Preparation and Physicochemical characterizations of emulsions using *Azadirachta indica* oil for improving and modulating the antioxidant efficacy of Ceftriaxone

**ABSTRACT**

For supplement the antioxidant ability of Ceftriaxone, an antibiotic drug, through encapsulation using Neem oil (*Azadirachta indica*) with Dodecyl Trimethyl Ammonium Bromide (DTAB) aqueous cationic surfactant was formulated. The prepared emulsions were characterized with physicochemical properties (PCPs) where differences in PCPs of neat and drug encapsulated emulsions were attributed to the drug loading efficacy, and have been quantified. Thereafter, Ceftriaxone encased emulsions were screened for antioxidant efficacy with free radical 2, 2'-diphenyl-1-picrylhydrazyl through spectrophotometric and physicochemical methods. With the DPPH assay, the antioxidant potential of pure drug and with emulsion were carried out where the formulation proved the increment in antioxidant activity of ceftriaxone. At first, the scavenging activity was analyzed with physicochemical method and the decrease in values of surface tension, viscosity and potential were made criteria for the radical scavenging activity, similar to DPPH assay. The reduced values of such properties of ceftriaxone encapsulated emulsions quantitatively verified the enhancement of antioxidant activity of ceftriaxone.

**Keywords:** Ceftriaxone, surfactants, emulsions, neem oil, DPPH.

INTRODUCTION

The derivatives of *Azadirachta indica* as bioactive compounds have been found effective including neem oil in kerosene, neem oil water emulsion, Neem Azal, neem cream and emulsifiable concentrate Azad EC 4.5, etc (Boschitz and Grunewald, 1994). Mentioned activities fascinated us to choose the neem oil for the emulsion formulation in the present study to evaluate the antioxidant ability of Ceftriaxone, an antibiotic drug through encapsulation. Ceftriaxone sodium (Figure 1) is chemically (6R,7R)-7-[(2Z)-(2-amino-4-thiazolyl)[methoxyimino]acetyl]amino]-8-oxo-3-[[[1,2,5,6-tetrahydro-2-methyl-5,6-oxo-1,2,4-triazin-3-yl]thio]-methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (Budavari, 2001; Tariq et al., 2010; Shah et al., 2013; Sun et al., 2011; Kale et al., 2011).
It is a third generation, semi-synthetic cephalosporin antibiotic. Ceftriaxone is a derivative of 7-
aminocephalosporic acid. Ceftriaxone sodium is a long acting, broad-spectrum cephalosporin antibiotic for
parenteral use (Kumar et al., 2010; Shrivastava et al., 2009).

It has shown in vitro activity against a wide range of
Gram-negative and Gram-positive micro-organisms (Shrivastava et al., 2009). It has stability towards most
beta-lactamases, both penicillinases and cephalosporinases, of Gram-positive and Gram-negative
bacteria, is higher (Nirav et al., 2012; Jat et al., 2010). Thus, the trend is that the industries look for ways to reduce the
impacts of their activities on the environment (Jat et al., 2011; 2012; Pal et al., 2001; The Merck Index, 2006).

Structurally, the presence of nitrogen containing six and
five membered rings and carbonyl moiety, impart
Ceftriaxone, the pH dependent functional abilities and an
electrophilic character, thereby making this a proficient
free radical scavenger. With such relevance, the emulsions
have proven effective and reliable carriers due to their
flexible compositions in terms of dispersed phase and
dispersion medium (Subhashis et al., 2010; Thakur et al.,
2013; Mc Clements and Rao, 2011; Mason et al., 2006;
Gibaud and Attivi, 2012).

Emulsions hold immense potential for improving
therapeutic efficacies and concomitant biological activities
of the drug (Thakur et al., 2013; Mason et al., 2006; Gibaud
and Attivi, 2012; Anuchapredea et al., 2012; Afornali et al.,
2013; Hamoudah et al., 2010). Thus, the present study is
an attempt to enrich the antioxidant activity of Ceftriaxone
through encapsulation in biocompatible emulsions. Seeing
this factual position, the neem oil and aqueous
dodecyltrimethylammoniumbromide (DTAB), a regular
ingredient of cosmetic preparations, have been chosen for
bio-emulsion preparation.

**MATERIALS AND METHODS**

The commercially available Neem oil was procured and
other chemicals such as DTAB, Ceftriaxone and DPPH free
radical were procured from Sigma Aldrich having 99.99% purity level. The chemicals were used as received
and Milli-Q water of conductivity 10⁻⁷ S cm⁻¹ was used.
Glassware were cleaned and dried to absolute dryness and
thereafter, was checked with anhydrous CuSO₄. A little
pinch of the CuSO₄ was spread inside the flasks, beakers,
measuring cylinders and glass pipettes that did not change
its color owing to a level of absolute dryness as any water
molecules were present.

**Preparation of formulations**

0.1 to 0.5 ml oil was separately taken into 50 ml RB flask
using a pipette. Thereafter, a 50 ml 0.001 M aqueous DTAB
solution was added in each of the formulations with 1000
rpm stirring. The stirring was further continued for 45
min, under similar conditions in each case.

**Ceftriaxone encapsulated emulsions**

For Ceftriaxone loading, initially, 10 uM (in methanol) was
dissolved in Neem oil, before the oil-drug mixture was kept
for magnetic stirring at 1000 rpm, so as to enable complete
solubilization. This process of adding Ceftriaxone in Neem
oil is referred to as drug encapsulation and this oil, only,
has been further used to prepare drug loaded emulsions
(DLEs).

**Experimental measurements**

**Physicochemical characteristics**

Densities were measured using Anton Paar DSA 5000 M
Density meter, with the respective accuracies being ±10⁻⁴ g
cm⁻³ and ±0.5 m/s, respectively. The instrument was
calibrated with water and dry air (DMA, manual; Anton
Paar, Graz, Austria). The viscosities and surface tensions of
drug encapsulated and neat formulations were measured
using Borosil Mansingh Survismeter, with the respective
accuracies of ±0.01 mN/m and ± 1x10⁻⁴ mPa·s (Man, 2006,
2007). The pH and potential were checked using laboratory instruments in India.

**Measurement of drug loading efficacy**

The drug loading efficacies for Ceftriaxone were
determined through the comparison of densities, surface
tension, viscosities, pH and potentials of with and without
drug loaded formulations. The quantitative limits were,
therefore measured in terms of relative % encapsulation,
surface availability and binding potential using densities,
surface tension and viscosities respectively. The
mathematical equations employed for calculating these
attributes are as discussed ahead (Parth et al., 2014):

\[
\text{% Encapsulation of Ceftriaxone} = \left( \frac{\rho_{\text{encapsulated formulation}} - \rho_{\text{unloaded formulation}}}{{\rho_{\text{unloaded formulation}}}} \right) \times 100
\]

(1)

\[
\text{% Surface Encapsulation of Ceftriaxone} = \left( \frac{\gamma_{\text{encapsulated formulation}} - \gamma_{\text{unloaded formulation}}}{{\gamma_{\text{unloaded formulation}}}} \right) \times 100
\]

(2)

\[
\text{% Binding Potential} = \left( \frac{\eta_{\text{encapsulated formulation}} - \eta_{\text{unloaded formulation}}}{{\eta_{\text{unloaded formulation}}}} \right) \times 100
\]

(3)
Table 1: reports components of formulations with and without drug.

<table>
<thead>
<tr>
<th>Quantity of oil (ml)</th>
<th>Components of formulations with and without drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration of Aq. DTAB (M)</td>
</tr>
<tr>
<td>0.10</td>
<td>0.001</td>
</tr>
<tr>
<td>0.20</td>
<td>0.001</td>
</tr>
<tr>
<td>0.30</td>
<td>0.001</td>
</tr>
<tr>
<td>0.40</td>
<td>0.001</td>
</tr>
<tr>
<td>0.50</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Figure 1: Structure of Ceftriaxone.

Where: \( \rho_{\text{encapsulated formulation}} \), \( \gamma_{\text{encapsulated formulation}} \) and \( \eta_{\text{encapsulated formulation}} \) are the values of densities, surface tension and viscosities for drug encapsulated formulations while the similar expressions with subscript unloaded formulation represent the densities, surface tensions and viscosities of blank formulations (without drug).

Measurements of antioxidant potential

Spectrophotometric method

Antioxidant activities of the drug encapsulated formulations were evaluated through a free radical scavenging effect of stable DPPH*, investigated through spectrophotometric titration, as reported in the literature with a slight modification (Patel et al., 2011; Enujiugha et al., 2012). For screening RSAs, pure DPPH* solution was mixed with DLEs in 1:1 ratio. Thereafter, on vigorous shaking, these samples were kept in dark for an incubation period of 45 min. The RSAs were evaluated as a measure of comparative % decrease in absorbance of pure DPPH* at \( \lambda_{\text{max}} = 517 \) nm, ascertained through the measurement of absorbance of DPPH + DLE 1:1 mixture, at the same wavelength. The measurements were made with Spectro 2060 plus model UV/Vis spectrophotometer. Initially, base line correction for the neat emulsion was made. The respective RSAs were thereafter calculated using the equation:

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

(4)

\( A_0 \) and \( A_s \) are the absorbance of pure DPPH* and DLEs, measured at 517 nm.

Physicochemical methods

The RSAs were evaluated through physicochemical analysis for the first time. In this method, the free radical interaction with drug in terms of RSA was determined through comparative analysis of physicochemical properties. The overall interaction with free radicals, surface scavenging and bulk scavenging activities were determined through the comparison of densities, surface tension and viscosities of pure DPPH and DPPH+DLEs. The mathematical equations employed for analysing these attributes are:

\[
\% \text{Overall interaction} = \left( \frac{\rho_{\text{DPPH}} - \rho_{\text{DPPH+DLE}}}{\rho_{\text{DPPH}}} \right) \times 100
\]

(5)
RESULTS AND DISCUSSION

Neem oil/ Aq. surfactant emulsions: Physicochemical characterizations

Figure 2 shows a milky coloured emulsion after one hour stirring of mixture of oil and aqueous DTAB. The neat DLEs carrying Ceftriaxone encapsulated Neem oil and aq. surfactant were critically analysed in light of PCPs in order to understand and study the dissolution and dispersion pattern of Ceftriaxone. In realization of this, the characteristic densities, surface tensions, viscosities, pH and potential of the formulations were estimated and evaluated.

Density is a primary tool for physicochemical characterization and Figure 3 shows the density of neat DLEs analysed. The neat formulation densities decreased with the amount of oil. This may be due to the higher amount of the oil which increased the hydrophobicity into ternary system (Oil+Water+Surfactant) that caused weaker interaction among the components and resulted in lower densities. In the case of DLEs the densities got increased with the amount of oil followed by reverse trend of neat formulations that confirmed the loading of the drug or DLEs.

\[
\begin{align*}
\% \text{ Surface Scavenging} &= \left( \gamma_{DPPH} - \gamma_{DLE+DPPH} \right) \times 100 \\
\% \text{ Bulk Scavenging} &= \left( \eta_{DPPH} - \eta_{DLE+DPPH} \right) \times 100 \\
\end{align*}
\]

(6)

(7)

There \( \rho \), \( \gamma \) and \( \eta \) are respective values of densities, surface tension and viscosities.

A potential study

The RSA was also analysed with potential studies with and without drug encapsulated emulsions as compared to potential of DPPH. The % scavenging effect was then analysed as:

\[
\% \text{ Scavenging activity} = \left( \frac{\text{Pot}_{DPPH} - \text{Pot}_{DLE+DPPH}}{\text{Pot}_{DPPH}} \right) \times 100
\]

(8)
Ceftriaxone having several hydrophilic groups decreased the hydrophobic effect of the oil and increased the interaction inferring the effectiveness of the drug with the oil. The analysis of pH of neat DLEs inferred the acidic nature of the oil which led to the acidic nature from basic form (Figure 4). Since ceftriaxone have acidic groups, therefore, the pH of neat formulation is higher than the pH of DLEs because the drug increases the acidic character of formulation. Especially with the 0.2 and 0.3 ml of oil the pH of DLEs decreased much as compared to others; such concentration may be noticed as effective concentration for a particular formulation. The decrease in pH for DLEs from neat formulation inferred the drug loading. The surface tension and viscosity as structural parameters accurately determine interaction with respect to metal ions responsible for the quality of emulsions. Surface tension and viscosity data from 0.1 to 0.5 ml with 0.1 ml interval for neat DLEs are calculated as per literature (Man, 2006, 2007). For the neat formulation both the surface tension and viscosity decreased while for the DLEs the same were found to increase with respect to the amount of oil. The increase and decrease in both parameters for the same solutions can be explained on the basis of ASIAM model (Ameta and Singh, 2015).

Surface tension is a cohesive property where the molecules or particles sustain their cohesivity with a
Table 2: Experimental pH, potential, $V$, density, $\rho$, viscosity, $\eta$, and surface tension, $\gamma$ of neat emulsion (without drug), Drug loaded emulsion (DLEs) and DPPH + DLEs at $T=303.15$ K.

<table>
<thead>
<tr>
<th>Quantity of oil (ml)</th>
<th>pH</th>
<th>$V$</th>
<th>$\rho/\pm10^{-6}g/cm^{3}$</th>
<th>$\eta/\pm10^{4}mPa.s$</th>
<th>$\gamma/\pm10^{2}mN/m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O</td>
<td>7.20</td>
<td>0.120</td>
<td>0.994034</td>
<td>0.7225</td>
<td>70.38</td>
</tr>
<tr>
<td>DTAB</td>
<td>7.40</td>
<td>0.063</td>
<td>0.907271</td>
<td>0.5780</td>
<td>49.08</td>
</tr>
<tr>
<td>0.1</td>
<td>8.87</td>
<td>0.030</td>
<td>0.818256</td>
<td>0.6036</td>
<td>31.45</td>
</tr>
<tr>
<td>0.2</td>
<td>8.82</td>
<td>0.027</td>
<td>0.815243</td>
<td>0.5881</td>
<td>30.46</td>
</tr>
<tr>
<td>0.3</td>
<td>8.79</td>
<td>0.026</td>
<td>0.809232</td>
<td>0.5816</td>
<td>26.55</td>
</tr>
<tr>
<td>0.4</td>
<td>8.47</td>
<td>0.026</td>
<td>0.803222</td>
<td>0.5598</td>
<td>25.42</td>
</tr>
<tr>
<td>0.5</td>
<td>8.38</td>
<td>0.022</td>
<td>0.799211</td>
<td>0.5504</td>
<td>25.00</td>
</tr>
<tr>
<td>DLEs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>5.70</td>
<td>0.136</td>
<td>0.810989</td>
<td>0.5475</td>
<td>26.94</td>
</tr>
<tr>
<td>0.2</td>
<td>5.52</td>
<td>0.132</td>
<td>0.812365</td>
<td>0.5706</td>
<td>28.02</td>
</tr>
<tr>
<td>0.3</td>
<td>5.46</td>
<td>0.128</td>
<td>0.813965</td>
<td>0.5938</td>
<td>29.20</td>
</tr>
<tr>
<td>0.4</td>
<td>5.40</td>
<td>0.125</td>
<td>0.814896</td>
<td>0.6167</td>
<td>30.03</td>
</tr>
<tr>
<td>0.5</td>
<td>5.33</td>
<td>0.116</td>
<td>0.816562</td>
<td>0.6380</td>
<td>30.94</td>
</tr>
<tr>
<td>DPPH + DLEs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPPH</td>
<td>5.99</td>
<td>0.21</td>
<td>0.793569</td>
<td>0.6546</td>
<td>43.57</td>
</tr>
<tr>
<td>0.1</td>
<td>5.42</td>
<td>0.164</td>
<td>0.822256</td>
<td>0.6491</td>
<td>33.52</td>
</tr>
<tr>
<td>0.2</td>
<td>5.45</td>
<td>0.147</td>
<td>0.821246</td>
<td>0.6103</td>
<td>36.22</td>
</tr>
<tr>
<td>0.3</td>
<td>5.65</td>
<td>0.144</td>
<td>0.819336</td>
<td>0.6044</td>
<td>37.36</td>
</tr>
<tr>
<td>0.4</td>
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<td>0.136</td>
<td>0.815148</td>
<td>0.5880</td>
<td>38.48</td>
</tr>
<tr>
<td>0.5</td>
<td>6.46</td>
<td>0.123</td>
<td>0.812258</td>
<td>0.5793</td>
<td>40.47</td>
</tr>
</tbody>
</table>

Uniform molecular arrangement in liquid phase. The cohesivity is developed due to intermolecular interaction among the water+surfactant+oil. The data (Table 2) revealed that this interaction is weaker than pure water inferring weaker cohesivity in three component systems. Initially, the oil disrupts the aqueous surfactant interaction which is stronger than oil-surfactant-water interaction. On increasing the amount of oil, the surface tensions and viscosities got decreased; it infers that number of oil molecules favors the development of the three component interacting systems.

Figure 5 shows surface tension and viscosity with the amount of oil varied linearly due to composition effect on interactions. In the case of DLEs, the viscosity and surface tension behaved inversely as compared to neat formulation where these were increased with respect to amount of oil. It inferred that the ceptriaxone having more hydrophilicity strengthened the interaction of oil-surfactant-water, and confirmed the loading of the drug into the formulation. Such variations in surface tension and viscosity for the neat DLEs furnish significant information about the emulsion system for conducting the drug delivery process.

Quantitative drug loading efficacy (QDLE)

As drug is loaded into any system it is distributed everywhere in the solution, and this distribution is analyzed in terms of QDLE using Equations 1, 2 and 3 where drug encapsulation, surface loading and bulk loading were studied using density, surface tension and viscosity respectively. Figure 5 shows an analysis of QDLE where increment and decrement in binding potential, surface encapsulation and total encapsulation were observed (-ve sign reflects decrease in the respective value). With the increasing amount of oil the increase in surface encapsulation was found to increase to 0.4 ml, which may be as a result of maximum concentration in surface activity and after that the same was found to decrease.

Ceftriaxone affinities, relative encapsulation efficiencies and activities with oil and surfactants were evaluated for relative changes in PCPs (Table 2) (Gibaud and Attivi,
Hence, the encapsulation efficacy of Ceftriaxone was quantitatively evaluated by analysis of relative changes in densities ($\rho$), with Equation (1). Figure 6 shows the percentage encapsulation of Ceftriaxone where a negative sign depicts that drug works as structure breakers while a positive sign shows it as a structure maker (Ameta and Singh, 2015). The higher densities of drug with oil and aqueous DTAB solutions reflect that the Ceftriaxone interacts strongly with the components of systems since the densities of drug loaded formulations with the surfactants were less than those of corresponding aqueous surfactant solutions (Table 2 and Figure 3). These experimental trends predict that the surfactants probably developed micro micelles and captured Ceftriaxone altogether. A maximum drug encapsulation

![Figure 5: Surface tension and viscosity analysis for the neat and DLEs.](image1)

![Figure 6: Quantitative drug loading efficacy: drug encapsulation, surface loading and bulk loading studies using density, surface tension and viscosity respectively.](image2)
(2.27%) was achieved at 0.5 ml of oil. Similarly, surface tension is a surface property of liquids, rendering the surface molecules to gain high energy as a result of being enclosed with fewer neighbours (Soni and Kuttan, 1992). Thereby, the surface tension variations of drug loaded formulations with those of blank formulations enable a quantitative estimation of surface availability of the drug, calculated with Equation (2). These values appeared as numerically negative with the amount of 0.1 and 0.2 ml of oil, whereas for 0.3, 0.4 and 0.5 ml, the corresponding values were positive. A positive sign for the characteristic surface availability reflects that the drug is segregated out of formulation components and gradually acquired a concentrative existence at the surface. This infers that the introduction of drug reorients the emulsion in a way that the oppositely charged poles aggregate on the Ceftriaxone as a core and stronger binding force that enhanced the γ values in Ceftriaxone loaded emulsions are developed.

This is particularly significant, with respect to optimizing the drug release potential of DLEs when the formulations exert and express themselves under the physiological conditions of temperature and ion-selective environment. It can be further experienced through the RSAs, which are higher. A maximum relative increase of 23.76% is attained in surface tension at 0.5 ml of oil. From both variations, it is very much evident that the interactions in DLEs are highly sensitive and thereby modulate the surface tension of the ternary mixtures so as to bring the saturated drug molecules over to the surface. The structural and dimensional vectors and factors of constituents of ternary systems result in the development of a canonical box enclosing ceftriaxone to not only bind but also to facilitate its transportation.

On comparison of surface tension values of blank and drug encapsulated formulations, it is inferred that the surface tensions decreased upon the drug encapsulation, with a stronger dispersion of drug. The viscosities (η) of DLEs enable the estimation of mutually prevalent extent of cohesive and intermolecular forces (IMFs) (Ostertag et al., 2012). As such, a quantitative estimation of corresponding interconversion of cohesive forces into IMFs was made in accordance with Equation (3) through a gradual interaction of the structural domains.

An increase in viscosities depicts that the cohesive forces operating among the solute molecules are broken with the gradual development of IMFs, on the breaking of previous structural interactions after an addition of drug. Hence, the chosen surfactant works as ideal environments for optimizing the antioxidant and thus, the resultant therapeutic efficacy of the drug. This is so as in all these formulations; the viscosities get increased in process pave way for the breaking of cohesive forces within the interaction environment and their corresponding gradual replacement by IMFs between the drug and surfactant. It is reflected by relative encapsulation values of the drug within the interaction media, which also highlights how the drug acts as a structure breaker in such formulations, irrespective of the operational quantity of oil.

Investigation of enhanced antioxidant potencies through DPPH* assay

DPPH* is an odd electron compound, possessing a strong characteristic UV/Vis absorption maximum at 517 nm, due to the availability of empty space in its orbital that further aids in transition of the electron to higher energy states (Maiti et al., 2007; Marczylo et al., 2007). This electronic behavior enables the DPPH* to develop a violet colour in solution. However, it becomes colourless on being neutralized on foraging of its electron by H+ made available through free radical scavenging antioxidant. In course of this neutralization, the DPPH* molecule achieves a paramagnetic status, mainly responsible for exhibiting a characteristic specific EPR signal (Ameta and Singh, 2015). Owing to this, DPPH* activity evaluation was used to estimate RSAs of DLEs through measurement of relative % decrease in absorbance at 517 nm, in accordance with Equation (4).

Figure 7 shows the corresponding percentage scavenging activities. For the study of a critical impact of emulsions mediated antioxidant activity, the RSAs of pure Ceftriaxone were also evaluated separately. The interactions of antioxidants with DPPH* proceed at variable rates and relative kinetics, depending on relative ease of antioxidant activity mechanism and a corresponding effect of UV light (Kitawat et al., 2013; Ameta et al., 2013). A decrease in absorbance depicts different modes of antioxidant activity. Thus, a particular antioxidant causing a reduction of initial DPPH absorbance by 50% (Kitawat et al., 2013; Ameta et al., 2013) was chosen as a characteristic standard for antioxidant potential determination in an impact wise manner.

Figure 7 depicts data for free RSAs of prepared DLEs, presented as means ± SD of three determinations. Here, pure Ceftriaxone showed nearly 23% RSAs respectively, while upon encapsulation it showed a maximum of 86% with respect to the amount of oil. So, an enhancement in the antioxidant ability of Ceftriaxone was observed when it was encapsulated within emulsions. However, with DTAB, three bulky –CH3 groups result in the stearic hindrance around the hydrophilic part, ultimately resulting in stronger hydrophobic interactions due to London dispersive forces. This leads to a formation of larger aggregates in the formulation since the drug with oil leads to the manifestation of additive hydrophobicity in the medium in an entropically driven mode.

Further, it was also observed that an optimum concentration effect with respect to encapsulating efficacy and activity of Ceftriaxone seems to play a key role in optimizing its therapeutic efficacy. As the effect of Ceftriaxone’s antioxidant efficacy through emulsions was studied without temperature fixation, this leads to a reliable speculation that the emulsions possess an oil
content dependent RSA which characteristically modulates the interaction and corresponding free radical saturation potential (Figure 7).

Investigation of antioxidant potencies through physicochemical properties

The antioxidant potential study or interaction with DPPH free radical was also investigated through measuring the difference in the data of PCPs. Similar to spectrophotometric method, the decrease in values of surface tension and viscosity of DPPH+DLEs from pure DPPH was made as a basis for RSA. The viscosity and surface tension of pure DPPH solution were 0.6546 {\text{Ps}} and 43.57 {\text{m N m}} respectively, when the same values were compared with the values of DLEs+DPPH solutions and these were found to be less (Figure 8). With the analysis of
surface tension the same values decreased only for 0.1 ml oil and afterward increased indicating the effect of the concentration of the oil. Analysis of viscosity revealed that these continuous decreases with increasing amount of oil indicate better physicochemical tool for analysis of RSA.

The percentage difference in density, surface tension and viscosity of DPPH and DPPH+DLEs were calculated as per Equations 5, 6 and 7; these are referred to as overall interaction, surface scavenging and bulk scavenging (Figure 9). The –ve values for overall interaction show strong interaction which is increased with respect to the amount oil. Figure 10 shows the percentage comparative analysis of bulk and surface scavenging where they are showing reverse trend infers that as surface scavenging is decreased the bulk scavenging is increased with the amount of oil. Initially, surface scavenging is high because at first the interaction occurs at surface and then slowly interacts in bulk.

**Investigation of antioxidant potencies through potential analysis**

Similarly, the RSA is also analyzed with potential study;
initially, the potential of pure DPPH was taken and compared with the potential of DPPH + DLEs. With respect to spectrophotometry, the decrease in potential of DPPH was made criteria for the RSA analysis. Potential of pure DPPH was 0.210 V, and decrease in the same was noticed by the addition of DLEs (Figure 11), where zero shows potential of pure DPPH.

**Conclusion**

Our study proved that the encapsulation of ceftriaxone within the physically active formulations leads to the optimization of its activity on an in-vitro basis. The formulated emulsions supplement the immune protective attributes of ceftriaxone, that otherwise remain impaired due to its rapid clearance from the systemic tissue environment.

The investigation of the ceftriaxone loaded formulations using physicochemical accounts for the fact that, in general, with an increase in oil, the corresponding parameter goes on increasing. Also, the physicochemical trends reflect the fact that an optimum of oil and surfactant combination is essential for making the emulsions as ideally feasible with a viewpoint to augment the antioxidant efficacy of ceftriaxone, due to the existence of a canonical ensemble. The incorporation of neem oil in the current study is also evidently justified through the symmetric distribution and interaction patterns of PCPs. Even though improved free radical scavenging activity is obtained with such formulations, further, there is a sharp modulation and optimization of physicochemical features of formulations, leading to the best efficiency towards an enhanced antioxidant effect. The reliable and promising outcomes of this study have been taken in due course, where in absence of DPPH the antioxidant analysis could be carried out with physicochemical properties.

The radical scavenging activities (RSA) of the encapsulated Ceftriaxone, have thus, confirmed an improvement in its antioxidant efficacy and the analysis of PCPs has depicted its loading efficacy and relative binding ability. Therefore, our study could not only furnish better understanding of enhancing the RSA of Ceftriaxone but also lead to the design of novel formulations with other surfactants and co-surfactants, thereby enabling better stability.

**ACKNOWLEDGMENT**

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**Figure 11:** Radical scavenging activity through potential analysis.
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