Sains Malaysiana 52(6)(2023): 1711-1721
http://doi.org/10.17576/jsm-2023-5206-08

Effect of Interference Study on Carrageenan Detection using Ultraviolet Visible Spectrophotometry

(Kesan Kajian Gangguan terhadap Pengesanan Karagenan menggunakan Spektrofotometri Ultraungu Tampak)

Muhamad Eqmal Izman Mohd Fadli[1], Wan Elina Faradilla Wan Khalid[1,*] & Sharina Abu Hanifah[2]

[1]School of Chemistry and Environment, Faculty of Applied Sciences, Universiti Teknologi MARA Cawangan Negeri Sembilan, Kampus Kuala Pilah, Pekan Parit Tinggi, 72000 Kuala Pilah, Negeri Sembilan, Malaysia
[2]Department of Chemical Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

Received: 15 September 2022/Accepted: 17 May 2023

ABSTRACT

Carrageenan is one of the most prominent hydrocolloids in the food industry used as a thickener and additive to improve the texture of food products. However, the detection of carrageenan in the food product is still limited as many interferences in the food matrix can interfere with the signal obtained. This research aims to study the effect of interference species on a simple and rapid quantitative detection of carrageenan by using a cationic dye which is methylene blue. Methylene blue will form a complex with carrageenan at 565 nm due to the hypsochromic shift of the methylene blue peak at 664 nm with a color change from blue to bluish purple. The optimization and analytical performance of carrageenan-methylene blue complexes were characterized by using UV-Visible Spectrophotometer. A dynamic linear concentration range for carrageenan detection was obtained in the range of 70-100 ppm (R^2 = 0.9837) with a limit of detection (LOD) estimated at 38.37 ppm. The reproducibility study was found to give a satisfactory relative standard deviation (RSD) value of 1.64-1.94%. Selectivity experiments were carried out where the methylene blue demonstrated acceptable selectivity towards carrageenan with no significant interference from sucrose and glucose.

Keywords: Complexation; free solution; methylene blue

ABSTRAK

Karagenan ialah salah satu hidrokoloid yang popular dalam industri makanan yang selalunya digunakan sebagai pemekat dan aditif untuk memperbaiki tekstur produk makanan. Walau bagaimanapun, pengesanan karagenan dalam produk makanan adalah masih terhad kerana banyak gangguan dalam matriks makanan yang boleh mengganggu isyarat yang diperoleh. Penyelidikan ini bertujuan untuk mengkaji kesan spesies gangguan terhadap pengesanan kuantitatif karagenan yang mudah dan cepat dengan menggunakan pewarna kationik iaitu metilena biru. Metilena biru akan membentuk kompleks dengan karagenan pada 565 nm disebabkan oleh peralihan hipsokromik puncak metilena biru pada 664 nm dengan perubahan warna daripada biru kepada ungu kebiruan. Pengoptimuman dan prestasi analisis kompleks karagenan-metilena biru telah dicirikan dengan menggunakan Spektrofotometer Ultraungu Tampak. Julat kepekatan linear dinamik untuk pengesanan karagenan diperoleh dalam julat 70-100 ppm (R^2 = 0.9837) dengan had pengesanan (LOD) dianggarkan pada 38.37 ppm. Kajian kebolehulangan didapati memberikan nilai sisihan piawai relatif (RSD) yang memuaskan iaitu 1.64-1.94%. Uji kaji kepilihan telah dijalankan dan metilena biru menunjukkan kepilihan yang boleh diterima terhadap karagenan tanpa gangguan ketara terutamanya daripada sukrosa.

Kata kunci: Larutan bebas; metilena biru; pengkompleksan

Introduction

Food industry has always been a multi-billion dollars business. New products are developed rapidly to further compete and tap into this industry (Sacks et al. 2020). Growing demand for processed foods is a global trend due to the population growth and the conflicting

economic trends around the world (Chen et al. 2020). This has made one of the branches in the food industry which is the hydrocolloid market to be relatively popular and successful. One of the most popular hydrocolloids used in the food industry is carrageenan due to its great physical and biological properties (Goeff & Guo 2019).

Generally, carrageenan is widely added into confectionery products and processed food as a food hydrocolloid (Hotchkiss et al. 2018). Carrageenan is commonly used in food products to improve appearance, flavour, taste and colour qualities, to impart processing benefits such as freeze-thaw stability, sliceability, retain freshness, and increase shelf life, as well as to increase yield (Goeff & Guo 2019). Even though carrageenan was considered safe to consume by the U.S. Food and Drug Administration (FDA), some studies have shown that consuming carrageenan can affect human health (Tobacman et al. 2008). Controversial studies made by a researcher named Tobacman that started in the late 1990s have shown that consuming carrageenan can cause gastrointestinal effects or inflammation. Although the result of the studies was not able to be replicated, it has started a conversation about the harmful effect of consuming carrageenan and has become the topic of interest to researchers to study the harmful effect of consuming carrageenan toward the human body (Liu et al. 2021).

The Scientific Committee for Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) have both approved carrageenan for human consumption. The SCF had no objections to carrageenan being used in infant formula for up to 0.3 g/L in 2003. In addition, JEFCA stated in 2015 that 'the use of carrageenan in newborn formula up to 1000 ppm is not of concern' (Younes et al. 2018). Nowadays, studies have found that carrageenan can inhibit proteolysis which is the breakdown of proteins or peptides into amino acids by the action of enzymes in the gastrointestinal tract especially toward children and adults with short bowel syndrome (Liu et al. 2021). Carrageenan have also been shown to regulate gut permeability and intestinal barrier function in the human body (Liu et al. 2021).

The quantitative measurement of carrageenan in food remains a difficult problem for analysts. Low carrageenan content in food products, and a lot of interfering chemicals including alginate, agar, glucose, calcium, and sodium chloride can make it hard to analyse the carrageenan content in food products. Generally, there are a lot of methods and techniques such as high-performance liquid chromatography (HPLC), Fourier transform infrared spectroscopy (FTIR), histochemical method and spectrophotometric titration to determine carrageenan in the food product. However, most of the analysis cannot be carried out *in-situ* and trained personnel is required to handle the instruments (Ling & Heng 2014; Ziółkowska et al. 2021). Moreover, all the conventional methods such as chromatographic technique, infrared spectroscopy and Raman spectroscopy usually are very time consuming and costly (Sun et al. 2021). For example, HPLC method may suffer from long analysis time and costly due the large amount of solvent use (Luxminarayan et al. 2017). In this study, methylene blue (MB) was used as the indicator for the study of carrageenan-MB complexation. Although MB is considered toxic and non-biodegradable, MB which is a cationic dye is widely used for adsorption study and are able to interact with polyanion (Alsubaie et al. 2021; Khan et al. 2022). The used of MB as the indicator for carrageenan detection offered a simple and quick method. The present paper reports a simple spectrophotometric method for the determination of carrageenan. Although, there are similar studies has been conducted by Ling and Heng (2014), in our study, we focus and highlight on the effect of interference species on the selectivity of the detection method.

Materials and Methods

Materials

Methylene blue, kappa-carrageenan, glucose, sucrose and sodium alginate was obtained from R&M Chemical. Deionized water was used throughout the measurements.

Preparation of kappa-carrageenan solution

Stock solution of kappa-carrageenan (1000 ppm) was prepared by weighing 0.1 g of kappa-carrageenan and adding 100 mL of deionized water. The solution was then heated at 75-80 °C and stirred continuously until the kappa-carrageenan was fully dissolved. The working solution (10-200 ppm) were freshly prepared with the required dilution.

Absorption spectra of MB solution and reaction of MB with carrageenan

All the absorption spectra was recorded using UV-Vis Spectrophotometer. About 2 mL of MB (0.020 mM) and 2 mL of carrageenan (100 ppm) was mixed and was analysed at the wavelength range of 200-800 nm.

EFFECT OF MB CONCENTRATION

A series of different MB concentrations (0.001 mM, 0.005 mM, 0.01 mM and 0.020 mM) solutions were prepared in 25 mL volumetric flasks. A fixed concentration of carrageenan (100 ppm) was then reacted with 0.001 mM MB followed by 0.005 mM, 0.01 mM and 0.020 mM.

EFFECT OF KAPPA-CARRAGEENAN CONCENTRATION

A series of different carrageenan concentrations (10-200 ppm) solutions were prepared in a 25 mL volumetric flask. A fixed concentration of MB (0.020 mM) was then reacted with 10 ppm of carrageenan solution at a volume ratio of 1:1. Graph of absorbance versus different concentration of carrageenan was plotted at 565 nm and 664 nm. Limit of detection (LOD) was calculated (Taha et al. 2022).

$$\mathrm{LOD} = \frac{3.3\sigma}{\mathrm{S}}$$

where **σ** is the standard deviation of the blank; and S is the slope of the calibration curve.

STABILITY STUDY OF MB SOLUTION AND REPRODUCIBILITY STUDY OF CARRAGEENAN-MB COMPLEX

For the stability study of the MB solution, the absorbance of the 0.020 mM MB solution was recorded for every 1 hour interval for the duration of ten hours. Then, the relative standard deviation (RSD) was calculated. For the reproducibility study, 0.020 mM MB was reacted with 70 ppm and 100 ppm carrageenan and the absorption spectra was recorded at the wavelength 565 nm. The same procedure was repeated eight times and the RSD was calculated.

INTERFERENCE STUDY

An interference study was performed to analyse the absorbance of the carrageenan-MB complex when carrageenan was used with other posibble interference species. The possible interference that were used in this study include sucrose, glucose and alginate. Interference study was carried out separately by reacting carrageenan with 0.020 mM MB and carrageenan with other possible inteference species. In addition, the response of the carrageenan solution (70-100 ppm) with the presence of sucrose (70-100 ppm) and 0.020 mM MB also was recorded. The same step was repeated for glucose and alginate species. Then, the linear range were tested using t-test statistical analysis. For a t-test statistical analysis, the value was concluded based on the p-value. The p-values that are less than 0.05 displayed that there is a significant difference between the absorbance value.

RESULTS AND DISCUSSION

CHEMICAL INTERACTION BETWEEN CARRAGEENAN AND METHYLENE BLUE

Based on Figure 1, the maximum absorbance for free MB was found at wavelength 664 nm. The reaction between carrageenan and MB displays a hypsochromic shift from 664 nm to 565 nm (Herfurth & Ulrich 2017). Complexation of the carrageenan with MB at 565 nm produce a colour change from light blue to bluish purple (Ziółkowska et al. 2021). This happened due to the nature of the MB as a cationic dye and carrageenan as a polyanion. The organosulfates located in the carrageenan structure will interact with the nitrogen in MB to form the carrageenan-MB complex (Lapwanit, Sooksimuang & Trakulsujaritchok 2018) (Figure 2). In this study, 565 nm was chosen as the working wavelength for the subsequent analysis.

EFFECT OF MB CONCENTRATION

The effect of MB concentration on the absorbance was performed in the range of 0.001-0.020 mM. The absorbance is proportional to the concentration where the absorbance of MB was found to be increased with the increasing of MB concentration in accordance with Beer's Lambert Law. A higher concentration of MB will lead to deviation from Beer's Lambert Law (Bhanvase and Barai, 2021). In this study, 0.020 mM MB was chosen as the optimum concentration and this concentration was used throughout the analysis.

EFFECT OF KAPPA-CARRAGEENAN CONCENTRATIONS

Figure 3(a) illustrates the absorption spectrum and Figure 3(b) shows the response curve of the MB-carrageenan compound with different concentrations of carrageenan (0-200 ppm). The absorbance of the compound forms at 565 nm increase with the increasing of carrageenan concentration while there is a decreasing trend in the absorbance at 664 nm with the R^2 value of 0.9837 and 0.9213, respectively. This is due to the formation of the dimer form of the carrageenan-MB compound at 565 nm (Lapwanit, Sooksimuang & Trakulsujaritchok 2018). Figure 3(a) also displays one isosbestic point at

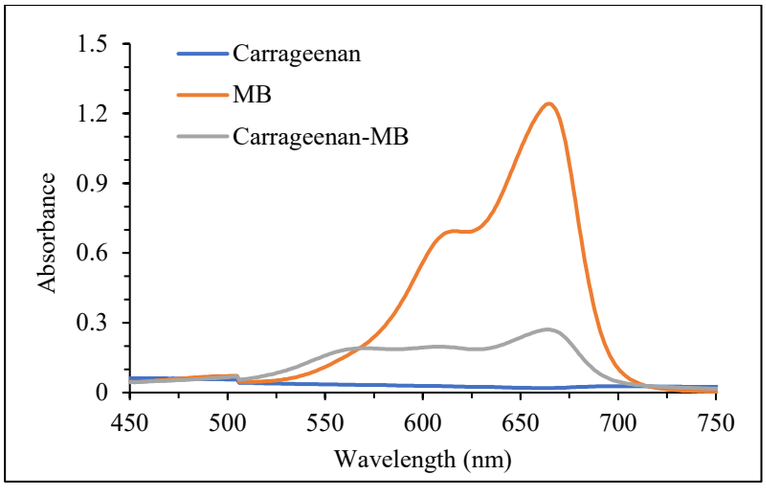

FIGURE 1. Absorption spectrum of carrageenan, methylene blue and carrageenan-MB with carrageenan and MB concentration of 1000 ppm and 0.020 mM, respectively

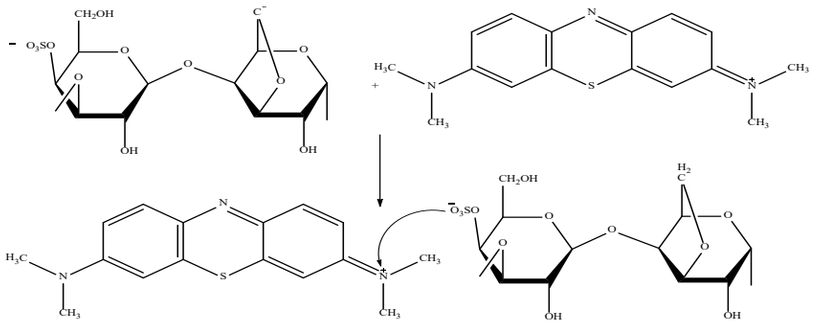

FIGURE 2. The formation of carrageenan-MB compound

588 nm where the presence of isosbestic points in the spectrum indicates that the compound's ion associates are stoichiometric and the carrageenan ions is fully used in the formation of the compound (Ziółkowska et al. 2021). Isosbestic point is the point where the wavelength, wavenumber, or frequency at which a sample's total optical activity remains constant during a chemical reaction (Hemdan, Jebaly & Ali 2019). The absorbance value at these wavelengths remains constant and is unaffected by carrageenan concentration. Based on Figure 3(b), the absorbance signal reaches a saturation point when the concentration of carrageenan is higher than 100 ppm where the signal at this point becomes plateau. This signifies that the complex formation between carrageenan and MB are maximum at this point. The dynamic linear response was found in the range of 70-100 ppm with linear regression value (R^2) of 0.9837 and LOD value of 38.37 ppm.

STABILITY STUDY OF MB SOLUTION AND REPRODUCIBILITY STUDIES OF CARRAGEENAN-MB COMPLEX

The photostability study was carried out to determine any possibility of photodecomposition of the MB reagent when the MB reagent was exposed to light for 10 h. The RSD value obtained was 0.252% which demonstrated that the MB reagent is stable and can be used when

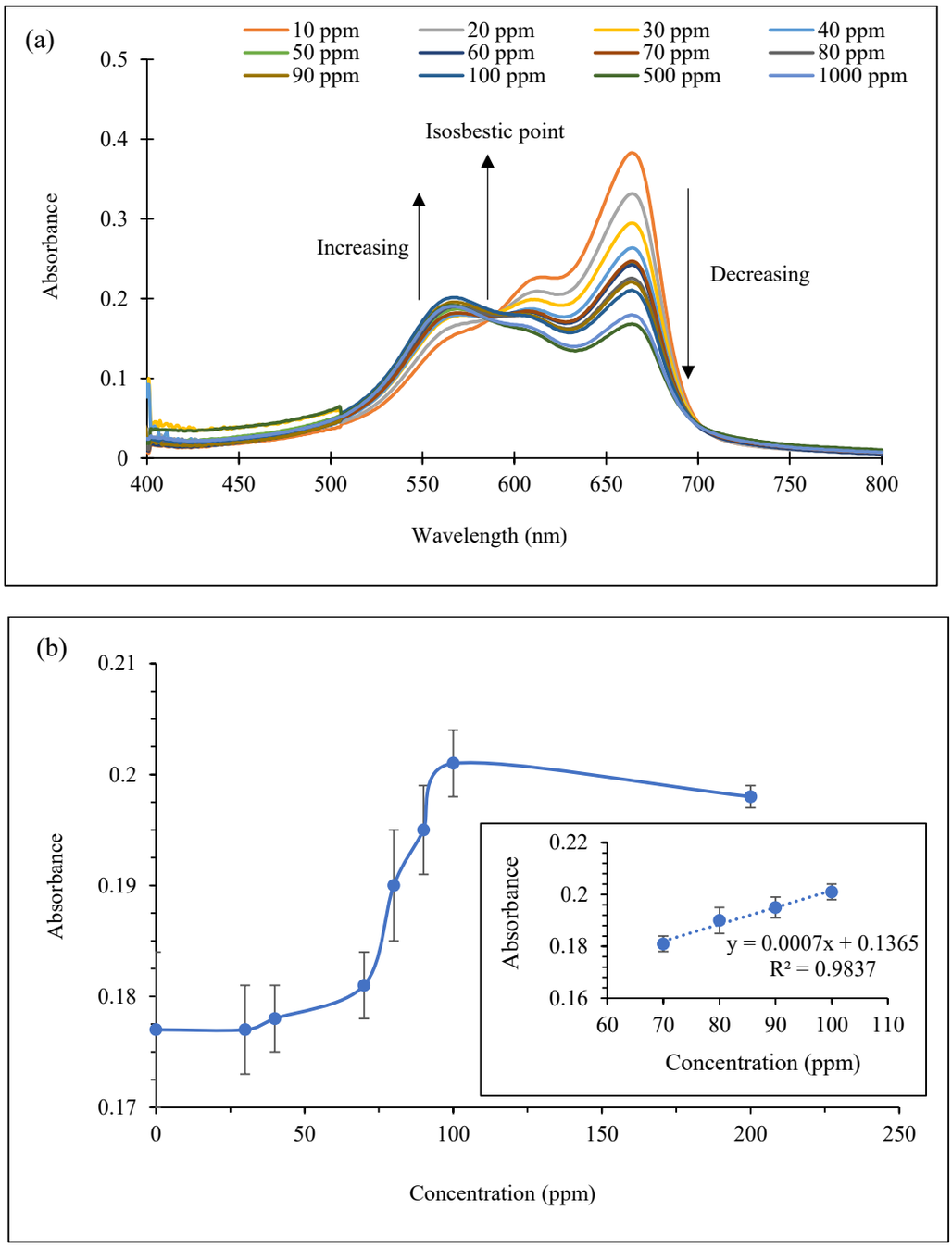

FIGURE 3. (a) Plot of absorbance versus wavelength at different carrageenan concentrations (10-1000 ppm) with 0.020 mM MB (b) Plot of absorbance versus concentration of carrageenan (0-200 ppm) react with 0.020 mM MB

exposed to light for up to 10 h. Reproducibility study was carried out by measuring the absorbance of eight replications of MB-carrageenan complex solution. The reproducibility study of eight replications of MB-carrageenan complex solution yields an acceptable RSD value of 1.94% and 1.64% for 70 ppm and 100 ppm,

respectively. The RSD value of less than 5% displayed that the reproducibility of the method is considered good and precise (Taylor 2018).

INTERFERENCE STUDY

Based on Figure 4(a), sucrose and glucose show almost similar absorption spectrum with MB alone with a prominent peak at 664 nm. Hence, sucrose and glucose can be considered not to interfere with the carrageenan detection as no hypsochromic shift occurred. Both carrageenan-MB and alginate-MB complex produce almost similar colours due to the formation of the dimer form of the complex at 565 nm and 600 nm for carrageenan-MB and alginate-MB, respectively (Lipatova, Makarova & Mezina 2016). Although both carrageenan-MB and alginate-MB complex produce almost similar colour, no absorption peak was shown at 565 nm for the alginate-MB complex. The alginate-MB complex shows two absorbance peaks at 600 nm and 664 nm.

In order to confirm whether the presence of alginate interfered with the signal of the carrageenan-MB complex, the dynamic linear range study was carried

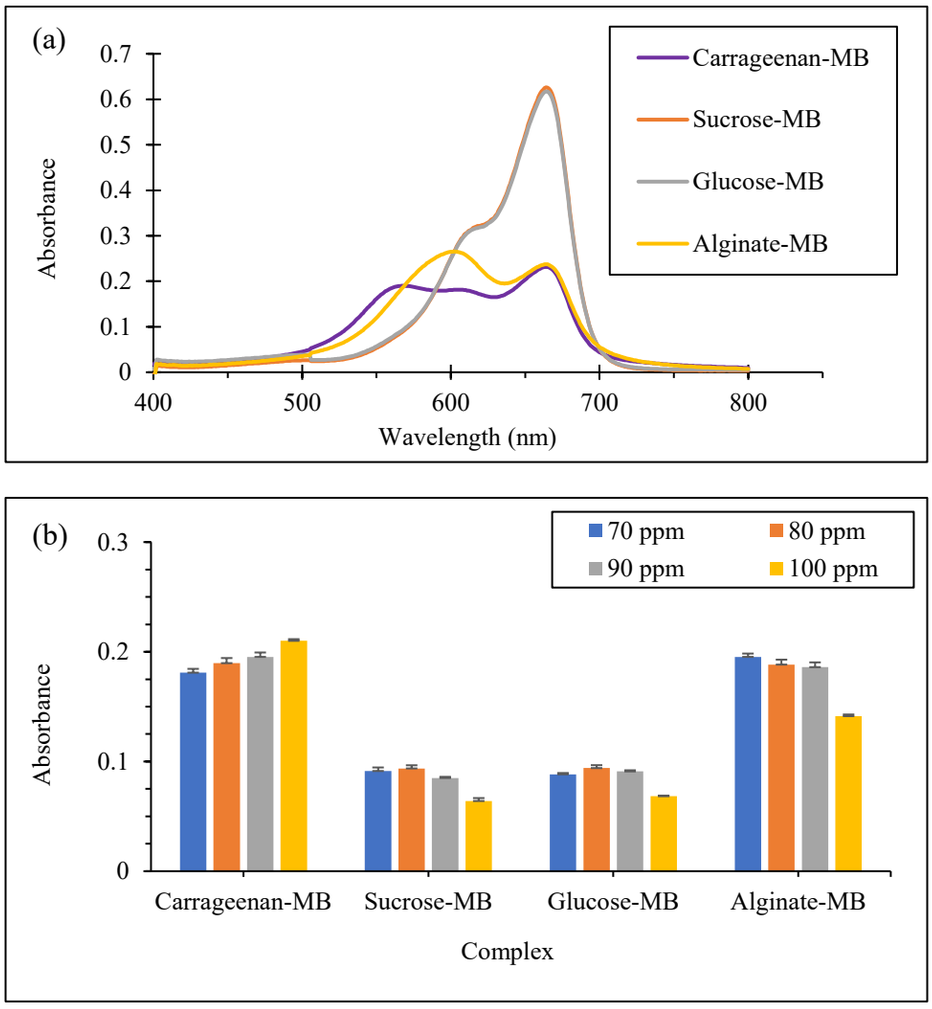

FIGURE 4. (a) Plot of absorbance versus wavelength for the reaction of carrageenan and interference species with MB. The concentration of carrageenan/interference species and MB were fixed at 100 ppm and 0.020 mM, respectively (b) Interference study at carrageenan and interference species concentration of 70-100 ppm. The signal was recorded at 565 nm

out. The absorbance of the carrageenan-MB complex at 565 nm increases with the increase of the carrageenan concentration and is significantly higher than the sucrose-MB and glucose-MB complex. Alginate can be considered to interfere with carrageenan detection although it does not exhibit any peak at 565 nm. This is because alginate is also a polyanion and can react with MB through charge-charge interaction (Lee & Mooney 2012; Makhado et al. 2020). Even though alginate-MB also displayed absorbance value at 565 nm, the trend is not similar with carrageenan where in the increasing of alginate concentration, the absorbance of the alginate-MB complex decreases. In order to test whether there are any significant differences between the absorbance value of the carrageenan-MB complex with the interference species, t-test statistical analysis was performed (Wan Khalid et al. 2019).

Based on Table 1, p-values that are less than 0.05 show that there is a significant difference between the absorbance value of the carrageenan-MB complex and interference-MB complex. For both sucrose-MB and glucose-MB complex, the p-value is less than 0.05 which means that there is a significant difference between the sucrose and glucose complex and the carrageenan-MB complex, thus accepting the null hypothesis. The difference in the absorbance of carrageenan-MB complex with sucrose-MB MB and glucose-MB complexes showed that sucrose and glucose are not interfere with the absorbance of the carrageenan-MB complex. For the alginate-MB complex, the p-value for both 565 nm and 664 nm are more than 0.05. This shows that the null hypothesis is rejected where there is no significant difference in absorbance value between the carrageenan-MB complex and the alginate-MB complex. Based on Figure 4(b), it displayed that no significant difference in the absorbance value when the alginate concentration is less than 100 ppm. As explained earlier, alginate-MB did not produce any significant peak at 565 nm while it produces a peak at 600 nm.

Based on Figure 5, there are two prominent absorption peaks located at 565 nm and 664 nm for the carrageenan-MB complex, carrageenan+sucrose-MB complex and carrageenan+glucose-MB complex. The carrageenan+alginate-MB complex is the only complex that has two significant peaks at 600 nm and 664 nm. In addition, there are no peaks at 565 nm for carrageenan+alginate-MB, however, the absorbance is maximum and nearly close to carrageenan absorbance. This may be due to the formation of dimer alginate-MB that is more likely than the formation of dimer carrageenan-MB. Moreover, this may occurred due to the presence of carboxylate (COO^-) groups in the alginate polymer which is more likely to bind with the cationic MB dye than organosulfate ($-OSO_3^-$) groups in the carrageenan polymer matrix (Król et al. 2016; Lapwanit, Sooksimuang & Trakulsujaritchok 2018).

It is pertinent to point out that the absorbance value for all interference species at 565 nm are slightly lower than the absorbance of carrageenan alone. In addition, there are some fluctuations in the absorbance value of all the interfering species especially at 90 ppm and 100 ppm. Moreover, the presence of alginate is considered to interfere with the carrageenan measurements since there is no clear increasing trend displayed especially in the presence of alginate. As for Figure 6, carrageenan-MB complex displays a lower absorbance value than the carrageenan+interference complex at 664 nm.

TABLE 1. p-value of interference-MB complex and carrageenan-MB complex at 565 nm and 664 nm

Complex	565 nm	664 nm
Sucrose-MB	0.047	1.294×10^{-5}
Glucose-MB	0.042	3.53×10^{-5}
Alginate-MB	0.228	0.092

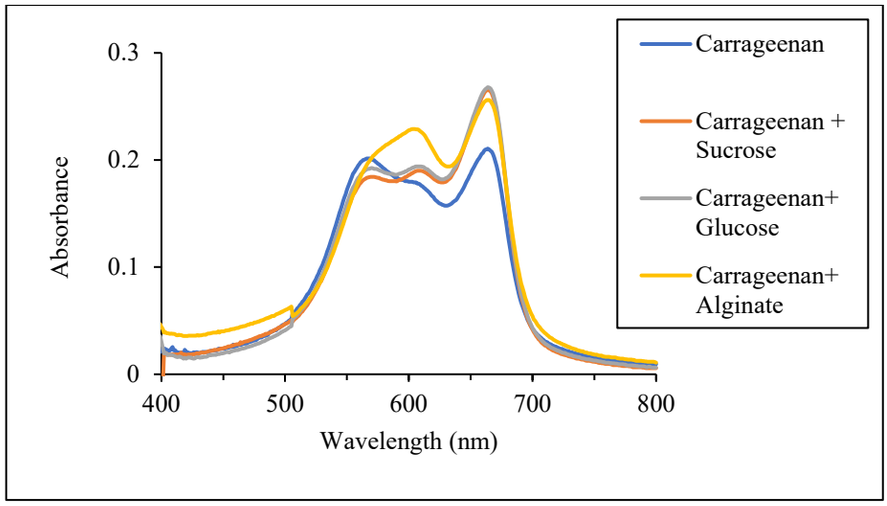

FIGURE 5. Plot of absorbance versus wavelength for carrageenan with interference species. Carrageenan and interference species concentration were fixed at 100 ppm with 0.020 mM MB

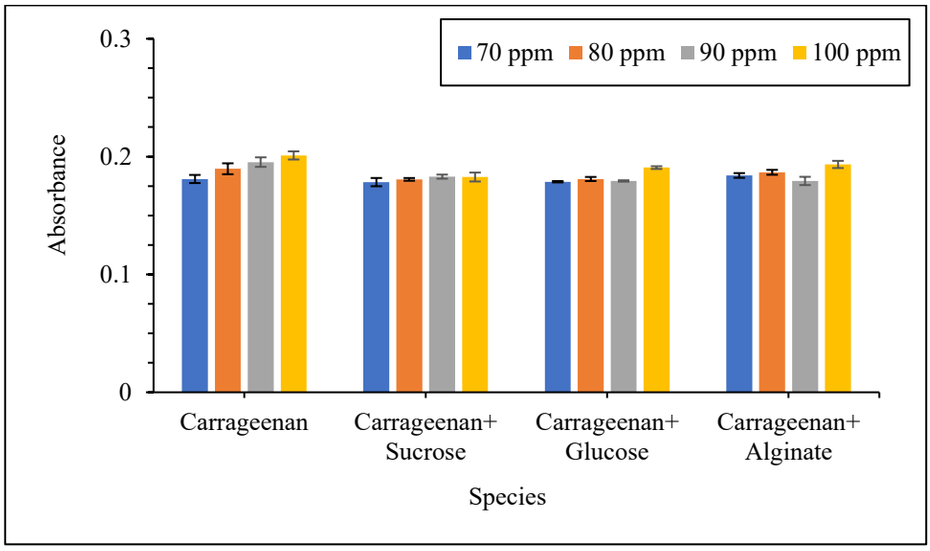

FIGURE 6. Interference study at interference species concentration in the range of 70-100 ppm. The signal was recorded at 565 nm

Based on the Table 2, carrageenan+alginate-MB have shown to give the lowest R^2 value. This means that in the presence of alginate, the determination of carrageenan will be affected the most even though there are no peak at 565 nm.

As mentioned earlier, t-test was conducted to determine whether the presence of interference species together with carrageenan will give effect to the linear range of the carrageenan detection at 565 nm and 664 nm.

TABLE 2. The linear correlation and R^2 of carrageenan and interference species

Species	Linear correlation	R^2
Carrageenan	y = 0.0007x+0.1365	0.9837
Carrageenan+sucrose	y = 0.0002x+0.1681	0.8464
Carrageenan+glucose	y = 0.0030x+0.1532	0.6294
Carrageenan+alginate	y = 0.0002x+0.1683	0.2082

TABLE 3. p-value of carrageenan+interference complex when compared to carrageenan species at 565 nm and 664 nm

Species	565 nm	664 nm
Carrageenan+sucrose	0.047	4.75×10^{-4}
Carrageenan+glucose	0.042	3.76×10^{-4}
Carrageenan+alginate	0.228	0.02

Based on Table 3, the carrageenan+sucrose and carrageenan+glucose show a p-value of less than 0.05 at 565 nm. This shows that there is a significant difference in the absorbance value between the carrageenan species and carrageenan+sucrose and carrageenan+glucose complex. This means that sucrose and glucose are considered to interfere with the intensity of the absorbance value of the dimer carrageenan-MB. There are some contradictions between the R^2 value and the p-value as the p-value suggest that sucrose and glucose will interfere with the detection of carrageenan. This happened may be due to possibility of glucose to reduce the MB and decolorize the MB into a light blue thus changing the absorbance intensity at 565 nm (Anderson et al. 2012). The p-value shows that no interference from alginate and as can be seen in Figure 5, no significant peak displays for alginate at 565 nm. However, the absorbance intensity of the carrageenan and carrageenan-alginate are almost similar due to alginate that have a high affinity for MB in solution (Figure 5) (Ling & Heng 2014). Generally, the interference from the interfering species can be avoided or minimized by using masking agents. Alginate can be masked by adding calcium chloride in the sample. Alginate will react with calcium chloride to form calcium alginate gel that can be removed from the sample using filtration process (Haldar & Chakraborty 2019).

Table 4 displays the analytical performance of this work with the previous spectrophotometric study for carrageenan detection. Bartlová et al. (2021) used new MB and titration process for carrageenan detection. Although the LOD of this method is very low, they used a large amount of reagent in each titration process. The spectrophotometric study using MB and toluidine blue developed by Ziółkowska et al. (2017) also used titration process for determination of carrageenan. As for Dürüst et al. (2011), they used protamine, poly-L-lysine and poly-L-argine reagents for carrageenan detection. In their study, they also produce a low LOD, but the reagent used is quite costly. Although our work can detect higher concentration of carrageenan, but basically this approach is cheap as compared to Dürüst et al. (2011) where they use synthetic amino acid such as poly-L-lysine and poly-L-argine. In addition, our work can be considered involved a very simple approach. In the study by Bartlová et al. (2021), they use titration and the determination process involved stirring.

TABLE 4. Analytical performance of this work with previous spectrophotometric study for carrageenan detection

Reagent	Linear range (ppm)	LOD (ppm)	Reference
Methylene blue	70.0-100.0	38.7	This work
New methylene blue	7.0-100.0	7.0	Bartlová et al. (2021)
Protamine, poly-L-lysine and poly-L-argine	1.0-40.0	1.0	Dürüst et al. (2011)
Methylene blue and toluidine blue	5.5-44.1	16.6	Ziółkowska et al. (2017)

Conclusions

A spectrophotometric detection of carrageenan based on MB dye was developed. Based on the results, it shows that the chemical interaction of carrageenan and MB will produce a carrageenan-MB complex that will shows two prominent peaks at 565 nm and 664 nm. The complex will undergo a color change from light blue to bluish purple. In addition, the absorption spectrum of the carrageenan-MB complex illustrates a hypsochromic shift from 664 nm to 565 nm with an isosbestic point at 588 nm. The carrageenan-MB complex shows a good reproducibility with RSD value of 1.64-1.94%. Based on the t-test, sucrose and glucose are considered not interfere with the detection of carrageenan when they were analysed separately. When the sucrose and glucose were each mix with carragenan, they are considered interfered with the intensity of the absorbance at 565 nm. As a conclusion, MB indicator has demonstrated acceptable selectivity towards carrageenan. No significant interference was found from sucrose and glucose species.

Acknowledgements

The authors wish to thank Makmal Kimia Instrumentasi and Makmal Mikrobiologi Universiti Teknologi MARA Cawangan Negeri Sembilan for the services and facilities to carry out the work.

References

Alsubaie, N., Alshamrani, R., Domyati, D., Alahmadi, N. & Bannani, F. 2021. Methylene blue dye adsorption onto polyoxometalate ionic liquid supported on bentonite: Kinetic, equilibrium and thermodynamic Studies. *Open Journal of Physical Chemistry* 11(02): 106-127.

Anderson, L., Wittkopp, S.M., Painter, C.J., Liegel, J.J., Schreiner, R., Bell, J.A. & Shakhashiri, B.Z. 2012. What is happening when the blue bottle bleaches: An investigation of the methylene blue-catalyzed air oxidation of glucose. *Journal of Chemical Education* 89(11): 1425-1431.

Bartlová, M., Ziółkowska, D., Pospiech, M., Shyichuk, A. & Tremlová, B. 2021. Determination of carrageenan in jellies with new methylene blue dye using spectrophotometry, smartphone-based colorimetry and spectrophotometric titration. *Food Science and Technology* 41: 81-90.

Bhanvase, B. & Barai, D. 2021. Stability of nanofluids. *Nanofluids for Heat and Mass Transfer.* Massachusetts: Academic Press. pp. 69-97.

Chen, X., Zhang, Z., Yang, H., Qiu, P., Wang, H., Wang, F., Zhao, Q., Fang, J. & Nie, J. 2020. Consumption of ultra-processed foods and health outcomes: A systematic review of epidemiological studies. *Nutrition Journal* 19(1): 1-10.

Dürüst, N., Meyerhoff, M.E., Ünal, N. & Naç, S. 2011. Spectrophotometric determination of various polyanions with polymeric film optodes using microtiter plate reader. *Analytica Chimica Acta* 699(1): 107-112.

Goeff, D. & Guo, Q. 2019. Food structure development: The interplay between processing routes and formulation elements. *Part A Food Structure Development: The Interplay Between Processing Routes and Formulation Elements*. pp. 1-28.

Haldar, K. & Chakraborty, S. 2019. Investigation of chemical reaction during sodium alginate drop impact on calcium chloride film. *Physics of Fluids* 31(7): 072102.

Hemdan, S.S., Jebaly, A.M.A. & Ali, F.K. 2019. Importance of isosbestic point in spectroscopy: A review 62(1): 1-21.

Herfurth, J. & Ulrich, J. 2017. Analysis of hydrocolloids in crystalline material. *Chemical Engineering and Technology* 40(7): 1261-1267.

Hotchkiss, S., Brooks, M., Campbell, R., Philp, K. & Trius, A. 2018. The use of carrageenan in food. In *Protests and Riots Past: Present and Future Perspectives,* edited by Pichette, A. pp. 47-75.

Khan, I., Saeed, K., Zekker, I., Zhang, B., Hendi, A.H., Ahmad, A., Ahmad, S., Zada, N., Ahmad, H., Shah, L.A., Shah, T. & Khan, I. 2022. Review on methylene blue: Its properties, uses, toxicity and photodegradation. *Water* 14(2): 242.

Król, Z., Malik, M., Marycz, K. & Jarmoluk, A. 2016. Characteristic of gelatine, carrageenan and sodium alginate hydrosols treated by direct electric current. *Polymers* 8(8): 275.

Lapwanit, S., Sooksimuang, T. & Trakulsujaritchok, T. 2018. Adsorptive removal of cationic methylene blue dye by kappa-carrageenan/poly(glycidyl methacrylate) hydrogel beads: Preparation and characterization. *Journal of Environmental Chemical Engineering* 6(5): 6221-6230.

Lee, K.Y. & Mooney, D.J. 2012. Alginate: Properties and biomedical applications. *Progress in* Polymer *Science* 37(1): 106-126.

Ling, Y.P. & Heng, L.Y. 2014. Reflectance based sensor for carrageenan utilizing methylene blue embedded acrylic microspheres. *Sensors and Actuators, B: Chemical* 192: 247-252.

Lipatova, I.M., Makarova, L.I. & Mezina, E.A. 2016. A spectrophotometric study of the compoundation between methylene blue dye and sodium alginate. *Russian Journal* of *General Chemistry* 86(9): 2226-2231.

Liu, F., Hou, P., Zhang, H., Tang, Q., Xue, C. & Li, R.W. 2021. Food-grade carrageenans and their implications in health and disease. *Comprehensive Reviews in Food Science and Food Safety* 20(4): 3918-3936.

Luxminarayan, L., Neha, S., Amit, V. & Khinchi, M.P. 2017. A review on chromatography techniques. *Asian Journal of Pharmaceutical Research and Development* 5(2): 1-8.

Makhado, E., Pandey, S., Modibane, K.D., Kang, M. & Hato, M.J. 2020. Sequestration of methylene blue dye using sodium alginate poly(acrylic acid)@ZnO hydrogel nanocomposite: Kinetic, isotherm and thermodynamic investigations. *International Journal of Biological Macromolecules* 162: 60-73.

Sacks, G., Riesenberg, D., Mialon, M., Dean, S. & Cameron, A.J. 2020. The characteristics and extent of food industry involvement in peer-reviewed research articles from 10 leading nutrition related journals in 2018. *PLoS ONE* 15: 1-15.

Sun, Y., Zhu, X., Shen, X. & Wang, W. 2021. Determination of carrageenan in livestock and poultry meat by ultrahigh-performance liquid chromatography-tandem mass spectrometry. *International Journal of Analytical Chemistry* 2021: 5277453.

Taha, A.M., Said, R.A.M., Mousa, I.S. & Elsayed, T.M. 2022. Simultaneous determination of ofloxacin and bromfenac in combined dosage form using four different spectrophotometric methods. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 273: 121066.

Taylor, J.K. 1983. Validation of analytical methods. *Analytical Chemistry* 55(6): 600A-608A.

Tobacman, J.K., Bhattacharya, S., Borthakur, A. & Dudeja, P.K. 2008. The carrageenan diet: Not recommended. *Science* 321(5892): 1040-1041.

Wan Khalid, W.E.F., Mat Arip, M.N., Jasmani, L. & Heng, L.Y. 2019. A new sensor for methyl paraben using an electrode made of a cellulose nanocrystal–reduced graphene oxide nanocomposite. *Sensors* 19(12): 2726.

Younes, M., Aggett, P., Aguilar, F., Crebelli, R., Filipič, M., Frutos, M.J., Galtier, P., Gott, D., Gundert-Remy, U., Kuhnle, G.G., Lambré, C., Leblanc, J-C., Lillegaard, I.T., Moldeus, P., Mortensen, A., Oskarsson, A., Stankovic, I., Waalkens-Berendsen, I., Woutersen, R.A., Wright, M., Brimer, L., Lindtner, O., Mosesso, P., Christodoulidou, A., Ioannidou, S., Lodi, F. & Dusemund, B. 2018. Re-evaluation of carrageenan (E 407) and processed *Eucheuma* seaweed (E 407a) as food additives. *EFSA Journal* 16(4): e05238.

Ziółkowska, D., Kaniewska, A., Lamkiewicz, J. & Shyichuk, A. 2017. Determination of carrageenan by means of photometric titration with methylene blue and toluidine blue dyes. *Carbohydrate Polymers* 165: 1-6.

*Corresponding author; email: wan_elina@uitm.edu.my