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Preparation, characterisation, and drug release from thermoresponsive microspheres

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Abstract

An emulsion polymerisation method for the preparation of thermoresponsive hydrogel microspheres based on *N*-isopropylacrylamide is described in this paper. It was found that microsphere size distribution could be altered by varying the rate of stirring during the polymerisation process and also by modifying the concentration of lecithin. Microspheres were imaged in the fully hydrated state using environmental scanning electron microscopy (ESEM). Thermoresponsive release of a model drug from the microspheres was found to be a function of microsphere size, with improved control as microsphere size increased.

Keywords: Responsive drug delivery; Thermoresponsive polymer; Microsphere; Environmental scanning electron microscopy

1. Introduction

There has been much interest in the development of pulsed and self-regulated drug delivery systems (Kost and Langer, 1991, 1992). The majority of controlled drug delivery research has focussed on systems where release rates are a simple function of time, e.g., zero-order, however, it has been demonstrated that for many therapeutic agents (e.g., peptides, proteins, hormones, vaccines) such kinetics may not be desirable and improved therapeutic effects may be achieved by modulating drug output in a precisely controlled manner. Chronotherapeutic approaches have been proposed where it has been suggested that optimal therapeutic effects may be achieved by adjusting drug delivery patterns to varying demands dependent on physiological requirements (Hrushesky et al., 1991). Additionally, it is likely that many therapeutically active peptides will require delivery to the body in a precisely controlled manner which may be optimum when delivery mirrors physiological release profiles, e.g., cytokines, hormones (Lee, 1990). Interest in the development of responsive drug delivery systems has lead to studies involving the use of polymers from which the release may be altered by an external energy source (stimuli). The energy sources which have been utilised to alter release from these systems include electricity

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(Grodzinsky and Grimshaw, 1990; D'Emanuele and Staniforth, 1993), ultrasound (Kost et al., 1989), oscillating magnetic fields (Edelman et al., 1985), and thermal energy (Bae et al., 1987; Hoffman, 1987).

The utilisation of temperature changes as a signal for modulating drug release from polymers has attracted much interest recently (Bae et al., 1987; Hoffman, 1987). It has been proposed that thermoresponsive drug delivery systems may control release in response to changes in body temperature and therefore act as self-regulating systems (e.g., for the release of antipyretic drugs), or, alternatively, release drugs in response to changes produced in the environment of the polymer (e.g., external heater, or heat generated by ultrasound). It has also been suggested that thermoresponsive polymers may be used as polymeric membranes in transdermal patches which can act as on-off switches. Certain hydrogels, such as crosslinked poly(*N,N'*-alkyl substituted acrylamides), possess a lower critical solution temperature (LCST) when placed in solution. When the temperature of the polymer is raised above the LCST a phase separation occurs within the polymer accompanied by a dramatic shrinkage in volume. It is this gel collapse phenomenon that has been utilised in the development of thermoresponsive drug delivery systems.

The majority of studies involving the application of thermoresponsive polymers for responsive drug delivery have concentrated on the use of devices in the form of discs or slabs. However, it would be desirable to develop microsphere formulations based on thermoresponsive hydrogels. There has been considerable interest in the preparation, characterisation, and biomedical applications of microspheres. Microsphere formulation offers the advantage in the design of implantable systems as they permit insertion into the target site without the need for surgery (Langer, 1990). Matsuo and Tanaka (1988) employed an inverse suspension polymerisation method to prepare microspheres using the thermoresponsive hydrogel *N*-isopropylacrylamide and performed a study on the kinetics of swelling and shrinking of microspheres. Park and Hoffman (1990) employed a similar method to study the immobilisation of ß-galactosidase in thermoresponsive microspheres for use in a packed bed reactor.

In the present study we report on a novel method for the preparation of thermoresponsive microspheres for the release of drugs which results in the production of discrete and spherical microspheres. A method is described which was used to obtain high drug loadings into microspheres and the effect of temperature changes on the release of drug from microspheres is described.

2. Materials and methods

2.1. Materials

Acrylamide (AAm) was obtained from Fluka Chemicals Ltd (Gillingham, UK). *N*-Isopropylacrylamide (NIPAAm) was obtained from Polysciences Inc. (Northampton, U.K.). *N,N*-Methylenebisacrylamide (MBAAm), *N,N,N',N'*-tetramethylethylenediamine (TEMED), and ammonium persulphate (AP) were obtained from Aldrich Chemical Co. Ltd (Gillingham, UK). Indomethacin was obtained from Sigma Chemical Co., (Poole, UK). Water used was freshly distilled. Other solvents used were of analytical reagent grade.

2.2. Preparation of microspheres

Microspheres were prepared by an emulsion polymerisation method. The aqueous disperse phase was prepared by adding 10% w/w monomer (NIPAAm), 1.0% w/w comonomer (AAm), and 0.2% w/w crosslinker (MBAAm) to 30 ml of distilled water. The solution was stirred under nitrogen gas for 30 min. 100, 200, 250, or 300 mg lecithin (as emulsifier and stabiliser) was added to 200 ml of cyclohexane and stirred under nitrogen gas for 30 min. 50 mg of ammonium persulphate was dissolved in the aqueous solution 30 s before adding to a flask containing the continuous phase (cyclohexane with lecithin) whilst stirring under nitrogen gas. An emulsion was formed by stirring the mixture for 1–2 min. 400 μl of TEMED was then added to the emul-

sion to initiate polymerisation. Stirring was continued for 25 min under nitrogen gas. Microspheres were separated and washed firstly with ethanol, followed by distilled water. They were then left to soak in water for 1 week, changing the water daily (to remove initiator and unreacted monomer).

The effect on the size distribution of microspheres of modifying emulsifier concentration and stirring rate was examined. The range of lecithin concentrations and stirring rates examined in the present study are shown in Table 1.

2.3. Size distribution measurement

The size distribution of microspheres was determined using a gradient sieving method. A series of 38 mm British Standard laboratory test sieves (Endecotts Ltd, London, UK) with a range of sieve sizes were placed in series in a descending order of mesh size (nine sieves were used with a mesh size ranging from 125 to 1400 μm). The assembled sieves were held together in a steel tube. The fully hydrated microspheres were placed on the top sieve and water was passed through the sieves for 10 min to allow the microspheres to separate on the basis of their size in the fully hydrated state. The sieves were removed, separated, and left to dry at room temperature for 2 days, then further dried at 55°C overnight.

2.4. Study of microsphere morphology

It has not been possible to image thermoresponsive polymers in their natural hydrated state in the past using conventional scanning electron microscopy (SEM). Sample preparation techniques required and the high vacuum conditions used for SEM preclude the imaging of hydrated samples in their natural state. Sample preparation may also lead to problematic artefact production. To overcome this problem, the relatively new technique of environmental scanning electron microscopy (ESEM) was employed which permits the imaging of samples in their natural state. In contrast to conventional SEM, most samples may be imaged in the ESEM without any sample preparation or modification. Additionally, samples which behave as electrical insulators (the majority of materials of pharmaceutical interest) do not require the deposition of an electrically conducting coat (e.g., gold) (D'Emanuele, 1992). The technique is therefore ideal for the imaging of hydrogels in their fully hydrated state. It is also possible to visualise the swelling and deswelling of the hydrogels by altering the temperature and/or water vapour pressure in the sample chamber. A small amount (approx. 5 mg) of the hydrated microspheres was placed on a sample stub and imaged at 15°C using the ElectroScan E-3 (ElectroScan Corp., Wilmington, MA).

2.5. Drug loading and release experiments

Indomethacin was used as a model drug in release experiments. 100 mg of dry microspheres were immersed in 25 ml of an 8% solution of indomethacin in acetone. The microspheres were left to soak in the solution for 3 days. This permitted the hydrogels to swell and for the uptake of indomethacin into the hydrogel network. It was found that a drug loading of 125% w/w could be achieved using this method (based

Table 1
Microsphere formulations

	NIPAAm (g)	AAm (g)	MBAAm (g)	AP (mg)	TEMED (μl)	Lecithin (mg)	Stirring rate (rpm)
M1	3	0.3	0.06	50	400	100	150
M2	3	0.3	0.06	50	400	200	150
M3	3	0.3	0.06	50	400	200	250
M4	3	0.3	0.06	50	400	250	150
M5	3	0.3	0.06	50	400	250	250
M6	3	0.3	0.06	50	400	300	150

on dry polymer weight). The drug loaded microspheres were removed from the solution and dried carefully to ensure homogeneous drying and to minimise drug migration. The microspheres were placed on a nylon mesh fixed onto a perforated teflon film which was mounted inside a thermostated vacuum flask. To minimise drug migration to the surface of microspheres, a controlled drying procedure was implemented under low vacuum: 3 h at −20°C, 3 h at −5°C, 6 h at 5°C, and 12 h at 25°C.

Sörensen buffer (pH 7.4) was chosen as the medium for drug release experiments. Microspheres (20 mg) were placed in a mesh basket connected to a motor (Crouzet, Farnborough, UK) and rotated at 50 rpm (similar to the dissolution apparatus described in USP XXI). The mesh basket was immersed in a beaker containing 500 ml Sörensen buffer. The beaker was placed in an ethylene glycol/water bath (Julabo F10, Seelbach, Germany) to control the temperature. The amount of indomethacin released into the medium was determined using a flow-through UV spectrophotometer (Applied Biosystems 785A, Foster City, CA) at a wavelength of 268 nm. Buffer was pumped into the spectrophotometer at a rate of 2 ml per min and returned to the beaker (Gilson Minipuls 3, Anachem, Luton, UK). The water bath temperature and data acquisition from the UV spectrophotometer was under microprocessor control (Archimedes 420/2, Acorn Computers, Cambridge, UK). In drug release experiments the temperature was cycled below and above the LCST of hydrogels, and the amount of drug released recorded as a function of time.

3. Results and discussion

3.1. Preparation of microspheres

The emulsion polymerisation method used was found to be efficient, reproducible and resulted in the preparation of discrete, hydrated microspheres. It was found that emulsifier concentration and stirring rate were critical, and determined the size distribution of microspheres. Additionally, it was found that using less than 1% w/v lecithin resulted in aggregation of microspheres. The method was successfully used to prepare microspheres in the size range 200–1200 μm.

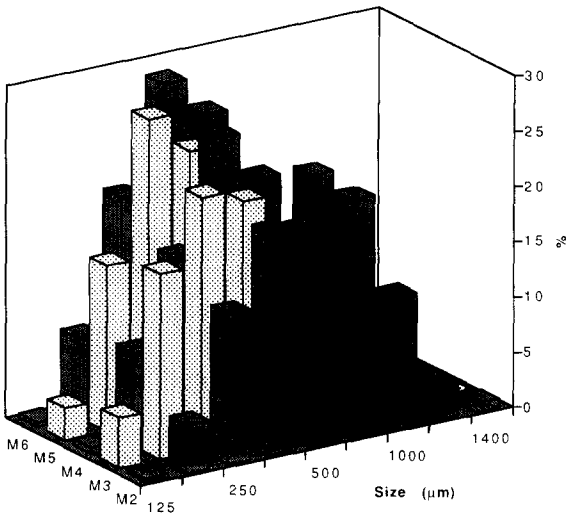

Fig. 1. Effect of lecithin concentration and stirring rate on microsphere size distribution.

3.2. Size distribution of microspheres

The gradient sieving was method was successfully used to measure the size distribution of microspheres in their hydrated state. The effect of emulsifier content and stirring rate on size distribution of microspheres is shown in Fig. 1. It

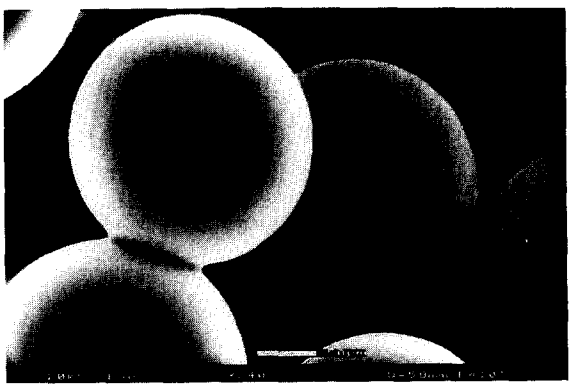

Fig. 2. Electron scanning micrograph of fully hydrated microspheres (formulation M4).

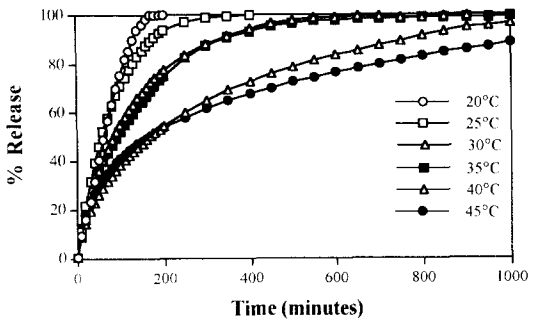

Fig. 3. Effect of temperature on the release of indomethacin from M3 microspheres.

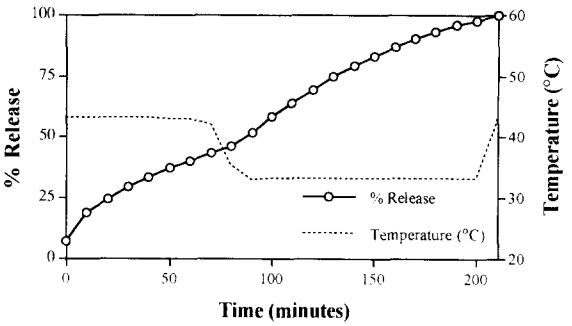

Fig. 5. Effect of temperature cycling on drug release from microsphere formulation M4.

can be seen that microsphere size decreases with an increase of stirring rate from 150 to 250 rpm. It was also found that lecithin concentration also modified the size distribution. Microsphere size decreases with increasing lecithin concentration.

3.3. Scanning electron microscopy

Microspheres were imaged in the fully hydrated state using ESEM. The microspheres were found to be discrete, spherical, and with a smooth surface for all formulations except M1, where the microspheres were aggregated. Fig. 2 shows an example of the morphology of hydrated microspheres. It was also possible to dehydrate microspheres by controlling the environment of the sample chamber.

3.4. Drug release from microspheres

Fig. 3 shows the effect of temperature on the release from microspheres. As expected with this type of thermoresponsive polymer, the release rate of indomethacin decreases as temperature increases. It was also found that a relatively large burst effect occurred at the start of release with about 40% of drug released within the first 2 h, irrespective of the temperature. This is in contrast to the release of indomethacin from polymer discs where no significant burst effects were found and release occurred over much longer periods (Dinarvand and D'Emanuele, 1992). These observations can be explained by the large surface area of microspheres compared to discs.

The effect of temperature cycling on drug release for formulations M2, M4, and M6 is shown in Fig. 4, 5 and 6, respectively. It was found that control over the release was difficult to achieve using temperature cycling, unlike with devices fabricated in the form of discs (Dinarvand and D'Emanuele, 1992). In the case of discs it was possible to obtain effective on-off effects on drug release by the use of temperature cycling. In the present study, it was observed that improved

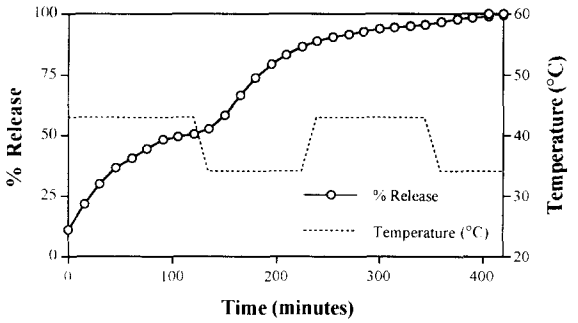

Fig. 4. Effect of temperature cycling on drug release from microsphere formulation M2.

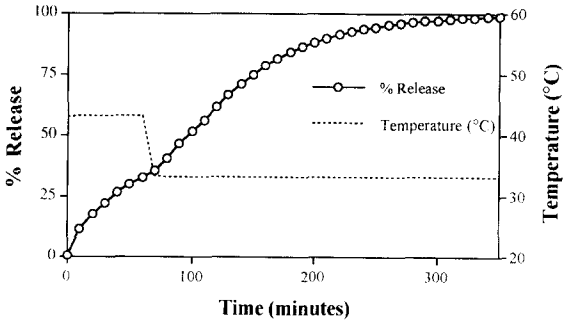

Fig. 6. Effect of temperature cycling on drug release from microsphere formulation M6.

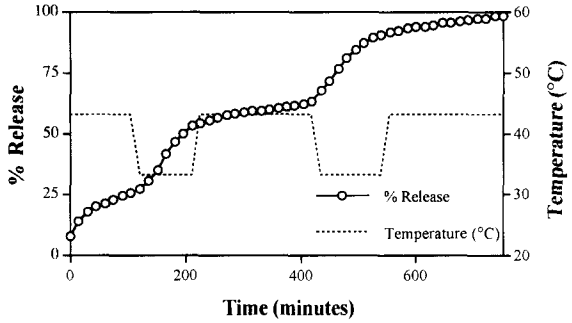

Fig. 7. Effect of temperature cycling on drug release from microsphere formulation M2 with a size greater than 100 μm.

thermoresponsive behaviour was found with formulation M2 than with other formulations. This was thought to be related to the larger size of these microspheres. To examine the effect of larger microsphere size, M2 microspheres were sieved and release studies performed on microspheres with sizes greater than 1000 mm. Fig. 7 shows that thermoresponsive control is much more effective with the larger size fraction of M2.

4. Conclusions

Microspheres were prepared by a microemulsion polymerisation technique. The method was shown to be an efficient and rapid method for the preparation of microspheres. The morphology of hydrated microspheres was studied using environmental scanning electron microscopy (ESEM). Hydrated microspheres were found to be discrete and circular entities. Although an on-off release pattern could be achieved, the initial burst release of drug from microspheres was significant. Drug release from microspheres was found to be rapid and difficult to control using temperature cycling, however, thermoresponsive release behaviour was found to improve significantly as microsphere size was increased. Further studies are required to prolong the release and improve the thermoresponsiveness of microsphere formulations.

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