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Studies on the development of a microencapsulated delivery system for norbormide, a species-specific acute rodenticide

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Abstract

Norbormide, a selective rat toxicant, was microencapsulated to both mask the flavour and delay the release until after a lethal dose has been ingested by the rat. To this end, gelatine microspheres containing norbormide were made and over coated with either shellac resin or an equal mixture of shellac and Eudragit RS® in a fluid-bed coating machine. The microcapsules absorb water, swell and burst to release their contents. In rats an 8 h window is available to delay the release of encapsulated material. In initial experiments, a shellac coating of 20% w/w was established as suitable for delaying the release. A capsule size range of 200–400 μm was selected, from capsule mastication experiment, for oral gavaging and feeding studies in rat. Oral gavage study has demonstrated for the first time that a substantial delay in release of a lethal dose of an acute poison has been achieved by microencapsulation. Feeding test has demonstrated that there is a fine balance between the size and density of the capsules in the bait to overcome mastication of the capsules by rats. A combination of shellac and Eudragit RS® resins is a viable polymeric wall material to control the rate of penetration of water in to microcapsules. © 2002 Published by Elsevier Science B.V.

Keywords: Norbormide; Acute rodenticide; Microcapsules; Gelatine microspheres; Delayed release

1. Introduction

Norbormide is selectively toxic to rodents of the genus rattus, *Rattus norvegicus*, *R. hawaiiensis* and *R. rattus*, which makes this poison environmentally safe and acceptable for rodent control (Roszkowski et al., 1964). The compound selectively acts on the microvasculature of rats (Roszkowski, 1965; Bova et al., 1996). Rats as a species lack the emetic centre and this may be the reason why they sample food, especially novel food, such that they do not, as far as possible, ingest a toxic dose. This sampling strategy appears to be an evolutionary trait that the rat has developed for survival. Norbormide is an acute poison, that is to say, it is a quick acting poison with rapid onset of toxic symptoms. Therefore, the rats develop a learnt aversion to this poison after consuming sub lethal doses during sampling, which is referred to as poison bait shyness (Kusano, 1975; Shimizu, 1983). Norbormide is also

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relatively unpalatable to rats (Greaves, 1966; Ogushi and Iwao, 1970; Rennison et al., 1968). Microencapsulation of norbormide has been demonstrated to overcome palatability problems, but the quick in vivo release led to bait shyness (Greaves et al., 1968).

Modern microencapsulation technology could provide the opportunity to both mask the flavour and delay the release of the compound until after a lethal dose has been ingested. Contrary to drug delivery systems, where the objective is generally to produce sustained release thus maintaining therapeutic levels of the active ingredient in the blood for a protracted period, in vertebrate pest control, it is often necessary to have a sudden surge and absorption of the toxicant from the gastrointestinal tract (GIT). Therefore, the microcapsules must readily breakdown, preferably after a time lag, in the target region of the GIT and release the active ingredient in a concerted fashion ready for absorption. Concerted release of the active ingredient is also desirable from a humane perspective, because rapid onset of toxic effects leads to a quick death. Unlike opportunistic carnivores, which bolt their food, rats generally masticate their food prior to swallowing. Therefore, it is also important to have the size of the microcapsules small enough to survive mastication to maintain the integrity of the capsules in the GIT to achieve the intended release of the active ingredient. Previous studies in our laboratory on the behaviour of microspheres in the GIT of rat, have established that a time window of about 8 h, is available for delaying the release of active ingredients and that the degradation of microcapsules based on pH and enzymatic action is inappropriate for the delivery of acute toxicants such as norbormide (Nadian et al., 1994). Therefore, another delivery mechanism was required. Uncrosslinked gelatine microspheres absorb water and swell to double their size when placed in water. This property of gelatine was used to design the delivery system by controlling the rate of water penetration into polymer coated gelatine microcapsules. The capsules burst on absorption of water and the gelatine dissolves at body temperature of the rat to produce a concerted release of the toxicant. The development and evaluation of such a delivery system is reported here.

2. Materials and methods

2.1. Materials

Gelatine (300 bloom), iron(II,III) oxide, Heavy liquid paraffin, Span 85®, 2-propanol, *n*-hexane, polyethylene glycol 400 (PEG 400) were purchased from Sigma-Aldrich (Dorset, UK). Eudragit®(RS) Rohm (Germany), hydroxypropylmethylcellulose (HPMC E4M®) Colorcon (UK) were obtained as gifts. Shellac (bleached) was a personal gift from Mr Manfred Penning (Germany). Norbormide was synthesised in our laboratory. Rats were, 10-week-old females, obtained from our in-house Wistar derived strain. EPA diet was made up by mixing 5% w/w icing sugar into sieved maize meal.

2.2. Preparation of gelatine microspheres

Plain gelatine microspheres and gelatine microspheres containing either iron oxide (magnetically responsive microspheres) or norbormide were prepared by modification of a published method (Leucuta, 1986). Typically, a fine suspension of either iron oxide or norbormide in 31% w/v aqueous gelatine solution at 60 °C was emulsified into heavy liquid paraffin, maintained at the same temperature, containing Span 85® as the emulsifying agent. Once the droplet size of the emulsion was adjusted to the required size, the dispersion was cooled to 4 °C and maintained at this temperature for 30 min with continued agitation. The resultant gelled microspheres were dewetted with 2-propanol, filtered, washed with *n*-hexane, air-dried and sieved (Fritsch® analysette3, Fritsch GmbH, Idar-Oberstein, Germany) to obtain free flowing individual microspheres.

2.3. Film coating of gelatine microspheres

Film coating of gelatine microspheres was carried in a laboratory model R&D fluid-bed coating machine (Coating Place Inc., Verona, WI) equipped with a bottom pneumatic spray nozzle and a 4 cm acrylic coating chamber. To produce microcapsules, the microspheres were coated with either 6% w/v shellac in 2-propanol or a 6% w/v

solution, containing an equal mixture of shellac and Eudragit RS polymer in an equal mixture of acetone and 2-propanol. The amount of norbormide in the microspheres and microcapsules was quantified by UV spectroscopy. The microcapsules were crushed in a pestle and mortar, dissolved in warm one molar hydrochloric acid, diluted appropriately and the absorbance at 301 nm measured. Gelatine and other formulation material did not interfere with the analysis. Typically a loading of 21% by weight of norbormide in the microcapsules was obtained.

2.4. Microcapsule bursting experiments

Gelatine microspheres in the size range of 300–350 μm were coated with shellac resin and samples were taken when 14, 28, 42 and 49% w/w coating, with respect to microsphere, had been applied. The samples were air dried and small aliquots added to distilled water at 25 °C and the number of capsules bursting as a function of time monitored.

2.5. Determination of capsule size surviving mastication by rats

Magnetically responsive gelatine microspheres coated with 28% w/w shellac resin were fractionated into three size range, 200–300, 300–400 and 400–500 μm, by sieving. Three groups of five female laboratory rats were individually caged and allowed to feed on EPA diet containing a known amount of one of the above size range of microcapsules per group. Soon after feeding, each animal was sacrificed, the contents of its stomach dispersed in 50 ml of water at 40 °C, stirred thoroughly with a glass rod and allowed to stand for 15 min. All intact capsules were harvested with a small rod magnet covered with paraffin film, washed with hot water, filtered through a sieve to remove free iron oxide particles, dried and weighed. Three 1-g aliquots of each of the three baits containing magnetically responsive microcapsules were taken, the capsules harvested from each aliquot as described above and their weight determined. The percentage of capsules masticated in each size range by the rats was estimated as follows:

$$\text{Percentage of microcapsules masticated} = [(A - B)/A] \times 100$$

where; A is the mean weight of capsules present in the amount of bait consumed by rats in a particular group, B is the mean weight of capsules recovered from the stomach of rats in the corresponding group.

2.6. Oral gavage experiments

Three rats per dose were gavaged with one lethal dose of (a) a solution of norbormide in one molar hydrochloric acid; (b) a suspension of micronised norbormide solid in PEG 400; (c) a suspension of norbormide microspheres (100–150 μm) in PEG 400 and (d) a suspension of norbormide microspheres (250–300 μm) in corn oil. Three female laboratory rats per dose were also gavaged with one of the following suspensions in aqueous HPMC: norbormide microcapsules (300–350 μm) with 20% w/w shellac coating (sample A) and norbormide microcapsules (250–300 μm), with 20% w/w coating of shellac and Eudragit RS® polymer (sample B). Rats received microcapsules equivalent to 1–6 times the lethal dose of norbormide.

2.7. Feeding experiments

Test baits were made up by mixing either sample A or sample B microcapsules into sieved maize meal containing 5% w/w icing sugar (EPA diet). Sample A capsules were incorporated at 5 and 2.6% w/w and sample B at 10 and 2.15% w/w into the bait. Rats, individually caged and starved overnight, were allowed to feed on test bait for a short period of about 15 min. Test baits were then replaced by plain EPA diet. Water was available ad libitum.

3. Results and discussion

Microcapsule bursting experiments were carried out, to study the permeability of shellac resin coats to water, with the view to establish the level of coating that had to be applied to gelatine

microspheres for the mastication and toxicity studies. The results of microcapsule bursting studies are summarised in Fig. 1. Fifty percent of gelatine microspheres coated with 14 and 28% w/w shellac resin burst within 3.5 and 10 h respectively.

Based on this result, a coating of 28% w/w shellac was selected for microcapsule mastication studies. This level of coating allowed sufficient time to harvest and quantify the capsules, that had not been masticated, from the rat stomach. Mastication of the capsules damages the polymeric coating, which permitted the rapid absorption of water by gelatine, resulting in the capsules swelling and bursting. The swollen gelatine either dissolve in the stomach of the rat or when placed in hot water. Therefore, only the capsules that had not been damaged in the feeding process are harvested from the rat stomach. Fig. 2 shows the results of mastication experiment. Substantially less (14%) microcapsules in the 200–400 μm range were masticated by rats. Hence microcapsules in this range were selected for toxicity studies.

Previous study has shown that an 8 h window is available to delay the release of active ingredients in the rat (Nadian et al., 1994). Therefore, a 20% w/w coating of shellac resin was applied to gelatine-norbormide microspheres to produce sample A. Results of oral gavage experiments are given in Table 1. The Standard oral toxicity test for norbormide as a solution in 0.1 molar hydrochloric acid (Poos et al., 1966) and the time to onset of signs and death shown are typical. Since

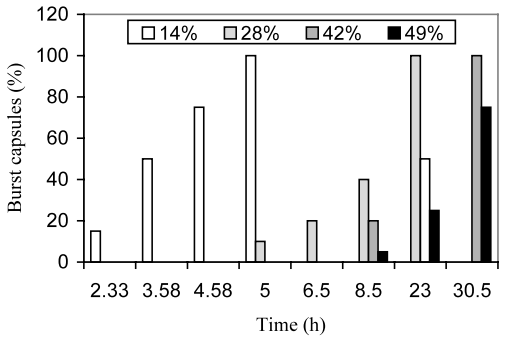

Fig. 1. Stability of 14, 28, 42 and 49% (w/w) shellac resin coated gelatine microcapsules in water at 25 °C.

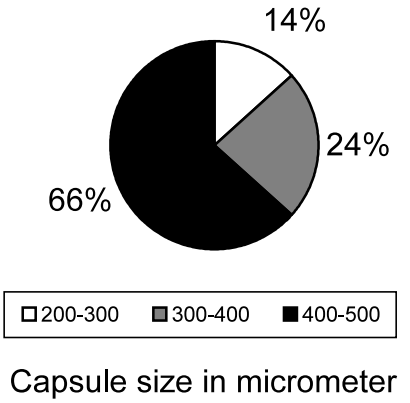

Fig. 2. Mean percentage of 28% (w/w) shellac resin coated gelatine microcapsules masticated by rats.

norbormide exists as micronised powder in the gelatine matrix of the microcapsules, rats were dosed with a suspension of micronised powder, which was shown to be equivalent in potency to that of the solution. The results also show that norbormide is released readily from the gelatine microspheres and absorbed by the rat, even when lipophilic corn oil is employed as the dosing vehicle. When rats were gavaged with sample A, no fatality occurred even with 6 lethal doses, which is indicative of the sample A capsules failing to release in vivo. HPMC E4M was employed as the dosing vehicle for the microcapsules because PEG 400 interacted adversely with the capsule coating. All the rats gavaged with sample B showed no sign of toxicity during the 2.5 h observation period, but rats which received 4 and 7 lethal doses died overnight. This clearly demonstrates for the first time that the release of a lethal dose of an acute toxicant has been delayed substantially in vivo. Table 2 shows the results of rat feeding tests. In feeding tests all of the rats fed on bait containing 5% sample A microcapsules developed signs of poisoning within 30 min and died within an hour. This is akin to gavaging with unencapsulated material, indicating that sufficient amount of capsule wall had been damaged through mastication by the rats, resulting in the release of at least 1 lethal dose of norbormide. The amount of sample A capsules in the bait was reduced to 2.5% to overcome the problem of mastication, but here

Table 1
Norbormide oral gavage experiment

Sample of norbormide	Number of lethal doses	Onset of signs (min)	Time to death (min)	Remarks
Solution in 0.1M HCl	1	30	45	
Suspension of micronised solid in PEG 400	1	30	45	
Gelatine microspheres (100–150 μm)	1	30	45	Dosing vehicle: PEG 400
Gelatine microspheres (250–300μm)	1	40	60	Dosing vehicle: corn oil
Sample A	1			No effects on rats Dosing vehicle: corn oil
Shellac coated gelatine microcapsules (300–350 μm)	2			No effects on rats
	3			Dosing vehicle: 1% HPMC E4M
	4			
	5			
	6			
Sample B	2			No effects on rats Dosing vehicle: 1% HPMC E4M
Shellac:Eudragit RS::1:1	3			
Coated gelatine microcapsules (250–300 μm)	4		Over night	No signs manifested during 150 min post dosing observation period
	5		Survived	
	6		Survived	
	7		Over night	

again all the rats died. Therefore, the capsules in the 300–350 μm range are readily masticated by rats. When rats were fed on bait containing 10% sample B, all of the rats died as per sample A, indicating that the capsules, albeit smaller, are being masticated at the given loading in the bait. When sample B loading was reduced to 2.15%, rats consuming baits equivalent to 8 lethal dose died as before. However, a rat which had consumed only the equivalent of 5 lethal dose manifested minor signs of poisoning 40 min after feeding, but recovered and was free of toxic signs during the 2.5 h observation period. This animal was very ill the following morning.

The above results indicate that the amount of capsules chewed by rats could be reduced or totally eliminated by reducing both the capsule size and the density of the capsules in the bait. An oral gavage study in rat has demonstrated that for the first time that a substantial delay in the release of a lethal dose of norbormide, an acute poison, has been achieved by microencapsulation. The feeding study has demonstrated that there is a fine balance between the size of microcapsules and their density in the bait to overcome mastication by rats. A combination of shellac and Eudragit RS® resins has been shown to be a viable polymeric wall material to control the rate of penetration of water into microcapsules.

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Table 2
Norbormide feeding tests

Sample of norbormide	Number of lethal doses	Onset of signs (min)	Time to death (min)	Remarks
Sample A	16.7	20	35	Microcapsule loading in bait: 5% w/w
Shellac coated gelatine microcapsules (300–350 μm)	15	28	40	
	5.6	20	40	Microcapsule loading in bait:
	7.5	30	35	2.5% w/w
	4.8	30	180	
Sample B	26	20	30	Microcapsule loading in bait: 10% w/w
Shellac:Eudragit RS::1:1 coated gelatine microcapsules (250–300 μm)	11	25	35	
	8.5	30	60	Microcapsule loading in bait:
	8	20	35	2.15% w/w
	4.8	40 (minor signs)		Minor signs after feeding, but recovered and ate a lot of plain food. Very ill following morning

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