Label-Free Detection of Proteins from Dried-Suspended Droplets Using Surface Enhanced Raman Scattering

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Table 1 shows the physicochemical properties of the proteins used in the study. The hydrodynamic radius and zeta potential of the proteins in the suspension were investigated by DLS (Zetasizer) at room temperature. The system contains a 4 mW He-Ne laser with a wavelength of 633 nm. The scattered light was collected by an avalanche photodiode detector with an angle of 173°. The refractive index and absorption of the protein solutions were assumed as 1.45 and 0.001, respectively.

Table 1 Physicochemical properties of the proteins.

Protein	Isoelectric point (pI)	Molecular weight (kDa)	Zeta potential (mV)	Hydrodynamic radius (nm)	Property
HSA	4,7-5,2	66,5	-28,9	7,937	Acidic
Transferrin	~5,5	76	-18,4	15,55	Acidic
Myoglobin	6,8-7,3	17,6	-17,6	4,259	Acidic
Cyt *c*	10-10,5	12,3	+22,5	2,01	Basic
Avidin	~10	66	+27,1	3,53	Basic
Lysozyme	11,4	14,3	+5,9	2,1	Basic

Figure 1 shows the photographs of suspending drying droplets on both CaF_2 and PDMS surfaces. The hydrophobicity of the CaF_2 and PDMS is very similar as concluded from the contact angle measurements, which were found 90º and 95º for CaF_2 and PDMS, respectively.

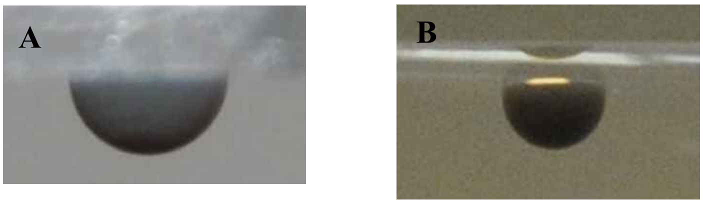

Fig. 1 The photographs of hanging drying droplets on both CaF_2 (A) and PDMS (B) surface.

Fig. 2 shows ten SERS spectra obtained from negatively, HSA, and positively charged protein, cyt *c*, containing suspended dried droplets and white light images of the droplet areas where these spectra were collected. The reproducibility of the SERS spectra was found satisfactory after testing the spot-to-spot spectral variations on the region where the SERS spectra obtained. The spectra presented on Fig. 2 are raw and no baseline correction was made.

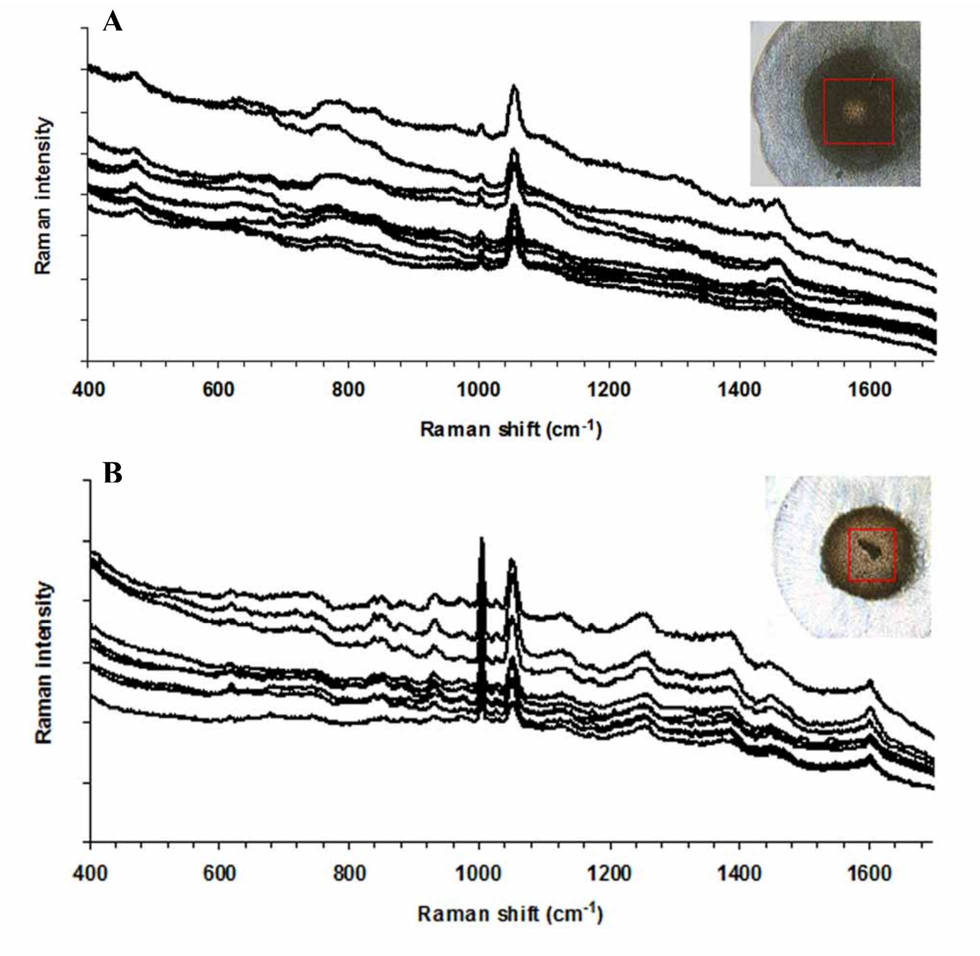

Fig. 2 Ten SERS spectra obtained from HSA (A) and cyt *c* (B) containing suspended dried droplets and white light images of the droplet area where these spectra were collected. The protein concentration is 50 μg/mL for both protein-AgNP mixtures.

Fig. 3 shows the five SERS spectra obtained from negatively, HSA, (A) and positively charged protein, cyt *c*, (B) containing suspended dried droplets. Each SERS spectrum on the graphs is the average of ten SERS spectra, which were randomly collected from the middle regions of five different droplets. The spectra presented on Fig. 3 are raw and no baseline correction was made.

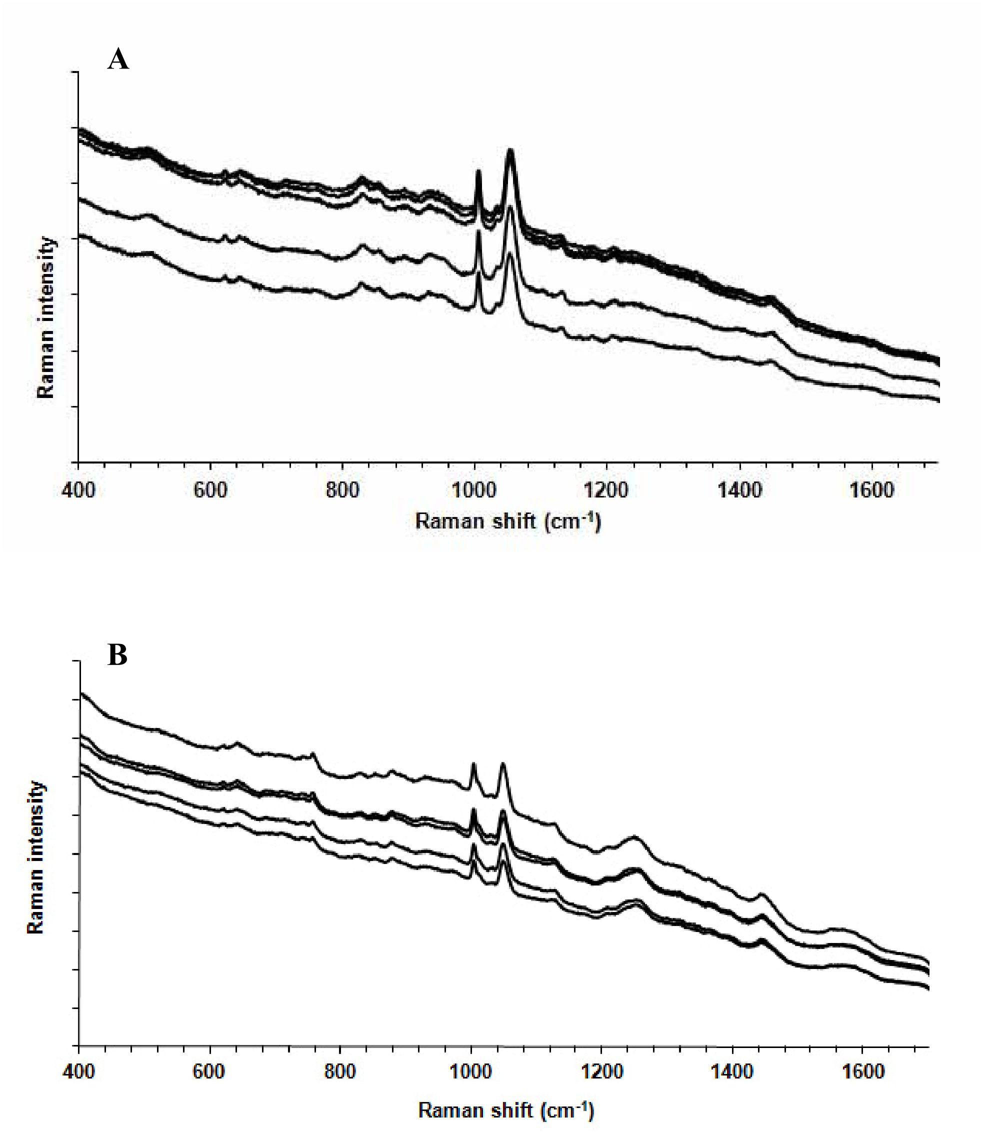

Fig. 3 Five SERS spectra obtained from negatively, HSA (A) and positively charged protein, cyt *c*, (B) containing suspended dried droplets. Each SERS spectrum on the graphs is the average of ten SERS spectra, which were randomly collected from the middle regions of five different droplets. The protein concentration is 50 μg/mL for both protein-AgNP mixtures.

Table 2 Band assignments for SERS spectra of proteins.

HSA	Transferrin	Myoglobin	Cyt *c*	Avidin	Lysozyme	Band Assignments
471	---	---	---	---	---	---
---	---	---	524	---	---	S-S str.[1]
---	---	---	---	540	---	Cys[2]
---	618	618	---	618	---	C-C twist.[3]
---	646	---	643	---	---	Tyr[4]
631	710	700	710	---	669	Met[5]
712			738			
756	755	---	---	758	755	Trp[6]
---	827	---	---	---	827	Pro, Tyr[4]
838	---	838	838	---	---	vibration of amine groups[7]
852	852	852		852	852	Tyr, Pro[4]
---	---	---	879	---	---	Trp, Pro[4]
928	928	928	928	928	928	Pro, Val[8]
950	950	---	---	950	950	Pro, Val[9]
---	---	---	970	---	---	Ser[6]
	---	---	---	980	---	Trp[6]
1001	1001	1001	1001	1001	1001	Phe[6]
1029	1029	1028				
1053	1053	1053	1053	1053	1053	C-O, C-N str.[3]
1088	---	---	---	1090	---	C-C str.[10]
---	1123	1123	1083	---	---	C-N str.[1]
			1123			
---	---	---	1171	1171	---	Try[6]
---	1204	---	---	---	---	Try+Phe[6]
---	---	---	---	---	1224	Amide III[11]
---	---	---	1250	---	---	Amide III[6]
---	---	---	---	1237	---	Gly, Pro[12]
				1280		
---	---	1335	---	---	---	Trp[6]
---	---	1368	1368	---	1355	Trp[6]
---	1441	---	---	---	---	CH_2 sciss.[6]
1453	---	---	---	---	---	Gly[6]
---	---	---	1444	---	1444	CH_2 sciss.[6]
---	---	---	1582	---	---	Phe[6]
---	---	1600	---	1602	---	COO^- asym. str.[6]

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