
- To apply the method to real samples including tap water, well water and river water.

1.4. Significance of the study

The findings of the study could have the following significances:

- It can provide alternative method for determination of Cr (VI) from water samples.
- It can be used as baseline data on determination of Cr (VI) present in water samples.
- The results of the study could be used as a source of information for further researcher who wants to conduct a research on similar area.

2. REVIEW OF RELATED LITERATURE

2.1. Sources of chromium

Chromium is an element commonly found in rocks, animals, plants, soil, rivers and seawater, dusts and volcanic fumes, at a concentration of approximately 100 mg/kg [12]. Chromium in the environment can be of natural origins, such as rock and soil erosion, volcanic eruptions, or of anthropogenic origins, through the use of this chemical element in a range of industrial activities such as metallurgy (steel, aluminum and alloys), refractories (cement, glass and clay) and chemical industries (leather tanning, wood preservation and pigments) [13]. These industries contain Cr (III) and Cr (VI) at a concentration ranging from 10 to 100 mg/L [14-15]. Long-term release of such wastewater may result in the accumulation of heavy metals in soil or water.

2.2. Health Effects of Chromium

Trivalent chromium is the only form of chromium known to play a beneficial biological role for human and animal at levels of 50 to 200 µg/day. This trivalent chromium is supplied as stable complex of insoluble macro molecule at normal blood pH. The major advantage of trivalent chromium is the maintenance of normal glucose tolerance. Cr (III) may also stabilize biological proteins in their proper configurations. Although Cr (III) is an essential nutrient, exposure to high levels via inhalation, ingestion or dermal contact may cause some health effects. Chromium in general is listed by EPA as a class “A” human carcinogen [1, 4].

Cr (VI) is easily penetrates biological membranes because it has high oxidizing potential; and therefore it is more toxic than the trivalent. It is easily sorbed by gut or body walls because of its higher solubility. Cr (VI) can cause mutagenic effect by penetrating into the cell walls in the living beings on nucleic acid, and therefore they cause genetic corruption. Hexavalent chromium is also corrosive and causes chronic ulceration of skin surface [16]. Besides, over the long term, they cause lung cancer, nephritis in kidneys and respiratory tract mucous.

In addition, when such wastewaters, discharged to watercourses, cause the living population in water to be poisoned, and the pollution they create destroys the balance in water environment. Chromium compounds are among dangerous wastes, and they have several harmful impacts on

soil, water and living beings due to their toxic effects. For example, Cr (III) content between 10-100 mg/L in soil affects the micro-organism population and reduces CO₂ formation; therefore it affects the biological reactions in soil in the negative way. Wastewaters that include chromium reduce the efficiency of the soil when discharged to agricultural land, and prevent the growth of the plants in these lands [17].

2.3. Determination of Cr (VI)

Several methods have been studied and used for chromium determination in water samples such as liquid-liquid extraction, solid phase extraction, dispersive liquid-liquid microextraction, cloud point extraction, etc prior to its determination by different spectroscopic [3, 6, 8, 14, 15], chromatographic[18] and electrochemical techniques [19-20]

2.3.1. Liquid-liquid extraction

Liquid-liquid extraction is one of the most extensively studied and widely applied methods in pre-concentration and separation procedures for the determination of trace elements due to its simplicity, convenience, wide scope, etc [2]. This procedure based on the distribution of analyte between two essentially immiscible solvents. The formation of an uncharged chemical species in aqueous phase by either chelation or ion-association is essential for extraction into suitable organic solvent. The distribution ratio of a particular analyte is usually defined as the ratio of its total concentration in organic phase to that in aqueous phase at equilibrium. The proper choice of extractant, pH or acidity of the aqueous phase, immiscible solvent, masking agents, salting out agents, and modifiers are important [9-11].

Separation and pre-concentration procedures using solvent extraction generally result in a high enrichment factor due to the difference between the volumes of aqueous and organic phases. Analytical procedures for extraction and pre-concentration Cr (VI) by LLE and determination by atomic spectrometric techniques have been widely applied [14, 15]. Nevertheless, this technique exhibits several shortcomings, such as the emulsion formation, the use of large sample volumes and toxic organic solvents and hence, the generation of large amounts of pollutants makes liquid-liquid extraction expensive, time consuming and environmentally unfriendly. Therefore, miniaturization of conventional liquid-liquid extraction is needed.

2.3.2. Solid phase extraction

Solid phase extraction (SPE) is another widely utilized technique for the heavy metal extraction and pre-concentrations. The basic principle of SPE is the transfer of analytes from aqueous phase to the active surface of solid phase [21-22]. The sorbents play an important role in SPE process hence the current researches in SPE are mainly focused on the development of new sorbents.

Up to now, a variety of sorbents have been reported as materials for the adsorption of chromium such as activated carbon [23], inorganic materials [24], nanomaterial's [25], chelating resins and other organic sorbents [26].

2.3.3. Dispersive liquid-liquid microextraction

Recently, a new procedure termed as dispersive liquid– liquid microextraction (DLLME) has received much attention for sample pretreatment. Simplicity of the operation, rapidity, low sample volume, low cost, and high enrichment factor are the main advantages of this method. This technique based on the use of an appropriate extractant, i.e., a few microliters of an organic solvent with high density such as tetrachlorometane, chloroform, carbon disulphide, nitrobenzene, bromobenzene, chlorobenzene or 1,2-dichlorobenzene, and a disperser solvent with high miscibility in both extractant and aqueous phase such as methanol, ethanol, acetonitrile or acetone. Liquid–liquid dispersions play an essential role in separation processes and reaction systems. This is because the large interfacial area due to dispersion facilitates mass transfer and reaction rate [3]. Dispersive liquid–liquid microextraction has been successfully applied to the pre-concentration of chromium from various samples [3, 14, 7 and 8].

2.3.5. Cloud point extraction

Separation and pre-concentration based on cloud point extraction (CPE) are becoming an important and practical application of surfactants in analytical chemistry [27-28]. The technique is based on the property of most non-ionic surfactants in aqueous solutions to form micelles and become turbid when heated to a temperature known as cloud point temperature. Above cloud point temperature, the micellar solution separates into a surfactant-rich phase of a small volume and a diluted aqueous phase, in which the surfactant concentration is close to the critical micellar

concentration (CMC). Any analyte solubilized in the hydrophobic core of the micelles will separate and become concentrated in the small volume of the surfactant-rich phase.

The small volume of the surfactant-rich phase obtained with this methodology permits the design of extraction schemes that are simple, cheap, highly efficient, speedy and lower toxicity to the environment than those extractions that use organic solvents. CPE also provides results comparable to those obtained with other separation techniques. Accordingly, any species that interacts with the micellar system, either directly (generally hydrophobic organic compounds) or after a prerequisite Derivatization reaction (e.g., metal ions after reaction with a suitable hydrophobic ligand) may be extracted from the initial solution and may also be pre-concentrated. CPE has been widely used to separate and pre-concentrate organic compounds and metal ions [29-33].

2.4. Salting out assisted liquid-liquid extraction

Aqueous solutions containing salts are of increasing importance and influence on separation processes in chemical engineering [34]. Aqueous liquid-liquid equilibrium is the results of intermolecular forces, mainly of the hydrogen-bonding type; addition of a salt to such systems introduces ionic strength (forces) that affect the thermodynamic equilibrium [34]. When the mutual solubility decreases due to the salt addition, the size of the two-phase region increases, and this effect is called “salting out”[34].

Salt has a great effect on LLE. The addition of non-volatile solute to a solvent mixture changes the interaction among the various solvent solutes molecules resulting in shifting their phase equilibrium even to the extent of eliminating the solutrope in LLE. The salt mainly affects the solubility of organic component in an aqueous-organic solvent mixture [34]. The distribution of solute between two liquid phases mainly depends upon the concentration of electrolyte. The electrolyte will remain in the phase in which it is most soluble and the other solute will be transferred to the phase that it is poor in electrolyte [34]. In general, this method can be influenced by different variables.

2.4.1. Selection of the type and volume of organic solvent

Selecting an appropriate extracting solvent is based on two important parameters, the solubility of the target compound and the penetrability into the matrix must be considered [35]. Due to miscibility of extracting solvent in water, SALLE was applied for extraction, pre-concentration and clean-up of polar compounds from water or liquid samples.

Therefore, it is important to choose an appropriate extraction solvent with suitable polarity for maximum analyte or analytes extraction during SALLE steps [35]. The volume of the extraction solvent also plays a great role on the extraction performance of the SALLE procedure. At low volumes, phase separation between organic solvent and aqueous phases may not be clear and thus, collections of the organic phase will difficult [36]. On the other hand, at higher volumes, the volume of organic phase obtained after phase separation is higher than its initial volume, indicating the existence of dissolved water in the organic phases [37].

It was observed by the investigation of SALLE for phenyl urea pesticides analysis in water under the following extraction condition; using 3 mL water sample containing 0.1 mol/L citrate buffer (pH 2) and 25% NaCl (w/v) and 2 mL of each organic solvent and with exception of methanol, in which the two phase system was not observed [38]. The result depicted acetonitrile is the best extraction solvent for the pesticides investigated.

2.4.2. Effect of salt type and amount

In SALLE, water molecules preferentially form hydration sphere around the salt ions leading to separation of organic phase rich in analytes [35]. Thus the nature of salt is responsible for strong hydration to enhance the extraction efficiency. Concentration of the salt is also important factor affecting the extraction efficiency of the method. The concentration of the salt must be large enough to induce the required phase separation. The quantity of salt added must be enough to clearly separate the two phases, but should not be added in excess beyond saturation to avoid adsorption of analytes on solid phase [39].

The effect of ionic strength on the extraction efficiency of SALLE was evaluated in the determination of methylene blue and sunset yellow in waste water and food samples using salting-out assisted liquid-liquid extraction. In order to obtain phase separation and the optimum

extraction efficiency, several salts, i.e. $(\text{NH}_4)_2\text{SO}_4$, NaCl, K_2HPO_4 , and Na_2CO_3 were tested. Their experimental results demonstrated that $(\text{NH}_4)_2\text{SO}_4$ provided higher extraction efficiency than other salts. This may be due to stronger ability of salting-out and more solubility of $(\text{NH}_4)_2\text{SO}_4$ in water [35].

2.4.3. Effect of extraction time

Extraction time is also another vital parameter in SALLE since mass transfer is time dependent. The extraction time was studied in Salting-out assisted liquid-liquid extraction coupled with hydrophilic interaction chromatography for the determination of biguanides in biological and environmental samples from 30-75 second. There was no significant increase in extraction recovery over the investigated period which was expected as the two phases were completely miscible before the addition of the salt. Therefore, 30 second was chosen for further studies [36].

2.4.4. Effect of pH

The environmental pH determines and influence the ionization status and transfer of analytes from aqueous phase to organic phase [38]. This parameter should consistent with the isoelectric point of the analyte. In the isoelectric point net charge of analyte is zero and thus mass transfer to organic phase increases. Low and high pHs may decreases extraction efficiency due to decrease in the stability of nonpolar or medium polar organic pollutant and increasing its surface charge [35].

2.4.5. Effect of centrifugation speed and time

Centrifugation is another important parameter in SALLE and it accelerates the phase separation [37]. Centrifugation of the sample solution influences kinetics of the extraction and enhances contact between the organic solvent and aqueous solution and thus facilitates formation of the two-phase system in the SALLE technique [39].

3. MATERIALS AND METHODS

3.1. Chemicals and reagents

Potassium dichromate ($K_2Cr_2O_7$) obtained from Sigma Aldrich, chelating agent 1, 5-diphenylcarbazide or DPC obtained from (Kiran light laboratories, Mumbai, India) , organic solvents acetonitrile (99.8%) and ethanol (99.8%) were obtained from Sigma Aldrich (Germany), methanol (96%) and acetone(99.9%) were purchased from Carlo Erba (Paris, France), sulphuric acid (H_2SO_4) 98%, Purchased from Merk, Darmstadt, Germany, phosphoric acid (H_3PO_4) from Riedel-de Hein, ACS, Germany) and nitric acid (HNO_3) 65%, Blulux laboratories, LTD, Potassium dihydrogen phosphate (KH_2PO_4) and di potassium hydrogen phosphate (K_2HPO_4) were purchased from the Beijing Chemical Reagents Company (Beijing, China). Salts that are used as salting out agents (NH_4) $_2$ SO_4 purchased from Fisher Scientific Company (Pittsburgh, USA), NaCl, $MgSO_4$ and Na_2CO_3 obtained from BDH Chemicals Ltd (Poole, England) were used in this study.

3.2 Instruments and apparatus

UV–Vis absorption spectrum of the complex was measured in 1 cm quartz cuvette using Specord 200 double beam UV-Vis spectrophotometer (analytic Jena, Germany), Centrifuge (1790_{xg}, 4000 r/min) obtained from Gemmy Industrial Corporation, Taipei, Taiwan, filter paper (Whatman® no. 542, 110 mm) obtained from Whatman International Ltd (England). Apparatus such as, falcon centrifuge tubes (15 mL), medical syringe with special long needle and common glass were used in this study.

3.3 Standard and working solution preparation

Stock solution of chromium (VI) with a concentration of 1000 mg/L was prepared by dissolving 2.83 g $K_2Cr_2O_7$ in 1000 mL of 0.01 mol/L HNO_3 and then stored in amber bottle at 4 °C for a maximum of two days. The intermediate solution 100 mg/ L was prepared from the stock solution by dilution. Then 5 mg/L was prepared from the intermediate solution by dilution to prepare the required the desired concentration level for the daily use and preparation of the seven concentration levels, form 0.25 - 2 mg/L for construction of calibration curve. The stock solution

1000 mg/L of the chelating agent, DPC, was also prepared in acetone and kept in amber bottle at less than 4 °C when not in use. Phosphate buffer (pH 2) was prepared by dissolving proper amount of KH_2PO_4 and the K_2HPO_4 , 2 mol/L HNO_3 , and 3 mol/L H_2SO_4 , 3 mol/L KOH were prepared in water.

3.4. SALLE procedure

5 mL aqueous solution containing 2 mg/L Cr (VI) was transferred to 15 mL falcon centrifuge tube. Then, 0.5 mL of 0.05 mol/L H_2SO_4 and 400 μL of 1000 mg/L of DPC were added, respectively. After complete complexation was confirmed by formation of red violet color, 0.5 mL of phosphate buffer was added for adjusting pH of a solution. Consequently, 1.5 mL of acetonitrile as extraction solvent and 3 g $(\text{NH}_4)_2\text{SO}_4$ as salting out agent were added. The content was shaken by hand until the added salt was completely dissolved. Then, the content was centrifuged for 3 min at 4000 rpm to enhance the phase separation between aqueous and organic phases. Finally, the upper organic phase containing target analyte was carefully taken by syringe and transferred to quartz cuvette for subsequent UV-Vis analysis at 540 monitoring wavelength [14 - 15].

4. RESULTS AND DISCUSSION

4.2. Optimizations of SALLE parameters

4.2.1. Effect of pH on complexation

Sample pH is important parameter that need to be optimized since it highly influence the formation of metal-ligand complex, which could directly affects the extraction efficiency of Cr(VI)-DPC complex by the method under the study [40]. The effect of pH on the formation of the metal-ligand complex was investigated by varying pH of the sample solution from 1–7. Figures 1 demonstrate the extraction efficiency of the method at various pH of sample solution.

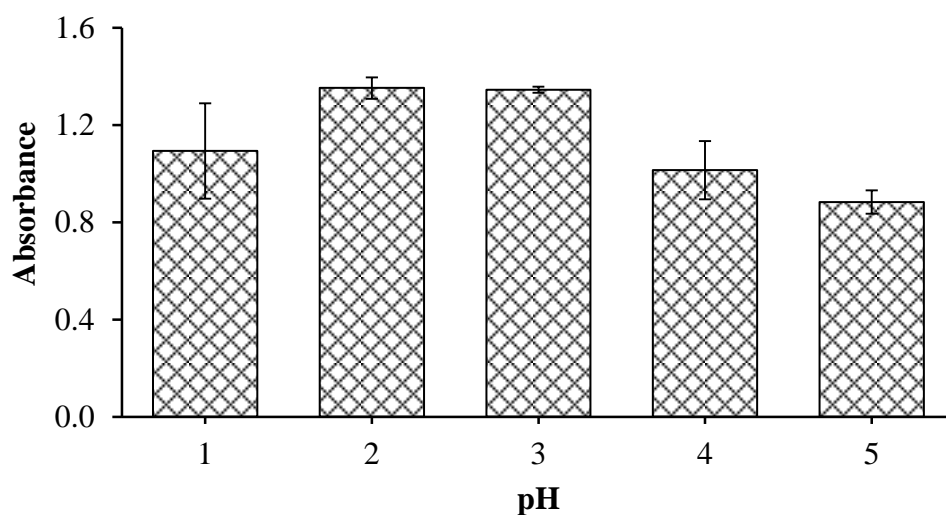


Figure 1. Effect of the pH. Conditions: sample volume: 5 mL, Cr (VI): 2 mg/L, DPC: 1000 mg/L (400 μ L), acetonitrile: 1.5 mL, $(\text{NH}_4)_2\text{SO}_4$: 3 g, extraction time: 3 min

The obtained results showed that up on addition of the reagent, 1, 5-diphenylcarbazide, to the solution a red violet complex were formed in the pH range 1 - 5. But, at higher pH, i.e., pH 6 and 7, change of color was not observed up on addition of the reagent, indicating that at pH higher than 5 DPC could not form complex with Cr (VI). As can be seen (Figure 1), the absorbance of the complex increased as pH rises up to pH 2 and then, started to gradually decrease at pH higher than 3. But, the observed absorbance at pH 2 and 3 were almost similar, indicating that DPC

form complex with Cr (VI) at pH 2-3 as also indicated in literature [2]. Therefore, pH 2 was selected as optimum pH and used throughout the study.

4.2.2. Effect DPC volume

The effect of DPC concentration on the formation of complex was investigated by adding various volumes of 1000 mg/L DPC ranging from 100 μ L - 600 μ L into 5 mL sample containing constant concentration of Cr (VI), 2 mg/L. Figure 2 shows the effects of the volume of DPC on the extraction efficiency of the method.

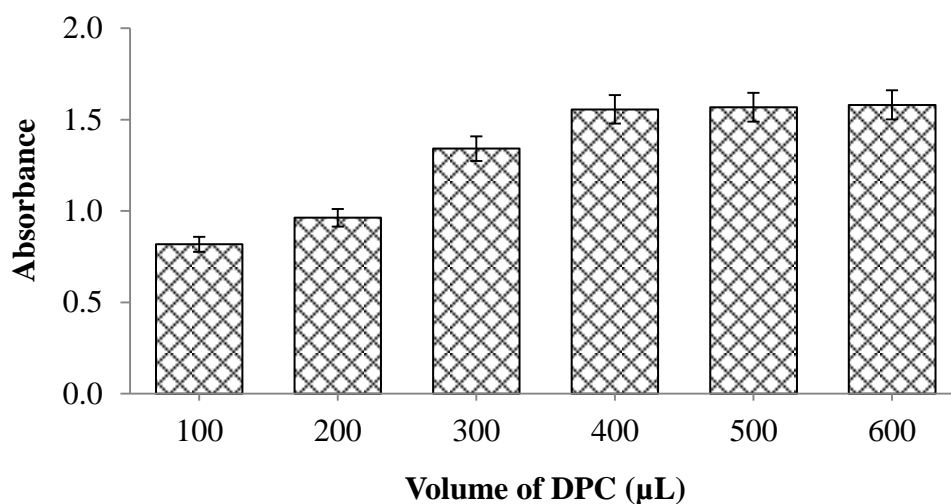


Figure 2. Effect of DPC volume. Conditions: pH: 2 and others are mentioned in Figure 1.

The results showed that fast increase in the absorbance of the resulting extracts upon increasing the volume of the ligand from 100 – 400 μ L. However, beyond 400 μ L, absorbance of the resulting extracts remained nearly constant.

This implies that at lower volume, below 400 μ L, the metal to ligand ratio has not been fulfilled the exact stoichiometric ratio, this indicating that the presence of excess Cr (VI) in the solution. Therefore, in further studies 400 μ L of 1000 mg/L DPC was used throughout the study.

4.2.3. Selection of extraction solvent

Selection of organic solvent is one of the critical parameters influencing the SALLE procedure. The organic solvent used for the extraction should be highly polar, miscible in water and induce

phase separation up on addition of appropriate amount of salt [41-42]. Accordingly, in this study solvents such as acetonitrile, acetone, ethanol and methanol were evaluated, using 2 mL of each as extraction solvent. It was observed that when ethanol and methanol were used, adequate phase separation was not formed. But, when acetonitrile and acetone were used phase separation was observed, but the absorbance of the resulting extract was higher when acetonitrile was used than the extract of acetone. Literature also showed that acetonitrile is the most commonly used solvent in SALLE [36-38]. Therefore, acetonitrile was selected as extraction solvent in further experiments.

4.2.4. Effect of extraction solvent volume

The extraction solvent volume also plays significant role on the extraction efficiency of the SALLE procedure. Generally, the optimum extraction solvent volume should offer high extraction efficiency as well as sufficient volume of the organic phase for the subsequent analysis [43]. The volume of acetonitrile was varied over the range of 0.5 – 3.0 mL and the obtained results are displayed in Figure 3.

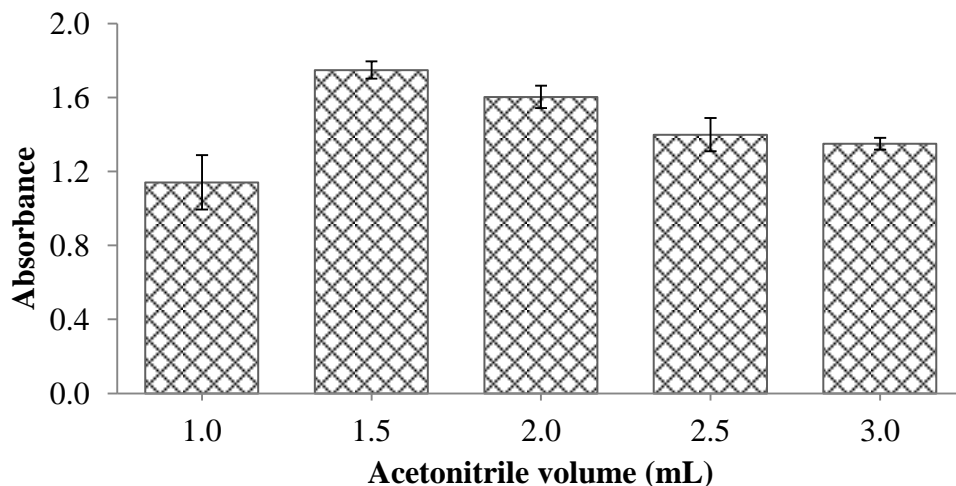


Figure 3. Effect of extraction solvent volume. Conditions: volume of DPC; 400 μ L and others are mentioned in Figure 2.

It was observed that phase separation was not observed when 0.5 mL of acetonitrile was used. But, when higher volumes, greater than 1 mL acetonitrile were used appropriate level of phase separation was observed. The extraction efficiency of the method also increased up to 1.5 mL

and then decreased at higher volumes of absorbance of the target analytes decreased when volume of acetonitrile is greater than 1.5 mL which may be attributed to dilution of the organic phase. The highest absorbance were obtained with 1.5 mL of acetonitrile and thus used as optimum extraction solvent volume.

4.2.5. Effect of the Salt Type

The addition of a salt into the sample solution cause increase in ionic strength of the sample solution and facilitates the transfer of target analytes from the aqueous phase to the organic solvent phase [44-45]. In order to evaluate this effect, 2 g of each of the salts; sodium chloride (NaCl), magnesium sulphate (MgSO_4) sodium carbonate (Na_2CO_3) and ammonium sulphate ($(\text{NH}_4)_2\text{SO}_4$), were added as potential salting-out agents. The result revealed that, sodium chloride and magnesium sulphate cannot form adequate phase separation; while Na_2CO_3 and $(\text{NH}_4)_2\text{SO}_4$ were formed adequate phase separation with absorbance of 1.0 and 1.4 respectively. Therefore, $(\text{NH}_4)_2\text{SO}_4$ is suitable salting-out agent and used for further experiments.

4.2.6. Effect of salt amount

The amount of salt is also important factor affecting the extraction efficiency of the SALLE method. The concentration of the salt must be large enough to induce the required phase separation. The quantity of salt added must be enough to clearly separate the two phases, but should not be added in excess beyond saturation to avoid adsorption of analytes on solid phase [46]. The amount of $(\text{NH}_4)_2\text{SO}_4$ was varied from 0.5 to 3.5 g (10 - 70%), at seven points (0.5, 1, 1.5, 2, 2.5, 3, 3.5) Figure 4 below.

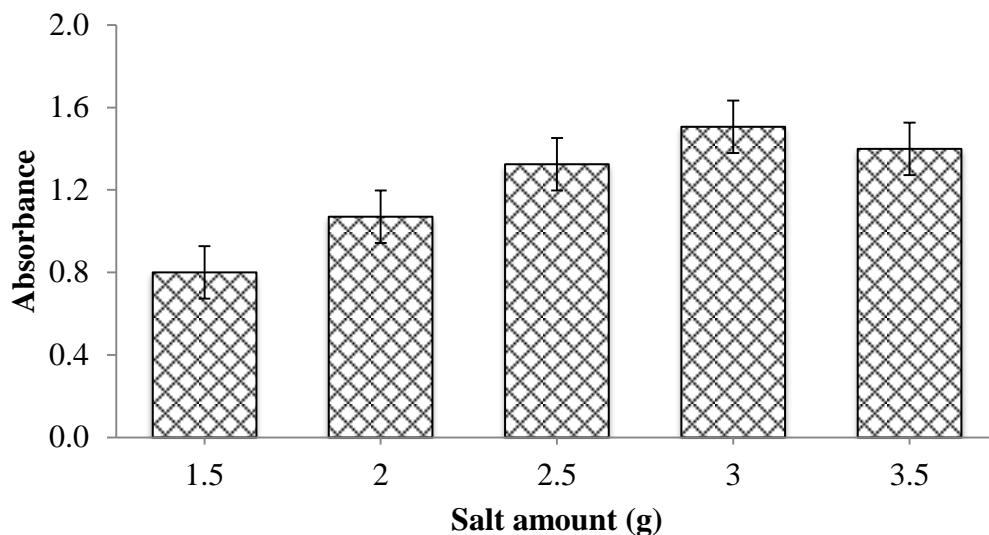


Figure 4. Effect of salt amount. Conditions: Volume of acetonitrile: 1.5 mL and other parameters mentioned in Figure 3.

It was observed that when smaller amounts, i.e., 0.5 g and 1 g salt were added, phase separation was not observed. On the other hand, the absorbance of the target analytes increases from 1.5 g up to 3 g (because the phase separation or amount of extraction solvent is increasing) and then slightly decreases at 3.5 g (this may be due to the adsorption of target analyte to the salt because it takes more time of shaking to dissolve completely). Therefore 3 g of $(\text{NH}_4)_2\text{SO}_4$ was used throughout this study.

4.2.7. Effect of extraction time

The effect of extraction time was investigated in the range of 1-11 min. The obtained result revealed that good extraction efficiency (enrichment) was obtained at 3 min and then it almost remained constant after 3 min (Figure 5). Therefore 3 min was chosen for further study.

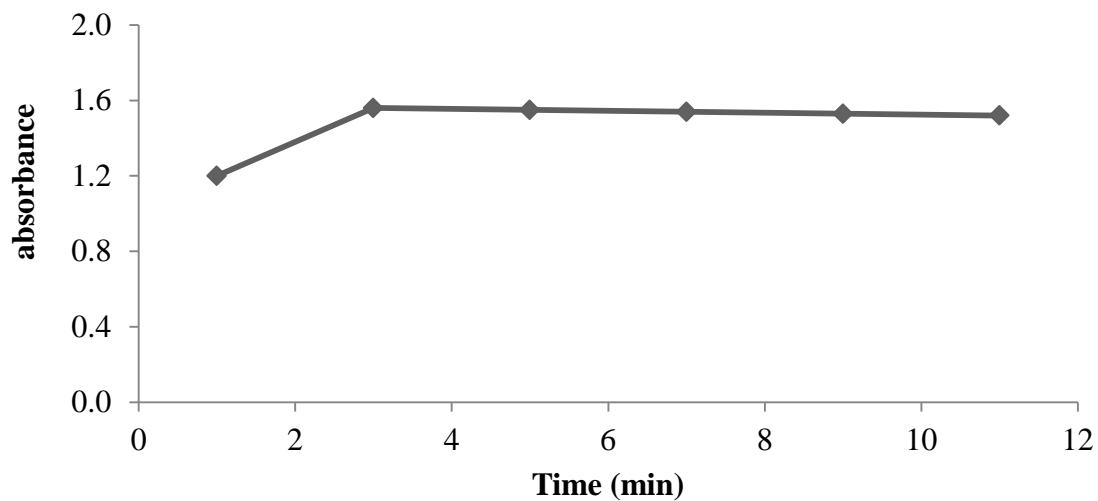


Figure 5. Effect of time. Condition: 3 g of $(\text{NH}_4)_2\text{SO}_4$ and other mentioned in Figure 4

4.2.8. Stability of the complex

The stability of the complex in the extraction phase (acetonitrile) was studied in the range of 20-160 min with the interval of 20 min by keeping the extracted analyte in dark until the analysis carried out using UV-Vis spectrophotometry. (Figure 6).

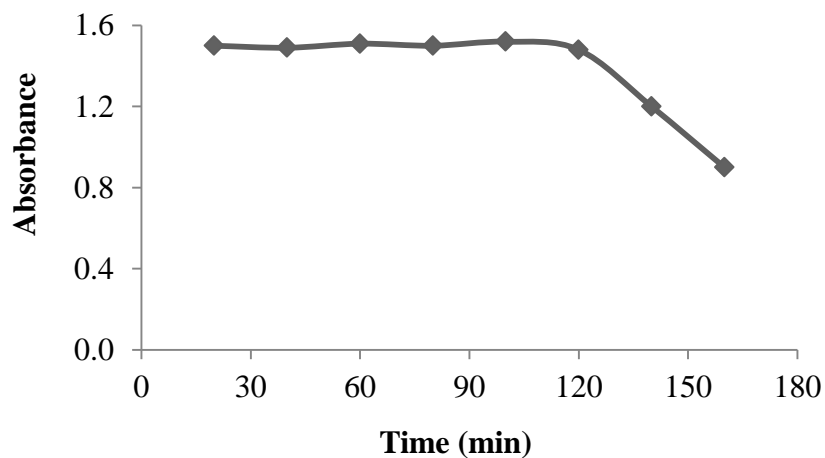


Figure 6. Stability of the complex. Condition: extraction time: 3 and other mentioned in Figure 5

The result shows that, the absorbance of the complex was almost constant until 120 min but beyond 120 min the absorbance was started to decrease. This may be due to the degradation of complex [14]

4.2.9. Effect of interferences ions

One of the most significant features of each developed method is selectivity of the method. It is obvious that good recovery can be obtained in presence of cations and anions that they are exist in real samples, which are problematic, and separation step are obligatory [5]. Therefore, the selectivity of this method was investigated by the determination of 2 mg/L Cr (VI) in presence of some cations that are added at different concentration level ranging from 50 -500 mg/L (Table 1). These cations are selected based on earlier reported literatures [3, 14, and 15]. The tolerance limit of interfering ions at each concentration was taken with percent relative recovery (%RR) \pm 5%.

The relative recovery (%RR) was calculated as: $A_{sp}/A_{unsp} \times 100\%$

Where: A_{sp} is absorbance of target analyte at each spiked concentration of interferences.

A_{unsp} is absorbance of unspiked (original) concentration.

As can be seen, the method is free from the interference of a large number of cations. It was observed that, among cations which tested only Cr (VI) form coloured complex with 1, 5-diphenylcarbazide.

Table 1. Effect of interferences for determination of 2 mg/L Cr (VI) with 1, 5-diphenylcarbazide

Concentration Added in mg/L	Interfering metals			
	Fe %RR \pm RSD	Copper %RR \pm RSD	Vanadium %RR \pm RSD	Cadmium %RR \pm RSD
0 (unspiked)	100 \pm 1.8	100 \pm 2.1	100 \pm 0.8	100 \pm 2.2
50	97.8 \pm 1.7	95.6 \pm 0.9	97.5 \pm 6.4	96.8 \pm 3.7
100	99.0 \pm 1.8	97.1 \pm 0.7	98.5 \pm 3.8	97.8 \pm 0.3
200	96.6 \pm 1.5	96.0 \pm 0.6	97.7 \pm 4.0	103.2 \pm 2.6
300	95.3 \pm 0.9	98.8 \pm 0.5	104.4 \pm 0.9	103.3 \pm 0.1
500	96.3 \pm 0.5	96.0 \pm 4.9	98.7 \pm 3.8	104.7 \pm 3.0

4.3. Method Validation

4.3.1 Calibration curve construction

The calibration curves were constructed by eight various concentration levels (blank, 0.25 mg/L, 0.5 mg/L, 0.75 mg/L, 1 mg/L, 1.25 mg/L, 1.5 mg/L and 2 mg/L) by using the developed optimum conditions. Each concentration levels were extracted in duplicate (experimental replicates) and each extract was then detected in triplicate (instrumental triplicate). Then, the calibration curves were obtained by plotting concentration against corresponding absorbance.

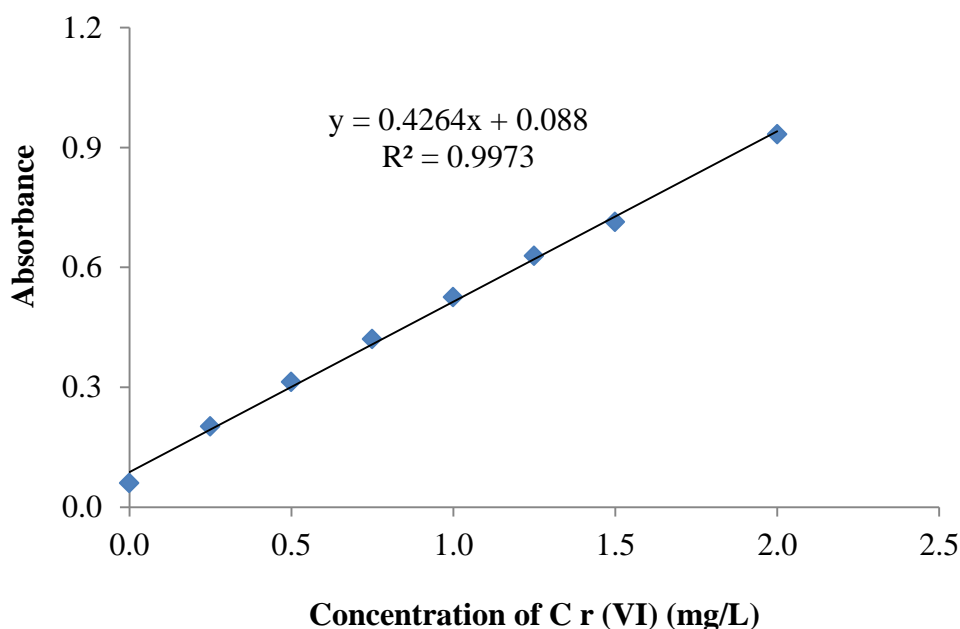


Figure 7: Calibration curve of Cr (VI).

According to the experimental result obtained from figure 5 above, the wide linearity range coefficient of determination (R^2) for the target analyte was 0.9973 achieved.

4.3.2 Limit of detection, Limit of Quantification and enrichment factor.

The LOD and LOQ were calculated by dividing 3 and 10 times of standard deviation of the blank measurements ($n = 10$) to slope of the calibration curve, was 0.004 mg/L and 0.015 mg/L respectively. Enrichment factor usually obtained by dividing the absorbance of extracted analyte

and absorbance of the initial concentration of the analyte in the aqueous sample. The enrichment factor was 5.8. Details of the figures of merits of the proposed method are presented in Table 2.

Table 2. Figures of merit of the propose SALLE-UV-Vis method for extraction and determination of Cr (VI)

LDR (mg/L)	R²	LOD (mg/L)	LOQ (mg/L)	EF
0.25-2.00	0.9973	0.004	0.015	5.8

4.3.3 Precision study

Precision of the proposed SALLE method was investigated in terms of intra-day precision (repeatability) and inter-day precision (between laboratories reproducibility). Intra-day precision was studied by extracting the Cr (VI) samples at two different concentration levels (0.75 and 1.25 mg/L) and each concentration levels was extracted in duplicates and each extract also was detected in triplicate on the same day (in early morning and evening). Under the same experimental conditions, inter-day precision of the method was also evaluated at two concentration levels earlier used for intra-day precision studies, for five consecutive days with the same time interval. The relative standard deviation (RSD) of the Intra-day (repeatability, n = 4) and inter-day (reproducibility, n = 20) precisions of the proposed method are shown in table 3.

Table 3: Intra- and inter-day precisions (RSD) of the proposed method

	Intra-day (n = 4)		Inter-day (n = 20)	
Spiked Conc, mg/L	Level 1	Level 2	Level 1	Level 2
RSD, %	0.5	1.3	6.2	8.7

Level 1=0.75 mg/L, Level 2= 1.25 mg/L

4.3.4 Analysis of real sample and recovery studies

The proposed method applicability's in the analysis of Cr (VI) in water samples was evaluated under optimum conditions. Before subjecting to the developed method each samples was filtered through filter paper and kept at ambient condition. The proposed method was validated for accuracy by extracting target analytes from different sources of water samples including tap water (taken from Jimma university analytical laboratory), well water (taken from Jimma town kebele 05) and river water taken from Jimma town Awetu River).

The samples were spiked at two concentration level 0.75 and 1.25 mg/L and each concentration levels was extracted in duplicates and each extract also was detected in triplicate for investigation of accuracy of the method in real sample analysis. For each of the real samples duplicate of blank samples (unspiked) were also extracted and analyzed by the proposed method. The obtained results shown in table 4 demonstrated that the amount of Cr (VI) in tap water and well water were not detected (below detection limit) and detected in river water with concentration of 0.06 mg/L before spiking (unspiked) with known amount of Cr (VI). Good recoveries ranging from 89.70 to 97.34%, with RSD% less than 9% were obtained after spiking with known amount of Cr (VI). This show that the values are within the acceptable range and the method can be successfully applied to real samples.

Table 4. Determination of Cr (VI) by proposed method in different real samples

Levels	Tap water	River water	Well water
Level 1	97.3 ± 0.1	89.0 ± 8.7	90.0 ± 4.7
Level 2	94.8 ± 0.1	88.0 ± 2.5	91.0 ± 1.1
Conc. Cr (VI)	ND	0.06 ± 1.00	ND

Level 1=0.75 mg/L, Level 2= 1.25 mg/L, ND= not detected

4.4. Comparison of the applied method with other reported methods

The extraction efficiency of the proposed SALLE method has been compared with related analytical techniques reported in the literatures employed for the extraction of Cr (VI) from

water samples. The comparison was made in terms of calibration curve (linearity), precision (% RSD), limit of detection (LOD) and relative percentage recovery (% RR), Table 5.

Table 5: Comparison of the proposed SALLE with other methods

Analytical methods	Matrix	LDR (mg/L)	R²	LOD (mg/L)	Precision (%RSD)	Volume (mL)	Ref.
SALLME-IL-FAAS	Environmental water and food grade salts	300- 1500	0.9994	0.001	< 2	10	[14]
DLLME-DES-UV-VIS	Water and waste water samples	500-2000	0.9951	0.0015	< 6	10	[15]
SPE-FAAS	Natural water	10-50	-	0.045	<4	250	[47]
ILDLLBME	Serum, whole blood	3-440	-	0.008	< 5	1	[48]
DLLME-UV-Vis	Water and waste water samples	0.10- 0.52	0.987	0.03	< 7	5	[3]
SALLE-UV-Vis	Water and waste water samples	0.25-2	0.9973	0.004	< 9	5	Current study

As it can be seen from Table 5, it was noted that the method developed in this study has provided better linearity, similar or better LOD and comparable precision relative standard deviation (% RSD) cited for comparison and smaller sample volume than the other methods. The technique utilizes moreover classical laboratory equipment as well as less toxic organic solvent (acetonitrile), which can be found in common research laboratories. Based on the experimental findings, the developed SALLE-UV-Vis method can be considered as one of the preferred alternative, having promising future for selective and quantitative extraction of Cr (VI) from water samples considered in this study.

5. CONCLUSION AND RECOMMENDATION

In this work, SALLE method was proposed for extraction and/or pre-concentration of Cr (VI) in water samples prior to its determination by UV-Vis spectrophotometry. Various optimization parameters influencing the extraction efficiency of the methods were seriously studied and the optimum conditions were established. Utilizing the optimal conditions, the proposed SALLE method exhibited its usefulness for the extraction of Cr (VI) using nontoxic organic solvent (acetonitrile) from water samples with acceptable analytical performances, precisions, limit of detection and quantification and relative recoveries.

5.1. Recommendation

The method salting out induced liquid-liquid extraction (SALLE) was proposed for extraction and/or pre-concentration of Cr (VI) in water samples prior to its determination by UV-Vis spectrophotometry. The obtained results demonstrated that the developed method was fully trusted best sample preparation method due to its low cost, simple, rapid as well as it pronounced good enrichment factors for sample extraction, pre-concentration and determination of the target analyte Cr (VI) from water samples and other related matrices. Although, the method has advantages in rapidity, low cost, simplicity it need milliliters (1.5 mL) of extraction solvent acetonitrile which is relatively expensive in developing country. Therefore it is better to decrease the volume of extraction solvent from milliliter to microliter.

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