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Research paper

Reduction in burst release of PLGA microparticles by incorporation into cubic phase-forming systems

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ABSTRACT

A high initial burst release of an phosphorothioate oligonucleotide drug from poly(lactide-co-glycolide) (PLGA) microparticles prepared by the w/o/w solvent extraction/evaporation was reduced by incorporating the microparticles into the following glycerol monooleate (GMO) formulations: 1) pure molten GMO, 2) preformed cubic phase (GMO + water) or 3) low viscosity in situ cubic phase-forming formulations (GMO + water + cosolvent). The in situ cubic phase-forming formulations had a low viscosity in contrast to the first two formulations resulting in good dispersability of the microparticles and good syringability/injectability. Upon contact with an aqueous phase, a highly viscous cubic phase formed immediately entrapping the microparticles. A low initial burst and a continuous extended release over several weeks was obtained with all investigated formulations. The drug release profile could be well controlled by the cosolvent composition with the in situ systems.

1. Introduction

Phosphorothioate oligonucleotide drugs have attracted special interest as a novel class of therapeutic agents for the treatment of viral infection, cancers and genetic disorders. However, the oligonucleotides have poor biological stability due to short half-life ranging from 15 to 60 min after oral or systemic administration [1]. In order to improve the in vivo efficacy, repeated administration of oligonucleotide would be required in order to provide a sustained pharmacological effect. The repeated administration can be overcome by encapsulating oligonucleotide within biodegradable polymeric microparticles. The release of oligonucleotides from microparticles would provide the controlled delivery at a predetermined rate to the target tissue over a period of time and protect from degradation (e.g. enzymes), thus providing improved bioavailability.

One challenge in the development of biodegradable microparticles is often a too high initial drug release ("burst effect"), in particular with highly water-soluble drugs [2]. A higher initial drug release reduces the effective lifetime of the system and could lead to toxic effects [3]. This problem is particularly relevant to protein and peptide drugs [4].

The multiple emulsion (w/o/w) solvent evaporation technique has been widely applied for the microencapsulation of macromolecular drugs within biodegradable polymeric microparticles [5–9]. A high initial burst from microparticles prepared by the w/o/w emulsion method is usually attributed to a rapid diffusion of the drug through water-filled pores and channels within the microparticles [10] or to drug present close to the surface [11,12]. In addition, the external and internal morphology of the microparticles is significantly influenced by the initial burst and release behaviour [13].

Approaches to reduce the initial burst include the modification of formulation [9], changing process conditions [14], extraction of the drug close to the surface [15], surface modification [16] or an additional coating [17]. Poly (D,L-lactide-co-glycolide) microspheres of Zn-human growth hormone were suspended in a reverse thermal gelation solution, which formed a gel around the microparticles at body temperature (37 °C). This contributed to a significant reduction of the initial burst [18].

Glycerol monooleate (GMO) forms various lyotropic liquid crystalline structures. Anisotropic (hexagonal and lamellar) or isotropic (very viscous cubic phases) structures are formed depending on the ratio of GMO/water/additive and temperature [19]. The cubic phase is a suitable carrier for both hydrophilic and lipophilic drugs because of its amphiphilic nature [20–25]. GMO is non-toxic, biocompatible and biodegradable [26,27] and shows good chemical and physical stability of incorporated drugs and proteins [25]. Drug release from the cubic phase is diffusion-controlled and follows the square root of time kinetics [22]. However, the cubic phase is highly viscous and thus difficult to handle or to inject. As an alternative, low viscosity three-component formulations composed of GMO:water:cosolvent were developed [23] . Upon injection into aqueous media, the cosolvent leaches out and a viscous cubic phase is formed in situ. The Camurus AB, Sweden have been marketing glycerol monooleate based formulation for Parodontitis use (e.g. Elyzol® dental gel), and several products for parenteral use are in phase II studies.

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The objective of this study was to reduce the initial burst release of an oligonucleotide model drug from PLGA microparticles by their incorporation into GMO-containing formulations (molten GMO, preformed cubic phase, in situ cubic phase-forming systems).

2. Materials and methods

2.1. Materials

The following materials were used as received and were at least of reagent grade: phosphorothioate oligodeoxynucleotide (ISIS Pharmaceuticals Inc., Carlsbad, CA, USA), poly (D,L-lactide-co-glycolide) (PLGA, Resomer® RG 755, Mw 64,286 Da, inherent viscosity 0.6; Boehringer Ingelheim KG, Ingelheim, Germany), polyvinyl alcohol (PVA, Mowiol® 40–88, Clariant GmbH, Frankfurt, Germany), glycerol monooleate (GMO, GMOrphic® 80; Eastman Chemical Company, Kingsport, TN, USA), polyethylene glycol 300 (PEG 300, Lutrol® 300, BASF AG, Ludwigshafen, Germany), propylene glycol (PG, BASF AG, Ludwigshafen, Germany), ethanol, methylene chloride, potassium dihydrogen phosphate, sodium hydroxide, sodium chloride, sodium azide (Merck KGaA, Darmstadt, Germany), dialysis bag (cellulose ester, MWCO:100,000, Spectra/Por® CE, Spectrum Medical Industries Inc., Houston, Texas, USA).

2.2. Preparation of microparticles by the w/o/w-method

Standard formulation: An aqueous solution of the oligonucleotide (0.033, 0.060 and 0.075 g drug in 0.25 ml water) was emulsified into a solution of the polymer (0.30 g PLGA RG 755 in 4.0 ml methylene chloride) by probe sonication (Sonoplus® HD 250, Bandelin Electronic, Berlin, Germany) for 30 s. This primary w/o emulsion was then dispersed into 800 ml external aqueous phase (0.25% w/v PVA containing 0.25 M NaCl) under propeller stirring (Heidolph Elektro, Kehlheim, Germany) for 5 min and then further agitated for 1 h with a magnetic stirrer (Jankle and Kunkel GmbH & Co., IKA Labortechnik, Staufen, Germany) for the solidification of the polymeric particles by solvent extraction/evaporation. The microparticles were separated from the external aqueous phase by wet sieving (stainless steel test sieves, 50 and 100 μm), washed with 200 ml water, dried in a desiccator for 48 h and then stored in refrigerator.

2.3. Drug content

Triplicate samples of microparticles (10–12 mg, accurately weighed) were dissolved in 8 ml 0.5 M NaOH followed by agitation in a horizontal shaker (IKA HS 501 digital horizontal Shaker, Janke & Kunkel & Co. IKA Labortechnik, Staufen, Germany) for 12 h. The drug concentration in the aqueous phase was determined by UV-spectrophotometry at $\lambda = 260$ nm (Shimadzu UV 2101 PC UV–vis scanning spectrophotometer, Kyoto, Shimadzu Japan) [6]. The polymer did not interfere at the wavelength used. The actual drug loading and encapsulation efficiency were calculated as:

Actual drug loading (%) = (mass extracted drug/mass of microparticles) × 100 %

Encapsulation efficiency (%) = (actual drug loading/theoretical drug loading) × 100 %.

2.4. Incorporation of microparticles into different GMO matrices

Microparticles were incorporated into three different GMO matrices: (1) GMO molten in a water bath at 45 °C and thoroughly mixed with the microparticles (20%, 30% and 40% w/w microparticles); (2) a cubic phase obtained by mixing of molten GMO (3.5 g) and water (1.5 g) on the surface of a Petri-dish by spatula. This cubic phase was transferred into tightly closed glass vials, stored for 24 h at room temperature for equilibration and was subsequently mixed with the microparticles as described above. The phase transformation (cubic to lamellar phase) upon the incorporation of the microparticles was observed under a polarized light microscope (Axioskop, Carl Zeiss Jena GmbH, Jena, Germany); (3) the low viscosity in situ cubic phase-forming formulations were obtained by mixing of molten GMO, water and cosolvents (PEG 300, propylene glycol, ethanol) in glass vials and stored tightly closed for 24 h for equilibration at room temperature (Table 1). Microparticles were dispersed into the formulations prior to drug release experiments. The microparticles and in situ cubic phase-forming formulation were filled in separate syringes. The syringes were connected, and the microparticles were dispersed in the liquid phase by moving the syringe plungers forward and backward 10 times.

2.5. Optical microscopy

The liquid crystalline phases with/without drug or microparticles were identified by polarized light microscopy (Axioskop, Carl Zeiss Jena GmbH, Jena, Germany). The lamellar phase could be identified by its anisotropic textures (birefringence). The cubic phase was of isotropic nature (transparent) under the polarized microscope.

2.6. In vitro drug release

The in vitro drug release was performed ($n = 3$) in 0.1 M phosphate buffer, pH 7.4, with 0.1% sodium azide as preservative in a horizontal shaker (37 °C, 75 spm; GFL 3033, Gesellschaft für Labortechnik GmbH, Burgwedel, Germany). 10–12 mg microparticles (accurately weighed) was placed into 8 ml prewarmed release medium; 0.1–0.2 g (accurately weighed) of GMO or preformed cubic phase containing microparticles was placed in a Teflon sample holder and 0.1–0.2 g of the in situ cubic phase-forming formulations containing microparticles was filled in dialysis bags. The Teflon sample holder and dialysis bags were then immediately put into 25 ml prewarmed release medium and treated as described above. At predetermined time intervals, 2 ml samples were withdrawn and replaced with fresh medium. The drug concentration was measured spectrophotometrically at $\lambda = 260$ nm (Shimadzu UV 2101 PC UV–vis scanning spectrophotometer, Kyoto, Shimadzu Japan) [6].

3. Results and discussion

Microparticles with different oligonucleotide loadings (10%, 17% and 20%) were prepared by the multiple emulsion (w/o/w) solvent evaporation method. The encapsulation efficiency was 95.10 ± 2.43, 89.26 ± 1.12 and 89.35 ± 0.10, respectively. The oligonucleotide-containing microparticles had a high initial burst, which increased with increasing drug loading (Fig. 1). The burst

Table 1
Composition of the in situ cubic phase (ICP) forming formulations

Solvent type	Composition, %		
	Solvent	GMO	Water
EtOH	35	35	30
PEG 300	50	35	15
PG	70	20	10
PEG 300-EtOH*	50	35	15

* Ratio 1:1.

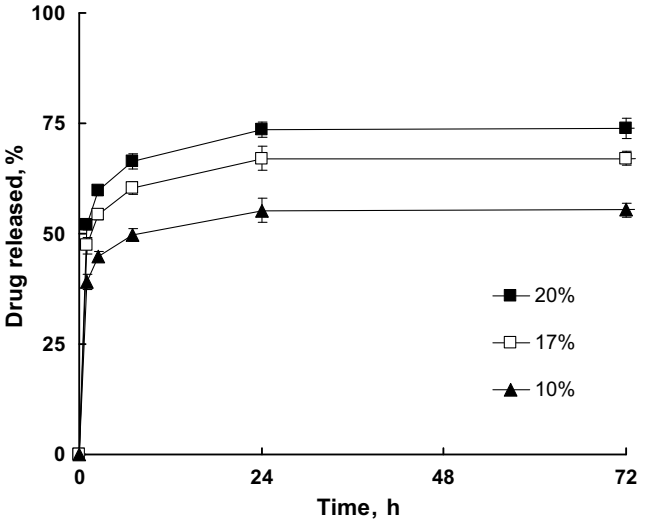

Fig. 1. Effect of theoretical drug loading on drug release from microparticles (n = 3).

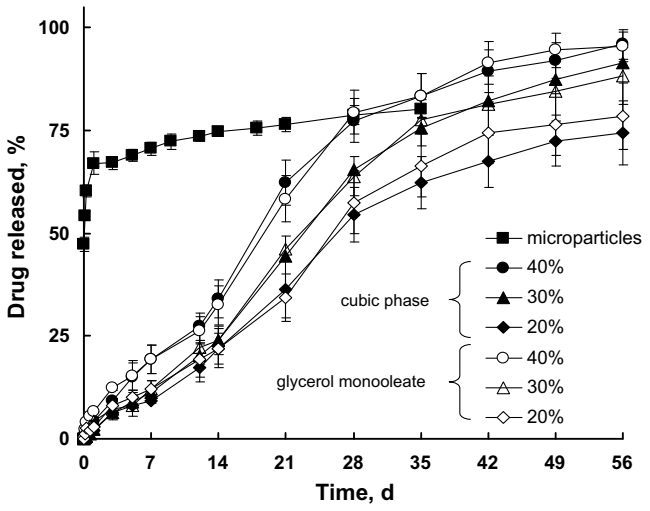

Fig. 3. Effect of amount of microparticles dispersed into molten glycerol monooleate or preformed cubic phase on drug release (n = 3).

is due to the rapid diffusion of the highly water-soluble drug through pores present in the microparticles (also surface pores) (Fig. 2).

Several approaches based on the incorporation of microparticles into GMO were investigated in order to reduce the burst release. These approaches included the incorporation of the microparticles into (1) molten GMO, (2) preformed cubic phase or (3) GMO-based liquid formulations, which formed a cubic phase in situ. In all cases, the microparticles were finally entrapped into the cubic phase, which was expected to reduce the burst release.

The burst release from microparticles could be significantly reduced by dispersing the microparticles in GMO or preformed cubic phase (Fig. 3). Upon dispersion of the microparticles into the preformed cubic phase, it first turned from a highly viscous and isotropic phase into a less viscous, anisotropic (lamellar) phase, probably due to water uptake by the microparticles. However, this lamellar phase or the pure GMO matrices uptake water upon contact with release medium at 37 °C and immediately transformed into a cubic phase. The fully swollen cubic phase surrounding the microparticles built up a diffusional barrier thus reducing the initial burst release (Fig. 3). The release profile was sigmoidal in shape and an extended release over 56 days was obtained. The drug release increased with increasing microparticle content because of a decreased portion of the surrounding matrix. The drug release from microparticles incorporated into GMO only or into a preformed cubic phase followed a similar pattern. This suggests that both systems converted spontaneously into a fully swollen cubic phase after contact with the release medium and that the drug release occurred through water channels of the swollen cubic phase [18].

Low viscosity formulations are essential for injectable drug delivery systems with regard to patient compliance. The cubic phase itself is too viscous to be injected. An injectable system can be achieved by using in situ cubic phase-forming formulations based on GMO, water and water-miscible cosolvents like ethanol, propylene glycol, polyethylene glycol 300 or mixtures thereof [22]. These formulations were of low viscosity and isotropic at room temperature (clear solutions observed under a polarized light microscope). The presence of a cosolvent prevented the formation of a viscous cubic phase. The microparticles could be very easily dispersed therein with a two syringe system just prior to use. The exception was the ethanol-based in situ cubic phase-forming formulation (GMO/water/EtOH, 35/30/35), wherein the microparticles aggregated. The PLGA microparticles softened due to the plasticizing effect of ethanol or ethanol/water mixture and formed aggregates. The glass transition temperature (T_g) of dried PLGA microparticles (44.23 ± 0.47 °C) decreased after incubation (1 min) into ethanol or ethanol/water mixture (1:1) to 11.51 ± 0.87 °C and 15.33 ± 0.06 °C, respectively. Therefore, a mixture of PEG 300 and ethanol in a ratio of 1:1 (GMO/water/PEG/EtOH ratio 35/15/25/25) was used. Upon injection into the aqueous medium, the cosolvent diffused out of the formulations and the transformation into the cubic phase occurred.

The release from drug-only loaded in situ cubic phase-forming formulations based on propylene glycol (GMO/water/PG ratio 20/

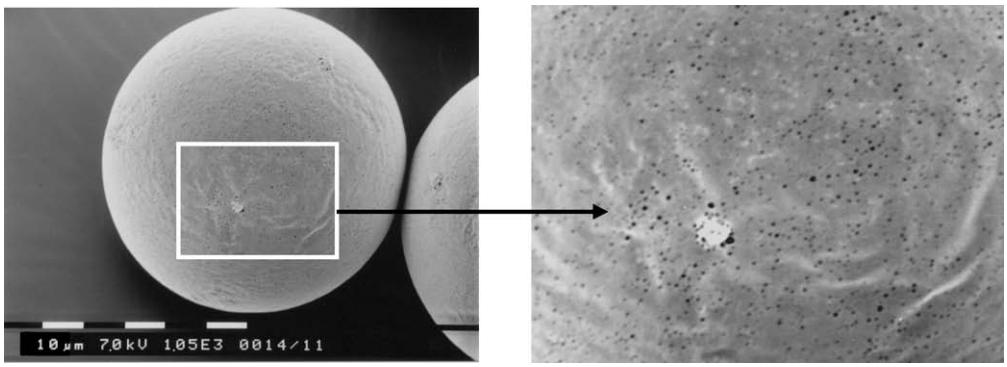

Fig. 2. Scanning electron micrographs of drug-containing microparticles before dissolution studies.

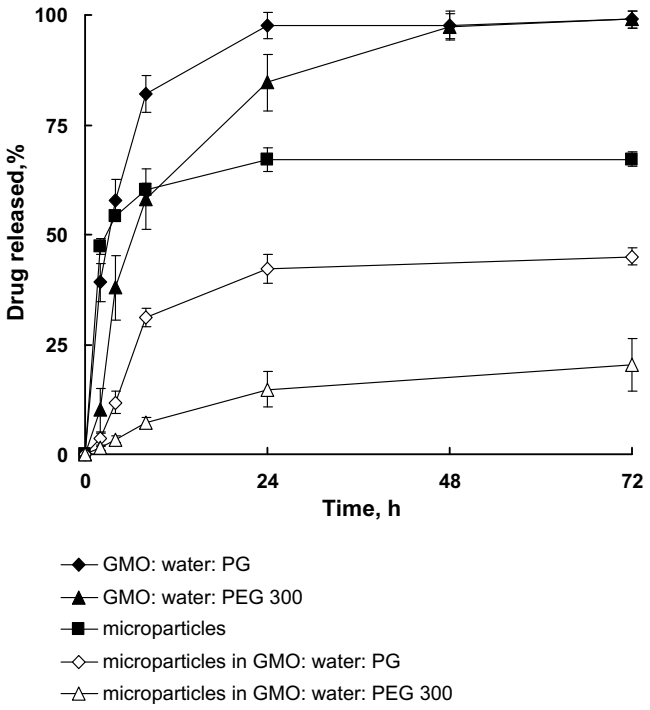

Fig. 4. Drug release from drug- or microparticle-containing in situ cubic phase-forming systems (20% w/w microparticles; GMO:water:PG, 20:10:70; GMO:water:PEG 300, 35:15:50; $n = 3$).

10/70) and polyethylene glycol (GMO/water/PEG 300 ratio 35/15/50) was rapid and complete within 48 h (Fig. 4, release up 72 h). Both cosolvents are completely water-miscible and leached out immediately upon contact with the release medium resulting in a matrix formation. The release was significantly reduced when microparticles were loaded into the in situ cubic phase-forming formulations (Fig. 4). Being an additional barrier for water diffusion, the cubic phase protected the microparticles from rapid contact with the release medium. Therefore, the release was controlled by both the microparticle and cubic phase matrix. The initial burst from microparticles loaded into the PG (70 % w/w)-based formulation was higher than from the PEG 300 (50 % w/w)-based formulation because of lower amount of GMO in PG-based formulations (Table 1).

The extent and rate of drug release from microparticles could be well controlled by the cosolvent composition. For example, a more rapid drug release profile over a one week period was obtained when PEG 300 was partially replaced by ethanol (GMO/water/PEG 300/ethanol, 35/15/25/25) (Fig. 5). The faster release can be explained by the better solubility of GMO in ethanol and additionally by the reduced viscosity of the PEG 300/ethanol system compared to PEG 300 only. The solubilities of GMO at room temperature in ethanol, ethanol/PEG (1/1) and PEG were 6.20, 4.03 and 2.31 g/ml, respectively.

As already observed above, an increased drug release was also observed with the in situ systems with increasing microparticle amount (Fig. 6).

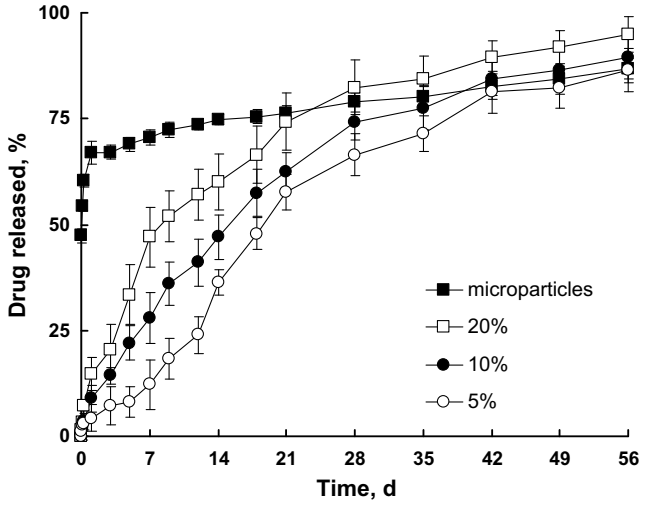

Fig. 6. Effect of microparticle concentration (5%, 10% and 20% w/w) incorporated into in situ cubic phase-forming systems on drug release (GMO:water:PEG 300, 35:15:50; $n = 3$).

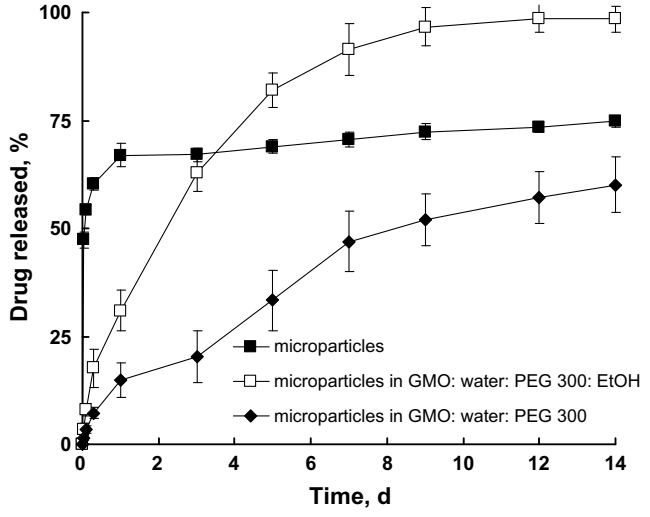

Fig. 5. Effect of solvent mixture used to prepare in situ cubic phase systems on drug release (GMO:water:PEG 300:EtOH, 35:15:25:25) and (GMO:water:PEG 300, 35:15:50; $n = 3$).

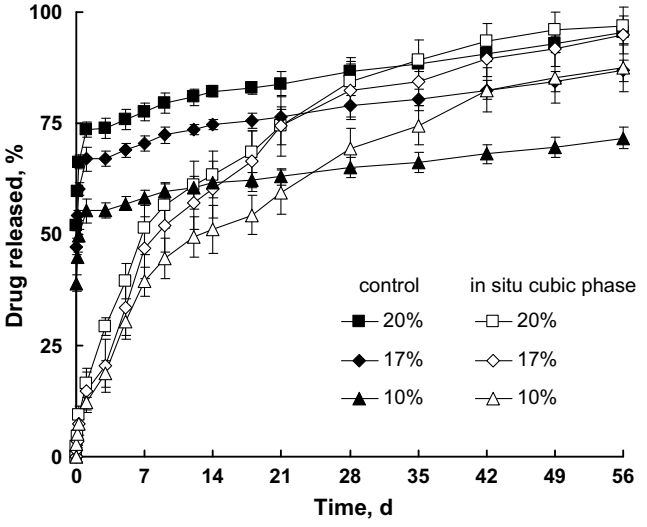

Fig. 7. Effect of drug loading of the microparticles on the drug release from microparticles incorporated in in situ cubic phase-forming systems (GMO:water:-PEG 300, 35:15:50; $n = 3$).

The initial burst release from microparticles increased with increasing drug content (Fig. 7, control), while no major difference in the much slower initial release was observed after incorporation of the microparticles into GMO/water/PEG 300 (35/15/50)-based in situ cubic phase-forming systems (Fig. 7). In the later release stages, the drug release decreased to some extent with lower loadings.

4. Conclusion

Microparticles incorporated into GMO or in a preformed cubic phase were surrounded by a fully swollen cubic phase upon contact with aqueous medium. This cubic phase matrix served as an extended release matrix and reduced the initial burst significantly. In addition, microparticles were incorporated into low viscosity in situ cubic phase-forming formulations, rapidly transforming into a viscous cubic phase after contact with aqueous fluids [23,28]. Microparticles could be easily dispersed within these formulations and extended release over several weeks was achieved.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejpb.2008.07.008.

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