

## Review Using 2,4-dinitrophenylhydrazine in spectrophotometric determination

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### Abstract

2,4-Dinitrophenylhydrazine (2,4-Dnphh) is substituted hydrazine. It is structure contains hydrazine which is the main active functional group. 2,4-Dnphh is an important reagent in analytical chemistry it is used firstly in the identification (qualitative analysis) of carbonyl groups (aldehyde and ketones compounds). 2,4-Dnphh is react with aliphatic and aromatic carbonyl compounds to produce the corresponding 2,4-dinitrophenyl-hydrazone. The aromatic carbonyls give red precipitates compared with aliphatic carbonyls giving more yellow color. The two colored products were achieved according to the addition-elimination reaction, the reaction included nucleophilic addition of amino group (-NH<sub>2</sub>) to the carbonyl group(C=O), then the elimination of the H<sub>2</sub>O molecule. Also, a modification of 2,4-Dnphh to produce a derivative can be used as a chemical sensor for some colorimetric detection of various compounds. Recently 2,4-Dnphh is used in quantitative spectrophotometric estimation of various compounds via oxidative coupling reaction and diazotization-coupling reaction, and also 2,4-dinitrophenylhydrazone-compound prepared from the reaction of 2,4-Dnphh with compound to be determined give good results in injection to the column of high-performance liquid chromatography. The present review included all methods as possible in the literature including the uses of 2,4-Dnphh in the qualitative and quantitative determination of various compounds.

### Introduction:

2,4-Dinitrophenylhydrazine is one of the important organic compounds has the chemical formula [ C<sub>6</sub>H<sub>3</sub>(NO<sub>2</sub>)<sub>2</sub>NHNH<sub>2</sub>]. 2,4-Dnphh is a solid compound with orange-red color, it is chemical structure (Figure 1) contains two nitro groups in ortho and para positions to hydrazine so it is substituted hydrazine.

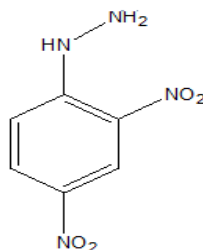
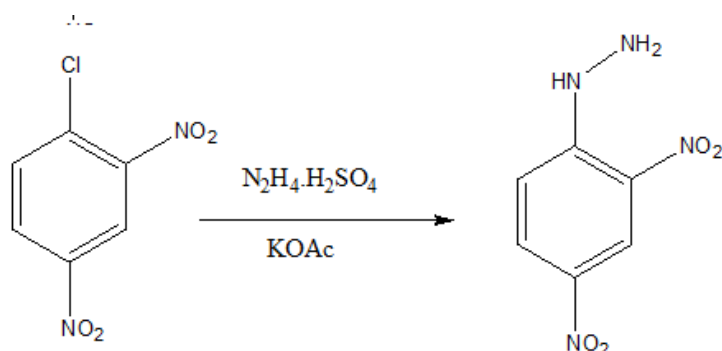


Fig. 1: The chemical structure of 2,4-Dnphh.

2,4-Dnphh can be prepared by the reaction of 2,4-dinitrochlorobenzene with hydrazine sulphate in the presence of  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$  and KOAc (Scheme 1)[1].



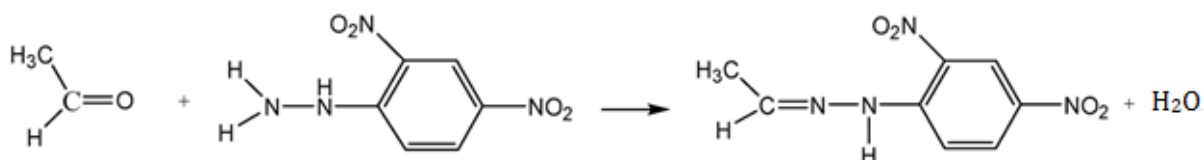
**Scheme 1:** Preparation of 2,4-Dnphh.

Dissolving 2,4-Dnphh in aqueous solution to prepared Brady's reagent, to be used for the identification of carbonyl compounds (aldehyde and ketone).

Other researchers prepared Brady's reagent or Borche's reagent, by dissolving 2,4-dinitrophenylhydrazine in a mixture solution containing methanol and a few milliliters of concentrated sulfuric acid[2].

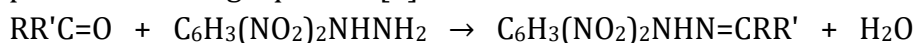
The formation of a yellow, orange or red precipitate is indicated as a positive test of the presences of carbonyl groups. The aromatic carbonyls give red precipitates compared with aliphatic carbonyls give a more intense yellow color.[3]

2,4-Dnphh can reacts with aldehydes and also with ketones to form a 2,4-dinitrophenylhydrazone. An example reaction with ethanal [4] (Scheme 2).



**Scheme 2:** Reaction of 2,4-Dnphh with ethanal.

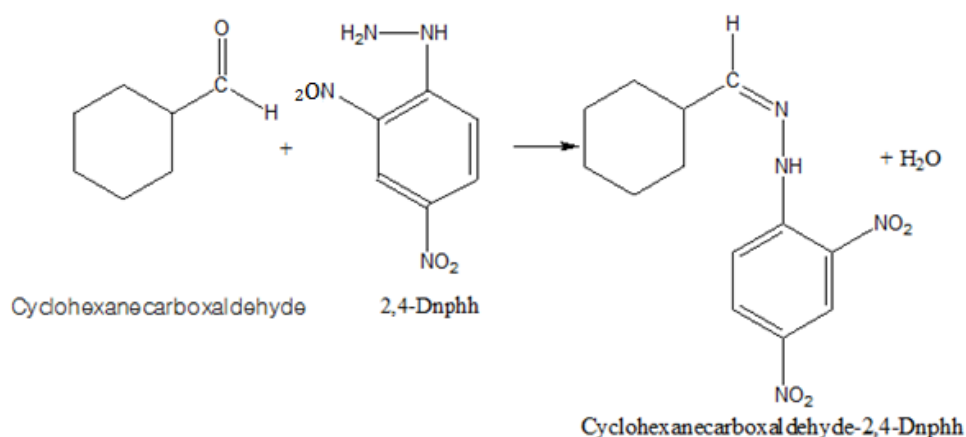
The condensation reaction to form the product ( hydrazone) from the reaction of ketone with 2,4-Dnphh included loss of water, this suggested mechanism can be considered as an example of addition-elimination reaction, of the amino group ( $-\text{NH}_2$ ) undergo nucleophilic addition to the carbonyl group( $\text{C}=\text{O}$ ), then the elimination of a water molecule, as per the following equation:[5].



Recently a modification of the derivative 2,4- dinitrophenylhydrazine dithiocarbamate - silver nanoparticles can be used as am chemical sensor for some colorimetric detection of various compounds such as omeprazole [6].

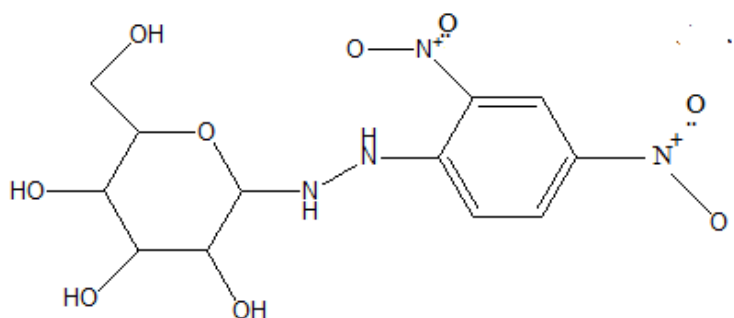
The liquid carbonyl compounds, for example, cyclohexanone can react with 2,4-Dnphh to produce solid derivatives. The reaction happens at one of the two  $\text{NH}_2$  groups. The ion-pair

electrons of the NH<sub>2</sub> group attached to the carbonyl is less nucleophilic compared to those of the other NH<sub>2</sub> group (Scheme 3) [7].



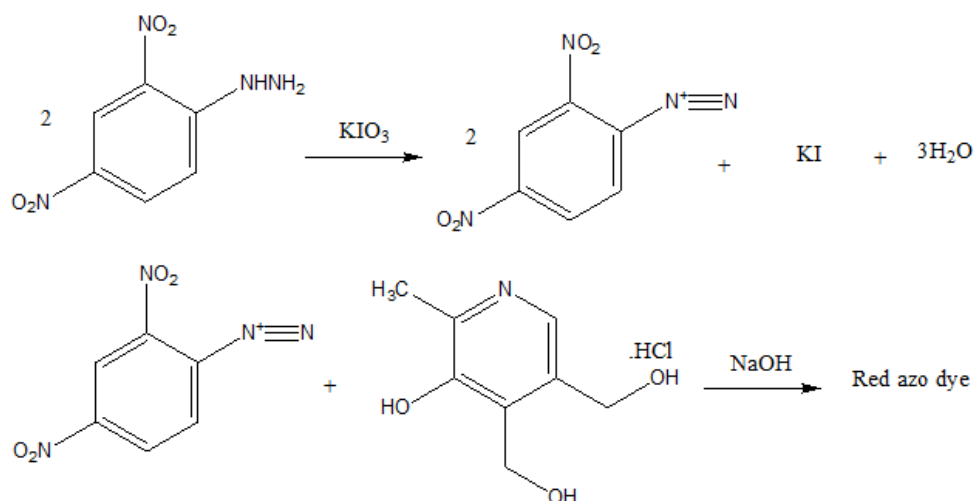
**Scheme 3:** The suggested reaction of cyclohexanone with 2,4-Dnphh.

2,4-Dnphh has been often used as a derivatizing agent for carbonyl compounds, including reducing compounds for example glucose to form 2,4-Dnphh- glucose derivative (Scheme 4), acetaldehyde, and various carbonyl groups, and these derivatives can easily determination by HPLC techniques. [8-10].

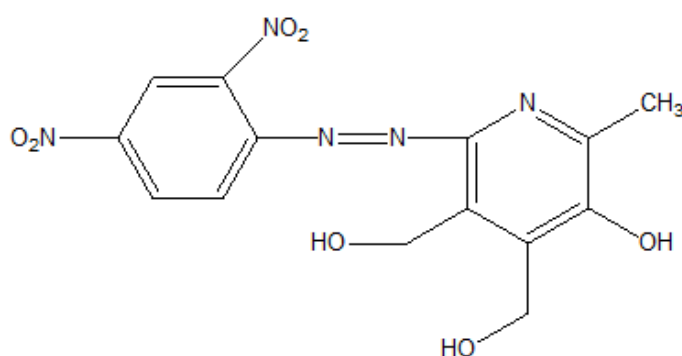


**Scheme 4:** 2,4-Dnphh- glucose derivative.

It has been shown that 2,4-Dnphh can be oxidized by various oxidizing agents such as potassium iodate or sodium periodate to produce diazotized 2,4-Dnphh as an intermediate compound which later can be under go coupling reaction with other compounds such as pyridoxine hydrochloride in alkaline medium to produce red azo dye that has the highest absorption at the wavelength of 522 nm. The proposed reactions as shown below in Scheme 5 [11].



**Scheme 5:** The proposed reactions to produce the red azo dye  
The suggested structure of red azo dye is shown below.



Through the literature survey, many researchers used 2,4- Dnphh reagent in the estimation of various compounds. The reaction mechanism was explained concerning oxidation and coupling reaction, relying mainly on the use of the oxidizing agent in the first step of the reaction, and below Table 1 includes these types of reactions

**Table 1:** Using 2,4- Dnphh in determination various compound via oxidative coupling reaction.

Compound	$\lambda_{\text{max}}$ , nm	Linearity $\mu\text{g/ml}$	$\epsilon$ L/mol.cm	Reference
Fungicide dithianon	550	.....	.....	[12]
Selenium(IV)	520	0.03-3.5	$3.1 \times 10^4$	[13]
Vanadium	495	0.02-3.5		[14]
Vitamin C	280	.....	0.0323	[15]
Atorvastatin	479	4-10	$558.65 \times 10^3$	[16]
Ezetimibe	457	4-12	$409.34 \times 10^3$	
Acyclovir& Ganciclovir	414 450	20-60 5-25	.....	[17]
Modafinil	476	2-7	$3.3297 \times 10^5$	[18]
Carbamazepine	485	4-50	$6.7335 \times 10^3$	[19]
Bisacodyl	522	2-20	6505	[20]
Mefenamic acid	528	2-8	$3.6873 \times 10^4$	[21]
Sulphadiazine	486	3-15	$2.23 \times 10^4$	[22]
Propranolol hydrochloride	481	1-35	6224.16	[23]
Catechol	385	1-40	$5.0866 \times 10^3$	[24]
Resorcinol	404	1-25	$4.404 \times 10^3$	
Lansoprazole	484.5	1-30	13260.132	[25]
Nateglinide	480	30-100	$0.331054 \times 10^3$	[26]
Pregabalin	461	50-450	$0.273824 \times 10^3$	[27]
Clopidogrel	412	10-30	$0.8817 \times 10^4$	[28]
Gemfibrozil	421	2-10	$2.6081 \times 10^4$	

While Table 2 also includes the estimation of various compounds, the researchers suggested an explanation for the reaction based on the principle of diazotization and coupling, depending on the interpretation of the formation of corresponding diazonium salt of 2,4-Dnphh when the reagent was oxidized by the oxidizing reagents.

**Table 2:** Using 2,4- Dnphh in determination various compounds via diazotization and coupling reaction.

Compound	$\lambda_{\text{max}}$ , nm	Linearity $\mu\text{g/ml}$	$\epsilon$ L/mol.cm	Reference
Salbutamol	540	2.5-17	$2.19 \times 10^4$	[29]
Ritodrine	510	2-29	$0.74 \times 10^4$	
Amoxicillin Isoxsuprine	520	4-33	$1.00 \times 10^4$	
	520	5-30	$8.04 \times 10^4$	
Thymol	570	0.25-10	$2.2 \times 10^4$	[30]
Diclofenac sodium	600	0.5-40	$2.0994 \times 10^4$	[31]
Cefadroxil	515	7.5-75	$7 \times 10^3$	[32]
Oxymetazoline hydrochloride	607	2.5-30	7242	[33]
Sulfathiazole	492	2-28	$1.1437 \times 10^4$	[34]
Paracetamol	430	0.5-18.4 mg.mL-1	$2.776 \times 10^4$	[35]
Tetracycline hydrochloride	360	0.1-9	$1.262 \times 10^4$	[36]
Dopamine	560	5-50	$7.9 \times 10^4$	[37]
Sulphamethoxazole	435	2-14	14412.77	[38]

Table 3 included the compounds determined via condensation reaction with 2,4-Dnphh to produce the corresponding hydrazone.

**Table 3:** Using 2,4- Dnphh in determination various compounds via condensation reaction.

Compound	$\lambda_{\text{max}}$ , nm	Linearity $\mu\text{g/ml}$	$\epsilon$ L/mol.cm	Reference
Josamycin	411	10-160	.....	[39]
Eplerenone	430	5-35	$3.177 \times 10^4$	[40]
Sitagliptin phosphate	400	2-10	$0.2485 \times 10^{-4}$	[41]
Cilostazol	355	2-20	$2.2980 \times 10^4$	[42]
Haloperidol	456	3.6-10.8	$2.84 \times 10^4$	[43]
Artemisinin	310	0.5-60	$2.930 \times 10^4$	[44]
Dihydroartemisinin	300	0.5-50	$2.8 \times 10^4$	
Macrolide antibiotics	542-545	5-40	$1.48 \times 10^4$	[45]
	523-526	5-35	$2.59 \times 10^4$	
	539-542	5-35	$2.10 \times 10^4$	
Tolperrisone	520	2.5-15	$2.4 \times 10^4$	[46]
Lamivudine	438	5-35	.....	[47]
Reactive carbonyl in proteins and peptides	370	.....	.....	[48]

## Conclusion

2,4-Dinitrophenylhydrazine is an important reagent used to identify (qualitative) of carbonyl groups (aldehyde and ketones compounds), and also it is used in quantitative spectrophotometric determination of various compounds via various types of reactions, such as oxidative coupling, diazotization and coupling reaction and reacted with aliphatic and aromatic carbonyl compounds to produce the corresponding 2,4- dinitrophenyl-hydrazone (addition-elimination) reactions.

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## مراجعة: استخدام 4،2- ثنائي نيتروفينيل هيدرازين في التقدير الطيفي

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قسم الكيمياء، كلية العلوم، جامعة الموصل

## الخلاصة:

يعد 4،2- ثنائي نيتروفينيل هيدرازين من معوضات الهيدرازين. إذ يحتوي في تركيبه على الهيدرازين وهو المجموعة الوظيفية الرئيسية النشطة. ويعد كاشف مهم في الكيمياء التحليلية إذ تم استخدامه أولاً في التشخيص النوعي لمجموعات الكربونيل (مركبات الألديدات والكيلونات). وذلك بتفاعله مع مركبات الكربونيل الأليفاتية والاروماتية لإنتاج 4،2- ثنائي نيتروفينيل-هيدرازون المقابل. تعطي مركبات الكربونيل الاروماتية راسب حمراء مقارنة مع مركبات الكربونيل الأليفاتية التي تعطي على الاغلب اللون الأصفر. تم التحقق من الناتجين ذو اللونين ووجدا انهما يسيران وفقاً لتفاعل الإضافة والحذف، يتضمن التفاعل اضافة المجموعة الأمينية ( $-NH_2$ ) إلى مجموعة الكربونيل ( $C=O$ ) ، ثم عملية حذف جزيئة ماء. أيضاً يمكن استخدام الكاشف 4،2- ثنائي نيتروفينيل هيدرازين لإنتاج مشتق بوصفه مستشعر كيميائي يستخدم للكشف اللوني للمركبات المختلفة. تم استخدام 4،2- ثنائي نيتروفينيل هيدرازين مؤخرًا في التقدير الطيفي الكمي للمركبات المختلفة عن طريق تفاعل الاقتران التأكسدي وتفاعل الأزوتة والاقتران، وكذلك 4،2- ثنائي نيتروفينيل هيدرازون المحضر من التفاعل مع المركب المراد تحديده يعطي نتائج جيدة في الحقن في عمود كروماتوغرافيا السائل عال الأداء. تضمنت المراجعة الحالية جميع الطرائق المتوفرة في الأدبيات التي تضمنت استخدامات 4،2- ثنائي نيتروفينيل هيدرازين في التقدير النوعي والكمي للمركبات المختلفة.

## معلومات البحث:

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## الكلمات المفتاحية:

4،2- ثنائي نيتروفينيل

هيدرازين، الديهايدات

وكيتونات، طرائق طيفية، الاكسدة

والاقتران، الأزوتة والاقتران

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