Real Time Detection of Photoreactivity in Pharmaceutical Solids and Solutions with Isothermal Microcalorimetry

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Purpose. In this study an irradiation cell made as an accessory for an isothermal microcalorimeter is introduced, and its suitability for detection photoreactivity in pharmaceutical solutions and solids is demonstrated. The pharmaceuticals employed are chosen as sample materials to evaluate the usefulness and stability of the irradiation cell.

Methods. An irradiation cell has been constructed and tested in an isothermal microcalorimeter with pharmaceutical solutions and solids known to be sensitive to daylight or UV light. Light is produced with an Xe-arc lamp, split into two parts and introduced into calorimetric vessels with optical light cables. One of the vessels containing the reference sample gives the response to the heat absorbed by the material (radiant power), and the other vessel containing the sample material gives the response also to the photoreaction. The two irradiation cells are positioned in the sample sides of two separate twin microcalorimetric units.

Results. Nifedipine and L-ascorbic acid were found to be photosensitive in solutions and solid states, the extent of the degradation depending on the irradiation intensity and wavelength. The threshold values of the wavelength for the photoreactions, as well as the wavelengths for the maximum reaction rates, were estimated via the scanning irradiation measurements. The ability of photons with different energies to produce heat in the photosensitive reaction of nifedipine was calculated using constant λ measurements.

Conclusions. The technique introduced offers a rapid and versatile method to study the photosensitivity of materials in any state. In the measurements, various conditions can be simulated and thus provide information on the real behavior of materials.

KEY WORDS: photoreactivity; photochemistry; isothermal microcalorimetry; nifedipine; ascorbic acid.

INTRODUCTION

External factors such as heat, moisture, oxygen and acidity of the surrounding atmosphere, together with light, may dramatically affect the instable behavior of materials individually or via co-action. The factors can cause both physical and chemical changes in the material. Many pharmaceutical drug molecules are known to be sensitive to light and to degradate chemically under energetic light. Photosensitivity of molecules is often studied in solutions, and the changes in the concentrations have been examined by chromatography (1–4) and spectrophotometry (2,5). Discoloration of powders has been examined by colorimetry (6,7). When a new molecule or photostabilization of a photolabile drug with packaging material, films and colorants is under study, it would be advantageous to detect the effects of light in the sample material in real time. Furthermore, a kinetic study demands a long observation time with ordinary techniques to get reliable results if the degradation reaction is slow. If the kinetic study is made with solutions, the results are difficult or impossible to extrapolate to the reaction in the solid state. Thus, every form of existence must be studied separately, and, if possible, in the form identical to the actual drug formulation.

Thermodynamical examination of photosensitive reaction with calorimetric measurement techniques has been applied already for years (8). Different types of calorimeters have been developed for different purposes and the field of applications covers not only biology and organic chemistry but also materials science. However, to observe slow and low-energetic reactions, few, hardly any instruments have been developed; the sensitivity and stability of the apparatus would be of great importance.

Nifedipine (dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl) pyridine-3,5-dicarboxylate, $C_{17}H_{18}N_2O_6$) is a well-known photolabile substance, which decomposes concurrently into four components when exposed to daylight or UV light (9–11). The photodegradation of nifedipine has been widely studied in solution and solid state as small crystals, and the degradation mechanism has been closely examined. Moreover, the possibilities to protect nifedipine from light have been studied intensively (12,13).

L-Ascorbic acid (3-oxo-L-gulofuranolactone, $C_6H_8O_6$) also undergoes photochemical degradation upon exposure to light (14), but the rate is slower when compared with nifedipine in solution. Beside the photostability of ascorbic acid as a pure solution, also the effects of dyes (15) and sweetenings (16) have been studied and the influence of hydroxyl radicals in the solution has been determined.

In this paper an irradiation cell made as an accessory instrument to the commercial isothermal calorimeter is introduced, and its suitability for use in the detection of photoreactivity in pharmaceutical liquids and solids is investigated. The materials employed are chosen as sample materials to evaluate the usefulness and stability of the irradiation cell.

EXPERIMENTAL

The irradiation cell was designed for use with the commercial isothermal heat conduction microcalorimeter TAM 2277 (Thermometric AB, Sweden) which is described elsewhere (17). A diagrammatic drawing is presented in Fig. 1; the cell was engineered to fit into the commercial 4 ml ampoule microcalorimetric unit. The design was based on the invention presented in (18) and (19). Light was produced with a 75 W Xe-arc lamp (Hamamatsu, Japan) which was mounted in an Oriel 60115 lamp house. From the house the light was introduced through a grating monochromator (Oriel 77250) via focusing mirrors and a shutter, after which the light enters two identical 1 mm optical cables. The wavelength can be controlled with a PC driven stepper motor (Oriel 77325). The band width used in this study was 23 nm (FWHM) (Fig. 2). The spectral irradiances in Fig. 2 were measured at the end of the light cable before entering the cell using another monochromator (Oriel) with a CCD array detection. The light cables from the monochromator

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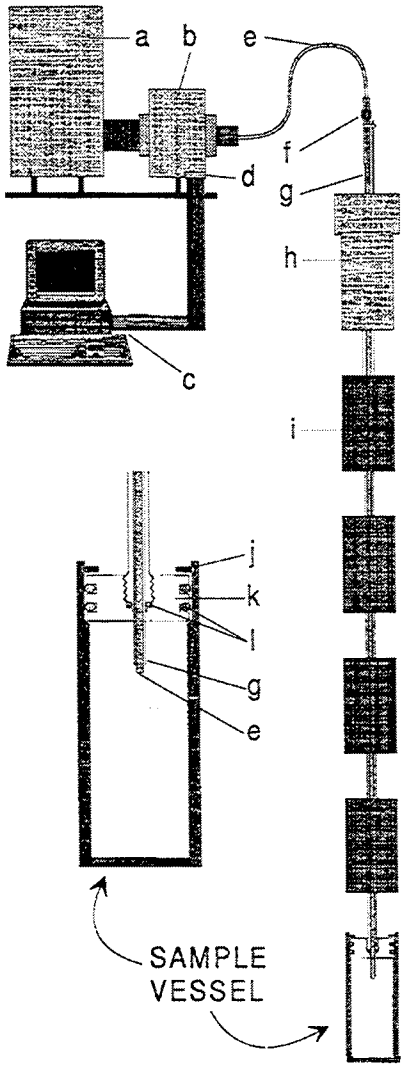

Fig. 1. Diagrammatic construction of the irradiation cell. The beam from the monochromator is split into two parts and conducted into identical irradiation cells. a, lamp house; b, monochromator; c, computer; d, step motor; e, light cable; f, SMA connector; g, insertion tube for light cable; h, blastic holder; i, heat exchanger; j, locking ring; k, teflon lid; l, O-ring.

were connected with SMA connectors to the identical cables entering the irradiation cell and the sample vessel. The insertion light cable was fed into a stainless steel tube all the way down to the vessel. A silicone sock at the upper end of the tube was used to prevent convection. The lower end of the cable was moulded into the tube with epoxy resin to prevent evaporation. The ends of the cables were polished. To prevent thermal conduction to the shaft tube, the lid of the vessel was made from teflon, and it was jointed to the irradiation cell with thread and was removable. The insertion tube was fixed tightly to the teflon lid with an O-ring. The stainless steel sample vessel was sealed to the lid by the use of two O-rings and was fastened with a circlip locking ring. After closing the ampoule, the extra pressure generated by pushing the teflon lid into place was removed by pulling away a constantan string left between the vessel and the lid during the closing.

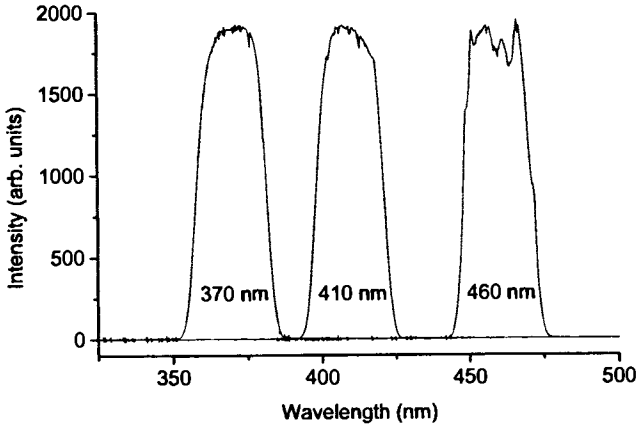

Fig. 2. Spectral irradiance for the light of 460 nm, 410 nm and 370 nm used in the irradiation of the samples at a constant wavelength. The intensities for the wavelengths are not comparable with each other.

During the actual measurement two separate and technically identical irradiation cells are positioned in the sample sides of two twin calorimetric units. Hermetically sealed stainless steel vessels filled with a proper reference material, e.g., ethanol or water, are positioned in the reference sides. One irradiation cell is employed as the actual photocalorimetric reaction vessel that gives the response to both the thermally active reactions (including the photosensitive reactions) and absorption of the light. The other irradiation cell operates as a reference cell giving a response only to the light absorption, i.e., radiant power. This arrangement enables the detection of changes in the intensity of light during the measurement and calculation of the energy of light fed into the sample vessel. Prior to the measurements with samples, the reference runs must be performed for both of the cells filled with the appropriate reference material, to calculate the correction parameters to get the signals for the absorption of the split light beams equal (cf. Theory section). With the reference runs the irradiance power lost by the back irradiation through the fiber, which is dependent on the optical properties of the vessel, can also be estimated. In this study, empty vessels for solid samples, 2.5 ml ethanol for nifedipine solutions, and 2.5 ml water for ascorbic acid solutions, were used in the reference runs. The microcalorimetric response from the reference cell filled with 2.5 ml ethanol for irradiation of 460 nm and 410 nm is shown in Fig. 3. The heat flow curves in Fig. 3 are obtained with different irradiation cells, indicating slight differences between them. However, the baseline stability is fairly good, especially when the aging of the lamp and the effects of the surroundings on the intensity are considered, the signal noise and drift being within the limits of ± 0.5 μW over 40 hours. The data points have been recorded every 60 seconds as an average of 10 seconds.

The irradiation cells constructed can be equipped with two gas tubes inserted in the shaft tube of the cell. Through the gas tubes the sample can be perfused with various gases to get information about, e.g., the influence of oxygen in the oxidation process. However, the system employed in this study was closed, even with the powder samples. In this case, the desired humidity can be generated in the vessel with a miniature chamber filled with purified water or saturated salt solution. It should be borne in mind at this point that all the extra things inside the vessel affect the absorption of the light.

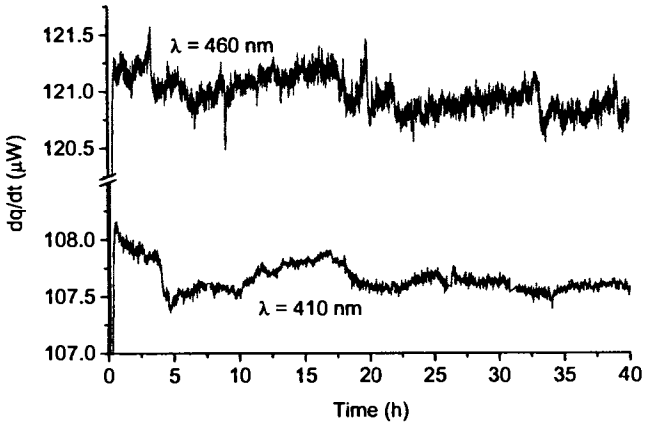

Fig. 3. The heat flow signals from the photoinert side at wavelengths of 460 nm and 410 nm when the vessel is filled with 2.5 ml ethanol.

The sample materials, nifedipine and L-ascorbic acid, were purchased from Sigma. Ethanol was 99.5% concentrated and water was deionized. The samples for the measurements were prepared and handled in a darkened room under red light. In the preparation of solutions with various concentrations the weighing was performed to an accuracy of 1%. With nifedipine solutions, 40 mg powder was dissolved in 2.5 ml ethanol, and with L-ascorbic acid solutions, 100 mg powder was dissolved in 2.5 ml water. The solutions were stored in the dark at room temperature for 24 hours before the measurements and were used as samples as such. When powders were being studied, a standard load of 100 mg was employed with both of the materials.

THEORY

As the beam of light is split into two parts after the monochromator, the parts entering the light cables differ from each other in intensity and wavelength. As the monochromatizing is performed with a grating, the spatial wavelength distribution is not symmetric in the exit slit but varies from one side to the other. Thus, the wavelength distributions between the light cables are not equal if the ends of the cables are not positioned properly in regard to the exit slit. In a calorimetric measurement, where monochromatic light is employed, this is observed as a difference in the levels of the heat flow signals due to the energy difference of the light quants. The same effect is observed when the intensities of the split beams differ. In a scan measurement, when the wavelength is varied linearly during the measurement, the difference in the wavelength distribution is observed as an overall shift in the heat flow curve retaining the same shape. With the help of reference runs with empty ampoules, or filled with ethanol or water, the correction for the heat flow signal from the photoinert side ($(dq/dt)_{ref}$) should be made as

$$\left(\frac{dq}{dt}\right)_{ref.corr} = \left(\left(\frac{dq}{dt}\right)_{ref} - k_t\right) \cdot k_p \qquad (1)$$

where the time axis is corrected with k_t (wavelength shift) and the heat flow axis with k_p (intensity correction). The correcting factors are determined so that the heat flow signals $(dq/dt)_{ref}$ and $(dq/dt)_{sample}$ from the irradiation cells in the reference run coincide. When the measurements are performed with monochromatic light the factor $k_t = 0$.

The intensity of the light used for the irradiation of the sample varies during the measurement and between the measurements due to aging of the lamp and the optical fibers. Also, changes in the component arrangements, e.g., replacement of light cables or the lamp, affect the intensity. Thus, it would be advantageous for correct comparison of photoinduced processes at various wavelengths to express the progress of the photoreaction not as a function of time, but as a function of the total intensity of the irradiation. With the use of the measurement arrangement described above, it is simple to determine the total irradiation intensity I_t (7) absorbed by the sample to time t as

$$I_t = \int_0^t \left(\frac{dq}{dt}\right)_{ref} dt \qquad (2)$$

for monochromatic light. On the other hand, the number of the photons n in the irradiation can be determined, since for the monochromatic light the energy is achieved from

$$E_\lambda = \frac{h \cdot c}{\lambda} \qquad (3)$$

(h = Planck's constant, c = speed of light and λ = wavelength) and the total irradiation intensity from

$$I_t = n \cdot E_\lambda \qquad (4)$$

Since the wavelength distribution is symmetric, Equation (3) can be used to estimate the energy of irradiation in this study. Using the equations above, the ability of the quants with different energy to cause the photoreaction can be estimated.

RESULTS AND DISCUSSION

Scan Measurements

Various scanning rates and ranges were used, but scanning the range from 700 nm to 280 nm in 1110 min was found to be the most practical, since the slow rate enables reasonable resolution and the entire measurement cycle could be carried out in 24 hours in this way. When the samples were loaded in the vessels and the irradiation cells were lowered into the measurement positions of the calorimetric units, it was waited until the heat flow signals reached steady base levels and a light of 700 nm was conducted into the vessels. The lamp was turned on several hours before the shutter was opened and light was conducted into the vessels. This was done to minimize the fluctuations in intensity due to warming of the components of the lamp house, monochromator and power supply. As the heat flow signals reached their steady state at 700 nm the step motor was turned on and the scanning was started. After the scans the steady heat flow levels at 280 nm were checked and the light was disconnected by closing the shutter to obtain the base levels after the scans. The base levels were used as the baselines and were corrected for the actual heat flow signals. The maximum heat flows during the scans were obtained at 467 nm for the Xe-lamps used, and varied from 130 μW to 330 μW depending on the optical arrangements. The measurements were performed at 25°C if not otherwise stated.

Nifedipine

A typical scan measurement for the nifedipine solution at 25°C is shown in Fig. 4 where the heat flow signal from the

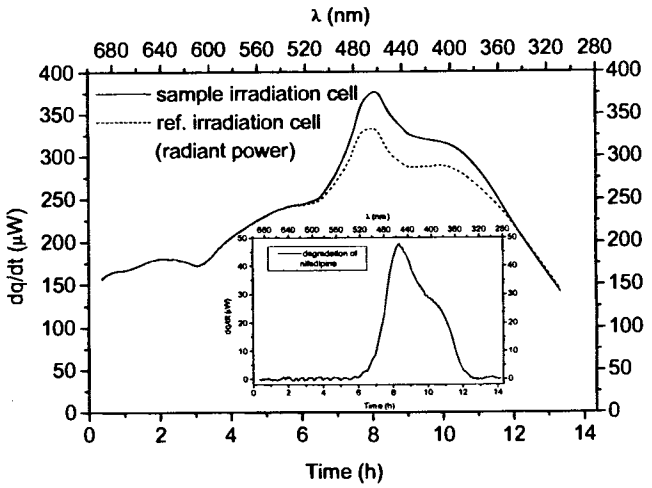

Fig. 4. Scan measurement for the photoreaction of nifedipine in solution. In the inserted figure, the signals from the sample and the reference irradiation cell have been subtracted. The reference material used was 2.5 ml ethanol.

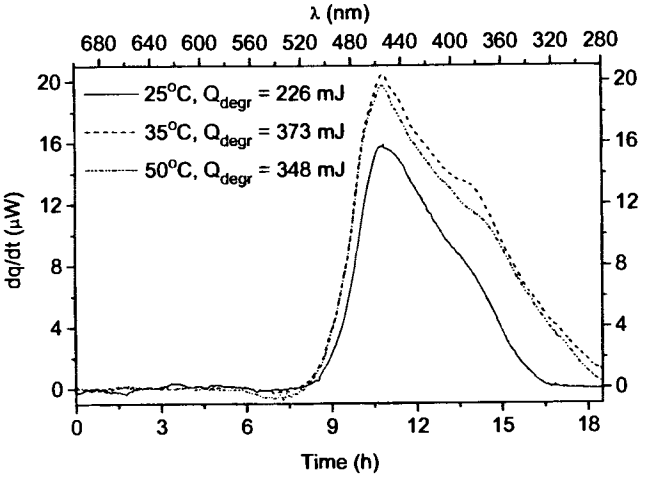

Fig. 5. Photoreaction of nifedipine at 25°C, 35°C and 50°C during scan measurements. Corresponding reaction heats are 226 mJ, 373 mJ, and 348 mJ.

reference cell filled with 2.5 ml ethanol has been corrected according to Equation (1) to correspond to the intensity changes of the irradiation in the sample cell during the scan. In this particular case, the radiant power obtained was high compared to the other measurements presented in this paper, which was mainly due to the replacement of the broken lamp. When the heat flow signals from the sample and the reference cells have been subtracted from each other, the response from the phosensitive reaction of nifedipine is obtained. Integration of the photoreaction peak gives the heat for the photoreaction process during the irradiation, and if the total heat evolved in the process is known, the extent of the process can readily be calculated. The more intensive the irradiation, the more extensive the photoreaction and the greater the thermal response obtained in the calorimetric measurement. The threshold wavelength for the light-induced reaction is at ca. 510 nm, which after the reaction rate rises rapidly to reach its maximum at 455 nm. After this, the rate reduces along with the irradiation intensity and returns back to zero level before the end of the scan. When a subsequent scan is performed with the same sample solution, an identical photoreaction signal is obtained but the reaction heat has slightly increased. Thus, it is evident that the decrease in the photoreaction signal at short wavelengths is not due to a significant loss of the concentration of nifedipine. Moreover, the decrease in the irradiation intensity cannot explain the behavior alone. It seems that nifedipine molecules are sensitive to light only in the range of 510 nm–280 nm, this result being in keeping with results reported earlier (13).

Chemical reactions are normally accelerated by temperature and the dependence of the reaction rate on temperature is expressed as the activation energy obtained from the Arrhenius equation. The identical scan measurements with nifedipine solution were performed not only at 25°C but also at 35°C and 50°C to investigate the influence of temperature on the photoreaction of nifedipine (Fig. 5). The threshold value of 510 nm is slightly shifted into longer wavelengths, and the heat of the photoreaction is increased at higher temperatures. This seems to be due to the overall acceleration of the reaction, but especially due to the rising of the tail in the photoreaction signal at short wavelengths. As the irradiant power was here quite unchanged (±2%) from one scan measurement to another, temperature presumably has an influence on the efficiency of the irradiation.

Materials behave in different ways under light depending on their state. In solutions, photodegradation can proceed rapidly through the entire sample, but in solid state the penetration of photons into the material is the main restricting factor. Also, the surroundings of the molecules in solutions differs from the solid state, giving the different opportunities for the formation of the free radicals required in the photodegradation. The photoreaction of nifedipine powder (room moisture ca. 35% RH) is presented in Fig. 6. The threshold value of 510 nm is observed, but the maximum is shifted to a shorter wavelength of 390 nm. The reason for the behavior presumably differs from that in solution, and could be due to the limited penetration of the irradiation into the sample. Moreover, the shifting of the maximum is also under further study.

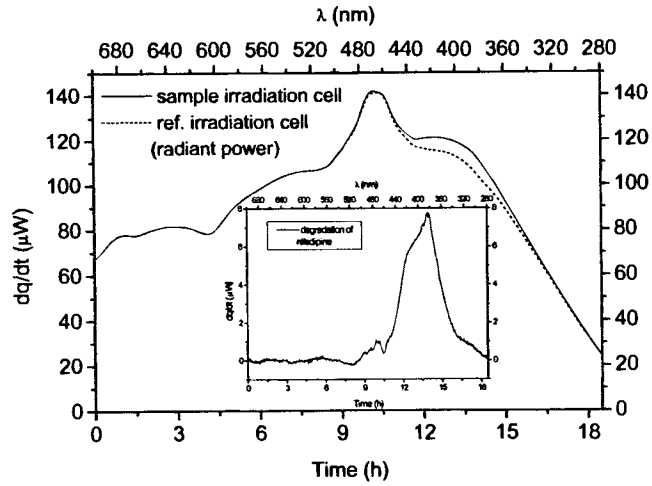

Fig. 6. Scan measurements for the photosensitive reaction of nifedipine powder containing room moisture. The reference irradiation cell was empty.

L-Ascorbic Acid

Nifedipine is stable in the dark and photolysis progresses rapidly under proper illumination conditions, so the process is optimum to study with calorimetry. On the contrary, ascorbic acid oxidizes easily in aqueous solution (20) and the oxidation is catalyzed by radiation and metal ions. Ascorbic acid undergoes the oxidation process also in the solid state and the extent and the rate of the oxidation is affected by the moisture content of the sample (21).

A scan measurement for the aqueous solution of ascorbic acid is presented in Fig. 7. The reference level produced by the oxidation progressing in dark conditions is determined before and after the irradiation scan, and has been subtracted from the response from the sample irradiation cell. The contribution of light-independent oxidation to the signal obtained from the scan measurement was approximated as linear. This was well grounded for the sample stored for 24 h with the separate measurement performed with ascorbic acid solution closed in a stainless steel vessel. Light starts to catalyze a photoreaction from 450 nm on and the maximum is reached at 320 nm. The decline in photoreaction thereafter is mainly caused by the loss of irradiation intensity.

The influence of light on ascorbic acid in the solid state is minor but obvious. The maximum is reached earlier than with solution at 340 nm but this is presumably affected by the poor penetration of light through the product layer.

Constant λ Measurements

The scan measurements were made to rapidly screen the possible photoinduced reactions and dependence of the reaction upon the light wavelength. To obtain a closer insight into the effect of different wavelengths on the photoreaction the measurements can be performed with constant wavelengths.

Nifedipine

The heat generation during the photoreaction of nifedipine in ethanol solution strongly depends on the intensity of irradiation and the wavelength used. The measurements were made

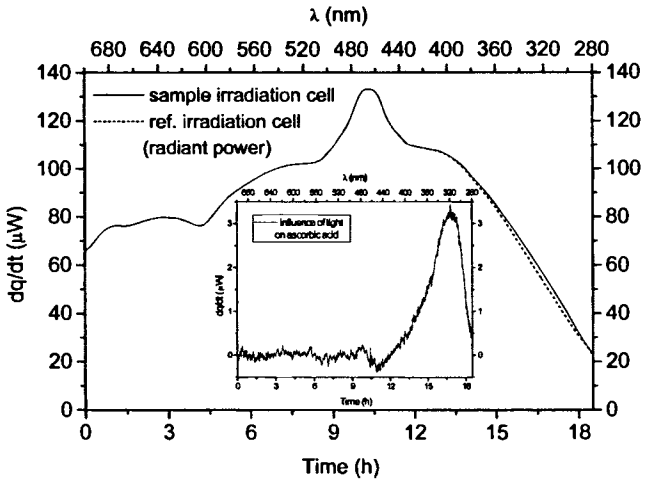

Fig. 7. Scan measurements for the influence of irradiation on photoreaction of ascorbic acid in solution. 2.5 ml water was used as the reference material.

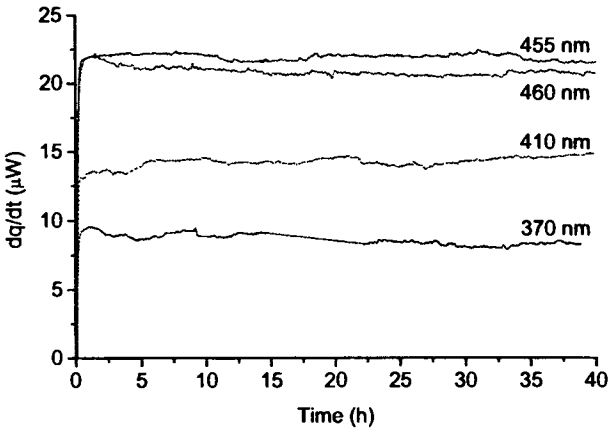

Fig. 8. Photoreactivity of nifedipine in solution at wavelengths of 460 nm, 455 nm, 410 nm, and 370 nm. The time zero corresponds to the opening of the shutter.

with wavelengths of 460 nm, 455 nm, 410 nm and 370 nm, the heat flow level caused by irradiation being 121μW, 123μW, 108 μW and 92 μW, respectively (cf. Fig. 3). For all the measurements, the calorimetric responses to the photoreaction of nifedipine remained on a steady level during the course of the measurements (Fig. 8), making any predictions about the total reaction heat and the duration of the photoreaction impossible without any knowledge of the changes in concentration. However, monitoring the efficiency of various radiations to cause the photoinduced reaction of nifedipine is possible via Equations (2–4) in the Theory section. Integrating the thermal response from the reference irradiation cell, the energy value absorbed by the sample is acquired, and the cumulative heat evolved in the photoreaction can be obtained by integrating the subtracted signal (Fig. 8). As the energy of light quants is known, the reaction heat can be expressed as a function of photons in irradiation (Fig. 9). This gives information about the ability of different wavelengths to interact with the molecule. However, as the photoproducts may depend on the wavelength used, and thus the total heat evolved in the photoreaction may vary as a function of the wavelength, this can lead to erroneous conclusions. Thus, it would be advantageous to monitor the

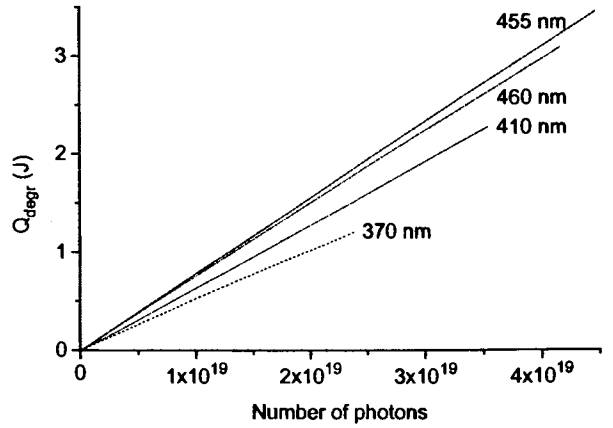

Fig. 9. Efficiency of light quants with various energies (460 nm, 455 nm, 410 nm, and 370 nm) to produce heat in the photosensitive reaction of nifedipine in solution.

degradation reaction in so far as the total reaction heat can be assessed, or to make the chemical assay after microcalorimetric measurement, which would also help to validate the measuring method.

L-*Ascorbic Acid*

The aqueous solution of ascorbic acid was also irradiated at a constant wavelength of 320 nm that was observed to produce the maximum thermal response in the scan measurement. As it had been detected with nifedipine solutions, the steady level reached (ca. 3 μW) remained until the end of the measurement (40 h).

CONCLUSIONS

The irradiation cell proved to be useful and valuable in the detection of the photo-sensitivity of pharmaceuticals in both solutions and solids. With a rapid screening, a threshold value for the wavelength for the photoreactivity can be obtained, together with an estimation for the wavelength causing the highest reaction rate. For example, when various possibilities to protect material from light are under investigation this kind of measuring arrangement can have advantages as a rapid and reproducible tool.

In this study, radiant power of ca. 120 μW produced a heat flow signal of 22 μW from the photosensitive reaction of nifedipine, giving the efficiency factor of 0.18. As the irradiation intensity can be raised up to 1000 μW with proper optical arrangements, and if the sensitivity of the microcalorimeter in these measurements is taken as 0.5 μW, a photoreaction with efficiency of 0.0005 can be detected. This means that a photoreaction 360 times less thermally active than that of nifedipine can be detected. In addition, the effect of different environmental factors on the photoreactivity, e.g., relative humidity and temperature, can readily be monitored, and thus information about the real behavior of the material can be acquired.

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