Superparamagnetic maghemite nanoparticles from solid-state synthesis – Their functionalization towards peroral MRI contrast agent and magnetic carrier for trypsin immobilization

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A B S T R A C T

Nearly monodispersed superparamagnetic maghemite nanoparticles (15–20 nm) were prepared by a one-step thermal decomposition of iron(II) acetate in air at 400 °C. The presented synthetic route is simple, cost effective and allows to prepare the high-quality superparamagnetic particles in a large scale. The as-prepared particles were exploited for the development of magnetic nanocomposites with the possible applicability in medicine and biochemistry. For the purposes of the MRI diagnostics, the maghemite particles were simply dispersed in the bentonite matrix. The resulting nanocomposite represents very effective and cheap oral negative contrast agent for MRI of the gastrointestinal tract and reveals excellent contrast properties, fully comparable with those obtained for commercial contrast material. The results of the clinical research of this maghemite–bentonite contrast agent for imaging of the small bowel are discussed. For biochemical applications, the primary functionalization of the prepared maghemite nanoparticles with chitosan was performed. In this way, a highly efficient magnetic carrier for protein immobilization was obtained as demonstrated by conjugating thermostable raffinose-modified trypsin (RMT) using glutaraldehyde. The covalent conjugation resulted in a further increase in trypsin thermostability ($T_{50}$ 61 °C) and elimination of its autolysis. Consequently, the immobilization of RMT allowed fast in-solution digestion of proteins and their identification by MALDI–TOF mass spectrometry.

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1. Introduction

Due to its spinel structure with two magnetically nonequivalent interpenetrating sublattices, maghemite, $\gamma$-Fe$_2$O$_3$, exhibits a strong magnetic behavior which has been used practically in various biomedical and biological applications including magnetic resonance imaging (MRI) contrast enhancement, biomagnetic separations, hyperthermia treatment or magnetic drug targeting [1–11]. Such an extensive use of maghemite nanoparticles originates from their nontoxicity, biocompatibility, biodegradability, low particle dimension, large surface area and suitable magnetic properties. Nevertheless, for in vivo and in vitro applications, the appropriate surface modification of the particles (functionalization) represents a key point to prevent them from agglomeration, chemical destruction and/or to bind further substances (drugs, cells, etc.) to them [12–18]. The functionalization process can be achieved either in one chemical step [19–21] or in two steps with primary synthesis of high-quality magnetic cores and their secondary surface modification [22,23].
The use of maghemite nanoparticles as a negative contrast agent in MRI constitutes one of the most frequent applications in medicine. SuperParamagnetic Iron Oxides (SPIO), including nanoparticles of maghemite and/or magnetite, enhance T1 but predominantly T2 relaxation time [1,2,4–6]. Advances in MRI including an implementation of high gradient scanners and availability of several contrast agents have led to an increasing use of this diagnostic technique for the evaluation of abdominal diseases such as tumors and inflammatory, especially in children [27]. Moreover, the conventional upper and lower endoscopic techniques allow visualization of only the esophagus, stomach plus duodenum and colon plus terminal ileum, respectively, leaving the larger part of the small bowel indiscernible to the gastroenterologists [28]. On the other hand, noninvasive MRI enables to visualize the small bowel. For the purposes of the MRI bowel diagnostics, the negative contrast agents are more frequently used than the positive ones [29]. Negative bowel agents decrease the extent of noise and motion artifacts related to the bowel peristalsis [27,30]. Among the commercially available negative contrast agents, magnetic iron oxide nanoparticles (Lumirem, Abdoscan) [31–36] are often used for the peroral bowel MRI diagnostics. They are usually coated with an insoluble material (siloxane, polystyrene, etc.) and suspended in viscosity-increasing agents (starch or cellulose) to prevent particles from aggregation and to ensure the homogenous contrast distribution throughout the bowel [1,37]. In this work, we describe a highly efficient negative contrast agent composed of superparamagnetic nearly monodispersed maghemite nanoparticles incorporated in a layered aluminosilicate mineral (bentonite). As proved by the results of clinical tests, such a magnetic composite, prepared in two simple steps, could be a very cheap contrast agent for peroral diagnostics of the small bowel.

Another important bioapplication of iron oxide nanoparticles is based on their use as a magnetic solid support to immobilize different types of biomolecules including enzymes, antibodies, other proteins and oligonucleotides [38,39]. An important example is the immobilization of trypsin [40], which plays a key role in mass spectrometry (MS)-driven proteomics [41]. In general, trypsin (EC 3.4.21.4) is the protease of choice for protein identification and analysis by peptide mass fingerprinting or peptide sequencing because of its efficiency, reliability, and cleavage specificity [42–44]. However, two major drawbacks hamper the application of trypsin in proteomics. The enzyme shows only a marginal thermostability at 37 °C and undergoes a rapid autolysis at basic pH, even in the presence of stabilizing calcium ions [45]. The low thermal stability often limits the application of trypsin in medical and biotechnological processes. A considerable advancement in the enhancement of its thermal stability has been achieved by conjugation with water-soluble polymers or oligosaccharides such as raffinose [45]. Eliminating or reducing the influence of trypsin autolysis in mass spectra can be realized by immobilization of trypsin on a magnetic carrier [41,46]. The immobilization provides many advantages including shorter digestion time, improved storage properties, higher thermal and pH stability, and the possibility of repeated use after magnetic separation from the reaction mixture [47,48]. Up to now, magnetic microparticles have been most frequently used as a magnetic carrier for trypsin [43,49]. To our best knowledge, there are only a few reports dealing with such an application of magnetic nanoparticles [41,50]. The authors used amine-functionalized magnetic nanoparticles as a magnetic carrier, which was consequently covalently attached to trypsin using glutaraldehyde. The immobilized trypsin exhibited an excellent digestion efficiency as demonstrated with the peptide mapping analysis of three model proteins. However, the reflection of the trypsin immobilization to its thermostability has not been reported yet.
A drop of very dilute suspension was placed on a carbon-coated grid and allowed to dry by evaporation at ambient temperature. Scanning electron micrographs were performed on field emission SEM (Hitachi, located at Palacky University in Olomouc, Czech Republic) at 0.7 and 4.0 kV. 57Co Mössbauer spectrum was measured in a constant acceleration mode with a 57Co(Rh) radioactive source. To distinguish between maghemite and magnetite, the measurements were carried out at 20 K (below the blocking temperature of the superparamagnetic nanoparticles) using closed He cycle cooling system (located at Palacky University in Olomouc, Czech Republic). Surface area of the as-prepared nanopowder was determined by nitrogen adsorption at 77.4 K by the static volumetric technique on a Sorptomatic 1990 (Thermo Finnigan) instrument (located at Palacky University in Olomouc, Czech Republic). Thermogravimetric measurements of the initial iron(II) acetate (TG/DTA) were performed in air with a temperature increase of 5 °C/min in the range of 25–400 °C using an Exstar 6000 (Seiko Instruments Inc., located at Palacky University in Olomouc, Czech Republic). A superconducting quantum interference device (SQUID, MPMS XL-7, Quantum Design, located at Palacky University in Olomouc, Czech Republic) was used for the magnetic measurements. Hysteresis loops were recorded at 2 and 300 K in the range of external magnetic fields from –7 T to 7 T.

2.3. Bioapplications of nanocomposites

2.3.1. MRI applications with MB nanocomposites

In the framework of the small bowel clinical research with the bentonite–maghemite nanocomposite contrast agent, 1000 ml of contrast suspension was administrated orally to the patient. In the optimized conditions, the final contrast dispersion consists of 400 mg of maghemite nanoparticles, 4 g of bentonite matrix, 500 ml of fresh fruity juice (HAMI-fresh squeezed apple/carrot juice), 500 ml water and 80 µl of polyethylene glycol (PEG-4000).

MRI examinations were performed on a 1.5 T MRI scanner (GE SIGNA HORIZON Lx., located at MEDIHOPE, Prostejov, Czech Republic). For signal detection, the multiphase array coil TORSOPA was employed. The coil was wrapped around the patient in the prone position both for MRI enterography and for magnetic resonance cholangiopancreatography (MRCP).

For purposes of MRI enterography, the patient was sipping 1000 ml of the prepared contrast agent 60 min before investigation. The measurement was based on the acquisition of a 2D T2SSFSE sequence in coronal and axial planes with the following parameters: axial plane – TR/TE = 2027/79.7 ms, FOV 38 × 38 cm², slice thickness of 5 mm, spacing (sp) 1.0, and matrix 256 × 256; and coronal plane – TR/TE = 2579/255.9 ms, FOV 48 × 48 cm², slice thickness of 5 mm, sp 0.0, and matrix 384 × 320.

In the case of MRCP, the patient was sipping 500 ml of the contrast agent 30 min before investigation. Again, a 2D T2SSFSE sequence was acquired in coronal and axial planes: axial plane – TR/TE = 2500/120 ms, FOV 40 × 40, slice thickness of 5 mm, sp 2.0, and matrix 256×256; coronal plane – slice thickness 20 mm, TR/TE = 6000/500 ms, FOV 34×34, and sp0.0. Both measurements were carried under the breath-hold condition.

The clinical study was approved by the Institutional ethics commission located in Teaching hospital and F.D. Roosevelt Policlinic in Banska Bystrica, Slovakia. Informed consent was obtained from all patients who participated in this clinical study.

2.3.2. Testing of immobilized RMT for protein digestion

The feasibility and performance of the raffinose–modified trypsin immobilized on magnetic carriers (MCRMT composite) were demonstrated by in-solution digestion of bovine serum albumin (BSA). MCRMT composite (1 mg) was dispersed in 500 µl of 50 mmol l⁻¹ NH₄HCO₃ under continuous sonication. A stock solution of BSA (2 mg ml⁻¹) was prepared in 50 mmol l⁻¹ NH₄HCO₃. Before trypsin digestion, the dissolved BSA was denatured by heating at 95 °C for 15 min followed by rapid cooling in a water–ice bath. After that, 10 µl of the BSA solution was put into a test tube and consequently, 20 µl of the MCRMT suspension and 70 µl of 50 mmol l⁻¹ NH₄HCO₃ were added. No reduction/alkylation was performed. After incubation of the reaction mixture at 37 °C for 60 min, MCRMT was separated using NdFeB permanent magnet. The obtained supernatant was analyzed by MS. MALDI probes were prepared by the dried droplet method using an MSP AnchorChip 600/96 microScout target (Bruker Daltonik, located at Palacky University in Olomouc, Czech Republic) and a saturated solution of α-cyano-4-hydroxycinnamic acid in acetonitrile:0.1% TFA (1:2, v/v) as a matrix. Measurements were performed in positive ion reflectron mode on a Microflex MALDI–TOF LR200 mass spectrometer (Bruker Daltonik, located at Palacky University in Olomouc, Czech Republic). The instrument was calibrated externally with a mixture of peptide standards (Bruker Daltonik). Mass spectra were accumulated from 100 to 200 shots at a laser repetition rate of 10 Hz (the m/z range was 500–4000) and processed by FlexAnalysis 2.4 and Biotools 3.0 software (Bruker Daltonik). Protein identification was achieved by database search using the program Mascot Server 2.2 (Matrix Science).

The thermostability of unmodified bovine trypsin, RMT and MCRMT were determined by measuring residual activities after incubation in 20 mmol l⁻¹ sodium acetate buffer, pH 5.25, at different temperatures (20, 37, 45, 55, 65 and 75 °C) for 30 min. Trypsin activity was determined using a chromogenic substrate N-benzoyl-

3. Results and discussion

3.1. Maghemite nanoparticles from iron(II) acetate precursor

Although the thermal decomposition of suitable Fe-bearing precursors in high boiling solvents (thermolysis) represents a frequent approach to synthesize magnetic (magnetite, 

**Fig. 2.** XRD pattern (a) and TEM micrograph (b) of maghemite nanoparticles synthesized from iron(II) acetate precursor. Insets: detail of cubic crystal and SAED pattern.
maghemite) nanoparticles [53–58], the thermally induced solid-state conversions are only rarely applied [59]. This is related with the difficult control of solid-state reactions occurring usually at higher temperatures and thus resulting in polymorphous mixtures and broad size distributions. A solid-state synthetic route, described here, is based on direct oxidative decomposition of iron(II) acetate in air leading to nearly monodispersed single-phase superparamagnetic maghemite nanoparticles. The conversion of the iron(II) acetate takes place in one step as deduced from TG/DTA analysis (not shown) and is completed at about 300°C. The total weight loss of 54.1%, determined from TG curve, corresponds well to the formation of the Fe₂O₃ phase (a theoretical weight loss of 54.1%).

Detailed isothermal experiments performed at selected temperatures in the range of 320–400°C for 1 h proved that maghemite is the only formed Fe₂O₃ polymorph. At 400°C, well-developed cubic shape crystals with a narrow size distribution (20–25 nm) can be prepared in accordance with XRD (see Fig. 2a) and TEM data (Fig. 2b). The cubic spinel structure is further confirmed by selected area electron diffraction (SAED) pattern (see Fig. 2b, insets). The ⁵⁷Fe Mössbauer spectrum of the as-prepared nanopowder, recorded at 25 K (Fig. 3a), reveals a sextet with hyperfine parameters (δ = 0.45 ± 0.01 mm/s, ΔE₀ = 0.00 ± 0.01 mm/s and Bhf = 51 ± 0.3 T) corresponding well to maghemite [59]. In the spectrum, no traces of divalent iron were identified, which excludes the presence of magnetite.

![Mössbauer spectrum measured at 25 K (a) and hysteresis loops taken at 300 and 2 K (b) of the as-prepared maghemite nanoparticles.](image)

![SEM image of maghemite nanoparticles adsorbed at the bentonite surface (a); the inset shows a detailed view of individual maghemite nanoparticles; SEM image of the free unmodified bentonite clay is given for a comparison (b).](image)

![Comparison of the negative contrast in the phantom experiments. Top: MB suspension; and bottom: commercial contrast agent (Lumirem). Right: schematic representation of the different functionalization of SPIO nanoparticles.](image)
The magnetic properties of the as-prepared nanomaghemite were monitored by measurement of hysteresis loops at 2 and 300 K (Fig. 3b). The maximum magnetization \( M_{\text{max}} \) at the highest applied field is 53.18 emu/g, which is lower than that reported for bulk maghemite (84 emu/g) [60]. This phenomenon is usually observed in nanoparticle interacting systems [61]. Such a reduction of \( M_{\text{max}} \) can be ascribed to surface effects arising from broken symmetry and reduced coordination of atoms lying at the surface of the maghemite particle and also to a high degree of interparticle interactions. At 300 K, the hysteresis loop reveals a superparamagnetic character, which means that the maghemite nanoparticles are still not in a blocked state within the time scale of the magnetization measurements.

In summary, we have developed a one-step and cost effective synthetic route allowing to prepare nearly monodispersed superparamagnetic maghemite nanoparticles of well defined structure and cubic shape. The magnetic nanoparticles from the iron(II) acetate precursor can be produced in a large scale and are well dispersible in water. Thus the prepared nanopowder is a promising candidate for the consequent functionalization and applications in biomedicine and biochemistry as discussed below.

3.2. MB nanocomposite as an MRI contrast agent for imaging of gastrointestinal tract

Functionalized superparamagnetic iron oxides (SPIO) are well recognized as negative contrast agents with a high applicability in MRI of the liver, spleen, brain, lymph nodes and macrophages [62,63], where they are used by an intravenous way. Concerning their peroral application in the abdominal diagnostics, there are only two commercial agents commonly used (Lumirem, Abdoscan), which consist of agglomerates of maghemite/magnetite nanoparticles coated by siloxane and polystyrene, respectively. The synthesis of these composites is relatively difficult with a low yield resulting in their high price and, thus, limiting their use in daily diagnostic practice.

To overcome this drawback, we have used a montmorillonite clay (bentonite) as an insoluble matrix to incorporate the as-prepared maghemite nanoparticles from ferrous acetate on the surfaces of the mineral layers [64]. Bentonite is natural, low-cost, readily available and biocompatible aluminosilicate mineral endowed with unique swelling, sorption, ion exchange and intercalation properties. It carries a layer negative charge, which is compensated by ion exchangeable positive cations [65]. The clay is known to be essentially nontoxic and nonadsorbable, established by the Food and Drug Administration as “Generally Recognized As Safe” (GRAS). Moreover, bentonite itself can act as a T2 relaxation agent [66], similarly to SPIO nanoparticles [1]. The introduced simple procedure (see Experimental) based on the attachment of positively charged maghemite nanoparticles to the negatively charged surface of the bentonite clay (in aqueous environment) resulted in a contrast agent with superior properties in imaging of the small bowel.

In the present magnetic nanocomposite, the maghemite nanoparticles are well dispersed on the bentonite surface without any indications of massive agglomeration (see Fig. 4). This fact was also reflected by the homogeneous negative MRI contrast, as proved by the phantom experiments.

Fig. 6. MR enteroclysis in a healthy patient after application of MB contrast agent. T2-weighted image.

Fig. 7. MRCP image of a patient with a tumor of the head of the pancreas acquired without the application of the negative contrast agent (a) and of a patient after hepaticojejunal anastomosis with hepatolithiasis; here the small bowel signal is fully suppressed due to the application of the MB contrast agent (b).
Fig. 5 compares the negative signal intensity of the MB water suspension (top) with a commercial contrast agent – Lumirem (bottom). In the outer flasks, only water, as a blind sample, is used (a white surrounding of the contrast suspensions). For the purposes of the phantom experiments, the optimized concentration of 0.32 g/l of maghemite nanoparticles was used in both cases. As a key conclusion, the negative contrast effect of both phantoms is comparable and very high. The absence of any artifacts in MRI image of the MB phantom allows to assume a homogenous delination of the maghemite nanoparticles incorporated in the bentonite matrix within the whole volume of water in the inner flask.

For the use in clinical diagnostics, it was necessary to optimize the volume and composition of the final dispersion containing, besides the MB contrast agent in water, also a freshly squeezed juice and PEG 4000. The optimized concentrations of all ingredients (see Experimental) resulted from several pre-clinical tests. Among various tested juices, the commercial apple/carrot freshly squeezed juice for children (HAMI brand) was selected as a viscous liquid, good tasting, and healthy drink. Moreover, the MB material is well miscible with this juice and the maghemite nanoparticles are fully dispersed in the resulting suspension. In standard gastrodiagnostics, the contrast agent must not be digested during the medical examination and moreover, it should be fully secreted from the body without any chemical change. Therefore, we used polyethyleneglycol (PEG) as a standard water absorbing agent. The PEG prevents absorption of the water from the bowel lumen through the bowel wall.

The first stage of the clinical research with the MB contrast agent was focused on the evaluation of the contrast effect of the small bowel in healthy patients. Fig. 6 demonstrates an MR enteroclysis obtained in a representative healthy patient from the volunteer group. Evidently, the whole small bowel reveals homogenously reduced signal intensity in T2-weighted images. The lumen is hypo-even anti-tense, aboral side-ileum is homogenously anti-tense. All patients tolerated the contrast agent very well, without any side effects.

In the second stage of clinical tests, we examined a group of 50 patients with various abdominal diseases, mainly with a pancreatic tumor or choledocholithiasis (a disease afflicting the bile duct). In all examined patients, the observed suppression of the small bowel signal (the high-quality homogeneous negative contrast) helped significantly in MRI diagnostics of the particular abdominal field, while various artifacts originated from the small bowel signal were observed in the images without MB application. For illustration, Fig. 7a shows an MRCP image of a patient (women, age 84) with a tumor at the head of the pancreas acquired without contrast agent. There are disturbing hypertensive artifacts visible, which originate from the duodenum loop coinciding with the choledoch and pancreatic terminal (see arrows in Fig. 7a). After MB application, these artifacts are reliably removed. Similar promising results were obtained for a patient after hepaticojejunal anastomosis with hepaticolithiasis (women, age 41), where the gall-stone was much clearly observable (see the dark field inside the ring in Fig. 7b) using the prepared contrast agent (see arrows in Fig. 7b). Now the investigations of the group of patients are being gradually completed and statistically evaluated.

To conclude this part, we have developed an inexpensive negative oral contrast agent, which can be prepared in a large scale in two simple steps. This agent, exploiting the attachment of the superparamagnetic maghemite nanoparticles from ferrous acetate to the surface of the montmorillonite (bentonite) matrix, provides excellent...
negative contrast of the small bowel resulting in superior MRI diagnostics of the adjacent abdominal areas (pancreas and bile duct).

3.3. MCRMT nanocomposite for applications in proteomics

The well-known application of trypsin in proteomics is limited by two key factors including its low thermal stability and rapid autolysis under common working conditions (37°C, pH 8.0). The presence of autolytic trypsin fragments in a protein sample digest makes the interpretation of the MALDI-TOF peptide mass fingerprint more difficult especially when the original sample concentration is low. This has a negative impact on the sample identification by database search. As has been shown, the problem of trypsin autolysis can be eliminated by immobilization of the enzyme on magnetic nanoparticles enabling, in this way, the magnetic separation of the enzyme. Concerning the ways to increase trypsin thermostability, a significant progress in our laboratory has been achieved by its chemical modification with periodate-oxidized raffinose [45].

Here we describe immobilization of a raffinose-modified bovine trypsin (RMT) on the suitably functionalized maghemite nanocarrier with the aim to improve the thermostability of trypsin and to stimulate its performance in digestion of protein samples. Initially, we functionalized the magnetic nanoparticles by chitosan [51] allowing the covalent binding of trypsin via primary amino groups and using glutaraldehyde as a linking agent (see Fig. 1).

A TEM image of the prepared MCRMT complex is shown in Fig. 8a, where the chitosan–RMT shell (2–4 nm) is well observable. Compared to the cubic crystals observed in the TEM image of the as-prepared maghemite nanoparticles (Fig. 2b), the functionalization by chitosan and consequent attaching of trypsin led to rather globular-shaped composite nanoparticles. It is worthy to mention that the chitosan shell itself is relatively narrow and does not considerably affect the morphology of particles [51].

The magnetic characteristics of the MCRMT complex can be clearly deduced from its hysteresis loops measured at 2 and 300 K (Fig. 8b). At 300 K, no hysteresis is observed, indicating that the assembly of MCRMT nanocomposites behaves in a super-paramagnetic manner similarly to the as-prepared maghemite nanopowder. In addition, when comparing the hysteresis loops of maghemite nanoparticles alone (Fig. 3) with that of the MCRMT complex, we can see that the curve for the MCRMT system saturates even at a lower applied magnetic field than that of the starting maghemite nanoparticles. This unambiguously confirms that the chitosan–trypsin shell on the surfaces of the maghemite nanoparticles effectively suppressed the interparticle interactions which generally cause magnetic disorder within the nanoparticle mainly at its surface. Thus, the MCRMT system exhibits a more homogeneous magnetic response towards an applied magnetic fields compared with the as-prepared maghemite nanoparticles.

The key results providing information on the thermostability of the prepared MCRMT complex and on the applicability of the chemically modified and immobilized trypsin are shown in Fig. 9. The temperature dependences of the residual activity for free trypsin, RMT and MCRMT (Fig. 9a) illustrate the gradual increase in the thermostability with the determined $T_{50}$ values of 37, 48 and 61°C, respectively. The value determined for the MCRMT complex is the highest published so far and offers the possibility of substantial shortening of the digestion time with respect to common overnight digestion protocols by an increased reaction rate at higher temperatures (such as 55°C). The ongoing research work is underway in our laboratory.

From the viewpoint of the practical applications of the MCRMT complex, it is very important to emphasize its proved high efficiency in protein digestion and the absence of its autolytic peptides in the reaction mixture. Indeed, we did not register any characteristic autolytic peaks of trypsin in MALDI–TOF MS of a digested standard protein (Fig. 9b).

4. Conclusions

We describe the thermally induced oxidative decomposition of iron(II) acetate in air as a simple and large scale synthetic route towards magnetic iron(III) oxide nanoparticles with a narrow size distribution and well defined cubic shape. The prepared maghemite nanoparticles can be easily attached to the surface of the bentonite clay resulting in a highly efficient negative oral contrast agent for MRI diagnostics of the small bowel and adjacent abdominal areas including pancreas and choledoch. Additionally, we have exploited chitosan-coated maghemite nanoparticles as a carrier for immobilization of raffinose-modified trypsin. Such immobilized trypsin exhibits the highest thermostability published so far and is applicable for rapid protein digestion at 37–55°C and the subsequent peptide analysis by MALDI–TOF MS. Peptide mass fingerprints of
BSA, which was used as a standard protein, show the absence of trypsin autolytic peptides, which makes the trypsin-functionalized magnetic nanocomposite a promising tool for proteome research.

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References


