Design of X-Ray Fluorescence and Ion Beam Analysis for Blood Drops Solidified as Homogeneous Thin Films (HTSF™) for Fast, Small Volume, Accurate Diagnostics

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GOAL: So-called '*super-hydrophilic'* (Fig.1c) and '*hyper-hydrophilic'* (Fig.1d) coatings, labeled 'HemaDrop™ [1] are applied on small volume blood collection devices called InnovaStrip™. Their goal is to solidify *within minutes* µL-sized blood drops into Homogeneous Thin Solid Films (HTSFs™) on InnovaStrip™ substrates so that rapid Small Volume Blood Diagnostics (SVBD) can be conducted within 20 min of blood collection. Fast, accurate analysis in the solid state, such as X-Ray Fluorescence (XRF), and Ion Beam Analysis (IBA) yields whole blood compositions with relative errors well within medical standards of <10%. Six blood components are selected for benchmark analysis per blood test: five blood electrolytes (Na, Cl, K, C, Mg) and blood Fe because of their ubiquity in diagnostics blood tests.

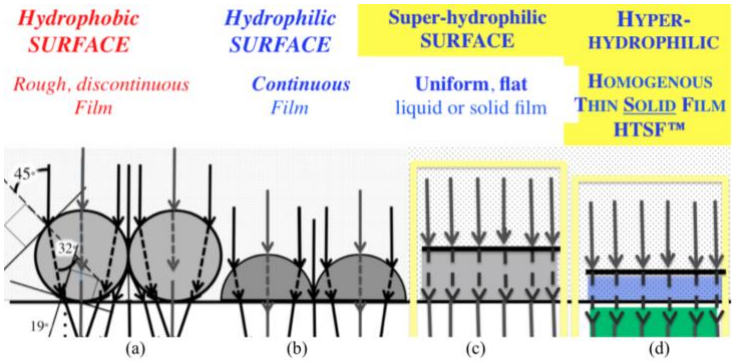
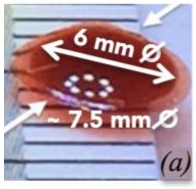


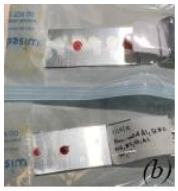
Fig. 1. Concept of Super-hydrophilic and hyper-hydrophilic behavior. *The topography of liquid films is shown for four interactions models (a-d) on solid surfaces. (a) Hydrophobic surfaces yield 'beading' without wetting (b) Hydrophilic surfaces yield continuous films with wetting but with residual 3D topography (c)* ***Super-hydrophilic*** *surfaces* ***flatten*** *drops into uniform 2D films which can dry within min. (d)* ***Hyper-hydrophilic*** *surfaces* ***flatten*** *too but* ***absorb*** *rapidly H_2O in the applied drops to solidify them into Homogeneous Thin Solid Films (HTSF)[1-5] within minutes. Both (c) and (d) yield flat, smooth, stable 2D solid blood thin films which are suitable for solid sate analysis[1-5]. analysis.*

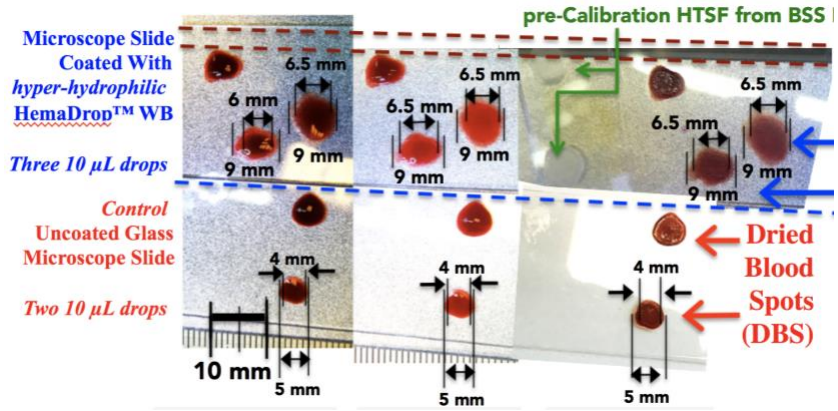
METHODOLOGY FOR MICRO-FLUIDIC STUDY OF µL BLOOD DROPS ON SOLID SURFACES AND RESULTS

The microfluidic behavior of µl blood drops is investigated using 120 InnovaStrip™ (Fig.1b), with 60 uncoated and 60 HemaDrop™ coated ones, 30 with *super-hydrophilic* (Fig.2) and 30 *with hyper-hydrophilic* surface (Fig.3) to design HSTF are consistently produced on HemaDrop™ instead of dried blood spot (DBS)



5 µL Blood Drop wetting an Hyper-hydrophilic coating, HemaDrop™ WB. An 8 mm diameter plasma outer ring with 0.75 mm width, and an inner whole blood center with a 6 mm diameter form consistently for each drop tested





(c) time= 0 s **(d) time = 93 s** **(e) time = 15 min 45 s**

1. Whole Blood HTSF™ solidified on Hyper-hydrophilic HemaDrop™. Optical Microscopy images and dimensional study of 10 µL human blood drop solidifying at 25°C, H_R = 30% are compared on uncoated glass slides as **Dried Blood Spot (DBS)** on the lower slides in **time lapse Fig.2c-e** with **HTSF** on hyper-hydrophilic HemaDrop™ WB coated InnovaStrip™ in Fig,2 ab, upper slide in **time-lapse in Fig.2c-e**.

Fig. 2a-e. ***(a)*** *Micro-fluidic analysis for 10 µL whole blood drops on hyper-hydrophilic coatings* ***(b)*** *Example of coated Al-clad InnovaStrip™* ***(c-e) Time-lapse:*** *Lower slide: Two blood drops* ***dry as DBS*** *for 15 min 45s in (c-e) on uncoated glass slides. Their non-uniform topography exhibit craters and coagulation with an outer diameter of 5 mm*

Upper Slide Two flattened blood drops solidify on super-hydrophilic HemaDrop™ coated slides ***within 93s as HTSF*** *in (d). After 15 min 45s they meet the criteria as usable HTSF for XRF, IBA and XPS analysis. They reach an outer diameter of 9 mm, with at least 6.5-7 mm of usable, homogeneous area for analysis.*

[1] N. Herbots, N. Suresh *et al.* US & International Patents Pending (2016-20).
[5]N. Herbots, N. Suresh, *et al.* (2019). MRS Adv. 1-25. 10.1557/adv.2019.398.

2. Blood Plasma HTSF™ solidified on Super-hydrophilic HemaDrop™ - The rapid flattening and rapid spreading of 10 µl drops of super-hydrophilic coatings lead to larger HTSF diameter, where the less viscous plasma spreads more rapidly that the whole blood, so that two uniform blood phases circular regions are consistently created as depicted in the six HTSF in Fig. 3a-b during XPS and XRF analysis. A more detailed optical and compositional analysis shows that super-hydrophilic coatings can be designed to lend to phase separation into three distinct regions: whole blood, plasma, and serum as seen in Fig.3(e).

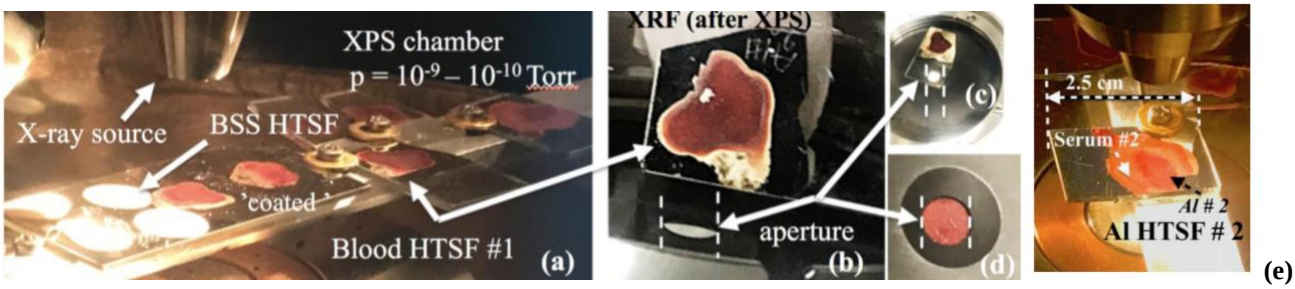
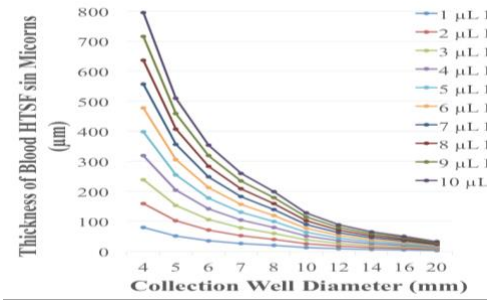


FIG. 3 *(a) Exposure at p =10^{-9} - 10^{-10} Torr of six blood HSTFs on super-hydrophilic Al-cladded slides with four BSS calibration. The images show how the HTSF extend into ~12 mm in diameter, and exhibit clear separation between whole blood HTSF in the center and plasma HTSFs in outer rings (b) Blood HTSF #1 in (a) as shown after 36 hours' exposure to UHV in (b), and marked with jointed arrows. In (c-d) HTSF #1 is then analyzed by XRF and the results are listed in Table VII.*

COATINGS, HTSF, COLLECTION WELLS AND ASPECT RATIO DIMENSIONING FOR OPTIMIZING QUANTITATIVE ANALYSIS BY XPS, XRF, IBA ON INNOVASTRIP ™

Aqueous volume of whole blood are ~ 55% depending on hydration levels while 45% of blood volumes consists red and white blood cells. Since red blood cells average 6-8µm, Whole Blood HTSFs have to be at least 8 µm thick to yield a representative blood Fe content. White blood celss average 12-17µm, about twice as much, so HTSFs have to be at least that thick to yield representative compositions for blood proteins for example, by measuring the N content which scales directly with protein content.

(a)



(b)

(c)

Fig. 4 (a) *Transmitted light Optical Microscopy to image HTSF thickness homogeneity* **(b)** *Computed HTSF thicknesess as a function of µL-size blood drop volumes and well diameters to optimize HTSF thicknes. (It has to be divided by about a factor 2 to represent the estimated thickness for solidified HTSF™ after water adsorption)* **(c)** *Collection wells on InnovaStrip™.*

Method *Quantities*	*Materials*	Elements identified by the analysis method (Atomic %) C	N	O	Na	Cl	K	Ca	Fe	Mg	Al	Si	S
XPS	Al Clad	Avg_{BSS}	62.49	0.09	36.12	1.00	0.10	0.15	0.08	0.02	-	-	-	-
		$Std.dev_{BSS}$	± 2.18	± 0.06	1.54	± 0.82	0.04	± 0.11	± 0.07	0.02	-	-	-	-
		Rel.err$_{BSS}$	*± 3%*	*± 64%*	*± 4%*	*± 82%*	*± 44%*	*± 69%*	*± 80%*	*± 65%*	-	-	-	-
		$Avg_{B.H}$	72.83	6.94	19.72	0.07	0.15	0.27	0.07	0.03	-	-	-	-
		$Std.dev_{B.H}$	± 6.03	±1.96	8.25	±0.01	±0.05	±0.17	±0.05	±0.03	-	-	-	-
		Rel.err$_{B.H}$	*± 8%*	*± 28%*	*± 42%*	*± 20%*	*± 34%*	*± 64%*	*± 67%*	*± 94%*	-	-	-	-
		Avg_{Serum}	69.07	5.12	24.70	0.06	0.38	-	50.0	0.11	-	-	-	-
		$Std.dev_{Serum}$	± 4.28	± 1.27	± 6.27	± 0.01	± 0.37	-	± 0.34	± 0.01	-	-	-	-
		Rel.err$_{Serum}$	*± 4%*	*± 25%*	*± 25%*	*± 13%*	*± 59%*	-	*± 89%*	*± 35%*	-	-	-	-
RBS	TiN & Si(100)	$Avg_{C.B.100K}$	70.01	18.84	8.9	0.92	1.18	-	-	0.11	-	-	-	-
		$Std.dev_{C.B.100K}$	±1.17	±1.32	±1.62	±0.18	±0.06	-	-	±0.01	-	-	-	-
		Rel.err$_{C.B.100K}$	*±2%*	*± 7%*	*±17%*	*±21%*	*±6%*	-	-	*±9%*	-	-	-	-
		$Avg_{C.B.400K}$	69.96	18.95	8.84	0.98	1.18	-	-	0.11	-	-	-	-
		Rel.err$_{C.B.400K}$	*±0%*	*±1%*	*±1%*	*±10%*	*±2%*	-	-	*±2%*	-	-	-	-
XRF	Al clad & Glass slide	Avg_{B}	-	-	-	5.71	24.09	9.92	31.89	3.18	0.59	1.84	11.24	11.0
		$Std.dev_{B}$	-	-	-	±0.47	±1.28	±0.68	±1.60	±1.56	±0.17	±0.13	±0.8	±0.6
		Rel.err$_{B}$	-	-	-	*±9%*	*±5%*	*±7%*	*± 5%*	*± 49%*	*± 40%*	*± 7%*	*±7%*	*± 6*

TABLE 1: *Comparison of XPS, RBS and XRF for C, N, O, Na, Cl, K, Ca, Fe, in absolute atomic %, with their relative errors, by measuring each on systematic sets of three measurements.*

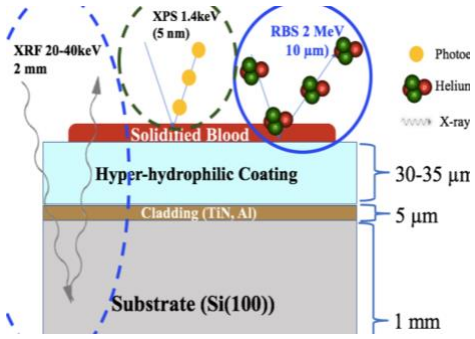


Fig. 5. *Depths probed by XRF, XPS and IBA and their role of coatings, collections wellsand InnovaStrip dimensional design*