Supporting Information

for

Light responsive single amino acid-based supramolecular hydrogel for photo-controlled vitamin release

Divya Chauhan, Chandan Maity*

(Organic)Material Science and Engineering Laboratory, Centre for Nanobiotechnology, Vellore Institute of Technology (VIT), Vellore Campus, Tamil Nadu-632014, India.

Email: chandanmaitylab@gmail.com, chandan.maity@vit.ac.in (CM)

Instrumentation and characterization:

Nuclear magnetic resonance (NMR) spectra (^{1}H NMR and ^{13}C NMR) were acquired using a Bruker Avance III 400 MHz spectrometer at 298K using residual protonated solvent signals as internal standard. Thin layer chromatography (TLC) was performed on Merck Silica Gel 60 F254 TLC plates, and were visualized under UV light of 254 nm wavelength. Fourier-transform infrared spectroscopic (FTIR) analysis was performed using the Thermo Fisher Scientific, Thermo Nicolet iS50 with an inbuilt ATR. The spectra were recorded in the range of 4000 cm^{-1} to 400 cm^{-1} with resolution of 2 cm^{-1}. The spectra were obtained by averaging over 32 scans. High-resolution mass spectrometry (HRMS) was performed using an Acetonitrile, XEVO-G2-XS-QTOF system. Field Emission Scanning Electron Microscope (FE-SEM) was recorded by Thermo Fischer FEI QUANTA 250 FEG. Transmission electron microscopic (TEM) images were recorded with a G2-20 TWIN (Operating voltage 200 kV) (FEI–TECNAI) transmission electron microscope. rating voltage 200 kV) (FEI–TECNAI) transmission electron microscope. UV-Vis absorption spectra were recorded on a diode-array spectrophotometer (Shimadzu UV-2600) at room temperature. The cell path length was 1.0 cm. Fluorescence spectra was recorded by using Hitachi F 7000 Fluorescence Spectrophotometer instrument with a 1.0 cm path length quartz cuvette. Irradiation experiment conducted using Kessil PR160L-370 nm Gen 2 UV light. All the hydrogel samples which are lyophilized using an Alpha 1,2 LD-plus Martin Christ lyophilizer. Confocal laser scanning microscopic (CLSM) images were taken using an Olympus Confocal Laser Scanning Microscope-Fluoview Fv3000 at a 10x magnification. The rheology measurements were conducted on Anton Paar MCR 92 rheometer operating in oscillatory mode, with a parallel plate geometry. All pH measurements were done using Aceteq pH meter (Model: PH-035).

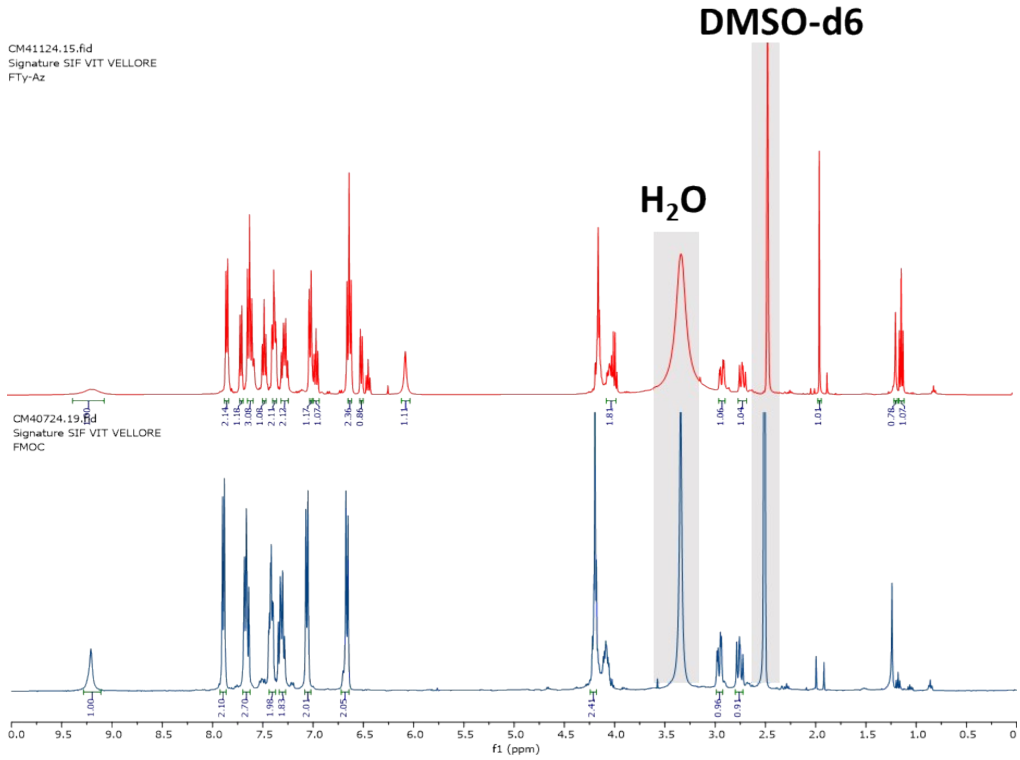


Figure S1: Comparative ^{1}H NMR spectra of FmocY (bottom) and FmocY-Azo (top) in DMSO-d_6 at room temperature.

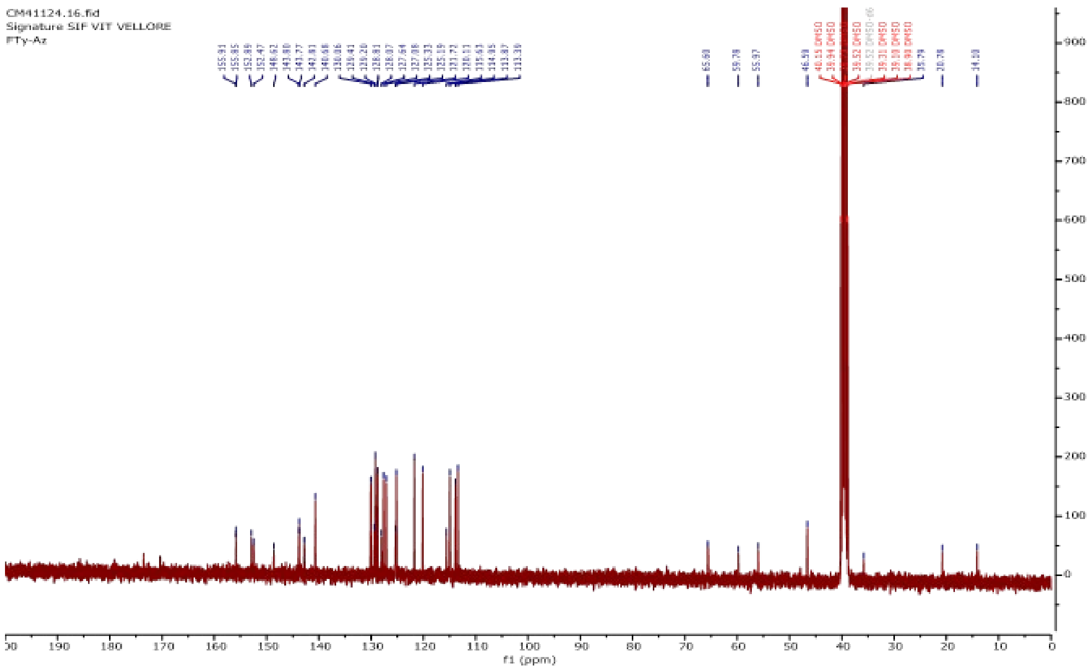


Figure S2: ^{13}C NMR spectrum of FmocY-Azo in DMSO-d_6 at room temperature.

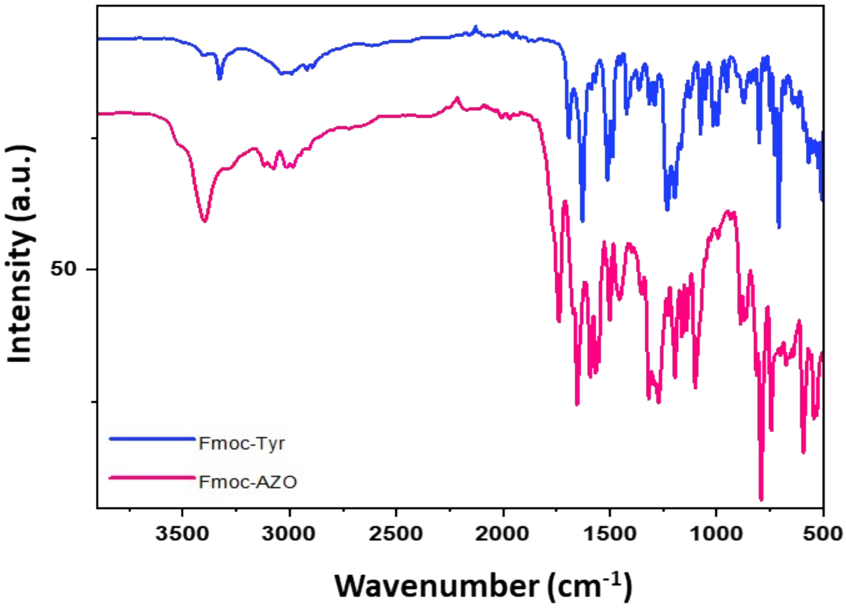


Figure S3: Comparative IR spectrum of FmocY (blue line) and FmocY-Azo (pink line).

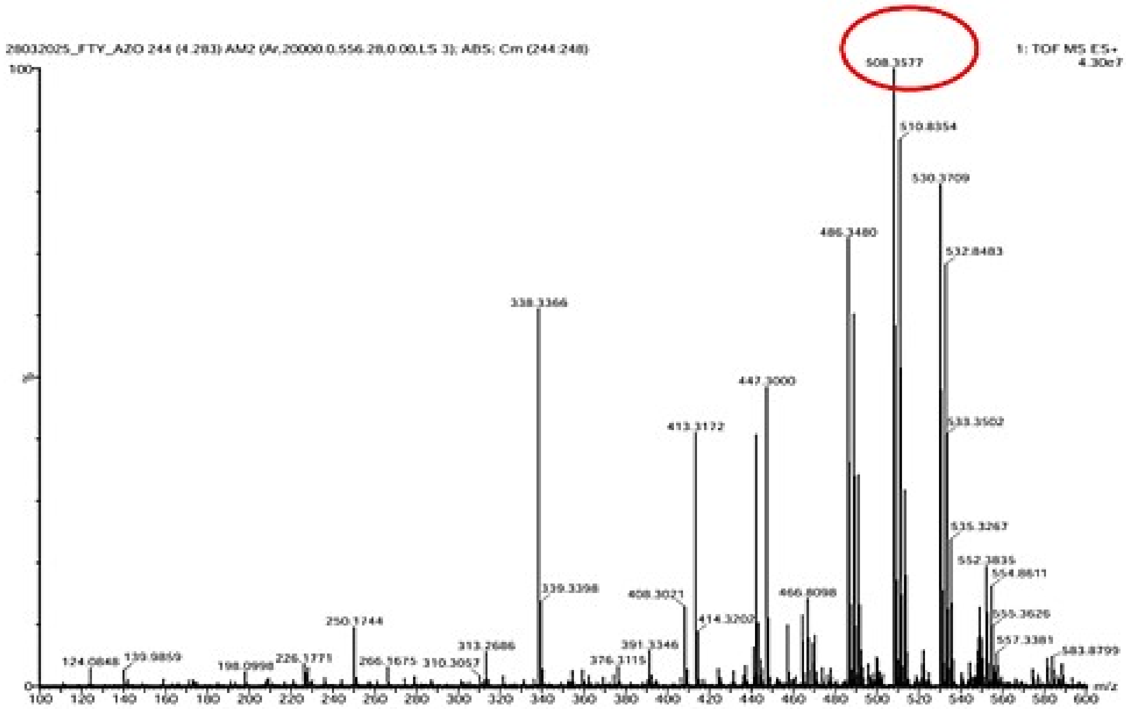


Figure S4: Mass spectrum of FmocY-Azo.

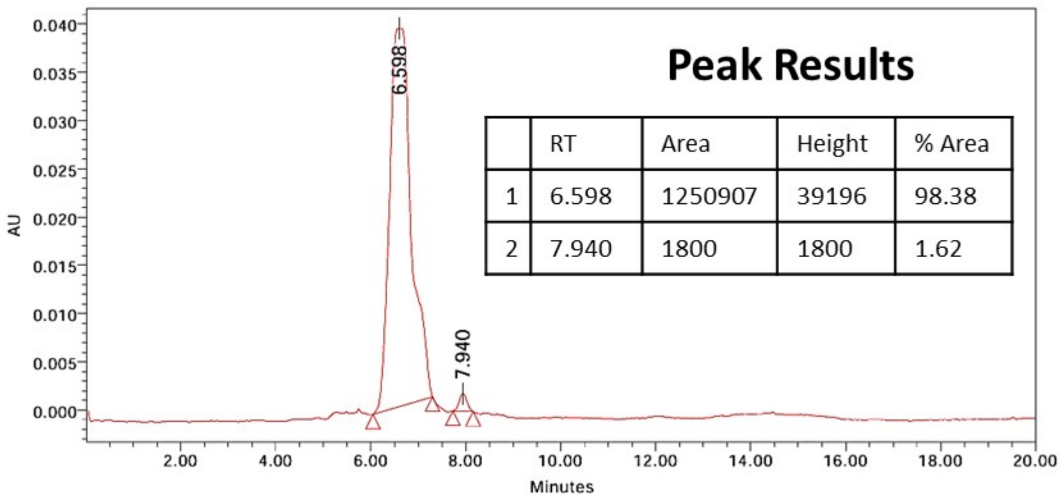


Figure S5: HPLC profile of FmocY-Azo.

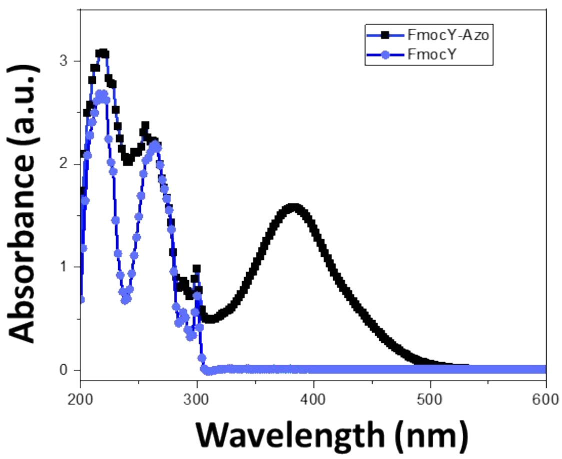


Figure S6: Comparative absorption spectra of FmocY (violet line), and FmocY-Azo (black line) in DMSO (conc. = 2x 10^{-4} M) at room temperature.

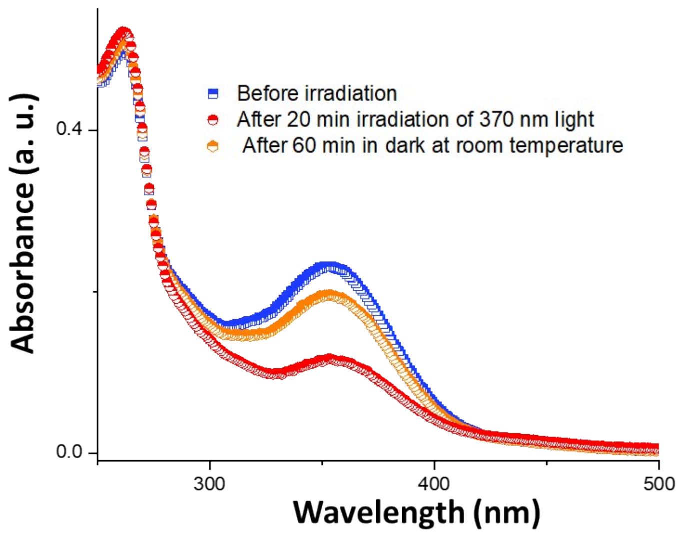


Figure S7: UV-Vis absorption spectra of FmocY-Azo in >99 % water (conc. = 5 x 10^{-5} M) before light irradiation (blue square), upon irradiation of 370 nm light for 20 minutes (red circle), and after keeping in the dark at room temperature for 60 minutes (orange hexagon).

Preparation of Hydrogel and determination of minimum gelation concentration (MGC).

Table S1: Optimization data for FmocY-Azo hydrogelation at different pH.

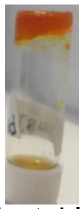
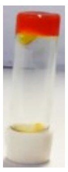
pH of Buffer solution	4	5	6	7	7.4	8
Conc. of FmocY-Azo (in mM)	5.4	5	5.7	6.0	6.3	6.5
Time required for gelation	45 min	45 min	80 min	90 min	120 min	> 2 h
Observation	 Stable & strong	Stable & strong	 Stable & strong	 Stable	 Stable	 Unstable

Table S2: Optimization data for FmocY-Azo hydrogelation at pH = 5

Conc. of FmocY-Azo (mM)	4.5	4.7	4.9	5
Observation	 Unstable	 Unstable	 Stable, but weak	Stable & strong

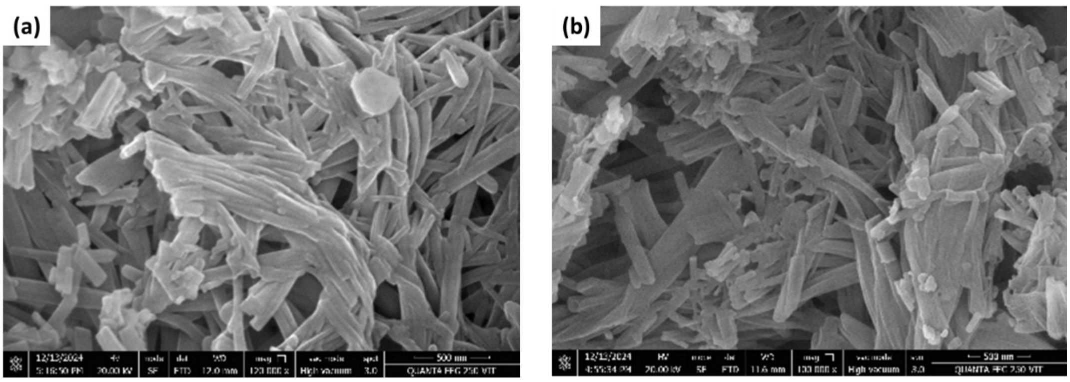


Figure S8: FESEM micrograph of the hydrogel prepared at (a) pH = 7, and (b) pH = 8. Scale bar is 500 nm.

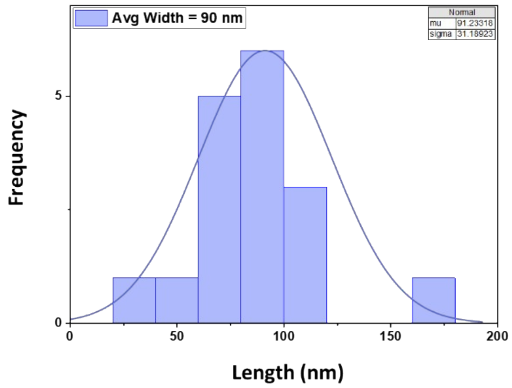


Figure S9: Distribution of fiber diameter from TEM image (Figure 3f).

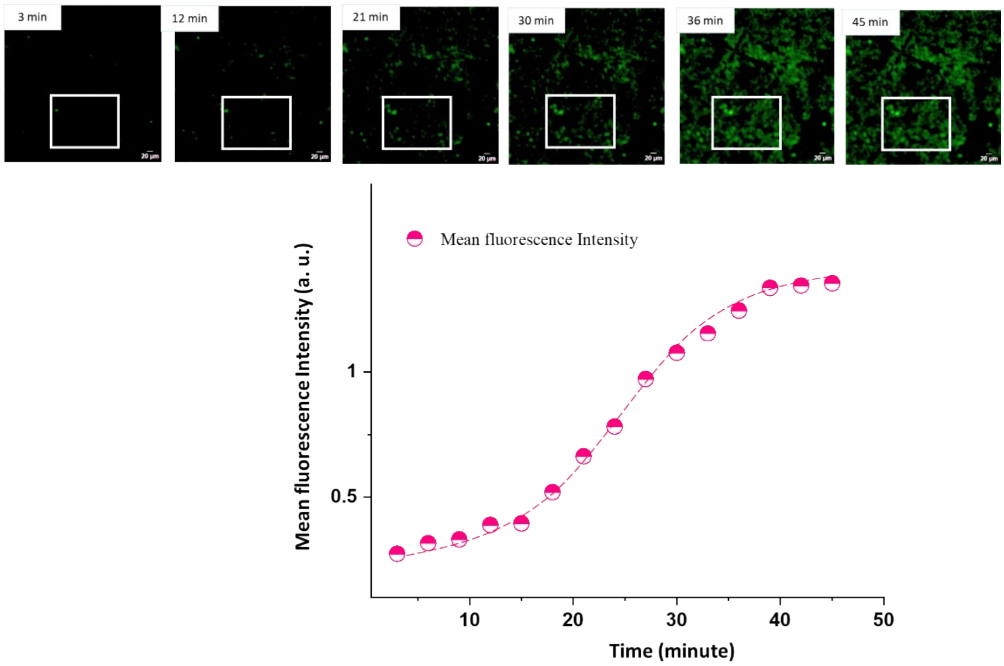


Figure S10: The CLSM images were imported into ImageJ software, and the specific region was selected. Fluorescent intensity of the rectangular zone was measured with time. Mean fluorescence intensity was measured with time showing change in fluorescence intensity over time. The mean value was plotted using Origin software. The line is drawn to guide the eyes.

Table S3: Thixotropic behaviour at different pH.

pH of medium	MGC (mM)	Recovery time (in hour)
4	5.4	Not recovered
5	5.0	~5
6	5.7	6
7	6.0	Not recovered
8	6.5	Not recovered

Light-induced gel↔sol transitions:

Table S4: Optimization data for Gel to sol of FmocY-Azo hydrogel upon light irradiation

Conc. of FmocY-Azo (mM)	Time required for gel→ sol conversion upon light irradiation
5.0	30 min
5.5	60 min
6.0	130 min
6.5	>200 min
7.0	>200 min

Vitamin B_{12} (VB_{12}) entrapped with FmocY-Azo hydrogel:

Table S5: Optimization for VB_{12} trapped FmocY-Azo hydrogel in pH 5.0

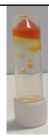
Conc. of FmocY-Azo with VB_{12} (mM)	5.0	5.4	5.7	5.9	6.0
Observation	 unstable	 unstable	 weak	 weak	 stable

Table S6: Optimization for VB_{12} trapped FmocY-Azo hydrogel in pH 7.4

Conc. of FmocY-Azo with VB_{12} (mM)	6.3	6.6	7.0	7.4	7.8
Observation	 No gelation	 unstable	 Unstable	 weak	 stable

Light-induced release of VB_{12}:

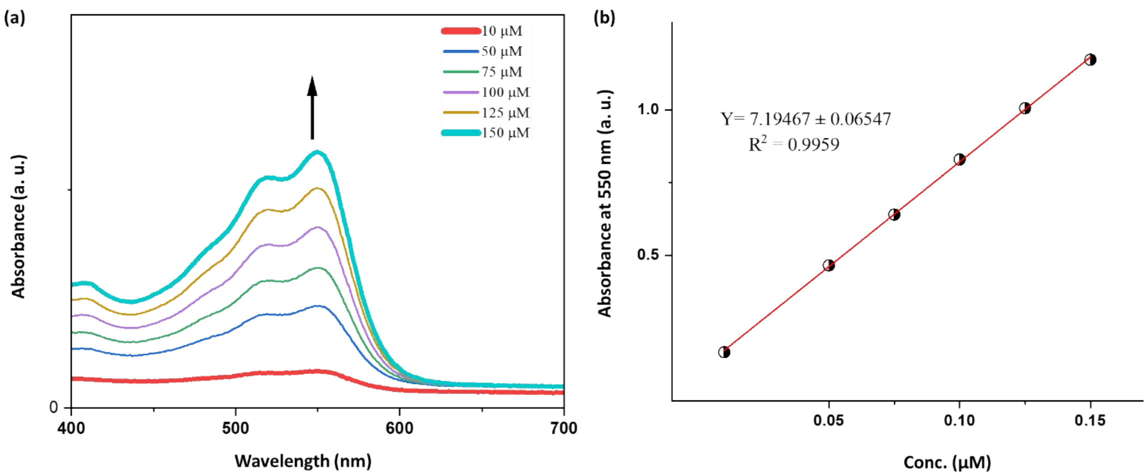


Figure S11: UV-Vis absorption spectra of VB_{12} with increasing concentration, the calibration graph for VB_{12} recorded at 550 nm.

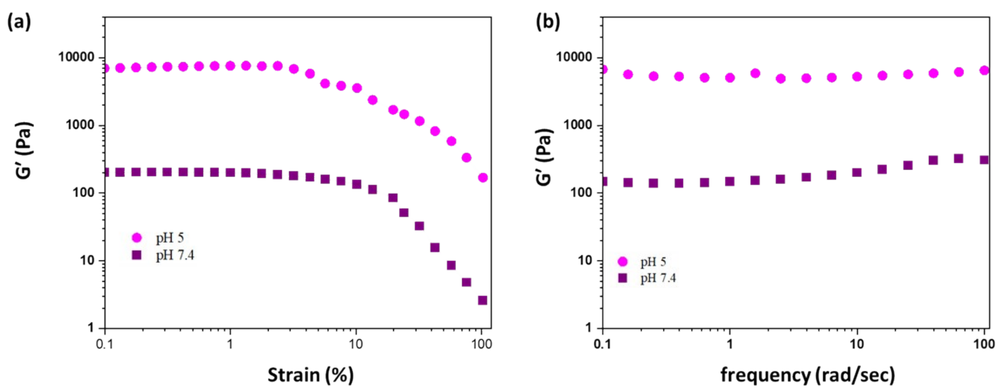


Figure S12: Rheology measurement of the gel prepared at pH = 5.0 (purple circle) and at pH 7.4 (magenta square): (a) strain sweep performed at 6 rad/sec, (b) frequency sweep performed at 0.1 % strain.

Kinetic study for the release VB_{12}:

Table S7: Equation for release study[S1]

Equation for release study	Equation
Zero Order	$Qt = k_o t + Q_o$
First order	$\log C = \log C_0 + K_t/2.303$
Higuchi	$Q = KH\sqrt{t}$
Korsmeyer-Peppas	$\log (M_t/M_\infty) = \log k + n \log t$

Absorbance at 550 nm was plotted as the dependent variable with time (t) as independent variable, and the graph was fitted in Origin 2022 to obtain the correlation coefficient (R^2). The best fits of VB12 release are given in **Figure S13** and **Figure S14** for pH = 5.0.

Table S8: Result of release Model in term of R^2 at pH=5.0

Release Model	**VB_{12} release without light irradiation**	**VB_{12} release with light irradiation**
Zero-Order	$R^2 = 0.99874$	$R^2 = 0.99873$
First-Order	$R^2 = 0.98515$	$R^2 = 0.98839$
Higuchi	$R^2 = 0.97724$	$R^2 = 0.98818$
Korsmeyer-Peppas	$R^2 = 0.98803$	$R^2 = 1.0$

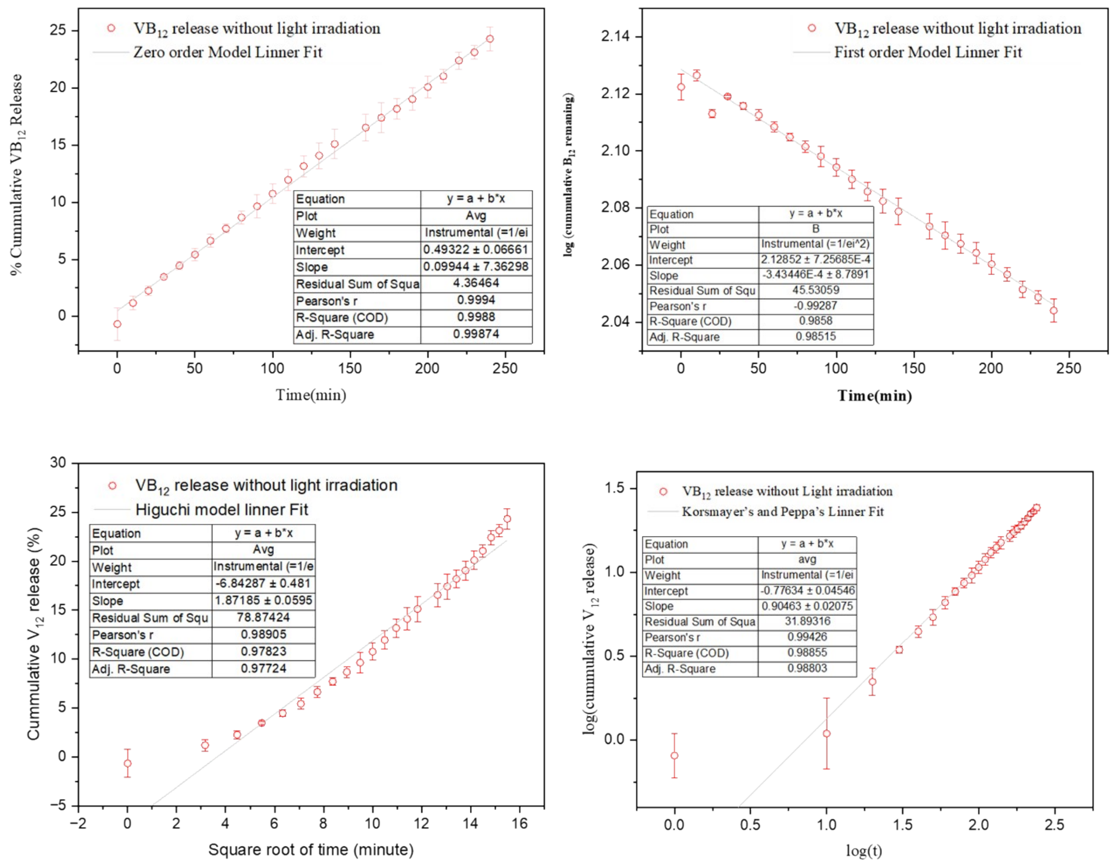
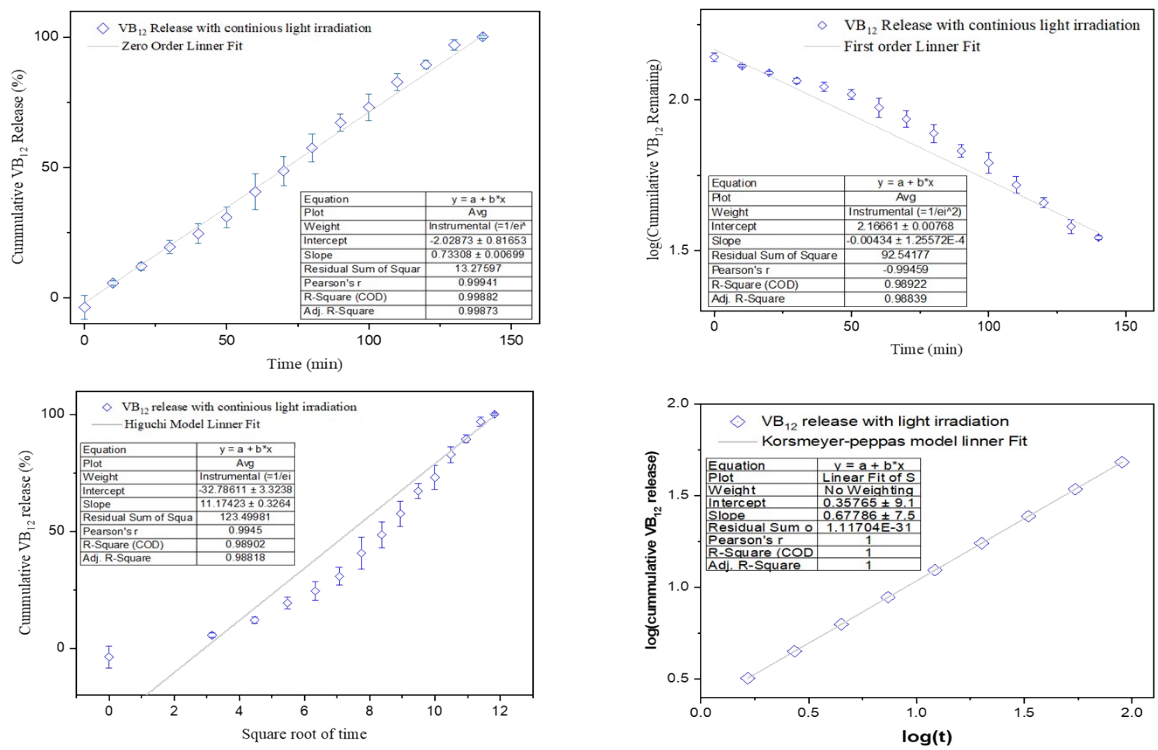


Figure S13: Kinetic studies for the release of VB_{12} at pH = 5.0 without light irradiation, based on



four models.

Figure S14: Kinetic studies for the release of VB_{12} at pH = 5.0 in presence of light irradiation, based on four models.

References

[S1] S. Dash, P. N. Murthy, L. Nath, P. Chowdhury. Kinetic modeling on drug release from controlled drug delivery systems. *Acta. Pol. Pharm.* 2010, **67**, 217-23.