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RESEARCH PAPER

The Influence of Polysorbate 80 on the Radiochemical Synthesis of a PET Tracer in the FASTlab

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ABSTRACT

Purpose The aim of current study was to investigate the influence of a common non-ionic surfactant, polysorbate 80 (PS80), on radioactive labelling process of a novel PET tracer, [^{18}F]Flutemetamol.

Methods Ferrous oxidation-xylenol orange (FOX) assay, in addition to UV/VIS and ^{1}H NMR spectroscopies were applied to characterise the composition of the PS80 solution after storage. Multivariate Curve Resolution (MCR) and PLS analysis was used to establish correlation between quality of the PS80 solution and the RCP obtained after labelling.

Results The levels of unsaturated fatty acid moieties of PS80 were negatively correlated to RCP of [^{18}F]Flutemetamol after synthesis. This explains the slight increase in RCP when stored PS80 solutions were applied in the synthesis. The mechanism behind this observation is suggested to be related to radiation induced radical formation in the unsaturated fatty acids, which subsequently causes instability of the PET tracer. UV/VIS spectroscopy was demonstrated to have the ability as a possible control tool for quality assurance of the studied radioactive labelling process.

Conclusions The presence of unsaturated fatty acid moieties in PS80 was found to be one of the most important factors responsible for the reduction in RCP of [^{18}F]Flutemetamol after synthesis.

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KEY WORDS FASTlab · [^{18}F]Flutemetamol · polysorbate 80 · radioactive labelling

ABBREVIATIONS

MCR	Mulitivariate curve resolution
PET	Positron emission tomography
PLS	Partial least squares
PS80	Polysorbate 80
RCP	Radiochemical purity

INTRODUCTION

Radiopharmaceuticals have been synthesised and successfully used both for the purpose of therapy and medical diagnosis for several decades (1,2). In addition to traditional cancer therapy and diagnosis, radiopharmaceuticals also play an important role in the diagnosis of many common diseases such as autoimmune disorder (3), psychoneurotic disorder (4) and bacterial infection (5). One of the most useful tools in nuclear imaging that has become increasingly common during the last decades is Positron Emission Tomography (PET). For clinical applications, the radioactive half-life of the nuclide is essential. The half-life should be short (preferably minutes to hours) to avoid extended radiation to the patient and minimize the waste disposal challenges. However, it should be long enough to offer flexibility in terms of preparation and distribution of the product, as well as scheduling of treatment. Compared to other radionuclides, fluorine-18 fulfills many of these criteria and also possesses several other favourable nuclear properties for PET imaging (2). The isotope's radioactive half-life (110 min) is long enough to allow tracer synthesis, delivery and imaging procedures that may last over hours. Furthermore, interchangeability between hydrogen and fluorine allows the possibility to include the radionuclide in biomolecules.

Several radiopharmaceuticals based on fluorine-18 have been demonstrated with promising results (6,7). One example is the PET tracer [^{18}F]Flutemetamol for detection of fibrillar amyloid-β in the brain, diagnosing Alzheimer's disease (8,9). The amyloid marker is a fluorinated analogue of the widely studied [^{11}C]Pittsburgh compound B. Replacement of ^{11}C with ^{18}F increased the half-life of the tracer five-fold (10). Clinical studies in human beings have provided evidence of convincing diagnostic efficacy in differentiating between people with Alzheimer's disease and healthy controls (11,12). For clinical purposes, [^{18}F]Flutemetamol is generally manufactured by a fully automatic synthesis module like FASTLab™ provided by GE Healthcare. In these synthesis platforms, virtually all necessary reactant vials and purification cartridges are embedded in a disposable cassette. The advantage of radioactive tracer synthesis on-site is elimination of the need for hands-on manipulation of the radiopharmaceuticals and thus diminished radiation exposure to the operators (13). Figure 1 illustrates the steps involved in synthesis of [^{18}F]Flutemetamol. Among the last steps in the synthesis is addition of an amphiphilic excipient followed by filtration through a sterilising grade filter. The filtration step is included as a part of the sterility assurance of the product. Since the PET tracer is designed to cross the blood–brain barrier, it is lipophilic (eLogD = 3.2) (14). Inclusion of an amphiphilic excipient, in this case Polysorbate 80 (PS80), in the formulation has two functions: 1) to prevent adsorption of [^{18}F]Flutemetamol to the filter material during filtration by competitive binding to the filter material and 2) to solubilise the lipophilic PET tracer by micelle formation. In order to preserve these functions, intact PS molecules are a prerequisite, and loss or degradation of the PS80 molecules is generally associated with compromised activity.

Characterisation of the chemical stability of PS80 by liquid chromatography–mass spectrometry (LC-MS) has previously been described by our group (15). The results of the stability studies under accelerated conditions indicated the possibility for stability challenges during storage. In the present study, the nature and extent of the reported degradations are studied by various accelerated storage experiments. A method for fast and easy evaluation of the quality of PS80 prior to its use in the process could be of importance. We therefore also evaluated the possibility of using a combination of UV/VIS spectroscopy and Mulitivariate Curve Resolution (MCR) as a tool for quality control of the PS80 prior to the radiochemical labelling reaction. This was performed by assessing the method's ability to reveal changes relevant for the functional stability of PS80, both with respect to adsorption prevention and solubilisation. To our knowledge, this aspect has not previously been described in the literature.

MATERIALS AND METHODS

Preparation of Polysorbate 80 Solution

In this study, the PS80 solutions were prepared by dissolving appropriate amounts of PS80 in an 18.82 μM aqueous phosphate buffer containing 1% Sodium chloride. Oxygen dissolved in the water was removed by bubbling with nitrogen gas for 30 min prior to preparation.

Retention of [^{19}F]Flutemetamol by Sterilising-Grade Filter

To determine the concentration range of PS80 necessary to obtain a robust process during manufacturing of the tracer, formulations with varying concentrations of the surfactant (in the range of 0–1.0% w/v) were tested towards loss of [^{19}F]Flutemetamol by filtration through sterilising-grade filters. To avoid operator radiation exposure, the retention of the tracer in this study was estimated using the non-radioactive [^{19}F]Flutemetamol. Supor 200 sterilising-grade filters provided by Pall Corporation were used in this study. Solutions containing PS80 in the range of 0.06–1.0% (w/v) were prepared according to the procedure given in the previous paragraph. The concentrations of [^{19}F]Flutemetamol dissolved in different PS80 solutions were 20, 75 and 130 ng/ml, while the concentrations of NaCl and the phosphate buffer were kept constant. The sample solutions were carefully drawn into a 10 ml syringe and then expelled through the sterile filter, without priming or conditioning the filter. The

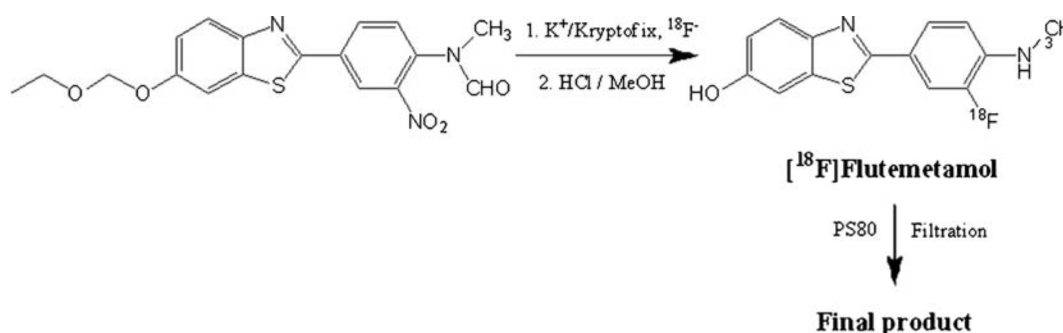


Fig. 1 Synthesis scheme of [^{18}F]Flutemetamol.

content of [^{19}F]Flutemetamol in the filtrate was then quantified by the HPLC method described below.

Quantification of [^{19}F]Flutemetamol by High-Performance Liquid Chromatography (HPLC)

The chemical and radiochemical purity of the samples were assessed by high-performance liquid chromatography. The chromatographic separation of the analyte was performed in a ZORBAX StableBond C18 column (4.6×50 mm, particle size 1.8 μm) with an injection volume of 20 μl. The mobile phase consisted of 0.1% (v/v) formic acid in water (mobile phase A) and 0.1% (v/v) formic acid and 20% (v/v) water in acetonitrile (mobile phase B). A gradient program was employed, starting with 40% mobile phase B and increasing linearly to 70% over 5.5 min, then to 90% over the next 0.8 min. The mobile phase was held isocratic for 0.9 min and then back to 40% mobile phase B during 0.1 min and held isocratic at 40% mobile phase B for the rest of the time. The total run time for each sample was 10 min. The flow rate was 1.5 ml/min and the column was kept at 40°C. For quantification of the substance both an ultraviolet and radioactivity detector were applied.

Stability Study of Polysorbate 80 Solution

For the stability study, sample solutions of 0.67% (w/v) PS80 were applied. 37.2 ml of the prepared PS80 solutions was introduced into 55 ml glass vials and sealed. A long term stability study was performed to study the impact of stored PS80 solution on labelling of [^{18}F]Flutemetamol. In this study, samples were stored at −20°C and 5°C for up to 48 weeks. Samples from this study were withdrawn and used in labelling of the PET tracer according to an in-house schedule. Neither spectroscopic analysis nor the FOX procedure was performed on samples collected from the long term stability study. In order to generate varying amounts of PS80 degradation products, a short term stability study including accelerated conditions was also conducted. The sample vials were stored in temperature-controlled chambers at −20°C, 5°C, 25°C and 40°C for up to 12 weeks. One vial from each storage condition was withdrawn from this short-term stability study every 2 weeks and analysed by UV/VIS spectroscopy, FOX analysis and ^{1}H NMR spectroscopy. Detailed description of the analytical procedures is given below. With the exception of ^{1}H NMR spectroscopy, the samples were analysed immediately after collection. Samples collected for ^{1}H NMR analysis were stored at −20°C until analysis.

Peroxide Determination of Polysorbate 80 Solutions

The stability of PS80 is closely linked with peroxide formation. Peroxide determination was performed with a modified version of the FOX (ferrous oxidation with xylenol orange) reagent (16). Briefly, a stock solution of FOX reagent containing 1.0 mM xylenol orange, 2.5 mM ferrous ammonium sulphate, 1.0 M sorbitol and 250 mM sulphuric acid was initially prepared. A 150 μl aliquot of the sample was diluted with 450 μl Milli-Q water, followed by addition of 600 μl of the FOX reagent stock solution. The reacting solution was incubated at room temperature for 30 min protected from light. Any peroxides present in the solution would mediate oxidation of Fe^{2+}-ions to Fe^{3+}-ions followed by complexation with xylenol orange and thereby colour development. A light protection procedure was included to prevent light induced peroxide formation during incubation. Absorbance values in the range of 400 to 700 nm were recorded with a Unicam UV500 UV-Visible spectrophotometer. The absorbance value at 560 nm was used for calculation of the hydrogen peroxide equivalent (PE) as described in the literature (16). Nine hydrogen peroxide (H_2O_2) standards were prepared to confirm linearity of the calibration curve of hydrogen peroxide equivalents in the range 0 to 200 μM (R=0.993). One point calibrations with at least two freshly prepared solutions of 90 μM H_2O_2 were measured each day of analysis to adjust for day-to-day variations. The total peroxide amount in the samples was estimated by the ratio $PE = A_{sample} \times 90\ \mu M / A_{90\mu M}$, where PE is the hydrogen peroxide equivalent of the sample, A_{sample} and $A_{90\mu M}$ are the absorbance value of the sample and calibration solution of 90 μM hydrogen peroxide after incubation with FOX reagent, respectively. In order to adjust for possible colour formation by the buffer, the absorbance from a pure buffer system mixed with the FOX stock solution was subtracted.

Proton Nuclear Magnetic Resonance (^{1}H NMR) Analysis of Polysorbate 80 Solutions

In order to identify the UV/VIS absorbing moieties in PS80 and to elicit the mechanism behind their effect during radioactive labelling of [^{18}F]Flutemetamol, ^{1}H NMR spectra of PS80 in sample solutions were recorded. A Varian ^{Unity}Inova 500 spectrometer with a 5 mm inverse detection z-gradient probe was used for the spectral acquisitions. These were performed at 25°C with WET water suppression (17). The samples were prepared by thawing and transferring 630 μl of the solution and 70 μl of D_2O as field-frequency lock, to a 5 mm NMR tube. The sequence repetition time was 6 s which is equivalent to 6xT_1 of the most slowly relaxing resonance. Spectra were referenced by fixing the methyl resonance of fatty acid chains to **δ** 0.7779 ppm.

UV/VIS Spectroscopy of Polysorbate 80 Solutions

This technique was included due to a combination of rapid process time and the possibility for obtaining a good

prediction of the labelling outcome. All UV/VIS spectra of PS80 solutions were obtained on a Unicam UV500 UV-Visible spectrophotometer equipped with a quartz cell with 1 cm path length. To prevent oversaturation of the absorbance peaks, 100 µl aliquots of the sample solutions were diluted with 900 µl Milli-Q water. The software Vision Security version 2.03 was used for spectral acquisition. Absorbance values in the range from 190 nm to 450 nm were recorded. Two scans were performed on each sample. The arithmetic mean value of each wavelength of the two cycles was calculated. A baseline offset was subtracted to compensate for unspecific differences in spectral offset and background prior to multivariate analysis.

Synthesis of [^{18}F]Flutemetamol

The radiochemical labelling of [^{18}F]Flutemetamol was performed on a commercially available synthesis module, FASTlab™ (GE Healthcare), with disposable pre-assembled cassettes. The fully automated system is designed for fluorinations with cyclotron-produced [^{18}F]fluoride. Different parameters in the device were controlled by the software package. The schematic illustration for the synthesis of [^{18}F]Flutemetamol is presented in Fig. 1. The concentration of PS80 solution applied in this study was 0.67%.

Measurement of the Radiochemical Purity

Radiochemical purity (RCP) of the final product was measured using HPLC. RCP is the percentage of the total radioactivity that is present as [^{18}F]Flutemetamol (measured by HPLC radioactivity detector). Chemical impurities were quantified against standards of known concentration using a UV detector operating at 330 nm.

Multivariate Methods

Multivariate Curve Resolution

Multivariate Curve Resolution (MCR) was performed on the UV/VIS spectra in the range 190–450 nm to estimate both the number of chromophores present in the test solution during storage and their relative concentrations. Prior to the MCR analysis, the spectra were subject to baseline correction and restrained to non-negative concentration profiles.

Correlation Between MCR and ^{1}H NMR Spectroscopy

PLS regression between ^{1}H NMR spectra obtained and concentrations of the chromophoric groups estimated by MCR were performed in order to identify the chemical structure of the groups. The ^{1}H NMR spectra were selected as predictors and the estimated concentration of each chromophoric components obtained by MCR analysis as response variable. During PLS regression, the large water resonance and the expected peak for the PEG units were both removed to avoid suppression of other possibly informative structural variables. The latter was also excluded from the predictor variable set since this functional group is not expected to give absorption in the investigated UV/VIS range.

Correlation Models Between RCP After Labelling and the Quality of Polysorbate 80

The two sets of stability data were applied separately to elicit the effect of stored PS80 on the radioactive labelling of [^{18}F]Flutemetamol. Firstly, for the long-term stability study, the storage time, storage temperature, initial radioactive concentration (RAC) of the fluorine-18 solution and both first order interaction and quadratic terms were included as predictor variables in a PLS regression. Secondly, data from the short-term stability study was modelled in an attempt to explain the observed effect. The MCR values obtained, RAC, FOX values and their first order interaction terms and quadratic terms were included as predictor variables. For both datasets, radiochemical purity (RCP) was selected as the response variable. The predictor variables were all centred and scaled to unit variance prior to analysis. Jack-knife estimates of the uncertainty of the predictor variables were used as exclusion criteria to remove statistically insignificant variables one by one.

RESULTS AND DISCUSSIONS

[^{19}F]Flutemetamol Retention by Sterilising Grade Filter

To evaluate the adsorption preventing effect of PS80 during sterile filtration of [^{18}F]Flutemetamol, formulations with different concentrations of the surfactant were applied. As presented in Fig. 2, application of insufficient levels of surfactant may result in more than 20% loss of [^{19}F]Flutemetamol during sterile filtration. Similar effects may be observed for adsorption to the tubings of the medical device (data not shown). This demonstrates the importance of including surfactant in order to secure an acceptable yield after sterile filtration. Presence of >0.3% (w/v) PS80 reduces the adsorption to below 5%. To ensure sufficient amount of PS80 throughout the process and the shelf-life of the solution, a surfactant concentration exceeding 0.3% (w/v) should consequently be present in the formulation. For the PS80 solutions employed in the present study this corresponds to a degree of degradation of more than 50%.

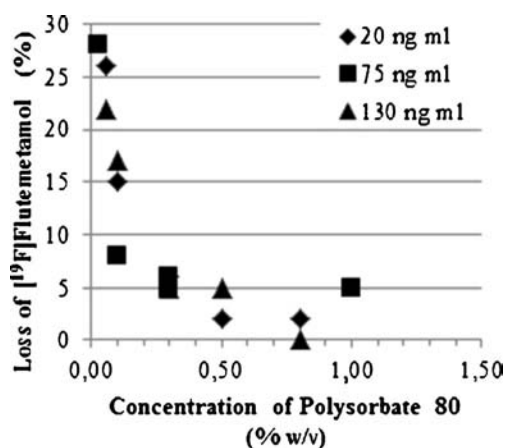


Fig. 2 Concentration of Polysorbate 80 in the solution *versus* percentile loss of [^{19}F]Flutemetamol after filtration. Concentrations of [^{19}F]Flutemetamol investigated were 20, 75 and 130 ng/ml.

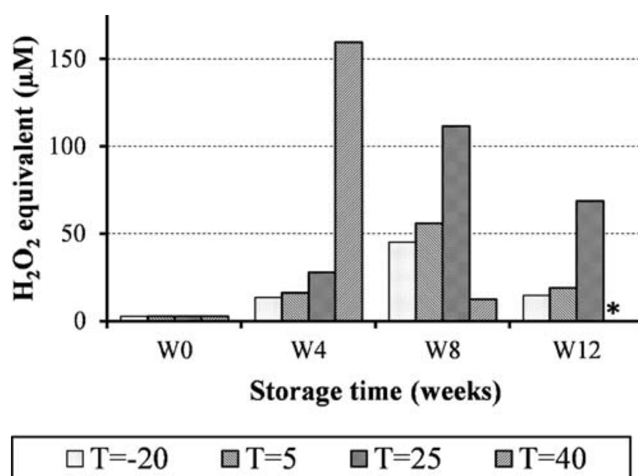


Fig. 3 Peroxides in PS80 solution during storage at −20°C, 5°C, 25°C and 40°C up to 12 weeks. (*measurement not performed for that sample).

The filter material might, in general, adsorb surfactants, active substances as well as other components in the solution. The factors determining the degree of adsorption towards the filter material are amongst others, size, lipophilicity and the charges of both the compounds and the material. In the current experimental conditions, no charge should be present either on the tracer, the PS80 or the polyethersulfone filter material. The predominant force for filter retention should therefore be hydrophobic interactions. However, further studies have to be conducted to elucidate the exact mechanism behind this observation.

Characterisation of Stored PS80 Solutions by FOX Analysis

The peroxide levels in the solutions during storage were monitored since autoxidative degradation of the surfactant has been shown to be pronounced and the presence of peroxides causes adverse impact on product stability in general (18,19). The peroxide formation in the samples stored at different temperatures for up to 12 weeks is shown in Fig. 3. The amount of peroxides increased gradually during storage at temperatures ≤25°C and reached a maximum after storage for 8 weeks. Storage at 40°C on the other hand, resulted in a very rapid increase in peroxide level throughout the first 4 weeks of storage, followed by a sharp decline in the consecutive weeks. The peroxide level then remained low for the rest of the study period. These results were generally in agreement with previous observations (18–20). However, Kishore *et al.* reported insignificant formation of peroxides during storage at 25°C. This is contrary to our observation of a substantial build-up of peroxides (Fig. 3). Differences in pH and PS80 concentration between the investigated solutions might be a possible explanation. Despite the disagreement, a bell-shape curve has commonly been reported. The sharp decline in peroxide level may be explained by the rate of terminating reactions, i.e. the rate of peroxide decomposition exceeds the rate of peroxide formation. This might be due to limitations with respect to reactants, i.e. oxygen and reactive sites of the molecule.

The influence of peroxide formation on the synthesis and stability of [^{18}F]Flutemetamol will be discussed later in this manuscript.

Characterisation of PS80 Solution by ^{1}H NMR Spectroscopy

Typical ^{1}H NMR spectra of the PS80 samples are presented in Fig. 4. The most pronounced difference between fresh and stored samples is the absence of four resonances at δ 5.13–6.15 ppm (labelled 2 in Fig. 4). These resonances correspond to the protons of conjugated double bonds. The results thereby indicate degradation of this group during storage at 40°C. In addition, a decrease in the resonances at δ 5.22 ppm and δ 1.92 ppm (labelled 3 and 10 in Fig. 4) were also observed after storage at an elevated temperature. These signals can be attributed to protons situated in isolated double bonds and methylenes alpha to isolated double bonds respectively. Minor displacement of resonances at δ 2.20 ppm and a decrease in the resonance at δ 4.10 ppm (labelled 8 and 5 in Fig. 4) can also be detected at the same storage condition. These peaks correspond to methylenes adjacent to the carboxylate in the fatty acid chains and methylenes adjacent to the carboxylate in the polyoxyethylene chains, respectively.

Integration of the peaks provides an estimate of the number of isolated double bonds and ester bonds of the fatty acid moiety. The results showed a loss of 19% and 13%, respectively, after storage at 40°C for 8 weeks. With the exception of the conjugated double bonds, no remarkable degradation of the aforementioned functional groups was observed during storage at ≤25°C.

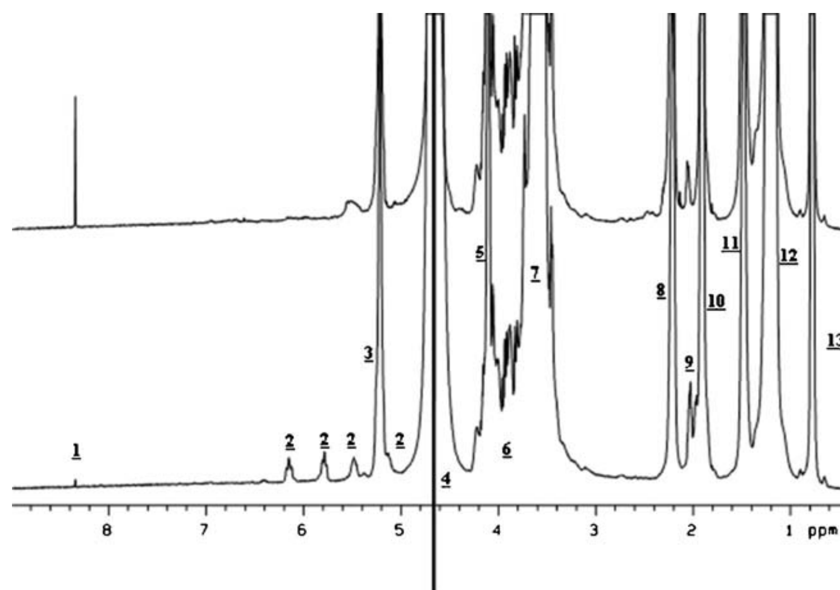


Fig. 4 ^{1}H NMR spectra of PS80 solutions; upper, stored at 40°C for 4 weeks, lower, freshly prepared solution. Key to assignments: *1* Formic acid, *2* Conjugated double-bond protons in fatty acid chains, *3* Isolated double-bond protons in fatty acid chains, *4* Water resonance (with 'wet' solvent suppression), *5* Polyoxyethylene (POE) methylene at carboxylate, *6* Sorbitan unit, *7* Polyoxyethylene (POE) units, *8* Methylenes alpha to carboxylate in fatty acid chains, *9* Methylenes alpha to conjugated double bonds in fatty acid chains, *10* Methylenes alpha to isolated double bond in fatty acid chains, *11* Methylenes beta to carboxylate in fatty acid chains, *12* Methylenes remote from carboxylate and double bonds in fatty acid chains, *13* Methyls of fatty acid chains.

The ^{1}H NMR spectra obtained for samples stored at different conditions were further correlated to the UV/VIS results to reveal the identity of the different UV/VIS absorbing moieties of the substance and is discussed later in this manuscript.

Characterisation of PS80 Solution by UV/VIS Spectroscopy

UV/VIS spectra collected at different time points for samples stored at 25°C and 40°C are shown in Fig. 5 and illustrate the characteristic changes in the spectra which take place during storage at temperatures above 5°C. To estimate the number of chromophoric groups present in the solution as well as to assess the changes in these groups during storage, the UV/VIS spectra were subject to Multivariate Curve Resolution (MCR) analysis. The analysis suggested that three chromophores contributed to the final spectra of the sample solution. The estimated pure spectra of these groups in the range 190–350 nm are shown in Fig. 5c, denoted as peaks A–C respectively. The estimated changes in concentration of these chromophores during storage are presented in Fig. 6. Please note that the estimated relative concentration is on an arbitrary scale since the true concentration of the chromophore will be affected by the unknown specific absorptivity of each functional group. Mainly due to decreased reaction rates, storage at −20°C and 5°C did not result in noticeable changes in the composition of the chromophoric groups. The most remarkable change was the decrease in peak C, with an absorption maximum at 236 nm. A minimum level of that chromophore was reached after storage at 40°C for 4 weeks. A considerable degradation was also observed during storage at 25°C, with the remaining content being reduced to about 50% of the original value. The wavelength of the absorbance maximum indicates that conjugated dienes in the fatty acid chain, present in the solution, might be responsible for the observed UV/Vis absorption (15,21). Correlation between ^{1}H NMR spectra of the samples and the MCR result also supports this interpretation (Please see section below). For the two other chromophores, only minor changes were observed at temperatures ≤25°C. Storage at 40°C, on the other hand, produced a reduction in the sharp peak at 202 nm (peak A), and an increase in the peak at 221 nm (peak B).

Correlation Between ^{1}H NMR and MCR Results

Several structural determining methods may be applied in order to elucidate the molecular changes of PS80 during storage. Examples of such methods are ^{1}H NMR spectroscopy, as presented earlier in this manuscript, and mass spectrometry. Absorption spectroscopy was selected in this study due to the facile quantification of different groups present in the sample. Furthermore, this technique offer considerably shorter process time and is more commonly available in laboratories and clinics compared to the advanced structural determining methods mentioned above. However, due to the fundamental properties of this technique, only the presence of chromophoric groups may be detected. Not to mention that

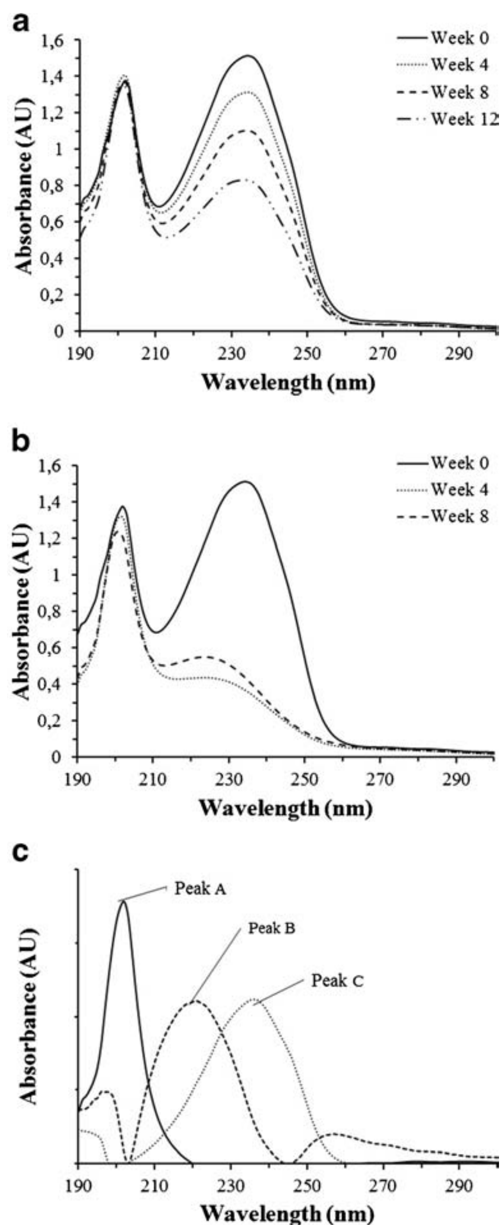


Fig. 5 UV/VIS spectra of Polysorbate 80 solutions stored at **a** 25°C and **b** 40°C for up to 12 weeks. **c** The estimated pure spectra of chromophores present in the solution.

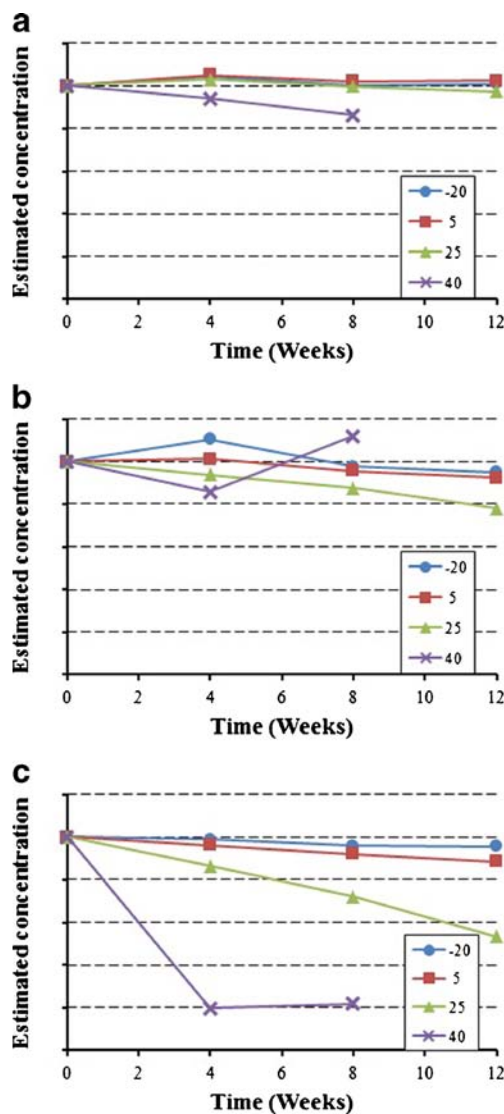


Fig. 6 The estimated concentrations of chromophore peak A (**a**), peak B (**b**) and peak C (**c**) present in the PS80 solutions during storage at −20°C, 5°C, 25°C and 40°C.

the compositional change of the substance either due to variation between manufacturers or between batches will affect the absorption spectrum of a particular PS80 solution. It is therefore vital to verify that the observed differences in the UV-spectra reflect changes relevant to the functional stability of PS80.

To obtain structural information about the chromophoric groups revealed by the MCR analysis and thereby possibly obtaining explanatory information relevant for the stability studies, PLS regression with ^{1}H NMR spectra as explaining variables was performed. In the regression study, peak C in Fig. 5c (absorbance maxima at 236 nm) shows strong correlation to resonances at δ 2.03, 5.79 and 6.15 ppm. These signals correspond to methylenes alpha to conjugated double bonds (2.03 ppm) and two of the conjugated double-bond protons in the fatty acid chains (5.79 and 6.15 ppm). These results thus support the identity of peak C suggested by the UV/VIS spectra as being conjugated double bonds in the fatty acids (Table I).

Table I The Suggested Identity of Chromophores in PS80

Peak	Absorption maximum (nm)	Suggested identity
A	202	Isolated double bond
B	221	Enone
C	236	Conjugated diene

A similar correlation study performed for peak A shows positive correlation to resonances at **δ** 5.22 and 1.92 ppm. As discussed in previous section, these peaks can be attributed to the isolated double bonds in the fatty acid. This observation suggests that isolated double bonds present in PS80 solutions are stable during storage at temperature ≤25°C, which is in concordance with the ^{1}H NMR result.

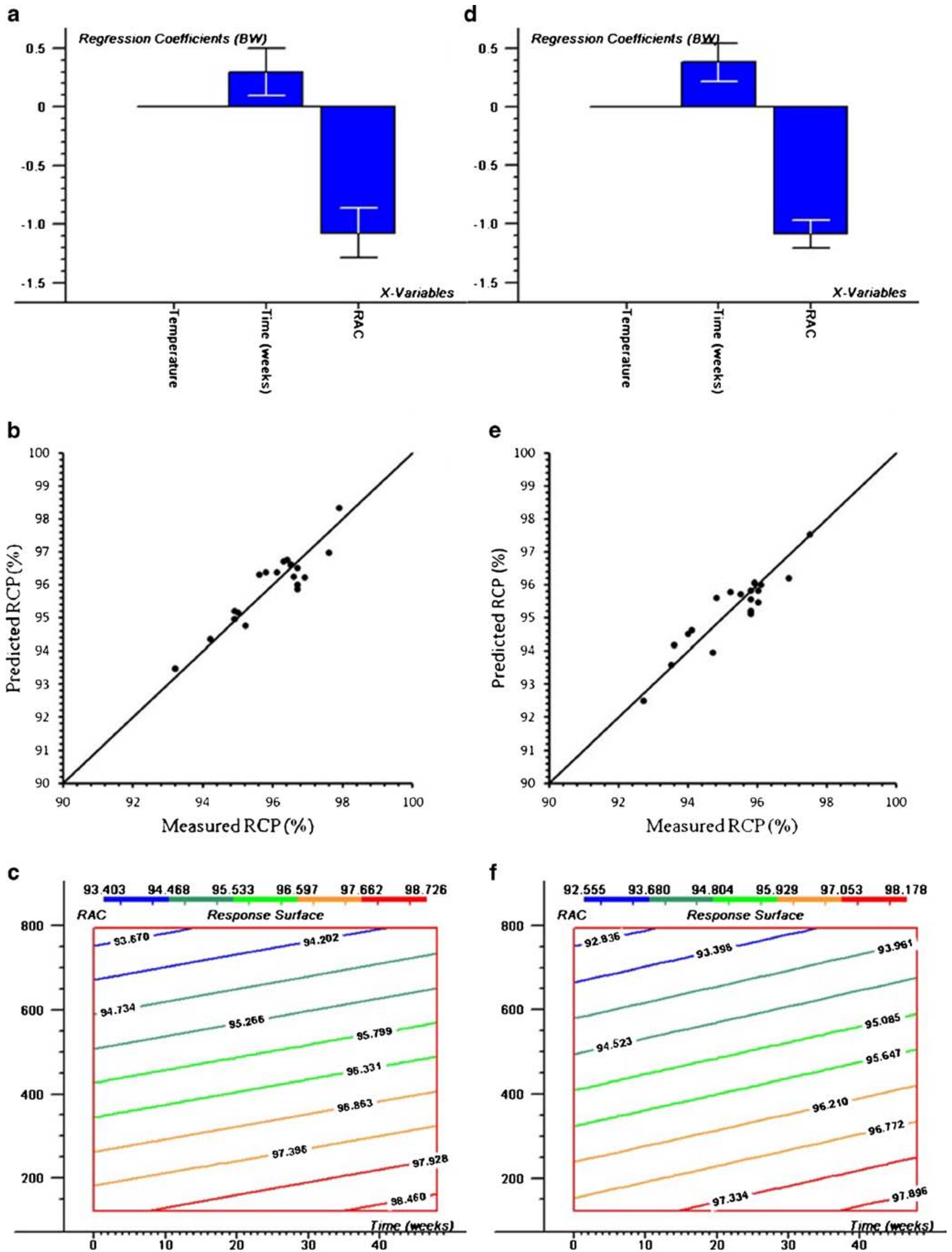
According to Fig. 6b, the quantity of the chromophore responsible for peak B was stable when stored below room temperature. On the other hand, a slight tendency to increase over time was observed for storage at an elevated temperature. Based on this observation and the wavelength of the peak appearing in MCR analysis, this substance is likely to be one of the degradation products of the diene system. Possible degradation products might be unsaturated fatty acid chains with hydroxy-, hydroperoxide-, keto- and epoxy-group, as revealed in earlier studies (15,22). Identification of the chemical structure for the compound by correlation to the ^{1}H NMR spectra turned out to be challenging. This is both due to the low level of substance present in the sample and to the low variation in concentration obtained throughout the time of study. The pure spectrum suggested by MCR analysis shows absorption maxima at 220 nm. This corresponds to a $\pi \rightarrow \pi^*$ transition, modified by vicinal substitutions. Possible degradation products of PS80 described in the literature with this absorption property are unsaturated fatty acid moieties with either hydroxy-, hydroperoxide- or carbonyl-groups in the vicinity of the double bond. PLS correlation with the ^{1}H NMR spectra divided into several sequences shows that the quantity of peak B is positively correlated to double-bond protons with a carbonyl-group in **α**-position to the group (**δ**=6.70 and 5.98 ppm) in the high end of the spectra, and to methylenes in **α**-position to an olefin group (**δ**=2.06 ppm) in the low end of the spectra. According to these findings, the peak B is likely to be an enone (conjugation between a carbonyl group and a double bond), which is a secondary degradation product of peroxides produced by lipid peroxidation. If this is the case, a chemical shift at ~3 ppm is expected to be found, corresponding to methylene in **α**-position to the carbonyl-group. However, this was not observed in the ^{1}H NMR spectra obtained. Possible peaks appearing in this region might be overshadowed by the broad peak of polyoxyethylene (POE) units around **δ** 3.6 ppm. Further investigation on samples containing higher level of this degradation product as well as chromatographic isolation of this compound should be performed to confirm the molecular structure.

Impact of Polysorbate 80 Degradation on Radiochemical Labelling of [^{18}F]Flutemetamol

The main purpose of PS80 in the formulation was to solubilise the tracer [^{18}F]Flutemetamol. The ability of PS80 to perform its function might, however, be altered during storage; affecting the RCP. In order to investigate if PS80 degradation would affect the RCP of the final product, synthesis of [^{18}F]Flutemetamol was performed with both stored and freshly prepared PS80 solutions. Modelling with the long-term stability data shows that the RCP measured immediately after synthesis (T0) and 6 h after synthesis (T6) were well explained by the model (R^2=0.93). In these analyses, RAC and storage time of PS80 were significant variables (Fig. 7a and d). As shown in the figure, the radioactivity applied during the synthesis, represented by RAC, appeared to have a significant, negative impact on the RCP. Radiolysis (please see below) of the tracer both during and after synthesis is a plausible explanation for this observation. Furthermore, the storage temperature turned out to be insignificant in this analysis, indicating no additional protection from storage in a freezer compared to a refrigerator. Interestingly, the storage time of PS80 showed a slight positive effect on RCP. The observed effect of storage time is slightly more evident when measured at T=6 compared to T=0 (Fig. 7a and d). This can be explained by partial degradation of the fatty acid moieties in PS80 during the storage as described below.

Mechanism. In an attempt to reveal the mechanism of the observed benefit from storage of PS80, the chemical composition of the PS80 solutions as described by the estimated concentrations of the chromophores obtained by MCR analysis, and the peroxide level of the solutions estimated by FOX analysis were correlated to the observed RCPs of the short term storage set, as this type of information was not available for the long term set. The obtained yields measured at the two different time points were well explained by the two models as shown in Fig. 8. When RCP was measured immediately after the synthesis (T0), only the level of peaks A (202 nm) and C (236 nm) were significant. For correlation with RCP-T4, also peak B (221 nm) appeared to be significant. All the aforementioned peaks contributed negatively to the observed RCP (Fig. 8a and d). In the previous analysis, the RAC applied during the synthesis had a significant negative impact on the yield (Fig. 7). However, no significance of this variable was observed for the short-term storage data set. A likely explanation for this observation is the small variation in applied RAC for this stability data set (313–431 MBq/ml) compared to the larger range for the long-term stability data set (122–793 MBq/ml). Another interesting finding was the lack of significant effect of the measured peroxide levels, especially since the composition of the fatty acids evidently has a

Fig. 7 Regression model for labelling of [^{18}F]Flutemetamol described by ▶ storage conditions of polysorbate 80 solutions in the long term study. **a** and **d** Significant regression coefficients, **b** and **e** Predicted *vs.* measured plot, **c** and **f** Response surface plot. **a**, **b** and **c** are when the radiochemical purity (RCP) was measured at T=0. **d**, **e** and **f** are the corresponding plots when RCP was measured 6 h after end of synthesis.


a
Regression Coefficients (BW)
0.5
0
-0.5
-1.0
-1.5
X-Variables
Temperature
Time (weeks)
RAC
d
Regression Coefficients (BW)
0.5
0
-0.5
-1.0
-1.5
X-Variables
Temperature
Time (weeks)
RAC
b
Predicted RCP (%)
Measured RCP (%)
e
Predicted RCP (%)
Measured RCP (%)
c
93.403 94.468 95.533 96.597 97.662 98.726
RAC
Response Surface
93.670
94.202
94.734
95.266
95.799
96.331
96.863
97.396
97.928
98.460
Time (weeks)
f
92.555 93.680 94.804 95.929 97.053 98.178
RAC
Response Surface
92.836
93.398
93.961
94.523
95.085
95.647
96.210
96.772
97.334
97.896
Time (weeks)

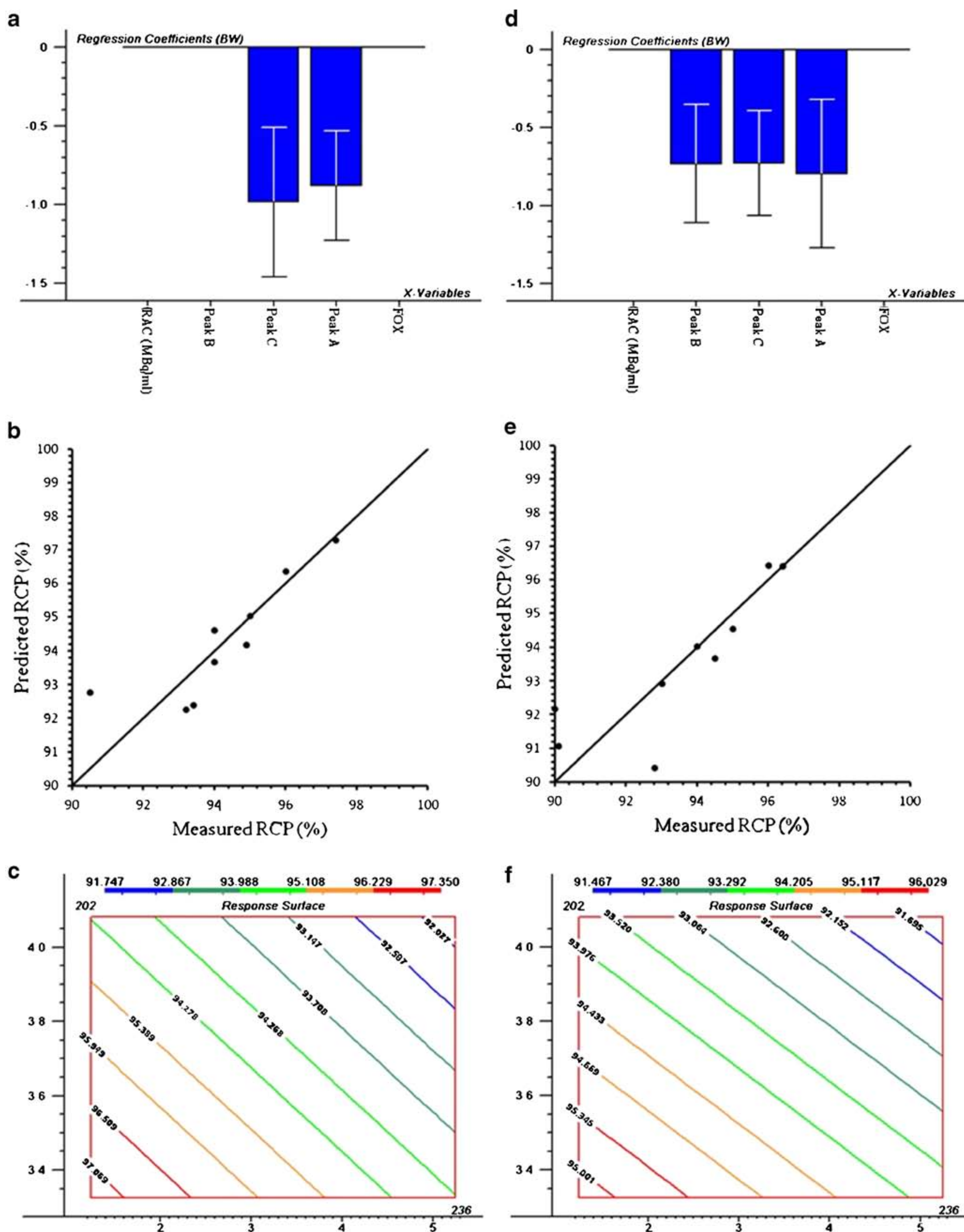


Fig. 8 Regression model for labelling of [^{18}F]Flutemetamol described by chemical properties of polysorbate 80 solutions in the short term study. **a** and **d** Significant regression coefficients, **b** and **e** Predicted *vs.* measured plot, **c** and **f** Response surface plot. **a**, **b** and **c** are when the radiochemical purity (RCP) was measured at $T=0$. **d**, **e** and **f** are the corresponding plots when RCP was measured 4 h after end of synthesis.

significant influence on the PET tracer degradation. These results may be explained by the relative proximity between the different species in the solution. One possible degradation pathway during radiosynthesis is free radical formation by the applied radioactivity in the surrounding species (radiolysis) (23). In our system, the PS80 included will form micelles with hydrophobic cores in which the lipophilic PET tracer will be solubilised. The majority of the peroxides present in the sample are likely to be hydrogen peroxides, which will be located in the aqueous environment surrounding the micelles. Direct contact between these peroxides and the tracer, with subsequent hydrogen peroxide induced degradation is therefore less plausible. The oxygen radicals formed in the fatty acid chains, on the other hand, might have direct access to the PET tracer and cause oxidative instability. As shown above, the peaks at 202 and 236 nm can be attributed to fatty acids with isolated and conjugated double bonds respectively. Yao *et al.* (24) showed by calculation that the unsaturated fatty acid ester substituents in PS would be more susceptible to autoxidation than ethylene oxide groups, and peroxyl radicals can propagate the oxidation of olefins by direct attack at the double bond. Thus, one possible degradation mechanism is that upon radiation exposure, the unsaturated fatty acids in the PS80 are oxidized with accompanying radical formation (either directly or indirectly). These radical bearing groups may subsequently transfer the free radical to the PET tracer and thereby causing oxidative degradation of the drug. This can explain the negative contributions to the labelling yield from both peak A and C. Furthermore, as observed in Fig. 6c, gradual degradation of the conjugated dienes occurred even for samples stored at temperature down to 5°C. Thus, the positive contribution of storage time of PS80 observed in Fig. 7 a and d might partly be explained by reduction in the dienic systems of the samples during storage.

Another interesting feature observed in the model is the contribution of peak B (221 nm) in Fig. 8. In correlation to the RCP, the level of this substance is significant when correlated to RCP measured at $T=4$, but not at $T=0$. As discussed above, the peak B is associated with oxidative degradation of the fatty acids. The observed contribution of this substance in the labelling process might be two-fold: 1) The oxidative degradation of the unsaturated fatty acid give rise to oxygen containing functional groups in the vicinity of olefinic groups. This modification could either increase the reactivity of the group with a subsequent increase of the extent of radiolysis, causing oxidative degradation of the PET tracer as described in the previous paragraph. 2) Scissoring of the fatty acid chains is often a consequence of oxidation of the chain. Shortening of fatty acids in non-ionic surfactants will form smaller micelles. This might result in an up-concentration of radioactivity within the micelles and thus the PET tracer is more likely to undergo radioactive degradation.

Impact of Polysorbate 80 Degradation on Solubilisation of [^{18}F]Flutemetamol

As mentioned in the previous section, one of the main functions of PS80 was to solubilise the PET tracer. In addition to the degradation mentioned above, two other routes of polysorbate degradation are extensively described in the literature: hydrolysis of the ester bond causing release of free fatty acids, and auto-oxidation of the molecule resulting in formation of peroxides and oxidation products. Kishore *et al.* has given an extensive overview of the mechanistic pathway of the PS degradations (19,22). With respect to the properties of PS80 as solubiliser and preventing retention of [^{18}F]Flutemetamol during filtration, several of the suggested degradation pathways of the surfactant may potentially interfere with this activity. Hydrolysis of the ester bond as well as oxidative fragmentation of both the fatty acid moiety and the POE chains might alter this property or even offset the observed benefit of storage of the sample solutions. As described in our previous manuscript (15), very little hydrolysis of the ester bonds was observed for the test solutions stored at 40°C for up to 8 weeks. Estimates based on the ^{1}H NMR spectra showed only ca. 13% degradation after storage under the aforementioned condition. Furthermore, formation of POE chain esters of fatty acids resulting from autoxidation was observed in the same study. As discussed by Kishore *et al.* (22), the presence of degradation products from PS may be tolerated to a certain extent.

Based on the degradation rate of C18:1 containing PS80-species found by Hvattum *et al.*, the cumulative degradation of those species at 40°C can be described by a linear regression line ($y=-2.05$ t $+100$) where t is number of weeks of storage (15). According to that analysis, extrapolation to 12 weeks' storage would result in 24.6% degradation of PS80 molecules containing C18:1. Additionally, all of the C18:2 species, which were estimated to be about 9% of all fatty acids in the samples, will be oxidized within this time of storage. The remaining intact PS80 molecules after storage at 40°C for 12 weeks, including assumption of random hydrolysis of the fatty acid moiety, can be estimated to be 60% of the initial content. This corresponds to a PS80 concentration of 0.30% (w/v). According to Fig. 2, this level of PS80 will still provide an acceptable level of post filtration recovery of [^{19}F]Flutemetamol. Furthermore, the possible degradation products are potentially of amiphiphilic structure and might contribute to solubilisation and preventing tracer adsorption to the filter material and tubings. Storage at ≤25°C, on the other hand, showed remarkably lower degradation of both the conjugated and isolated double bonds. The number of intact PS80 molecule after storage at these conditions will be considerably higher than when stored at 40°C. Therefore, it is reasonable to assume that degradation of PS80 has a minor impact on the retention of [^{18}F]Flutemetamol

during sterile filtration and that the observed effect on the labelling yield is due to the chemical degradation of the tracer itself both during and post synthesis as discussed above.

Comparison Between Polysorbate 80 and Other Polysorbates

In our study, PS80 was selected as surfactant. As specified in the European Pharmacopeia and showed by Hvattum *et al.* (15), the fatty acid moiety of PS80 might be a mixture of different fatty acids. The poly-unsaturated components, which could considered as impurities of PS80, contributed negatively to the studied labelling process. Therefore, application of another surfactant or Polysorbate, without or with limited unsaturation, might be advantageous. One of the candidates evaluated earlier by our team is PS20, which is specified to contain less than 14% unsaturated fatty acids. Initial studies demonstrated that the prevention of adsorption of [^{19}F]Flutemetamol to the filter was comparable to PS80 (data not shown). Furthermore, it has been reported in the literature that solutions of PS80 exhibited both higher oxidisability and level of accumulated peroxide than PS20 solutions (19,24). These indicate that PS80 is more susceptible to autoxidation and thus more liable when included in a formulation compared to PS20. Presence of unsaturated fatty acid moiety in PS80 was suggested as one of the reasons to these observations (25).

Regarding the fatty acid composition, PS40 might also be a relevant candidate as a substitute to PS80. This surfactant is specified to contain over 90% palmitic acid, which is a saturated fatty acid. However, PS40 is not as commonly used as PS20 and PS80 (26). Alternatively, commercial product of PS80 where the conjugated fatty acid impurities have been removed before ester formation is also available. Both the supplier and Wuelfing *et al.* showed that this product shows no spectral features at $\lambda > 210$ nm (27). Therefore, formulation with this PS80 could also limit the observed adverse effect during labelling.

CONCLUSIONS

Inclusion of a certain level of PS80 is necessary to avoid lost of the lipophilic PET tracer [^{18}F]Flutemetamol during the radioactive labelling process. The chemical composition of the surfactant, thereby the presence of unsaturated fatty acid moieties, was found to be one of the most important factors responsible for the reduction in RCP of the labelling process. However, this was shown not to be due to compromised functionality of PS80, but to chemical degradation of the tracer itself.

With respect to the suitability of UV/VIS spectroscopy as a possible tool for quality assurance, we conclude that by combining spectroscopy with MCR, features relevant for the functional stability of PS80 may be revealed.

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