

Spectrophotometric Determination of Dibenzoylmethane by Diazocoupling Reaction in Pharmaceutical Preparations

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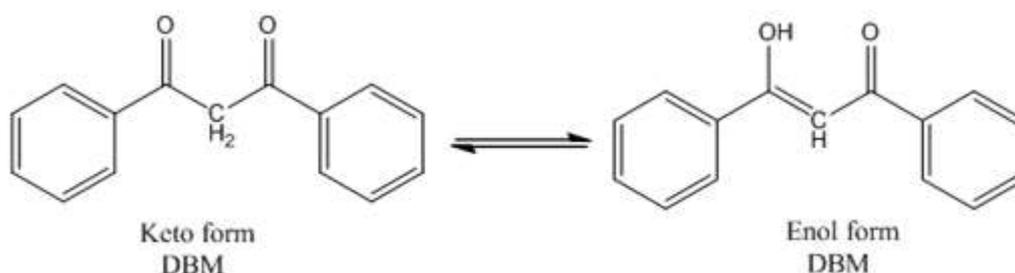
Abstract—A simple, sensitive, rapid and new spectrophotometric method for the determination of Dibenzoylmethane (DBM) in aqueous solution was developed. The method was based on the coupling of DBM with diazotized *p*-nitroaniline in basic medium and in the presence of Triton X-100. The orange-red dye formed was water-soluble, stable and shows maximum absorption at 524 nm. Beer's law was obeyed over the range 5-500 $\mu\text{g}/25\text{ml}$, i.e., 0.2-20 ppm with a molar absorptivity of $3.04 \times 10^4 \text{ l.mol}^{-1}.\text{cm}^{-1}$ and Sandell's sensitivity index of $0.0074 \mu\text{g.cm}^{-2}$, a relative error of -2.915 to 1.295 % and relative standard deviation of ± 2.215 to ± 3.848 %. The study of the interferences showed that the method was selective. The proposed method seemed effective and fast spectrophotometric

approach which has been applied successfully to determine DBM in some water samples, because there were no pharmaceutical preparations for the application of the method.

Keywords— Dibenzoylmethane, Spectrophotometry, *P*-nitroaniline, Diazocoupling.

I. INTRODUCTION

Dibenzoylmethane (DBM) or 1,3-diphenyl - 1,3-propanedione was the name given to the compound having the following structure (Reichardt, 1988).



The drugs (DBM and Trazodone) were carefully used to treat cancer and depression respectively, it was found that both of these drugs can cross the blood-brain barrier and restore correct protein production and this was great for Alzheimer's disease. (Halliday, et al., 2017). Dibenzoylmethane (DBM) is an important β - diketone which is widely used in polyvinyl chloride (PVC) and acrylonitrile-butadiene styrene (ABS) resin, as new auxiliary stabilizer (Ara and Khan, 2014).

DBM has the potential to prevent the formation of polycyclic aromatic hydrocarbon in vivo, (MacDonald et al, 2001), (Lin, et al., 2001). DBM forms the list of materials that are globally approved for use especially those for topical application to the skin. It protects almost any surface against damage from UV radiation and in conjugation with UV sunscreen, (Maier, et al., 2001). DBM plays an important role in the preparation of different compounds e.g. the formation of 1,5- diketones by photochemical reaction between DBM and o-quinones (Chang et al., 1998). The (DBM) has been shown to exhibit antineoplastic effects in prostate cancer cell lines by induction of cell cycle arrest, (Khor, et al, 2009). (Kockler, et al., 2014), investigated the effect of TiO_2 particle size on the photo stability of the chemical UV-filters of

butylmethoxydibenzomethane (BMDMB) and octocrylene formulated in a micro emulsion. The dibenzoylmethane derivatives protect dopamenergic neurons, these derivatives displayed neuro protective functions both in cell culture and animal models of Parkinson's disease (Takano, et al., 2007). (Bellucci, et al., 2011) explored that introducing large amounts of alpha synuclein into cell cultures results in the initiation of the un-folded protein response, so there is evidence that (DBM) might be neuro protective for Parkinson's disease. (Julie, et al. 2012) found that a sudden loss of protein associated with the connections between neurons occurred at 9 weeks post infection.

Consequently, it was quite clear that DBM was a very important compound, however, the methods available for its determination were limited. Visual and potentiometric titrations in methanol were used to measure DBM under nitrogen atmosphere (Afrawal, et al. 1975). The spectrophotometric method was used to determine DBM as its metal chelates using several metal ions such as Fe (III) but this method suffer from several disadvantages (Incitti, S. et al., 1966). DBM was determined in carbon tetrachloride as its Cu(II) chelate by measuring the absorbance of the organic

phase at 340 nm but this method has a low selectivity (Sorghie, and Fisel, 1972). (Heydarain, 2011) described a thorough conformational analysis of (DBM) in order to obtain detailed information on the geometrical parameters, and to estimate the barrier high for proton transfer and hydrogen bond strength. (Lin, et al 2005) examined the metabolic fate of DBM by dependent cytochrome p-450 enzymes in mouse liver indentified by NMR, GC and MS techniques which explained the potential role of DBM as a modulator of the cytochrome.

A highly sensitive and simple (HPLC) assay has been developed and validated for the quantification of (DBM) in rat plasma, the utility of the assay was confirmed by the successful analysis of plasma samples from DBM pharmacokinetics studies in the rats after oral and intravenous administrations (Shen, et al. 2007). These findings promote to search for a new, sensitive, selective and simple alternative approach for the analysis and determination of DBM in aqueous solution spectrophotometrically .

II. EXPERIMENTAL

2.1. Instruments and Apparatus

All measurements were performed using Shimadzn UV-Visible recording spectrophotometer UV-160, with 1-cm matched silica cells. The pH measurements were performed on philiphs PW 9420 pH meter with a combined - glass electrode.

2.2. Reagents: All chemicals used were of the Highest Purity Available

2.2.1. *The stock DBM solution, (1000 µg/ml).* A 0.1 gm amount of DBM was dissolved in 15 ml of ethanol in 25 ml volumetric flask. The solution was then kept in a brown bottle. This solution was stable for at least 3 months.

2.2.2. *The working DBM solution, (100 µg/ml).* This solution was prepared by diluting 1 ml of the stock solution to 100 ml

with 5% ethanol in a volumetric flask. Then the solution was kept in a brown bottle.

2.2.3. *Triton x-100 solution 1%.* This solution was prepared by diluting 1 gm of Triton x-100 (BDH) with warmed distilled water, then after cooling the solution was completed to 100 ml in a volumetric flask.

2.2.4. *Diazotized p-nitro aniline reagent solution.* This solution was prepared by dissolving 0.3453 gm of the reagent (BDH) in about 40 ml warmed distilled water. Then 8 ml of 5 M HCl was added and the solution was heated, the mixture was then transferred to a 100 ml volumetric flask and was cooled to (0 - 2C°) in an ice bath. A 0.1725 gm of NaNO₂ (BDH) is added and the mixture was stirred vigorously. After 5 minutes, the solution was made up to volume in a 100 ml volumetric flask with cold distilled water. The solution was kept in a brown bottle.

2.2.5. *Sodium hydroxide solution (1M) (Fluka).* This solution was prepared by appropriate dilution of the concentrated solution with distilled water.

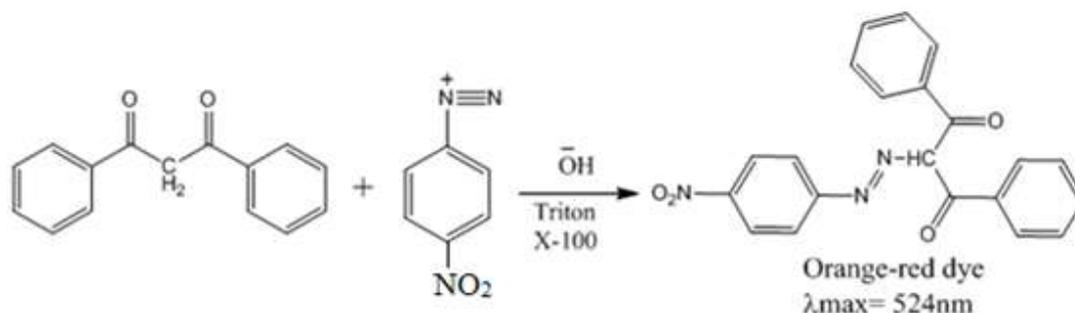
2.2.6. *Foreign compound solutions.* All these compound were prepared by dissolving the accurate amount in its suitable solvent and diluting the volume to the mark. (Water was the main solvent, sometimes ethanol is used instead).

2.2.7. *Tap water sample.* This water was taken from Bartella place in Mosul city. It was filtered and then 1, 3 and 5 ml of the waters were each treated with 1 ml of 0.2 M EDTA. Then 25, 50 and 400 µg of DBM were added and the recommended procedure was applied.

2.2.8. *Well water sample.* This water was taken from Bartella city, and treated as tap water samples.

III. PRINCIPLE OF THE METHOD

The method involves the coupling of DBM with diazotized p-nitroaniline to form an intense coloured dye in a basic medium and in the presence of Triton X-100.



IV. RESULTS AND DISCUSSION

For subsequent experiments, 50µg of DBM were taken in 25 ml final volumes.

4.1. Study of the Optimum Reaction Conditions

The effect of various parameters on the intensity of the coloured dye were investigated and the reaction conditions have been optimized.

4.1.1. Effect of base

The experimental data shows that the coloured dye was developed only in alkaline medium. Many (strong and weak) bases with different amounts were examined and the results were shown in Fig. 1.

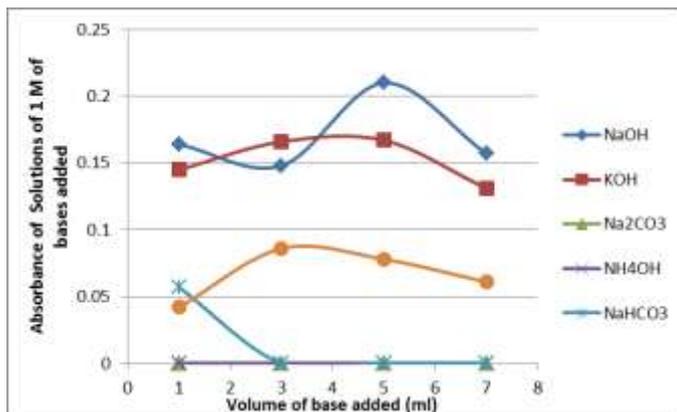


Fig. 1. Effect of various bases on the absorbance

The results above indicate that the coloured dye needs strong base medium. Therefore, 5 ml of 1 M NaOH solution was recommended for subsequent experiments.

4.1.2. Effect of surfactant

The effects of several types of surfactants on colour intensity of the dye in different amounts were investigated as shown in Fig. 2.

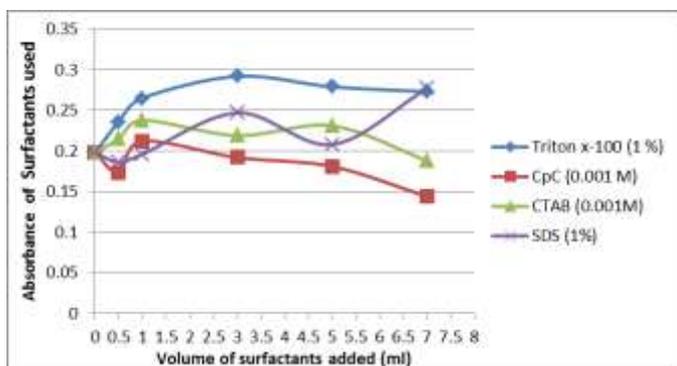


Fig. 2. Effect of surfactants on absorbance

* CpC: Cetylpyridinium chloride.

CTAB: Cetyltrimethyl ammonium bromid.

SDS: Sodium dodecylsulphate

The results above show that the addition of Triton x - 100, CpC and CTAB increases the intensity of the dye; while Triton x -100 addition causes the highest intensity with a good value of colour contrast. Therefore, 3 ml of 1% Triton x -100 solution was used in the subsequent experiments.

4.1.3. Order of surfactants addition

Three orders of Triton x-100 addition are examined as shown in Table 1.

TABLE 1. The order of addition Triton X-100

Order	Absorbance	λ_{max} , nm
I	0.283	527
II	0.227	527
III	0.206	527

(I) = DBM + Triton x-100 (T) + p-nitroaniline (R) + base (B)

(II) = DBM + R + T + B

(III) = DBM + R + B + T .

From the above results, order I was recommended.

4.1.4. Effect of diazotized p-nitroaniline

Several Diazotized agents were investigated such as p-phenylazoaniline, 8-amino phthalene-2-sulphonic acid and p-

nitroaniline. Diazotized p-nitroaniline reagent gives the best results (sensitivity and contrast of colour reaction). Also, Diazotized p-nitroaniline gives a water soluble dye in contrast to p-phenylazoaniline which gives an insoluble dye in aqueous solution. Further, Diazotized p-nitroaniline reagent gives at least two times as sensitive a dye as diazotized 8-aminonaphthalene-2-sulphonic acid, under the same preliminary experimental conditions. Then, the effect of the amount of the diazotized p-nitroaniline reagent on the maximum absorbance of the dye formed is next examined and the results are shown in Fig. 3.

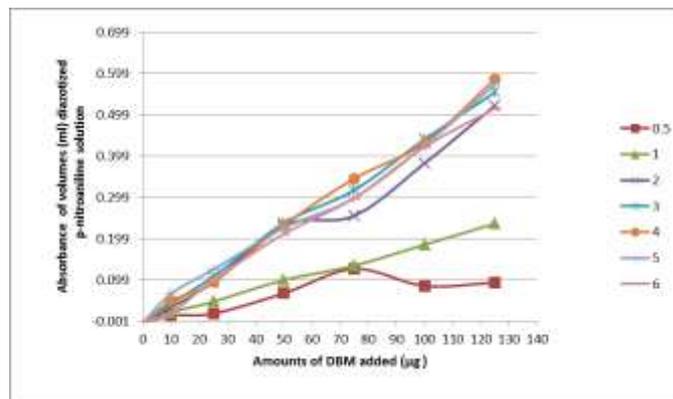


Fig. 3. Effect of diazotized p-nitroaniline reagent amount on absorbance
r* = correlation coefficient

From the values of r and ϵ in the Figure above, a 3ml of diazotized p-nitroaniline reagent was recommended for the subsequent experiments

4.1.5. Effect of time and amount of DBM

The effect of time on the development and stability period of the coloured dye is investigated under optimum experimental conditions described before. The formation of the coloured dye being practically complete immediately and the absorbance of the species remain constant for the least 2 hours, as shown in Fig. 4.

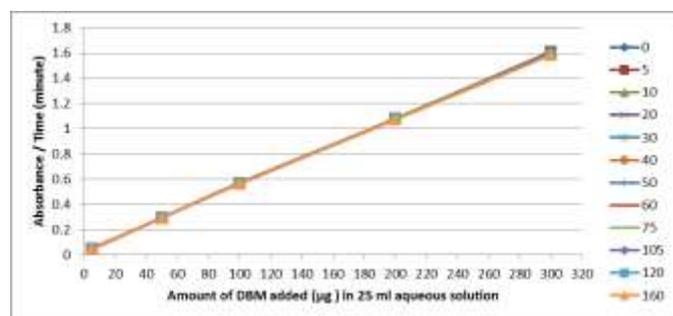


Fig. 4. Effect of Time and DBM amount on absorbance

The results above show that the dye colour develops completely after five minutes and remains stable for at least 2.5 hours.

4.2. Final Absorption Spectra

The absorption spectra of the coloured dye formed from coupling of DBM with diazotized p-nitroaniline in basic medium, and the presence of Triton x-100, against its

corresponding reagent blank shows maximum absorption at 524 nm in contrast to reagent blank which shows absorbance (0.001) at the above λ max.

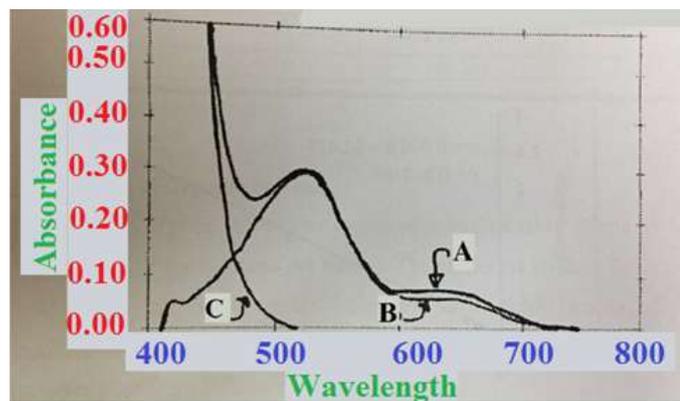


Fig. 5. Absorption spectra of 50 µg DBM / 25 ml measured against: (A) blank, (B) distilled water and (C) blank measured against distilled water.

The wavelength of maximum absorption at 524 nm is selected for subsequent experiments.

4.3. Recommended Procedure and Calibration Graph

To a series of 25 ml volumetric flask, an aliquots covering the range of 5 - 500 µg DBM are transferred 3 ml of Titron x-100, 3 ml of diazotized p-nitroaniline reagent and 5 ml of NaOH solution are then added, the volumes are made to the mark with distilled water. The reaction mixtures are mixed and the absorbencies are measured immediately at 524 nm against the reagent blank. The calibration graph shown in Fig. 6 is linear over the range 5-500 µg/25 ml, i.e. 0.2 - 20 ppm. A higher concentration shows negative deviation from Beer's law. The apparent molar absorptivity is found to be $3.04 \times 10^4 \text{ l.mol}^{-1}.\text{cm}^{-1}$ and Sandell sensitivity index is $0.0074 \mu\text{g}.\text{cm}^{-2}$.

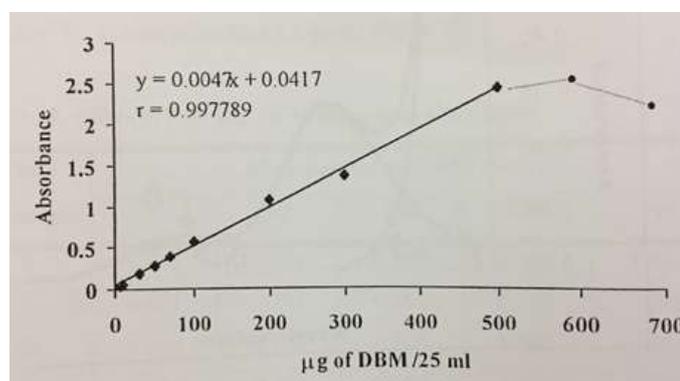


Fig. 6. Calibration graph of DBM determination using diazotized p-nitroaniline reagent

4.4. Accuracy and Precision

To check the accuracy and precision of the method is determined at five different concentrations. The results shown in Table 2 indicate that the method is satisfactory.

TABLE 2. Accuracy and Precision of the method

µg DBM	Relative error % *	Recovery % *	Relative standard deviation % *
10	-2.90	97.1	± 3.9
30	-2.30	97.7	± 3.8
50	1.10	101.10	± 2.7
200	0.50	100.50	± 3.2
500	1.30	101.30	± 2.2

* Average of five determinations.

4.5. Effect of Organic Solvents

Different organic solvents are examined the coloured dye. The results are on the spectrum are given in Table 3. The solvent water is still chosen in the subsequent experiments because of its cost, availability and best results.

TABLE 3. Effect of organic solvents.

Solvents	λ_{max} (nm)	$\epsilon \times 10^4 \text{ l.mol}^{-1}.\text{cm}^{-1}$
Acetic acid	Turbid	-
Acetone	531	3.60
Dimethyl sulphoxide	539.5	3.37
Ethanol	524.5	3.21
Formic acid	404	1.78
Methanol	518	3.22
2- Methoxyethanol	409	Unstable
1- propanal	525	3.0
Pyridine	Turbid	-
Tetrahydrofuran	Turbid	-
Water	524	3.04

4.6. Study of Interferences

The effect of various amount of foreign compounds are examined. The results are shown in Table 4.

TABLE 4. Effect of Interferences

Foreign ions	Amount added, µg	Recovery, %
Acetaldehyde	15000	101.79
Acetic acid	10000	96.17
Acetone	500	100.37
Acetylacetone	200	99.25
Acetophenone	500	104.60
Acetylacetone	25	127.08
Aniline	25	106.03
Ascorbic acid	100	102.82
Benzoylacetone	25	129.93
Cyclohexanone	10000	100.0
Cyclopentanone	100	100.0
Dimedone	25	103.17
Dimethylformamide	500	100
Dimethylmalonate	25	125
Dimethylsulphoxide	500	100.71
1,4-Dioxane	10000	95.40
Ethanol	10000	101.48
Ethylacetoacetate	100	101.06
Formaldehyde	10000	98.38
Formic acid	10000	98.94
Lactic acid	10000	100.0
Malonic acid	5000	98.86
Methanol	10000	101.11
Oxalic acid	10000	97.70
Phenol	25	203.09
1-Propanol	10000	96.17
2-Propanol	10000	99.29
Succinic acid	10000	96.68
Thenoyltrifluoroacetone	50	105.28

The above data show that acetyl acetone, aniline, Benzoylacetone, dimethylmalonate and phenol are serious interferences, since most of them are α or β - dicarbonyl compounds.

4.7. Application of the Method

To test its applicability, the present method has been used to determine DBM in tap and well waters.

4.7.1. Tap water

Table 5 shows the results obtained by the present method for the assay of DBM in tap water.

TABLE 5. Determination of DBM in tap water

DBM added, μg	Recovery % of DBM / ml of tap water		
	1	3	5
25	102.13	100.74	100.67
50	99.98	99.92	99.88
400	99.45	99.08	99.84

The results in the above table show that DBM can be determined in tap water without any treatment.

4.7.2. Well water

Table 6 Shows the results obtained by the present method for the assay of DBM in well water.

TABLE 6. Determination of DBM in well water

DBM added, μg	Sample	Recovery % of DBM / ml of Well water		
		1	3	5
25	A	94.6	92.12	90.35
	b	100.29	99.26	97.71
50	A	93.88	91.04	89.97
	b	99.30	98.92	94.96
400	A	92.29	91.39	87.73
	b	99.10	101.66	100.82

a: sample without treatment.

b: sample with 1 ml of 0.2M EDTA added.

The results in the Table show that the recovery of DBM is improved by adding 1 ml of 0.2 M EDTA added

4.8. Comparison of the Method

Table 7 shows the comparison between analytical variable for the present method and literature method.

TABLE 7. Comparison between analytical variable for the present method and literature method

Analytical parameter	Present method	Literature method*
pH	Basic medium	Acidic medium
Temperature	R.T.	-
Development time (min)	Immediately	40
λ_{max} (nm)	524	518
Reagent	Diazotized p-nitroaniline	Iron (III) alum
Beer's law range (ppm)	0.2 - 20	4 - 280
Molar absorptivity ($1.\text{mol}^{-1}.\text{cm}^{-1}$)	3.04×10^4	1085
Relative error (%)	(-2.915) - (1.295)	-
RSD (%)	$< \pm 3.848$	-
Colour of the dye	Orange - red	Red - violet

* S. Incitti and A. L. Ginestra, Analyst, (1966), 91, 814 - 816

V. CONCLUSION

In conclusion this study has demonstrated a simple, selective, sensitive and new spectrophotometric method for the determination of DBM in aqueous solution. The method was based on coupling of DBM with diazotized p-nitroaniline basic medium and in the presence of Triton x-100 forming orange - red dye and showing maximum absorption at 524 nm with a molar absorptivity of $3.04 \times 10^4 \text{ l.mol}^{-1}.\text{cm}^{-1}$ and Beer's law is obeyed over the range 5-500 $\mu\text{g}/25\text{ml}$, i.e., 0.2-20 ppm. Because there were no pharmaceutical preparations the proposed method was applied successfully to determine DBM in different water samples.

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