Inclusion Complexation between α-Cyclodextrin and Oligo(ethylene glycol) Methyl Ether Methacrylate

Marcos Mariano,* Oigres Daniel Bernardinelli, Rafael Pires-Oliveira, Guilherme A. Ferreira, and Watson Loh*

ABSTRACT: The preparation of inclusion complexes based on α-cyclodextrin (α-CD) and oligo(ethylene glycol) methyl ether methacrylate (OEGMA) was investigated aiming to reveal complexation particularities and thermodynamic and kinetic aspects as a function of the oligomer architecture. Small-angle X-ray scattering and isothermal titration calorimetry measurements revealed that oligomer molecular weight controls both the kinetics and thermodynamics of inclusion. Unlike linear ethylene glycol polymers, OEGMA groups possess a methacrylate group, which seems to act as a stopper, affecting their mode of complexation. Nuclear magnetic resonance spectra and relaxation measurements support the fact that methacrylate groups lie outside the α-CD ring and that a full sequential complexation of the oligomer ethylene oxide groups is not observed. These results allied to the temperature sensitivity of these oligomers and enable possible routes for chemical modifications and design of new stimuli-responsive materials.

1. INTRODUCTION

Supramolecular interactions have been used as a tool to create systems that are not based on covalent bonds, and, as a consequence, can assemble into reversible structures. Nowadays, thermoresponsive and self-healing gels are common examples of this approach. Earlier investigations on the topic are focused on the most basic phenomena and inclusion complexation (IC), which ultimately lead to the fabrication of fundamental molecular machines.1,2 The achievement of the so-called inclusion complexes is based on host–guest interactions, where a macrocycle motif is able to thread specific molecules inside its cavity.

Both chemical affinity and adequate size affect the thermodynamic parameters that govern the formation of such complexes. As frequently discussed in the literature, van der Waals and hydrophobic interactions are the main factors involved in this complexation phenomenon, although hydrogen bonding and steric effects can also influence this process.3 Several host–guest combinations have been used to prepare, for instance, polyrotaxanes (and pseudorotaxanes), which have been proposed as biomimetic systems to study different biologically relevant processes, such as drug delivery and other uses of colloidal systems.4,5

After pioneer work published by Harada and co-workers,6,7 who used polyethylene glycol (PEG) as guest molecules to be included into cyclodextrin (CD) hosts, the association of macrocycles with polymers proved to be useful to control their molecular properties in solution. Recently, IC of thermoresponsive polymers has sparked a great deal of interest because of the possibility of controlling their lower critical solution temperature (LCST) through the complexation of their responsive moieties.8–10 Formed by the cyclization of six glucose units, α-cyclodextrin (α-CD) possesses a truncated cone ring shape with an inner cavity, displaying a maximum diameter of 0.53 nm and height of 0.78 nm. These dimensions are compatible with the size of stretched ethylene oxide (EO) units present in different polymers and oligomers, among which one is oligo(ethylene glycol) methyl ether methacrylate (OEGMA).

This oligomer and its derived polymers were extensively studied by Lutz and collaborators10,11 because of their biocompatible and thermoresponsive properties, ideal to be used in sustainable drug release12 and injectable hydrogels13 or even to preserve protein structures.14 Unlike PEG, the EO moieties present in poly oligo(ethylene glycol) methyl ether methacrylate (POEGMA) are not in the polymer backbone but appear as side chains, and their size can be used to control polymer LCST by tuning the EO chain length.15 Despite the great potential of POEGMA in the preparation of smart gels...
formed as a laminated structure.18 The crystalline character of nm, which indicates that particles observed are probably CD inclusion complexes normally present a thickness of 10 Previous studies revealed that the layer of individual PEG/OEGMA ratios, calculated as some complex di polymers.16,17 Systems based on cyclodextrins and POEGMA are until now limited to the preparation of some hydrogels based on di-block monomers ( ) and other biomedical materials, few information is available about its complexation with macrocycles (such as α-CD). Besides the presence of EO groups, OEGMA behavior is also influenced by the presence of a methacrylate group which occupies one end of the oligomer chain. Because the formation of pseudorotaxanes is dependent on macrocycle–polymer end group interactions, the role played by the methacrylate group is essential to understand the IC of these oligomers. In this paper, we will discuss the complexation mechanism of POEGMA oligomer molecular weight. According to Bragg’s Law, this peak indicates d = 4.5 Å and may be ascribed to a tubular structure of the formed crystals. At the same time, fading of the peak at 2θ = 21.6° suggests the absence of pristine cyclodextrin in these solid samples. It can also be observed that samples EO20-CD1 and EO45-CD1 display a new secondary peak at 2θ = 19.2°. The same peak was previously observed by Zou et al.,21 studying a copolymer containing an OEGMA block ( ) and OEGMA ( ) complexed with α-CD. The position of such a peak is consistent with the diffraction pattern of PEG crystals, suggesting the presence of free EO (1:1 samples) units that can form crystalline domains upon drying.21 Similarly, Travel et al.22 reported that the crystallinity of free EO moieties is affected by the amount of α-CD present. For all samples, free EO groups seem to be present. As observed in Figure 1c, an increase in the amount of α-CD (higher CD/OEGMA ratio) leads to higher mass yield of the complex, with maximum yields around 70–80 wt % being achieved (Table 1). These values are almost twice those described in the literature for ICs based on star-poly(ethylene glycol) molecules24 but very similar to those reported for long PEG-based molecules containing a bulk end group, where a plateau is described at high CD concentration.25 If precipitate washing is not fully efficient in removing free CD, these values may be slightly biased toward higher CD/OEGMA ratios. Longer EO moieties lead to an increase in IC mass yield, suggesting that the oligomer size affects complexation efficiency, indicating that the exclusion of high-energy water molecules from the CD cavity may not be the only force driving this process, as previously proposed by Takahashi et al (2016).19 Besides, the size of the oligomer also modifies the kinetics of incorporation, as established from visual observation of mixtures. The arising cloudiness and, in some cases, complex

**Figure 1.** Analyses of solid IC through optical microscopy (a), XRD patterns of 1:1 IC complexes (b), and mass yield of ICs as a function of CD/ OEGMA ratio (c). Dashed lines are guides to the eyes.

**Table 1.** Composition of Samples and Characteristics of the Resulting Precipitates

| composition (OEGMA-α-CD) | EO units in OEGMA | complex ID | CD/OEGMA ratio (n) | yield range (wt %) | phase separation time | complex aspect |
|--------------------------|------------------|------------|-------------------|-------------------|----------------------|--------------|
| OEGMA<sub>9518</sub>/α-CD | 2                | EO<sub>2</sub>-CD<sub>1</sub> | 1                 | 30 minutes/hours | powder               |              |
| OEGMA<sub>9518</sub>/α-CD | 4–5              | EO<sub>4</sub>-CD<sub>1</sub> | 1–2               | 23–48 hours       | powder               |              |
| OEGMA<sub>9518</sub>/α-CD | 20               | EO<sub>20</sub>-CD<sub>1</sub> | 1–10              | 25–84 days        | powder/gel           |              |
| OEGMA<sub>300</sub>/α-CD  | 45               | EO<sub>45</sub>-CD<sub>1</sub> | 1–20              | 28–77 weeks/no separation | gel             |              |

“Subscript values on OEGMA represent its nominal molecular weight (g mol<sup>-1</sup>). Minimum and maximum mole ratio between α-CD and the OEGMA chain used to prepare different ICs. This is also represented as the subscript in the complex ID, as EO<sub>n</sub>-CD<sub>c</sub>. Referring to different CD/ OEGMA ratios, calculated as Y% = \( \frac{\text{wt}_{\text{IC}}}{\text{wt}_{\text{PEGMA}} + \text{wt}_{\text{CD}}} \).”

“Dependent on sample composition. Pictures of the suspensions and more comprehensive data can be found in the Supporting Information, Figure S1 and Table S1.”

and other biomedical materials, few information is available about its complexation with macrocycles (such as α-CD). Systems based on cyclodextrins and POEGMA are until now limited to the preparation of some hydrogels based on di-block monomers.16,17

Besides the presence of EO groups, OEGMA behavior is also influenced by the presence of a methacrylate group which occupies one end of the oligomer chain. Because the formation of pseudorotaxanes is dependent on macrocycle–polymer end group interactions, the role played by the methacrylate group is essential to understand the IC of these oligomers. In this paper, we will discuss the complexation mechanism of POEGMA oligomers (i.e., OEGMAs) and their potential application in polymer synthesis and in modulation of thermal properties. For this purpose, we present results from di polymer synthesis and in modulation of thermal properties.
precipitation can vary from minutes to weeks depending on OEGMA molecular weight. These phase separation phenomena seem to be related to the ability of longer molecules to produce nonstoichiometric complexes (i.e., complexes whose composition differs from the expected EO/CD ratio). Earlier literature describes the sequential complexation of α-CD units along linear polymer chains to form “necklace” structures called pseudorotaxanes (PRs).20 A ratio of 1 unit of α-CD for each 2 units of EO (2:1) is usually described in the literature as the value of oligomer saturation during the formation of such complexes.27

To further explore such multiple complexations, different ratios between α-CD and OEGMA were used in order to obtain saturated PRs. The visual inspection of these samples follows the same tendency previously described here and in the literature.24 Solutions containing longer oligomers take longer times to display cloudiness. Concerning the CD/EO ratio, higher amounts of α-CD lead to a faster increase of suspension turbidity. However, longer oligomers (EO10-CD4 and EO45-CD2) do not present the formation of a well-defined solid precipitate at the investigated compositions. For these ICs, after following certain samples for 5 weeks, the formation of a consistent white gel-like material is observed. Here, it is worth to note that visual observations (e.g., turbidity and precipitations) are related to the aggregation of formed complexes because early stages of threading cannot be verified by the visual aspect of the samples.28

2.2. Nuclear Magnetic Resonance Techniques. In order to understand IC structures and the role of different moieties in complexation, several nuclear magnetic resonance (NMR) techniques were explored.

2.2.1. 1H Nuclear Magnetic Resonance. Initially, the spectra of pristine components were obtained. As observed in Figure S2, the α-CD spectrum shows characteristic glucose peaks at 3.4 ppm (m, H-3), 3.8 ppm (m, H-5), 3.9 ppm (m, H-2), and 5.0 ppm (s, H-1), similar to those described by Schneider et al.29 The oligomers (e.g., EO2) present peaks at 1.9 ppm (s, CH3), 3.2 ppm (s, CH3), 3.6 ppm (m, −CH2), 4.2 ppm (t, −CH2), 5.6 ppm (s, CH), and 6.1 ppm (s, CH). As also demonstrated in Figure S2, ICs spectra are found to be very similar to the superposition of α-CD and OEGMA peaks.

Furthermore, sample stoichiometry was complementarily determined by 1H NMR and gravimetric methods. Such a complementary approach was necessary because of limitations of each method. Gravimetry fails to predict sample composition at lower α-CD concentration because an unknown amount of oligomers and macrocycles can be lost during sample washing. On the other hand, the 1H NMR approach leads to a deviation of nominal expected composition in samples containing higher amounts of α-CD. This can be related to its inefficient washing as medium viscosity increases (gel phases), as previously discussed in the literature.30

Furthermore, at these compositions, larger experimental errors are related to the decrease of signal/noise ratio in longer ICs (related to the low intensity OEGMA peak), which can vary up to 25%. Figure 2 exhibits the relationship between the expected (nominal) and experimental composition of the complexes estimated from both techniques.

As can be observed, experimental and nominal compositions are similar at lower CD/OEGMA ratios (i.e., until 5:1 values), independent of the oligomer size, following the expected 2:1 (EO/CD) ratio. At higher CD/OEGMA ratios, both techniques present the expected deviations. This disagreement between nominal and real sample compositions was previously observed and discussed in the literature for linear molecules.31

Furthermore, we use the relaxation of the oligomer in low-field NMR to investigate this observation in the present system, as discussed in the next section.

2.2.2. Low-Field NMR. Once threaded inside the macrocycle cavity, oligomers present different molecular dynamics. Such a property can be followed in order to obtain valuable information about host–guest complexation. NMR is applied to obtain the spin–spin relaxation time (also named transverse relaxation) of protons present in the system (1H-T2). Such relaxation times can be related to material mobility and structure.32,33

The addition of α-CD units to an oligomer solution restrains, at first, molecule diffusion because of steric limitations arising in a crowded environment. With time and advancement of oligomer inclusion, EO groups are stretched and threatened in a different chemical environment.31,34 These new conditions lead protons present in OEGMA to exhibit new spin–spin relaxation times (T2), which can be expressed in terms of relaxation times of free (T2f) and bonded (T2b) protons, according to eq 1 and as illustrated in Figure 3.35

\[
\frac{1}{T_2} = \frac{1}{T_{2f}}(1 - p) + \frac{1}{T_{2b}}p
\]

where p is the probability to find a bonded molecule. This equation suggests that two different contributions can be observed in relaxation time spectra if the difference between individual contributions of each term is large enough.

As observed in Figure 3a–c, the distributions of relaxation times from α-CD and oligomers are modified after inclusion, eventually reaching intermediate values. This behavior can be clearly observed for EO15-CD4 samples (Mn 300 g·mol⁻¹). Because of its rigid structure, relaxation times of α-CD are short, around 85 ms. For bulky oligomers, T2 varies from 150 to 800 ms according to the sample molecular weight, as shown in Figure 3d. Intermediary values found for different samples (200–600 ms) are related to relaxation of α-CD after exclusion of high-energy water molecules from its internal cavity. Such an effect is combined with the reduction of oligomer mobility after inclusion. These results show that inner cavity interactions among the host and guest are not strictly rigid,
which fits the expectations of necklace formation (where a certain level of flexibility is necessary). The decay in OEGMA-\(T_2\) is much smaller than the one previously reported in the literature for EO groups trapped inside TiO\(_2\) nanotubes, where molecule relaxation decreases almost 40 times.\(^{36}\) Again, this difference indicates some malleability of molecules in the ICs investigated in the present work.

2.2.3. NOESY. During this study, we attempted to prepare inclusion complexes between \(\alpha\)-CD and similar methacrylic acid but no change in appearance or turbidity was observed. Furthermore, the position of OEGMA methacrylic groups was analyzed by NMR techniques which did not reveal signs of complexation. Figure 4 shows the NOESY–\(H^1\) NMR spectrum of a CD/OEGMA complex. The spectrum shows some interactions between OEGMA protons (e.g., 4.25 ppm) with internal protons of the macrocycle (around 3.8 ppm) but very weakly with its external ones (e.g., 5 ppm). It corroborates the results presented by Takahashi et al.\(^{18}\) In addition, spatial proximity between methacrylate protons (Figure 4b) and internal hydroxyl groups of cyclodextrin (mostly between 3 and 4 ppm) is not observed, which indicates the absence of interaction between these molecules. Overall, these results suggest that methacrylate protons are spatially distant, probably in the external part of the cavity, as illustrated in Figure 4c. As a consequence, it seems that methacrylate groups do not participate in IC, despite their influence on neighbor protons, as shown in low-field NMR results discussed above.

2.3. ITC Experiments. Because many properties of the complex are dependent on oligomer molecular weight, inclusion kinetics and thermodynamics was studied as a function of oligomer size. Isothermal titration calorimetry (ITC) was used in order to measure the heat involved in the addition of \(\alpha\)-CD to an aqueous solution of OEGMA, which could provide information about the kinetics and energetics of this incorporation.\(^{37}\)

Figure 3. Relaxation time distribution (\(T_2\)) for different inclusion complexes (a–c), OEGMA-\(T_2\) at different molecular weights (d), and examples of \(T_2\)-decay for EO\(_{20}\)-CD\(_n\) and EO\(_{45}\)-CD\(_n\) (e). Red lines in figures (d,e) are exponential fits. A complete list of relaxation times calculated in this work can be found in the Supporting Information (Table S2).

Figure 4. 2D-NOESY spectra of IC-A (a), OEGMA methacrylate peaks (b), and proposed position of the methacrylate group with respect to the cyclodextrin ring (c). \(H^1\) NMR spectra of the individual oligomer, \(\alpha\)-CD, and IC can be found in Figure S2.
ITC curves associated with the formation of these inclusion complexes vary significantly with the molecular weight of the OEGMA used. In general, the peaks associated with this interaction (after discounting the heats of dilution of CD) are all exothermic, indicating a favorable enthalpic interaction involved in the inclusion of EO groups into the CD cavity. For the measurements with small OEGMA (n = 2), the interaction is fast, as confirmed by the quick return to the baseline. However, as the molecular weight of OEGMA increases (as discussed below), the process becomes slower and intervals between injections were increased from minutes to hours. This finding agrees with the visual observation of time scales for the appearance of precipitates resulting from IC discussed above. It is noteworthy that more sensitive ITC is capable of monitoring early processes involved in the incorporation of EO groups into CD, while visual observation is less sensitive and detects only macroscopic phase separation.

2.3.1. Fitting ITC Data for IC. The complexation of OEGMA_{188} and α-CD presents the simplest ITC data, as shown in Figure 5a. Because the endothermic heat of dilution of cyclodextrin in water was subtracted, the resulting negative enthalpy values can be ascribed to be related to the formation of the EO2-CD1 complex.

The observed decrease in heat exchanged as titration advances is a consequence of the oligomer saturation, giving rise to a sigmoidal curve, as shown in Figure 5b. Using experimental heat of interaction (ΔH) and different binding models, the fitting of such a curve allows the determination of parameters such as association constants (K), stoichiometry of inclusion (N), entropy (ΔS), and Gibbs energy (ΔG) of EO2-CD1 formation. Equations 2−4 show these relations.

\[ K = \frac{[\alpha - CD \cdot OEGMA]}{[\alpha - CD][OEGMA]} \]  
\[ ΔG = RT \ln K \]  
\[ ΔG = ΔH - TΔS \]  

In the case of OEGMA_{188}, it was possible to fit the experimental ITC data using a model that assumes only one set of binding sites (the CD cavity), as shown in Figure 5c, producing a stoichiometry of CD/OEGMA for the complex close to 1:1.

However, as shown in Figure 5c, the profile of interaction enthalpy values as a function of CD/OEGMA mole ratio varies with the oligomer molecular weight. Not only this but also the shape of heat peaks becomes more complex with the increase of oligomer size (as can be observed in Figures S3 and S4). Clearly, the simple model that accounted for the formation of EO2-CD1 was not suitable to describe these more complex interaction processes, especially for the earlier indications, suggesting the absence of defined stoichiometry for this IC. These inclusion processes were therefore re-investigated by running ITC experiments with longer intervals between injections to account for the slow incorporation kinetics. Results for these experiments are shown in Figure 6a that
contains different titration profiles according to the oligomer nature.

In the case of EO2-CD and EO4-CD systems, heat peaks are narrow and no sign of secondary processes is observed. However, curves associated to these two samples show strong perturbations (abrupt changes in the baseline) around 10–20 h, which is probably related to the precipitation of solid particles. Moreover, Figure 6b shows in detail the aspect of the third injection peak (prior to any sign of precipitation) for different oligomers. These peaks clearly show that for EO2 and EO4, secondary events occur following the oligomer inclusion, leading to broader peaks that are consistent with slow processes. This confirms the observations obtained with other techniques in the present study, suggesting a slow incorporation of oligomers with longer EO chains.

### 2.4. Small-Angle X-ray Scattering

Self-assembly of cyclodextrin complexes can lead to the formation of different structures. Simpler organizations such as channel tube and cage-like ones are well reported in the literature, but the structures. Simpler organizations such as channel tube and loose aggregates are being formed, presenting low colloidal stability and tendency toward phase separation. Because of the size of these objects and the q range available during these experiments, further detailed information about such objects could not be obtained.

#### 2.4.2. In Situ Complexation

The progressive formation of ICs and aggregates was followed for the longer complexes (i.e., EO20-CD and EO45-CD) through in situ SAXS measurements. In this case, α-CD and OEGMA solutions were mixed shortly before injection into the sample holder, and scattering profiles were acquired during 1 h. As shown in Figure 8, the SAXS curves show distinct profiles for the two oligomers investigated and for the longest EO chain (EO45), features that evolve during the acquisition time. For the IC of EO20, the SAXS profiles remained, basically, unchanged during this period of time, suggesting that complexation was accomplished before the first minute after mixing (before data collection), although intensity modification with time is observed. The curves for IC with EO45, however, display a broad peak centered at 0.5 nm⁻¹, whose intensity increases with time, along with the intensity of scattering at lower q values. Scattering data obtained for EO20-CD present a power law behavior with an exponent of 3.6 that remains almost constant with time, suggesting that the structure is already present in the first measurement (after 1 min of sample preparation). On the other hand, EO45-CD samples initially display a power law exponent of 2.2 (close to that present by pristine α-CD samples, Figure S5), which progressively increases to a value of 3.4 after 60 min. The appearance of a second peak at higher q values (around 3.5 nm⁻¹) is also observed in Figure 8c,d. This peak is promptly observed in the EO20-CD sample, remaining constant during the measurements, but it is barely observed after 60 min in sample EO45-CD.

Based on the visualization of pseudopolyrotaxanes structures reported in the literature,⁴⁰ we attempted to fit these SAXS curves to a pearl-necklace model, producing the solid lines represented along the scattering data in Figure S6, with good agreement, as discussed in the next section. Concerning α-CD units present on PRs, the dimensions obtained by the fits are similar to those found in pristine α-CD suspensions (i.e., 0.8 nm), fitted using the cylinder model, which also agree with CD dimensions.

### 3. Discussion

Based on the obtained results, we propose that OEGMA complexation by α-CD follows the same general mechanism demonstrated for PEG molecules. It means that PR formation should take place by the accommodation of stretched EO units into the macrocycle cavity. However, several particularities arise for OEGMA monomers, mostly because of the role of the methacrylate group as a stopper at one end of the oligomer chain. Further details on IC derived from the current experimental data are discussed below.

#### 3.1. Mechanism of Inclusion Complex Formation

As illustrated in Figure 9a, the α-CD structure possesses an internal cavity that can be filled with different molecules. It means that PR formation should take place by the accommodation of stretched EO units into the macrocycle cavity. However, several particularities arise for OEGMA monomers, mostly because of the role of the methacrylate group as a stopper at one end of the oligomer chain. Further details on IC derived from the current experimental data are discussed below.
EO moieties that are present in OEGMA molecules (Figure 9b) possess an adequate size to be included into the $\alpha$-CD cavity. Along with the formation of inclusion complexes (Figure 9c), the solution becomes turbid and complexes tend to precipitate as a crystalline white solid in the case of short oligomers. By modifying oligomer size, their ability to form solid precipitates, complexation yield, and initial clouding times are modified.

The ability of longer oligomers to form gels fits well with the description of Sabadini et al.\textsuperscript{43} about partially threaded PEG chains, which are able to form a supramolecular gel through the interaction of complex $\alpha$-CDs. The presence of soluble structures consistent with pseudopolyrotaxanes and necklace structure formation is also suggested by these authors.

An important difference in the present study is the influence of the methacrylate end group over the incorporation into $\alpha$-CD and the complexation mechanism. Most of the inclusion complexes are based on PEG-OH, which can be included via both edges of the polymeric chain. In addition, there are no rigid groups in the PEG structure in temperatures above 280 K, providing enough chain mobility to adopt different conformations during inclusion.\textsuperscript{30,44} This seems fundamental during $\alpha$-CD complexation, where PEG chains possess two times the length of relaxed ones because of the stretching effect imposed by cavity restrictions. Such flexibility seems unlikely to be found in methacrylate groups because of their sp$^2$ bonds. Furthermore, the methacrylate group is also larger than the EO groups and may act as a stopper in one of the extremities of the oligomer chain. We found no evidence to suggest that these bulkier end groups enter the $\alpha$-CD cavity. In this case, IC should be formed by on-side inclusion into $\alpha$-CD, as illustrated in Figure 2. Together with oligomer size, this behavior can alter the oligomer chain dynamics and influence inclusion efficiency. Some calculations reported by Serres-Gómes et al.\textsuperscript{25} suggest that one-side complexes can be even more thermodynamically favorable.

The role played by small nonlabile sp$^2$ molecules as stoppers for $\alpha$-CD polyrotaxanes has not been reported in the literature, to the best of our knowledge. However, there are reports suggesting that methacrylate makes the threading of a PEG-b-PDMAEMA block copolymer into $\alpha$-CD difficult.\textsuperscript{45} In early 2000s, a series of papers described the synthesis of methacrylate-based polymers prepared through an IC methodology.\textsuperscript{46−48} As a general rule, these publications are based on the preparation of IC between $\beta$-CD and methyl methacrylate monomers (usually resulting in a yellow solution\textsuperscript{17}) which are polymerized by ATRP. Some of these studies suggest that methacrylate groups are included in the $\beta$-CD cavity and show the interactions between internal $\beta$-CD protons and $\equiv$CH$_2$ from methacrylate through 2D-NMR (NOESY) measurements.\textsuperscript{46,49} Such an interaction is not observed here because methacrylate groups are too rigid and bulky to fit inside the smaller $\alpha$-CD cavity.

3.2. Complex Stoichiometry. As exhibited in Figure 3, complexes based on longer oligomers, where sequential inclusion of $\alpha$-CD is allowed, present bimodal distribution of

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**Figure 8.** Time evolution of SAXS scattering profiles for (a) IC-C$_5$ and (b) IC-D$_5$ systems. Figures (c,d) contain a zoom at a higher $q$ region ($3−4$ nm$^{-1}$) for same samples. Curves were recorded at times 1, 5, 10, 20, and 60 min after mixing.

**Figure 9.** Structure of the $\alpha$-CD monomer and molecular dimensions (a), structure of the OEGMA monomer (b), and inclusion complex representation of the OEGMA oligomer inside the $\alpha$-CD cavity (c).
T₂ values at average CD/OEGMA ratios. In these samples, both free and bonded species coexist and are associated with two values of T₂. At higher CD/OEGMA ratios, most of the EO groups are inside (or near to) the α-CD cavity and only IC T₂ is observed. Figure 3e also shows that long oligomers (Mn > 300 g·mol⁻¹) display T₂ values reaching a plateau after the inclusion of a certain number of α-CDs. This behavior indicates the saturation of the oligomer chains or, at least, that α-CD units are equally distributed along the OEGMA chain, restricting its relaxation. These saturation values are similar to those shown in Figures 1c and 2 for higher amounts of α-CD as determined by gravimetry, corroborating that saturation of oligomer chains occurs with a smaller number of α-CD units than expected (or that would be physically achievable). Similar observation was previously discussed by Joseph et al. for PEO grafted onto polystyrene particles, where only one extremity of the EO chains is free in solution and, hence, available for interaction. Altogether, these results corroborate the proposed role of the methacrylate group as a stopper, when present at one extremity of the oligomer chain. During the present experiments, varied T₂ values were observed for oligomers with different molecular weights. An exponential growth (Figure 3d) of relaxation times with an increase in molecular weight suggests that the methacrylate group affects mostly closer EO units, causing short oligomers to present smaller T₂ values. With the increase of molecular weight, longer oligomers escape from this influence for being further apart. As a consequence, the methacrylate group becomes isolated at one chain extremity and cannot affect the majority of EO groups, as illustrated in Figure 2.

3.3. Inclusion Thermodynamics. Calorimetric measurements were performed in order to assess this inclusion thermodynamics. Results shown in Figure 5a,b reveal that data for the formation of the EO2CD1 inclusion compound could be fitted using a simple binding model that assumes one type of sites. The obtained value of N (0.81) is similar to the one found by H₁-NMR (around 0.7), suggesting that such a model successfully describes the 1:1 (CD/OEGMA) IC ratio between the macrocycle and the simplest oligomer. Model data also reveal that IC is a spontaneous (ΔG < 0) and entropically driven process, associated with displacement of water solvation models from the CD cavity (ΔH = ΔS).50

Data presented in Figure 5c show the modification of the measured heat profile according to oligomer molecular weight upon α-CD addition. Assuming full complexation for the first α-CD injections (first points in Figure 5c), average ΔH values for inclusion vary from −1.9 to −0.3 kJ·mol⁻¹ as EO molecular weight increases from 188 to 2050 g·mol⁻¹. Such values are in the same range of values reported for other inclusion complexes described in the literature for similar molecules, as some alcohols, determined by calorimetric methods.5 The observed enthalpy dependency on oligomer size can be a result of incomplete binding for longer EO chains.

The coexistence of different calorimetric events along the observed process can be associated with the changes observed in the shape of the peaks.51 Here, along with a decrease in peak intensity, secondary events are observable (red arrows) for longer OEGMA chains (EO20-CD₅ and EO45-CDₐ, Figure 6b) after α-CD injection. These secondary peaks (indicated in Figure 6b) are broader than the main ones and appear in earlier injections for EO20-CDₐ, in comparison to EO45-CDₐ samples. For shorter oligomers (EO₂-CD₁ and EO₄-CDₐ), complete inclusion occurs in the first minutes after each injection. On the other hand, longer EO chains display longer inclusion times, sometimes over 1 h. The formation of new complexes per time is reduced for these longer samples, and no complete inclusion (consumption of all species to form PRs) is observed. This occurs because the threading of new α-CDs units depends on the sliding of previously threaded ones along the molecular chain. A proposed mechanism is illustrated in Figure 10, agreeing with the previous description of the threading process put forward by Lo Nostro et al.52 and reinforcing the effect of oligomer molecular weight on the inclusion process.

In the described scenario, random coil conformations (typical of long segments in solution) make complexation difficult, decreasing the resulting exchanged heat at short periods. Further studies should be performed in order to corroborate or refuse such an effect.

3.4. Pearl-Necklace Structure. The structure of the obtained ICs was investigated by SAXS in order to understand the influence of oligomer size and number of CD units available to complex during complexation and on the final structure of the obtained complex. As observed in Figure 7, scattering profiles change progressively as a function of sample composition. Data analyses reveal a power law behavior with the formation of large structures. Samples’ n index (H(q) vs q⁴) increases from pristine α-CD to EO45-CD₂₀, which could indicate that these particles are progressively becoming more anisotropic. This explanation agrees with the model of longer PRs being formed as a function of OEGMA size at a time scale which possibly could not be determined because of the experimental conditions.

A pearl-necklace model was used to estimate the number of pearls per chain for both complexes after 60 min (Figure S6). According to this model, we have found that not only pearl-to-pearl distances are larger at EO45-CD₂₀ (longer chain) but also that EO20-CD₅ displays around 6 CD units per chain (at complete complexation), while EO45-CD₂₀ contains only 3 CD units per OEGMA chain.

These results suggest that PRs based on EO₂₀ are able to form, align, and self-assemble more rapidly than those based on the longer oligomer, corroborating the rapid aggregation.
and precipitation mechanisms revealed by ITC experiments and the slower kinetics of complexation of the longer oligomer. Moreover, the formation of larger structures (lower q values, around 0.5 nm⁻¹) is observed in the EO₄₅-CD₉ complex (Figure 8b). Such an observation is probably related to the rapid formation of α-CD self-assembled structure, which potentially follows a cage-like to tubular structure described in earlier studies.²⁶,³³,³⁴ SAXS time evolution shown in Figure 8b also reveals that the 0.5 nm⁻¹ peak reaches a maximum in intensity before decreasing. It suggests that equilibration of the samples can lead to scattering patterns similar to those observed in Figure 7 for all samples, after the appropriate time. It is also possible to observe that the time scale where this maximum is reached depends on the concentration of α-CD present in the suspension, as observed in Figure S7. As expected, the process is faster in samples containing higher CD/EOEGMA ratios. The same behavior was reported by Serres-Gómez et al.²⁵

Finally, the peak observed at higher q values (around 3.5 nm⁻¹) should be related to the oligomer threading (as suggested by XRD results, Figure 1b). Notably, it appears earlier and displays better definition for shorter oligomers than for longer ones (arrows in Figure 8c,d, respectively). As EΟ₂₀-CD₉ complexes show a well-defined peak in the first measurement (less than 1 min after sample preparation), the EΟ₄₅-CD₉ system only exhibits the same peak position 30 min after EOEGMA-CD mixing. Such a difference in complexation times corroborates kinetic observation based on ITC results, where longer inclusion times are clearly observed for larger oligomers. This seems to be a consequence of the random coil formation of longer chains in solution, which hinders the prompt sequential inclusion of different α-CDs in the same oligomer chain.

4. CONCLUSIONS

Besides the large amount of data available about PEG/CDs inclusion complexes, this is the first study which investigates the complexation mechanisms between EOEGMA chains and this macrocycle. Here, we demonstrate that ITC formation and characteristics are highly dependent on the EO oligomer molecular weight and α-CD concentration. These parameters strongly influence complex appearance and formation kinetics, leading to solids or gel-like materials according to sample composition.

Larger amounts of α-CD accelerate complex formation and aggregation. Calorimetric experiments (ITC) corroborate the visual observations which suggest that longer complexation times are required for inclusion of high M₀ oligomers and also reveal that complexation close to 1:1 (EO/CD) is attained only for the shortest EO chain. Structural analyses suggest that threading proceeds to the formation of pearl-necklace structures which vary with the oligomer EO chain, confirming their close relationship to PEG pseudopolyrotaxanes.

SAXS experiments indicate a modification in ordered α-CD structures, already present prior to oligomer inclusion, in the presence of longer guest molecules. This is probably related to the progressive accommodation of new α-CD units at long oligomer chains, taking place after certain time intervals.

Although these complexes present several similarities with traditional PEG-based structures, mostly because of EO characteristics, the influence of methacrylate groups leads to certain particularities. The obtained results suggest that the methacrylate group lies outside the α-CD cavity after complexation, acting as a stopper and favoring a one-side complexation mechanism. The availability of such a functional group can be interesting for some applications because it can be used to tune oligomer polymerization in the presence of inclusion complexes, allowing for LCST control and the creation of potentially thermoresponsive molecular machines.

5. MATERIALS AND METHODS

5.1. Materials. EOEGMA (with nominal molecular weights of 188, 300, 950, and 2050 g·mol⁻¹) and α-CD were purchased from Sigma-Aldrich. In this work, EOEGMA oligomers were purified by passing them through a basic alumina oxide column to remove inhibitors and α-CD was used as received. All solutions were prepared with Milli-Q grade water (18.2 MΩ cm).

5.1.1. Inclusion Complex Preparation. In a typical procedure, two equal parts of aqueous solutions containing α-CD (50 ± 5 mg·mL⁻¹) and the desired oligomer (variable concentration) are mixed together at ambient conditions (around 25 °C). After system stabilization, the precipitate was thoroughly washed with water in order to remove non-complexed molecules. Typical equilibration times for these samples depend on oligomer molecular weight. Short ones lead to complete solid precipitation in some minutes, while longer ones lead to the formation of a white suspension stable for several days. At least 24 h after washing, the supernatant is removed and the system is dried in an oven at 60 °C. Samples are weighted and kept in a desiccator. Finally, the dry mass of precipitates was used to determine gravimetric yield. Complex stoichiometry was estimated by assuming that only α-CD is lost during sample washing, resulting in a sample composed of the initial amount of oligomers and a measurable mass of CD.

Herein, the samples used are represented by the following nomenclature: EOₓ-CDₓ where x represents the average number of EO units in the oligomer side chain and n represents the mole ratio of α-CD to EOEGMA chain used for sample preparation. For example, sample EO₄₅-CD₁ contains 1 mol of α-CD to 1 mol of the EOEGMA oligomer with an Mₙ of 2050 g·mol⁻¹. All samples are listed in Table 1.

5.1.2. Optical Microscopy. The observation of solid particles was performed in a NIKON HS505 microscope under environmental conditions. The particles were simply collected over a glass plate and directly observed.

5.1.3. X-ray Diffraction. The XRD patterns were recorded on a Shimadzu XRD 7000 X-ray diffractometer at 40 kV and 30 mA with Cu Ka radiation (λ = 0.154 nm) in the range of 2θ = 5–35° for samples using a fixed time mode at a scan speed of 2°·min⁻¹ in steps of 0.02°.

5.1.4. Isothermal Titration Calorimetry. Experiments were conducted in a MicroCal VP-ITC (Northampton, MA, USA) at 25 °C. Aliquots ranging from 10 to 40 μL (typically for short and long experiments, respectively) were added stepwise by an automatic injection syringe containing 270 μL of a concentrated α-CD solution (50 mmol·L⁻¹) injection into the reaction cell of 1.43 mL, containing either water or EOEGMA solutions (25 mmol·L⁻¹). Experiments were performed with intervals varying from 5 to 270 min, which were previously checked to ensure appropriate baselines. ITC data were treated with the associated Origin 7.0 software.

5.1.5. High-Field NMR. ¹H-NMR measurements were performed at 40 °C on a Bruker ADVANCE 500 spectrometer with a proton frequency of 499.87 MHz. Accumulations of 16 spectra were used with D₂O as the solvent. 2D NOESY
measurements were performed at 40 °C on a Bruker ADVANCE 400 spectrometer with a proton frequency of 400.18 MHz, using the standard three-pulse sequence. For all the samples, mixing times (T_m) of 200 ms and 1 s were used with 16 accumulations in order to allow better discrimination between intra- and intermolecular interactions.

5.1.6. Relaxation NMR (TD-NMR). Water holding was performed on a Bruker Minispec mq20 NMR analyzer (Bruker Company, USA) with a proton resonance frequency of 20 MHz. The samples prepared with D_2O (20 mg/mL) were placed in an 8 mm-diameter glass tube and inserted into the NMR probe, and a temperature of 39.8 ± 0.2 °C was stabilized for 15 min. The spin–spin relaxation time, T_2, was measured using the Carr–Purcell–Meiboom–Gill sequence, with 90 and 180 proton pulses of 8.4 and 16.7 μs, respectively, and an echo time of 160 μs. The data were recorded in triplicate, in which 30,000 echoes were acquired with 32 scan repetitions with intervals between subsequent scans. The fitting of the CPMG decay curves was performed using multi-exponentials.

5.1.7. Small-Angle X-ray Scattering. SAXS measurements were taken at the SAXS1 beamline of the Brazilian Synchrotron Light Laboratory, LNLS, in Campinas, Brazil. The samples were positioned in a cell with two flat mica windows, and a thermal bath connected to the sample holder was used for temperature control (at 25.0 ± 0.5 °C). The X-ray wavelength was 1.608 Å, and the sample-to-detector distance was around 0.6 m, calibrated using silver behenate as a reference. The X-ray scattering function I(q) was obtained by fitting the scattering data to the “pearl-necklace model” for samples named EO 20-CD5 and the “strands (OEGMA-platform)” for the sample named CD. Additionally, a power law model was used to fit the experimental data to the “pearl-necklace model” for samples named EO 20-CD5 and EO 50-CD5 and the “cylinder model” for the sample named CD, which are described in detail in refs 59–63, respectively. Additionally, a power law model was used to fit the experimental data of sample EO 50-CD5 at q > 2 nm⁻¹. To fit these curves, the scattering length density values were set to 9.4 × 10⁻⁶ Å⁻² for water, 0.135 × 10⁻⁶ Å⁻² for the pearls (cyclodextrin), and 7.8 × 10⁻⁶ Å⁻² for the strings (OEGMA oligomers), calculated using SASView software. Extra information can be found in the SAXS section of the Supporting Information.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00741.

Images and qualitative information about physical aspects of the described systems, NMR, extra calorimetric data, and SAXS curves (PDF)

AUTHOR INFORMATION

Corresponding Authors
Marcos Mariano — Institute of Chemistry, University of Campinas (UNICAMP), 13083-970 Campinas, São Paulo, Brazil; orcid.org/0000-0003-2374-5198; Email: marcos.mariano1@outlook.com

Watson Loh — Institute of Chemistry, University of Campinas (UNICAMP), 13083-970 Campinas, São Paulo, Brazil; orcid.org/0000-0002-8049-3321; Email: wloh@unicamp.br

Authors
Ogires Daniel Bernardinelli — Institute of Chemistry, University of Campinas (UNICAMP), 13083-970 Campinas, São Paulo, Brazil
Rafael Pires-Oliveira — Institute of Chemistry, University of Campinas (UNICAMP), 13083-970 Campinas, São Paulo, Brazil; orcid.org/0000-0003-1855-5322
Guilherme A. Ferreira — Institute of Chemistry, University of Campinas (UNICAMP), 13083-970 Campinas, São Paulo, Brazil; orcid.org/0000-0002-4932-3666

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c00741

Notes
The authors declare no competing financial interest.

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