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RESEARCH PAPER

Feasibility of Capsule Endoscopy for Direct Imaging of Drug Delivery Systems in the Fasted Upper-Gastrointestinal Tract

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ABSTRACT

Purpose To develop a minimally-invasive method for direct visualization of drug delivery systems in the human stomach and to compare the obtained results with an established *in vitro* model. The method should provide the capsule rupture, dispersion characteristics, and knowledge regarding the surrounding physiological environment in the stomach.

Methods A capsule endoscopic method was developed. The disintegration time, dispersion characteristics and the impact of the physiological environment on different lipid based delivery systems in different gelatin capsules in the fasted stomach of nine healthy volunteers were visualized. Biorelevant dissolution studies using a USP II apparatus and a droplet size analysis of the released SNEDDS were performed.

Results Visualization of the behavior of both hard and soft gelatin capsules formulations was possible. The disintegration and dispersion of EP oil in a soft capsule and SNEDDS in a hard shell capsule were visualized. The *in vitro* release rates were different from the *in vivo* release rates of the soft capsule due to volume, fluid composition and motility differences but not for the hard capsule containing SNEDDS.

Conclusions A minimally-invasive capsule endoscopic method was developed for direct visualizing of drug delivery systems in the human stomach and maybe later, in the duodenum.

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KEY WORDS capsule endoscopic visualization · direct imaging · fasted gastric behavior · gelatin capsules · humans · lipid based drug delivery

INTRODUCTION

The actual behaviour of oral dosage forms in the environment of the gastro-intestinal tract (GIT) is not very well elucidated. More knowledge is needed in order to fully understand how the conditions in the GIT can impact the fate of oral dosage forms and thereby also if the currently existing *in vitro* models are predictive for this behavior. The characteristics of the GIT, such as pH, bile salts, enzyme activities, gastric emptying rate, intestinal motility, hydrodynamics, shear rates, and fasted or fed state significantly impact release, dispersion, dissolution and absorption of the drug and the performance of the delivery system (1,2).

Only a few studies have visualized lipid filled capsules in the human stomach and intestine using imaging techniques (3). Lipid based formulations are developed in order to overcome the obstacle of poor bioavailability due to low solubility, which is a dominating characteristic of many new drug compounds (4,5). The approach generally used in lipid based drug delivery systems is to dissolve the drug in a vehicle, such as oils, emulsions or self-emulsifying formulations (6). Lipid based delivery systems are normally dosed in hard or soft gelatin capsules (7). Differences between soft and hard gelatin capsules are filling procedures, compatibility with liquid formulations, and stability (7). Additionally soft gelatin capsules contain plasticizer, gelatin, and water, whereas hard gelatin capsules only consist of gelatin and water. High content of moisture may change the chemical stability and a dissolution property of both soft and hard gelatin capsules (8–10). The composition of the lipid based drug delivery system, as well as the type of capsule employed influence the *in vivo* behavior.

In pharmaceutical development, methods to study the behavior of the more sophisticated delivery system in the

GIT without affecting the physiological process are required. In humans *indirect* visualization techniques have been successfully used in order to improve the understanding of GIT physiology and the interaction and behaviour of oral drug delivery systems. These techniques include 1) γ-scintigraphy, 2) Radiology (X-ray) including magnetic resonance imaging (MRI), 3) Radiotelemetry (pH-capsules) and 4) Ultrasonography (11–15). The *indirect* visualization techniques have been used to study e.g. gastric volume, GIT transit time of different dosage forms, stomach emptying time, pH, and intragastric distribution of drug delivery systems (13–18). Additionally, the disintegration of gelatin capsules and distribution of the contained lipid based drug delivery system in the human stomach and the gastric emptying of emulsion have been visualized (3). The *indirect* visualization studies have provided useful knowledge, but are all associated with some limitations e.g.; the visualization of the oral dosage form in the GIT is from the outside of the body, the use of γ-scintigraphy requires that the volunteers are exposed to ionization radiation, and MRI requires use of contrast agents and the measurements are disturbed by gastric and intestinal motion. These disturbances can only be avoided by breath holding and by suppression of the motility using anticholinergic drugs (19,20).

A direct visualization method has been established using gastroscopes (11,21,22). The method has shown that direct visualization can increase the understanding of dosage form and drug behavior in the upper GIT. Endoscopy provides real-time visual examination of dosage form interactions with the mucosal tissue (11,21,22). In the 1970ties, Hey *et al.* showed that the use of gastroscopy could contribute to a better understanding of the behavior of a dosage form in the stomach. In the study, the disintegration pattern of a tablet and a gelatin capsule formulation of the APIs, pivmecillinam and pivampicillin were studied (21,22). It was found that the capsule formulation induced gastrointestinal bleeding and mucosal erosion, whereas this did not occur with the tablet. It was visualized that the capsule adhered to the mucosa, leading to local release of the APIs, while the tablet formulation disintegrated over a large area in the stomach (21,22). Even though this useful information was obtained, gastroscopy is seldom used in the visualization of dosage forms in humans; the technique is invasive and volunteers experience a lot of discomfort during the examination as the gastroscope consist of a fiber optic tube that have to pass through the esophagus into the stomach (11). During the procedure insufflation of air into the stomach is needed, which might influence the behavior of a dosage form and the volunteers are placed in the left lateral position potentially leading to misleading results (22). Lastly, administration of water, which is recommended upon intake of dosage forms, is restricted during gastroscopy (22).

The purpose of this study was to develop a minimally-invasive method for direct visualization of dosage form behavior in the human stomach without changing the physiological environment. It was decided to evaluate the behavior of liquid filled soft and hard gelatin capsules. The method should provide information on the capsule rupture time, dispersion characteristics, and knowledge regarding the surrounding physiological environment that might impact the performance of an oral dosage form in the stomach. The *in vivo* observations were compared with the dispersion studies of the capsules in an optimized USP II setup.

MATERIALS AND METHODS

Materials

Evening Primrose (EP) oil in soft gelatin capsules obtained from Futura, Denmark. Maisine 35–1 was purchased from Gattefossé (Saint Priest, Canada). Cremophor RH 40 was obtained from BASF (Massagua, Canada). Ethanol (Ph. Eur. grade) was purchased from VWR (Herlev, Denmark). Hard gelatin capsules (size#2) purchased from Universal capsules (ACG North America LCC). Histoacryl ® purchased from Braun (Ann Arbor, MI USA). Sesame oil, pepsin from porcine gastric mucosa and Taurocholic acid sodium salt hydrate (NaTC) were all purchased from Sigma-Aldrich (Saint Louis, MO, USA). Sodium chloride (NaCl) and sodium hydroxide pellets (NaOH) were purchased from Merck (Damstadt, Germany). Phosphatidylcholine (Lipoid S PC) was purchased from Lipoid (Ludwigshafen, Germany). Purified water was obtained from a Millipore Milli-Q Ultrapure Water Purification System (Billeria, MA, USA).

Preparation of Formulations

Oil Filled Soft Capsules

EP oil formulation in a soft gelatin capsule, obtained from Futura, Denmark.

Oil Filled Hard Capsules

Three hundred fifty milligram pure sesame oil was filled into hard two-piece gelatin capsules (size#2, Universal capsules, ACG North America LCC) the day before used.

Self- Nano Emulsifying Dryg Delivery Systems- SNEDDS Filled Capsules

A long chain SNEDDS (LC-SNEDDS) was prepared inspired by Thomas *et al.* but substituting soy bean oil with sesame oil (23). The ingredients (Table I) were weighed into glass vials,

Table I The Composition of the Long Chain Self-Nano Emulsifying Drug Delivery Systems – LC-SNEDDS

Excipients	Composition of LC-SNEDDS (%, W/W)
Sesame oil	27.5
Maisine 35-1	27.5
Chremophor RH 40	35
Ethanol	10

which were closed with Teflon sealed screw caps followed by vortex mixing until a homogenous mixture was obtained. The mixture was stored for equilibration at 37°C over night. The formulations were used within 1 week after preparation.

Three hundred fifty milligram SNEDDS were filled into two-piece hard gelatin capsules (size#2, Universal capsules, ACG North America LCC) on the day before use.

In Vitro Studies

Preparation of Fasted State Simulated Gastric Fluid, FaSSGF

The simulated gastric media, FaSSGF was prepared according to Vertzoni *et al.* (24). Table II shows the composition of the media. The solution was stirred overnight and pH was adjusted to 1.6 with HCL. Purified water was added to a final volume of 1000 ml and the FaSSGF medium was stored at 5° until used. Additionally, a similar FaSSGF medium was prepared according to the above mentioned and pH was adjusted to 2.9 (25).

In Vitro Release Studies

In vitro release studies were conducted using a USP II apparatus, with 300 ml of fasted state simulated gastric fluid – FaSSGF (24) (37°C, $n=3$). A rotation speed of 75 rpm was selected as this has shown to correspond to the hydrodynamic conditions of the upper gastrointestinal tract of Labradors (26). Vertzoni *et al.* state that the realistic volume simulating the fasted state volume present in the stomach should be in the range 250–300 ml. Therefore, a total volume of 300 ml was used for these *in vitro* release studies (24). The initial release of oil or SNEDDS from the model formulation and the total disintegration of the capsules were visually observed.

Table II The Composition of the FaSSGF Medium

Components	Concentration
Pepsin	0.1 mg/ml
NaTC	80 μM
Phospholipids	20 μM
NaCl	34.2 mM

Similarly a preliminary study was performed to evaluate the initial release of EP oil from the capsule fixed with dental floss using USP II apparatus, 300 mL FASSGF and a rotation speed of 100 RPM (37°C, $n=3$).

Droplet Size Measurements

The droplet size of the dispersed SNEDDS from *in vitro* release studies was measured by dynamic light scattering on a Malvern Zetasizer, nanoseries ZS ZEN 3600 from Malvern Instruments AS (Worchestershire, United Kingdom). The mean droplet size was calculated on the basis of the volume size distribution using Dispersion Technology Software, Nanoseries and HPPS. DTS version 4.10 from Malvern Instrument AS.

In Vivo Visualization Studies

Subjects

The endoscopic visualization studies was performed at University Hospital of Copenhagen, Herlev and Gentofte, Denmark. Ten healthy subjects (5 male and 5 female) between 24 and 63 years old volunteered for the study. The volunteers were not allowed to take any medication on the day of the study and had no history of gastrointestinal diseases. The volunteers were not allowed to eat or drink, 6 and 2 h prior to the study, respectively. One study was excluded due to failure of following the study protocol.

Endoscopic Visualization in Humans

The capsule endoscopic system comprises a digital colon capsule endoscope (26 mm * 11 mm), eight electrodes connected to a data recorder, and computer software for real-time monitoring. The electrodes were fastened to the abdominal wall of the volunteers and sequential images were captured by the endoscopic capsule camera and transmitted and saved by a data recorder. Colon capsule endoscope (Pillcam – Fig. 1) from Given Imaging was tightened to a soft string of dental floss (Colgate Total) with a minimum length of 1.0 m to retain and control the camera in the fasted stomach and to avoid passage of the pylorus due to the peristalsis. Histoacryl® glue was used to ensure that the dental floss was fixed to the smooth capsule endoscope. A capsule (EP oil formulation, sesame oil formulation or SNEDDS formulation) was additionally fastened with dental floss just in front of the capsule endoscope. The capsule endoscope and a model formulation were swallowed with 200 ml of water. Additionally, 100–150 ml of water were available for ingestion to avoid discomfort for the healthy volunteer. During the observation, the volunteers were standing or sitting in an upright position and holding the dental floss to ensure that the capsule endoscope

Fig. 1 A capsule endoscopic method for visualization of dosage forms in the stomach. In the figure a lipid capsule (model formulation) is fixed in front of a colon Pillcam (Given Imaging) with a soft string (dental floss).

and capsule did not travel with peristalsis from the stomach to the intestine. The dosage form was video-recorded by the capsule endoscope, and each different formulation was evaluated in three volunteers.

The capsule rupture time and dispersion characteristics were evaluated for each model formulation. The capsule rupture time was measured from when the endoscopic capsule and model formulation was swallowed to the first SNEDDS or oil was observed released. Additionally, the surrounding physiological environment was visualized and observed in order to demonstrate what impact it would have on the behavior of the oral dosage form in the stomach (location inside the stomach, motility etc.). After the model formulation was dissolved the dental floss was cut. Thus, the capsule endoscope traveled with peristalsis throughout the GIT and was discarded.

Discomfort Score System

After completion of the study, the volunteers were asked to evaluate their discomfort during the study. The volunteers gave a grade between 1 and 10, where 1 corresponded to easy tolerated and 10 to extremely uncomfortable. Additionally, the volunteers were asked whether they would consider participating in a similar study again.

Ethics

The study was approved by the Ethical Committee of Denmark, Copenhagen, Denmark and followed the convictions of the Declaration of Helsinki (H-1-2011-115). All volunteers gave their written informed consent to the experimental procedure.

RESULTS

Capsule Endoscopy

Capsule endoscopic method was developed to visualize dosage forms in the human stomach (Figs. 2, 3 and 4). A preliminary *in vitro* release experiment using the USP II apparatus has shown that the use of the dental floss to attach the model formulation (EP oil) to the capsule endoscope has no effect on the capsule rupture time (Table III).

Discomfort Score System

The study was concluded as being minimally-invasive for the volunteers. Nine volunteers evaluated the discomfort of the study. Five graded participation in the study as easy (grade 1), three graded the study as "2", and one volunteer graded the study as "4". All volunteers confirmed that they would volunteer for a similar study again.

In Vivo Visualization

Three capsules with different content (EP oil, sesame oil or SNEDDS) were visualized in the human stomach using capsule endoscopy.

Soft Gelatin Capsule Formulation Containing γ-Linoleic Acid Rich Oil

Figure 2 illustrates the capsule rupture, disintegration process and dispersion process of EP oil containing capsule in the stomach. Figure 2a shows the EP oil capsule in the esophagus. The average transit time through the esophagus was measured to be 35 s ± 13.7 s (n=3). In Fig. 2b the capsule has just entered the stomach. It was observed that the EP oil capsule was submerged in the gastric fluid during its entire residence in the stomach (Fig. 2a–i). The first EP oil droplet released was observed after 1 m 52 s ± 19 s (Table III) indicating capsule rupture. This is illustrated in the Fig. 2c. In Fig. 2d more EP oil was released through pores in the soft gelatin capsule. The soft gelatin capsule adhered to the gastric mucosa in the stomach and was kneaded by the folds of the gastric mucosa (Fig. 2e and h). The dispersion of oil was captured and illustrated in Fig. 2f. The melting process of the soft gelatin capsule is captured over time (Fig. 2g). In Fig. 2i the gelatin capsule has totally disintegrated and only the dental floss loop is left in front of the capsule endoscope.

Hard Gelatin Capsule Containing Sesame Oil

The capsule rupture and dispersion processes of sesame oil containing hard gelatin capsule in the stomach are illustrated in Fig. 3. The esophageal transit time was 27.0 s ± 14.5 s (n=3) (Fig. 3a). When entering the stomach the transparent nature of the hard gelatin capsule and sesame oil made it difficult to visualize the behavior of the formulation (Fig. 3b). Therefore, the observed initial time of release is uncertain. In Fig. 3b–d the hard gelatin capsule are surrounded by air bubbles in the surface of the gastric fluid, possibly due to that the capsule is floating in the gastric fluid. In Fig. 3e the hard gelatin capsule is fully submerged indicating capsule rupture and release of some sesame oil. In Fig. 3e and f dispersed oil droplets were captured.

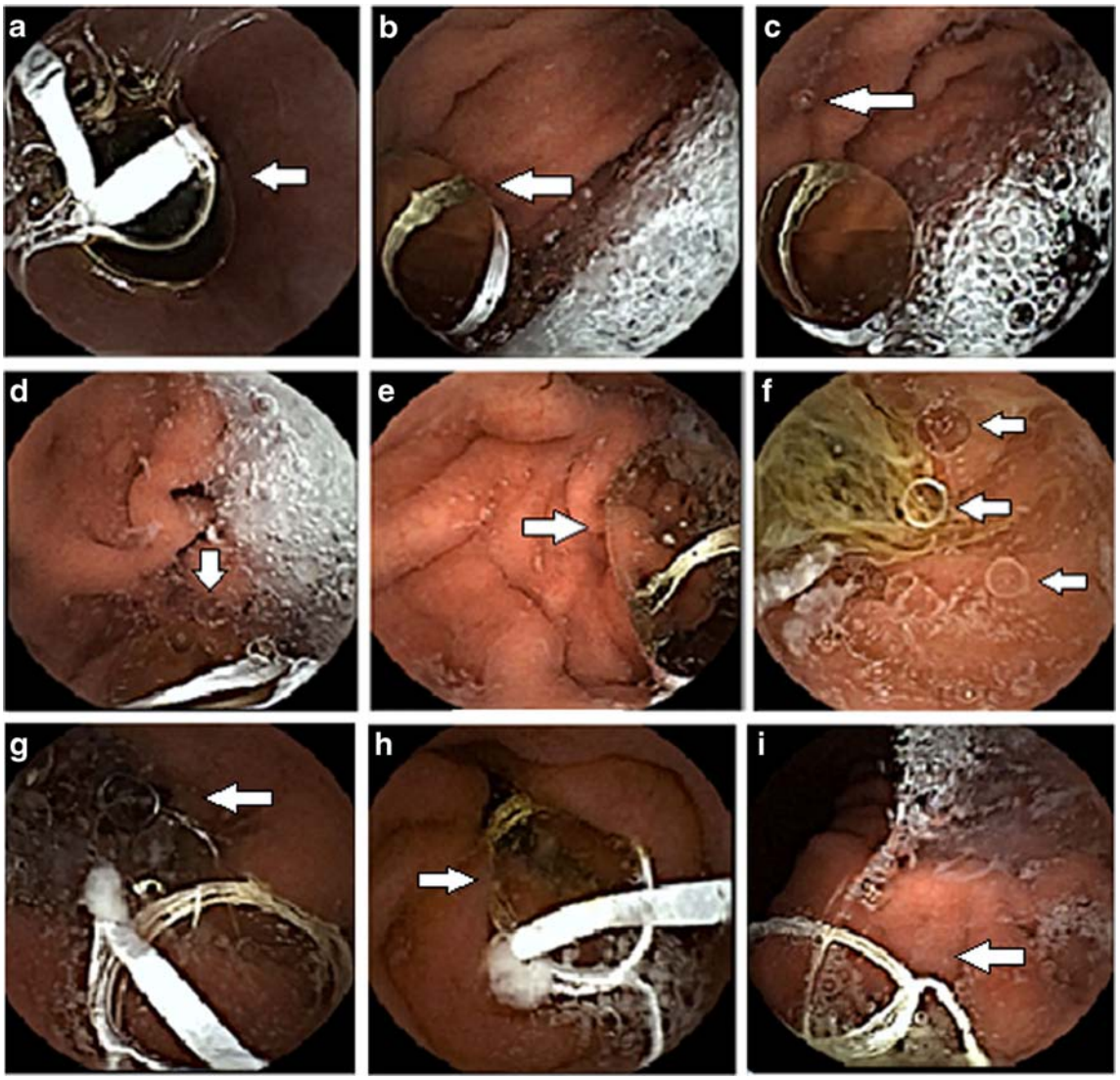

Fig. 2 Capsule endoscopic method used for direct visualization of a soft gelatin capsule containing EP oil. The capsule rupture and dispersion pattern were followed over time in the fasted stomach of volunteers ($N=3$) and illustrated for one.

Hard Gelatin Capsule Containing SNEDDS

Direct visualization of the disintegration process and dispersion process of SNEDDS in a hard gelatin capsule in the human stomach is illustrated in Fig. 4. The transit time through the esophagus was 23.7 s±3.8 s for the capsule endoscope and hard gelatin capsule formulation containing SNEDDS ($n=3$) (Fig. 4a).

In Fig. 4b the hard gelatin capsule containing SNEDDS just entered the stomach. The hard gelatin capsule containing SNEDDS was floating in liquid surface in the stomach and is illustrated by examples in Fig. 4 similar to the hard gelatin capsule containing sesame oil (Fig. 4c, e and f). The release and dispersion of the SNEDDS was captured over time. The release of SNEDDS was easy to detect with the capsule endoscope because the dispersed SNEDDS turned into a bluish optically clear and slightly opalescent dispersion when getting in contact with the gastric fluid (Fig. 4h). The first release of SNEDDS from the hard gelatin capsule was observed after 5 m 34 s± 2 m 42 s ($n=3$) (Table IV) indicating capsule rupture. The first release of SNEDDS in the illustrated study is presented in Fig. 4c and d. As SNEDDS was released the hard gelatin capsule became fully submerged as illustrated in Fig. 4 after 17 m 41 s±9 m 24 s ($n=3$) (Fig. 4g). During the study, an increasing amount of SNEDDS was released as the gelatin capsule melted (Fig. 4). Over time the SNEDDS was dispersed in the gastric content (Fig. 4h). In Fig. 4i the last release of SNEDDS was observed and the gastric fluid appeared as a bluish and clear dispersion of SNEDDS, 34 m 41 s ±3 m 29 s ($n=3$).

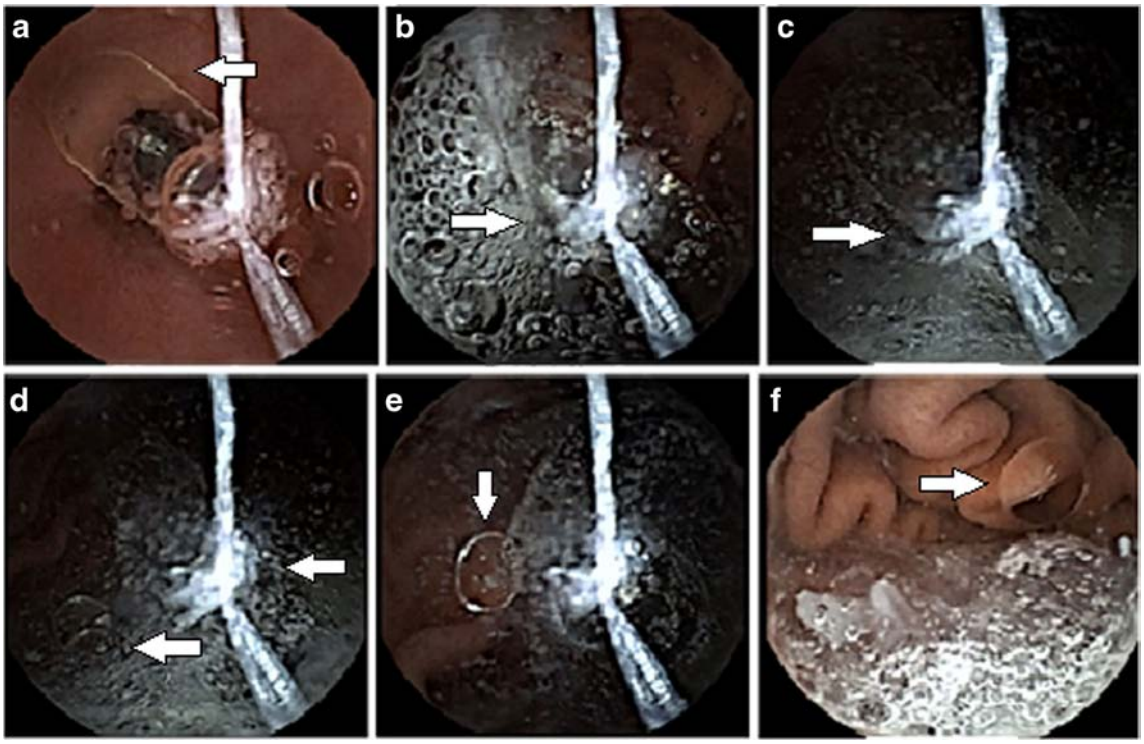

Fig. 3 Capsule endoscopic method used for direct visualization of a hard gelatin capsule containing sesame oil. The capsule rupture, disintegration and dispersion pattern were followed over time in the fasted stomach of volunteers ($N=3$) and illustrated for one.

In Vitro Visualization

The results of the *in vivo* visualization studies were compared with the *in vitro* release studies performed for different lipid based drug delivery systems. The *in vitro* release studies showed that the soft gelatin capsule containing EP oil was sinking into the FaSSGF medium in the dissolution apparatus, whereas the hard gelatin capsules containing sesame oil and SNEDDS was floating at the liquid surface (Fig. 5). The hard gelatin capsule formulations started sinking as sesame oil or SNEDDS were released from the capsule (Fig. 5).

The initial release was determined to be when the first oil droplet or SNEDDS was observed released from the capsule indicating capsule rupture. For the soft gelatin capsule containing EP oil the initial release was 1 m 17 s±6 s *in vitro* (Table IV and Fig. 5). Gastric pH is known to be highly variable even in fasted state (1,27–29), therefore a dispersion study evaluating capsule rupture at pH of 1.6 and 2.9 was performed. These *in vitro* dispersion studies showed that the capsule rupture and release from soft gelatin capsule containing EP oil were independent of the pH present and were measured to be 1 m 17 s±6 s at pH 1.6 and 1 m and 25 s± 07 s at pH 2.9. The *in vitro* initial release from the hard gelatin capsule containing sesame oil and SNEDDS were 1 m 0 s± 13 s and 9 m 3 s±3 m 24 s, respectively (Table IV and Fig. 5). The average particle size of the dispersed SNEDDS was measured to be 94.3±2.0 nm with a polydispersity index of 0.27±0.03.

DISCUSSION

Imaging by capsule endoscopy is widely used for diagnosing and screening diseases in the GIT, but is in the present study shown also to enable direct visualization of dosage forms in the human stomach. The capsule endoscopic method has minimized the invasive nature of direct visualization (gastroscopy) of dosage forms in humans and was well tolerated and accepted by the volunteers included in this study (30–33). Additionally, the method provides advantages such as increased angle of view, depth of field, image numbers, and enables longer duration of the visualization procedure up to 8 h, which makes the technique ideal for visualizing drug delivery systems in the stomach and maybe, later in the duodenum (32,33). In contrast to gastroscopy, insufflations of air are not needed during visualization when using the capsules endoscopic method. Additionally, the use of gastroscopy limits the possibility of observing drug delivery systems in the small intestine, but this is believed to be possible using the capsule endoscopic method (30). Additionally, the method visualized how the actual physiological environment impacts the performance of capsule formulations. However,

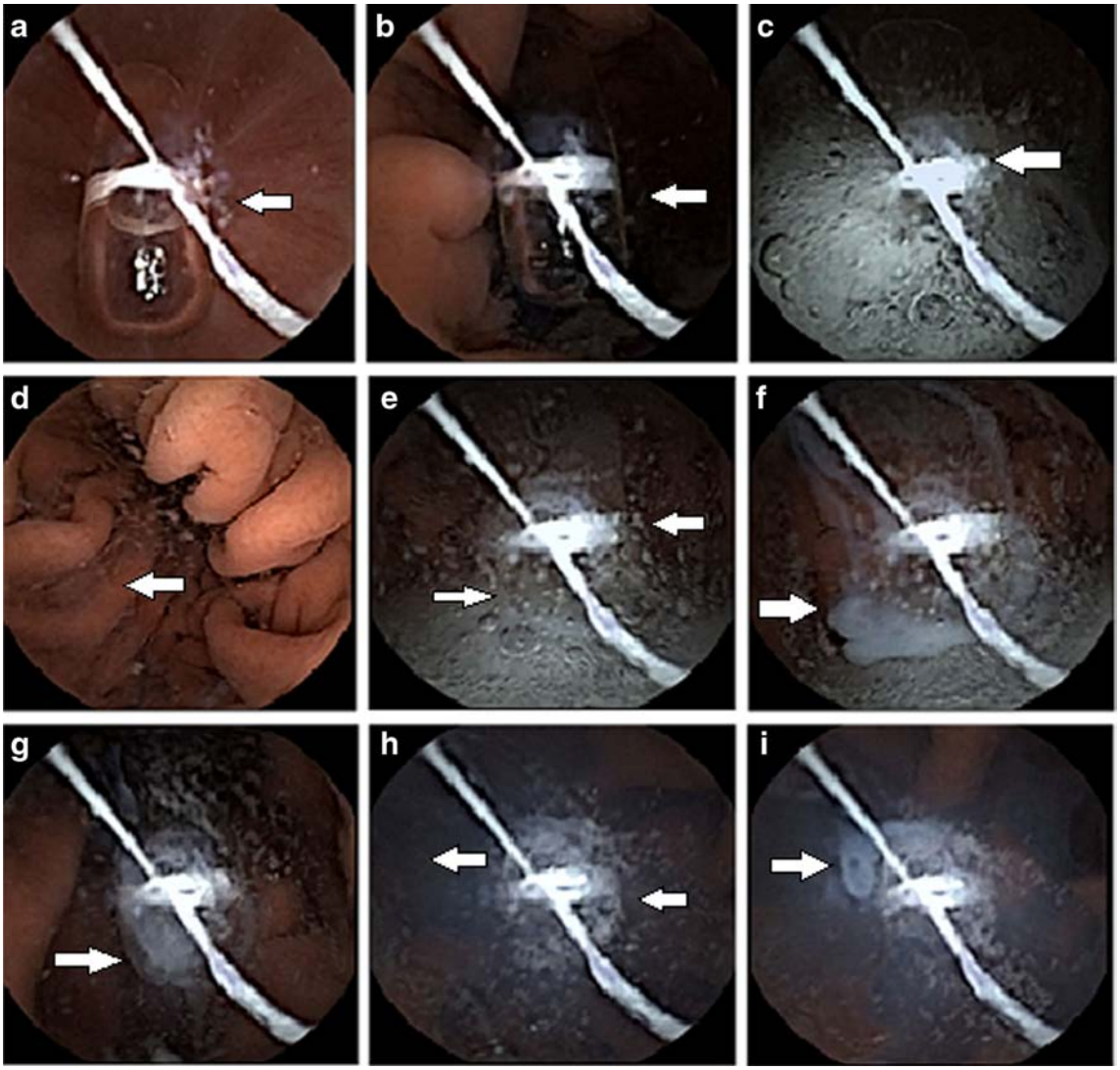

Fig. 4 Capsule endoscopic method used for direct visualization of a hard gelatin capsule containing LC-SNEDDS formulation. The capsule rupture, disintegration and dispersion pattern were followed over time in the fasted stomach of volunteers ($N=3$) and illustrated for one.

the use of dental floss to fix the dosage form in front of the capsule endoscope and to retain the capsule endoscope and the dosage form in the stomach might reduce the intragastric mobility of the dosage form under investigation.

The present capsule endoscopic method made it possible to visualize the behavior of both hard and soft gelatin capsules formulations in the stomach. The esophageal transit times for both the soft and hard capsule formulations were measured to be 35 s±13.7 s, 27.0 s±14.5, and 23.7 s±3.8 s. Chisaka *et al.* measured a significantly faster mean esophageal transit time of smaller gelatine capsule formulation (size #4) to be 6.0±2.4 s ($n=14$), compared to the present study (34). The attachment of the dosage form to the relatively large capsule endoscope might impact the transit time and aerodynamics of the dosage forms. Further, the volume of water administered and the age differences of the subjects ingesting the dosage forms have earlier been shown to affect the esophageal transit time (34,35).

Table III The Effect of Dental Floss on the Initial Release from an EP Oil Formulation

	Dental floss	pH = 1.6
Initial release	With	1 m 17 s±6 s
	Without	1 m 16 s±15 s

It was observed that the soft gelatin capsule formulation was submerged during its entire residence in the stomach (Fig. 2), whereas the hard gelatin capsules containing sesame oil or SNEDDS were floating on top of the ingested liquid in the stomach although the capsule was fastened to the heavy capsule camera (Figs. 3 and 4). This is due to the density of the capsule and to air present in the hard gelatin capsules, as it is not possible to fill the capsules completely. After some of the

Table IV Initial Release Observed from EP Oil Formulation, Sesame Oil Formulation or SNEDDS Formulation *In Vivo* and *In Vitro*

	ɣ-linoleic acid rich oil formulation	Sesame oil formulation	SNEDDS formulation
Initial release *in vivo* ($n=3$)	1 m 52 s ± 19 s	NA	5 m 34 s ± 2 m 42 s
Initial release *in vitro* ($n=3$)	1 m 17 s ± 6 s	1 m 0 s ± 13 s	9 m 3 s ± 3 m 24 s

Data evaluated using Student *t*-test using Graphpad Prism . *NA* not available

content of oil or SNEDDS was released from the hard gelatin capsule, the capsule became fully submerged in the liquid content of the stomach, indicating that no air is left in the capsule. This *in vivo* observation was confirmed *in vitro* where it was observed that the soft gelatin capsule sank and remained submerged during an entire *in vitro* release study (Fig. 5b). Confirmatory, the hard gelatin capsule formulations were floating at the liquid surface and sank after releasing some oil or SNEDDS (Fig. 5a).

It was not possible to observe the initial rupture of the hard gelatin capsule containing sesame oil *in vivo* due to its transparent nature. The initial rupture of the soft gelatin capsule containing EP oil and hard gelatin capsules containing SNEDDS was observed after 1 m 52 s ± 19 s and 5 m 34 s ± 2 m 42 s, respectively. In comparison, Brown *et al.* and Digenis *et al.* showed that the initial disintegration of hard gelatin capsules containing acetaminophen and amoxicillin were 8 m ± 2 m and 7 m ± 5 m, respectively using gamma-scintigraphy (36,37). Wilding *et al.* found that a sumatriptan formulation encapsulated in a gelatin capsule had an initial and a complete disintegration time of 8.0 ± 5.6 m and 15.9 ± 7.4 m, respectively. The disintegration of these gelatin capsules were found to be complete in the stomach prior gastric emptying (38). The nature of the content (e.g. liquid or powder) in the hard gelatin capsules can also impact the initial rupture of the capsules.

The capsule endoscopic method made it possible to visualize the dispersion properties of both oil formulations EP oil and the SNEDDS formulation. The dispersion of EP oil was clear due to its yellowish color and the size of the released oil droplets. The released SNEDDS was easy to detect although it had a droplet size below 100 nm due to the SNEDDS formulation providing a bluish optically clear and slightly opalescent dispersion when in contact with gastric fluid. The basic principle of a SNEDDS is the ability to form fine oil-in-water nanoemulsion under gentle agitation following dispersion in aqueous phases. These self-emulsifying properties require a low energy input, which is provided by the motility and agitations in the GIT (39,40). The oil formulation containing sesame oil was difficult to visualize due to its very transparent color. Therefore, for future visualization of lightly colored or transparent oil formulations, it would be recommended to add a color excipient that is lipid soluble to enable visualization of the capsule rupture and dispersion in the GIT.

The capsule rupture and dispersion studies were additionally investigated *in vitro* in order to evaluate how well the release studies performed in the USP II apparatus correspond to the *in vivo* situation. The *in vivo* conditions observed in this study are not simulated by the conventional *in vitro* USP II apparatus, e.g. due to differences in the available dissolution volume and motility. *In vitro* the initial release and capsule rupture time of the soft gelatin capsule formulation containing EP oil was significant faster than *in vivo* (Table IV). No significant differences were observed for the initial release and capsule rupture time from the hard gelatin capsule formulation containing SNEDDS, which might be due to that the formulation is floating at the liquid surface and therefore is not affected by the paddle rotations in the same manner as the soft gelatin capsule formulation which sank immediately after added to the dissolution chamber. Similarly, Kalantzi *et al.* showed that the *in vitro* disintegration time of unstressed hard gelatin capsules containing amoxicillin, using the USP II, 100 rpm and 500 mL FASSGF, corresponded to the *in vivo* gastric disintegration time, as assessed by scintigraphy (41). In

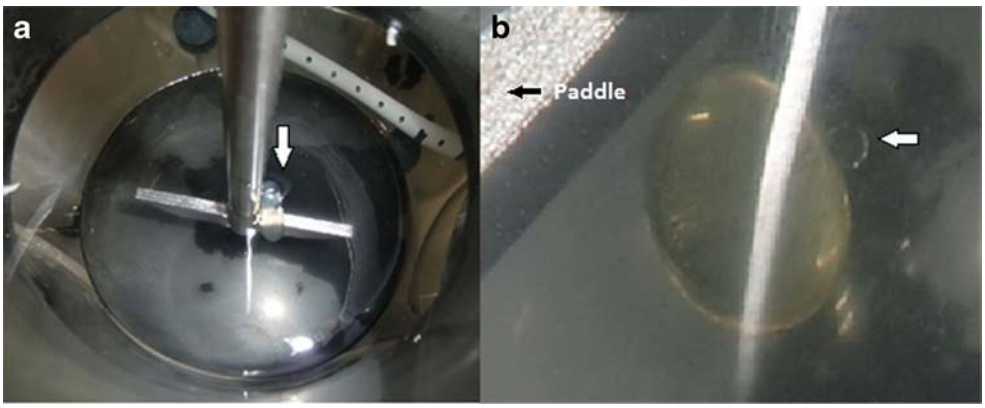

Fig. 5 *In vitro* initial position and release from (**a**) hard gelatin capsule containing SNEDDS formulation and (**b**) soft gelatin capsule formulation containing EP oil.

a standard dissolution setup a sinker would normally be used when evaluating a floating capsule, this will, however, render the *in vitro* situation less *in vivo* relevant. Based on this, the use of sinkers should be avoided when evaluating capsule formulations, if attempting to simulate the *in vivo* conditions. In the present study an optimized dissolution setup was used with *in vivo* relevant dissolution volume and shear rates (24,26). Thus, it can be anticipated that an increased difference between the *in vitro* and *in vivo* behavior would be apparent using the standard dissolution setup where 900 mL and a rotation speed of 50–100 rpm are recommended (42).

The hydrodynamics and strain distribution have been shown to be very different in the USP II apparatus. The release from drug delivery systems might be highly position dependent in the dissolution apparatus (43,44). Kakura *et al.* showed that the strain rates around the paddle are high and might therefore induce faster initial release from the soft gelatin capsule formulation than that observed *in vivo* where only slow movements are present (43). In contrast, no significant differences in the initial release from the floating SNEDDS formulation were observed *in vitro* and *in vivo*. This is likely to be due to similar strain being present in the liquid surface *in vitro* and *in vivo*. Kukura *et al.* showed that the strain is low near the rod of the paddle at the liquid surface in the USP II corresponding the position of the SNEDDS formulation during the *in vitro* release study even at 100 rpm (43).

The capsule endoscopic method provides a direct *in vivo* visualization method that enables investigation of the behavior of dosage forms under the actual conditions of the GIT. This method enables investigation of the behavior of new and sophisticated drug delivery systems in humans. Additionally, the method can be used as control to support the reliability of *in vitro* studies and to increase the understanding of the conditions that dosage forms are subjected to in the stomach. Thus, enabling the development of more *in vivo* relevant *in vitro* models.

CONCLUSION

A minimally-invasive and well tolerated capsule endoscopic method was developed. The method provides clear visualization of the performance of lipid filled soft and hard gelatin capsules in the stomach. With this method, direct visualization can be performed under normal fasted gastric conditions without changing the environment. The method enables evaluation of the disintegration time, dispersion characteristics, and enhances the understanding of how drug delivery systems behave in the actual physiological environment in the stomach.

The *in vitro* release rates determined by established biorelevant dissolution methods were different from *in vivo* release rates of the EP oil capsules due to volume, fluid composition and motility differences, but not for the hard capsules containing SNEDDS. It should be noted that the use of less biorelevant *in vitro* settings, such as USP recommendations, might induce larger differences from the *in vivo* release rates.

The capsule endoscopic method is likely to be able to visualize the performance of other types of drug delivery systems in both the fasted stomach and duodenum. *In vivo* visualization by capsule endoscopes is a novel and valuable tool for evaluation of dosage form performance in the stomach.

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