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Extraction of Vitamin B12 from Aqueous Solution by using Solid-Liquid Extraction and Ultra Sonic Frequency

Shaymaa I. Saeed

Department of Chemistry, College of Science, University of Kerbala, Karbala, Iraq;
*e-mail: shaimaa.ibrahim@uokerbala.edu.iq

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A new method to uptake vitamin B12 in aqueous solution as pharmaceutical preparations by adsorption on the solid phase surface by using a multi-layer graphene oxide derivative compounds while the ultrasonic frequency wave was used to increase the efficiency of the extraction of drug from aqueous solution. Interlayer drug extraction conditions were optimized by studying a number of conditions that affect the extraction rate, including pH, ultrasound frequency, residence time of the solution inside the ultrasound device, the effect of the amount of graphene oxide derivative, and vitamin B12 concentration. The effect of temperature was also studied and a number of thermodynamic factors were generated. From the results, it was found that the best weight for the economically useful graphene oxide derivative is 0.01 grams at a concentration of 20 ppm relative to the concentration of the vitamin using an acidic medium of 2.1. The best temperature is 40 ^{0}C and within 50 Hz of the frequencies of the ultrasound device during the incubation period of 50 minutes. The proposed method was also applied to pharmaceutical samples containing vitamin B12, and the extraction rate was 95.35 %, with a very small error rate of 0.0465.

Keywords: vitamin B12, solid phase extraction, sonochemistry, fluorinated graphene oxide

Introduction

Vitamin B12 plays an important biological role in DNA synthesis. The deficiency of vitamin B12 is a common cause of some diseases such as anemia and pancytopenia [1]. B12 is also considered one of the cofactors that participate in the synthesis of enzymes and many fatty acids, which makes its deficiency a role in many neurological symptoms and effects on health. What is in excess of the body's needs is stored in the liver, so in some sick cases, the reserve is used to fill the deficiency [1]. Many studies have been conducted on the effects of vitamin deficiency on human health [2, 3]. Associated with folic acid and other B vitamins, a relatively inexpensive way of releasing plasma homocysteine levels, that might be a way to decrease CVD risk [4-6]. Sonochemistry is a potent field of study where a large amount of ultrasonic energy (20–10 MHz) causes molecules to conduct a chemical reaction [7, 8]. In the liquid phase, where the sonochemical reaction occurs. There are instances where the reaction yields both nanoamorphous and nanocrystalline particles. In the ring region where the reaction occurred, temperature is the primary factor. The temperature is higher than the bulk temperature but lower than the temperature inside the collapsing bubble [9, 10].

Ultrasonic cavitation, caused by the formation and collapse of bubbles in liquids exposed to high-intensity ultrasonic waves, is the source of both the physical and chemical effects of ultrasound [11, 12]. Graphene is a relative novelty as a material of high interest and potential [13].

Graphene oxide history dates back several decades to some of the first investigations into the chemistry of graphite. [14]. Numerous oxygen functionalities are present in graphene oxide, including carboxyl and ketone groups at the edges and 1,2-epoxide and alcohol groups on the basal planes [15, 16]. One of the best chemical methods for changing the physiochemical characteristics of carbon compounds is fluorination since it makes additional substitution simple to carry out [17,18]. The C-F bond has superior thermal and oxidative stability [19]. Additionally, fluorine gives C-F bonds high polarity and low surface free energy because it is the most electronegative element in the Periodic Table. Graphene oxide (GO) and fluorinated graphene (FG) differ in their properties because of the special characteristics of covalent fluorine atoms. FG is a novel two-dimensional carbon material that has fluorine atoms that have been covalently functionalized [20, 21].

Here, a new modification analytical method for Vitamin B12 extraction from an aqueous solution by adsorption on the multilayer material as the Functionalized and Modified Fluorinated graphene oxide (A-MFGO) that has a big hole between the solid phase layer; it's very effective in occlusion of the vitamin B12 molecules and remove from the matrix solution.

Materials and methods

Materials and Apparatuses

All the chemicals utilized in this study were sourced from Merck or Fluka. Vitamin B was used at a concentration of 250 mg/mL. 1 M NaOH stock solution was prepeared by dissolving 2000 mg of NaOH in distilled water. Hydrochloric acid was used in a concentration of 1 M.

The A-MFGO sample was analyzed using a variety of instruments. The FTIR (Fourier Transform Infrared Spectroscopy) instrument from Shimadzu, Japan was used to identify functional groups and chemical bonds in the sample. The morphology of the A-MFGO was studied by capturing a surface image using Hitachi's cold field SEM. XRD (X-ray diffraction) analysis was performed over an angle range from 0 to 120 degrees using the INEL Equinox 3000 device with CuK radiation at 30KV to gain insights into phase purity and crystal structure. Additionally, a UV-Vis spectrophotometer from Kyoto, Japan (UV-1800) was used in the analysis.

Synthesis of the adsorbent. A-MFGO was synthesized according to the literature procedure (see Figure 1) [21].

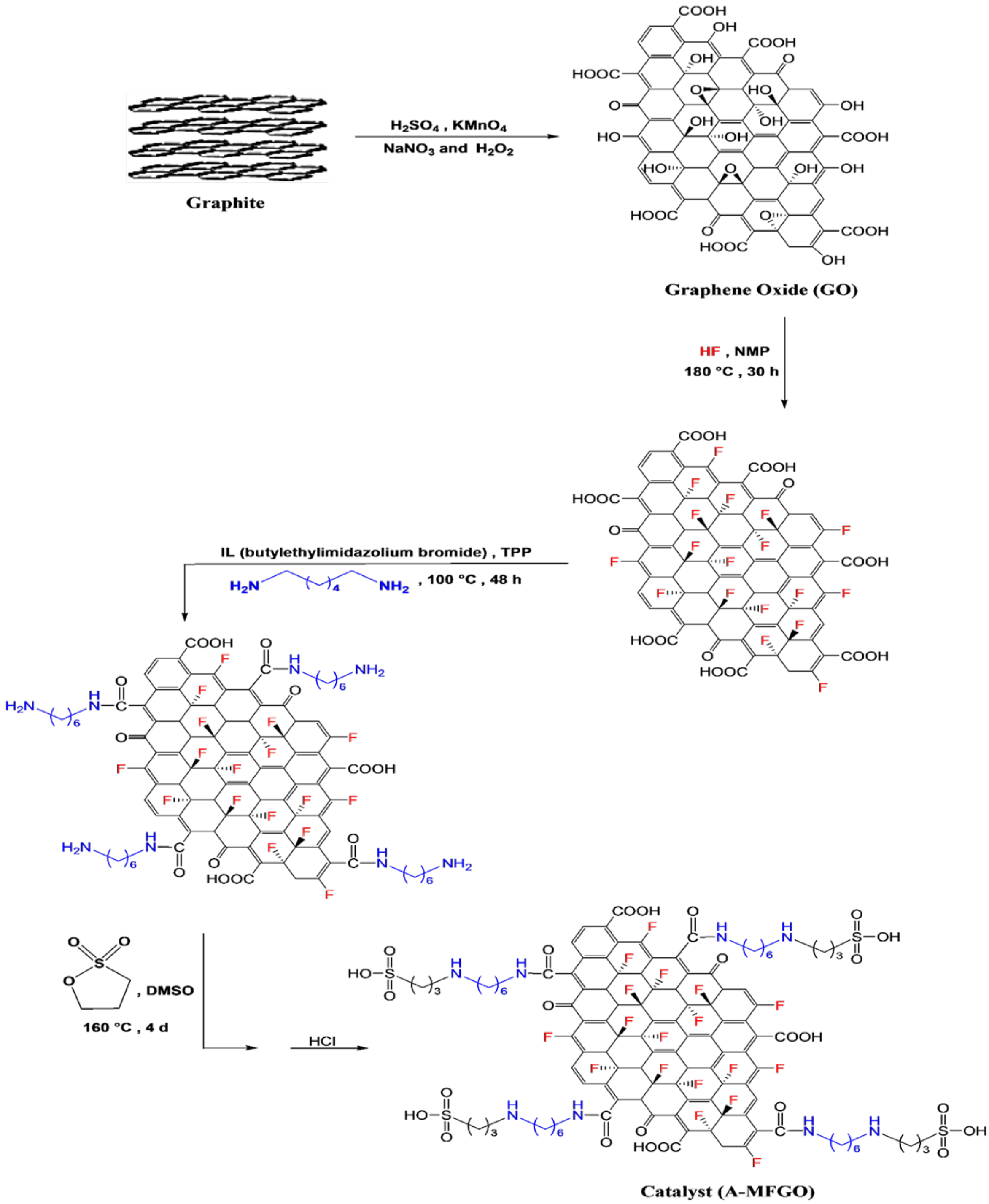

Figure 1. Synthetic routes of A-MFGO.

Solid phase extraction procedure

Solid phase extraction was used to extract vitamin B12 from its aqueous solutions using a polymer nanocomposite prepared by modifying and functionalizing fluorinated graphene oxide. The SPE method involved using 10 mL of B12 solution with a concentration of 20 ppm. The solution was adjusted to the optimal pH, and then 0.01 g of functionalized and modified fluorinated graphene oxide was added. An ultrasound device was used at a temperature of 40 °C, exposing the solution to 50 Hz frequencies for 50 minutes. After this, the solid material was obtained through the filtration process; the precipitate was washed several times and then dried at laboratory temperature. The resulting material was characterized using FTIR and SEM techniques to determine the extent to which the shape of A-MFGO changed after vitamin B12 extraction.

Optimization of the adsorption conditions

To optimize the adsorption process, we investigated theIn order to optimize the adsorption process, we conducted a series of experiments to study the impact of adsorbent weight, temperature, pH, and vitamin B12 concentration. We used 0.001 to 0.01 grams of A-MFGO for extracting 15 ppm of vitamin B12 at room temperature, stirring at 50 Hz for 60 minutes. Additionally, we performed extraction tests at temperatures of 293, 303, 313, and 323 K with a stirring rate of 50 Hz and a duration of 60 minutes to observe the effect of temperature. We also varied the stirring rate from 20 to 80 Hz with a duration of 50 minutes to assess the impact of frequency. Furthermore, we examined the impact of vitamin B12 concentration using solutions with concentrations ranging from 5 to 25 ppm at a pH range of 2.1 to 12.0.

Results and discussion

Characterization of the adsorbent (A-MFGO)

The stretching of [O-H] bonds is evident in the broad band at 3419 cm^{-1} in the FTIR spectrum image of A-MFGO Figure 2(a). The [C=O] bond can be stretched using the peak at (1721 cm^{-1}), whereas, in carboxylic acids, the [C-O] bond is found at (1383 cm^{-1}). [C=O] bonds in the amide group were responsible for the peak at (1636 cm^{-1}), while [C-F] bonds were found at (1167 cm^{-1}). Additionally, the [S=O] bond in the [$-SO_3H$] group is responsible for the peak at (1035 cm^{-1}), while the [C-S] bond is responsible for the peak at (741 cm^{-1}) [22].

Through XRD technology, it is possible to know the properties of the nanomaterial, its crystal size, and the phase that the material passes through [23]. Figure

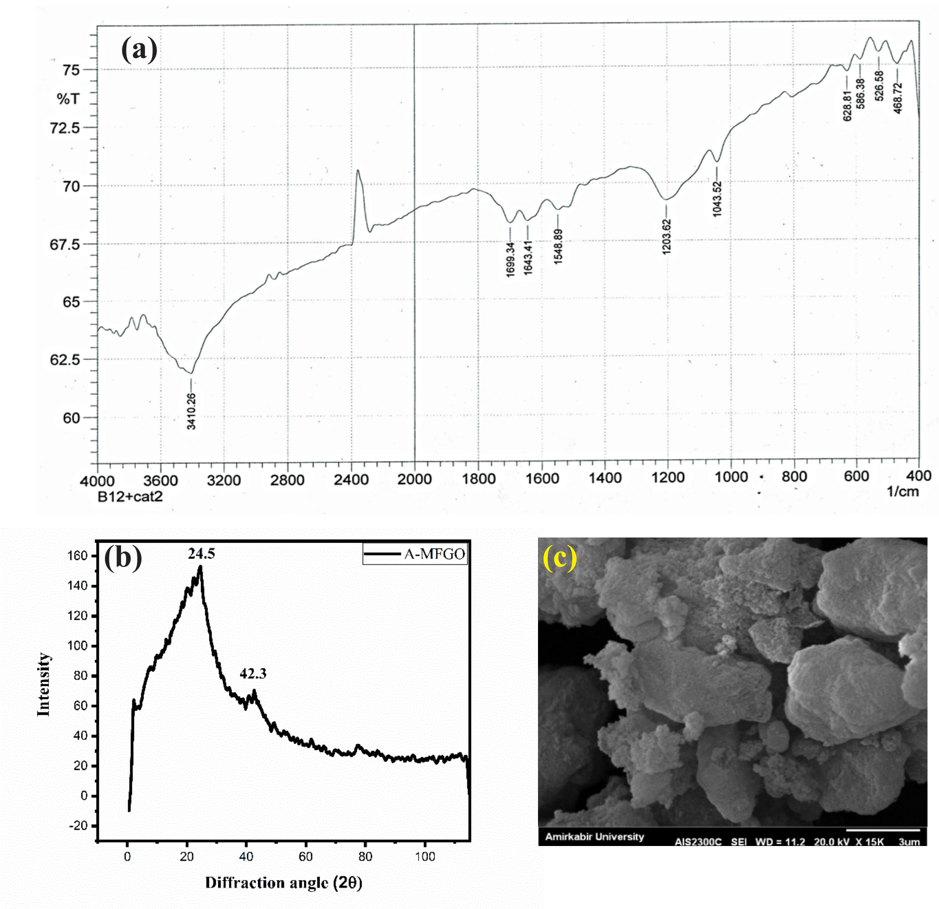

Figure 2. (a) FTIR spectra, (b) XRD measurement, and (c) SEM image of A-MFGO.

2(b) of A-MFGO shows its peaks at (24.5) and (42.3) with a d-spacing of (3.62) and 3.66 A, respectively. The absence of other peaks at different places in the spectrum means that the compound is in an amorphous form.

SEM is a valuable technique for analyzing the surface morphology of samples. Figure 2(c) depicts an SEM image of A-MFGO, revealing large particles with low rank, indicating the presence of bulk carbon. where the images showed the presence of agglomerated aggregates with irregular shapes, which indicates amorphous particles [24]. The prepared compound was analyzed using the SEM technique shown in Figure 3(c), where the images showed the presence of agglomerated aggregates with irregular shapes, which indicates amorphous particles [25].

Optimization conditions of the adsorption

Effect of the adsorbent weight

The results of this factor are shown in Figure 3, where it was found that the extraction percentage increases with increasing weight of the A-MFGO. Based on the findings, it has been found that 0.01 grams of (A-MFGO) was economically sufficient to extract vitamin B12.

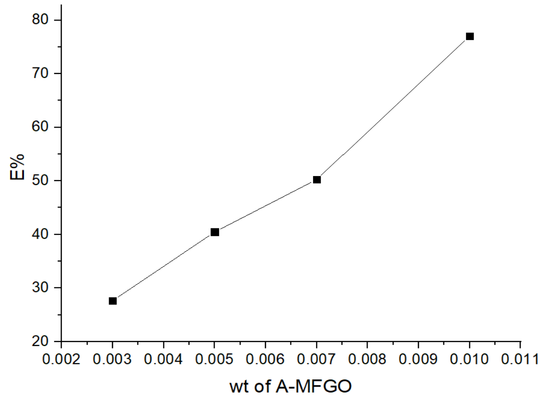

Figure 3. Impact of A-MFGO added weight on the extraction process.

Effect of the temperature

It was found that as the temperature rises, the extract percentage gradually increases until it reaches its maximum value at 40 °C, at then point it decreases at 50 °C. In order to continue the task, the ambient temperature of 313 K was selected. Figure 4 illustrates how the temperature affects the extraction process.

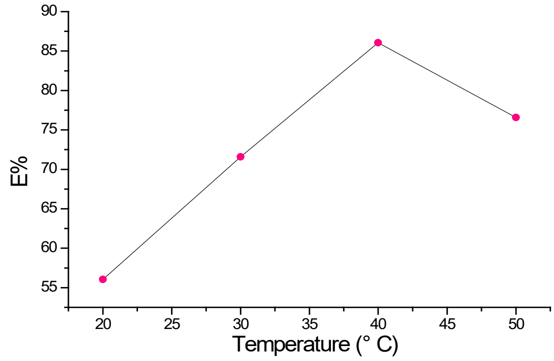

Figure 4. The effect of temperature on the extraction process.

Furthermore, measurements of the extraction equilibrium constant and thermodynamic parameters, such as enthalpy change (ΔH), Gibbs free energy (ΔG), and entropy (ΔS), were made in order to comprehend the mechanism and viability of the extraction process. Figure 5 shows the Van’t Hoff relationship displayed in log D versus 1 /T. It produces a straight line with the slope (-ΔH) (2.303 R) that corresponds to it. After that, Table 1 displays all values, where the universal gas constant is denoted by R (8.314 J mol^{-1} K^{-1}) and the absolute temperature by T.

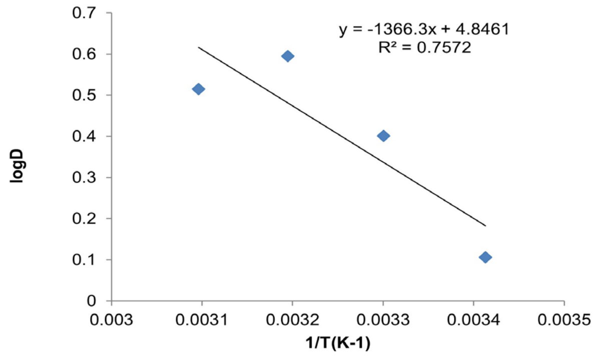

Figure 5. Relationship between log D and 1/T.

Effect of shaking time

The impact of shaking time on the ultrasound device was investigated after establishing the optimal conditions indicated by the weight and temperature of A-MFGO. The findings are presented in Figure 6, revealing that the optimal duration for extracting vitamin B12 from the solution was determined to be 50 minutes.

Table 1. Thermodynamic parameters of the extraction process.

(D)	(lnD)	(t/°C)	(T/K)	(ΔH/KJ mol^{-1})	(ΔG/KJ mol^{-1})	(ΔSKJ mol^{-1})
1.275449	0.2343	20	293	26.160	-0.570755	0.091231
2.518519	0.9237	30	303		-2.326931	0.094016
5.716981	1.3699	40	313		-3.56487	0.094968
3.269663	1.1847	50	323		-3.181419	0.09084

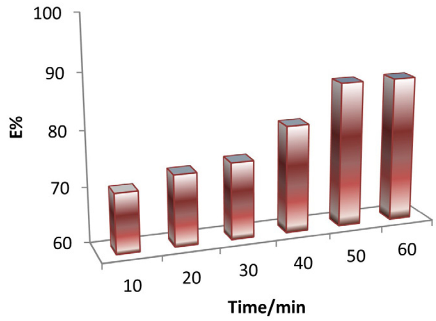

Figure 6. The effect of shaking time on the extraction process.

Effect of frequency

According to Figure 7, the extraction rate increases as the frequency rises until it reaches its peak at 50 Hz. After this point, the extraction values decline at higher frequencies. This could be due to the destruction of vitamin B12 at high frequencies in the solution, or it might be related to the process of separating the vitamin from the detector at higher frequencies. As a result, a frequency of 50 Hz was selected as the preferred value for the subsequent studies.

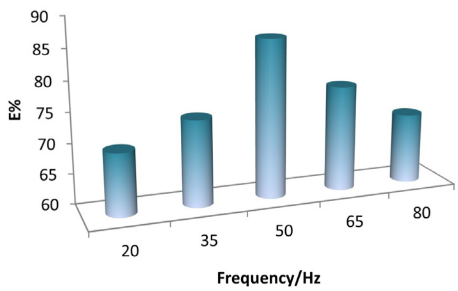

Figure 7. The effect of frequency on the extraction process.

Effect of vitamin B12 concentration

As shown in Figure 8, results indicated that the extraction process increased as the concentration of vitamin B12 increased, reaching its peak at 20 ppm. Afterward, the extraction values began to decrease slightly, possibly due to the saturation of the detector's surface with the extracted compound.

The effect of pH

Figure 9 illustrates the extraction behavior of vitamin B12 as a function of pH. The results indicate that the acidic medium yields the highest extraction rate, while the neutral medium has the lowest. Furthermore, the basic medium shows the greatest decrease in extraction rate. Therefore, a pH of 2.1 has been identified as the ideal pH for further study.

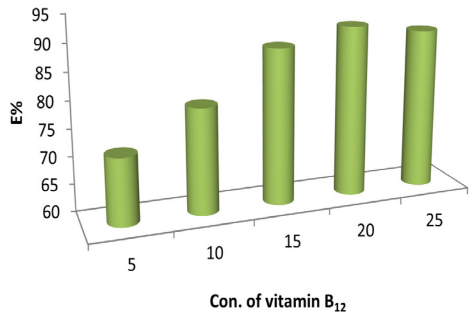

Figure 8. The effect of vitamin B12 concentration.

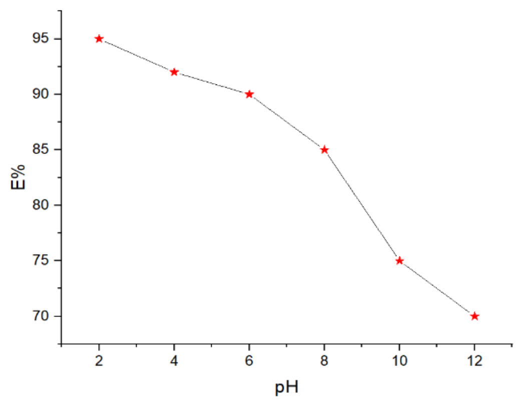

Figure 9. The effect of pH on the extraction process.

Analytical applications

Table 2 displays the results obtained when applying the method under optimal conditions for extracting vitamin B12 from the medication Neurorubine, a mixture of B vitamins.

Conclusions

An easy-to-use, quick, and precise spectrophotometric technique was evaluated for extracting vitamin B12, in both pure and pharmaceutical forms. This technology's primary benefits for routine analysis in quality control are its short analysis time and low cost.

Table 2. Result of vitamin B12 extraction from a drug sample.

Information of pharmaceutical drug	The value of concentration theoretical	The value of concentration prepared	The value of concentration extracted	E%	Error ratio
Neurorubine	0.333 mg /mL	20 mg /L	19.07 mg /L	95.35	0.0465

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References

1. Lyon, P.; Strippoli, V.; Fang, B.; Cimmino, L. B Vitamins and One-Carbon Metabolism: Implications in Human Health and Disease. Nutrients 2020, 12 (9), 1–24.
2. Layden, A. J.; Täse, K.; Finkelstein, J. L. Neglected Tropical Diseases and Vitamin B12: A Review of the Current Evidence. Trans. R. Soc. Trop. Med. Hyg. 2018, 112 (10), 423–435.
3. Fritz, J.; et al. A Systematic Review of Micronutrient Deficiencies in Pediatric Inflammatory Bowel Disease. Inflamm. Bowel Dis. 2019, 25 (3), 445–459
4. Mathew, A. R.; Schwenkenbecher, P.; Suhs, K.-W.; Raab, P.; Stangel, M.; Skripuletz, T. Vitamin B12 Deficiency and the Nervous System: Beyond Metabolic Decompensation—Comparing Biological Models and Gaining New Insights into Molecular and Cellular Mechanisms. Int. J. Mol. Sci. 2024, 25 (1).
5. Doets, E. L.; In ‘t Veld, P. H.; Szczecińska, A.; Dhonukshe-Rutten, R. A.; Cavelaars, A. E.; van ‘t Veer, P.; Brzozowska, A.; de Groot, L. C. Systematic Review on Daily Vitamin B12 Losses and Bioavailability for Deriving Recommendations on Vitamin B12 Intake with the Factorial Approach. Ann. Nutr. Metab. 2013, 62 (4), 311–322.
6. Andrès, E.; Zulfiqar, A. A.; Serraj, K.; Vogel, T.; Kaltenbach, G. Systematic Review and Pragmatic Clinical Approach to Oral and Nasal Vitamin B12 (Cobalamin) Treatment in Patients with Vitamin B12 Deficiency Related to Gastrointestinal Disorders. J. Clin. Med. 2018, 7 (10), 1–13.
7. Elsupikhe, R. F.; Shameli, K.; Ahmad, M. B.; Ibrahim, N. A.; Zainudin, N. Green Sonochemical Synthesis of Silver Nanoparticles at Varying Concentrations of κ-Carrageenan. Nanoscale Res. Lett. 2015, 10 (1), 1–8.
8. Martínez, R. F.; Cravotto, G.; Cintas, P. Organic Sonochemistry: A Chemist’s Timely Perspective on Mechanisms and Reactivity. J. Org. Chem. 2021, 86 (20), 13833–13856.
9. Dheyab, M. A.; Aziz, A. A.; Jameel, M. S. Recent Advances in Inorganic Nanomaterials Synthesis Using Sonochemistry: A Comprehensive Review on Iron Oxide, Gold and Iron Oxide Coated Gold Nanoparticles. Molecules 2021, 26 (9).
10. Li, Z.; et al. A Power-Triggered Preparation Strategy of Nano-Structured Inorganics: Sonosynthesis. Nanoscale Adv. 2021, 3 (9), 2423–2447.
11. Yusof, N. S. M.; Babgi, B.; Alghamdi, Y.; Aksu, M.; Madhavan, J.; Ashokkumar, M. Physical and Chemical Effects of Acoustic Cavitation in Selected Ultrasonic Cleaning Applications. Ultrason. Sonochem. 2016, 29, 568–576.
12. Karthikesh, M. S.; Yang, X. The Effect of Ultrasound Cavitation on Endothelial Cells. Exp. Biol. Med. 2021, 246 (7), 758–770.
13. Li, J.; Zeng, H.; Zeng, Z.; Zeng, Y.; Xie, T. Promising Graphene-Based Nanomaterials and Their Biomedical Applications and Potential Risks: A Comprehensive Review. ACS Biomater. Sci. Eng. 2021, 7 (12), 5363–5396.
14. Adetayo, A.; Runsewe, D. Synthesis and Fabrication of Graphene and Graphene Oxide: A Review. Open J. Compos. Mater. 2019, 9 (2), 207–229.
15. Guo, S.; Garaj, S.; Bianco, A.; Ménard-Moyon, C. Controlling Covalent Chemistry on Graphene Oxide. Nat. Rev. Phys. 2022, 4 (4), 247–262.
16. Liu, D.; Bian, Q.; Li, Y.; Wang, Y.; Xiang, A.; Tian, H. Effect of Oxidation Degrees of Graphene Oxide on the Structure and Properties of Poly(vinyl alcohol) Composite Films. Compos. Sci. Technol. 2016, 129, 146–152.
17. Roshchina, T. M.; Glazkova, S. V.; Khrycheva, A. D.; Makotchenko, V. G.; Pantyukhin, M. L. Effect of Fluorination on the Surface Properties of Carbon Materials as Probed by Gas Chromatography. Moscow Univ. Chem. Bull. 2007, 62 (2), 67–71.
18. Chronopoulos, D. D.; Bakandritsos, A.; Pykal, M.; Zbořil, R.; Otyepka, M. Chemistry, Properties, and Applications of Fluorographene. Appl. Mater. Today 2017, 9, 60–70.
19. Giannetti, E. Thermal Stability and Bond Dissociation Energy of Fluorinated Polymers: A Critical Evaluation. J. Fluor. Chem. 2005, 126 (4), 623–630.
20. Bi, X.; et al. Fluorinated Graphene Prepared by Direct Fluorination of N, O-Doped Graphene Aerogel at Different Temperatures for Lithium Primary Batteries. Materials (Basel) 2018, 11 (7), 1–11.
21. Hanoon, H. D.; Kowsari, E.; Abdouss, M.; Ghasemi, M. H.; Zandi, H. Highly Efficient and Simple Protocol for Synthesis of 2, 4, 5-Triarylimidazole Derivatives from Benzil Using Fluorinated Graphene Oxide as Effective and Reusable Catalyst. Res. Chem. Intermed. 2017, 43 (7), 4023–4041.
22. Li, M.; Zhang, X.; Wang, S.; Hu, X.; Zhao, Y.; Xie, J.; Xiong, Y.; Xu, K.; Cheng, F.; Chen, J. Relating Catalysis between Fuel Cell and Metal-Air Batteries. Matter 2020, 2 (1), 32–49.
23. L. Ed, E. X-ray Characterization of Materials; Wiley-VCH: New York, 2003.
24. Chen, J.; Yan, W. Q.; Lam, C. M.; Zeng, C. C.; Hu, L. M.; Little, R. D. Electrocatalytic Aziridination of Alkenes Mediated by n-Bu4NI: A Radical Pathway. Org. Lett. 2015, 17 (4), 986–989.
25. Huseynov, E.; Garibov, A.; Mehdiyeva, R. TEM and SEM Study of Nano SiO2 Particles Exposed to Influence of Neutron Flux. J. Mater. Res. Technol. 2016, 5 (3), 213–218.