An Interaction Study of Chloro and Alkyl Substituted Benzylamine with DPPH• through UV Spectrophotometric and Physicochemical Methods at 
\[ T = (298.15, 303.15 \text{ and } 308.15) \text{ K} \]

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Received November 2013

ABSTRACT

Radical scavenging activity (RSA) of chloro and methyl substituted benzylamine derivatives (BADs) has been studied using 1, 1-diphenyl-2-picrylhydrazyl free radical (DPPH•) through spectrophotometric and physico-chemical techniques at \[ T = (298.15, 303.15 \text{ and } 308.15) \text{ K} \]. New experimental data on the density, sound velocity, isentropic and apparent molal compressibility of selected BADs + DPPH• solutions as a function of temperature and concentration are reported. The results are discussed with regards to structure-activity relationship (SAR) principles of BADs. The relative deviations in RSAs varied with structural potentials of BADs which were analyzed by making a comparative study for both the spectrophotometric and physicochemical results.

KEYWORDS

SAR; RSAs; BADs; Spectrophotometric; Physicochemical Data

1. Introduction

Free radicals in food, chemicals and even in living systems are produced by an oxidation process playing an important role in processes of food spoilage, materials degradation and many other modes of deactivation [1-4]. To prevent oxidation, several kinds of antioxidants mainly organic compounds and other metal complexes are being used for last few decades [5]. Recently, there has been a growing interest regarding antioxidants usage for medicinal purposes in the recent years [6]. Evidently, it has also been suggested that antioxidants diminish the jeopardy for chronic diseases like those of cancer and heart disease [7]. In biological systems, highly reactive free radicals and oxygen species are present which may oxidize nucleic acids, proteins, lipids or DNA, and can initiate degenerative disease [2-4,5]. To calculate their efficiency in vitro, several methods are being used which measure the RSA of antioxidants against free radicals [8-10]. Among the several methods, a rapid, simple and inexpensive method based on free radical DPPH scavenging effect is often used to quantify antioxidants in complex biological systems reported in the recent years [9]. Many inorganic metal complexes have been reported for their antioxidant potential [11-16]. The RSAs of metal complexes depend on the ligands which are attached with the metal through coordination bonding and pliancy of their functional groups [12,13]. Thus, the RSA of metal complexes are highly affected with ligands and also enhances the anticancer activity of the complexes [14]. Recently, BADs as ligands have been used to prepare anticancer complexes of platinum against solid tumor, and also complexes have been shown impressive antioxidant activities [17]. Since, BADs as ligands were used in study reported by Ameta et al. [17], those have great potential towards catalytic or biological applications [18]. Thus, with this in mind, the alkyl and chloro substituted benzylamine as BADs (Table 1) are selected for analysis of their antioxidant potential through in vitro method. Generally, the interaction is always temperature dependent and strength of interaction is determined with the physicochemical properties of the compounds [19]. Thus, in present study, we determined RSAs of chosen BADs at \[ T = (298.15, 303.15 \text{ and } 308.15) \text{ K} \], to bring to the foreground some of these aspects, including the peculiarities of the BADs. Thus, an input of physicochemical properties of BADs + DPPH• mixture such as density,
speed of sound, isentropic and apparent molal compressibility data could be found as useful materials in understanding of the antioxidant mechanism and may lead to develop new correlations or predictive models.

2. Experimental

2.1. Materials

Table 1 reports chemicals, used as received, and Milli-Q water (Millipore SAS 67/20 Mosheim) of 10⁻⁷ S·cm⁻¹ was used for solutions. Glassware were cleaned with standard methods and dried to absolute dryness checked with an anhydrous CuSO₄. Few pinch of CuSO₄ was spread in flasks, beakers and others which did not change color due to a level of absolute dryness and considered as a level of dryness.

2.2. Experimental Measurements

The antioxidant activities were assessed on a basis of free radical scavenging effect of stable DPPH● with spectrophotometric titration [17,20]. The 0.01% DPPH● stock solution was prepared in ethanol/water (1:1) and for preparing a sample solution, 5 mL DPPH● solution was mixed with 5 mL BADs solutions followed by vigorous shaking and incubated in dark. Before measuring absorbance, the sample solutions were incubated at chosen temperature for an hour in dark. In measurement of absorption spectra, initially blank spectra for ethanol/water were taken then spectrophotometric titration was performed with chosen concentration of BADs. Absorbance was measured at 517 nm with Spectro 2060 plus model UV/V is spectrophotometer, and RSAs were determined as a decrease in absorbance of DPPH●. Their densities and sound velocities were measured by Anton Paar Density and Sound velocity meter DSA 5000 M with ±10⁻³ kg·m⁻³ accuracy [21]. For each measurement, the tube was cleaned with acetone and dried by passing dry air through the tube by inbuilt air pump. Results and discussion

2.3. Spectrophotometric Study

Figure 1 contains absorption and scavenging activities (%) of BADs at chosen temperature where the RSA was determined as a result of decrease in absorbance of DPPH● [17,20] calculated with following formula:

\[
\text{Scavenging activity} \% = \left( \frac{A_0 - A_s}{A_0} \right) \times 100
\]  

The \(A_s\) is absorbance of DPPH● with BADs and \(A_0\) is an absorbance of DPPH● without BADs. The data calculated for antioxidation are presented as means ± SD of three determinations. The odd numbered electrons in free DPPH● give a strong absorption maximum at 517 nm due to availability of empty space in orbital for transition and impart it a purple colored appearance which turns to yellow to form a reduced DPPH-H (Figure 1) [17,20,22,23].

The change in UV with position of them, the absorbances have decreased in compare to the pure DPPH●. Therefore, a sample amount can lower an initial absorbance of DPPH● solution by 50% and has been chosen as an endpoint in measuring antioxidant activity [20]. The % RSAs of BADs have affected with concentration as well as with temperature where BADs have shown their RSAs as \(\text{BADs}_{303.15}\ K > \text{BADs}_{308.15}\ K > \text{BADs}_{298.15}\ K\) for chosen concentrations (Table 2, Figure 2).

It inferred that at 303.15 and 298.15 K maximum and minimum %RSA respectively, BADs are active to scavenge the free radicals. At 303.15 K the hydrogen donating activity is maximum which stabilized the free radicals.

Table 1. Benzylamine derivatives and scavenging agent studied in this work.

| Structure | Name |
|-----------|------|
| \[ \text{Phenylmethylamine} \] | Phenylmethanamine |
| \[ (2-chlorophenyl)methanamine \] | (2-chlorophenyl)methanamine |
| \[ (3-chlorophenyl)methanamine \] | (3-chlorophenyl)methanamine |
| \[ (4-chlorophenyl)methanamine \] | (4-chlorophenyl)methanamine |
| \[ N\text{ methyl(phenyl)methanamine} \] | N methyl(phenyl)methanamine |
| \[ N, N \text{ dimethyl(phenyl)methanamine} \] | N, N dimethyl(phenyl)methanamine |

Figure 1. Mechanism of DPPH● free radical for antioxidant activity.
Table 2. Absorbance and scavenging activities of BADs at $T = (298.15, 303.15$ and $308.15)$.

| BADs | mM  | 298.15 K | 303.15 K | 308.15 K | 298.15 K | 303.15 K | 308.15 K |
|------|-----|----------|----------|----------|----------|----------|----------|
| BA   | 10  | 0.494    | 0.370    | 0.453    | 52.68    | 62.66    | 60.26    |
|      | 20  | 0.517    | 0.387    | 0.474    | 50.48    | 60.95    | 58.43    |
|      | 30  | 0.518    | 0.398    | 0.483    | 50.38    | 59.84    | 57.25    |
|      | 40  | 0.426    | 0.320    | 0.396    | 59.20    | 67.71    | 65.63    |
|      | 50  | 0.553    | 0.470    | 0.529    | 47.03    | 52.57    | 49.52    |
| 2CBA | 10  | 0.537    | 0.431    | 0.506    | 48.56    | 56.51    | 53.71    |
|      | 20  | 0.558    | 0.464    | 0.520    | 46.55    | 53.18    | 50.16    |
|      | 30  | 0.697    | 0.577    | 0.665    | 33.24    | 41.78    | 38.02    |
|      | 40  | 0.561    | 0.439    | 0.529    | 46.26    | 55.70    | 52.85    |
|      | 50  | 0.523    | 0.429    | 0.496    | 49.90    | 56.71    | 53.92    |
| 3CBA | 10  | 0.459    | 0.357    | 0.426    | 56.03    | 63.98    | 61.65    |
|      | 20  | 0.521    | 0.402    | 0.481    | 50.10    | 59.43    | 56.82    |
|      | 30  | 0.512    | 0.411    | 0.479    | 50.96    | 58.53    | 55.85    |
|      | 40  | 0.561    | 0.459    | 0.530    | 46.26    | 53.68    | 50.70    |
|      | 50  | 0.590    | 0.501    | 0.563    | 43.49    | 49.45    | 46.19    |
| 4CBA | 10  | 0.520    | 0.425    | 0.487    | 50.19    | 57.11    | 54.35    |
|      | 20  | 0.554    | 0.470    | 0.530    | 46.93    | 52.57    | 49.52    |
|      | 30  | 0.548    | 0.467    | 0.521    | 47.51    | 52.88    | 49.84    |
|      | 40  | 0.509    | 0.409    | 0.477    | 51.25    | 58.73    | 56.07    |
|      | 50  | 0.533    | 0.451    | 0.501    | 48.95    | 54.49    | 51.56    |
| MBA  | 10  | 0.486    | 0.386    | 0.453    | 53.45    | 61.05    | 58.54    |
|      | 20  | 0.501    | 0.394    | 0.464    | 52.01    | 60.24    | 57.68    |
|      | 30  | 0.547    | 0.434    | 0.513    | 47.61    | 56.21    | 53.38    |
|      | 40  | 0.591    | 0.491    | 0.562    | 43.39    | 50.45    | 47.26    |
|      | 50  | 0.610    | 0.540    | 0.596    | 41.57    | 45.51    | 42.00    |
| MMBA | 10  | 0.502    | 0.394    | 0.477    | 51.92    | 60.24    | 57.68    |
|      | 20  | 0.526    | 0.441    | 0.476    | 49.62    | 55.50    | 52.63    |
|      | 30  | 0.540    | 0.462    | 0.498    | 48.28    | 53.38    | 50.38    |
|      | 40  | 0.505    | 0.393    | 0.515    | 51.63    | 60.34    | 57.79    |
|      | 50  | 0.500    | 0.399    | 0.467    | 52.11    | 59.74    | 57.14    |

DPPH 0.01% 1.044 0.991 0.931 - - -

*Estimated uncertainties in absorbance and % scavenging activities were less than ±1 × 10^{-3} and ±0.01 respectively. The uncertainties in temperature are ±0.01°C.*
radicals while at 298.15 K it is minimum, may be, due to
temperature dependent structural changes create such
environment. For comparing individual % RSAs, their
values were compared with each other and found as
2CBA > 3CBA > 4CBA > BA and MMBA > MBA at all
T = (298.15, 303.15 and 308.15) K. It inferred that the
2CBA is highly effective to scavenge the free radicals
due to negative inductive effect of chlorine with an in-
crease in hydrogen donating ability which stabilized the
free radicals. But in case of 3CBA and 4CBA the posi-
tion of chlorine in BA has changed where the negative
inductive effect works as 2C > 3C > 4C, therefore, 2CBA
showed higher scavenging effect and BA showed mini-
mum. In comparison of MMBA and MBA, since positive
inductive effect increases electron density on nitrogen,
therefore, two methyl group of MMBA increased much
electron density on nitrogen than one methyl group of
MBA that increased hydrogen donation ability more.
Thus, MMBA showed higher RSA than MBA.

2.4. Physicochemical Study

Both BADs and DPPH molecules have hydrophilic and
phobic parts, therefore their molecular interactions are
analyzed with physicochemical properties (PCPs). Hence,
density, apparent molal volume and sound velocity as
PCPs for BADs solutions with DPPH\(^*\) at \(T = (298.15,
303.15 \text{ and } 308.15)\) K, have been critically analyzed.
For example, the density decreased on an increase in tem-
perature and concentration of BADs + DPPH\(^*\) solutions
except MMBA + DPPH\(^*\) solution but increased from
pure DPPH\(^*\). For instance, the density for 10 mM of BA

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**Figure 2.** % Scavenging activities of BADs at \(T = (298.15, 303.15 \text{ and } 308.15)\) K where (A), (B), (C), (D), (E) and (F) are stand for BA, 2CBA, 3CBA, 4CBA, MBA and MMBA.
increased by 0.948391 g/cm³ from 0.933242 g/cm³ (pure DPPH*) at 298.15 K while it decreased by 0.945097 and 0.944585 g/cm³ for 20 mM at 303.15 K respectively. Densities decreased with the concentration and temperature for BADs except MMBA, due to interaction with DPPH* which has produced a lower denser medium where positions of chlorine on BA were critical. Also due to negative and positive inductive effects of chlorine and methyl groups respectively, made -NH bond weaker in BADs. In case of MMBA, density increased with concentration while decreased with temperatures, it was may be that two methyl groups created a compact environment which made the medium strong denser. Their limiting values (ρ₀) where the structural contribution was found maximum for BA with trend as ρ₀BA > ρ₀3CBA > ρ₀MBA > ρ₀2CBA > ρ₀4CBA > ρ₀MMBA at chosen temperatures (Figure 3 and Table 3).

Thus, the substitution of chloro or methyl groups has decreased interaction of BADs with DPPH* due to weaker intermolecular forces. Apart from density, the apparent molal volume, ρ₂*=, was calculated as below.

\[ \rho₂*=1000 \left( \frac{\rho₀-\rho}{M} \right) + \frac{M}{\rho} \]  

(2)

m and M are molality and molecular weight of BADs while ρ₀ and ρ are densities of pure DPPH* and BADs + DPPH* solutions respectively. The ρ₂* data explained molecular interactions that elucidated a net concentration effect of BADs with DPPH* interactions. Analysis of ρ₂* data has inferred strength of interactions where all BADs + DPPH* systems showed negative ρ₂* values except at few concentrations, due to stronger interaction of DPPH* + BADs molecules occupied less space and caused high internal pressure. Figure 4 shows a variation in ρ₂* where it increased with concentration and decreased (except MMBA) with temperature and negative ρ₂* inferred a contraction produced on interaction between BADs and DPPH* molecules.

![Figure 3. Densities of BADs + DPPH interacting mixture at T = (298.15, 303.15 and 308.15) K.](image-url)
Table 3. Limiting values of density, $\rho^0$, apparent molal volume, $^{#}_2V^*_V$, and apparent molal compressibility, $^{\phi}_{Ko}$, at (298.15, 303.15 and 308.15) K.

|                | 298.15 K | 303.15 K | 308.15 K |
|----------------|----------|----------|----------|
|                | $\rho^0$/(g.cm$^{-3}$) | $^{#}_2V^*_V$/(m$^3$mol$^{-1}$) | $^{\phi}_{Ko}$/($m^2$N$^{-1}$) |
| BA             | 0.951254 | -2611.55 | -0.0062  |
| 2CBA           | 0.940925 | -928.77  | -0.0029  |
| 3CBA           | 0.947944 | -2160.22 | -0.0060  |
| 4CBA           | 0.938524 | -595.37  | -0.0023  |
| MBA            | 0.944669 | -2183.13 | -0.0057  |
| MMBA           | 0.933718 | 2.84     | -0.0006  |

In case of MMBA, the $^{#}_2V^*_V$ values decreased with concentration and increased with temperature towards positive values, it may be that both methyl groups have positive inductive effect, and combining positive inductive effect of both methyl groups of MMBA created much electron density on nitrogen as well as indirectly on hydrogen of -NH bond. Therefore a stronger interaction developed between MMBA and DPPH molecules. The structural contribution of BADs to this contraction have studied with a comparative analysis of their limiting values (Table 3) found as

$^{#}_2V^*_V$$_{BA} > ^{#}_2V^*_V$$_{2CBA} > ^{#}_2V^*_V$$_{3CBA} > ^{#}_2V^*_V$$_{4CBA} > ^{#}_2V^*_V$$_{MBA}$

Since, negative $^{#}_2V^*_V$ values due to solutions of BADs in a given amount of DPPH$^*$ have a smaller volume than the same amount of pure DPPH$^*$ inferred that, the structural contraction is stronger for BA and weaker for MMBA. The physical reason is that nearby DPPH$^*$ molecules are strongly attracted to their molecules so that they occupied less space. With the increasing concentration, the $^{#}_2V^*_V$ values have increased (Table 3), which showed that BADs molecules occupy more space on interaction with DPPH$^*$ while in case of MMBA it is reversed. The interaction between BADs molecules and DPPH$^*$ is also determined with their sound velocities data and its derivatives where it decreased with concentration as well as with temperatures. Due to two kinds of interaction such as hydrophobic-hydrophobic and hydrophilic-hydrophilic interaction of BADs and DPPH$^*$ molecules develop different zone of interactions which heterogenised the interaction system. And as a result of internal pressure developed that determined with speed of sound data for gaining information about interaction of BADs and DPPH$^*$ Equation (3). $n$ is number of molecules, $^{#}_2V^*_V$ is apparent molal volume and $T$ for specific temp.
The density and sound velocity data were fitted in Newton-Laplace equation to get isentropic compressibilities, $K_s$, as Equation (4). The $\rho$ is density and $u$ is sound velocity of the BADs + DPPH$^*$ solutions. The $K_s$ values for BADs + DPPH$^*$, are positive at all temperatures and attributed to a breaking or stretching of interaction bonds in a self-associated DPPH$^*$. It inferred that $K_s$ have increased when BADs molecules interacted to DPPH$^*$. In general, the isentropic compressibility increases with an increase in temperature at a fixed composition due to an increase in thermal agitation which makes the solution more compressible [19]. In the present study, $K_s$ increased with an increase in temperature and decreased with an increase in concentration of BADs for BADs + DPPH$^*$ system. The $K_s$ of the BADs+ DPPH$^*$ mixture is found lower than pure DPPH$^*$ solution, it may be due to presence of BADs, the interaction of DPPH$^*$ molecules has broken which led to development of weaker BADs-DPPH$^*$ interaction than DPPH$^*$-DPPH$^*$. Therefore, the compactness decreased and a system compressed lesser. Since, the interaction of BADs with DPPH$^*$ varied with different BADs which has analyzed with density, isentropic apparent molal volume and compressibility. For checking compressibility at the molecular level, the apparent molal compressibility, $\phi K_s$ are calculated with equation as under.

$$
\phi K_s = \frac{1000}{m \cdot \rho_0} \left( \rho_0 K_s - \rho_0 \kappa_s + \frac{u_k M}{\rho_0} \right)
$$

Figure 4. Apparent molal volumes of BADs + DPPH interacting mixture at $T = (298.15, 303.15$ and $308.15)$ K.
The m and M are molality of solution and molecular weight of taken BADs. The \( \rho \), \( \rho_0 \) and \( K_\text{s}^0 \) are density and isentropic compressibility of BADs + DPPH\(^\ast\) solution and pure DPPH\(^\ast\) solution respectively. Table 3 reports the values of \( \phi K_\text{s} \) are negative indicate stronger interaction between BADs and DPPH\(^\ast\) at structural or molecular level. The increase in \( \phi K_\text{s} \) primarily explains that changes with structure lead to change in ultrasonic velocity as well as a definite contraction upon mixing which in turn dependent on the structural contributing strength. The increase in \( \phi K_\text{s} \) value indicates a dominant contribution from structure-breaking effect in BADs + DPPH\(^\ast\) by the stronger intermolecular force. Since, DPPH\(^\ast\) molecules have cohesive forces between self-associated DPPH\(^\ast\) molecules and when interacted with BADs, this cohesivity is disrupted to make the interaction bond, thus, friccohesity is a real model of structure breaker effect in BADs-DPPH\(^\ast\) by molecular forces. The breaking of intermolecular interaction of DPPH\(^\ast\) molecules induced by different BADs is reflected by \( \phi K_\text{s} \) values. The overall structural contribution of BADs is measured by regressing the \( \phi K_\text{s} \) values where the trend of \( \phi K_\text{s} \) is found as \( \phi K_\text{s} \) \( \text{BA} > \phi K_\text{s} \) \( 3\text{CBA} > \phi K_\text{s} \) \( \text{MBA} > \phi K_\text{s} \) \( 2\text{CBA} > \phi K_\text{s} \) \( 4\text{CBA} > \phi K_\text{s} \) \( \text{MMBA} \) for chosen temperatures (Figure 5 and Table 3).

The BA due to absence of any functional group on benzene ring showed higher \( \phi K_\text{s} \) than others like chloro and methyl groups attached benzene ring such as 2CBA, 3CBA, 4CBA, MBA and MMBA. Since, two methyl groups on the nitrogen of MMBA occupy a certain amount of space, and on interaction they come too close together with an associated cost in energy due to overlapping electron clouds. Thus, the presence of two methyl groups on nitrogen in MMBA developed steric hindrance to interact with DPPH\(^\ast\) molecules with lower \( \phi K_\text{s} \) among all the BADs. It also showed an effect of functional group on the compressibility at the molecular level which can be categorized as -3Cl > -2Cl > -4Cl and -\( \text{CH}_3 \) > -(\( \text{CH}_3 \))\(^2\).

![Figure 5](image_url)

Figure 5. Apparent molal compressibilities of BADs + DPPH interacting mixture at \( T = (298.15, 303.15 \text{ and } 308.15) \text{ K} \).
3. Conclusion

This study defined the SAR of chloro and methyl substituted BADs by analyzing their antioxidant activities thermodynamically using DPPH· scavenging effect through spectrophotometric and physicochemical methods. The chloro substituted BADs showed higher antioxidant activities than methyl where position of chloro and methyl substitute affected antioxidant activity as 2CBA > 3CBA > 4CBA and MMBA > MBA respectively, which inferred that their SAR varied with chloro and methyl groups. These thermodynamic physicochemical results may be of significance in evolving a mechanism for effective biodegradable agents, bioremediation, drug efficacy and protein interacting capacity.

Acknowledgements

Authors are highly thankful to Central University of Gujarat, Gandhinagar for financial, infrastructural support and experimental facilities.

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