

**SALTING-OUT EXTRACTION OF CATECHOL
AND HYDROQUINONE FROM AQUEOUS SOLUTIONS
AND URINE SAMPLES**

UDC 577.118; 547.562.2

**Goran M. Nikolić¹, Jelica M. Perović²,
Ružica S. Nikolić², Milorad M. Cakić³**

¹Department of Chemistry, Faculty of Medicine, University of Niš,
Braće Tasković 81, 18000 Niš, Serbia and Montenegro

²Department of Chemistry, Faculty of Sciences, University of Niš,
Višegradska 33, 18000 Niš, Serbia and Montenegro

³Faculty of Technology, University of Niš,
Bulevar oslobođenja 124, 16000 Leskovac, Serbia and Montenegro

Abstract. *The influence of ammonium, sodium, potassium, and magnesium chlorides and sulfates on the extraction of catechol and hydroquinone with diethyl and diisopropyl ether was studied. All the salts investigated in this study largely increased the extraction efficiency of both catechol and hydroquinone from aqueous solutions, magnesium salts being the most efficient salting-out agents. The extraction efficiency in the presence of magnesium salts in comparison to the extraction efficiencies from the salt-free aqueous solutions increased from 14%, in the case of catechol extraction with diethyl ether, up to about 90%, in the case of hydroquinone extraction with diisopropyl ether. The extraction from spiked urine samples proved that investigation of salting-out effect in aqueous solutions could be used as a good model system for the reliable choice of optimal conditions for catechol and hydroquinone extraction from real samples.*

Key words: *salting-out, extraction, catechol, hydroquinone*

INTRODUCTION

Biological monitoring for exposure to volatile organic compounds is currently the subject of very intensive research [1]. Determination of catechol (1,2-benzenediol) and hydroquinone (1,4-benzenediol) in human urine received considerable attention due to its possible use in the biological monitoring of low-level benzene exposures [2-5]. Because of the low concentration of these two benzene metabolites in human urine an extraction step is usually included in the sample preparation for analysis. However, both catechol

and hydroquinone are poorly extracted with organic solvents most commonly employed in routine work (e.g. diethyl ether). In such cases the use of salting-out may greatly enhance the extraction efficiency of various organic compounds from aqueous solutions and thus improve the sensitivity and precision of their determination. There are some data previously published about the influence of different salts on the extraction of these two phenols by *n*-hexanol [6] and butanols [7], and Jones *et al.* investigated various solvent/salt combinations [8]. In this paper we investigate the influence of ammonium, sodium, potassium, and magnesium chlorides and sulfates on the extraction efficiency of catechol and hydroquinone with diethyl and diisopropyl ether, which are among the most frequently used solvents for the extraction of various phenolic compounds. The choice of inorganic salts and organic solvents studied was aimed at direct practical application of the obtained results.

EXPERIMENTAL

Reagents

Stock solutions of catechol and hydroquinone (1 g dm^{-3}) were prepared on a daily basis by dissolving the exactly weighted amount of substance in water. The pH value of these solutions was adjusted to approximately 2 by the addition of HCl. Saturated aqueous solutions of inorganic salts were prepared by directly dissolving salts in water and were kept at least 24 h (with occasional shaking) in contact with excess of salt before used. Catechol, hydroquinone, diethyl ether, and diisopropyl ether were purchased from Merck (Darmstadt, Germany), while inorganic salts used in this study were purchased from local suppliers. All the reagents used were of analytical grade purity and were used without further purification.

Procedure

The conventional shake-flask method was employed for the determination of distribution ratios of catechol and hydroquinone between organic and aqueous phase because it proved to be the most reliable method for such kind of determination [9]. Two cubic centimeters of stock solution were diluted with 18 cm^3 of water or saturated salt solution and extracted with ether in a separatory funnel by vigorous shaking for 1 h. After the phases were allowed to separate, the absorbance of aqueous phase was measured at 277 and 290 nm for catechol and hydroquinone, respectively. All the extractions were performed at two volume ratios of aqueous and organic phase; 1:1 and 2:1. Distribution ratio (*D*) of phenolic compounds was calculated according to the equation:

$$D = \frac{A_0 - A}{A} \times \frac{V_{aq}}{V_{org}}$$

where A_0 is the absorbance of aqueous phase before extraction, A is the absorbance of aqueous phase after extraction, and V_{aq} and V_{org} are the volumes of aqueous and organic phase, respectively. All extractions were performed in triplicate and the relative error of *D* value determinations was calculated by using the following simple expression:

$$R(\%) = \pm \frac{D_{\max} - D_{\min}}{D_{\max} + D_{\min}} \times 100$$

where D_{\max} and D_{\min} were maximum and minimum D values obtained for each ether-phenolic compound-aqueous phase system, respectively.

Extraction efficiency (E) was calculated according to the equation:

$$E(\%) = \frac{D}{D + V_{aq} / V_{org}} \times 100.$$

A SPECORD UV/VIS spectrophotometer (Carl Zeiss, Jena, Germany) was used for the absorbance measurements.

RESULTS AND DISCUSSION

The D values obtained for catechol and hydroquinone extraction with diethyl and diisopropyl ether in the presence of various salts are presented in Table 1.

Table 1. Distribution ratio values for systems investigated in this study.

Salt	Diethyl ether extraction		Diisopropyl ether extraction	
	D	R (%)	D	R (%)
Catechol				
No salt	6.6	2.02	3.5	1.47
NH ₄ Cl	14.5	2.17	5.4	1.82
(NH ₄) ₂ SO ₄	40.7	1.76	13.6	2.24
NaCl	34.0	2.09	12.4	2.26
Na ₂ SO ₄	21.6	0.94	8.0	1.52
KCl	18.2	1.16	6.3	1.96
K ₂ SO ₄	10.4	1.12	4.4	0.73
MgCl ₂	92.0	1.62	30.7	1.84
MgSO ₄	86.9	2.13	25.6	2.41
Hydroquinone				
No salt	1.7	1.71	0.8	1.93
NH ₄ Cl	4.2	0.70	1.4	1.24
(NH ₄) ₂ SO ₄	10.5	1.13	3.1	0.96
NaCl	10.4	1.25	3.3	1.34
Na ₂ SO ₄	6.0	0.54	1.9	1.02
KCl	5.5	0.92	1.7	0.90
K ₂ SO ₄	2.7	1.03	1.0	0.81
MgCl ₂	16.0	2.04	5.6	1.87
MgSO ₄	16.7	1.39	5.1	1.56

Figure 1 shows extraction efficiencies for the phenol-salt-organic solvent systems investigated in this study.

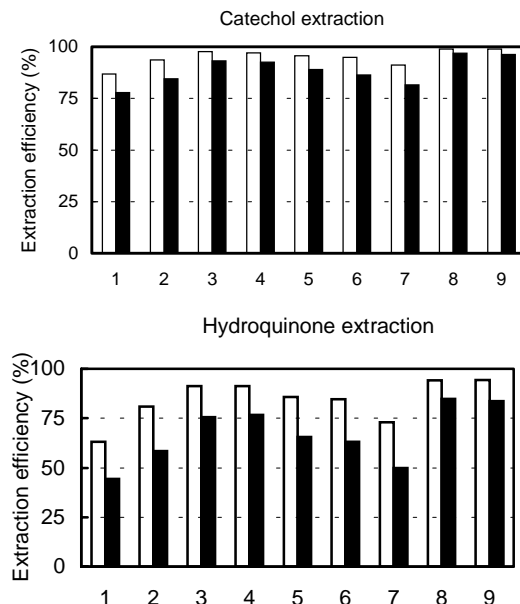


Fig. 1. Extraction efficiencies for phenol-inorganic salt-ether systems investigated in this study at $V_{aq}/V_{org} = 1:1$. Light bars present data for diethyl ether and black bars for diisopropyl ether extraction. Salt numbering: 1 = No salt, 2 = NH_4Cl , 3 = $(\text{NH}_4)_2\text{SO}_4$, 4 = NaCl , 5 = Na_2SO_4 , 6 = KCl , 7 = K_2SO_4 , 8 = MgCl_2 , 9 = MgSO_4 .

All the salts investigated in this study enhanced extraction efficiency of both catechol and hydroquinone from aqueous solutions with either diethyl or diisopropyl ether. The influence of salts on the extraction efficiency decreased in the following order:



The greatest salting-out effect of magnesium chloride and sulfate is obviously related to the properties of Mg^{2+} ion. Although Mg^{2+} ion may form labile complexes with catechol in weakly alkaline aqueous solutions [10], such possibility certainly did not have any significant influence in this study because the salting-out order of salts for catechol is very similar to that of hydroquinone which has no ability of complex formation with Mg^{2+} ion. Figure 2 shows scatter diagram of $\log D$ values for catechol and hydroquinone extraction with diethyl and diisopropyl ether.

Approximately linear relationship of these $\log D$ values suggests that inorganic salts investigated in this study did not influence the mechanism of extraction. Thus it seems reasonable to ascribe the greatest salting-out effect of magnesium salts to the highest ionic potential of Mg^{2+} ion. It is interesting to note that in the previous studies of salting-out effect in the extraction of catechol and hydroquinone [6-8] magnesium salts have not been investigated at all.

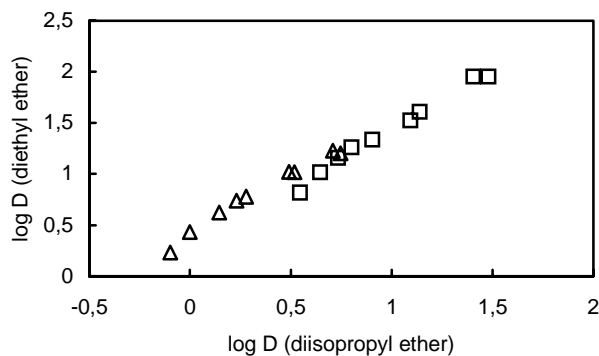


Fig. 2. Scatter diagram of logD values for catechol (squares) and hydroquinone (triangles) extraction with diethyl and diisopropyl ether.

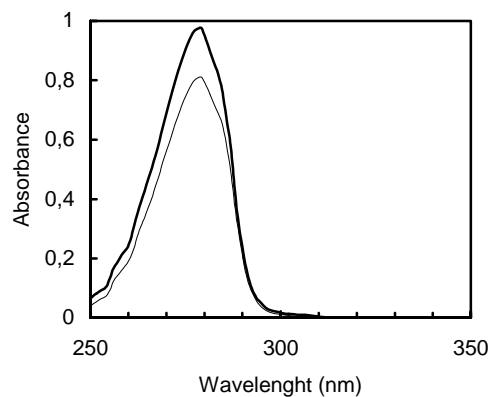


Fig. 3. UV spectra of diisopropyl ether extracts of urine samples spiked with catechol for salt-free (thin line) and NaCl saturated sample (thick line). Diisopropyl ether extracts of unspiked urine samples were used as references.

Extraction efficiency in the presence of magnesium salts in comparison to the extraction efficiencies from the salt-free aqueous solutions increased from 14%, in the case of catechol extraction with diethyl ether, up to about 90%, in the case of hydroquinone extraction with diisopropyl ether. The increase of extraction efficiency for magnesium salts was even greater than the increase observed with NaCl and Na₂SO₄ which are the most common salting-out agents used during the sample preparation by either liquid-liquid extraction [3,4] or solid phase microextraction [11] for the analysis of various phenolic compounds. This extraction efficiency increase in the case of hydroquinone extraction with diisopropyl ether went up to 30%.

In order to check whether the results of salts influence on the extraction of catechol and hydroquinone from aqueous solutions are applicable to the real samples we investigated the diisopropyl ether extraction of these compounds from spiked urine samples. Figure 3 shows UV spectra of diisopropyl ether extracts ($V_{aq}/V_{org} = 1:1$) of urine samples spiked with catechol (0.1 g dm^{-3}) for NaCl saturated and salt-free samples.

According to the data obtained for the extraction from aqueous solutions an absorbance increase of 18.9% for NaCl saturated sample in comparison to salt-free sample was expected. Actually, we observed an absorbance increase of 20.3% for this system. For the extraction of catechol from KCl saturated urine sample the absorbance increase of 11.9% was observed (expected value - 10.9%), and for the extraction of hydroquinone from NaCl saturated urine sample the absorbance increase of 68.5% was observed (expected value - 72.2%) in comparison to salt-free sample. As can be seen, reasonably good agreement between the measured and calculated absorbance increase for the diisopropyl ether extracts of salt saturated urine samples in comparison to salt-free samples was obtained for a wide range of values (~10% to ~70%). We believe these results proved that the study of salting-out effect in aqueous solutions could be used as a good model system for a reliable choice of optimal conditions for the extraction of catechol and hydroquinone from real samples. Some preliminary results of this study have already been used for the optimization of catechol determination in human urine by electron spin resonance spectroscopy [12].

CONCLUSIONS

Based on the results of this study we may recommend $MgCl_2$ or $MgSO_4$ as salting-out agents for the catechol and hydroquinone extraction instead of NaCl and Na_2SO_4 which are commonly used during the sample preparation for the analysis of various phenolic compounds. We also demonstrated that the study of salting-out effect in aqueous solutions could be used for the reliable choice of optimal conditions for the extraction of catechol and hydroquinone from real samples.

Acknowledgement: Ministry of Science and Technologies of the Republic of Serbia is gratefully acknowledged for financial support of this research.

REFERENCES

1. R. Heinrich-Ramm, M. Jakubowski, B. Heinzow, J.M. Christensen and E. Olsen, Biological Monitoring for Exposure to Volatile Organic Compounds (VOCs). *Pure Appl. Chem.*, **72** (2000) 385.
2. O. Inoue, K. Seiji, M. Kasahara, H. Nakatsuka, T. Watanabe, S.-G. Yin, G.-L. Li, S.-X. Cai, C. Yin and M. Ikeda, Determination of Catechol and Quinol in the Urine of Workers Exposed to Benzene. *Br. J. Ind. Med.*, **45** (1988) 487.
3. B.L. Lee, H.Y. Ong, C.Y. Shi and C.N. Ong, Simultaneous Determination of Hydroquinone, Catechol and Phenol in Urine Using High-Performance Liquid Chromatography with Fluorimetric Detection. *J. Chromatogr.*, **619** (1993) 259.
4. P. Hotz, P. Carbannelle, V. Haufroid, A. Tschopp, J.P. Buchet and R. Lauwerus, Biological Monitoring of Vehicle Mechanics and Other Workers Exposed to Low Concentrations of Benzene. *Int. Arch. Occup. Environ. Health*, **70** (1997) 29.
5. N. Rothman, W.E. Bechtold, S.-N. Yin, M. Dosemeci, G.-L. Li, Y.-Z. Wang, W.C. Griffith, M.T. Smith and R.B. Hayes, Urinary Excretion of Phenol, Catechol, Hydroquinone, and Muconic Acid by Workers Occupationally Exposed to Benzene. *Occup. Environ. Med.*, **55** (1998) 705.
6. Ya.I. Korenman and T.N. Ermolaeva, Ekstraktsionnoe vydelenie dioksibenzolov iz vodnykh sred. *Zh. Prikl. Khim.*, **62** (1989) 114. (in Russian)
7. Ya.I. Korenman, T.N. Ermolaeva, E.V. Bobrinskaya and L.A. Kharitonova, Ekstraktsiya poligidroksibenzolov iz vodnykh rastvorov gidrofil'nykh spiritami. *Zh. Prikl. Khim.*, **67** (1994) 1569. (in Russian)

8. L.A. Jones, J.B. Prabel, J.J. Glennon, M.F. Copeland and R.J. Kavlock, Extraction of Phenol and its Metabolites from Aqueous Solution. *J. Agric. Food Chem.*, **41** (1993) 735.
9. H. Tanaka, Y. Yamamoto and H. Chuman, Determination of the Distribution Coefficients of Phenol Derivatives and Others Based on a Volume Ratiometry Coupled with Shake-Flask Method. *Anal. Sci.*, **18** (2002) 485.
10. G.M. Nikolić, R.S. Nikolić and P.I. Premović, Spectrophotometric Study of Catechol Oxidation by Aerial O₂ in Alkaline Aqueous Solutions Containing Mg(II) Ions. *Spectrosc. Lett.*, **31** (1998) 327.
11. M. de Fatima Alpendurada, Solid-Phase Microextraction: A Promising Technique for Sample Preparation in Environmental Analysis. *J. Chromatogr. A*, **889** (2000) 3.
12. G.M. Nikolić, P.I. Premović, M.Ž. Abramović, B.Lj. Milić and J.M. Čanadović-Brunet, Quantitative Analysis of Catechol in Human Urine by ESR Spectroscopy. *Eur. J. Clin. Chem. Clin. Biochem.*, **33** (1995) A80.

UTICAJ ISOLJAVANJA NA EKSTRAKCIJU KATEHOLA I HIDROHINONA IZ VODENIH RASTVORA I URINA

**Goran M. Nikolić, Jelica M. Perović,
Ružica S. Nikolić, Milorad M. Cakić**

Ispitivan je uticaj amonijum, natrijum, kalijum i magnezijum hlorida i sulfata na ekstrakciju katehola i hidrohina dietil i diizopropil etrom. Sve soli ispitivane u ovom radu su znatno povećale efikasnost ekstrakcije i katehola i hidrohina iz vodenih rastvora, pri čemu su najbolji efekat izoljavanja pokazale magnezijumove soli. Efikasnost ekstrakcije u prisustvu magnezijumovih soli u odnosu na efikasnost ekstrakcije iz vodenih rastvora bez dodatka soli povećana je od 14% (kod ekstrakcije katehola dietil etrom) do čak 90% (kod ekstrakcije hidrohina diizopropil etrom). Ekstrakcija uzoraka urina sa dodatkom fenolnih jedinjenja potvrdila je da se ispitivanje efekta izoljavanja iz vodenih rastvora može koristiti kao dobar model sistem za pouzdan izbor optimalnih uslova za ekstrakciju katehola i hidrohina iz realnih uzoraka.