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A 'biorelevant' approach to accelerated in vitro drug release testing of a biodegradable, naltrexone implant

Sunil S. Iyer [a], William H. Barr [b], H. Thomas Karnes [a,*]

[a] *Department of Pharmaceutics, School of Pharmacy, Virginia Commonwealth University, Richmond, VA 23298-0533, USA*
[b] *Center for Drug Studies, School of Pharmacy, Virginia Commonwealth University, Richmond, VA 23298-0533, USA*

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Abstract

The development of a 'biorelevant' approach for accelerating drug release from an implant is described. A miniature, capillary system has been shown previously to be suitable for real-time release tests for a biodegradable, naltrexone implant. Whereas the real-time study under physiological condition was essential for evaluation of the system, the accelerated (short-term) method provides for a faster assessment of in vitro drug release that would be useful in product development and quality control.

Increased temperature was employed as the mechanism for accelerating drug release. Release rates were investigated and compared using modifications of two devices: the flow-through cell and the new, potentially more 'biorelevant' capillary device. The data generated for accelerated release using both devices through 45 days indicated approximately two-fold and four-fold increases in release rates at 45 and 55 °C, respectively, as compared to the real-time release rate. The similar activation energy values for both devices obtained from Arrhenius plots demonstrated that the release mechanism had been consistent; and that the rates of release could be used for long-term prediction. The rate of release reverted to that observed in real-time data, however, upon a reduction of temperature to 38 °C. The results demonstrated that temperature was the sole factor involved in modification of the release rate in vitro. The profiles using both systems followed zero-order kinetics after an initial period of burst release.

Keywords: Naltrexone implant; Modified Hank's balanced salts solution; Capillary and flow-through devices; Accelerated in vitro release testing

1. Introduction

Most depot formulations made from biodegradable polymers release the incorporated drug over an extended period of time (Eldrige et al., 1991). An estimation of the real-time release rate is critical for characterization of these dosage forms. The process however, consumes significant time spanning weeks or months for sustained release parenteral dosage forms. This is disadvantageous in early research, and therefore not conducive for efficient management of the product development pipeline. Accelerated in vitro release tests are also desirable for quality control, particularly for setting specifications for releasing batches.

Accelerated stability testing has been employed to predict the shelf life of drugs and dosage forms (Baertschi and Jansen, 2005). Method applications to accelerate dissolution rates of oral dosage forms have been published (Scott, 2002). Several parameters, such as changes in temperature, pH, solvent, ionic strength, surfactants, agitation rate, enzymes, and the application of microwaves alter the rate of in vitro dissolution/release (Iyer et al., 2006). The primary consideration during application of these tests is that only the rate of drug release should increase as a function of changes in the selected parameter; the mechanism of drug release, however, should not be altered (Burgess et al., 2002). Furthermore, the accelerated test should mimic physiological conditions at the site of administration (termed 'biorelevance') to the extent possible.

For biodegradable matrices, temperature is most often selected as the parameter of choice since it has been proven to induce maximum effects on release rates (Scott, 2002). The application of accelerated tests for in vitro release of subcutaneous implants requires prior consideration of several factors (Table 1). These factors represent changes that could be induced as a result of employing stressed conditions for the test. As an

* Corresponding author. Tel.: +1 804 8283819; fax: +1 804 8288359.
E-mail address: tom.karnes@vcu.edu (H.T. Karnes).

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Table 1
Factors influencing application of stress conditions for accelerating in vitro drug release from subcutaneous implants

Selection of an appropriate parameter for stress induction
The mechanism controlling the release of drug from the dosage form
The stability of the medium
The stability of the drug in the dosage form, and in the medium once released
The robustness of the in vitro release apparatus to withstand the applied stress
Determination of the sampling interval and the duration of the study
The solubility of the drug, the dose rate and the influence of 'sink' conditions

example, variables that control the release rate from matrix-based drug delivery systems are the composition of the polymer and porosity changes (Bergsma et al., 1994). The variables are influenced at elevated temperature to shorten degradation time for polylactides (Aso et al., 1994), and to demonstrate drug release mechanisms (Cha and Pitt, 1989; Kaniwa et al., 1995). However, as a function of the composition of the polymer, drug release is also accompanied by a softening of the polymer due to enhanced hydration and degradation (Pitt, 1990). Therefore, it is important to select and adjust the parameter only to an extent such that the integrity of the dosage form matrix is not compromised.

Stabilities of the medium and the drug are also significant factors that need consideration. The components of the medium are likely to undergo degradation under the stress conditions employed. The degradation products formed would accumulate in the medium if a closed system, in which the same medium is kept recirculating through the release apparatus into the reservoir for the complete duration of study, is used. Several confounding events can then occur in the system. Drug release may be influenced by possible deviations in pH, buffer capacity, or osmolality from initial values of a medium freshly prepared. In addition, a faster degradation of the dosage form matrix may be induced due to bulk processes, such as 'autocatalysis', whereby the liberated free carboxylic end groups catalyze further cleavage of ester groups of polycaprolactones. This could result in complicated release mechanisms, deviating from the objective of keeping the mechanism consistent with real-time studies.

Another important factor for consideration during method optimization for accelerated release is the robustness of the test apparatus to withstand the applied stress conditions. This requires that the apparatus must have well-defined components that do not undergo deterioration. Depending upon the exposure of parts to the flow of medium, all pieces of tubing, connectors, and filters, O-rings, etc. have to be checked for abrasion under test conditions.

Further insight can be gained by an investigation of the drug's stability under elevated temperatures. Verification of the validity of using these conditions might include an Arrhenius plot after obtaining release rate profiles from linearized release profiles (Makino et al., 1985). The question that remains, however, is what constitutes a significant change in the dissolution profile, since defined limits for the consideration of percent drug remaining in the medium after a designated test time are not available in any guidance (Storey, 1996).

Naltrexone, an opiate receptor antagonist (Resnick et al., 1974), has been recognized as a good candidate for formulation as an implant to increase patient compliance and ultimately improve treatment effectiveness (Brewer, 2002; Hulse et al., 2004). In earlier studies (Iyer et al., 2007; Iyer et al., in press), we investigated a 'biorelevant' approach to real-time drug release testing of a biodegradable, subcutaneous implant of naltrexone. Design modifications of a capillary device and a flow-through cell were employed for release rate investigations, and the data suggested that a better representation of in vivo drug absorption may be achieved in vitro using the capillary device. In addition, a detailed characterization of the modified Hank's balanced salts solution had established its potential for in vitro drug release studies. Although the use of accelerated methods for dissolution rate studies of oral dosage forms (Quist and Östling, 2002) are available, no published information is available on application of this technique for subcutaneous implants.

In this study, we describe an approach based on elevated temperature to accelerate naltrexone release from the implant. This is the first publication on accelerated release testing for a subcutaneous implant dosage form, although published approaches for tablets are available. Two elevated temperatures, 45 and 55 °C, were investigated for increasing the rate of drug release. Modified flow-through cells and previously separated capillary devices for real-time release were utilized.

2. Materials and methods

2.1. Materials

Naltrexone hydrochloride (USP Grade, working standard) for the assay was obtained from Sigma (St. Louis MO, USA). The capillary device (Cellmax™) was procured from Spectrum Labs, CA, USA. The sagittal saw for the study was generously provided by Stryker Corporation, MI, USA. Analytical grades of triethylamine, ammonium hydroxide and trifluoroacetic acid, and Hank's balanced salts (1 × 10 l) and HEPES buffer (10 mM) were purchased from Sigma (St. Louis, MO, USA). Sodium hydroxide was procured from Fisher Scientific (Fairlawn, NJ, USA). HPLC grade Acetonitrile was purchased from Burdick & Jackson (Honeywell International, Inc. MI, USA). Water was obtained in-house using the Nanopure Diamond water system (Barnstead International, IO, USA).

2.2. Selection of temperature as the parameter for accelerating in vitro release rate

The modified Hank's balanced salts solution was pumped at the same flow rate as employed in the real-time study (Iyer et al., in press). The 'biorelevant' flow rate had been calculated to be 1.06 ml/min on the basis of shear stress (0.07–20 dyne/cm^2) that endothelial cells are exposed to in vivo (Redmond et al., 1995). Although an enhancement of flow rate within the biorelevant range was possible as an option to accelerate drug release using the flow-through cell, it could not be expected to result in significant changes in release rates with the capillary device. This was because the flow of medium entering a capillary device

would be split between 50 capillaries within the device. Also, since the implant was placed in the extracapillary space (representative of the subcutaneous interstitium) as compared to the flow-through cell, the medium would not flow directly across its length. Any increase of flow rate in the circulating loop would not have translated into a corresponding flow rate increase at the site in which the implant was positioned. Therefore, flow rate was not considered as a useful parameter. pH and ionic strength of the medium could also be potentially modified to accelerate drug release. However, a reduced ionic strength would denote a reduction in buffer capacity of the medium and could result in the medium to deviate from physiological pH range when used over a prolonged period of time. Polycaprolactone and poly(glycolic acid) are degraded by enzymes, such as esterase and elastase Park et al. (1993). The enhanced degradation of the polymer could increase the release rate of drug. However, the addition of enzymes could potentially lead to other challenges, such as development of selective analytical methods capable of detecting a larger number of degradation products generated by the enzyme itself. Furthermore, a difficulty exists in maintaining enzyme activity constant throughout the period of study. The use of surfactants would also suffer from similar disadvantages as enzymes, in terms of maintenance of exact concentration and detection of additional degradation products.

Therefore, temperature was selected as the parameter of choice. Two elevated temperatures, 45 and 55 °C, were utilized for this purpose, and a comparison of data obtained using the modified flow-through cell and capillary device were compared with the real-time release rates at 38 °C. The 55 °C was selected as the highest temperature based upon the melting point range of the polymer, 59–64 °C (Pitt et al., 1980; Pitt, 1990).

2.3. Calculation of 'sink' conditions

The solubility of naltrexone freebase in Hank's balanced salts solution at 32 °C is 5.42 mM Stinchcomb et al. (2002). This reported value was used for calculation of 'sink' conditions. Based upon a conservative factor of 3 times the solubility of the drug, the threshold concentration for a departure from sink conditions at 32 °C would be 1.63×10^{-2} M or 5.54 mg/ml. Since release rates from implants were expected to be much slower and assuming that the solubility of naltrexone does not vary much as a function of temperature, the value indicated fair flexibility provided for accumulation of drug in the release medium prior to replacement.

2.4. Additional modifications of system assembly

All components of the system used previously for investigation of real-time release rate were employed also for this study. No changes were required, except that out of the six flow-through cells and six capillary devices used previously at 38 °C, three each were utilized at the two elevated temperatures investigated. Two water baths calibrated to provide temperatures of 45 and 55 °C inside the media reservoirs were utilized for this purpose. The flow path and location of the system components (pump, tubing, etc.) relative to each other were modified suitably. This involved providing new connections to all 12 channels of the pump in order to divert them to the desired devices.

The other system components that including reservoirs, fraction collectors, glass beads, and filters were the same as employed in the previous paper (Iyer et al., in press). The pump was calibrated for a flow rate of 1 ml/min.

2.5. Test material

The naltrexone implants employed earlier in the real-time release study (Iyer et al., in press) were utilized for this investigation. Important information about the persistence of an intact membrane sheath and a homogenous core after 90 days of real-time testing had been obtained through scanning electron microscopy. These implants were obtained from Durect Corporation, CA, USA. The monolithic implant consisted of a biodegradable core of naltrexone: polycaprolactone enclosed in a sheath of poly-(DL-lactide:caprolactone). The implant itself was fabricated by melt-extrusion and the ends of the cylinder were sealed with the same polymer that had been used for the membrane sheath. The implants retained their dull grey appearance following the real-time in vitro study. Fig. 1 is a photograph of the implant after the real-time in vitro release study.

2.6. Accelerated in vitro drug release experiments

The flow-through and capillary systems were modified to accommodate the implants as described earlier (Iyer et al., in press). The flow-paths were assembled as described in Section 2.4. A final check of the flow rate was conducted before

Fig. 1. Photograph of the biodegradable naltrexone implant after real-time release investigation of 90 days.

reservoirs containing fresh media pre-heated to the selected temperatures were placed in the water bath, and the flow path closed. For the accelerated study, three flow-through and three capillary devices containing an implant were studied at 45 and 55 °C. Since one implant had been sacrificed for microscopic evaluation following real-time studies, it was decided to employ two implants for the flow-through cell at 55 °C. When the system was ready, the pump was switched on. The side ports of the capillary device were kept open initially to allow air in its extracapillary space to escape. As soon as the medium made its way up, the ports were closed to enable the medium to exit via the end port into the reservoir. The system was operated continuously except that the pumps were stopped during replacement of buffers. Samples were collected every 24 h for 45 days, and were stored below −20 °C until analysis.

Periodic checks were conducted for the temperature inside the vessel. For reservoirs at 45 and 55 °C, the flow rate and pH of the media were checked every 4th and 3rd day, respectively, as determined by Iyer et al. (2007). Media replacement was also conducted on those days. For medium replacement, the outlet from the reservoir was removed. After the medium in the loop drained back in the reservoir, the pump was stopped. The stopper was removed, and the needles and filter were rinsed thoroughly with water to prevent any carryover of naltrexone. Reservoirs containing fresh medium pre-heated to 45 °C or 55 °C, as the case may be, was placed in the water bath, and the stopper was replaced. The pump was switched back on and as described previously, side ports of the capillary device were manipulated to allow entrapped air to escape. The complete procedure required less than 10 min of pump stoppage time. No fluctuations in flow rate were observed throughout the study, because no clogging of the filters occurred. At the end of 45 days, the implants were taken out of the release devices, and allowed to dry at room temperature and the weights were recorded.

It was decided to cross-validate the approach in terms of changes in the implant matrix during the accelerated study that could potentially influence the mechanism of release. This was based on the assumption that if at all any matrix changes had taken place, a reversal of the temperature back to real-time condition (38 °C) would result in a different release profile from that reported initially (Iyer et al., in press). Therefore using both types of devices, the study was extended into a real-time investigation at 38 °C to cross-check if satisfactory release profiles were still obtained. A single water bath was used for this purpose, as had been described in the earlier paper (Iyer et al., in press).

3. Results and discussion

3.1. System performance

The system worked efficiently throughout the study period. At the end of Day-20, the peristaltic pump tubing was checked for abrasions to avoid possible leakage. Replacement of tubing was not required during the study. No visible contamination of microorganisms was observed indicating the efficiency of the antimicrobial agent, Primocin. The pump performance was rugged in terms of a constant maintenance of flow rate during the entire study period. No flow fluctuations due to clogging of filters in the flow-through device were observed, also indicating that the dosage form had retained its integrity. This was further verified at the end of the study by removal of the dosage form from the devices and measuring its dimensions.

On one occasion while the capillary devices was running at 55 °C, a minor leak (approximately less than 2 ml of medium had been lost) was detected at the spot where the system had been resealed. This was effectively sealed within 0.5 h, however, and a concentration factor for the lost amount of naltrexone was employed in further calculations for that particular device.

Media replacement proceeded smoothly with a 10-min pump stoppage time. The pH was observed to vary up to only ±0.05 units as measured for the medium immediately after each replacement; thereby indicating that the modified Hank's balanced salts solution had adequate buffer capacity for accelerated study of the implant. A visible examination of media performed periodically indicated the absence of microbial contamination.

3.2. Sample analysis

The samples were analyzed by a validated high performance liquid chromatography (HPLC) method described elsewhere (Iyer et al., 2007). The peak areas at 204 nm for naltrexone were used for quantification. No chromatographic interference was observed from any degradation product. The calibration curves were linear in the range of 0.16–20 μg/ml ($r^2 > 0.99$) using a weighting factor of 1/concentration, and the precision and accuracy of quality control samples processed and analyzed along with the samples were all within 5% of the nominal concentration. The results were expressed as micrograms per milliliter of medium, that were used for calculation of cumulative drug released by taking into account the volume of medium in the reservoir and a correction factor for the amount of drug lost at each sampling point.

A R.S.D. of 19.43% ($n = 6$) was obtained for mean concentrations of naltrexone in samples withdrawn 12 h after each consecutive replacement of medium. Any additional peaks that would have represented degradation of drug, dosage form, or capillary material, were not observed. Assuming drug release to follow zero-order kinetics, this result is consistent with the fact that: (a) solid-state degradation of the drug in the implant, if any, was negligible, and (b) no carryover in terms of non-specific binding of naltrexone to the flow path existed.

3.3. Analysis of accelerated in vitro release data

A comparison of the accelerated release profiles obtained for 45 days using the modified flow-through and capillary devices is made in Fig. 2. Overall, the rates of release of naltrexone using the flow-through cell were 0.22 mg/12 h (95% CI: 0.198, 0.233), and 0.39 mg/12 h (95% CI: 0.348, 0.429), respectively, at 45 and 55 °C. The rates of release of naltrexone using the capillary device were 0.09 mg/12 h (95% CI: 0.077, 0.102), and 0.18 mg/12 h (95% CI: 0.161, 0.201), respectively, at 45 °C and 55 °C. The capillary device resulted in lower rates

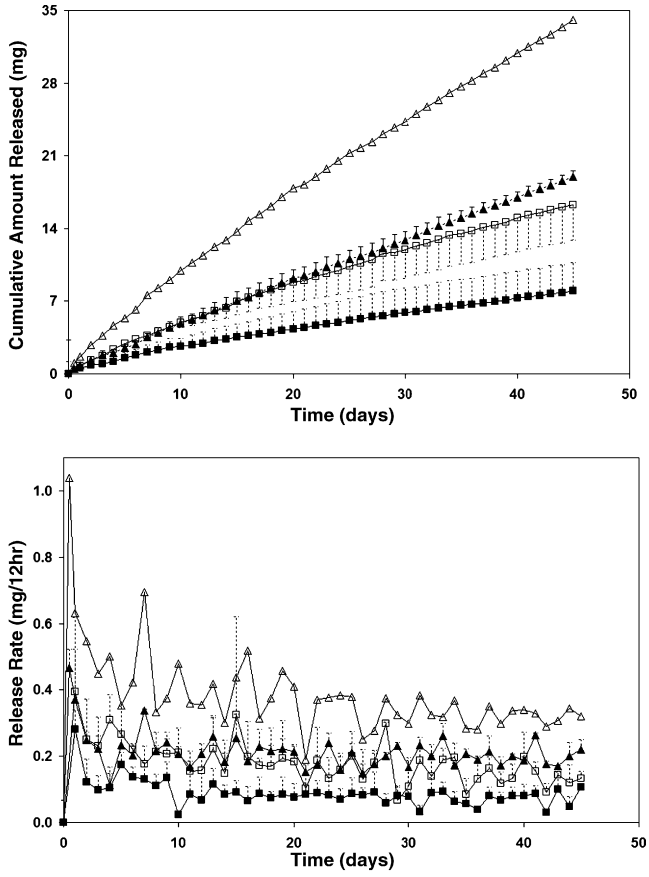

Fig. 2. (a) Cumulative release plot and (b) release rate plot for naltrexone implants under accelerated conditions using the modified flow-through and capillary devices ($n = 3$). Key: (△) flow-though cell at 55 °C*; (▲) flow-though cell at 45 °C; (□) capillary deivce at 55 °C; (■) capillary device at 45 °C (error bars represent standard deviations; *$n = 2$).

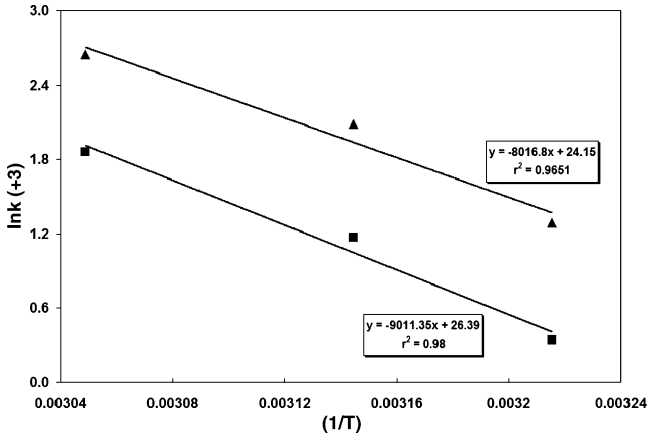

Fig. 3. Arrhenius relationships of release rates using the modified flow-through ($n = 5$) and capillary devices ($n = 6$) at 38, 45 and 55 °C. Key: (▲) flow-though cell; (■) capillary device.

of release at corresponding temperature levels, consistent with those observed in real-time data. This can be explained by a lower rate of flow of the medium in extracapillary space, thus effectively simulating a barrier to diffusion that would exist in vivo.

For both the devices and at both temperatures, the values mentioned above represented a significant ($p < 0.0001$) increase in the overall rate of release from the corresponding real-time rates. This proved that the increased temperature had succeeded in accelerating the release rate from the implant in vitro.

Table 2 represents mean release rates of naltrexone from the implants using both devices at 38, 45, and 55 °C, in periods following initial burst when steady zero-order profiles were observed. At 45 °C, the release rate using the flow-through cell and capillary device increased 2.2-fold and 2.0-fold, respectively, as compared to the corresponding rates at 38 °C; whereas at 55 °C, the rates increased 3.9-fold and 4.0-fold with the corresponding devices. These observations show that, irrespective of the type of device used, temperature was the sole critical parameter that determined increase in release rate.

Furthermore, an initial period of burst release was observed consistent with the phenomenon observed during real-time release. The percolation-limited diffusion theory (Tzafriri, 2000) offers a possible explanation for this phenomenon. A pool of mobile drug molecules would exist on the dried polymer surface. As soon as fresh medium flows into the pool, naltrexone release would follow immediately and account for the phase of burst release. For the flow-through cells, mean peak release rates of 1.04 and 0.47 mg/12 h were observed at 55 and 45 °C, respectively; whereas for the capillary device, the rates were 0.37, and 0.28 mg/12 h, respectively, at the two temperatures. The time for peak burst release at both temperatures, however, remained 0.5 and 1 day with the flow-through and capillary devices, respectively, as comparable to corresponding observations in real-time release profiles. This observation shows that although the magnitudes of release rates were different, no indication of a change in release mechanism had occurred.

To demonstrate the validity of the temperatures used, an Arrhenius relationship was investigated for both devices using the zero-order rate constants. A good linear relationship (Fig. 3) was found for both devices. The energy of activation (E_a), as calculated from the slopes was 16.62 and 17.84 kcal/mol, for the

Table 2
Comparison of mean release rates (mg/12 h) of naltrexone at different temperatures

Condition	Type of device		Statistical significance (p)
	Flow-through	Capillary	
38 °C (Data beyond Day-25)	0.09 (0.04)	0.04 (0.03)	<0.0001
45 °C (Data beyond Day-10)	0.20 (0.03)	0.08 (0.02)	<0.0001
55 °C (Data beyond Day-10)	0.35 (0.07)	0.16 (0.05)	<0.0001

Values in parentheses represent standard deviations.

flow-through cell and capillary device, respectively. The similar activation energy values obtained for both the devices demonstrated that the release mechanism had been consistent; and that the rates of release could be used for long-term prediction. Also, the E_a values were greater than 5.65 kcal/mol, the activation energy earlier reported (Iyer et al., 2007) for naltrexone in the same medium under similar conditions. Although activation energies cannot be assumed to be truely additive, we can speculate that the higher values of E_a are a result of contributions from degradation and/or erosion of the polymer.

It is recommended that the specifications for accelerated release should include a determination of at least 80% of the cumulative amount released (Burgess et al., 2002). Also, for a prediction between accelerated and real-time release, it has been suggested that the time to reach a cumulative release of approximately 100% be used to determine whether a relationship can be established for products with different real-time release rates. A major objective for any product is to provide a thorough characterization of the release profile in vitro, the practical aspects of the recommendation are questionable, especially when sustained release dosage forms with drug release over a period of months are investigated. Furthermore, the Arrhenius plots for both devices showed that a prediction for long-term accelerated release from this implant could be made. Therefore, it was decided to terminate the study after 45 days, during which the release rates were determined to be consistent with zero-order kinetics.

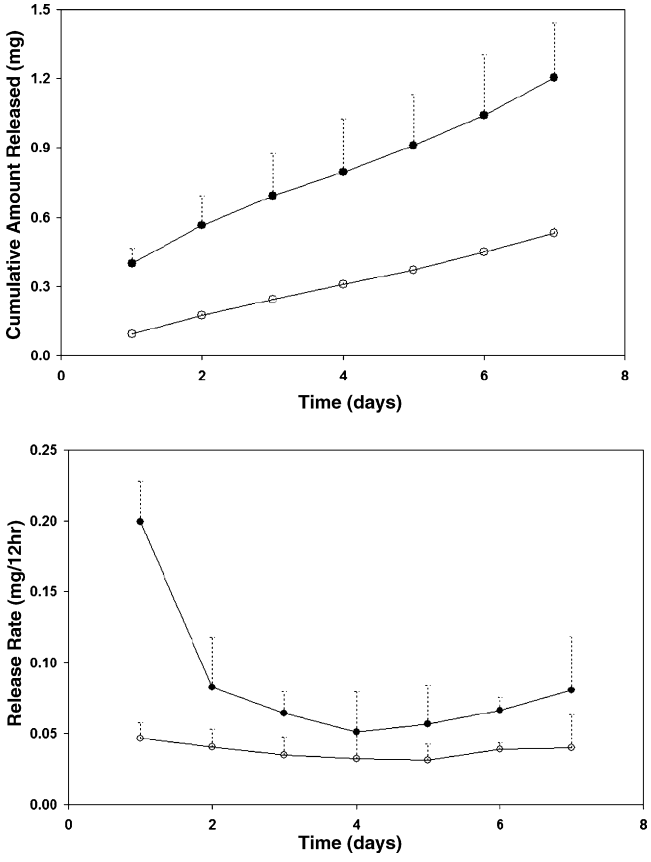

Fig. 4. (a) Cumulative release plot and (b) release rate plot for naltrexone implants under real-time conditions using the modified flow-through ($n = 5$) and capillary devices ($n = 6$) to cross-validate the accelerated release study. Key: (●) flow-though cell; (◯) capillary device.

Fig. 4 is a representation of the profiles obtained after reverting the test temperature back to 38 °C. A phase of burst release more prominent for the flow-through cell was observed. Release rates decreased correspondingly with the flow-through cell [0.07(±0.013) mg/12 h] and capillary device [0.04(±0.004) mg/12 h]. This observation demonstrates that the integrity of the dosage form was maintained throughout the studies and the release rate mechanism followed zero-order kinetics after an initial stage of burst release.

4. Conclusion

This study investigated the application of a 'biorelevant' approach to accelerate naltrexone release from a biodegradable implant. The capillary system was employed without any major modification from that used for real-time study and remained stable throughout the investigation at elevated temperatures. The modified Hank's balanced salts solution has been shown to be suitable for accelerated release rate investigation of the implant. Sample analyses using a validated stability-indicating HPLC method resulted in zero-order in vitro release profiles. Drug release using both devices increased by identical magnitudes without affecting the rate mechanism. The results demonstrated the controlled use of temperature to be a useful parameter to investigate accelerated drug release from implant dosage forms. Further investigations with implants having different release rate characteristics would validate the approach. Upon validation, the methodology will be very useful for long-term prediction of drug release from implants, or for setting specifications as part of quality control, depending upon the stage of development of the test product. Decisions regarding the duration for which accelerated release studies need to be conducted in order to allow adequate characterization of drug release need to be made on a case-by-case basis.

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References

Aso, Y., Yoshiyoka, S., Wan Po, L., Terao, T., 1994. Effect of temperature on mechanisms of drug release and matrix degradation of poly(D,Llactide) microspheres. J. Controlled Release 31, 33–39.

Baertschi, S.W., Jansen, P.J., 2005. Stress testing: a predictive tool. In: Baertschi, S.W. (Ed.), Pharmaceutical Stress Testing. Predicting Drug Degradation. Drugs and the Pharmaceutical Sciences, vol. 153. Marcel Dekker Inc., New York, pp. 13–50.

Bergsma, J.E., Rozema, F.R., Bos, R.R.M., Boering, G., De Bruijn, W.C., Pennings, A.J., 1994. In vivo degradation and biocompatibility study of in vitro pre-degraded polymerized polylactide particles. Biomaterials 16, 267–274.

Brewer, C., 2002. Serum naltrexone and 6-beta-naltrexol levels from naltexone implants can block very large amounts of heroin: a report of two cases. Addict. Biol. 7, 321–323.

Burgess, D.J., Hussain, A.S., Ingallinera, T.S., Chen, M.L., 2002. Assuring Quality and Performance of Sustained and Controlled Release Parenterals: Workshop Report. AAPS Pharm. Sci., 4, article 7.

Cha, Y., Pitt, C.G., 1989. The acceleration of degradation-controlled drug delivery from polyester microspheres, 8, 259–265.

Eldrige, J.H., Staas, J.K., Meulbroek, J.A., Tice, T.R., Gilley, R.M., 1991. Biodegradable and biocompatible poly(DL-lactide-co-glycolide) microspheres as an adjuvant for staphylococcal enterotoxin B toxoid which enhances the level of toxin neutralizing antibodies. Infect. Immun. 59, 2978–2986.

Hulse, G.K., Arnold-Reed, D.E., O'Neil, G., Chan, C.-T., Hansson, R., O'Neil, P., 2004. Blood naltrexone and 6-β-naltrexol levels following naltrexone implant: comparing two naltrexone implants. Addict. Biol. 9, 59–65.

Iyer, S.S., Barr, W.H., Karnes, H.T., 2006. Profiling in vitro drug release from subcutaneous implants: a review of current status and potential implications on drug product development. Biopharm. Drug Dispos. 27, 157–170.

Iyer, S.S., Barr, W.H., Karnes, H.T., 2007. Characterization of a potential medium for 'biorelevant' in vitro release testing of a naltrexone implant, employing a validated stability-indicating HPLC method. J. Pharm. Biomed. Anal. 43, 845–853.

Iyer, S.S., Barr, W.H., Dance, M.E., Coleman, P.R., Karnes, H.T. A 'biorelevant' system to investigate in vitro drug released from a naltrexone implant. Int. J. Pharm, in press.

Kaniwa, N., Shameem, M., Katori, N., Aoyagi, N., Kojima, S., 1995. The suitable temperature for the evaluation of controlled release products in vitro. Pharmazie 50, 53–55.

Makino, K., Arakawa, M., Kondo, T., 1985. Preparation and in vitro degradation properties of polylactide microcapsules. Chem. Phar. Bull. 33, 1195–1201.

Park, K., Shalaby, W.S.W., Park, H., 1993. Biodegradable hydrogels for drug delivery. Technomic Publishing Co., Inc., Lancaster, PA, pp. 189–232.

Pitt, C., Marks, T.A., Schindler, A., 1980. Biodegradable drug delivery systems based on aliphatic polyesters: application to contraceptives and narcotic antagonists. In: Baker, R. (Ed.), Controlled Release of Bioactive Materials. Academic Press, Inc., New York, pp. 19–43.

Pitt, C., 1990. Poly-ε-caprolactone and its copolymers. In: Chasin, M., Langer, R. (Eds.), Biodegradable Polymers as Drug Delivery Systems, Drugs and the Pharm. Sciences Series, vol. 45. Marcel Dekker, Inc., New York, pp. 71–120.

Quist, P.O., Östling, G., 2002. Accelerated dissolution testing for improved quality assurance. J. Pharm. Biomed. Anal. 28, 1081–1089.

Redmond, E.M., Cahill, P.A., Sitzmann, J.V., 1995. Perfused transcapillary smooth muscle and endothelial cell co-culture-a novel in vitro model. In vitro Cell Dev. Biol. 31, 601–609.

Resnick, R.B., Volavka, J., Freedman, A.M., Thomas, M., 1974. Studies on EN-1639A (naltrexone): a new narcotic antagonist. Am. J. Psychiatry 131, 646–650.

Scott, P., 2002. Process analytical technology: applications to the pharmaceutical industry. Dissol. Tech., 6–9.

Stinchcomb, A.L., Swaan, P.W., Ekabo, O., Harris, K.K., Browe, J., Hammel, D.C., Cooperman, T.A., Pearsall, M., 2002. Straight-chain naltrexone ester prodrugs: Diffusion and concurrent esterase biotransformation in human skin. J. Pharm. Sci. 91, 2571–2578.

Storey, D.E., 1996. The role of dissolution testing in the design of immediate release dosage forms. Drug Info. J. 30, 1039–1044.

Tzafriri, A.R., 2000. Mathematical modeling of diffusion-mediated release from bulk degrading matrices. J. Controlled Release 63, 69–79.