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Spiropyrans for light-controlled drug delivery†

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The supramolecular assembly of acetylsalicylic acid to a merocyanine–Zn complex translates into a photo-controllable drug delivery system. Herein, we present the synthesis and spectroscopic analysis of a three-component assembly combining a spiropyran derivative, a zinc(II) cation, and acetylsalicylic acid. The ON/OFF system can be modulated by safe and easily affordable stimuli, for the specific delivery of more than one active compound. Our results confirm the formation of a ternary system in solution, where the behaviour is dominated by photo-induced responses, and pave the way for the use of such smart platforms for medicinal chemistry purposes.

Introduction

The study of new stimuli-responsive nano-devices is gaining great attention in the nanomedicine realm. The careful design of nano-carriers opens new opportunities for smart platforms responsive to different endogenous or exogenous stimuli.[1] In this context the development of new light-controlled systems is a challenging and promising field of investigation for new applications in the biological and biomedical fields. The possibility of regulating systems with a low invasive tool like visible light is promising for photo-pharmacological purposes.[2] Light stimuli can be controlled in space and time with high precision and can be easily modulated in terms of intensity and precise operational wavelength. Moreover, the assembly of the coordination complexes of light-controlled scaffolds with particular ligands presenting intrinsic properties itself represents a new route to study multi-stimuli-responsive materials for applications in the biological realm.[3]

Among the photochromic and photo-switchable molecules, spiropyrans (SP)[4,5] have attracted a lot of attention. Spiropyrans are well-known molecular switches that undergo reversible structural transformation in response to external stimuli.[6,7] This class of compounds has been intensively studied and used for the assembly of hybrid photo-switchable structures based on polymers,[8,9] nanoparticles,[10,11] biomolecules[12,13] and carbon nanomaterials among others.[14–16] The photochromism of spiropyrans was highlighted in the1950s;[17] this phenomenon is evidenced by a significant visible color change and it is due to the different molecular properties of the two isoforms of these molecules. The reversible isomerization process consists in the breakage of the spiro C–O bond followed by the *cis–trans* isomerization of the double bond. The two isomers are significantly different:

(i) The closed ring form, called spiropyran (SP), is the more stable isomer and shows a perpendicular spatial disposition of the indolenine and benzopyran entities, connected by a spiro junction. In this conformation the molecule doesn't present π-conjugation, doesn't contain charged groups and does not absorb light above 400 nm.

(ii) The open less stable form, named merocyanine (MC), presents a planar conformation and an extended π-conjugation pattern. In this isomeric form, MC absorbs and emits visible light.

The isomerization from SP to MC can be achieved using a variety of external stimuli such as light, heat, protons and metal ions.[18] Spiropyrans have been investigated in the last decade for the formation of metal complexes with several metal ions differing in softness/hardness, coordination or binding properties that are responsible for the final geometry of the complex. We have designed and synthesized a library of spiropyran derivatives that can act as sensors for biologically important cations with the goal to obtain probes selective for specific cations, with increased chelating stability, but maintaining the ON/OFF switchability properties fundamental for the development of multi-use sensors. We have focused our attention on derivatives that don't need UV light for isomerization, as UV light could represent an invasive stimulus for applications in the biological realm. We have developed a highly selective spiropyran based ion receptor for the naked-eye detection of Zn(II), which is fully reversible upon visible

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irradiation.[19] We have obtained crystallographic evidence that emphasizes how metal binding can stabilize an open form of an SP-based ligand in the solid state.[20]

Recently we have studied the coordination chemistry of a spiropyran ester derivative (SP-E), containing an *N*-substitution in the indolenine moiety and a NO_2 group in the benzopyran portion, which stabilizes the formation of the MC isomer. Three different metal ions have been investigated, namely Mg^{2+}, Cu^{2+} and Zn^{2+}, and the interactions of the MC isomer with the metal ions have been studied by spectroscopic and crystallographic analysis.[21] The obtained information constitutes an essential starting point for the development of more complex assemblies for potential drug delivery systems.

With this in mind we have investigated the behaviour of SP-E complexed with Zn^{2+} in the presence of acetylsalicylic acid (ASA). We have completed several chemical and physical studies that constitute a preliminary investigation for the development of a potential prodrug delivery system (Fig. 1).

Zinc has been selected as the cation because it is an essential element for human beings: it plays an antioxidant role and it is the cofactor for more than 200 enzymes; moreover it is a structural element in several proteins; its deficiency can lead to issues in the regular biochemical processes of cells and these effects can clearly have an influence on the immune system and the production of hormones among others.[22] The zinc cation possesses anti-inflammatory properties and its use has been tested as an adjuvant agent in cancer prevention.[23] Recently, chronic inflammation has been shown to be associated with the development of many forms of cancer, and for this reason, the possibility to control the inflammatory response to tissue damage has become a key factor for cancer treatment and prevention.[24]

ASA has been chosen as a nonsteroidal anti-inflammatory drug (NSAID) because of its anti-inflammatory activity, also in combination with zinc.[25] This Active Pharmaceutical Ingredient (API) has been shown to decrease the incidence of several tumor types[26] and its geometry allows its interaction with metal ions, in particular with zinc.[27]

Here we report the studies carried out in acetonitrile (MeCN) solutions of the complex systems containing SP-E, zinc(II) cation and ASA, the possible formation of a ternary system between these species, and its reversibility features. We additionally highlight how this assembly could be investigated and studied for further applications for the development of light-triggered drug delivery devices.

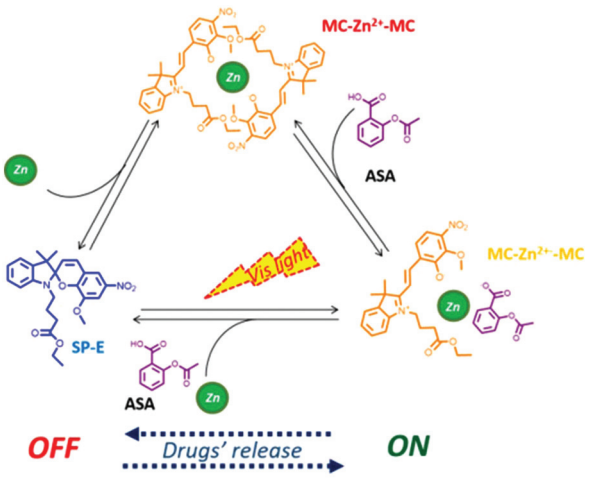

Fig. 1 Schematic representation of the light-modulable spiropyran based drug delivery device.

Results

In this work we present a chemical–physical investigation of the behaviour of our photochromic spiropyran (SP-E) molecule in acetonitrile solution in the presence of the zinc(II) cation, using $Zn(ClO_4)_2{\cdot}6H_2O$ as the zinc source and acetylsalicylic acid (ASA) as the Active Pharmaceutical Ingredient (API). The perchlorate hexahydrate salt of zinc has been selected because of its solubility properties in the MeCN medium in addition to its weak coordinating features that will not interfere with the complex interactions between the other components.

Design

The proposed system, summarized in Fig. 1, presents a photoswitchable subunit that is characterized by isomerization properties already investigated by our group in the presence of Zn^{2+}. The presence of a methoxy group and an O^- in the nitrophenyl portion of the SP-E molecule in its MC form contributes to the coordination with metal cations.[21]

The location of the strong electron-withdrawing nitro group in the 6′ position, which stabilizes the open MC form, has highlighted SP-E as a promising candidate for our goal. ASA coordinates with Zn^{2+} in a manner similar to that already reported.[28] These three compounds can be used to assemble a ternary reversible drug delivery system that can be formed under dark conditions and disassembled under visible light stimulus, allowing the controlled release of both APIs.

The studies reported here mainly involve a UV/Vis spectroscopic characterization of the system itself; these investigations highlight the system's features, its reversibility properties and its stability over time. These data constitute useful and necessary information for the development of such a successful nano-delivery system.

Synthesis

The SP-E molecule was synthesized following a published procedure optimized by our group with two steps consisting of the N-alkylation of the appropriate indolium moiety followed by the condensation with 3-methoxynitrosalicylaldehyde.[21] The synthetic scheme and ^1H NMR and MS characterization are reported in the ESI.†

UV/Vis absorption spectroscopy

The UV/Vis absorption spectra of spiropyrans can provide precise information related to the isomerism between the

closed and the open forms of the molecule, and they are also a useful tool to indicate and control the binding phenomena.

The isomerization and binding events, which generate the MC isomer in solution, are highlighted by a significant change in the photochromic properties of the compounds: the system in solution shows an absorption band in the visible region that is absent when the SPs are just dissolved in the selected solvent without the introduction of binding agents or the application of external stimuli.[4]

We have found that a MeCN solution containing the SP-E, ASA and Zn(II) cation in a 1 : 1 : 1 ratio with a 5×10^{-5} M concentration each presents a significant absorption area in the visible region (yellow line, Fig. 2). In this region, between 400 and 500 nm, the complexes between MC and metal complexes are usually found. The shape of the absorption spectra in this area is different compared to that observed for a MeCN solution containing SP-E and the Zn(II) cation in a 1 : 1 ratio (red line, Fig. 2) at the same concentration. The absorption spectrum of a solution containing SP-E and ASA in a 1 : 1 ratio (blue line, Fig. 2) doesn't show any absorption in this region, just a very weak band around 585 nm is found, due to the presence of a small fraction of the MC isomer, which increases when the solution is exposed to UV light. For these reasons, we decided to investigate the highlighted area around 430 nm that appears in the presence of all the three components.

Additionally, the three solutions present different colors (see photo insets in Fig. 2). Increasing the addition amount of ASA equivalents changes the color of the solution significantly from orange to yellow (details are shown in the ESI, Fig. S1†). The new absorption shoulder observed at around 430 nm presents a hypsochromic shift compared to the MC-Zn^{2+} band with its maximum at 490 nm. This is the first evidence of the formation of new species that we investigated through titration experiments for the whole system.

The experiments were performed using a 5×10^{-5} M concentration of the titrated compounds and progressive addition of 1×10^{-2} M solution of the titrant compounds. The analyzed samples present a concentration of 5×10^{-5} M for each component in MeCN solution. Two strategies were followed: (i) addition of an increasing amount of $Zn(ClO_4)_2 \cdot 6H_2O$ to a 1 : 1 ratio of SP-E and ASA and (ii) an addition of an increasing amount of ASA to a 1 : 1 ratio of SP-E and $Zn(ClO_4)_2 \cdot 6H_2O$ (consisting of a MC-Zn^{2+} solution previously equilibrated overnight). The obtained data are shown, respectively, in Fig. 3a and b.

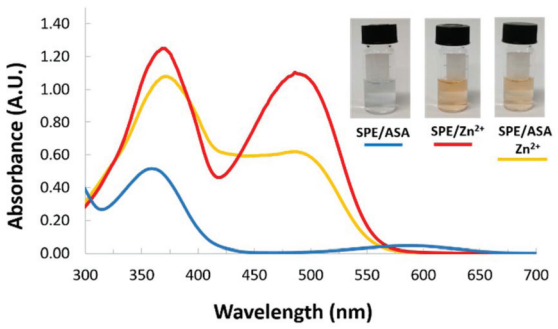

Fig. 2 Absorption spectra of MeCN solutions of SP-E in the presence of equimolar, 5×10^{-5} M, amount of Zn^{2+} (red line); ASA (blue line); ASA/Zn^{2+} (yellow line).

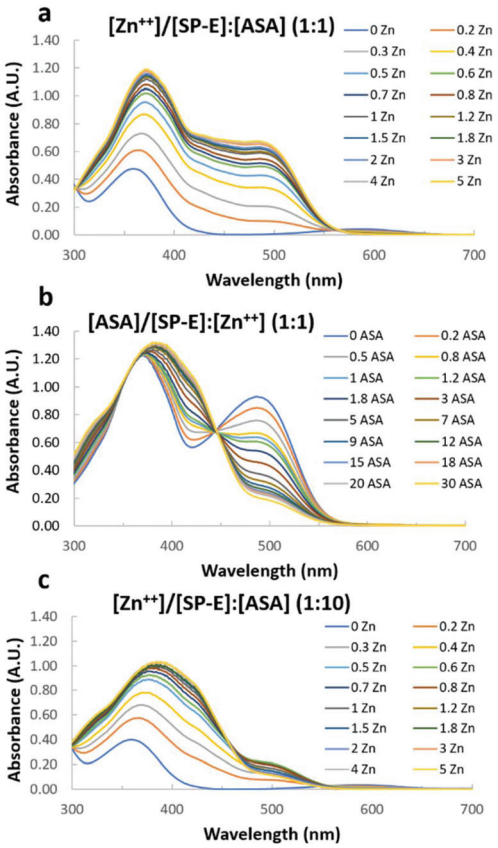

Fig. 3 UV/vis absorption spectra changes upon the addition of increasing amounts of titrating agents: (a) $Zn(ClO_4)_2 \cdot 6H_2O$ to a 1 : 1 ratio of SP-E and ASA; (b) ASA to a 1 : 1 ratio of SP-E and $Zn(ClO_4)_2 \cdot 6H_2O$; and (c) $Zn(ClO_4)_2 \cdot 6H_2O$ to a 1 : 10 ratio of SP-E and ASA.

Moreover, considering the results obtained by the strategies (i) and (ii) we performed another titration experiment (iii) achieved by the addition of increasing amounts of $Zn(ClO_4)_2 \cdot 6H_2O$ to a 1 : 10 ratio of SP-E and ASA (Fig. 3c). The spectra were measured after the equilibration of the ternary system with the aim to prove also its stability over time. The formation of the complex is not immediate; this process is correlated to the rate of the isomerization from the SP to the MC isomer and is reported in the irradiation experiments discussed in the next paragraph. To prove this, we measured the spectra after 3 h from the addition of the titration agent to ensure the investigation of the whole complex after its complete formation. Moreover, the whole complex is stable for 1 month (with no signs of precipitation and no variation in the absorption spectra or in the color). Data confirming these results and experimental details are reported in the ESI (Fig. S2†).

During the titration we analyzed the spectroscopic behaviour of the two absorption bands at 430 nm and 490 nm, which could correspond to the ternary system and the MC complex with the zinc(II) cation, respectively.

It is clear from Fig. 4 and 5 how the presence of ASA influences the equilibria in solution of the whole environment, and the titration spectra, analyzed at the selected wavelengths of 430 nm and 490 nm, highlight how the equilibria are different.

The absorbance spectra (Fig. 3) show different shapes depending on which titration agent is added. The different titration tests present different features and the comparison of the two selected wavelengths (430 nm and 490 nm) confirms these different trends. The (iii) titration experiment has been conducted in order to determine the properties of the system in a situation where the ASA component was in excess compared to the other components considering that in titration (ii) the amount of ASA needed to achieve the stability of the titration seemed to be 10 times more.

To conduct a deeper investigation of the system, UV/Vis measurements have also been conducted in MeCN solutions containing the SP-E molecule and a preformed acetylsalycilic acid-zinc(II) salt; these data are reported in the ESI (Fig. S4†)

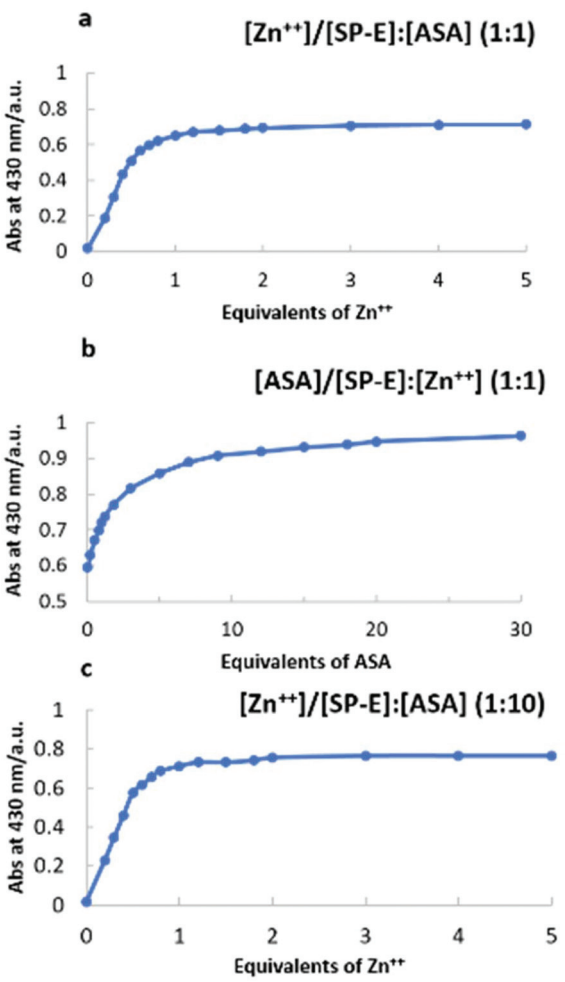

Fig. 4 Titration curves for the performed experiments analyzed at 430 nm upon the addition of increasing amounts of (a) $Zn(ClO_4)_2{\cdot}6H_2O$ to a 1 : 1 ratio of SP-E and ASA; (b) ASA to a 1 : 1 ratio of SP-E and $Zn(ClO_4)_2{\cdot}6H_2O$; and (c) $Zn(ClO_4)_2{\cdot}6H_2O$ to a 1 : 10 ratio of SP-E and ASA.

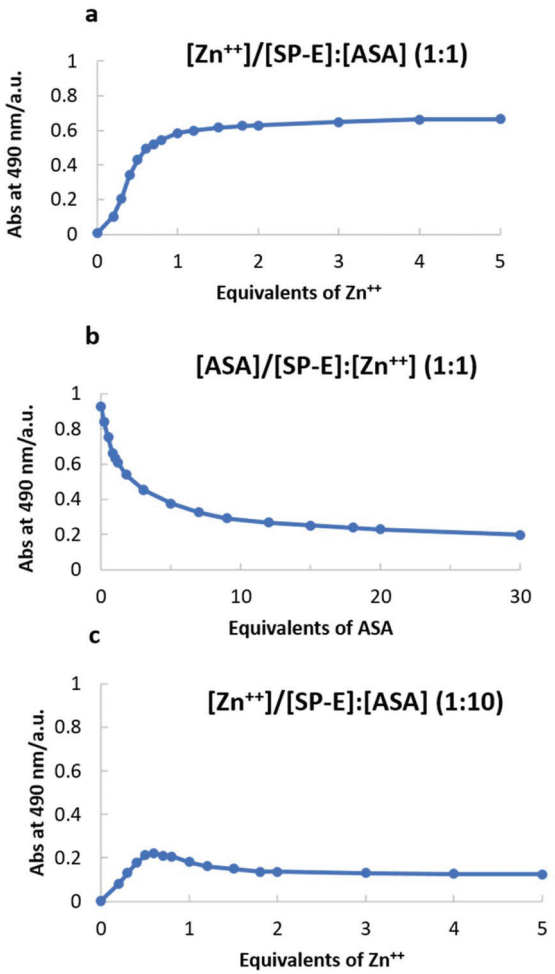

Fig. 5 Titration curves for the performed experiments analyzed at 490 nm upon the addition of increasing amounts of (a) $Zn(ClO_4)_2{\cdot}6H_2O$ to a 1 : 1 ratio of SP-E and ASA; (b) ASA to a 1 : 1 ratio of SP-E and $Zn(ClO_4)_2{\cdot}6H_2O$; (c) $Zn(ClO_4)_2{\cdot}6H_2O$ to a 1 : 10 ratio of SP-E and ASA; an enlarged version of (c) is reported in the ESI, Fig. S3.†

and will be deeply discussed in the Discussion section. The comparison measurements were performed with the aim to highlight how important it is to insert in the systems all the components without pre-interaction/pre-complex formation, with the goal to allow the formation of the systems between the three necessary elements, *i.e.* SP-E, ASA and the Zn(II) cation, representing the objective of this study.

Light-responsiveness of the system

The species in solution were studied to analyze their kinetic properties and the reversibility features that constitute a key point for the development of a light-triggered release. For these purposes we prepared solutions containing SP-E, ASA and $Zn(ClO_4)_2{\cdot}6H_2O$ in a 1 : 1 : 1.5 ratio and SP-E, $Zn(ClO_4)_2{\cdot}6H_2O$ and ASA in a 1 : 1 : 15 ratio (Fig. 6a and b). This corresponds to the plateau region in the titration experiments and ensures the presence of the ternary system from the spectroscopic viewpoint. After the sample preparation we allowed its equilibration by storing the sample in the dark

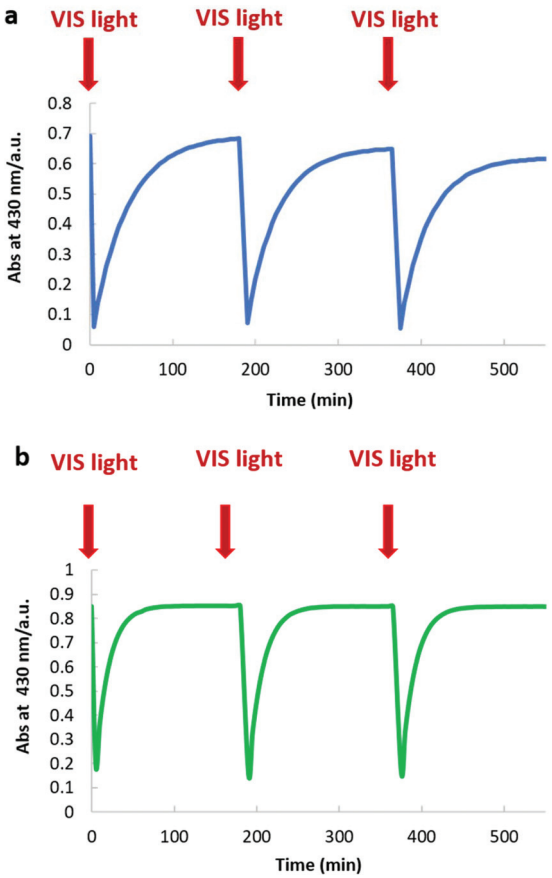

Fig. 6 The formation of the new ternary system is monitored at 430 nm for 3 hours in three consequential dark/light cycles for both the plateau region of the titration experiments: (a) SP-E : ASA : Zn^{2+} 1 : 1 : 1.5 and (b) SP-E : Zn^{2+} : ASA 1 : 1 : 15.

overnight, enabling the formation of the system. After the first measurement, the solution was irradiated for 5 min with a low-power LED torch (150 mW, 12 lumen) to evaluate the conversion of the SP moiety from the open to the closed isomer, resulting in the disappearance of the absorption in the visible region and the photochromic change of the solution. After the removal of the visible light source the samples were maintained under dark conditions and the re-formation of the complex was registered by measuring the sample every 5 minutes for 3 hours. The systems are completely reformed in 2 hours and they don't seem to degrade during these dark/light cycles, as shown in Fig. 6 where 3 sequential dark/light cycles are shown.

It is interesting to observe how the monitored reaction of the re-formation of the system is faster in the presence of excess of ASA; indeed the plateau region is achieved after around 70 min, while without the excess of ASA it reached after 120 min. This behaviour suggests that an excess of ASA helps in the formation of the ternary system. These cycling experiments were repeated on 3 different samples for the sample SP-E : ASA : $Zn(ClO_4)_2$ 1 : 1 : 1.5 to prove the repetitiveness in the formation of the ternary system and the results show a high rate of reproducibility with no significant statistical variation (details of the three measurements and the recorded spectra are reported in the ESI Fig. S5 and S6†).

Fluorescence spectroscopy

The MC isomer of the spiropyran molecule presents a π-electron delocalized system: this generates fluorescence emission in the visible region. In our design the ternary system is formed with the spiropyran in its MC isomer, and to demonstrate its presence we have analyzed the emission properties of the whole system. A solution containing the three interacting elements at 5×10^{-5} M concentration in a 1 : 1 : 1.5 ratio of SP-E : ASA : Zn^{2+} in MeCN was equilibrated in the dark for 4 h and the fluorescence was evaluated using a λ_{exc} = 430 nm, which corresponds to the absorption region of the ternary system and λ_{exc} = 490 nm, which corresponds to the absorption region of the complex between SP-E and Zn^{2+} (Fig. 7). The resulting spectrum shows that the sample presents fluorescence in the visible region with emission at λ_{max} = 603 nm with the excitation at 430 nm and λ_{max} = 616 nm with the excitation at 490 nm. An analogue measurement was also performed for the sample containing SP-E : Zn^{2+} : ASA in a 1 : 1 : 15 ratio in MeCN using the same excitation wavelengths at 430 nm and 490 nm (data reported in the ESI in Fig. S7†). The collected data suggest that the ternary system is present in its MC isomer form along with the SP-E : Zn^{2+} complex as also shown in the absorption spectra, while the MC isomer alone in MeCN solution presents an emission with λ_{max} at 670 nm, after its excitation at a wavelength of 585 nm that corresponds to the MC absorption maximum for the molecule alone in its MC form in MeCN.

High resolution mass spectrometry (HRMS)

MALDI-TOF mass spectrometry studies were conducted to prove the existence of the ternary system. This soft ionization technique has been chosen because it also allows the detection of fragile complexes. The data shown here (Fig. 8) highlight how in a solution of SP-E : $Zn(ClO_4)_2$: ASA in MeCN the peaks indicating the presence of 2SP + Zn + ClO_4^- and SP + Zn + ClO_4^- entities were found at 1067 *m*/*z* and 615 *m*/*z*, respect-

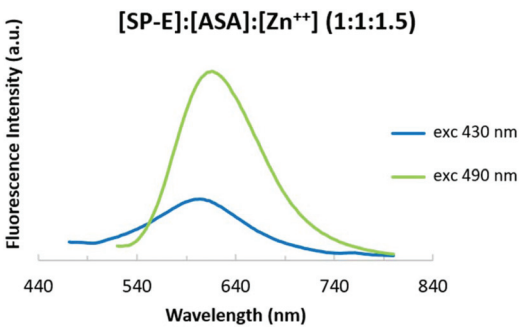

Fig. 7 Fluorescence emission spectrum of the SP-E : ASA : Zn^{2+} sample in a 1 : 1 : 1.5 ratio, concentration 5×10^{-5} M, λ_{exc}: 430 nm and 490 nm.

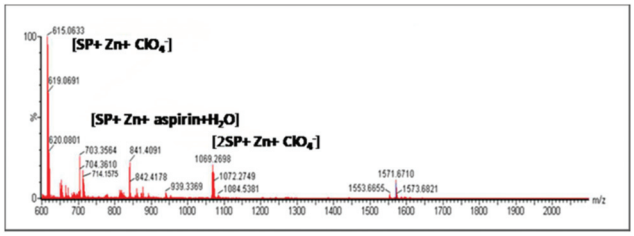

Fig. 8 High-resolution mass spectrum of the sample containing SP-E/ASA/Zn^{2+}.

ively. The same peaks are found in a solution of SP-E + $Zn(ClO_4)_2$ in MeCN, and the data are presented in the ESI (Fig. S8†). The presence of the peak at 714 *m*/*z* in the SP-E + $Zn(ClO_4)_2$ + ASA sample suggests the presence of a SP-Zn : ASA : H_2O system; indeed this *m*/*z* peak presents the exact value required for the precise SP : Zn : ASA : H_2O system itself and it is not found in the sample containing only SP-E + $Zn(ClO_4)_2$ (Fig. S7†).

Discussion

Our preliminary studies on this three component system were carried out to investigate the use of spiropyran photochromic entities as real tools for visible light activated release of drugs. The results obtained from steady-state UV/Vis absorption spectroscopy clearly indicate the formation of a new species among the three components in MeCN solution. The formation of a new band with a maximum around 430 nm, belonging to this new species, is deeply investigated in terms of formation and stability over time. We have conducted several control experiments among the three components (SP-E, ASA and Zn^{2+}) separately or in combination with each other to get more preliminary information and to reinforce our results. The data are reported in the ESI (Fig. S9 and S10†) and they are accompanied by a visual colorimetric overview of the photochromic features of all the possible combinations between the three elements in MeCN solutions. Titration experiments conducted between SP-E and ASA and between ASA and Zn^{2+} indicate that the new highlighted species is formed only in the presence of all the three components. Moreover, additional titration experiments were conducted with a preformed ASA–zinc(II) salt that was synthesized accordingly to a reported procedure,[29] with the only variation being the source of the salt ($Zn(ClO_4)_2 \cdot 6H_2O$ instead of $Zn(SO_4)_2 \cdot 7H_2O$). The titration experiments were conducted by adding this pre-synthesized salt to a constant amount of SP-E at 5×10^{-5} M concentration, following the same procedure used for all the reported titration experiments. The colour of the MeCN solution was observed to vary: in the analysis conducted with the preformed ASA–Zn(II) salt was pink coloured, while in the analyses performed with ASA or $Zn(ClO_4)_2$ titrants was orange. This analysis shows a different behaviour compared to that of the already performed ones as we can clearly observe in the reported spectra as shown in the ESI (Fig. S4†). The new ternary species is slower in its formation and the amount of the ASA–Zn(II) complex needed to reach a plateau region is higher (around 120 equivalents); moreover the system is not stable over time (precipitation was observed). The precipitated material in the samples is analyzed by MS spectrometry and basically consists of ASA, and the residual solutions analyzed after the precipitation phenomena show the absorbance spectra comparable to those before the precipitation which mainly show only the presence of the MC–Zn^{2+} complex. The results of these analyses suggest that the three components are not in the appropriate ratio to form the ternary system we want to investigate; indeed probably the zinc contribution is too low to form the ternary system and the zinc present is mainly involved in the formation of the salt with ASA and in the formation of the binary system. These data underline how our proposed ternary system shows better properties. The UV-Vis absorbance spectroscopy data collected and the titration curves analyzed at 430 nm and 490 nm highlight how, in the titration performed using ASA as the titrating agent, the two species show a competitive formation feature. While the absorption shoulder at 430 nm increases during the experiment the band at 490 nm decreases (Fig. 5): this can be interpreted as the formation of a new ternary system between the three elements despite the MC–Zn^{2+} complex. A deeper analysis on the plotted data shows that the stability, *i.e.* the plateau region, is achieved at different values depending on the titrating agents. In the case of zinc titration, the amount of equivalent needed to reach the plateau is around 1.2, while in the case of the ASA titration, the amount of equivalent is ten times more, indeed about 12 equivalents are necessary to reach a stable value. These data show how the binary system (SP-E : Zn^{2+}) and the ternary one (SP-E : ASA : Zn^{2+}) compete with each other with respect to their formation, where the one with two components is more favourite. Zinc titrations performed in samples containing ASA in excess (10 : 1 ratio to SP-E) have shown the expected trends. By the plotted data we can observe that in this experiment, while the species at 430 nm increases until a plateau constant region, the species at 490 nm presents a slight increase until around 0.6 equivalents, and it then decreases to a constant value at around 1.8 equivalents (Fig. S3† shows an enlargement of these data to highlight the phenomena). This trend could be an additional information that in the presence of an excess of ASA, in the case of zinc titration, the species at 490 nm seems to be limited in its formation because zinc is involved in the formation of the ternary system that is in competition with the formation of the binary one. The results obtained by MS spectrometry highlighted the presence of a SP-E : Zn^{2+} : ASA species in a 1 : 1 : 1 ratio; therefore we believe that the SP-E and ASA in a 1 : 1 ratio represent a good starting point to demonstrate qualitatively the presence of the ternary system in solution along with with the MC–Zn^{2+} complex. Our investigations have pointed out how an excess of ASA increases the formation of the ternary species from the quantitative view point; these data will be taken into consideration in future analyses. The suc-

cessful results obtained for the three component system in terms of stability, repetitiveness and visible light response/reformation evidence how the key feature of a light-regulated system is achieved for our proposed drug-delivery platform. The fluorescence emission properties of the SP-E/ASA/Zn^{2+} system upon excitation at the corresponding absorbance band highlight how the photochromic ligand is present in its MC isomer. Moreover, the emission properties of the SP-E/ASA/Zn^{2+} system excited at 490 nm confirm that the two binary and ternary systems have different properties and are both present in solution in their MC isomers. The emission spectra recorded in excess of ASA show the presence of MC isomers for both the wavelength used for the excitation (Fig. S7†). The emission spectra seem to be affected by the complexation phenomena, the excitations at 430 nm and 490 nm cause an excitation of both complexes (binary and ternary) that could be the reason for the slightly different positions of the peaks' maximum position. HRMS results show the formation of molecular assembly between the three components dissolved in MeCN; indeed the mass peaks belonging to both SP-E/Zn^{2+} and SP-E/ASA/Zn^{2+} complexes are observed. Analyzing this preliminary information, the investigated multicomponent system seems to contain several entities in solution and the investigations of their equilibria is useful for the final application of this unit.

Conclusions

The reported preliminary investigations highlight the great potential of our photo-responsive drug-delivery system. The assembly of a photochromic system, structured by the interactions between a metal cation, correlated to a pharmaceutically active compound, and its binding geometry with a spiropyran moiety, constitutes a promising scaffold for the simultaneous light-regulated delivery of two active agents (ASA and zinc(II) cation). The techniques used to perform the analysis have provided the fundamental preliminary data necessary to carry out deeper investigations. The system shows good spectroscopic features and its essential visible light reversibility has been proven. Further investigations involving X-ray crystallography and NMR spectroscopy could represent the next steps to define the formation of a three-component complex system in its whole feature and space orientation. These systems could be used as APIs for new medical devices such as bandages that could release the medicaments over the necessary areas only when the strips are removed from their envelope and they are applied over the skin under visible light. Moreover, they could also be investigated for internal body medicaments and their release and consequently activation can be obtained by the co-operation of optical fibers driving visible light stimuli.

The results obtained thus far show the possibility to "switch" the system with a suitable and safe external stimulus like visible light and open the way for achievable applications of smart responsive materials based on this photochromic system with final drug delivery goals.

Experimental

All starting materials and solvents were purchased in high-purity grade from Sigma Aldrich and used without further purification. Silica gel for flash chromatography (70–230 mesh, 60 a) was purchased from Carlo Erba Reagents (Italy). Solvents for UV/Vis absorption and fluorescence emission spectroscopic studies were purchased in the spectrophotometric or HPLC grade. 1 cm path length quartz cuvettes were used for UV/Vis absorption and fluorescence emission spectroscopic analyses. For solution spectroscopy studies, 1.00×10^{-2} M mother solutions of SP-E, acetylsalicylic acid and the metal salt in acetonitrile were freshly prepared and diluted to the appropriate concentration prior to the analysis. Absorption spectroscopy studies were performed on an Agilent Cary 8454 UV/Vis diode array spectrophotometer. Fluorescence spectra were recorded on a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer with 3 nm excitation and emission slits, 1 nm resolution, and 0.1 nm s^{-1} scan speed. The effect of visible irradiation/dark cycles were repeated three times to test the system's stability and was evaluated by means of absorption spectroscopy by using a Cary 6000i UV/Vis/NIR spectrophotometer; the solutions were exposed to broadband visible-light illumination by using a low-power LED torch (Varta EasyLine Trilogy, white LED, 150 mW, 12 lumen) and were allowed to equilibrate in the dark inside the spectrometer chamber while performing the measurement. Data were acquired every 5 min for 36 consecutive turns. HRMS spectra were recorded by MALDI-TOF MS; spectra were acquired using a Waters MALDI-Q TOF Premier spectrometer. The instrument was operated in the positive or negative reflectron mode as required and all samples consisted of MeCN solutions used for the UV-Vis absorption and fluorescence emission spectroscopy.

Conflicts of interest

There are no conflicts to declare.

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