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Simple pH-Triggered Control over Hydrogel Formation by Acetyl Valine

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Abstract

This paper reports on the use of acetyl-L-valine (Ac-Val) as an effective and precise pH modifier for inducing hydrogel formation. Ac-Val offers several advantages: it is fully water-soluble, overcoming dissolution issues, and allows for stock solution preparation to fine-tune trigger volume and final material pH. As a weaker carboxylic acid compared to inorganic acids, Ac-Val enables more controlled pH variation. For comparison, a commercial lactic acid (LA) solution was also evaluated. The reliability of Ac-Val as a pH modifier was tested on three amino acid derivatives — Boc-Dopa(Bn)$_2$-OH, Lau-Dopa(Bn)$_2$-OH, and Pal-Phe-OH, all known to be efficient gelators. These molecules, sharing common structural features, form gels varying in transparency, robustness, and elasticity. Notably, Pal-Phe-OH is a supergelator. A key benefit of Ac-Val lies in its ability to cause an instantaneous pH modification, allowing for precise pH adjustment before the gel network forms. This pH-change approach with Ac-Val demonstrates broad applicability, enabling the creation of gels with tailored pH values for various acidic molecules, which is particularly valuable for applications like drug delivery where specific pH environments are crucial.

Keywords: Ac-Val; hydrogels; pH change; self-assembly; trigger

1. Introduction

The aim of this work is to envision a straightforward, tunable and easily reproducible technique to prepare hydrogels at defined pH values, with a particular focus on biocompatible hydrogels, specifically those within the physiological pH ranging around 7. This aspect is of paramount importance when the hydrogel is intended for biological applications, such as drug delivery systems. Such applications invariably require a well-defined pH environment, which may exhibit considerable variability depending on the specific target tissue and the intended application [1]. Indeed, it is crucial to acknowledge the heterogeneity of pH values across various anatomical locations within the body [2], including substantial alterations in the baseline pH due to the presence of tumors and infections [3–5]. Finally, pH may highly impact on the drug release [6–8].

Low molecular weight gelators (LMWGs) offer a wide tunability of the chemical structure and of the conditions necessary to the gelation process. These compounds form supramolecular structures thanks to non-covalent interactions between aromatic rings, proton donors and acceptors, and hydrophobic moieties [9–13]. LMWGs are often designed to respond to specific triggers, such as ultrasound sonication, addition of a non-solvents or of salts, pH changes, enzymes [14–19]. When the LMWG is an amino acid derivative, a common strategy is to exploit its free carboxylic group: the addition of an acid to the alkaline solution of the gelator will trigger gel formation [20]. This reversible sol/gel process driven by the pH may be exploited for drug delivery purposes [21–26].

To this end, identifying a suitable gelator is undoubtedly crucial, but the selection of an appropriate pH modifier is equally important. Indeed, if the pH reduction is done by the addition of a strong acid, such as hydrochloric acid, the pH is immediately altered with the rapid formation of the protonated form of the gelator, which is insoluble in water [17]. This excessively fast

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transformation leads to the formation of aggregates or precipitates, hindering the creation of a homogeneous fibrous network. The formation of a gel, suitable for subsequent applications or modifications, requires a sufficient amount of time for the creation of fibrils that self-assemble into fibers, trapping the solvent in the process.

A valuable alternative to this process was proposed by Adams [27] who suggested the use of δ-gluconolactone (GdL) as a slow pH modifier. Indeed, the hydrolysis of GdL yields gluconic acid, which reduces the pH of the solution over time, allowing the sample to achieve a uniform pH and promoting the slow formation of fibrils through the self-aggregation of the insoluble acid. However, with this method the final pH is reached after several hours, by which time the gel has already formed. Furthermore, GdL is a solid and, upon addition, requires a certain period for dissolution, which can affect the homogeneity of the final gel. Inspired by the same hydrolysis concept of GdL, the same group also proposed organic anhydrides as possible alternatives to slowly trigger the pH shift [28].

Another strategy involves the use of buffer solutions at defined pH ranges, but this approach suffers from the presence of inorganic salts in the solution, which could interfere with the self-aggregation process of the gelator and alter the properties of the final gel [29].

In a previous work, we established that free amino acids could serve as triggers for gel formation, yielding gels with distinct final pH values determined by the amino acid selected [30]. Expanding on our understanding of gelation triggers, we have also reported the use of Boc-L-Ala-Aib-L-Val-OH, a tripeptide with a free carboxylic acid, to acidify and trigger the gelation of Boc-L-Dopa(Bn)$_2$-OH solutions. Our findings showed that by varying the mixing ratios of these two molecules, we could effectively fine-tune both the mechanical properties and the final pH of the gels [31].

In this paper, we report our recent findings regarding the use of a single acetylated amino acid, acetyl-L-valine (Ac-Val), as a pH modifier. This molecule can be easily synthesized and purified and is fully water-soluble. It can be added as aqueous solution, thus overcoming the issue of delayed dissolution during gel formation.

For comparative purposes, we also evaluated the use of a commercial lactic acid (LA) solution. Unfortunately, commercial LA solutions typically lack a clearly defined concentration. This aspect can affect the reproducibility of the gel formation.

2. Results

The use of a solution of Ac-Val as reliable and reproducible pH modifier has been tested on three different amino acid derivatives that behave as efficient gelators when submitted to the pH change method: Boc-Dopa(Bn)$_2$-OH (Figure 1**A**) [32,33], Lau-Dopa(Bn)$_2$-OH (Figure 1**B**) [34] and Pal-Phe-OH (Figure 1**C**) [35,36]. It has been previously reported that gelators **A** and **C** are fully biocompatible [37,38] and can be used to prepare materials for drug delivery or tissue engineering. We included in our study also gelator **B**, as it is very similar to the others, so it is reasonable to think that it is biocompatible too.

These three molecules contain aromatic rings, one stereogenic center, one carboxylic group, and one lipophilic side chain (Figure 1), and form materials that differ in term of transparency, robustness and elasticity. In addition, Pal-Phe-OH **C** is a supergelator, able to form gel in concentration down to 0.025% w/v while Boc-L-Dopa(Bn)$_2$-OH **A** and Lau-Dopa(Bn)$_2$-OH **B** form gels in the 0.3-0.5% w/v concentration range. In the light of that, we studied the impact of the pH modifier always at 0.5% w/v gelators concentration to better compare the results.

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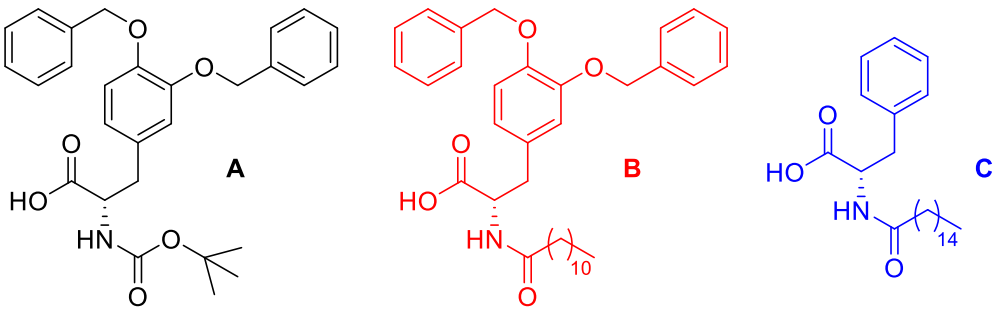

Figure 1. Chemical structure of the gelators: Boc-Dopa(Bn)$_2$-OH **A** (black), Lau-Dopa(Bn)$_2$-OH **B** (red) and Pal-Phe-OH **C** (blue).

As an initial investigation into the suitability of Ac-Val as an effective pH modifier, we examined the gelation of the three gelators using 1.3 equivalents of GdL, LA, and Ac-Val as triggers. This specific amount was chosen based on our prior research, where 1.3 equivalents of GdL were predominantly employed.

To prepare the gels, the gelator was suspended in Milli-Q® H_2O, and one equivalent of NaOH (0.1 M aqueous solution) was added. The resulting solution was stirred and sonicated until the complete dissolution of the gelator was achieved. Subsequently, the trigger was introduced: Ac-Val and LA were added as aqueous solutions, while GdL was added in its solid form. In all cases, the final volume was adjusted to yield a 0.5% w/v concentration of the gelator. Following the addition of the trigger, the solutions were gently stirred for a few seconds and then left undisturbed for 16 hours.

As anticipated, all the samples successfully formed gels (as depicted in Figure S1), and their mechanical properties were subsequently analyzed and compared using rheological analysis. The corresponding results are presented in Figure 2.

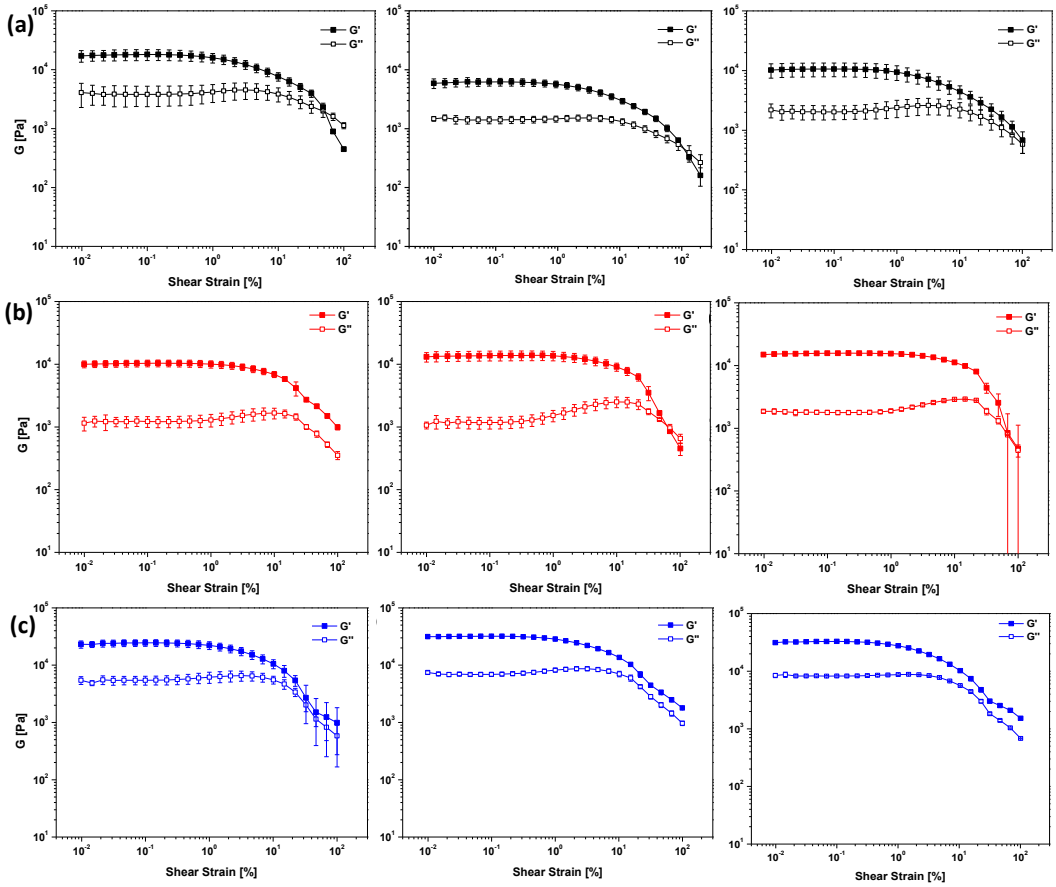

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Figure 2. From top to bottom: results for amplitude sweep tests of the hydrogels of Boc-Dopa(Bn)$_2$-OH **A** (black), Lau-Dopa(Bn)$_2$-OH **B** (red) and Pal-Phe-OH **C** (blue), triggered with GdL (left), LA (middle) and Ac-Val (right), always 1.3 equiv. The experiments were repeated in triplicate and results are expressed as mean ± standard deviation.

Comparison of the amplitude sweeps revealed that the gels formed using the three triggers exhibited comparable strength, with storage moduli (G') ranging from 10^4 to 10^5 Pascal. A confirmation of these results has been obtained by the analysis of the gelation time of the nine samples, by recording the time sweep analysis of their formation during the first 2 hours (Figure S2).

These results seem to indicate that Ac-Val allows the formation of gels comparable to the GdL ones. A key benefit of Ac-Val is the ease of precise dosing as it can be added as a solution at desired concentration. In contrast, the use of LA presented notable challenges, as foreseen. The lack of a clearly defined concentration in the commercial samples required several repetitions of the experiments to achieve the desired pH range of 4.5 to 5, to obtain hydrogels comparable with the samples obtained adding GdL or Ac-Val.

To better check the properties of the materials, we prepared the corresponding aerogels by freeze-drying the sample and we analyzed the samples by SEM (Figure 3).

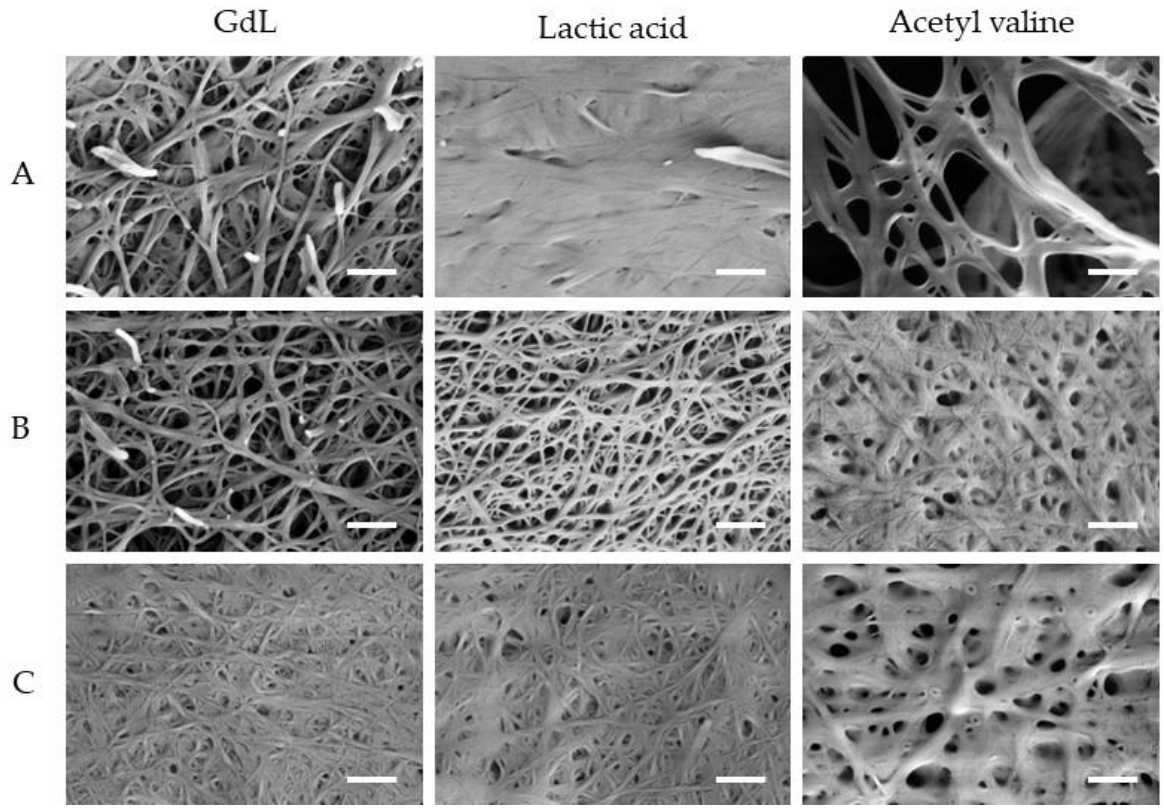

Figure 3. Scanning electron microscopy images of the aerogels of Boc-Dopa(Bn)$_2$-OH **A** (top), Lau-Dopa(Bn)$_2$-OH **B** (middle) and Pal-Phe-OH **C** (bottom), triggered with GdL (left), LA (middle) and Ac-Val (right), always using 1.3 equiv. Scalebar: 600 nm.

The SEM analysis showed a fibrillar morphology being present in all samples. Among the gelators, gelator **A** forms thicker fibrils with a lower interconnectivity, which correlate with the poorer rheological properties of these gels. On the other hand, gelators **B** and **C** form fibrils with comparable size and interconnectivity with a higher propension for gelator **B** to form a porous network.

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Observing the different gels formed using different triggers, gelator **A** shows always fibrils of comparable size with no relevant amorphous materials observed covering the fibrils. Gelator **B** shows a maximum fibrillar thickness between 70 and 90 nm with all the triggers tested, with a small amount of amorphous material being observed covering the fibrils prepared using Ac-Val as a trigger. Finally, gelator **C** shows comparable fibrils when triggered with GdL or LA, both exhibiting clear fibrils with similar maximum thickness (49 ± 8 and 50 ± 10 nm respectively, N = 10). On the other hand, when triggered using Ac-Val the gelator forms thicker fibrils (with a maximum thickness of 120 ± 30 nm, N = 10) while also exhibiting an amorphous phase covering the fibrils. It has to be taken into account that the higher thickness observed may be an artifact due to the amorphous phase deposited on the fibrils.

The samples have been analyzed also by ATR-FTIR spectroscopy (Figure S3) and we could not detect any substantial difference among the absorption bands.

Freeze-dried gels were also analyzed using X-ray powder diffraction (XRPD) to evaluate their crystal structure (see Figure S4-S5-S6). All samples showed a crystal pattern with a different one for each of the three gelators. Despite that, all the gelators showed the most intense diffraction peak at low angle corresponding to about 18 Å for gelator **A**, 29 Å for gelator **B**, and 32 Å for gelator **C**. Each of these lengths is comparable with the length of the gelators' molecule fully extended.

Gelator **A** (Boc-Dopa(Bn)$_2$-OH, Figure S4) showed no relevant crystallographic differences when triggered using LA or Ac-Val but exhibits a completely different crystal structure when triggered using GdL. This last pattern shows a split of the main peak, located around 4.8° (18.4 Å) in the LA and Ac-Val triggered gels, into two diffraction peaks at 4.7° (18.8 Å) and 5.1° (17.1 Å) respectively. This split suggests this polymorphic form present two different conformations of the gelator, one more extended and one more compact while the LA and Ac-Val crystal form has mostly a single conformation.

Contrary, the gel obtained using gelator **B** (Lau-Dopa(Bn)$_2$-OH, Figure S5) shows no relevant difference in crystal structure when prepared using the different triggers.

The gels prepared using gelator **C** (Pal-Phe-OH, Figure S6) also shows similar diffraction patterns. Nonetheless, while the two gels prepared using GdL and Ac-Val show a similar level of crystallinity, a higher crystallinity was observed when LA is used. Using the Scherrer equation it was possible to calculate the crystallite size on the main peak, corresponding to 29.4 nm for the gel prepared using LA and 15.6 nm for the gels prepared using GdL and Ac-Val. When observing the main peak, a small shift is also visible between samples. The sample prepared using LA is the one with the higher angle, 2.92° (30.0 Å), followed by the one prepared using Ac-Val, 2.83° (31.1 Å), and the one prepared using GdL, 2.70° (32.7 Å). This trend suggests how the three triggers induce a different average extension of the molecule, being the one triggered using GdL the one with the higher extension. Other small shifts with the same trend are visible in the diffraction patters as reported in the Figure S6.

From the comparison of the results obtained using the three different triggers, we can conclude that Ac-Val is a valid trigger for forming gels *via* the pH change method, with the three gelators.

To obtain stable gels at neutral pH for drug delivery applications, we prepared gels with the three gelators, adding decreasing amounts of trigger, to increase the final pH of the gels. The gelator was suspended in Milli-Q® H_2O, and NaOH (1 equivalent, 0.1 M aqueous solution) was added. The solution was stirred and sonicated until the gelator was completely dissolved. Then, Ac-Val was added as an aqueous solution. The final volume of each sample was adjusted to achieve a 0.5% w/v gelator concentration. Following the trigger addition, the solutions were gently stirred for a few seconds and then left to stand for 16 hours. All twelve additional samples formed gels (Figure S7), and their mechanical properties were analyzed and compared using rheological analysis. The results are shown in Figure 4.

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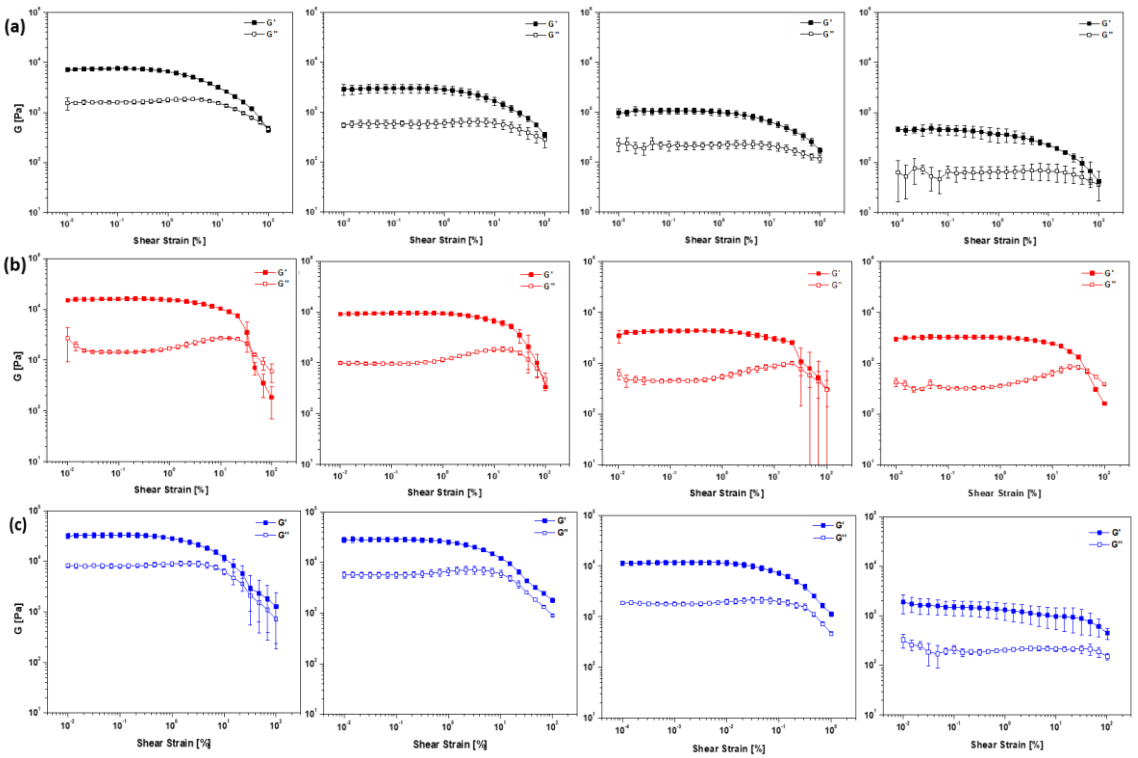

Figure 4. From top to bottom: results for amplitude sweep tests of the hydrogels of Boc-Dopa(Bn)$_2$-OH **A** (black), Lau-Dopa(Bn)$_2$-OH **B** (red) and Pal-Phe-OH **C** (blue), triggered with Ac-Val: from left to right 1.15 equiv., 1.00 equiv., 0.85 equiv. and 0.70 equiv. The experiments were repeated in triplicate and results are expressed as mean ± standard deviation.

The formation of a complex network that produces the gel formation was confirmed by SEM analysis of the corresponding aerogels (Figure 5). The results showed how decreasing the number of equivalents of Ac-Val used induce a fibrillar thickening and shortening in gelator **A**, leading to a lower interconnectivity and consequently a weaker gel. Contrary, gelator **B** shows always a comparable fibrillar morphology with a gradual decrease in the amorphous phase covering the fibrils. Finally, gelator **C** shows comparable morphologies when using 1.15 and 1.00 equivalents and a decrease in the amorphous phase when using 0.85 equivalents. Contrary to gelator **B**, a drastic morphological change is observed when using 0.70 equivalents with fibrils barely visible and the amorphous phase being the main component of the hydrogel. For both gelator **B** and **C** this decrease in the amorphous phase associated with the fibrils aligns with the decrease in the rheological properties of the gel. This may suggest that, when associated with a well-formed fibrillar network, the amorphous phase is a critical component of the hydrogel that may act as a binder between the fibrils, thus reinforcing the hydrogel network.

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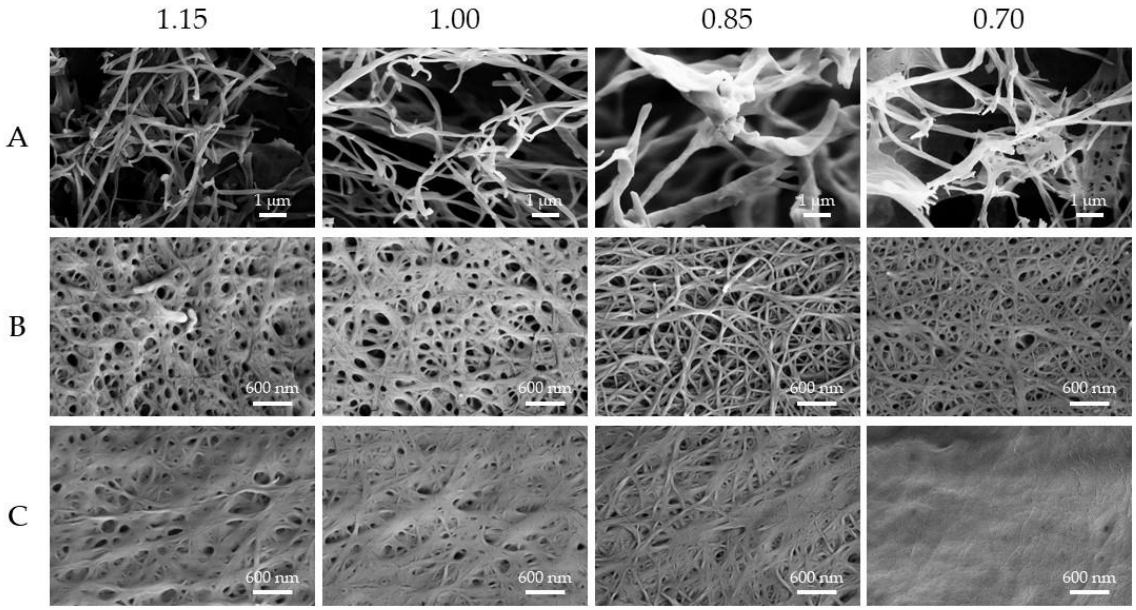

Figure 5. Scanning electron microscopy images of the aerogels of Boc-Dopa(Bn)$_2$-OH **A** (top), Lau-Dopa(Bn)$_2$-OH **B** (middle) and Pal-Phe-OH **C** (bottom), triggered with Ac-Val: from left to right 1.15 equiv., 1.00 equiv., 0.85 equiv. and 0.70 equiv.

The samples were also analysed using ATR-FTIR spectroscopy (Figure S8), and as expected, no significant differences were observed in the absorption bands.

The XRPD analyses of the samples highlighted small changes in the crystallographic structures when different equivalents of Ac-Val were used. The gelator **A** showed a shift of the main peak from 4.8° (18.3 Å) to 5.0° (17.8 Å) when moving from 1.00 eq. to 0.80 eq. of trigger. This may indicate how a higher pH may result in a more compact conformation of the gelator. A similar trend was observed for the peak at 9.6°, exhibiting a shift to 9.7°. Gelator **B** showed no shift in the main peak but one of a low intensity signal from 14.9° to 13.9° when moving from 1.15 equivalents to 1.00 equivalents. Similarly, gelator **C** shows only a small shift of the signal from 4.0° to 4.2° when moving from 1.15 equivalents to 1.00 equivalents. It is interesting to notice how this shift brings the signal is the same position as when GdL is used. No relevant changes in the overall crystallinity, or the main peak crystallite size, was observed decreasing the amount of trigger for any of the gelators.

Table 1 summarizes the results obtained for gel formation with the three gelators. In all cases gels were formed, although their strength decreased as the pH increased, approaching physiological values. We can thus confirm that Ac-Val provides a highly tuneable approach, allowing for precise control of the desired pH by adjusting the equivalents added to the gelator. Specifically, the addition of 0.7 equivalents consistently resulted in gels, close to physiological pH, suitable for drug-delivery applications. Furthermore, by simply adjusting the Ac-Val equivalents, gels with any pH value between 4 and 7 can be easily formed. Carefully tuning the conditions of gel formation and using higher concentrations of gelator (i.e. 1% or 2% w/v), it is also possible to make gels with final pH reaching 7.5-7.6.

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Table 1. Summary of the properties of the gels obtained by varying the equivalents of trigger (Ac-Val) with the three different gelators, **A** (Boc-Dopa(Bn)$_2$-OH), **B** (Lau-Dopa(Bn)$_2$-OH) and **C** (Pal-Phe-OH).

Gelator	Trigger	Trigger equiv.	G′ (KPa)	G″ (KPa)	pH
A	GdL	1.3	18.14 ± 3.82	3.82 ± 1.44	4.39 ± 0.10
	LA	1.3	6.21 ± 0.93	1.41 ± 0.19	5.06 ± 0.03
	Ac-Val	1.3	10.64 ± 2.73	2.03 ± 0.53	4.28 ± 0.12
		1.15	7.54 ± 0.43	1.59 ± 0.13	4.67 ± 0.09
		1.00	3.02 ± 0.59	0.59 ± 0.11	5.40 ± 0.05
		0.85	1.06 ± 0.15	0.22 ± 0.05	6.07 ± 0.06
		0.70	0.45 ± 0.10	0.07 ± 0.05	6.62 ± 0.11
B	GdL	1.3	13.71 ± 1.28	1.18 ± 0.24	4.33 ± 0.04
	LA	1.3	10.35 ± 2.46	1.22 ± 0.25	4.48 ± 0.17
	Ac-Val	1.3	15.70 ± 1.01	1.79 ± 0.14	4.23 ± 0.05
		1.15	15.98 ± 1.06	1.44 ± 0.06	4.56 ± 0.13
		1.00	9.48 ± 0.73	0.96 ± 0.06	5.18 ± 0.05
		0.85	4.46 ± 0.30	0.42 ± 0.03	5.40 ± 0.04
		0.70	3.26 ± 0.31	0.32 ± 0.03	6.57 ± 0.09
C	GdL	1.3	24.54 ± 3.22	5.44 ± 0.95	4.15 ± 0.03
	LA	1.3	32.02 ± 1.96	6.89 ± 0.50	4.54 ± 0.05
	Ac-Val	1.3	32.87 ± 0.82	8.24 ± 0.26	4.09 ± 0.01
		1.15	32.97 ± 3.62	8.05 ± 0.73	4.40 ± 0.04
		1.00	28.50 ± 2.83	5.68 ± 0.79	4.73 ± 0.03
		0.85	11.71 ± 1.17	1.78 ± 0.14	5.36 ± 0.06
		0.70	1.15 ± 0.41	0.17 ± 0.70	6.69 ± 0.10

To investigate whether the addition of less than one equivalent of the trigger could induce fibril formation, we conducted ^{1}H NMR spectroscopy. This technique is highly informative [39–41] because molecules incorporated into fibers are no longer in solution, thus are not visible in the NMR spectrum. We acquired ^{1}H NMR spectra of the three gels formed by adding Ac-Val (0.7 equiv.) to a solution of the gelator in D_2O/NaOD. These spectra were recorded 16 hours after the trigger addition, once the gels had set. For comparison, we also recorded ^{1}H NMR spectra of Ac-Val in D_2O and of the three gelators dissolved in D_2O/NaOD before the trigger was introduced (Figures S12-S14). In all three hydrogel samples, the spectra displayed only the peaks corresponding to Ac-Val, indicating that this molecule does not participate in network formation. Conversely, the peaks associated with

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the gelators were absent, confirming their involvement in the network structure, even with only 0.7 equivalents of the trigger. This result suggests that the final pH is the primary determinant for fibril formation in gelators containing a carboxy group. Figure 6 presents a plot summarizing the strength of the hydrogels as a function of their pH, which can assist users in selecting the appropriate amount of Ac-Val to achieve the desired final pH.

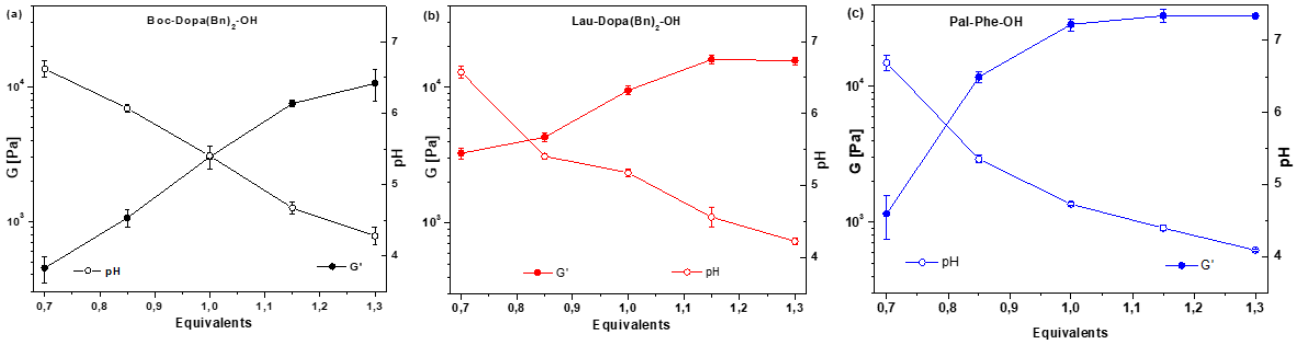

Figure 6. Correlation between G′ and pH of hydrogels of Boc-Dopa(Bn)2-OH (black), Lau-Dopa(Bn)2-OH (red) and Pal-Phe-OH (blue) triggered with variable equivalents of Ac-Val.

The three samples exhibited gel-like behaviour, as demonstrated by the rheological analysis presented in Figure 4 and summarized in Table 1.

To further characterize these promising materials, we evaluated their stability under heating (temperature sweep) and after shaking (thixotropy). Thixotropy is the property of a gel to become temporarily fluid when shaken or stirred (G″ > G′), then they quickly recover the solid state (G′ > G″) [42,43].

The results, shown in Figure 7, confirm that all three materials are both thixotropic and thermally stable. The gels formed with Boc-Dopa(Bn)2-OH (black) and Lau-Dopa(Bn)2-OH (red) remained stable up to 85 °C, while the gel derived from Pal-Phe-OH (blue) melted at approximately 80 °C. In all cases, these gels demonstrate sufficient stability for biomedical applications, as in drug delivery systems.

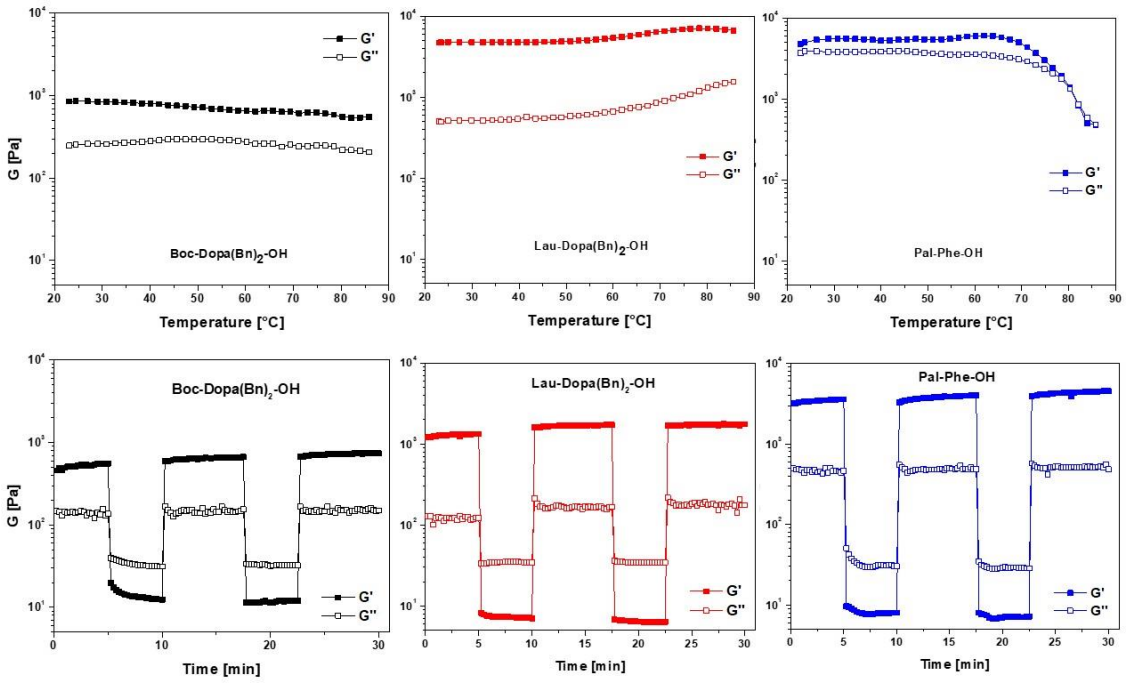

Figure 7. Top: results for temperature sweep tests of the hydrogels of Boc-Dopa(Bn)2-OH **A** (black), Lau-Dopa(Bn)2-OH **B** (red) and Pal-Phe-OH **C** (blue), triggered with Ac-Val (0.70 equiv). Bottom: Top: results for

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thixotropy tests of the hydrogels of Boc-Dopa(Bn)$_2$-OH **A** (black), Lau-Dopa(Bn)$_2$-OH **B** (red) and Pal-Phe-OH **C** (blue), triggered with Ac-Val (0.70 equiv).

3. Materials and Methods

Chemicals – All chemicals and solvents were purchased from Sigma-Aldrich (Germany), apart from L-Dopa (TCI) and palmitic acid (Acros Organics).

General Remarks for the Synthetic Procedure - All reactions were carried out in dried glassware and all compounds were dried *in vacuo.*

The gelators **A**, **B** and **C** were prepared according to the procedures described in previous papers [32,35,44]. All the characterization data matched the literature values.

The SEM analyses were acquired with a LEO 1530 FEG (Zeiss, Oberkochen, Germany) using a voltage of 5 kV, an aperture of 60 μm, and a working distance of 7 mm. Prior analyses the samples were frozen with liquid nitrogen and freeze-dried. Prior analyses a portion of the resulting aerogel was glued on a carbon tape and coated with about 20 nm of gold.

High quality infrared spectra (64 scans) were obtained at 2 cm^{-1} resolution with an ATR-FTIR Agilent (Santa Clara, CA, USA) Cary 630 FTIR spectrometer. pH meter XS pH 8 PRO Basic (XS Instruments, Carpi (MO), Italy) equipped with XS Sensor Standard T BNC was used to measure the samples pH. NMR spectra were recorded with a Varian (Palo Alto, CA, USA) Inova 600 spectrometer at 600 MHz (^{1}H NMR).

XRPD analyses were performed using an Empyrean from Malvern Panalytical (Malvern, Worcestershire, United Kingdom), equipped with a rotating flat holder (with a rotation time of 2 s), using Cu Kα radiation generated at 40 kV and 40 mA (λ = 1.54056 Å). The diffractograms were collected between 2° and 30° using a step size of 0.04° and 400 s of counting time.

The rheological analyses were performed using an Anton Paar (Graz, Austria) MCR 102 rheometer. A cup and vane measuring system was used, setting a gap of 2.2 mm. The gel samples were prepared in 7 mL Sterilin Cups® that fit in the rheometer and were left to rest for 16 h at room temperature before their use.

Oscillatory amplitude sweep experiments were performed at 23 °C using a Peltier control system and the data points were collected (γ: 0.01 – 100%) using a constant angular frequency of 10 Hz. Time Sweep experiments were performed at 23 °C (controlled by an integrated Peltier system) using a constant shear strain (γ) of 0.5% and a constant angular frequency (ω) of 10 rad/s, collecting 1 point every minute. Temperature sweep analyses were performed on 2 mL gels prepared in glass vials that fit the rheometer, at a constant γ (0.2%) and ω (10 rad/s), by increasing the temperature using a linear ramp from 23 to 85 °C, with a rate of 3 °C/min.

Synthesis of Ac-Val - Ac-Val was synthetized using an acetylation procedure previously reported in the literature [45]. A suspension of L-valine (2.38 g, 20.3 mmol) and acetic anhydride (5.0 mL, 5.40 g, 52.9 mmol) in methanol (9.0 mL) was stirred under reflux for 6 h. After cooling to rt all volatiles were removed under reduced pressure and the reaction crude was washed twice with diethyl ether and twice with hexane, under sonication. The resulting colorless solid was collected by filtration and dried in vacuo. Yield: 2.31 g (14.5 mmol, 71.5%), colorless solid. Mp: 166–168 °C. ^{1}H NMR (400 MHz, MeOD) δ: 4.32 (1 H,d, J = 5.7 Hz, NHCH), 2.16 (1 H, dsept, J = 6.9, 5.6 Hz, CH(CH$_3$)$_2$), 2.01 (3 H, s, C(O)CH$_3$), 0.97 (6 H, dd, J = 6.9, 2.7 Hz); ^{13}C NMR (101 MHz, MeOD) δ: 174.9 (C), 173.5 (C), 59.1 (CH), 31.6 (CH), 22.3 (CH$_3$), 19.5 (CH$_3$), 18.3 (CH$_3$). The data are consistent with the literature [46].

Procedure for Gel Preparation - The gels used for the rheological analysis were prepared in 7.0 mL Sterilin Cups®, using the following procedure: the gelator was suspended in Milli-Q® H$_2$O and 1.0 equivalents of NaOH (0.1 M, aq) were added. The solution was stirred and sonicated until the complete dissolution of the gelator. To trigger the formation of the gel, three different triggers were used: Ac-Val, lactic acid and glucono-δ-lactone (GdL). Ac-Val and lactic acid were added in volume while GdL was added by weight, according to the gelators equivalents required to trigger the gel formation. Acetyl Valin solutions were prepared dissolving in 1 mL of Milli-Q® H$_2$O respectively 1.3,

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1.15, 1.0, 0.85 and 0.7 equivalents. Lactic Acid solutions were prepared by diluting a solution 11.88 M. After the addition of the trigger the solutions were gently stirred and then placed at rest for 18 h.

Procedure for Gel Preparation for ^1H NMR analysis - The gels used for the ^1H NMR analysis were prepared using the following procedure: the gelator 0.5 %w/V was suspended in D_2O and 1.0 equivalent of NaOD (0.1 M, aq) was added. The solution was stirred and sonicated until the complete dissolution of the gelator. Before adding the trigger, the ^1H NMR spectra was acquired. To trigger the formation of the gel a small volume of Ac-Val (0.7 equivalents) dissolved in D_2O was added to the NMR tube containing the dissolved gelators. After the addition of the trigger, the NMR tube was placed at rest for 18 h and then the ^1H NMR spectra of the gel was acquired.

4. Conclusions

This study delineates the utility of Ac-Val as an innovative pH-responsive hydrogelation trigger. The efficacy of Ac-Val was tested with three gelators, allowing the formation of hydrogels with tunable pH values, that makes them good candidates for their use in drug delivery systems.

This molecule exhibits complete aqueous solubility and prolonged solution stability (weeks), facilitating the preparation of stock solutions across a broad concentration range. Its aqueous addition ensures immediate and homogeneous dispersion within the gelator solution, leading to rapid formation of macroscopically homogeneous gel networks. This may lead to the fabrication of hydrogels with tunable final pH values, a feature of significant value for applications requiring precise pH microenvironments, particularly within drug delivery systems.

Mechanistically, the carboxylic acid functionality of Ac-Val induces an instantaneous pH adjustment at the desired pH, as LA, in contrast with GdL, that exhibits slow, progressive pH reduction during the gelation process. Although XRPD analyses of the aerogels suggest that the three triggers may induce different organization of the molecules in the fibril formation, the final gels show similar properties at the same pH.

In conclusion, this acid-catalyzed hydrogelation strategy demonstrates broad applicability, capable of inducing gelation across a diverse array of molecules possessing acidic moieties.

Supplementary Materials: Figure S1. Photographs of gels triggered with 1.3 equiv. of GdL, LA and Ac-Val; Figure S2. Time sweep of gels triggered with 1.3 equiv. of GdL, LA and Ac-Val; Figure S3. ATR-IR spectra of aerogels obtained with gels triggered with 1.3 equiv. of GdL, LA and Ac-Val; Figure S4. XRPD analyses of Boc-Dopa(Bn)$_2$-OH aerogels obtained with gels triggered with 1.3 equiv. of GdL, LA and Ac-Val; Figure S5. XRPD analyses of of Lau-Dopa(Bn)$_2$-OH aerogels obtained with gels triggered with 1.3 equiv. of GdL, lactic acid and Ac-Val; Figure S6. XRPD analyses of Pal-Phe-OH aerogels obtained with gels triggered with 1.3 equiv. of GdL, LA and Ac-Val; Figure S7. Photographs of gels triggered with decreasing equivalents of Ac-Val; Figure S8. ATR-IR spectra of aerogels obtained with gels triggered with decreasing equivalents of Ac-Val; Figure S9. XRPD analyses of Boc-Dopa(Bn)$_2$-OH aerogels obtained with different equivalents of acetyl valine; Figure S10. XRPD analyses of Lau-Dopa(Bn)$_2$-OH aerogels obtained with different equivalents of acetyl valine.; Figure S11. XRPD analyses of Pal-Phe-OH aerogels obtained with different equivalents of acetyl valine; Figure S12. ^1H NMR spectra of hydrogels obtained from Boc-L-DOPA(Bn)$_2$-OH triggered with Ac-Val (0.7 equiv.) in D_2O; Figure S13. ^1H NMR spectra of hydrogels obtained from Lau-L-DOPA(Bn)$_2$-OH triggered with Ac-Val (0.7 equiv.) in D_2O; Figure S14. ^1H NMR spectra of hydrogels obtained from Pal-Phe-OH triggered with Ac-Val (0.7 equiv.) in D_2O.

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3.3 "Introduction of innovative doctorates that meet the innovation needs of companies and promote the recruitment of researchers from companies".

Data Availability Statement: The authors confirm that the data supporting the findings of this study are available within the article and its Supplementary Materials.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Ac-Val	acetyl-L-valine
Phe	Phenylalanine
Dopa	3,4-dihydroxyphenylalanine
Lau	lauroyl
Pal	palmitoyl
LMWGs	Low molecular weight gelators
GdL	glucono-δ-lactone
Boc	tert-butyloxycarbonyl
SEM	Scanning Electron Microscope
NMR	Nuclear Magnetic Resonance
LA	Lactic Acid
XRPD	X ray powder diffraction
ATR-IR	Attenuated total reflection infrared spectroscopy

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