Synthesis and Characterization of Gefitinib and Paclitaxel Dual Drug Loaded Cockle Shell (Anadara granosa) Derived Calcium Carbonate Nanoparticles †

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Abstract: Calcium carbonate nanoparticles have salient properties, such as biocompatibility, pH responsiveness, and the ability to alkalize a tumor, thereby reducing metastasis. A combination therapy regimen is normative for breast cancer, and besides its side effects, toxic vehicles are required for certain drugs. This study is aimed to transform the readily available Blood cockle shells (Anadara granosa) to calcium carbonate nanoparticles (CSCaCO3NP), loading them with Gefitinib (GEF) and Paclitaxel (PTXL). Facile top-down synthesis of CSCaCO3NP is comprised of grinding, sieving, and stirring with Tween 80, followed by filtration and finally dry milling for 120 h. A ratio of 1 + 0.5:25 of GEF+PTXL: CSCaCO3NP in an equal admixture of DMSO and 0.05% Tween 80 buffer was used for drug loading. Loading content (%) and encapsulation efficiency (%) for GEF and PTXL in dual drug-loaded NP (GEF-PTXL-CSCaCO3NP) was 1.98 ± 0.11, 50.01 ± 2.18 and 0.92 ± 0.01, 45.60 ± 0.32. Field emission scanning electron micrographs revealed that the nanoparticles were almost spherical with the average diameter (nm) measuring 63.96 ± 22.3 and 87.20 ± 26.66 for CSCaCO3NP, and GEF-PTXL-CSCaCO3NP, respectively. The Dynamic Light Scattering data gives the average diameter of CSCaCO3NP and GEF-PTXL-CSCaCO3NP as 179 ± 10.9 (nm), and 274 ± 23.22 (nm), and Zeta potential was −17 ± 1.15 (mV) and −10.30 ± 1.7 (mV), respectively. Fourier-transform Infrared spectroscopy proves that CSCaCO3NP have been loaded with the drugs. X-Ray Diffraction data indicate that the aragonite phase is unaltered. N2 adsorption-desorption isotherms reveals that CSCaCO3NP are mesoporous and that the surface area was reduced from 10.68 ± 0.22 to 9.88 ± 0.24 m2/g after drug loading. For the first time, this work will describe the process that enabled to synthesize CSCaCO3NP, which was used as a carrier to load GEF and PTXL and its salient characteristics.

Keywords: calcium carbonate nanoparticles; Gefitinib; Paclitaxel; dual drug loading; XRD; FTIR; mesoporous
1. Introduction

Blood cockle shells (Anadara granosa) are bivalve shellfish that grow along the shallow waters of the coastline of southeast Asia, east Asia, and south Asia including Malaysia [1,2]. Cockle shell derived inorganic calcium carbonate nanoparticles (CSCaCO3NP) have been in focus in recent years due to its physicochemical and biocompatible properties. Their availability, low cost, safety, biocompatibility, pH-sensitive property, and slow biodegradability makes CSCaCO3 nanoparticles the right candidate to be nominated as the preferred drug delivery system. In the past various drugs have been successfully loaded onto CaCO3NP ranging from hormones [3], antibiotics [4], to even chemotherapeutic drugs [5]. Dual drug-carrying nanoparticles have more advantages since the nanoparticles help in improving the problem of solubility of hydrophobic drugs by carrying them and releasing them mostly at the tumor milieu. Paclitaxel (PTXL) and Gefitinib (GEF) are the two hydrophobic drugs aimed to be loaded together onto the pH-dependent CSCaCO3NP. The CSCaCO3NP decomposes slowly in the normal physiological pH (7.4) while decomposing more quickly in the acidic pH (<6.5) of the tumor environments [6]. When designing novel drug delivery systems, the physicochemical characterization of nanoparticles is very essential in determining their long-term stability and also their biological effects on tissues [7]. The major parameters required for physicochemical characterization of nanomaterials are shape, size, poly dispersive index, surface charge, composition, and purity [8].

2. Methods

The synthesis of CSCaCO3NP is comprised of two major steps. The first step was the synthesis of microparticles from cockle shells [9]. The second step was preparing a suspension with 2 g of the 75-µm of CSCaCO3 powder with 20 mL of deionized water and 1 mL of Tween 80. This suspension was stirred at 1000 rpm for 2 h and filtered. The filtrate was centrifuged for 10 min at 14,000 rpm and the supernatant was discarded. The pellet was dispersed upon the addition of 20 mL deionized water and then washed twice with deionized water. The collected nanoparticles were dried and then ball milled at 120 rpm for 120 h. The resultant particles (CSCaCO3NP) were characterized before loading the drugs.

For synthesizing dual drug loaded GEF-PTXL-CSCaCO3NP, three groups, namely GEF1-PTXL, GEF2-PTXL, and GEF3-PTXL were assigned with concentration of GEF and PTXL being 400 µg + 200 µg, and varying concentrations of CSCaCO3NP 10,000 µg, 15,000 µg, and 20,000 µg, respectively. PTXL and GEF dissolved in DMSO were added to 5 mL of CSCaCO3NP suspension prepared with 0.05% Tween 80 buffer and DMSO in a 50:50 (v/v) ratio. The loading was achieved by continuous stirring at 200 rpm overnight at room temperature. The individual suspensions were centrifuged at 14,000 rpm for 10 min, followed by washing the pellet with deionized water and drying. The supernatant obtained after centrifugation contained the un-entrapped GEF and PTXL, which was used to indirectly determine the amount of drug-loaded onto the nanoparticles using UV-Vis spectrophotometer. The encapsulation efficiency (EE%) and loading content (LC%) were determined as the average measurement of 3 independent measurements[10].

Physicochemical characterization of CSCaCO3NP and GEF-PTXL-CSCaCO3NP were conducted by High resolution Transmission electron microscopy (HR-TEM), Field emission Scanning electron microscopy (FESEM), Zeta potential and hydrodynamic diameter detection, Powder X-ray diffraction (PXRD), Fourier-transform infra-red spectroscopy (FT-IR) and BET analysis. For HR-TEM, the sample was added to 3 mL of 100% acetone and sonicated for 30 min. A drop of the sonicated solution was placed onto a carbon-coated copper grid and excess fluid was wicked off with a filter paper, followed by drying at room temperature for an hour and examined. For FESEM, the sample was dispersed onto 12 mm diameter aluminum sample holders using conductive carbon paint and then coated with the Platinum layer under vacuum and examined. These images were analyzed using ImageJ software. For Zeta potential, Polydispersity Index, and Hydrodynamic diameter analysis, 0.4 mg of the sample was dispersed in 15 mL deionized water and then
sonicated for 30 min. After sonication, the sample was again double diluted with deionized water. Then, the sample was injected into disposable cuvettes, and hydrodynamic diameter along with the Poly-dispersity Index were measured using Zetasizer Nano ZS (Ver.7.11; Malvern Instruments Ltd., Malvern, UK). All measurements were also carried out in duplicate of three independent experiments. PXRD patterns were obtained using the Shimadzu XRD-6000 powder diffractometer configured with Cu X-ray tube with 1.540562 Å. The samples were scanned at the rate of 40/min with diffraction angles from 4.0207° to 89.9527° at room temperature. The data were analyzed with X’pert HighScore Plus software. The samples were investigated using the Fourier infrared spectrophotometer (Model 100 series, Perkin Elmer, Shelton, CT, USA) over the range of 4000 to 400 cm⁻¹ at a 2 cm⁻¹ resolution and averaging 64 scans/s. The obtained data were analyzed with OriginPro 9.0. For determining the surface area, nitrogen absorption and desorption experiments were carried out with Micromeritics. The generated data were analyzed using BET (Brunauer-Emmett-Teller) and BJH (Barrett-Joyner-Halenda) models to determine the BET specific surface area and BJH mean pore size. Statistics were calculated using OriginPro 9.0, and Microsoft excel (Microsoft, Redmond, WA, USA) for analyzing the mean and SD.

3. Results and Discussion

The structural integrity and physicochemical properties of intact nanoparticles must be preserved throughout the formulation process until the finished product. Milling technology has been applied to synthesize poorly water-soluble compounds [11]. The current top-down technique, used to synthesize the CSCaCO₃NP from the cockle shell is laborious. On the other hand, this method is more effective and less expensive than the use of a high-pressure homogenizer technique via a microemulsion system to synthesize CSCaCO₃NP by Kamba et al. [12].

The method followed for loading the drugs is facile and efficient. Drug-loading and drug-encapsulation percentages are very vital parameters in the synthesis of nanomedicines [13]. The Loading content and encapsulation efficiency of the three groups are tabulated (Table 1). Loading content (%) and encapsulation efficiency (%) for GEF and PTXL for GEF1-PTXL was 1.98 ± 0.11, 50.01 ± 2.18 and 0.92 ± 0.01, 45.60 ± 0.32. GEF1-PTXL is a suitable group, since it possesses higher encapsulation efficiency and the highest loading content of GEF, and PTXL, respectively. The loading of drugs into the nanoparticles is also governed by the surface area available on the CSCaCO₃ nanoparticles. Another important factor is the water solubility of the drugs employed [14]. The lower loading content of less than 10% is usually observed for inorganic carrier-based nanoparticles [13]. A similar result is reflected in the loading content obtained in the current experiment. In addition to the above-stated facts, other factors to be considered for lower values are the physical and electrostatic interactions during the drug loading process. The CSCaCO₃ NP is negatively charged, and so will contribute to electrostatic repulsion leading to lower loading content. The obtained encapsulation efficiency can be compared to the data obtained by Ibiyeye et al. where CSCaCO₃ NP was loaded with Thymoquinone/Doxorubicin, and the obtained values of this study follow a similar trend [15]. The observed trend in the encapsulation efficiency (%) for CSCaCO₃NP was similar to the data obtained for single drug loading like Doxorubicin [3,5,7] and Docetaxel [5] loaded onto the same type of cockle shell-derived CaCO₃NP, but the loading content is lower in this study, when compared to other research.
Table 1. Loading content (%) and Encapsulation efficiency (%) of various groups of GEF-PTXL-CSCaCO3NP.

| Groups    | Drugs          | CSCaCO3NP (µg) | Loading Content (%) | Encapsulation Efficiency (%) |
|-----------|----------------|----------------|---------------------|-----------------------------|
| GEF1-PTXL | GEF (400 µg)   | 10,000         | 1.98 ± 0.11         | 50.01± 2.18                 |
|           | PTXL (200 µg)  |                | 0.92 ± 0.01         | 45.60 ± 0.32                |
| GEF2-PTXL | GEF (400 µg)   | 15,000         | 1.14 ± 0.23         | 42.95 ± 8.98                |
|           | PTXL (200 µg)  |                | 0.50 ± 0.08         | 37.45 ± 5.73                |
| GEF3-PTXL | GEF (400 µg)   | 20,000         | 1.12 ± 0.19         | 45.03±10.37                 |
|           | PTXL (200 µg)  |                | 0.44 ± 0.08         | 43.93 ± 7.25                |

The TEM micrographs of CSCaCO3NP demonstrated a spherical shape and a size of 52.36 ± 15.82 nm (Figure 1a). The FESEM micrographs of CSCaCO3NP revealed particles of 63.96 ± 22.3nm and GEF-PTXL-CSCaCO3NP exhibited spherical shape and relatively uniform size of 87.20 ± 26.66 nm. (Figure 1b). This size is comparable to the results of Ibiyeye et al. [15]. The zeta potential of the CSCaCO3NP was -17 ± 1.15 (mV) and GEF-PTXL-CSCaCO3NP was -10.30 ± 1.7 (mV) (Figure 1c). The negative Zeta potential is in concurrence with other works [4,5], and the poly-dispersity index (PDI) was on average 0.3 for both the nanoparticles (Figure 1c). The Hydrodynamic diameters of CSCaCO3NP and GEF-PTXL-CSCaCO3NP are 179 ± 10.9 (nm) and 274 ± 23.22 (nm), respectively (Figure 1c), which was larger than the Doxorubicin loaded CSCaCO3NP obtained by Danmaigoro et al. [16] and Hamidu et al. [17]. The overestimation of the hydrodynamic diameter from DLS was upto 17 to 31%, when compared with the values from the electron micrographs [4,16,17]. This type of discrepancy has been observed as the methods used to measure the size is different. Another vital point to be noted is that the dispersant used for the DLS measurement also plays a role in altering the values [18], the aggregation or opsonization of particles in the deionized water medium [19], as compared to TEM, where the particles were measured in a dry state.

PXRD patterns of CSCaCO3NP and GEF-PTXL-CSCaCO3NP indicate that both the nanoparticles possess aragonite crystalline signature (Figure 1d), and showed the highest score with that of the aragonite phase of CaCO3. This results are in agreement with other researchers where various drugs, like Vancomycin [20], Doxorubicin [16], Thymoquinone, and Doxorubicin [15] were loaded onto CSCaCO3NP. FT-IR spectra is a result of the absorption of electromagnetic radiations at frequencies that correlate to the vibration of a specific set of chemical bonds form within a molecule [21]. The FT-IR spectra of CSCaCO3NP demonstrated vibrational band assignments at 1445, 1084, 856, and 714 cm⁻¹ (Figure 1e). The largest and strongest band exhibited at 1445 cm⁻¹ is attributed to the C-O stretching band. Other peaks at 1084 and 856 cm⁻¹ are attributed to CO₃²⁻ in the molecular structure of the calcium carbonate. The derived spectra are similar to the spectra obtained by other researchers for cockle shell derived CaCO3NP [16]. The spectral absorption peaks of GEF-PTXL-CSCaCO3NP demonstrated new vibrational band assignments at 952.84 (cyclohexane), 1024.20 (C-F stretch), 2918.30 (C-H stretching) and 3435.22 (amomatic amine and OH⁻ stretch) cm⁻¹ [22,23] (Figure 1e). The largest and strongest band exhibited by CSCaCO3NP at 1445 cm⁻¹ remained unaltered in spectra of GEF-PTXL-CSCaCO3NP, indicating that the alkyl group is unaffected. All these changes indicate that the drugs are adsorbed onto the nanoparticles.
Figure 1. Characteristics of CSCaCO₃NP and GEF-PTXL-CSCaCO₃NP (a) TEM micrograph of CSCaCO₃NP with 50 nm particles of spherical shape and relatively even size; (b) FESEM micrograph of GEF-PTXL-CSCaCO₃NP with 87 nm on average and relatively spherical shape and (c) Zeta Potential, Particle size distribution (1), and hydrodynamic diameter (2) of CSCaCO₃NP, and Zeta Potential, Particle size distribution (3), and hydrodynamic diameter (4) of GEF-PTXL-CSCaCO₃NP; (d) PXRD patterns demonstrates aragonite crystalline phase in both the nanoparticles and labelled are the miller indices planes of the synthesized crystals; (e) FT-IR pattern of CSCaCO₃NP and formation of new peaks (green box) in the spectra of GEF-PTXL-CSCaCO₃NP.

From the Nitrogen adsorption and desorption experiments, the isotherm obtained for CSCaCO₃NP and the GEF-PTXL-CSCaCO₃NP is Type IV, based on the classification of BET system. This type of isotherm is characterized by the “hysteresis loop,” where, capillary condensation occurs, with an initial loop formed by the mono-multi layer adsorption, a 2nd loop by the desorption of gases. This type of isotherm indicates that the nanoparticles are mesoporous [24,25]. The H1 hysteresis loop is formed when the adsorption and desorption curves are almost vertical and approximately parallel to each other and it signifies the presence of pores with cylindrical geometry and uniform pore size. The surface area of the synthesized CSCaCO₃NP and GEF-PTXL-CSCaCO₃NP are 10.68 ± 0.22 and 9.88 ± 0.24 (cm²/g), which is higher than the surface area obtained by Danmaigoro et al. [16] and Hammadi et al. [5]. The BJH mean pore size diameter of the synthesized...
CSCaCO₃NP and GEF-PTXL-CSCaCO₃NP was 5.21 and 5.23 (nm), which is slightly higher than the pore size obtained by Danmaigoro et al. [16].

4. Conclusions

In conclusion, the simple blood cockle shells, a by-product of the food industry, was converted into a potential nanoparticulate drug carrier. The blood cockle shell-derived CaCO₃NP is purely aragonite, porous, and contains a large surface area compared to the particle size. Specifically, in this work, we have proved that cockle shell-derived CaCO₃NP can be loaded with chemotherapeutic drugs. After loaded with Gefitinib and Paclitaxel, the physicochemical characterization data revealed that the drugs were successfully loaded onto the nanoparticles and the aragonite phase of the cockle shell-derived CaCO₃NP has remained unaltered.

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References

1. Faulkner, P. Morphometric and taphonomic analysis of granular ark (Anadara granosa) dominated shell deposits of Blue Mud Bay, northern Australia. J. Archaeol. Sci. 2010, 37, 1942–1952, doi:10.1016/j.jas.2010.02.021.

2. FAO. Aquatic Species Distribution Map Viewer. Fish. Aquac. Dep. Available online: http://www.fao.org/figis/geoserver/factsheets/species.html%0A (accessed on 6 August 2020).

3. Jaji, A.Z.; Zakaria, Z.; Mahmud, R.; Loqman, M.Y.; Hezmee, M.N.M.; Isa, T.; Fu, W.; Hammadi, N.I. Synthesis, characterization, and cytocompatibility of potential cockle shell aragonite nanocrystals for osteoporosis therapy and hormonal delivery. Nanotechnol. Sci. Appl. 2017, 10, 23–33, doi:10.2147/NSA.S113030.

4. Idris, S.B.; Kadir, A.A.; Jesse, F.F.A.; Ramanoon, S.Z.; Basit, M.A.; Zakaria, Z.A.; Zakaria, M.Z.A.B. Synthesis, characterization, and in vitro release of oxytetracycline loaded in pH-responsive CaCO₃ nanoparticles. J. Appl. Pharm. Sci. 2019, 9, 19–27 doi:10.7324/JAPS.2019.91103.

5. Hammadi, N.I.; Abba, Y.; Hezmee, M.N.M.; Razak, I.S.A.; Jaji, A.Z.; Isa, T.; Mahmod, S.K.; Zakaria, M.Z.A.B. Formulation of a Sustained Release Docetaxel Loaded Cockle Shell-Derived Calcium Carbonate Nanoparticles against Breast Cancer. Pharm. Res. 2017, 34, 1193–1203, doi:10.1007/s11195-017-2135-1.

6. Maleki Dizaj, S.; Barzegar-Jalali, M.; Zarrintan, M.H.; Adibkia, K.; Lotfipour, F. Calcium carbonate nanoparticles as cancer drug delivery system. Expert Opin. Drug Deliv. 2015, 12, 1649–1660, doi:10.1517/17425247.2015.1049530.

7. Hosokawa, M.; Nogi, K.; Naito, M.; Yokoyama, T. Basic properties and measuring methods of nanoparticles. In Nanoparticle Technology Handbook, 1st ed.; Hosokawa, M., Nogi, K., Naito, M., Yokoyama, T., Eds.; Elsevier: Amsterdam, Netherlands, 2007; pp. 1–166.

8. Crist, R.M.; Grossman, J.H.; Patri, A.K.; Stern, S.T.; Dobrovolskaia, M.A.; Adiseshiah, P.P.; Clogston, J.D.; McNeil, S.E. Common pitfalls in nanotechnology: Lessons learned from NCI’s Nanotechnology Characterization Laboratory. Integr. Biol. 2013, 5, 66–73, doi:10.1039/c2ib20117h.

9. Islam, K.N.; Ali, M.E; Bakar, M.Z.B.A.; Loqman, M.Y.; Islam, A.; Islam, M.S.; Rahman, M.M.; Ullah, M. A novel catalytic method for the synthesis of spherical aragonite nanoparticles from cockle shells. Powder Technol. 2013, 246, 434–440, doi:10.1016/j.powtec.2013.05.046.

10. Fu, W.; Noor, M.M.H.; Yusof, L.M.; Ibrahim, T.A.T.; Keong, Y.S.; Jaji, A.Z.; Zakaria, M.Z.A.B. In vitro evaluation of a novel pH sensitive drug delivery system based cockle shell-derived aragonite nanoparticles against osteosarcoma. J. Exp. Nanosci. 2017, 8080, 1–22, doi:10.1080/17458080.2017.1287965.

11. Chen, H.; Khemtong, C.; Yang, X.; Chang, X.; Gao, J. Nanonization strategies for poorly water-soluble drugs. Drug Discov. Today 2011, 16, 354–360, doi:10.1016/j.drudis.2010.02.020.

12. Kamba, A.S.; Ismail, M.; Tengku Ibrahim, T.A.; Ibrahim, T.; Zakaria, Z.A.B.A. pH-Sensitive, Biobased Calcium Carbonate Aragonite Nanocrystal as a Novel Anticancer Delivery System. Biomed. Res. Int. 2013, 2013, 1–10, doi:10.1155/2013/587451.

13. Shen, S.; Wu, Y.; Liu, Y.; Wu, D. High drug-loading nanomedicines: Progress, current status, and prospects. Int. J. Nanomed. 2017, 12, 4085–4109, doi:10.2147/IJN.S132780.
14. Govender, T.; Riley, T.; Ehtezazi, T.; Garnett, M.C.; Stolnik, S.; Illum, L.; Davis, S.S. Defining the drug incorporation properties of PLA-PEG nanoparticles. *Int. J. Pharm.* **2000**, *199*, 95–110, doi:10.1016/s0378-5173(00)00375-6.

15. Ibiyeye, K.M.; Zakaria, M.Z.A.B.; Nurdin, N.; Mokrish, A. Combine Drug Delivery of Thymoquinone-Doxorubicin by Cockle Shell-derived pH-sensitive Aragonite CaCO₃ Nanoparticles. *Nanosci. Nanotechnol. Asia* **2020**, *10*, 518–533, doi:10.2174/2210681209666190508122540.

16. Danmaigoro, A.; Selvarajah, G.T.; Noor M.H.M.; Mahmud, R.; Zakaria, M.Z.A.B. Development of Cockleshell (*Anadara granosa*) Derived CaCO₃ Nanoparticle for Doxorubicin Delivery. *J. Comput. Theor. NanoSci.* **2017**, *14*, 5074–5086, doi:10.1166/jctn.2017.6920

17. Hamidu, A.; Mokrish, A.; Mansor, R.; Razak, I.S.A.; Danmaigiro, A.; Jaji, A.Z.; Zakaria, M.Z.A.B. Modified methods of nanoparticles synthesis in pH-sensitive nano-carriers production for doxorubicin delivery on MCF-7 breast cancer cell line. *Int. J. Nanomed.* **2019**, *14*, 3615–3627, doi: 10.2147/IJN.S190830

18. Souza, T.G.F.; Ciminelli, V.S.T.; Mohallem, N.D.S. A comparison of TEM and DLS methods to characterize size distribution of ceramic nanoparticles. *J. Phys. Conf. Ser.* **2016**, *733*, 1-5, doi: 10.1088/1742-6596/733/1/012039.

19. Gaumet, M.; Vargas, A.; Gurny, R.; Delie, F. Nanoparticles for drug delivery: The need for precision in reporting particle size parameters. *Eur. J. Pharm. Biopharm.* **2008**, *69*, 1–9, doi:10.1016/j.ejpb.2007.08.001.

20. Saidykhian, L.; Rukayadi, Y.; Umar Kura, A.; Yazan, L.S.; Zakaria, M.Z.A.B Development of nanoantibiotic delivery system using cockle shell-derived aragonite nanoparticles for treatment of osteomyelitis. *Int. J. Nanomed.* **2016**, *11*, 661, doi:10.2147/IJN.S95885.

21. Coates, J. Interpretation of Infrared Spectra, A Practical approach. In *Encyclopedia of Analytical Chemistry*; Meyers, R.A., Ed.; John Wiley & Sons, Ltd.: Chichester, UK, 2000; pp. 10815–10837.

22. Renuga Devi, T.S.; Gayathri, S. FTIR and FT-Raman spectral analysis of Paclitaxel drugs. *Int. J. Pharm. Sci. Rev. Res.* **2010**, *2*, 106–110.

23. Talari, A.C.S.; Martinez, M.A.G.; Movasaghi, Z.; Rehman, S.; Ur Rehman, I. Advances in Fourier transform infrared (FTIR) spectroscopy of biological tissues. *Appl. Spectrosc.* **2017**, *52*, 456–506, doi:10.1080/05704928.2016.1230863.

24. Sing, K.S.W. Reporting Physiosorption data for gas/solid systems with special Reference to the Determination of Surface Area and Porosity. *Pure Appl. Chem.* **1982**, *54*, 2201–2218.

25. Thommes, M.; Kaneko, K.; Neimark, A.V.; Oliver, J.P.; Rodriguez-Reinoso, F.; Rouquerol, J.; Sing, K.S.W. Physiosorption of gases, with special reference to the evaluation of surface area and pore size distribution (IUPAC Technical Report). *Pure Appl. Chem.* **2015**, *87*, 1051–1069.