



Data Article

Data on the characterization of a platelet lysate and of the system in which it is included (nanoparticles/hydrogel), intended for the treatment of wounds [☆]

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ABSTRACT

Platelet lysate has attracted attention for different biomedical applications, including biological processes where cellular proliferation and migration have been altered. Spectroscopic properties of a platelet lysate obtained from human platelets were performed in order to be incorporated in polymeric nanoparticles and then into a Pluronic[®] F127 hydrogel, intended for wound healing (more details can be found at <https://doi.org/10.1016/j.ejps.2020.105231> [1]). The platelet lysate (PL) was assessed by ultraviolet, infrared and circular dichroism spectroscopy. The developed hydrogel was also analyzed by infrared spectroscopy to evaluate if the Pluronic[®] F127 structure was maintained when the nanoparticles or platelet lysate-loaded nanoparticles were included. The sol-gel transition temperature of the hydrogel was determined

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through its thermal behavior and by dynamic light scattering.

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Specifications table

Subject	Pharmaceutical Science
Specific subject area	Characterization of a therapeutic protein mixture (platelet lysate) by using color reactions and spectroscopy techniques such as ultraviolet, infrared and circular dichroism. The PL is included into nanoparticles/hydrogel and this system is characterized. The system is intended for the healing of chronic wounds.
Type of data	Graph Figure Table
How data were acquired	MB3000 ABB FTIR spectrometer (Quebec, Canada), U5100 Hitachi UV-VIS spectrophotometer (Chatsworth, CA), J-815 Perkin-Elmer Jasco Circular dichroism spectrophotometer (Easton, MD), Q20 calorimeter (TA Instruments, New Castle, DE, USA), Zetasizer Nano ZS90 (Malvern, Worcs, UK). On the other hand, the color reactions (Bradford and Biuret) data were acquired by using visual criteria and by photographic evidence.
Data format	Raw data & analyzed data
Parameters for data collection	The parameters considered to collect the data were based on the type of spectroscopy or technique with the following conditions: Ultraviolet: Samples in a 10 mm quartz cell, scan speed 40 nm/min, start and end wavelength of 200 nm and 400 nm respectively. Infrared: Samples in a horizontal ATR zinc selenide cell, 150 scans and a resolution of 16, wavenumber interval from 500 to 4000 cm^{-1} . Circular dichroism: Samples in a 1 mm cell with a standard sensitivity, bandwidth of 1.00 nm, scan speed 10 nm/min, temperature of 25.04 °C, start and end wavelength of 250 nm and 195 nm respectively, with 0.5 nm intervals, registering 110 points per reading. Calorimetry: Samples of 10 mg were placed in Tzero® aluminum pans (TA Instruments, New Castle, DE, USA). The analysis was carried out under the following conditions: sample equilibrium at 0 °C, isotherm for 1 min and a heating ramp of 1 °C/min until 70 °C. Brownian motion loss temperature: This was determined by taking advantage of the measurement principle of the dynamic light scattering technique, measuring the particle size on a ramp from 5 to 30 °C, recording the temperature where the value of particle size drops drastically, indicating that the system has gelled. Color reactions (Bradford and Biuret): Visual change of color was taken as the parameter that evidences the effect.
Description of data collection	Data were collected from the readings obtained with platelet lysate solutions and the hydrogels, using the techniques mentioned above.
Data source location	Facultad de Estudios Superiores-Cuautitlán/UNAM, Estado de México, México
Data accessibility	With the article
Related research article	Sergio Bernal, Sergio Alcalá, Doris Cerecedo and Adriana Ganem Platelet lysate-loaded PLGA nanoparticles in a thermo-responsive hydrogel intended for the treatment of wounds European Journal of Pharmaceutical Sciences https://doi.org/10.1016/j.ejps.2020.105231

Value of the data

- The data obtained here will contribute to the understanding of the spectroscopic characteristics of a platelet lysate.

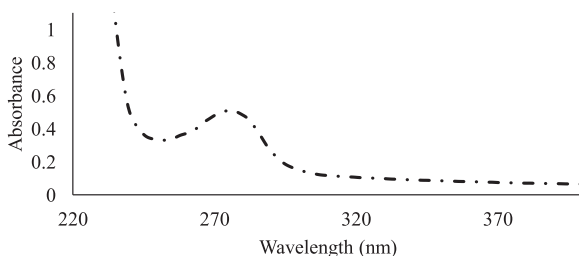


Fig. 1. PL UV spectrum.

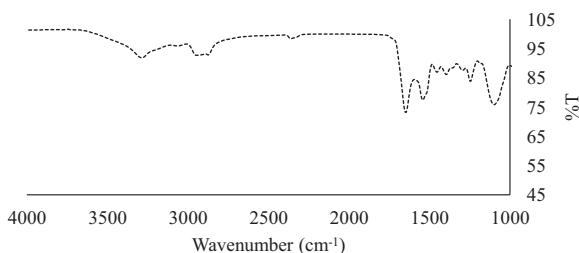


Fig. 2. PL IR spectrum.

- The data can be used by researchers and as basic data of further research in the inclusion of proteins into nanocarriers and/or hydrogels.
- The data could provide the basis for the development of new drug delivery systems for use in future topical applications.

1. Data description

The following results contribute to the understanding of the chemical and physical properties of a platelet lysate, which was later included in polymeric nanoparticles, and which in turn were incorporated into a hydrogel (HG). The complete system was developed for use in wound management. The platelet lysate was characterized through several analytical techniques such as ultraviolet, infrared and circular dichroism spectroscopy, as well as by colorful reactions. All these techniques were focused, on the one hand, on corroborating the presence of the protein structures that make up the lysate, and on the other hand, on studying the thermal and rheological behavior of HG. The latter, with the intention of verifying that it complied with important technological characteristics for its administration in wounds. Finally, the validation of a method by UV spectrophotometry is presented, which was used for the quantification of total proteins in the platelet lysate [1].

Characteristics of proteins can be detected through various spectroscopic techniques such as ultraviolet (UV) [2], infrared (IR) [3] and circular dichroism (CD) [4], as well as by colorful reactions [5] which are indicative of the presence of these biopolymers. The figures show the UV (Fig. 1), IR (Fig. 2), and CD (Fig. 3) spectra, as well as the colorful behaviour (Fig. 4) of a platelet lysate (PL) obtained from human platelets.

Poloxamers exhibit thermoreversible behavior in aqueous solutions, and this property has been used for different biomedical applications, including drug delivery [6]. Fig. 5 shows the IR behavior of a 20% Pluronic® F127 hydrogel (BASE HG), the hydrogel including empty nanoparticles (NP HG), and the hydrogel with PL-loaded nanoparticles (NP+PL HG).

The sol-gel transition temperature was determined for hydrogels by the following methods: (i) Micellization temperature: Thermograms were performed by Differential Scanning Calorime-

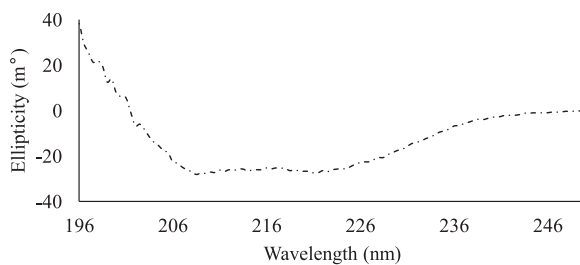


Fig. 3. PL CD spectrum.

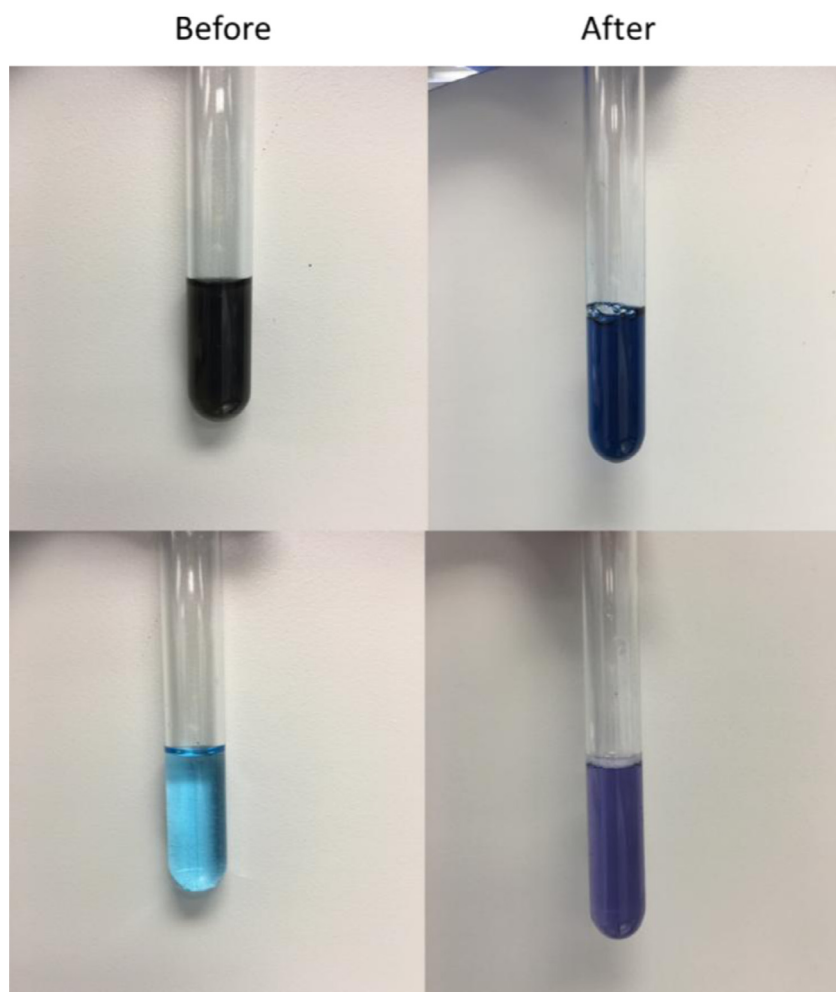


Fig. 4. Colorful reactions for PL in the presence of Bradford's reagent (top) and Biuret's reagent (bottom) before and after being added.

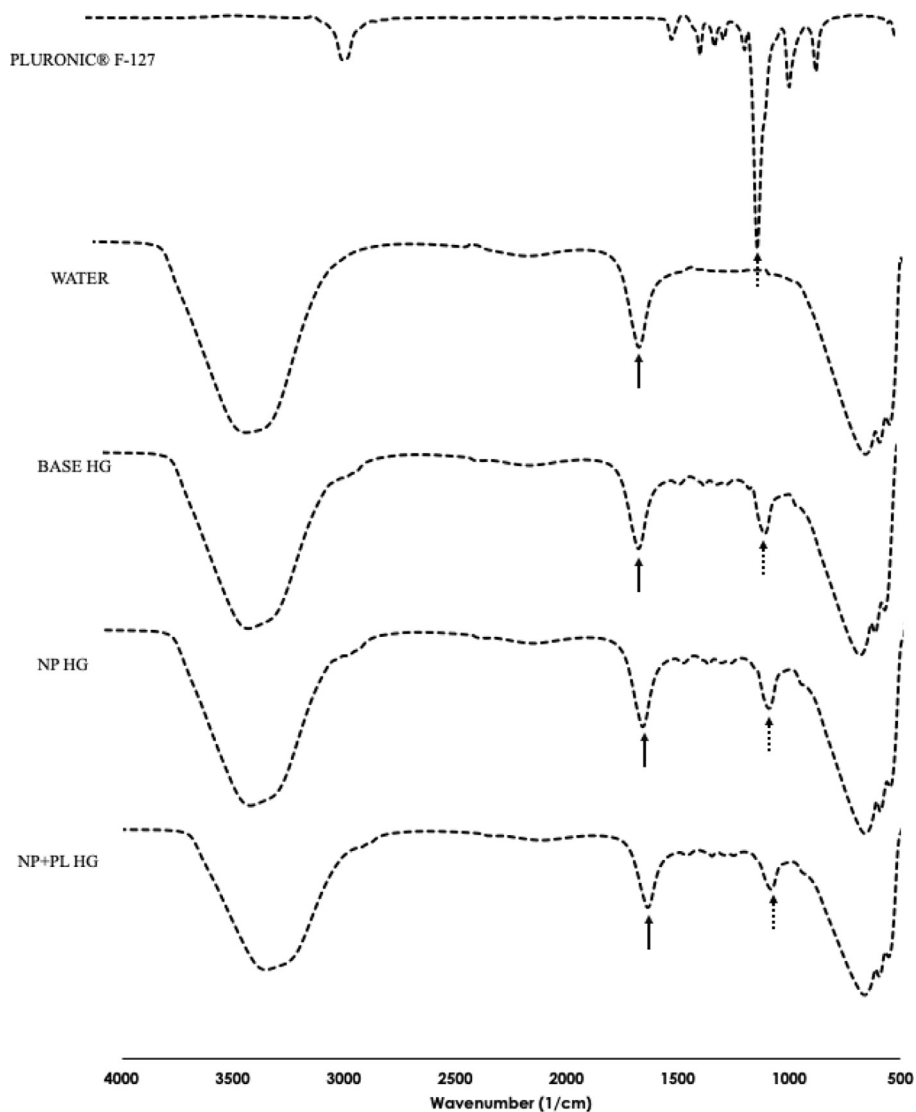


Fig. 5. IR spectra of different hydrogels: 20% Pluronic® F127 hydrogel (BASE HG), the hydrogel including empty nanoparticles (NP HG), and the hydrogel with PL-loaded nanoparticles (NP+PL HG). The dashed arrows show the characteristic symmetric stretching band for the C–O group of Pluronic® F-127, while the solid arrows indicate a band that represents one of the water vibrational modes.

try (DSC) using a Q20 calorimeter (TA Instruments, New Castle, DE, USA). The analysis was made for the polymer (Pluronic® F-127) and for the different hydrogels with and without nanoparticles (Fig. 6). (ii) Brownian motion loss temperature: This was determined by taking advantage of the measurement principle of the Dynamic Light Scattering technique using a Zetasizer Nano ZS90 (Malvern, Worcs, UK), recording the temperature at which the particle size value dropped dramatically, indicating that the system has gelled, and that, therefore, the equipment was not able to measure the particle size (Fig. 7).

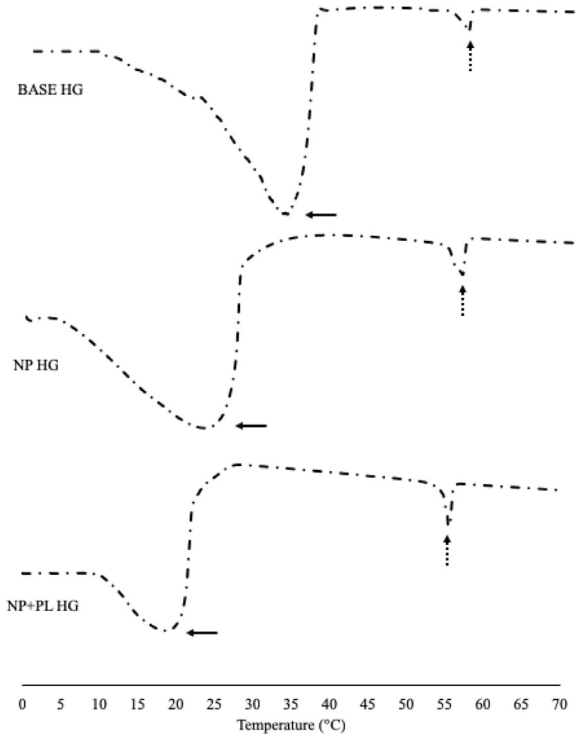


Fig. 6. Thermograms for the different HG systems. BASE HG = without nanosystem; HG containing unloaded nanoparticles (NP HG); HG containing PL-loaded NP (NP+PL HG). Dashed arrows show a thermal transition of Pluronic® F-127 (melting temperature), while the solid arrows indicate the thermal transition associated with the micellization temperature of the different HG systems.

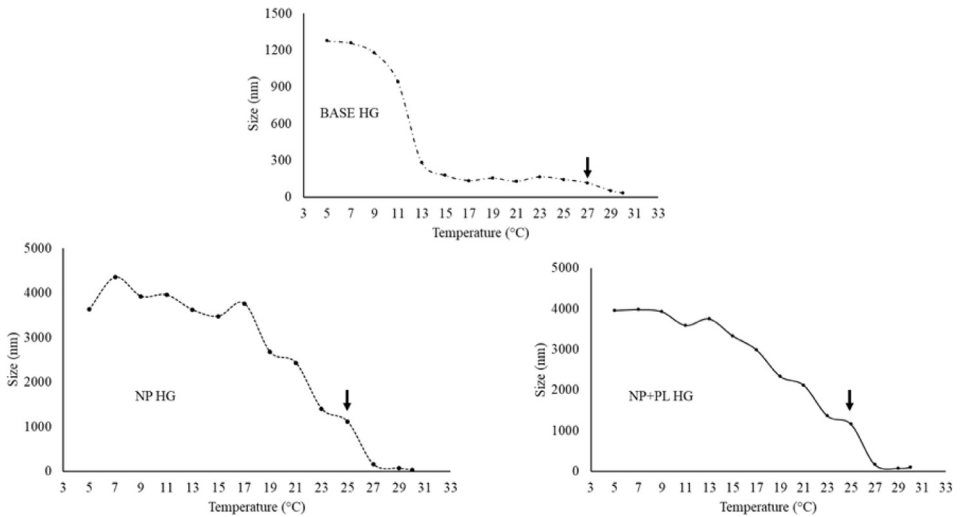


Fig. 7. Brownian motion loss temperature determined by Dynamic Light Scattering for the HG systems. BASE HG = without nanosystem; HG containing unloaded nanoparticles (NP HG); HG containing PL-loaded NP (NP+PL HG). The arrows indicate the point at which the different HG systems lose their fluidity due to an increase in temperature.

Table 1

Validation parameters for the UV method intended for the quantification of PL at a wavelength of 280 nm.

Validation characteristics	Results	Acceptance criteria
Range	90–300 µg/ml	—
Linearity		
• Correlation coefficient (<i>r</i>)	0.9983	$r \geq 0.99$
• Coefficient of determination (r^2)	0.9966	$r^2 \geq 0.98$
• y-intercept	$0.00584 \pm 3.94 \times 10^{-3}$	CI preferably should include 0
• Slope	$0.00063 \pm 1.98 \times 10^{-5}$	CI should not include 0
Precision		
• Repeatability (three points of the validated range, intraday),%	C.V. ₁ = 0.35, C.V. ₂ = 0.19, C.V. ₃ = 1.9	C.V. (%) ≤ 2
• Intermediate precision (two linear regression curves, interday)	No significant difference ($p > 0.05$)	t-student test at 95% confidence
Accuracy (% recovery)	101.5 ± 1.73	CI should include 100% or% Recovery ≥ 97
Specificity	None of the components, different from the PL, showed absorption at 278 nm	No absorption of components different from PL must be observed at 278 nm
Detection limit	13.60 µg/ml	—
Quantitation limit	41.35 µg/ml	—

CI: confidence interval; the CI was performed at 95% ($n=3$ for linearity intervals, and $n=6$ for accuracy interval).

PL was determined through the different stages of the work by a UV method, which was validated based on the Validation Guide of Analytical Methods of the Biological Pharmaceutical Chemists College from Mexico [7]. Table 1 shows the results obtained.

2. Experimental design, materials, and methods

UV spectroscopy: A scan from 200 to 400 nm of a PL solution in phosphate buffer pH 7.2 (100 µg/ml) was performed, using a quartz cell of 10 mm at a scan rate of 40 nm/min.

IR spectroscopy: Lyophilized PL or the different hydrogel formulations were placed on a horizontal ATR zinc selenide cell registering the infrared spectra in an interval from 500 to 4000 cm^{-1} , using an infrared spectrometer ABB-MB3000 FTIR (Quebec, Canada). Measurements represented an average of 150 scans with a resolution of 16. Experiments were performed in triplicate.

CD spectroscopy: A sample of a PL solution in phosphate buffer pH 7.2 (100 µg / ml) was placed in a 1 mm cell, obtaining a scan from 250 to 195 nm, with 0.5 nm intervals, using J-815 Perkin- Elmer Jasco Circular dichroism spectrophotometer (Easton, MD). The following conditions were maintained: Standard sensitivity, a bandwidth of 1.00 nm, a scan speed of 10 nm/min, and a temperature of 25.04 °C, registering 110 points per reading.

Determination of the sol-gel transition temperature:

- Micellization temperature: Thermograms were performed by Differential Scanning Calorimetry (DSC) using a Q20 calorimeter (TA Instruments, New Castle, DE, USA). The analysis was made for the polymer (Pluronic® F-127) and for the different hydrogels, placing approximately 10 mg in Tzero® aluminum pans (TA Instruments, New Castle, DE, USA). The analysis was carried out under the following conditions: sample equilibrium at 0 °C, isotherm for 1 min and a heating ramp of 1 °C/min until 70 °C.
- Brownian motion loss temperature: This was determined by using a Zetasizer Nano ZS90 (Malvern, Worcs, UK), measuring the particle size on a ramp from 5 to 30 °C.

Colorful reactions: A volume of 1.5 ml of a PL solution (1 mg/ml) was poured into a test tube. Then, 1 ml of the Bradford or Biuret reagent was added, detecting the color change, after 10 min in which the reaction was carried out (Bradford: red → blue and Biuret: blue → purple).

Validation of the UV spectrophotometric method used to quantify the PL loaded in the nanoparticle/hydrogel systems, as well as during the in vitro release studies: This was carried out according to the Validation Guide of Analytical Methods of the Biological Pharmaceutical Chemists College from Mexico [7].

CRediT authorship contribution statement

Sergio Bernal: Conceptualization, Writing - original draft. **Sergio Alcalá:** Validation, Formal analysis. **Adriana Ganem-Rondero:** Project administration, Formal analysis, Writing - review & editing.

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Conflict of Interest

None

References

- [1] S.A. Bernal, S. Alcalá, D. Cerecedo, A. Ganem, Platelet lysate-loaded PLGA nanoparticles in a thermo-responsive hydrogel intended for the treatment of wounds, *Eur. J. Pharm. Sci.* 146 (2020), doi:[10.1016/j.ejps.2020.10523](https://doi.org/10.1016/j.ejps.2020.10523).
- [2] N.J. Anthis, G.M. Clore, Sequence-specific determination of protein and peptide concentrations by absorbance at 205nm, *Protein Sci.* 22 (2013) 851–858, doi:[10.1002/pro.2253](https://doi.org/10.1002/pro.2253).
- [3] A. Barth, Infrared spectroscopy of proteins, *Biochim. Biophys. Acta* 1767 (2007) 1073–1101, doi:[10.1016/j.bbabbio.2007.06.004](https://doi.org/10.1016/j.bbabbio.2007.06.004).
- [4] S.M. Kelly, T.J. Jess, N.C. Price, How to study proteins by circular dichroism, *Biochim. Biophys. Acta* 1751 (2005) 119–139, doi:[10.1016/j.bbapap.2005.06.005](https://doi.org/10.1016/j.bbapap.2005.06.005).
- [5] C.V. Sapan, R.L. Lundblad, N.C. Price, Colorimetric protein assay techniques, *Biotechnol. Appl. Biochem.* 29 (Pt 2) (1999) 99–108, doi:[10.1111/j.1470-8744.1999.tb00538.x](https://doi.org/10.1111/j.1470-8744.1999.tb00538.x).
- [6] S. Nie, W.L. Hsiao, W. Pan, Z. Yang, Thermoreversible Pluronic F127-based hydrogel containing liposomes for the controlled delivery of paclitaxel: in vitro drug release, cell cytotoxicity, and uptake studies, *Int. J. Nanomed.* 6 (2011) 151–166, doi:[10.2147/IJN.S15057](https://doi.org/10.2147/IJN.S15057).
- [7] National College of Biological Pharmaceutical Chemists from Mexico A.C., Validation Guide of Analytical Methods. Available at <https://www.colegioqfb.org.mx/recursos/cipam>.