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Research Article

A Novel Light-Responsive Azobenzene—*N*-Succinylchitosan Hydrogel Bead for Kojic Dipalmitate Delivery

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ABSTRACT

A simple light-responsive azobenzene-*N*-Succinylchitosan (AZO-NSC) polymer has been investigated for its ability to act as a drug carrier. The AZO-NSC hydrogel bead was prepared by the ionic gelation method for the controlled delivery of kojic dipalmitate. The structure and surface morphology of the hydrogel were characterized by FTIR and SEM, respectively. The hydrogel showed good light-responsive. The release of the encapsulated drug from the hydrogel was regulated by (*trans–cis*) photoisomerization of azobenzene moiety. The *in vitro* release behavior of drug from these hydrogel systems is revelative of the potential of the hydrogel for controlled drug delivery.

INTRODUCTION

The past few years, more attention has focused on the development of new drugs in conjunction with site specific and controlled drug delivery systems. The latter studies have mainly focused on biopolymers, which are responsive to physiological changes such as pH, temperature and external stimuli such as light and, at the same time, can release an adaptable dosage of the therapeutic agent [1]. This kind of biopolymer gels can undergo a discrete and reversible volume phase transition upon changes in their environmental conditions. However, the kinetics of these volume phase transitions induced by the temperature, the pH, or an electric field are usually limited by thermal diffusion or ion diffusion. In contrast to these variables, the imposition of light can be performed instantly [2]. Therefore, it is clearly desirable for the phase transition of a gel to be controllable with light.

In general, a light-responsive water soluble polymer consists of a photoreceptor such as a photoisomerizable N=N moiety, and a hydrophilic polymeric backbone.

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The *trans–cis* photoisomerization in the chromophores results in a change in the physical (or chemical) properties such as morphology and degree of swelling of the biopolymers [3-6]. The above property of light-responsive water soluble polymers makes them promising material for applications in drug delivery systems.

Among the light-responsive groups, azobenzene functionality, in particular, has attracted special attention because of its ability to undergo reversible N=N *trans–cis* isomerization under UV–vis light as well as the fact that the azobenzene moiety is cleavable by enzymes (azoreductases) produced by microflora of the gastrointestinal tract [7-11]. When azobenzene chromophores are incorporated in the main chain or attached to the side chain of polymers, the conformation changes of azobenzene induced by the isomerization shall produce a concomitant change in the physical properties of the azobenzene containing polymers. This has led to the use of azobenzene derivatives as a light-responsive trigger for control of polymers properties. Indeed, now there exist a large number of literatures about azobenzene-induced changes of physical properties of a variety of polymers [12-18].

In this study, we have developed a light-responsive hydrogel, based on 4-carboxy

azobenzene (AZO) and *N*-Succinylchitosan (NSC). NSC, which is a water-soluble derivative of chitosan, has unique characteristics in vitro and in vivo such as biocompatibility, low toxicity and long-term retention in the body. NSC is valuable as a drug carrier to prepare its conjugates with many kinds of agents due to having $-NH_2$ and –COOH groups [19-20].

On the basis of our previous work on *N*-Succinylchitosan-g-polyacrylamide/ attapulgite composite hydrogel [21], *N*-Succinylchitosan/alginate hydrogel bead for nifedipine delivery [22], and nifedipine-loaded pH sensitive alginate–chitosan hydrogel beads [23], novel AZO-NSC hydrogel beads were prepared. Then, the beads were used as a light-responsive controlled release system for the delivery of kojic dipalmitate. Kojic dipalmitate is commonly used in cosmetics due to its inhibition of melanin production. kojic dipalmitate loaded AZO-NSC hydrogel beads can absorb UV light with certain wavelength and then release the drug, to block the production of melanin, bleach skin and prevent skin against sun tan and ultraviolet radiation. The morphology and infrared spectrum of the beads, swelling characteristics, and release properties of kojic dipalmitate from the beads under UV irradiation were also studied, and the results were analyzed using a semi-empirical equation to reveal the drug release mechanism.

MATERIALS

N-Succinylchitosan (MW is 3×10^5, degree of substitution is 66%) was acquired from Lanzhou Institute of Chemical Physics, the Chinese Academy of Sciences (Lanzhou, China). 4-carboxy azobenzene was purchased from J&K Chemical Ltd (China). *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDAC) was purchased from Shanghai Medpep Co., Ltd (China). kojic dipalmitate(purity～98.0) was purchased from Beijing Brilliance Biochemical Co., Ltd (China). All other chemicals and reagents used were of analytical grade.

Synthesis of AZO-NSC Polymer

0.08015g NSC was dissolved in 2ml distilled water, followed by addition of EDAC 0.02348g. The resulting mixture was agitated slowly on a shaker. A solution of 0.00807g AZO in 1ml *N*, *N*-dimethylformamide (DMF) was added to the above NSC mixture and slowly agitated on a shaker overnight. The reaction mixture was then precipitated in 20ml of acetone. The precipitate was isolated by filtration. It was then washed with acetone, diethyl ether and ethanol, and subsequently dried in air overnight.

Preparation of AZO-NSC Hydrogel Beads

The AZO-NSC hydrogel beads were prepared by dropping aqueous AZO-NSC into a calcium chloride solution. Aqueous solution of AZO-NSC polymer was prepared by dissolving the appropriate amount of polymer in deionized water. The prepared aqueous AZO-NSC solution was then dropped into a gently stirred 4% calcium chloride solution through a 0.45mm syringe needle at a dropping rate of 1.2 ml/min. The beads were allowed to crosslink with Ca^{2+} in solution with gentle stirring at room temperature overnight. The calcium-crosslinked beads were rinsed with deionized water for several times to remove unreacted calcium chloride on surface and subsequently dried in air overnight.

Preparation of Kojic Dipalmitate Loaded AZO-NSC Hydrogel Beads

15mg kojic dipalmitate was dispersed in AZO-NSC solution. The other processes were the same as the preparation of AZO-NSC blank beads.

Determination of Encapsulation Efficiency and Loading Efficiency

The kojic dipalmitate loaded beads (10 mg) were pulverized and incubated in 10ml tetrahydrofuran (THF) at room temperature for 3h. The amount of free kojic dipalmitate was determined in the clear supernatant by UV-spectrophotometry (UV-2401PC, Shimadzu, Japan) at 254nm. Supernatant from the empty beads (without kojic dipalmitate) was taken as blank. The encapsulation efficiency (%) is the percentage of kojic dipalmitate contained within the hydrogel bead in relation to the initial amount employed. The loading efficiency (%) is defined as the weight percentage of loaded drug based on the weight of hydrogel bead. All samples were analysed in triplicate.

$$encapulation\ effciency(\%) = \frac{kojic\ dipalmiate\ loaded\ amount}{kojic\ dipalmitate\ initial\ amount} * 100 \quad \textbf{(1)}$$

$$loading\ efficiency(\%) = \frac{kojic\ dipalmiate\ loaded\ amount}{weight\ of\ drug\ loaded\ hydrogel\ bead} * 100 \quad \textbf{(2)}$$

Scanning Electron Microscopy

The surface morphology of air-dried hydrogels was determined using a scanning electron microscope (JSM-5600LV, JEOL, Ltd). Before

observation of SEM, all samples were fixed on aluminum stubs and coated with gold.

FTIR Spectroscopy

Fourier transform infrared (FTIR) spectra of the samples were taken from KBr pellets. The FTIR spectra over the wavelength range 4000~400cm^{-1} were recorded using a FTIR spectrometer (Thermo Nicolet, NEXUS, TM, USA), the number of scans per spectra is 16.

Photoisomerization of AZO-NSC Hydrogel

Photoisomerization of AZO-NSC hydrogel in aqueous dispersion (2 mg/ml) was investigated using 365nm irradiation. The absorbance at 325 nm, which corresponds to the Π–Π* transition (*trans* azobenzene moiety), decreased with time, and the photostationary state was reached within 15 min of irradiation. The dispersions were then kept in the dark and absorbance was recorded at different time intervals to determine the reversible isomerization process.

Swelling Characteristics of AZO-NSC Hydrogel Beads

Swelling characteristics studies were carried out to examine the effect of UV irradiation on the size of the hydrogels in aqueous. A dry sample of hydrogel beads (0.01017 g) were immersed in 5 ml deionized water at room temperature, and one of the samples was placed in the dark while the second one was irradiated with UV light (365nm). At specific time intervals, the samples were removed from the swelling medium and were blotted with a piece of paper towel to absorb excess water on the surface. The time taken for weighing the sample was kept to a minimum to minimize the error because of evaporation of water. Swelling ratio (SR) of the sample was calculated according to the following expression [24].

$$SR(\%) = [(W - W_o)/W_o] * 100 \quad (3)$$

Where W is the weight of the swollen test sample and W_0 is the weight of the dried test sample. This experiment was repeated three times to establish the correctness of finding.

Release Studies

The in vitro kojic dipalmitate release properties from the hydrogel beads were determined as follows: The kojic dipalmitate loaded hydrogel beads were suspended in aqueous media (20ml). One of the beakers containing the hydrogels was kept in the dark while the second one was placed inside the UV reactor, under irradiation (365 nm). 2 milliliters of the samples were taken from each of the beakers and was assayed for kojic dipalmitate through high-performance liquid chromatographic (HPLC) (Agilent 1100, USA), at different time intervals. The dissolution medium was supplied with 2ml fresh solution to maintain the total volume. The drug release percent was determined using Equation (4). The data points represent the mean ± standard deviation of three independent experiments performed.

$$Drug\ release(\%) = \frac{Rt}{L} * 100\% \quad (4)$$

Where L and Rt represent the initial amount of drug loaded and cumulative amount of drug released at time t.

Release Kinetics.

The mathematical models, first-order $\ln[1 - M_t/M_\infty] = -kt$, Higuchi $M_t/M_\infty = kt^{1/2}$, and zero-order $f(t) = kt$ equations were fitted to individual dissolution data with linear regression by SPSS 11.0 for Windows. The drug release mechanisms of hydrogel beads were described by a semi-empirical equation.

RESULTS AND DISCUSSION

Synthesis of AZO-NSC Polymer

AZO-NSC polymer was synthesized successfully by amidation reaction of the amino groups on NSC with the carboxyl groups on AZO. The reaction scheme is shown in Figure 1. The conjugation of the AZO with NSC backbone was ascertained by FTIR spectroscopy.

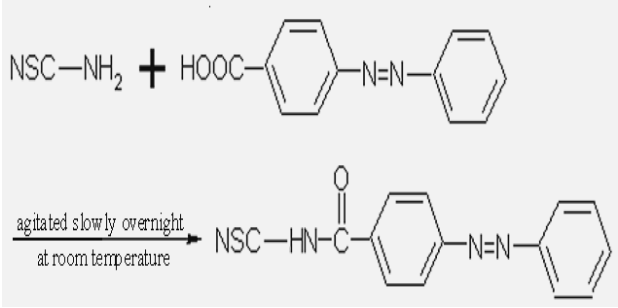

Figure 1: Synthesis of AZO-NSC polymer

Preparation of Kojic Dipalmitate Loaded AZO-NSC Hydrogel Beads

Having carboxylate ions (-COO$^-$) on polymer chains, NSC polymer should be a good candidate for Ca^{2+} crosslinked hydrogel beads. Gel formation was observed upon addition of aqueous AZO-NSC into a calcium chloride solution. This indicated that ionic crosslinks between the carboxylate ions (–COO$^-$) on AZO-

NSC can also be established by Ca^{2+}. Kojic dipalmitate was dispersed in AZO-NSC solution, and then the mixture solution was dropped into calcium chloride solution. Through this method, kojic dipalmitate can be encapsuled in the cavities of the hydrogel beads. The encapsulation efficiency (%) is 86.64±0.54. The loading efficiency (%) is 10.47±0.32.

Morphology Observation

Morphological study of AZO-NSC beads was investigated in this section. It can be seen in Figure 2 (a, b), the shape of kojic dipalmitate loaded AZO-NSC beads in the wet state was spherical and the surface was smooth. The color of the test beads appeared stramineous. After drying in air, the test beads had a rough surface with large wrinkles. The SEM pictures of hydrogel beads were illustrated in Figure 2 (c, d, e). As can be seen, the surface morphology was regular. Detailed examination of the surface structure revealed cracks and wrinkles caused by partly collapsing the polymer network during dehydration.

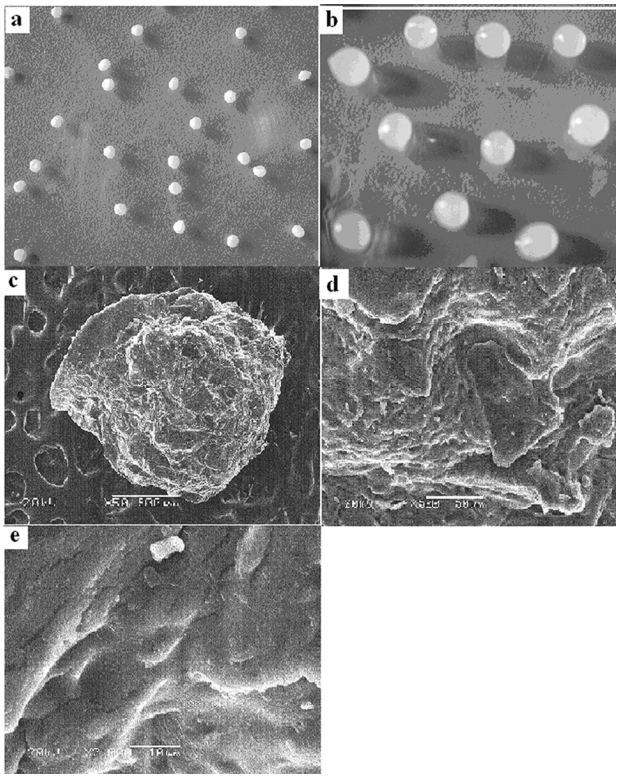

Figure 2: Photographs of: (a) dry kojic dipalmitate loaded AZO-NSC beads, (b) wet kojic dipalmitate loaded AZO-NSC beads. SEM micrographs of surface morphology of dry kojic dipalmitate loaded AZO-NSC beads: magnification (c×50); (d×500); (e×2000).

FTIR Spectroscopy

The FTIR spectra of *N*-Succinylchitosan, 4-carboxy azobenzene, kojic dipalmitate, AZO-NSC blank beads, kojic dipalmitate loaded AZO-NSC beads are shown in Figure 3. The FTIR spectrum of *N*-Succinylchitosan showed stretching vibration of –OH and $–NH_2$ at 3421 cm^{-1}, the weak band of $–CH_2$ stretching at 2924 cm^{-1}, the C=O stretching of amide I band at 1657 cm^{-1}, and the amide II band at 1569 cm^{-1}[25]. The peak at 1406 cm^{-1} belongs to $–COO^-$ symmetric stretching vibration, the peaks observed at 1068 and 1029 cm^{-1} were the secondary hydroxyl group (characteristic peak of –CH-OH in cyclic alcohols, C–O stretching) and the primary hydroxyl group(characteristic peak of $–CH_2$-OH in primary alcohols, C–O stretching)[26].

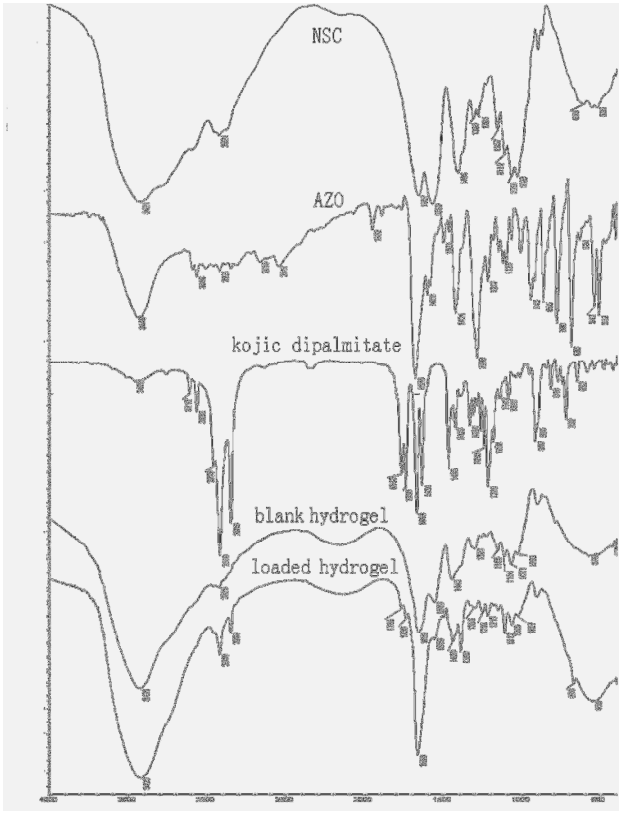

Figure 3: The FTIR spectra of *N*-Succinylchitosan, 4-carboxy azobenzene, kojic dipalmitate, AZO-NSC blank beads, kojic dipalmitate loaded AZO-NSC beads.

4-carboxy azobenzene showed the following distinct peaks: (1)The sharp peak at 1424 cm^{-1} arises from the aromatic N=N stretching vibration ; (2) the aromatic ring stretching bands at 1603 cm^{-1}; The peak at 688 cm^{-1} belongs to the out-of-plane deformation of azobenzene ring; the sharp peaks observed at 1288 $cm^{-1}$was the in-of-plane deformation of azobenzene hydrogen; (3) The peaks at 1678 cm^{-1} were the characteristic absorption band of the C=O

stretching vibration; The absorption bands at 3446 cm^{-1} was the stretching vibrations of O-H.
For the AZO-NSC blank beads, three bands at 1658 cm^{-1}, 1558 $cm^{-1}$and 1300 cm^{-1} are corresponding to C=O,N-H and the C-N stretching vibrations of –CONH-, respectively. Compared to the FTIR spectra of *N*-Succinylchitosan , the characteristic absorption bands of –CONH- became broad, which indicated that new amide bonds were formed between NSC and AZO.

In the FTIR spectra of kojic dipalmitate, the peaks at 1739 cm^{-1}, 1218 $cm^{-1}$and 2918 cm^{-1} were the characteristic absorption band of kojic dipalmitate. Those peaks were observed in the FTIR spectra of kojic dipalmitate loaded AZO-NSC beads, which confirmed that kojic dipalmitate was physically filled in the polymeric network.

Photoisomerization of AZO-NSC Hydrogel

Photoisomerization of AZO-NSC hydrogel dispersed in aqueous solution was investigated through irradiation at 365 nm. Before irradiation, the hydrogel consisted of only the *trans* form of the AZO moiety because it is thermodynamically more stable than the *cis* form. At room temperature, UV irradiation of the *trans* isomer converted it to the *cis* isomer. Figure 4 shows the maximum adsorption at 325 nm corresponding to Π–Π* transition (N=N *trans*), where the intensity is observed to decrease as the time of UV exposure increases. While new peaks appear at 420 nm due to the n-Π* transitions of the *cis* isomer, and the intensity increases as the time of UV exposure increases.

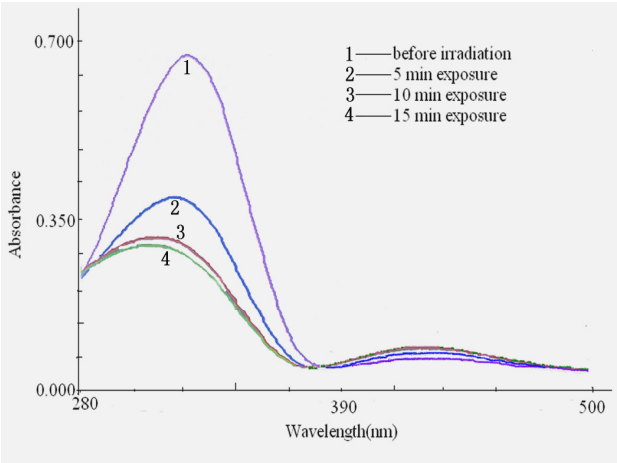

Figure 4: UV–vis spectra of *trans*-to-*cis* isomerization in AZO-NSC hydrogel

In aqueous dispersion of hydrogels, photostationary state was reached within 15 min of irradiation (no further change takes place in the intensity of the band at 325 nm). The reversible *cis–trans* isomerization in the hydrogels was studied in order to establish the stability of the *cis* form within the polymeric network, which is essential for sustained release of drugs. This was done by keeping the hydrogels dispersion in the dark. As the *cis* isomer is thermodynamically less stable, it spontaneously isomerizes back to the *trans* form due to thermal energy. The reverse *cis–trans* isomerizations at different time intervals were also shown in Figure 5. The results establish the fact that the reversible *cis–trans* isomerization process was relatively slow.

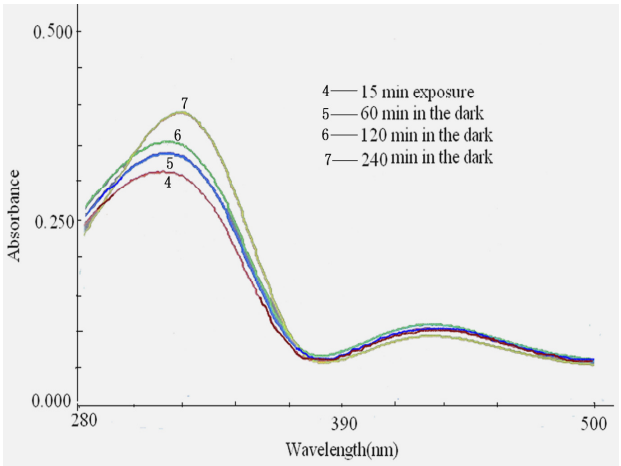

Figure 5: UV–vis spectra of *cis*-to-*trans* reversion in AZO-NSC hydrogel

Swelling Characteristics of AZO-NSC Beads

The swelling results of AZO-NSC beads were shown in Figure 6. The maximum swelling ratios of AZO-NSC beads under irradiation were much higher than those in the dark. The swelling behaviors of AZO-NSC beads in the dark may be attributed to the hydration of the hydrophilic groups of AZO-NSC polymer [27]. In aqueous medium, the hydrophilic groups, including amino groups, carboxyl groups and hydroxyl groups, can hydrate. In this case, free water penetrates inside the beads to fill the inert pores among the polymer chains, and subsequently the beads swell. When AZO-NSC beads under UV light irradiation, azobenzene isomerize (*trans–cis*), the hydrophobic interactions in cross-linked azobenzene side-chains decrease, and then the inner hydrophobic cavities expanded (swelled). Disintegration of test beads was observed at the end of swelling.

Table1: Parameters of the mathematical models and descriptive statistics of regression for the dissolution data of kojic dipalmitate loaded AZO-NSC beads under different conditions

Different conditions	$ln[1-Mt/M\infty]= -kt$	$Mt/M\infty = kt^{1/2}$	$F(t)=kt$
under UV	$ln[1-F(t)]$=0.0021t+4.5167 r=0.9646	$F(t)$=3.4648 $t^{1/2}$- 8.3764 r=0.9813	$F(t)$=0.1228t+12.07 r=0.9432
in the dark	$ln[1-F(t)]$=0.0015 t+4.5144 r=0.9687	$F(t)$=2.8591 $t^{1/2}$- 6.4614 r=0.9863	$F(t)$=0.1008t+10.518 r=0.9423

*$Mt/M\infty$ is the fractional release of the drug at time *t;* r^2, determination coefficient; *k*, dissolution rate constant

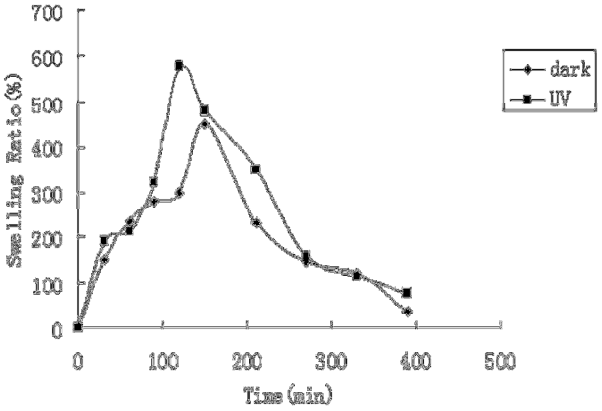

Figure 6: The influence of UV light irradiation on the swelling characteristic from AZO-NSC hydrogel beads

Release Properties of Kojic Dipalmitate from AZO-NSC Beads

A comparison of the release behavior of loaded samples under different conditions revealed that the rate of release of the encapsulated active molecules from the *trans*-isomerism of hydrogels was slower as compared to that from the *cis*-isomerism (Figure 7). In each set of experiments under UV and in the dark, the experimental parameters were kept the same. With the *trans–cis* isomerization in the hydrogels, the hydrophobic interactions in cross-linked azobenzene side-chains (in hydrogels) decrease with concurrent expansion (swelling) of the inner hydrophobic cavities, resulting in increase of diffusion rate of the drug through the polymer matrix. For all examined hydrogel beads, significant early release of kojic dipalmitate was observed. This could be because of the heterogeneous distribution of kojic dipalmitate in the gel beads. Diffusion and migration of kojic dipalmitate may occur during the drying process as water moved to the gel surfaces and evaporated. kojic dipalmitate may diffuse by convection with the water, leaving an uneven drug distribution across the gel, with higher concentrations at the surface, which leaded to early burst-like release as in Figure 7. The cumulative release values of kojic dipalmitate became constant after 500 min for each group of beads. The amount of kojic dipalmitate released from the hydrogel beads cannot approach 100%, this is because some drug molecules may be entangled within the hydrogel network, and those cannot be released unless polymer matrixes are degraded completely.

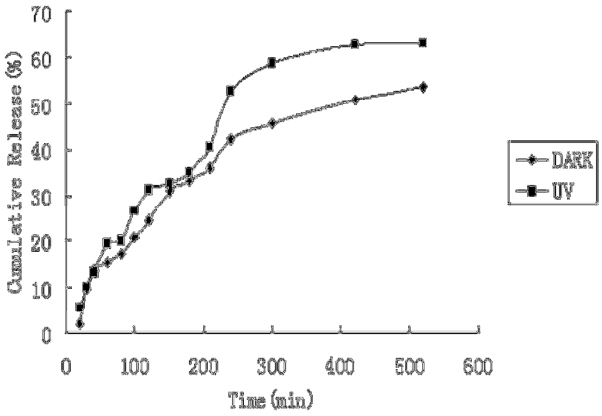

Figure 7: The cumulative release curves of kojic dipalmitate from AZO-NSC hydrogel beads under UV and in the dark.

The swelling behavior of the beads under UV irradiation is mainly attributed to the hydration of the hydrophilic groups of NSC moieties and the hydrophobic interactions between the AZO moieties. When the AZO moieties are in the more planar *trans* conformation, hydrophobic interactions and stacking between the AZO groups are favored. When the AZO moieties are photoisomerized to the skewed *cis* conformation, the hydrophobic association and the stacking between *cis* AZO groups are inhibited. It appears that, in aqueous media, a higher degree of swelling occurs loosening the hydrophobic interactions and the efficient isomerization in the AZO moieties thus causes a faster release of the drug molecule. This is attributable to the fact that, in aqueous media, the gels get effectively hydrated and swell adequately to cause a greater degree of *trans–cis* photoisomerization leading to faster rate of diffusion of the entrapped species from the hydrophobic pockets of the gel network.

Release Kinetics

It can be seen in Table 1, drug release model fitted to Higuchi equation, which could not explain drug release mechanisms. In many experimental situations, including the case of drug release from swellable polymeric systems, the mechanism of drug diffusion deviates from the Fickian equation and follows a non-Fickian (anomalous) behavior. In these cases the following general equation or its logarithmic form can be used [28-29].

$$M_t/M_\infty = kt^n \qquad (5)$$

Where M_t/M_∞, is the fractional release of the drug at time *t, k* is the constant related to the structural and geometric characteristic of the device, and *n* is the swelling exponent, indicative of the drug release mechanism. For spheres, values of *n* between 0.43 and 0.85 are an indication of both diffusion controlled drug release and swelling controlled drug release (anomalous transport). Values above 0.85 indicate case-II transport which relate to polymer relaxation during hydrogel swelling. Values below 0.43 indicate that drug release from polymer was due to Fickian diffusion [30-31]. The results are shown in Table 2. The release of drug from AZO-NSC beads took place by both diffusion through the swollen matrix and relaxation of the polymer.

Table 2: Estimated parameters and drug release mechanism of kojic dipalmitate loaded AZO-NSC beads under different conditions: Kinetic constants (*k*), diffusional exponents (*n*), correlation coefficient (*r*), and drug transport mechanism

Different conditions	*n*	*k*	*r*	Drug transport mechanism
under UV	0.7352	0.8132	0.982	anomalous transport
in the dark	0.8261	0.4250	0.926	anomalous transport

*Kinetic constants (*k*), diffusional exponents (*n*) and correlation coefficients (*r*) by linear regression of log ($Mt/M\infty$) vs log*t*; k is the constant related to the structural and geometric characteristic of the device; *n* is the diffusional exponents, indicative of the drug release mechanism.

CONCLUSION

In this study, we report on a new class of light-responsive hydrogels based on non-covalently crosslinked AZO-NSC hydrogels for controlled release of drugs. It emerges that the in vitro release behavior of drug from these polymeric hydrogel systems is influenced by their photoisomerization properties that in turn regulate hydrophobic characteristics of the hydrogels. These findings admit of potential therapeutic applications of hydrogels systems in which controlled release of the drug molecule from the matrix is central towards the light-responsive character of the moiety embedded in the polymer matrix.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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