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Smart Polyelectrolyte Hydrogels: a Novel Platform for Drug Delivery

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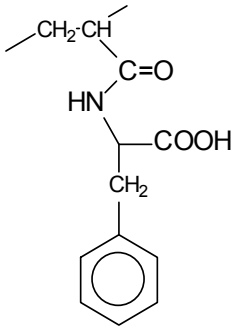
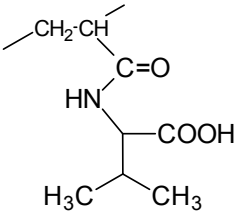
Three kinds of vinyl hydrogels with α-aminoacid residues have been considered as potential platforms for the deliver of several therapies (pain, diabetes, and mood) when loaded with appropriate drugs. The presence of ionizable groups of the L-valine, L-phenylalanine and L-histidine residues is able to modify the swelling properties of the hydrogel on the basis of its pK_a values. A greater basicity constant of the functional groups improves a greater loading of the drug and a longer sustained-release pattern due to a strong polymer-drug ionic interaction. This occurs for the insulin and the paroxetine loaded on carboxylate hydrogels, and diclofenac loaded on zwitterionic hydrogels. The pH stimulation improves the swelling of the hydrogel and increases 'on demand' the drug availability. A further remote stimulus based on alternating magnetic fields (AMF) on hydrogels containing embedded magnetic nanoparticles ($CoFe_2O_4$) allows a greater release rate of insulin and paroxetine for several pulses at physiological pH.

1. Introduction

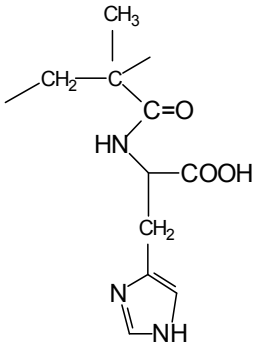
In recent years we have proposed some polyelectrolyte hydrogels, of anionic and ampholyte nature, capable of complexing, besides metal ions, with metal-based drugs and drug molecules containing amino functionality (Casolaro M. and Casolaro I., 2013, 2016a, 2016b). The main polymer structure bearing residues of α-aminoacids (L-phenylalanine, PHE, L-valine, AVA, and L-histidine, Hist) was cross-linked with different cross-linking agents and copolymerized with *N*-isopropylacrylamide (NIPAAm).



poly(PHE)



poly(AVA)



poly(MHist)

In some cases, magnetic nanoparticles have been incorporated to make the hydrogel a multi-responsive nanocomposite sensitive to both direct (pH, temperature, ionic strength) and remote (alternating magnetic fields, AMF) stimulations (Casolaro et al., 2014). In the ionized state some of these hydrogels exhibit an exceptionally high degree of swelling, the magnitude of which can be monitored by the ionic strength, temperature and pH. In fact, anionic hydrogels show different critical conditions at which the whole swollen mass completely collapses at pH 4 and pH 5, due to the compounds bearing the L-valine and the L-phenylalanine residues, respectively (Casolaro et al., 2012a, 2012b). Typically, the swollen hydrogel undergoes a rapid collapse in the presence of cationic drugs and this collapse is the more rapid and complete

the larger is the basicity constant of the drug itself. The main drug-polymer interaction was reported to be of ionic nature, although hydrophobic interactions cannot be excluded (Casolaro et al., 2015). It follows that the drug-polymer strength affects the whole drug release process; the free drug, that is in equilibrium with the adduct, must spread through the network to be used for therapeutic purposes. A further advantage is the load of the drug within the hydrogel, which preserves its effectiveness over time. This was evidenced by previous results for the 'in vitro' release study of anticancers (cis-platinum, doxorubicin) (Casolaro et al., 2011, 2014), glaucoma treatment (pilocarpine) (Casolaro et al., 2012a) and, not finally, of antidepressants (citalopram, trazodone, paroxetine, duloxetine) (Casolaro et al., 2015, 2016b). In all cases, the typical release profiles showed that the drug concentration is maintained in the therapeutic window for an extended period of time, ensuring the sustained therapeutic action. In some cases, the need to promote a drug concentration in the solution is ensured by the increasing temperature and by the application of the appropriate AMF stimulation; thus, 'on demand' may be released a greater amount of drug to the target organ to alleviate the medical needs of the moment and to restore a normal state of health. Besides the sustained release formulation, the pulsatile system may benefit from a therapy mainly because it is linked with the circadian rhythms of the body (Ohdo S., 2010). Researchers have addressed the question of creating new stimuli-responsive hydrogels as carriers for drug delivery systems because their unique physicochemical and biological properties can protect sensitive drugs (Campbell et al., 2015). Based on the externally triggered ability, the use of stimuli-responsive hydrogels can be associated with the events of the circadian rhythm that cause the release of a drug at predetermined intervals.

2. Experimental

Materials. The hydrogels used in this study were synthesized as previously reported from synthesized and commercial monomers (Casolaro et al., 2012a, 2013 ,2014). The synthetic monomers include *N*-acryloyl-L-phenylalanine (PHE), *N*-acryloyl-L-valine (AVA), *N*-acryloyl-L-histidine (Hist), *N*-methacryloyl-L-histidine (MHist). Commercial monomers include *N*-isopropylacrylamide (NIPAAm) and *N,N'*-ethylene-bisacrylamide (EBA). The hydrogels obtained by radical polymerization, such as homopolymers or copolymers with NIPAAm, are summarized below. PHE-Nip3: poly(PHE-*co*-NIPAAm) at a unitary PHE/NIPAAm molar ratio and cross-linked with 2 mol% EBA; the hydrogel was synthesized in the presence of a magnetic nanoparticles dispersion (2.00 mL of $CoFe_2O_4$ from Colorobbia, Italy). PHE-Nip2: the hydrogel is similar to the former but containing about 1/3 of the magnetic nanoparticle dispersion (0.60 mL). AVA-2: poly(AVA) cross-linked with 2 mol% EBA. Hist-10: poly(Hist) cross-linked with 10 mol% EBA. MHist: poly(MHist-*co*-NIPAAm) at 95/5 molar ratio NIPAAm/MHist and cross-linked with 2 mol% EBA. All other materials were supplied by Sigma-Aldrich and the drugs used were purified by methanol solutions of their respective commercial tablets (Casolaro M. and Casolaro I., 2015, 2016b).
Measurements. The pH measurements were carried out with a potentiometric apparatus (TitraLab90, Radiometer Anal.) and automatically recorded with a Windows-based software (TimTalk 9). A combined glass pH electrode (Red Rod) and a temperature sensor (T201) carefully measured the pH and the temperature of the solution. The spectrophotometric measurements were made with a Specord 210 spectrophotometer (Analytik Jena) equipped with 10 mm quartz cuvettes. In addition, the alternating magnetic field (AMF) measurements have been supported by an AG 1006 amplifier/generator (T&C Power Conv., Inc., Rochester, NY, USA) operating at 50W and 20kHz of power and frequency, respectively; the cell used for this purpose has been previously described and consists of a solenoid winding with honeycomb cell (Casolaro et al., 2014).

3. Results and Discussion

Protonation thermodynamics. The thermodynamic properties for the protonation of basic hydrogel sites have shown a marked polyelectrolyte character. In any case, the equilibrium constants (pK_a) vary with the degree of protonation α of the whole macromolecule. The gradual decrease of pK_a in relation to the decreasing pH is reported in Figure 1 that shows, unlike the zwitterionic Hist hydrogel, a sudden interrupted linearity at critical pHs 5 and 4, for carboxyl hydrogels with residues of PHE and AVA, respectively (Casolaro et al., 2004, 2014). Such critical pH values are related to the partial neutralization of the ionized carboxyl groups that allows the shrinking of the macromolecular structure, as hydrophobic interactions outweight the electrostatic repulsion. During protonation in 0.15 M NaCl, the pK_a decreases with increasing α (pH decreasing) till α reaches about 0.3 (pH 5) and 0.7 (pH 4), respectively for PHE and AVA hydrogels. Above the critical α values, the electrostatic forces are superimposed by the hydrophobic ones, due to the presence of isopropyl and phenyl groups present in the side chain of the macromolecular structure. Contemporaneously, the values of the equilibrium degree of swelling $EDS=(W_w-W_d)/W_d$ (where W_w and W_d is the weight of wet and dry hydrogel, respectively) sharply decreases in the narrow critical pH range. Thus, the compact structure of the hydrogel

shows a phase separation that remains with the lowest EDS value even at low pH. On the other hand, the Hist hydrogel, showing the isoelectric point near to pH 5, further hydrates and swells at low pH because of the protonated imidazole nitrogen. The lower EDS value of the hydrogel Hist in comparison to the hydrogel PHE and AVA (Figure 1) is attributable to the larger cross-linking content (10 mol% of EBA in Hist versus 2 mol% of EBA in PHE and AVA).

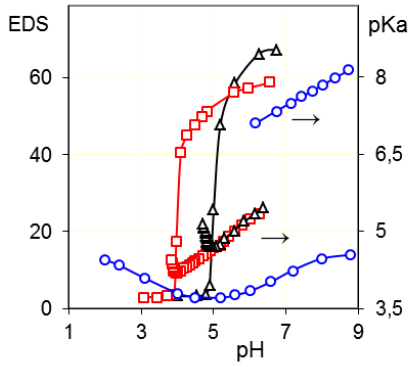


Figure 1: Equilibrium degree of swelling (EDS) and basicity constants (pKa) in relation to pH of the hydrogels PHE-Nip3 (dark triangles), AVA-2 (red squares), and Hist-10 (blue circles) in 0.15 M NaCl and 25 °C.

Diclofenac release. Among the simplest and most interesting carboxylic acids capable of ionically interacting with the imidazole nitrogen of hydrogel Hist, diclofenac was preferred. The latter, as a non-steroidal anti-inflammatory drug, has analgesic properties especially at low doses and is widely used for both oral and cutaneous administration (Altman et al., 2015). Briefly, we report some preliminary results of the 'in vitro' release of diclofenac from the hydrogel Hist-10 loaded with the drug. In addition, a copolymerized NIPAAm hydrogel containing only a low amount of MHist was used to compare the release kinetics in acidic environment (pH 4.60) with subsequent pulses at pH 7.40. Figure 2 shows essentially two different diclofenac release profiles:

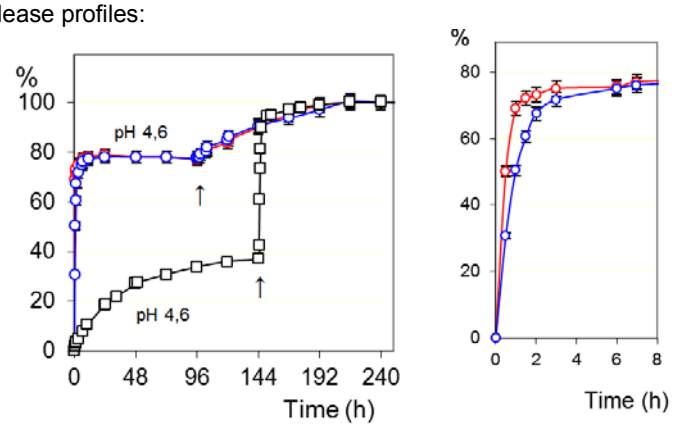


Figure 2: Release (%) of diclofenac in PBS (pH 7.40) and in acetate (pH 4.60) buffer solutions from the hydrogel MHist (inset: without AMF, blue circles; with AMF, red circles) and Hist-10 (dark squares) at 25 °C.

at pH 4.60, Hist-10 hydrogel releases a percentage of drug in the set time of six days, which is about half than that observed for the MHist hydrogel, the latter being depleted in its loading in a few hours. The next pH boost at 7.40 has an immediate effect of complete discharge of the drug from Hist-10 hydrogel in a few hours, while releasing the drug from the hydrogel MHist is more sustained over a few days. This different behavior of the two hydrogels can be attributed to the greater compactness of Hist-10 at pH 4.60 in which the drug forms a stable and slightly soluble adduct; conversely, the MHist hydrogel containing a few ionic units is affected by higher hydration. Consequently, the increase of pH at 7.40 favors the Hist-10 ionization and swelling more rapidly, allowing a faster release. Furthermore, the additional effect of a magnetic stimulation on the hydrogel MHist produces a small and significant increase in release of diclofenac only in the early hours and at pH 4.60, i.e. full load. All this is supported by the EDS values as measured at pH 4.60 for Hist-10 (EDS 5.4) and MHist (EDS 13 and 14 with AMF and No-AMF, respectively); at the end of experiments at pH 7.40, EDS became 9.4 for Hist-10 and 17 for MHist (in both AMF and No-AMF conditions).

Insulin release. The most well-known function of insulin is to regulate the level of blood glucose by reducing blood glucose and activating various metabolic and cellular processes. It is administered in various ways and in different body areas to avoid the creation of lipo-hypertrophy. There are several side effects associated with subcutaneous seizure, including metabolic, ophthalmic and systemic. Researchers are studying useful systems for a right administration of insulin to avoid overdosage (Zhao et al., 2017). Among the many insulin-controlled release systems, hydrogels have attracted the attention of many researchers in the past and still nowadays (Huynh et al., 2009). Hydrogel use is very versatile as it allows the release of the drug following a glucose-induced glucose oxidase (GOD) enzyme reaction. An anionic hydrogel can incorporate an insulin reserve and contain the GOD immobilized on the carboxyl groups. The presence of glucose permits the reaction of gluconic acid to be transformed into a weak acid, promoting the protonation of the ionized carboxyl groups and the consequent collapse of the composite hydrogel system. A hydrogel that responds specifically to changes in glucose concentrations or other external stimuli is well suited for its utility. For the purpose of resuming and following our previous study on insulin release (Ito et al., 1989), this section presents an initial approach to the release of insulin from the proposed hydrogels considering their response to the pH solvents and the effect of the alternating magnetic field. Figure 3 shows the pulsed release of insulin in acidic medium (pH 2.9) with timely-increasing pulses of 2, 3, and more hours at pH 7.40 in PBS. As was expected, at pH 2.9 the insulin loaded hydrogel is compact and this does not allow the release of the large drug molecule.

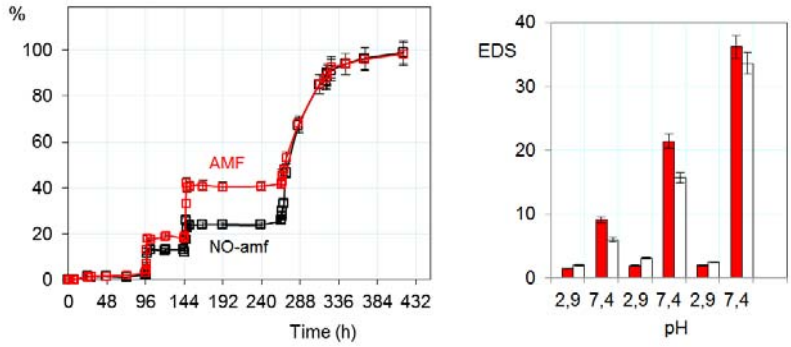


Figure 3: Left: Pulsed release (2h and 3h pulses, PBS pH 7.40) of insulin from the hydrogel PHE-Nip2 in 0.15 M NaCl (at pH 2.90) with the presence (red squares) and the absence (black squares) of AMF. Right: Equilibrium degree of swelling (EDS) measured at the end of each pH pulses with (red) and without (dark) AMF applications.

Only at pH 7.40, the ionization of the hydrogel involves a regular swelling with the possibility of immediate release of insulin. The return to pH 2.9, a prompt hydrogel collapse causes blocking of any leakage of insulin molecules. The release profile becomes flat for as long as the pH is set to pH 7.40. Once again, the second pulses at pH 7.40 (for 3h) reveals a prompt presence of insulin in solution. Returning to the pH solution 2.9 again blocks insulin trapped in the collapsed hydrogel. Finally, at the third prolonged pulse of pH 7.40, the release of insulin becomes regular and extended for more than a week. The application of AMF shows a clear increase in insulin release at the various pH pulses considered. The difference can be attributed to the energy induced by the AMF that can cause an improved permeability control mechanism due to oscillation or vibration of the network-embedded $CoFe_2O_4$ magnetic nanoparticles. This in turn may cause twisting and/or displacement of the polymeric chains, resulting in an enhancement of the diffusion process. According to other researchers, the magnetically-induced deformation of the hydrogel is a result of the elastic oscillation or vibration of the embedded magnetic nanoparticles resulting in mechanical deformation of the hydrogel to squeeze out the drug (Hu et al., 2008).

Release of antidepressants. Depression is a common illness worldwide and can happen to anybody; it causes mental anguish and affects people's ability to carry out everyday tasks (Murray et al., 1997). Recently, the World Health Organization has estimated more than 300 million people to be affected and, for this purpose, has launched a one-year campaign to ensure that more people with depression both seek and get help. Fortunately, depression can be prevented and treated adequately. For the treatment of the disease, three antidepressant (paroxetine, duloxetine, vortioxetine) drugs, that are specific and used today in the clinical practice, were chosen and considered for the release studies. The pulsed release of the paroxetine from the hydrogels PHE-Nip3 was analyzed in a period of time long enough (at least two weeks) in two acidic pH solutions (pH 2.9 and 4.6). At set intervals of time, the external acid solution was replaced by a physiological pH solution (PBS, pH 7.40) for a short period of time (1h or 2h). Moreover, the presence of the ionized drug

substantially contributes to maintain the hydrogel in the collapsed state because of its ionic interaction with the carboxylic groups. This is evident in any case since the formation of the drug-hydrogel adduct leads to the complete collapse of the ionic and swollen hydrogel in presence of the hydrochloride drug solution. Additionally, despite the non-swellability at acid pH, the hydrogel loaded with the drug is wettable and capable of releasing the drug itself in a slow way and controllable by external stimuli. In Figure 4 is shown the comparison of the pulsed release of the paroxetine from the hydrogel PHE-Nip3 in two different aqueous solutions of pH 2.9 and 4.6, with the pH pulse 7.4 for 1h. In addition to pH 4.6, it is shown a comparison with the pulse of pH 7.4 for 2h. In all cases, the additional stimulation of the alternating magnetic field (AMF) is compared.

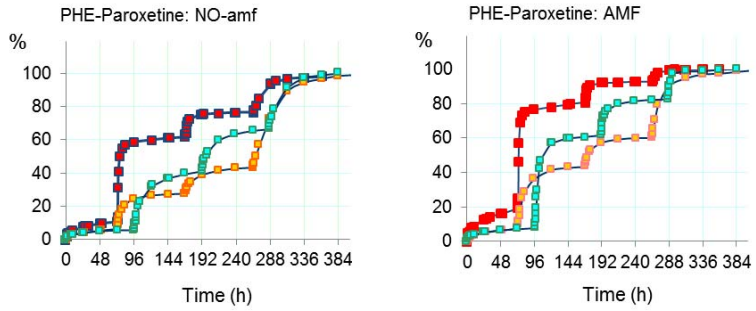


Figure 4: Release of paroxetine (%) from the hydrogel PHE-Nip3 in buffered solutions (pH 4.60: orange and light blue; pH 2.9: red) with PBS (pH 7.40) pulsed variations of 1h (orange, red) and 2h (light blue) without (left panel) and with (right panel) AMF applications at 25 °C.

From the beginning it is immediately evident that the release of the drug is greater in the more acidic solution (pH 2.9) and that the released quantity becomes significantly higher when an appropriate external AMF is applied. In general, the application of the AMF produces an increase in the percentage of release, especially at the first stage of the pulsed release. On the other hand, the release into solution at pH 4.6 is significantly lower for a long period of time. Only the change of external solution to pH 7.4 (first pH pulse of 1h) leads to a significant increase in the paroxetine release which is even greater if the residence time in contact with the hydrogel is doubled to two hours: compared with 1-hour, pulses of 2-hours ensure a higher percentage of the drug in solution. The return to conditions of acidic pH (2.9 and 4.6) restores the release for the successive period of days, while the further pulse to pH 7.4 reproduces the burst in drug release. Finally, in the last pH pulse performed at stage 3, the PBS solution at pH 7.4 has been in contact for a long period of days to allow the complete release of the drug from the hydrogel. This sustained release has required a few days, despite the speeding of the AMF force. All the stepwise process is to be related to chemical equilibria of all ionic/ionizable species involved and their base strength. Paroxetine, being a strong base (pK_a 9.77), remains mostly in the protonated state in all considered pH conditions (Casolaro M. and Casolaro I., 2016b, 2017). This species, interacting ionically to some extent with the ionized COO^- groups of the swollen hydrogel, allows the collapse of the whole structure with the formation of a drug-polymer adduct of chemical stability. Possible pH variation of a solution containing the adduct may shift the protonation equilibrium of the hydrogel towards the ionised form if the $pH>pK_a$ (pK_a of the hydrogel) with the consequence of an increase in swellability and a greater release of the drug in solution; on the other hand, at $pH<pK_a$ the hydrogel involves a charge neutralization with a consequent shrinking phenomenon and a greater availability of the free drug in solution. All this is consistent with the data reported in Figure 4. Of course this depends on the pH value, as the gradual ionization of the hydrogel promotes its swelling. In all the intermediate stages, the EDS follows a trend connected to the pH and to the amount of the drug released at that stage.

4. Conclusion

New stimuli-responsive hydrogels based on α-amino acid residues can be considered as promising platforms for chrono-therapeutic applications, as they respond to external triggers. The complications of circadian rhythms generally require a chrono-pharmacotherapy which can be easily accomplished by means of a suitable pulsed drug delivery system (Peppas and Leobandung, 2004). The pulsed release of drugs is a new delivery system that certainly provides an increase in therapeutic benefit to patients suffering from chronic diseases, because it delivers the drug in the right amount at the right time and place. Though the possible biomedical applications are remarkable, in this study we wanted to preliminarily investigate the pulsed release of different drugs. The pulsed release at physiological pH of some drugs is significantly higher in the presence

of a low-frequency AMF. The gradual and pulsed variation of the pH implies a reversible swelling-collapsing mechanism of the hydrogel, which improves a higher release rate of the drug in dependence also on the time of the applied pulse. The mechanism by diffusion is improved in all cases when the hydrogel is pulsed at pH 7.4 and is then collapsed at a pH lower than the critical one. On the basis of these preliminary observations we conclude that the proposed polyelectrolytes hydrogel platforms can be used to design useful chrono-pharmacotherapeutic systems based on the circadian rhythm of the body (Sonis, 1992). Among these systems, one that may deserve a certain interest is the design of an artificial pancreas. To this purpose, a study for the release of insulin from smart hydrogels containing the immobilized enzyme glucose oxidase should be undertaken for future outlook.

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References

Altman R., Bosch B., Brune K., Patrignani P., Young C., 2015, Advances in NSAID development: evolution of diclofenac products using pharmaceutical technology, Drugs 75, 859-877.

Campbell S., Maitland D., Hoare T., 2015, Enhanced pulsatile drug release from injectable magnetic hydrogels with embedded thermosensitive microgels, Macro Lett. 4, 312-316.

Casolaro M., Bottari S., Cappelli A., Mendichi R., Ito Y., 2004, Vinyl polymers based on L-histidine residues. Part 1. The thermodynamics of poly(ampholyte)s in the free and in the cross-linked gel form, Biomacromolecules 5, 1325-1332.

Casolaro M., Del Bello B., Maellaro E., 2011, Hydrogel containing L-valine residues as a platform for cisplatin chemotherapy, Coll. Surf. B: Biointerfaces 88, 389-395.

Casolaro M., Casolaro I., Lamponi S., 2012a, Stimuli-responsive hydrogels for controlled pilocarpine ocular delivery, Eur. J. Pharm. Biopharm. 80, 553-561.

Casolaro M., Casolaro I., 2012b, Multiple stimuli-responsive hydrogels for metal-based drug therapy, Polymers 4, 964-985.

Casolaro M., Casolaro I., 2013, Multiple stimuli-responsive hydrogels based on α-aminoacid residues for drug delivery, vol. 2, 199-227: Smart Materials for Drug Delivery, Eds. Alvarez-Lorenzo C., Concheiro A., RSC Publ., Cambridge, UK.

Casolaro M., Casolaro I., Bottari S., Del Bello B., Maellaro E., Demadis K.D., 2014, Long-term doxorubicin release from multiple stimuli-responsive hydrogels based on α-aminoacid residues, Eur. J. Pharm. Biopharm. 88, 424-433.

Casolaro M., Casolaro I., 2015, Controlled release of antidepressant drugs by multiple stimuli-sensitive hydrogels based on α-aminoacid residues, J. Drug Deliv. Sci. Techn. 30, 82-89.

Casolaro M., Casolaro I., 2016a, Stimuli-responsive hydrogels bearing α-aminoacid residues: a potential platform for future therapies, J. Biom. Eng. Med. Dev. 1, 111.

Casolaro M., Casolaro I., 2016b, Polyelectrolyte hydrogel platforms for the delivery of antidepressant drugs, Gels 2, 24.

Casolaro M., Casolaro I., 2017, Pulsed release of antidepressants from nanocomposite hydrogels, Biol. Eng. Med. 2, 1-8.

Hu S.H., Liu T.Y., Huang H.Y., Liu D.M., Chen S.Y., 2008, Magnetic-sensitive silica nanospheres for controlled drug release, Langmuir 24, 239–244.

Huynh D.P., Im G.J., Chae S.Y., Lee K.C., Lee D.S., 2009, Controlled release of insulin from pH/temperature-sensitive injectable pentoblock copolymer hydrogel, J. Controlled Rel. 137, 20-24.

Ito Y., Casolaro M., Kono K., Imanishi Y., 1989, An insulin-releasing system that is responsive to glucose, J. Controlled Rel. 10, 195-203.

Murray C.J., Lopez A.D., 1997, Alternative projections of mortality and disability by cause 1990-2020: global burden of disease study, Lancet 349, 1498-1504.

Ohdo S., 2010, Chrono-drug-delivery focused on biological clock: intra- and inter-individual variability of molecular clock, Adv. Drug Deliv. Rev. 62, 857-858.

Peppas N.A., Leobandung W., 2004, Stimuli-sensitive hydrogels: ideal carriers for chronobiology and chronotherapy, J. Biomater. Sci., Polym. Ed., 15, 125-144.

Sonis W.A., 1992, Chronobiology of seasonal mood disorders, vol. 15, 89-114: Clinical guide to depression in children and adolescents, Ed. Shati M., American Psychiatric Association, Washington, DC.

Zhao L., Wang L., Zhang Y., Xiao S., Bi F., Zhao J., Gai G., Ding J., 2017, Glucose oxidase-based glucose-sensitive drug delivery for diabetes treatment, Polymers 9, 255.