Extraction, Identification and Determination of di-(2-ethylhexyl) Phthalate (DEHP) Plasticizer in Some Stored Blood Samples Bags Using Different Spectroscopic Techniques.

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Abstract

The di-(2-ethylhexyl) phthalate (DEHP) was extracted using different solvents from plastic blood bag. The extracted product was identified using FT-IR, NMR (1H and 13C), DEPT, COSY, HMBC and HSQC_TOCSY spectrometry. The extracted plasticizer was tested in complex formation with Fe^{2+} and Cr^{3+} using UV-visible spectrophotometric method. The migration of the plasticizer from the blood bags to the blood was studied and determined during different storage times depending upon the formation of complexes with Fe^{2+} and Cr^{3+}, and the change in the concentration of Fe^{2+} and Cr^{3+}.

Key words: Extraction, identification, determination, DEHP, blood bag samples, different spectroscopic techniques.

Introduction

Plastics have found wide usage in medical and paramedical applications as well as for food containers and wrapping materials. In the past, much attention was directed at the physical, chemical, and mechanical properties of these materials in meeting the requirements for developing suitable products. However, more recently, attention has been focused on the potential toxic liability of these products when they are used for medical purposes or as contaminants of the food or materials which may come into contact with the human body [1].

The use of plasticized polyvinyl chloride (PVC) for toys and medical devices has been under attack from various environmental and healthcare activity groups. Their concerns are related to the contention that, under certain conditions, small amounts of the plasticizer may leave the flexible PVC compound referred to as leaching, migrating, or extraction. This finding has raised questions of possible hazard for man exposed to such levels of plasticizer. The extracted plasticizer could then enter the human body and cause damage ranging from hormone disruption to cancer [2].

The first poly (vinyl chloride) blood bag containing di-2-ethyl hexyl phthalate (DEHP) as plasticizer was introduced in medicine in 1950. Since then, millions of liters of blood collected in plastic have been transfused [1]

Plasticizers work by embedding themselves between the chains of plastic polymers, spacing them apart (increasing of the "free volume"), thus making them softer. They usually have low molecular weight and thus have the tendency to migrate from packing material into the surrounding. Since the plasticizer is not bound to the plastic resin, it can migrate into foods then begin ingested and absorbed into body, as well as during blood transfusion from PVC blood bags [2].

Phthalates are suspected to be endocrine distributing chemicals exhibiting carcinogenic action, for example DEHP induce liver tumors and testicular atrophy in rats [3]. Due to plasticizer potential risks for human health and environment, several phthalates have been listed as priority substances by many national and regulatory organizations [4].

Many techniques have been used to determine phthalates in several types of material such as serum and blood bags [5], like high performance liquid chromatography [6], liquid-liquid extraction and solid-phase extraction [7], capillary gas chromatography with flame ionization [8], electron capture or mass spectrometer detection[9]. All these techniques are highly performance, but very expensive and need more time for many steps.

Iron is involved in energy metabolism as an oxygen carrier in hemoglobin and as a structural component of cytochromes in electron transport [10]. Iron concentration in blood 50-150 µg/L. The most common nutritional deficiency disease Worldwide is Iron deficiency anemia [11].

Chromium is an essential trace element and has a role in metabolism of (proteins, lipids, nucleic acids, carbohydrates, and mineral substances) [12]. It seems to have an effect in hormonal regulation as in the action of insulin and thyroid health [13]. Chromium concentration in blood is in the range 0.7-28.0 µg/L [14]. However, there are problems with conditions of chromium deficiency including diabetes cardiovascular heart Disease (CHD) and cardiovascular heart disease (CHD) [10].

The present study describes extraction, identification of phthalate plasticizer using infrared spectrometry, proton and C13 spectrometry and determination of phthalate plasticizer in the blood samples bags that are used in Erbil city hospitals depending upon its complexation with Fe^{2+} and Cr^{3+}ions present in the blood.

Materials and methods

Reagents

All chemicals used were of analytical reagent grade.

- Methanol (99.9%), ethanol (99.9%), THF, ethyl acetate, Chloroform, CS_2, o-xylene, benzene, petroleum ether (40-60ºC), n-hexane.
- Stock ferrous solution (1000ppm): 0.3511g of Ammonium ferrous sulfate was dissolved in 2ml of concentrate H_2SO_4 then diluted to 50ml with ethanol to obtain 1000ppm of ferrous ion. Working standard solutions were freshly prepared by diluting the stock solution with ethanol.
- Stock chromic solution (1000ppm): 0.3184g of Chromic sulphate was dissolved in 2ml of concentrate H_2SO_4 then diluted to 50ml with ethanol to obtain 1000ppm of Chromic ion.
- Diethyl hexylphthalate solution (1%).
- Concentrated sulfuric acid.

Samples:

- Blood samples bags were obtained from Nanakely hospital [RuhsatSahibi: Meyda Medical (Turkey)].
- Blood samples were obtained from Central blood storage in Erbil.

Extractions of the (DEHP) plasticizer

Blood bag plastics were cut and grinded. Different solvents (H_2O, methanol, ethanol, THF, benzene, petroleum ether, n-hexane, o-xylene, CS_2, ethyl acetate, chloroform) have been used to extract the plasticizer from blood bags. Plastic sample (10g) was treated different solvents in soxhelt apparatus with the extraction period 6hr and 33 cycle (6cycle/hr.). The plasticizer was separated from the solvents by distillation [15, 16].

Preparation of blood serum samples

The serum samples have been taken from blood bags with different saving time: fresh blood, blood samples after (1, 6, 11, 15, 20, 24, 31) days and even after the expired date of blood bag plastics (44 days). The serum samples were centrifuged for 20min, then ethanol was added to precipitate proteins.

Results and Discussion

Extraction of the plasticizer (DEHP):

For DEHP extraction 11 different solvents were used, the gravimetric measurement shows that the percentage of extraction increases as the solvents change from polar to non-polar and the maximum extraction was with ethyl acetate. The results are shown in Table (1) and Figure (1).

Figure (1) shows the maximum absorption spectra of the extracted DEHP using different solvents in the case of using ethyl acetate as a solvent.

Identification of DEHP

To identify DEHP various spectroscopic techniques have been used. Figure (1) shows the normal spectra of the extracted DEHP using different solvents with maximum absorption at 325 nm. It seems that ethyl acetate and chloroform best solvents for the extraction of DEHP. Ethyl acetate was selected as the solvent for extraction of EDHP.

The skeleton of the extracted DEHP confirmed by spectroscopic methods like FT-IR, 1H-NMR, 13C-NMR and 13C-DEPT-135 that show that the extracted plasticizer is di-(2-ethylhexyl phthalate) (DEHP). The IR spectrum Figure(2) showed a band at (1721) cm^{-1} attributed to (C=O) group of esters, two strong bands at 1255cm^{-1}, 1098cm^{-1} referring to (O=C-O-C) bonds, and a characteristic band at 965cm^{-1} refer to vinyl esters bond. Chemical shifts, multiplicities and *J* values of protons were determined from 1H-NMR spectrum and further confirmed by 2D- COSY. Numbers, multiplicities and 1H - ^{13}C correlations were determined from 1D ^{13}C-NMR (Table 2), DEPTQ and 2D HSQC and HMBC (Figurs3-9). The assignment of all protons and carbons was further confirmed by HSQC-TOCSY 2D spectrum (Figure 10).

C1, C2 and C3 all give signals in ^{13}C NMR in the range typical for aromatic carbons. DEPTQ shows an inverted signal for C3, which makes it a quaternary aromatic C. HSQC assigns the proton at 7.46 to C1 and the second one at 7.63 to C2. This is further confirmed by HMBC, which shows that the proton at 7.63 is the one next to C3. C4 is a quaternary C (a very short inverted signal in DEPTQ and no protons attached from HSQC), and it's clear from the chemical shift 167 that it should be an ester (or amide). HMBC reveals correlation between this carbon and H5, also a strong correlation with the aromatic proton H2 and a weaker one with H1.

Both DEPTQ and HSQC present C5 as CH2 (secondary C). HMBC correlates it to H6, H7, H11 and C4. The next carbon in the series C5, on the other hand is seen as a primary carbon (CH), bonded to the proton at 1.61, and correlated to protons H5, H7, H11 and H12. The lineup of carbon and protons in the alkyl side chains was determined from COSY which relates CH2 – CH3 (C11 and C12) together, and similarly puts carbons from C6 through to C10 in a linear arrangement.

All the above assignments were further confirmed by HSQC-TOCSY which shows the aromatic protons to be in one system separated from all the other protons.

^1H NMR (400 MHz, $CDCl_3$) δ 7.63 (dd, *J* = 5.7, 3.3 Hz, 1H), 7.46 (dd, *J* = 5.7, 3.3 Hz, 1H), 4.15 (ddt, *J* = 11.0, 6.0 Hz, 2H), 1.61 (tt, *J* = 12.3, 6.1 Hz, 1H), 1.35 (dq, *J* = 7.3, 2.3 Hz, 2H), 1.31 – 1.20 (m, 6H), 0.85 (t, *J* = 7.5 Hz, 3H), 0.82 (t, *J* = 6.9 Hz, 3H).

δ C (101 MHz, $CDCl_3$) 167.77, 132.45, 130.90, 128.81, 79.42, 77.36, 77.04, 76.73, 68.15, 38.72, 30.36, 28.93, 23.74, 23.00, 14.07, 10.97.

The analysis mentioned insure the identification of the plasticizer as di-(2-ethylhexyl phthalate) (DEHP).

Complex Formation

The study of the ability of DEHP to react with Fe^{2+} and Cr^{3+} ions, which are present in the blood, depends upon the complex formation of Fe^{2+} and Cr^{3+} with DEHP. The effect of the medium type and the concentration of DEHP on the formation of the complexes were studied at 325 nm for Fe^{2+} and 365 nm for Cr^{3+}.

1. Metal ion concentration

Two metal ions were tested in complex formation Fe^{2+} and Cr3+. The selection of the metal ion was according to its existence in the blood.

In Fe^{2+} and Cr^{3+} solutions, the concentration of (10ppm) was selected from series of conc. (5, 10, 15, 20, and 25) ppm, which shows good results for complex formation.

2. Concentration of phthalate plasticizer

The effect of different volumes of DEHP (1.0%) (0.1, 0.2, 0.3, 0.4, and 0.5) mls were tested. In the case of Fe^{2+} ions, 0.2ml of DEHP gave best results and for Cr^{3+} 0.4ml of DEHP gave best results.

3. Bases

For basic medium different 0.1N bases were tested (sodium hydroxide, potassium hydroxide and sodium carbonate, ammonium hydroxide and aniline), a colloidal solution was formed in the case of inorganic bases. The aromatic amine (aniline) was weaker base than NH_3, aniline leads to more clear solution.

4. Acid

For acidic medium 0.1N HCl was used.

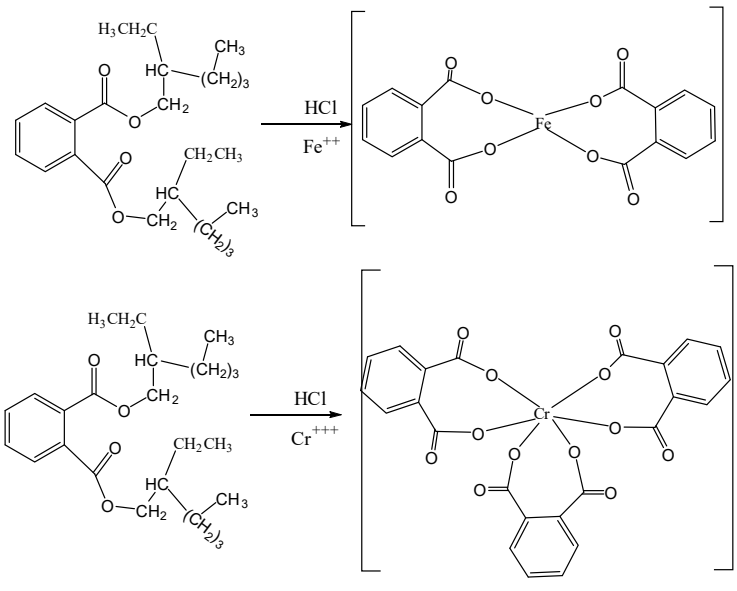
Ethanol has been investigated as a medium for complex formation rather than water because of the hydrophobicity of phthalate plasticizer [17].

Calibration curve

Using the previously optimum conditions, the final absorption spectra shown in Figures (11),(12),(13),and(14) two straight lines of calibrations curves were obtained, the maximum absorption of Fe^{2+}complex was 325 nm and for Cr^{3+} complex was 365nm, whereas, the blank has no absorbance in these regions. with a slightly colored system that followed Beer's law over the concentration range of Fe^{2+} ion (0.5-5ppm) and correction coefficient 0.9992, also Cr^{3+} was followed beer's law over the concentration range (0.5-7ppm) and correlation coefficient 0.9994.

Nature of the complex

The jobs method was used to study the stoichiometry of Fe^{2+} and Cr^{3+} complexes with DEHP which showed that the ratio of Fe^{2+} and Cr^{3} are 1:2 and 1:3 respectively as follow,(Figures 15(a)and(b)respectively).



Application of the method

The proposed method was applied to determine the quantity of phthalate plasticizer that migrate from blood plastic bag into the blood during its storage by study the ability of complex formation of phthalate plasticizer in acidic and basic media with Fe^{2+} and Cr^{3+} ion that present in blood. Since the blood is slightly alkaline with a pH between 7.35 and 7.45, a decrease in pH value from 7.30 to 6.0 cause hemoglobin hemolysis.

This may explain by hemolysis of the red cells that increases due to processing and during storage and is maximum during the first week.

Conclusion

The aim of this study is to extract a maximum percent of plasticizer using solvents with different polarity from plastic blood, typically, the total plasticizer in PVC is about 20%for minimum flexibility to 60%to avoid exudation, maintain flexibility, and for economy, preferably 35-50%, based on the weight of polymeric substance. Our study shows that the maximum percent of plasticizer extraction were in using with polarity range (4.4-2.5), and identify the type of the extracted plasticizer that is effective in the suppression of the auto hemolysis of the stored blood which is di (2-ethylhexyl) phthalate (DEHP) in plastic blood bag.

Noticeably, in our study, the concentration of di-(2-ethylhexyl) phthalate (DEHP) was increased in the blood samples followed by decreasing of the absorbance of the considered complex of plasticizer with Fe^{+2}&Cr^{+3} especially in the normal &basic medium, since the plasticizer has been effective to decrease the rate of hemolysis during storage. The mechanism of hemolysis suppression by DEHP is unknown, but it could be assuming that plasticizer may be embody into and stabilize the lipid bilayer of the RBC membrane, because of the possibility that plasticizer molecules having the two carboxy-ester groups oriented to the same direction of the ortho position may form a pair at the inner and outer sides of the RBC membrane and act as one component of the lipid bilayer. On the other hand, in case the orientation of the acyl groups is different from each other, the plasticizer molecules may be also embodying into the lipid bilayer, but not form such a pair. And the degree of hemolysis suppression effect is due in part to the concentration of the plasticizer [18].

In acidic medium, the absorbance increase and complex formation increase due to increase in hemolysis of red blood cell and realize its components when the serum acidified with 0.1 N HC1 instead of un acidified serum, Stored blood for transfusion has a low pH.. The anticoagulant contains citric acid. When mixed with the blood the pH drops. PCO_2 rises because of the action of acid on bicarbonate ions. Most of the pH drop is due to this CO_2 which does not escape from the stored blood plastic bags. The non-respiratory pH of the stored blood is not as low as the actual pH. The pH of stored blood does not fall progressively if stored for up to three weeks at 4[19].

We decide to study the extent of hemolysis up to Day 28 of storage only since the hemoglobin at day 40 of storage appear straw colored to slightly red at the low end and visibly red at the upper end and most of RBC units get issued within this period, but there is an absorbance for the complex in this period this show the effectiveness of DEHP plasticizer to improves RBC storage by reducing hemolysis. In a concern of this the toxicity of DEHP has been questioned, thus in our Application Notes demonstrates that, for the blood acceptors it is better to take blood bag with minimum period of storage or from blood donators after proper testing is performed to reduce the risk of transfusion hemolysis.

It is recommended for use of blood product that is five days old or less, to "ensure" optimal cell function [20]. Also, some hospital blood banks will attempt to accommodate physicians' requests to provide low-aged RBC product for certain kinds of patients (e.g. cardiac surgery) [21].

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Table (1): The percentage of DEHP extraction using different solvents.

Solvents	Polarity	Absorbance	% Extraction
H_2O	10.2	0.161	1.09
Methanol	5.1	0. 862	17.3
Ethanol	5.2	0. 926	19.2
THF	4	1.15	44.9
Ethyl acetate	4.4	1.31	47.8
Chloroform	4.1	1.27	46.2
Carbon disulfide	0	1.18	44.7
o-Xylene	2.5	1.15	43.3
Benzene	2.7	1.01	42.0
Petroleum ether (60-80 °C)	0.1	0.908	19.4
n-Hexane	0	0. 839	15.7

Table (2): The ^{1}H- nmr and ^{13}C-nmr data of di-(2-ethylhexyl) phthalate (DEHP).

Label	δ ^{1}H	Multiplicity	*J* Hz	δ ^{13}C	Multiplicity
C1	7.46	dd	5.74, 3.26	130.9	CH
C2	7.63	dd	5.75, 3.26	128.81	CH
C3	n/a	n/a	n/a	132.45	C
C4	n/a	n/a	n/a	167	C
C5	4.15	Ddt (or m)	11.02, 11.02, 6.00	68.15	CH_2
C6	1.61	tt	12.25, 6.12	38.72	CH
C7	1.28	*	*	30.36	CH_2
C8	1.24	*	*	28.93	CH_2
C9	1.23	*	*	23	CH_2
C10	0.82	t	6.92	14.07	CH_3
C11	1.34	dq	7.26, 2.30	23.74	CH_2
C12	0.85	t	7.48	10.97	CH_3

* Haven't been determined because of signal overlap.

Table (3): Change in the Absorbance of blood samples with the ability of complex formation of phthalate plasticizer with Fe^{2+}.

Days of storage	Absorbance of 5 ml of serum	ppm	Absorbance of 1ml of serum +0.2ml DEHP (acidic media)	ppm	Absorbance of 1ml of serum+0.2ml DEHP(basic media)	ppm
Fresh	0.738	19.83	0.317	5.76	0.219	2.49
1	1.410	42.27	0.368	7.47	0.201	1.89
6	1.404	42.10	0.379	7.83	0.184	1.32
11	1.178	34.51	0.391	8.24	0.176	1.05
15	0.813	22.33	0.403	8.64	0.143	0. 050
20	0.675	17.70	0.411	8.90	0.115	0. 986
24	0.432	9.60	0.417	9.10	0.097	1.59
31	0.243	3.29	0.423	9.30	0.071	2.46
44	0.147	0.0835	0.428	9.47	0.054	3.02

Table (4): Change in the Absorbance of blood samples with the ability of complex formation of phthalate plasticizer with Cr^{3+}.

Days of storage	Absorbance of 5 ml of serum	ppm	Absorbance of 1ml of serum +0.4ml DEHP (acidic media)	ppm	Absorbance of 1ml of serum +0.4ml DEHP (basic media)	ppm
Fresh	0.820	36.51	0.185	5.29	0.252	8.59
1	1.703	79.91	0.205	6.28	0.243	8.15
6	1.524	71.11	0.264	9.18	0.235	7.76
11	1.321	61.13	0.333	12.57	0.217	6.87
15	0.987	44.72	0.357	13.75	0.202	6.13
20	0.795	35.28	0.367	14.24	0.164	4.27
24	0.456	18.62	0.376	14.69	0.125	2.35
31	0.174	4.76	0.391	15.42	0.106	1.42
44	0.082	0. 236	0.398	15.76	0.095	0.875

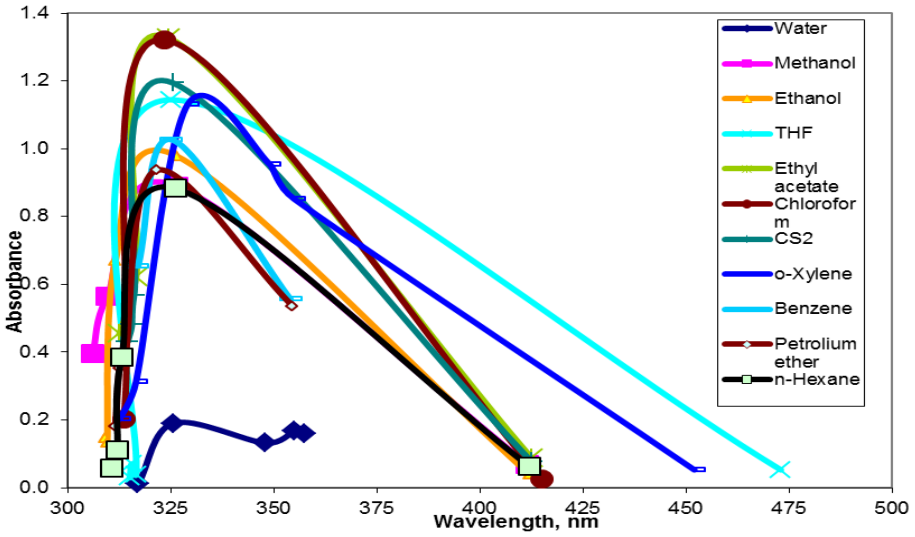

Figure (1): Effect of different solvent on the extraction of DEHP.

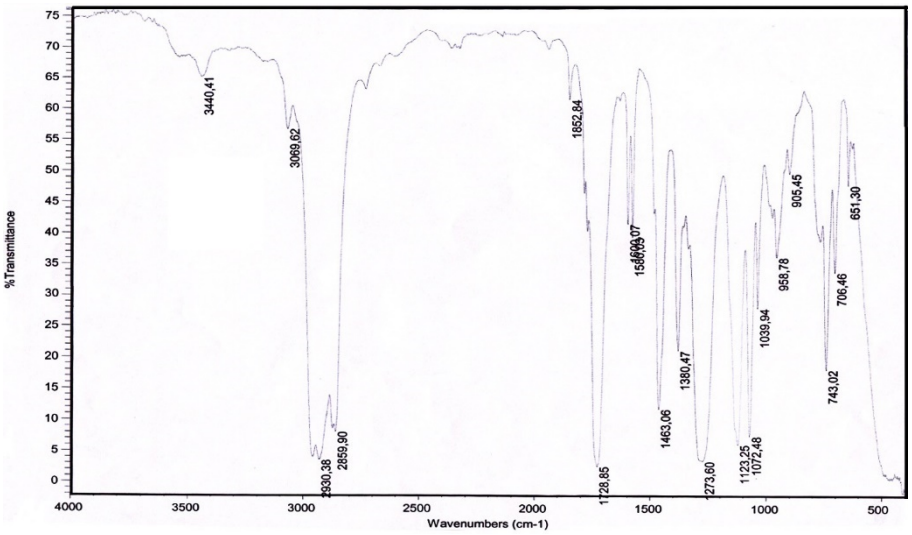

Figure (2): IR spectrum of di-(2-ethylhexyl) phthalate.

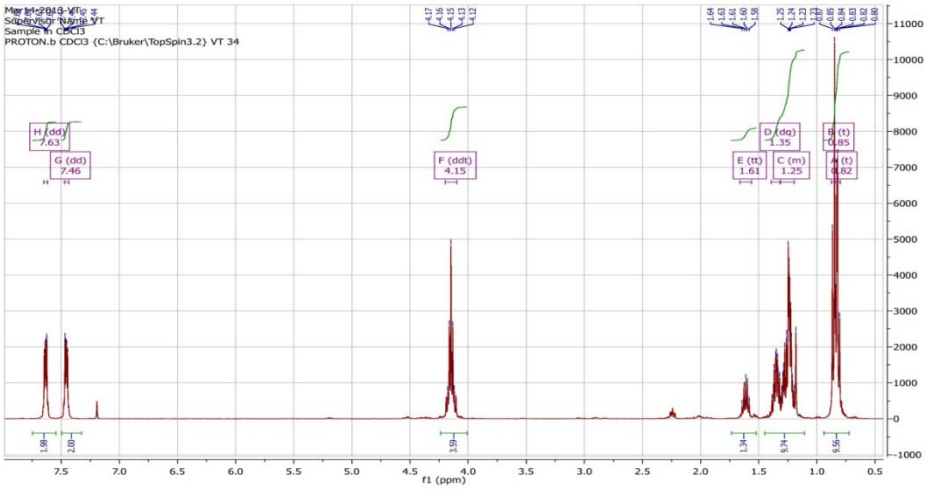

Figure (3): NMR (^{1}H) spectrum of di-(2-ethylhexyl) phthalate.

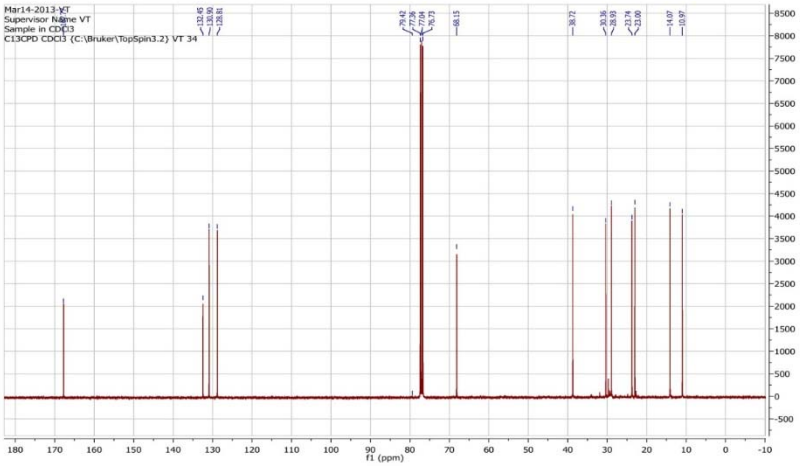

Figure (4): ^{13}C-nmr spectrum of di-(2-ethylhexyl) phthalate.

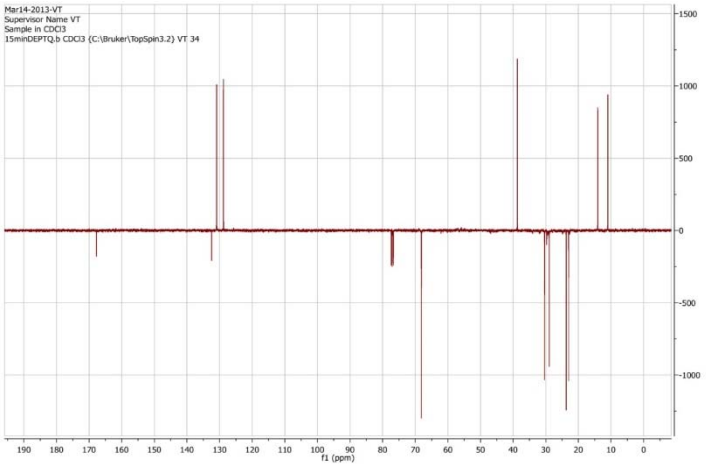

Figure (5): Dept135 spectrum of di-(2-ethylhexyl) phthalate.

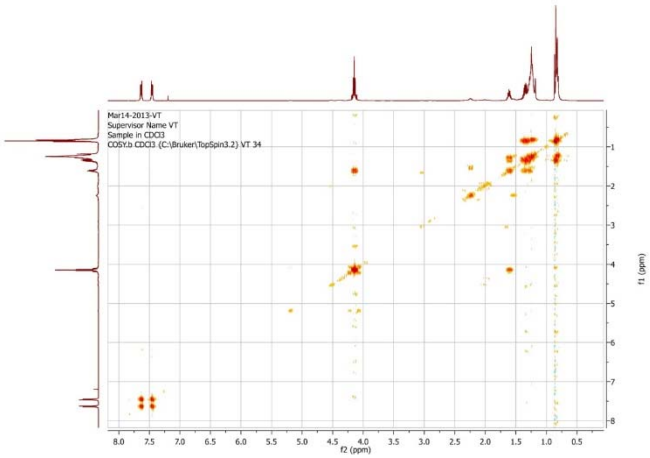

Figure (6): COSY.spectrum of di-(2-ethylhexyl) phthalate.

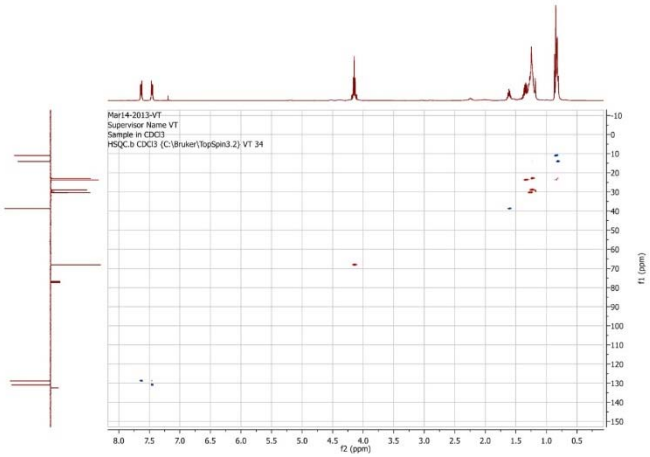

Figure (7): HSQC.spectrum of Di-(2-ethylhexyl) phthalate.

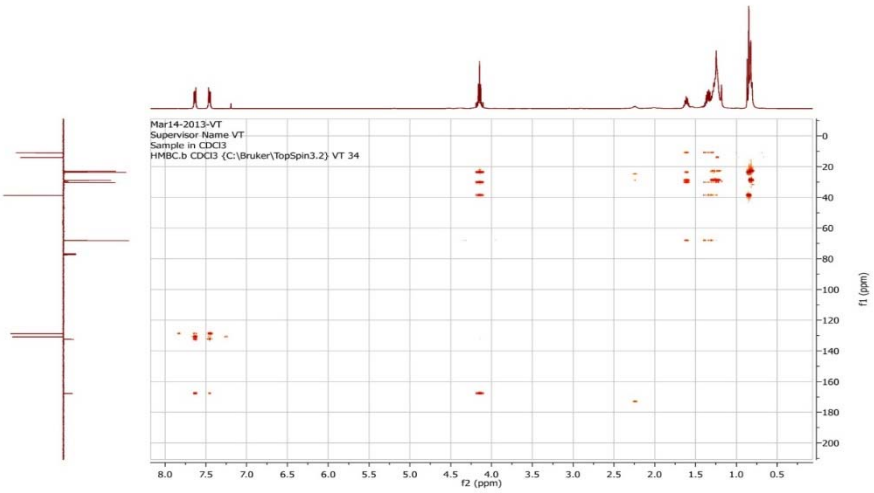

Figure (8): HMBC.spectrum of di-(2-ethylhexyl) phthalate.

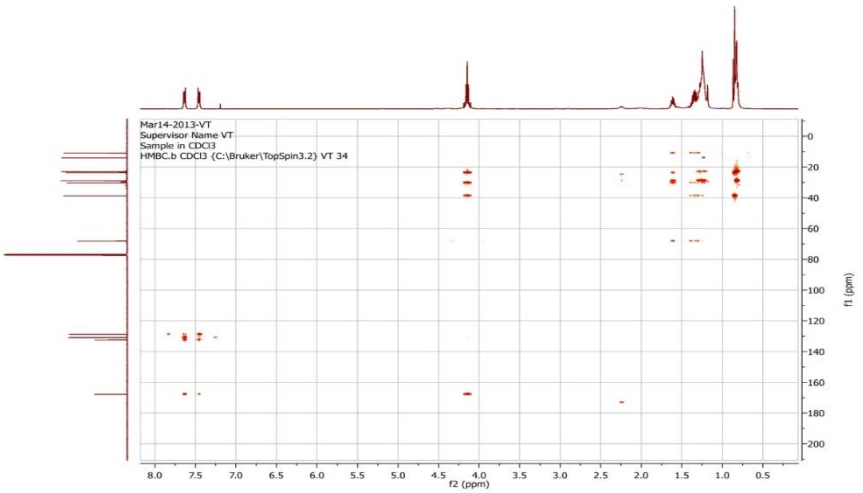

Figure (9): HMBC.spectrum of di-(2-ethylhexyl) phthalate.

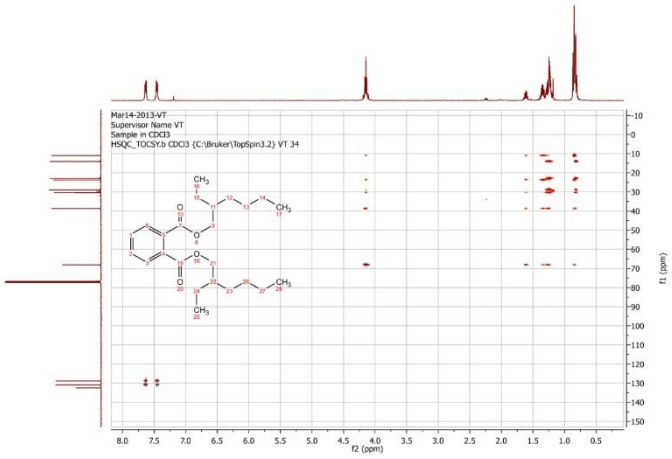

Figure (10): HSQC-TOCSY spectrum of Di-(2-ethylhexyl) phthalate.

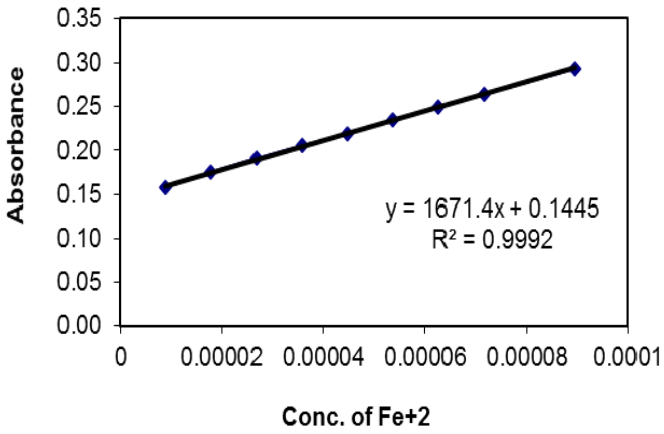

Figure (11): Calibration curve of Fe^{2+}.

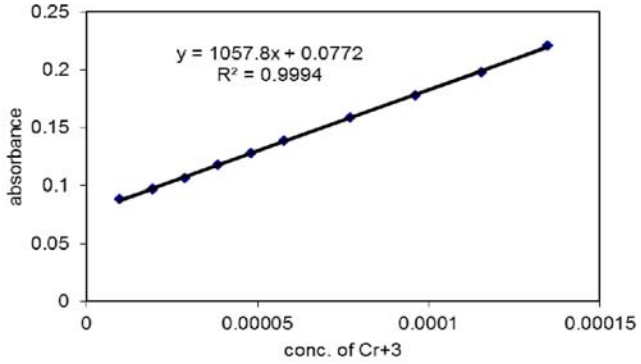

Figure (12): Calibration curve of Cr^{3+}.

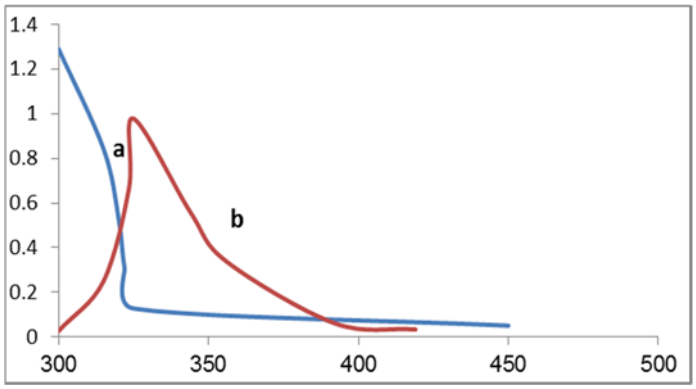

Figure (13): Absorption spectra of: a. Blank solution against ethanol, b: Fe^{2+} complex with DEHP against blank.

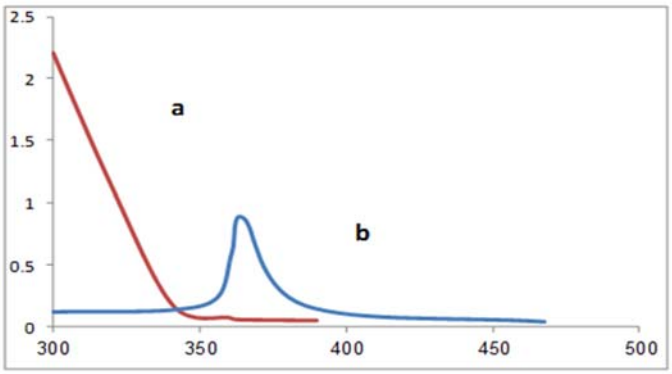

Figure (14): Absorption spectra of: a. Blank solution against ethanol, b: Cr^{3+} complex with DEHP against blank.

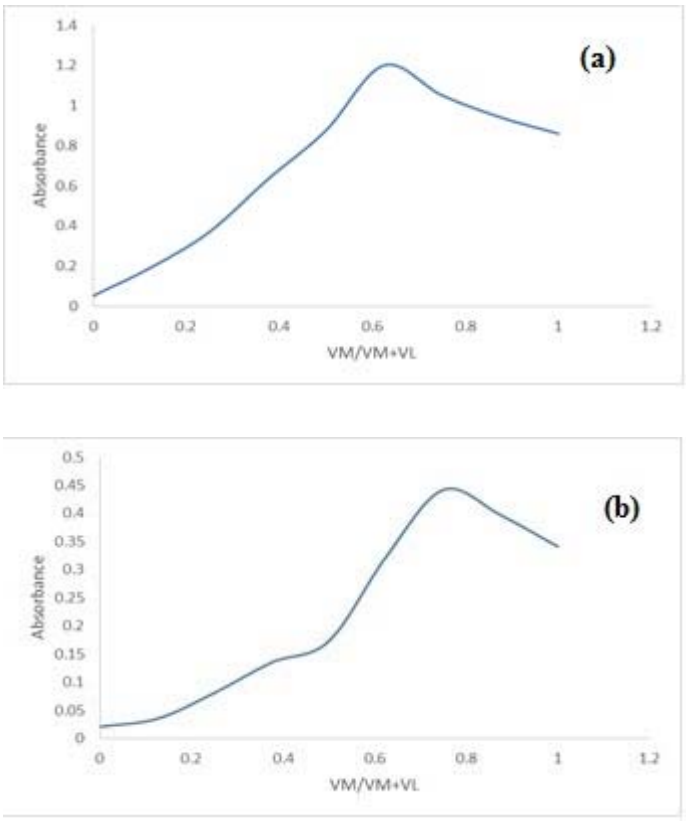

Figure (15): Stiochiomctry of:(a)Fe^{2+} Complex,(b)Cr^{3+}Comlex

استخلاص و تعيين و تقدير الملدن ثنائى –(2-أثيل هكسيل) فثاليت في بعض اكياس حفظ الدم باستعمال تقنيات مطيافية مختلفة

داريا جليل
نبيل عادل
قسم الكيمياء/كلية التربية/جامعة السليمانية

استلم في:20/أيلول/2015 ،قبل في:28/كانون الأول/2015

الخلاصة

تتضمن هذه الدراسة استخلاص الملدن من اكياس الدم باستعمال مذيبات مختلفة. و ثم تشخيص الملدن المستخلص بوساطة الطرائق الطيفية التالية: طيف الأشعة تحت الحمراء و طيف الرنين النووي المغناطيستقةي بأنواعه (COSY, ^{1}H-NMR , ^{13}C-NMR,,DEPT HSQC, HMBC, HSQC_TOCSY) وتم اختيار قابلية الملدن لتكوين معقد مع كل من ايونات الحديد(II) والكروم (III) وذلك عن طريق مطيافية الاشعة المرئية وفوق البنفسجية.
وفي هذه الدراسة اختبرنا قابلية انتقال الملدن من اكياس الدم الى الدم عن طريق تكوين معقد مع ايونات الحديد والكروم الموجودة في الدم، ودراسة التغيير في تراكيز الايونات مع زيادة مدة الخزن وعلاقته مع قابلية الملدن للانتقال.

الكلمات المفتاحية:استخلاص، تشخيص، تقدير،DEHP،نماذج أكياس دم، تقنيات مشتقة الطيف