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Research paper

# Controlled release of antidepressant drugs by multiple stimuli-sensitive hydrogels based on α-aminoacid residues

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ABSTRACT

Two slightly cross-linked hydrogels bearing L-phenylalanine (Phe-Nip3) or L-valine (Ava2) residues of a copolymeric and homopolymeric vinyl structure were considered for their potential application in the psychiatric treatment of depression. Two antidepressant drugs (citalopram and trazodone) were loaded into hydrogels and their controlled release behavior monitored for several days at 25 °C in two buffer solutions of different pHs (PBS pH 7.4 and acetate pH 4.6). The different basicity constants (logKs) of the involved substance determine a different electrostatic effect between the drug ionized positively and the negatively charged hydrogel. Both the hydrogels loaded with citalopram showed a greater binding effect with respect to trazodone. In fact, for the same hydrogel, the release of citalopram in PBS (4 days) was slower than trazodone (24 h). In addition, at pH (4.6) < logK the release of the drug was much slower and durable, due to the lower capacity of ionization and swelling of the hydrogel. Additionally, the magnetic nanoparticles ($CoFe_2O_4$) embedded into the hydrogel Phe-Nip3 were an additional remote control for drug release through the stimulation of an appropriate alternating magnetic field (AMF, 20 kHz and 50 W). In these conditions, the kinetics of the drug released was substantially increased.



## 1. Introduction

Hydrogels are very versatile materials in various fields of application, especially for the controlled release of drugs [1]. They are also developed in biomedicine for vocal fold regeneration and soft tissue repair and augmentation [2,3]. Their characteristic resides, as is well known, in the control of the size of three-dimensional polymer network which involves a swelling/collapse following the absorption/release of water molecules of the system in which it is located [4]. The polyelectrolyte hydrogels, that is made up of macromolecules bearing ionic charges, are well suited for use as a carrier of drugs whose molecule is of opposite charge, or based on metal [5–12]. These hydrogels are able to interact electrostatically with the molecule of the drug, thereby trapping the drug in a stoichiometric way [11].

We recently reported several crosslinked polymer systems useful for their potential application in the biomedical and pharmaceutical industries [13–16]. The materials are made from vinyl polymers bearing residues of α-aminoacids (L-phenylalanine, L-leucine, L-valine, L-histidine) crosslinked with *N,N′*-ethylene-bisacrylamide or polyetilenglicol–dimethacrylate [13,14,16,17]. The residues of the carboxylic acid and/or amine (ampholytic) groups allow to form stable complexes with either metal ions and drug molecules of opposite charge [10–12,18,19]. In all cases, the hydrogels provide pH- and temperature-responsiveness due to the presence of ionic/ionizable functional groups and of hydrophilic amido and hydrophobic isopropyl/phenyl groups, respectively [11,13,14,16–19].

Therefore, we investigated the usefulness of application for loading and release of anticancer molecules (cisplatin, doxorubicin) [10,11,19] and molecules for the treatment of glaucoma (pilocarpine) [18]. A greater force of electrostatic interaction between the hydrogel and the drug has always led to highlight a slow release of the drug in buffer solution (PBS, pH 7.40) and the release itself is prolonged for a long time while preserving the activity of the drug [19].

Following our interest in the application of polyelectrolyte hydrogels in controlled release of drugs, we decided to contribute to the development of therapeutic systems in the field of depression [20,21]. Few results are reported in the literature though depression affects 350 million people around the World, causing

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850,000 deaths every year. According to recent estimates, in 2020 it will be the second leading cause of work disability, because of the scientifically proven correlation between job loss, poverty and the disease, with a raise of 0.79% in the suicide rate for each 1% increase in unemployment installments [22,23]. Yet, with the right diagnosis, drug treatment and social support, remission could be almost always possible.

In this work we report the results obtained by two anionic hydrogels, bearing carboxyl groups of the L-phenylalanine and L-valine residues, that interact with amino ionized antidepressant (citalopram, trazodone) drugs (Fig. 1).

Citalopram is used to treat depression [24]. Citalopram is an antidepressants belonging to the class of selective serotonin reuptake inhibitors (SSRIs) [25]. It is thought to work by increasing the amount of serotonin, a natural substance in the brain that helps maintaining mental balance. Citalopram is one of the more recent molecule and, according to numerous studies, the most selective and, therefore, it shows fewer side effects. It is widely used because of its high tolerability, in depression and panic disorder at doses ranging from 10 to 40 mg daily. If the response is inadequate the dose may be increased up to a maximum of 60 mg per day. The antidepressant effect is usually seen in 2–4 weeks after initiation of therapy. Citalopram not directly stimulates serotonin (which is a neurotransmitter receptors), but inhibits the re-uptake of serotonin in the synaptic cleft by the presynaptic terminal. In this way, when the re-uptake is inhibited, the concentration of serotonin in the synaptic cleft remains high for longer.

Trazodone is an antidepressant molecule behaving as a serotonin receptor antagonist and reuptake inhibitor (SARI) [26–28]. It seems to be widely accepted that trazodone is a safe drug with few side effects, if compared to other classes of antidepressants. Its most common side effects are daytime sleepiness and excessive sedation, headache, dizziness and hypotension; priapism is a rare but serious side effect. Furthermore, trazodone combined 5HT2A receptor antagonism and blockade of serotonin transporter (SERT) leads to positive clinical implications in terms of tolerability (it prevents the occurrence of side effects in the initial and long-term treatment with SSRIs, especially anxiety, insomnia and sexual dysfunction). However, there is also a theoretical rationale suggesting the possibility of a synergic antidepressant effect in the case of pharmacological association between SSRIs and trazodone.

As can be seen from Fig. 1, both drug molecules contain tertiary nitrogen atoms and then are subjected to ionization. Their strength

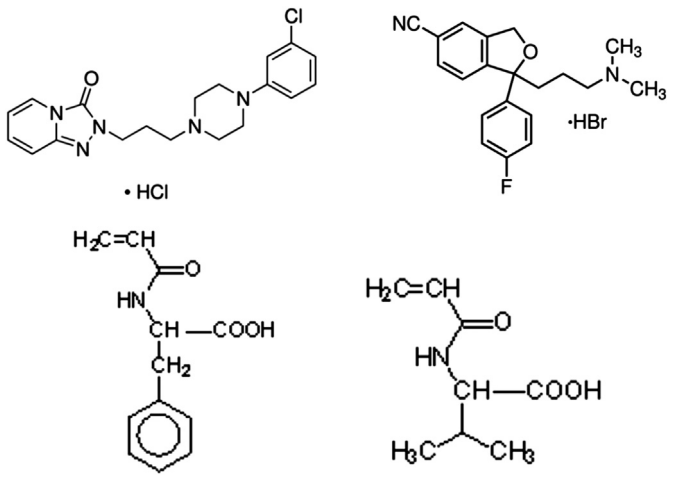


**Fig. 1.** Chemical structures of trazodone hydrochloride, citalopram hydrobromide, *N*-acryloyl-L-phenylalanine, and *N*-acryloyl-L-valine.

of electrostatic interaction with the hydrogel will depend mainly on the $pK_a$ of the functional groups. A higher value of $pK_a$ ensures greater stability of the complex polymer-drug and will determine a subsequent slower release under physiological conditions. Furthermore, the release of the drug can occur 'on-demand' if an extra stimulus is present in the hydrogel. For example, magnetic nanoparticles (NPs) embedded in the hydrogel matrix allow an additional remote-control mechanism to trigger the drug release by a non-contact force, which is superior to the traditional stimuli such as pH or temperature [19,29]. Hence, the magnetic carriers loaded together with citalopram or trazodone can promote local accumulation or can be guided to the site-specific target and then provide a sustained drug release under the application of an external alternating magnetic field (AMF).

## 2. Materials and methods

### 2.1. Materials

The two hydrogels (Phe-Nip3 and Ava2) used in this study were prepared according to a previously reported procedure [18,19]. While the hydrogel Ava2 was an homopolymer prepared by the *N*-acryloyl-L-valine, the hydrogel Phe-Nip3 was an equimolar copolymer obtained by two monomers (*N*-acryloyl-L-phenylalanine and *N*-isopropylacrylamide) embedded of cobal ferrite ($CoFe_2O_4$) magnetic nanoparticles [30]; both hydrogels were cross-linked with 2 mol% of *N*,*N*′-ethylene-bisacrylamide (EBA). Citalopram hydrobromide was supplied by ratiopharm® (Germany) as 40 mg tablets; the active ingredient was separated from the other ingredients in the tablet, dissolving it in 40 mL of methanol under magnetic stirring. Subsequently, after decantation, the solution was filtered and placed to dry. A white powder was recovered. The purity of the obtained drug was determined by potentiometric titration with a standard solution of sodium hydroxide. The trazodone hydrochloride was provided by the Angelini *Co*. (Italy), as a solution (Trittico) of 25 mg/mL active ingredient. Alternating magnetic field (AMF) measurements were performed with the AMF apparatus previously described [19]. The monitoring functions (frequency and power) were provided by the model AG 1006 amplifier/generator (T&C Power Conversion, Inc.), that applied an electric field of 50 W at 20 kHz to the solenoid winding with honeycomb cell. Spectrophotometric measurements were performed with a Specord 210 spectrophotometer (Analytikjena) equipped with 10 mm quartz cuvettes. The Agilent Cary 630 FTIR, equipped with the Agilent diamond ATR accessory, was used to record infrared spectra.

### 2.2. Methods

#### 2.2.1. Potentiometric measurements

Potentiometric titrations were carried out with a TitraLab 90 titration system (Radiometer Analytical), as previously reported [31]. The TitraLab 90 is supported mainly by the TIM900 Titration Manager and connected to a Windows-based software, for remote control (TimTalk 9). Measurements of pH and temperature was insured by the combined pH electrode (Red Rod) and the temperature sensor (T201), respectively. TIM900 was calibrated with two buffer solutions (Radiometer) of known pH value (pH 7.00 and 4.01) before each potentiometric titration. Potentiometric measurements were carried out in a thermostated glass cell (25 °C) filled with 50 mL of a NaCl aqueous solutions (0.15 M and 0.01 M). For the hydrogels, a weighed and finely crushed solid material (15–30 mg) was dispersed under stirring, together with a known excess amount of standard sodium hydroxide solution, under a presaturated nitrogen stream. When the pH of the solution was stable, and