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Insulin-induced conformational transition of fluorescent diblock copolymer: A perspective of self-assembly between protein and micellar solution of smart copolymer

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Instrumentation and measurements

The Fourier transform infrared (FTIR) spectrum was recorded on an iS 50 FT-IR (Thermo-Fisher scientific) spectrometer. The bubble-free samples were placed into an IR cell with two ZnSe windows. All of the samples were pre-equilibrated prior to measurements. A chromel-alumel K-type thermocouple was provided for continuous monitoring of the temperature inside the sample chamber. Each IR spectrum reported here was an average of 200 scans using a spectral resolution of 4 cm^{-1}. Omnic software was used to analyze the FTIR spectra. Ultraviolet-visible (UV-vis) absorption spectra of the copolymers were recorded from 190 to 800 nm by means of a double beam UV-visible spectrophotometer (UV-1800, Shimadzu Co., Japan) at room temperature. An aliquot of sample solution was transferred uniformly into the quartz cell of 1 cm path length. The spectrophotometer had matched quartz cells, with spectral bandwidth of 1 nm and wavelength accuracy of ±0.3 nm with automatic wavelength correction. Fluorescence intensity measurements of aqueous copolymer solution in the absence and in the presence of insulin were carried out using a Cary Eclipse fluorescence spectrophotometer (Varian optical spectroscopy instruments, Mulgrave, Victoria, Australia) with an intense Xenon flash lamp as light source. Emission spectra were recorded with PMT voltage of 720 V. Scan speed was kept at 1200 nm min^{-1}. Quartz cuvette (QC) containing sample was placed in multi cell holder, which is electro-thermally controlled at precise temperature by a peltier. The temperature control of the peltierthermostated cell holders is extremely stable over time, with a typical precision of ±0.05 K. prior to measuring each sample solution was left for 30 min undisturbed at all temperatures to attain thermodynamic equilibrium.

Hydrodynamic diameter (d_H) of copolymer aggregates in the absence and in the presence of insulin were measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK), equipped with He-Ne (4 mW, 632.8 nm). In built thermostatic sample chamber enables to maintain the desired temperatures within a temperature range of 2-90 ^{0}C with a great accuracy. This instrument measures the movement of particles

under Brownian motion and converts this motion into size (d_H) by using the Stokes-Einstein equation as follows

$$d_H = \frac{kT}{6\pi\eta D} \quad (1)$$

where k is the Boltzmann's constant, T is absolute temperature, η is viscosity, and D is diffusion coefficient. All data were obtained by the instrumental software. All the reported values are an average of three measurements of the sample. X-ray diffraction patterns were recorded using X-ray diffractometer (Model No.D8 DISCOVER).

Time resolved Fluorescence spectroscopy

Fluorescence lifetime measurements were performed using a Fluorocube TCSPC system from Horiba Scientific, Japan. The excitation wavelength for time resolved measurements was 275 nm to selectively excite the polymer and the emission intensities were recorded at the λ_{max} 430, 310 and 430nm for three respective polymers. Slit widths of 15 nm were used on both the excitation and emission monochromators. The multi-exponential decay curves were analyzed using Data Analysis Software (DAS v6.3) provided with the instrument.

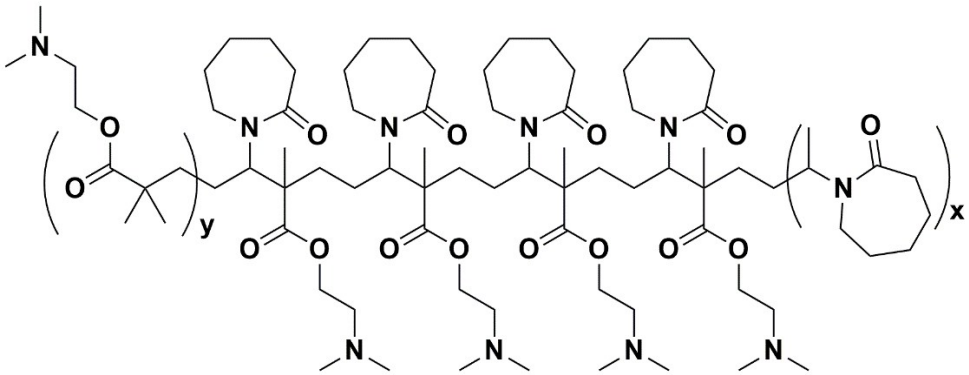
SEM, TEM and AFM measurements

Scanning electron microscopy analysis was performed using JEOL Japan Model: JSM 6610LV with tungsten or LaB6 filament electron source and operating voltage of 10 KV. Elemental analysis was performed using EDS software. Morphological characterization was carried out using Talos, Thermo-scientific with outstanding high resolution TEM images with energy dispersive x-ray spectroscopy (EDS) signal detection. WITec alpha 300 RA in non-contact mode was employed for atomic force microscopy (AFM). Instrument is equipped with research grade optical microscope with 6x objective turret, fibre coupling, LED white-light source for Kohler iluumination of AFM tip and sample and active vibration isolation system. AFM images were analyzed and roughness parameter was calculated using project four data evaluation software.

Differential scanning calorimetry measurements

Differential scanning calorimetry measurements were performed with the aid of NANO DSC instrument (TA Instruments, USA). It is equipped with a sample and reference cell containing ~ 0.650 µL cell volume. The changes in heat flow were recorded as a function of temperature against a pressure of 3 atm with scan rate of 2 °C/min. All the samples were equilibrated for 15 minutes. The sample was degassed with the degassing system provided with instrument. Before starting the experiment, water-water scans were performed for baseline reproducibility within the specified temperature range. All the data obtained was finalized by NANO analyzer software.

(a)



(b)

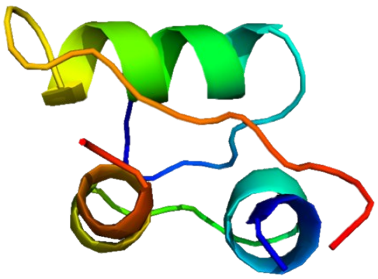


Fig. 1S. Schematic diagram of (a) $PVCL_x$-$PDMAEMA_y$ (with varying ratio of x and y) and crystal structure of (b) Insulin, which was downloaded from the protein data bank and processed with the PyMOL viewer software.

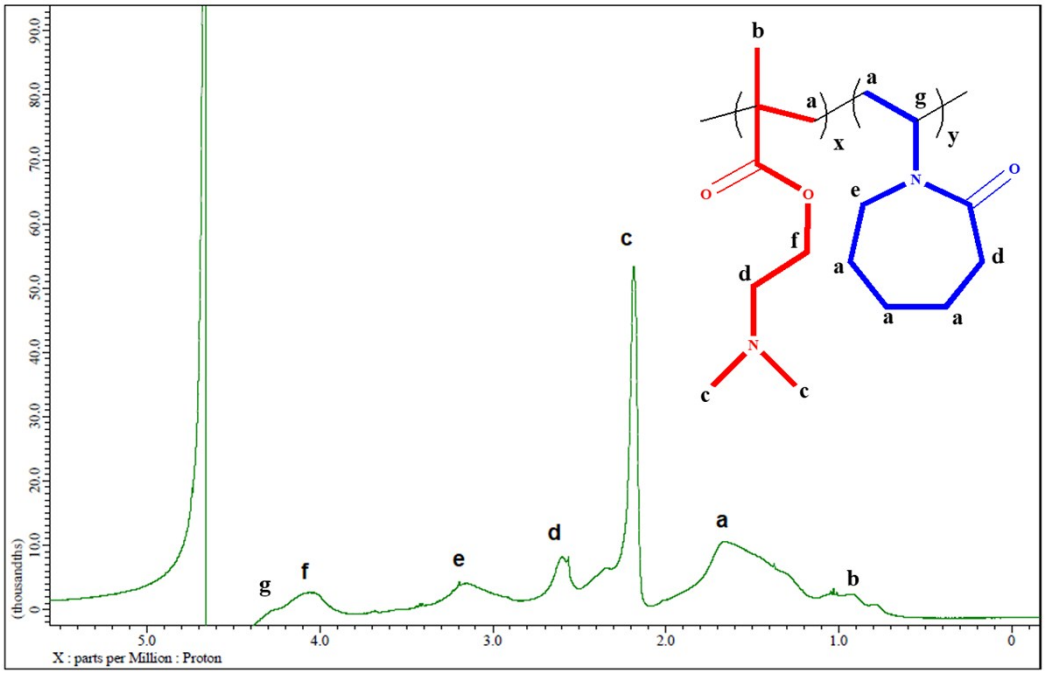


Fig. 2S. ^{1}H NMR spectrum of PVCL-*r*-PDMAEMA in D_2O.

Table S1: Molecular characteristics of PVCL-*r*-PDMAEMA copolymer

Sample	M^{a}_{n} (g/mol)	M^{a}_{w} (g/mol)	M_w/M^{a}_{n}
$PVCL_{30}$-$PDMAEMA_{70}$	10376	10386	1.0001
$PVCL_{50}$-$PDMAEMA_{50}$	10571	10587	1.0015
$PVCL_{70}$-$PDMAEMA_{30}$	11626	11643	1.0015

[a]Measured by GPC in THF

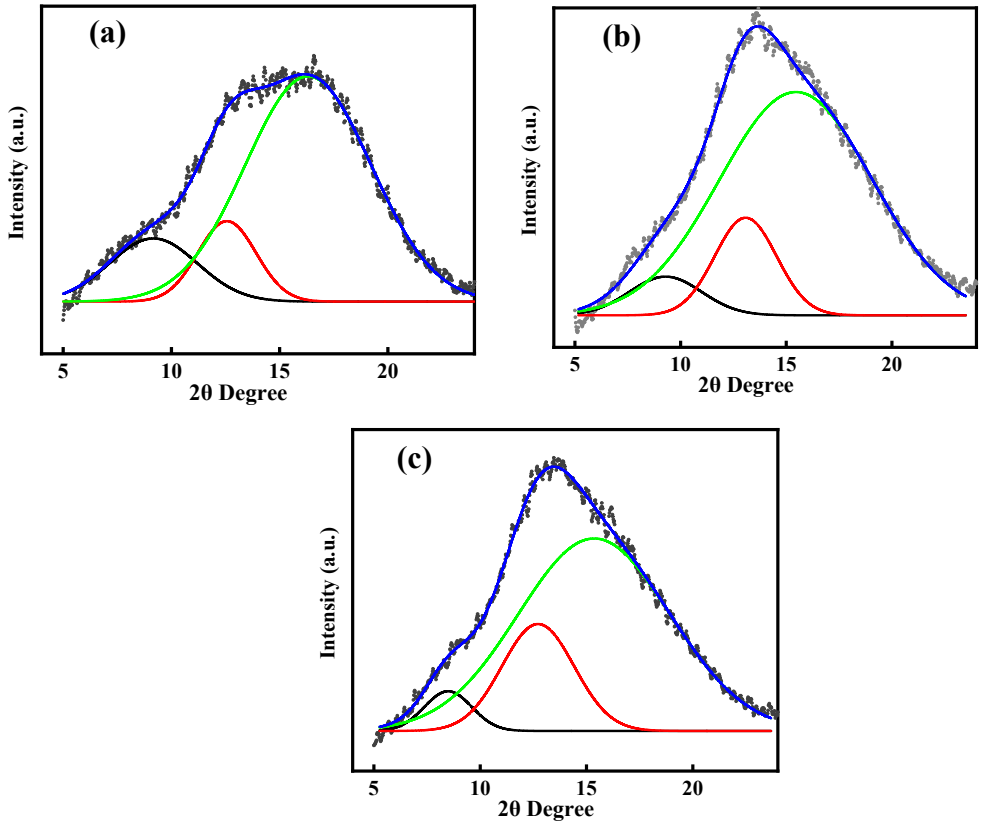


Fig. 3S: Deconvoluted XRD pattern for first intensity maximum peak (a) $PVCL_{30}$-$PDMAEMA_{70}$, (b) $PVCL_{50}$-$PDMAEMA_{50}$ and (c) $PVCL_{70}$-$PDMAEMA_{30}$.

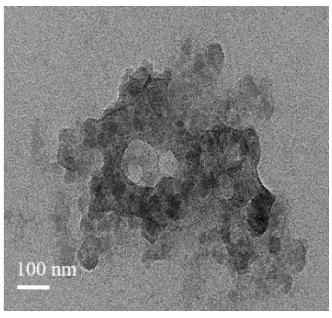


Fig. 4S: TEM image of diblock copolymer $PVCL_{30}$-*r*-$PDMAEMA_{70}$ showing formation of pentagonal shape arranged copolymer.

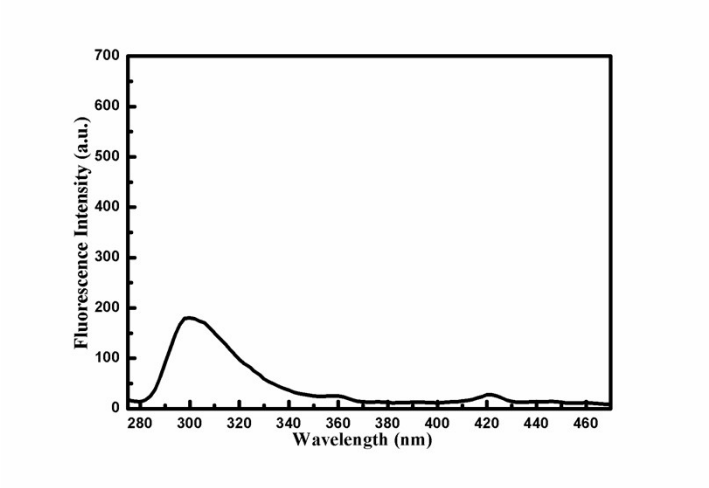
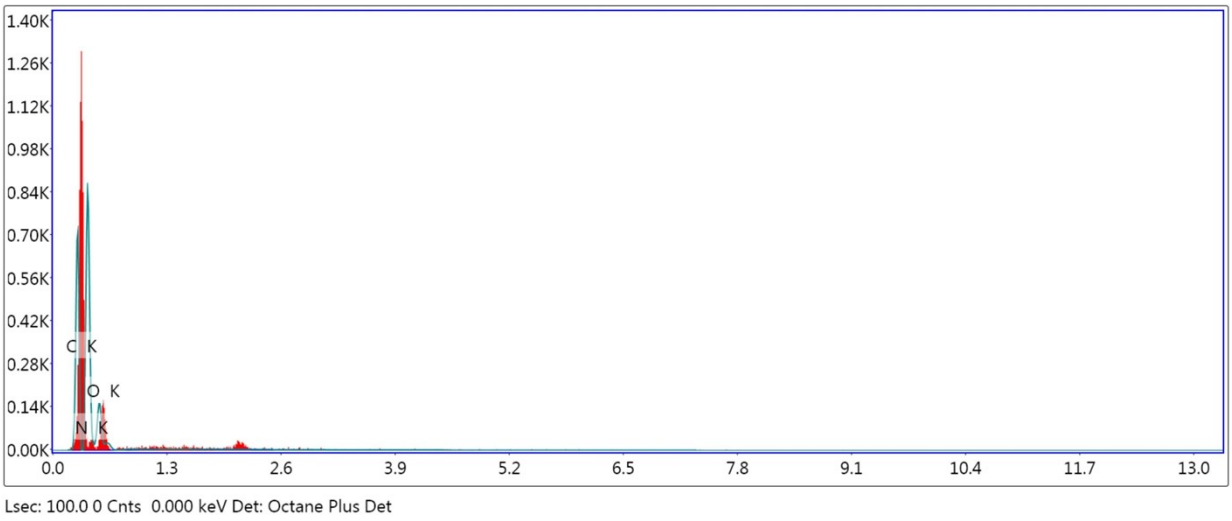


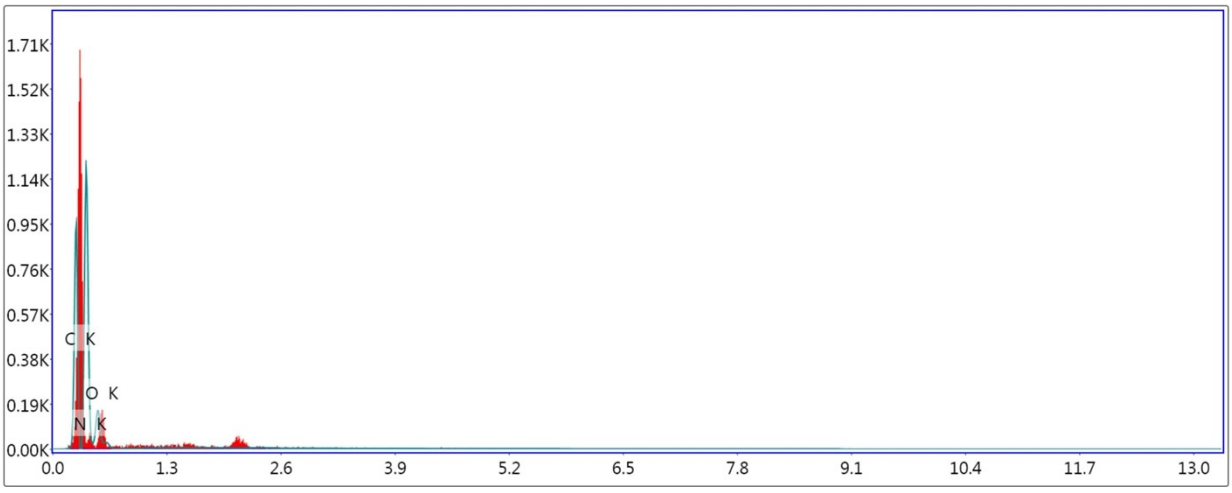
Fig. 5S: Steady-state fluorescence spectroscopy of insulin aqueous solution at room temperature.

(a)



Element	Weight %	Atomic %	Net Int.
C K	21.06	24.16	34.63
N K	64.32	63.26	42.3
O K	14.62	12.59	7.59

(b)



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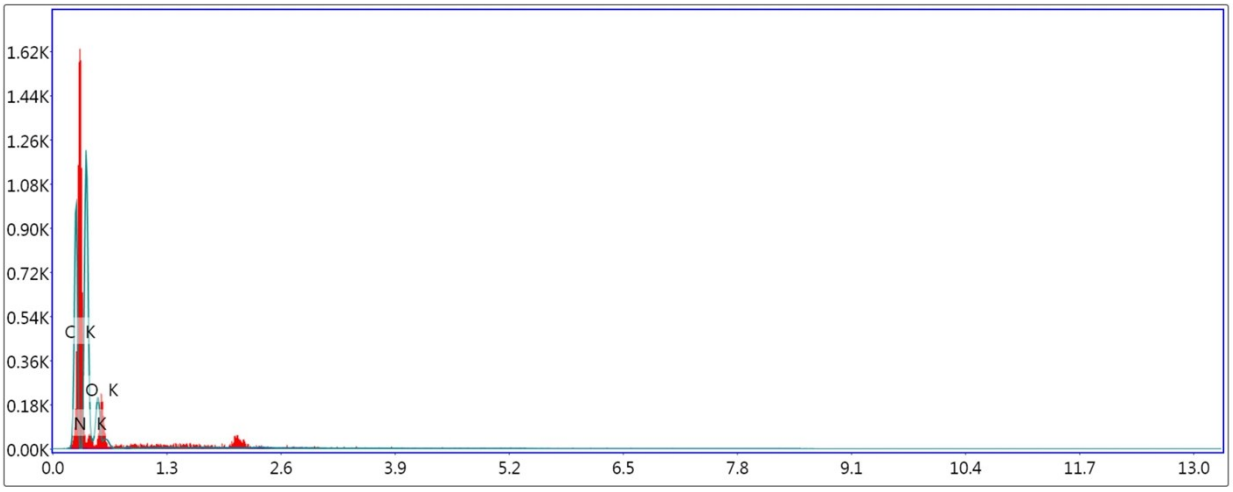
Element	Weight %	Atomic %	Net Int.
C K	20.87	23.87	46.12
N K	67.24	65.93	59.25
O K	11.89	10.21	7.89

(c)



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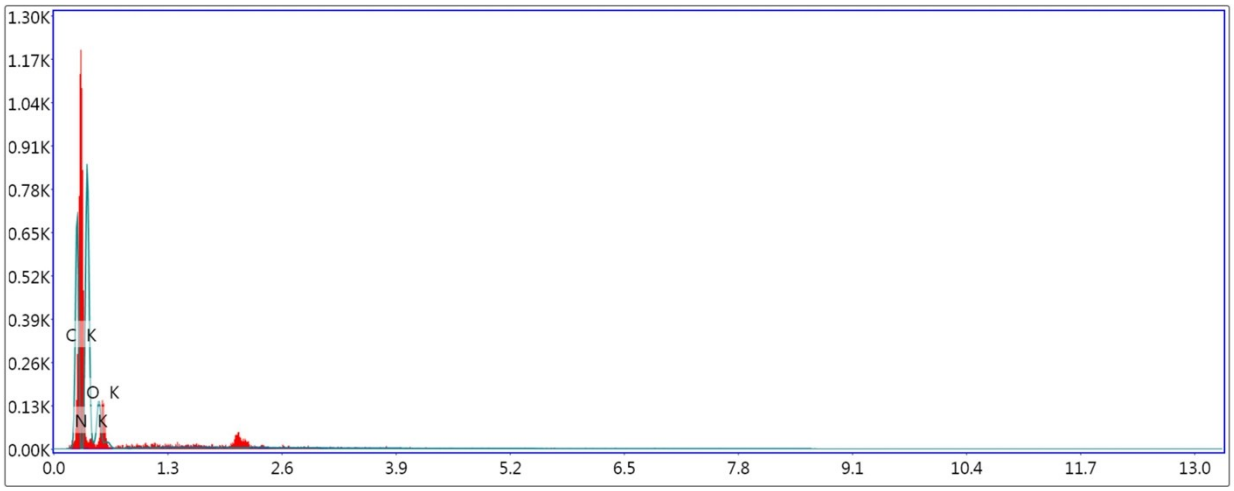
Element	Weight %	Atomic %	Net Int.
C K	21.23	24.32	48.73
N K	64.77	63.63	58.89

O K	14	12.05	10.04

(d)



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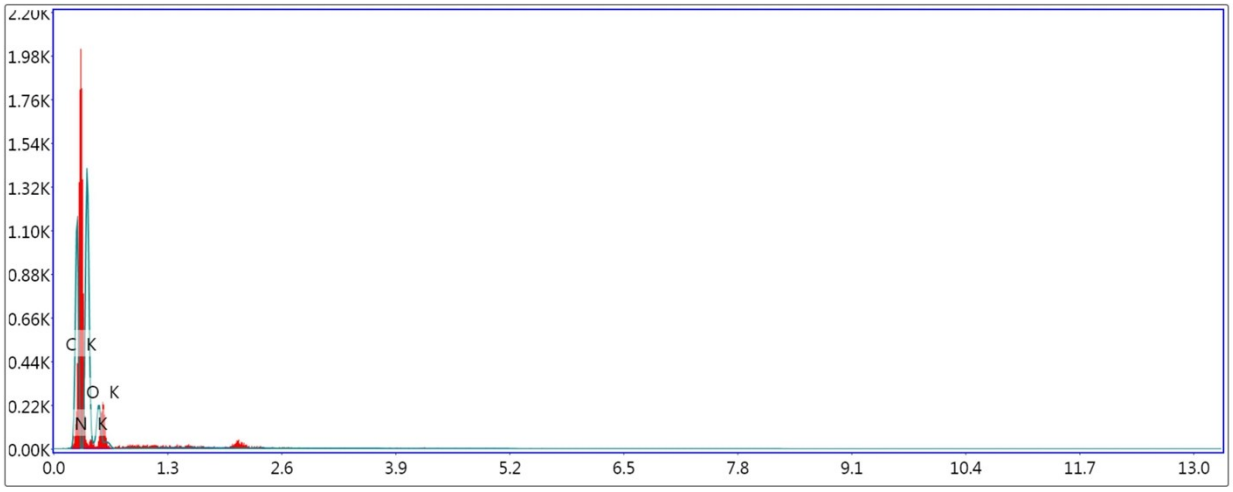
Element	Weight %	Atomic %	Net Int.
C K	21.03	24.11	33.67
N K	64.89	63.78	41.51
O K	14.08	12.11	7.04

(e)

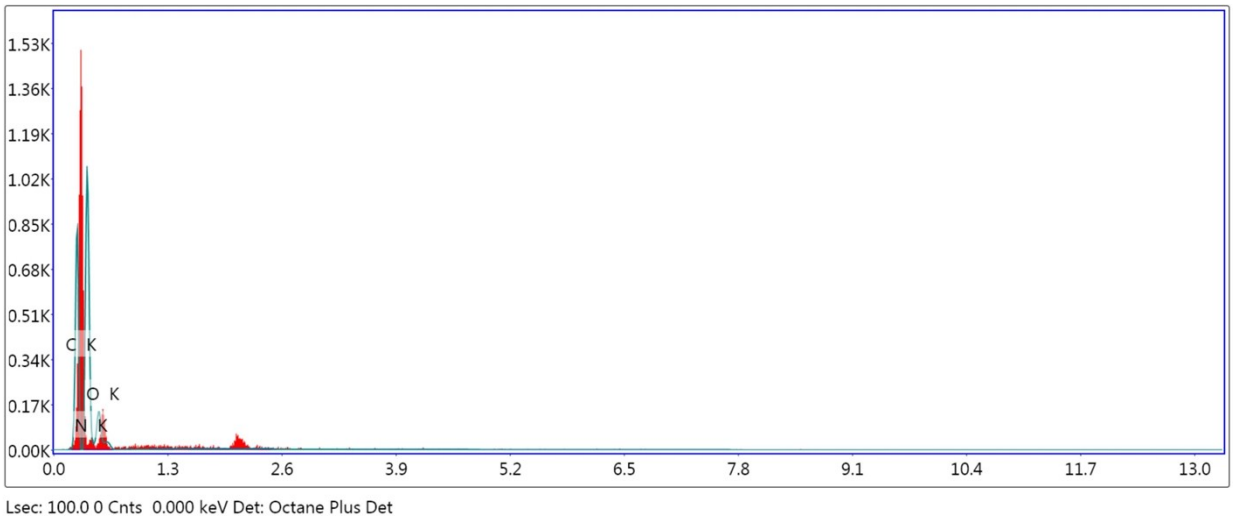


Lsec: 100.0 0 Cnts 0.000 keV Det: Octane Plus Det

Element	Weight %	Atomic %	Net Int.

C K	21.04	24.09	55.27
N K	65.7	64.51	68.69
O K	13.26	11.4	10.74

(f)



Element	Weight %	Atomic %	Net Int.
C K	20.92	23.91	40.39
N K	67.37	66.04	51.75
O K	11.71	10.05	6.77

Fig. 6S.EDAX pattern of PVCL-*r*-PDMAEMA with and without insulin. Panel 'a' represents $PVCL_{30}$-$PDMAEMA_{70}$, panel 'b' represents $PVCL_{50}$-$PDMAEMA_{50}$, panel 'c' represents $PVCL_{70}$-$PDMAEMA_{30}$, panel 'd' represents $PVCL_{30}$-$PDMAEMA_{70}$+insulin, panel 'e' represents $PVCL_{50}$-$PDMAEMA_{50}$+insulin and panel 'f' represents $PVCL_{70}$-$PDMAEMA_{30}$+insulin.

Table S2:

Roughness parameter for AFM studies:

Parameter	$PVCL_{30}$-$PDMAEMA_{70}$	$PVCL_{50}$-$PDMAEMA_{50}$	$PVCL_{70}$-$PDMAEMA_{30}$
Number of Pixels	65536	65536	65536
True Area [µm²]	25.0708	25.0876	25.9778
Reference Area [µm²]	24.8051	24.8051	24.8051
SDR [%]	1.1923	1.21281	5.00977
SDQ [nm]	0.156862	0.15641	0.323694
SSC [1/µm]	0.217902	0.167681	0.200397
Average [nm]	2.85874E-10	-1.56677E-09	6.61833E-09
SA [nm]	3.72782	11.0388	25.7774
SQ [nm]	5.58937	15.3394	35.1614
SSK	-1.00573	0.0205351	0.241726
SSU	10.3635	6.48052	4.49659
Peak-Peak [nm]	85.2009	168.501	303.803