The saturation equation - an analytical expression for partial saturation during wicking flow in paper microfluidic channels

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Abstract

The design and fabrication of paper based microfluidic devices is critically dependent on modelling fluid flow through porous paper membranes. A commonly observed phenomenon is partial saturation, i.e., regions of the paper membrane not being filled completely due to pores of different sizes. The most comprehensive model till date of partial saturation during wicking flow in paper is the Richard’s equation. However, solution of the Richard’s equation requires numerical solvers like COMSOL, which makes it largely inaccessible to the paper microfluidics and lateral flow assay community. There is therefore a need for a simple and appropriate model of partial saturation in paper membranes, easily usable by the wider research community. In the current work, we present an approach to model paper membranes as a bundle of parallel capillaries whose radii follow a two parameter log normal distribution. Application of the Washburn equation to the bundle provides a distribution of fluid fronts, which can be used to calculate saturation. Using this approach, we developed the “saturation equation” – an explicit analytical expression to calculate saturation as a function of space and time in 1D wicking flow. Experimentally obtained data for spatiotemporal saturation for four different paper materials were fit to this analytical model to obtain parameters for each material. Results obtained from this analytical model match both experimental data as well as numerical results obtained from the Richards equation well. The availability of an explicit analytical expression for partial saturation will enable incorporation of the critical phenomenon of partial saturation in the design of paper microfluidic devices.
Introduction

Medical diagnostics has been a critical area of research, especially after the global pandemic. There is a specific emphasis on rapid, accessible, and cost-effective diagnosis, and the need for the development of point-of-care tests has dramatically increased\(^1\). Paper is used as one of the substrates to conduct these tests, one of the major reasons being negative capillary pressure driven flow, eliminating the need for external pumps or power sources, making it point-of-care compatible\(^2,3\). However, a big challenge is the design and fabrication of these devices, which involves trial and error for optimization\(^3\). A key aspect of this process includes understanding flow behavior in these membranes. There have been multiple attempts to develop models for fluid imbibition in porous materials\(^3\)–\(^10\). The most commonly used models for fluid flow include the Lucas Washburn Equation\(^11\) and Darcy’s law\(^5,7,8\). Lucas Washburn assumes paper to be a bundle of capillaries having the same pore diameter and provides the location of the fluid front in the membrane as a function of time, while Darcy’s law is a phenomenologically derived constitutive relation that relates flowrate to the pressure gradient across the membrane.

In recent years, studies on fluid flow through papers microfluidic channels have revealed the phenomenon of “partial saturation”, wherein although the fluid front reaches a certain location in some time (referred to as apparent fluid front from now on), the membrane is not completely saturated till that point, i.e., there are pores in the membrane that have not been filled completely\(^12\)–\(^14\). Both Darcy’s law and Washburn model fail to account for partial saturation. Some attempts have been made to adapt these models to account for partial saturation. Notably, Cummins et al\(^5\) developed a novel method to predict spatiotemporal variation of saturation by experimentally measuring pore size distribution and applying Darcy’s law to capillaries of different sizes.
However, experimental measurement of pore size distribution is cumbersome and not accessible to many researchers. Moreover, the mathematical implementation of the technique is challenging, preventing its usage by the larger paper microfluidics community.

Another method, and perhaps the most powerful tool that has emerged to model partial saturation in paper membranes is the Richard’s equation\textsuperscript{15}. Widely used for modelling flow in soil, the technique has recently been used by several researchers to model flow through paper membranes. The Richard’s equation relies on using empirical functional relationships for capillary pressure and permeability as a function of saturation, e.g., Brooks and Corey\textsuperscript{16}, and Van Genuchten\textsuperscript{17}. Buser\textsuperscript{18}, and subsequently our group\textsuperscript{10}, have described experimental methods to measure capillary pressure as a function of saturation. However, we have observed that while modelling flow in paper using the Richard’s equation, parameters of these empirical relationships often need to be tweaked or guessed to get an accurate prediction. For instance, in a previous publication\textsuperscript{10} from our group, parameters were obtained by fitting experimental data to the Van Genuchten formulation for modelling flow through a nitrocellulose membrane. One of the Van Genuchten parameters, $\alpha$, had to be changed from a value of 0.1 to 1 to get the model to match exactly with the experimental results. In another work, Wang et al.\textsuperscript{19} included an additional term in the Richard’s equation to account for the dynamic change in capillary effect in paper and showed an improvement in performance of the model, but there were still discrepancies between the model predictions and experimental data. It is possible that the difference in microstructure of paper and soil, the latter being the medium for which such empirical models were originally developed, makes them less applicable to paper membranes\textsuperscript{20}. In addition to the above, and importantly so, the solution of the highly nonlinear Richard’s equation requires numerical solvers, e.g., COMSOL, which ultimately
restricts its usage by the wider community. Therefore, despite being a critical phenomenon that may affect the performance of assays in paper\textsuperscript{10}, partial saturation has remained a mere theoretical concept. The reason that the Washburn equation and Darcy’s law are so popular among paper microfluidics researchers is that they are simple analytical expressions that do not require numerical solvers. Therefore, there is a need for a model that is as simple to use as the Washburn equation, and yet incorporates partial saturation.

In this article, we overcome this gap by developing an explicit analytical expression for spatiotemporal saturation during 1D wicking in paper. We accomplish this by modelling paper membranes as bundles of parallel capillaries, the flow in each capillary being governed by the Lucas Washburn equation. We propose that the radii of these capillaries follow a two-parameter log normal size distribution. The model was tested on 4 different paper materials; the two parameters for each material were evaluated by fitting experimental data for spatiotemporal variation of saturation to the analytical expression. With only 2 parameters per material, the model accurately predicts spatiotemporal partial saturation in 1D wicking flow, and the results are comparable to those produced by the Richards equation. By the provision of an explicit analytical expression relating saturation to space and time in 1D wicking, this model promises to significantly increase access to modelling partial saturation among paper microfluidics researchers.

**Experimental Section**

**Paper Materials and Characterization**

Four different commercially available paper materials were used. Three grades of nitrocellulose (FF80HP Plus, FF120HP, FF170HP Plus), and Whatman filter paper grade 1, were procured from
Wipro GE Healthcare Pvt. Ltd., (Whitefield, Bengaluru, India). Paper strips of desired dimensions were drawn using AutoCAD (Autodesk, San Rafael, CA, USA) and then cut using a 50W CO₂ laser cutter (Universal Laser Systems, Scottsdale, AZ, USA). The porosity of each material was obtained by measuring the difference between dry weight and weight at 100% saturation of 2 cm × 0.4 cm pieces of the membrane. These values were further validated with the porosity previously reported by Rath et al.¹⁰

**Measurement of spatiotemporal variations of saturation**

The experimental setup was similar to the one used in a previous publication from our group.¹⁰ Rectangular paper strips (1 cm x 10 cm) were cut for each paper membrane using the laser cutter and placed on a backing card (2 cm x 11 cm). The backing card was graduated with marks every 1 cm made using the laser cutter. Deionized water (DI water) was introduced into the strip using a reservoir connected to one end. The strip was placed inside a custom-made acrylic humidification chamber. A hole was drilled at the top of the chamber and at one of the sides - the top hole was to provide an inlet to introduce sample (DI water) into the reservoir, while the hole on the side was to insert a humidity probe for measuring the relative humidity. The humidity inside was maintained above 80% at all times by introducing wet paper towels in the chamber, in order to minimize evaporation losses. The sample was introduced into the reservoir, and the flow was stalled after a certain amount of time by disconnecting the strip from the reservoir. Thereafter, the disconnected strip was quickly cut at the graduations, and weights were measured. These weights were used to get saturation, as 

\[
S_e = \frac{W - W_{dry}}{W_{sat} - W_{dry}}
\]

which was then plotted as a function of distance from the fluid source at a given time. Similar experiments were conducted at different time points to get the spatiotemporal variation of saturation. At each time, 3 or 4 experimental replicates were conducted.
Estimation of Washburn Coefficients

Washburn coefficients for each paper membrane were obtained by tracking the fluid front over time during wicking. Rectangular strips, 1 cm x 6 cm, of the four paper membranes were cut with a laser cutter and placed on a backing card. The backing card was graduated with marks every 5 mm using the laser cutter. DI water was introduced into a fluid reservoir connected at one end of the paper strip, and the time taken by the apparent fluid front to cover every 5 mm was noted.

Capillary Pressure and Permeability Measurements

The output of the newly developed model in this work was compared to the output of the better-established Richard’s equation. To assist in Richard’s equation modelling, capillary pressure as a function of saturation, and permeability at 100% saturation, were measured experimentally using techniques described previously\textsuperscript{10}. Permeability as a function of saturation is then calculated using a theoretical method described previously\textsuperscript{10}. The details of this are mentioned in Electronic Supplementary Information (ESI S1).

Theory and Modelling Section

The Lucas Washburn equation is one of the simplest models to describe wicking flow in paper membranes, and its functional form is:

\[
L^2 = \frac{\gamma r_{pore} t}{2\nu} \tag{1}
\]

where, \(r_{pore}\) is the effective radius of the pore, \(\gamma\) is the surface tension of the fluid-air interface (including contact angle dependence), \(\nu\) is the dynamic viscosity of the fluid, and \(L\) is the length travelled by the fluid in time \(t\). In this form of the equation, paper is modelled as a bundle of
capillaries, all having a single pore radius, $r_{\text{pore}}$. Therefore, it predicts a sharp fluid front at length $L$ in the paper at time $t$, and fails to account for partial saturation. The profile for saturation, $\theta$, according to the Lucas Washburn equation is as follows:

$$\theta(x, t) = \begin{cases} 1, & x \leq L(t) \\ 0, & x > L(t) \end{cases}$$

(2)

**Figure 1.** Paper modelled as a bundle of parallel capillaries of varying diameters.

Here, we propose that this limitation may be overcome by modelling paper as a bundle of capillaries of varying diameters, characterized by a known size distribution. Application of the Lucas Washburn Equation to each capillary then leads to a distribution of lengths travelled in the capillaries, as shown in Figure 1. Fluid travels longer lengths in pores with larger diameters, as inferred from equation (1). Figure 2 shows that the non-uniform size distribution of capillaries has
an implication that there are sections of the membrane where smaller capillaries are empty, while larger capillaries are filled, making these sections partially saturated.

Now, an appropriate size distribution for these parallel capillaries needs to be selected. While the model does not demand an accurate size distribution, it is still necessary for the distribution to be realistic. Cummins et al.\textsuperscript{5} performed mercury porosimetry to get pore size distribution of filter paper, and their findings suggest a distribution of pore sizes that is skewed towards smaller pore sizes compared to a normal distribution. A realistic distribution would also be the one where the random variable (pore size) can hold only positive values. One of the simplest approaches in this context could be the use of a log-normal distribution, which generally fits well for skewed distributions and has been commonly used for porous materials\textsuperscript{21–27}. The log-normal distribution considering pore radius, $r$, as the random variable is given as follows:

$$f(r) = \frac{1}{r \sigma \sqrt{2\pi}} \exp\left(-\frac{(\ln(r)-\mu)^2}{2\sigma^2}\right)$$ \hspace{1cm} (3)

This expression is for the fraction of capillaries that have a radius in between $r$ and $r + dr$. Here, there are two parameters, $\mu$ (which is the natural logarithm of the median radius) and $\sigma$ (standard deviation of the natural logarithm of the pore radius), the defining characteristics of the distribution. Based on equation (1), the length traversed by fluid in a capillary of radius $r$ can be written as:

$$L = A \sqrt{rt}, \text{ where } A = \sqrt{\frac{y}{2v}}$$

By performing a variable transformation, and substituting $r = \frac{L^2}{A^2t}$, the distribution in (3) gets transformed to:
\[
f(L) = \frac{2}{L \sigma \sqrt{2\pi}} \exp \left( -\frac{(\ln(L^2) - (\mu + \ln(\lambda^2)))^2}{2\sigma^2} \right)
\]  

(4)

This represents the fraction of capillaries in which fluid has travelled a length between \( L \) and \( L + dL \) at time, \( t \). The fraction of capillaries where fluid has travelled lengths between \( L_1 \) and \( L_2 \) is given as:

\[
F(L_1 \leq L \leq L_2) = \int_{L_1}^{L_2} f(L) \, dL
\]  

(5)

Hence, to obtain the fraction of capillaries where fluid has travelled lengths between 0 and \( x \), the relation is given as:

\[
F = \int_0^x f(L) \, dL
\]  

(6)

Equation (6) represents the fraction of capillaries in which fluid has travelled a length between 0 and \( x \). So, if we place ourselves at any position, \( x \), this is the fraction of pores that are not saturated because fluid has not yet reached that point. It follows that the complementary fraction of pores (1 – \( F \)) are saturated at position \( x \).

The volume occupied by this fraction of unsaturated pores is given as:

\[
V_{unsaturated} = \int_0^x \pi r^2 L f(L) \, dL
\]  

(7)

Total volume occupied by all pores can be calculated as:

\[
V_{total} = \int_0^\infty \pi r^2 L f(L) \, dL
\]  

(8)

Hence, the volume occupied by saturated pores is given as \( V_{saturated} = V_{total} - V_{unsaturated} \), and saturation is given as fraction of the total volume that is saturated, i.e., \( \theta = \frac{V_{saturated}}{V_{total}} \). Thus, saturation can be written as:

\[
\theta = 1 - \frac{\int_0^x \pi r^2 L f(L) \, dL}{\int_0^\infty \pi r^2 L f(L) \, dL}
\]  

(9)
The solution to this integral involves multiple variable changes and substitutions and is shown in the ESI S2. After the integration, Equation (9) finally reduces to:

$$\theta(x, t) = \frac{1}{2} \text{erfc} \left( \frac{\ln(x^2) - \mu \ln(A^2t) - \frac{5}{2}\sigma^2}{\sqrt{2}\sigma} \right)$$

(10)

Where erfc stands for the complementary error function, which can be readily calculated in even basic programs like Microsoft Excel. Here, parameters $\mu$ and $\sigma$ come from log-normal distribution of pore radii. In this work, these parameters have been estimated considering pore radii to be in units of $\mu$m. Also, the parameter $\mu$ represents the natural logarithm of the median radius in the distribution. Let us assume $\mu = \ln(r_m)$, so the numerator in equation (10) becomes $\ln\left(\frac{x^2}{r_mA^2t}\right) - \frac{5}{2}\sigma^2$. The term inside the logarithm must be dimensionless. The constant $A$ comes from Lucas Washburn equation, and the units of $A^2$ are m/s. Time, $t$, must be in seconds, and the distance from the fluid source, $x$, must be in meters. Since, $r_m$ is in $\mu$m, thus we multiply $10^6$ to $x^2$ in order to make this term dimensionless. Thus, equation (10) can be finally written as:

$$\theta(x, t) = \frac{1}{2} \text{erfc} \left( \frac{\ln(10^6x^2) - \mu \ln(A^2t) - \frac{5}{2}\sigma^2}{\sqrt{2}\sigma} \right)$$

(11)

Equation (11) is an analytical expression for saturation, which we would now on refer to as the “Saturation equation”. This equation returns the saturation as a function of space, $x$, and time, $t$, for any porous material, which can be represented by two parameters: $\mu$ and $\sigma$. In this work, values used for capillary pressure, $\gamma$, and viscosity, $\nu$, are 0.072 N/m and $8.9 \times 10^{-4}$ Pa.s, respectively, assuming the fluid to be water. Accordingly, the value used for $A = \sqrt{\gamma/2\nu}$ was 6.36 m$^{0.5}$s$^{-0.5}$. 

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Results and Discussion

Effects of Parameters

As a demonstration of the output of the saturation equation, profiles for saturation obtained using equation (1) for arbitrary parameter values of $\mu = 2$ and $\sigma = 0.5$ are plotted in Fig. 2A. These profiles show that regions near the fluid source ($x=0$) are fully saturated ($\theta = 1$), and there is a nonlinear decline in saturation with increasing distance. These profiles have similar shapes as reported in the literature\textsuperscript{5,10}. Experimental data for these profiles will subsequently be shown in this article.

The effect of parameters $\mu$ and $\sigma$ on the saturation profiles at a fixed time, $t = 0.5s$, is demonstrated next. Note that for a log normal distribution, the mean is given by $\exp\left(\mu + \frac{\sigma^2}{2}\right)$ and variance is given by $(\exp(\sigma^2) - 1)(\exp(2\mu + \sigma^2))$. Increasing $\mu$ at a fixed value of $\sigma$ would imply an increase in the mean pore size. This leads to the fluid fronts reaching longer distances at a fixed time point (Fig. 2B). On the other hand, when $\mu$ is kept fixed and $\sigma$ is increased, it primarily increases the variance. For the limiting case of $\sigma = 0$, the profile manifests itself as a step change in saturation as all capillaries would be of the same size, and the fluid fronts in all capillaries would be at the same location (Fig. 2C; blue plot). As $\sigma$ increases, the diversity of pore sizes increases, and fluid fronts deviate more from the step change and become more diffuse (Fig. 2C).
**Figure 2.** Illustration of the output of the saturation equation. (A) Saturation profiles at different time points as predicted by the Saturation Equation for $\mu = 2, \sigma = 0.5$; (B) Effect of $\mu$ at $\sigma = 0.5$ and $t = 0.5\, s$; (C) Effect of $\sigma$ at $\mu = 2$ and $t = 0.5\, s$

**Parameter estimation**

To get the parameters $\mu$ and $\sigma$ for each material, experimental data for spatiotemporal variation of saturation was fit to the saturation equation (Equation 11) using the `curve_fit` function from the `scipy.optimize` package in Python. The full data set was split into two sets in an 80:20 training:test ratio. Datapoints in the training set were used to obtain the parameters, while the ones in the test set were used to check the performance of the model on unknown datapoints. Goodness-of-fit metrics, $R^2$, mean absolute error (MAE), and mean squared error (MSE) were calculated for both the training and test data sets. Further, to test the robustness of the model to changes in the training and test datasets, a well-known machine learning algorithm called k-fold cross validation was used, where a different fraction of the training data was used for training each time. The number of folds used was 4, in accordance with the size of the training data available. Before splitting into folds, the dataset was shuffled with a random state of 42. Metrics $R^2$, MAE, and MSE were calculated for each fold and their average values are reported.

Fitted parameters thus obtained are shown in Table 1. Based on these parameters, the pore size distributions obtained for each membrane are shown in Fig. 3. Table 2 shows the mean, median,
and standard deviation of the log-normal distribution, calculated using the fitted parameters obtained. It is observed that the mean pore size follows the trend: NC FF80 HP Plus > NC FF120 HP > NC FF170 HP Plus > filter paper (Table 2). Experimentally measured wicking flow rates follow the same trend, i.e., fastest in NC FF80 HP Plus and slowest in filter paper. This is evident from the Washburn Coefficients which have been experimentally measured as $4.36 \times 10^{-3}$, $3.68 \times 10^{-3}$, $3.27 \times 10^{-3}$, and $2.78 \times 10^{-3}$ ms$^{-0.5}$ (ESI S3). This corroborates the measured trend of mean pore sizes as fluid is expected to flow faster through membranes with larger pore sizes. Note that the distribution shows much smaller pore sizes for these membranes as compared to what is reported in literature$^5,10,28$. For example, the mean pore size for grade 1 filter paper estimated by the model is 0.08 µm while it is reported to have pore sizes in the range of 1-20 µm by Cummins et al$^5$. This is because here we assume the paper membrane to be a bundle of rigid, straight, and parallel capillaries. In reality, paper is a tortuous network of interconnected capillaries of varying pore cross sectional shapes and sizes. The distribution in Fig. 3 is thus hypothetical and limited only to the use of $\mu$ and $\sigma$ as parameters to be used in the saturation equation (Equation 11) for the modelling of partial saturation. Technically, one could attempt to include tortuosity, interconnection, and swelling in this model to predict a more accurate pore size distribution, but that is not the objective of this work.

\[ \text{Table 1: Model parameters} \]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NC FF 80 HP Plus</th>
<th>NC FF120 HP</th>
<th>NC FF 170 HP Plus</th>
<th>Whatman Filter Paper Grade 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$</td>
<td>$-1.34 \pm 0.1$</td>
<td>$-2.04 \pm 0.07$</td>
<td>$-2.24 \pm 0.11$</td>
<td>$-2.72 \pm 0.22$</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>$0.43 \pm 0.22$</td>
<td>$0.46 \pm 0.13$</td>
<td>$0.49 \pm 0.17$</td>
<td>$0.68 \pm 0.13$</td>
</tr>
</tbody>
</table>
Figure 3. Pore size distributions generated by the model for (A) NC FF80 HP Plus (B) NC FF120 HP (C) NC FF170 HP Plus (D) Whatman Filter Paper Grade 1. The dotted line shows the peak of the probability density function corresponding to median pore radius.

Table 2: Pore size distribution characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NC FF 80 HP Plus</th>
<th>NC FF120 HP</th>
<th>NC FF 170 HP Plus</th>
<th>Whatman Filter Paper Grade 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Radius (μm)</td>
<td>0.29</td>
<td>0.15</td>
<td>0.12</td>
<td>0.08</td>
</tr>
<tr>
<td>Median Radius (μm)</td>
<td>0.26</td>
<td>0.13</td>
<td>0.11</td>
<td>0.07</td>
</tr>
<tr>
<td>Standard Deviation (μm)</td>
<td>0.13</td>
<td>0.07</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

The goodness-of-fit metrics: $R^2$, MSE and MAE are reported in Table 3. $R^2$ values for all four membranes for the training data set were $> 0.94$. The high quality of fitting indicates that the saturation equation (Equation 11) adequately fits the experimental variation in spatiotemporal saturation during wicking flow. For the test data set, $R^2$ values for all membranes were $> 0.85$ (and $> 0.9$ for NC FF80, NC FF120 and NC FF170), demonstrating the strong predictive value of the saturation equation. Finally, for k-fold validation, average $R^2$ values were $> 0.9$ for all materials, which indicates that the parameters are robust to changes in the training data set. It is evident from
Table 3 that the $R^2$ values for filter paper, although satisfactory, are inferior compared to all three nitrocellulose materials. This is likely because of challenges in obtaining experimental spatiotemporal saturation data for filter paper. While the nitrocellulose membranes are pre-casted on a plastic laminate, filter paper lacks this laminate and was instead directly placed on a cardboard backing card, which could affect wicking flow. Moreover, at larger time stamps, filter paper was observed getting delaminated close to the fluid source, which could have added to experimental artefacts.

**Table 3: Metrics for model performance**

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Metric</th>
<th>NC FF 80 HP Plus</th>
<th>NC FF120 HP</th>
<th>NC FF 170 HP Plus</th>
<th>Whatman Filter Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training set</td>
<td>$R^2$</td>
<td>0.959</td>
<td>0.978</td>
<td>0.954</td>
<td>0.943</td>
</tr>
<tr>
<td></td>
<td>MAE</td>
<td>0.053</td>
<td>0.042</td>
<td>0.062</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td>MSE</td>
<td>0.007</td>
<td>0.003</td>
<td>0.007</td>
<td>0.008</td>
</tr>
<tr>
<td>Test set</td>
<td>$R^2$</td>
<td>0.958</td>
<td>0.917</td>
<td>0.98</td>
<td>0.862</td>
</tr>
<tr>
<td></td>
<td>MAE</td>
<td>0.06</td>
<td>0.034</td>
<td>0.04</td>
<td>0.097</td>
</tr>
<tr>
<td></td>
<td>MSE</td>
<td>0.007</td>
<td>0.003</td>
<td>0.004</td>
<td>0.013</td>
</tr>
<tr>
<td>k-fold</td>
<td>$R^2$</td>
<td>0.948</td>
<td>0.97</td>
<td>0.93</td>
<td>0.908</td>
</tr>
<tr>
<td>average</td>
<td>MAE</td>
<td>0.057</td>
<td>0.044</td>
<td>0.07</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>MSE</td>
<td>0.008</td>
<td>0.003</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Comparison of modelled saturation profiles with experiments**

A comparison between experimentally measured spatiotemporal saturation values and those predicted by the saturation equation using fitted parameters is shown for NC FF80 (Fig. 4A), NC FF120 (Fig. 4B), NC FF170 (Fig. 4C), and filter paper (Fig. 4D). The model accurately captures the shape of the saturation profiles at all time points. Note that the saturation profiles are sharper.
at earlier time points and become more diffuse at later time points, which is well captured by the model. This shows that the phenomenon of partial saturation becomes more prominent at later times. The impact of $\sigma$ can be seen by comparing the saturation profiles in filter paper with the nitrocellulose membranes. A $t = 5$ min, the apparent fluid front for filter paper reached 10 cm and yet the part of paper that had 100% saturation was shorter than 3 cm (Fig. 4D). In contrast, for FF120 (Fig. 4B) and FF170 (Fig. 4C), at the same time ($t = 5$ min), the apparent fluid fronts were at $< 9$ cm and the part of the paper having 100% saturation had crossed 3 cm for both materials. This shows that filter paper has wider partially saturated regions than nitrocellulose membranes. This is reflected in the larger value of $\sigma$ for filter paper compared to all three nitrocellulose membranes (Table 1).
Figure 4. Performance of the saturation equation. A-D: Comparison of spatiotemporal saturation profiles generated experimentally (blue markers) to those generated by the saturation equation (orange line) for NC FF80HP Plus (A); NC FF120HP (B); NC FF170HP Plus (C); and Whatman Filter Paper Grade 1 (D). All error bars represent standard deviations (N=3).

Comparison of modelled saturation profiles with Richard’s Equation

Modelling flow using Richard’s equation involves measuring the capillary pressure and permeability as a function of saturation and fitting these data to the Van Genuchten formulation to obtain Van Genuchten parameters. Permeability values at 100% saturation obtained experimentally for NC FF80 HP Plus, NC FF120 HP, NC FF170 HP Plus and grade 1 filter paper were
$1.4 \times 10^{-13}, 9.02 \times 10^{-14}, 6.1 \times 10^{-14}$ and $1.2 \times 10^{-13} \text{ m}^2$ respectively. Using the `curve_fit` tool from `scipy.optimize` package in Python, the Van Genuchten parameters were obtained by fitting the measured data to the Van Genuchten formulation. The estimated parameters are shown in Table 4. Note that the parameter $l$ in Table 4 relates permeability to saturation. There are no established methods to calculate or measure permeability as a function of saturation, so we resorted to a theoretical method previously developed by us (ESI S1).

### Table 4: Van Genuchten Parameters

<table>
<thead>
<tr>
<th>Membrane</th>
<th>$n$</th>
<th>$\alpha$</th>
<th>$l$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC FF80 HP Plus</td>
<td>2.28</td>
<td>0.13</td>
<td>16.9</td>
</tr>
<tr>
<td>NC FF 120 HP</td>
<td>1.96</td>
<td>0.2</td>
<td>41.3</td>
</tr>
<tr>
<td>NC FF 170 HP Plus</td>
<td>2.13</td>
<td>0.15</td>
<td>58.2</td>
</tr>
<tr>
<td>Whatman Filter Paper Grade 1</td>
<td>1.3</td>
<td>0.74</td>
<td>2.1</td>
</tr>
</tbody>
</table>

To get spatiotemporal variations for saturation, Richard’s equation was solved using COMSOL Multiphysics using the Subsurface Flow module. The Richard’s equation solves for capillary pressure as function of space and time; capillary pressure, in turn, is assumed to have a 1:1 correlation with saturation. Spatiotemporal saturation is thereby obtained from the solution of the equation. Initial and boundary conditions on capillary pressure, $\Psi$, for solving the Richard’s equation were as described previously. Details of the COMSOL simulation are provided in ESI S4. It must be noted that the parameter $l$ had to be reduced by 1 or 2 orders of magnitude for all membranes for the model results to match well with the experimental observations. This points towards the unavailability of an appropriate method to evaluate the parameter, $l$, and it is usually assumed to be around 0.5 while solving Richard’s equation. Moreover, the permeability at
100% saturation had to be slightly changed for all the membranes. The final parameters used in the COMSOL model to obtain spatiotemporal saturation profiles that matched experimental profiles are given in Table 5. Table 5 also provides the boundary condition imposed at the inlet ($\psi(Se = 1)$) and the initial condition ($\psi(t = 0)$) for each membrane. The meshing conditions were as reported by Rath et al.\textsuperscript{10}

The plots comparing the spatiotemporal variations of saturation in NC FF 80 HP Plus, NC FF120 HP, NC FF170 HP Plus, and filter paper obtained: i) using Richard’s equation, ii) using the saturation equation, and iii) experimentally are shown in Fig. 5. It is evident that the performance of saturation equation is comparable to the Richard’s equation. Moreover, as shown here, a few parameters in Richard’s equation need to be manually adjusted to get the solutions to match with the experimental results. For the saturation equation, this problem does not arise because the parameters are estimated using the spatiotemporal data of saturation itself.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>$\alpha$</th>
<th>$n$</th>
<th>$l$</th>
<th>$\kappa_s$ (m$^2$)</th>
<th>$\psi(Se = 1)$ (kPa)</th>
<th>$\psi(t = 0)$ (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF80 HP Plus</td>
<td>0.13</td>
<td>2.28</td>
<td>1.7</td>
<td>$8.7 \times 10^{-12}$</td>
<td>-10.97</td>
<td>-92.32</td>
</tr>
<tr>
<td>FF120 HP</td>
<td>0.2</td>
<td>1.96</td>
<td>4.13</td>
<td>$5 \times 10^{-13}$</td>
<td>-21.49</td>
<td>-98.29</td>
</tr>
<tr>
<td>FF170 HP Plus</td>
<td>0.15</td>
<td>2.13</td>
<td>0.58</td>
<td>$8.85 \times 10^{-14}$</td>
<td>-39.59</td>
<td>-103.74</td>
</tr>
<tr>
<td>Filter Paper</td>
<td>0.74</td>
<td>1.3</td>
<td>0.21</td>
<td>$1.7 \times 10^{-12}$</td>
<td>-10.97</td>
<td>-95.77</td>
</tr>
</tbody>
</table>

Table 5: Parameters for COMSOL Simulation
Figure 5. Comparison of the saturation equation with the Richard’s equation. Spatiotemporal profiles of partial saturation measured experimentally (blue markers), using the saturation equation (orange line), and the Richard’s equation (green line) for NC FF80HP Plus (A); NC FF120HP (B); NC FF170HP Plus (C); and Whatman Filter Paper Grade 1 (D). All error bars represent standard deviations (N=3).

Conclusions

While partial saturation might be a critical phenomenon that significantly affects performance of paper-based assays, it has largely been ignored by the paper microfluidics community due to absence of simple and accessible mathematical tools to incorporate it. In this work, we present an
analytical expression, known as the saturation equation, to obtain spatiotemporal variations of saturation in paper membranes. The parameters in the equation can be evaluated by performing simple experiments and a data fitting exercise. We anticipate that this equation will be adopted widely by paper microfluidics researchers, and that by using this equation, estimations of partial saturation during wicking flow would be as facile as it has been to estimate the distance travelled by fluid fronts using the Washburn equation. Although currently restricted to use in 1-dimension (1D; like the Washburn equation), the saturation equation still has wide applications in paper microfluidics because a large body of work in the field utilizes lateral flow assays and dipstick assays that feature 1D flow. Moreover, for popular devices like μPADs (microfluidic paper analytical devices) that feature the distribution of fluid from a single inlet into multiple channels containing analysis zones, the saturation equation may be used individually on each distribution channel. The saturation equation may thus facilitate using the understanding of partial saturation to develop paper-based devices in a more efficient manner.

Author Contribution Statement

Satvik Verma: Conceptualization, Methodology, Investigation, Software, Formal analysis, Writing – Original Draft; Bhushan J. Toley: Conceptualization, Methodology, Writing – Review & Editing, Supervision, Funding Acquisition

Conflict of Interest

The authors do not have any conflict of interest to declare.
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References


18 J. R. Buser, 2016.


24 R. Fu, T. Zhang and X. Wang, *Desalination*, 2023, **549**, 116318.


