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Note

The use of election paramagnetic resonance spectroscopy in early preformulation experiments: the impact of different experimental formulations on the release of a lipophilic spin probe into gastric juice

Beate Bittner*, Hugues Isel, Richard J. Mountfield

Pharmaceuticals Division, F. Hoffmann-La Roche Ltd., Basel, Switzerland

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Abstract

The lipophilic spin probe TEMPOL-benzoate was dissolved in different experimental formulations, including polyethylene glycol 400 (PEG 400), Miglyol, glycerol monooleate (GMO), and Cremophor RH-40. Samples were measured by electron paramagnetic resonance (EPR) spectroscopy before and after addition to human gastric juice. The distance between the first and the third peak in the EPR spectrum ($2a_N$) was measured to monitor the polarity of the spin probe's microenvironment. Moreover, the ratio between the signal amplitudes of the second and the third peak (*a/b* ratio) was used to monitor the mobility of the spin probe in a certain formulation. Thus, by calculating $2a_N$ and the *a/b* ratio of the EPR spectra it was possible to determine a potential release of the spin probe from different formulations into gastric juice. It was found that oily and surface-active vehicles (Miglyol, Cremophor RH-40, and GMO) were more suitable to protect a lipophilic compound from being released within a gastric environment than PEG 400. Our results demonstrate that EPR spectroscopy seems to be a promising tool in early preformulation experiments to monitor the release of spin probes from formulations of different nature. This kind of experiment can be of value for the optimization of exploratory formulations.

Keywords: Preformulation; EPR spectroscopy; Polyethylene glycol 400; Miglyol; Glycerol; Monooleate; Cremophor RH-40; Oral drug delivery

1. Introduction

In early preformulation, compounds to be formulated in an oral drug delivery device are typically lipophilic, barely soluble or insoluble in water [1]. Therefore, the choice of a suitable vehicle in which to dissolve the drug at reasonably high concentrations represents a major challenge in the field of preformulation. However, it is well documented that oils and co-solvents exhibit the potential to increase the solubility of lipophilic compounds [2], thus in many cases a variety of these vehicles have been investigated as oral formulations [3]. Unfortunately, the solubilization of a lipophilic compound in this kind of vehicle does not automatically guarantee high oral bioavailability. Most of the vehicles do not prevent the test compound from being rapidly released from the formulation and thereafter from being precipitated in the stomach and intestinal aqueous environments. For example, many compounds are soluble at low pH in the stomach but are relatively insoluble at intestinal pH. A release of the test compound in the stomach would result in precipitation of the compound upon entering the intestine.

To circumvent these problems, surface-active ingredients such as Cremophor or oleic acid can be incorporated into formulations. Due to their amphiphilic character, these additives stabilize the formulation droplets generated in the stomach, therefore preventing a precipitation of the incorporated compound.

The sensitivity of EPR spectra of spin probes such as stable nitroxide radicals to the microviscosity and micropolarity of the surrounding vehicle can be used to investigate the mechanisms of drug delivery [4,5].

Therefore, the aim of the current investigation was to find out whether it is feasible to monitor by EPR spectroscopy the release of the lipophilic spin probe TEMPOL-benzoate from different experimental formulation ingredients in human gastric juice. The formulations investigated were Miglyol, PEG 400, GMO, and Cremophor RH-40.

* Corresponding author. Pharmaceuticals Division, F. Hoffmann-La Roche Ltd., CH-4070 Basel, Switzerland. Tel.: +41-61-688-0412; fax: +41-61-688-8689.

E-mail address: beate.bittner@roche.com (B. Bittner).

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2. Materials and methods

2.1. Materials

The lipophilic spin probe TEMPOL-benzoate (4-benzoic-2,2,6,6-tetramethyl-piperidine-1-oxyl) was supplied by Sigma (Buchs, Switzerland). TEMPOL-benzoate has a molecular weight of 276.31 g/mol and a melting point of 103°C, the octanol/water partition coefficient is greater than 100, indicating a very low solubility in aqueous media [6]. Miglyol 816 (Hoffmann-La Roche Ltd., Basel, Switzerland), PEG 400 (Fluka Chemika, Buchs, Switzerland), Cremophor RH-40 (Hoffmann-La Roche), and GMO (Eastman, Kingsport, USA) were used as experimental formulations. All other materials used were of analytical grade.

2.2. Preparation of formulations

TEMPOL-benzoate was dissolved in either PEG 400, Miglyol, GMO, or Cremophor RH-40 by vortexing for ca. 2 min (final concentration: 1 mM; total volume of formulation: 500 μl).

2.3. EPR spectroscopic investigations

Formulations were measured before and after the addition of human gastric juice (obtained from healthy volunteers, Kantonsspital, Basel, Switzerland, 100 μl formulation + 300 μl human gastric juice). This mixture was vortexed for 2 s and measured immediately by EPR (1.1 GHz, L-band EPR spectroscope, surface coil equipped, Magnettech GmbH, Berlin, Germany).

3. Results and discussion

Several characteristics of the EPR spectrum can be used to ascertain the environment of spin probes [4,5]. The molecular structure of the spin probe TEMPOL-benzoate and its mesomeric forms are shown in Fig. 1. Due to charge-dipol interactions the mesomeric form I is favored in a polar environment, whereas form II will predominate in a non-polar environment. The distance between the first and the

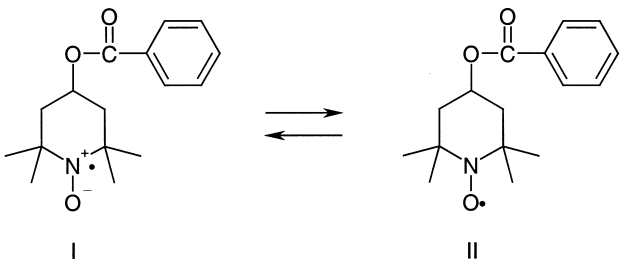

Fig. 1. Molecular structure and mesomeric forms of the nitroxyl radical TEMPOL-benzoate (4-benzoic-2,2,6,6-tetramethyl-piperidine-1-oxyl). Form I is favored in polar environments, form II is favored in non-polar environments.

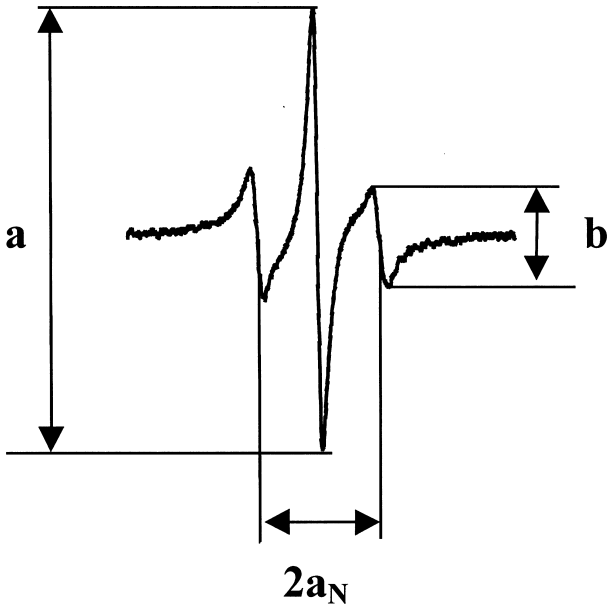

Fig. 2. $2a_N$ (distance between the first and the third peak) and the ratio *a/b* between the signal amplitudes of the second and the third peak can be calculated from the spectral shape.

third peak in the EPR spectrum ($2a_N$) is directly proportional to the spin density on the nitrogen atom of the nitroxyl moiety (Fig. 2). Thus, $2a_N$ is sensitive to the polarity of the environment of the spin probe. Consequently, $2a_N$ of tempol-benzoate in gastric juice (a polar environment) will be higher than in a lipophilic environment (non-polar formulation).

The mobility of the spin probe in a certain environment can be monitored by means of the *a/b* ratio between the signal amplitudes of the second and the third peak as shown in Fig. 2. Both the dissolution in a higher viscous environment and the incorporation into micellar structures can contribute to a reduced mobility of the spin probe. Lower mobility of the spin probe leads to less averaging of the anisotropy and therefore to an increase in the ratio *a/b* and to decreased signal amplitudes. For example, the measurement of the nitroxyl radical in gastric juice (representing low viscosity), in which there is a high mobility of the dissolved compounds, results in an isotropic spectrum characterized by three absorption lines with comparable signal amplitude. This spectrum is the result of an almost complete averaging of the anisotropic hyperfine coupling of the electron spin with the nitrogen nucleus. Consequently, a release of the model compound from the formulation into the surrounding environment can be visualized by calculation of the *a/b* ratio (Fig. 3).

In the current investigation, the spin probe was dissolved in different vehicles, which are commonly used as ingredients to formulate compounds for intravenous or peroral administration in animal studies [7–9]. Thus, the co- solvent PEG 400, the medium-chain triglyceride Miglyol, the long-chain monoglyceride GMO and the surfactant Cremophor

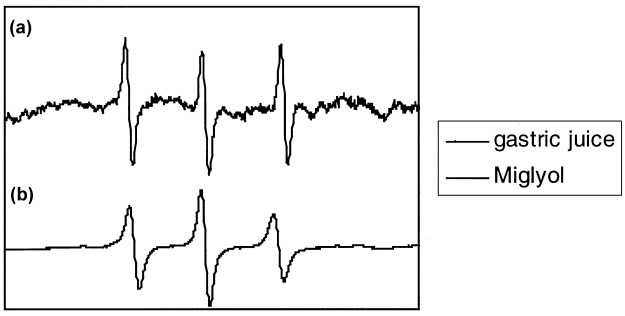

Fig. 3. EPR spectra of TEMPOL-benzoate in gastric juice (a) and in Miglyol (b).

RH-40 were selected as typical vehicles with different inherent properties. All samples were measured in the presence and in the absence of gastric juice.

For example, the above vehicles differ in their behavior in the presence of water. Thus, in contrast to PEG 400, Miglyol is not soluble in water, and forms droplets in the presence of aqueous phase, whereas GMO, which is also not miscible with water, starts swelling after addition of aqueous phase. Finally the amphiphilic Cremophor RH-40 is water soluble, and forms micellar structures above a certain concentration. Additionally, at room temperature, PEG 400 and Miglyol are fluids, while Cremophor RH-40 and GMO are semisolid substances.

As demonstrated in Table 1, the *a/b* ratio of the TEMPOL-benzoate spectrum in the different vehicles varies significantly, indicating a different mobility of the spin probe due to alterations in the microenvironment. In pure gastric juice the mobility of TEMPOL-benzoate does not seem to be restricted since the *a/b* ratio of 1.02 indicates an almost isotropic spectrum. The isotropy of the TEMPOL-benzoate spectra generated in the different vehicles in the absence of gastric juice decreases in the following order: PEG 400 > Miglyol > Cremophor RH-40 > GMO. An increase in the viscosity of the vehicle alone can not be the only explanation for this observation, since the viscosity of PEG 400 is 80–115 cP, while that of Miglyol is only 28 cP. A possible interpretation of the result might be the partial incorporation of the spin probe into micellar structures formed by the residual amphiphilic fatty acids inside the triglyceride Miglyol. Thus, the mobility of TEMPOL-benzoate would decrease compared to the probe in PEG 400.

Table 1
Influence of the vehicle composition on the spectral characteristics of TEMPOL-benzoate[a]

Formulation	Gastric juice	*a/b*	$2a_N$ (mT)	SD (mT)
PEG 400		1.25	3.52	0.00
PEG 400	+	1.09	3.70	0.01
Cremophor RH-40		1.73	3.46	0.02
Cremophor RH-40	+	1.70	3.50	0.01
Miglyol		1.70	3.42	0.00
Miglyol	+	1.70	3.43	0.01
GMO		1.82	3.52	0.00
GMO	+	1.29	3.52	0.00
Gastric juice		1.02	3.76	0.01

[a] *a/b* reflects mobility and $2a_N$ polarity. SD, standard deviation. $n = 3$ for each determination.

The *a/b* ratio in the spectrum of the spin probe solution in Cremophor RH-40 is in the same range as that of the Miglyol vehicle and that of the GMO spectrum is even higher. Thus, both the viscosity of the vehicles and an incorporation of the spin probe into micellar structures generated by the amphiphilic Cremophor RH-40 and GMO molecules might contribute to an overall low mobility of TEMPOL-benzoate.

To determine the impact of a gastric environment on the microenvironment of the spin probe, the different exploratory formulations were added to human gastric juice as described above, and the resulting mixtures were measured by EPR spectroscopy. In the case of PEG 400, a significant decrease in the *a/b* ratio to a value close to that of a spin probe solution in neat gastric juice was observed. Moreover, the $2a_N$ value was increased, indicating an increase in polarity of the spin probe microenvironment. Thus, both the mobility of the spin probe and the polarity of the microenvironment in the formulation/gastric juice mixture did not differ significantly from the values obtained for the TEMPOL-benzoate solution solely gastric juice. These data might be explained by the miscibility of PEG 400 with aqueous media, resulting in a release of the spin probe into the gastric juice.

Concerning the spin probe solution in Miglyol, neither the *a/b* ratio, nor the $2a_N$ value changed significantly after mixing with gastric juice. Since TEMPOL-benzoate is highly lipophilic, it is unlikely that it was released from the oily phase into the aqueous environment. Moreover, Miglyol is not miscible with aqueous media and therefore, this vehicle seems to be suitable in protecting the release of an incorporated compound already in the stomach. A release from this kind of vehicle is more likely to occur within an intestinal environment where digestion of the oil by pancreas lipase and co-lipase has occurred [10]. The resulting digestion products are mono- and diglycerides and free fatty acids, which all have an amphiphilic character. Moreover, due to the secretion of bile salts in to the intestinal lumen [11], a transfer of a lipophilic compound from the oily vehicle into the aqueous environment is facilitated by the formation of mixed micelles from bile salts and the digestion products.

In the case of TEMPOL-benzoate in Cremophor RH-40, again both the *a/b* ratio and the $2a_N$ value of the spectrum did not change significantly as a consequence of the addition of gastric juice. Due to its amphiphilic character Cremophor RH-40 seems to protect the lipophilic molecule from being released into the aqueous environment. As already described for the Miglyol vehicle, the release of a lipophilic molecule into an aqueous environment is not likely to occur

in the absence of sufficiently high amounts of amphiphilic molecules in the aqueous phase.

The monoglyceride GMO also has amphiphilic characteristics, but the EPR spectra obtained after mixing with gastric juice are not comparable to those of the Miglyol and the Cremophor RH-40 vehicles. The $2a_N$ value remained unchanged, thus indicating that TEMPOL-benzoate was not released into the aqueous environment, but interestingly, a significant decrease in the *a/b* ratio was observed. GMO is a polar, bioadhesive, insoluble, lipid monoolein that has swelling properties and forms highly ordered cubic phases in the presence of water [10]. Thus, the compound does not yet seem to be released, but the spectral shape is rendered into a more isotropic form, indicating that the transformation into a cubic, isotropic structure had been initiated.

However it has to be mentioned that distribution of the spin probe between the different liquid phases might also occur, but for the overall release characteristics these processes are of minor importance. Moreover, since in vivo a formulation does not represent a static system due to both decomposition during digestion processes and the formation of micellar structures with naturally occurring bile salts, real equilibrium between definite phases will not be observed.

From the data described above it can be concluded that EPR spectroscopy seems to be a suitable analytical tool for the characterization of oral formulations in vitro. Results indicated that depending upon the nature of a vehicle, a model lipophilic spin probe was released quite differently. In combination with other techniques (X-ray diffraction, NMR spectroscopy, microscopy), EPR spectroscopy could also be used to explain phase transitions in micellar vehicles as demonstrated for the GMO system. Additionally, this type of investigation applied in early preformulation experiments could be used to study the impact of the percentage composition of a mixture of different vehicles on the release of incorporated compounds.

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