

# Investigation of Moisture Sorption Properties of Gelatin/Poly (Aniline)/Films

Bajpai SK<sup>1\*</sup>, Sanjay Awasthi<sup>2</sup>, Ajay Singh Utiye<sup>2</sup> and Bharat Mishra<sup>3</sup>

<sup>1</sup>Polymer Research Laboratory, Department of Chemistry, Govt. Model Science College, Jabalpur (M.P)-482001, India

<sup>2</sup>Department of Physics, Govt. Model Science College, Jabalpur (M.P) – 482001, India

<sup>3</sup>Department of Physical Sciences, MGCGV Chitrakoot (M.P)-485334 India

Received: May 11, 2017; Accepted: August 01, 2017; Published: August 08, 2017

\*Corresponding author: Bajpai SK, Professor Polymer Research Laboratory, Department of Chemistry, Govt. Model Science College, Jabalpur (M.P) – 482001, India, Tel:+919993220651; E-mail: sunil.mnlbpi@gmail.com

## Abstract

This work describes moisture sorption behavior and other physico-chemical properties of gelatin/poly (Ani) films. The glyoxal (Glox) - cross linked gelatin (Gel) films have been loaded with aniline, followed by their *in-situ* oxidative polymerization to yield Gel/poly (Ani) composite films. The moisture uptake behavior of the plain Gel and Gel/poly (Ani) films has been studied under various Relative Humidity (RH) and at different temperatures. The data obtained has been interpreted in the terms of GAB model. The thermodynamic parameters such as  $q_{st}$  and  $sd$  have also been determined using Clausius-Clapeyron equation. It was found that both, the  $q_{st}$  and the  $sd$  decreased with moisture contents. Finally, the films exhibited fair biocompatible behavior.

**Keywords:** Films; Protein; Gelatin; Cross-linking; Swelling;

## Nomenclature

Gel-Gelatin; Poly (Ani)-Poly aniline; Glox- Glyoxal; RH - Relative Humidity; ECP- Electrical Conducting Polymers; APS - Ammonium Per sulphate; EMC-Equilibrium Moisture Content ;ACD- Acid Citrate Dextrose; OD- Optical Densities; GAB - Guggenheim-Anderson-de Boer.

## Introduction

In the last decade, a great emphasis has been made on using Electrical Conducting Polymers (ECP) for biomedical applications. Responsiveness of some tissues to electric stimuli makes the biocompatible CP's particularly attractive for several biomedical applications. Availability of such materials may provide solutions to many problems in neural biology/medicine [1]. Such materials have been shown to modulate activities of nerve, cardiac, skeletal muscle, and bone cells. They stimulate cell growth, migration, and adhesion; enhance DNA synthesis and protein secretion. One of the widely studied polymers is poly (aniline). There have been several reports, published recently, describing synthesis and applications of poly (aniline) [2,3]. Poly aniline is a promising conducting polymer with still increasing application potential in biomedicine. Its surface modification can be an efficient way how to introduce desired functional groups and to control its properties while keeping the bulk characteristics of the material unchanged [4]. They have also been employed as electrochemical sensors [5,6]. Recently, poly (aniline) has also been doped with metal oxide nano particles to obtain light tunable devices [7].

Although, poly (aniline) based films appear to have a variety of applications in various applied fields, still there is lack of literature related with the biomedical applications of poly (aniline) films. One of the reasons is that poly (aniline) films have poor solubility, fusibility and processibility [8]. Moreover, it has very poor hydrophilic nature which limits its applications for wound dressings and drug delivery [9]. One of the strategies to minimize the above mentioned drawbacks of poly (aniline) is to prepare its blends with some hydrophilic biopolymer so that the resulting polymer can have better hydrophilic nature and may be employed in biomedical field. For example, most recently [10] nonwoven mats of electro

spun poly (lactic acid)/poly aniline blend nano-fibers have been prepared and characterized by various analytical techniques. Similarly, Merlini, et al. [11] have reported electro spun mats by blending Emeraldine Base (EB) or DBSA-doped Poly aniline (PANI.DBSA) with poly (vinylidene fluoride) (PVDF). However, in the methods, used to prepare blends, the two polymer solutions are mixed under specific conditions so as to prepare the blend with required properties. Contrary to this, in our previous communication [12], we developed a new method which involved *in-situ* polymerization of aniline within the gelatin (Gel) film to obtain uniform distribution of aniline within the gelatin film. We also investigated characterization and water absorption behavior of these films. In continuation, we here by report moisture absorption and biocompatibility of these films.

## Experimental

### Materials

The monomer Aniline (Ani), the initiator Ammonium Per Sulphate (APS), polymer gelatin (Gel) and its cross linker glyoxal (Glox) were obtained from Hi Media Chemicals. Mumbai, India and used as received. The various salts, such as KOH,  $CH_3COOK$ ,  $K_2CO_3$ ,  $Mg(NO_3)_2$ , NaCl, KCl and  $K_2SO_4$  were obtained from SRL, Pune, India and were analytical grade. The Relative Humidity (RH), produced by the above mentioned salts, was adopted from the Ref. [13] and the values are given in Table I. We used de-ionized water throughout the experiments.

### Preparation of glyoxal-cross linked- gelatin Gel films

A pre-calculated quantity of gelatin (Gel) was added in to definite volume of water and left for 12 h under moderate stirring to ensure complete dissolution. The solution, thus obtained, was filtered using a sieve so as to remove minute impurities and finally an almost transparent pale yellow solution of gelatin was obtained. The solution was made up to a definite volume so as to get a 10 % (w/vol) Gel solution. In order to prepare cross linked film, 20 ml of gelatin solution was taken in a beaker and to it 0.5 ml of 40 % (vol/vol) solution of Glox was added under mild stirring and the total volume was made up to 25 ml with distilled water. The above cross linking reaction mixture was transferred in to Petri plate and kept in an electric oven (Temp star, India) at 60°C for a period of 5 h to ensure complete cross linking. The film, thus obtained, were equilibrated for 6 h in refreshing distilled water to remove un-reacted chemicals, and then dried till constant weight.

### Preparation of Gel/poly (Ani) film

In the present work poly (Ani) has been synthesized *in-situ* within the gelatin film via oxidative polymerization. We followed the same procedure as reported in our previous work. In brief, aniline was dissolved in pre-determined quantity of 2M HCl under chilled conditions and thereafter definite volume of 2 % (wt/vol) APS solution was added. Finally, this polymerization mixture was kept overnight in a reaction chamber for a period of 12 h to ensure complete formation of poly (Ani) within the glyoxal-cross linked gelatin film. The Gel/poly (Ani) film was taken out, washed with water and then allowed to dry at 40°C till constant weight.

### Determination of Moisture Sorption Isotherm

The moisture uptake of plain gelatin and Gel/poly (Ani) films was investigated using weight measurement approach as described elsewhere [14]. The in-lab built apparatus was employed to study the weight gain by the films under various relative humidity environments (see Figure 1). A completely dried piece of pre-weighed film was placed in the small crucibles and put in the poly (propylene) chamber as shown in Figure 1. Indeed, a number of such chambers were employed, filled with different saturated salt solutions to produce definite RH environments as shown in Table I. The films were taken out after every 24 h till they attained constant weight. The moisture uptake of films was determined from the following expression and expressed as g per g dry film.

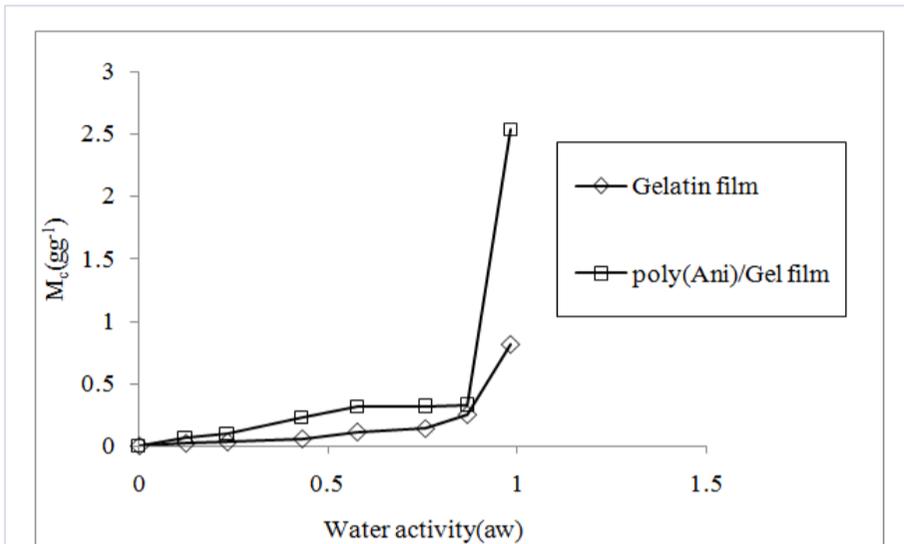


Figure 1: Moisture sorption isotherms for Gel and poly (Ani)/Gel films at 30°C

Table 1: The water activities ( $a_w$ ) of saturated salt solutions 30°C

Salt	$a_w$ at 30°C
KOH	0.0738
CH <sub>3</sub> COOK	0.2161
K <sub>2</sub> CO <sub>3</sub>	0.4317
Mg(NO <sub>3</sub> ) <sub>2</sub>	0.514
NaCl	0.7509
KCl	0.8362
K <sub>2</sub> SO <sub>4</sub>	0.97

$$\text{Moisture uptake (g/g dry film)} = \frac{W_e - W_0}{W_0} \text{ g/g dry film} \quad \dots(1)$$

All the measurements were made in triplicate and average data are shown

### Blood compatibility

The plain Gel and Gel/poly (Ani) films were investigated for thrombogenicity and hemolytic potential according to the procedure reported in the International Standard Organization (ISO) (ISO 10993-4, 1999). A 0.9 % solution of NaCl, with final pH adjusted to 7.4, was employed in the study [17]. All the measurements were made in triplicate and the average data were used.

### Thrombus formation test

In a typical experiment as suggested elsewhere [18], the test sample, having surface area of almost 1 cm<sup>2</sup>, was equilibrated in 0.8 % saline for a period of 24 h at 37°C. Now, the film was taken out and 0.95 ml of Acid Citrate Dextrose (ACD) and 0.03 ml of calcium chloride were added on its surface. After a time period of 45 min, the blood clotting process was stopped by adding 4.0 ml of water and the clot thus formed was fixed by the addition of 2.0 ml of 36 % formaldehyde solution. The film was weighed to know the amount of clot formed on its surface. The positive and negative controls were taken as glass beaker without sample and glass beaker without sample and blood [19]. For comparison, a commercial paraffin gauge (G/1289 India) was also used.

The thrombus percentage was calculated as follow:

$$\text{Thrombose (\%)} = \left[ \frac{\text{Weight of test sample} - \text{Weight of (-) control}}{\text{Weight of (+) control} - \text{Weight of (-) control}} \right] \times 100\%$$

### Heamolysis test

The hemolysis tests were performed as described in American Society for Testing and Materials (ASTM) [20]. The film samples (area 1 cm<sup>2</sup>) were kept in 7mL of 0.9% saline solution taken in test tubes. After 24 h of incubation at 37°C, the film samples were taken out, and 0.05 ml of Acid Citrate Dextrose (ACD) blood was placed on the surface of each film. After 15 min, 10ml 0.9% saline solution was added and the films were kept in the incubator at 37°C for 3 h. Positive and negative controls were prepared by adding the same amount of ACD blood to 10 mL of distilled water and 0.9% saline, respectively. Each tube was gently inverted twice to make contact of the blood with the film. After incubation, each fluid was transferred to a suitable tube and centrifuged at 100 rpm for 15 min. The hemoglobin released by Hemolysis was measured by the Optical Densities (OD) of the supernatants at 545 nm using a UV-visible spectrophotometer (Shimadzu, Genesis 10-S). The percentage of hemolysis was calculated as follows [21].

$$\text{Heamolysis (\%)} = \left[ \frac{\text{OD of test sample} - \text{OD of (-) control}}{\text{OD of (+) control} - \text{OD of (-) control}} \right] \times 100\%$$

According to ASTM F 756-00, materials can be classified in to three different categories according to their percent heamolysis. Materials with > 5% Heamolysis are classified as haemolytic; while 2% – 5% are classified as slightly haemolytic and < 2% is considered as a non-haemolytic material.

## Results and Discussion

### Moisture Sorption Behavior

Figure 1 shows the experimental moisture uptake data, for the film samples Gel and poly (Ani)/Gel at 30°C. It can be seen that both of the curves are sigmoid in shape, exhibiting type-II characteristic isotherms. Such curves are typical for most of the biopolymers milk proteins [22], soy proteins [23] polysaccharides such as gum cordial [24] chitosan [25], cellulosic materials [26] etc. It may be seen that Gel film shows a higher moisture uptake at all water activities as compared to the Gel/poly (Ani) film. This may be attribute to the fact that gelatin is a hydrophilic polymer with a number of polar groups along the macromolecular chains. These groups interact with incoming vapor molecules and fix them along their active polar sites, thus causing a higher uptake. On the other hand, the Gel/poly (Ani) film has poly (aniline) network as one of the constituent which does not interact with vapor due to absence of polar sites along its aromatic structural network. However, the observed moisture uptake may simply be due to the presence of gelatin molecules. On the isotherms, obtained, three zones are noted; zone – I ( $a_w$ : 0.0 to 0.2), zone – II ( $a_w$ : 0.2 to 0.7) and finally zone – III ( $a_w$ : 0.7 to 1.0). In the region I (termed as monolayer sorption region). The EMC increases with water activity due to the presence of a large number of polar groups like –COOH, –NH<sub>2</sub> along the gelatin molecules. These groups act as strong binding sites for incoming water vapor molecules. The water vapor molecules are bound very strongly to the active polar sites available. Falade and Aworh [27]. In the zone – II also termed as multilayer sorption region, Moisture content increases appreciably with water activity and at a faster rate. In this zone, though sorption takes place at less active site, but there is unfolding of macromolecular chains due to water sorption. This results in exposure of new active sites to the incoming vapor molecules, thus enhancing the moisture uptake. However, this is more pronounced for pure gelatin film. Finally, in the zone – III (usually termed as capillary condensation zone) there is sharp increase in EMC which may be attributable to diffusion of moisture into voids and capillaries within the film matrix. The water in this zone is in the Free State. These isotherms show that substrate adsorbed proportionately more water towards the later part of the curves.

The moisture sorption behavior of above film samples was analyzed using three parameters GAB (Guggenheim-Anderson-de Boer), which is a theoretically derived isotherm model [28] as given below:

$$M = \frac{M_o C K a_w}{(1 - K a_w)(1 - K a_w + C K a_w)} \quad \dots(1)$$

Where,  $M_o$  is the monolayer moisture content,  $C$  is a constant related to the first layer heat of sorption and  $K$  is a factor related to the heat of sorption of the multi-layer. In order to determine the parameters of GAB isotherm model, GAB equation was re-arranged into a second degree polynomial equation, as shown below:

$$\frac{a_w}{M} = \alpha a_w^2 + \beta a_w + \gamma \quad \dots(2)$$

Where,

$$\alpha = \frac{K}{M_o} \left[ \frac{1}{C-1} \right] \quad \dots(3)$$

$$\beta = \frac{1}{M_o} \left[ 1 - \frac{2}{C} \right] \quad \dots(4)$$

and

$$\gamma = \frac{1}{M_o C k} \quad \dots(5)$$

A non-linear regression analysis of  $a_w/M$  as  $a_w$  yielded polynomial of second order as shown in

The coefficients  $\alpha$ ,  $\beta$  and  $\gamma$  were thus obtained from this polynomial equation and substituted one by one to obtain GAB constants. The related parameters for GAB model are given in Table 2. The parameters of GAB model provide important information about the behavior of substrate in the presence of moisture. The value of monolayer moisture content ( $M_o$ ) is indicative of the quantity of water molecules that are strongly bound to the active sites present on the surface of the substrate. The value of monolayer moisture content ( $M_o$ ) is of particular interest since its value indicates the amount of water that is strongly adsorbed to specific sites at substrate surface and is considered as the optimum value to assure stability of substrate material. Therefore,  $M_o$  is recognized as the moisture content affording the longest time period with minimum quality loss at a given temperature. Below it, rates of deteriorative reactions are minimum. Hence, at a given temperature, the safest water activity level is that corresponding to  $M_o$  or lower. The values of monolayer content  $M_o$ , as shown in Table 2, are 0.0260 and 0.0986 g/g for, poly (Ani)/Gel and pure Gel films respectively. The higher value of  $M_o$  for the pure Gel film may be attributable to the presence of various polar groups along the gelatin chains, thus providing more sites for attachment of water vapor molecules as compared to the poly (Ani)/Gel film which contains poly (aniline) as one of the constituents. The absence of polar groups in poly (Ani) contributes towards lowering of moisture absorption.

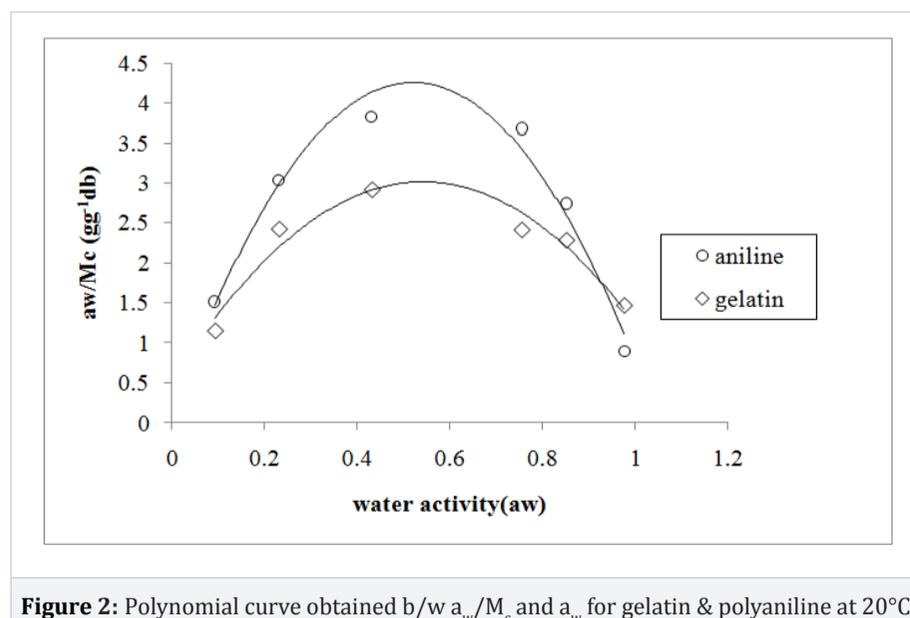


Figure 2: Polynomial curve obtained b/w  $a_w/M_c$  and  $a_w$  for gelatin & polyaniline at 20°C

Table 2: Data showing the parameters of GAB isotherm for Gelatin and poly (Ani)/Gel based films

GAB parameters	Gel/poly(Ani) film	Gel film
K	0.9491	0.4765
C	62.9687	11.1336
$M_o$ (g g <sup>-1</sup> db)	0.0620	0.0986
R <sup>2</sup>	0.9659	0.9390

The GAB constant  $C$  describes adsorbent – adsorbate interactions and it is reported that [29] the parameter  $C$  should fulfill following relations: for  $C > 2$  the GAB model should yield a sigmoidal shape curve with point of inflection (type II of Brunauer's (1943) classification): and for  $0 < C < 2$  the isotherm is of the type-III only (isotherm without point of inflection). In this study, the value of  $C$  was greater than 2 for both of the film samples and the isotherm curves obtained were also sigmoidal. Thus supporting the above predictions. Finally, the value of  $K$  provides a measure of the interactions between the molecules in multilayer with the adsorbent and tends to fall between the energy values of the molecules in the monolayer and that of liquid water. The prescribed range for  $K$  values is  $0 < K \leq 1$ . As can be seen, the values of  $K$ , obtained for both of the film samples fall within the prescribed range.

### Effect of temperature on moisture uptake

Temperature of the humid environment plays a significant role in governing the moisture uptake of the polymeric films. The moisture absorption isotherms of plain Gel and poly (Ani)/Gel films at 283, 293 and 303 K are shown in Figure.3 (A) and (B) respectively. It can be seen that both of the films, namely Gel and poly (Ani)/Gel, show sigmoidal type-II curves at all the temperatures. In addition they show negative trends, i.e. the moisture uptake decreases with the increase in temperature. This may simply be explained as follows: When the temperature is low, the inter molecular attractive forces between the active polar sites within the film matrix and the water vapor molecules are strong due to less kinetic energy of vapor molecules. However, as the temperature increases, the kinetic energy of incoming water vapor molecules increases and therefore they are not so strongly bound to the active sites available, thus causing a decrease in the equilibrium uptake.

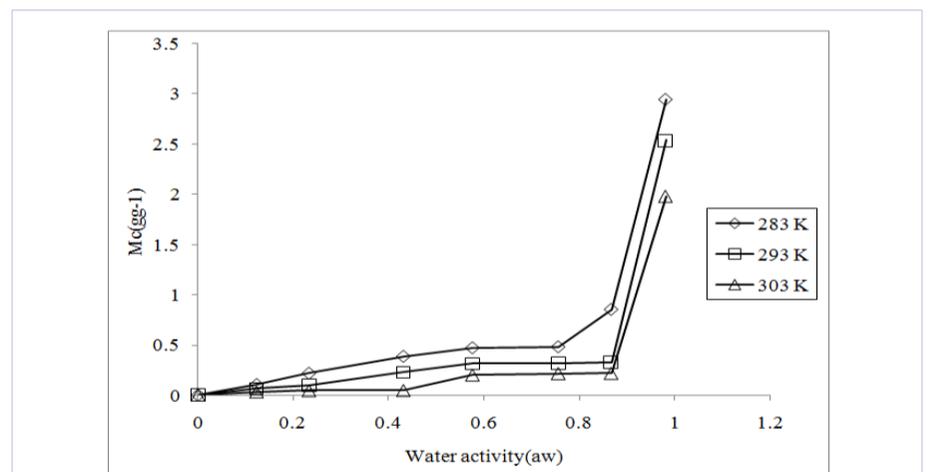


Figure 3A: Effect of temperature on moisture uptake of Gel films

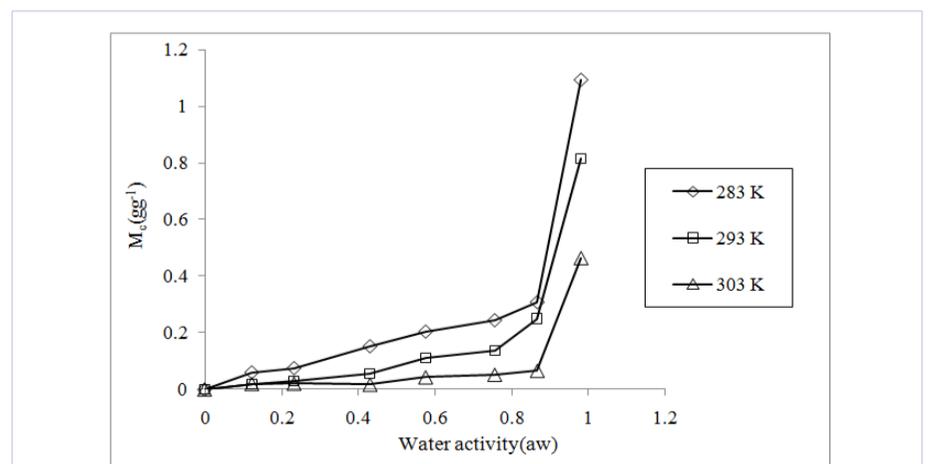


Figure 3B: Effect of temperature on moisture uptake of poly (Ani)/ Gel films

### Evaluation of thermodynamic parameters

The application of thermodynamic principles to sorption isotherm data has been used to obtain more information about the properties of water bound to the active sites of the film matrix, and physical phenomena on the film surfaces and sorption kinetic parameters. These parameters are very significant, particularly in case when the film is to be used for packaging of food stuff or similar products. The net isosteric heat of sorption ( $q_{st}$ ) is defined as the total heat of sorption ( $q_{st}$ ) minus the heat of vaporization of water at the system temperature [30]. The net isosteric heat of sorption or differential enthalpy shows the energy requirement for removing moisture from food material (water - solid binding strength) and has a practical use in complete drying calculations and modeling of energy [31]. Moreover, entropy change also plays an important role in the energy analysis of food processing systems and the differential entropy of sorption,  $S_d$ , can be calculated using Gibbs- Helmholtz equation [32]. For calculation of  $q_{st}$  and  $S_d$ , various values of moisture contents from 0.75 to 2.0 and 0.06 to 0.44 ( $gg^{-1}$  dry basis) were used for Gel and poly (Ani)/Gel films respectively.

Through these values, the corresponding values of  $a_w$  were determined at different temperatures. By plotting  $\ln a_w$  versus  $1/T$ , for a specific moisture content of the material and then determining the slope ( $-q_{st}/R$ ) and intercept ( $S_d/R$ ), the net isosteric heat ( $q_{st}$ ) and differential entropy ( $S_d$ ) of sorption were obtained using the following equation:

$$-\ln a_w = \frac{q_{st}}{R} \frac{1}{T} - \frac{S_d}{R} \quad \dots(6)$$

Where,  $q_{st}$  is the net isosteric heat of sorption ( $kJ mol^{-1}$ ),  $S_d$  is the differential entropy ( $kJ mol^{-1}K^{-1}$ ),  $R$  the universal gas constant ( $kJ mol^{-1} K^{-1}$ ),  $T$  is the absolute temperature, (K) and  $a_w$  is the water activity. The respective plots between  $\ln a_w$  and  $1/T$  for Gel and poly (Ani)/Gel films are shown in Figure. 4(A) and (B) respectively. Using the slopes and intercepts of linear plots, displayed in Figure.4, values of isosteric heat of sorption  $q_{st}$  and differential entropy  $s_d$  were evaluated. The data obtained indicated that  $q_{st}$  decreased from 78.94 to 2.34  $kJ/mol^{-1}$  with the increase in  $M_c$  from 0.2 to 0.9  $g/g$  db for native Gel film while the decrease in  $q_{st}$  was from 48.95 to 3.53  $kJ/mol^{-1}$  with the increase in  $M_c$  from 0.04 to 0.4  $g/g$  db for Gel/poly(Ani) film respectively (see Figure.5 (A) and (B)).

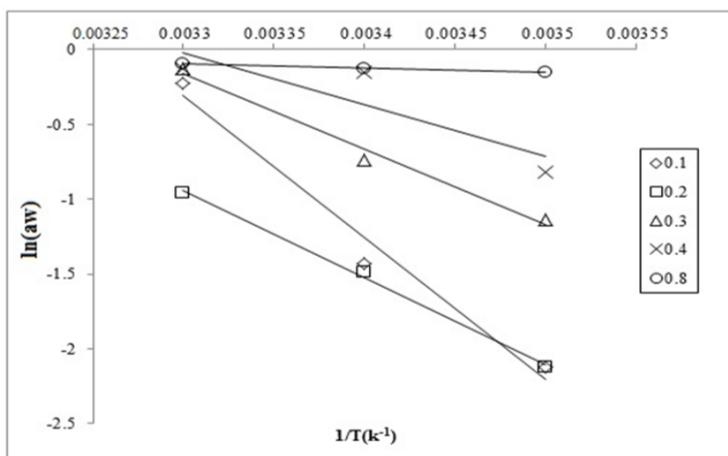


Figure 4A: Variation in  $M_c$  with  $1/T$  for the Gel film

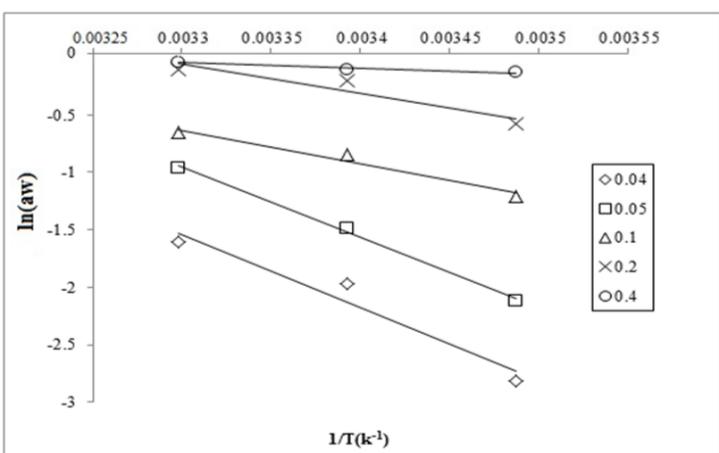


Figure 4B: Variation in  $M_c$  with  $1/T$  for the Gel/poly (Ani) film.

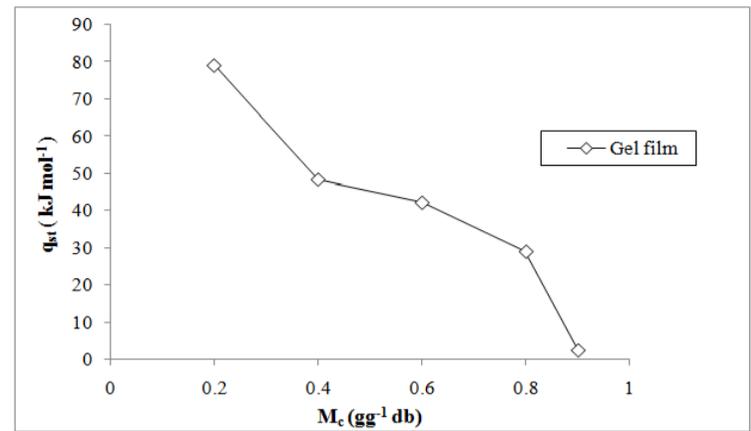


Figure 5A: Variation in  $q_{st}$  with  $M_c$  for the film Gel

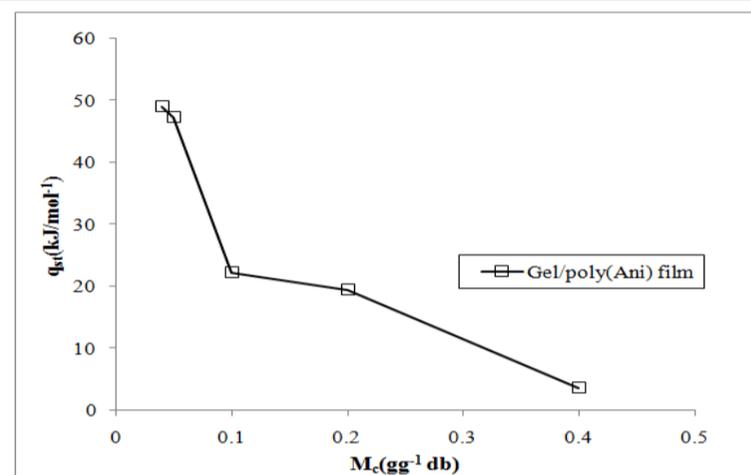


Figure 5B: Variation in  $q_{st}$  with  $M_c$  for the film Gel/poly (Ani).

This decrease can be attributed to the fact that initially sorption occurs at the most active sites, thus giving rise to greatest interaction energy. As the moisture content increases, the sites available for water vapor sorption decreases, thus resulting in lower values of  $q_{st}$  [33]. At low moisture content, higher  $q_{st}$  values could be due to strong interaction between water molecules and hydrophilic groups of starch molecules. Almost similar results have also been reported elsewhere [34]  $q_{st}$  ( $kJ mol^{-1}$ ).

The differential entropy of sorption  $S_d$  versus moisture content plot is also shown in Figure.6 (A) and (B). It is clear that differential entropy also decreases with increasing moisture content. The value of  $s_d$  decreased from 0.257 to 0.006 for the increase in  $M_c$  from 0.2 to 0.9  $gg^{-1}$  db in the case of native Gel film. On the other hand, the observed decrease in  $q_{st}$  from 0.152 to 0.011  $kJ/mol^{-1}$  for the corresponding increase in  $M_c$  from 0.04 to 0.4 was noticed for Gel/poly (Ani) film. These results show strong dependence of net isosteric heat of sorption  $q_{st}$  and differential entropy of sorption  $S_d$  on moisture content. Similar trends have been also reported elsewhere [35].

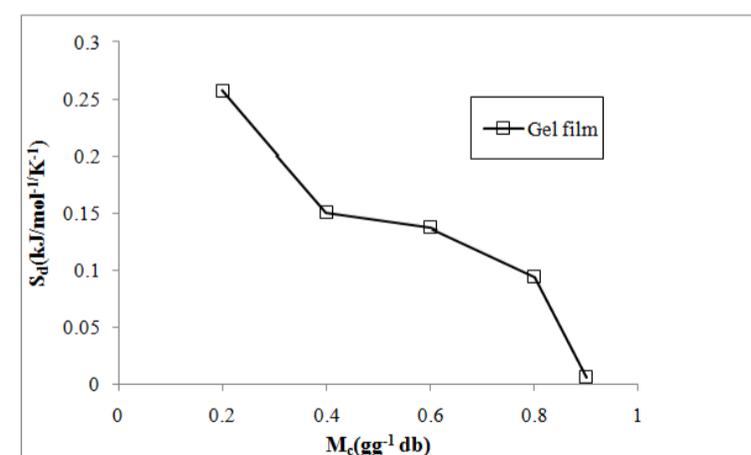


Figure 6A: Variation in  $s_d$  with  $M_c$  for the film Gel

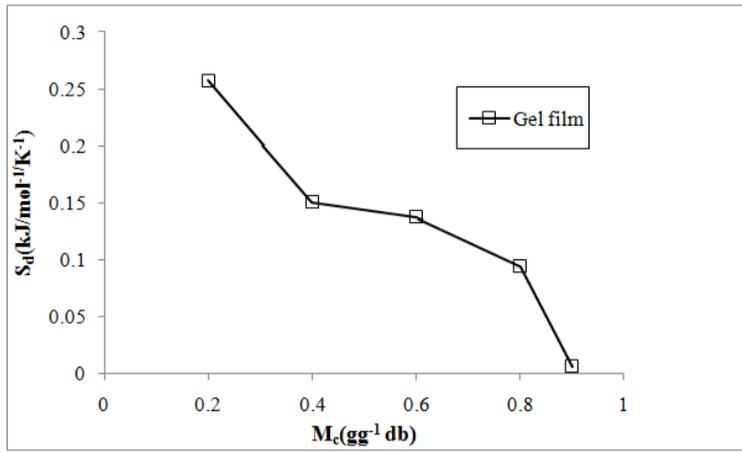


Figure 6B: Variation in  $s_d$  with  $M_c$  for the film Gel/poly (Ani).

### Entropy-Enthalpy compensation theory

According to compensation theory, there exists a good correlation ship between  $S_d$  and  $q_{st}$  [36]. This is expressed mathematically as :

$$Q_{st} = T_{\beta} S_d + \Delta G_{\beta} \quad ..(7)$$

Here,  $T_{\beta}$  is the isokinetic temperature and the free energy  $\Delta G_{\beta}$ , provides a criterion to evaluate if water sorption is a spontaneous ( $-\Delta G_{\beta}$ ) or non-spontaneous process ( $+\Delta G_{\beta}$ ).

The isokinetic temperature ( $T_{\beta}$ ) is the temperature at which all the sorption reaction will take place at the same rate. This is a characteristic property of the material surface [37], and it represents the slope of the enthalpy-entropy linear relationship. The linear plots between  $q_{st}$  and  $S_d$  for plain Gel and Gel/poly (Ani) films are shown in Figure. 7(A) and (B) respectively. It may be noticed that there

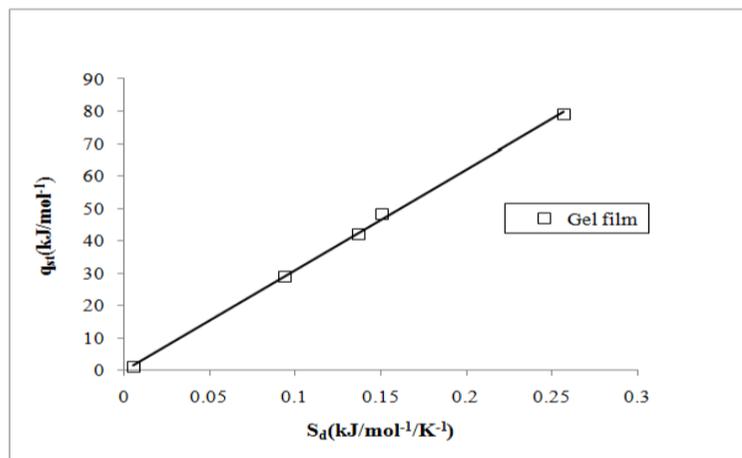


Figure 7A:  $S_d$  versus  $q_{st}$  plot for Gel film

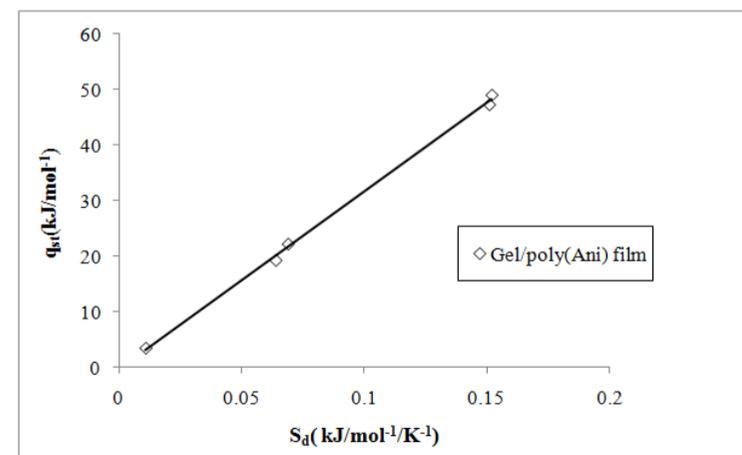


Figure 7B:  $S_d$  versus  $q_{st}$  plot for Gel/poly(Ani) film.

exists a fair correlation between  $q_{st}$  and  $S_d$  as indicated by the higher values of  $R^2$  for both of the films. In this work, values of  $T_{\beta}$  for the native Gel and Gel/poly (Ani) films were found to be 311.41 and 318.61 K respectively. The values of  $\Delta G_{\beta}$  were found to be -0.3278 and -0.2378 kJ/mol respectively. The negative values are indicative of the spontaneous moisture sorption process for both of the films. This is simply attributable to the fact that presence of gelatin as main constituents in both of the films causes a predominance of the polar active sites within the film matrices and therefore water vapor molecules are sorbed spontaneously on the films. In addition, the value of  $\Delta G_{\beta}$  is more negative for plain Gel film due to presence of more active sites within the film matrix as compared to the Gel/poly (Ani) film.

### Thrombus formation test

An anti-thrombogenic activity of paraffin gauge, film samples Gel and Gel/poly (Ani) was determined by measuring the weight of a dry blood clot. The weights of blood clots were  $0.004 \pm 0.0002$ ,  $0.004 \pm 0.0004$ , and  $0.005 \pm 0.0003$  g respectively. In addition, the weight of clot on glass film was 0.30 g. Thus, it shows that less amount of clot was formed on the films, thus confirming the biocompatibility and excellent anti-thrombogenicity of the films [38].

### Hemolysis test

The % Hemolysis for the commercial paraffin gauge, Gel and Gel/poly (Ani) films was determined to be  $0.83 \pm 0.05$ ,  $1.35 \pm 0.09$  and  $1.07 \pm 0.08$  respectively. As the values obtained were less than 2 %, the samples can be treated as fairly non-hemolytic.

### Conclusion

It may be concluded from this study that gelatin based film undergoes an appreciable change in its water absorption properties due to incorporation of poly (aniline) network within the film matrix. The mono layer moisture content  $M_o$ , was found to decrease from 0.0986 g/g to 0.0260 as the Gel film was loaded with poly (Ani) network within the matrix. The moisture uptake decreased with the increase in temperature for both of the films, namely Gel and poly (Ani)/Gel. The net isosteric heat of sorption,  $q_{st}$  decreased from 78.94 to 2.34 kJ/mol<sup>-1</sup> with the increase in  $M_c$  from 0.2 to 0.9 g/g db for native Gel film while the decrease in  $q_{st}$  was from 48.95 to 3.53 kJ/mol<sup>-1</sup> with the increase in  $M_c$  from 0.04 to 0.4 g/g db for Gel/poly (Ani) film respectively. The hemolysis test indicated that both of the samples were non-hemolytic. The films have potential to be used for biomedical applications.

### References

- Kotov NA, Winter JO, Clements IP, Jan E, Timko BP, Campidelli S, et al. Nanomaterials for Neural Interfaces. *Advanced Materials*. 2009;21(40):3970-4004. doi:10.1002/adma.200801984
- Xie J, Zong C, Han X, Ji H, Wang J, Yang X, et al. Redox-Switchable Surface Wrinkling on Polyaniline Film. *Macromol Rapid Commun*. 2016;37(7):637-642. doi:10.1002/marc.201500700
- Park CS, Kim DH, Shin BJ, Tae HS. Synthesis and Characterization of Nanofibrous Polyaniline Thin Film Prepared by Novel Atmospheric Pressure Plasma Polymerization Technique. *Materials (Basel)*. 2016;9(1):1-12. doi:10.3390/ma9010039
- Humpolíček P, Kuceková Z, Kašpárková V, Pelková J, Modic M, Junkar I, et al. Blood coagulation and platelet adhesion on polyaniline films. *Colloids Surf B Biointerfaces*. 2015;133:278-285. doi:10.1016/j.colsurfb.2015.06.008
- Fomo G, Waryo TT, Sunday CE, Baleg AA, Baker PG, Iwuoha EI. Aptameric Recognition-Modulated Electroactivity of Poly (4-Styrenesulfonic Acid)-Doped Polyaniline Films for Single-Shot Detection of Tetrodotoxin. *Sensors*. 2015;15(9):22547-22560. doi:10.3390/s150922547
- Menegazzo N, Boyne D, Bui H, Beebe TP, Booksh KS. DC Magnetron Sputtered Polyaniline-HCl Thin Films for Chemical Sensing Applications. *Anal Chem*. 2012;84(13):5770-5777. doi:10.1021/ac301006f
- Kaitsuka Y, Goto H. Preparation of Polyaniline/ZnO Films by Electrochemical Polymerization. *Open Journal of Polymer Chemistry*. 2016;6(1):1-7. doi:10.4236/ojpcem.2016.61001
- Bhadra J, Al-Thani NJ, Madi NK, Al-Maadeed MA. Effects of aniline concentrations on the electrical and mechanical properties of polyaniline polyvinyl alcohol blends. *Arabian Journal of Chemistry*. 2017;10(5):664-672. doi:10.1016/j.arabjcc.2015.04.017
- Qu M, Zhao G, Cao X, Zhang J. Biomimetic fabrication of lotus-leaf-like structured polyaniline film with stable superhydrophobic and conductive properties. *Langmuir*. 2008;24(8):4185-4189. doi:10.1021/la703284f
- Al-Jallad M, Atassi Y. Preparation of nonwoven mats of electrospun poly (lactic acid)/

- polyaniline blend nanofibers: A new approach. *Applied Polymer Science*. 2016;133(29). doi:10.1002/app.43687
11. Merlini C, Pegoretti A, Araujo TM, D.A.S Ramoa S, Schreiner WH, Barra GMDO. (2016) Electrospinning of doped and undoped-polyaniline/poly(vinylidene fluoride) blends. *Synthetic Metals*. 2016;213:34–41. doi:10.1016/j.synthmet.2015.12.024
  12. Awasthi SK, Bajpai SK, Utiye AS, Mishra B. Gelatin/poly (aniline) composite films: Synthesis and characterization. *Journal of Macromolecular Science, part a: Pure and Applied Chemistry*. 2016;53(5):301–310. doi:10.1080/10601325.2016.1151650
  13. da Silva JB, Pereira FV, Druzian JI. Cassava starch-based films plasticized with sucrose and inverted sugar and reinforced with cellulose nanocrystals. *J Food Sci*. 2012;77(6):N14–N19. doi:10.1111/j.1750-3841.2012.02710
  14. Bennaceur S, Draoui B, Touati B, Benseddik A, Saad A, Bennamoun L. Determination of the Moisture-Sorption Isotherms and Isotheric Heat of Henna Leaves. *Journal of Engineering Physics and Thermophysics*. 2015;88(1):52-62. doi:10.1007/s10891-015-1167-9
  15. Ahn JY, Kil DY, Kong C, Kim BG. Comparison of Oven-drying Methods for Determination of Moisture Content in Feed Ingredients. *Asian-Australas J Anim Sci*. 2014;27(11):1615–1622. doi:10.5713/ajas.2014.14305
  16. Oliveira EG, Rosa GS, Moraes MA, Pinto LAA. Moisture sorption characteristics of microalgae *Spirulina platensis*. *Brazilian Journal of Chemical Engineering*. 2009;26(1):189–197. doi:10.1590/S0104-66322009000100018
  17. Li QC, Cohen KA, Zhuang G. A Capillary Gas Chromatographic Procedure for the Analysis of Nine Common Residual Solvents in Water-Insoluble Bulk Pharmaceuticals. *Journal of Chromatographic Science*. 1998;36(3):119-124. doi.org/10.1093/chromsci/36.3.119
  18. US Pharmacopeia XXIII, 1994
  19. Ferreira P, Pereira R, Coelho JFJ, Silva AFM, Gil MH. Modification of the biopolymer castor oil with free isocyanate groups to be applied as bio adhesive. *International Journal of Biological Macromolecules*. 2007;40(2):144–152 doi:10.1016/j.ijbiomac.2006.06.023
  20. American Society for Testing and Materials, ASTM F 756-00(2000) Standard practices for assessment of haemolytic properties of materials Philadelphia, 156-165doi: 10.1520/F0756-08
  21. Kamoun EA, Kenawy ERS, Tamer TM, El-Meligy MA, Eldin MSM. Poly (vinyl alcohol)-alginate physically cross linked hydrogel membranes for wound dressing applications: Characterization and bio-evaluation. *Arabian Journal of Chemistry*. 2015;8(1):38-47. doi:10.1016/j.arabjc.2013.12.003
  22. Khojare AS. Moisture sorption hysteresis in sandesh at 200C. *Asian J. Dairy & Food Res*. 2014;33(3):179-182. doi:10.5958/0976-0563.2014.00598.3
  23. Rachtanapun P, Suriyatem R. Moisture Sorption Isotherms of Soy Protein Isolate/ Carboxymethyl Chitosan Blend Films. *Journal of Agricultural Science and Technology*. 2012;50-57.
  24. Haq MA, Jafri FA, Hasnain A. Effects of plasticizers on sorption and optical properties of gum cordia based edible film. *J Food Sci Technol*. 2016;53(6):2606-2613. doi:10.1007/s13197-016-2227-7
  25. Quyen DTM, Joomwong A, Rachtanapun P. Relationship between Solubility, Moisture Sorption Isotherms and Morphology of Chitosan/methylcellulose Films with Different Carbendazim Content. *Journal of Agricultural Science*. 2012;4(6):187-196. doi: 10.5539/jas.v4n6p187
  26. Rachtanapun P, Tongdeesoontorn W. Effect of glycerol concentration on sorption isotherms and water vapour permeability of carboxymethyl cellulose films from waste of mulberry paper. *As J Food Ag-Ind*. 2009;2(04):478-488.
  27. Falade KO, Aworh OC. *European Food Research and Technology*. 2004;218:278-283.
  28. Prothon F, Ahrne L. Application of the Guggenheim, Anderson and De Boer model to correlate water activity and moisture content during osmotic dehydration of apples. *Journal of Food Engineering*. 2004;61(3):467–470. doi:10.1016/S0260-8774(03)00119-5
  29. Blahovec J, Vanniotis S. Investigation of Moisture Sorption, Permeability and Drug Release Behavior of Carrageenan/Poly Vinyl Alcohol Films *Food and Bioprocess Technology*. 2008;1:82-906. doi:10.15228/2014.v04.i03.p07
  30. Torgul H, Arslan N. Moisture sorption isotherms and thermodynamic properties of walnut kernels. *Journal of Stored Products Research*. 2007;43(3):252-264. doi:10.1016/j.jspr.2006.06.006
  31. McMinn WAM, Magee TRA. Thermodynamic properties of moisture sorption of potato. *Journal of Food Engineering*. 2003;60(2):157-165. doi:10.1016/S0260-8774(03)00036-0
  32. Torgul H, Arslan N. Moisture sorption isotherms and thermodynamic properties of walnut kernels. *Journal of Stored Products Research*. 2007;43(3):252-264. doi:10.1016/j.jspr.2006.06.006
  33. Tsami E, Maroulis ZB, Marinos-Kouris D, Saravacos GD. Heat of sorption of water in dried fruits. *International Journal of Food Science and Technology*. 1990;25(3):350- 359. doi:10.1111/j.1365-2621.1990.tb01092
  34. Muhtaseb-Al AH, McMinn WAM, Magee TRA. Water sorption isotherms of starch powder Part I: Mathematical description of experimental data. *Journal of Food Engineering*. 2004;61(3):297-307. doi:10.1016/S0260-8774(03)00133
  35. Muhtaseb-Al AH, McMinn WAM, Magee TRA. Water sorption isotherms of starch powder Part II: Thermodynamic characteristics. *Journal of Food Engineering*. 2004;62(2):135-142. doi:10.1016/S0260-8774(03)00202-4
  36. Igathinathane C, Womac AR, Sokhansanj S, Pordesimo LO. moisture sorption thermodynamic properties of corn stover fractions. *American Society of Agricultural and Biological Engineers*. 2007;50(6):2151-2160.
  37. Aguerre RJ, Suarez C, Viollaz PE. Enthalpy-entropy compensation in sorption phenomena: Application to the prediction of the effect of temperature on food isotherms. *J Food Sci*. 1986;51(6):1547-1549. doi:10.1111/j.1365-2621.1986.tb13856
  38. Kurhade S, Momin M, Khanekar P, Mhatre S. Novel Biocompatible Honey Hydrogel Wound Healing Sponge for Chronic Ulcers. *International Journal of Drug Delivery*. 2013;5:353-361.