Chemical Science International Journal

30(7): 15-23, 2021; Article no.CSIJ.71530
ISSN: 2456-706X
(Past name: American Chemical Science Journal, Past ISSN: 2249-0205)

Remediation of Metformin Hydrochloride in Aqueous Solution Using Locally Sourced Seaweed (*Fucus spiralis*) Through HPLC

J. I. Bungudu[1*] and Lorrie M. Murphy[2]

[1]Department of Pure and Industrial Chemistry, Faculty of Science, Federal University Birnin Kebbi, Kebbi State, Nigeria.
[2]Chemistry Unit, School of Natural sciences, Bangor University, Wales, United Kingdom.

Authors' contributions

This work was carried out in collaboration between both authors. Author JIB and wrote the first draft while author LLM performed proof reading and editing. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CSJI/2021/v30i730240
Editor(s):
(1) Thomas P. West, Texas A&M University, Texas.
Reviewers:
(1) GHALEM Said, Tlemcen University, Algeria.
(2) Melih ONAY, Van Yuzuncu Yil University, Turkey.
Complete Peer review History: https://www.sdiarticle4.com/review-history/71530

Original Research Article

Received 02 June 2021
Accepted 07 August 2021
Published 12 August 2021

ABSTRACT

Metformin hydrochloride is an anti-hyperglycaemic drug that is widely prescribed in the management of noninsulin diabetes mellitus (NIDDM). However, metformin does not undergo complete metabolism in the body thereby excreting significant amount through urine and eventual discharged into the water bodies. Therefore, this work investigates the possibility bio-sorption of metformin by *Fucus spiralis* seaweed. High performance liquid chromatography (HPLC) and FITR was used for quantifying metformin biosorption. The result shows that Fucus spiralis is a potential biosbent for metformin removal in aqueous solution. The highest removal was up to 74% at 50 µg/mL. It can be mentioned here that this study is the first of it kind in testing seaweed for metformin biosorption.
In conclusion, biomass *(Fucus spiralis*) was tested for its efficiency in metformin removal in aqueous solution. Adsorption studies revealed that *F. spiralis* can be used as potential adsorbent for metformin uptake. Very limited literature investigates the application of seaweeds species for pharmaceutical remediation. Remediation of waste and surface water using readily available

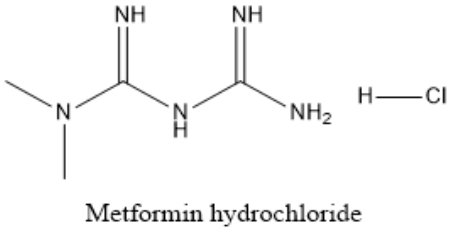
**Corresponding author: E-mail: jibungudu@gmail.com;*

adsorbent such as seaweed will be useful as it relates to human health and environmental contamination. HPLC was used in this study but other spectroscopic technique such as UV/vis could be explored to ascertain the optimized method. Further studies would be needed to test other algal species for metformin bio sorption.

Keywords: Metformin hydrochloride; Fucus spiralis; seaweed; HPLC and FTIRl.

1. INTRODUCTION

Recently, pharmaceuticals products that contribute to saving millions of lives has been reported to have a harmful effect on human and the environment as environmental contaminants, these contaminants including metformin has been reviewed [1]. Metformin (actually formulated as metformin hydrochloride) is N, N-dimethyl biguanide hydrochloride [2] and this drug is widely prescribed drug used in the treatment of patients with type 2 diabetes mellitus [3].



Metformin hydrochloride

Fig. 1. Structure of metformin hydrochloride

Metformin is among the most prescribed medication use across the UK and reaching many people [4]. Metformin hydrochloride is widely prescribed drug due to it oral anti-hyperglycaemic agent used in the management of noninsulin dependent diabetes mellitus (NIDDM). However, it must be noted that much of this drug does not undergo significant metabolism (>90%) [5] in the body and is excreted a such through the urine [6]. Research has shown how metformin breaks down under stress into a stable guanylurea (amidinourea and dicyandiamidine) that could potentially have an ecological impact on the environment [6]. This has prompted an international concern leading to many studies to test for metformin and other drugs as a potential new emerging pollutant in the waterways. Metformin has been detected in high concentrations in the surface waters in France [7] with range of 200 – 735 ng/L, United State of America 0.11 -0.15 µg/L [8], Netherlands (0.3 – 1.6 µg/L) [9], Belgium (0.6 ± 0.2µg/L) 9 and Germany (130 -1700 ng/L) [10]. Considering the above concern, therefore, there is need for an efficient and less costly system for removal of metformin from wastewater. In the review by Patel et al. a number of different methods such as photolysis [11], UV-degradation [12], reverse osmosis [13], adsorption [14] and nanofiltration [15] have been noted as used for the remediation from aqueous systems [1]. However, many of these methods were limited by toxic sludge removal, high operating and capital costs, incomplete removal, maintenance and skilled personal [1], while adsorption technologies appears to be low cost alternative, easily applicable in developing nations where is lack of advanced technology, capital and skilled manpower.

Searching peer reviewed papers indicated that no studies for metformin bio-sorption using seaweed have been published. However, some pharmaceuticals have been reported to be remediated by seaweeds. Two different pharmaceutical pollutants Venlafaxine (VLF) and Fluoxetine (FLX) was investigated using a brown seaweed *Bifurcaria bifurcate* for bio-sorption efficiency in an aqueous solution [16]. The adsorption studies shows that removal was efficient at lower pH values (5-10). The maximum adsorption capacities reported in those studies were 12 ± 3 and 22 ± 4 µmol/g for VLF and FLX respectively. Similar work also investigated the removal of propranolol using *Sargassum filipendula* alginate extract in an aqeous media [17]. This research reported 93% removal efficiency and therefore remaining *Sargassum filipendula* extract maybe a potential bio sorbent for propranolol environmental remediation. High performance liquid chromatography (HPLC) [18] and spectrophotometric techniques [19] have been previously successfully developed for analysing and evaluating metformin.

The major analytical techniques used in metformin analysis include NMR [20], HPLC [21], GC [22], PVC matrix membrane sensors [23], UV-Vis [24], capillary electrophoresis [25], potentiometric titrations etc.24 However, many of these methods are only used in human biological samples and in pharmaceutical for purity determination and many of them are either time consuming or very expensive to run large samples [19].

In this study we tried to explore the use of HPLC technique to test the possibility of using seaweeds in metformin removal from an aqueous solution. The work of Kar and Choundhury [18], et al. a HPLC method was adapted for this study.

2. MATERIAL AND METHODS

2.1 Instrumentation

Sample analysis was carried out on a Dionex 3000 HPLC column. The volume of organic phase (80%) and buffer solution (20%) was used to make the mobile phase. Also, a v/v 62:38 organic phase was made from the mixture of methanol: acetonitrile, respectively. Monopotassium phosphate solution (20mM) was used as buffer and the pH adjusted to 4.0 ± 0.05 using o- phosphoric acid. Before the samples was injected into the C18 column separation of components, the mobile phase was degassed and filtered. The room temperature of 23°C was used as experimental temperature and with mobile phase fixed flow of 1.0 mL/min. Sample injection was at 20 µL in triplicate using an auto sampler for solvent delay of 2 mins. The UV/Vis was set at 230 nm for analysis. HI 2211 benchtop pH meter pH was used for calibration.

2.2 Sample Preparation

Brown seaweeds *Fucus spiralis* used in this study were collected along the coastal area of north Wales beach near Llandudno location (53˚19´17.9" N 3˚51´01.3" W). The dried crude seaweed was digested using the procedure also in chapter 3. Initial mass of *ca.* 0.1 g and 0.5 g (accurately weighed) of the seaweeds (*Fucus spiralis* and *Ascophyllum nodosum)*, at 25°C, 400 rpm after which it was allowed for 24 hours contact time was used to test for 50 mg/L metformin hydrochloride bio-sorption in 15 ml tube using high performance liquid chromatography (HPLC).

Chemical reagents were all bought from Fischer Scientific or Sigma-Aldrich. All solvents were analytical grade. Pure metformin hydrochloride sample (98 %) for HPLC analysis was used without further purification**.**

2.3 Stock Solutions and Standards Preparation for *Fucus spiralis* Bio-Sorption of Metformin

Pure metformin hydrochloride was used to prepare the stock standards solution by dissolving 10 ± 0.3 mg metformin hydrochloride into volumetric flask using HPLC methanol grade. Different set of standards was prepared (10 µg/ml - 100 µg/ml) from 100 µg/ml stock solution. These known set of standards were used to calculate the unknown sample concentration using calibration curve. Standards solution were filtered using 0.2 µm membrane filter and injection was carried out in triplicate.

3. RESULTS AND DISCUSSION

In this analysis, a calibration curve is required from the statistical point of view. The method reported in the work of Kar and Choundhury, et al. [18] was adapted. The method was easy, simple, and reproducible when using HPLC for calibration curve. Curve fitting for the metformin standards concentration range 0 to 100 µg/ml (Fig. 2) was found to give R^2= 0.999 1 fitted with a linear regression.

This shows that the high value of the correlation coefficient and the value of Y- close to zero indicate good linearity of the calibration curve (Fig. 2) To calculate the detection and quantification limit, a regression analysis of the data for the blank samples (n=10) was conducted using the formulas (1) and (2).

LOD = 3.3 multiply by Standard deviation of the blank (1)

LOQ = 10 multiply by Standard deviation of the blank (2)

The values in Table 1 shows various LOD and LOQ of metformin from the published literature and the work in this thesis which differ from each other. The LOD and LOQ are in the range 0.05 – 1.00 µg/ml and 0.47 – 3.00 µg/ml respectively. Ref 27-29. Factors such as ground noise and analyte signals may contribute The different varriations in LOD and LOQ reported above may be attributed to various factors such background noise, analyte signal, column types, flow rate sample loading and detection [26].

There is no available literature to relate the higher retention time at lower concentrations but the retention time of the blank (methanol) solvent in methanol, acetonitrile and water mobile phase is found to be around 2.1 in this work i.e 0.0 µg/ml. Trouble shooting issues relating to column and instrument sensitivity has been associated with shifts in retention time among the

concentrations range [26]. To carry out further analysis to determine the possible metformin biosorption, test on metformin was conducted on both fresh samples containing only metformin (50 µg/ml) from 80% organic and 20% buffer (mobile phase), prepared within 2 hours and measured on same day and incubated sample of 50 µg/ml metformin with 0.1 g of *F. spiralis* and measured overnight to find if there was any absorption of the metformin during the incubation period. The two samples were run under same conditions as per instrument setting. According to the Fig. 3 below, the retention time and peak area in fresh metformin was observed at 2.305 min and 390.57 mAU*min respectively. In the work of [27], similar value of 2.18 retention time (min) for metformin were reported.

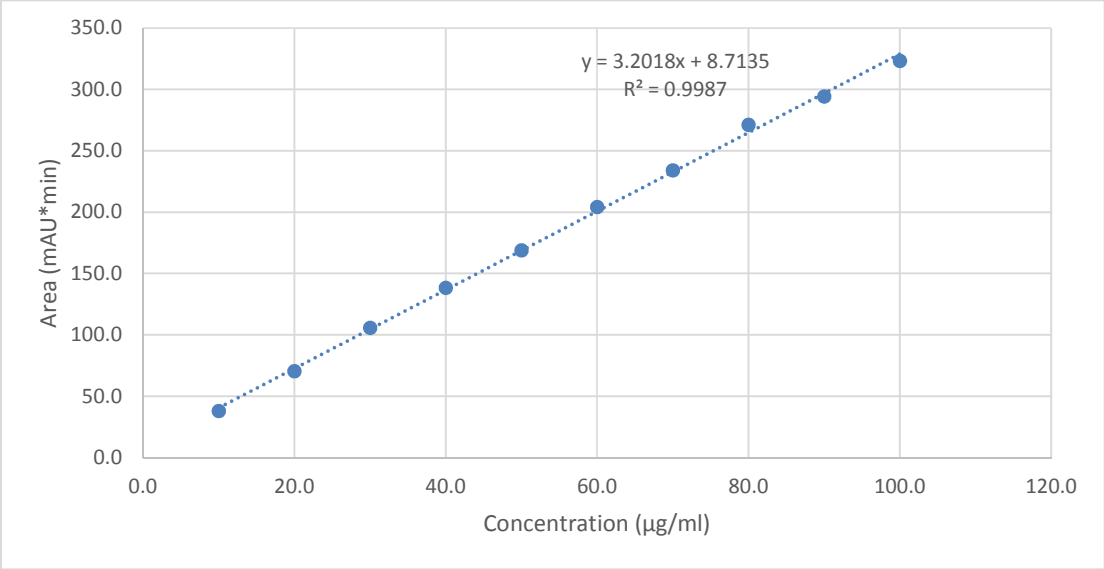


Fig. 2. Linear regression calibration graph produced using HPLC method (section 5.1) different concentrations of metformin hydrochloride (10-100 µg/ml

Table 1. LOD and LOQ of metformin

LOD (µg/ml)	LOQ (µg/ml)	References
0.80	2.45	[27]
0.05	0.47	[28]
1.00	3.00	[29]
0.32	0.96	Present study

Table 2. Standard solution raw data obtained from HPLC and calculated average of area and retention time

Conc.(µg/ml	Retention time (min) R= retention time				Area (mAU*min) A= Area			y
	R1	R2	R3	Average R	A1	A2	A3	Average A
10.0	4.5	4.5	4.6	4.5	37.1	37.5	39.3	38.0
20.0	4.6	4.6	4.6	4.6	70.7	70.4	70.1	70.4
30.0	4.6	4.6	4.7	4.6	106.0	105.6	105.7	105.7
40.0	4.7	4.7	4.7	4.7	138.9	138.2	137.8	138.3
50.0	4.7	4.7	4.8	4.7	170.2	169.5	166.8	168.9
60.0	4.8	4.8	4.8	4.8	205.0	204.1	203.4	204.2
70.0	4.8	4.8	4.8	4.8	234.6	233.9	233.5	234.0
80.0	4.8	4.8	4.8	4.8	271.9	271.1	270.4	271.1
90.0	4.8	4.8	4.8	4.8	294.7	294.2	293.9	294.2
100.0	4.8	4.8	4.8	4.8	323.0	323.3	323.3	323.2

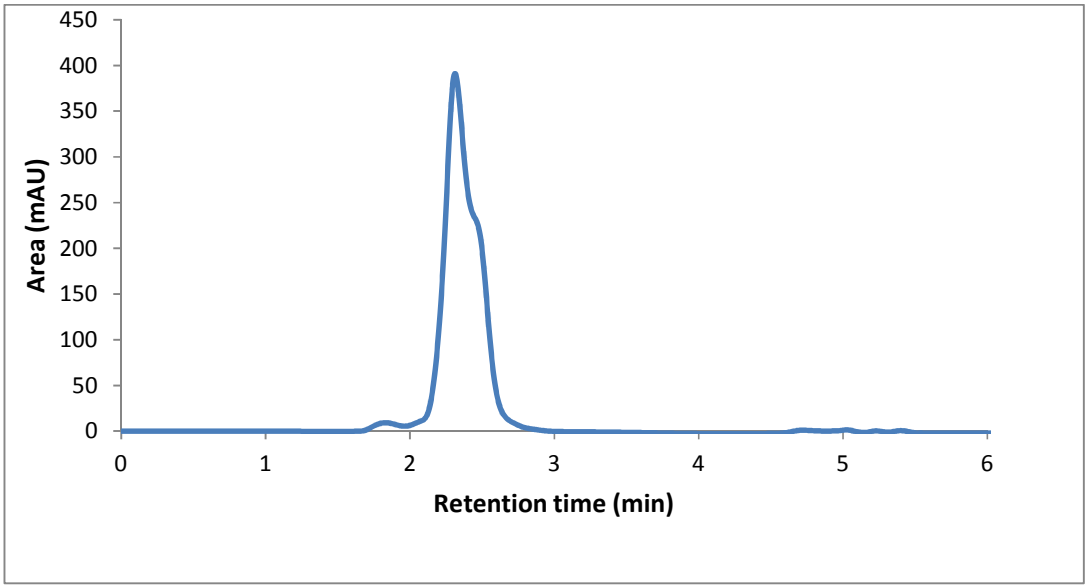


Fig. 3. 50 µg/ml of fresh metformin in 15 ml tube at 25°C for 24 hours contact time

It can also be seen in Fig. 4 that there is shift in both retention time and peak area for incubated metformin. The retention time was recorded at 2.135 min and peak area at 358.67 mAU*min. This variation maybe attributed to retention on ions exchangers is a function of charge and type of stationary phase, the type of concentration of ions in the mobile phase, the degree of ionization of the analyte as well as analyte molecular size [28]. Quantification to determine the concentration of metformin being absorbed by the sample was possible even though we have only one visible peak and two shoulder areas.

An additional experiment was conducted under the following conditions: concentration range 50 – 250 µg/ml of metformin (in triplicate), 0.1± 0.05 g of *F spiralis*, 0.05 L in 15 ml tube, at 25°C for 24 hours contact time to test for various possible of metformin absorption.

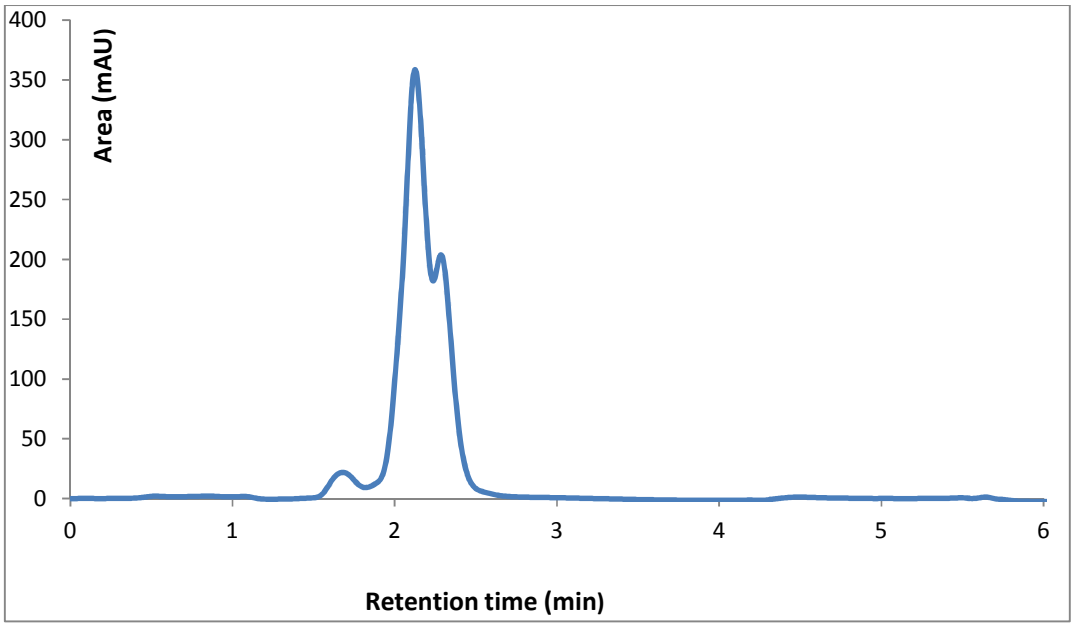


Fig. 4. 50 µg/ml metformin, 0.1 g *F. spiralis* in 15 ml tube at 25°C for 24 hours contact time

3.1 Metal Removal Efficiency

Biosorption capacity (q_e), the amount of metal adsorbed per gram of biosorbent, can be calculated at equilibrium in mg/g using the following equation.

$$Q_e = (C_o - C_e)V/m \quad (1)$$

Where

C_o is the intial concentration of metal ions in each solution (mg/L)

C_e is the concentration of metal ions in the solution (mg/L), V is the final volume of the solution (in litres) and m is the mass or weight of biosorbent measured in grams.

Moreover, metformin uptake can also be calculated in percentage as cited in chapter 4 using the following equation;

$$\% \text{ removal} = (C_o - C_e)/ C_o .100 \quad (2)$$

The highest value of biosorption capacity (q_e) of 103 ± 5 mg/g was observed in 150 µg/ml sample while the lowest was recorded with 70. 62 ± 3 mg/g of 50 µg/ml. Fig. 5 below shows the percentage removal of metformin for *Fucus spiralis* in which the highest removal was observed in sample 50 µg/ml with removal efficiency of 73.86 ± 2.66 % and the lowest removal was observed with sample 250 µg/ml of 42.68± 0.13 %.

The gradual increase in percentage removal from sample concentrations 250 – 50 µg/ml highlighted the possibility of *F. spiralis* reaching the peak of metformin removal with maximal removal at lower concentrations. At lower concentrations, the metformin tends to bind all the available active sites but as concentration increases the percentage removal became lower until all the active sites have been occupied by *F. spiralis*. In a similar work where the effect of initial ascorbic acid and lactose concentration was tested. The result shows that ascorbic acid removal was higher at lower concentrations [29]. *F. spiralis (*SF) removal efficiency in metformin uptake can be related to the work of Moogouei and Borghei, et al. [30] where a plant species including *Amaranthus retroflexus, Ricicnus communis, Brassica napus, Celosia cristata, Helianthus anuus and Phragmites australis* all shows potential removal capacity of metformin in solution of 20 and 50 mg/L of up to 69.53 ± 2.25 and 65.7 ± 1% respectively. In our research, different range of concentration were tested while varying concentration may influence the metformin uptake, the seaweeds has shown a potential in removing metformin from an aqueous solution at each sample concentration.

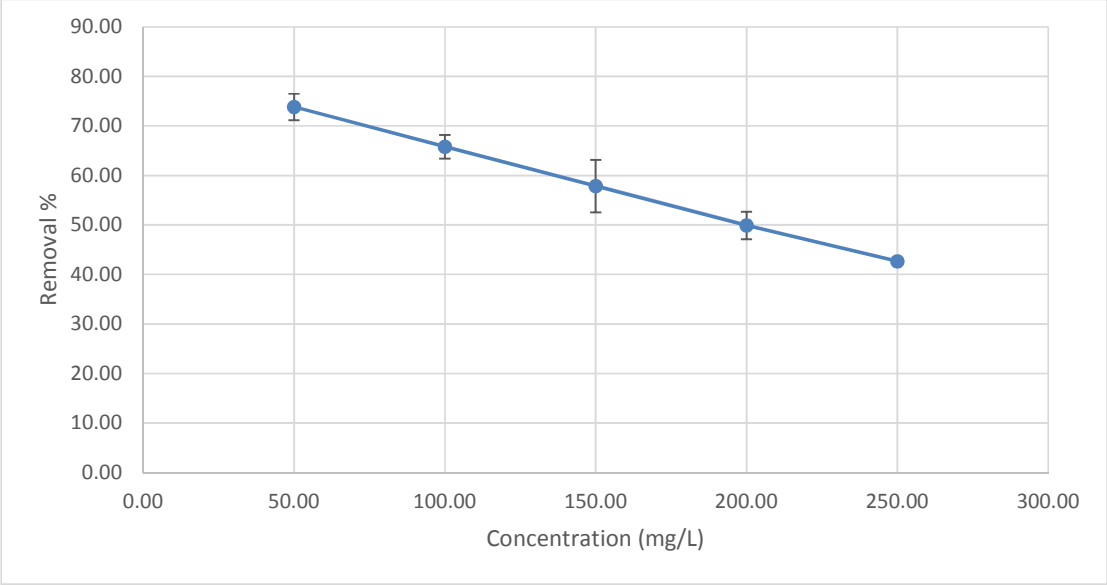


Fig. 5. Application of HPLC for metformin uptake under control condition where n= 5; Initial concentration range 50 – 250 µg/ml (in triplicate), 0.1± 0.05 g of *F. spiralis*, 0.05 L volume in 15 ml tube, at 25°C for 24 hours contact time and pH 3 ± 0.05

3.2 Adsorption Isotherm Study

Adsorption study was conducted in which the most common isotherm models of Freundlich, 1907 and Langmuir, 1918 for prediction of water and wastewater treatment samples was evaluated [31]. The Langmuir isotherm is mathematically represented as follows:

$$q_e = q_{max}{}^{b}C_e/(1+bC_e) \quad (3)$$

Where

q_e (mg/g) is the observed biosorption capacity at equilibrium.
q_{max} (mg/g) is the maximum biosorption capacity corresponding to the saturation capacity otherwise known as total binding sites of biomass,
C_e (mg/L) is the equilibrium concentration and
b (L/mg) is a coefficient related to the affinity between the sorbent and sorbate
(*b* is also known as energy of adsorption).

The linear relationship can be obtained by plotting ($1/q_e$) vs. ($1/C_e$):

$$1/q_e = 1/(bq_{max}C_e) + 1/q_{max} \quad (4)$$

The parameters *b* and *q* are determined from slope and intercept, respectively. This equation can be used to compare the individual biosorbent by its respective q_{max} value calculated from fitting the Langmuir isotherm model to that of the experimental data. The Langmuir isotherm tested (Fig. 5) proved the favourability of metformin adsorption on the *F. spiralis* (R^2 = 1) and this indicates that the biosorption process perfectly fit Langmuir model.

3.3 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy is an analytical technique that provide an insight into the possible changes in molecular vibrations within molecules and measurement of the ways in which bonds vibrates. It can also explain the present of impurities with minimal or no sample pre-treatment [32]. The raw sample of metformin hydrochloride in pure form was run and recorded over the frequency 400-4000 cm^{-1} and is represented in Fig. 7. Various bands observed in the spectra along with the assignment were shown in Table 2. The N-H stretching of C=N-H group occurs in the region 3400 – 3100 cm^{-1} but due to the presence of hydrogen bond, the frequency of this vibration tends to decrease [33].

The small intensity bands at 3153 and 3145 cm^{-1} have been assigned to C-H stretching. N,N di-substituted guanidine hydrochlorides have medium intensity bands at 150 -1530 cm^{-1} due to in-plane NH_2 deformation [34].

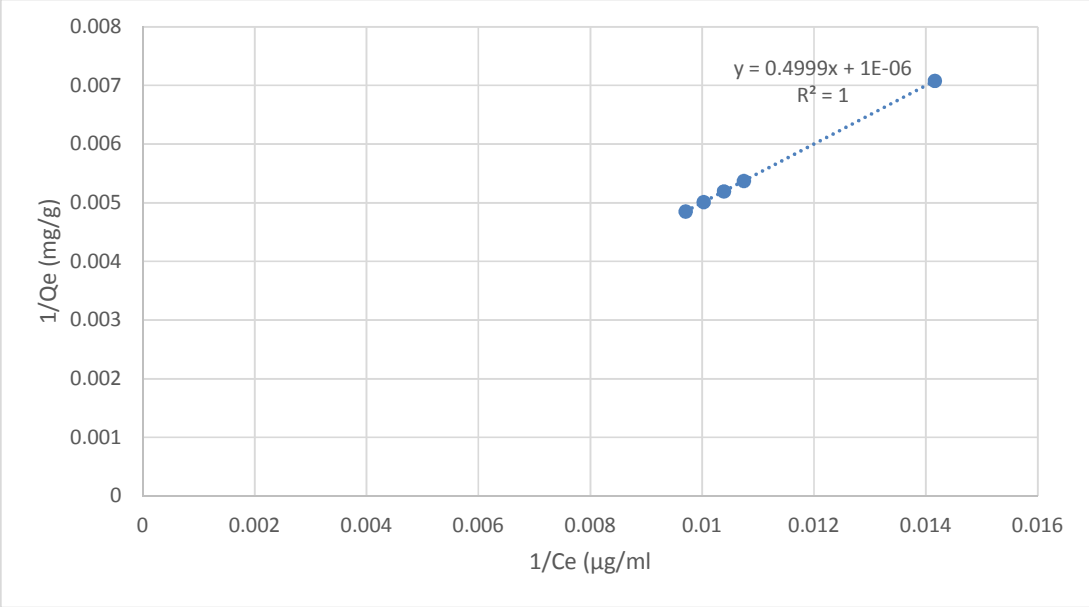


Fig. 6. Langmuir isotherm plot for metformin showing linearity

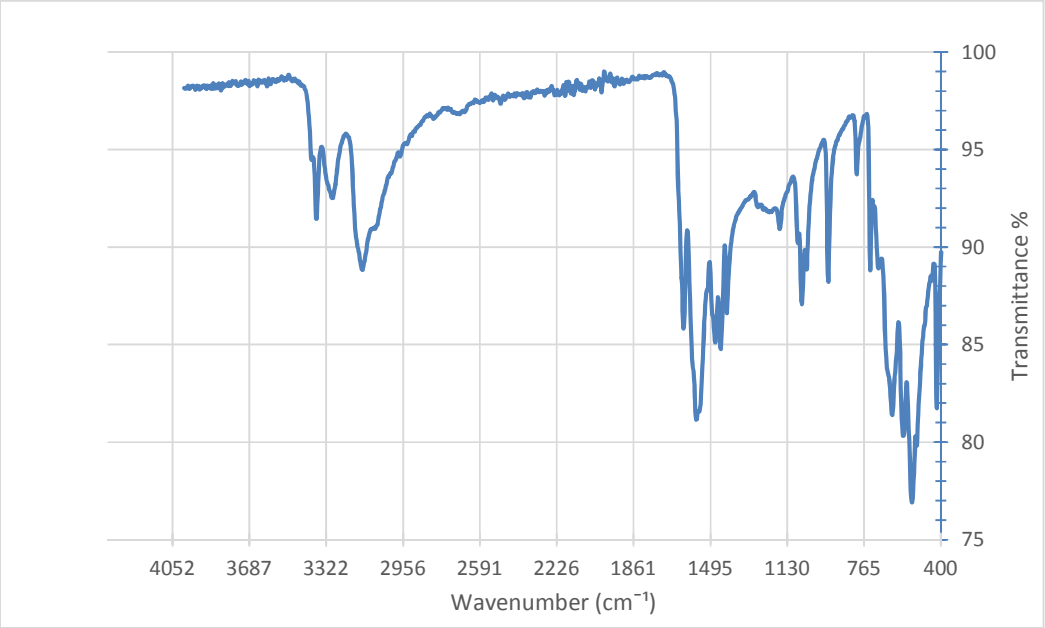


Fig. 7. FT-IR spectra of pure metformin hydrochloride

Table 3. Vibrational band assignment of pure metformin hydrochloride

Frequency (cm^{-1})	Assignments
3369 (m)	N-H stretch
3153 (n, s)	C-H stretch
3145 (s)	C-H stretch
1627 (m)	N-H bend
1559 (m)	C-C stretch (in-ring)
1063 (m)	C-N stretch
938 (s)	N-H bend
736 (m)	C-H rock
639 (b,s)	-C = -C: C-H bend
541 (m)	C-Br stretch

4. CONCLUSION

In conclusion, marine alga biomass *(Fucus spiralis*) was tested for its efficiency in metformin removal in aqueous solution. Adsorption studies revealed that *F. spiralis* can be used as potential adsorbent for metformin uptake. Very limited literature investigates the application of algal species for remediation. Remediation of waste and surface water using readily available adsorbent such as algae will be useful as it relates to human health and environmental contamination. HPLC was used in this study but other spectroscopic technique such as UV/vis could be explored to ascertain the optimized method. Further studies would be needed to test other algal species for metformin bio sorption.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Patel M, Kumar R, Kishor K, Mlsna T, Pittman CU, Mohan D. Chem. Rev. 2019;119:3510–3673.
2. Teresa PD, Klepser B, PHARM D, Michael W Kelly. Am. J. Heal. Pharm. 1997;54:893–903.
3. Rojas LBA. Gomes MB. Diabetol. Metab. Syndr. 2013;5:1–15.
4. Wilkinson S, Douglas I, Stirnadel-Farrant H, Fogarty D, Pokrajac A, Smeeth L, Tomlinson L. BMJ Open. 2018;8:1–7.
5. Scheen AJ. Clin. Pharmacokinet. 1996;30:359–371.

6. Trautwein C, Berset JD, Wolschke H, Kümmerer K. Environ. Int. 2014;70:203–212.
7. Vulliet E, Cren-Olivé C. Environ. Pollut. 2011;159:2929–2934.
8. Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, et al. Environ. Sci. Technol. 2002;36:1202–1211.
9. Houtman CJ, ten Broek R, de Jong K, Pieterse B, Kroesbergen J. Environ. Toxicol. Chem. 2013;32:2449–2459.
10. Scheurer M, Sacher F, Brauch HJ, Environ J. Monit. 2009;11:1608–1613.
11. Giokas DL, Vlessidis AG. Talanta. 2007; 71:288–295.
12. Andreozzi R, Marotta R Paxéus N. Chemosphere. 2003;50:1319–1330.
13. Sirés I, Brillas E. Environ. Int. 2012;40: 212–229.
14. Bui TX, Choi H. Chemosphere. 2010; 80:681–686.
15. Yoon Y, Westerhoff P, Snyder SA, Wert EC, Yoon J. Desalination. 2007;202:16–23.
16. Silva A, Stawiński W, Romacho J, Santos LHMLM, Figueiredo SA, Freitas OM, et al. Environ. Eng. Sci. 2019;36:573–582.
17. Coelho CM, de Andrade JR, da Silva MGC, Vieira MGA. Environ. Sci. Pollut. Res. 2020;27:16599–16611.
18. Kar M, Choudhury PK. Indian J. Pharm. Sci. 2009;71:318–320.
19. Arayne FA, Sultana MS, Zuberi N, Siddiqui MH. Indian J. Pharm. Sci. 2009;71:331–335.
20. Assaad ESPBVHN, Ahmad ASEl-aini K. 1988;208:321–324.
21. Stepensky D, Friedman M, Raz I, Hoffman A. Drug Metab. Dispos. 2002;30:861–868.
22. Goedecke C, Fettig I, Piechotta C, Philipp R, Geissen SU. Anal. Methods. 2017;9:1580–1584.
23. Khaled E, Kamel MS, Hassan HN, Abd El-Alim SH, Aboul-Enein HY. Analyst. 2012;137:5680–5687.
24. Hassan SSM, Mahmoud WH, Elmosallamy MAF, Othman AHM. Anal. Chim. Acta. 1999;378299–311.
25. Lai EPC, Feng SY, Chromatogr J. B Anal. Technol. Biomed. Life Sci. 2006;843:94–99.
26. Şengül Ü. J. Food Drug Anal. 2016;24:56–62.
27. Martín J, Buchberger W, Santos JL, Alonso E, Aparicio I, Chromatogr J. B Anal. Technol. Biomed. Life Sci. 2012;895–896:94–101.
28. Lundanes L, Greibrokk E, Reubsaet T. Chromatography : Basic principles, sample preparations and related methods, John Wiley and Sons, Incorporated, 1st edn; 2013.
29. Abdel–Gawad SA, Abd El–Aziz HM. Air, Soil Water Res.
DOI: 10.1177/1178622119848761
30. Moogouei R, Borghei M, Hosseini S, Tajadod G. Int. J. Environ. Sci. Technol. 2018;15:593–598.
31. Abdel-Gawad SA, Baraka AM, El-Shafei MM, Mahmoud AS. J. Environ. Biotechnol. Res. 2016;5:18–28.
32. Farouk F, Moussa BA, Azzazy HMES. Spectroscopy. 2011;26:297–309.
33. Colthup WS, Daly NB LH. Introduction to infrared and raman spectroscopy, Academic press, New york., 2nd edn;1975.
34. LJ B. Infrared spectra of complex molecules, Chapman and Hall,London, New York, London, 3rd edn; 1975.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/71530