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RESEARCH PAPER

# Long-Acting Poly(DL:Lactic Acid-Castor Oil) 3:7-Bupivacaine Formulation: Effect of Hydrophobic Additives

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## ABSTRACT

***Purpose*** To reduce formulation viscosity of bupivacaine/poly (DL lactic acid co castor oil) 3:7 without increasing bupivacaine release rates.

***Methods*** Poly(DL lactic acid) 3:7 was synthesized and bupivacaine formulation prepared by mixing with additives ricinoleic acid or castor oil. *In vitro* release measurements identified optimum formulation. Anesthetized ICR mice were injected around left sciatic nerve using nerve stimulator with 0.1 mL of formulation. Animals received 10% bupivacaine-polymer formulation with 10% castor oil (p(DLLA:CO)3:7–10% bupi-10% CO) or 15% bupivacaine-polymer with 10% castor oil (p(DLLA:CO)3:7–15% bupi-10% CO). Sensory and motor block were measured.

***Results*** Viscosity of 10% and 15% bupivacaine-p(DLLA:CO) 3:7 formulations was reduced using hydrophobic additives; however, castor oil reduced bupivacaine release rates and eliminated burst effect. Less than 10% of the incorporated bupivacaine was released during 6 h, and less than 25% released in 24 h *in vitro*. *In vivo* formulation injection resulted in a 24 h motor block and a sensory block lasting at least 72 h.

***Conclusions*** Incorporation of hydrophobic low-viscosity additive reduced viscosity in addition to burst release effects. Bupivacaine-polymer formulation with castor oil additive demonstrated prolonged sensory analgesia *in vivo*, with reduced duration of motor block.

**KEY WORDS** bupivacaine-polymer · castor oil · prolonged analgesia · ricinoleic acid · viscosity

## INTRODUCTION

Postoperative pain is a major problem for the healing process after surgery. Fifty percent of discharged out-patients suffer moderate pain during the first 24–48 h (1). Pain is mediated by sensory fibers, and their action can be blocked by the presence of local anesthetic agents on the sodium ion channels, which prevents the spread of depolarization and may increase the refractory period. In order to produce an effect, the local anesthetic must diffuse across the nerve sheath in the form of an uncharged free base (non-ionized form) (2). Once the local anesthetic has left the sodium channel, depolarization returns to normal. Bupivacaine is a clinically effective amide local anesthesia agent commonly used in all invasive analgesia techniques: epidural, spinal, local nerve blockade, and wound infiltration. It has a relatively rapid onset of action, and duration of action up to 24 h when used in local infiltration. Encapsulation of massive doses of bupivacaine could provide continuous slow release of the drug to increase the duration of the analgesia (3,4). Bupivacaine delivered directly in massive doses in non-encapsulated form

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would be lethal. Several delivery systems were evaluated (liposomes, liquid injectable polymers, lipospheres, lipid-protein-sugar particles, PLGA micro and nanospheres) (5–9); however, only liposomes and injectable liquid polymers met the desired criteria in terms of formulation administration, degradation rates of the carrier, and *in vivo* efficacy (10–14). Clinical application of a bupivacaine-polymer formulation has potential advantages over liposomes. Liposome manufacturing lacks reliability and reproducibility due to oxidation and hydrolysis, which result in leaking of the encapsulated drug. In addition, low weight-to-volume ratio decreases the payload which can be incorporated (15). On the contrary, incorporation of bupivacaine into polymer carrier is easy and reliable and offers high loading potential. Previously, we demonstrated that poly(lactic acid co castor oil 3:7) loaded with 10% bupivacaine and 15% bupivacaine formulation prolonged *in vivo* efficacy of bupivacaine up to 48 (12) and 96 (13) hours, respectively. However, these formulations were viscous (about 10,000 cP) at room temperature, which hindered their injectability. It was hypothesized that addition of low viscosity hydrophobic liquids could reduce the formulation viscosity, improving the injectability of the formulation.

In addition, increasing carrier hydrophobicity by increasing hydrophobic drug loads reduces bupivacaine release rates and doubles *in vivo* efficacy (12–14,16). It was previously demonstrated that the bupivacaine release rates have been reduced using oil depot formulations (17). Through increasing carrier hydrophobicity to improve injectability, a reduction of the bupivacaine release rate could prolong sensory analgesia whilst reducing the duration of the motor block (differential nerve blockade), since previous *in vivo* testing demonstrated a relatively long duration of motor block seen with our formulations.

## MATERIALS AND METHODS

### Materials

Poly(DL lactic acid) 3:7 designated as p(DLLA-CO) 3:7 was synthesized as previously described (18). The polymer has Mw = 2900 and Mn = 2300 and is a clear liquid at room temperature. Castor oil European Pharmacopoeia (Eur Ph) (CO) was obtained from Florish (Haifa, Israel). DL lactic acid (DL-LA) was purchased from J. T. Baker (Deventer, The Netherlands). Ricinoleic acid (99%) was prepared by hydrolysis of castor oil as previously described (8). Bupivacaine HCl USP was purchased from Eurotrade, Commerce, S.L.

$CDCl_3$ for NMR was purchased from Sigma-Aldrich (Rehovot, Israel). All solvents and salts were analytical grade from Aldrich or Biolab (Jerusalem, Israel).

### Preparation of Formulation and *In Vitro* Drug Release

Bupivacaine free base was prepared from bupivacaine hydrochloride by alkaline precipitation and filtration as previously described (12). Two additives: ricinoleic acid (RA) or castor oil (CO) were selected as potential liquefying agents, and their effect on previously reported polymer-bupivacaine formulations was examined *in vitro* and *in vivo*. The formulations were prepared by directly mixing all components at room temperature in two steps: (1) ricinoleic acid or castor oil (10% and 20% wt/wt) was mixed with the polymer until homogeneous mix was formed, (2) bupivacaine (10, 15 or 20% wt/wt) was incorporated in the mix paste by trituration as previously described (12,13).

The GPC evaluations were performed on a gel permeation chromatography (GPC) system consisting of a Waters 1515 Isocratic HPLC Pump, with 2410 Refractive Index detector (RI) (Waters, MA), a Rheodyne (Coatati, CA) injection valve with a 20 μL loop. Samples were eluted with chloroform through a linear Styrogel column, 500Å-pore size (Waters, MA), at a flow rate of 1 mL/min. The molecular weights were determined relative to polystyrene standards (Polyscience, Warrington, PA) with a molecular weight range of 500 to 12000 using BREEZE 3.20 version, copyright 2000.

*In vitro* drug release studies were conducted by injecting 0.1 ml the bupivacaine-additive-polymer formulation in 50 ml of dissolution medium (phosphate buffer 0.1 M, pH 7.4) at 37°C with constant shaking (100 rpm), where it formed a droplet in the buffer. At each time point the release buffer was fully removed and replaced with fresh buffer solution and the bupivacaine concentration determined by HPLC. Bupivacaine concentrations in buffer solutions were determined using a C18 reverse phase Hypersil GOLD Phenyl column (250×4.6 mm, 5 μm) (Thermo Scientific). A mixture of 50% acetonitrile: 50% 0.01 M $H_3PO_4$ pH = 3.3 at a flow rate 1 ml/min was used as eluent and UV detection at 210 nm (injection volume 60 μl, run time 10 min). All experiments were done in triplicate.

### Validation and Calibration

The validity of the analytical procedure was established through a study of specificity, linearity, and accuracy according to the compliance criteria laid down in the ICH Guidelines (19). The linearity of the analytical procedure was evaluated by plotting the detector response (peak area) against analyte concentration. Linear regression analysis was applied to calculate the slope, intercept, and linear correlation coefficient ($R^2$). The accuracy was established by quantitative determination of the bupivacaine amount in quality control samples and was expressed as percent recovery by the assay of a known amount of

analyte in the samples (19). The limit of detection (LOD) was calculated as signal-to-noise ratio of 3:1, and the limit of the quantification (LOQ) was determined as signal-to-noise ratio of 10:1 (19). Calibration curves for bupivacaine in release medium were obtained by programmed injection of different aliquots (10–100 μl) of a standard solution with increments of 10 μl. The concentration of the standard solution was 10 μg/ml.

### Viscosity Measurements

Viscosity of polymers was measured using a Brookfield LVDV-III programmable viscometer coupled to a temperature-controlling unit. T-shaped spindle TF96 was used. The viscosity was measured at constant temperature (22°C) at shear rate of 31.3 $s^{-1}$.

### *In Vivo* Efficacy of the Formulations

The study received approval from the ethics committee of the Hebrew University Hadassah Medical School (National Institutes of Health approval number: OPRR-A01-5011) for performance of animal studies (ethics committee research number: MD-83.02-4). Female ICR mice weighing approximately 30 g were housed ten in a cage with free access to food and water. The animal room was light cycled (12 h light, 12 h dark), and the temperature was 22°C.

The animals were anesthetized with volatile anesthetic Isoflurane solution to facilitate identification and injection of the formulation at the sciatic nerve as previously described (12). The sciatic nerve was identified using a nerve stimulator (StimuplexR B.Braun Melsungen AG, Germany) at 0.2 mA and 1 Hz via a needle of 22 G diameter (14). One group of animals received a formulation containing 10% bupivacaine and 10% castor oil (p(DLLA:CO)3:7–10% bupivacaine-10% CO), and the second group received 15% bupivacaine and 10% castor oil (p(DLLA:CO)3:7–15% bupivacaine-10% CO). These solutions were chosen due to their optimum *in vitro* release characteristics (low viscosity and constant bupivacaine release). Each animal received a single injection (0.1 ml) of the formulation in one leg and 0.1 ml 0.9% saline solution on the contralateral side. Efficacy tests included both sensory and motor evaluation as previously described (14). Sensory tests were performed using the Hargreave's hot plate to measure time to withdrawal of the tested leg at predetermined time points (20). Motor block was measured using a composite score of limp, splay, grip, and proprioception, where a score of 0 = total motor block and 4 = full motor function. Four groups of five animals were used at 24 and 72 h. Each leg was tested 5 times at one single time period, thus a total of 10 tests per animal.

### Statistical Analysis

Data were analyzed to delineate a statistical difference between the drug (polymer-bupivacaine) and the control group (normal saline). Hargreave's (sensory blockade) scores were analyzed using a mixed model analysis of variance. The primary outcome was whether drug affected the Hargreave's score (measure of sensory blockade). Two experiments were conducted, and different animals were used at each time point. Drug, experiment, and hour were considered as fixed effects, and animal (nested within experiment and hour) was considered to be a random effect. The SAS PROC Mixed (Version 8.02) procedure was used to perform the analyses. Motor response variables grip, splay, and proprioception were all binary in nature, and the scores combined as previously described (12). The question of interest was whether drug affected these variables. A composite variable (Y) was created by summing these variables for each observation. Response variable Y was analyzed using a mixed model analysis of variance, with drug and hour defined as fixed effects and animal defined as a random effect. A $p$ value $< 0.05$ was considered significant and all tests were two-tailed.

## RESULTS

### Formulation Characterization

The ricinoleic acid and castor oil additives blended homogeneously with the polymer. Gel permeation chromatography (GPC) was used to determine if the additives form covalent bonds with the polymer or bupivacaine. Formation of covalent bonds between the bupivacaine and the polymer would affect the release rates of the drug and might influence the pharmacological efficacy due to chemical modification of the bupivacaine with polymer chains. Figures 1 and 2 show the GPC chromatograms of the formulations following addition of 10% ricinoleic acid (Fig. 1) or castor oil (Fig. 2).

It was previously shown that bupivacaine was incorporated in the polymer without affecting the polymer molecular weight (12). After addition of 10% wt/wt ricinoleic acid or castor oil, all the formulation components were separated during GPC analysis, and the polymer and bupivacaine molecular weights remained unchanged, confirming absence of covalent conjugation between the polymer and the drug.

### Viscosity

The viscosity of 10% and 15% bupivacaine formulations was reduced using additives (Fig. 3). Both ricinoleic acid

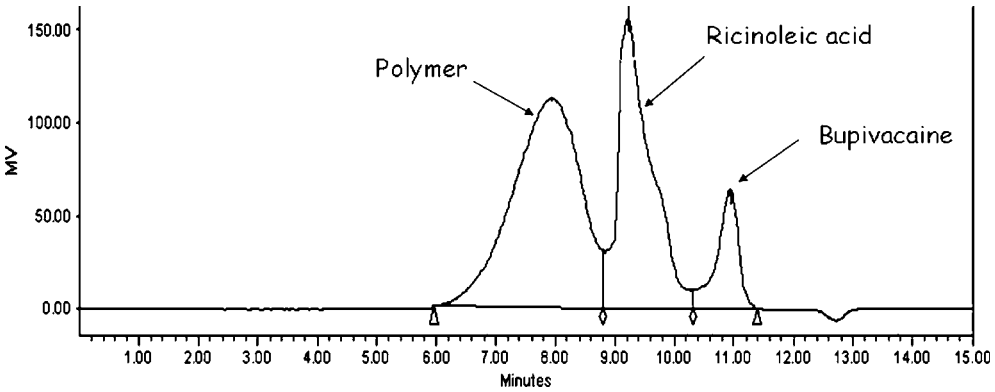


**Fig. 1** Gel permeation chromatogram of p(DLLA:CO) 3:7-bupivacaine 10% formulation-10% w/w ricinoleic acid. 0.1 ml of the formulation was dissolved in 0.5 ml of chloroform and evaluated by GPC.

and castor oil reduced the viscosity of the formulations 2–3-fold compared to the reference formulations, from 6,700 cP (10% bupivacaine) or 10,300 cP (15% bupivacaine) to 4,000 cP, which is close to the viscosity of the blank polymer (2,700 cP). No significant difference was observed between the effects of the two additives.

### *In Vitro* Bupivacaine Release from Formulations

Our previously reported 10% formulation showed burst release with both 10% and 15% formulations reaching "plateau" values after 48 h. Addition of ricinoleic acid or castor oil induced first-order prolonged release profiles up to 7 days (Figs. 4 and 5).

The effect of castor oil on the release profile of bupivacaine from both 10% to 15% formulations was similar and correlated directly to bupivacaine and castor oil contents. Total drug release from 10% to 15% bupivacaine formulations was lower for 20% castor oil (85% and 76%) compared to 10% castor oil (62% and 48%). For the formulations with similar castor oil content, the release was lower as the bupivacaine content was higher, in agreement with our previous observations (3,5,6). No burst effect was observed for any evaluated castor oil formulation (less than 10% of the drug released in 6 h).

However, ricinoleic acid effect was different depending on the initial bupivacaine loading. Addition of 10% ricinoleic acid to 10% bupivacaine formulation prolonged the release (88% compared to 60%); however, the formulation exhibited burst release. Increasing ricinoleic acid content to 20% decreased the initial release rates (20% of the drug released in 24 h) and the total release (60%). Addition of 10% ricinoleic acid to 15% bupivacaine formulation reduced the release rates at all time points, but addition of 20% ricinoleic acid to 15% bupivacaine formulation increased the initial drug release (7 h).

### Selection of Formulation for *In Vivo* Experiments

The selection of the formulation for *in vivo* evaluations was based on two criteria: (1) slower drug release rates with no burst effect, and (2) viscosity of the formulation. Since the observed viscosity values were similar for all evaluated formulations (both with ricinoleic acid and castor oil), the selection was based on the bupivacaine release profiles. Burst effect *in vitro* correlates with *in vivo* toxicity, and the formulation without burst effect has a greater potential for *in vivo* efficacy (13). The castor oil-containing formulations showed lowest release rates with direct correlation to castor oil content, while the release profiles of the ricinoleic acid-containing formulations were not consistent with respect to additive content. Based on these observations, castor oil was selected as the optimum additive. Since the *in vitro* drug

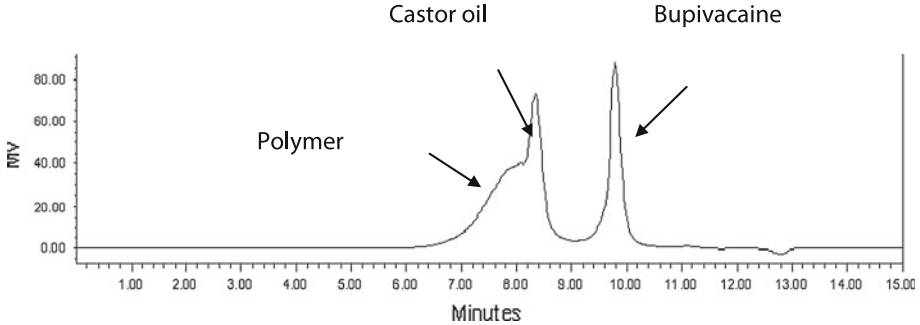


**Fig. 2** Gel permeation chromatogram of p(DLLA:CO) 3:7-bupivacaine 10% formulation with 10% w/w castor oil as additive. 0.1 ml of the formulation was dissolved in 0.5 ml of chloroform and evaluated by GPC.

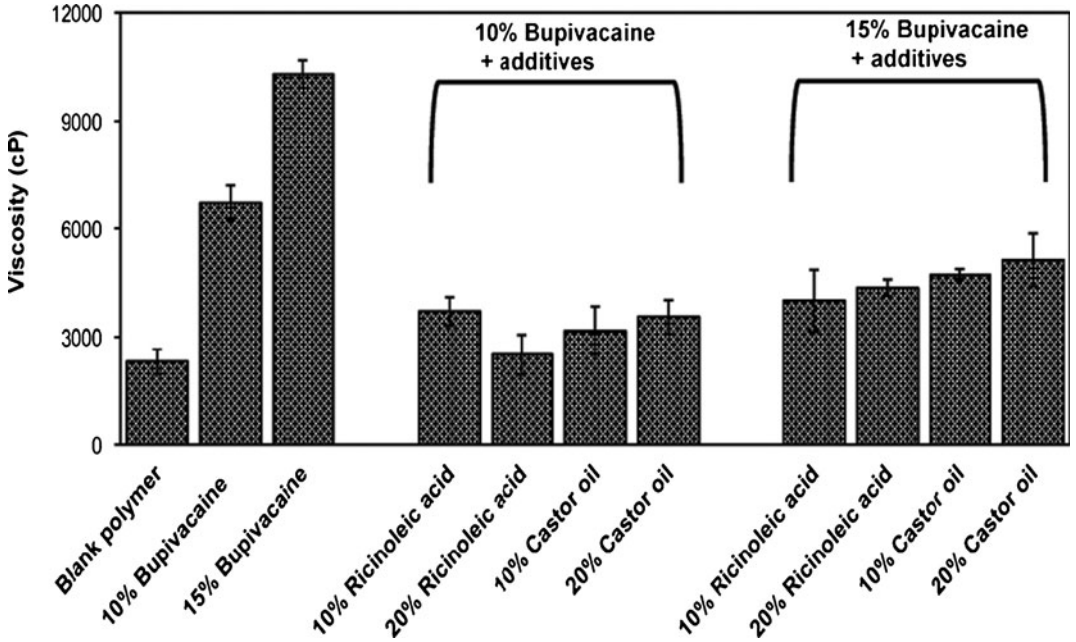


**Fig. 3** Viscosity of poly(DLLA:CO) 3:7 and the poly(DLLA:CO) 3:7-bupivacaine formulations with ricinoleic acid or castor oil as additives measured at 22°C at shear rate of 31.3 $s^{-1}$.The formulation values are statistically significantly different ($p \leq 0.0001$) in comparison to formulations + additives (for both 10% w/w and 15% w/w formulations) or blank polymer.

release (up to 72 h) for both 10% and 20% castor oil formulations was similar (for each bupivacaine content), no advantage for increasing castor oil content in the formulation was observed. Thus, the castor oil content was established as 10% w/w for both 10% and 15% bupivacaine-polymer formulations for *in vivo* efficacy evaluation.

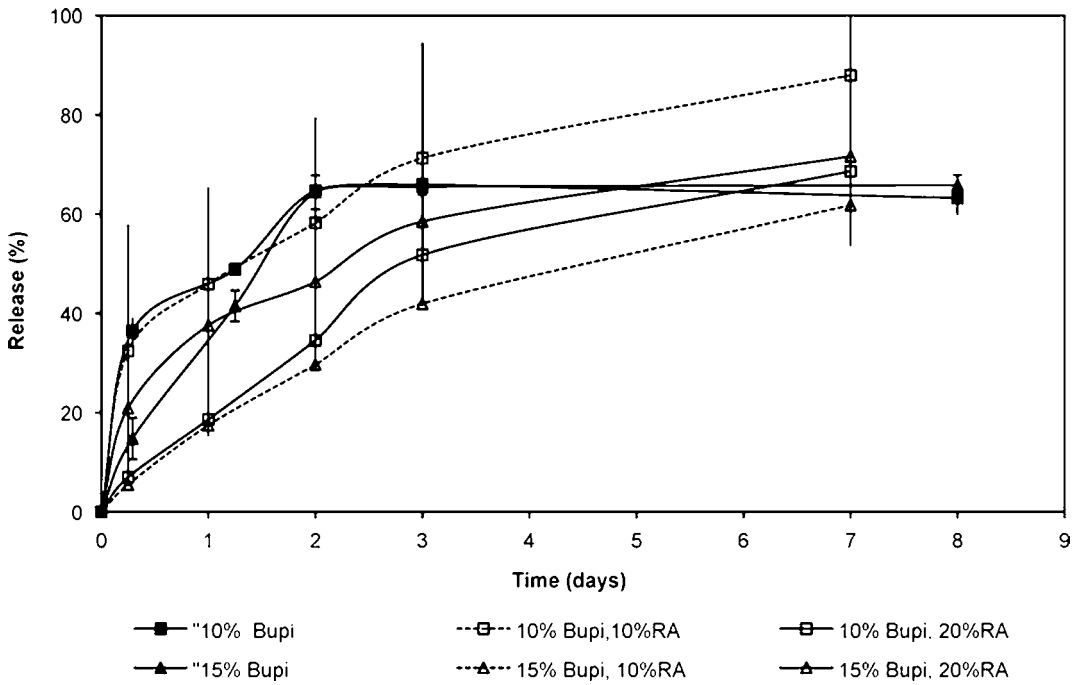


**Fig. 4** *In vitro* release of Bupivacaine free base from p(DLLA-CO) 3:7 (10 or 15% w/w) with 10 or 20% w/w ricinoleic acid (RA) as additive. Bupivacaine release was conducted in a 0.1 M phosphate buffer (pH 7.4) at 37°C. Bupivacaine free base content in the releasing medium was determined by HPLC. 10% Bupivacaine: 10% w/w bupivacaine no additive, 10% Bupivacaine: 10% CO: 10% w/w Bupivacaine, 10%w/w castor oil as additive, 10% Bupivacaine: 20% CO: 10% w/w Bupivacaine, 20% w/w castor oil as additive, 15% Bupivacaine: 10% w/w Bupivacaine no additives, 15% Bupivacaine: 10% CO: 15% w/w Bupivacaine, 10% w/w castor oil as additive, 15% Bupivacaine: 10% CO: 15% w/w Bupivacaine, 20% w/w castor oil as additive.

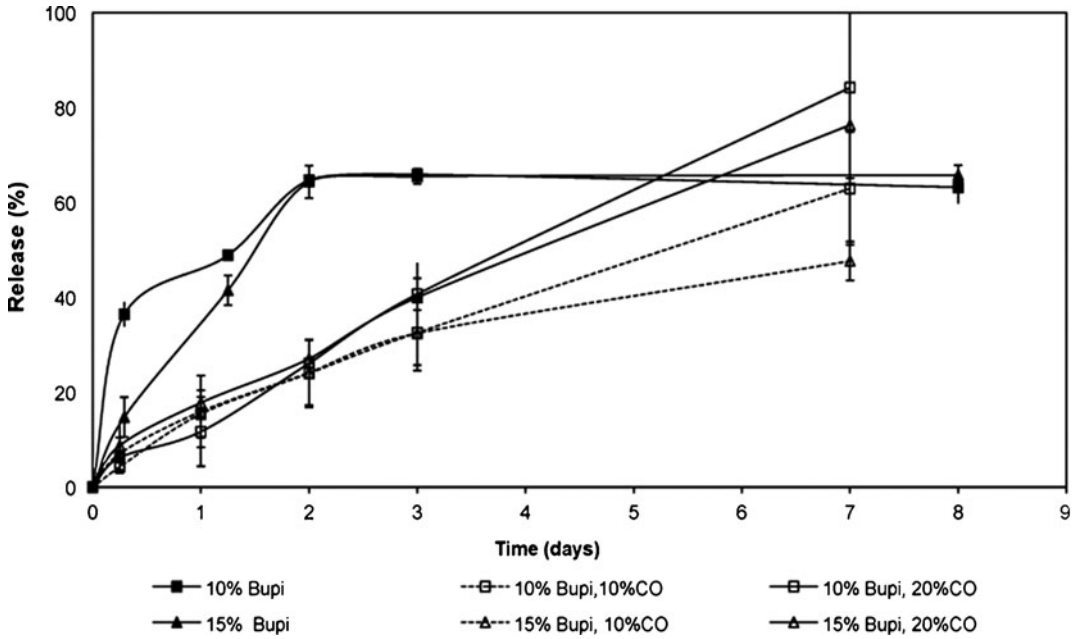


**Fig. 5** *In vitro* release of Bupivacaine free base from p(DLLA-CO) 3:7 (10 or 15% w/w) with 10% or 20% w/w castor oil (CO) as additive. Bupivacaine release was conducted in a 0.1 M phosphate buffer (pH 7.4) at 37°C. Bupivacaine free base content in the releasing medium was determined by HPLC. 10% Bupi: 10% w/w Bupivacaine no additive. 10% Bupi, 10% CO: 10% w/w Bupivacaine, 10% w/w castor oil as additive. 10% Bupi, 20% CO: 10% w/w Bupivacaine, 20% w/w castor oil as additive. 15% Bupi- 10% w/w Bupivacaine no additives. 15% Bupi, 10% CO: 15% w/w Bupivacaine, 10% w/w castor oil as additive. 15% Bupi, 10% CO: 15% w/w Bupivacaine, 20% w/w castor oil as additive.

### *In Vivo* Efficacy

#### *Sensory Tests*

The mice received 0.1 ml of polymer-local anesthetic-additive formulation and were monitored for 72 h post injection. In each group, animals demonstrated a significantly increased time to withdrawal in the drug-formulation leg compared with the reference leg on the hot plate up to 72 h post injection (Fig. 6a and b). For the 10% bupivacaine formulation, the response time increased to 58.8±12.36 s, compared to 41.1±9.13 s for the reference leg at 48 h, $p<0.0001$, after injection and remained the same at 72 h, 56.76±7.1 s *versus* 35.68±4.1 s, $p<0.0001$. For the 15% bupivacaine formulation, a similar pattern was observed: 57.3±12.53 s *versus* 41.7±14.33 s at 48 h, $p=0.001$, and 53.3±4.66 s *versus* 34.8±7.64 s at 72 h, $p<0.0001$. These results demonstrate significantly prolonged sensory block for at least 72 h for both tested formulations.

#### *Motor Tests*

Motor block was present in all animals at 24 h (15% drug leg 1.22±0.67 *versus* 4.0±0 in control leg, $p\leq0.0001$, 10% drug leg 1.57±0.79 *versus* 4.0±0 in control leg, $p\leq0.0001$) (Fig. 7). The 15% bupivacaine formulation showed a small non-significant difference in motor function at 48 h: 3.78±0.67 compared with 4.0±0 in control leg, $p=0.2959$, and there was no difference at 60 h. For 10% bupivacaine formulation, both legs were normal by 48 h.

## DISCUSSION

The current study demonstrated that hydrophobic additives of castor oil and ricinoleic acid can be used as viscosity-reducing agents and in addition alter the release characteristics of incorporated bupivacaine in poly(DL: Lactic acid co castor oil) polymer. Furthermore, the *in vivo* activity of the bupivacaine-poly(DL:Lactic acid co castor oil) was improved by relative reduction of the motor block duration whilst demonstrating prolonged sensory analgesia.

Use of additives to improve injectability through reduction of viscosity or modify drug release rates from polymer-based formulation has been previously explored. Additives have been evaluated as potentially beneficial to physical properties of polymer carrier or to incorporated drug release pattern. These additives can be divided into two subcategories: hydrophilic and hydrophobic. The hydrophilic additives include PEGs (21,22), gelatin, albumin, methylcellulose (23), L-tartaric acid dimethyl ester (DMT), Pluronic(R) F127; 2-hydroxypropyl derivative of beta-cyclodextrin (HPB), methyl derivative of beta-cyclodextrin (MMB) (24). Hydrophobic additives include phospholipids (22), ricinoleic acid (21), beewax (24) and oleic acid (25). All the hydrophilic and hydrophobic

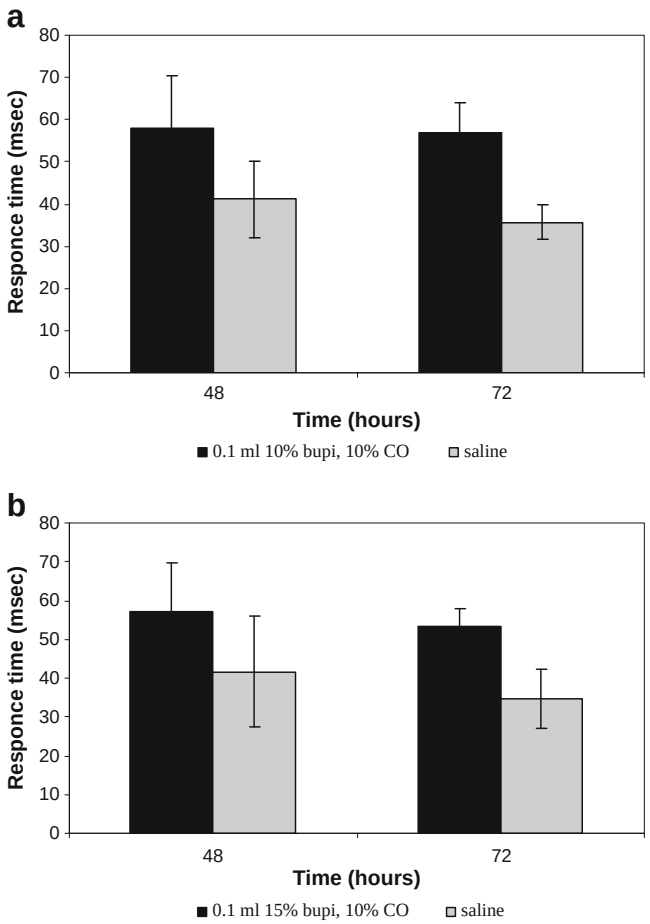


**Fig. 6** Graph of withdrawal latency after administration of 0.1 ml polymer formulation containing (**a**) 10% w/w bupivacaine-10% castor oil or normal saline and (**b**) 15% w/w bupivacaine-10% castor oil or normal saline. The data are represented as means ± STD (units are 1/10 of a second). In all presented time points the formulation values are statistically significantly different ($p \leq 0.0001$) in comparison to normal saline.

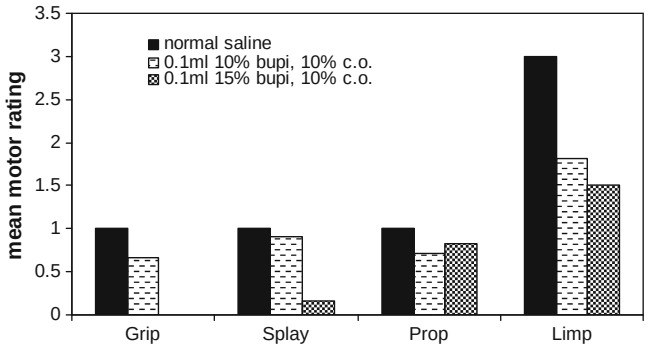


**Fig. 7** Graph of motor block produced by administration of polymer formulation containing 10% bupivacaine-10% castor oil or 15% bupivacaine-10% castor oil 24 h after injection. Grip, proprioception and limp (present or absent) were measured on a scale 0 = none, 1 = present. Splay was assessed according to a 4-point scale: 4–normal, 3–intact dorsiflexion of foot with impaired ability to splay toes when elevated by the tail, 2–toes and foot plantar flexed with no splaying ability, 1–loss of dorsiflexion, flexion of toes, and impairment of gait.

additives successfully altered physical properties of the formulations; however, the influence on drug release was different between these two groups. Hydrophilic additives overall enhanced the drug release, whereas for hydrophobic additives the effect depended on the additive nature.

Phospholipids and ricinoleic acid slightly increased release of paclitaxel from poly(sebacic co ricinoleic acid) carrier (21). Beeswax did not increase the drug release (21). Oleic acid reduced the release rates of the model drugs (propranolol HCl and quaiphenesin) *in vitro* (25).

High release rates and burst release result in drug concentrations near or above their toxic level (26,27), causing *in vivo* toxicity (12). Previously, we demonstrated that the release rates of bupivacaine from 15% formulation were in the safe range both in the terms of efficacy and toxicity (13). Since it was necessary to maintain or reduce the drug release rates, only hydrophobic additives were considered for the current study. Ricinoleic acid and castor oil were selected since they are completely miscible with the polymer and would form a homogenous mixture. Addition of ricinoleic acid and castor oil reduced viscosity compared to previously reported formulations (both 10% and 15%). Measurements of bupivacaine release *in vitro* demonstrated that both ricinoleic acid and castor oil altered the drug release profile, inducing first-order release pattern. However, the effect of ricinoleic acid was not uniform for all tested formulations, enhancing or reducing the release depending on bupivacaine and ricinoleic acid contents. On the other hand, castor oil served as a reliable bupivacaine retainer. It was hypothesized that the release of the bupivacaine would depend on three parameters: viscosity, nature/hydrophobicity of the additive, and its concentration. There was no significant difference in the viscosity of the formulations with the different additives, suggesting that the effect of reduced viscosity on the bupivacaine release rates was negligible. Several publications showed lipid-based formulations for sustained release of bupivacaine. Larsen *et al.* evaluated bupivacaine free base dissolved in Viscoleo/castor oil (2:1) both *in vitro* and *in vivo* (28). The formulation had low viscosity; however, sustained release for up to 50 h *in vitro* was reported, and it was suggested that the release rates are controlled by the attainment of the equilibrium between the oily vehicle and the aqueous phase. Potentially, the transport from the oil to aqueous phase is the rate-limiting step in drug release from oily formulations, which compensates for the low viscosity and potential spread of the formulation (17,29). Similar results were obtained by Kranz *et al.* (30). Polymer carrier was loaded with bupivacaine and emulsified into external oil phase. The external oil phase decreased the release of bupivacaine due to slow rate of bupivacaine transport from oil to aqueous phase. It was shown that the release rates depend on polymer concentration and

polymer:oil phase ratio (30,31). The results obtained in the current study correlate well with the study by Kranz *et al.* (30), since all formulations showed first-order release. Castor oil may form an external lipid phase, and the release mechanism is similar to the described in the literature. However, the difference between the ricinoleic acid and castor oil effect may be attributed to their structures. Ricinoleic acid is a charged carboxylate anion single chain molecule that may form a salt with bupivacaine. This can affect the transport of the bupivacaine from the formulations and thus its release, as was shown with the increase in release of paclitaxel from poly(sebacic co ricinoleic acid) carrier (21). On the other hand, castor oil is a three-arm branched lipid with no charge. Similar results were previously reported for other amphiphilic additives (25). Since bupivacaine content in the formulation directly affects the release rates (12–14), it is a combination of the bupivacaine and ricinoleic contents, which direct the release from ricinoleic acid containing formulations. The increase in bupivacaine release marked ricinoleic acid as not suitable for further *in vivo* evaluations, and only castor oil-containing formulations were evaluated.

*In vivo* administration of the formulation demonstrated that addition of castor oil altered the efficacy of both 10% and 15% bupivacaine formulations by maintaining a prolonged sensory block. Castor oil is FDA approved for injections and is used as solvent in several FDA-approved formulations. For the 10% bupivacaine formulation, the sensory block was prolonged to 72 h compared to 48 h without castor oil, and for the 15% bupivacaine formulation, the duration of the motor decreased to 24 h while preserving a prolonged sensory blockade (72 h). The similarity of the *in vivo* efficacy profiles implies that castor oil is a key factor in determining *in vivo* release rates. The decrease in motor block duration compared to prolonged sensory block marked the 15% bupivacaine formulation as potentially more suitable for clinical application.

Use of hydrophobic additives may affect additional characteristics of the formulation. Increase in hydrophobicity of the carrier reduces the degradation rates of the polymer and the formulation both *in vitro* and *in vivo* (21,32,33). With prolonged degradation rates, the polymeric implant might remain in the injection site after the clinical need. The ramifications of this should be addressed in future formulation characterization and evaluation.

## CONCLUSIONS

The addition of castor oil to p(DLLA:CO)3:7-bupivacaine formulations reduced the viscosity, which improved *in vivo* efficacy results. Future study of this drug delivery system will aim to refine the drug release rates to eliminate burst effect, while maintaining efficacy and safety profile of the formulation.

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