



Cite this: *Polym. Chem.*, 2025, **16**, 5127

Received 24th October 2025,  
Accepted 20th November 2025

DOI: 10.1039/d5py01007a

rsc.li/polymers

Water-soluble polymers are extensively studied materials in the field of biomedical chemistry research. This study investigates the behavior of methacrylamide-based homopolymers and copolymers with linear, two-arm, and star-like structures in aqueous solutions. The research focuses on how the polymer structure affects its solution behavior. The study uses intrinsic and shear viscosity measurements to examine the influence of these structures on solution behavior at both molecular and macroscopic levels.

## Introduction

Water-soluble polymer carriers designed to deliver active moieties to a tumor or inflammatory tissue have been extensively investigated in the last few decades.<sup>1,2</sup> Some of the most studied polymer materials belonging to this group are polymers based on *N*-(2-hydroxypropyl)methacrylamide (HPMA).<sup>3–6</sup> HPMA-based polymer materials take advantage of their biocompatibility, excellent non-fouling properties with biological materials, structural variability, and excellent water-solubility. The high hydrophilicity and water-solubility of HPMA-based polymers are important properties that make them highly functional materials in drug delivery and *in vitro* diagnostics (IVD).<sup>7–9</sup>

The solution behavior of macromolecules is strongly related to the compactness and shape of the polymer coil. These properties, determined at the molecular level, significantly impact the material's behavior at the macroscopic scale. From an application perspective, the solution behavior determines the potential use of macromolecules in human medicine or related fields. One issue is related to production and troubles arising from the formulation processes, such as the dissolution rate and the viscosity properties of stock solutions.

<sup>a</sup>Institute of Macromolecular Chemistry, Department of Biomedical polymers, Czech Academy of Sciences, Heyrovského nám. 2, 162 00 Prague 6, Czech Republic. E-mail: kostka@imc.cas.cz

<sup>b</sup>Institute of Macromolecular Chemistry, Department of Polymer Processing, Czech Academy of Sciences, Heyrovského nám. 2, 162 00 Prague 6, Czech Republic

Secondly, it emphasizes the biological availability of active pharmaceutical ingredients (APIs), where the compactness of polymer coils can affect their availability towards enzymatic reactions and nonspecific noncovalent interactions. Therefore, we studied in detail the solution behavior of linear and star-like polymers and the influence of their molecular weight and structure on solubility in aqueous buffers.

Intrinsic viscosity is the most relevant variable for describing the behavior of a polymer solution at the molecular level. This is because intrinsic viscosity reflects the true viscosity properties of a polymer molecule independently of its concentration in solution.<sup>10</sup> It is used in various industries to describe a product's storage stability and quality. Intrinsic viscosity is also a relevant testing parameter in the pharmaceutical industry (e.g., for the molar mass determination of products such as sodium hyaluronate). As such, it is listed in several monographs of the EU Pharmacopoeia and US Pharmacopeia.<sup>11</sup>

Shear viscosity is one of the transport coefficients of liquids and describes the current of transverse momentum.<sup>12</sup> Provided that it governs the macroscopic flow of liquids, it plays an essential role in treating flows in chemical engineering and other fields. In addition, the shear viscosity of a solution is often regarded as a measure of the rates of dynamic processes of solutes dissolved in the solvent. It is, therefore, important and interesting to understand the shear viscosity of liquids in terms of microscopic structure and intermolecular interaction.

## Results and discussion

HPMA-based homopolymers and copolymers with *N*-(tert-butoxycarbonyl)-*N'*-(6-(methacryloylamino)hexanoyl)hydrazine (Ma-Acap-NHNH-Boc), featuring linear and star structures, were synthesized within the range of 8 to 1350 kg mol<sup>–1</sup> using previously described methods.<sup>13–15</sup> The physicochemical characteristics, such as molar mass, dispersity, and intrinsic viscosity of the synthesized samples, along with their structures, are detailed in the SI (Tables S1–S4, Fig. S1–S5). All



synthesized polymers contained identical chain end groups to eliminate the influence of those functional groups on solution behavior. The primary aim of the study was to determine the effects of molecular weight and structure, along with the varying concentration of polymers, on solution behavior at both the molecular and macroscopic levels (Fig. 1). Size exclusion chromatography (SEC) with viscometric, MALS, and dRI detectors was employed to measure the intrinsic viscosity of dilute polymer solutions, while a rotational rheometer was used to assess the shear viscosity of semi-concentrated polymer solutions, both physiological solutions.

### Intrinsic viscosity

To confirm the suitability of PBS buffer for the measurements with HPMA polymers, we plotted a conformation plot, dependence of RMS radius on molar mass, for linear polymers with molar masses above  $100 \text{ kg mol}^{-1}$ ; see Fig. 2.<sup>16</sup> The slope of

the fitted curve was calculated as 0.51, proving that the polymer coil behaves like a random coil through the entire molar mass range.

We investigated the relationship between intrinsic viscosity and molar mass, along with the Mark–Houwink plots (MHPs), for all synthesized materials (see Fig. 3A). The solution behavior of HPMA homopolymers and copolymers containing 6 mol% of comonomer units based on Ma-Acap-NHNH-Boc, regardless of being protected or deprotected, was found to be similar to that of HPMA homopolymers (refer to SI Fig. S6). The calculated slopes of the Mark–Houwink plots for homo- and copolymers yielded a precise value of 0.65, confirming a random coil conformation for the copolymers as well.<sup>17</sup> The consistent behavior of the HPMA copolymers suggests that they can maintain their solution behavior even when functional groups suitable for the attachment of biologically active molecules are present.

The random coil conformation behavior changes were not observed when the aromatic linker was employed in the two-arm structure (see the red dot in Fig. 3B). However, significant changes were observed when the polymer's structure was changed from linear to star-like.

Importantly, the star-like polymer structures with at least four arms exhibit different solution behavior than linear polymers of the same molecular weight. For details, see SI Tables S3 and S4. The number and length of the polymer arms strongly impact the solution behavior of the star-like polymers. The intrinsic viscosity of four-armed star-like HPMA polymers becomes independent of molecular weight, and the solution behavior of the material is close to that of a solid sphere for which the slope of the MHP acquires zero value. Nevertheless, the slope values for 4-armed star-like polymers with  $M_w < 100 \text{ kg mol}^{-1}$  were slightly positive, being dependent on arm length, 0.14 for SC-SA ( $18 \text{ kg mol}^{-1}$  polymer arms) and 0.18 for SC-LA ( $37 \text{ kg mol}^{-1}$ ), respectively (see Fig. 3B). As the molar mass of the star-like polymers increases above  $100 \text{ kg mol}^{-1}$ , the slope value changes to zero for both SC-SA and SC-LA (see Fig. 3C).

Based on the observation that linear fits work well for both linear and star-like polymers (SI Fig. S7), their size increases proportionally with molar mass, but at a slower rate for star-like structures compared to linear polymers. To determine the compactness of the polymer molecule, we can use the  $R_g/R_h$  ratio, which indicates shape and compactness. For linear polymers, this ratio is above one, indicating elongated particles. For both SC-SA and SC-LA polymers, this ratio is significantly below 0.77, which corresponds to a particle with a dense core decorated by long, sparse chains. Please refer to SI Fig. S8 for further details.

Understanding changes in the conformation of polymer molecules in solution is crucial for the *in vivo* application of the polymer and interpreting results from biological experiments. A few years ago, Etrych *et al.* published a paper describing the elimination rate of polymer carriers dependent on polymer molar mass and architecture.<sup>18</sup> Based on the results, HPMA-based carriers are eliminated from the organism *via* the

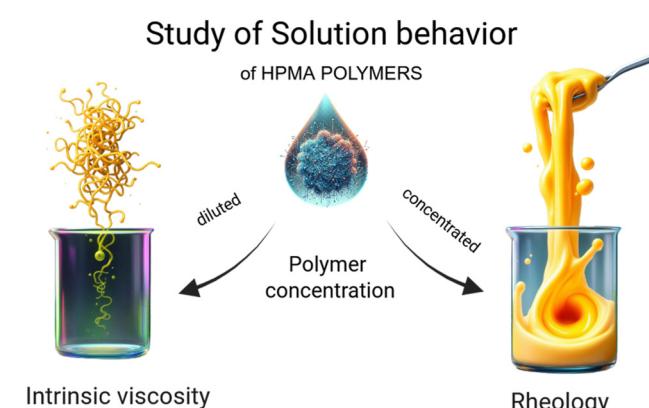


Fig. 1 Graphical representation of the studied polymer properties at the molecular and macroscopic levels, where we used intrinsic viscosity measurements for molecular level behaviour in diluted samples and rheology for macroscopic observation of solution behaviour at higher concentrations.

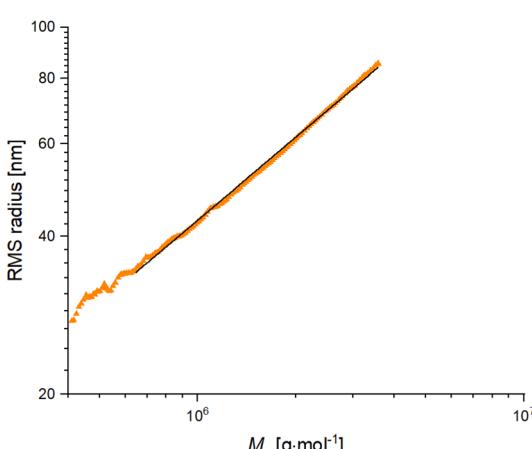
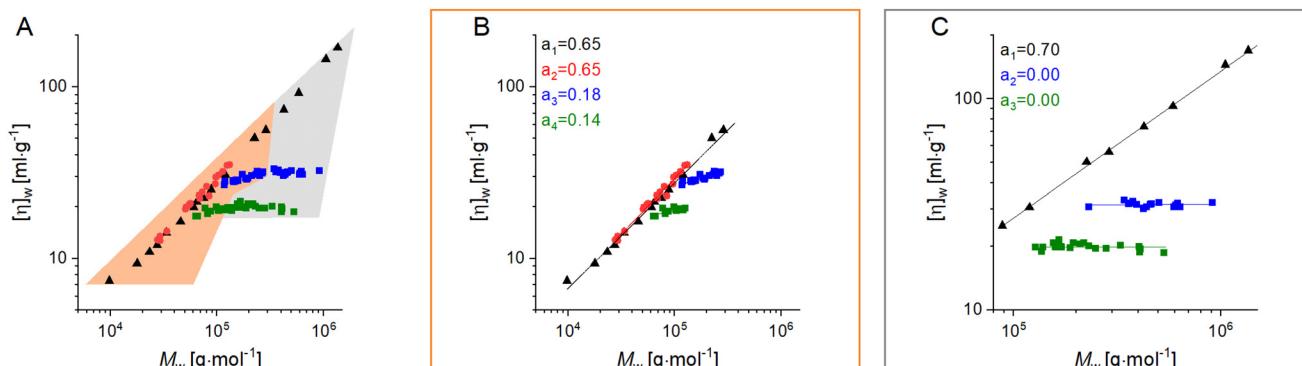


Fig. 2 Conformation plot showing the dependence of the RMS radius of linear HPMA-based homopolymers on molecular weight. Orange – curve from the MALS detector; black – the fitted curve.





**Fig. 3** Mark–Houwink plots. Intrinsic viscosity of HPMA-based polymers with different molar masses and architectures. Linear homopolymers (LH, black), linear two-arm polymers (DH, red), star-like polymers with  $18 \text{ kg mol}^{-1}$  polymer arms (SC-SA, green) and star-like polymers with  $37 \text{ kg mol}^{-1}$  polymer arms (SC-LA, blue). (A) Mark–Houwink plot for all synthesized types of polymers – LH, DH, SC-SA, and SC-LA. The orange and grey colored areas in graph A are zoomed in on (B) and (C), respectively. In (B) and (C), slope values  $a$  are calculated separately for each group of polymer.

kidneys up to molar fractions of  $69 \text{ kg mol}^{-1}$  in the case of linear polymers and up to molar fractions of  $53 \text{ kg mol}^{-1}$  in the case of star-like carriers. Also, the cumulative urinary elimination of linear conjugates was two times higher than that of star-like polymers. These findings suggest that the flexibility and conformation of the polymer chain may play a crucial role in its suitability for biomedical applications.

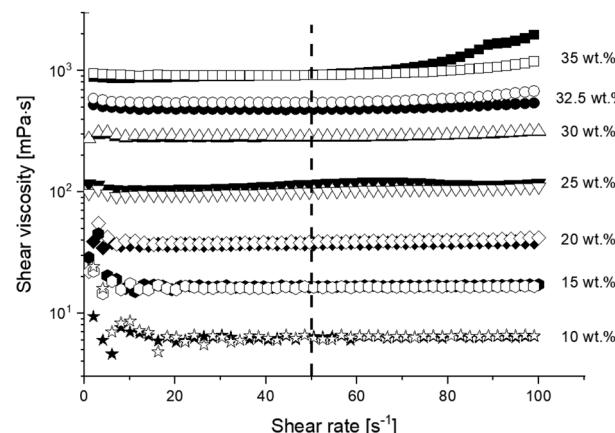
### Shear viscosity

The above-mentioned study examined solution behavior at the molecular level in highly dilute solutions. The rheological behavior of four homopolymers and one star-like copolymer with a molecular weight of  $464 \text{ kg mol}^{-1}$  was studied in dilute and semi-concentrated solutions (10–45 wt%). The solutions' viscosity was measured. The homo-polymers had molecular weights ranging from 10 to  $65 \text{ kg mol}^{-1}$ . Polymers with a molar mass of about  $50 \text{ kg mol}^{-1}$  are restricted to a concentration below 35%.

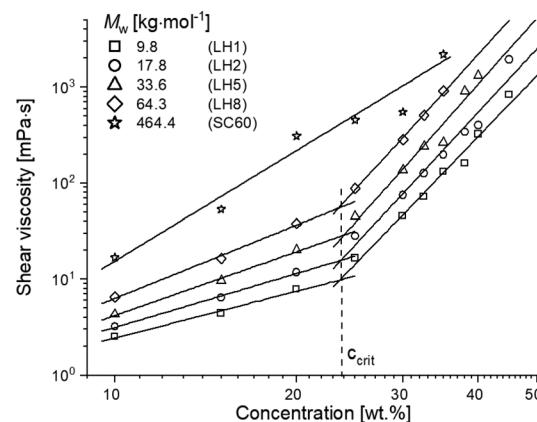
The rheological behavior of the polymer solutions has been studied in a steady shear mode. All solutions prepared from polymers with different molecular weights show Newtonian behavior, *i.e.*, the viscosity values are independent of the shear rate applied in the whole measured range. Flow curves of solutions containing the polymer with a higher molecular weight of  $64.3 \text{ kg mol}^{-1}$  are displayed in Fig. 4.

The absence of shear thinning implies that there are no intertwinings of polymer chains in the solutions. A small viscosity increase at high shear rates was observed in some measurements, particularly those at higher concentrations. This effect is caused by the evaporation of water during the measurement, and thus, it is considered a measurement artefact. The reproducibility of the measurements was very good, with differences between individual measurements lower than 10%.

To evaluate the effects of molecular weight and concentration on solution viscosity, values at a shear rate of  $50 \text{ s}^{-1}$  were selected. Fig. 5 shows these values on a log–log scale, which is commonly used to differentiate between dilute and



**Fig. 4** Flow curves of polymer solutions with  $M_w = 64.3 \text{ kg mol}^{-1}$  at  $25 \text{ }^\circ\text{C}$  at various solution concentrations (10–35 wt%). Experiments were performed in duplicates (empty and filled marks).



**Fig. 5** Concentration dependence of shear viscosity for polymer solutions with different molecular weights (the straight lines are linear fits of the experimental values on a log–log scale).

semi-concentrated solutions. As anticipated, the viscosity of the solutions increases with polymer concentration and molecular weight. For linear polymers at lower concentrations, the dependencies can be accurately described by a linear function, with coefficients of determination  $R^2$  exceeding 0.99. At polymer concentrations greater than 25 wt%, the dependencies become significantly steeper. In this concentration range, linear fits still show reasonable accuracy, with  $R^2$  values between 0.96 and 0.99.

A critical concentration  $c_{\text{crit}}$ , at which the linear macromolecules in the solution begin to interact and thus cause a more significant increase in shear viscosity with concentration, was determined from the extrapolated linear fits of both concentration ranges. The critical concentration was close to 24 wt%, independent of the molecular weight of the dissolved linear polymers. This can be explained by the fact that in the given polymer–solvent system, the macromolecules at the same concentration occupy the same total hydrodynamic volume regardless of their size. However, the molecular weight of the dissolved polymer affects how the shear viscosity increases with concentration. At lower concentrations of up to 20 wt% of the polymer, where no or minimal interactions between polymer coils are expected, the viscosity increases more rapidly as the molecular weight rises. This can be seen in Fig. 6, where the slopes of the linear fits describing the concentration dependencies of viscosity are plotted as a function of polymer molecular weight. In contrast, no clear trend is observable at concentrations above the critical concentration, and viscosity increases similarly for all studied molecular weights of linear polymers.

Besides polymers with a linear structure, a polymer with a star-like architecture and a molecular weight of 400 kg mol<sup>-1</sup> has been studied. In this case of the star-like polymer, no transition can be identified in the concentration dependence of shear viscosity. All measured data up to 35 wt% of the

polymer, higher concentrations cannot be prepared due to insufficient solubility, correspond to a linear fit with  $R^2 = 0.97$  (Fig. 5).

Although the fit quality is somewhat poorer than that of linear polymers, the critical concentration of the star-like polymer, at which polymer molecules begin to interact in the solution, is shifted to higher values well above 30 wt%. This finding is expected because star-like macromolecules occupy smaller hydrodynamic volumes compared to linear ones (cf. Fig. S7 in the SI). The impact of different architectures of dissolved polymer chains is also illustrated in Fig. 6. The viscosity of solutions of a star-like polymer as a function of concentration increases with a slope of 3.83, which is significantly lower than expected for a linear polymer with the same molecular weight. The determined slope of 3.83 for the star-like polymer with 464.4 kg mol<sup>-1</sup> actually corresponds to that of a linear polymer with a molecular weight of approximately 142 kg mol<sup>-1</sup>. For a linear polymer with the corresponding molar mass (464 kg mol<sup>-1</sup>), the theoretical slope value should be 8.6 (cf. Fig. 6, grey column). This finding reinforces the conclusion that the concentration dependence of shear viscosity sensitively reflects the hydrodynamic volume of dissolved polymer chains.

## Conclusions

This study describes consistently and deeply the solution behavior of HPMA-based polymers with different architectures at both molecular and macroscopic levels for the first time. The MHP shows that linear or two-arm structures maintain a flexible conformation of a random coil with a slope value of 0.65. Conversely, starting with four arms, star-like structures adopt a solid sphere conformation, even when relatively long polymer arms are attached to the core. The intrinsic viscosity value of star-shaped polymers with a molecular weight of less than 100 kg mol<sup>-1</sup> is dependent on their molar mass, with a value of  $\alpha = 0.14$ . However, the intrinsic viscosity value becomes independent of molar mass for polymers with higher molecular weights. Intrinsic viscosity measurements can reveal differences in the shape or conformation of polymer carriers, which can aid in predicting their behavior. For instance, the polymer molecule's stretchability or flexibility could be a crucial property of the material being investigated in biological systems.

Rheological measurements confirmed that the concentrated solution up to 45 wt% exhibited Newtonian behavior. The viscosity values remained independent of the applied shear rate throughout the measuring range. The highly concentrated solutions can be stored and utilized immediately after dilution to the experimental concentration, making them advantageous for infusion applications. The critical concentration of the shear viscosity slope, calculated to be approximately 24 wt% for linear polymers, regardless of their molecular weight in the biologically relevant molar masses, was determined solely for linear polymers.

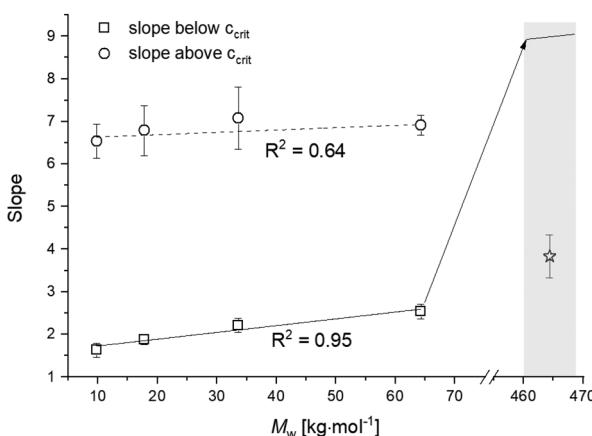


Fig. 6 The slopes of the linear fits describing the concentration dependence of viscosity as a function of polymer molecular weight (a star represents the value for solutions of star-like polymers). The grey column shows the theoretical slope value for a linear polymer with a molar mass corresponding to that of the star-like polymer.



## Author contributions

LeK, LiK, and ZS contributed to the analysis, investigation, methodology, drafting, and final writing; MH and MN contributed to the investigation and analysis; LiK and TE contributed to funding acquisition, writing, and final revision.

## Conflicts of interest

There are no conflicts to declare.

## Data availability

The data supporting this article have been included as part of the supplementary information (SI). It contains a complete materials and methods section, including the characteristics of all prepared and used polymers. Supplementary information is available. See DOI: <https://doi.org/10.1039/d5py01007a>.

## Acknowledgements

This work was supported by the Czech Science Foundation (project no. 23-05642S) and by the National Institute for Cancer Research (Programme EXCELES, ID project no. LX22NPO5102) – funded by the European Union – Next Generation EU.

## References

- 1 K. Ulbrich, K. Hola, V. Subr, A. Bakandritsos, J. Tucek and R. Zboril, *Chem. Rev.*, 2016, **116**, 5338–5431.
- 2 J. Kopecek and J. Yang, *Adv. Drug Delivery Rev.*, 2020, **156**, 40–64.
- 3 H. Krakovičová, T. Etrych and K. Ulbrich, *Eur. J. Pharm. Sci.*, 2009, **37**, 405–412.
- 4 A. Libanska, E. Randarova, S. Skoroplyas, M. Bartos, J. Lunackova, F. Lager, G. Renault, D. Scherman and T. Etrych, *J. Controlled Release*, 2023, **353**, 30–41.
- 5 X. M. Liu, S. C. Miller and D. Wang, *Adv. Drug Delivery Rev.*, 2010, **62**, 258–271.
- 6 A. K. Pearce, A. B. Anane-Adjei, R. J. Cavanagh, P. F. Monteiro, T. M. Bennett, V. Taresco, P. A. Clarke, A. A. Ritchie, M. R. Alexander, A. M. Grabowska and C. Alexander, *Adv. Healthcare Mater.*, 2020, **9**, e2000892.
- 7 F. Surman, T. Riedel, M. Bruns, N. Y. Kostina, Z. Sedlakova and C. Rodriguez-Emmenegger, *Macromol. Biosci.*, 2015, **15**, 636–646.
- 8 C. Rodriguez-Emmenegger, M. Houska, A. B. Alles and E. Brynda, *Macromol. Biosci.*, 2012, **12**, 1413–1422.
- 9 S. Rani and U. Gupta, *Drug Discovery Today*, 2020, **25**, 997–1012.
- 10 W. Lu, J. Mays, M. I. Malik, J. Mays and M. R. Shah, in *Molecular Characterization of Polymers*, Elsevier, 2021, pp. 261–280. DOI: [10.1016/B978-0-12-819768-4.00008-7](https://doi.org/10.1016/B978-0-12-819768-4.00008-7).
- 11 G. Nahler, in *Dictionary of Pharmaceutical Medicine*, Springer Vienna, Vienna, 2009, pp. 69–69. DOI: [10.1007/978-3-211-89836-9\\_515](https://doi.org/10.1007/978-3-211-89836-9_515).
- 12 R. B. Bird, *Appl. Mech. Rev.*, 2002, **55**, R1–R4.
- 13 M. Hrochova, L. Kotrchova, M. Frejkova, R. Konefal, S. Gao, J. Fang, L. Kostka and T. Etrych, *Acta Biomater.*, 2023, **171**, 417–427.
- 14 L. Kostka, L. Kotrchova, V. Subr, A. Libanska, C. A. Ferreira, I. Malatova, H. J. Lee, T. E. Barnhart, J. W. Engle, W. Cai, M. Sirova and T. Etrych, *Biomaterials*, 2020, **235**, 119728.
- 15 V. Raus and L. Kostka, *Polym. Chem.*, 2019, **10**, 564–568.
- 16 K. Šolc and W. H. Stockmayer, *J. Chem. Phys.*, 2003, **54**, 2756–2757.
- 17 W. W. Yau and S. W. Rementer, *J. Liq. Chromatogr.*, 1990, **13**, 627–675.
- 18 T. Etrych, V. Subr, J. Strohalm, M. Sirova, B. Rihova and K. Ulbrich, *J. Controlled Release*, 2012, **164**, 346–354.

