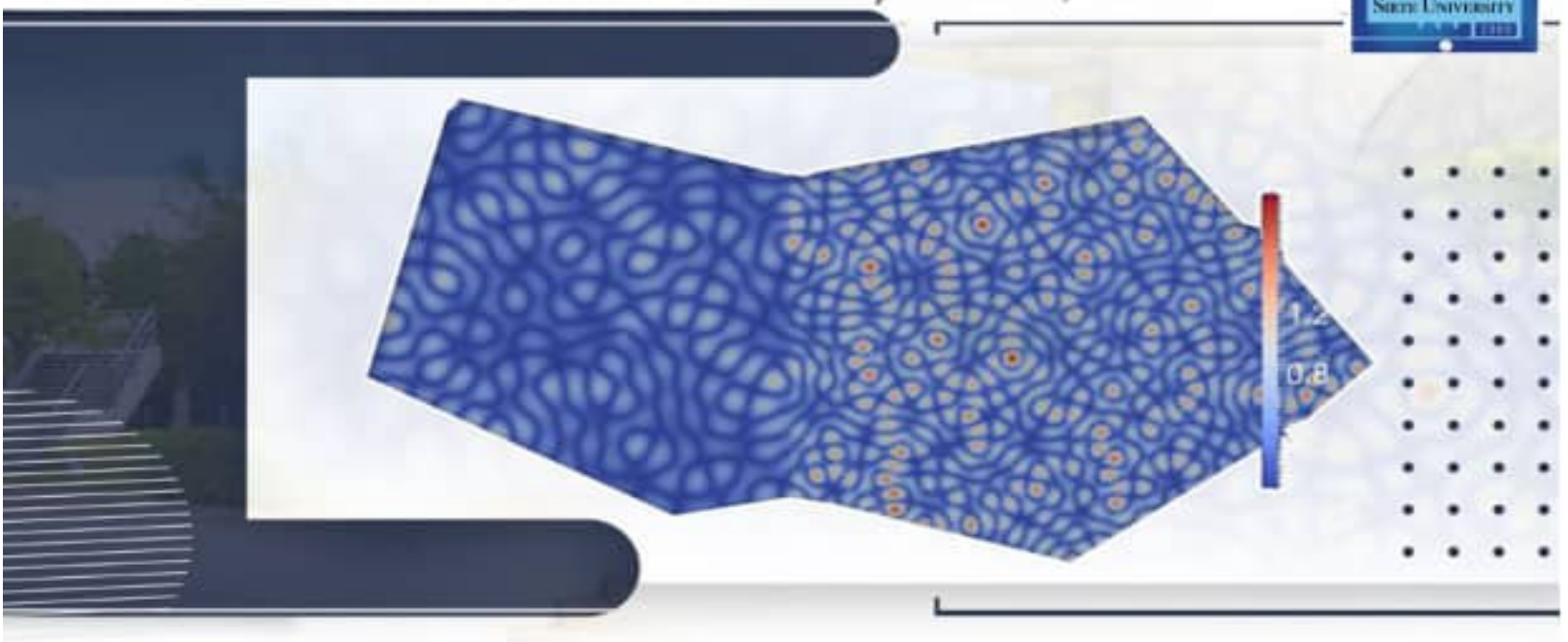
eISSN: 2789-858X

Scientific Journal for the faculty of Science - Sirte University

DOI: 10.37375/issn.2789-858X - Indexed by Crossref, USA



Volume2 Issue2 October 2022

Bi-annual Peer-Reviewed, Indexed,and Open Accessed e-Journal

SJFSSU

Legal Deposit Number@National Library(Benghazi): 990/2021

sjsfsu@su.edu.ly
journal.su.edu.ly/index.php/JSFSU

Scientific Journal for the Faculty of Science-Sirte University Vol. 2, No. 2 (2022) 38-45

Scientific Journal for the Faculty of Science-Sirte University

Journal home page: http://journal.su.edu.ly/index.php/JSFSU/index

DOI: 10.37375/issn.2789-858X

Muco-adhesion Evaluation of Polysaccharides in Simulated Physiological Fluids

Hana A. S. Binhamad[1] and Ramadan Diryak [2]

[1]*Chemistry Department, Science Faculty, Omar al-Mukhtar University, al-Bayda, Libya.*

[2]*Medicine Faculty, Sirte University, Sirte, Libya.*

DOI: https://doi.org/10.37375/sjfssu.v2i2.510

ARTICLE INFO:

Received 19 August 2022.

Accepted 04 September 2022.

Published 27 October 2022.

Keywords: Gellan gum, Methylene blue, Retention time, Gellan gel, Drug delivery.

A B S T R A C T

Gellan gum is a microbial exopolysaccharide, water-soluble polymers secreted by microorganisms during fermentation. The biopolymer gellan gum is a relatively recent addition to the family of microbial polysaccharides that is gaining much importance in food, pharmaceutical and chemical industries due to its novel properties. The purpose of this work is to investigate the impact of physiological fluids on both the physical and chemical properties of gellan gum, and to understand the role of polymers gel in muco-adhesion and drug delivery to prolong the residence time of the drug inside the body. Muco-adhesion measurements of retention time were performed using bespoke retention apparatus to determine the retention of labelled gellan gum dose. The physiological fluids used in this work are artificial gastric juice, artificial saliva fluid, and artificial tears fluid. Results of this work show that in general the viscosity of gellan increased with high concentration and the gel formation is strong with artificial gastric juice (HCL) and weak gel formation with artificial saliva and tears but the retention time is longer with saliva and tears than with artificial gastric juice.

1 Introduction

Hydrocolloids or gums is a type of polysaccharide which is commercially available and it is used in food industries as stabilizers, crystallization inhibitors, thickening and gelling agents and in some non-food industries it is used as encapsulating agent. Due to the strong interlinked connections in the molecules, polysaccharides have a characteristic strength. In nature it is present in plant cell wall as a component or exist as extracellular substance e.g., Gellan gum. Gellan gum is a high molecular weight compound with a negative charge on its molecule. It is produced by microorganism named *Sphingomonas elodea* as a fermentation product. It can also be produced commercially on demand with a consistent quality (Jansson et al., 1983).

Physical characteristics of gellan Gum

The gelatinization of gellan gum is dependent on ambient temperature and cationic strength. The process of gelatinization involves the formation of double helical junctions which further form complexes with cations and hydrated with water molecules by hydrogen bonds.

The gelatinization of gellan gum in aqueous environment is a two-step process. In step one double helices of random coil chains are formed leading to aggregation of pairs of double helices, this is also called Coil-helix transition. In step two it makes electrostatic interaction with co-existing cations in the solutions and this is affected by varying the pH of the solution.

Chemical Characteristics of gellan Gum

The molecular structure of it consists of a tetra saccharide repeating unit made of glucose, glucouronic acid and rhamnose residue in a ratio of 2:1:1. In the negative charged molecule two acyl substituent; glyceryl at O (2)

Corresponding author: E-mail: hana.1983@yahoo.com

and an acetyl at O (6) are present on the 3 linked glucose molecule (Fig. 1). The gellan gum can deacetylate by alkali treatment.

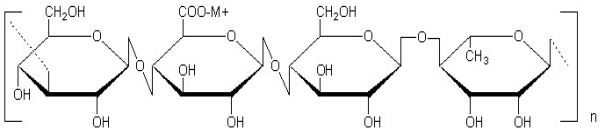


Figure (1). The structure of Gellan Gum (Matricardi et al., 2009)

Gellan gum has been characterized into the three groups on the following basis: Polysaccharide content, the percentage of O-acetyl substitution on the molecule, and the protein content (including the nucleic residues and other organic nitrogen sources).

Acyl content has a profound impact in the physical characteristics of gel. Gels with high acyl content are very soft, elastic and not brittle whereas gels with low acyl content are firm, brittle and are inelastic. Both forms of acyl gels (high content which is the native form and the synthetics low content form) have similar linear structure having a tetra saccharide unit repeat. The tetra saccharide contains three different molecules which are glucose, rhamnose and glucouronic acid with a molar ratio of 2:1:1. Glucose residues in the molecule have an acetyl group and L-glyceryl groups on it (Jansson et al., 1983). The molecular formula of gellan gum can vary as it depends on the glucouronic acid neutralization with various salts. The anionic nature of the gum is due to the presence of the carboxylate groups in molecular structure which gives it the ability to undergo gelatinization in the presence of monovalent or divalent cations. The gum form complexes with them by electrostatic interactions. It has been reported by Sanderson and Clark (1983) that the gum has greater affinity for the divalent cations.

Physiological Fluids

The weight of water in the human body is in the range of 45-75%. The percentage of it varies with age and gender. The adipose tissue of the human body holds up to 10% water whereas the lean tissues contain approximately 70 to 75 percent of water. The total body water is with varying percentage in intracellular fluid and extracellular fluid. Transcellular fluid like cerebrospinal fluid, humours of eye, digestive secretions and the renal tubular fluid secretions contain 1-2% of body water.

Gastric Juice

Several factor like hormonal (by gastric hormones), neural and intestinal factors affect the secretion of gastric acid from the stomach. Food intake and the deficient level of glucose in the brain result in the reflex secretion of gastric juice. The reflex system has optic, gustatory and olfactory afferent nerves which are responsible for partial conditions reflex and the impulse then flows through an efferent vagus nerve. When the HCl secretion is on the peak the pH of the stomach drops to ca. 0.8. The food being swallowed buffers the pH and increases it up to 1.8 to 4. This is the optimum pH for the enzymatic action of pepsin and gastric lipase. The low pH provides a bactericidal effect and it also denatures dietary protein making it easier to be degraded of protein enzymes.

Saliva

The human saliva is a complex mixture of different fluids; it is secreted by a set of major and minor salivary glands. There are three major salivary glands which are parotid, sublingual and submandibular glands. All these glands are under the control of autonomic nervous system. A human has salivary secretion of 1.5 litres in a day. It has slightly alkaline pH which is 7.4. Some organic and inorganic substances which are suspended in water make the saliva. It has glycoprotein Mucin along with some enzymatic proteins like lipase and salivary amylase. Some other compounds like lactoferrins, cystatin histatins, immunoglobulins and some thiocyanate ions are also present (Humphrey and Williamson, 2011).

Tear Fluid

A smooth ocular surface is responsible for the good visual activity. This property is provided to eye by Tear fluid or TF which covers the whole ocular surface and protects it. It serves as a barrier, lubricant, nutrient and an anti-microbial protectant. It improves the optical properties of eye and the maintenance of a normal Tear fluid is thus important for a smooth ocular surface.

Tear fluid lipids and proteins give high surface pressure and thus help in the stability of it. The average value of TF surface tension was found to be 70 mN/m (Glasgow et al., 2010). Tan and his fellows reported in 1993 that during sleep all major protein and water secretion is inhibited but Immunoglobulin A is the exception and it's secreted during the sleep. Na^{+}, K^{+}, Cl^{-}, HCO^{3-} and low levels of Mg^{+2} and Ca^{+2} are the principal electrolytes in the basal tear fluid. The TF is usually isotonic in nature with serum but it has got a higher concentration of K+ ions comparing to it (Gilbard, 1994).

In this paper the effect of the physiological fluids discussed above on the gellan gum will be discussed. Muco-adhesion of gel on the living tissue will be discussed in the context of bio-responsiveness in drug delivery systems.

Muco-adhesion

Muco-adhesion phenomenon is defined as a process in which two molecules, one of which is biological in nature interact with another with an interfacial force for an extended period of time. It's also defined as the ability of biological material or a synthetic material to stick on a tissue for a longer period of time. The tissue on which mucoadhesive adhere are actually mucous membrane. There are two steps which are involved in the muco-adhesion phenomenon. In the first step a contact between

the mucous membrane and the mucoadhesive polymer is established. This can be achieved by either good wetting of mucoadhesive surface or by promoting swelling of mucoadhesive substance. Once a good contact is established between them, the second step starts in which mucoadhesive penetrates the tissue surface or an interpenetration of mucoadhesive chains with the mucosal ones occurs the two surfaces are then stabilized by low energy chemical bonds (Duchene et al., 1988).

Muco-adhesion was used as a novel strategy for the improvement of therapeutic effect of various drugs in the early 1980s. Some water-soluble polymers have bio-adhesive properties i.e., they adhere to the mucous membrane on hydration. This property of bio-adhesive polymers was employed for designing mucoadhesive drug delivery systems. These polymers on the basis of this property can target the desired drug to the target tissue and keep it there for a longer period of time. The idea of using Mucoadhesive in drug delivery system was the result of need to localize drugs at target site for an extended period of time to optimize the action of delivered drug (Nagai and Machida, 1985).

The absorption of drug is constrained by the residence time of it at the target site e.g., in ocular drug delivery system a residence time of two minutes is available for the absorption of drug right after the instillation of it in the eye. But most of the drug washes away due to the solution drainage and rendering it unavailable for the absorption. This created the need development of drug delivery system which will keep the target at the target site for an extended period of time. Innovative formulations were developed with the use of gellan gum for the drug delivery to the target sites. The presence of gellan gum ensures the bioavailability of the drug for effective absorption by the tissue by adhering it to mucosal linings. From the research it has been observed that this gum is a very effective carrier and some attractive formulations were formulated that make the delivery of drug to the target tissues very specific (Kamath and Park, 1994). Following are the features that made the basis for the gellan gum drug delivery system:

- The delivery of the drug to the pharmacological specified sites can be controlled by using this gum.
- Another remarkable feature of it is that it increases the permeability of the cell membrane and thus helps in the effective drug uptake by the cell membranes.
- It stabilizes the drug product which is supposed to be delivered in the body.
- The solubility of the dug product is also increased in the body fluids (e.g., intestinal fluid) due to the presence of gellan gum.
- The drug metabolism is also reduced by its use thus it prevents the elimination of drug from the body.

Mao et al. (2000) explained the most remarkable feature of gellan which is its swelling ability which can be triggered by altering the environment surrounding to the delivery system (Fig. 2). The environmental factors that have an effect on the efficiency of the gel are pH, temperature or ionic strength. These factors can either swell or shrink the delivery system if changed from their normal values. The gellan gum is a pH sensitive compound and the functioning of the drug delivery having gellan gum can be monitored or optimized by changing the ambient pH. The most effective pH for it is alkaline. At alkaline pH the gel swells and releases the drug content inside it whereas at low pH the delivery system is collapsed. This property of gellan gum makes it an ideal candidate to be used in oral drugs in which it is rendered ineffective on its passage to the stomach where the pH is too low. When it reaches the upper intestine with high pH, the drug is released here to perform its function (Huang, 2004)

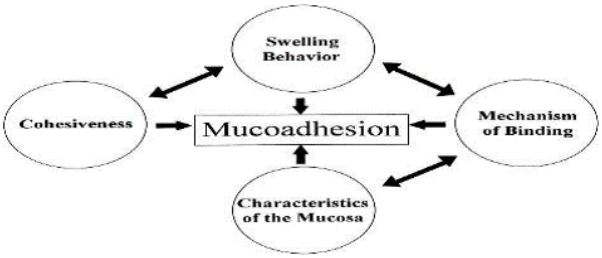


Figure (2). Schematic presentation of effects influencing Muco-adhesion. (Bernkop-Schnürch & Steininger 2000)

2 Materials and Methods

Gellan gum polysaccharide (Kelcogel) was from CP Kelco (Leatherhead, UK). Six well plates and dialysis tube supplied from Sigma-Aldrich (Poole UK). All other chemicals which were used in this study were supplied by the chemicals store, University of Huddersfield.

Gellan Gum Solutions

Three different concentrations of gellan gum solutions were prepared (0.5, 1.0 and 1.5 % (w/v) by slowly adding a weighed amount of gellan gum to hot deionised water (80°C) with continuous stirring until fully dissolved and then left at room temperature to cool before it was transferred into a 100 ml volumetric flask.

Gastric Juice (artificial)

Artificial gastric fluid was prepared from concentrated hydrochloric acid (HCl) mixed with deionised water in 1000 ml volumetric flask to produce a 0.1M solution and was adjusted to pH 1.2 using concentrated NaOH or HCl.

Artificial Saliva Fluid

The preparation of artificial saliva was developed by (Parker et al., 1999) and prepared in g/l by using 0.34 g

KH_2PO_4, 0.43 g Na_2PHO_4, 1.5 g $KHCO_3$, 0.58 g NaCl, 0.14 g $MgCl_2$, 0.22 g CaCl and 0.03 g Citric Acid dissolved together in a 1-liter deionised water and then the pH of solution was adjusted to 6.7 using concentrated of NaOH or HCl.

Artificial Tears Fluid

The preparation of artificial tear fluid was adopted from a tear fluid analysis (Stjernschantz and Astin, 1993) and made using 6.8 g NaCl, 2.2 g NaHCO, 0.084 g CaCl, 1.4 g KCl in 1 l of deionised water.

Methylene Blue Stock Solution

The stock solution was prepared by dissolving 0.1 mg/ml of Methylene blue crystals (methylthioninium chloride) in 100 ml volumetric flask with deionised water and then seven different amounts from the stock were taken (0.5,1, 2,4,6,8 and 10 m) respectively to prepare different concentration from the stock as standards for plotting standardization curve. AUV-V is spectrophotometer in scanning mode was used to identify the peak maxima (λ max) for Methylene blue which was measured at 658nm. This wavelength was used to measuring the absorbance of the standards.

Preparation of Methylene Blue Labelled Gel for Retention Time Measurement

Labelled gel was prepared by dissolving the gellan powder and Methylene blue crystal in deionised water (hot water 80°C) while stirring until completely dissolved. Four different concentrations from Methylene blue were prepared (0.01, 0.025, 0.05 and 0.1 mg/ml) to find out the suitable concentration for the experiment which was 0.1 mg/ml. The three different concentrations from gellan gel were prepared by the same way that mention above (0.5, 1.0 and 1.5 g %) after finish preparation the gel leaved at room temperature for 24 hours before measure the retention time.

Retention Time Measurement

Measurements of retention time were performed using bespoke retention apparatus shown in Fig. 3 (Batchelor et al., 2002). The apparatus used to determine the retention of labelled gellan gum dose on dialysis tube as a tissue. A Perspex® mounting block was manufactured with dimensions 100 mm length by 60 mm width and 15 mm deep. A groove was cut into this block in a central position; the dimensions of the groove were 100 mm long by 12 mm wide and 5 mm deep. The mounting block was permanently attached to a clamp that was able to rotate through approximately 120°. The clamp was attached to a stand and placed within a temperature and humidity-controlled environment, an adapted Gallenkamp industrial humidity cabinet (model BR185H). This apparatus allowed the temperature and

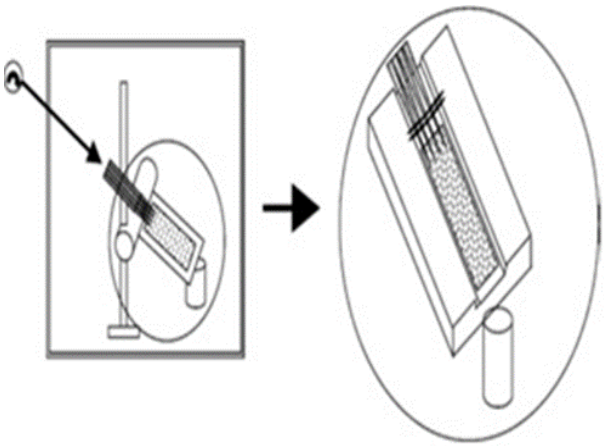


Figure (3). Apparatus used to determine the retention of a *vivo* tissue in presence three different type of artificial physiological fluids (Gastric juice HCl, saliva and tears).

humidity to be controlled and maintained via the water bath. The cabinet had sealed glove access to enable procedures to be performed during the experiment. The washing medium delivered to the tissue was supplied via a peristaltic pump (Watson Marlow model 202). The flow was split into three channels to provide an even distribution of the media over the entire tissue section. labelled gellan gum on dialysis tube as a tissue instead The Methylene blue labelled gellan gel (0.5, 1.0 and 1.5 %) were prepared and kept in room temperature for 24 hours before measuring retention. The retention time was examined for each concentration of the gel with the three fluids.

A 60 mm by 12 mm longitudinal section of dialysis tube (used as a model for epithelial tissue) was cut and placed into the groove cut on the stand and fixed with super glue. Then 1 ml of the gel sample was taken by syringe and put on the dialysis tube and the artificial fluid was allowed to flow over the gel controlled by an automatic pump at a rate of 4 ml per minute. The eluted physiological fluid was collected at time intervals up to 60 minutes and the absorbance was then measured at 658nm.

Calculation of Retention Time

The retention time can be calculated by using the equation from the calibration curve of Methylene blue standards.

Y = 197.42 x -0.0146, where Y is the absorbance value

Concentrations of the Methylene blue were calculated in different period of time, concentration versus absorbance was plotted on the curve.

3 Results

By using UV spectrophotometer, it can be able to identify the suitable wavelength for Methylene blue solution. The UV spectrophotometer allows a user to identify electronic transitions within the various regions of the

electromagnetic spectrum. UV can be measured by a spectrophotometer most readily when it is in the 400 to 700 nanometre (nm) region to quantify and determine the features of colour perception. The wavelength which will be used to measuring the absorbance for standards is (658 nm). Table 1 shows the absorbance for all concentration of standards which were taken at 658 nm for plotting a calibration curve.

The calibration curve of Methylene blue for seven different standards which give good linearity and the coefficient of determination (R^2) = 0.98 which is close to one (Fig. 4). The equation obtained from the curve is used to calculate the retention time.

Table (1). shows the absorbance for all concentration of standards

Concentration mg/ml	Absorbance (658nm)
0.0005	0.102
0.001	0.205
0.002	0.407
0.004	0.764
0.006	1.090
0.008	1.393
0. 01	2.141

Calculation of Retention Time

1- Labelled Gel with Artificial Gastric Juice (HCL)

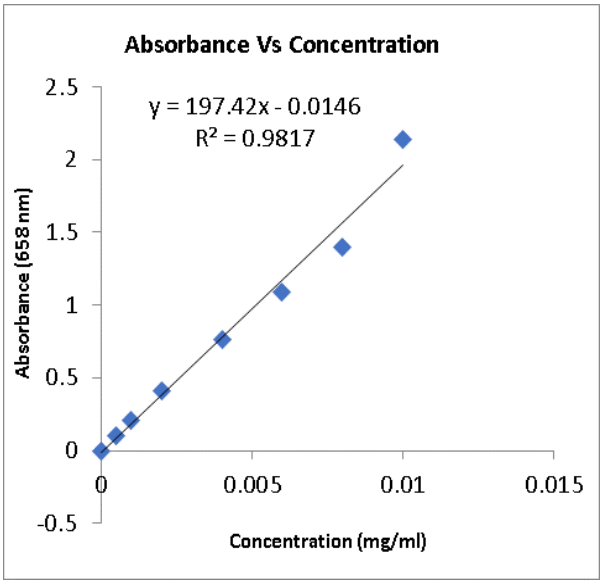


Figure (4). Calibration curve of Methylene blue standards

Calculation and comparing between the retention time of Methylene blue in three different concentrations of gel:

1- 0.5 % w/v labelled gel:

Absorbance (658nm) = 0.329, Amount of all volume collected = 204 ml

Y= 197.42X - 0.0146

X = 0.329 + 0.0146/197.42 = 0.0017 mg/ml

Dilution factor= 0.0017 × 204 = 0.35 mg/ml

Retention concentration = 1 - 0.35 = 0.65 mg/ml (65%)

2- 1.0 % w/v labelled gel:

Absorbance (658nm) = 0.374 Amount of all volume collected = 244 ml

Y= 0.374, X = 0.374 + 0.0146/197.42 = 0.002 mg/ml

Dilution factor = 0.002 × 244= 0.49 mg/ml

Retention concentration = 1 - 0.49 = 0.51 mg/ml (51%)

3- 1.5 % w/v labelled gel:

Absorbance (658nm) = 0.421 Amount of all volume collected = 244ml

Y= 0.421,

X = 0.421 + 0.0146/197.42 = 0.0022

Dilution factor = 0.0022 × 244= 0.54 mg/ml

Retention concentration = 1 - 0.54 = 0.46 mg/ml (46%)

2- Labelled Gel with Artificial Saliva

Calculation and comparing between Retention and Removed amount of Methylene blue in different concentration of gel with saliva

1- 1.0 % w/v labelled gel:

For all volume (124ml) = 0.141+0.0146/197.42= 0.0008 Amount

Dilution factor= 0.0008×124 = 0.1 mg/ ml Remaining concentration = 1- 0.1 = 0.9 mg/ ml (90%)

2- 1.5 % w/v labelled gel:

Amount for all volume (184ml) = 0.192+0.0146/197.42= 0.001

Dilution factor = 0.001×184 = 0.18 mg/ ml Remaining concentration = 1- 0.18 = 0.82 mg/ ml (82%)

3- Labelled gel with artificial tear

Calculation and comparing between Retention of Methylene blue in different concentration of gel

1- 0.5 % w/v labelled gel:

Amount for all volume (124ml) = 0.186+0.0146/197.42= 0.001

Dilution factor= 0.001×124 = 0.124 mg/ ml
Remaining concentration = 1- 0.124 = 0.88 mg/ ml (88%)

2- 1.0 % w/v labelled gel:

Amount for all volume (124ml) = 0.171+0.0146/197.42= 0.0009.

Dilution factor= 0.0009×124 = 0.112 mg/ ml
Remaining concentration = 1- 0.112 = 0.89

mg/ ml (89%)

3- 1.5 % w/v labelled gel:

Amount for all volume (184ml) = 0.232+0.0146/197.42= 0.0012

Dilution factor= 0.0012×184 = 0.221 mg/ ml
Remaining concentration = 1- 0.221 = 0.78 mg/ ml (78%)

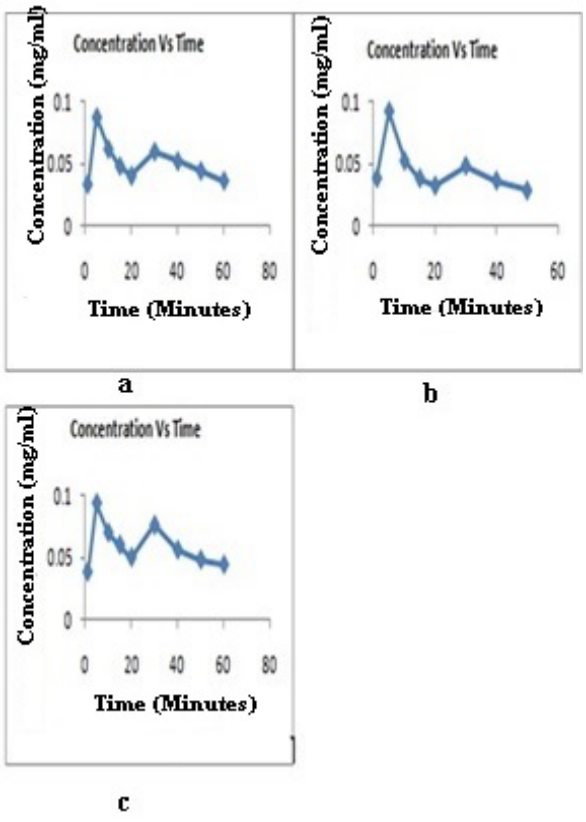


Figure (5). Concentrations of gellan gel forming with artificial gastric juice (HCL) in three different concentrations (a: 0.5, b: 1.0 and c: 1.5 %)

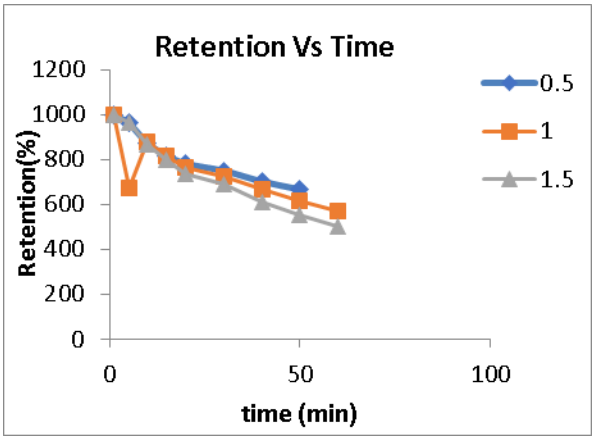
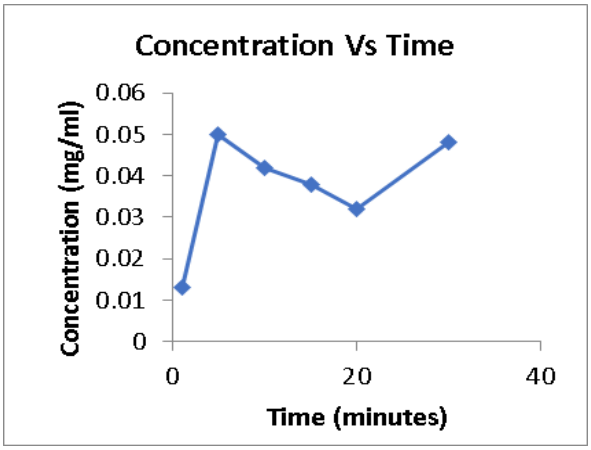
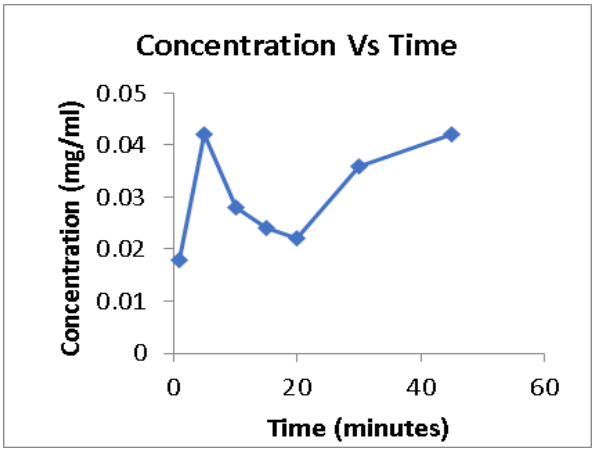


Figure (6). Comparing between retention amount of Methylene blue in different concentrations of labelled gel



(a)



(b)

Figure (7). Concentration of Methylene blue in various concentrations of labelled gel (a: 1.0 and b: 1.5 %)

4 Discussion

Figs. 5, 7, and 9 show the concentrations of Methylene blue which removed from the gel throughout 60 minutes intervals time by flowing physiological fluids over the gel. The results which can be obtained from the graphs show that the adhesion of Methylene blue stain with artificial saliva and tear better than with artificial gastric juice (HCL).

Figs 6, 8, and 10, illustrates the retention time of Methylene blue with three types of physiological fluids that mentions above in three different concentrations (0.5 %, 1%, 1.5 %) w/v. The graphs show that the retention time increases with low concentrations of labelled gel and decreases with high concentrations in three types of fluids.

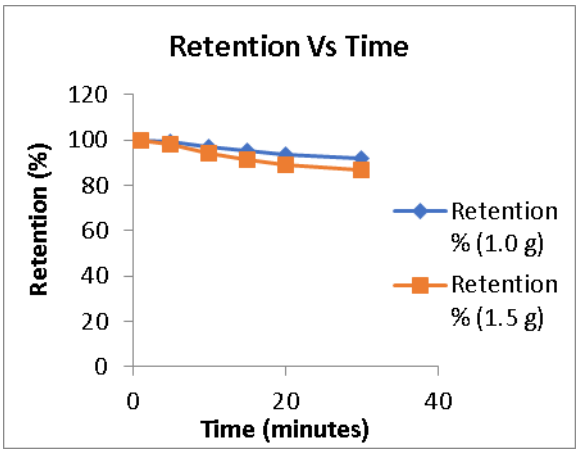


Figure (8). comparing between retention amounts of Methylene blue in different concentrations of labelled gel

5 Conclusion

Gellan gum is a bacterial polysaccharide, discovered through the screening of thousands of bacteria and prepared commercially by aerobic submerged fermentation from *Sphingomonas elodea*. The rheological behaviours like viscosity and oscillation effected by temperature, concentration, shear rate and stress.

The polymer as secreted by the microorganism contains approximately 1.5 acyl substituent per tetra saccharide repeating unit. The strength of gellan gum gels increases with the cations mono and divalent (Ca^{+}, k^{+}, H^{+} and Mg^{+}) which present in physiological fluids such as gastric juice, saliva and tear fluid. Muco-adhesive polymers can be used as means of improving drug delivery through different routes like gastrointestinal and ocular.

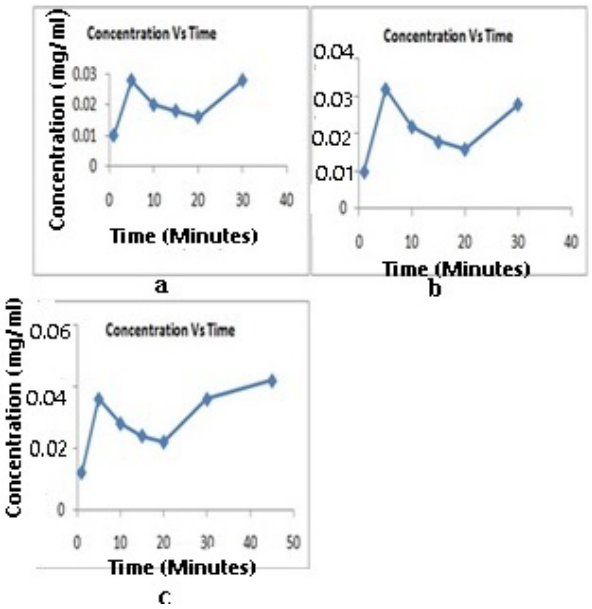


Figure (9). shows concentrations of gellan gel forming with artificial tear in three different concentrations (a:0.5, b:1.0, and c:1.5 %)

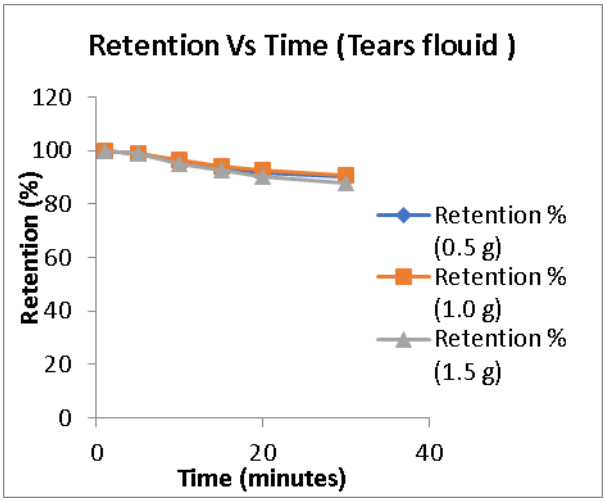


Figure (10). shows comparing between retention amounts of Methylene blue in different concentrations of labelled gel

References

Batchelor, H. K., Banning. D., Dettmar, P.W., Hampson, F.C., Jolliffe, I. G., Craig, D. Q. M., (2002) An in vitro mucosal model for prediction of the bio-adhesion of alginate solutions to the oesophagus, *International Journal of Pharmaceutics*, 238: 1–2, 123-132.

Bernkop-Schnürch, A., & Steininger, S. (2000). Synthesis and characterisation of mucoadhesive thiolated polymers. *International journal of pharmaceutics*, ***194***(2), 239–247.

Duchene, D., Touchard F., Peppos, N.A., (1988). Drug Dev, *1nd Pharm*, 14:283.

Gilbard, J. P., Human tear film electrolyte concentrations in health and dry-eye disease, *Int Ophthalmol Clin.* 1994, 34:27-36.

Glasgow, B. J, Gasymov, O. K., Abduragimov, A. R., Engle, J. J., Casey, R. C., (2010) Tear lipocalin captures exogenous lipid from abnormal corneal surfaces. *Invest Ophthalmol Vis Sci.* 51:1981-7.

Huang, Y., (2004). Gelling temperatures of high acyl gellan as affected by monovalent and divalent cations with dynamic rheological analysis, *Carbohydr. Polym*, 56, 27-33.

Humphrey, S. P., Williamson, R. T., (2001). A review of saliva: normal composition, flow, and function. *The Journal of Prosthetic Dentistry*, Vol. 85, 162.

Jansson, P. E., Lindberg, B., and Sandford, P. A. (1983) Structural studies of gellan gum, an extracellular polysaccharide elaborated by Pseudomonas elodea, *Carbohydr. Res.*, 124, 135-139.

Kamath, K. R, Park, K., (1994). Mucosal adhesive preparations, in *Encyclopaedia of Pharmaceutical Technology*; J. Swarbrick and J. C. Boylan, eds., Vol. 10 Marcel Dekker, New York, 133.

Mao M., Tang J., Swanson B.G. (2000). Texture properties of high and low acyl mixed Gellan gels, *Carbohydr, polmr*., 41, 331-338.

Matricardi, P., Cencetti, C., Ria, R., Alhaique, F., and Coviello, T. (2009). Preparation and Characterization of Novel Gellan Gum Hydrogels Suitable for Modified Drug Release, *Molecules*, 14, 3376-3391.

Nagai T, Machida Y. (1985). Mucosal adhesive dosage forms, *Pharm Int*, 6: 196-200.

Sanderson, G. R., Clark, R. C., (1983). Gellan gum, *Food Technology*, 37, 62–70.

Stjernschantz, J., Astin, M., (1993). Anatomy and physiology of the eye physiological aspects of ocular drug therapy, In: *Biopharmaceutics of Ocular Drug Delivery*, Edman, P. ed., CRC Press, Boca Raton, pp. 1–25.

Scientific Journal for the Faculty of Science-Sirte University - SJFSSU

sjsfsu@su.edu.ly
journal.su.edu.ly/index.php/JSFSU