Vol.03, Issue 02 (2024): 01-09
DOI: 10.58921/jse.03.02.068

Journal of Sustainable Environment
ISSN (p): 2710-2386, ISSN (e): 2957-9228

Published by
SMI UNIVERSITY

Extraction & Assessment of Physicochemical Parameters of *Rubus Laciniatus* by Using Classical and Instrumental Technique

Atya Hassan[1*], Madhi Kazmi[2], Safia Hassan[1], Farkhanda Hassan[1], and Aneela Wahab[1]

[1] Department of Chemistry Federal Urdu University of Art, Science and Technology, Karachi, Pakistan

[2] China University of Geosciences Wuhan, School of Environmental Studies. Wuhan, Hubei, China

ABSTRACT

In this work extraction process was used to study the various parameters of *Rubus Laciniatus*. Dye extracted from the fruits of sample in aqueous medium. Acidic and basic characteristics of dye were determined by classical method as well as pH technique. Conductivity method was used to evaluate the molar and specific conductance at 25±1^{0}C. Viscometer was used to determine the viscosity of extracted dyes from 10±1^{0}C to 50±1^{0}C. Further, elucidated the different type of parameters such as relative, specific and reduced viscosity respectively. Inspite, of these graphical and theoretical methods was applied for the determination of intrinsic viscosity. Surface tension of dye measured at 25±1^{0}C by distinct methods. By using surface tension, the surface excess concentration was examined with the help of Gibbs adsorption isotherm modal. Activation energy and frequency factor estimated from viscosity at 283±1k$^-$ to 323±1k$^-$. Gibbs free energy and activation enthalpy determined by co-relating the activation energy and frequency factor.

Key Words: Intrinsic viscosity, Surface tension, Activation energy**.** Frequency factor, Gibbs free energy, Conductivity method

1. INTRODUCTION

In 1856, basic dye synthesized by Wiliam Parkins. Synthetic dyes were replaced by natural dyes due to carcinogenic behaviour, ulceration of skin, hemorrhage, several irritations of skin and dermatitis (Joshi et. al, 2013). In contrast, the natural dyes are safe and eco-friendly but mostly unstable and oxidize easily, resulting discoloration (Chao et. al, 2017). *Rubus* genus contain diverse 700 species with chromosome number 7 (Graham and Brennan 2018). From which *laciniatus* is one species. Rubus laciniatus commonly known as *cut-leaf blackberry*. This plant belongs to *Rosaceae* family, commonly found in Pakistan. Fruits of blackberry of *Rubus* contain 7.6 g dietary fiber, 46.1 mg calcium (Ca), 282.2 mg potassium (K) and 30.2 g vitamin C (Perkins & Collins 2001). Cut-leaf blackberry contains 83-326 mg/100 g of anthocyanin in fresh fruits. This is a type of blackberry which contain the cyanidin 3-glucoside and cyanidin 3-rutinoside as a major and minor anthocyanins respectively, (Stintzing, et, al. 2002). The molecular weight of cyanidin 3-glucoside is 449.388g/moles with a molecular formula $C_{21}H_{21}O_{11}^+$. The molecular structure of cyanidin 3-glucoside is depicted in figure 1. Beside this various aroma compounds such as 2, butanedione, 2-heptanol, l-carvone, thiophene, dimethyl-tri-sulfide are present (Qian, and Wang 2006) in Thorn-less blackberry.

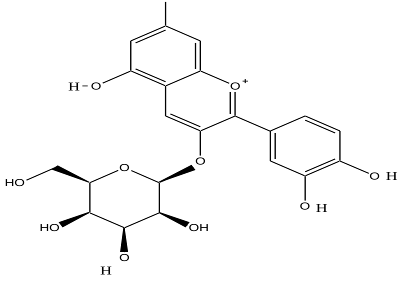

Figure 1: Chemical structure of Cyanidin 3-O-β-D-glucoside

Literature review reveals that various work has been reported regarding the behaviour of different natural dyes solution. Different thermodynamics and kinetics parameters of some organic and natural dyes were reported by some workers. Such as the kinetics of hydrolysis of bi-functional reactive dyes **(**Liqi, 1989). The kinetics and thermodynamics study were performed on natural dye Curcumin on PLA fiber (wu et, al. 2013). Adsorption kinetics and study of Dragon food skin was done (Hamid, & Skinah 2017). Effect of solvent polarity on non-radiative processes in xenthene dyes:Rhodamine B in normal alcohol (Casey, & Quiitevis, 1988). Cytological study in *Rubus genus* (Haslop-Harrison 1953). Aroma compounds e.g. Preliminary aroma comparison of Marion and Evergreen blackberry by dynamic headspace/OSME techniques was studied **(**Klesk, and Qian 2003)**. P**athogenic study was done by different researchers on evergreen blackberry, such as Phragmidium violaceum on Rubus Armeniacus and Rubus Laciniatus in British Columbia (Callan et. al. 2011). Similarly, the texanomical, Phyto geographic, and Phytosociological studied carried out on different Rubus species in north-west Europe **(**Haveman, 2017)**.** Surface tension and absorption spectroscopic measurement of monolayers of surface-active azo and stilbene dye (Heesemann, 1980). But from these physical parameters of dyes like pH, viscosity, density, fluidity, surface tension, conductance, activation energy, Thermodynamics, surface excess concentration, and so many others, only few parameters were reported and have very little attention were paid to workers.

In present work conductivity and pH metry techniques are used for the characterization of dye solution. The conductivity method helps to explain the mobility, transfer of charge particle and total dissolved solid (TDS) in solution. However, the pH metry technique helps to study the behaviour of solution such as acidity, alkalinity etc. Furthermore, it helps to assess the stereochemistry and stability of dye while both parameters behold on pH. In the view of chemist pH is dominant physicochemical parameter which formally elaborate the nature and reactivity of dye. The pH directly influences the conductance as well as kinetics and thermodynamic of dye. Kinetics and thermodynamics competition takes place due to variation in pH.

2. EXPERIMENTAL

2.1 Material

Analytical grade agents were used in this study NaOH (E.Merck), $H_2C_2O_4$ (E.Merck), Distilled water, deionized water,

2.2 Collection of plant material

The dark purple hued fruit of evergreen blackberry was collected from the North Karachi, Metropolitan city of Pakistan.

2.3 Preparation of Raw Material and dye

After collection of samples, it was washed by distilled water and then by de-ionized water for abolition of impurities. After washing the dye extracted from sample and different physical characteristics were studied.

2.4 Methods

For the measurement of density, the relative density bottle (R.D Bottle) is used. Electrical analytical weight balance (Mettler tolado MS-TS) used for the detection of masses of substances. The conductance of dye examined through conductivity meter (janway 4510, Germany). Ostawld U-tube viscometer (Germany) used for noting the rate flow. Via glass stalagmometer surface tension is determined. Hot plate stirrer (china J.k.I) is used for heating purpose. For the evaporation of solvents, the Rotary evaporator (Buchi Rotavapor R-210) was used. pH analysis was done by the pH meter (3510 jenway, Germany).

3. RESULT & DISCUSSIONS

3.1 Study of variation in hues using titrimetric method

The colour change was observed by titrimetric method. For this purpose, the 0.18M standard NaOH was used for the titration of dye. Variable hues were observed during titration by the addition of different volume of standard base which was shown in (Table 1). At 0.0mL, the purple hued of dye was turned into the yellow hued at 60.10mL. Its means no. of OH^- ions gradually rise then acidic dye neutralized at 60.10mL. Addition of base is responsible for variation in colours at different volumes.

Table 1, Different hues at different volumes

Volume of titrant (mL)	Hues of analyte
0.00	Purple
3.00	Indigo blue
5.00	Dark green
5.30	Light green
21.20	Fubile green
39.00	Dark yellow
61.10	Light yellow

3.2 Effect of concentration of dye on pH

The alkalinity or acidity of solution is characterized by pH. The change in concentration effect the pH of solution. The observed pH of dye was 5.485 at 25±1^{0}C. This value indicated the dye was weak acid. In dilute concentrations of dye the pH was gradually decreased illustrated in table 2.

As the increased in concentration, increased the H^+ ions of dye. The equilibrium between the flavylium cation (AH^+), quinoidal base (A) are responsible for change in colour (Coutinho, et, al. 2015). Solvent medium effect the multi-equilibrium of anthocyanin. Cyanidin-3-glucoside is a glucolated form of anthocyanin (Khoo et, al. 2017) at pH 5.07, in cyanidin-3-glucoside, equilibrium is shifted from AH^+ to quinoidal base A, hemiketal and chalcone. The positive charge in flavylium cation delocalized at all pyrilium moieties, especially carbon atoms 2 and 4 are most charged atoms. In neutral solution the deprotonation of acidic OH^- group give purple hue or some time hydration of AH^+ give colorless hemiketal form. The carbon 2 and 4 are charged defective carbons of AH^+ and more susceptible for nucleophilic reaction. When pH moves to neutrality the quinoidal base undergo deprotonation. As a result of deprotonation, the pH of dye at different dilute solution was decreased reflected in Figure -1. Now hemiketal form undergo cis-chalcone form by tautomeric process. It was observed that at low pH the hydration had rapidly occurred then tautomerization.

Table 2: Effect of concentration of dye at different concentration on pH

S.No	% solutions	pH
1	1	5.327
2	2	5.296
3	3	5.037
4	4	4.980
5	5	4.905
6	6	4.820
7	7	4.822
8	8	5.190
9	9	4.715
10	10	4.690

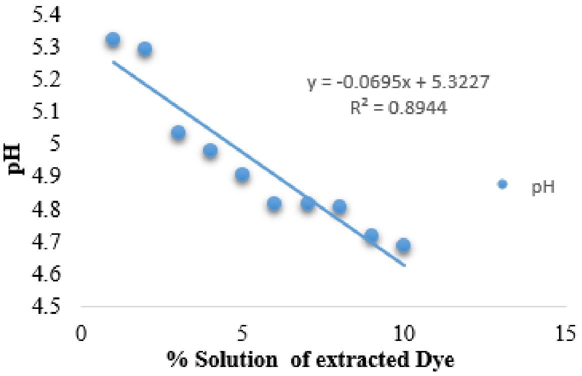

Figure 2: Plot of pH of dye vs. % solution of extracted at 25±1°C.

3.3 Determination of titration curve by Conductometric titration

The conductometric analysis performed at 25±1°C. The amount of energy in term of current was measured. The conductometric titration was performed by using standard solution of NaOH (0.18 M) against the extracted dye. The conductivity was increased by the addition of each drop of base (NaOH) presented in (Table 3).

By the addition of NaOH (0 mL to 3 mL) insignificant change were observed. After 4 mL to 16 mL addition of NaOH, conductivity was hike. This response showed that this dye behaved as very weak acid. The Figure 3 specified that addition of 4rth mL of base enhanced the conductance. Conductance constantly increased with rise in volume. The molar conductance of different percent solutions was measured at 25±°C.

Λ_{mol} = ƙ/c..................(1)

Table 3: Conductometric titration of dye with stand. NaOH at 25±1 °C.

S.No	Volume (mL)	Conductance (mS)	S.No	Volume (mL)	Conductance (mS)
1	0	0.00085	10	9	17.8
2	1	0.00085	11	10	20.7
3	2	0.00099	12	11	24.7
4	3	0.165	13	12	26.9
5	4	2.8	14	13	28.8
6	5	5.8	15	14	30.6
7	6	8.9	16	15	33.0
8	7	11.5	17	16	35.60
9	8	14.6			

The specific conductance of dye at dilute solutions depicted in table 3, sharply increased with increased in concentration. The increased conductivity in dilute solutions as a function of concentration depicted in table 4. Antocyanins contain flavylium cation (AH+), in aqueous solution the flavylium cation electronic excitation take place as a result in transfer of proton to water (Ferreira de silva et, al. 2004). By this transfer, the specific conductance of dye increases in dilute solutions. But the conductance of 7% dye solution fluctuated may be due to the polarization or variation in temperature.

Table 4: Specific and Molar conductance of dye at different solutions.

Concentration (% solution)	**Sp. Conductance (μS)**	**Molar Conductance (μS mol^{-1}) (Λ_m = k/C)**
1	44.5	44.5
2	70.9	35.4
3	73.2	24.4
4	99.0	24.75
5	115.0	23.0
6	136.6	22.7
7	134.7	19.2
8	138.3	17.2
9	193.0	21.4
10	203.0	20.3

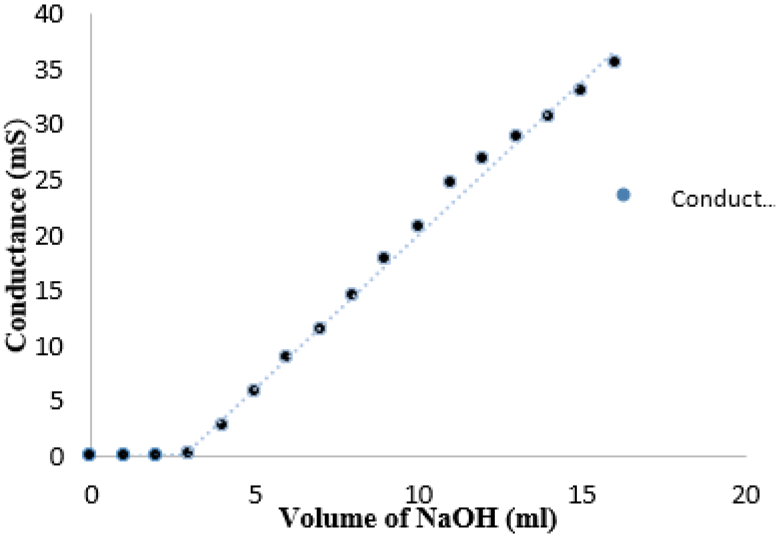

Figure 3: Plot of conductance of dye vs. volume of NaOH by Conductometry titration at 25±1°C.

3.4 Viscosity (η) of dye

Flow rate is an important parameter through which flow nature of fluid is examined. The viscosity of dye investigated at 25±1°C depicted in table 5.

Table 5: Different forms of viscosity of dye at different % solutions**.**

Conc. (%solutio)	**Shear rate(Ɣ) (s^{-})**	**Viscosity (dyn-s/cm^2)**	**Rel. Viscosity (η_{rel})**	**Sp.viscosity (dyn-s/cm^2) (-tive sign as common)**	**Red.viscosity (cm^{3}g-) (-tive sign as common)**
1	1.17	8.5	0.95	0.05	0.05
2	2.3	8.6	0.96	0.04	0.02
3	3.4	8.7	0.97	0.03	0.01
4	4.6	8.7	0.98	0.02	0.005
5	5.5	9.0	1.004	0.004	0.00066
6	6.7	8.9	1.011	0.011	0.0022
7	8.0	8.70	0.977	0.1	0.014
8	9.1	8.73	0.98	0.02	0.0025
9	10.2	8.80	0.98	0.02	0.0022
10	11.0	9.05	1.011	0.011	0.0011

The viscosity of dye was 9.2 dyne-s/cm^2. Distinct forms of viscosity also measured at different concentrations referred in table 5. The reported value of water flow rate is 8.9 dyens/cm^2 at 25±1°C. In dilute solution the increased in concentration, raise the inter-molecular friction, as a result the viscosity increased. The velocity of molecules in layer forms changed with the changed in concentrations. This shear rate is proportional to concentration. Here the concentration act as a shear stress (τ). In dilute solution of dye, the sharply increased in concentration deformed the layers of molecules in Viscometer. The Figure 4 showed the Newtonian nature of dye.

The apparent viscosity is a function of shear rate (Falcao et, al. 2009) at 25±1^{o}C. where the shear rate (γ) is explained by eq. (2), where viscosity in PaS.

$$\eta=Ʈ \div Ɣ \qquad \text{..............} \ (2)$$

The apparent viscosity (in pa.s) is the relation between shear stress and shear rate.

$$Ɣ=Ʈ / \eta \qquad \text{............} \ (3)$$

The Sp. Viscosity of dye randomly decreased illustrated in table 5. This variation occurs due to the change in concentration. At 7% dilute solution Sp. Viscosity is 0.1dyne-s/cm^2. It depends upon the pH and ionic strength. With the increase in ionic strength the reduced viscosity is decreased (Ray et al. 1996).

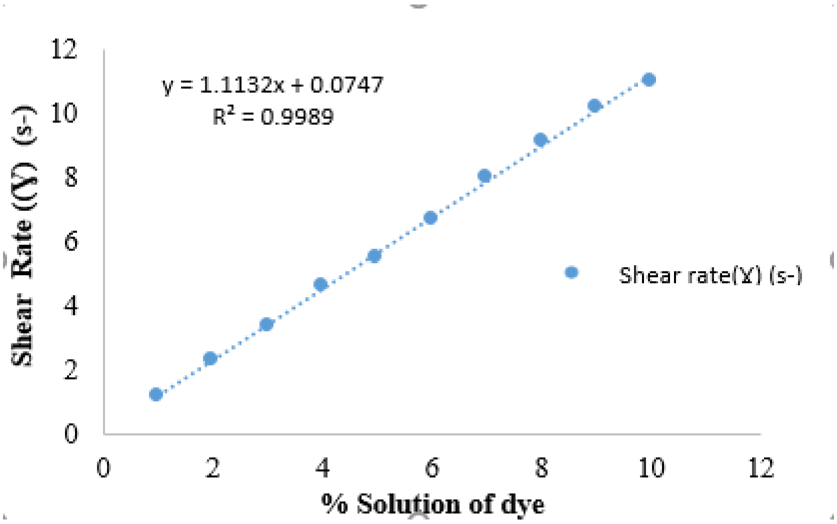

Figure 4: Shear rate of dye vs.at different concentrations of extracted dyes.

3.5 Intrinsic viscosity [η] of dye

The determination of viscosities of dilute solutions give knowledge about molecular characteristics (Visakh et, al. 2006) of analyte. Normally Intrinsic viscosity measured by applying Huggin's equation. The intrinsic viscosity of dye was calculated by single point measurement method (Abdel Azim et, al. 1998) via applying ''Solom and Ciutas equation,

$$[\eta]=(2)^{0.5}/C\{\eta_{sp}-\ln\eta\}^{0.5} \ \text{..................} \ (4)$$

By solving the equation 6 intrinsic viscosity was found to be 0.0025. The low value of intrinsic viscosity is due to the dependence on the ionic strength. The number of molecules of anthocyanin i-e cyanidin-3-glucoside are responsible for the decrease in intrinsic viscosity of dye.

3.6 Activation energy and Thermodynamics Dye

Minimum amount of energy required to activate the atoms or molecules to undergo reaction is called activation energy. Arrhenius equation used for the activation energy measurement,

$$\eta= Ae^{E0/RT} \qquad \text{......................} \ (5)$$

E_0 in equation 5 represent the activation energy. The plot of *ln (η)* against reciprocal of kelvin temperature (*1/T*) show linear behaviour in eq. (6) and figure 5.

By measuring the pre-exponential factor (frequency factor), Arrhenius temperature (Messaadi, et, al. 2015) calculated via equation 6.

$$T_A=-E_0/R\ln(A) \text{..................} \ (6)$$

The activation energy from figure 5 was -7.563kj/moles. The natural antilog of pre-exponential factor was 24.5. This factor depends on temperature because the collision of molecules is dependent upon the frequencies of molecules. The Arrhenius temperature calculated that was 37.1k$^-$. Increase in temperature enhance the rate of reaction. The rate constant used to examine the rate of reaction. Some reaction proceeds more slowly at high temperature.

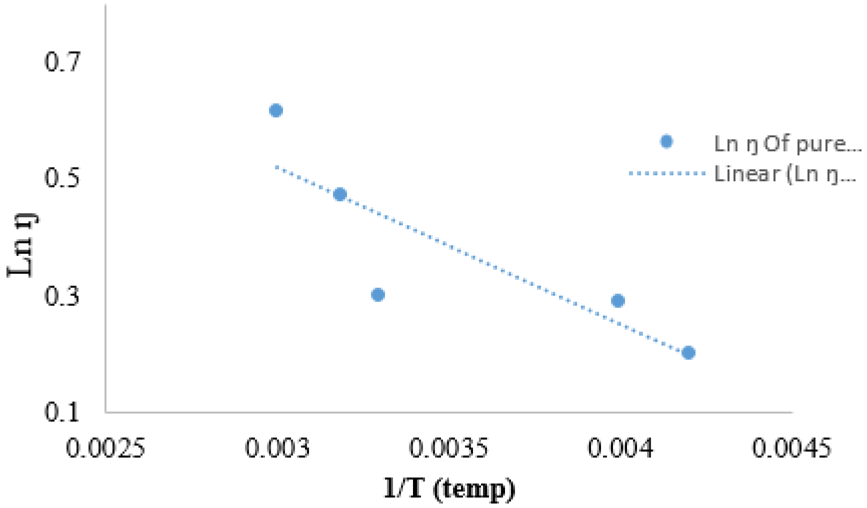

Figure 5: Plot of ln η vs. Temperature inverse (T^{-1})

Negative value of activation energy suggests the dye degrade or react in elementary steps. The enthalpy of dye is calculated with the help of activation energy in table 6. At high temperature the collision between the molecules reduces due to escalation in temperature, molecules move away from the collision zone as a result the negative activation energy are observed. Enthalpy of dye at different temperature suggested that the dye contain cyanidin-3-glucoside degrade rapidly with temperature. The Gibbs energy of dye measured from the following equation,

$$\Delta G=-RT \ln k \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

The negative value of Gibbs energy showed spontaneity feature of dye. The randomness of dye in aqueous medium was investigated via equation 8

$$\Delta S=\Delta H-\Delta G/T \ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

The positive value indicates that the randomness of dye in aqueous medium increased. Raising temperature reduced the colour intensity of cyanidin-3-glucoside. Degradation of cyanidin-3-glucoside increased with increasing in temperature (Corrales et, al. 2008).

Table 6: Activation energy, Rate constant and Activation enthalpy from viscosity of pure dye at different temperature

Temp (K)	Temp (K^{-})	Viscosity of H_2O (m pa-s)	Ln η of pure dye	Activation energy (kJ/moles)	Rate constant	Enthalpy (kJ/moles)	Gibbs energy (kj/moles)	Entropy (kj/moles.k^{-1}) (10^{-3})
283	0.003	1.308	0.29	-2.5	24.3	-4.8	-7.5	9.5
293	0.0034	1.002	0.020	-3.1	24.3	-5.5	-7.7	7.4
303	0.0033	0.797	0.269	-2.5	24.3	-5.0	-8.0	9.9
313	0.0039	0.653	0.470	-1.96	24.3	-4.5	-8.3	12.1
323	0.0039	0.547	0.616	-1.5	24.3	-4.1	-8.5	13.6

3.7 Surface tension (Ɣ) of dye

When the cohesive forces of dye molecules are greater than the adhesive forces of air the forces in bulk lead to surface as a result tension exert on the surface due to unequal forces and surface tension phenomenon occur. The surface tension of pure dye was 0.90N-m^{-1} while the surface tension of water at 25C^{0} was 0.07197N-m^{-1} or 71.97dyne/cm. Different methods were applied for determination of surface tension of solution at different concentrations. The general trend was observed in relative surface tension in table 7. Relative Surface tension decreased with increased in concentration. By increasing concentration of dye, the adhesive forces overcome to cohesive forces which depressed the surface tension. The surface tension of different solutions of dye were also measured by different standard stalagmometers. Due to the difference in width and size of capillary of stalagmometer the difference in values observed.

3.8 Surface excess concentration (Γ)

The related covered area of surface by analyte is surface excess concentration. It was estimated at 25±1^{o}C from

Gibbs adsorption isotherm.

$$\Gamma = -\ 1/RT.d\gamma/d\ln C\ \ldots\ldots\ldots\ldots\ldots\ldots.(9)$$

The surface excess concentration was -0.01816mmoles/cm^2 depicted in figure 6.

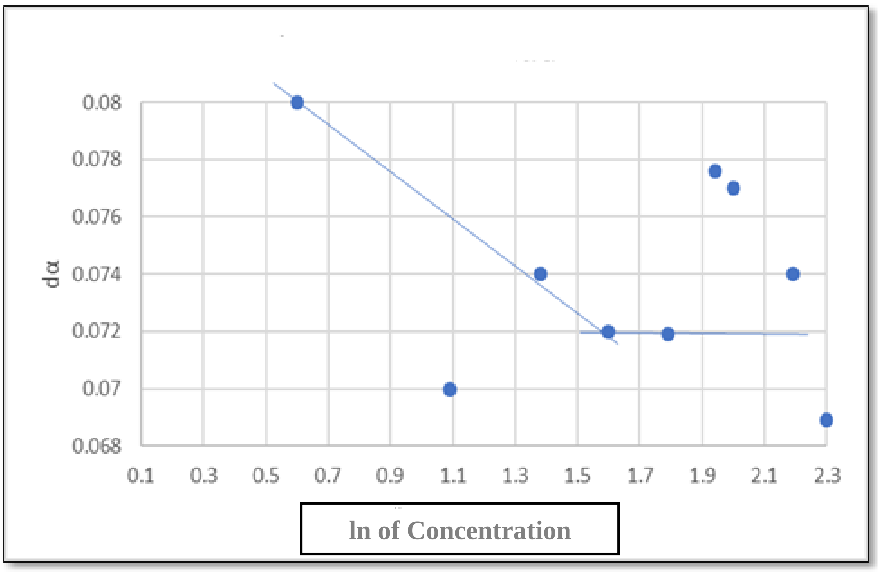

Figure 6: The surface excess concentration of dye

The negative value of surface excess concentration means that the solute with higher concentration in bulk than at surface contrary to positive adsorption of solute accumulate mostly in bulk (Ali shah, & Bilal 2015). The electrostatic attraction between ions of dye with water, additional pull was developed, other than H-bonding in between the water molecules, is exerted on surface of water which responsible for increasing surface tension. The minimum area per dye molecule was calculated by surface excess concentration (Sansanwal, 2006).

$$A_{min}=10^{14}/N_A.\Gamma_{max}\ \ldots\ldots\ldots\ldots\ldots\ldots\ (10)$$

The molecules of dye per unit area was -9.1μm^2. Natural dyes have an ability to reduce the surface tension in surfactant solutions (Chandravanshi, S. K. Upadhyay 2012). The dye under study was also act itself as a surfactant because it also reduced the surface tension in aqueous medium. It can act as micellar aggregate for the reduction of surface tension. Because it has ionic hetrocyclic ring structure which enable it to form micelles. The standard gibbs energy for formation of micelles in dye aqueous medium from figure 6 was calculated by using equation 11

$$\Delta G_{mic}^{*}=2.303RT\ (\log CMC-\ \log\acute{\omega})\ \ldots\ldots\ldots.\ (11)$$

$\acute{\omega}$ is the molar concentration of water at 25±1^{0}C. The CMC concentration of dye from graph 4 was 1.6moles. The molar concentration of water is 55.5moles/L. The value of ΔG_{mic}^{*} was -8.78kj/moles energy was required to dye for micelles formation. Standard gibbs energy of adsorption calculated as

$$\Delta G^{*}_{ads}=.\ \Delta G_{mic}^{*}-6.023e^{-1}A_{mi.}\ \Pi\ldots\ldots.\ (12)$$

While the standard Gibbs energy for adsorption was -8.929kj/moles. Lower value of standard Gibbs energy for the adsorption than micellar standard Gibbs energy suggests that the micellization process take place in bulk.

Table 7: Surface tension of dye solutions by different standard stalagmometer**.**

Conc. (V/V%)	**R.S.T (N-m-1)**	**D.W.M (N-m-)**	**D.C.M (N-m-)**	**By 5mL Standard stalagmometer**	**By 2.5mL standard stalagmometer**
1	1.16	0.08	0.08	120.4	60.0
2	1.14	0.082	0.082	115.7	57.87
3	1.08	0.077	0.077	111.5	55.7
4	1.03	0.074	0.074	144.9	53.5
5	1.01	0.072	0.072	104.1	52.4
6	1.00	0.0719	0.0719	104.1	52.13
7	1.079	0.0776	0.0776	111.4	55.7
8	1.08	0.077	0.077	111.7	55.9
9	1.04	0.074	0.074	107.3	553.69
10	0.958	0.0689	0.0689	98.9	49.44

4. CONCLUSION

It is concluded that natural dye isolated from the *fruit of Rubus laciniatus,* which is acidic in nature. Conductometry as well pH analysis ratified, the dye was weak acid. Due to structural changing of dye, pH of solution varied at different concentration. The viscosity of dye was 0.029dynes/cm^2. By applying shear stress, the shear rate constantly increases and dye act as Newtonian fluid. The dye under studied was surface active nature.

5. ACKNOWLEDGMENT

Authors would like to thank chairperson Department of Chemistry, Federal Urdu University of Arts, Science and Technology Gulshan-e-Iqbal Campus Karachi, Pakistan for the fulfilment of this Project.

6. REFERENCES

Abdel Azim, A., Atta, M., Farhat, S., Boutros, Y., (1998). Determination of intrinsic viscosity of polymeric compound through single specific viscosity measurement, J. Great Britain., 39, 6827-6833.

Ali shah, A., Ali, K., Bilal, S., (2013). Surface tension, Surface excess concentration, Enthalpy and entropy of surface formation of aqueous salt solutions, *J. Colloids and surface A: Physicochemical and engineering aspects*., 417,183-190.

Casey, K., Quiitevis, E., (1988) Effect of solvent polarity on nonradiative processes in xenthene dyes:Rhodamine B in normal alcohal, *J.of Physical chemistry*., 92, 6590-6594.

Callan, B. E., Wall, R., Dale, P., and Joshi, V. (2011). Phragmidium violaceum on *Rubus armeniacus* and *R. laciniatus* in British Columbia, *J. North American Fungi*., 6, 1-5.

Chao, Y. C., Ho, T. H., Cheng, Z. J., and. Kao, L. H. (2017). A study on combining natural dyes and environmentally friendly mordant to improve colour strength and ultraviolet protection of textiles, *J. Fibers and polymers*, 18, 1523-1530.

Chandravanshi, S., Upadhyay, S. K. (2012). Natural dye surfactant interaction: and surface parameters, *J. Coloration technology.,* 128, 300-.

Corrales, M., Lindauer, R., Butz, P., Tauscher, B. (2008). Effect of heat/pressure on cyanidin-3-glucoside ethanol model solution, *J. Physics. Conference series.*, 121, 1-6.

Coutinho, I. B., Freitas, A., Maçanita, A. L., and C.Lima, J. (2015). Effect of water content on the acid–base equilibrium of cyanidin-3-glucoside. *Food Chemistry*., 172, 476–480.

Falcao, A., Chaves, E., Falcao, L., Gauche, C., Barreto, P., Terazinha, M. (2009). Rheological behaviour and colour stability of anthocyanins from Merlot and Bordo graps in jam modal system, J. Cienciae Tecnologia de alimetos., 29, 857-862.

Ferreira de silva, P., Lima, J., Quina, F., Macanita, A. (2004). Excited state electron transfers in anthocyanins and related flavylium salts, *J. Physical Chemistry*., 108,10133-10140.

Graham, J., and Brennan, R. (2018). Raspberry Breeding, challenges and advances, Springer nature Switzerland AG, Switzerland.

Hamid, N., Skinah, M. (2017). An adsorption kinetics and thermodynamics study of dyeing Betacyanin extracted from Dragon fruit skin onto the spun silk and acrylic yarn, *J. Basic and Applied Sciences,* 11, 33-41.

Haslop-Harrison, Y., (1953). Cytological studies in the genus *Rubus L, J. New phytologist*., 52(1), 22-39.

Haveman R., (2017). Concealed Diversity: taxonomical, phytogeographical and phytosociological notes on brambles (*Rubus L. Suben. Rubus)* in Northwest Europe Wageningen University, ISBN 978946341019, 10-200.

Heesemann, (1980). Studies on Monolayers. 1. surface tension and absorption spectroscopic measurements of monolayers of surface-active azo and stilbene dyes. J. Chemischer Informations dienst., 11(27).

Joshi, R. k., Purohit, M. C., and Joshi, S. P. (2013). Improvement of traditional system of dyeing on wool fiber using eco-friendly natural dyes. *J. Applicable Chemistry*., 2, 841-849.

Khoo, H., Azlan, A., Tang, S., Lim, S., (2017). Anthocyanidin and Anthocyanin coloured pigment food, pharmaceutical ingredient and potential health benefit, *J. Food and nutrient research.*,61, 1-2.

Klesk, K., and Qian, M. (2003). Preliminary Aroma Comparison of Marion *(Rubus spp. hyb)* and Evergreen (*R. laciniatus L.*) Blackberries by Dynamic Headspace/OSME Technique. *J. Food Science.,* 68(2), 697–700.

Liqi, H. (1989). Kinetics of hydrolysis of some model bifunctional reactive dyes. *Dyes and Pigments.*, 10(3), 195–215.

Messaadi, A., Dhouibi, N., Hamda, H., Bin muhammad belgacem, F., Abdelkader, Y., Ouerfelli, N., Hichem hamzaoui, A. (2015). A new equation relating viscosity, Arrhenious temperature and activation energy for some newtonian classical solvents, *J. Chemistry.*, 2015, 1-12.

Perkins, P., Collins, J. K. (2001). Contribution of nonvolatile phytochemicals to nutrition’s and flavor, *J. HortTechnology*., 11, 539-546.

Qian, M. C., and Wang, Y. (2005). Seasonal Variation of Volatile Composition and Odor Activity Value of“Marion” *(Rubus spp. hyb*) and“Thornless Evergreen” (*R. laciniatus L*.) Blackberries. *J. Food Science.*, 70(1), 13-19.

Ray, F., Ferreira, M. A., Facal, P., Machado, A.A.S.C. (1996). Effect of Concentration, pH and ionic strength on viscosity of solution of soil fulvic acid, *J. Canadian Chemistry*., 74, 295-299.

Sansanwal, P.k. (2006). Effect of co-solutes on the physicochemical properties of surfactant solutions, *J. Scientific and industrial research.*, 65,57-64.

Stintzing, F. C., Stintzing, A. S., Carle, R., Wrolsted, R. E. (2002). A novel zwitter ionic anthocyanin from evergreen blackberry, *J. Agricultural and food Chemistry*., 50, 396-399

Visakh, P. M., Morlnes, M. J. M. (2015). Polyethlene-Based blends, Composites and nanocomposites, Chapter 8 (Molecular weight determination of polyethylene terephtalate), Wley Scrivener, Beverley

Wu, J., Guo, H., Ke, J., Fan, J. (2013). Studies on kinetics and thermodynamics on PLA fiber, *J. Fibers and textiles.*, 38, 424-426.