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Four Injection–Single Detector Merging Zone Continues Flow Injection Analysis for Determination of Ascorbic Acid in Bell and Chili Peppers

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FOUR INJECTION–SINGLE DETECTOR MERGING ZONE CONTINUES FLOW INJECTION ANALYSIS FOR DETERMINATION OF ASCORBIC ACID IN BELL AND CHILI PEPPERS

Abstract: Vitamin C can improve the immune system and cause chronic inflammatory diseases. A significant proportion of ascorbic acid can be obtained from fruits, vegetables, and food products. The selection of bell and chili pepper samples was made according to increasing use at the present time due to Coronavirus in 2019 and 2020. This paper deals with a merging zone (MZ based on four-hand mead injection/single detection using a spectrophotometer. Merging zone (MZ) systems resulting from different reactions via flow injection analysis (FIJ) would be beneficial for identifying the simultaneous reaction faster and at a lower cost. Specifically, the MZ area and ferroin complex resulting from the reaction between Fe (III), ascorbic acid (AA), and 1,10-Phenanthroline under acetic acid conditions. The system of the MZ system was modelled under continuous FIJ and determination of AA. The method performed AA at a range of (1□10-3 - 32□10-3□L), with a correlation coefficient of 0.9822 and DL 12.5ng. The obtained results from bell and chili peppers show excellent agreement with the results from the SCI Ό analysis.

Keywords: Peppers, Ascorbic acid, merging zone, flow injection, SCI Ό analysis

Introduction

Presently, ascorbic acid (vitamin C) can be used to improve the immune system and chronic inflammatory diseases (Anitra C. Carr 2017; Sorice *et al.* 2014). A significant proportion of ascorbic acid can be obtained from fruits, vegetables, and food products(Maki *et al*., 2011; Id *et al*., 2018). Flow injection analysis offers high flexibility regarding the utilisation of micro quantities of materials and low cost (Al-badri 2007; Shakir and Al-badri 2008b; Al-badri and Al-bayati 2014). Consequently, 100 references are offered to determine ascorbic acid using flow injection analysis with different detection techniques (Yebra-Biurru 2000). Despite this, few studies (Shakir and Al-badri 2008a; Muhammad 2019) have indicated that the merging zone system can be based on the number of injection units, not only by one carrier and one injection but also by one carrier and double injection. For instance, (Shakir and Al-badri 2008a) reported that the carrier is an orthophosphate ion, and ascorbic acid and molybdenum are injected to synthesise the molybdenum blue complex. Therefore, the merging zone continued flow injection analysis for the determination of ascorbic acid.

To study the merging zone in the determination of ascorbic acid during flow injection analysis, it is important to evaluate the concentration of ascorbic acid using more than three solutions and spectrophotometry. A range of studies (Fadhel 2012; Revanasiddappa and Veena 2008; Janićijević 2012; Permanganate 2018b; Al-anbakey 2018; Silver *et al.* 1986; Desai 2019; Sultan and Hassan 2009) related to the determinations of ascorbic acid used spectrophotometer as a detector. These studies report that the first step is based on the oxidation of ascorbic acid into dehydroascorbic acid using different materials. For example, bromine water (Janićijević 2012; Desai 2019), potassium permanganate (Sultan and Hassan 2009; Permanganate 2018b; Fadhel 2012), dichromate (Fadhel

2012)(Revanasiddappa and Veena 2008), Se(IV) in an acid medium (HCl 2M/1ml) (Revanasiddappa and Veena 2008), and mixing of [FeNH4(SO4)2. 12H2O]and K3Fe(CN)6 around 2.5ml (100 μg/mL) and 3ml(100 μg/mL), respectively, in an acid solution of HCl-pH=4 (Al-anbakey 2018), and by mixing FeCl3 with methanol and low acid HCl (Silver *et al.* 1986). Therefore, the oxidation of ascorbic acid is a complex problem. The core objective of this study was to investigate and explain the merging zone of the ascorbic acid reaction. Ferric iron and 1,10-Phenanthroline (Zhu *et al.* 2018) under acetic acid conditions (Rubiales *et al.* 2018) were selected for this investigation. Then, flow injection analysis modelling with a hand-mead spectrophotometer was carried out to explain the ascorbic acid content in bell and chili peppers and pharmaceuticals through a merging zone system.

Material and Methodology

Material

1. Ascorbic acid Samples;

In this study, three vitamin C samples were taken as different types of peppers (food) as models as well as pharmaceutical models (models locally manufactured in the Iraqi market, Samarra Factory). These samples were selected according to increasing use at the present time due to COVID-19 (Name *et al.* 2020; Shakoor *et al.* 2021). The vitamin C samples of bell and chili papers change the paper in juice and then filter to obtain pure liquid (Nerdy 2018; Permanganate 2018a). Meanwhile, the vitamin C samples from the pharmaceutical preparation procedure entail two stages: first, the weight of each tablet. The second was ground to a fine powder (Sud and Kamath 2013). In order to prepare the standard solution 1000 ppm by dissolve 0.1 g of ascorbic acid, completed in volumetric flask up to 1000 mL with ultrapure deionised water (18.2 MΩcm at 25°C) (Sud and Kamath 2013). The application solution and addition method (Steliopoulos 2015) were used in the analysis (to determine the concentration of the samples). The standard solution was added in different orders; for instance (0,3,5,7,9, and 11mL) were added to six 25mL volumetric flasks, then each one was filled and mixed well with 5mL of unknown sample concentration and after that, was completed with ultrapure deionised water (18.2 MΩcm at 25°C)

2. Fe (III) solution. Prepared from $Fe(NO_3)_3.9H_2O$ by dissolveing 3.620g in 83.6 ml of with concentration completed up to $Fe(NO_3)_3.9H_2O$ 500 mL with ultrapure deionised water (18.2MΩcm at 25°C);

3. 1, 10-Phenanthroline. Prepare 1.515×10^{-2} by dissolving 0.75g in deionised water, then transferred to volumetric flasks and completed up to 250 mL with ultrapure deionised water (18.2MΩcm at 25°C) (Muhammad 2019; Esma 2005);

4. Acetic acid. Prepare $(2.8Mol.L^{-1})$2.8 Mol.L^{-1} from concentration (density1.05 g.cm-3 with 99.5%).

Methodology

1. Experimental set-up;

A spectrophotometer was used to investigate the best spectral scanning complex(Ali *et al.*, 2015), which is the absorbance and wavelength (nm) of the samples. The spectral scanning complex of dehydroascorbic acid is shown in Figure (1). The curves in the figures clearly show that the absorbance of the complex started to decrease as the wavelength increased. λ=510nm; therefore, the λ flow injection analysis in this study was 510nm. The obtained results are in accordance with previous studies (Vakh *et al.* 2017; Güçlü *et al.* 2005; Ali *et al.* 2015) that have wavelength λ =510nm.

2. Merging zone via flow injection analysis experiments;

3. A hand-mead injection/single detection method was employed for the MZ-FIJ experiments. The experimental system consisted of four injections/single detections, three-line carrier samples and reagents with peristaltic pumps, four mixing coils, and detesters, as depicted in Figure 2;

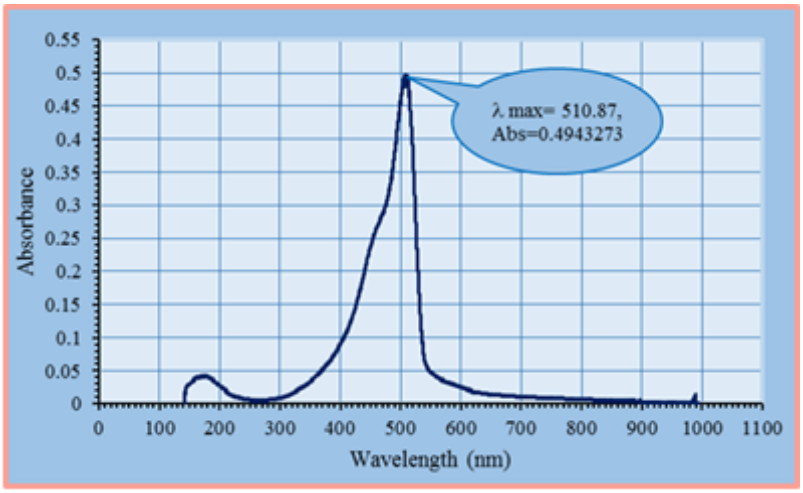

Figure 1. The wavelength (nm) of dehydroascorbic acid complex.

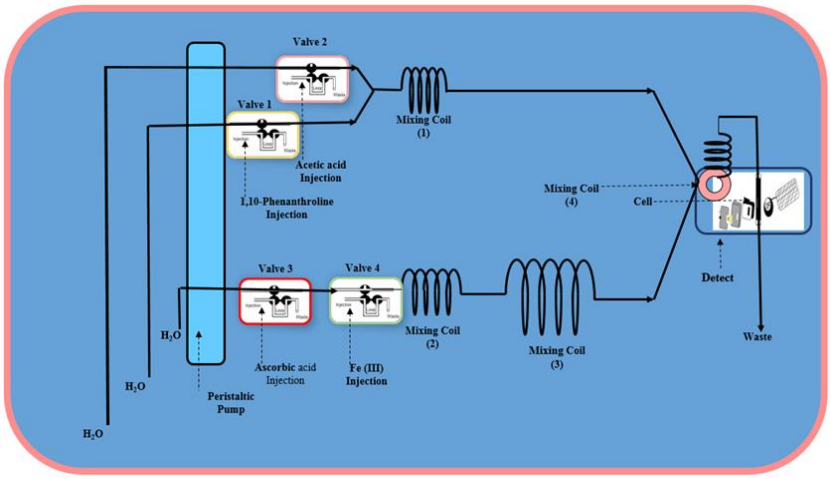

Figure 2. Schematic diagram of the merging zone via flow injection analysis experiments.

A set of five experiments was designed to optimise the best optimisation of chemical and physical parameters. These experiments are in line with those experiences by (Makahleh and Saad 2011)(Kuznetsov 2018). Experiments were performed to further understand the merging zone technique during flow injection analysis of the dehydroascorbic acid complex.

Each experimental test was conducted using ultrapure deionised water (18.2 MΩcm at 25°C) as a carrier solution. In these experiments, each analysis was performed in triplicate, and the average of the results was calculated. The details of each experiment are presented in Table (1).

In these experiments, the reaction time is an important parameter, which is the time between the injection of the sample and the end of the response signal of the sample. The merging zone via the flow injection analysis experiment was calibrated using a dehydroascorbic acid complex.

Based on the results of the optimisation of chemical and physical parameters and ascorbic acid in the range of (1×10^{-3} - 32×10^{-3}µL), in order to determine the correlation coefficient and detection limit. The method was applied to two types of bell and chili peppers as food samples, and pharmaceuticals, model vitamin C samples using the standard addition method, and then the obtained results were compared with other analytical results from SCI˙O analysis using statistical methods.

Table 1: The details of each experiment to investigate the optimisation of chemical and physical (Flow injection operational) parameters.

Exp. N	Investigated the effect of	Parameters use
1	Sample volume	Four injection volume, 50,100,150,200 µL.
2	Concertation of ascorbic acid, and Fe(III)	Ascorbic acid (AA) range 2.5-15µg/L×10^3, and Fe (III) range2-20 µg/L.
3	Total flow rate	Total flow rate range1-8mL.min-^{1}.
4	Reagents volume	Total injection volume rang 10-50 µL.
5	Mixing coil length	For each coil range 20-120cm.
6	Interactions and Interferences	Pharmaceutical substances that may be present with the vitamin, such as vitamins, metal ions, reductive acids, amino acids, and sugars.

4. The statistical method;

Two types of statistical analysis, the T-test and F-tests (Miller 2018), were used to compare the vitamin C content in food samples and pharms samples. The methods were based on the mean and standard deviation of the results obtained from the experimental test with other analytical results from the SCI Ό analysis.

Results and Discussion

5. Optimisation of physical (flow injection operational) parameters;

6. The obtained results showed improvement in terms of sample volume (100 µL), reagent volume (30 µL), and lengths of mixing coil (50, 50, 70, and 40 for 1, 2, 3, and 4, respectively) comparing to other studies (Muhammad 2019; Maki *et al.* 2011). Noteworthy, chosen results consider the decrease in dispersion with an increase in the absorbance and repeatability of the results and also the peak shape (Trenggamayunelgi *et al.*, 2019). The absorbance values varied significantly among the total flow rates. The best absorbance was chosen as a parameter for the successive experiment 6 ml.min-^{1} as depicted in Figure 3.

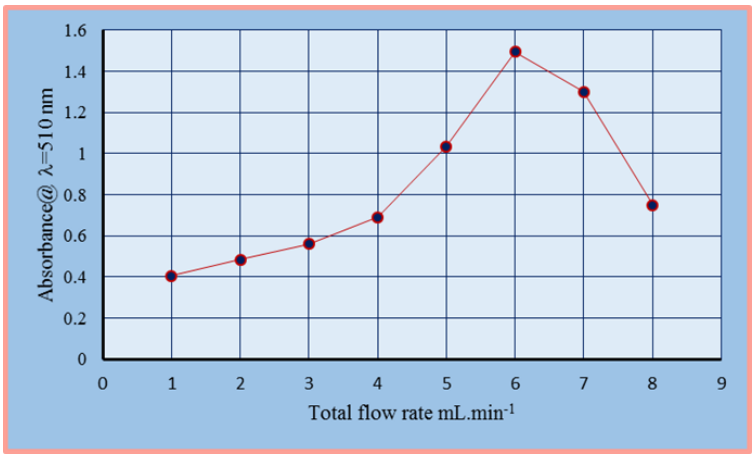

Figure 3. The graph of absorbance@ λ=510 nm, and total flow rate mL.min^{-1}

1. Optimisation of chemical (flow injection operational) parameters;

1.1 Optimisation of the concentration of Fe (III);

The concentration of Fe (III) was performed to assess the effect of volume of Fe (III) with different concentrations range2-20 µg/L and also, the different concentration range of ascorbic acid (AA) range 2.5-15µg/L×10^3. The results concentration shifts are observed as shown in Figure 4; this is caused by an increase in the concentration of Fe (III), with increased volume. As it is known, the result of the reaction increased with vitamin C (Al-anbakey 2018; Ali *et al.* 2015; Güçlü *et al.* 2005; Abdullah 2012).

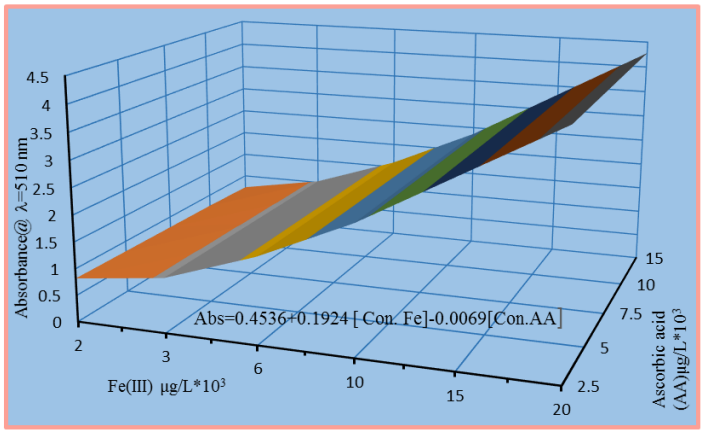

Figure 4. The graph studied the volume of Fe (III) with different concertations.

1.2 Optimisation concentration of 1,10-Phenanthroline;

1, 10-Phenanthroline as an indicator for Fe (III) - $phen_3$ complex was optimised to assay the influence of increasing concentration vitamin C (Lau and Luk 1987; Al-anbakey 2018). Five series total volume (10, 20, 30, 40 and 50) µL of 1,10-Phenanthroline concentrations 1.515×10^{-2} µg/L and acetic acid 2.8 Mol.L^{-1} with concentrations range 2.5-7. 5µg/L×10^3 of ascorbic acid were prepared, as can be seen in Figure 5. Further, the total volume of 1, 10-Phenanthroline and acetic acid was chosen by the highest absorbance @ λ=510 and good shape peak to measure the linearity of ascorbic acid concentration.

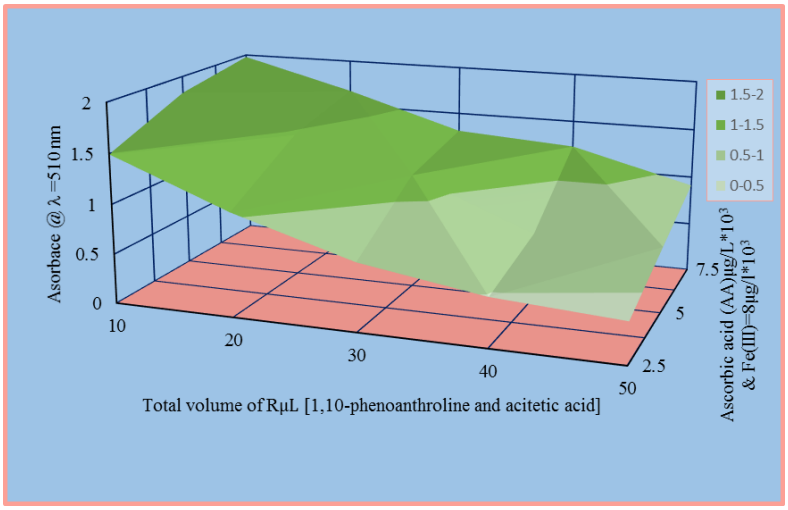

Figure 5. The graph studied the volume and concertation of 1, 10-Phenanthroline.

1.3 Linearity measurement of ascorbic acid Concentration;

The flow injection merging zone (MZ based on four-hand mead injection/single detection using a spectrophotometer) processes were based on the obtained experimental data outline above (i.e. operational conditions such as sample loop of 100 µL, the flow rate of 5.8 mL/min, and length of mixing coil of 50 cm, and also chemical conditions for concentration Fe (III) 8µg/L×10^3 and concentration of 1,10-Phenanthroline concentrations 1.515×10^{-2} µg/L and acetic acid 2.8 Mol.L^{-1} with the total volume of 1, 10-Phenanthroline and acetic 10 µL). This method gives results to be linear. The results of the linearity ascorbic acid range (1×10^{-3} to 32×10^{-3}µL) are depicted in Figure 6 with a correlation coefficient of 0.9822 and DL of 12.5ng.

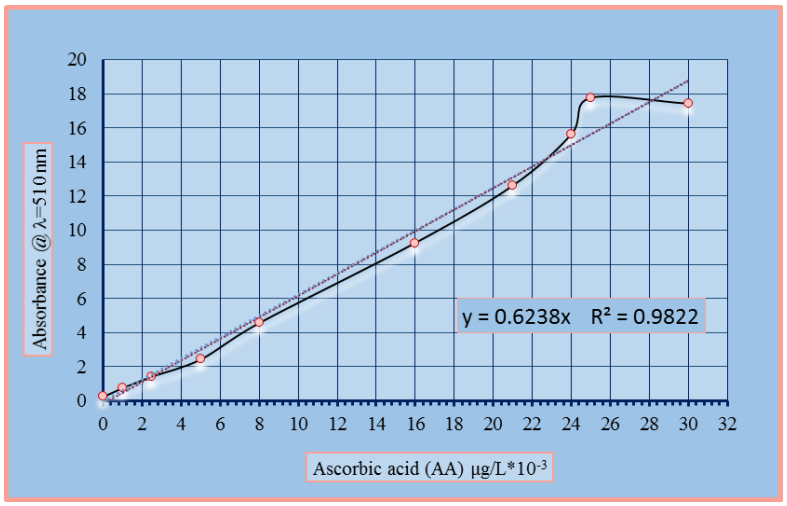

Figure 6. The calibration graphs of vitamin C.

Application

Two established samples were used in order to:

7. Demonstrate the efficiency of the proposed system;
8. Determining the level of vitamin C in pharmaceuticals and pepper.

These will provide a good opportunity to replace medicinal tablets containing vitamin C with peppers. The results obtained from the MZ-FIJ experiments were in good agreement with those obtained by the SCIˑO analysis of pharmaceutical samples, as shown in Table2.

Table 2: Compared the content of vitamin C by use MZ-FIJ experiments and SCIˑO analysis.

Samples	MZ-FIJ experiments	SCIˑO analysis	t-value	f-value
1 Samarra (500mg)	4.02	4.06	1.41	2.17
2 Samarra (500mg)	3.98	3.89	1.13	1.35
3 Samarra (500mg)	6.2	6.1	1.57	3.18

There was no significant difference between the MZ-FIJ experiments and the SCIˑO analysis based on the results of the t-test and F-test. The calculation of three pharmaceutical samples of t value and f value (1.41, 1.13, 1.57 and 2.17, 1.35, 3.18 respectively) confirm no significant difference in accuracy and precision between methods. Therefore, MZ-FIJ experiments were performed on the four pepper samples. The result showed that the average levels of vitamin C (mg/100g) were 86.3, 18.5, 135.6, and 195.9 for chili, green bell, orange bell, and red bell, respectively, as illustrated in Figure7.

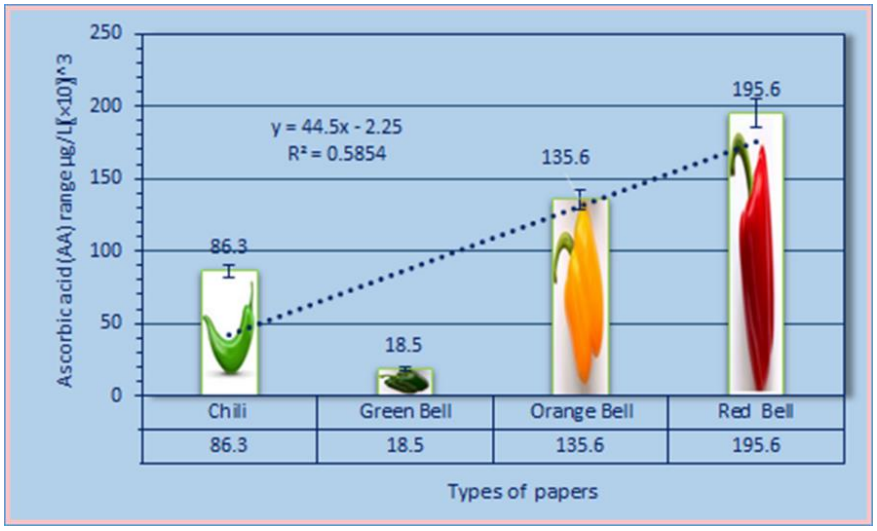

Figure 7. The graph explains the concertation of vitamin C in four types of peppers.

Conclusion

This study provided a flow injection analysis model that offers high flexibility regarding the utilisation of micro quantities of materials and low cost. The level of vitamin C in pepper ranges from high to low as the red bell, orange bell, green bell, and chili green, respectively. The results showed that the level of vitamin C in the red bell pepper is equivalent to the dose of vitamin C that the body needs every day in order to prevent the hyperactivation of immune cells (Shakoor *et al.* 2021) with COVID-19 and 20. Therefore, one red pepper is a day-resistant virus every day.

References

Abdullah, A.M., 2012. Determination of Ascorbic Acid (Vitamin C) by Direct Injection Enthalpimetry (DIE) Technique. *Ibn Al-Haitham Journal for Pure and Applied Science No.* 25(1).

Al-anbakey, A.M., 2018. Sensitive Spectrophotometric Method For Determination Of Vitamins (C And E). *International Journal of Pharmaceutical Sciences and Research* 9(8), pp. 3373–3377.

Al-badri, S.B., 2007. Newly Developed System For The Analysis Of Micro Quantities Of Molybdenum (VI) Using Flow Injection Analysis. *Baghdad Science Journal* 4(2), pp. 180–190.

Al-badri, S.B. and Al-bayati, Y.K., 2014. A new system of technology integration in the areas of analysis and continuous injection Jeriana use spectrometer plant locally to estimate the drug paracetamol. *Al-Nahrain Journal of Science* 17(April), pp. 1–9.

Ali, N.W. et al., 2015. Novel spectrophotometric methods for determination of Salicylamide and Ascorbic acid in their binary mixture. *Journal of the Chemical Society of Pakistan* 36(6), pp. 988–995.

Anitra C. Carr, S.M., 2017. Vitamin C and Immune Function. *Nutrients* 9(1211), pp. 1–25.

Desai, A.P., 2019. UV Spectroscopic Method for Determination of Vitamin C (Ascorbic Acid) Content in Different Fruits in South Gujarat Region. *International Journal of Environmental Sciences & Natural Resources* 22(2), pp. 41–44. Available at: https://juniperpublishers.com/ijesnr/IJESNR.MS.ID.556056.php.

Esma, T., 2005. neocuproine reagent in beverages and pharmaceuticals Spectrophotometric determination of ascorbic acid using copper (II)– neocuproine reagent in beverages and pharmaceuticals. *Talanta* 65(April), pp. 1226–1232.

Fadhel, D.H., 2012. Spectrophotometric Determination of Ascorbic Acid in Aqueous Solutions. *Journal of Al-Nahrain University* 15(3), pp. 88–94.

Güçlü, K. et al., 2005. Spectrophotometric determination of ascorbic acid using copper(II)-neocuproine reagent in beverages and pharmaceuticals. *Talanta* 65(5), pp. 1226–1232.

Id, Y.Z. *et al.*, 2018. A Review of the Extraction and Determination Methods of Thirteen Essential Vitamins to the Human Body : An Update from 2010. *Molecules* 23(1484), pp. 1–25.

Janićijević, Č., 2012. Spectrophotometric analysis of total ascorbic acid content in various fruits and vegetables. *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina* 38, pp. 39–42.

Kuznetsov, V. V., 2018. Flow injection analysis: An approach via linear non-equilibrium thermodynamics. *Talanta* 187(May), pp. 237–245. Available at: https://doi.org/10.1016/j.talanta.2018.04.048.

Lau, O. and Luk, S., 1987. Spectrophotometric Determination of Ascorbic Acid in Canned Fruit Juices, Cordials, and Soft Drinks with Iron (III) and 1, 10-Phenanthroline as Reagents. *Journal of Association of Official Analytical Chemists* 70(3), p. 10.

Makahleh, A. and Saad, B., 2011. Flow injection determination of free fatty acids in vegetable oils using capacitively coupled contactless conductivity detection. *Analytica Chimica Acta* 694(1–2), pp. 90–94.

Maki, T. *et al.*, 2011. Flow injection fluorometric determination of ascorbic acid using perylenebisimide-linked nitroxide. *Talanta* 85(4), pp. 1730–1733. Available at: http://dx.doi.org/10.1016/j.talanta.2011.06.054.

Miller, J.M.J.C., 2018. *Statistics and chemometrics for analytical chemistry*.

Muhammad, H.Y., 2019. Double injection–single detector flow injection spectrophotometric method for simultaneous determination of ascorbic acid and paracetamol in pharmaceutical formulations. *Iranian Chemical Society ISSN* (1735–207X J).

Name, J.J. *et al.*, 2020. Zinc, Vitamin D and Vitamin C: Perspectives for COVID-19 With a Focus on Physical Tissue Barrier Integrity. *Frontiers in Nutrition* 7(March), pp. 1–14.

Nerdy, N., 2018. Determination of Vitamin C in Various Colours of Bell Pepper (Capsicum annuum L.) by Titration Method. *ALCHEMY Jurnal Penelitian Kimia* 14(1), p. 164.

Permanganate, U.P., 2018a. Spectrophotometric Analysis of Vitamin C in Different Matrices. *European International Journal of Science and Technology* 7(February), pp. 1–16. Available at: https://www.researchgate.net/publication/322888847%0ASPECTROPHOTOMETRIC.

Permanganate, U.P., 2018b. Spectrophotometric analysis of vitamin c in different matrices spectrophotometric analysis of vitamin c in different. *European International Journal of Science and Technology* 7(2304–9693), pp. 70–84.

Revanasiddappa, H.D. and Veena, M.A., 2008. Sensitive Spectrophotometric Methods for the Determination of Ascorbic Acid. *E-Journal of Chemistry* 5(1), pp. 10–15.

Rubiales, D. *et al.*, 2018. Editorial: Advances in legume research. *Frontiers in Plant Science* 9, p. 246.

Shakir, I.M. and Al-badri, S.B., 2008a. Determination of Ascorbic acid Via Semi automated On–line merging zone technique. *National Journal of Chemistry*, 29(1), pp. 68–75.

Shakir, I.M. and Al-badri, S.B., 2008b. Flow injection analysis photometric determination of iron (iii), using new homemade micro photometer. *Iraqi Journal of Science* 1(49), pp. 18–28.

Shakoor, H. *et al.*, 2021. Immune-boosting role of vitamins D, C, E, zinc, selenium and omega-3 fatty acids: Could they help against COVID-19? *Maturitas* 143, pp. 1–9.

Silver, J. *et al.* 1986. Studies on the reactions of ferric iron with ascorbic acid. A study of solution chemistry using Mössbauer spectroscopy and stopped-flow techniques. *Inorganica &mica Acta, 12.5* 125(October), pp. 97–106.

Sorice, A. *et al.*, 2014. Ascorbic Acid : Its Role in Immune System and Chronic Inflammation Diseases Ascorbic Acid : Its Role in Immune System and Chronic Inflammation Diseases. *Mini-Reviews in Medicinal Chemistry* 14(5), pp. 1–9.

Steliopoulos, P., 2015. Extension of the standard addition method by blank addition. *MethodsX* 2, pp. 353–359. Available at: http://dx.doi.org/10.1016/j.mex.2015.09.001.

Sud, S. and Kamath, A., 2013. Methods of Size Reduction and Factors Affecting Size Reduction in Pharmaceutics. *International Research Journal of Pharmacy* 4(8), pp. 57–64.

Sultan, S.M. and Hassan, Y.A.M., 2009. Sequential Injection Titrimetric Analysis of Vitamin C in Drug Formulation Using Potassium Permanganate. *Journal of Flow Injection Analysis* 26(1), p. 53.

Trenggamayunelgi, F.S. *et al.*, 2019. Development of flow injection-spectrophotometry method for hydroquinone determination based on the formation of blue starch-iodine complex. *IOP Conference Series: Materials Science and Engineering* 546(3).

Vakh, C. *et al.*, 2017. Simultaneous determination of iron (II) and ascorbic acid in pharmaceuticals based on flow sandwich technique Journal of Pharmacological and Toxicological Methods Simultaneous determination of iron (II) and ascorbic acid in pharmaceuticals based on flow sandwich technique. *Journal of Pharmacological and Toxicological Methods* 73(October), pp. 56–62. Available at: http://dx.doi.org/10.1016/j.vascn.2015.03.006.

Yebra-Biurru, M., 2000. Review Flow injection determination methods of ascorbic acid. *Talanta* 52(3), pp. 367–383.

Zhu, J. *et al.*, 2018. Factors affecting the determination of iron species in the presence of ferric iron. *Applied Water Science* 8(8), pp. 1–4. Available at: https://doi.org/10.1007/s13201-018-0876-6.