Monitoring of Partitioning of Poorly Water-Soluble Drugs during *In Vitro* Lipolysis by Electron Paramagnetic Resonance

Selena Di Maio[1], David E. Budil[2], Rebecca L. Carrier[1]
[1]*Department of Chemical Engineering,* [2]*Department of Chemistry and Biological Chemistry, Northeastern University*

Introduction

Ingested lipids could offer valuable opportunities for enabling oral drug delivery, as approximately 40-70% of all new drug candidates have been estimated to have very poor water solubility[1] and are expected to exhibit low bioavailability when orally dosed. For this class of drug molecules, whose dissolution in water is likely the limiting step of overall oral absorption, the influence of ingested lipids on oral absorption has been associated with complex, poorly characterized interactions between drugs and several colloidal nanostructures, taking place during lipid digestion[2]. In particular, emulsion droplets, micelles and vesicles influence dissolution kinetics and they are able to maintain larger quantities of hydrophobic drugs in solution, increasing the solubilization power of the gastrointestinal (GI) tract contents. Furthermore, the distribution of drugs within oil, aqueous and micellar phases affects their absorption rate. Despite the recognized capability of ingested lipids to impact several processes associated with the overall absorption of hydrophobic drugs[3], the fate of co-administered drugs remains unclear and unpredictable.

In order to investigate the role of lipid-based colloidal particles in the partitioning process of poorly water-soluble drugs during lipid digestion, an updated *in vitro* lipid digestion model[4] has been designed based on knowledge of gastrointestinal contents[5]. The presence and the size of colloidal species have been evaluated by dynamic light scattering (DLS), while kinetics of drug partitioning between colloidal phases (oil, micellar, aqueous) formed during *in vitro* digestion has been tracked by electron paramagnetic resonance (EPR). This is a non-invasive technique that has the capability of detecting and quantifying radicals acting as spin probes. Nitroxides are well-known paramagnetic molecule, which can be selected according to their physicochemical properties as models for hydrophobic drugs. Since EPR spectra are highly sensitive to changes in environment micropolarity and microviscosity, the relocation of the selected spin probe within different phases can be monitored in real-time. In addition, EPR spectrum fitting can be used to quantify the amount of paramagnetic species in each phase.

Experimental Method

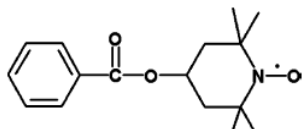

Figure 1: Molecular formula of the stable nitroxide radical TEMPOL Benzoate (TB).

The proposed *in vitro* lipolysis model consists of a bio-relevant medium, reflecting GI contents in the fed state, which was prepared in maleate buffer at pH 6.5 and included lecithin 4 mM and sodium taurodeoxycholate 12 mM as a model of human bile. The lipid digestion experiments were performed at 37°C and initiated by adding lipase/colipase enzymes and soybean oil as lipid substrate. Samples were collected at specific time intervals before and during *in vitro* lipid digestion experiments and analyzed by DLS and EPR techniques. Since only paramagnetic molecules are visible to EPR, the spin probe TEMPOL benzoate (TB), shown in Figure 1, was selected as a model for poorly water-soluble moderately lipophilic drug with an octanol/water partition coefficient (log P) of 2.46. In lipolysis experiments coupled with EPR, TB was added to the bio-relevant medium before the beginning of the lipolysis process and tracked between phases before and as the digestion proceeded. EPR spectra of TB in separate environments (maleate buffer, bio-relevant medium, soybean oil) were recorded at 37°C and used to obtain simulation parameters for resolving EPR multi-component spectra of pre and post-lipolysis samples. Simulation of the EPR spectra was performed by means of Multicomponent, a LabVIEW program for fitting multi-component EPR spectra of nitroxide spin probes[6].

Results and Discussion

The dynamic evolution of the size of colloidal particles formed during the *in vitro* lipolysis of soybean oil at pH 6.5 and 37°C was monitored by DLS. The bio-relevant medium contained micelles with a hydrodynamic diameter less than 20 nm. Detected particles suddenly increased to 200 nm after the addition of lipase/co-lipase enzymes and the lipid substrate, which might indicating the formation of vesicles during the enzymatic hydrolysis of lipids, in agreement with previous in vivo studies concerning human intestinal fluids contents[7]. However, additional investigation of colloidal particles' morphology and composition are needed to enable modeling mathematically their role during the oral drug absorption.

In order to determine the partitioning of the drug model TB between aqueous, micellar, and oil phases before and during lipid digestion, real-time compound tracking measurements were performed before and during *in vitro* lipolysis of soybean oil at pH 6.5 and 37°C by means of EPR. The recorded EPR spectra of pre and post-digestion samples represented complex spectra containing up to three different components (aqueous, oil, micellar), which were resolved by spectral simulation. In order to reduce the amount of variable parameters during the simulation process, EPR spectra of TB in the separate environments were first fitted in order to determine the relative simulation parameters, which were used to fit complex EPR spectra of TB recorded at different times before and during *in vitro* lipolysis. In the EPR spectrum of TB dissolved in the bio-relevant medium, 85% of the TB molecules were associated with mixed micelles. After the addition of soybean oil, EPR spectra of pre-lipolysis samples (Figure 2) indicated increasing partitioning of TB into the oil phase (up to 30%), while the amount of TB associated with mixed micelles decreased to 67% over a time scale similar to that of lipid digestion. During lipolysis experiments, EPR spectra showed that drug partitioning into the micellar phases suddenly increased to 74% within the first 15 minutes of the parallel digestion process, while it decreased to 67% at the end of lipolysis. The higher TB partitioning into the micellar phase in the early stage of lipolysis, followed by its decrement in the late stage, might be associated with the formation and evolution of more complex colloidal particles, such as vesicles, which are expected to show increased drug association. Previous studies[8] have reported that vesicles were likely formed after few minutes of the lipolysis start, and then able to evolve throughout the lipid digestion process. The proposed *in vitro* lipolysis model coupled with EPR analysis was able to track and quantify in real-time the model drug between the different phases formed during lipid digestion. Therefore, the method represents a helpful tool to develop kinetics of digestion, drug partitioning, and, in future studies, drug absorption profiles, using mathematical models to predict overall oral absorption of drug molecules in the presence of lipids.

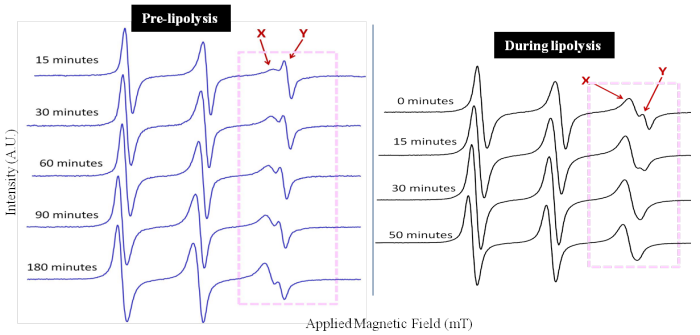

Figure 2. EPR spectra before and during the *in vitro* lipolysis process: X denotes the hydrophobic phases (oil and micelles), while Y denotes the aqueous phase.

References

1. D.J. Hauss, 1st ed. Informa Healthcare, New York, 2007.
2. C. J. H. Porter, *et al*, *Nature Rev.,* 6 (2007) pp. 231-248.
3. P. G. Welling, *Annu. Rev. Nutr*., 16 (1996) pp. 383-415.
4. L. Sek, *et al, J Pharm. Biom. Analysis,* 25 (2001) pp. 651-661.
5. S. Di Maio, *et al*, *J Control Release*, 151 (2011) pp. 110-122.
6. https://sites.google.com/site/altenbach/labview-programs/epr-programs.
7. O. Hernell, *et al*, *Biochemistry* 29 (1990) pp. 2041–2056.
8. D. G. Fatouros, *et al*, *Pharma Res.*, 26 (2009) pp. 361-374.