# Liquid-Liquid Microextraction of Vanadyl Porphyrins by Nano-baskets

B. Mokhtari*, K. Pourabdollah†

*Razi Chemistry Research Center (RCRC), Shahreza Branch, Islamic Azad University, Shahreza, Iran*

The novelties of this approach are introducing the self-settled dispersive liquid-liquid microextraction technique to remove the centrifuging step, conducting the dispersive liquid phase microextraction in complex organic systems, applicability of water as disperser phase, and nano-extraction of charged porphyrins by nano-baskets of calixcrown, which act as the settling agents as well as the inclusion ligands. Four proton di-ionizable diacid conformers of 25,26-bis(carboxymethoxy)calix[4]arene-27,28-crown-3; -crown-4; -crown-5; and -crown-6 in the cone conformation were synthesized and used. The related parameters including ligand concentration, volume of water disperser, salt effect, and extraction time were optimized. The linear range, detection limit (S/N = 3) and precision (RSD, n = 6) were determined to be 0.2–50, 0.07 $\mu gL^{-1}$ and 5.3%, respectively. The established method was applied to determine the target compound in five samples of live crude oil, were sampled from an Iranian offshore field. Owing to the overall differences (such as organic media, inclusion extraction, water-soluble ligands, etc), the comparison of the proposed method with the traditional liquid-liquid microextraction was inapplicable. These results revealed that the new approach is competitive analytical tool and an alternative of the traditional methods in the crude oil and related systems.

**Keywords:** Nano-baskets, Microextraction, Vanadyl Porphyrins, Calixcrown.

PACS numbers: 87.85.Qr, 87.85.Rs

## 1. INTRODUCTION

Determination of metalloporphyrins in crude oil is of interest in understanding the geochemical origins of petroleum reservoirs, the diagenetic and the catagenetic pathways in the oil formation, and the maturation, depositional [1] and environmental reconstruction studies [2]. Alfred Treibs discovered the petroleum porphyrins (petroporphyrins) in 1934 [3]. Vanadyl porphyrins were the first petroleum biomarkers [4] and they are molecular fossils of tetrapyrrolic pigments such as bacteriochlorophylls and chlorophylls [5]. Vanadium in crude oil causes corrosion problems that derive from the formation (in the combustion chamber of power plants) of sodium vanadates (with low melting point), which react with the metal surface of the superheaters and form the metal oxide [6].

According to the literature reports [4], five main types of porphyrins along with their homologues are present in crude oil including etioporphyrins (Etio), deoxophylleoerythroetioporphyrin (DPEP), tetrahydrobenzo DPEP, benzo-Etio and benzo-DPEP. Up to 30% of vanadium and 25% of nickel in Tatarstan crude oil are found as porphyrin complexes. Ion et al. [7] analyzed the vanadyl porphyrin distribution of Romanian petroleogenetic rocks by UV-vis spectrophotometry, FTIR, ICP-atomic emission spectrometry, ESR and X-ray fluorescence spectroscopy. Holden and coworkers [5] developed the method of high-resolution reflectance spectroscopy for determination of porphyrins (low levels) in the kerogen fraction. After that, Premovic et al. [8] used the electron spin resonance (ESR) to quantify the high levels of vanadyl porphyrins in kerogens.

Saitoh et al. [9] used a series of preliminary separation procedures for preconcentration of metalloporphyrins and determination by reversed-phase high-performance liquid chromatography (HPLC). Ali et al. [2] extracted the nickel and vanadyl porphyrins in residue of Saudi Arabian Crude Oils. The nickel porphyrins were separated from vanadyl porphyrins using adsorption chromatography on alumina and silica gel by solvents of increasing polarity. They monitored the chromatographic separation by UV-vis spectrophotometry.

New aspects of analytical chemistry in crude oil and reservoir engineering are summerized manly as chemometrics [10], sample preparation and microextraction [11] techniques.

New sample-preparation methods, which are easy to use, inexpensive, fast, environmental friendly and compatible with a range of analytical instruments, are outspreaded. More recently, efforts have been placed on the development of the dispersive liquid–liquid microextraction (DLLME) [12] procedure, which is based on a ternary component solvent system. The dispersion of extraction solvent by disperser solvent within the aqueous solution leads a large contact area between the aqueous phase and the extraction solvent. Other examples of sample preparation by DLLME have been presented for trace determination of pesticides in soils, organophosphorus pesticides in water, nickel and Cu(II) in water, and chlorobenzenes in water.

Flocculation, which is the first action of the demulsifier on an emulsion, involves the joining of the small water droplets. When magnified, the flocks take on the appearance of fish-egg bunches. If the emulsifier film surrounding the water droplets is very weak, it will break under this flocculation force and the coales-

---

* mokhtari@iaush.ac.ir
† pourabdollah@iaush.ac.ir

cence, which is the rupturing of the emulsifier film and the uniting of water droplets, will take place without further chemical action. Once the coalescence begins, the water droplets grow large enough to settle out.

Asphaltenes are the high polar fraction of the petroleum and play an important role in the formation and stabilization of the water in crude oil emulsions [13]. The goal of demulsifier action is to offset the stabilization of emulsion both from the kinetic and thermodynamic points of view [14]. Demulsifiers are molecules that aid the separation of water from oil and prevent formation of water in oil mixture. Some demulsifiers are polymers, others have calixarene and calixcrown structures. Calixarenes and calixcrowns have been subjected to extensive researches [15] and reviews [16] in development of extractants, transporters, stationary phases, electrode ionophores, optical sensors and medical researches over the past decades.

In this paper, a novel approach, entitled self-settled dispersive liquid phase microextraction, was introduced and is used for determination of vanadium porphyrins in live crude oil. The main objectives of this work are applicability of water as disperser phase, removing the centrifuging step, settling the water dispersed droplets by calixcrowns, conducting the dispersive liquid phase microextraction in complex organic systems, and inclusion microextraction of charged porphyrins by ionizable calixcrowns. This method deals with twin role of calixcrowns scaffolds as the settling and the complexing agent.

## 2. EXPERIMENTAL

### 2.1 Chemicals and reagents

Doubly distilled and deionized water (DDW) with a specific resistivity of 18 MΩcm, from a Milli-Q water purification system (Millipore, Bedford, MA), was used as disperser. According to the literature methods [4, 9], the vanadyl porphyrins were separated from the oil matrix and were used as standard solutions. The oil samples were collected from one of the Iranian offshore oil fields and their chemical characteristics are presented in Table 1.

### 2.2 Analytical Apparatus

Determinations The extractions and injections were performed by microsyringe (Agilent, CA, USA) bearing an angledcut needle tip (needle id: 0.11 mm and glass barrel id: 0.6 mm). Atomic absorption spectrometer of Shimadzu (model AA-670G) with deuterium lamp background correction and a graphite furnace atomizer (GFA-4B) was used. A reversed phase (RP) $C_{18}$ column (4.6mm diameter, 100 mm length, 2 µm macropore size, and 13 nm mesopore size) was obtained from Merck (Darmstadt, Germany). A RP-$C_{18}$ guard column was fitted upstream of the analytical column. The mobile phase was optimized to be 45:55, v/v methanol–water and was delivered by an HPLC pump (Waters LC-600). The UV detection wavelength was set at 254 nm and the flow rate of the mobile phase was adjusted to be 3 ml/min.

**Table 1** – Chemical characteristics of blend crude oil.

| Component | Stream Liquid (*mol %*) | Flashed Gas (*mol %*) | Flashed Liquid (*mol %*) |
|---|---|---|---|
| $N_2$ | 00.04 | 00.31 | 00.00 |
| $CO_2$ | 00.35 | 02.78 | 00.00 |
| $H_2S$ | 00.57 | 04.52 | 00.00 |
| $CH_4$ | 03.76 | 30.14 | 00.00 |
| $C_2H_6$ | 01.50 | 12.02 | 00.00 |
| $C_3H_8$ | 03.77 | 21.90 | 01.18 |
| $i$-$C_4H_{10}$ | 01.92 | 07.25 | 01.16 |
| $n$-$C_4H_{10}$ | 07.23 | 12.40 | 06.49 |
| $i$-$C_5H_{12}$ | 02.79 | 03.21 | 02.73 |
| $n$-$C_5H_{12}$ | 03.80 | 02.81 | 03.94 |
| $C_6$ | 07.30 | 01.86 | 08.08 |
| $C_7$ | 08.76 | 00.68 | 09.91 |
| $C_8$ | 09.61 | 00.12 | 10.97 |
| $C_9$ | 08.77 | 00.00 | 10.02 |
| $C_{10}$ | 06.75 | 00.00 | 07.71 |
| $C_{11}$ | 03.60 | 00.00 | 04.12 |
| $C_{12+}$ | 29.48 | 00.00 | 33.69 |

| | |
|---|---|
| Total Sulfur | 00.85/*mass %* |
| Asphaltenes | 00.32/*mass %* |
| Waxes | 05.00/*mass %* |

### 2.3 Sample Preparation

5.0 mL pre-washed crude oil was placed into a 10-mL screw-cap glass centrifuge tube with conic bottom. 100.0 µL of distilled water (as dispersive solvent) containing 0.001 g calixcrown (as extraction ligand) was rapidly transferred into the above-mentioned centrifuge tube and was gently shaked.

The calixcrown demulsifier caused the dispersed fine droplets of the extraction phase be sedimented at the bottom of the conical test tube. The volume of the sedimented phase was determined using a 50.0 µL microsyringe, and was completely transferred to another 100 µL centrifuge tube. After evaporation of the water under a gentle nitrogen flow, the residue was redissolved in 25 µL LC-grade methanol and injected into HPLC for analysis.

## 3. RESULTS AND DISCUSSION

The effects of all parameters (that can probably influence the performance of extraction) were investigated. They were (1) the concentration of demulsifier or extraction ligand (calixcrown), (2) the volume of disperser (water), (3) the extraction time and (4) the salt addition that were investigated and optimized in order to achieve the higher enrichment factor and recovery of vanadyl porphyrins from the samples of live oil.
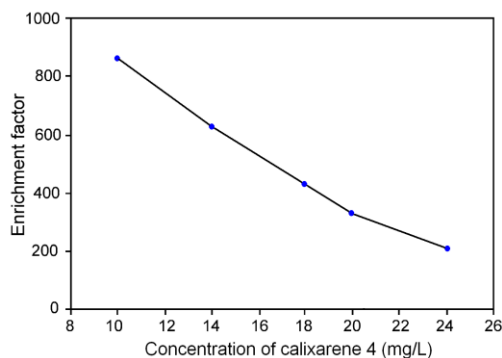
### 3.1 Effect of the calixcrown concentration

In order to evaluate the effect of calixcrown concentration on the extraction efficiency and the separation of phases, the following experiments were performed using 0.50 ml DDW containing different concentrations of calixcrown (9.5, 14.5, 19.5 and 24.5 mgl$^{-1}$). Figures 1–3 depicts the traces of recovery of vanadyl porphyrins, enrichment factor and volume of sedimented phase versus the calixcrown concentration, respectively.

As illustrated in Figure 3, the extraction recovery is almost increasing (from 62.0–95.5%) owing to the quan-

tity extraction and high distribution coefficient of vanadyl porphyrins in the conditions of high concentration. Obviously, in Figure 2, the enrichment factor decreases from 880 to 200. Thus, 10 μL of vanadyl porphyrins was selected in order to obtain high enrichment factor, and hence low detection limit and high recovery. According to Figure 3, by increasing the calixcrown concentration from 10 to 26 mgL⁻¹, the volume of sedimented phase increases (6.0–20.5 µL).
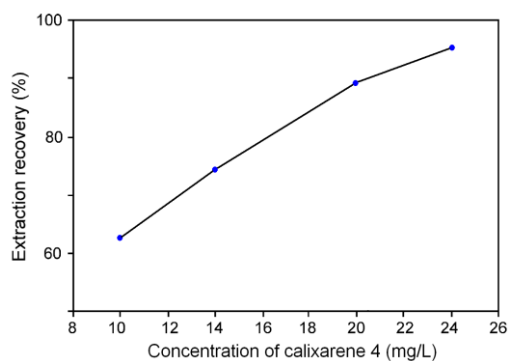


**Fig. 1** – Effect of the calixcrown concentration on the recovery of vanadyl porphyrins. Extraction conditions: oil sample volume, 5.00 ml; disperser solvent (water) volume, 0.50 ml; at ambient temperature



**Fig. 2** – Effect of the calixcrown concentration on the enrichment factor of vanadyl porphyrins. Extraction conditions: as with Figure 1; concentration of calixcrown, 10 mgL⁻¹
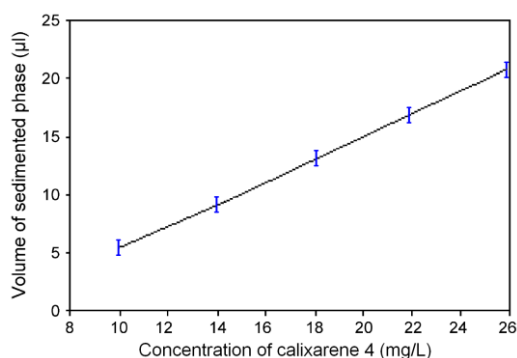


**Fig. 3** – Effect of calixcrown concentration on the volume of sedimented phase (N = 3). Extraction conditions: as with Figure 1

### 3.2 Effect of the disperser volume

As discussed above, water was selected as the best disperser solvent; hence, it was necessary to optimize

the disperser volume. As a rule, the water disperser (at low volumes) cannot disperse the extracting calixcrown properly. In the other hand, in such conditions, the cloudy solution is not formed completely.

For obtaining optimized volume of water, some experiments were conducted using different volumes of water (0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 ml) containing 10.0, 12.5 16.0 and 24.0 µgL⁻¹ vanadyl porphyrins, respectively. It is necessary to change the volume of vanadyl porphyrins by changing the volume of water in order to obtain constant volume of sedimented phase in all experiments. Figure 4 illustrates the trace of vanadyl porphyrin's recovery versus the volume of water. Base upon the results, a 0.50 ml water was chosen as the optimum volume of disperser.
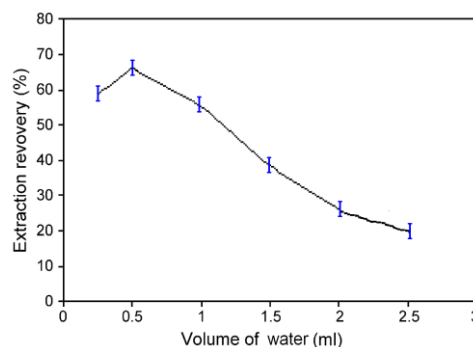


**Fig. 4** – Effect of the volume of water disperser on the recovery of vanadyl porphyrins obtained from DLLME (N = 3). Extraction conditions: as with Figure 1
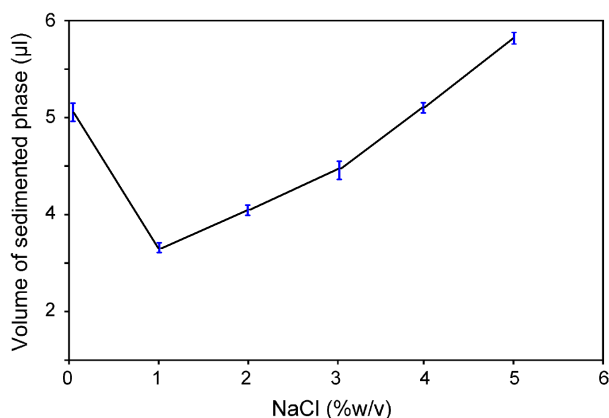
### 3.3 Effect of the extraction time

The interval time between the injection of the water disperser (containing the extraction ligand) and starting to decant was defined as the extraction time. The effect of extraction time on the performance of DLLME is a key factor, which is evaluated here. Different extraction times in the range of 0 to 90 min (with constant experimental conditions) were investigated. Base upon the results, the extraction regime is time-independent since an infinitely large surface area is available between the aqueous phase (extraction solvent) and the oil media. According to Figure 5, this method is very fast and this is a common advantage of DLLME.
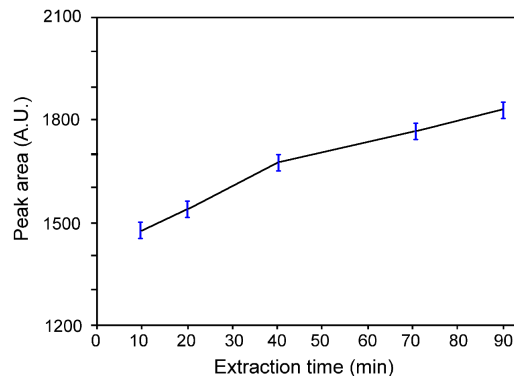


**Fig. 5** – Effect of extraction time on the peak area of vanadyl porphyrins obtained from DLLME (N = 3). Extraction conditions: as with Figure 1

### 3.4 Effect of the salt addition

The influence of ionic strength on the performance of DLLME was studied by adding different amounts of NaCl (0–5%), while the other experimental conditions were kept constant. Figure 6 presents the effect of increasing the ionic strength on the volume of sedimented phase of vanadyl porphyrins. Obviously, by increasing the ionic strength (from 0 to 1%) the volume of sedimented phase decreases and then by more increasing (from 1 to 5 %), the volume of sedimented phase increases.



**Fig. 6** – Effect of salt addition on the volume of sedimented phase obtained from DLLME (N = 3). Extraction conditions, as with Figure 1

### 3.5 Real sample analysis

The matrix effects on the extraction were also evaluated by studding the applicability of this approach to determine the concentration of vanadyl porphyrins in the crude oil samples taken from one of the Iranian offshore fields. The samples were extracted using DLLME method and analyzed by HPLC–UV. The samples were spiked with vanadyl porphyrin standards at different concentration levels to investigate the matrix effects. A typical chromatogram representing the vanadyl porphyrins is depicted in Figure 7.
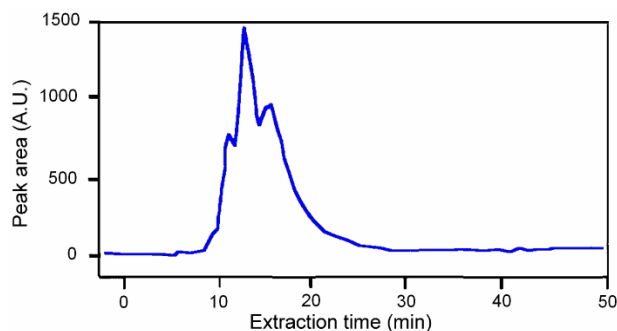


**Fig. 7** – Chromatograms of decanted water and spiked samples at concentration level of 12.5 µgL$^{-1}$ vanadyl porphyrins

### 4. CONCLUSIONS

This study introduced a new approach and a self-settled DLLME method combined with HPLC–UV for separation, preconcentration and determination of vanadyl porphyrins in crude oil. This method deals with twin role of calixcrown scaffolds as the self-settling and the complexing agent. Removing the centrifuging step and performing the inclusion microextraction of vanadyl porphyrins by means ionizable calixcrown was the novelty of this project to enhance the preconcentration speed and extraction the vanadyl porphyrins

The results of this study revealed that the proposed approach is acceptable for preconcentration of vanadyl porphyrins from crude oil samples. The linear range, detection limit (S/N = 3) and precision (RSD, n = 6) were determined to be 0.2–50, 0.07 µgL$^{-1}$ and 5.3%, respectively

### REFERENCES

1. K. Pourabdollah, A. Zarringhalam Moghaddam, R. Kharrat, B. Mokhtari, *Energy Fuels* **24**, 4396 (2010).
2. M.F. Ali, H. Perzanowski, A. Bukhari, A.A. Al-Haji, *Energy Fuels* **7**, 179 (1993).
3. A. Treibs, *Annales des Chemie* **509**, 103 (1934).
4. P.I. Premovic, L.S. Jovanovic, *Fuel* **76**, 267 (1997).
5. P.I. Premovic, T. Allard, N.D. Nikolic, I.R. Tonsa, M.S. Pavlovic, *Fuel* **79**, 813 (2000).
6. F.G. Lepri, B. Welz, D.L.G. Borges, A.F. Silva, M.G.R. Vale, U. Heitmann, *Anal. Chim. Acta* **558**, 195 (2006).
7. S.M. El-Sabagh, *Fuel Process. Technol.* **57**, 65 (1998).
8. H. Salehizadeh, M. Mousavi, S. Hatamipour, K. Kermanshahi, *Iranian J. Biotechnol.* **5**, 226 (2007).
9. P.I. Premovic, I.R. Tonsa, D.M. Dordevic, M.S. Pavlovic, *J. Serb. Chem. Soc.* **65**, 113 (2000).
10. K. Saitoh, H. Tanji, Y. Zheng, *Anal. Sci.* **17**, 1511 (2001).
11. K. Pourabdollah, B. Mokhtari, *Spectrochim. Acta A* **84**, 22 (2011).
12. M. Rezaee, Y. Assadi, M.R. Millani, E. Aghaee, F. Ahmadi, S. Berijani, *J. Chromatogr. A* **1116**, 1 (2006).
13. J.B. Ramalho, N. Ramos, E. Lucas, *Chem. Chemical Technol.* **3**, 53 (2009)
14. M. Rondon, P. Bouriat, J. Lachaise, *Energy Fuels* **20**, 1600 (2006)
15. B. Mokhtari, K. Pourabdollah, *J. Coord. Chem.* **64**, 3081 (2011)
16. B. Mokhtari, K. Pourabdollah, N. Dalali, *J. Incl. Phenom. Macrocycl. Chem.* **69**, 1 (2011)