Biodegradable Polymeric Film for Food Packaging

Raminder Kaur  
Delhi Technological University

Neelam Yadav*  
Delhi Technological University

ABSTRACT

Food packaging films that show post-consumer biodegradability are rarely explored by researchers. The present study was carried out to investigate the physicomechanical characteristics of ethyl cellulose (EC) based films to be used in food packaging. Ethyl cellulose was plasticized with different percentage of polyethylene glycol (PEG). The samples of standard dimensions were subjected to different testing such as soluble matter content, moisture content, oil permeability, surface morphology, mechanical testing etc. The data obtained was analysed to decide the moderate percentage of plasticizer that can be used to provide a rational explanation of a perfect quality specimen. It has been revealed that too high or too low percentage of plasticizer was not appropriate for a good film. Tensile stress analysis was used to estimate the mechanical properties of the films. The results have shown an increase in tensile properties with increase in coalescence temperature. Temperature-dependent plastic deformation was observed for coalescence temperatures above 50°C.

KEY WORDS

Ethyl cellulose, Plasticizer, Biopolymers, Food packaging.

*Raminder Kaur  
Corresponding Author  
rkwaliadtu@gmail.com
INTRODUCTION

Packaging protects food from the external environment and provides information to the consumers about the food inside the package. Foods are packaged to deliver safe, wholesome and nutritious food to the consumers and it gives a high significance of link between packaging and consumer protection [1]. Plastics commonly used in food packaging include polyethylene (both HDPE and LDPE), polypropylene, polyvinyl chloride, polyethylene terephthalate and polystyrene [2]. These plastics used in food packaging, are derived from petroleum and generate greenhouse gases, thus cause a huge impact on environment. Moreover, they also interact with food material and may release toxic chemicals into food [3]. In addition, the quality of the packaged food is likely to be deteriorate during its storage due to change in its natural ingredients, which may include the loss in the moisture content or volatile odour. However, this deterioration can be reduced by selecting passable food packaging or coatings. Many researchers have reported the enrichment of these packaging films by the addition of other active agents such as O₂ and CO₂ absorbers, antimicrobial agents and antioxidants etc. in order to enhance their utility [4-7]. However, the food packaging films which show post-consumer biodegradability, without causing much burden on the environment were not much explored [8]. Like other polymeric products [9-15], researches are now focused on the development of the bio-based performance end products in the food packaging, with an attention to make them environment friendly. Most widely used bio-based polymers are the cellulose derivatives, such as methyl cellulose, cellulose acetate, hydroxypropyl cellulose and ethyl cellulose, that, have a backbone of cellulose with repeating structure of anhydrous glucose units, each having three reactive -OH (hydroxyl) sites [16]. Ample of work has been done in the past for the preparation and investigation of modified atmosphere packaging films based on protein, lipid and polysaccharide, which provide barrier to the volatile component of food as well as its moisture content [17,18]. However, the main concern is that, these bio-degradable packaging films lack many desirable characteristics of packaging materials to be used at a commercial scale. Various approaches may be used to overcome these problems, including the modification of raw material or the incorporation of plasticizers to improve their film forming characteristics. The type of plasticizer and its concentration can modify the different physical properties of the films [19]. The present study has been conducted to synthesize ethyl cellulose based bio-degradable films with improved physical properties by the incorporation of polyethylene glycol as plasticizer. The ethyl cellulose films were synthesized by taking different ratios of ethyl cellulose and polyethylene glycol and their various properties are studied.

MATERIALS AND METHOD

2.1 Materials

Ethyl cellulose containing ethoxy content of about 48-49.5% was used for film formation. Ethanol (99.9%) was used as solvent for dissolving ethyl cellulose to form a 2.5% w/v solution with viscosity about 18-24 cp. Polyethylene glycol (MW 400) was used as plasticizer. All the chemicals used were of AR grade and were supplied by CDH (P) Ltd., New Delhi.

2.2 Method

2.2.1 Preparation of Ethyl Cellulose Film

All films were prepared by simple casting method. A 2.5% (w/v) solution of ethyl cellulose in ethanol was prepared by constant stirring at 100 rpm for 20-25 minutes using a magnetic stirrer at room temperature. After the formation of a homogenized solution, polyethylene glycol (PEG); as a plasticizer was added to it. The ethyl cellulose and polyethylene glycol
glycol used were in the ratio 4:1, 2:1 and 4:3 for different samples i.e. Sample 1, 2 and 3 respectively. The cast solution was then gently poured into a petridish (diameter = 4 cm) avoiding any bubble formation. The content of the petridish was then dried overnight at room temperature on a levelled surface, thus, resulting in a spotless film that was manually peeled unbroken from the casting surface.

2.3 Assessment of Ethyl Cellulose Films for their Suitability as Food Packaging Material

The samples of ethyl cellulose films containing different ratio of PEG were assessed for the physical and chemical properties so as to be used in a food packaging. Five replicates of each sample was tested for assuring the reproducibility of the results.

2.3.1 Film Appearance

The appearance of the ethyl cellulose films having different ratio of polyethylene glycol was assessed by its texture and uniformity.

2.3.2 Film Thickness

All ethyl cellulose films i.e. Sample1, Sample 2 and Sample 3 were acclimatised at 24°C ± 2°C and 75% RH for 24 hours and the thickness was determined using micro-meter, which recorded upto exactness of ± 0.001mm. The thickness measurements was done at 6 diverse points of the ethyl cellulose films. Mean value was used to estimate the thickness of the resulted ethyl cellulose films [20, 21].

2.3.3 Soluble Matter (SM)

The soluble matter percentage of the resulted films were estimated by immersing the samples (2.0 cm in diameter each) in 50 ml of distilled water, which was slowly stirred for a duration of 24 hrs. The samples were removed and dried in oven for 24 hrs. The following equation was used to calculate the amount of soluble matter in the resulted films [22].

\[
\text{Soluble matter} \% = \left( \frac{\text{Initial dry weight} - \text{Final dry weight}}{\text{Initial dry weight}} \right) \times 100 \quad (1)
\]

2.3.4 Moisture Content (MC)

The moisture content of all samples was calculated by measuring the weight loss of films by drying it in a furnace at 105°C ± 3°C for approximately 12 hrs, till a constant weight had been achieved (dry sample weight). Moisture content was determined as percentage loss in mass during drying of the films using following equation [23, 24].

\[
\text{Moisture content} \% = \left( \frac{\text{Initial mass} - \text{Final mass}}{\text{Initial mass}} \right) \times 100 \quad (2)
\]

2.3.5 Scanning Electron Microscopy (SEM)

The microstructural examination of ethyl cellulose films was done using a scanning electron microscope (SEM). Film samples to be tested for SEM were confirmed for no water present in them. For the purpose, they were kept in desiccators with P2O5 for 2 weeks. Further, ethyl cellulose films were frozen using liquid nitrogen and then a cryo-fractured cross section of these films were gold sputtered (placing gold layer of about 100 Å so as to escape charging up of the films under electron beam) and then observed under scanning electron microscope (Hitachi S3700 SEM) at 10 kV as accelerating voltage [21].

2.3.6 Mechanical Properties

The wet films were dried in a levelled- forced air drying oven, pre-set at a desired coalescence temperature (The temperature at which the components of miscible solution attract each other) for 24hrs [25]. Mechanical properties like tensile strength (σut), elongation at break (EAB %), elastic modulus (E) and work of failure for these ethyl cellulose films were determined by ASTM method, using an Universal Testing Machine (Micro UTM KLA Tencor T150 UTM with a gauge length of 2 mm to 10 mm and a load variable of 50mN to 20 kN.).The samples of each ethyl cellulose film was
cut into a rectangular shape having a measurement of 60 mm x 60 mm and with an average thickness of 1 mm. The samples was griped on the device with a gap of 1 mm and pulled away at a speed of 0.1 mm/s and load of 1 N. The curves plotted between stress (Tension force (F) / cross sectional area(S)) and strain (change in length (ΔL) / original length (L₀)) were used to determine the tensile properties as per equation 3 and 4, given below [24, 26].

\[ \sigma_{ut} = \frac{F}{S} \]  \hspace{1cm} (3)

\[ EAB = \frac{\Delta L}{L₀} \times 100\% \]  \hspace{1cm} (4)

Equation 3 was used to calculate the elastic modulus of the ethyl cellulose films, which was calculated by taking the ratio of the maximum force exerted on the film by the cross sectional area and is represented in MPa. Equation 4 represents the percentage change in strain.

Equation 5 is used to calculate the elastic modulus which is the ratio of stress and strain at elastic limit

\[ E = \frac{\sigma}{\epsilon} \]  \hspace{1cm} (5)

Where, \( \sigma \) denotes stress and \( \epsilon \) denotes strain at elastic limit

\[ \text{Work of failure} = \frac{(\text{Area under curve} \times \text{cross head speed})}{(\text{film thickness} \times \text{film width})} \]  \hspace{1cm} (6)

Work of failure represented by Equation 6 is a function of work done in breaking the film specimen and is representative of the film thickness.

Applied load is an important parameter to be considered, especially when comparing the results of two studies, since the strain or percent elongation of a polymeric film is normally inversely proportional to the rate of strain and the parameter changes due to various reasons namely sample preparation method, coalescence temperature and also on coating process variables.

\[ 2.3.7 \text{ Anti-permeability for Oils} \]

To test the anti-permeability of ethyl cellulose films (incorporated with different percentage of PEG) for liquid oils and oily foods, the samples of resulted films of size 2 cm x 2 cm was firmly pressed on the open end of the test tube (inner diameter- 25 mm, outer diameter-27 mm) containing liquid oil (soya bean oil). Further, the test tube was placed upside down on the filter paper, and was observed for the permeability of the oil through the ethyl cellulose films as time went. Observations were repeated on 5 samples of similar composition, for a duration of 3 days storage under normal conditions of temperature, pressure and relative humidity [27].

\[ 2.3.8 \text{ Reliability, Collapsibility and Water-solubility of Ethyl Cellulose films} \]

The samples of the ethyl cellulose films were considered reliable, as they maintained the integrity of appearance, when peeled from petri dish. Also, the samples of resulting ethyl cellulose films were creased for 20 times at the same line to test its plasticity or collapsibility. Water solubility of the samples were examined by immersing a square piece of each film in 100 ml of distilled water, without stirring, at a temperature of 25°C. Water solubility of ethyl cellulose film was estimated by observing the time taken for the film to totally dissolve in water [21].

\[ \text{RESULTS AND DISCUSSIONS} \]

\[ 3.1 \text{ Film appearance} \]

The samples of ethyl cellulose films formed on a flattened surface was peeled off unspoiled from the petri-dish. The appearance of the sample 1 was hazy and that of sample 3 was translucent while the appearance of sample 2 was opaque, as seen from Figure 1. However the texture of all the samples was smooth and homogeneous and showed no breakage while peeling from the surface. These same samples were used for further testing.
Fig. 1: Appearance of surface of Ethyl cellulose film containing different ratio of ethyl cellulose and polyethylene glycol in ethanol (a) EC: PEG-4:1 (b) EC: PEG-2:1 (c) EC: PEG-4:3
3.2 Films Thickness

The samples of resulted films were measured for the film thickness at 6 different points (centre of each film, at centre of each quarter of the film and edges of the film). Average value of all the points was calculated to determine the thickness of each ethyl cellulose film. The mean thickness of various samples was measured as 1.010 mm with a standard deviation of 0.01, 1.017 mm with standard deviation of 0.04 and 1.014 mm with a standard deviation of 0.02 for sample 1 (EC:PEG-4:1), sample 2 (EC:PEG-2:1) and sample 3 (EC:PEG-4:3) respectively.

3.3 Soluble Matter (SM)

The samples of the resulted ethyl cellulose films immersed in distilled water for 24 hrs, showed no swelling, as well as, they maintained their structure. The result of soluble matter among different categories of ethyl cellulose film was maximum for the ethyl cellulose film containing EC: PEG ratio 4:3, which showed approximately 75.00% total soluble matter and minimum for the ethyl cellulose film containing EC: PEG ratio 2:1, having approximately 56.25% total soluble matter. However, the soluble matter for ethyl cellulose film containing EC: PEG ratio 4:1 showed the total soluble matter of about 60.97%. The mean values of initial and final weights (with standard deviation) for each sample were as given in Table 1. The soluble matter content for ethyl cellulose films of different EC: PEG compositions is as shown in Figure 2.

Sample of ethyl cellulose film with EC: PEG 2:1 ratio showed a slight lower moisture percentage (about 10% decrease) as compared with the sample having EC: PEG 4:1 ratio, while the ethyl cellulose film having EC: PEG 4:3 ratio showed a great increase in moisture content. Figure 3 gives the moisture content for ethyl cellulose films of different EC: PEG compositions.
compositions. The low moisture percentage of EC: PEG 2:1 can be explained as a result of interference of water structure in the binding condition. The mean values of weights (mg) and moisture content (%) of different EC: PEG compositions are given in Table 2.

### 3.5 Scanning Electron Microscopy (SEM)

The microstructural examination of the ethyl cellulose films was qualitatively studied using scanning electron microscopy (SEM). The images of the surface and cross section of EC films revealed the effects of addition of different percentage of PEG. Figure 4 show the SEM images of PEG plasticized ethyl cellulose films with EC: PEG ratio 4:1, EC: PEG ratio 2:1 and EC: PEG ratio 4:3 respectively. The surface image of both ethyl cellulose films with EC:PEG ratio 4:1 and 2:1 showed unbroken, compact and smooth traits without any micro-cracks, which meant polyethylene glycol has been easily incorporated into ethyl cellulose as plasticizer. However the EC film with ratio 4:3 showed few cracks and the surface was not apparently smooth, which might be due to decreased polymer-polymer cohesiveness, with increases content of plasticizer, leading to the deterioration of the quality of film. After observing all the three films, it is inferred that the EC: PEG ratio of 2:1 and EC: PEG ratio of 4:1 both are considered suitable for uniform and good quality film.

The structure of the film is affected by the organization of various constituents in the film making solution and the interaction taking place between the constituents during the drying of the ethyl cellulose film. The microstructural analysis of the film is also helpful to know the mechanism of water vapor permeability across the films and the arrangement of components in the film and their effect on mechanical properties of the film [28].

---

**Table 2: Mean values of weights (mg) and moisture content (%) of different EC: PEG Compositions**

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>EC:PEG</th>
<th>INITIAL WEIGHT (mg)</th>
<th>FINAL WEIGHT (mg)</th>
<th>MOISTURE CONTENT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4:1</td>
<td>0.043</td>
<td>0.016</td>
<td>62.79</td>
</tr>
<tr>
<td>2</td>
<td>2:1</td>
<td>0.022</td>
<td>0.011</td>
<td>50.00</td>
</tr>
<tr>
<td>3</td>
<td>4:3</td>
<td>0.044</td>
<td>0.014</td>
<td>68.18</td>
</tr>
</tbody>
</table>

---

**Fig. 3: Moisture Content for Ethyl Cellulose Films of Different EC: PEG Compositions**
Fig. 4: SEM Images of PEG 400 Plasticized Ethyl Cellulose Films (a) EC: PEG ratio: 4:1; (i) surface of the film, (ii) cross section of the film (b) EC: PEG ratio: 2:1; (i) surface of the film, (ii) cross section of the film. (c) EC: PEG ratio: 4:3; (i) surface of the film, (ii) cross section of the film.
3.6 Mechanical Properties

Characteristic features of the load-time or typical stress-strain profiles from tensile tests are routinely used to characterize the polymer properties. Toughness is directly proportional to the area under a load-time or load-displacement curve which is quantitated as energy. The higher the amount of energy a polymeric film can absorb prior to break under load, the higher is its toughness. Elastic modulus is an indicator of the elasticity of the film, with lower values corresponding to higher elasticity. On the other hand, tensile strength is an indicator of the film strength, with larger values corresponding to the stronger films. Ideally a film coating must be both strong and elastic and, therefore, must yield a higher tensile strength-to-elastic modulus ratio. This ratio has been used as an indicator of the overall mechanical quality of the films.

Figure 5 depicts the plot of tensile strength as a function of coalescence temperature for the samples prepared. The tensile strength of the films increased with a corresponding increase in coalescence temperature and did not reach a plateau within the temperature range used in this investigation. It was observed that sample 2 showed best characteristics in comparison to other samples. At temperatures above 60°C, no appreciable differences were observed in the tensile strengths of the films cast from all formulations. This could possibly be due to evaporation of the plasticizers from the films at elevated temperatures. Mean values of tensile strength of various samples at different coalescence temperatures is as given in Table 3.

Mean values of work of failure of various samples at different coalescence temperatures are given in Table 4. The values of work of failure

---

**Figure 5: Plot of Tensile Strength Vs Coalescence Temperature (Sample1-EC: PEG - 4:1; Sample 2- EC: PEG-2:1; Sample 3 – EC: PEG-4:3).**

**Table 3: Mean values of tensile strength of various samples at different coalescence temperature**

<table>
<thead>
<tr>
<th>Coalescence temperature in (°C)</th>
<th>Tensile strength in (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAMPLE 1 (sd)</td>
</tr>
<tr>
<td>30</td>
<td>0.007 (0.00015)</td>
</tr>
<tr>
<td>40</td>
<td>0.013 (0.0017)</td>
</tr>
<tr>
<td>50</td>
<td>0.016 (0.00114)</td>
</tr>
<tr>
<td>60</td>
<td>0.016 (0.00125)</td>
</tr>
<tr>
<td>70</td>
<td>0.0149 (0.00143)</td>
</tr>
</tbody>
</table>
plotted as a function of coalescence temperature for all the three samples are shown by Figure 6. No significant difference was observed in the work of failure for the films cast from formulation EC:PEG ratios 4:1 and 2:1. The work of failure value for the formulation with EC:PEG ratio 4:3 is almost approaching zero at lower coalescence temperature i.e. at 30°C. At higher coalescence temperatures, the higher values of work of failure depict a significantly higher absorption of energy, possibly due to the complete penetrate of plasticizer molecules into the polymeric network. From the load-time profiles, it was observed that the films at and above 50°C exhibited distinct and prolonged plastic deformation with the resultant increase in the area under the curve. This could be due to strong interaction between the neighboring polymer particles at and above the coalescence formulation.

The influence of coalescence temperature on the elastic modulus values of the films is shown in Figure 7. Plot of applied load with time is used to calculate the value of elastic modulus. Higher value of load carrying ability indicate a high value of elastic modulus, thus reflects the strength of the film. In general, it is observed that as the coalescence temperature get increased, the elastic modulus of the films increased almost linearly, for all the samples of the polymeric films, until they achieved a peak. A decline in the values of elastic modulus values was further observed, at elevated coalescence temperatures, which is due to the softening of the film at elevated temperature. Mean values of load

<table>
<thead>
<tr>
<th>Coalescence temperature in °C</th>
<th>Work of failure (Nm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAMPLE 1 (sd)</td>
</tr>
<tr>
<td>30</td>
<td>5 (0.67)</td>
</tr>
<tr>
<td>40</td>
<td>4 (0.45)</td>
</tr>
<tr>
<td>50</td>
<td>5.5 (0.167)</td>
</tr>
<tr>
<td>60</td>
<td>15 (0.253)</td>
</tr>
<tr>
<td>70</td>
<td>30 (1.67)</td>
</tr>
</tbody>
</table>

Fig. 6: Work of Failure vs Coalescence Temperature of Different Ethyl Cellulose Films (Sample1-EC: PEG - 4:1; Sample 2- EC: PEG - 2:1; Sample 3 – EC: PEG - 4:3).
Table 5: Mean values of load carrying capacities for various time periods

<table>
<thead>
<tr>
<th>Time in (sec)</th>
<th>Applied load (Elastic Modulus in MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample 1</td>
</tr>
<tr>
<td>0</td>
<td>0.3 (91.52)</td>
</tr>
<tr>
<td>2</td>
<td>0.3 (91.52)</td>
</tr>
<tr>
<td>5</td>
<td>0.35 (106.78)</td>
</tr>
<tr>
<td>10</td>
<td>1.1 (335.58)</td>
</tr>
<tr>
<td>12</td>
<td>0.3 (91.52)</td>
</tr>
<tr>
<td>15</td>
<td>0.3 (91.52)</td>
</tr>
<tr>
<td>20</td>
<td>0.31 (94.57)</td>
</tr>
<tr>
<td>25</td>
<td>0.3 (91.52)</td>
</tr>
<tr>
<td>30</td>
<td>0.31 (94.57)</td>
</tr>
<tr>
<td>35</td>
<td>0.3 (91.52)</td>
</tr>
<tr>
<td>40</td>
<td>0.31 (94.57)</td>
</tr>
</tbody>
</table>

Fig. 7: Applied Load Vs Time of Different Ethyl Cellulose Films (Sample1-EC: PEG - 4:1; Sample 2- EC: PEG - 2:1; Sample 3 – EC: PEG - 4:3) used for calculation for elastic modulus.
carrying capacities for different ethyl cellulose films at various time periods is as given in Table 5.

The independent interpretation of these three parameters, i.e., tensile strength, work of failure, and elastic modulus may not always lead to meaningful conclusions with respect to the in situ performance of the films. The mechanical properties of the films was observed to be dependent on the type of the plasticizer and the coalescence temperature. With an increase in the coalescence temperature, the films exhibited a characteristic transition in their mechanical properties, i.e., from a soft and weak system (as evident from lower values of tensile strength, strain at break and elastic modulus) to a hard and tough system (as evident from higher values of tensile strength, strain at break, and elastic modulus). The optimal values of the tensile strength-to-elastic modulus ratio obtained for films of all three formulations dried at 50°C indicate that the films coalesced at or around this temperature may be less susceptible to physical defects.

3.7 Anti-permeability for Oil

The bio polymeric films used for packaging food, especially oily foods or liquid oil containing edibles, should be anti-permeable for oils, otherwise leakage of oil from packaging films will cause decline in the appearance, flavor and shelf life of packaged foods. So, the anti-permeability test of different sample of ethyl cellulose was carried out. The result showed no trace of oil leakage through the ethyl cellulose films, thus offering good anti-permeability for oil [29].

3.8 Reliability, Collapsibility and Water-solubility of Ethyl Cellulose Films

Samples of ethyl cellulose film formed by casting method showed complete integrity while peeling off from the surface of the petri-dish. The thickness of the resulted films are as reported in Table 1. The folding test performed for finding the collapsibility of ethyl cellulose films showed that 20 times folding of film at a particular trace did not showed any fissure in sample1 and sample 2 but in sample 3 slight marking was observed. The visual test showed that the sample 2 had a good flexibility of all the films. Further the water solubility of the samples of ethyl cellulose films with different ratio of EC: PEG also indicated a delayed water solubility in sample 2 in comparison to sample 1 and sample 3. The comparison of the properties of ethyl cellulose films with different EC: PEG ratios is as given in Table 6.

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>EC:PEG</th>
<th>APPEARANCE</th>
<th>THICKNESS (mm)</th>
<th>COLLAPSIBILITY (folding at a point)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4:1</td>
<td>Hazy</td>
<td>1.02</td>
<td>NO FISSURE</td>
</tr>
<tr>
<td>2</td>
<td>2:1</td>
<td>Opaque</td>
<td>1.01</td>
<td>NO FISSURE</td>
</tr>
<tr>
<td>3</td>
<td>4:3</td>
<td>Translucent</td>
<td>1.01</td>
<td>SLIGHT FISSURE</td>
</tr>
</tbody>
</table>

CONCLUSION

The ethyl cellulose films with different amount of polyethylene glycol, as plasticizer, had been casted to be used as a bio-degradable food packaging material. Among the different EC: PEG ratios used, the film with EC: PEG ratio 2:1 was found to have better suitability for food packaging applications and showed a very good flexibility and collapsibility. The soluble matter and moisture content of the film with EC: PEG ratio 2:1 was found to be minimum, among all the films tested. The developed film showed a maximum tensile strength of 0.0165 MPa at a coalescence temperature of 50°C and a maximum work of failure of magnitude 29.5 Nm/sec was observed at temperature of 70°C. The
film also exhibited a maximum load of 2.65 kN for a substantially good amount of time. The film was hence found to be mechanically stable of handling sufficient amount of load. The three parameters, characterising mechanical strength of the film namely tensile strength, work of failure, and elastic modulus were found to be good for the reported polymeric films.

CONFLICT OF INTEREST

The work presented in this manuscript has been carried out for the research purpose only and there is no conflict of interest involved in it.
REFERENCES


