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Research Paper

In Situ-Forming Oleogel Implant for Rivastigmine Delivery

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Purpose. To provide a simplified dosing schedule and potentially reduce side effects associated to peak plasma concentrations, an *in situ*-forming oleogel implant was studied for the sustained-release of rivastigmine.
Materials and methods. The gel was prepared by dissolving 5–10% (*w*/*w*) *N*-stearoyl L-alanine methyl ester (SAM) organogelator in safflower oil containing either dissolved rivastigmine or its dispersed hydrogen tartrate salt. Rheological analysis, differential scanning calorimetry, and infrared spectroscopy were carried out to assess the impact of drug incorporation on the oleogel; this was followed by *in vitro* and *in vivo* release studies.
Results. A weakening of intermolecular interactions was suggested by gel-sol transition temperature drops of 10–15°C upon incorporation of dissolved drug. Meanwhile, the dispersed drug salt induced minimal or no changes in transition temperature. Gels containing dispersed rivastigmine had the lowest burst *in vitro* (<15% in 24 h). *In vivo*, the 10% SAM formulation containing dispersed rivastigmine provided prolonged drug release within the therapeutic range for 11 days, with peak plasma levels well below the toxic threshold and up to five times lower than for the control formulation.
Conclusions. This study established SAM gels to be a promising option for sustained-release formulations in the treatment of Alzheimer's Disease.

KEY WORDS: Alzheimer's disease; implant; organogel; rivastigmine; sustained release.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia amongst elderly and is estimated to afflict 18 million people worldwide (1). Findings of the significant cholinergic deficit in AD patients (2,3) led to the common treatment involving cholinesterase inhibitors (ChEIs) that facilitate neurotransmission in remaining cholinergic neurons (4) and delay the decline of functional and cognitive ability (5). ChEIs are presently administered orally, once to twice daily (6). In unsupervised older adults suffering of AD, treatment compliance (7) and adherence (8,9) might be problematic, which could potentially compromise the already modest efficacy of ChEIs (10). Adverse effects are reported to be the main reason for treatment cessation (8), whereas there is evidence that these can be reduced with more frequent, smaller doses (11). These observations led to the hypothesis that a ChEI sustained release formulation would entail a possible improvement in treatment adherence and compliance, as a result of decreased side effects and simplified dosing regimens, respectively, while alleviating responsibility and workload for caregivers.

Despite the potential benefits of a sustained release of ChEIs, there have only been few and preliminary investigations to this respect. A number of studies have investigated the encapsulation of huperzine A into biodegradable microspheres of poly(*d*,*l*-lactic acid), PLA, and poly(*d*,*l*-lactide-co-glycolide), PLGA (12–14). Huperzine A is a plant-derived ChEI that is currently approved for the treatment of AD in China. To date, sustained release formulations have been investigated for only two FDA-approved ChEIs. Release studies of tacrine from PLGA microparticles (15) showed the potential of drug delivery over prolonged periods. The major problem with tacrine is its marked hepatic toxicity, which has limited its use in favour of the better-tolerated second-generation ChEIs (rivastigmine, donepezil, and galantamine). Out of these, rivastigmine alone has been investigated for sustained release (transdermal patch) (16).

There has been increasing interest in developing parenteral sustained-release systems for long-term drug delivery, microparticles being the most studied of such systems (17). *In situ* forming implants, such as those composed of organogels, are also gaining in popularity due to the inherent advantages of their ease of preparation and administration (18). Inspired by the work of Bhattacharya *et al.* who demonstrated gelation of a wide variety of organic solvents by alanine-based gelators (19), our group further investigated the thermoreversible gelation of various pharmaceutical oils by alkyl-chain derivatized L-alanine at concentrations of 10% (*w*/*w*) or less (20,21). Gelling is induced by the self-assembly of gelator

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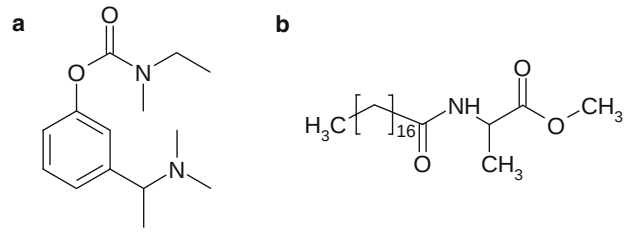

Fig. 1. Molecular structures of **a** rivastigmine base and **b** N-stearoyl L-alanine methyl ester (SAM).

molecules through van der Waals interactions and hydrogen-bonding between alkyl chains and polar head-groups, respectively. The network of gelator aggregates formed immobilizes the oil and yields a semi-solid system. When injected subcutaneously to rats, the oleogel degraded over several weeks and histological analysis revealed the implants to be biocompatible (21). A subsequent study showed the first use of these gels for the sustained release of leuprolide for 14–25 days (22). In the present work, *N*-stearoyl L-alanine methyl ester (SAM) oleogels containing rivastigmine (Fig. 1) were investigated as *in situ* forming implants for long-term drug-delivery, with potential use in AD treatment. The physico-chemical effects of drug incorporation into the gel were studied and formulations were optimized for minimal *in vitro* burst effect. Sustained release of rivastigmine from the injectable implants was demonstrated in a pharmacokinetic study on rats.

MATERIALS AND METHODS

Materials

N-methyl-2-pyrrolidone (NMP), amylamine, isopropyl alcohol, hydrogen peroxide, magnesium sulphate, sodium phosphate dibasic, trifluoroacetic acid (TFA), and sodium chloride were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). Dichloromethane and HPLC-grade acetonitrile (ACN) were obtained from ACP (St-Leonard, QC, Canada). Sodium azide was purchased from American Chemicals Ltd. (Montreal, QC, Canada). Super Refined safflower oil and rivastigmine hydrogen tartrate (RHT) were kindly provided by Croda Inc. (Toronto, ON, Canada) and Zentiva (Prague, Czech Republic), respectively. Rivastigmine base (RB) was extracted from RHT as described below. Deionised water was generated using a Millipore Milli-Q system (Bedford, MA). SAM was synthesized as previously described (21). RHT was tritium-labelled to a specific activity of 10 Ci/mmol by American Radiolabeled Chemicals Inc. (Saint-Louis, MO).

Preparation of Implants

Two types of gel formulations using different drug incorporation methods were investigated.

Dispersed-RHT Gels

For this type of formulations, the RHT powder ($d_n = 25 \pm 14$ μm, measured by light scattering in suspension on a Beckman Coulter LS230, Beckman Coulter, Mississauga, ON, Canada) was dispersed in safflower oil at concentrations up to 5% (*w*/*w*). SAM organogelator was added to the oil phase in concentrations up to 10% (*w*/*w*). The mixture was vortexed and heated until dissolution of the organogelator. Upon cooling, the solution set to a gel containing dispersed drug particles. Unless otherwise specified, 10% (*w*/*w*) NMP was subsequently added to formulations, in order to decrease the gel's viscosity and facilitate injection through conventional needles. After NMP addition, the gel was heated and vortexed again in order to reform a homogenous dispersion.

Dissolved-RB Gels

For this type of formulations, an extraction was first performed on RHT in order to isolate the RB moiety. This was done by dissolving the drug salt in sufficient deionised water and reacting it with 5 mol eq. of amylamine, thus neutralizing the base moiety and allowing the formation of an amylamine-tartrate ion pair. RB was extracted from the reaction mixture in dichloromethane, which was subsequently washed with three portions of water and dried over magnesium sulphate. The solution was filtered and the solvent evaporated to yield the RB (oily appearance). Product identity was confirmed by ^{1}H-NMR. The RB was directly dissolved in the safflower oil and the rest of the gel preparation was carried out as outlined above.

Characterization

Rheology

The rheological properties of the organogels were measured by an AR2000 (Advanced Rheometer 2000, TA Instruments, New Castle, DE), with parallel plate geometry (diameter of 40 mm). Formulations of 10% (*w*/*w*) SAM in safflower oil, with 4 and 5% (*w*/*w*) dispersed RB or dissolved RHT, respectively, were heated to 80°C and the formulations were placed between the parallel plates. The final material coat thickness between the plates was 650 to 750 μm. The film was cooled at 4°C to form the gel. To determine the linear regime of the gel, the oscillatory strain sweeps were performed at 1 Hz in the strain (γ) range of 0.003 to 50%. The linear regime is characterized by constant dynamic storage (G′) and loss (G″) moduli that are independent of strain amplitude. In this regime, G′ and G″ have been measured as a function of the angular frequency (0.1 to 10 Hz) at 25 ± 0.1°C. The gel-sol (T_{GS}) transition temperature was determined by measuring G′ and G″ variations at constant strain and angular frequency of 0.005% and 1 Hz, respectively, with temperatures scanned between 25 and 80°C at 1°C/min.

Differential Scanning Calorimetry (DSC)

Thermograms of dissolved-RB and dispersed-RHT gels varying in drug concentration were collected on a 2910 TA Instruments DSC system (New Castle, DE). The calorimeter was controlled with a Thermal Solutions (Version 1.4E) interface and peak integration was carried out using the Universal Analysis software (Version 2.5H), both from TA

Instruments Inc. The instrument was calibrated with indium. Formulations were weighed into aluminum pans that were subsequently sealed. Temperature was scanned between 5 and 90°C at 10°C/min. The reported gel-sol transition temperatures (T_{GS}) corresponded to the maximum of the endothermic peaks. Six replicate analyses were conducted per formulation.

Thermal Fourier-Transform Infrared (FTIR) Spectroscopy

Infrared spectra of gels varying in RB concentration were recorded as a function of temperature on a Bio Rad FTS-25 spectrometer (Bio-Rad Laboratories, Randolph, MA) equipped with a water-cooled globular source, a KBr beam splitter, and a deuterated triglycine sulphate detector. The concentration of the organogelator was fixed at 10% (*w*/*w*) SAM and the concentration of RB dissolved in the formulation was taken to be 0, 4, and 40% (*w*/*w*). Once prepared, the gel was liquefied by heating and deposited between two CaF_2 windows separated by a 5-μm Teflon spacer. This assembly was placed in a brass sample holder whose temperature was controlled by Peltier thermopumps. Starting at room temperature, the sample was heated up to 75°C in 5°-increments. At each temperature, the sample was equilibrated for 5 min before data acquisition. Each spectrum was the average of 80 scans with a nominal resolution of 2 cm^{-1}. Triplicates of each formulation were tested. Spectra were analyzed using the GRAMS software (Galactic Industry, Salem, NH).

Microscopy

Environmental scanning electron microscopy (ESEM) images were obtained on a Hitachi S-3000 variable pressure scanning electron microscope equipped with an environmental scanning electron detector (Hitachi, Tokyo, Japan). Accelerating voltage, operating pressure, and working distance were set to 20 kV, 120 Pa, and 9 mm, respectively. Samples studied were oleogels containing either RB, RHT, or no drug, prepared as previously described, as well as "dried gels" prepared by forming 10% SAM gels in toluene and evaporating the solvent overnight, directly on the analysis surface. Optical microscopy images of drug-free oleogels were obtained on an Axiovert S100 microscope (Carl Zeiss, Welwyn Garden City, UK) equipped with an integrated digital camera.

In Vitro Release of Rivastigmine

Release Study

Dissolved-RB and dispersed-RHT gels were prepared as described above. The organogelator concentration was varied for both types of gel up to 10% (*w*/*w*). NMP was added to some gels to test its effect on the release rate. The concentrations of all components in the formulation were calculated disregarding the amount of added NMP, since the solvent is expected to quickly diffuse out of the formulation upon contact with the release medium. The gels were loaded into cylindrical dialysis bags (MW cut-off 100 000 g/mol, 500 μl; DispoDialyzer, Fisher Scientific, Montreal, QC, Canada) using a syringe fitted with a 20-G, 1 1/2 needle. The dialysis bag was placed in 100 mL of phosphate-buffered saline (PBS; 104 mM NaH_2PO_4, 36 mM NaCl, 0.1% *w*/*v* NaN_3, pH 7.4) at 37°C and agitated at 135 rpm. Triplicate 1-ml samples were collected from the release medium at 15, 30 min, 1, 1.5, 2.5, 4, 6, 9, 12, 24, 48, 72 h, 7, and 14 days, with replacement of sampled volumes by fresh PBS buffer. Release kinetics were performed under sink conditions (knowing that the solubility of rivastigmine in PBS exceeds 1 mg/ml and the drug concentration in the release medium was at all times below 0.2 mg/ml). Collected samples were immediately analysed by high performance liquid chromatography (HPLC) using the method described below. Concentration values at each data point were corrected for sampling and dilution as previously described (23). Each formulation was tested in triplicate.

HPLC Method

In vitro release samples were analyzed using a modified liquid chromatography method previously published (24). A Gilson Model 302 HPLC system (Gilson, Middletown, WI) equipped with a Gilson 234 autoinjector, a Gilson 106 pump, and a Gilson 151 dual-wavelength UV-detector was used. An Altima guard column (C18, 4.6×7.5 mm, 5 μm; Mandel, Guelph, ON, Canada) was placed upstream of the analytical column, an XTerra (RP-18, 4.6×250 mm, 5 μm) purchased from Waters (Mississauga, ON, Canada). A mobile phase of water/ACN (78/22 *v*/*v*), containing 0.1% (*v*/*v*) TFA, was used at a flow rate of 1 ml/min. The injection volume and detection wavelength were 20 μl and 210 nm, respectively.

Pharmacokinetic Study

All experimental procedures involving animals were conducted following a protocol approved by the Animal Care Committee of the University of Montreal and complied with The Guide for the Care and Use of Laboratory Animals (NIH Publication no. 85-23, revised 1996). Male Long Evans rats (200–225 g; Charles River Inc. St-Constant, QC, Canada) were housed for 1 week under controlled conditions (12-h light/dark schedule, 24°C) prior to the start of experiments. Rat chow and tap water were provided *ad libitum*. All formulation components were sterilized individually. SAM was sterilized on dry ice by γ-radiation at 25 kGy using a ^{60}Co source (Nordion Inc., Laval, QC, Canada). Stability of the organogelator after sterilization was confirmed by mass spectrometry and ^{1}H-NMR. The oil and NMP were filtered on 0.2-μm polytetrafluoroethylene filters. RHT was dissolved in water; the solution was spiked with the appropriate amount of ^{3}H-labelled drug (25 μCi/formulation) and sterilized on a 0.2-μm nylon filter. Samples were lyophilized under sterile conditions to yield a homogeneously radiolabelled drug powder. Using the sterilized oil, SAM, NMP, and RHT, the final oil and gel formulations were prepared under aseptic conditions. A control RHT-saline solution was prepared by dissolving RHT in 0.9% NaCl solution, spiking the solution with radiolabelled drug (25 μCi/formulation) and sterilizing the final solution by filtration. The rats were divided into four groups ($n = 6$) and given a single s.c. injection of approximately 400 μl of the appropriate formulation in the lower dorsal area using a 20-G syringe. Rats were injected with the following approximate rivastigmine doses: 10 mg/kg for saline formula-

Table I. Gel Characterization: 10% (*w/w*) SAM Oleogels Containing Various Concentrations of Rivastigmine Incorporated by Dissolution or Dispersion were Characterized by Rheological Analysis, DSC, and IR (mean±SD)

Drug Incorporation Method	Rivastigmine Content % (*w/w*)	Rheology ($n=3$)		DSC ($n=6$)		IR ($n=3$)
		G′; G″ (kPa)	TGS (°C)	ΔHGS (J/g SAM)	TGS (°C)	TGS (°C)
Drug-free	0	8.3; 1.49	66.5 ± 0.3	1.65 ± 0.12	75.1 ± 1.8	77.8 ± 2.1
	4	22.5;1.4	54.7 ± 0.2*	1.57 ± 0.17	70.7 ± 1.2*	75.8 ± 4.1
Dissolution	20	n.d.	n.d.	1.31 ± 0.27	65.6 ± 1.6*·**	n.d.
	40	n.d.	n.d.	1.05 ± 0.18*	61.7 ± 2.8*·***·***	56.4 ± 0.6*·**
Dispersion	5	142; 19.2	64.0 ± 0.8*·**	1.42 ± 0.20****	73.4 ± 0.8 ***·****	n.d.

n.d. Not determined
*$p < 0.05$ *vs* drug-free gel
**$p < 0.05$ *vs* 4% (*w/w*) dissolved RB gel
***$p < 0.05$ *vs* 20% (*w/w*) dissolved RB gel
****$p < 0.05$ *vs* 40% (*w/w*) dissolved RB gel

tions and 18 mg/kg for oil and gel formulations. The exact amount of injected formulation was obtained by weight difference of syringes before and after injection. The tested formulations were a RHT-saline solution, as well as RHT dispersed in oil, in 5%, and in 10% SAM oleogels. Blood samples (200 µl) were periodically collected from the subclavian vein under isoflurane anaesthesia, and were subsequently weighed, digested using up to 1.5 ml Solvable® (PerkinElmer, Groningen, The Netherlands) and 500 µl isopropyl alcohol to ensure solubility. The samples were incubated at 60°C for 1–2 h to complete digestion, after which they were bleached using 1–1.5 ml hydrogen peroxide. Finally, 10-ml portions of HionicFluor scintillation cocktail (PerkinElmer) were added to each sample, which was vortexed, stored in the dark at 4°C for 24 h, and subsequently counted on a liquid scintillation analyzer (Tricarb 2100TR, Packard, Meridan, CT). Values of drug concentration in the blood (µg drug/g blood) were obtained from the radioactivity count (dpm) of each sample by reporting the latter to the total radioactivity-to-drug ratio (dpm/µg drug) injected.

Statistical Analysis

Tests for significant differences between means of T_{GS}, transition enthalpy (ΔH_{GS}) values, and pharmacokinetic parameters were carried out by one-way analysis of variance (ANOVA), followed where necessary by the Tukey *post-hoc* test. Differences were considered statistically significant for $p < 0.05$.

RESULTS AND DISCUSSION

Physicochemical Characterization of Oleogel

Rheological Analysis

The rheological analysis was initially performed as a function of applied strain (γ) for drug-free as well as 4 and 5% RB- or RHT-loaded oleogels, respectively, at a constant oscillation frequency of 1 Hz (Supporting information). A linear regime, corresponding to strain-independent storage and loss moduli (G′ and G″) was measured up to a critical strain (γ^c) value of 0.05%, beyond which a decrease in G′ and G″ was observed. Using a strain value within the linear regime (0.01%), G′ and G″ were measured at 25°C as a function of oscillation frequency (Table I) and were found to be relatively constant between 0.1 and 10 Hz (Supporting information). The G″ value of the drug-free oleogel was found to be larger than 1/10 of the G′ value, this being characteristic of a poorly elastic gel. Indeed, the oleogel exhibited a soft and waxy aspect. When RB was dissolved at 4% in the formulation, G′ and G″ values slightly increased but remained within the same range as the unloaded system. However, the incorporation of dispersed RHT produced a 15-fold increase of G′ and G′. These findings show that the dispersed drug improved the formulation's mechanical strength, as previously observed for other gels containing physically dispersed particles (25). Finally, the T_{GS} was determined at a strain of 0.005% and a frequency of 1 Hz (Supporting information). At room temperature, G′ was found to be higher than G″, a property characteristic of gel systems. As the temperature rose, G′ and G″ decreased at different rates and eventually crossed, marking the system's transition to the sol-state. As shown in Table I, RB

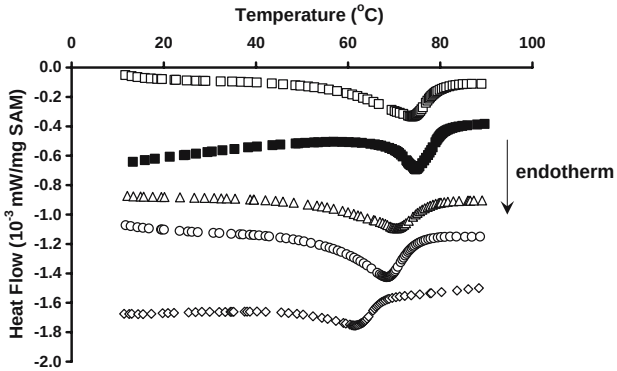

Fig. 2. DSC analyses showing gel-sol transitions for drug-free gels (*open square*), for gels containing 5% (*w/w*) dispersed RHT (*filled square*), and for gels containing RB dissolved at 4 (*open triangle*), 20 (*open circle*) and 40% (*w/w*) (*open diamond*). *Curves* are offset on the *y*-axis for improved clarity.

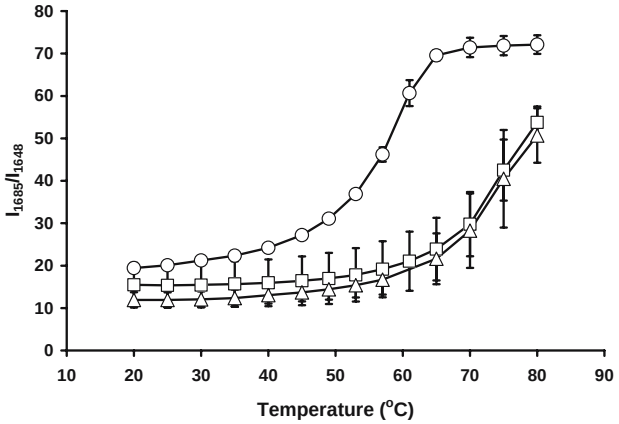

Fig. 3. FTIR analysis showing the proportion of free amide bonds in drug-loaded gels, as determined from the band intensity ratio of amide I peaks at 1,685 and 1,648 cm^{-1} (I_{1685}/I_{1648}), as a function of temperature for gels containing dissolved RB at different concentrations: 0 (*open square*), 4 (*open triangle*) and 40% (*w/w*; *open circle*; mean±SD, $n=3$).

significantly decreased the T_{GS} with respect to drug-free gels, while only a slight difference was observed for RHT-loaded formulations.

DSC

The effect of drug-loading on gel intermolecular interactions was evaluated based on gel-sol transitions obtained by DSC. For simplicity, only the behaviour of gels during heating cycles was investigated, however it has been previously shown that SAM oleogels undergo fully reversible transitions (21). Analysis of oleogels containing dissolved RB (Fig. 2) revealed that T_{GS} and enthalpies decreased as drug content was progressively increased from 0% (drug-free gels) to 4, 20, and 40% (*w/w*). The drop in T_{GS}, varying between 4 and 13°C, was found to be statistically significant between any of the gels containing dissolved RB and the drug-free gel (Table I). The DSC data corroborated well with observations from rheology measurements, both methods indicating a decrease in T_{GS} with increasing RB-loadings. Nevertheless, T_{GS} values determined by rheological analysis were found to be 10–15°C less than those determined by DSC, an expected difference explained by the measurement of different phenomena by the two techniques. DSC further showed that transition enthalpies decreased more moderately and were found to be significantly lower than for the drug-free gel only when extremely high concentrations of RB (40% *w/w*) were added, yielding a 39% decrease in this case. The observation of decreasing T_{GS} and ΔH_{GS} suggests a weakening of organogelator interactions by the addition of rivastigmine. Gels containing a dispersion of RHT salt (5% *w/w*) were also analyzed by DSC and were found to have similar T_{GS} and ΔH_{GS} values to the drug-free formulation. This indicates that, contrary to dissolved RB, the dispersed RHT crystals do not influence the gel structure at this concentration.

FTIR

To gain further insight into the effect of RB addition on the gelation process, hydrogen-bonding between SAM molecules in the absence and presence of dissolved drug was studied by FTIR spectroscopy as a function of temperature (Fig. 3). Two vibrational peaks of the amide I group were studied, indicating the presence of species identified as H-bonded (1,649 cm^{-1}) and free (1,685 cm^{-1}) amide carbonyl groups (21,26). It is known that the gel-sol transition is associated to a hydrogen-bond disruption between amide groups of organogelator molecules (21,26). This translates to an abrupt increase and a corresponding decrease in the peak intensities of the 1685 and 1649 cm^{-1} amide I components, respectively (see Supporting information, Fig. 2S). By plotting the peak intensity ratio of these two bands (I_{1685}/I_{1649}) against temperature, the T_{GS} was determined from the inflection point of the corresponding sigmoidal function (Table I). These T_{GS} values correlated well to those obtained by DSC. The drug-free and 4% (*w/w*) RB gels showed a sharp increase in the I_{1685}/I_{1649} ratio around 70°C, without the subsequent formation of a plateau, suggesting only partial network disruption for these gels at 75°C (the maximum accessible temperature for our experimental set-up). For gels with high RB concentrations (40% *w/w*), the FTIR study showed the H-bond network to be disrupted at lower temperatures (~55°C). The transition to the sol state appeared to be complete as the I_{1685}/I_{1649} ratio levelled off at around 65°C. Contrary to DSC, the IR study was not sensitive enough to show an interference of the dissolved drug with the oleogel structure at low drug-loading (4% *w/w*). However, at very high drug-loading, the lowering of the transition temperature is confirmed by both

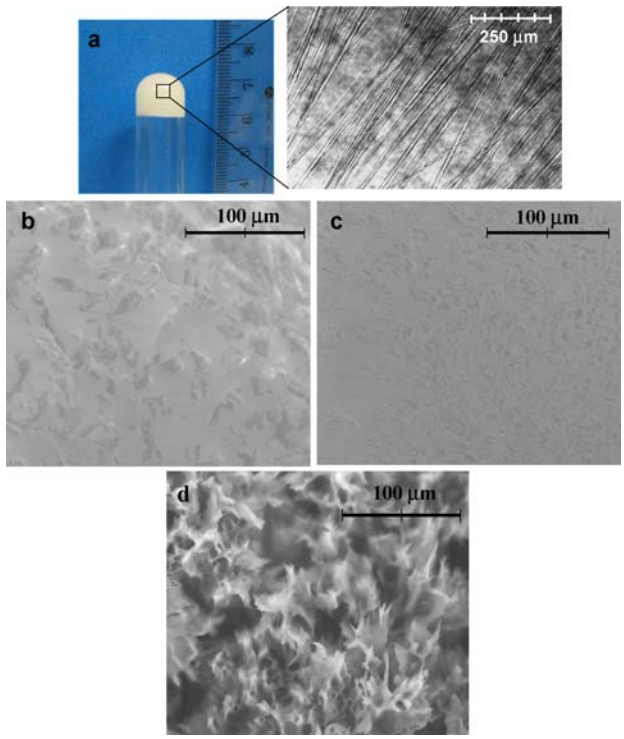

Fig. 4. **a** An inverted tube containing 10% (*w/w*) SAM gel is shown at room temperature to demonstrate the cohesiveness of the system. The *inset* shows an optical microscopy image of the oleogel network. ESEM images of a 10% (*w/w*) SAM **b** drug-free oleogel and **c** oleogel containing dissolved RB (5% *w/w*). Finally, an ESEM image is shown of **d** a dried formulation, obtained after complete solvent evaporation from an organogel of 10% SAM in toluene.

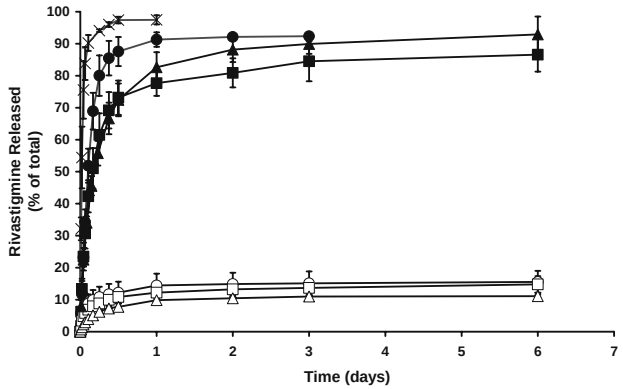

Fig. 5. *In vitro* release experiments for formulations varying in SAM and NMP content as well as in drug incorporation method (dissolution *vs* dispersion). A control RHT solution in PBS (*X*) was tested. Release experiments from formulations containing dissolved RB are represented by solid symbols: oil formulation (*filled circle*), 10% SAM gel with (*filled square*) and without NMP (*filled triangle*); release experiments from gels containing dispersed RHT are represented by hollow symbols: oil formulation (*open circle*) and 10% SAM gel with (*open square*) and without NMP (*open triangle*; mean±SD, $n=3$).

the DSC and IR studies. A hypothesis for the weakening effect of the dissolved drug on the gel network is the reduction of hydrogen-bonding between organogelator amide groups by competing interactions with the ester and amine functionalities of rivastigmine. Despite this weakening effect, the formulations remain in the gel state at physiological temperature, which permits their use as drug-loaded implants.

Microscopy

An optical microscopy picture of drug-free, 10% SAM oleogel (Fig. 4a) allowed visualisation of the continuous network of fibrous SAM aggregates responsible for gelation. Furthermore, samples of drug-free as well as RHT- and RB-loaded oleogels were analysed by ESEM (Fig. 4b,c). The advantage of this technique over conventional SEM is the potential of imaging wet and/or non-solid samples in their natural state, without sample preparation steps such as drying and metal coating. Micrographs depicted only the gel surface topography and did not allow the viewing of the underlying gelator network. Only the crests of SAM aggregates were seen protruding from the oil. The apparent aggregate size was found to be similar for drug-free gels (Fig. 4b) and those containing dispersed RHT (image not shown), while being noticeably smaller in gels containing dissolved RB (Fig. 4c). These data seem to corroborate with DSC and FTIR observations, suggesting that concurrent with the hydrogen-bond disruption between SAM chains by RB, a corresponding reduction in aggregate size is possible. In a further effort to visualise the gel network, the oil was replaced with a volatile continuous phase. Although the gel structure may be different between the toluene- and oil-based formulations, gelator network formation is common to both systems. After allowing complete solvent evaporation from organogels of 10% SAM in toluene, ESEM was carried out on the dried formulations (Fig. 4d). The micrograph clearly depicts the three-dimensional gelator network housing interconnected channels (dark areas) left empty by the evaporated solvent.

In Vitro Release

In vitro release kinetics were performed on oil and gel formulations (Fig. 5) containing either dissolved RB (solid symbols) or dispersed RHT (hollow symbols). No true correlation of the overall release profile with eventual *in vivo* behaviour is possible here given that spreading and enzymatic degradation of the implant do not occur in the chosen experimental set-up. The main purpose of this study was to optimize the formulation in terms of minimal burst release, so as to diminish the risk of adverse effects *in vivo*. It is known that side-effects to rivastigmine are considerably reduced if up-titration of the drug is slowed (27). As shown in Fig. 5, drug diffusion from the dialysis bag was not a rate-limiting factor, as illustrated by the complete release of drug from the control RHT solution within 6–9 h. NMP did not seem to play a significant role in the release mechanism, since formulations prepared with and without the organic solvent showed comparable burst releases (Fig. 5). Its incorporation in the formulation is however beneficial because it facilitates injection at room temperature by partially disrupting the interactions between organogelator molecules (21, 22). Formulations containing dispersed RHT had a much lower burst than gels containing dissolved RB, with about 10 *vs* 70–90%, respectively, of total rivastigmine released in 12 h. This is due to the limited solubility of the drug salt in the oil medium and consequently, the low amount of drug able to diffuse out of the implant. In fact, the release from these gels nearly stopped after the first day, with less than 15% of the total drug released, confirming diffusion of the drug out of the formulation to be minimal. The *in vitro* release study showed very little, if any, differences between gels varying in organogelator concentration, especially in the case of gels containing dispersed drug. Plourde *et al.* (22) have demonstrated that the release of leuprolide from SAM oleogels varied greatly with organogelator content. The drug used in this case was incorporated into the gels via a water-in-oil emulsion, which made the density of the gel fiber network critical to the retention of emulsified water droplets. However, in the case of a simple dissolution or dispersion of the

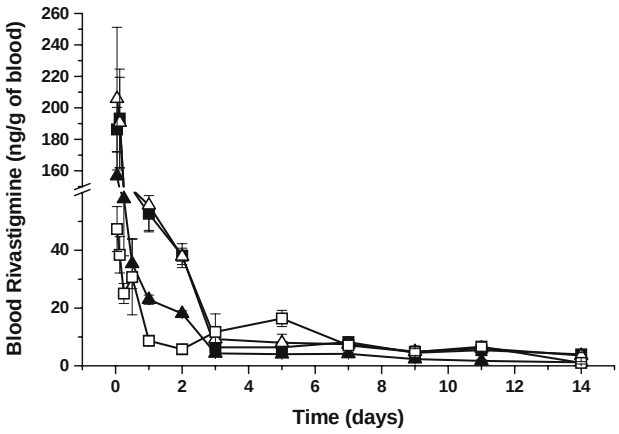

Fig. 6. Blood concentration of rivastigmine after the s.c. administration of a 10 mg/kg dose of saline RHT solution (*filled triangle*), and 18 mg/kg doses of RHT dispersed in oil (*filled square*), in 5% (*open triangle*), and in 10% (*w*/*w*) SAM gel (*open square*; mean±SEM, $n=6$). SEM rather than SD values were used for the error bars in order to reduce clutter and improve the clarity.

Table II. Pharmacokinetic Parameters of the Different Formulations Injected to Rats ($n = 6$)

Pharmacokinetic Parameter[a]	Formulation			
	Saline	Oil	5% SAM Gel	10% SAM Gel
AUC [ng·day·(g blood)$^{-1}$]	120 ± 20	250 ± 62	277 ± 101	140 ± 70
AUC/D_o [10^{-4} day·(g blood)$^{-1}$]	6.1 ± 1.4	8.4 ± 2.1	7.3 ± 4.1	4.2 ± 2.2
C_{max} (ng/g blood)	156 ± 37	204 ± 73	242 ± 102	48 ± 17*,**
T_{max} (min)	≤60	100 ± 62	120 ± 66	80 ± 49

[a] Values listed are the mean±SD.
*$p < 0.05$ *vs* oil formulation
**$p < 0.05$ *vs* 5% SAM gel formulation

drug in the gel, the rate-limiting mechanisms of release, namely drug dissolution and/or diffusion in the oily matrix, seem to be practically unaffected by the density of the gel network, within the investigated range.

Pharmacokinetic Study

Owing to their considerably smaller burst effect, dispersed-RHT gels were chosen for subcutaneous injection to rats. Gels varying in SAM concentration (5 and 10% *w/w*) as well as oil and saline control formulations were studied. It is to be noted that the rivastigmine dose injected in saline (10 mg/kg) was lower than for oil and gel implants (18 mg/kg) in order to avoid overdosing, since the former formulation allows rapid systemic absorption of the entire dose. Blood concentrations of rivastigmine were monitored over 2 weeks. In the days following implant injection, no dermal reaction or apparent signs of toxic effects related to rivastigmine (e.g. diarrhea, seizures) were observed. The pharmacokinetic profiles and parameters of the injected rivastigmine formulations are presented in Fig. 6 and Table II, respectively.

Values of area under the rivastigmine blood concentration *vs* time curve (AUC), obtained for days 0 to 14 by the trapezoid rule and normalized for injected dose (D_o), were not statistically different for the four formulations tested. This suggests a complete release of rivastigmine from all formulations within the two weeks studied. However, despite the lack of statistical differences, a trend of decreasing AUC/D_o can be observed with increasing organogelator concentration (8.4 *vs* 4.2×10^{-4} day·(g blood)$^{-1}$ for the oil *vs.* 10% SAM formulations, respectively). This observation suggests the possibility of slower, and potentially incomplete, release from the 10% SAM gels within the period studied.

As expected based on the *in vitro* experiments, the saline formulation was rapidly (<3 h) absorbed into circulation, producing an important burst, after which elimination drove blood levels down to baseline values within 3 days. Contrary to the *in vitro* study, there were marked differences among gels varying in SAM content. While both oil and SAM formulations showed sustained blood levels within the therapeutic range (1–400 ng/ml) (28) for 11 days, the 10% SAM oleogels proved largely superior to the oil and 5% SAM formulations in terms of minimizing burst release (C_{max} values up to five times lower). The low peak plasma concentration (48 ± 17 ng/g), being well below the toxic threshold (400 ng/ml), would allow the administration of higher rivastigmine doses, thus potentially increasing the delivery time. A hypothesis for the decrease in burst release in the case of 10% SAM formulations is the higher density of their constituting gelator network. The improved cohesiveness of these gels may reduce the subcutaneous spreading of the formulation. The potentially smaller surface area generated minimizes drug contact with the aqueous environment, contributing to a decrease in burst release.

In the time-period following the burst (days 3–7), rivastigmine plasma concentrations were maintained at higher levels in rats having received the 10% SAM implant (7–16 ng/g blood) compared to those having received oil or 5% SAM formulations (6–9 ng/g blood). Following the burst phase, it is thought that drug release is mostly driven by implant degradation by subcutaneous esterases and lipases (29–31), a process that may be affected by gel density. The question of enzymatic degradation will be further addressed in future studies.

The only other documented pharmacokinetic study involving the sustained release of rivastigmine was conducted in minipigs and demonstrated release of the ChEI from a dermal patch for up to 72 h, with bioavailability values 20–40 times higher than for oral administration, for which the authors reported only 0.5% bioavailability (16). Curiously enough, despite the advantages of sustained dosing regimens, no further studies were reported in the literature. The present work highlights the possibility of using L-alanine-based oleogels in the sustained release of rivastigmine for up to 11 days, demonstrating their potential use as *in situ*-forming implants for AD treatment. Future work will focus on optimizing the pharmacokinetic behaviour of these implants in the release of rivastigmine, notably by investigating release mechanisms involved and exploring the use of novel amino-acid-derived organogelators.

CONCLUSION

This study tested the sustained rivastigmine release from SAM oleogels. Physicochemical characterization contributed to the understanding of the effect of rivastigmine addition on intermolecular interactions within the gel. *In vitro* and pharmacokinetic experiments demonstrated the feasibility of using SAM oleogels for the sustained delivery of rivastigmine at therapeutic levels, and this for up to 11 days after subcutaneous injection to rats. Compared to the current once-a-day oral administration of rivastigmine, such a simplified dosing regimen has the potential to alleviate caregiver responsibilities as well as treatment-related adverse effects caused by high variations in plasma drug concentration.

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