Use of Gelatin–Acacia Coacervate Containing Benzocaine in Topical Formulations

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Abstract □ The in vitro release of a drug from topical formulations depends on the concentration of the drug in the formulation, the solubility of the drug in the base, the diffusion coefficient of the drug in the vehicle, and the partition coefficient of the drug between the vehicle, and the release medium. Incorporation of both complexing agents and cosolvents into such formulations has been used to enhance the in vitro release of a drug from topical formulations. In this investigation, a novel approach to enhance the in vitro release of benzocaine from different ointment formulations has been introduced. In this study, benzocaine was microencapsulated using gelatin–acacia complex coacervation technique. Various weight fractions of the coacervate, 5, 10, and 20% (w/w), were incorporated into both oleaginous and absorption bases. The in vitro release characteristics of benzocaine from the resulting ointments were studied using a modified USP Dissolution Apparatus 2. A plot of the cumulative amount of drug released (7–8%) per unit surface area versus $(\text{time})^{1/2}$ was linear. Microscopic studies of the formulations revealed that the coacervates maintained their integrity in the formulation during the preparation and storage of the dosage form. Differential scanning calorimetric (DSC) studies indicated that the drug existed in the crystalline state in all formulations including those at a low drug load (0.5% w/w). DSC was also used to determine the solubility of the drug in the formulation. The rate and extent of drug release was higher in the absorption base as compared to the oleaginous base.

Introduction

When striving to achieve therapeutic effectiveness in a topical formulation, two important factors are generally considered during the design of such formulations including (i) the rate of drug release from the vehicle used in the formulation and (ii) the ease or difficulty with which the drug will penetrate the skin barrier after its release from the base. Different mathematical models have been utilized in the literature to describe the in vitro release characteristics of a drug from topical dosage forms.[1−3] The rate of drug release from such formulations depends on various physicochemical factors including the solubility of the drug in the vehicle, the concentration of the drug in the formulation, and the diffusion coefficient of the drug in the topical vehicle.[1] However, when two-phase emulsion type topical formulations are utilized, one must consider the type of emulsion used in the formulation of the ointment and also the effect of micellar solubilization on the thermodynamic activity of the drug in various phases.[3−6] When the topical formulation matrix is an emulsion or suspension type, the effective diffusion coefficient of the drug must be used instead of diffusion coefficient.[3] In general, the rate of drug release from topical formulations has been enhanced by increasing the drug load, by changing the diffusivity of the drug in the vehicle, and by changing the drug's solubility in the vehicle. The solubility of a drug in the vehicle has been modified by incorporation of other components into the base, the addition of a cosolvent, changing the pH of the medium, and addition of a complexing agent.[7−9] The diffusivity of the drug has been altered by changing the microscopic viscosity of the vehicle.[1] In essence, the effect of different bases and their composition on the release of benzocaine from topical formulations has been extensively studied.[10−14] The present study attempts to enhance the release rate of a model drug benzocaine from ointments prepared from two different bases using a novel gelatin−acacia coacervation technique.

The gelatin−acacia complex coacervation method has been widely used for the microencapsulation of many hydrophobic drugs.[15−20] During the microencapsulation process, the coacervates formed are usually cross-linked and hardened by the addition of a dehydrating agent.[21] Aldehydes are generally used for this purpose. Cross-linking of the gelatin−acacia coacervate occurs through either a dimethylene ether bridge or methylene bridge.[21] In contrast, this investigation has used gelatin−acacia complex coacervates (without cross-linking) containing benzocaine in topical formulations to modify the release characteristics of this drug. In this study, benzocaine microcapsules prepared using the gelatin−acacia complex coacervation method were incorporated directly into the ointment bases by levigation prior to the hardening process of the microcapsule wall. Therefore, the objectives of this investigation were (i) to modify the release characteristics of benzocaine from various topical formulations by incorporation of the gelatin−acacia coacervate of the drug into base, (ii) to evaluate the effect of various base-types on the release characteristics of the drug, and (iii) to elucidate the mechanism of release from the resulting formulations.

Materials and Methods

Materials—Benzocaine and *n*-butyl PABA (Sigma Chemicals, Milwaukee, WS); gelatin, acacia, chloroform, *n*-octanol, hydrochloric acid (Fisher Scientific, Fairlawn, NJ); white petrolatum (Dayton Hudson, Minneapolis, MN); Aquaphor (Beiersdorf, Norwalk, CT) were used as received.

Preparation of the Benzocaine Microcapsules and the Ointments—Benzocaine (2 g) was dispersed in 100 mL of 1% (w/v) acacia solution in water, and the resulting suspension was then mixed with 1% (w/v) gelatin solution at 40 °C while stirring the solution. The pH of the 1% acacia solution was 4.5 and that of the 1% (w/w) gelatin solution was 4.0. The pH of the gelatin−acacia mixture (50:50 v/v) was 4.3. The pH of this mixture was adjusted to 3.9 with 0.1 N HCl and stirred for an additional 30 min. The coacervates were then centrifuged at 2000 rpm for 5 min in a IEC HN-II model centrifuge (International Equipment Company, Needham Hts, MI), and the supernatant was discarded. Different weight fractions of the coacervate were then incorporated into an oleaginous base (Petrolatum) and an absorption base

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(Aquaphor). Empty gelatin–acacia coacervates were prepared without benzocaine using the similar procedure described earlier for the benzocaine microcapsules. Physical mixtures of both bases with blank coacervate (microcapsules without drug) were prepared by mixing the coacervates with base by levigation. The resulting mixture will be described as the "physical mixture" in the text, hereafter. Ointments were prepared using the levigation method on an ointment slab. The water content of the coacervate without benzocaine and with benzocaine, prior to incorporation into ointment bases was determined using Karl Fisher titrimetry.

Analysis of Benzocaine—Benzocaine was analyzed by two methods. An HPLC method was used to study the in vitro release kinetics of the drug and its stability in the formulation. A Spherisorb C18, pH stable column (Phase Separations, Norwalk, CT), 15 cm in length was used. The column effluents were monitored at 254 nm (Shimadzu SPD-6A UV detector, Shimadzu. Koyoto, Japan), and the flow rate was maintained at 1 mL/min (Shimadzu LC-6A pump). The mobile phase consisted of methanol: phosphate buffer (58:42 v/v, pH 8.0). A UV/vis spectrophotometer (Perkin-Elmer, Lamda 400, Perkin-Elmer, Norwalk, CT) was operated at a wavelength of 290 nm. This method was used to determine the drug load in the formulation.

Drug Content in the Ointments—A known amount of the ointment was dissolved in chloroform, and the concentration of benzocaine in solution was determined using a calibration curve of the drug in chloroform. Standard curves were linear over the concentration range (0.5–40 μg/mL).

In Vitro Release Studies—The in vitro release of benzocaine from the ointments was carried out in a modified USP Dissolution Apparatus 2 (Hanson Research Corp., Chatsworth, CA). Five hundred milliliters of Sorensen's phosphate buffer pH 7.4 was used as the release medium, and temperature of the medium was maintained at 32 °C. The ointments (3.6–4.0 g) were placed in a specially designed aluminum cell (made in-house) with a diameter of 4.5 cm. The aluminum cells containing the ointments were carefully placed in the bottom of the dissolution medium. The paddles were rotated at 50 rpm. The release of drug from the surface (surface area = 15.90 cm^2) to the bulk of the release medium was studied. At predetermined time intervals, 1 mL of the release medium was collected and replaced with 1 mL of fresh buffer. The concentration of benzocaine in the solution was determined using the HPLC method described earlier. The data presented are the average of three formulations prepared independently.

Phase Contrast Microscopic Studies—The ointment samples were carefully smeared on glass slide and examined under a phase contrast microscope (Olympus, model CK2). This microscope was attached to a camera (OM-10) and used a Kodak Plus-X-Pan film with an ASA 125 automatic exposure.

Differential Scanning Calorimetry—DSC curves were obtained using a Shimadzu (DSC-50) differential scanning calorimeter attached to a Shimadzu (model C-R49) Chromatopac integrator. Samples (2–5 mg) were placed in the aluminum pans and heated at a rate of 10 °C/min with nitrogen purge at a rate of 20 mL/min.

Determination of Solubility of the Drug in the Ointment—The solubility of the drug in the ointment at its melting point was determined by DSC using a method described by Theeuwes et al.[22] The ointment sample (2–5 mg) was crimped into a nonhermatically sealed aluminum pan and heated in a DSC at a controlled rate (10 °C/min) with nitrogen purge. The enthalpy of fusion of the drug was determined and plotted against the known drug load.

Results and Discussion

The phase contrast microscopic studies of the petrolatum base containing benzocaine, empty microcapsules, and microencapsulated benzocaine is shown in Figure 1. Benzocaine crystals were evident in the ointment even at the lowest drug load (0.5% w/w) as shown in Figure 1a. Figure 1b depicts the micrograph of an ointment containing a physical mixture of 20% (w/w) of empty gelatin–acacia coacervate (without benzocaine) and 80% (w/w) of petrolatum after one month of storage in a refrigerator. Figure 1c represents the micrograph of ointment prepared from microencapsulated benzocaine in petrolatum. This study

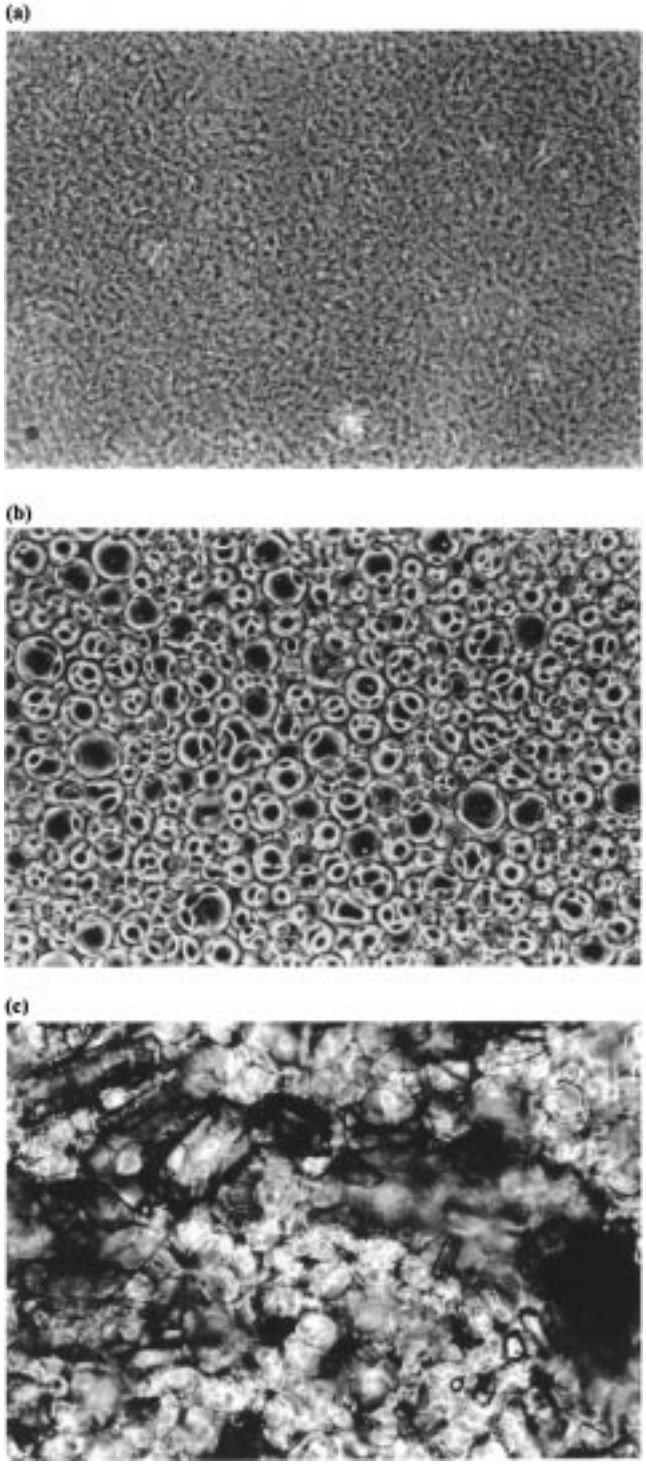

Figure 1—Photomicrographs of (a) benzocaine ointment in Petroatum (0.5% w/w); (b) physical mixture of empty microcapsules 20% (w/w) and Petrolatum 80% (w/w); (c) microencapsulated benzocaine in Petrolatum.

revealed that the coacervate maintains its integrity during preparation of the ointments and storage over a period of at least one month. The physical state of drug in the topical formulation is an important parameter, which in turn affects the drug's release rate from the topical vehicle. The rate of release of a drug from a formulation is always higher if the drug is present in the dissolved state rather than the dispersed state.[3,11] In this investigation, the physical state of the drug was determined by two independent methods. DSC was used to measure the enthalpy of fusion, if any, of the free crystalline drug in the formulation. No melting peak in the formulation indicated the drug was

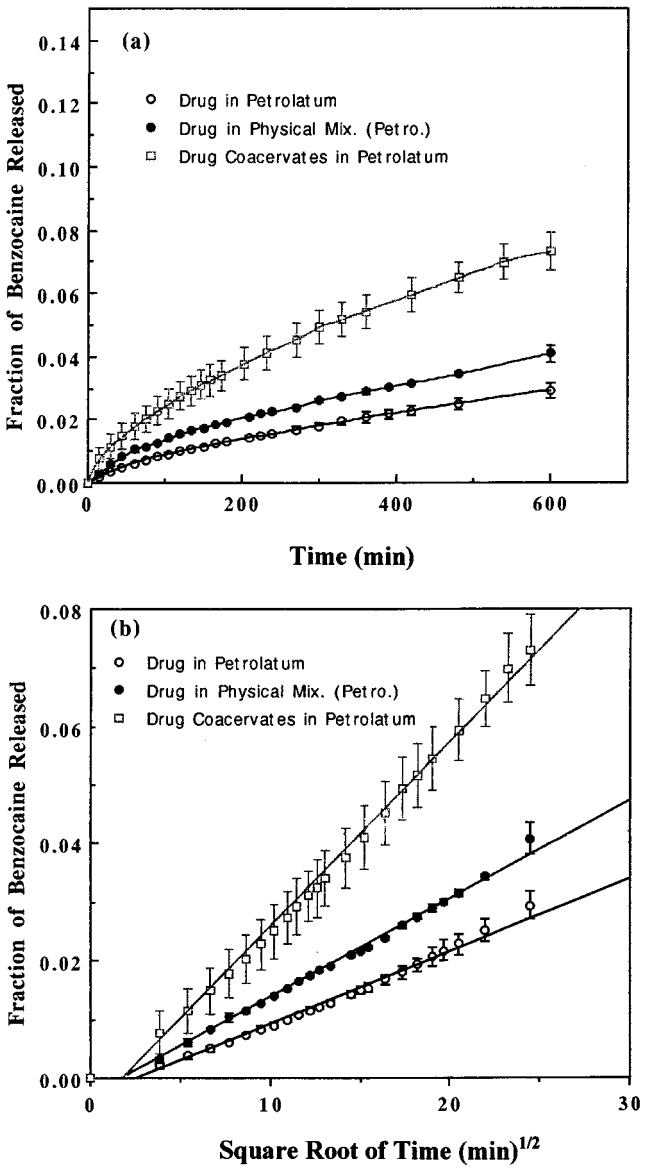

Figure 2—In vitro release of benzocaine from oleaginous (Petrolatum) ointments containing 2.9% (w/w) of benzocaine: (a) plot of cumulative amounts of benzocaine released versus time, and (b) plot of cumulative amounts of benzocaine released versus square root of time.

present in a molecularly dispersed state. The physical state of the drug in the formulation was further confirmed by visual inspection of the free crystalline drug in the formulation under a phase contrast microscope. DSC studies revealed that at even an extremely low drug load (0.5% w/w), an endothermic peak at 88 °C was observed in the DSC thermogram. This endothermic peak was attributed to the melting of benzocaine. Therefore, some of the drug was present in the crystalline state in the formulation at a drug load of 0.5% (w/w).

The fraction of benzocaine released from the ointments prepared from Petrolatum base is shown in Figure 2. In this study, benzocaine was incorporated into the petrolatum base as follows: (i) directly into Petrolatum, (ii) into a physical mixture of the empty microcapsules and Petrolatum (20:80 w/w), and (iii) microencapsulated benzocaine into Petrolatum. The benzocaine load was kept the same in all formulations. The cumulative amount of drug released from ointments containing microencapsulated benzocaine was found to be higher than the physical mixture or Petrolatum alone. A linear relationship was observed when the cumulative amount of drug released was plotted against the square root of time. The results of this study suggest that the release of benzocaine from these formulations occurred by a matrix diffusion control mechanism as described by Higuchi.[1] The slope of the lines (Figure 2b), which is a measure of the rate of drug released from the formulation, was then determined. In the case of the ointment containing coacervated benzocaine in Petrolatum, the release rate was found to be 2.5 times faster than the petrolatum ointment containing benzocaine alone. However, in case of the physical mixture the rate was only 1.4 times higher under similar test conditions.

Also of interest was the determination of the release kinetics of benzocaine from an absorption base. Aquaphor was selected for this study. Three different formulations were prepared in a similar manner as described earlier for Petrolatum. Interestingly, the ointment containing microencapsulated benzocaine also showed a higher rate and extent of benzocaine release. A plot of the cumulative amount of drug released vs the square root of time also showed a linear relationship indicating matrix diffusion control release. The rate of release of benzocaine from Aquaphor containing the coacervated drug was found to be 2.1 times faster than the Aquaphor containing the benzocaine alone. In the case of the physical mixture, the rate of drug release was approximately 1.4 times higher than the base containing benzocaine alone. Comparison of in vitro release profiles of benzocaine from these two bases revealed that in all three types of formulations studied, the release of benzocaine was always higher in Aquaphor than Petrolatum. The in vitro release profiles of benzocaine from Aquaphor and Petrolatum bases containing a similar drug load of benzocaine were then studied. Comparison of the slope of the cumulative amount of benzocaine released versus square root of time for these release profiles revealed that the rate of benzocaine release from the Aquaphor base is approximately 2.6 times higher than that of the Petrolatum base. The increase in drug release from Aquaphor as compared to Petrolatum is possibly explained by the fact that the solubility of the drug in petrolatum is higher than in Aquaphor, thereby held firmly by the vehicle, and the rate of release of drug was slow.[12] The presence of water in the formulation due to the incorporation of the coacervate could also contribute to the rate of release of the drug from these ointments. The water content of the empty coacervates and coacervate containing benzocaine prior to incorporation into the ointments were determined by Karl Fisher Titrimetry and found to be 82.5 and 78.5% w/w, respectively. Therefore, water content of the ointments was approximately 15 to 17% w/w (assuming 20% w/w of the coacervate in the ointment). Presence of water will provide rapid diffusion pathways of drug to get into the dissolution medium. Although benzocaine has very low solubility in water,[23] diffusion through the water pathway or aqueous coacervate pathway would be much faster than through the high viscosity, gellike Petrolatum or Aquaphor. This may explain the increased rate of drug release from ointments containing coacervates or from the physical mixtures.

The solubility of the drug in both bases and the physical mixture was determined by DSC. As mentioned earlier, the solubility of the drug in the matrix is determined by this method at the drug's melting point (approximately 88 °C). As such, the solubility of the drug in the Petrolatum base was determined from the intercept of the line obtained after plotting the drug-load in the formulation versus the heat of fusion. After heating, some samples were allowed to cool to room temperature and again reheated. Melting was observed at the same temperature during reheating indicating the drug was in the same crystalline form. Using this method, the solubility of benzocaine in the Petrolatum

was found to be 0.06 (w/w) (at 88 °C). Unfortunately, the determination of the solubility of benzocaine in Aquaphor, as well as in the physical mixtures, was not possible due to other thermal activity interference near the melting point of the drug from the matrix material.

Conclusions

(1) Gelatin−acacia coacervation method was used in the microencapsulation of benzocaine.

(2) Incorporation of the coacervate (without cross-linking) into Petrolatum or Aquaphor base substantially enhanced the rate of drug release from both bases.

(3) This enhanced release is possibly due to the increased diffusivity of the drug in the base, change in solubility, and the thermodynamic activity of the drug in base by inclusion of coacervates in the formulations.

(4) The release of benzocaine (7−8%, w/w) from all of the formulations occurred via a matrix-controlled diffusion mechanism.

(5) The absorption base showed a higher rate and extent of benzocaine release as compared to the oleaginous base.

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