Neutron Activation-Based Gamma Scintigraphic Imaging and Scintigraphy-Based Pharmacokinetic Modelling of Per Oral Controlled Release Drug Delivery

Janne Marvola

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Pharmacy of the University of Helsinki, for public examination in Auditorium 2 at Viikki Infocenter (Viikinkaari 11) on 16 May 2008, at 12 noon.

Helsinki 2008
Abstract


The per oral route of administration is the most convenient and commonly used means of drug administration. However, many drugs are ineffectively absorbed per orally, or the dosing frequency is inconveniently short. Controlled-release (CR) technologies offer means to optimize the resulting plasma concentration-time profiles of such drugs.

Transit of dosage forms in the gastro-intestinal (GI) tract is one of the major factors that determine their overall in vivo performance. Gamma scintigraphy is one of the most appropriate means of studying the fates of drug formulations in the human GI tract. For complex dosage forms imaging methods involving use of stable (non-radioactive) markers during preparation of the products are preferable. Samarium oxide can be incorporated in a formulation during normal manufacture and later it can be activated in a thermal neutron flux of a nuclear reactor to $^{153}\text{Sm}_2\text{O}_3$. A number of scintigraphic studies have been published during the last two decades, but only a few studies about neutron activation in relation to oral administration.

In per oral drug delivery, the rate and extent of drug absorption is determined by the drug, the formulation and the properties of the gastrointestinal tract. Modelling helps to reveal the relative importance of different factors and to predict the biopharmaceutical impact of formulation changes. So far, the published computational models have included only stomach and small intestine, but not colon, even though major part of drug release from the CR formulations takes place in the colon. Pharmacokinetic simulation models can be designed based on parameters in relation to the transit of the formulation and the physiological environment in the gut obtained from imaging studies.

In this study, neutron activation-based scintigraphic methods were developed and evaluated for various CR formulations in the human GI tract, from the oesophagus to the colon. The developed methods were successfully utilized in imaging the transit of dosage forms and in verifying drug release for a maximum of 24 h after administration. A total of 48 healthy volunteers were imaged in five clinical studies. The in vivo transit characteristics of capsule formulations were studied in the oesophagus. Muco adhesion of chitosan granule formulations was studied in the stomach. Sites and rates of drug release and disintegration of different CR capsules and tablets were investigated in the small intestine and the colon. In one imaging study, colon targeted drug delivery using a CR capsule formulation was also verified in vivo. Results of these studies revealed new information in relation to the fates of the studied dosage forms in the GI tract and provided a basis for planning subsequent in vitro and in silico studies for further development of these formulations. The developed imaging methods and equipment used were proven to be valuable tools in CR per oral drug delivery studies.

The fate of one CR caffeine tablet formulation was studied further by means of computational pharmacokinetic simulation modelling by designing an extended compartmental model that takes into account drug release, transit and absorption in the small intestine and the colon. Three colon segment compartments were added to the existing seven-compartment small intestinal transit and absorption model. The new model revealed that the inter-individual differences in the kinetics of the tablet are due to differences in drug metabolism, rather than in the dosage form transit.
Acknowledgements

This work was carried out at the Division of Biopharmaceutics and Pharmacokinetics, Faculty of Pharmacy, University of Helsinki.

I wish to express my warmest gratitude to the former head of the Division of Biopharmaceutics and Pharmacokinetics, Professor Martti Marvola, for his guidance into the world of pharmaceutical sciences as well as for providing excellent working facilities. I am also grateful to my two supervisors, Professor Marjo Yliperttula and Professor Arto Urtti, for their support and guidance through the challenges of this work during the last year and a half.

I am sincerely grateful to all my co-authors, especially Dr. Mia Sivén, Dr. Outi Honkanen, and M.Sc. Tuuli Marvola, for their enthusiastic collaboration during the clinical trials and subsequent data analysis. Professor Aapo Ahonen is acknowledged for his excellent help during the clinical phase.

I wish to express my gratitude to Dr. Kai Lindevall and M.Sc. Hanna Kanerva from Encorium Ltd. For their crucial input in building up the gamma-scintigraphic consortium, but in financial terms and in working hours. I extend my sincere thanks to Dr. Seppo Salmenhaara and staff of the reactor laboratory at VTT. Their expertise was of great value during neutron activation.

Professor Kristiina Järvinen and Dr. Pekka Suhonen are acknowledged for carefully reviewing the manuscript and for giving their valuable suggestions for its improvement.

I would like to express my warmest thanks to all my colleagues at the Division of Biopharmaceutics and Pharmacokinetics, at the Center for Drug Research and at the Industrial Pharmacy Discipline for scientific discussions and for creating a very pleasant working atmosphere as well as for friendship and various fun off-duty activities.

The Finnish Funding Agency for Technology and Innovation (Tekes), the Rector of the University of Helsinki and the Center for Drug Research, University of Helsinki, are acknowledged for financial support.

Finally, I wish to express my thankfulness to my parents, Leena and Martti, for their neverending encouragement and support throughout these years.

Helsinki, April 2008
## Contents

Abstract .......................................................... 3  
Acknowledgements ............................................. 4  
List of original publications ................................. 8  
Abbreviations ..................................................... 9  
1. Introduction .................................................... 10  
2. Theory and review of the literature ................. 12  
  2.1. Gamma scintigraphy .................................... 12  
    2.1.1. Gamma radiation .................................. 12  
    2.1.2. Gamma camera and image acquisition ....... 13  
    2.1.3. Image processing and data analysis ........ 14  
    2.1.4. Scintigraphic markers ......................... 14  
  2.2. Neutron activation ..................................... 16  
    2.2.1. Nuclear reactor .................................. 16  
    2.2.2. Properties of the markers .................... 17  
    2.2.3. Samarium .......................................... 18  
  2.3. Radiation safety ....................................... 19  
    2.3.1. Possible harmful effects of gamma radiation 19  
    2.3.2. ALARA ............................................. 19  
    2.3.3. Safety limits and recruitment of human volunteers 20  
    2.3.4. Radiation authorities and the Ethics committee 20  
  2.4. In vivo scintigraphic imaging ....................... 20  
    2.4.1. Gamma scintigraphic imaging methods ....... 20  
    2.4.2. Imaging of per oral dosage forms ............ 21  
  2.5. Modelling drug absorption and transit .......... 22
2.5.1. Compartmental pharmacokinetic models 22

2.5.2. Compartmental absorption and transit model (CAT) 23

3. Aims of the study 25

4. Materials and methods 26

4.1. Test formulations for per oral imaging studies (I–IV) 26

4.1.1. Samarium oxide (I–IV) 26

4.1.2. Preliminary in vitro test formulations 26

4.1.3. Test formulations for preliminary in vivo trials 26

4.1.4. The Egalet® system (I, V) 27

4.1.5. Microcrystalline chitosan granules (II) 27

4.1.6. HPMC and gelatin capsule formulations (III) 28

4.1.7. Enteric coated gelatin capsule formulations (IV) 28

4.2. Nuclear reactor and neutron activation 28

4.2.1. Neutron activation 29

4.2.2. Sampo program 29

4.3. In vivo imaging studies (I–IV) 29

4.3.1. Oesophageal imaging studies (III) 30

4.3.2. Imaging of formulations in the stomach (II) 31

4.3.3. Imaging of formulations in the small intestine (I) 32

4.3.4. Imaging of colonic drug delivery (I, IV) 32

4.4. Modelling of dosage form transit, drug release and absorption (V) 33

4.4.1. Stella™ program 33

4.4.2. Computational model 33

5. Results and discussion 35

5.1. Preliminary in vitro irradiation tests 35
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2. Preliminary imaging studies</td>
<td>37</td>
</tr>
<tr>
<td>5.3. Oesophageal imaging (III)</td>
<td>40</td>
</tr>
<tr>
<td>5.4. Imaging formulations in the stomach (II)</td>
<td>42</td>
</tr>
<tr>
<td>5.5. Imaging formulations in the small intestine and colon (I)</td>
<td>44</td>
</tr>
<tr>
<td>5.6. Imaging formulations in the colon (IV)</td>
<td>45</td>
</tr>
<tr>
<td>5.7. Scintigraphy-based PBPK simulation model (V)</td>
<td>46</td>
</tr>
<tr>
<td>6. Conclusions</td>
<td>48</td>
</tr>
<tr>
<td>References</td>
<td>49</td>
</tr>
</tbody>
</table>
List of original publications

This dissertation is based on the following studies, which are referred to in the text by their Roman numerals:


Reprinted with the permission of the publishers. Previously unpublished results are also represented.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALARA</td>
<td>As low as reasonably achievable (optimization principle)</td>
</tr>
<tr>
<td>AP</td>
<td>Anterior–posterior (image)</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under curve</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index (weight divided by squared height, kg•m⁻²)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Maximum (drug) concentration</td>
</tr>
<tr>
<td>CR</td>
<td>Controlled release</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EMR</td>
<td>Electro magnetic radiation</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration (United States)</td>
</tr>
<tr>
<td>FiR 1</td>
<td>Finnish Research Reactor 1, Nuclear reactor operated by VTT</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>GUI</td>
<td>Graphical User Interface</td>
</tr>
<tr>
<td>HPC</td>
<td>Hydroxy propyl cellulose</td>
</tr>
<tr>
<td>HPMC</td>
<td>Hydroxy propyl methyl cellulose, hypromellose</td>
</tr>
<tr>
<td>HUCH</td>
<td>Helsinki University Central Hospital</td>
</tr>
<tr>
<td>IAEA</td>
<td>International Atomic Energy Agency</td>
</tr>
<tr>
<td>ICRP</td>
<td>International Commission on Radiological Protection</td>
</tr>
<tr>
<td>IR</td>
<td>Immediate release (dosage form)</td>
</tr>
<tr>
<td>k&lt;sub&gt;a&lt;/sub&gt;</td>
<td>Absorption rate constant</td>
</tr>
<tr>
<td>LEGP</td>
<td>Low energy general purpose (collimator)</td>
</tr>
<tr>
<td>LEHR</td>
<td>Low energy high resolution (collimator)</td>
</tr>
<tr>
<td>MC</td>
<td>Methyl cellulose</td>
</tr>
<tr>
<td>MR</td>
<td>Modified release</td>
</tr>
<tr>
<td>PA</td>
<td>Posterior–anterior</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly (ethylene glycol)</td>
</tr>
<tr>
<td>PBPK</td>
<td>Physiology-based pharmacokinetic (model)</td>
</tr>
<tr>
<td>Ph.Eur.</td>
<td>European Pharmacopoeia</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>STUK</td>
<td>the Finnish Radiation Safety Authority (Säteilyturvakeskus)</td>
</tr>
<tr>
<td>S/N</td>
<td>Signal to noise ratio</td>
</tr>
<tr>
<td>t&lt;sub&gt;lag&lt;/sub&gt;</td>
<td>Lag time</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Time of maximum drug concentration</td>
</tr>
<tr>
<td>USP</td>
<td>United States Pharmacopeia</td>
</tr>
<tr>
<td>VTT</td>
<td>(VTT) Technical Research Centre of Finland</td>
</tr>
</tbody>
</table>
1. Introduction

The oral route of administration is typically considered the preferred and most convenient means of drug administration. The reality during drug discovery and development is that many compounds are ineffectively absorbed after oral administration, or that dosing frequency is inconveniently short. Eventhough lead optimization techniques can be used, it is in many cases not possible to find a physicochemically and pharmacokinetically ideal candidate. Modified (MR) or controlled-release (CR) formulation technologies offer an effective means to enhance the bioavailability and to optimize the resulting plasma concentration-time profiles of such drugs during formulation development (Charman and Charman, 2003). Site or time-specific drug delivery as well as potential delivery of e.g. protein and peptide drugs also calls for more advanced delivery systems.

It has become increasingly evident that in vitro studies are inadequate in relation to the development of modified-release per oral drug formulations. In vivo behaviour in man also needs to be investigated at an early stage of drug development. Pharmacokinetic studies alone do not give enough information about the fates of drug formulations inside a human body. Thus, imaging of drug delivery becomes necessary.

At present, one of the most appropriate means of studying the fates of formulations in the gastrointestinal (GI) tract is gamma scintigraphy (Wilson and Washington, 2000; Newman et al., 2003). For more complex dosage forms methods involving use of stable (non-radioactive) markers during preparation of the products are preferable. Samarium oxide can be satisfactorily employed in this connection (Parr et al. 1985). Following its incorporation in a formulation samarium is activated by a thermal neutron flux to yield $^{153}\text{Sm}_2\text{O}_3$. This method requires access to a suitable research-scale nuclear reactor.

Gamma scintigraphic imaging methods based on neutron activation can be utilized in per oral drug delivery studies to provide information about adhesion of formulations to the oesophagus, mucoadhesion or flotation of formulations in the stomach or site-specific drug release in the small intestine and colon (Dansereau et al., 1999; Washington and Wilson, 1999; Hebden et al., 1999). Majority of the gamma scintigraphic studies during the last two decades have utilized isotopes that are radioactive in the beginning of formulation preparation. Furthermore, many scintigraphic studies have been conducted in relation to nasal drug delivery or drug delivery to the eye rather than per oral administration route. Only a few neutron activation-based studies on per oral drug delivery have been presented (Parr et al., 1986; Parr et al., 1987; Digenis et al., 1990; Digenis et al., 1991; Habib and Sakr, 1999). Small number and limited access to suitable nuclear reactors obviously reduces the possibilities to carry out neutron activation-based imaging studies.

The rate and extent of per oral drug absorption is determined by the drug, the formulation and the physiology of the gastrointestinal tract. Computational models have been developed to understand the roles of different factors in the dosage form transit, drug release, and pharmacokinetics of drugs (absorption, distribution and elimination) in the small intestine. In the past, the small intestine was considered to be the most important site of drug absorption (Macheras et al., 1995). In general, this is true for immediate release dosage forms. However, major part of drug release takes place in the colon when CR delivery systems are
used (Wilson, 2003). This has to be taken into account while designing models to simulate drug delivery.

Pharmacokinetic simulation models can be designed based on the information about the physiological environments around the delivery system and knowledge of its transit parameters in the GI tract (Grass and Sinko, 2002). The most convenient way to obtain the required in vivo transit data for the model is conducting an imaging study of the delivery system simultaneously with a common pharmacokinetic study. These kinds of studies are referred to as ‘pharmaco-scintigraphy’. The combined data enables modelling of the dosage form behaviour and systemic pharmacokinetics of the drug simultaneously. Such physiologically-based models are useful in the analysis of the roles of the physiological factors and formulation parameters on inter-individual variance. Furthermore, they are useful in predicting in vivo behaviour of modified drug delivery systems. Implementation of scintigraphy-based pharmacokinetic modelling in the drug development processes may reduce the rate of product attrition in the expensive clinical drug development phases.

In this thesis, neutron activation-based scintigraphic methods were developed and evaluated to study several CR formulations in the human GI tract, from the oesophagus to the colon. The properties of one delivery system were explored further by means of pharmacokinetic simulations with a computational model that takes into account also drug release, transit and absorption in the colon.
2. Theory and review of the literature

2.1. Gamma scintigraphy

The first applied studies of gamma scintigraphy in the context of oral pharmaceutical dosage forms were carried out in the 1970's (Casey et al., 1976; Alpsten et al., 1976). The technique had already been used for many years in studying the physiology of gastrointestinal (GI) tract (Griffiths et al., 1966). The idea was originally to gain information in relation to the anatomy and the physiology of the human body by using radio nuclides that localize in specific organs. When using high enough activity levels, also radiotherapy for treatment of e.g. tumours became possible. Soon after, it was discovered that the same basic procedure can be utilized in drug studies. Pharmaceutical gamma scintigraphy takes a step forward beyond the traditional anatomical imaging because the movements of drug molecules or delivery systems are monitored continuously. Therefore, it is called functional imaging.

2.1.1. Gamma radiation

The nuclei of atoms consist of positively charged protons and neutral neutrons packed close to each other. The nuclei stay together, when the nucleic forces overcome the electromagnetic repulsion forces. Due to the superposition of different forces, only the nuclei with certain amounts of protons and neutrons are stable. Labile nuclei are radioactive, i.e. they are gradually transformed towards a stable state by emitting radiation. Radiation can be either emission of alpha or beta particles or electromagnetic gamma radiation.

Gamma radiation was first discovered by a French physicist, Henri Becquerel. In 1896, he found out that uranium minerals (actually radium-226) could expose a photographic plate through a heavy opaque paper. Roentgen had recently discovered x-rays, and Becquerel reasoned that uranium emitted some invisible light similar to x-rays. Gamma radiation originates when the nuclei transit from an excited state to a lower excited state or the ground state. Amount of energy emitted—the energy of gamma photons or quanta—depends on the emitting nucleus. Even though this electromagnetic radiation (EMR) comes from the nucleus it has basically the same properties as x-rays (EMR originating in electron transitions). Gamma radiation utilized in clinical or pharmaceutical studies has relatively low energy, which is comparable to the energy of x-rays. Yet, this gamma radiation penetrates well in the tissues. Rough energy, wavelength and frequency spectra of different EMR are presented in Figure 1.
Gamma radiation is ionizing and is, thus, potentially harmful to living organisms. In clinical studies, low energy gamma radiation is used to minimize the risk of damaging the cells and their DNA.

### 2.1.2 Gamma camera and image acquisition

Gamma imaging—or nuclear imaging as it is also referred to—produces images of distribution of radionuclides in objects. Gamma cameras in modern hospitals are based on the original design of Hal Anger in the late 1950’s at the University of California Berkeley (scintillation camera, the Anger camera) (Anger, 1958; IPSM, 1985). Modern gamma cameras have two heads and they can be used to obtained anterior and posterior images of the subject simultaneously. The head of a gamma camera consist of large sodium iodide crystal, 40 cm in diameter, activated with thallium to promote scintillation properties. Coupled to the crystal is an array of photomultiplier tubes which detect the scintillation pulses. Collimators, that act as the optics of the gamma camera, generally used in clinical scintigraphic studies are of the ‘low energy general purpose’ (LEGP) or ‘low energy high resolution’ (LEHR) parallel hole type. These collimators are typically optimized for gamma ray energies of 70–140 keV.

Imaging data from the camera can be collected as static images or as dynamic acquisition using computer and suitable software. Imaging periods typically last from one minute to an hour depending on the study. Scintigraphic images, or scintigrams, are typically planar digital images with (resolution) matrices of $128 \times 128$ or $256 \times 256$ pixels (Perkins, 1999). Gamma camera images are not high-resolution anatomical images and therefore high spatial resolutions are not needed. Typically, for an intestinal imaging study, one pixel corresponds to an area of approximately $2 \text{ mm} \times 2 \text{ mm}$ in the subject.

It is necessary to narrow down the energy spectrum of the detected pulses that are accepted in the data to minimize the negative effect of scattering on the quality of images. Energy windows are generally chosen to include the average gamma energy of the scintigraphic marker ±10–20%. Signal to noise ratios (S/N) of ten or more can easily be reached in pharmaceutical imaging studies, depending on the setup and marker radioactivity.
The detected background noise is mainly due to cosmic gamma radiation and radiation originating from the building materials (concrete) or the ground. In theory, the method is very sensitive, but when physiological time scales need to be considered in clinical imaging, a practical lower limit for radioactivity is set to around 100 kBq for a single object.

2.1.3. Image processing and data analysis

Once the images have been stored on the computer, mathematical operations may be performed to improve the visualization of the data (Sampson, 1994). Simple adjustments of threshold and saturation levels and different filters are usually utilized. The most important feature in analysing scintigrams is the ability to quantify the image data. While each pixel represents the number of gamma counts in the corresponding area of the subject, it is possible to quantify the amount of marker probe (incorporated in the dosage form) in that area at a given time. Regions of interest (ROI) may be drawn around the formulation (or the released probe) and curves of activity versus time plotted from each ROI.

A number of aspects need to be considered when quantifying data. The main points are (Perkins, 1999):

- The time of image acquisition (count rate is of primary interest)
- Background subtraction (to eliminate effect of background radioactivity)
- Decay correction (depending on the half-life of the nuclide)
- Gamma ray attenuation (depth in the subjects, different tissues)

The first three points are quite straightforward to take into account. Gamma rays are attenuated at depth inside the human body. The attenuation coefficient can be measured using a transmission source and subsequently used for an appropriate correction.

2.1.4. Scintigraphic markers

The half-life of isotopes used in clinical trials must be in realistic relation to the duration of the study including the preparation of the test formulations, storage of the formulations, and the actual imaging experiment. Physical half-lives of commonly used radionuclides are presented in Table 1. For example, commonly used technetium has a relatively short half-life (6 h) for long imaging studies while thorium has a longer than optimal half-life (3 d). Short half-lives make the study scheduling and the preparation of test formulations more difficult and may result in poor S/N ratio in later images. Thus, the initial activity levels may have to be raised resulting in larger absorbed radiation doses for the subjects. Long half-lives on the other hand also result in greater absorbed radiation doses.

When studies of controlled drug release are considered, the use of stable isotopes instead of readily radioactive markers is preferable for various reasons including:
− Safer and more straightforward preparation of test formulations as no radioactive material is present
− Possibility to study more complex delivery systems as manufacturing can be done with normal equipment
− Easier to get a uniform (or desired) distribution of the marker inside the test formulation

The uniform distribution of the marker is achievable because stable markers enable manufacturing with normal equipment and admixing the marker in other excipients. A wide variety of pharmaceutical dosage forms can be radioactively marked by using stable nuclides such as barium-138, samarium-152 and erbium-170 followed by neutron activation in a thermal neutron flux of a nuclear reactor (Parr et al., 1986; Parr et al., 1987; Awang et al., 1993). Properties of these marker nuclides are presented in Table 2. Samarium has a nearly optimal half-life (47 h) for controlled drug release studies that may last up to 24 h. It is feasible for easier timing of manufacturing and administration as well as for improved radiation safety to have the half-life in scale with the duration of imaging.

Table 1. Properties of single photon emitting radionuclides commonly utilized in traditional gamma scintigraphy (Perkins, 1999).

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Type of decay</th>
<th>Principle photon energy (keV)</th>
<th>Physical half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{99m}$Tc</td>
<td>Electron capture</td>
<td>140</td>
<td>6 h</td>
</tr>
<tr>
<td>$^{81m}$Kr</td>
<td>Isomer transition</td>
<td>191</td>
<td>13 s</td>
</tr>
<tr>
<td>$^{111}$In</td>
<td>Electron capture</td>
<td>173, 247</td>
<td>2.8 d</td>
</tr>
<tr>
<td>$^{123}$I</td>
<td>Electron capture</td>
<td>160</td>
<td>13 h</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>Beta emission</td>
<td>360</td>
<td>8 d</td>
</tr>
<tr>
<td>$^{201}$Th</td>
<td>Electron capture</td>
<td>78</td>
<td>73.1 h</td>
</tr>
</tbody>
</table>
Table 2. Properties of $^{138}$Ba, $^{170}$Er and $^{152}$Sm utilized in neutron activation based scintigraphy ('natural abundance' means the isotopic occurrence in nature) (Digenis et al., 1989).

<table>
<thead>
<tr>
<th>Stable nuclide</th>
<th>Natural abundance %</th>
<th>Neutron capture cross-section (barn)*</th>
<th>Radio-nuclide</th>
<th>Half-life</th>
<th>Gamma energies</th>
<th>Photon gain (%)</th>
<th>Daughter nuclide</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{138}$Ba</td>
<td>71.7</td>
<td>0.4</td>
<td>$^{139}$Ba</td>
<td>83 min</td>
<td>166</td>
<td>22</td>
<td>$^{139}$La (stable)</td>
</tr>
<tr>
<td>$^{170}$Er</td>
<td>14.9</td>
<td>9.0</td>
<td>$^{171}$Er</td>
<td>7.5 h</td>
<td>112</td>
<td>20</td>
<td>$^{171}$Tm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>124</td>
<td>9</td>
<td>$^{171}$Tm (T1/2 = 1.9y)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>296</td>
<td>28</td>
<td>$^{171}$Yb (stable)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>308</td>
<td>64</td>
<td>$^{171}$Yb (stable)</td>
</tr>
<tr>
<td>$^{152}$Sm</td>
<td>26.7</td>
<td>210</td>
<td>$^{153}$Sm</td>
<td>47 h</td>
<td>97</td>
<td>103</td>
<td>$^{153}$Eu (stable)</td>
</tr>
</tbody>
</table>

* 1 barn = $10^{-28}$ m²

2.2. Neutron activation

For complex dosage forms a method involving use of a stable isotope during preparation of the product is preferable. Thereafter, e.g. samarium oxide in a formulation can be activated in a thermal neutron flux to $^{153}$Sm$_2$O$_3$ via neutron capture. Half-life of $^{153}$Sm is 47 h, which is nearly ideal for studying controlled-release delivery systems in the entire GI tract. The neutron activation method requires access to a suitable research-scale nuclear reactor.

2.2.1. Nuclear reactor

Thermal neutrons are needed in neutron activation of stable nuclides such as barium, samarium and erbium. Neutrons relevant in activation processes are divided in thermal ($\sim 0.025$ eV), epithermal (0.025 to 1 eV) and fast (> 1 eV) neutrons, based on their kinetic energy. Too energetic neutrons are not capable to induce heavier nuclides by neutron capture because they bounce off when hitting the nucleus. Also epithermal neutrons can activate the nuclides, but are more likely to produce unwanted changes in other properties of the samples. There are several types of neutron sources (reactors, accelerators, and radioisotopic neutron emitters) available for neutron activation. Nuclear reactors are preferred due to their high neutron fluxes from uranium fission. A typical neutron energy spectrum of a nuclear reactor is presented in Figure 2. The thermal neutron flux consists of low-energy neutrons (below 0.5 eV) in thermal equilibrium with atoms in the moderator of the reactor. At room temperature, the energy spectrum of thermal neutrons is described by a Maxwell-Boltzmann distribution with a mean energy of 0.025 eV and the most probable velocity of 2200 m/s. In a typical
reactor irradiation position, 90–95% of the sample neutron bombardment is by the thermal neutrons.

The nuclear reactor should have low enough temperature at the irradiation chamber to avoid alterations of the physicochemical properties of the sample. Additionally the thermal neutron flux must be high enough to allow activation of small amounts of marker in a realistic time scale. Also, there should not be too high fast neutron flux or intensive core gamma radiation present to minimize the harmful effects.

2.2.2. Properties of the markers

Barium-138, samarium-152 and erbium-170 used in neutron activation-based gamma scintigraphy are available as isotope-enriched forms, but they are sometimes considered too expensive for research. Thus, use of natural abundance forms has also been studied with promising results (Watts et al, 1991; Ahrabi et al., 1999a; Ahrabi et al., 1999b). In the case of samarium, the naturally occurring amount of isotope $^{152}\text{Sm}$ is 26.7%. Thus, roughly four times as much marker must be introduced in the test formulation compared to the isotope enriched form. In practice, this means that a typical amount of the oxide in an oral formulation may rise from 0.5 mg to 2 mg.

The most important properties of stable marker nuclides are the following (Wilson and Perkins, 1992):

- Cross-section for neutron capture must be large enough
- Gamma quanta emitted by the daughter nuclide must have energies suitable for gamma imaging (100–400 keV)
- Neutron irradiation must not produce significant amounts of alpha or beta emitting daughter nuclides.

Currently available isotope-enriched scintigraphic markers are listed in Table 3. Also non-enriched forms can be used in scintigraphic studies. Natural abundance samarium oxide is discussed more closely later in this work.
Table 3. Physicochemical properties of $^{138}\text{Ba}$, $^{170}\text{Er}$ and $^{152}\text{Sm}$ (based on Digenis et al., 1989).

<table>
<thead>
<tr>
<th>Nuclide</th>
<th>Isotopic enrichment available (%)</th>
<th>Chemical forms available</th>
<th>Solubility in water (g/100 ml)</th>
<th>Density (g/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{138}\text{Ba}$</td>
<td>$&gt;99$</td>
<td>Carbonate</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nitrate</td>
<td>8.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloride</td>
<td>37.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sulphate</td>
<td>0.00022</td>
<td>3.51</td>
</tr>
<tr>
<td>$^{170}\text{Er}$</td>
<td>$&gt;96$</td>
<td>Oxide</td>
<td>0.00049</td>
<td>9.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nitrate</td>
<td>Freely soluble</td>
<td></td>
</tr>
<tr>
<td>$^{152}\text{Sm}$</td>
<td>$&gt;98$</td>
<td>Oxide</td>
<td>0.000054</td>
<td>7.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nitrate</td>
<td>58.95</td>
<td></td>
</tr>
</tbody>
</table>

2.2.3. Samarium

Samarium oxide has been used previously as a radiolabel in pharmaceutical gamma scintigraphic studies. The effects of introducing a lanthanide oxide in a tablet formulation were studied by Parr and Jay (1987). It was found out, that sufficiently small amount of the oxide (less than 3.3% (w/w) in the case of samarium) did not alter the drug release properties significantly. Radiolabelling of polymer micro granules has also been investigated (Watts et al., 1991, 1993a, 1993b). Eudragit® RS micro pellets were found to unaffected by the added samarium oxide (0.8% (w/w)).

As can be seen from Table 3, samarium oxide is poorly soluble in water and it is also chemically stable. Thus, samarium oxide is not absorbed in the GI tract. Rare earth elements (including samarium) have been studied as non-absorbable faecal markers and have been found to be totally non-absorbable in the GI tract with a recovery rate of 101% (Fairweather-Tait et al., 1997). This has positive effects on radiation safety but, on the other hand, samarium oxide behaves quite differently compared to soluble and absorbable drug substances usually delivered with controlled release formulations. Samarium oxide can act as a model drug in dosage forms that disintegrate by erosion but not so well in dosage forms that are e.g. based on a non-dissolvable matrix. Commercially marketed samarium oxide has a mean particle size of about 5 µm, but also a ‘nano powder’ with a particle size of 30–50 nm is available (Inframat Advanced Materials LLC, United States; American Elements, United States). Pharmaceutical experiments with the finely grinded powders and the effects of particle size of the powder on the release and transit of the marker, however, have not been reported.

Samarium has also been studied in other chemical forms including samarium trichloride and samarium tristearate for radiolabelling of parenteral oil-in-water emulsions. These lipophilic derivatives of the lanthanide can be considered as suitable neutron activatable...
excipients in emulsion formulations. Concerning the incorporation efficiency of lanthanide fatty acids and long-term stability of the emulsions, a load of 1 mmol/ml was maximal (Buszello et al., 1999). Radiolabelling of samarium nitrate solutions was successfully demonstrated by Awang and co-workers (1993). Samarium is also commonly used in therapeutical purposes as e.g. samarium 153-ethylene diamine tetramethylene phosphate in orthopaedics (Siegel et al., 2004).

2.3. Radiation safety

2.3.1. Possible harmful effects of gamma radiation

Gamma radiation is ionizing and, thus potentially harmful to living organisms. In clinical studies, low energy gamma radiation is preferable in order to minimize the risk of damaging cells and their DNA.

Gamma rays have adequate energy to pass through the human body without interactions with the tissues, but they can also ionize atoms in tissue or cause secondary ionizations by transferring energy to atomic particles such as electrons. The gamma rays can induce DNA alterations by interfering with genetic material of a cell. DNA double-strand breaks are generally considered to be the most significant mechanism by which radiation causes cancer and hereditary disease (Rothkamm and Löbrich, 1999).

After gamma-irradiation, and the possible breaking of the DNA double-strands, the cell can repair the damaged genetic material up to its capacity. However, it has been suggested that a chronic low-dose exposure could not be fought by the body as effectively as a high-dose exposure because repairing mechanisms work much slower in the case of a low-dose exposure (Rothkamm and Löbrich, 2003). For acute full-body equivalent dose, 1 Sv causes slight blood changes, 2–5 Sv causes nausea, hair loss, haemorrhaging and will cause death in many cases. For low-dose exposure, e.g. among nuclear power plant workers, who receive an average dose of 19 mSv, the risk of dying from cancer increases by two percent.

2.3.2. ALARA

ALARA or ‘As low as reasonably achievable’ is an optimization philosophy that must be obeyed in all radiation work. It states that the amounts of radiation must be kept to minimum with all practical means. It is therefore important that the necessary (or prescribed) amount of a radionuclide administered for diagnosis or research is not exceeded. Additionally, isotope activities must be selected so that imaging of the phenomena under investigation is possible but unnecessary radiation dose to a test subject is avoided. In other words, ALARA is not a dose limit but a process, which has the objective of attaining the study results at radiation doses as low as possible, much below the safety limits.
2.3.3. Safety limits and recruitment of human volunteers

The Finnish Radiation and Nuclear Safety Authority (STUK) published a guide for the use of radiation in nuclear medicine in 2003. The ST 6.3 guide states a few principles for the selection of test subjects (human volunteers). As a general rule, people under the age of 18 should not be included and effective absorbed doses should not exceed 10 mSv in one year. The number of subjects involved should also be kept as low as possible while still obtaining the wanted information from the study.

The International Commission on Radiological Protection uses four risk classes of clinical studies utilizing radiation (ICRP, 1991). The first category to consider (Category IIa in European Commission, 1998) is 0.1–1 mSv and is applied when the project supposedly adds knowledge that leads to health benefits to society. This category involves risks of the order of one in a hundred thousand. When absorbed dose is 1–10 mSv (Category IIb), the study should provide information that can be applied in diagnosis, cure or prevention of disease. This category involves risks to the irradiated individual of the order of one in ten thousand.

2.3.4. Radiation authorities and the Ethics committee

The basic provisions governing the medical use of radiation in Finland are set out in chapter 10 of the Radiation Act (521/1991, amendment 1142/1998). The act contains e.g. provisions governing the grounds for procedures involving exposure to radiation and the instructions to be followed when performing such procedures (STUK, 2005).

Radiation and Nuclear Safety Authority (STUK) is a regulatory authority that regulates the use of radiation and radioactive substances in health care, industry, research and education. A safety license granted by STUK is needed for the use of ionizing radiation.

Additionally, when conducting clinical drug trials, an approval from the Ethics committee is needed. The committee considers the likely health benefits of the intended study and makes its decision based on the radiation protection guidelines discussed earlier. All clinical drug trials in Finland require an approval from the National Agency for Medicines.

2.4. In vivo scintigraphic imaging

2.4.1. Gamma scintigraphic imaging methods

In vivo scintigraphic imaging methods have been widely used in studying the fates of different types of pharmaceutical dosage forms inside the human body. Most common are studies on the respiratory tract (Hardy et al., 1985; Vidgren, 1987; Clark et al., 1996; Pouton et al., 1996; Hardy, 1999). This is due to e.g. the fact that deposition of the inhaled powder has a key role in effective medication. Studies of oral, ocular and dermal drug delivery have widely been presented in the literature as well (Digensis et al., 1998; Wilding et al., 2001; Wilson, 1999). In developing e.g. controlled-release tablets, capsules, suppositories or aerosol
formulations, it is important to establish that any optimized system will perform correctly. Also, especially in the case of CR medication, thorough testing is required before a new product can be submitted to a regulatory authority for approval (Wilding et al., 2001). Normal procedures during CR formulation development include in vitro tests and in vivo pharmacokinetic studies with both fed and fasted human volunteers. Also methods like deconvolution of the drug plasma profiles are utilized in determining the in vivo drug release. In deconvolution, absorption behaviour of a dosage form can be calculated based on release data of the formulation and absorption kinetics of the drug substance. In some cases these methods do not suffice to give clear evidence of desired in vivo performance and more knowledge of the dosage form transit and the rate and the site of drug release is required for conclusions. Gamma scintigraphy provides a non-invasive means of acquiring such information under normal physiological conditions.

2.4.2. Imaging of per oral dosage forms

The gastrointestinal tract is the preferred site of absorption for most drugs. In per oral drug delivery, the formulation is administered through the mouth and oesophagus and usually reaches the stomach or later parts of the GI tract before release of drug starts. In brief, the drug needs to be released from the formulation, dissolved in the intestinal fluids and reach the site of absorption and be absorbed in order to be available to the human body.

Although much about the performance of a delivery system can be learned from in vitro dissolution studies, evaluation in vivo is essential in product development of modern sophisticated delivery systems to confirm that the desired or predicted transit and release behaviour actually takes place in vivo. The traditional method for evaluating the in vivo release of drugs from oral controlled-release systems is through the analysis of pharmacokinetic (PK) data i.e. \( C_{\text{max}} \) (maximum concentration), \( t_{\text{max}} \) (time of \( C_{\text{max}} \)), \( t_{\text{lag}} \) (lag time), \( k_a \) (absorption rate constant) and AUC (area under curve). In vivo drug absorption can be studied further using mass balance methods like Wagner–Nelson and Loo–Riegelman (Wagner, 1975). In vivo drug release profiles can also be calculated from the measured drug plasma profiles by applying deconvolution methods (Süverkrüp, 2000). To get direct evidence demonstrating precisely where the formulation releases the drug within the GI tract, imaging techniques like gamma scintigraphy can be utilized.

Gamma scintigraphy can be satisfactorily applied in delivery studies from the mouth to the rectum including all possible sites of per oral drug absorption: mouth, oesophagus, stomach, duodenum, jejunum, ileum and colon. For instance, in the buccal cavity, release characteristics of controlled-release systems including matrix tablets, lozenges and chewing gums have been studied (Davis et al., 1983; Wilson et al., 1987; Christrup et al, 1990). Oesophageal transit of different dosage forms has also been studied already in the 1980’s (Fell et al, 1983; Wilson et al, 1988; Robertson and Hardy, 1988). Transit of dosage forms through the GI tract has been of interest to many researchers. Gastric emptying, small intestinal and colonic transit of dosage forms in healthy volunteers (Davis et al., 1984; Coupe et al, 1991a) and vegetarians (Price et al., 1991) together with variations in physiological factors such as age, posture, time of dosing, exercise and bed rest (Coupe et al., 1991b) are well covered in
the literature. Imaging studies have revealed e.g. small intestinal transit to be faster than commonly referred to in the literature before, typically only two to three hours (Wilson, 2003). Verification of the sites of drug release has as well been widely successful. Gamma imaging can provide information in relation to the transit of the formulation, the site of disintegration and/or drug release and, especially when combined with pharmacokinetic sampling, absorption characteristics.

In the case of complex dosage forms, methods involving the use of stable isotopes followed by neutron activation are preferable. During the irradiation of the marker, also the entire dosage form undergoes stress caused by the neutron flux and the core gamma radiation at the irradiation location near the reactor core. It is well known that the irradiation procedures can e.g. accelerate drug release, especially from formulations that contain polymeric excipients (Watts et al., 1993; Waaler et al., 1997; Ahrabi et al., 1999). Thus, it is necessary—prior to the imaging study—to test the formulations in vitro to make sure that the drug release properties (or other physicochemical properties) have not significantly changed.

2.5. Modelling drug absorption and transit

2.5.1. Compartmental pharmacokinetic models

Prediction of per oral drug absorption is an important task in drug discovery and development. The rate and extent of drug absorption is determined by the drug, the formulation and the physiology of the gastrointestinal tract. Obviously, it is practically not possible to cover all the aspects by conducting clinical trials. Therefore, several computational models for transit, dispersion and absorption of drugs in human small intestine have been presented.

Traditionally, in pharmacokinetics, kinetic models with one or two compartments have been used in modelling absorption, distribution and elimination of drugs in the system. When characteristics of e.g. the per oral route of administration need to be taken into account, modelling of the human anatomy and physiology becomes necessary. These physiologically-based pharmacokinetic (PBPK) simulation models have recently been adopted throughout the drug discovery and development processes—also in the development and design of clinical trials. A model can be build by fitting the available data to a structural model. Later, the model can be used in simulations with different parameter values to predict behaviour under different conditions. A simulation model is, for the purposes of pharmacokinetics, a computational representation of the kinetic behaviour of molecules in humans or animals (Grass and Sinko, 2002). Models can be developed retrospectively based on e.g. pharmacoscintigraphic data. A thorough review of physiology-based modelling has been published by Grass and Sinko (2002) although imaging based modelling is not covered.

Simulation models can be single parameter models where a physiologically based parameter such as permeability is chosen and a dataset generated from e.g. a set of Caco-2 experiments. The data is then correlated to clinical data for the same drug (Grass, 1997).
More comprehensive multi-parameter models for the prediction of in vivo drug absorption include iDEATM (In vitro Determination for the Estimation of ADME) (Bohets et al., 2001) and the CAT (Compartmental Absorption and Transit) model (Yu et al., 1996; Yu and Amidon, 1999). Both models use a variety of parameters such as solubility, pKa and permeability and they also include physiological structures of the human GI tract including e.g. varying pH, fluid volumes and transit time in different parts of the tract. The models are applied in the prediction of per os drug absorption in the small intestine. They can also be used in analysis of the roles of the physiological factors and formulation parameters on drug plasma profiles and in predicting the profiles in different individuals.

2.5.2. Compartmental absorption and transit model (CAT)

The compartmental transit and absorption (CAT) model has been described to estimate the fraction of dose absorbed and the rate of drug absorption for passively transported drugs in immediate release (IR) products (Yu and Amidon, 1999). Previously, single-compartment models have been introduced for prediction of per oral drug absorption (Dressman et al., 1984; Sinko et al., 1991). Although gastric emptying and transit in the small intestinal transit flow are known to influence the rate and extent of per oral drug absorption, none of the previous models have fully considered these factors. The CAT model takes into account the simultaneous small intestinal transit flow, spreading of drug substance in the intestine and drug absorption based on a transit model by the same researchers (Yu et al., 1996).

The assumptions for the original CAT model include (Yu and Amidon, 1999):

1. Absorption from the stomach and colon is insignificant compared with that from the small intestine;
2. Transport across the epithelium of the small intestine is by passive diffusion;
3. Dissolution is instantaneous;
4. A drug transferring through the small intestine can be viewed as a process flowing through a series of compartments, with connecting linear transfer kinetics, and all compartments may have different volumes and flow rates, but have the same residence times (Yu and Amidon, 1998).

These initial assumptions limit the usefulness of the CAT model for controlled release (CR) medications, because the major part of CR drug release and absorption takes place in the colon.

A schematic diagram of the CAT model is presented in Figure 2. This model accounts for the transit in the stomach, duodenum, jejunum, and ileum, and the absorption in the duodenum, jejunum, and ileum, but not in the colon.
Figure 2. A schematic diagram of the CAT model with linear transit and passive absorption kinetics (Yu and Amidon, 1999). The small intestine is divided in seven segments, or compartments. In the model the drug is absorbed only from these seven compartments. Rate constants of gastric emptying (K_s), small intestinal transit (K_t) and intrinsic absorption (K_a) are depicted. Amount of drug in each compartment is marked with M_x.
3. Aims of the study

The general purpose of this study was to evaluate the usefulness of neutron activation-based gamma scintigraphy and its combination with pharmacokinetic modelling to evaluate per oral dosage forms in humans. The specific aims were:

1. To develop scintigraphic in vivo imaging methods based on the use of neutron-activated samarium oxide.

2. To evaluate the usefulness of those methods in the imaging of the dosage form transit in oesophagus, stomach, small intestine, and colon.

3. To obtain new information about the behaviour of different per oral CR drug delivery systems in the human GI tract.

4. To generate a computational model based on gamma imaging to estimate and predict the in vivo behaviour of per oral CR drug delivery systems.

5. To analyze the roles of the physiological factors and formulation parameters on the inter-individual variance on caffeine pharmacokinetics using the computational model.

To achieve these aims, imaging studies of oesophageal transit (III, previously unpublished results), drug disposition in the stomach (II, III), the small intestine (I) and the colon (I, IV) were conducted as shown in Figure 3. Additionally a computational model (V) was designed based on the scintigraphic results (I).

Figure 3. Schematic presentation of the human oesophagus and GI tract with the locations of imaging studies presented in publications I-IV.
4. Materials and methods

In this chapter, the materials and methods are briefly described. A more detailed description is found in the original publications I-V.

4.1. Test formulations for per oral imaging studies (I–IV)

4.1.1. Samarium oxide (I–IV)

Natural abundance samarium oxide was incorporated to the test formulations for preliminary in vitro testing and for imaging studies followed by activation in a thermal neutron flux of the FiR 1 nuclear reactor. Natural abundance of samarium-152 is 26.7%. Samarium oxide ($\text{Sm}_2\text{O}_3$) was purchased for the studies (Aldrich, United States).

4.1.2. Preliminary in vitro test formulations

For preliminary irradiation testing, several common pharmaceutical excipients were studied as powders in size-0 hard gelatin capsule shells. The excipients included four hypromellose qualities (Methocel® K100, K4M, K15M and K100M) (FMC BioPolymer, United States), lactose (Pharmatose DCL 21, DMV Pharma, the Netherlands), corn starch (Ph.Eur.), polyethylene glycol (PEG 6000) (Fluka, Switzerland), carbomer (Carbopol® 934P) (Noveon, United States), hydroxy propyl cellulose (HPC 2.0) (Hercules, Germany), methyl cellulose (Methocel® A 15 C) (Dow Chemical Company, United States) and alginic acid (Kelco, United Kingdom).

4.1.3. Test formulations for preliminary in vivo trials

Two formulations were designed for preliminary imaging studies: 1) ethyl cellulose-coated hard gelatin capsules, and 2) enteric-coated hard gelatin capsules. Formulations contained 6 mg of natural-abundance $\text{Sm}_2\text{O}_3$ (Aldrich, United States), of which 26.7% is $^{152}\text{Sm}_2\text{O}_3$, and lactose (Pharmatose DCL 21, DMV Pharma, the Netherlands) as required. The capsules were coated by dipping them several times in a 10% (w/w) ethanol solution of ethyl cellulose (Ph.Eur.) or Eudragit® L (Röhm GmbH, Germany). The increase in mass during coating was approximately 10%. Formulation 1 was intended to be a model for dosage forms required to pass through the entire human GI tract without disintegration. Formulation 2 was designed to mimic drug products that disintegrate rapidly in the small intestine at pH of 6.
4.1.4. The Egalet® system (I, V)

The Egalet® system is an injection-moulded drug delivery system (Egalet, Denmark). The constant release Egalet® system consists of an impermeable shell that encloses a plug of active drug. The shell is a non-degradable cylindrical tube open at both ends and is made of cetostearyl alcohol and ethyl cellulose. The matrix of the plug comprises a mixture of polyethylene glycol monostearates and polyethylene oxide (Bar-Shalom et al., 2003). The composition of the formulation can be seen in Figure 4. The drug is released based on the matrix erosion rather than diffusion from the matrix (Bar-Shalom et al., 2003). The formulation contained 8 mg of natural-abundance Sm₂O₃ (Aldrich, United States), and 50 mg of caffeine (Ph.Eur.). The study formulations (including Sm₂O₃) were manufactured by Egalet a/s.

![Figure 4. The constant-release Egalet® system consists of an impermeable shell enclosing an eroding plug of active drug (caffeine and samarium oxide) (Bar-Shalom et al., 2003). Initially the tablet is a filled tube (a), after administration it starts to erode at both ends (b) releasing its entire contents following zero-order kinetics (c).](image)

4.1.5. Microcrystalline chitosan granules (II)

Chitosan is a cationic polymer that forms gel in acidic environments e.g. in the stomach. The composition of the chitosan granules (F1, F2) used in the imaging study of retention in stomach was: 95% (F1) or 40% (F2) microcrystalline chitosan (MCCh) (Novasso, Finland) of mean molecular weight (Mw) 150 kDa, and 1.4–1.6% natural-abundance Sm₂O₃ (Aldrich, United States). Other excipients included lactose (Pharmatose DCL 21, DMV International, the Netherlands) and polyvinylpyrrolidone (PVP K25, Fluka Chemie, Switzerland). Also a reference lactose granule formulation (F3) without chitosan was prepared. The granules were manufactured by wet granulation. This process is described in more detail in the original publication (II). The granules were dispensed into hard gelatin capsules for the irradiation and the in vivo imaging.
4.1.6. HPMC and gelatin capsule formulations (III)

Hydroxypropyl methylcellulose (HPMC) capsules made of plant derived material and they have been studied as an alternative to hard gelatin capsules. Advantages of HPMC capsules include: lower moisture content, chemical inertness and mechanical integrity in dry conditions.

Hard size-0 HPMC capsule shells (Shionogi Qualicaps, Spain) filled with hypromellose (Ph.Eur., Methocel®, Dow Chemicals, United Kingdom) of two different viscosity grades, K100 and K4M, were studied in the oesophagus and the stomach. Natural-abundance Sm$_2$O$_3$ (Aldrich, USA) was used for radiolabelling. A size-0 gelatin capsule formulation was used as a standard. Main goal of the study was to obtain information about possible differences in release properties of the two capsule shells, but also information about the oesophageal transit of the capsules was of interest.

4.1.7. Enteric coated gelatin capsule formulations (IV)

The formulations in colonic drug delivery studies were based on hard size-0 HPMC capsule shells (Shionogi Qualicaps, Spain). They contained 96 mg of paracetamol (Ph.Eur.) and 4 mg of natural-abundance samarium oxide (Aldrich, USA) and either microcrystalline cellulose (MCC, Avicel PH 102, FMC BioPolymer, USA) or hypromellose (Methocel K4M, FMC BioPolymer, United States), as required. To obtain site-specific start of drug release in the vicinity of ileo-caecal junction the capsules were coated with the enteric methacrylate co-polymer Eudragit® S (Röhm, Germany). Eudragit® S is known to dissolve at pH 7 in the distal small intestine or the caecum (Hardy et al., 1987; Agyilirah and Banker, 1991). The gel-forming hypromellose inside the capsules enables prolonged drug release after the enteric coating is dissolved.

4.2. Nuclear reactor and neutron activation

In this work, a research nuclear reactor operated at Otaniemi was used for the irradiations (VTT Technical Research Centre of Finland, Espoo). The reactor has a thermal neutron flux of $1.1 \cdot 10^{12}$ n·cm$^{-2}$·s$^{-1}$ and the temperature at the irradiation ring around the core remains below 40°C at all times. The reactor is a water-cooled open tank (swimming pool) type TRIGA® Mk II reactor (General Atomics, United States) and it has a power capacity of 250 kW (STUK, 2007). The reactor has lately been equipped and slightly redesigned for clinical boron neutron capture therapy (BNCT), but also neutron activation analysis and irradiation and activation of samples is still conducted at the reactor laboratory for client companies (Auterinen et al., 2001). FiR 1 is considered to be a nearly optimal reactor for pharmaceutical activation purposes.
4.2.1. Neutron activation

Pharmaceutical samples were packed inside polyethylene capsules and lowered approximately five meters to the irradiation ring using a (fishing) jig. Typical irradiation times range from one minute to 15 min depending on the targeted activity and the amount of mother nuclide in the sample. Lead containers were used in transportation of irradiated formulations from the reactor to the hospital to minimize unnecessary exposure to gamma radiation.

The target samarium-153 activity of the formulations studied was 1.3 MBq. This corresponds to 1 mSv effective absorbed dose after per oral administration. Irradiation times were 2–6 min depending on the amount of samarium oxide per one formulation.

4.2.2. Sampo program

Energy spectra of gamma radiation were measured from the irradiated samples and analyzed at VTT Technical Research Centre using Sampo computer program (v. 4.00, Helsinki University of Technology and VTT) and a 8192-channel A/D (analog to digital) converter. A high purity germanium (HPGe) semiconductor detector model 7229P (Canberra, Belgium) was used. The Sampo program automatically detects energy peaks based on their energy and shape. The spectra were examined for radioactive impurities and for the activities of the marker nuclide isotopes.

4.3. In vivo imaging studies (I–IV)

Approvals of the Ethics Committee of the Hospital District of Helsinki and Uusimaa (HUS) were obtained for each in vivo imaging study presented in this thesis.

Healthy male volunteers participated in the gamma scintigraphic studies. Prior to the studies, each volunteer was examined physically, and subjected to routine haematological testing (Hb, HCR, B-Eryt, B-Leuc, ESR, S-Alat, S-Asat, S-AFOS, S-GT) and urine analysis (U-pH, U-Prot, U-Gluc). Each volunteer was informed about the possible risks and adverse effects of the study. Written informed consent to participation in the studies had been obtained. The investigations were carried out in accordance with the International Conference on Harmonization (ICH), Good Clinical Practice Guidelines and the Declaration of Helsinki (World Medical Organization, 1996) as revised in 2000. The National Agency for Medicine (Finland) and the Ethics Committee of Helsinki University Central Hospital (HUCH) approved the study protocols.

The imaging studies were carried out in the Clinical Physiology Division of Diacor Hospital (I) and the Nuclear Medicine Division of Helsinki University Central Hospital (HUCH) (II–IV). Both have radiation safety licenses issued by STUK. The study protocols were drawn up in accordance with the guidelines established by STUK, and the ALARA principle was observed. Total effective absorbed doses for the individual subjects did not exceed 1 mSv per year (I–IV). Gamma scintigraphic studies were carried out 48 h after neutron activation. This time period allowed the decay of unwanted radioisotopes, primarily
Materials and methods

Gamma spectra were measured from the irradiated dosage forms 24 h post-irradiation to ensure radioisotopic purity. Radioactivity of $^{153}$Sm was also measured prior to dosing in every single case.

The lower tip of the sternum and the iliac crests of each study subject were marked with a felt-tip pen, and markers containing $^{57}$Co were attached to the locations with adhesive tape. Imaging was performed with subjects in the supine position. Between imaging periods the subjects were allowed to move freely. Scintigrams were recorded at 103 keV (window width ±10%) using a Multispect 2 dual-head gamma camera (Siemens AG, Germany) (I) or a ADAC Forte dual-head gamma camera (ADAC Laboratories, United States) (II–IV). Collimators were of the LEHR type. The first meal was allowed four hours after ingestion of the formulation.

Data analysis based on the recorded scintigrams was done for each imaging study. Sequential scintigrams were used for each individual subject and the regions of interest (ROI) were drawn to represent e.g. oesophagus or stomach (II, III) or the remaining non-disintegrated formulation (I). The ROIs used were of fixed size for paired anterior and posterior (AP and PA) images. Count rates relating to the ROIs were calculated using Hermes 3.7 software (Nuclear Diagnostics, Sweden). Geometric means of counts in paired AP and PA images were used in the analysis. All count rates were corrected for background radioactivity and decay of the marker. Sequential scintigrams of each study subject were visually inspected to detect the anatomical location of the formulation at each time point.

4.3.1. Oesophageal imaging studies (III)

Procedure

One group of six healthy male volunteers participated in the oesophageal scintigraphic studies (III). The weights of the volunteers were 65–89 kg and their body mass indices (BMI) 19–26 kg·m$^{-2}$ (mean 22 kg·m$^{-2}$). The subjects were non-smokers. The studies were carried out at HUCH.

Each study subject received both formulations, one at a time on two separate study visits. A wash-out period of one week was held between the visits to remove the traces of remaining radioactivity from the GI tract. The formulations were administered in a sitting position with 180 ml of water. The subjects fasted for 12 h and they were not allowed to eat until 4 h after the dosage form administration when a standard meal was served. Following administration, anterior and posterior (AP and PA) images each of one minute duration were recorded continuously for 20 min, after which six one-minute scintigrams were recorded every 30 min for the next 7.5 h.

An additional in vivo study focusing only on possible stagnation of the capsules was also conducted. In this study 11 healthy, non-smoking, 18–40 year old male volunteers were imaged. Each study subject received both study formulations, a hard gelatin capsule and a HPMC capsule, one at each study visit (cross-over study) (Marvola et al., 2005). The formulations were administered in a supine position with 180 ml of water. The subjects drank
the water using a straw. This was a different procedure compared to the other three imaging studies. Following administration, AP and PA images each of one second duration were recorded continuously for five minutes.

Data analysis

Sequential scintigrams were used for each subject and the regions of interest (ROI) were drawn to represent the oesophagus and the stomach. The ROIs used were of fixed size for paired AP and PA images. Counts relating to the ROIs were calculated using Hermes 3.7 software (Nuclear Diagnostics, Sweden) and corrected for background and decay. Scintigrams were visually inspected to detect the oesophageal transit time.

4.3.2. Imaging of formulations in the stomach (II)

Procedure

Three groups of five healthy male volunteers participated in the scintigraphic studies of mucoadhesion. The weights of the volunteers were 62–97 kg and their BMI 19–27 kg·m$^{-2}$ (mean 23 kg·m$^{-2}$). The subjects were non-smokers. The studies were carried out at HUCH.

One study formulation (F1, F2 or F3 in Table 2, II) was administered to each volunteer in a sitting position, with 180 ml of water. Each volunteer received only one formulation. The subjects have fasted for 12 h and were not allowed to drink or eat during the study. AP and PA images each of one minute duration were recorded continuously for the first 30 min, after which six one-minute scintigrams were recorded every 15 min for the next three to four hours.

Data analysis

Sequential scintigrams were used for each subject and the ROIs were drawn to represent the stomach in each image. The ROIs used were of fixed size for paired AP and PA images. Counts relating to the ROIs were calculated using Hermes software and corrected for background and decay. Gastric emptying of the formulations was expressed in terms of remaining relative counts in each ROI as functions of time. Relative counts between 0.9 and 0.1 were used to determine the gastric emptying rate constant (k) by means of linear regression. Times at which half of the activity was cleared from the stomach were used in evaluation of gastric residence times.
4.3.3. Imaging of formulations in the small intestine (I)

Procedure

One group of six healthy male volunteers was enrolled in the small intestinal and colonic drug delivery study. Their weights varied from 70 to 87 kg and their BMI from 21 to 25 kg·m$^{-2}$. Additional four volunteers were enrolled in the preliminary imaging study conducted prior to imaging the actual formulation. All ten subjects were non-smokers. The studies were carried out at Diacor Hospital.

Each volunteer had fasted for at least 12 hours, and had been asked to abstain from foods and fluids containing xanthine or caffeine for 48 hours prior to drug administration. Xanthine and caffeine ingestion was also forbidden throughout the imaging period. At approximately 8 a.m. subjects in the sitting position were given one formulation with 180 ml of water. Formulation 1 (see 4.1.3.) was given to two subjects, Formulation 2 to two subjects and Formulation 3 (the Egalet® system) to six subjects. Following drug administration, ten AP and PA images of one minute duration were recorded at intervals of 0.5 (0–5 h), one (5–12 h) and three hours (12–24 h), for 24 h. Imaging was undertaken with subjects in the supine position. The first meal was allowed four hours after ingestion of the formulation. After that, the subjects were allowed to eat freely.

Data analysis

Stored scintigrams were used to determine the radioactivities of the products as a function of time. ROI relating only to counts originating from non-disintegrated drug formulations were drawn manually on AP and PA gamma images for each time point. Geometric means of counts relating to the two ROIs were calculated and corrected for background and decay. Scintigrams were visually inspected to detect gastric emptying, transit times through the small intestine and different parts of the colon.

4.3.4. Imaging of colonic drug delivery (I, IV)

Procedure (IV)

Six healthy male volunteers participated in the study (IV). Their weights were 63–101 kg and BMI 21–26 kg·m$^{-2}$. The lower tip of the sternum and the iliac crests of each study subject were marked with a felt-tip pen, and markers containing $^{57}$Co were attached to these locations with adhesive tape. The markers were removed after the first set of images in every time point so that they would not disturb detecting the irradiation sent by the formulations in the colon.

Scintigraphic imaging was carried out as a single-dose crossover study. Each study subject received both capsules (see 4.1.7.), one during each visit. Irradiated capsule containing either
MCC or HPMC as an excipient was administered to each volunteer in sitting position at approximately 8 a.m. after an overnight fasting for at least 12 h. The volunteers were allowed to eat and drink 4 h after the administration of the formulation. Following the administration, AP and PA images each of one-minute duration were recorded at intervals of one hour for 14 h in supine position. Between imaging times the subjects were allowed to move freely. Washout period between the two study visits was two weeks.

Also the previous small intestinal study (I) gave information in relation to the transit and drug release of delivery systems in the colon. These results were used as a basis in visual interpretation of the scintigrams.

Data analysis (IV)

Sequential scintigrams were visually examined to follow the transit through stomach and small intestine and to verify the location of the disintegration of the capsule shell. Also spreading of the contents and expected gel formation of HPMC was visually observed in the colon. Also the previous small intestinal study (I) and the unpublished studies with enteric and insoluble capsules gave information in relation to the transit and drug release of delivery systems in the colon. These results were used as a basis for visual interpretation of the scintigrams of the later study.

4.4. Modelling of dosage form transit, drug release and absorption (V)

The target formulation in the simulation modelling study was Egalet® constant release system (I). The Egalet® system was chosen for the study because it shows erosion-controlled zero-order release. Thus, it can be assumed that release rates of both caffeine and the insoluble scintigraphic marker (samarium oxide) are similar (I).

4.4.1. Stella™ program

The computational pharmacokinetic model was built using Stella™ modelling software (Stella v9.0.2, ISEE systems, Inc.). Stella program has a graphical user interface (GUI) by which computational models can be built by drawing. Pharmacokinetic compartmental models are represented by ‘stocks’ and ‘flows’ to which kinetic parameters are linked.

4.4.2. Computational model

The model was an extended version of the compartmental absorption and transit model (CAT) (Yu and Amidon, 1999). The original seven-compartment CAT model of small intestine was expanded to a ten-compartment model to include also colon. The additional
three compartments represent the ascending, transverse and descending parts of colon. Three compartments were used based on the tri-phasic \textit{in vivo} behaviour in the scintigraphic study \cite{1}. Unsolved drug was taken into account due to the slow erosion of the Egalet® system. Thus, additional series of compartments was introduced in the model to mimic the solid drug flow \cite{5, Figure 1}. The deconvolution based true absorption rate constant for caffeine in small intestine was obtained from Linnankoski and co-workers \cite{2006}. The rate of caffeine absorption from the colon was assumed to be at a lower level, half of the small intestinal value in the ascending and transverse colon, and one tenth in the descending colon. The assumption was made because the drug absorption may be slower in the transverse and descending colon \cite{1992, 2000}. The elimination of caffeine is included in the model. The elimination rate constant ($k_{el}$) was obtained from the literature \cite{1997}. Half-life of caffeine is known to vary greatly, from two to more than ten hours. Initially, an average half-life of four hours was used in the model.

The results of the Egalet® study \cite{1} were used in building the model. The pharmacokinetic data and the scintigraphic images from the same study provided an opportunity to build a model that includes both the gastrointestinal tract transit of dosage forms and caffeine pharmacokinetics. Dissolution rate constants were obtained from the scintigraphic data by taking into account the tri-phasic behaviour of gamma count rates observed \cite{1, Figure 3}. The dissolution rate constants were $5.5 \text{ mg·h}^{-1}$, $2.1 \text{ mg·h}^{-1}$ and $0.8 \text{ mg·h}^{-1}$ for the small intestine, ascending/transverse colon, and descending colon, respectively. These values were introduced into the extended model of small intestine and colon. The transit flow parameters for the solid drug (i.e. the formulation) were also directly obtained from the scintigraphic study results. The drug delivery system can be in only one compartment at a time while the dissolved drug is spread in many compartments. This was taken into account by moving the eroding delivery system in steps from one compartment to the next at the times defined on the basis of the scintigraphic evidence. In a similar manner, transit flow parameters related to the dissolved drug were calculated based on the measured small intestinal transit time of three hours. The structure of the model can be seen in Figure 1 \cite{5}.

At first stage, the average values from the gammascintigraphic study with six healthy volunteers were used. Scintigraphy-derived average transit times of the dosage form movements were integrated in the computational Stella model (the compartments on the left hand side in Figure 1 \cite{5}). Since stomach emptying took place within 30 min after administration, the absorption in stomach was not considered in the model. Thereafter, the transit in small intestine took 2.8 h, the formulation remained 4 h in the ascending colon, 6 h in the transverse colon, and 11 h in the descending colon.
5. Results and discussion

In this chapter the results of this thesis are summarized. More detailed discussion about the findings is found in the original publications. This chapter is focused on the evaluation of the scintigraphic methods as tools for studying the fate of formulations in different parts of the GI tract. The results are discussed here from this perspective.

Imaging methods used in the studies presented in the thesis are based on the use of samarium oxide. As mentioned earlier, samarium oxide is insoluble under normal physiological conditions and it is not absorbed from the GI tract. In practice, this means that the imaging results can be used to monitor the transit and disintegration of dosage forms but they cannot deliver information related to absorption, metabolism or excretion of drugs. From the safety point of view this is desired, because no radioactive material is absorbed from the GI tract and normally any residual activity present is cleared from the body within one day.

Neutron activation-based gamma scintigraphic studies have not been conducted with similar equipment or dosage forms prior to this work. Thus, during the work described here, methods were developed from the beginning for the reactor and other equipment used and for the selected radiomarker.

5.1. Preliminary in vitro irradiation tests

In vitro dissolution testing (Ph.Eur.) was carried out with the formulations (excipients as powders packed in gelatin capsules) both before the irradiation and after a 15-min neutron activation procedure. Since it was already known from the literature that some pharmaceutical excipients undergo changes in their physicochemical properties (see 5.4.2) resulting in different in vitro (and probably in vivo) behaviour while irradiated in a neutron flux, it was important to investigate the effects of irradiation on the excipients to be used in the formulations of the imaging studies. Results of the preliminary irradiation tests with some common pharmaceutical excipients are briefly summarized in Table 4. A 15-minute irradiation was assumed to be the likely maximum activation time. Thus, any detected changes in drug release-controlling properties of the excipients would be more obvious in this in vitro test.
Table 4. Irradiation tests with (n = 6) some common pharmaceutical excipients. Observed durations of total drug release (ibuprofen) are listed for untreated formulations. Effects of 15-min neutron irradiation are described by words for each excipient (unpublished).

<table>
<thead>
<tr>
<th>Excipient</th>
<th>Total drug release time</th>
<th>Effects of irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPMC</td>
<td>K100</td>
<td>7 h</td>
</tr>
<tr>
<td></td>
<td>K4M</td>
<td>15 h</td>
</tr>
<tr>
<td></td>
<td>K15M</td>
<td>15 h</td>
</tr>
<tr>
<td></td>
<td>K100M</td>
<td>15 h</td>
</tr>
<tr>
<td>Lactose</td>
<td>15 min (IR)</td>
<td>Virtually no change</td>
</tr>
<tr>
<td>Corn starch</td>
<td>15 min (IR)</td>
<td>No change</td>
</tr>
<tr>
<td>Carbomer</td>
<td>&gt; 15 h</td>
<td>0…3 h increased release rate; 3…15 h virtually no change</td>
</tr>
<tr>
<td>HPC</td>
<td>15 min (IR)</td>
<td>Virtually no change</td>
</tr>
<tr>
<td>MC</td>
<td>10 min (IR)</td>
<td>No change</td>
</tr>
<tr>
<td>Alginic acid</td>
<td>20 min</td>
<td>Slight increase in release rate</td>
</tr>
<tr>
<td>PEG</td>
<td>1.5 h</td>
<td>Substantial decrease in release rate</td>
</tr>
</tbody>
</table>

Figure 5. Scintigrams of six neutron activated capsule formulations inside a neck phantom each with 1.3 MBq radioactivity (photo of the computer screen). The imaging distance was approximately 15 cm and the acquisition time was one minute.

Regarding irradiation times, natural abundance samarium oxide was proven to be a valid choice for the selected methods. Isotope enriched form was also considered, but the use of natural abundance samarium seemed more reasonable considering the very high cost of the enriched form. Natural abundance samarium oxide had been used successfully for
radiolabelling pharmaceuticals in previous studies as well (Ahrabi et al., 1999; Ahrabi et al., 2000).

Gelatin capsules containing 4 mg of samarium oxide were irradiated for 5 min. Activity 48 h after irradiation was measured to be 1.3 MBq. This was the planned activity level at the beginning of clinical trials. Results of preliminary tests with the gamma camera (Multispect 2) are presented in Figure 5. Six capsules were imaged inside a neck phantom. The imaging matrix used was $256 \times 256$. The resulting scintigram was visually verified to be of good quality and hence it was decided that the in vivo studies with this kind of formulations could be started.

5.2. Preliminary imaging studies

Eudragit®-coated capsules fulfilled the in vitro disintegration specifications (Ph.Eur.) for an enteric product both before and after irradiation. Ethyl cellulose coated capsules, untreated or irradiated, did not disintegrate during the continued test for 20 h.

The two ethyl cellulose coated capsules did not disintegrate during 24 hours of transit through the gastrointestinal tract, which shows that the irradiation had not impaired the integrity of the ethyl cellulose coatings. At all times the capsules were easily identified as sharply outlined entities. Both capsules left the body within 24 h. The two Eudragit® L-coated capsules passed intact through the stomach and jejunum but disintegrated in the ileum, three to four hours after administration. Figure 6 shows a series of gamma images illustrating the fate of one Eudragit® capsule. The formulations left the stomach under fasting conditions within one hour, and the transit time through the small intestine was about two hours, i.e., shorter than the times commonly cited in the literature (Davis et al., 1986; Washington et al., 2001; Charman and Charman, 2003). There were no difficulties in detecting movement and disintegration of the capsules or the consequent spread of gamma-emitting material within the GI tract. Also the visual quality and signal-to-noise ratio were very good (Figure 6). The results of these two preliminary tests in four volunteers confirmed that our method worked as intended (unpublished results).

Measured gamma counts originating in an insoluble ethyl cellulose coated capsule were corrected for radioactive decay over time and plotted for one study subject as a function of time for AP and PA data separately in Figure 7 (orange and cyan lines, respectively). The gamma counts are detected from a circular 74-pixel ROI manually drawn around the formulation on a $256 \times 256$ image matrix. Gamma rays attenuate proportional to squared distance in air (or homogeneous tissue). Thus geometric mean should be used for combining AP and PA count rates. For comparison, both arithmetic and geometric mean counts are plotted in Figure 7 (magenta and dark blue lines, respectively). Geometric mean count rates were fairly constant throughout the 12-h period while arithmetic mean was less so. Slight fluctuation in the geometric mean is likely caused by varying attenuation properties of the tissues. It can be seen from the data that the radiation is attenuated more when the formulation is located in the small intestine than when the formulation is in the colon. The results, thus, suggest that the transit of an intact dosage form through the ileo-caecal junction
Results and discussion

(3–3.5 h after administration in this case) may be verified using the step-like increase in the count rate as an indicator (see Figure 7, geometric mean curve).

Radioactive purity of the neutron-activated samples was analysed at VTT prior to the imaging. The measurements were made for the preliminary study formulations (and subsequent imaging formulations) 24–36 h after irradiation. Results of one microcrystalline chitosan granule formulation (II) are presented in Figure 8. The energy peaks recognized by the Sampo program are marked in the graph. According to safety requirements less than 1% of the total measured radioactivity was allowed to derive from other nuclides than $^{153}$Sm. This requirement was fulfilled for each formulation with a clear marginal.

Figure 6. Scintigrams relating to one subject at 0, 1, 3, 3.5, 5 and 11 hours after administration of an Eudragit® L-coated capsule. Disintegration took place in the small intestine between 3 and 3.5 hours after administration (unpublished results).
Figure 7. Gamma counts originating from an ethyl cellulose-coated, insoluble capsule formulation as a function of time (corrected for decay). Orange: anterior gamma counts; cyan: posterior; dark blue: geometric mean of anterior and posterior; magenta: arithmetic mean; grey: average of the geometric mean over time (unpublished results).

Figure 8. Gamma energy spectrum of an irradiated formulation (MCCh granules (II)) 36 h after irradiation. Background radiation is contoured with the blue line. Energy peaks detected by the computer are marked with blue squares. Red squares are gamma energy peaks of $^{153}$Sm (69.7 and 103 keV) and violet squares are sum peaks of $^{153}$Sm.
5.3. Oesophageal imaging (III)

Oesophageal adhesion by dosage forms was recognised almost 40 years ago, when tablets containing potassium chloride caused oesophageal injuries (Pemberton, 1970). After that more than 70 drugs have been associated with similar injuries (Jaspersen, 2000). Usually food and pharmaceutical dosage forms pass through the oesophagus in 10–20 s (Channer, 1985; Bailey et al., 1987). This implies that the imaging schedule should measure accurately the first 30 s. In practice the subject must be imaged already during administration. When adherence or other adverse events are detected, image acquisition should continue without interruption for several minutes, depending on the case. Continued imaging is mandatory also for verification of the formulation arrival in the stomach.

Ingestion of formulation without water or with little water, particularly in association of recumbent position increases the risk of oesophageal adherence. However, because of the design of the gamma cameras used, the studies described in this work are conducted with the subjects in supine position. Hence, care must be taken in designing acquisition geometrics and time schedules for these methods.

The scintigrams were analysed for oesophageal and gastric residence times, small intestinal transit time and large intestine arrival time, initial and complete capsule disintegration times, and anatomical locations at each time point. These parameters are listed in Tables 1 and 2 (III). The first observation was that the HPMC capsules lodged in the oesophagus for 22–143 min on 4 of the 12 occasions. The incidence of stagnation (33%) was quite high although the subjects took 180 ml of water and remained in a sitting position for 30 s before lying down. That time should be long enough based on the information presented above. Figure 1 (III) shows how gamma counts were divided as a function of time between the oesophageal area and the stomach area in those cases where capsule stagnation was observed. In cases (a) and (b) the subjects took an extra dose of water (180 ml) 20 min after the administration. This led to an immediate detachment of the capsule and after 30 min almost all radioactivity was already found in the small intestine. In cases (c) and (d), no additional water was ingested. In previous scintigraphic studies, the research group found that also in one of ten subjects a hard size-0 gelatin capsule stuck to the oesophagus for 1.8 h although the amount of water ingested was 180 ml (Säkkinen et al., 2004). HPMC capsules have previously been studied at the Division of Biopharmaceutics and Pharmacokinetics by Honkanen and co-workers (2001, 2002a, 2002b). It was observed e.g. that the force required for the detachment of hard gelatine capsules from the isolated porcine oesophagus was almost 2.5 times greater than that for HPMC capsules, which would be an advantage for the HPMC capsules. However, the present results do not support that. Thus, it seems that scintigraphic imaging provides much more reliable results than current in vitro tests when oesophageal transit and sticking tendencies of dosage forms are considered. The possibility that the subjects became aware that the capsules might attach to the oesophagus and so swallowed the capsule more effectively the second time must yet be taken into account. Therefore, a further well-planned, double-blind, cross-over scintigraphic imaging study would be worth conducting.

Other results of the oesophageal study included that there was no evidence of the capsules having adhered to the gastric mucosa and that they were emptied from the stomach within two hours of ingestion. These results are discussed in the original publication (III).
The viscosity grade of HPMC did not affect the gastric residence time. Moreover, the initial release of the marker occurred almost at the same time for both formulations. Overall, the formulations behaved according to the assumptions.

The second scintigraphic study on oesophageal transit (Marvola et al., 2005) provided additional information about the stagnation of HPMC and gelatin capsule shells in the oesophagus. Sequential scintigrams were visually inspected to detect the speed and time of transit in the oesophagus and location of the product (oesophagus or stomach). Data was collected in a Table 5 for all 11 study subjects and animated reproductions of transit were generated using the recorded images. The results were compared with the previous data from similar formulations (III). No significant differences were detected in the transit of the two formulations. In fact in this study, the two verified stagnations took place when a gelatin capsule was administered. However, average transit times for both formulations were statistically the similar (18.2 s and 18.8 s). Thus, it was concluded that the previous finding of more likely stagnation of HPMC capsules in the original publication (III) might have been caused by other reasons (such as the earlier mentioned suspected differences in the behaviour of the test subjects) than differences in the formulations. Altogether 23 administered formulations were imaged in the oesophagus. The gamma scintigraphic methods used were concluded to be suitable for this kind of studies.

Table 5. Transit times through the oesophagus (unpublished results).

<table>
<thead>
<tr>
<th>Subject</th>
<th>HPMC capsule transit time (s)</th>
<th>Gelatin capsule transit time (s)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>25</td>
<td>50</td>
<td>Stagnation of gelatin capsule for 30 s</td>
</tr>
<tr>
<td>02</td>
<td>10</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>03</td>
<td>25</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>04</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>35</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>06</td>
<td>10</td>
<td>15</td>
<td>Stagnation of gelatin capsule for 7 s</td>
</tr>
<tr>
<td>07</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>08</td>
<td>25</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>09</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>35</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>18.2</td>
<td>18.8</td>
<td></td>
</tr>
</tbody>
</table>

Three scintigrams of study subject 01 are presented in Figure 9. From the left hand side image, it can be seen that the formulation moves down the oesophagus and is located in the upper part of the oesophagus within seconds after dosing. The two bright dots in the image are anatomical markers ($^{57}$Co) placed approximately at each end of the oesophagus. Markers
were removed 20 s after administration. The image in the middle presents the formulation in the middle part of the oesophagus, 16 s post dosing. On the right, the formulation is still observed to lodge in the lower end of the oesophagus at 45 s. For a dosage form, this is not the desired performance compared to the typical oesophageal transit times mentioned earlier.

![Images of oesophageal transit](image)

**Figure 9.** Oesophageal transit of a gelatin capsule in study subject 01, transit time is 50 s and a stagnation of 30 s was observed. Left: formulation is at the beginning of the oesophagus; middle: formulation is lodging in the middle part of the oesophagus; right: formulation has not cleared to the stomach at 45 s after dosing (unpublished results).

The neutron activation-based gamma scintigraphic method used was proven to provide valuable results when studying the transit of capsule formulations in the human oesophagus. In the case of $^{153}$Sm, the radioactive half-life of the isotope is longer than ideal for the short imaging protocols. This may result in slightly higher absorbed doses to the subjects due to samarium, but on the other hand it helps in minimizing radioactive doses from sodium and other irradiation produced isotopes with shorter half-lives. The Ethics committee did not see any problem in conducting this kind of studies with samarium oxide.

5.4. Imaging formulations in the stomach (II)

Preliminary studies with microcrystalline chitosan granules proved, that the properties of 150-kDa MCCh did not change during neutron irradiation (11, Figures 1a and 2a). Thus, it can be used in formulations for neutron activation scintigraphy. However, higher molecular weight MCCh (240 kDa) was more sensitive to radiation. Gels were formed less readily by this MCCh grade following irradiation, resulting in more rapid drug release. These findings are in accordance with previous results indicating that irradiation can result in degradation of polymers with glycoside linkages (e.g. ethyl cellulose, (Waaler et al., 1997), HPMC, and pectin (Ahrabi et al., 1999; in this work).

It has been suggested already more than 20 years ago that chitosan could be of particular value for controlling drug release from oral dosage forms (Hou et al., 1985). More recently, it has been suggested that chitosan formulations could also be used for gastro-retentive drug delivery (Shah et al., 1999; Remuñan-López et al., 2000; Hejazi and Amiji, 2002). Although the in vitro results have been encouraging, little information about in-vivo mucoadhesiveness
of chitosan formulations in the GI tract is available. Microcrystalline chitosan has previously been studied both in vitro and in vivo at the Division of Biopharmaceutics and Pharmacokinetics by Säkkinen and co-workers (2002, 2003a, 2003b). Chitosan could be ideal for use in formulations designed for prolonged drug release in stomach, since gel formation by chitosan takes place readily at acidic pH levels based on its cationic character.

The reference formulation passed rapidly through the stomach (II, Figure 5, Table 4). After a short lag time before onset of gastric emptying (II, Table 3) it took 15–60 min for the granules to be cleared from the stomach as small frequent boluses (II, Table 4). In contrast, passage of MCCh granules from the stomach was prolonged in three cases out of nine (II, Figures 3 and 4). Formulations were seen to lodge in the stomach for even 2.3 h. However, in the other six study subjects, radioactivity in the stomach declined rapidly (II, Figure 5). It was obvious from the scintigrams that in most cases adhesion to the gastric mucosa was not strong.

Four gamma images of one study subject are shown in Figure 10. Visual inspection of the sequential scintigrams verifies the adhesion of the granules to the gastric mucosa (Figure 10 C). Emptying of stomach to the pylorus can also be clearly seen (Figure 10 C and D) when examining subsequent image frames. An animated reproduction of the gastric emptying is
very elucidative to the eye of the investigator, but can not—for obvious reasons—be presented in print.

It is evident that the transit kinetics of MCCh formulations are not sufficiently reproducible for their use as a gastroretentive dosage form. Although results of animal studies suggest mucoadhesion, these polymers show little tendency to adhesion in man (Harris et al., 1990; Davis et al., 1993). Because there are always limitations on extrapolation of animal study results to human beings, the studies in human subjects are needed. In this study, gamma scintigraphy allowed us to obtain non-invasive direct information about the fates of the formulations in the GI tract. By looking at the scintigraphic images taken from the stomach, it is also evident, that, in some cases, a dual-isotope study with the other isotope diluted in the administered water (180 ml) would help to clarify the anatomical localization of the formulation. The same comment applies obviously to the oesophageal studies (III). The gamma scintigraphic methods used for the imaging of formulations in the stomach were verified to be suitable for this kind of studies as such.

5.5. Imaging formulations in the small intestine and colon (I)

The in vitro dissolution curve for caffeine from the Egalet® constant-release formulation is shown in Figure 2 (I). Drug release lasted for more than 10 h and closely followed zero-order kinetics, as the manufacturer had indicated. Irradiation changed the in vitro dissolution profile slightly. During the first hour the release was faster than from untreated formulations, but subsequently the rates were equal. It is well known that irradiation can accelerate drug release, especially from the formulations that contain polymeric excipients (Watts et al., 1993; Waaler et al., 1997). However, the changes in the dissolution profile were so minimal that imaging-based conclusions could still be drawn in relation to the fate of the product in the GI tract. Also, the detected differences in release properties are likely to affect the drug release only in the stomach and duodenum and not in the distal, more interesting parts of the small intestine and colon considering the time scale and pH dependency of the behaviour.

Figure 3 (I) shows the fate of the Egalet® system in one volunteer. The gastric residence time was one hour. Four hours after administration the formulation had reached the ileocaecal junction, i.e. the transit time through the small intestine had been three hours. It was evident that release of radioactive Sm₂O₃ during the first hour in the stomach was minimal, but that after it had begun it continued throughout the 24-hour imaging period. This can also been seen in Figure 4 (I) depicting the remaining radioactivity inside the formulation as average data from the six study subjects. When Egalet® dosage forms were in the small intestine, from one to three hours after administration, numbers of gamma counts in the ROIs declined linearly. The mean transit time through the small intestine of the Egalet® dosage forms was about two hours. This is slightly less than the commonly cited 3 ± 1 hours for single-unit dosage forms (Davis et al., 1986; Coupe et al., 1991). However, it has been reported that shorter transit times are not unusual, particularly in individuals who are regularly engaged in high-intensity sports (Wilson, 2003). Seven of the ten volunteers (four subjects in preliminary imaging studies and six Egalet® subjects) fell into this category.
Although the results of the Egalet® constant-release formulations are presented and discussed here with the focus on imaging of the small intestine, major part of the drug release from the Egalet® system takes part in the colon and, thus, valuable information about drug release from eroding systems in the colon can be obtained from this pharmacoscintigraphic study. Four hours after administration, the formulations had reached the colon. Subsequently, again, a linear decline in counts was observed until 12 hours after administration. During this time the units were in the ascending or transverse colon. The release rate was the lowest in the descending colon. Linear fitting to the mean values was carried out in three phases, using the method of least squares. Slopes of the fitted lines corresponding to the three phases were 43.9 cpm·h⁻¹ (small intestine, $R^2 = 0.85$), 16.5 cpm·h⁻¹ (ascending and transverse colon, $R^2 = 0.96$) and 6.4 cpm·h⁻¹ (descending colon, $R^2 = 0.92$). Without imaging techniques it would be very difficult to determine the effect of the location of the formulation on the release rate.

Based on Figure 5, an assumption can be made that the residence times in different parts of the colon would explain why there were two pharmacokinetic groups. A typical study subject from each of the two groups is presented (Figure 5). In subject 04 caffeine blood levels declined fairly quickly. In subject 05 caffeine concentrations remained high for longer. From gamma images, the total residence time in the ascending colon and transverse colon was about 7.5 hours in subject 04 but about 12 hours in subject 05. Based on these observations, slow transit through the proximal and central parts of the colon would seem to result in more prolonged drug absorption. This topic is discussed further in the results section of the modelling work. At this point it can be concluded that gamma scintigraphic methods provide valuable data in relation to drug delivery in the small intestine and colon and that they lay a foundation for further evaluation of in vivo dissolution and disintegration of drug formulations in the human intestine.

Based on the two different groups with distinctive difference in transit times and correspondingly in drug plasma profiles, it seems evident that variations in biological factors have much greater effects on the pharmacokinetic profile of a constant-release drug delivery system than any minor change in the characteristics of the dosage form. While the Egalet® system performed according to zero-order kinetics in vitro, the in vivo behaviour was strongly dependent of the individual characteristics of the GI tract.

5.6. Imaging formulations in the colon (IV)

In previous studies at the University of Helsinki, an enteric-coated capsule formulation has been developed for local treatment in the colon. Such formulation might be utilized, e.g. as a carrier for acetaldehyde binding compounds in prevention of alcohol induced colon carcinomas and as a carrier for β-lactamase in prevention of MRSA infections during drug treatment with β-lactam antibiotics (Väkeväinen et al., 2000; Harmoinen et al., 2004; Mentula et al., 2004; Marvola et al., 2008). In recent years, colon has been suggested and studied as a preferable site for peptide delivery (Chourasia et al., 2003). It has already been proven that hypromellose capsules coated with enteric polymer and containing a hydrophilic polymer as a diluent might be suitable for local colon-specific drug treatment (Marvola et al., 2008). It was
indirectly concluded that the formulation coated with Eudragit® S began releasing paracetamol at the ileo-caecal junction or in the ascending colon. This needed to be confirmed in vivo by imaging the drug delivery site.

In order to determine in vitro if neutron activation caused any changes to study formulations, irradiated capsules were subjected to dissolution testing before the gamma scintigraphic evaluation. The dissolution profiles of paracetamol from the capsules are presented in Figure 1 (IV). With these formulations, no significant difference between non-irradiated and irradiated formulations was seen.

The fate of Eudragit® S coated capsules containing MCC or HPMC K4M as diluent in one volunteer is presented in Figures 2 and 3, respectively (IV). The gastric residence time was less than one hour. Three to four hours after administration both formulations had reached the ileo-caecal junction, i.e. the transit time through the small intestine was approximately three hours. Both formulations remained intact in the stomach and the upper parts of the small intestine. They disintegrated and started to release samarium oxide four hours after administration either at the ileo-caecal junction or in the caecum. The capsules containing MCC released the marker momentarily to the ascending colon, whereas the capsules containing HPMC released the marker gradually spreading it to the entire colon. Eight hours after administration radioactivity of the MCC capsules was still mostly in the ascending colon and 14 h after administration it had only reached the hepatic flexure. The difference in behaviour of the two formulations was clearly visible in the scintigrams. Localization of the formulations was also straightforward based on the visual inspection of the images and the interpretation skills gained from the earlier scintigraphic studies. Controlled drug release was visually verified in the case of HPMC formulation as gel formation was detected: Five hours after administration three separate gel particles were visible. The gel particles distributed to the colon content so that 8 h after administration first gel particle was in the transverse colon, second one in the hepatic flexure and third one still in the upper parts of ascending colon. Ten hours after administration, all the gel particles were in the transverse colon and still clearly detectible. In the 12 and 14-h images, the gel has disintegrated and the radiation detected is spread more evenly to the colon. The study formulations behaved in similar manner in every study subject.

By comparing the results obtained from the gamma scintigraphic study to those previously obtained from the bioavailability studies (Marvola et al., 2008), it was possible to draw a conclusion that the formulations coated with Eudragit® S solution did in fact transport the probe compound, samarium oxide, to the ileo-caecal junction before releasing it. It was concluded that it is possible to make a capsule formulation suitable for local sustained-release treatment in colon utilizing enteric coating materials and hydrophilic polymers as diluents to decrease the drug release rate.

5.7. Scintigraphy-based PBPK simulation model (V)

The computational model was tested by varying several parameters related to the transit time of the delivery system. It was suggested in the scintigraphic study (I) that the differences in the transit times in specific parts of the intestine could explain the changes in the caffeine
concentrations. For simulations, two subjects (04 and 05) were chosen to represent the cases of low and high AUC. The transit times of these two subjects were included in the model together with the average data. The simulated and measured concentrations are shown in Figures 2 and 3 (V), respectively. The profiles of the in silico and in vivo concentration curves of each subject resemble each other. Simulated and measured AUC average values match each (V, Table I). However, the model built using the average elimination half-life for caffeine (4 h) does not explain the marked difference in levels of the concentration profiles between the subjects 04 and 05 even when their individual transit data was used to build the simulation model. This suggests that inter-individual differences in transit times alone cannot explain the different in vivo plasma concentration curves.

Sensitivity analysis of the model was conducted for changes in the elimination rate constant of caffeine. The elimination half-life of caffeine shows variability from two to more than ten hours due to differences in metabolism between individuals (Kaplan et al., 1997). These two extreme values of elimination half-life for caffeine were next included in the model with average gastrointestinal transit times. The impact of elimination half-life is presented in Figure 4 (V). It seems evident that differences in the elimination rate of caffeine would be the plausible explanation for the changes in AUC and drug concentration levels (V, Table 1, Figure 4), because the shapes of the in silico concentration curves are also modified so that they resembled the in vivo curves—quite like in the case of varying transit times. Furthermore, these simulations suggest that the elimination half-lives of 7.0 h and 2.1 h would yield the same cumulative AUCs that are seen in the in vivo study in subjects 04 and 05, respectively. Unfortunately, information about half-lives of caffeine in the study subjects is not available and, thus a further study of caffeine elimination using the same study subjects would be very interesting and could be the final evidence.

The computational model was additionally tested for sensitivity to changes in caffeine absorption rate. A value of 4.3 h⁻¹ (Linnankoski et al., 2006) was used as K₄ in the small intestine. Initially, the rate of absorption was decreased in ascending and transverse colon to 2.1 h⁻¹ (half of the value in small intestine) and then further to 1.1 h⁻¹ (one forth) in descending colon. The model was tested also by using the values 0.43 h⁻¹ (one tenth) in the ascending colon and 0.043 h⁻¹ (one hundredth) in the descending colon. The results are shown in Figure 5 (V). Insensitivity to the changes shows that the system is very robust. Also, the model worked remarkably well when average transit times and elimination rates were used and the results were compared to pooled experimental concentrations. It seems that computational simulation models based on gamma scintigraphic results can provide very useful information in relation to the behaviour of delivery systems in man. One can also say that computational modelling fully utilizes pharmacoscintigraphic data and provides the quantitative analysis tool to the investigator.
6. Conclusions

The following conclusions can be drawn based on the results of this study:

- The studied formulations can be satisfactorily radiolabelled utilizing samarium-153 oxide and neutron activation methods based on irradiation in the FiR 1 nuclear reactor. Required activity levels can be obtained without significantly altering the release properties of the studied formulations and without producing significant amounts of unwanted radioisotopes.

- The selected scintigraphic methods were suitable for studying controlled release oral formulations. The imaging methods enabled monitoring the formulations from the oesophagus to the sigmoid colon. Transit times and times of disintegration were obtained from the imaging studies in the stomach, the small intestine and the colon. Main limitation of the imaging methods used in this work is related to the samarium oxide marker. As samarium oxide is insoluble it may model poorly the release of typical drug substances if the release mechanism of the formulation is other than erosion.

- Small intestinal transit was found to be the same for all formulations and somewhat shorter than commonly cited in the literature.

- Controlled drug delivery with an eroding tablet formulation may be more affected by biological variation between individuals than by any minor changes in release characteristics of the formulations.

- Scintigraphy-based modelling of drug delivery to the human small intestine and colon provided a valuable new perspective towards factors interacting in intestinal transit and absorption of drugs. The model reveals that the inter-individual differences in the kinetics of a controlled release caffeine tablet are due to the differences in metabolism, rather than in the dosage form transit. Importantly, the computational model shows that caffeine absorption was not sensitive to changes in the absorption rate constant in the colon. Such sensitivity analysis can be useful in the design of new controlled release formulations.

- Gamma scintigraphy and simulation modelling based on scintigraphic results can be valuable tools in the design of per oral controlled release drug delivery systems and in providing evidence of in vivo behaviour to the regulatory authorities.
References


References


