TWO-ANTENNARY OLIGOGLYCINE COMPLEXES WITH INDOMETHACIN IN AQUEOUS MEDIA: FORMATION AND CHARACTERIZATION

Eduarda Encheva[1], Dimitrinka Arabadzhieva[1], Ivan Minkov[1,2], Anna Gyurova[1], Vesselin Petrov[3,4], Elena Mileva[1]

[1]*Bulgarian Academy of Sciences*
Institute of Physical Chemistry "Rostislaw Kaischew"
Acad. G. Bonchev St., Bl.11, Sofia 1113
Bulgaria, encheva@ipc.bas.bg (E.E.); dimi@ipc.bas.bg (D.A.)
any_gyurova@abv.bg (A.G.); mileva@ipc.bas.bg (E.M.)
[2]*Sofia University "St. Kliment Ohridski"*
Department of Chemistry and Biochemistry, Physiology, and Pathophysiology
Faculty of Medicine, 1 Koziak St., Sofia 1407
Bulgaria; ilminkov@med.uni-sofia.bg (I.M.)
[3]*Sofia University "St. Kliment Ohridski", Department of Physical Chemistry*
Faculty of Chemistry and Pharmacy, 1 James Bourchier Blvd.
Sofia 1164, Bulgaria, v.petrov@chem.uni-sofia.bg (V.P.)
[4]*Research, Development, Innovation Consortium, Sofia 1784, Bulgaria*

Received 17 April 2025
Accepted 21 June 2025
DOI: 10.59957/jctm.v60.i5.2025.4

ABSTRACT

Two-antennary oligoglycines are synthetic, biocompatible bola-amphiphiles composed of homologs with varying hydrophobic-to-hydrophilic ratios. These molecules exhibit a pronounced tendency for self-assembly in aqueous media, resulting in the formation of well-defined supramolecular structures known as tectomers. The present study is focused on one representative of the antennary olygoglycines and investigates its potential as a nanocarrier for hydrophobic drugs. The non-steroidal anti-inflammatory drug indomethacin is used as a model hydrophobic compound. To maximize the entrapment of the drug, we develop an experimental procedure, including the preliminary formation of tectomers at various pH values, followed by the addition of indomethacin. The obtained oligoglycine-indomethacin complexes are further characterized using UV-Vis spectroscopy, Dynamic Light Scattering, and Thin-Liquid-Film techniques. The results demonstrate that the two-antennary oligoglicine effectively captures indomethacin and forms stable complexes in aqueous media. We outline the optimal conditions for drug entrapment and propose hypotheses regarding the mechanisms behind the formation of the mixed complexes. This study advances the understanding of interactions between two-antennary oligoglycines and hydrophobic compounds and highlights their potential for biomedical applications.

Keywords: antennary oligoglycine, bola-amphiphiles, tectomer, indomethacin, hydrophobic-substance nanocarrier

INTRODUCTION

Two-antennary oligoglycines (C_xH_{2x}(-CH_2-NH-Gly_n)$_2$) are synthetic compounds that are classified as biocompatible amphiphiles [1 - 4]. They have two oligoglycine tails (antennae) of equal length, linked to both ends of a hydrocarbon chain. In aqueous solutions, these substances exhibit a self-assembly tendency, forming specific supramolecular nanostructures known as tectomers. The primary driving factor for the formation of these aggregates is the strong preference for the onset of highly coordinated intra- and intermolecular hydrogen bonding networks (Polyglycine II motifs (PG-II, 3_1-helix) [5 - 7]). The PG-II tendency is active

above a minimal length of the oligoglycine antennae (n ≥ 4) because H-bonding is a direction-specific interaction and a suitable mutual orientation of the carbonyl and amino groups in the neighboring antennae is needed so that a sterically stabilized H-bond to appear [3, 4]. The consequence is the formation of extra-stable supramolecular structures in the aqueous solution bulk.

The two-antennary oligoglycines have antennae terminated by amine groups, and the hydrocarbon segment usually has 5 - 10 carbon atoms. The presence of well-defined hydrophilic and hydrophobic portions determines the amphiphilic character of the molecules in aqueous media and presumes the possibility of amphiphilic self-assembly [8 - 11]. This tendency is coupled with the well-expressed PG-II option, and the precise balance between the two trends depends predominantly on the structure of the synthetic oligopeptide. It is also a function of the aqueous environment conditions, namely oligoglycine concentration, temperature, and the addition of various additives. Therefore, these substances belong to a group of smart materials whose properties can be precisely regulated and gradually altered by changing the complex fluid's conditions (e.g., pH, temperature, insertion of electrolytes, surfactants, etc.). The existence of self-assemblies, with well-defined and tunable properties, opens interesting prospects for their usage as capture agents and nano-transporters in aqueous media, with possible applications in medicine, pharmaceutics, for cleaning waste or industrial waters, etc.

The properties of several types of two-antennary oligoglycines have already been examined in earlier research [2 - 4, 12 - 14]. The focus of the present study is on one particular member of the oligoglycines - x = 8 and n = 4 (C_8H_{16}(-CH_2-NH-Gly_4)$_2$, T_2-C_8-Gly_4 (Fig. 1a). This substance is chosen because it has been previously systematically investigated at different concentrations of the oligoglycine, at temperature changes, and in the presence of added low-molecular-mass electrolyte [3]. It has been established that at ambient conditions ('native' pH = 5.1, 20°C), two types of bulk aggregates are formed in aqueous solutions: vesicular and disc-like tectomers. Besides, the size distributions of the nanostructures do not substantially change at varying oligoglycine content, temperature, and upon the addition of NaCl. Since T_2-C_8-Gly_4 is applied as hydrochloride (C_8H_{16}(-CH_2-NH-Gly_4)$_2$*2HCl), the self-assembled nanostructures are positively charged in the aqueous environment within a wide range of solution conditions; this fact gives additional opportunities related to the specific interrelations of hydrophobic and electrostatic interactions. Aside from the hydrophilic portions, the obtained supramolecular nanostructures have well-expressed hydrophobic regions. Thus, the tectomers are also expected to have a capacity to capture hydrophobic substances. So, aqueous formulations containing oligoglycine tectomers demonstrate significant potential as nanocarriers and/or drug delivery systems. These solutions are biocompatible, can be easily fine-tuned to adjust their structure-property relationships, and have the potential to achieve high entrapment efficiency.

Intending to examine the potential of T_2-C_8-Gly_4 as a hydrophobic-substance nanocarrier, we implemented a systematic investigation on mixed aqueous solutions of T_2-C_8-Gly_4 and the drug indomethacin (IMC) (Fig. 1b).

Indomethacin is a non-steroidal anti-inflammatory

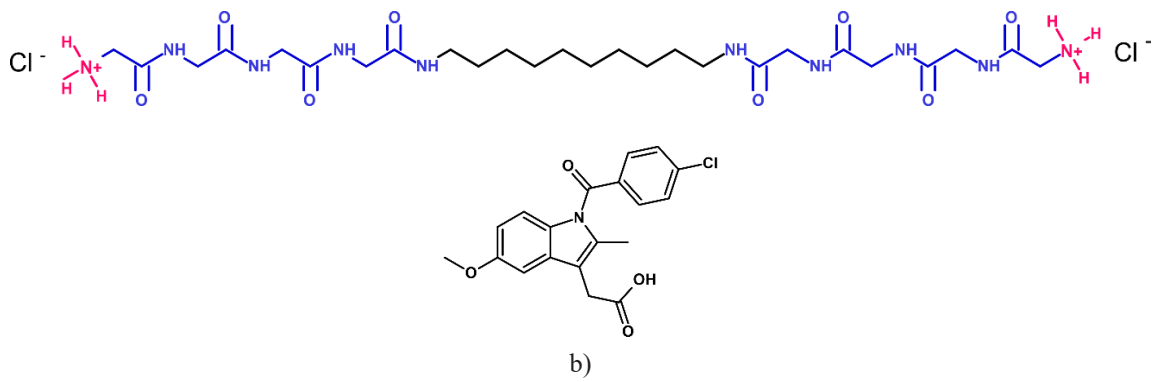

Fig. 1. Chemical structure of (a) two-antennary oligoglycine C_8H_{16}(-CH_2-NH-Gly_4)$_2$*2HCl and (b) indomethacin (IMC).

drug (NSAID), and it is widely studied as a model hydrophobic molecule for drug loading and delivery purposes [15, 16]. Due to its poor water solubility at ambient conditions, special strategies are required for its effective encapsulation and delivery [17]. IMC is known to exist in five polymorphic forms, and several solvates, each with different physicochemical properties that affect solubility, dissolution rate, stability, and ultimately, bioavailability [16 - 21]. Among these, the α- and γ-polymorphs are the most widely studied. The α-form contains three IMC molecules per asymmetric unit: two form a H-bonded dimer via carboxylic acid groups, and the third is linked to the dimer through a H-bond involving an amide carbonyl group. The γ-form consists of two IMC molecules joined by carboxylic acid H-bonds and allows for hydrophobic interactions, which may enhance its compatibility with amphiphilic carriers. In this study, we focus on the γ-polymorph of IMC due to its structural features that favour interaction with oligoglycine-based nanocarriers.

The major goal of the present investigation is to verify the possibility of IMC capturing by the tectomeric structures in aqueous solution formulations. The aim is to outline the specific conditions that might open suitable paths for using tectomers as drug nanocarriers. To achieve this goal, various experiments are performed using UV-Vis spectroscopy, Dynamic Light Scattering (DLS), and microscopic Thin-Liquid-Film (TLF) techniques. The specific tasks of the research are: (1) to find experimental evidence that the two-antennary oligoglycine may form stable T_2-C_8-Gly_4 + IMC complexes; (2) to outline the optimal conditions for the drug capturing in aqueous solution formulations; (3) to put forward some plausible hypotheses about the formation mechanisms of the mixed T_2-C_8-Gly_4 + IMC complexes.

EXPERIMENTAL

The two-antennary oligoglycine $C_8H_{16}(\text{-}CH_2\text{-}NH\text{-}Gly_4)_2{}^*2HCl$ used in the experiments is purchased from PlasmaChem (Berlin, Germany, purity 95 %). Based on the information obtained in previous studies, two concentrations are chosen as optimal for the experiments: 1.0×10^{-4} mol L^{-1} and 2.0×10^{-4} mol L^{-1} [3]. It has been established earlier that at these oligoglycine contents and at ambient conditions ('native' pH = 5.1, 20°C), the obtained tectomers in water solutions are predominantly vesicular. T_2-C_8-Gly_4 is added in a powdered form to aqueous media with controlled pH values in the range of 2.0 - 7.0.

The pH values are controlled by using buffers prepared according to the Britton-Robinson procedure [22]. The buffer contains three acids, namely boric (H_3BO_3, 99.99 % purity, Sigma Aldrich), phosphoric (H_3PO_4, purity ≥ 99.99 %, Sigma Aldrich), and acetic (CH_3COOH, 99.99 % purity, Sigma Aldrich), and sodium hydroxide (NaOH, 99.99 % purity, Sigma Aldrich). The pH-regulation also includes applying concentrated hydrochloric acid (HCl, 37 %, Merck KGaA, Darmstadt, Germany). Doubly distilled water (2D water) is used for the solution preparation.

Indomethacin (IMC, γ-form, purity ≥ 99 %, Sigma Aldrich) is used in the concentration range of 6.3×10^{-5} mol L^{-1} - 2.6×10^{-4} mol L^{-1}. Ethanol (EtOH, 99.8 % purity, Honeywell) is applied for the initial dissolution of the IMC. The procedure includes adding the powdered form of the substance to the ethanol. Then, a buffer with pH = 7.0 is added to the fluid sample so that the final concentration of EtOH in the samples becomes in the range of 2.5 % - 5 % (v/v).

The experimental procedure for the preparation of the investigated mixed systems (T_2-C_8-Gly_4 + IMC) is linked to the specific characteristics of both substances in an aqueous environment. The first challenge is related to the low and pH-dependent solubility of IMC, being essentially insoluble in water at pH < 7.0 [23]. The second issue concerns the possibility of pH-triggered regulation of the tectomer formation, as established for similar two-antennary oligoglycines of slightly longer tails, e.g., T_2-C_8-Gly_5, with possible effects on the size distributions of the tectomers [4, 12]. To achieve a suitable balance between the solubility of IMC and the formation of tectomers, as well as to create chances for maximum entrapment of the drug, a precise experimental procedure is developed (Fig. 2).

The proposed procedure is based on the preliminary onset of tectomers in the aqueous solutions, and then IMC is added to the sample. The substances are dissolved separately in an aqueous environment and then are mixed as samples of equal volume. The oligoglycine solutions have pH levels of 2.0, 4.0, or 7.0, respectively, and they are incubated for 24 h before mixing with the IMC solutions [2]. The applied indomethacin solution has

pH = 7.0 and contains 5 % (v/v) EtOH. The resulting aqueous mixtures have pH values of 4.1, 5.3, and 6.7, and are with reduced concentrations than the initial probes: the final concentrations are 1.0×10^{-4} mol L^{-1} for the oligoglycine, and 1.3×10^{-4} mol L^{-1} for IMC. The obtained samples are opalescent, and some sedimentation of larger entities is usually observed. To remove the largest particles, consecutive centrifugation at 3000 rpm for 30 min and filtration through a 450 nm filter is performed before further measurements.

All the samples are kept at a temperature of 20.0 ± 0.1°C, either in the thermostatic chamber of the applied instrumentations or in a separate thermostat Lauda Ecoline Re204 (Lauda Dr. R. Wobser GmbH&Co. KG, Germany) in between the experiments. The pH control is accomplished by a pH meter inoLab pH7110 (WTW, Weilheim, Germany).

UV-Vis spectroscopy is one of the often-used methods for the characterization of colloidal mixtures with potential medical applications [24]. According to previous studies, the absorbance spectrum of IMC possesses three characteristic absorbance maxima at wavelengths 205, 270, and 320 nm [23, 25]. It is known that no characteristic maxima within these wavelengths is observed in the spectrum of a similar type of the two-antennary oligoglycine, $C_8H_{16}(\text{-}CH_2\text{-}NH\text{-}Gly_5)_2$, $T_2\text{-}C_8\text{-}Gly_5$ [12]. These facts give grounds to presume that UV-Vis spectroscopy might be a reliable method for the analysis of the investigated systems and for the possible identification of the captured IMC in the mixed solutions. The studies are performed in the range of 200 - 800 nm, using a Cary 5000 UV-Vis-NIR spectrophotometer (Agilent Technologies, Inc., Santa Clara, CA, USA).

The size distribution of the bulk structural units is analysed by Dynamic Light Scattering with non-invasive backscattering (measuring angle 173°). Zeta potential (ζ-potential) is determined via mixed-mode measurement phase-analysis light scattering. All measurements are performed using a Zetasizer Pro (Malvern Panalytical Ltd., Malvern, UK) equipped with a He-Ne laser operating at a wavelength of 633 nm and a maximum power output of 10 mW. The mean particle size and the mean ζ-potential are calculated from at least ten measurements. All light scattering experiments are conducted at a controlled temperature of 20.0 ± 0.1°C.

The investigation of microscopic foam films from the single components and from the mixed systems is performed by the Scheludko-Exerowa micro-interferometric thin film liquid instrumentation [26, 27]. Thin-liquid film instrumentation is a classical technique for measuring surface forces related to the mutual impact of two fluid interfaces separated by thin (nanometer-thick) microscopic film. The major information that it provides is the following: (i) qualitative and quantitative

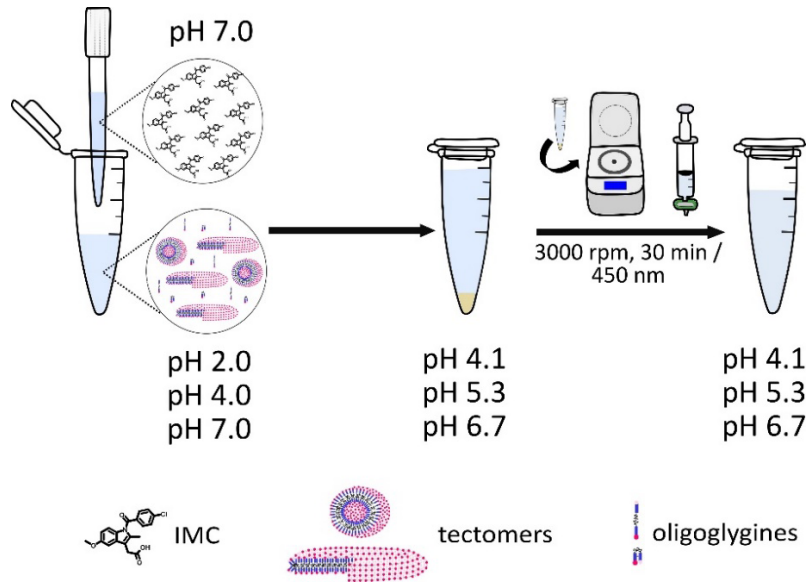

Fig. 2. Experimental procedure for preparing mixtures from indomethacin and $T_2\text{-}C_8\text{-}Gly_4$. The procedure includes mixing of equal volumes of solutions from the two components - $T_2\text{-}C_8\text{-}Gly_4$ and IMC.

data about the stability performance of microscopic TLFs, formation of black films, and determination of equilibrium film thickness values; (ii) outcomes about the drainage kinetics of TLFs, including the onset of black formations: black dots and black spots (onsets of local thinner portions within the films), formation of black films and their rupture, realization of metastable states and critical thickness values. These characteristics are functions of the structure-property relationships of the investigated samples. All experiments are executed under constant capillary pressure and with films of radii r = 100 nm. The samples are prepared and retained at a temperature of 20.0 ± 0.1°C before being uploaded into the measuring cell. Thereafter, the cell is put in the thermostatic chamber of the TLF instrumentation and kept for one hour before the start of the measurements.

RESULTS AND DISCUSSION

Due to the experimental procedure employed, the conditions for tectomer formation differ significantly before and after mixing the initially prepared T_2-C_8-Gly_4 and IMC samples. Consequently, the potential for IMC entrapment is also expected to vary. The experimental results support these assumptions, providing a reliable basis for comparing the performance of the mixed solutions with the properties of the individual components. This comparison enables the identification of the characteristic features specific for the T_2-C_8-Gly_4 + IMC systems.

Characterization by UV-Vis absorbance spectroscopy

It is established that the obtained absorbance spectra of the two single-component solutions (T_2-C_8-Gly_4 and IMC) are completely different in the range of 200 - 440 nm (Fig. 3). The spectra of the single-component T_2-C_8-Gly_4 in buffered aqueous solutions at several pH values are presented in Fig. 3a.

As shown in the spectra, T_2-C_8-Gly_4 does not exhibit characteristic absorbance maxima within the examined wavelength range. However, two notable features should be highlighted. First, a sharp increase in absorbance is observed at wavelengths below 240 nm, consistent with previous findings for the structurally similar oligoglycine T_2-C_8-Gly_5 [12]. Second, in the range of 240 - 440 nm, a progressive increase in absorbance is observed with rising pH. Specifically, at pH values below 4.0, the spectra remain close to the baseline; at pH 4.0, a slight absorbance becomes detectable; and at pH 7.0, a significant increase in the signal intensity is evident. This baseline shift may be recognized as tectomer formation in the solution. The presence of colloidal particles contributes to light scattering, which is registered as absorbance. Therefore, the observed spectral changes are likely to reflect the formation of T_2-C_8-Gly_4 tectomers, which is enhanced at higher pH values. Additionally, at pH < 7.0, the system may also contain individual oligoglycine molecules or smaller tectomeric assemblies that fall below the detection limit of UV-Vis spectroscopy. Similar pH-dependent self-assembly behavior has been reported for T_2-C_8-Gly_5 in aqueous media [2, 12].

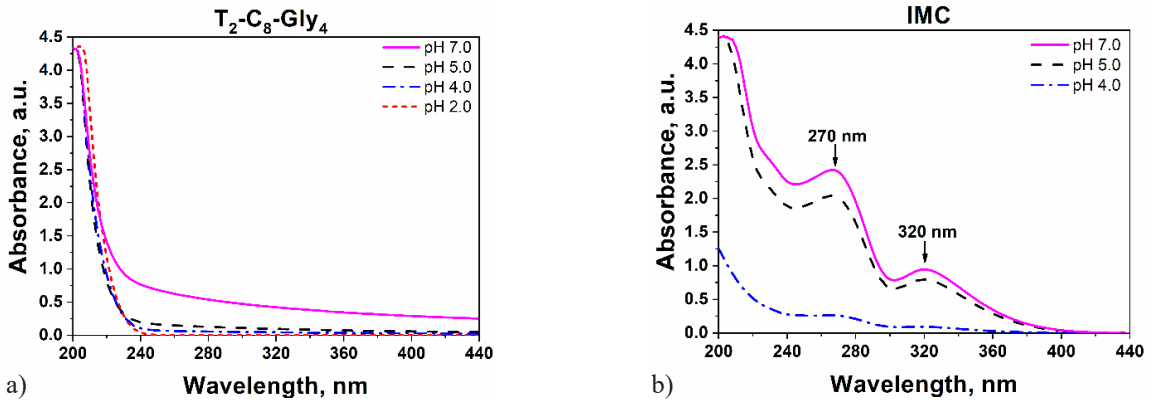

Fig. 3. Effect of pH on UV-Vis spectra of buffered solutions of (a) T_2-C_8-Gly_4 and (b) IMC. The concentration of T_2-C_8-Gly_4 is 2.0×10^{-4} mol L^{-1} and of IMC is 9.8×10^{-5} mol L^{-1}.

At lower pH values (pH < 7.0), T_2-C_8-Gly_4 is expected to form predominantly spherical vesicular structures. As pH increases, the size distribution might shift toward larger tectomeric aggregates. Regarding the effect of the concentration, test experiments at pH 2.0 showed that a twice lower concentration of T_2-C_8-Gly_4 results in only minor spectral deviations from the baseline. Thus, in line with previous observations at the compound's native pH, moderate variations in concentration are not expected to significantly impact the tectomer size distribution [3].

To evaluate the potential influence of ethanol on the self-assembly behavior of T_2-C_8-Gly_4, additional experiments are conducted using buffered solutions at pH 4.0 and pH 7.0 with 5 % (v/v) ethanol. At both pH values, the UV-Vis spectra of ethanol-containing solutions show no significant deviation from those of the corresponding buffered oligoglycine-only solutions without ethanol. These findings suggest that the presence of up to 5 % ethanol does not substantially affect the self-organization of T_2-C_8-Gly_4 under the experimental conditions. Instead, pH appears to be the dominant factor influencing the structural organization. Therefore, in all subsequent experiments, the ethanol concentration is maintained at a maximum of 5 % (v/v).

Indomathacin have characteristic absorbance maxima at 270 and 320 nm (Fig. 3b), and these spectral characteristics allow us to identify its presence in the mixed systems as well. At pH = 7.0, IMC is relatively soluble, leading to an increased absorbance signal. Lower pH values result in a decreased absorbance signal at the characteristic wavelengths, without a sensible shift or deformation of the peaks; only differences in the intensity of the bands due to the solubility changes are registered. The above observation follows the known pH-dependent solubility of IMC [23]; in the acidic environment, the substance becomes insoluble, resulting in crystallization and precipitation, while only a small quantity of the IMC entities remains dissolved in the solution, leading to lower absorbance values.

As previously noted, three solution systems with distinct final pH values (4.1, 5.3, and 6.7) are investigated. The experimental results for the mixed systems are presented in Fig. 4. For comparison, data from corresponding single-component systems adjusted to match the exact pH values of the mixtures are also shown. At pH 4.1, the absorbance spectrum of the IMC-only solution shows no detectable signal (Fig. 4a), indicating the absence of dissolved indomethacin under these conditions. The buffered single-component solution of T_2-C_8-Gly_4 also exhibits no absorbance in the 240 - 440 nm range. In contrast, the mixed solutions display clear absorbance peaks at 270 nm and 320 nm, confirming the presence of IMC in the system. These findings suggest that the absorbance in the mixtures arises from the formation of T_2-C_8-Gly_4 + IMC complexes. The contribution of free, dissolved IMC to the absorbance signal is considered negligible due to its extremely low solubility at pH 4.1. At this pH, IMC is expected to precipitate and is removed during the initial filtration step used to eliminate larger aggregates before the measurements.

A similar but more pronounced effect is observed at pH 5.3 (Fig. 4b), where the absorbance values are higher than those at pH 4.1. This indicates that a greater amount of indomethacin is incorporated into the T_2-C_8-Gly_4 + IMC complexes under these conditions.

At pH 6.7, the solubility of IMC significantly increases. As a result, following the filtration step, a larger portion of the drug remains dissolved in the bulk solution (Fig. 4c). As with the lower pH conditions, the mixed solution exhibits higher absorbance at the characteristic wavelengths compared to the IMC-only sample. Although the difference in spectra between the mixture and the IMC-only solution is smaller at pH 6.7, it still confirms the presence of T_2-C_8-Gly_4 + IMC complexes. In this case, the presence of free, unbound IMC molecules in solution is also quite probable.

To estimate the amount of indomethacin captured by the tectomers, a calibration curve for IMC at 320 nm is used. The results are presented in the form of the so-called entrapment efficiency (EE) [28 - 30]. EE is defined as the ratio of the amount of IMC in the carrier versus the concentration in the initial IMC + EtOH solution (Eq. (1)).

EE, % = (amount of IMC in a carrier) / (initial amount of IMC) × 100. (1)

The data are consistent with the qualitative trends observed in the UV-Vis spectra and indicate that the highest EE is achieved in the mixtures at pH 5.3 (Table 1). The second highest EE is observed at pH 6.7, while the least favourable conditions for IMC capture are found at pH 4.1. These findings suggest that oligoglycine-based aqueous systems offer enhanced potential for IMC

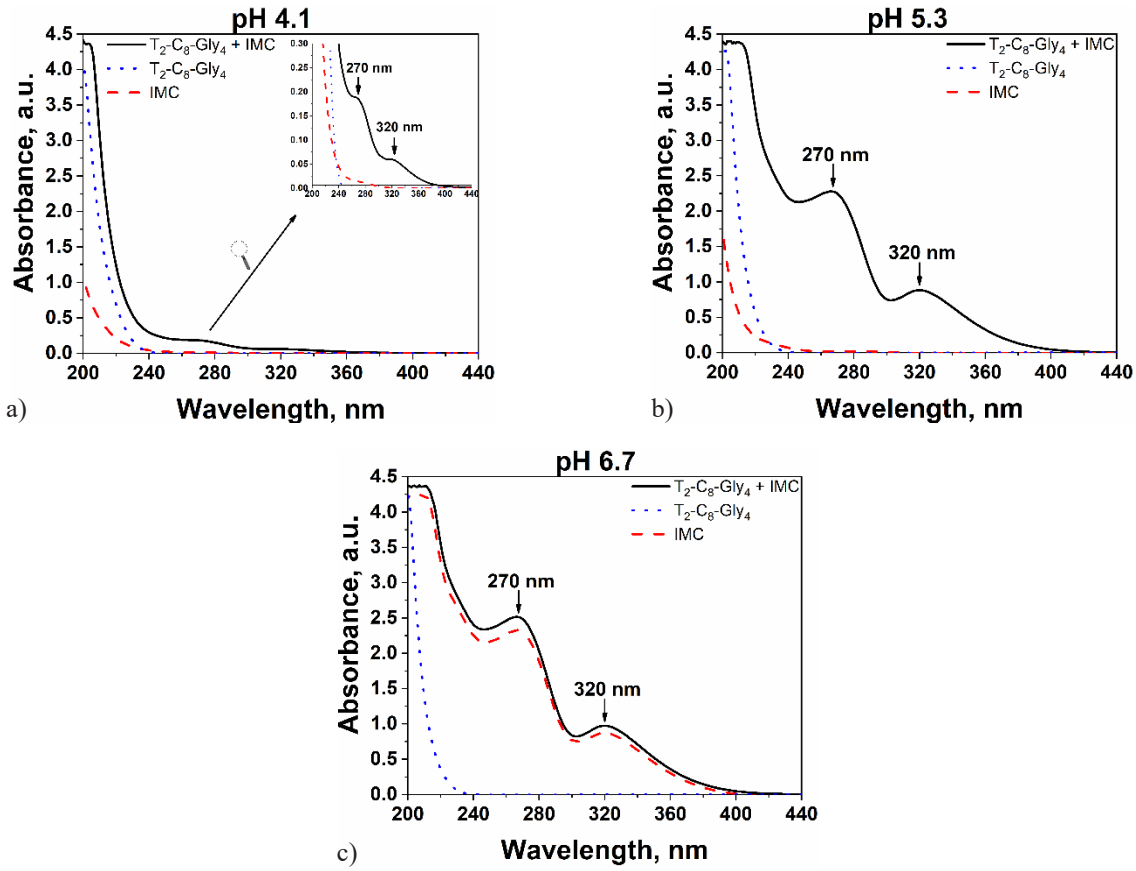

Fig. 4. Effect of pH on filtered solutions of mixtures, as well as on individual T_2-C_8-Gly_4 and IMC solutions, at varying pH values.

entrapment under intermediate pH conditions and may give additional application options for IMC entrapment by the oligoglycine aqueous solutions.

Characterization by dynamic light scattering

To complement the insights gained from UV-Vis spectroscopy and to characterize further the bulk properties of the studied systems, a series of additional DLS experiments are conducted. Specifically, the ζ-potential and electrical conductivity of T_2-C_8-Gly_4 solutions are measured across a range of pH values to evaluate the surface charge of the tectomeric structures and its pH dependence. The results of these measurements are presented in Fig. 5.

The ζ-potential measurements of T_2-C_8-Gly_4 show that the surface charge decreases as the pH increases. The same tendency was observed previously with another two-antennary oligoglycine studied [4]. In acidic media, the amino groups are almost fully protonated, and T_2-C_8-Gly_4 possess a positive charge, as evidenced by the high values of the ζ-potential under these conditions. As the pH increases, the amino groups are deprotonated, and the ζ-potential decreases. In aqueous media where the pH is acidic, the ζ-potential values are also high since T_2-C_8-Gly_4 is in the form of a hydrochloride, and its performance is like that in acidic media. Simultaneously with the ζ-potential measurements, the corresponding conductivity values are obtained and are presented in Fig. 5. For the buffer solutions at different pH levels, conductivity remains effectively constant, with only a slight increase observed at pH 7.0. This suggests that the primary contributor to the measured conductivity

Table 1. Entrapment efficiency of indomethacin at different pH.

	EE (T_2-C_8-Gly_4 + IMC), %
pH 4.1	5
pH 5.3	80
pH 6.7	9

is the buffer itself, rather than the tectomers. This interpretation is supported by the experiment conducted in distilled water, where the measured conductivity was practically zero.

The DLS analysis reveal that obtaining a well-defined size distribution for our mixed system is a complex task. The DLS results for the single components, as well as for the mixtures, show significant polydispersity. According to the data obtained before the filtration of the samples, the solutions contain particles with sizes varying in a wide range, between 100 nm to several microns. The 'large particles' fractions, above half a micron in size, demand filtration of the samples with a 450 nm filter. The UV-Vis study is performed after the filtration, where only fractions of particles of interest remain in the samples. However, after filtration, the concentration of the remaining particles in the samples becomes too low to obtain a DLS result. Therefore, representative results for the size distributions of T_2-C_8-Gly_4 + IMC samples before filtration at pH 4.1 and pH 5.3 are presented in Fig. 6.

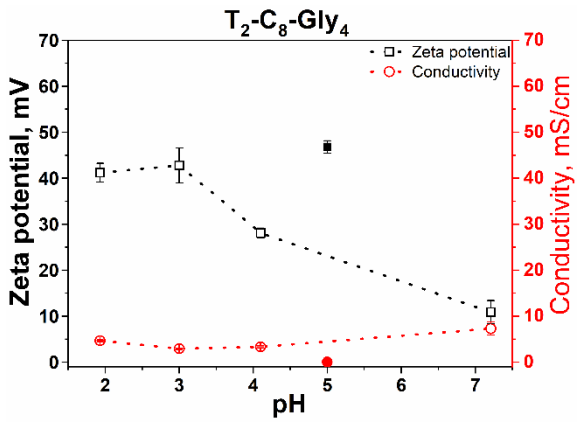

Fig. 5. Variation of ζ-potential (open black squares) and electrical conductivity (open red circles) of T2-C8-Gly4 as a function of pH, with reference values in destilled water (no bufer water (no bufer) shown with solid symbols.

At pH 4.1, the particle size distribution of the mixture reveals two distinct populations. The smaller particles show a noticeable shift compared to the T_2-C_8-Gly_4-only solution, indirectly indicating the formation of mixed complexes between hydrophobic IMC and tectomeric entities and reflecting a reorganization process that leads to stable T_2-C_8-Gly_4 + IMC complexes. Consequently, the particle distribution in the mixture differs from that of the solution containing tectomers only. In contrast, at pH 5.3, where the highest entrapment efficiency is observed, the mixture's particle size closely matches that of the tectomers alone, suggesting IMC capturing occurs without disrupting the pre-formed tectomer structure. These distinct DLS profiles across pH values imply that the mechanism of mixed complex formation varies with pH, mostly due to differing balances of electrostatic and hydrophobic interactions.

Characterization by thin-liquid-film instrumentation

The properties of the single-component solutions and their mixtures are investigated through foam films formation by the microinterferometric TLF techniques of Scheludko-Exerowa [26, 27]. Major characteristics of the microscopic foam films, such as stability, film lifetimes, and critical film thicknesses, are examined.

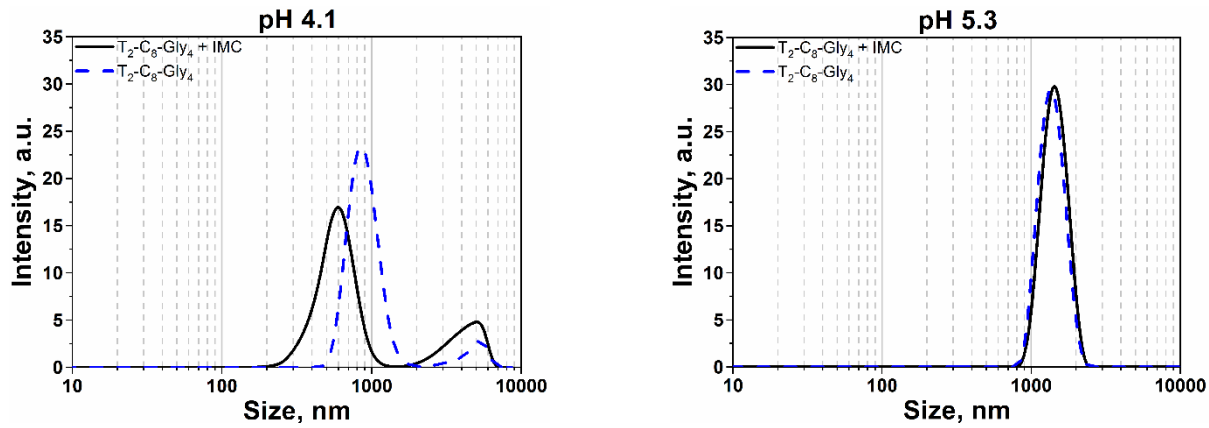

Fig. 6. Mean size distribution by intensity for unfiltered T_2-C_8-Gly_4 + IMC mixture solution at (a) pH 4.1 and (b) pH 5.3, compared with the T_2-C_8-Gly_4-only solution under similar conditions.

The effect of pH on the properties of T_2-C_8-Gly_4 thin liquid films is tested, in line with UV-Vis and DLS results. The representative snapshots of the microscopic foam films for the different pH values are shown in Fig. 7 (first row). At the investigated pH range, only unstable, relatively thick films are formed, which drain further with the initiation of black dots and then are followed by film rupture at $h_{cr} \geq 30$ nm.

The IMC-only solutions are examined under conditions that ensure sufficient solubility of IMC (pH = 6.7 and pH = 5.3). At pH = 6.7, the foam film drainage is characterized by the appearance of black spots, which progressively increase in size and number. At a concentration of 1.3×10^{-4} mol L^{-1}, corresponding to the concentration in the mixtures, common black films with a final thickness of approximately 16 nm are formed. These films rupture after the formation of a uniform black film and cannot remain stable under these conditions. This behaviour is likely due to an insufficient concentration of IMC to stabilize the films, a typical phenomenon observed with low molecular mass ionic or nonionic surfactants [30, 31]. At pH = 5.3, the films are even more unstable due to reduced IMC solubility. In this case, no black spots are observed during drainage, and the films rupture immediately after the appearance of the initial black spots.

Generally, the performance of foam films from buffered solutions of mixtures with pH = 4.1 and pH = 5.3 are qualitatively indistinguishable from the oligoglycine-only films at the corresponding pH values (Fig. 7, second row). However, the final thickness values are significantly different (Fig. 8a). This pattern is changed only in the case of pH = 6.7, where the film's time evolution becomes almost identical to that of the IMC-only solutions. The foam film drainage results in the formation of films with a critical thickness of 13 nm, which is close to the obtained 16 nm for IMC-only samples. Furthermore, upon the increase in pH, decrease of critical thickness is registered for the films obtained from both the mixtures and the IMC-only solution. Similar tendency is found for oligoglycine-only samples, while higher thickness values are observed. The film thicknesses for the mixtures are similar or equal to the critical thickness for the IMC-only samples.

The change in film lifetime for the mixed systems as a function of pH follows a similar trend to that observed for indomethacin-only films (Fig. 8b). At pH 6.7 and pH 5.3, additional stabilization is observed in the mixed-system films compared to those containing only IMC. This enhancement in film stability can be interpreted as indirect evidence for the formation of complexes between the tectomer and the drug.

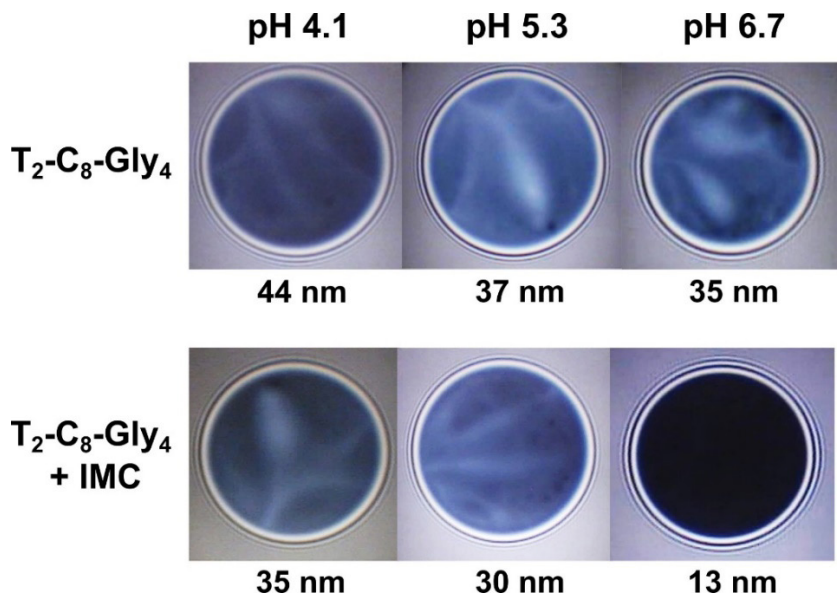

Fig. 7. Representative snapshots of thin liquid films before rupture for T_2-C_8-Gly_4 (first row) and the T_2-C_8-Gly_4 + IMC mixtures (second row) at different pH values.

Insights into T_2-C_8-Gly_4 + IMC interactions and complex formation

By analysing the data from the combined UV-Vis, DLS, and TLF methodologies, we can propose hypotheses regarding the interaction mechanisms in T_2-C_8-Gly_4 + IMC systems (Fig. 9). The behaviour and properties of these mixed fluid formulations are influenced by a delicate balance of interactions, including the tendency of T_2-C_8-Gly_4 to form PG-II structural motifs, hydrophobic and electrostatic interactions between T_2-C_8-Gly_4 and IMC, and the effect of pH on the structural units in both single-component solutions, and mixed systems.

When γ-IMC is dissolved in ethanol (99.8 %), it exists as single molecules, dimers, and small crystallites. The equilibrium between these structures shifts depending on the pH of the aqueous buffer added: at lower pH, non-charged IMC entities dominate, while higher pH favours the formation of charged species. Additionally, because of the 24-hour incubation of the oligoglycine-only solution before mixing with IMC, the IMC interacts predominantly with pre-formed oligoglycine tectomers. As already mentioned, zeta potential measurements of T_2-C_8-Gly_4-only samples as a function of pH show a decrease in the tectomer charge upon increasing the pH values (Fig. 5). Depending on the pH, the mixtures contain either predominantly vesicular T_2-C_8-Gly_4 tectomers or individual T_2-C_8-Gly_4 molecules. In an IMC-only aqueous solution at pH 7.0, IMC molecules are negatively charged, while dimers and microcrystals are uncharged. Consequently, T_2-C_8-Gly_4 + IMC complexes primarily form due to electrostatic interactions between the positively charged vesicular tectomers and single IMC molecules.

At lower pH values (4.1 and 5.3), IMC microcrystals can be shielded by single T_2-C_8-Gly_4 molecules through hydrophobic interactions, with charged IMC molecules potentially being trapped via electrostatic interactions with the positively charged oligoglycine tails (NH_3^+) on the attached T_2-C_8-Gly_4 molecules. Although this tendency is observed across the entire pH range, the highest entrapment efficiency is anticipated at intermediate pH (pH 5.3), as evidenced by the experimental data (Table 1). This is consistent with the pKa value (~ 4.5) of the carboxylic group in IMC [23, 32].

At pH 6.7, IMC is predominantly present in a fully dissolved form with minimal crystalline content. Consequently, hydrophobic interactions between IMC crystals and T_2-C_8-Gly_4 molecules are largely absent, favouring complex formation primarily through electrostatic interactions with dissolved IMC molecules. However, the significantly reduced surface charge density of the oligoglycine species at this pH leads to a decreased number of mixed complexes.

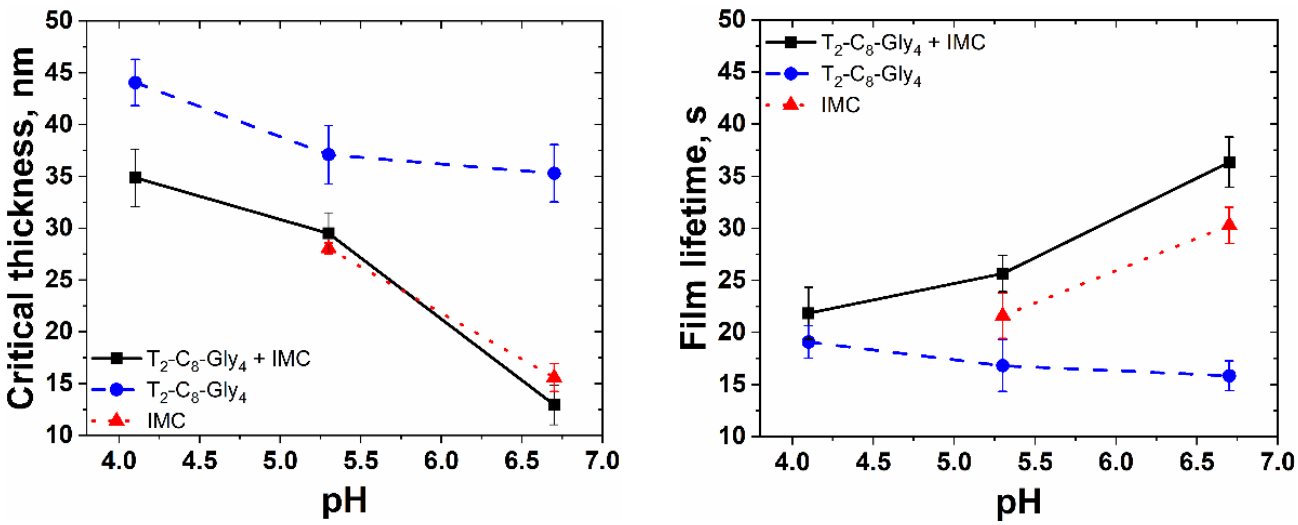

Fig. 8. Impact of pH on (a) critical thickness values and (b) lifetimes of foam films from mixtures, as well as the critical thickness values for films from single-component solutions.

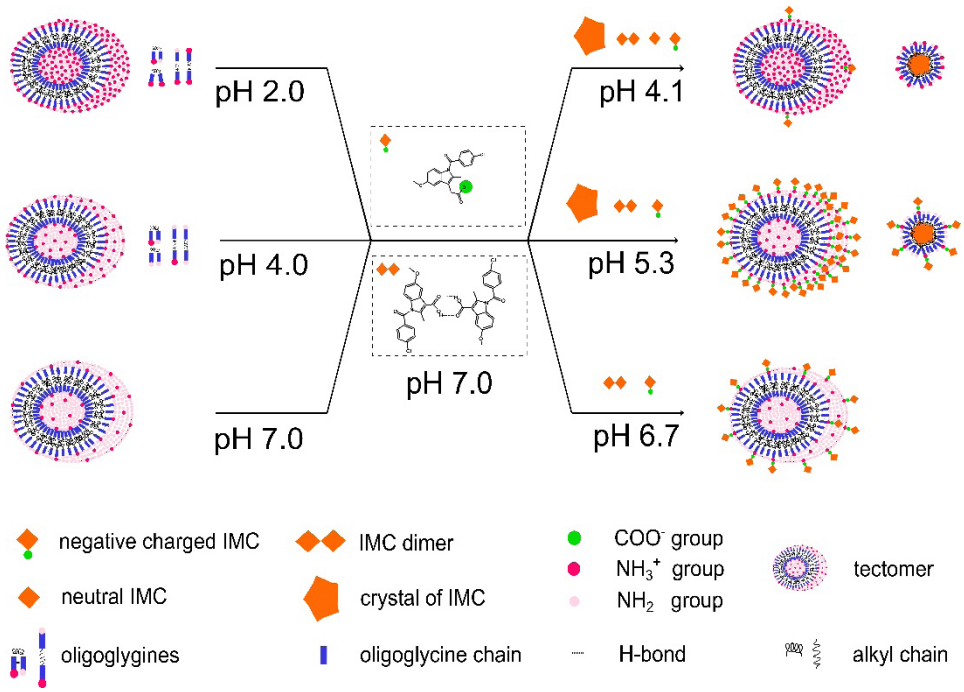

Fig. 9. Effect of pH on tectomer-IMC interactions and complex formation.

CONCLUSIONS

In this study, we employed a combination of UV-Vis spectroscopy, dynamic light scattering, and thin liquid film techniques to investigate the interaction behaviour between two-antennary oligoglycine (T_2-C_8-Gly_4) and the hydrophobic drug indomethacin (IMC) across a range of pH values, both in the bulk solution and at the air/solution interface. The results demonstrate that T_2-C_8-Gly_4 effectively captures IMC in aqueous media, forming stable supramolecular complexes. Among the tested pH conditions, pH 5.3 is identified as the most favourable for complex formation, yielding an entrapment efficiency of approximately 80%, as quantified directly via UV-Vis spectroscopy using entrapment efficiency (EE) calculations. While UV-Vis provided direct quantification of IMC incorporation, DLS and TLF measurements offered complementary, indirect evidence supporting the complex formation. Precisely, DLS measurements revealed shifts in particle size distribution in the mixed systems compared to the individual components, indicating structural reorganization upon the complexation. TLF analysis further supported these findings, demonstrating enhanced film stability in the mixed formulations. Based on experimental results, we propose a mechanism of complex formation driven by a combination of electrostatic and hydrophobic interactions.

Overall, this work advances the current understanding of interactions between two-antennary oligoglycine and the model hydrophobic drug and demonstrates the potential of T_2-C_8-Gly_4 as a versatile, biocompatible, non-toxic, and structurally tunable platform for a wide range of biomedical and environmental applications. The established methodology can be extended to other hydrophobic molecules with physicochemical characteristics similar to those of the indomethacin molecule. The present study highlights the broader potential of T_2-C_8-Gly_4, not only for the design of targeted drug delivery systems but also for environmental applications, such as water purification, where the selective capture of hydrophobic and charged contaminants is essential.

Acknowledgments

The present investigation is funded by the European Union-NextGenerationEU, project BG-RRP-2.012-0005-C01 and project BG-RRP-2.004-0008-C01. Research equipment of the Distributed Research

Infrastructure INFRAMAT, part of the Bulgarian National Roadmap for Research Infrastructures, supported by the Bulgarian Ministry of Education and Science, was used for some investigations in the present study. The authors would like to thank R&D&I Consortium at Sofia Tech. Park for technical assistance through project BG16RFPR002-1.014-0014-C01.

Authors' contributions: *E.M., D.A., I.M.: Conceptualization; E.M., D.A., I.M., E.E.: Methodology; E.E., I.M., D.A., A.G.: Formal analysis; E.E., D.A., I.M., A.G. investigation; E.M.: Resources; E.M., V.P.: data curation; E.E., I.M., E.M.: Writing - Original draft preparation; E.M., E.E., V.P.: Writing - review and Editing, ; E.E., D.A : Visualization; E.M.: Supervision; E.M., I.M., D.A.: Pproject administration; E.M: Funding acquisition.*

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