Controlled delivery of drugs through smart pH-sensitive nanohydrogels for anticancer therapies: synthesis, drug release and cellular studies

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ABSTRACT
Nanohydrogels were synthesized by microemulsion polymerization. These nanohydrogels were pre-designed to be pH and temperature stimuli-response materials, using N-isopropylacrilamide as a base monomer and 1-vinyl imidazole as ionizable comonomer. The pH sensitivity was confirmed by measuring the increase or decrease in volume in the nanoparticles by changing the pH of the medium. Nanoparticles were properly characterized by Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), nuclear magnetic resonance (NMR) and transmission electron microscopy (TEM). Glass-transition temperature increased with vinyl imidazole content, and nanoparticles with average diameter of 68 nm were obtained. Particle size decreases with the increase in pH. After characterization, nanohydrogels were functionalized with folic acid to take advantage that the folate receptor is overexpressed in different types of cancer cells. The nanoparticles were loaded with the drugs: metformin, terfenadine, and 5-fluorouracil. The amount of drug loaded and released by nanoparticles was measured by UV–vis spectroscopy. Then, cellular viability and internalization studies were performed obtaining promising results.

KEYWORDS
Delivery; nanohydrogels; nanoparticles; cancer cells; smart nanocarriers

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1. Introduction
Cancer is a generic term that names a broad range of diseases that can affect the human organism, being the main cause of death in developed countries. Nowadays the most common tools to fight against cancer are based on chemotherapy, although there are still lots of issues to solve. One of the most important matters to deal with is the mechanism of the therapeutic action that is employed at the present time, because it does not distinguish between healthy and damaged cells, causing adverse fatal effects in the human being.

The nanotechnology plays an important role in the resolution of these problems, not only combating the undesired side effects of the medical treatment but also improving the effectiveness of it. In particular, functionalized pH-sensitive nanohydrogels have already shown suitable properties regarding their applicability in cancer treatment. [1–2] Due to the functionalization of the nanoparticles with folic acid [3–7], they can target the malign cells (folic receptors that can be found in many carcinogenic cells) and cross the cytoplasmic barrier through an endocytosis process mediated by a receptor. Due to their response to lower pH, once the nanoparticles are inside the cell, they will swell and release the drug carried inside, just in the place it is required.[8–11]

In this work, we present a synthesis of poly(N-isopropylacrilamide-co-vinylimidazole). These nanoparticles could be functionalized with folic acid and used as drug delivery systems for cancer treatment. Polymers containing 1-vinylimidazole (VMDZ) are known for their swelling properties when pH changes.[12] If loaded with drugs, these polymers can swell at certain pH leading to the controlled release of the drug.[13–18] As these nanoparticles are loaded with drugs employed in cancer treatment as terfenadine or metformine [19], they could be a useful and less harmful methodology to fight against this disease. In the present research, different copolymer compositions of N-isopropylacrylamide (NIPA), as the base monomer, and VMDZ, as the comonomer, were synthesized in order to find the composition which will result in the best response toward the pH inside the cell. A full characterization of the copolymers as well their swelling and delivery performance was carried out. Cytotoxicity studies were performed on nanoparticles without any drug inside, and loading the nanoparticles with the drugs was determined to be carried out in four potentially cancer cell lines: breast cancer, neuroblastoma, colon cancer, and melanoma.
To track endocytosis, the nanogels were loaded with coumarin-6 and their capture by the cells was monitored by fluorescence confocal microscopy.

2. Experimental

2.1. Materials

Monomers: N-isopropylacrilamide (Across Organics, 99%) and 1-vinylimidazole (Sigma Aldrich, 98%). Cross-linker agent: N,N-methylenbisacrylamide (N MBA) (Fluka, >98%). Initiator: sodium metabisulfite (Merck, ≥95%). Surfactants: orbitan sesquiolate (ARLACEL83) (Sigma Aldrich) and polyoxyethylene sorbitol hexaoleate (ATLAS G-1086) (Sigma Aldrich). Organic phase: isoparaffinic oil (Isopar-M) (ExxonMobil Chemical). Drugs, terphenadine and 5-fluorouracil (5-FU) metformine hydrochloride, were purchased from Sigma Aldrich. The coumarin-6 and folic acid (98%) were provided by Sigma Aldrich. Water for all experiments was Milli-Q®.

2.2. Synthesis of nanoparticles

Nanogels were synthesized by inverse microemulsion polymerization at a controlled temperature of 25 °C. First, an aqueous phase was prepared (58 wt.% of the total microemulsion); this phase was a mixture of the cross-linker agent at different concentration ranges (N MBA) and water. The mixture was stirred for 2 h. Separately the organic phase was prepared (42% of the microemulsion); this phase was a mixture of isoparaffinic oil and surfactants and was stirred for 2 h. Then, the organic phase was added slowly to the aqueous phase to form the microemulsion. At this point (before the starting the copolymerization), the system is transparent and no phase separation was observed after centrifuging at 5000 rpm for 30 min.

To initiate the reaction, sodium metabisulfite aqueous solution was continuously added at constant flow (0.9 mL min⁻¹) using a Methrom Dosino 700 dosing unit. Nitrogen purge (4 mL min⁻¹ at 25 °C, 1 bar) was kept during all reaction time. Reaction mixture was immersed in a water bath kept at constant temperature (25 °C); as the reaction progressed, a temperature increase was observed (42 °C); polymerization was considered to be completed when temperature was back to its initial value. Peak temperature was reached in less than 1 min, and reaction time was 6 min. The polymer was obtained by destabilizing the microlatex by adding chloroform and pouring the mixture into acetone. The precipitate was rinsed with diethyl ether three times. Nanogels with different composition were prepared using different monomers ratios. Table 1 shows the formulations used to synthesize the particles.

2.3. Functionalization of nanoparticles with folic acid

Folic acid was easily covalently attached to the nanohydrogels via the reaction of the carboxyl groups of folic acid and the nitro pendant groups of the nanohydrogels in the presence of N-(3-dimethylaminopropyl)-N'-ethyl carbodiimide hydrochloride (EDC) carbodiimide through a conventional carbodiimide-mediated conjugation method.[20]

The amount of folic acid attached to the polymer was quantified using UV–visible spectroscopy of folate–nanohydrogel dispersions in phosphate buffer saline (PBS) at 363 nm.

2.4. Morphologic and size studies

TEM micrographies were collected using a JEOL JSM7000F electron microscopy at 10.0 kV, equipped with a field emission gang (FEG). An ultrathin coating of electrically conducting material is deposited by high vacuum evaporation or by low vacuum sputter coating of the sample. For these experiments, a gold film was deposited over the sample. Sphericity of the synthesized nanohydrogels was confirmed by this technique. The quasielastic light scattering (QlS) technique was employed to determine particle size and particle size distribution of the nanohydrogels. An AMTEC light scattering spectrophotometer was used at an angle of 90°. Intensity correlation function measurements were carried out using a Brookhaven BI-9000AT 522-channel digital correlator, equipped with a water-cooled argon-ion laser operated at 514.5 nm, as a light source. The dried powder samples were dispersed in water and acetone for 24 h. All measurements were made at 25 °C.

2.5. Differential scanning calorimetry analysis

Differential scanning calorimetry (DSC) thermograms were obtained using an automatic thermal analyzer system (DSC 2920 Modulated DSC, TA Instruments). Samples were crimped in standard aluminum pans and heated from –10 to 250 °C at a heating rate of 10 °C/min under constant purging of N2 at 20 mL/min. An empty pan, sealed in the same way as the sample, was used as a reference.

2.6. Nuclear magnetic resonance

The Nuclear Magnetic Resonance (NMR) spectra of nanohydrogels were obtained using deuterated DMSO as the solvent in a Bruker ACE instrument (250 MHz) at 20 °C; chemical shifts were measured relative to DMSO. The copolymer composition was determined using this technique and adding nitrobenzene as internal standard.
2.7. Drug loading experiments

For loading drugs into polymeric nanoparticles, 0.5 grams of nanohydrogel were weighed and placed in contact with 50 mL of an aqueous solution of known concentration of each drug. After this process, solutions were stirred while maintaining a pH of 5 (to ensure swelling of the polymer network and thereby allow passage of the drug into the hydrogel) for 24 h. Then, the pH was increased to 7.5 (to shrink the polymer network) to retain the drug within and was stirred for another 24 h. To obtain the nanoparticles, samples were centrifuged at 37 °C and at a speed of 10,000 rpm. To perform next tests, the nanoparticles were dried in an oven at a temperature of 50 °C during a period of 48 h and then stored in amber glass tubes to prevent possible changes of the drugs.

2.8. Drug release experiments

For drug release experiments, 10 mg of dry drug-loaded nanoparticles were placed in a vessel containing 50 mL of water at a constant temperature (25 °C ± 0.2) under magnetic stirring (250 rpm). A peristaltic pump operating at 50 rpm was used to maintain a constant flow between the release vessel and the spectrophotometer cell. To avoid passage of the nanoparticles to the spectrophotometric cell, a nanosized filter was used (Millipore brand). Spectrophotometer measurements began once the nanoparticles were in contact with the drug solution; solution absorbance was measured every 0.5 s during 40 min to obtain the drug release kinetics. Release kinetics equilibrium was reached once the absorbance did not change significantly.

The amount of released drug was determined by UV–vis spectroscopy (Cintra 303, GBC) with a quartz cuvette at absorbance wavelengths of 265 and 259 for 5-FU, and terfenadine, respectively. Previous scans from 200 to 600 nm were taken to select maximum absorption peak for each drug. The amount of drug at any selected time was calculated from the calibration curve. Metformin and 5-FU release were followed by UHPLC (ultra high pressure liquid chromatography). For chromatographic analysis, an equipment of high-resolution liquid chromatography (Acquity UHPLC Systems, Waters) coupled with a diode array detector was employed. 1.7 microns particle size Kinetex PFP column (Phenomenex 50 × 210 nm) was used. Formic acid/formate buffer was used at pH 3.5, 10 mM as mobile phase (aqueous phase) and methanol as organic phase. Calibration solutions for 5-FU and metformin were prepared at concentrations of 5, 10, 15, 20, and 30 mg/mL for 5-FU and 1, 2.5, 5, 10, 25, 50, 100, 200, and 400 mg/mL. A known volume of 2.5 mL of each solution was sequentially injected into the column.

2.9. Viability of cancer cells using nanohydrogels with and without drug in cancer cells

Cell viability was quantified by the cell viability assay XTT (Roche Molecular Biochemicals). This method is based on the metabolic activity of cells, which can be considered as an indicator of the cell viability. In metabolically active cells, reductase enzymes are responsible for the reduction of the tetrazoyl salts previously added to the culture to form formazan salts, showing a characteristic orange-yellowish color. These salts can be quantified with a plate spectrophotometer (SynergyHT, BioTek) at 490 nm.

Cells were seeded in 96 petri dishes, with a density of 1 × 10⁴ cell/spot in 100 μL of the culture media at 5% of SBF, and they are warmed inside the heater overnight to promote adhesion. Then, 100 μL of nanogel suspensions were added in the desired concentrations, and the culture was incubated inside the heater for 72 h. Cell viability was determined by colorimetric XTT assay, following the steps recommended by the supplier: after adding tetrazoyl salts to the culture and incubating the plate for 4–12 h, the absorbance of the samples is measured by plate spectrophotometer at 490 nm. Cell viability is calculated as the percentage of the cell viability with respect to control cells as follows: experimental absorbance/control absorbance × 100. These tests were conducted.

### Table 1. Formulations used to synthesize the particles.

| Sample | NIPA (%) | VMDZ (%) | NMBA (%) |
|--------|----------|----------|----------|
| M10.5  | 90       | 10       |          |
| M20.5  | 80       | 20       |          |
| M30.5  | 70       | 30       |          |
| M40.5  | 60       | 40       |          |
| M60.5  | 40       | 60       |          |
| M70.5  | 30       | 70       |          |
| M90.5  | 10       | 90       |          |
| M10.3  | 90       | 10       |          |
| M20.3  | 80       | 20       |          |
| M30.3  | 70       | 30       |          |
| M40.3  | 60       | 40       |          |
| M60.3  | 40       | 60       |          |
| M70.3  | 30       | 70       |          |
| M90.3  | 10       | 90       |          |
To determine the hydrodynamic radius size of the nanogels, they were dispersed in water and measured by QLS. Figure 2 shows that the major intensity is achieved for particles of 68 nm of diameter. The calculated polydispersity value (1.3) indicates that there is some polydispersity, which is in agreement with the data obtained by TEM and QLS.

1H-NMR analysis confirmed the presence of the NIPA and VMDZ in the copolymeric structure as it can be seen in Figure 3. The spectra show characteristic signals of the aromatic ring of VMDZ (7.2 ppm) and also the peaks corresponding to the isopropyl group of NIPA at lower chemical shifts (1.2 and 3.7 ppm). The integration of the area of the most characteristic peaks of the 1H-NMR spectra enabled the determination of the proportion of monomers present in the final nanogel. Moreover, an internal standard was added in order to calculate the real molar composition of each copolymer. The real molar composition is shown in Table 3.

Figure 4 shows the 1H-NMR spectrum in which the presence of the folate groups can be seen, and these peaks have been used to confirm the presence of these groups in numerous publications.[21] Similar results were obtained for the other functionalized copolymers.

### 3. Results and discussion

#### 3.1. Characterization

The synthesized samples were analyzed by DSC, showing values of glass-transition temperature (T_g) between 98 and 164 °C (Table 2). Those values are limited by their homopolymers, poly(NIPA) (T_g = 100 °C) and poly(VMDZ) (T_g = 175 °C).

An enrichment of the monomer VMDZ in the final structure is traduced as an increase in the T_g of the polymer. VMDZ presents a nitrogen atom, providing the polymer of polar sites either in the main chain or the side chains of the polymer, enlarging the intermolecular forces and causing an increase in the T_g values of the copolymer. Moreover, when a copolymer is being enriched with VMDZ, the amount of aromatic rings is also increasing, conferring rigidity to the structure which can also lead to a higher T_g.

TEM micrographies show a high polydispersity and that increasing polyVMDZ content larger particles are obtained (Figure 1).

| Sample | %NIPA | %VMDZ | %NMBA | T_g (°C) |
|--------|-------|-------|-------|----------|
| 1      | 90    | 10    | 5     | 98.1     |
| 2      | 80    | 20    | 5     | 111.6    |
| 3      | 70    | 30    | 5     | 127.2    |
| 4      | 60    | 40    | 5     | 131.1    |
| 5      | 40    | 60    | 5     | 146.5    |
| 6      | 30    | 70    | 5     | 151.3    |
| 7      | 10    | 90    | 5     | 163.2    |

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Figure 4 shows the 1H-NMR spectrum in which the presence of the folate groups can be seen, and these peaks have been used to confirm the presence of these groups in numerous publications.[21] Similar results were obtained for the other functionalized copolymers.

#### 3.2. Swelling properties

The decisive characteristic of these synthesized nanohydrogels lays on their response toward different values of pH, which will determine their suitability for controlled and targeted release therapies. To determine swelling behavior of the nanohydrogels, particle size and hydrodynamic diameter were measured at different pH on samples dispersed in water. Figure 5 shows that equilibrium water uptake increases at acid pH, and when the amount of VMDZ increases, the water uptake augments significantly. The drastic change in the hydrodynamic diameter of the particle at low pH is due to a transition from a collapsed state to a swelled and solvated condition, due to the repulsive electrostatic interactions between the generated cations with the decrease in pH. These results point out the importance of the composition and pH media to carry out drug loading and releasing studies.
3.3. Drug release studies

The pH values studied were the pH of the blood and physiological medium (7.4) and the pH of the cellular lysosomes and endosomes (5.5).

3.3.1. Release kinetics of terfenadine by UV–vis

A known volume of 10 mg of terfenadine-loaded nanogels were suspended in binary solutions of water–ethanol (70/30) at two different pH (7.4 and 5.5). Absorbance reading was performed at $\lambda = 259$ nm every 30 s for approximately 40 min. Figure 6 shows the release curves of terfenadine from the M60.40.3 sample at different pH. Much faster and higher amount released was obtained at the lower pH which can be explained by the higher swelling of the nanoparticles.

3.3.2. Release kinetics of 5-FU by UV–Vis

The release kinetic was performed taking 10 mg of nanoparticles loaded with 5-FU; these particles were suspended in MilliQ water at the desired pH. The kinetics were followed by measuring the absorbance at $\lambda = 265$ nm every 30 s during 40 min.

Figure 8 shows the release kinetics for M60.40. A great difference can be observed in the release kinetics depending on the pH of the medium. The amount of drug released at pH 7.4 was 0.96 ppm, much smaller than that released at pH 5.5 (6.2 ppm).

Figure 9 shows the amount of drug released by the sample M60 at different degrees of cross-linking agent (3 and 5) and pH (5.5–7.4). In a similar way than the release of terfenadine, the degree of cross-linking significantly affects the amount of drug released, higher degree of cross-linking agent and lower amount of drug released.

3.3.3. Delivery kinetics of 5-FU by UHPLC

To track the release rate of 5-FU from copolymeric nanogels of NIPA-co-VMDZ by UHPLC a calibration curve with standards of 5-FU in the two media in which will later be released, pH 7.4 and pH 5.5, was performed.

For release kinetics determination, 14 samples of 10 mg of nanoparticles each with a known amount of drug were placed in 14 different vials. Half of the samples were put in contact with an acid medium (pH 5.5), and half of the
having nanoparticles in the solution. This supernatant is again filtered before being injected into the UHPLC to avoid damaging the column. The chromatographic peaks obtained at pH 5.5 are shown in Figure 10.

### Table 3. Nanogels molar ratio measured by NMR.

| Internal standard (mg) | H\textsuperscript{1} signal A | H\textsuperscript{1} signal B | Sample (mg) | H\textsuperscript{1}/mg marquer | H\textsuperscript{1}/mg polymer | H\textsuperscript{1}A/mg polymer | H\textsuperscript{1}B/mg polymer | Deuterated solvent (mL) | NiPA (%) mol | VMDZ (%) mol |
|------------------------|---------------------|---------------------|--------------|----------------|----------------|----------------|----------------|----------------|-------------|----------------|
| 4.90                   | 0.37                | 0.7                 | 5.00         | 1.0           | 0.07           | 0.14           | 0.75           | 34.7           | 65.2        |
| 4.90                   | 0.47                | 0.8                 | 5.00         | 1.0           | 0.01           | 0.17           | 0.75           | 35.7           | 64.2        |
| 3.60                   | 0.68                | 1.7                 | 5.00         | 1.3           | 0.13           | 0.34           | 0.75           | 28.6           | 71.3        |
| 5.60                   | 0.36                | 0.6                 | 5.00         | 0.9           | 0.07           | 0.13           | 0.75           | 35.6           | 64.1        |
| 4.40                   | 0.605               | 0.8                 | 5.00         | 1.1           | 0.12           | 0.16           | 0.75           | 41.9           | 58.1        |
| 3.50                   | 1.05                | 0.04                | 5.00         | 1.4           | 0.21           | 0.008          | 0.75           | 91.1           | 8.8         |

samples were placed in a basic medium (7.4). Samples were stirred during different periods (0, 5, 10, 15, 20, 25, and 30 min). After the specified contact time, the supernatant is collected and filtered with a nanofilter to prevent having nanoparticles in the solution. This supernatant is again filtered before being injected into the UHPLC to avoid damaging the column. The chromatographic peaks obtained at pH 5.5 are shown in Figure 10.

**Figure 3.** \( ^{1}H\) NMR spectra of NIPA–VMDZ nanogels.

**Figure 4.** \( ^{1}H\) NMR spectrum of the sample M60.40.5 functionalized with folic acid.
metformin has confirmed its ability to inhibit the process of intracellular respiration, which results in tumor cell death.[23,24] Metformin calibration curves were obtained for each pH to be used for release tests.

To determine the release kinetics of metformine, 18 samples with a known amount of drug were placed in 18 vials. Nine samples were dispersed in an aqueous acid medium (pH 5.5), and the other nine were dispersed in an aqueous basic medium (pH 7.4). The samples were shaken and maintained in dispersion for different time periods (0, 5, 10, 15, 20, 25, 30, 35, and 40 min, respectively). After the specified contact time, the supernatant is collected.

The same procedure was performed on samples in contact with a basic medium to obtain the chromatogram shown in Figure 11. Inset shows an enlargement of the peaks.

Figures 10 and 11 show that areas with higher peaks are obtained as time goes on, indicating larger drug release. After measuring the areas, the drug released was calculated. The values obtained are plotted in Figure 12.

As expected according to the results of the releases followed by UV–vis, much higher drug release was obtained at pH 5.5.

3.3.4. Kinetic delivery of metformine by UHPLC

A third drug which is a potential anticancer agent, metformine, was chosen to study its release kinetics. Although their mechanism of action has not been fully elucidated,
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VMDZ (10, 30, 60, 90%). The tests depicted in Figure 16 show that none of the polymeric compositions affected negatively to the cell viability, obtaining values of 90–99% even for a maximum concentration of 100 μg/ml.

3.5. In vitro evaluation of the NIPA-co-VMDZ nanogels loaded with 5-fluorouracil and functionalized with folic acid

Cell viability on drug-loaded nanogels was studied. To evaluate the nanoparticles drug release efficiency inside the cell, these tests were conducted in four different types of cell families: dermal cancer cells (melanoma, MEL-OH), breast cancer cells (MCF-7), neuroblastomic origin cells and filtered using a nanoscale filter and injected into the chromatograph coupled to a UV–Vis detector. The chromatographic peaks obtained at λ = 232 nm and pH 5.5 and pH 7.4 are shown in Figures 13 and 14.

3.4. Cells viability studies

For the cell viability studies, murine melanoma B16F10 line was used due to its high proliferation rate. Moreover, being the origin of the cells murine, future in vivo test are expected to be simpler. As it can be seen in figure 15, Blind test were carried out with four nanogels of different composition of VMDZ (10, 30, 60, 90%). The tests depicted in Figure 16 shows that none of the polymeric compositions affected negatively to the cell viability, obtaining values of 90–99% even for a maximum concentration of 100 μg/mL.

Figure 9. 5-FU concentration (ppm) released by the M60 sample at different degrees of cross-linking agent and pH of the release medium.

Figure 10. Chromatograms of supernatant of M60.5 charged with 5-FU in acid medium (pH 5.5) at different release times.

Figure 11. Chromatograms of supernatant of the M60.5 sample charged with 5–FU in a basic medium (pH 7.4) at different release times.

Figure 12. 5-FU kinetic delivery curve from M60.40.5 sample obtained by UHPLC technique.

Figure 13. Chromatograms of supernatant of M60.5 sample charged with 5-FU at different release times.

Figure 14. Chromatograms of supernatant of the M60.5 sample charged with 5–FU in a basic medium (pH 7.4) at different release times.

Figure 15. 5-FU concentration (ppm) released by the M60 sample at different degrees of cross-linking agent and pH of the release medium.
cultures by confocal microscopy. Cells were seeded at a density of $12.5 \times 10^4$ cells/ml in 35 mm sterile Petri plates. Cells were incubated overnight in a CO$_2$ stove for proper adhesion. The culture medium of the plate was removed, and the conjugated nanogels suspension was added to the cells. Immediately they were observed in an inverted confocal microscope that allowed the observation of alive cells. Images were taken every 5 min for a period of 60 min. Some of the images are shown in Figure 18. It can be seen that almost all of the nanogels loaded with coumarin-6 were been internalized within the cell, having only a negligible amount out of them, so we can conclude that the internalization was due to endocytosis mediated by folate receptor.

(SMS-KCNR), and colon cancer cells (HCT8), all of analyzed cell lines are carcinogenic. For this study, the nanoparticles which gave best results in terms of viability, swelling, etc. in all previous studies were chosen (M6040.5).

Figure 17 shows that cell viability is reduced considerably when the sample is loaded with an anticancer drug 5-FU. In three of the types of tumors tested (MCF7, SMS-KCNR, MEL-HO), there is 60% of cell deaths and at least 40% cell death for the HCT8. Cell viability decreases with the suspension concentration of nanoparticles.

3.6. Study of the internalization of nanogels in tumor cells

To track internalization of nanogels in the cells, they were charged with coumarin-6 and were visualized in cell cultures by confocal microscopy. Cells were seeded at a density of $12.5 \times 10^4$ cells/mL in 35 mm sterile Petri plates. Cells were incubated overnight in a CO$_2$ stove for proper adhesion. The culture medium of the plate was removed, and the conjugated nanogels suspension was added to the cells. Immediately they were observed in an inverted confocal microscope that allowed the observation of alive cells. Images were taken every 5 min for a period of 60 min. Some of the images are shown in Figure 18. It can be seen that almost all of the nanogels loaded with coumarin-6 were been internalized within the cell, having only a negligible amount out of them, so we can conclude that the internalization was due to endocytosis mediated by folate receptor.
toward pH changes, once they are inside the cell (sudden pH change), they could easily release the desired drug. In particular, if tumor cell’s receptor was able to recognize specific functional groups present in the surface of nanogels, those nanoparticles could be used in cancer treatment, which could represent a breakthrough in cancer treatment achieving a controlled and targeted drug release mechanism.

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Disclosure statement

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4. Conclusions

NIPA-co-VMDZ nanohydrogels with different composition were synthesized by inverse microemulsion.

Nanoparticles with average diameter of 68 nm were obtained. The size decreases when increasing pH. Glass-transition temperature increased with vinyl imidazole content. Larger and faster release was obtained at pH 5.5 for all the drugs.

Taking into account their size and properly functionalized, the synthesized nanogels could participate in endocytosis process, and taking advantage of their behavior toward pH changes, once they are inside the cell (sudden pH change), they could easily release the desired drug. In particular, if tumor cell’s receptor was able to recognize specific functional groups present in the surface of nanogels, those nanoparticles could be used in cancer treatment, which could represent a breakthrough in cancer treatment achieving a controlled and targeted drug release mechanism.

Figure 18. Images of confocal spectroscopy of the studied samples loaded with coumarin-6.
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