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Contribution of process variables to the entrapment efficiency of propranolol hydrochloride within ethylcellulose microspheres prepared by the Solvent Evaporation Method as evaluated using a Factorial Design

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Abstract

The effects of the process variables, pH of aqueous phase, rate of addition of organic, polymeric, drug-containing phase to aqueous phase, organic:aqueous phase volume ratio and aqueous phase temperature on the entrapment of propranolol hydrochloride in ethylcellulose (N4) microspheres prepared by the solvent evaporation method were examined using a factorial design. The observed range of drug entrapment was 1.43 ± 0.02%w/w (pH 6, 25°C, phase volume ratio 1:10, fast rate of addition) to 16.63 ± 0.92%w/w (pH 9, 33°C, phase volume ratio 1:10, slow rate of addition) which corresponded to mean entrapment efficiencies of 2.86 and 33.26, respectively. Increased pH, increased temperature and decreased rate of addition significantly enhanced entrapment efficiency. However, organic:aqueous phase volume ratio did not significantly affect drug entrapment. Statistical interactions were observed between pH and rate of addition, pH and temperature, and temperature and rate of addition. The observed interactions involving pH are suggested to be due to the abilities of increased temperature and slow rate of addition to sufficiently enhance the solubility of dichloromethane in the aqueous phase, which at pH 9, but not pH 6, allows partial polymer precipitation prior to drug partitioning into the aqueous phase. The interaction between temperature and rate of addition is due to the relative lack of effect of increased temperature on drug entrapment following slow rate of addition of the organic phase. In comparison to the effects of pH on drug entrapment, the contributions of the other physical factors examined were limited.

Keywords: Solvent evaporation method; Propranolol hydrochloride; Entrapment efficiency; Factorial design; pH; Rate of addition; Temperature

1. Introduction

The solvent evaporation method is commonly used to microencapsulate water-insoluble drugs

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within water-insoluble polymers. Typically this process involves the dissolution or suspension of the drug within a polymeric solution of a volatile, aqueous phase immiscible, organic solvent and then, following emulsification of the polymeric phase within an aqueous phase, the organic solvent is removed using heat, freeze-drying or vacuum extraction. This results in the solidification of the polymeric material into drug-loaded microspheres. Following washing, the microspheres are subsequently collected using filtration, centrifugation or decantation and then dried (Beck et al., 1979; Bakan, 1986; Jones and Pearce, 1995).

Generally, the solvent evaporation method is inappropriate for the microencapsulation of water-soluble drugs as the associated efficiency of drug entrapment within the microspheres is low. This is primarily due to drug loss from the polymeric phase to the aqueous phase prior to solidification of the microspheres (Bodmeier and McGinity, 1988). The drug entrapment efficiency within microspheres produced using the solvent evaporation method is of fundamental importance as failure to achieve acceptable drug loadings may preclude the use of this method for economic reasons (Jones and Pearce, 1995). Consequently, elucidation of the contributions of process variables to the entrapment efficiency is of great interest. Previously, it was reported that the pH of the aqueous phase and concentration of emulsifier significantly contributed to the %w/w loading of propranolol hydrochloride within ethylcellulose microspheres prepared using the solvent evaporation method, whereas type of stirring, baffled or non-baffled, had an insignificant effect (Jones and Pearce, 1995).

There are several process variables which have been reported to influence the characteristics of microspheres produced by the solvent evaporation method including, aqueous solubility of the drug, type of organic solvent or solvent mixture, drug loading and type and concentration of emulsifier (Benita et al., 1984; Bodmeier and McGinity, 1987a; Bodmeier and McGinity, 1987b; Bodmeier and McGinity, 1988). Consequently, this study was designed to evaluate the effects of four specific process variables, namely ratio of polymeric phase to aqueous phase, temperature of aqueous phase, organic:aqueous phase volume ratio and rate of addition of the organic polymeric phase to the aqueous phase, on the entrapment efficiency of propranolol hydrochloride, a water soluble drug, within microspheres prepared by the solvent evaporation method. The experimental design used was factorial. We have previously applied factorial designs to elucidate the effects of primary variables on drug entrapment within microspheres in the solvent evaporation method and also to reveal any interactions between variables (Jones and Pearce, 1995).

2. Materials and methods

2.1. Chemicals

Propranolol hydrochloride was a gift from Oregin Ltd (NZ), Palmerston North, New Zealand, and was passed through a 180-μm sieve prior to use.

Gelatin type B was donated by Davis Gelatine NZ Ltd. (Christchurch).

Ethylcellulose N4, hydrochloric acid (GPR) and dichloromethane (GPR) were obtained from BDH Chemicals (Poole, England).

A Prideaux buffer system (Jordan, 1980) was used in the course of this study, the constituents of which, potassium hydroxide, anhydrous potassium chloride, glacial acetic acid, orthophosphoric acid and boric acid were purchased from BDH Chemicals (Poole, England) and were of AnalaR, or equivalent, grade.

2.2. Preparation of microspheres

Microspheres were prepared as previously reported (Wakiyama et al., 1982; Jones and Pearce, 1994; Jones and Pearce, 1995). In brief, ethylcellulose N4 (1 g) was dissolved in dichloromethane with stirring (Nuova 11 hot plate/stirrer, Medical Supplies Limited, NZ) and into this propranolol hydrochloride (1 g, < 180 μm) was thoroughly dispersed (final volume, 20 ml). This organic phase dispersion was added into an aqueous phase, composed of gelatin type B (0.5%w/v) at either pH 6.0 or 9.0 at the required temperature (25 or 33°C) with baffled stirring at 800 rpm (Heidolph R1RZ,

Wiartin, Ontario). The final emulsion volume was either 200 or 400 ml, corresponding to an organic:aqueous phase volume ratio of 1:10 and 1:20, respectively. The organic phase was added to the aqueous phase either dropwise (slow) or as a thin stream from a laboratory burrette over a 5-s period (fast). Stirring of the resultant o/w emulsion was continued until evaporation of dichloromethane occurred. The resultant microspheres were collected by filtration, washed with deionised water and dried in a desiccator for at least 48 h. The dried microspheres were sieved and those within the 250–500 μm size range retained and stored at room temperature for analysis.

2.3. Determination of the amount (%w/w) of propranolol hydrochloride entraped within microspheres

The percentage entrapment of propranolol hydrochloride within ethylcellulose microspheres was determined as previously described (Jones and Pearce, 1994; Jones and Pearce, 1995). Microspheres (50 mg) were placed in a mortar and throughly triturated with a pestle until fully pulverised. Propranolol hydrochloride was extracted from the microspheres into hydrochloric acid (50 ml, 0.1 M) in a 100-ml Erlenmeyer flask. The equipment was thoroughly rinsed with hydrochloric acid and the total mixture filtered through a Buchner funnel fitted with a sintered glass filter. Following further dilution with hydrochloric acid, the concentration of propranolol hydrochloride within the filtrate was determined spectrophotometrically (λ = 290 nm) using a Hewlett-Packard 8452A diode array spectrophotometer after reference to a pre-constructed calibration curve. The calibration curve for propranolol hydrochloride was linear over the range 0.5–10.0 mg ml^{-1} (r = 0.99 with zero intercept). The presence of ethylcellulose did not interfere with the analytical method. The theoretical maximum (100%) content of propranolol hydrochloride within the microspheres was 50%w/w.

2.4. Statistical analysis of results

In this study, the experiments were conducted to a factorial design. The effects of the variables on the % entrapment of propranolol hydrochloride within ethylcellulose microspheres were statistically analysed using analysis of variance, ANOVA (Statview, Abacus Concepts., CA, USA). Fischers PLSD test was employed to evaluate statistical differences between individual means. In all cases $p < 0.05$ was accepted to denote significance.

3. Results and discussion

In this study the effects of four principle process variables on the entrapment efficiency of propranolol hydrochloride within ethylcellulose microcapsules prepared by the solvent evaporation method were examined. The model water-soluble drug used in this study, propranolol hydrochloride, was the subject of previous studies (Jones and Pearce, 1994; Jones and Pearce, 1995) in which two initial process variables, namely pH and emulsifier (gelatin) concentration, were observed to statistically influence the entrapment efficiency of the solvent evaporation process. Consequently, in the present study, we have utilised gelatin type B (0.5%w/v) as the emulsifier as this concentration was associated with highest drug loading.

Table 1 shows the effects ($\pm$ S.D.) of the investigated process variables on the % drug loading of microspheres. The maximum drug loading was achieved at pH 9.0, 33°C, following slow addition of organic phase to aqueous phase at 1:10 phase volume ratio (16.63 $\pm$ 0.93%w/w), corresponding to a mean observed entrapment efficiency of circa 33.26%, whereas the minimum drug loading was observed under the following conditions, pH 6.0, 25°C, phase volume ratio 1:10 with fast addition (1.43 $\pm$ 0.02%w/w, mean observed entrapment efficiency circa 2.86%). The theoretical maximum drug loading was not observed under any of the conditions examined (50%w/w). Increased pH (6–9), decreased rate of addition (fast to slow) and increased temperature (25–33°C) were observed

Table 1
The effect of process variables on the entrapment (%w/w) of propranolol hydrochloride within ethylcellulose (N4) microspheres prepared by the solvent evaporation method

pH	Temp. (°C)	Phase volume ratio	Propranolol hydrochloride entrapment (%w/w ± S.D.) of microspheres prepared by the Solvent Evaporation Method using:			
			Slow addition rate		Fast addition rate	
			Batch 1	Batch 2	Batch 1	Batch 2
6	25	1:10	2.36 ± 0.06	2.01 ± 0.02	1.43 ± 0.02	1.44 ± 0.12
6	33	1:10	1.61 ± 0.11	1.86 ± 0.05	1.68 ± 0.04	1.53 ± 0.18
9	25	1:10	15.97 ± 0.10	15.64 ± 0.29	11.06 ± 0.31	12.59 ± 0.93
9	25	1:20	14.05 ± 0.93	14.40 ± 0.66	14.43 ± 0.71	14.38 ± 0.90
9	33	1:10	16.63 ± 0.92	16.36 ± 0.56	15.62 ± 0.06	15.99 ± 0.90
9	33	1:20	16.06 ± 0.91	15.55 ± 0.59	14.83 ± 0.82	15.55 ± 0.58

to significantly increase the entrapment of propranolol hydrochloride into ethylcellulose microspheres ($p < 0.05$) and, in addition, significant interactions were observed in the factorial design between the following parameters, pH and rate of addition, pH and temperature, and temperature and rate of addition.

The effect of phase volume ratio was examined at pH 9, at 25 and 33°C and at two rates of addition (Table 1). At the two extremes of phase volumes examined (1:10 and 1:20), the effect on drug entrapment was insignificant and, in addition, significant interactions between primary variables were not apparent.

To interpret the findings of this study, it is important to consider the events which occur in the solvent evaporation method ultimately resulting in the production of microspheres. After emulsification of the organic polymeric, drug containing phase in the external aqueous phase, the organic solvent at the surface of the droplets dissolves in the external phase, leading to an increase in the concentration of polymer in the organic solvent. At a critical point, the concentration of polymer exceeds its solubility in the organic solvent and, consequently, the polymer precipitates to produce microspheres (Bodmeier and McGinity, 1988; Watts et al., 1990; Jones and Pearce, 1995). Low entrapment efficiency of the solvent evaporation method reflects the ability of the drug to partition into the aqueous phase prior to microsphere solidification (Jones and Pearce, 1995). Consequently, several reports have addressed the effect of the pH of the aqueous phase on the entrapment of ionisable drugs within microspheres and have concluded that efficient entrapment may be performed whenever the pH of the aqueous phase does not promote drug ionisation (Bodmeier and McGinity, 1987a; Bodmeier and McGinity, 1987c; Watts et al., 1990; Jones and Pearce, 1994; Jones and Pearce, 1995). However, it has been reported that certain extremes of aqueous phase pH may be detrimental to microsphere structure and therefore manipulation of the pH of the aqueous phase to promote drug entrapment within microspheres may be inappropriate (Bodmeier and McGinity, 1987a). In this present study the % entrapment of propranolol hydrochloride at pH 9.0 was significantly greater than at pH 6.0, again which may be explained by the greater solubility at, and hence greater rate of partitioning of this drug into the aqueous phase at pH 6.0 compared with pH 9.0.

Previously it has been reported that the choice of organic solvent influenced the entrapment efficiency of the water-soluble drug quinidine sulphate. The use of solvents with greater aqueous solubility, e.g., dichloromethane (2.0% w/w aqueous solubility) resulted in greater drug entrapment than observed whenever organic solvents of low aqueous solubility, e.g., chloroform (aqueous solubility 0.8%w/w), were employed. The authors concluded that organic solvents of greater aqueous solubility caused rapid precipita-

tion of polymer at the droplet interface, and thus created a barrier to drug diffusion out of the forming microspheres (Bodmeier and McGinity, 1988). Similarly, in this present study the significant enhancement of drug loading following increased temperature and decreased rate of addition of organic phase to aqueous phase is suggested to be due to the resultant increase in the solubility of dichloromethane in the aqueous phase. Further clarification of the relative importance of the primary variables examined within this study is offered by analysis of the interaction terms within the factorial design. In the interactions between pH and rate of addition and between pH and temperature (Figs. 1 and 2, respectively), the effects of temperature (25 and 33°C) and rate of addition (slow and fast) on entrapment efficiency of propranolol hydrochloride are non-significant at pH 6 yet significant at pH 9. At pH 6, the rate of partitioning fom the organic to aqueous phases is relatively rapid and consequently increasing the temperature of the

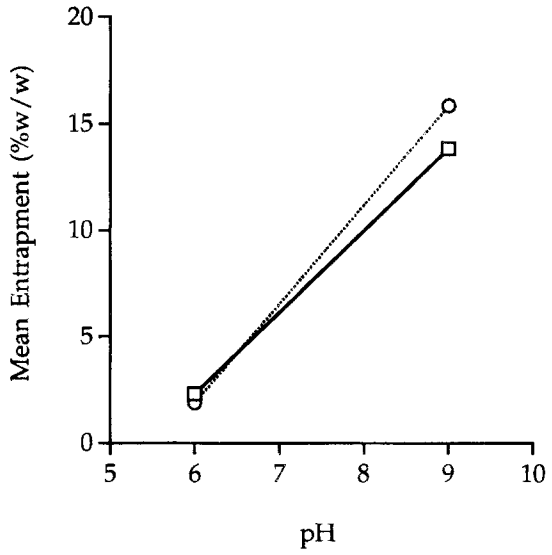

Fig. 2. Graphical illustration of the interaction between pH of the aqueous phase (pH 6 and 9) and temperature of the aqueous phase. The y-axis depicts the mean entrapment (%w/w) of propranolol hydrochloride within ethylcellulose microspheres prepared at 25 (□) or 33°C (○).

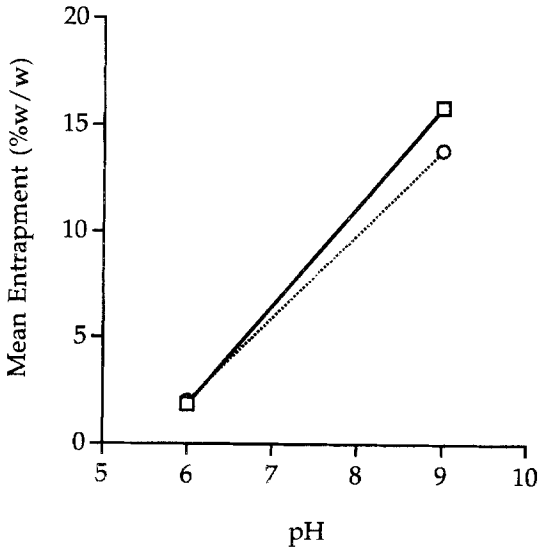

Fig. 1. Graphical illustration of the interaction between pH of the aqueous phase and rate of addition of organic polymeric, drug-containing, phase to the aqueous phase containing Gelatin type B (0.5%w/v as the emulsifier) at either pH 6 or 9. The y-axis depicts the mean entrapment (%w/w) of propranolol hydrochloride within ethylcellulose microspheres prepared following slow (□) or fast (○) rate of addition, respectively.

system from 25 to 33°C or decreasing the rate of addition failed to sufficiently enhance the rate of polymer precipitation prior to drug partitioning. The solubility of propranolol hydrochloride in the aqueous phase at pH 9 is lower than that at pH 6. As a result the rate and extent of partitioning will be decreased thus allowing sufficient time for temperature increase and rate of addition decrease to affect the solubility of dichloromethane and induce partial polymer precipitation prior to drug partitioning. In the interaction between temperature and rate of addition (Fig. 3), the differences between the drug loading of microspheres prepared at 25 and 33°C using a slow rate of addition are less than those prepared at these temperatures using a fast rate of addition. Therefore, whilst increased temperature may normally increase drug loading, this effect is dependent on the rate of addition of organic (drug containing) phase to the aqueous phase. This illustrates the greater importance of rate of addition than temperature of the aqueous phase on drug entrapment.

Given the observed increased drug entrapment at pH 9, the effect of organic:aqueous phase volume ratio (1:10 and 1:20) on propranolol hydrochloride entrapment at 25 and 33°C and at two rates of addition was further investigated. Increased aqueous phase volume will theoretically increase the aqueous solubility of dichloromethane and typically this may lead to increased polymer precipitation. The observed lack of effect of increasing the phase volume ratio from 1:10 to 1:20 may indicate that this increase was insufficient to significantly enhance the dissolution of dichloromethane in the aqueous phase to the extent where it significantly affects drug entrapment. It is conceivable that smaller organic:aqueous phase volume ratios will enhance drug entrapment however, at a practical level, this may be inappropriate due to the limitations of manufacturing vessel size.

In conclusion, this study has outlined the importance of aqueous phase pH, temperature and rate of addition of organic drug-containing polymeric phase to the aqueous emulsified phase on the subsequent entrapment efficiency of propranolol hydrochloride in ethylcellulose microspheres prepared by the solvent evaporation method. Statistically, pH of the aqueous phase was the most important determinant of drug loading, whereas rate of addition and temperature were primarily significant at pH 9, i.e., whenever drug ionisation is suppressed.

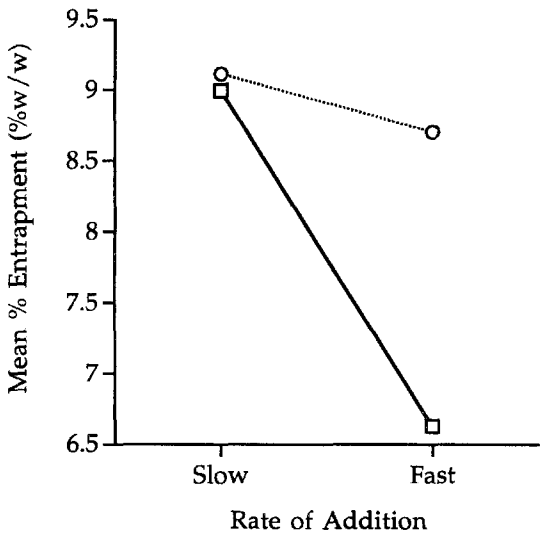

Fig. 3. Graphical illustration of the interaction between temperature of the aqueous phase (25, 33°C) and rate of addition of organic (drug-containing) phase to the aqueous phase (slow and fast). The y-axis depicts the mean ($\pm$ S.E.) entrapment (%w/w) of propranolol hydrochloride within ethylcellulose microspheres prepared at 25 (□) or 33°C (○).

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