In vitro characterization of a formulation of butorphanol tartrate in a poloxamer 407 base intended for use as a parenterally administered slow-release analgesic agent

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OBJECTIVE
To assess rheological properties and in vitro diffusion of poloxamer 407 (P407) and butorphanol–P407 (But-P407) hydrogels and to develop a sustained-release opioid formulation for use in birds.

SAMPLE
P407 powder and a commercially available injectable butorphanol tartrate formulation (10 mg/mL).

PROCEDURES
P407 and But-P407 gels were compounded by adding water or butorphanol to P407 powder. Effects of various concentrations of P407 (20%, 25% and 30% [weight of P407/weight of diluent] X 100), addition of butorphanol, and sterilization through a microfilter on rheological properties of P407 were measured by use of a rheometer. In vitro diffusion of butorphanol from But-P407 25% through a biological membrane was compared with that of a butorphanol solution.

RESULTS
P407 20% and 25% formulations were easily compounded, whereas it was difficult to obtain a homogenous P407 30% formulation. The P407 was a gel at avian body temperature, although its viscosity was lower than that at mammalian body temperature. The But-P407 25% formulation (butorphanol concentration, 8.3 mg/mL) was used for subsequent experiments. Addition of butorphanol to P407 as well as microfiltration did not significantly affect viscosity. Butorphanol diffused in vitro from But-P407, and its diffusion was slower than that from a butorphanol solution.

CONCLUSIONS AND CLINICAL RELEVANCE
But-P407 25% had in vitro characteristics that would make it a good candidate for use as a sustained-release analgesic medication. Further studies are needed to characterize the pharmacokinetic and pharmacodynamic properties of But-P407 25% in vivo before it can be recommended for use in birds.

In avian medicine for pain management. On the basis of a limited number of studies of psittacine birds, butorphanol appears to be the most effective opioid analgesic for members of the family Psittacidae; however, a very short period of antinociception (2 to 3 hours in Amazon parrots [Amazona spp]) limits its clinical use.1,2 Moreover, butorphanol has poor oral bioavailability and thus is usually administered parenterally (SC, IM, or IV as a continuous rate infusion).1 Repeated handling is stressful to birds, multiple injections may induce pain and cause repeated local muscle trauma, and placement of an IV catheter is not practical in all situations. Hence, there is a need for a sustained-release butorphanol formulation for use in psittacine birds.

Poloxamer copolymers, also known as poly(ethylene oxide-b-propylene oxide-b-ethylene oxide), are thermosensitive hydrogels.3 This group of copolymers consists of PEO and PPO blocks arranged in a triblock structure (ie, PEO-PPO-PEO), with a chemical formula of HO [CH_{2}-CH_{2}O]_{x} [CH(CH_{3})-CH_{2}O]_{y} [CH_{2}-CH_{2}O]_{z} OH.4 Gelation of poloxamer copolymers occurs by dehydration of PPO blocks. The PEO blocks remain hydrophilic between 0° and 100°C, whereas water solubility of
PPO blocks decreases dramatically as the temperature increases above 15°C. Increasing dehydration of PPO blocks results in aggregation of the copolymer molecules into micelles. Above the \( T_{\text{gel-sol}} \), micellar packing occurs and the compound becomes a gel; this phenomenon is perfectly reversible. Micellar packing is responsible for the high viscosity, partial rigidity, and slow dissolution of a gel, which make poloxamer copolymers highly effective sustained-release systems for both hydrophilic and hydrophobic drugs.

Poloxamer 407 is composed of approximately 70% PEO and 30% PPO. The \( T_{\text{gel-sol}} \) of P407 ranges from 15° to 35°C and is influenced by a number of factors. The \( T_{\text{gel-sol}} \) increases as P407 concentration decreases. Moreover, \( T_{\text{gel-sol}} \) of P407 can be influenced by the addition of drugs or other chemical agents. For example, sodium chloride, dimethyl sulfoxide, and vitamin B₁₂ have been found to decrease \( T_{\text{gel-sol}} \), whereas dicyclofenac, ethanol, and hydrochloric acid increase it. Other drugs, including morphine and lidocaine, apparently have no effect on \( T_{\text{gel-sol}} \).

Poloxamer 407 is frequently used as a sustained drug delivery system for medicinal agents because it has low toxicity, high solubility, and good drug-release capacity. It has been incorporated in a variety of formulations (oral, ophthalmologic, injectable, rectal, topical, and nasal) for use in humans and other species including rats, cattle, dogs, rabbits, and birds. To the authors’ knowledge, only 1 study has been conducted with birds. In that study, pharmacokinetics of P407-doxycycline administered IM to broiler chickens was compared with that of orally administered doxycycline (0.5% aqueous solution of doxycycline hyclate).

The objectives of the study reported here were to assess the case and reliability for compounding of P407 and identify the best concentration of P407 for use as the base for a sustained-release formulation of butorphanol, ensure that adequate gelation of P407 and of But-P407 occurs between 38° and 40°C (an approximation of avian core body temperature), compare rheological properties of But-P407 with those of P407 alone to ensure that the addition of butorphanol does not substantially alter gelation properties, determine whether sterilization of P407 via microfiltration affects \( T_{\text{gel-sol}} \) or viscosity, and determine the rate of butorphanol diffusion from P407 in vitro. We hypothesized that it would be possible to reliably compound P407, P407 compounds would have a higher viscosity at avian body temperature than at mammalian body temperature, in vitro viscosity and gelation properties of P407 and But-P407 would be similar, microfiltration would not alter the viscosity profile of P407, and in vitro diffusion of butorphanol from the gel would be slower than diffusion from a solution of butorphanol tartrate.

Materials and Methods

Sample

A P407 powder was purchased for use in the study. A commercially available injectable butorphanol tartrate formulation (10 mg/mL) was also purchased.

Preparation of poloxamer gel formulations

Powdered P407 was reconstituted by use of a cold method as previously described. Concentrations of P407 were expressed as a percentage as follows: (weight of P407/weight of diluent) X 100. The amount of P407 sufficient to yield a 20%, 25%, or 30% gel was added to a plastic syringe case that contained cold (5°C) deionized water or cold butorphanol tartrate. The density of butorphanol tartrate is the same as that of water (1 g/cm³). Ice packs were placed around the syringe case, and contents of the case were mixed by use of a vortex device (touch mode at 3,200 rpm) until a visually homogenous solution was obtained. Time to achieve the homogenous solution was 1 to 1.5 minutes for the 20% formulation and 3 minutes for the 25% formulation. For the 30% formulation, samples were placed on the vortex device for 5 minutes, during which time they were also intermittently shaken; manual stirring was also necessary to achieve a homogenous solution. After mixing with the vortex device was completed, all samples were refrigerated at 4°C for several minutes until the solution was clear of bubbles. Samples that were not macroscopically homogenous after refrigeration were placed on the vortex device for an additional 30 seconds and then again refrigerated for a few minutes. A subsample of the P407 25% formulation was placed in a freezer (−20°C) for 1 minute to prevent gelation and then sterilized by filtration through a 0.22-μm microfilter by use of a Luer-lock syringe. Preliminary experiments had revealed that autoclaving was an inappropriate method of sterilization of poloxamer powder because of evaporation of product.

The theoretical concentration of butorphanol in the But-P407 25% formulation was calculated by use of the difference in the volume of 6 samples before and after addition of the P407 powder to the butorphanol tartrate. Volume was measured with a graduated 6-mL syringe.

Rheological analysis

Rheology is the science of flow and deformation of matter under applied forces. Rheological analysis of the formulations was performed with a controlled stress–controlled rate rheometer. Viscosity was measured by use of the flow mode. The rheometer was calibrated in accordance with the manufacturer’s guidelines, and the geometry comprised a standard-size recessed-end concentric aluminum cylinder. For this geometry, stator inner radius was 15 mm, rotor outer radius was 14 mm, cylinder immersed height
was 42 mm, and recommended gap was 200 μm. Geometry inertia was 3.9 μN•m•s⁻². Samples were placed in a Peltier system consisting of a concentric cylinder, and tap water was used for cooling. Each sample (4 mL) was placed inside the cylinder, and 84 measurements of viscosity were obtained at increasing temperatures (4° to 45°C at a rate of 2°C/min). This was a standard rate and provided sufficient time for the product to change viscosity in parallel with the increase in temperature. The gelation time of P407 at room temperature (approx 20°C) is < 30 seconds for P407 concentrations > 17.5%. Shear rate was constant at 50/s, and the gap was set at 200 μm. The cylinder and geometry were cleaned with soap and water between subsequent samples, and the gap was recalibrated to 0 between samples.

Measurements were performed on samples of P407 20%, P407 25%, and P407 30% (5 samples/concentration) as well as on 3 samples of But-P407 25% to assess interassay variability of the preparation method and to compare the P407 25% and But-P407 25% samples (i.e., P407 25% with and without butorphanol). Measurements were also repeated 4 or 5 times on the same sample of P407 20%, P407 25%, and P407 30% to assess intra-assay variability of the rheometer at each P407 concentration. Three samples of P407 25% were passed through a 0.22-μm microfilter, and viscosity of each of these 3 sterile samples was measured. The T\text{sol-gel} corresponded to the inflection point on the curve of apparent viscosity as a function of the temperature.

### In vitro diffusion

A section of dialysis tubing was filled with 1 mL of But-P407 25%. Plastic scaling clips were used to seal each end of the tubing. The tubing was heated to 37°C with a heat lamp so that the formulations would become a gel. Tubing then was suspended in 100 mL of PBS solution in a beaker placed on a hotplate-stirrer. Temperature of the PBS solution was maintained at 38° to 39°C, and the PBS solution was stirred with a magnet rotating at 100 rpm. An aliquot (1 mL) of PBS solution was collected before immersion of the dialysis tubing (time 0) and 1, 2, 4, 6, and 10 hours after immersion. Each aliquot was replaced by 1 mL of fresh PBS solution. Aliquots were stored at −20°C until analysis (maximum storage time, 3 weeks). Butorphanol concentrations were measured in each sample. The experiment was repeated 3 times with freshly prepared formulations of But-P407 25%.

The same experiment was performed by use of a butorphanol solution. Butorphanol (8.3 mg [0.83 mL of butorphanol tartrate and 0.17 mL of sterile water]) was placed in the dialysis tubing to provide the same butorphanol concentration that was used in the aforementioned experiment. Cumulative diffusion of butorphanol for both formulations was calculated and graphed over time.

### Measurement of butorphanol concentrations

Butorphanol concentrations were quantitated in PBS solution by use of liquid chromatography-tandem mass spectrometry with modifications of a previously published method and butorphanol-d6 as the internal standard. A stock solution of butorphanol was diluted with methanol to create working standard solutions with concentrations of 0.01, 0.1, 1, 10, and 100 ng of butorphanol/μL. Calibrators were prepared by diluting the working standard solutions with water to achieve concentrations ranging from 1 to 80,000 ng/mL. Before analysis was performed, 0.1 mL of the in vitro sample was diluted with 400 μL of 5% acetonitrile in water, with 0.2% formic acid and butorphanol-d6 at 20 ng/mL. Samples were mixed in a vortex device for 2 minutes, and 30 μL of sample was injected into the mass spectrometer. Analytic instrumentation and methods were the same as those reported for analysis of plasma samples in another study. Accuracy (percentage of nominal concentration) was 98%, 99%, and 101% for quality control samples (3, 500, and 15,000 ng/mL, respectively). Precision (percentage relative SD) was 8%, 2%, and 1% for the quality control samples (3, 500, and 15,000 ng/mL, respectively). Accuracy and precision were considered acceptable on the basis of FDA guidelines for bioanalytical method validation. The assay was optimized to provide a limit of quantitation of 0.1 ng/mL and a limit of detection of 0.01 ng/mL.

### Statistical analysis

Viscosity-time curves were modeled by use of different equations. The initial portion of the curve (i.e., before gelation) was modeled by use of a nonlinear mixed regression model through a logistic growth curve. The second part of the curve (once a gel formed) was linear and thus was modeled by use of a mixed linear model. The T\text{sol-gel} and 95% CI was calculated as the inflection point of the logistic portion of each curve, which was the value on the x-axis of the sigmoid midpoint of the curve. Significant differences of T\text{sol-gel} among the various poloxamer preparations were assessed by comparison of their respective 95% CIs around the means. Values were considered significant at P < 0.05. Differences between viscosity at mammalian and avian core temperatures and among the various poloxamer preparations were assessed on the linear portion of the curves by use of linear mixed models and Tukey adjusted post hoc comparisons.

To assess differences in variability among the various formulations, the linear mixed models determined for the linear part of the curves were modified to account for heteroscedasticity among formulations. Dependence within samples was modeled by use of a compound symmetry covariance structure. Heteroscedasticity was modeled by use of different variances for each poloxamer formulation (different
variances per stratum variance function). Heteroscedasticity was tested against a homoscedastic model by use of a likelihood ratio test. Variances among poloxamer formulations were compared by assessing the 95% CIs of the weights of the variance per stratum in the variance function structure. A weight of 1 indicated that the variance was similar to the variance of the lowest poloxamer concentration (P407 20%). Other assumptions of the linear models were assessed graphically on residual plots.

In vitro pharmacokinetic diffusion was best modeled by use of an asymptotic function for the butorphanol solution and a polynomial regression for But-P407 25%. A program was used for the statistical analysis, and another program was used for mixed modeling.

### Results

#### Sample

The P407 20% and 25% formulations were easily created. Butorphanol (4 mL) mixed with 1.33 g of P407 yielded a median volume of 4.8 mL (interquartile range of volume, 4.725 to 4.875 mL) of But-P407 25%; thus, the median theoretical concentration of butorphanol in But-P407 25% was 8.3 mg/mL.

However, it was challenging to obtain a visually homogenous 30% formulation. In addition to standard mixing with a vortex device, this preparation also required manual mixing, which resulted in a loss of material on the stirrer. As a result, the volume of the product differed, the concentration of P407 probably was not consistently 30%, and the concentration

![Figure 1](image-url)

**Figure 1**—Mean ± SD viscosity (A) and the SD of viscosity (B) as a function of temperature for repeated measurements of single samples of P407 20% (circles; n = 5), P407 25% (squares; 4), and P407 30% (triangles; 4). Percentage of formulations was calculated as (weight of P407/weight of diluent) X 100.
of butorphanol in the final product could not be accurately established.

**Rheological analysis**

Intra-assay variability for apparent viscosity of the 3 concentrations of P407 was determined by use of a graph (Figure 1). Numeric values of the SD of measured viscosity as a function of temperature were also plotted. The greatest variability was at the inflection point on the curve that corresponded to $T_{\text{sol-gel}}$. The SD remained high above the $T_{\text{sol-gel}}$ for the 30% formulation, whereas it decreased for the other 2 formulations. Once gelation had occurred, the variance in viscosity was not significantly higher for P407 25% than for P407 20%. However, the variance for P407 30% was significantly ($P < 0.001$) larger, compared with results for P407 20% and P407 25%. Compared with the variance of viscosity for P407 20% and P407 25%, the variance of viscosity for P407 30% was approximately 2.6 times as great.

| Table 1—Mean and 95% CI values for $T_{\text{sol-gel}}$ as calculated from a logistic regression curve for 3 concentrations of P407, But-P407 25%, and sterilized P407 25%. |
|---|---|---|
| Formulation | $n$ | $T_{\text{sol-gel}}$ (°C) | 95% CI |
| P407 20% | 5 | 25.72 | 25.58–25.86 |
| P407 25% | 5 | 19.29 | 19.0–19.54 |
| P407 30% | 5 | 14.11 | 13.69–14.52 |
| But-P407 25%* | 3 | 16.92 | 16.66–17.16 |
| Sterilized P407 25%† | 3 | 19.08 | 18.79–19.36 |

Percentage of formulations was calculated as (weight of P407/weight of diluent) X 100.

*The diluent was butorphanol tartrate solution, rather than sterile water. †Sterilization was performed by filtration through a 0.22-µm microfilter.

Figure 2—Mean ± SEM viscosity (A) and the SD of viscosity (B) as a function of temperature for multiple samples ($n = 5$) of P407 20% (circles), P407 25% (squares), and P407 30% (triangles).
Interassay variability for apparent viscosity among 5 samples of each of P407 20%, P407 25%, and P407 30% was graphed (Figure 2). Numeric values of the SD of measured viscosity were also plotted. The SEM as a function of temperature was graphed to illustrate comparisons in mean viscosity among the various formulations. For each formulation, the variance among samples (interassay variability) was higher than for repeated measurements on the same sample (intra-assay variability). A clearly demarcated peak of variability at $T_{\text{sol-gel}}$ was evident for each formulation; at that temperature, the variance was significantly higher for P407 30% than for the other 2 formulations. Once gelation had occurred, the variance in viscosity was significantly ($P < 0.001$) higher for P407 25% than for P407 20% and for P407 30% than for the 2 lower concentrations. Compared with

the variance of viscosity for P407 20%, the variance was approximately twice as great for P407 25% and more than 4 times as great for P407 30%.

The values for $T_{\text{sol-gel}}$ differed significantly among each of the 3 P407 concentrations and decreased with increasing poloxamer concentration (Table 1). There were no significant differences in viscosity among the 3 concentrations over the range of 36° to 42°C.

Over the temperature range of 36° to 42°C, mean viscosity of each of the 3 formulations decreased, as determined on the basis of visual inspection of the data, particularly for P407 25% (Figure 2). There was no significant difference in viscosity among the 3 formulations at temperatures within this range, as determined by use of a linear mixed model. Comparison of the apparent viscosities at different temperatures

![Graph A](image1)

![Graph B](image2)

Figure 3—Mean ± SEM viscosity (A) and the SD of viscosity (B) as a function of temperature for multiple samples of P407 25% (squares; n = 5) and But-P407 25% (circles; 3).
within this range revealed that the viscosity of P407 25% was significantly ($P = 0.035$) higher at 36°C than at 40°, 41°, and 42°C; significantly ($P = 0.044$) higher at 37°C than at 41° and 42°C; and significantly ($P = 0.024$) higher at 38°C than at 42°C. Viscosity of P407 30% was significantly ($P = 0.009$) higher at 36°C than at 41° and 42°C. No significant changes over the temperature range were identified for the P407 20% formulation.

Effects of inclusion of butorphanol and the poloxamer on the viscosity of P407 25% were determined (Figure 3). The $T_{\text{sol-gel}}$ was significantly lower for But-P407 25% than for P407 25% (Table 1). Viscosity was not significantly different between But-P407 25% and P407 25% over the temperature range of 36° to 42°C; however, in this temperature range, the variance was significantly ($P < 0.001$) lower for But-P407 25%, compared with the variance for P407 25%.

Effects of filter sterilization on the viscosity of P407 25% were determined (Figure 4). The $T_{\text{sol-gel}}$ was not significantly different between unsterilized and sterilized P407 25% (Table 1). Neither the viscosity nor the variance was significantly different between unsterilized and sterilized P407 25% over the temperature range of 36° to 42°C.

**In vitro diffusion**

Diffusion of butorphanol from But-P407 25% into PBS solution was prolonged, compared with diffusion from the butorphanol tartrate solution (Figure 5). The cumulative amount of butorphanol that diffused (ie, was released) from the solu-

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**Figure 4**—Mean ± SEM viscosity (A) and the SD of viscosity (B) as a function of temperature for multiple samples of unsterilized P407 25% (squares; $n = 5$) and sterilized P407 25% (circles; 3). Sterilization was performed by filtration through a 0.22-µm microfilter.
tion after 10 hours was 85%, compared with 71% from But-P407 25%. Butorphanol diffusion from the poloxamer gel was steady and slow, whereas the rate of butorphanol diffusion from the solution decreased as a function of time.

The equation that best described the diffusion of butorphanol from the solution was an asymptotic function as follows: concentration of butorphanol = 84.61 + (0.0189 – 84.61)•e\(^{-0.0667\times\text{time}}\), where e is the mathematical exponential function. The polynomial equation that best described the diffusion of butorphanol from But-P407 25% was as follows: concentration of butorphanol = 1.86 + ([14.23×\text{time}] – [0.74×\text{time}^2]).

**Discussion**

In the present study, P407 could be compounded consistently at concentrations of 20% and 25%. Attempts to create a 30% gel were not successful; this formulation was difficult to create because it required additional time on the vortex device, required manual mixing, and was not as homogenous as the other 2 formulations. In addition, loss of product during compounding made it challenging to calculate the final concentration of butorphanol in the 30% gel.

Visual inspection of the results of repeated measurements of viscosity on a single sample over a range of temperatures revealed that variability was higher for the 30% formulation than for the 20% and 25% formulations. This variability among the formulations was influenced by several factors, including homogeneity of the sample, precision of the rheometer, and the operator. Thus, it was not surprising that the variability for the 30% formulation was the highest. Variability was higher when single measurements were made on different samples, which was evidence that consistency in preparation also influenced the variability in viscosity. The SD was particularly high for the P407 30% formulation.

For all 3 formulations, variability was highest at \(T_{\text{sol-gel}}\). This did not have biological relevance because \(T_{\text{sol-gel}}\) values for all formulations were below avian body temperature (between 38° and 40°C) and were within previously reported ranges. Values for \(T_{\text{sol-gel}}\) range from 23° to 26°C for P407 20%,\(^{5,35-38}\) from 13.8° to 25°C for P407 25%,\(^{4,5,29,35,37,38}\) and from 13° to 21°C for P407 30%.\(^{5,37}\) It was interesting to note the wide ranges reported, especially for P407 25%. Methods used in those studies to create poloxamer formulations were similar; however, 3 methods have been used to measure \(T_{\text{sol-gel}}\): rheological flow method, rheological oscillometry method, and magnetic stirrer method. The stirrer method consists of placing a magnetic stirrer in the formulation and determining the temperature at which the stirrer stops moving. Use of the 3 methods likely explained at least a portion of the variability among the studies.

Gelation time (the amount of time for the liquid poloxamer to become a gel when placed at a temperature above \(T_{\text{sol-gel}}\)) was not evaluated in the study reported here, but gelation time of P407 at room temperature is < 30 seconds for concentrations of P407 > 17.5% and is inversely correlated to the concentration of P407.\(^{32,39}\) A long in vivo gelation time might result in early loss of the drug from the carrier, which could result in a less effective slow release.\(^{4,40}\) Thus, P407 25% theoretically would be a better choice for drug delivery than would P407 20%.

Contrary to our hypothesis, P407 compounds did not have consistently higher viscosities at avian body temperatures than at mammalian body temperatures. For temperatures above the gelation temperature, viscosity did not continue to increase; instead, it reached a plateau (P407 20%) or decreased progres-
sively (P407 25% and P407 30%). There were no significant differences in the viscosity of P407 20% for temperatures between 36° and 42°C. For P407 25% and P407 30%, viscosity at some of the upper points in this temperature range was significantly lower than that at some of the lower points. However, viscosity did not decrease much, and the formulations remained a gel at the range of avian body temperatures, which indicated that they would still be appropriate for in vivo use in avian patients. The formulations remained a gel up to 45°C in the present study, which indicated that drug release variables should not change in stressed avian patients that might have a temporary increase in core body temperature as a result of handling. However, the mild differences in viscosity between mammalian and avian body temperatures, although not significant, might translate into measurable differences in slow-release pharmacokinetic properties between mammals and birds.

The initial results were used to select the most appropriate formulation for use in subsequent experiments. It was determined that P407 25% was the most appropriate formulation for further investigations into the effects of compounding with butorphanol and sterilization by microfiltration.

The T_{sol-gel} of But-P407 25% was significantly lower than that of P407 25%. Although the main excipient of the butorphanol tartrate solution used in the present study was water, it also contained citric acid monohydrate, sodium citrate, sodium chloride, and benzethonium chloride. Sodium chloride as well as benzalkonium chloride and benzethonium chloride can decrease T_{sol-gel} of P407.41 Therefore, it was possible that the excipients in the solution used in this study were responsible for a lower T_{sol-gel}. Results for the present study are in contrast with results of a study42 in which the addition of morphine (another opioid drug) to poloxamer did not modify T_{sol-gel}. However, the commercially available morphine used in that study42 did not contain the previously described excipients, and the method of preparation of the poloxamer formulation differed from that of the present study.

Although the lower T_{sol-gel} of But-P407 25% did not appear to be clinically relevant, it may be relevant during the manipulation of the formulation prior to injection. The product will become a gel at approximately 17°C, compared with approximately 19°C for P407 25%. However, most working spaces have an ambient temperature > 19°C, thus, both But-P407 25% and P407 25% would have to be kept cold (eg, on ice or refrigerated) prior to administration. Viscosity did not differ significantly between But-P407 25% and P407 25%; however, variance for the viscosity of But-P407 25% was lower than that of P407 25%. Possible explanations for this result included a difference in the number of samples evaluated (3 for But-P407 25% and 5 for P407 25%), which may have altered the precision for the estimation of the variance, and the fact that P407 powder dissolved better and in a more consistent manner in butorphanol than in water, which allowed for a more homogenous formulation.

The But-P407 formulation used in the present study was created by adding butorphanol tartrate directly to P407 powder, which was contrary to the method described for P407 in which the drugs were added to P407 and then reconstituted with water.4 To prepare a formulation with a final concentration of 25% by use of the previously described method, a more concentrated P407 product would need to be prepared and then mixed with butorphanol tartrate. However, in the authors’ experience, a P407 30% formulation cannot be reliably produced. Moreover, the method described by Dumortier et al41 would have yielded lower concentrations of butorphanol, which would make it less applicable for use in vivo because lower concentrations of butorphanol would require injection of high volumes of the gel to achieve a therapeutic dose. Use of butorphanol as the diluent for the P407 allowed for reliable creation of a 25% formulation with a high concentration of butorphanol (8.3 mg/mL), which should be appropriate for in vivo use.

Filtration of the P407 25% product through a 0.22-µm microfilter did not significantly affect T_{sol-gel} or viscosity at 40°C. Variance of the viscosity of the microfiltered samples was lower than that of the original product. It was extremely likely that the filtration process promoted the formation of a homogenous solution, perhaps by removing or breaking down larger aggregates. To the authors’ knowledge, the potential effects of microfiltration on viscosity of poloxamers have not been investigated; however, microfiltration sterilization of P407 was recommended by the manufacturer and has been used in multiple studies.43-45 Microfiltration has also been used to sterilize butorphanol formulations and was also recommended by the manufacturer.2,46-47 Thus, microfiltration appeared to be a suitable method for sterilizing a compounded But-P407 product intended for in vivo use.

Microfiltration is a method of drug sterilization approved by the US Pharmacopeia.48 A filter with a pore size of 0.2 or 0.22 µm must be used. Microfiltration also removes dead microbial cells from solutions, which is in contrast to results for chemical or thermal sterilization.49 Because cellular debris may have pyrogenic properties in vivo, microfiltration sterilizes a solution and also decreases potential pyrogenicity. Microfiltration may also remove viruses, but efficiency is variable.50

Results of the investigation of in vitro diffusion of the But-P407 25% formulation confirmed that the opioid was released from the gel and that the release was slower than for a standard solution of butorphanol tartrate. The specific numeric data may not accurately reflect an in vivo situation for a number of reasons. First, diffusion occurred through a synthetic membrane, which is extremely different from animal tissues. Moreover, physiologic fluids in which the gel will dissolve are continuously renewed in vivo, which would suggest a higher pick up by the law of
mass action, resulting in a higher release rate. Other factors influencing release might include destruction of the gel by macrophages and other inflammatory cells. Therefore, drug release would most likely be more rapid in vivo.

The But-P407 25%, which was created by directly mixing P407 powder with butorphanol tartrate solution, appeared to be a good candidate for a sustained-release analgesic medication for use in avian species. This preparation process resulted in a homogenous solution and minimally affected the rheological properties of P407 at avian body temperatures. Poloxamer 407 is inexpensive and readily available; however, studies are needed to characterize the pharmacokinetic and pharmacodynamic properties of this product in vivo as well as to determine stability of compounded opioid-poloxamer formulations over time before such products can be recommended for use in avian patients.

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Footnotes

a. BASF Corp, Sigma-Aldrich, Oakville, ON, Canada.
b. Torbugesic, Wyeth, Markham, ON, Canada.
c. BenchMixer, Benchmark, Edison, NJ.
d. Millex GP filter unit, 0.22-μm, Millipore Express PES membrane, Merck Millipore Ltd, Cork, Ireland.
e. AR 2000, TA Instruments, Mississauga, ON, Canada.
f. SnakeSkin dialysis tubing, 3.5K MWCO, 22 mm, ThermoFisher Scientific, Rockford, III.
g. Amresco, Solon, Ohio.
i. Toronto Research Chemicals, Toronto, ON, Canada.

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