Analytical Methods

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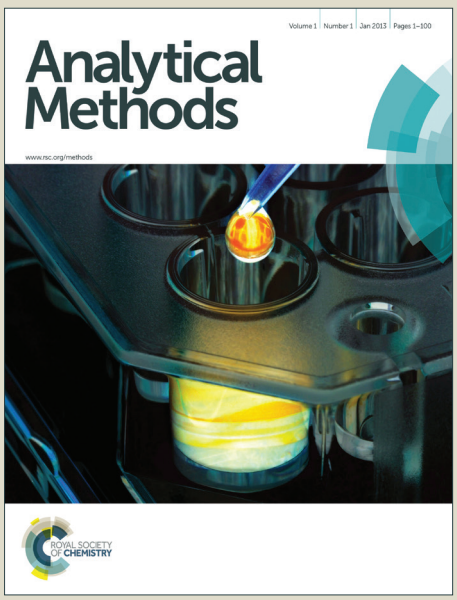

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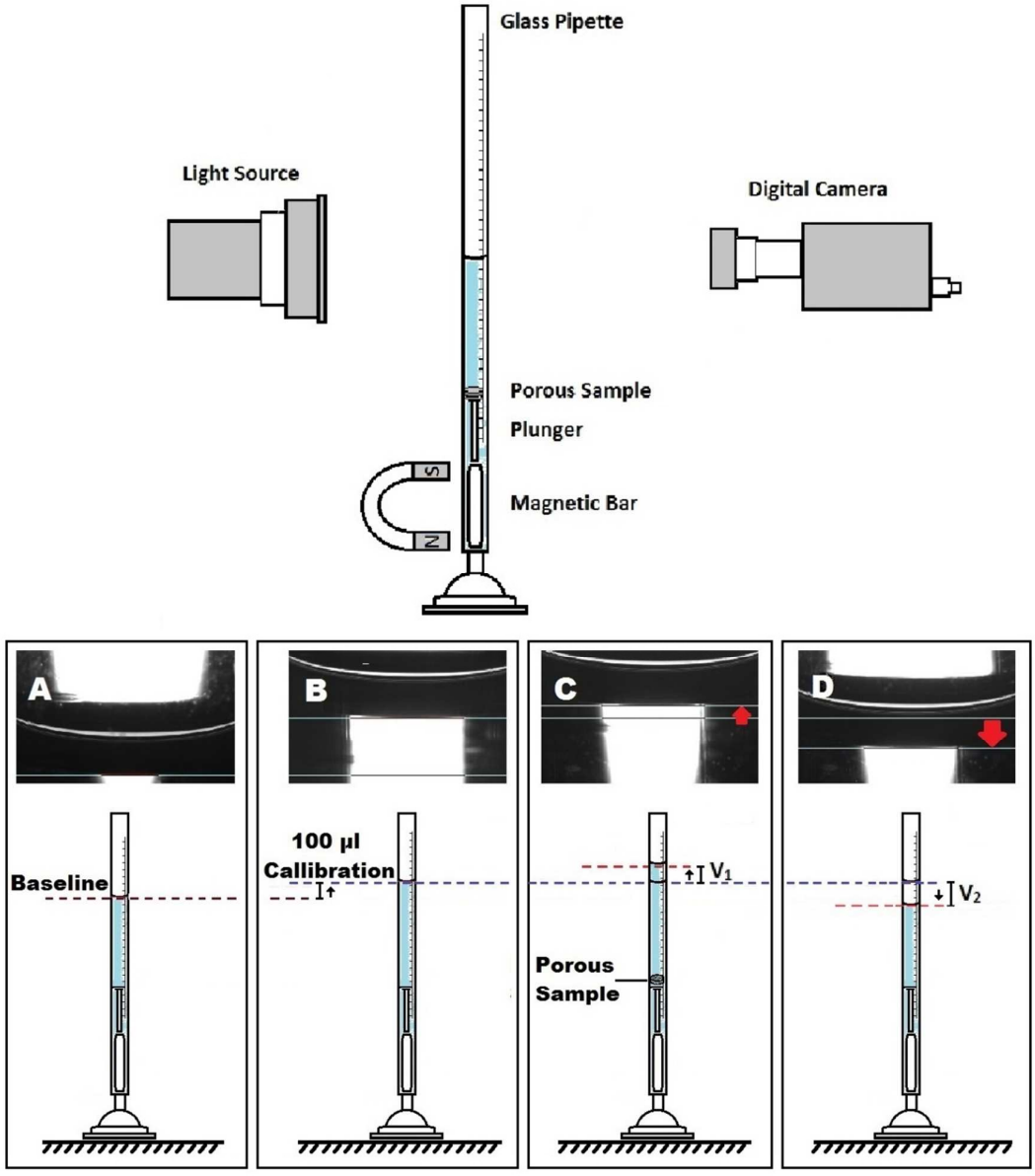
Graphical and textual abstract

Archimedes revisited: computer assisted microvolumetric modification of liquid displacement method for porosity measurement of highly porous light materials.



$$\text{Total Pore Volume} = \frac{V_2}{V_1 + V_2} \times 100$$

Archimedes revisited: computer assisted microvolumetric modification of liquid displacement method for porosity measurement of highly porous light materials.

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Abstract

Precise and accurate porosity measurement is essential in characterization of porous materials. Considering Archimedes' principle based liquid displacement methods of measuring porosity we have developed an excellent modified micro-volumetric method of porosity measurement. Changes in liquid level in a glass pipette after immersing and also removing the porous sample were recorded by a digital camera and analysed by ImageJ® software. Results of porosity measurement through micro-volumetric method were compared with micro-CT results. Bland-Altman analysis showed a much higher precision and accuracy for our micro-volumetric method (bias = -0.023, CI:]-0.459, 0.413[, SD = 1.96σ) compared to the micro-CT method (bias = 6.075, CI:]-20.993, 33.142[,SD = 1.96σ) . Our highly precise and accurate micro-volumetric method of porosity measurement is particularly applicable to small ultra-light highly porous materials.

Introduction

Porous materials find widespread applications almost in all the areas, covering engineering to medicine[1-4]. For these applications, the porosity and pore size have significant effect on

different properties of porous materials. For instance, porosity is a highly determinant factor in fabrication of three-dimensional (3D) scaffolds in tissue engineering to mimic the extracellular matrix as templates onto which cells of various tissues attach, proliferate, move and function[4, 5]. Pore density is important in fabrication of synthetic materials used in various applications such as filtration, bioreactors, analytical devices, prostheses and etc[6-8]. Therefore, it is essential to characterize the porosity of these materials precisely.

Due to the importance, various methods have been developed to characterize the porosity, such as (BET), Archimedes' principle based and computerized tomographic imaging techniques. Archimedes' principle based liquid[9, 10] and gas[11-13] displacement as well as computerize tomographic imaging[14] methods of porosity measurement are routinely used for porous material characterization. Eligibility of application of each technique for different porous materials depends on their physiochemical properties. Although micro-CT is assumed as a gold standard technique for porosity measurement especially in biomedical field, it is not applicable to non-opaque materials. Cost and time-consuming process of data acquisition especially at high resolution rendering and the need for high performance imaging machinery and users are still important concerns and hence measuring the porosity through other methods is considered. Probable invalid equation due to absence of a truly linear region, shrinkage of some samples (especially biomaterials and elastomers), need of degassing thermal preparations which may affect sample architecture as well as time consuming process, limited nitrogen gas adsorption with highly porous solid samples[15], restrictions with the minimum sample size (higher amount of sample is needed for porous samples)[16] and cost are some limitations with BET surface area analysers. Cost-effectiveness and feasibility have been the main reasons that Archimedes' principle based liquid displacement methods are still favourable. However, determining the porosity of non-opaque small sized highly porous light materials through these methods has always been a big concern. However Archimedes' principle based and micro-computerized tomography (micro-CT) techniques are comparatively better. In micro-CT, pore distributions from several tomographical images, generally developed

by X-ray, are combined together to get total porosity of small 3-D scaffolds. Variations of Archimedes' principle based liquid displacement method are still favourable due to their simplicity, inexpensiveness and ease of use for determination of porosity of solid materials with irregular shapes. Suspension, level, and overflow methods are three different modifications of liquid displacement using the same Archimedes' principle. It has been shown that suspension technique is the most preferred method in terms of accuracy and precision[17].

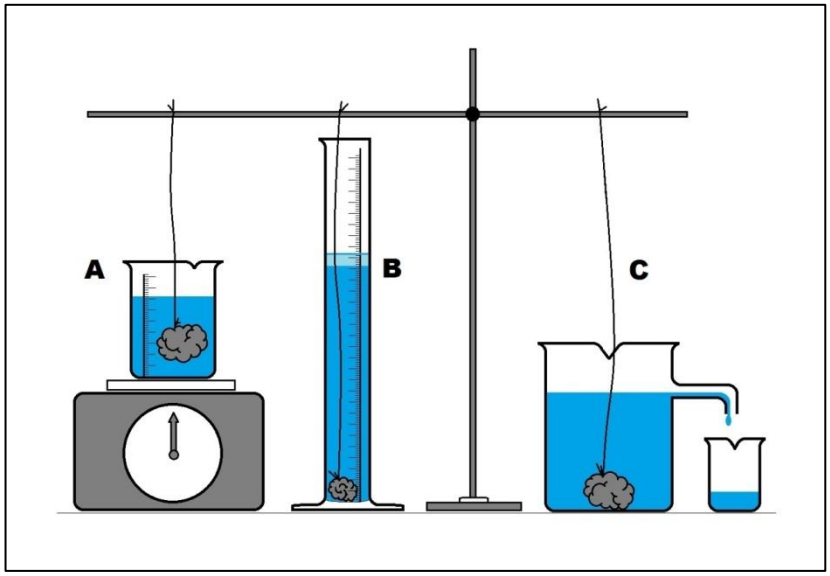

Figure 1: Schematic diagram of the A) suspension, B) level and C) overflow methods of measuring volume.

However, there are some limitations associated with these methods. For instance, the accuracy of each method depends on how delicate the weight or volume changes are read. On the other hand, precision and accuracy of the method depend on the readability of the weighing scale or volumetric readings. Moreover, the sample is to be suspended stationary in the liquid by means of a string line which can affect the readings by exerting a tension effect. Considering the weight and geometry of the sample, the string weight and volume may also not be negligible. Thus, suspension method cannot be used for small and very lighter objects. Overflow method on the other hand may not be applicable to very light and highly porous materials due to their geometry and the limitations caused by surface tension.

Therefore, there is still an unmet need for a much precise method for porosity measurement of ultra-light highly porous materials. In this study, we aim to develop a very

precise and accurate modification of the "Level" method, which is consistently applicable for very small and light objects up to the volume of only few microliters.

Materials and Methods

Standard Porous Samples

To assess the accuracy and precision of the method three standard porous samples were prepared. Two groups of porous alumina made of alumina/poly(ethylene oxide) composites with different ratios were fabricated by means of sintering. Briefly, 5.45 g and 5.62 g of poly(ethylene oxide) (density: 1.21 g/cc) were mixed homogeneously with 0.86 g and 0.81 g of Al_2O_3 (density: 3.95 g/cc), respectively and then the mixture powder was pelletized in a stainless steel mould at room temperature at 100 MPa uniaxial pressure using a hydraulic press. The pellets (ϕ10 mm $\times$ 5mm) were then sintered at 1000°C temperature for 3 hours in nitrogen atmosphere to get 65% and 70% porous alumina structures, called alumina1 and alumina2, respectively. Sintered porous glass bars (R & H Filter Co. Inc, Georgetown, USA) with dimensions of 4 mm x 5 mm x 50 mm and porosity of 61.5%, as supplied by manufacturer, also were selected as the third standard porous material (Figure 5A).

Experimental set up

A 10 ml glass pipette with an internal diameter of 7.2 mm was used for the experiment. The narrow tip of the glass pipette was cut and the other side was sealed with a rubber cap. A small magnetic bar (29x6 mm, 3 g) along with a modified insulin syringe plunger was inserted in the pipette to assist in taking out the soaked sample from the glass pipette. (Figure 2A)

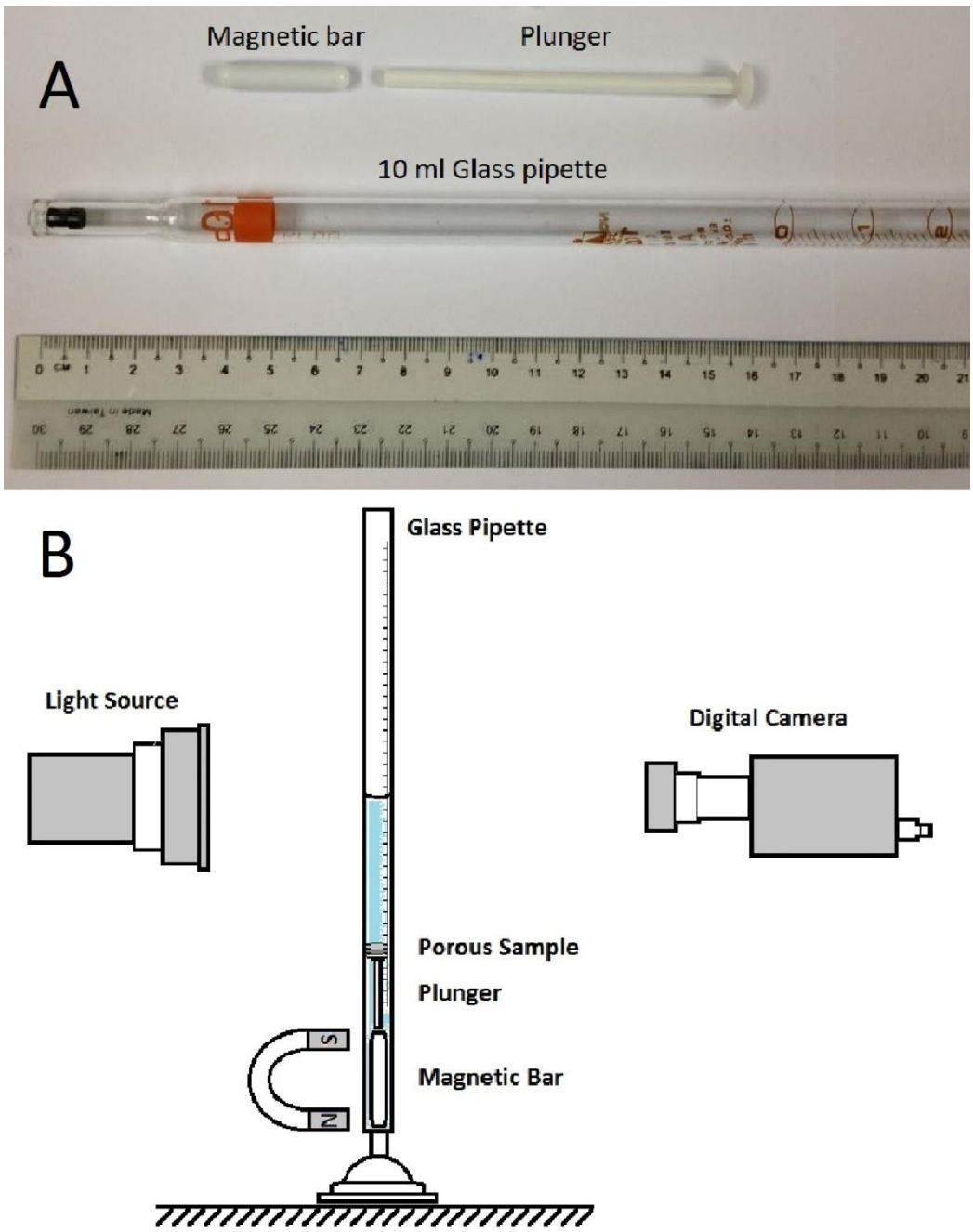

Figure 2: components of Microvolumetric liquid level displacement method for porosity measurement: A) magnetic bar, plunger from insulin syringe, and 10 ml glass pipette end sealed with rubber cap 2) Set up and vertical alignment of the measuring pipette and its internal components.

An *OCA 15EC* optical contact angle measuring instrument, *dataphysics*®, Germany was used to capture the images more perfectly during test. The sample table for contact angle measurement was replaced with the glass pipette, which was totally aligned and fixed vertically in between light source and the camera. (Figures 2B and 3)

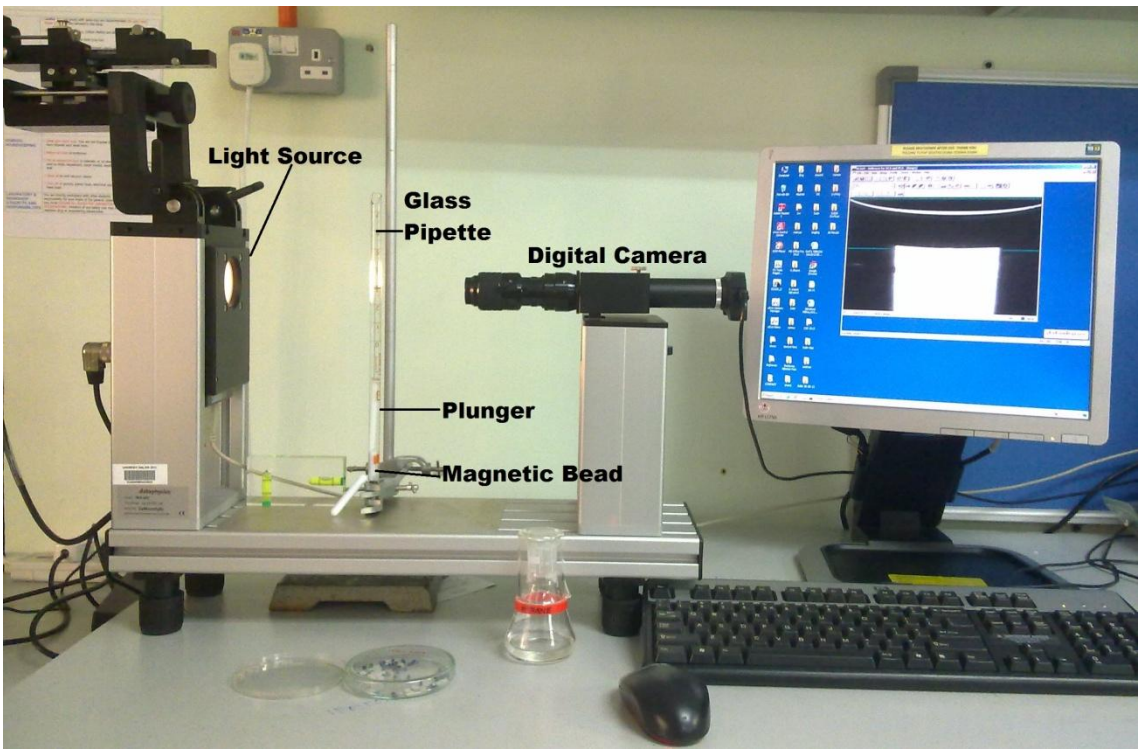

Figure 3: Laboratory set up for modified microvolumetric level displacement method for porosity measurement. The measuring glass pipette with its components aligned vertically in between the light source and the digital camera. The monitor shows the liquid level with the level cursor line.

The glass pipette was filled up to the lens vision level with liquid hexane (Fisher Scientific # H/0421/PB17), which can be clearly observed on the computer monitor. Hexane is as an inert, cheap, relatively safe and colourless liquid with a high evaporation rate (8.3). This volatility of hexane is an advantage since the liquid can easily reach into the sample very fast through its nano- or micro- channels without using any external pressure. In addition, when the sample is taken out from the liquid, the glass pipette wall dries faster, preventing the next sample to stick to the wall and not sinking into hexane. Using hexane is optional not obligatory and according to the solubility kinetics of the porous material which is being tested, the liquid in the glass pipette can be selected. The digital level marker was adjusted at the liquid level after proper focusing and the image was captured on the computer screen (Figure 4A). Using a micropipette, 100 µl of hexane was added to the previous level (Figure 4A) and the second image was captured with a new liquid level marker. The distance between the two markers was later used for scale calibration with the image processing software (Figure 4B). This calibration step is most important to validate our technique as precise and accurate. The new level was assigned as the new reference baseline.

Volumetric Measurements

Using a fine tip tweezer and side pinching with agility, the sample was moved into the standing pipette and let to sink. Once the sample was sat on the plunger surface, the elevation of the liquid level inside the pipette from the baseline was marked with a level cursor on the computer and the image was captured (Figure 4C). An external U-shaped permanent magnet was used to pull up the suspended sample, which was sitting on top of the plunger, with help of inside magnetic bar in the glass pipette, and then it was removed with the tweezer from the glass pipette. The plunger was left to settle back down the pipette. The liquid level marker was established and the last image was captured (Figure 4D).

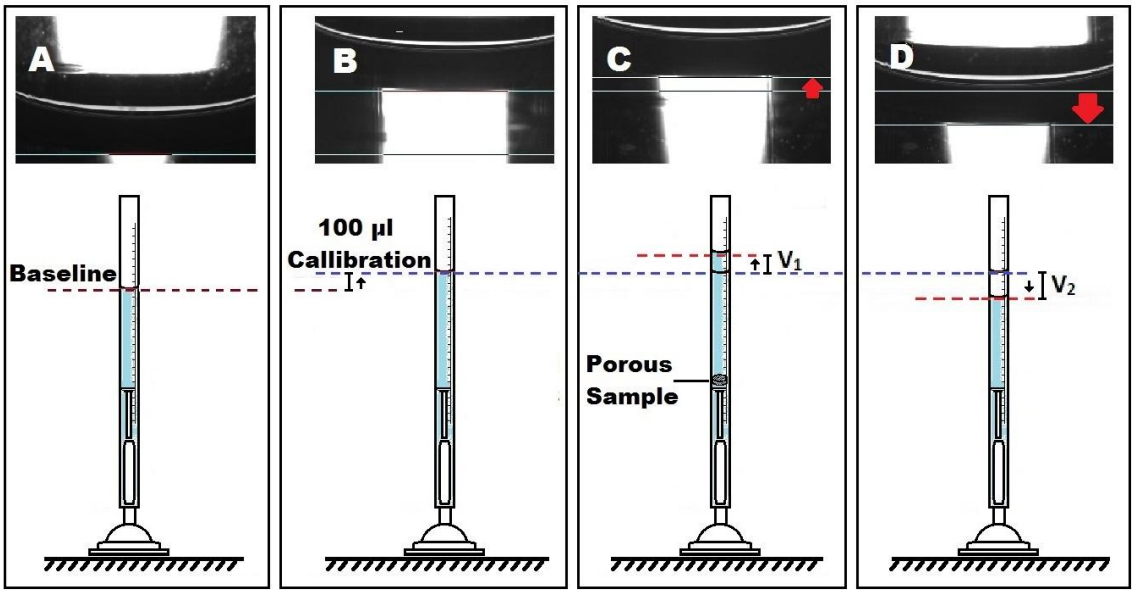

Figure 4: Microvolumetric level method for porosity measurement: A) Baseline establishment, B) Level calibration with 100 µl volume and second level establishment, C) V1: level elevation after immersing the sample, and D) V2: Volume depletion after removing the sample.

Changes in the hexane level in the pipette from the baseline after calibration were recorded. When the sample was immersed in the pipette, it sank into the hexane and sat on top of the plunger surface and the hexane level elevated to a higher level compared to the baseline. This volume change was recorded by the digital camera and called (V_1), representing the absolute volume of the sample. As the camera was already focused on the liquid level in the glass pipette, the duration of time between dropping the sample into the glass pipette, sinking, settling on the plunger surface, and capturing the image was not too long to affect the accuracy of the experiment due to fast evaporation effect. After removing the sample the

hexane level droped to a lower level compared to the baseline. The image of this volume depression which was called (V_2) and represented the pore volume in the sample was captured by the camera using a SCA20 software version 4. 2. 4. (*dataphysics®* Instruments). Changes in the hexane level were precisely measured through length measurement using ImageJ® 1.47v software. The volume calibration image was used to convert length to volume through imageJ® software.

Percentage of total pore volume was calculated as:

$$\text{Total pore volume} = \frac{V_2}{V_1+V_2} \times 100\ \%$$

Where, V_1 is volume change after immersing the sample (absolute volume = volume of solid matter without pores) and V_2 is volume change after removing the sample (pore volume).

Micro-CT

Micro-CT has widely been used as one of the best techniques to show the total pore volume and porosity present in a material with complex geometry[1-3, 18]. Therefore, three replicates of each standard porous group were subjected to micro-CT imaging for porosity analysis using a SkyScan-Bruker 1076 *in vivo* micro-CT (parameters: voltage: 59 kV, current: 100 uA, filter: Al 0.5 mm, resolution: 18 µm, rotation angle: 180°, rotation step: 0.7°, and number of scan slice: 100). Samples were mounted on polystyrene foam prior to CT scanning.

Field Emission Scanning Electron Microscopy (FESEM)

FESEM was performed using a Quanta™ 250 FEG – FEI microscope to confirm the outer surface porosity of the fabricated porous samples.

Statistical Analysis

A Bland-Altman analysis[19, 20] was used to assess agreement between two methods of porosity measurement with the actual values. XLSTAT® version 2014.1.05 package for Excel® (2007) was used for data analysis. A range of agreement was defined as mean bias ±2 standard deviation (SD).

Results and Discussion

Actual size of the sintered porous pellets from three different porous materials is shown in Figure 5A. Micro-CT and FESEM images shown in Figures 5B and 5C-E clearly show the porous structure of representative porous alumina1 and alumina12, and glass samples.

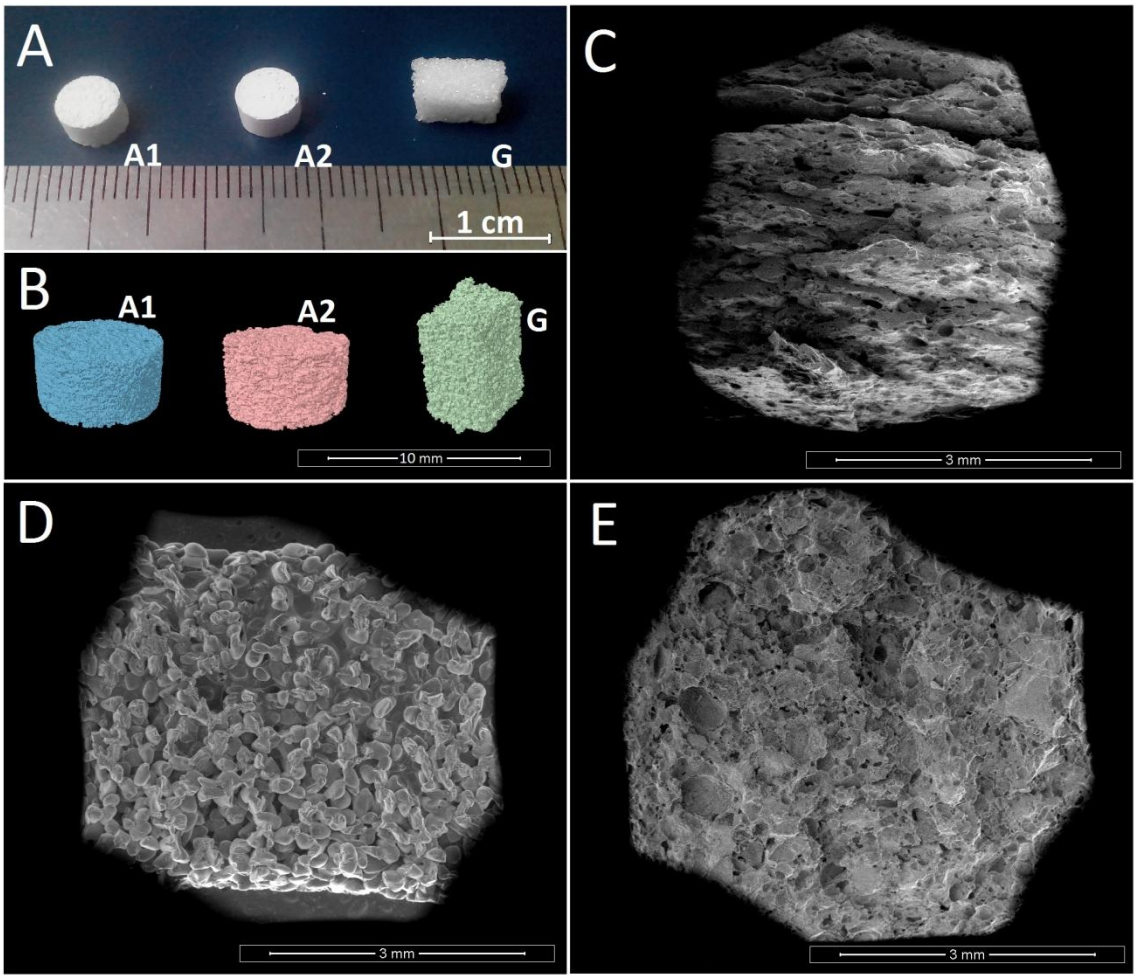

Figure 5: Digital photographs (A), Micro-CT 3D rendering images (B), and FESEM micrographs (C-E) of representative porous alumina1, glass, and alumina2 samples respectively.

The original results of porosity measurement by micro-volumetric and micro-CT methods along with the actual porosities for two types of porous different sintered alumina scaffolds and glass scaffolds are illustrated in Table 1. As it can be seen, total mass volume of the samples are to the scale of only few hundred microliters and volume measurements have been done to the resolution of less than one microliters.

Table 1: Micro-volumetric and micro-CT porosities compared with the actual porosity, mean and difference between each method and the actual value.

Samples	V_1 (µl)	V_2 (µl)	Actual Porosity (%)	Porosity from micro-volumetric (%)	Mean: Actual and Micro-volumetric	Difference: Actual and Micro-volumetric	Porosity from micro-CT (%)	Mean: Actual and Micro-CT	Differen and M
Alumina 1-1	59.2	112.5	65	65.5	65.3	-0.5	40.6	52.8	2
Alumina 1-2	55.6	107.5	65	65.9	65.5	-0.9	74.1	69.6	-
Alumina 1-3	56.6	109.5	65	65.9	65.5	-0.9	79.1	72.0	-1
Alumina 2-1	66.7	142.2	70	68.1	69.0	1.9	73.3	71.6	-
Alumina 2-2	61.9	131.9	70	68.0	69.0	2.0	83.4	76.7	-1
Alumina 2-3	55.6	119.4	70	68.3	69.1	1.7	84.3	77.1	-1
Glass 1	172.8	274.5	61.5	61.4	61.4	0.1	55.9	58.7	5
Glass 2	241.4	389.9	61.5	61.8	61.6	-0.3	70.9	66.2	-
Glass 3	107.5	170.8	61.5	61.4	61.4	0.1	79.6	70.5	-1

Figure 6 compares the measured porosity through micro-volumetric and micro-CT methods with the actual porosity of porous glass, alumina1, and alumina2 materials. While the values obtained from micro-volumetric method are almost equal to the actual porosities, with small standard deviations, the values gathered from micro-CT are different from actual porosity and have very big standard deviations.

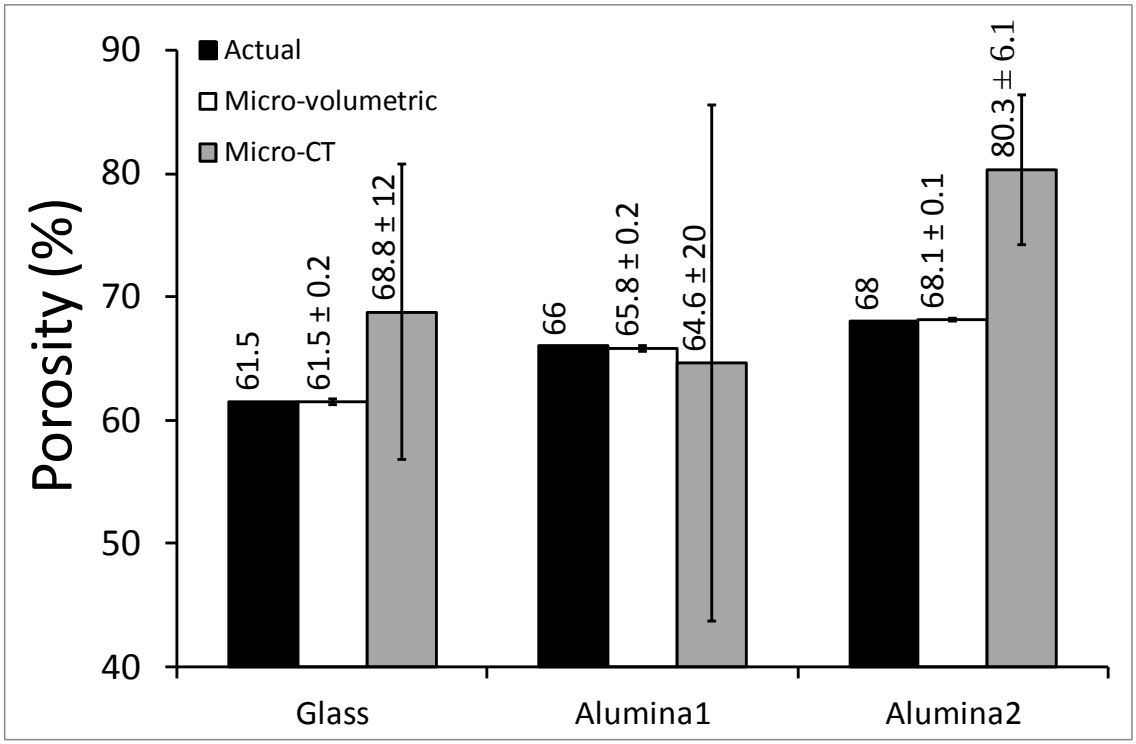

Figure 6: Comparison between the measured porosity through micro-volumetric and micro-CT methods with the actual porosities of porous glass, alumina1, and alumina2.

Figure 7A shows the scatter plot of measured porosity through micro-volumetric and micro-CT methods versus actual porosity values. There is a strong correlation coefficient of R^2=0.92 for micro-volumetric and actual porosities while that of micro-CT method and the actual porosities is only 0.14, indicating a weak correlation between the micro-CT and actual porosities. Figure 7B represents the Bland-Altman plot of the difference versus mean porosities of each pairs of data, indicating an almost consistent difference at various average porosities between the micro-volumetric and actual porosity compared to the changing difference for different values of mean porosity between micro-CT and the actual.

Figure 7C also shows the Bland-Altman plots of the data with the bias and precision lines for both micro-volumetric and micro-CT methods. The accuracy (bias= -0.023) and precision

(confidence interval: -0.459 , 0.413) for micro-volumetric method is much higher than that of micro-CT method (Bias = 6.1, CI: -21 , 33.1).

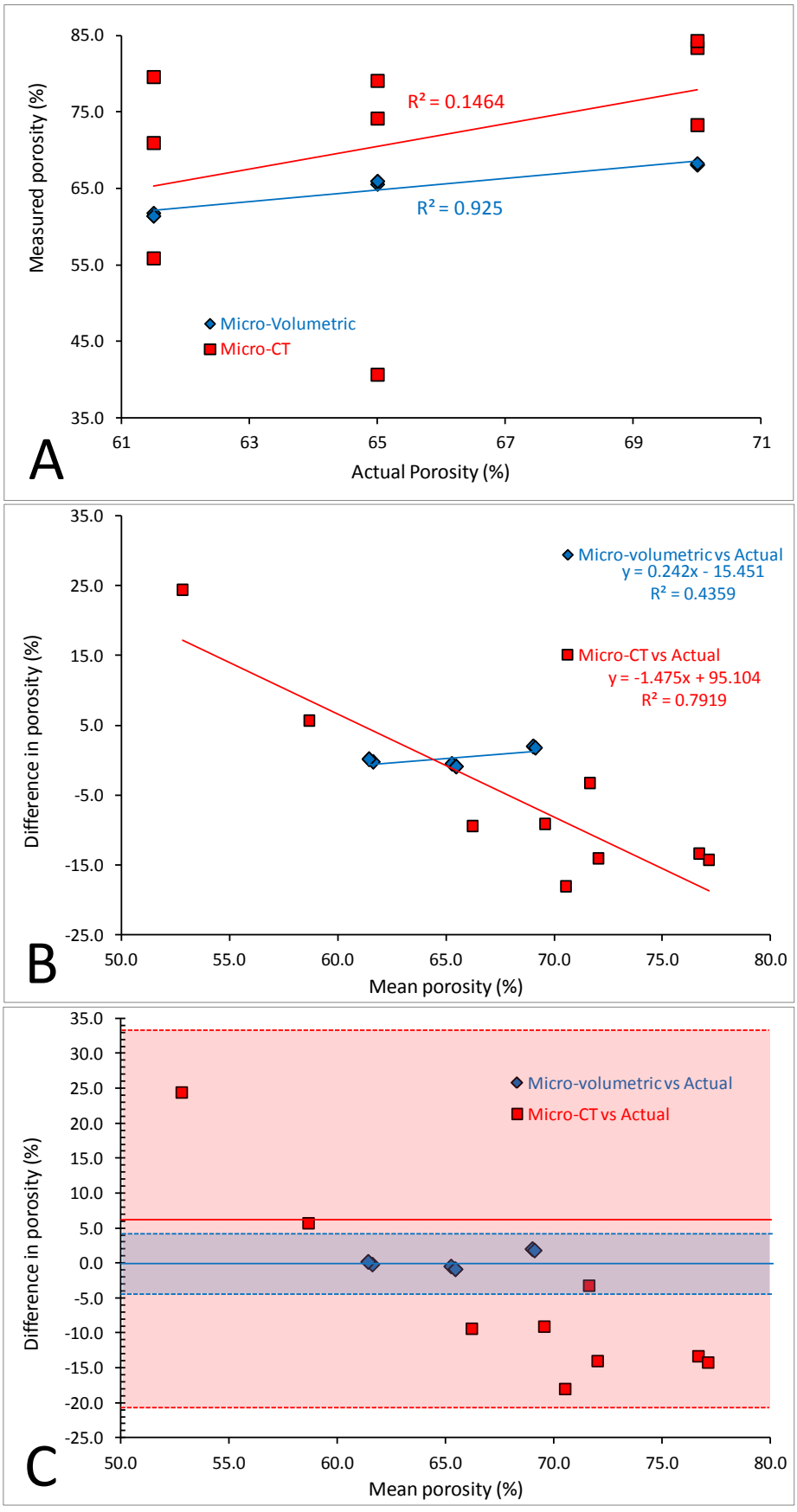

Figure 7: A) Scatter plot of measured versus actual porosity and the corresponding regression coefficients for Micro-volumetric and Micro-CT methods, and B) scatter plot of difference versus mean porosity of Micro-volumetric and Micro-CT with actual porosity, and C) Bland-Altman plots of the data showing the level of agreement between measured and actual porosities. The solid lines indicate the accuracy and the square doted lines represent the precision of the two techniques. 95% of the data are expected to fall within ±1.96σ.

Conclusions

We have presented a revised modification of Archimedes' principle based liquid level displacement method to increase the accuracy and precision of porosity measurements of small light highly porous materials for many advanced applications. Our method consists of using a pipette as a more delicate measuring cylinder along with the improved sample loading and removal system, and taking advantage of digital imaging and computer assisted image processing to increase the performance of liquid level change readings.

Our findings show that the precision and accuracy of our modification of Archimedes' method of porosity measurement is much higher than that of Micro-CT. Although each group of our standard samples were fabricated with constant conditions, micro-volumetric method shows similar results with a small standard deviation for each type of construct, while micro-CT method fails to do so. We achieved an excellent precision and accuracy in porosity measurements. The readability of microvolumetric method is down to 1 µl volumes.

This method is applicable to any porous material regardless of size, opacity, lightness, shape and degree of porosity. There is no need for degassing or any other sample preparation in our method. Since the volume of the ultra-light weighed sample is measured by the present microvolumetric method, it would be extremely useful in forensic test where the amount of sample is very precious. This modified microvolumetric method is comparatively faster than the other methods since it has no extra sample preparation or other processing steps. Taking advantage of the magnetic bead/plunger escalator technique instead of a variable size string to suspend the sample, high performance lens with integrated continuous fine focusing ability, and adjustable observation and camera tilt angle, minimize the measurement errors and brings in a high precision and accuracy to the measurements.

Microvolumetric level method is one of the most accurate and precise methods of porosity measurement for ultra light highly porous materials. Simple design, feasibility, open source ImageJ® software and straight forwarded results all are the benefits of this modification.

Acknowledgement

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