UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

Luciana Aparecida Corá

A Biosusceptometria AC aplicada à tecnologia farmacêutica

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Tese apresentada ao Instituto de Biociências de Botucatu, Universidade Estadual Paulista "Júlio de Mesquita Filho", como exigência parcial para obtenção do Título de Doutor em Ciências Biológicas (Área de Concentração: Farmacologia).

Orientador: Prof. Adj. José Ricardo de Arruda Miranda

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Resumo e Abstract

"Considero feliz aquele que quando se fala de êxito busca a resposta em seu trabalho." Emerson

Resumo

Corá, L.A. **A Biosusceptometria AC aplicada à tecnologia farmacêutica.** 2008. 84 p. Tese Doutorado – Instituto de Biociências de Botucatu, Universidade Estadual Paulista "Júlio de Mesquita Filho".

A administração oral de drogas é uma prática comum na terapia e as formas farmacêuticas sólidas são amplamente utilizadas. A variação no perfil de absorção ao longo do trato gastrintestinal (TGI) humano e a possibilidade de liberar drogas em diferentes regiões são os maiores desafios para o desenvolvimento de novos produtos. Desse modo, avaliar formas farmacêuticas sólidas *in vivo* fornece um entendimento mais profundo quando um efeito sistêmico ou local é desejado. Geralmente, estes estudos são realizados por meio da cintilografia e técnicas biomagnéticas. A Biosusceptometria de Corrente Alternada (BAC) é uma técnica que merece destaque por suas características, acurácia dos resultados obtidos e versatilidade. A BAC propiciou imagens do processo de desintegração de comprimidos tanto *in vitro* quanto no estômago humano, introduzindo outra perspectiva na análise desse processo. Os resultados também foram correlacionados com sucesso com aqueles obtidos por metodologias específicas, garantindo uma análise mais acurada dos parâmetros físicos envolvidos com a desintegração de comprimidos. A utilização da BAC permitiu avaliar a motilidade gastrintestinal e o processo de desintegração de cápsulas de hidroxipropilmetilcelulose (HPMC) revestidas no cólon humano. Além disso, também foi possível investigar a influência do estado prandial no esvaziamento gástrico e no trânsito gastrintestinal de um sistema multiparticulado magnético. Todos esses trabalhos fortaleceram a BAC como um método alternativo na pesquisa farmacêutica demonstrando seu potencial para avaliar diferentes processos, apesar das suas limitações. Sintetizando, a BAC é uma ferramenta valiosa, com a vantagem de ser livre de radiação e inócua aos voluntários, e vasta aplicabilidade na pesquisa farmacêutica, farmacológica e fisiológica.

Palavras-chave: Biomagnetismo, trânsito gastrintestinal, formas farmacêuticas sólidas, desintegração.

Abstract

Corá, L.A. **AC Biosusceptometry applied to pharmaceutical technology.** 2008. 84 p. Tese Doutorado – Instituto de Biociências de Botucatu, Universidade Estadual Paulista "Júlio de Mesquita Filho".

Oral administration is widely accepted route for drug delivery and solid dosage forms are commonly administered. The variation of absorption profiles along the human gastrointestinal tract (GIT) and the ability to target drugs by adequate dosage forms to distinct sites is the challenge in the pharmaceutical development of solid dosage forms. An understanding of the factors involved in drug absorption and how the gastrointestinal variables can interfere with this process is important to develop more reliable drug delivery systems. The performance of pharmaceutical dosage forms must be fully investigated *in vivo* to provide more reliable information when a local or systemic effect is desirable. Generally, *in vivo* investigation on the behavior of dosage forms has been made by using gamma-scintigraphy and biomagnetic techniques. AC Biosusceptometry (ACB) deserves consideration due to its features, accuracy and versatility. By using ACB technique, it was possible to monitor the disintegration process through acquisition of magnetic images *in vitro* and in human stomach. The results also were successfully correlated with those obtained with standard methods which provided a more reliable analysis on the physical parameters involved in the disintegration process of tablets. ACB allowed evaluating the gastrointestinal motility and the disintegration of hydroxipropylmethylcellulose (HPMC) coated capsules in human colon. Moreover, it was possible to investigate the gastric emptying and gastrointestinal transit of a magnetic multiparticulate system under influence of prandial state. All these studies have contributed to establish the ACB as an alternative method for pharmaceutical research and, despite some limitations, it was feasible to evaluate different pharmaceutical processes. In summary, ACB is a radiation free and non-invasive technique with wide applicability in pharmaceutical, physiological and pharmacological researches.

Key words: Biomagnetism, gastrointestinal transit, solid dosage forms, disintegration.

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Introdução

"A mente que se abre a uma nova idéia jamais voltará ao seu tamanho original."
Albert Einstein

Introdução

Considerando que as variáveis fisiológicas referentes ao trato gastrintestinal (TGI) humano influenciam significativamente a absorção e a biodisponibilidade de fármacos administrados por via oral, tornou-se imperativo implementar métodos de análise capazes de caracterizar o comportamento de formas farmacêuticas *in vivo*. Nesse contexto, os métodos fundamentados no Biomagnetismo, destacando a Biosusceptometria AC (BAC), tornaram-se alternativas viáveis para a pesquisa farmacêutica. A inserção da BAC nesse campo de estudo veio de encontro à necessidade de utilizar metodologia de baixo custo, livre de radiação ionizante, não invasiva e capaz de avaliar processos farmacêuticos não apenas *in vitro*, como também no TGI humano.

O contínuo aperfeiçoamento da BAC, culminando no desenvolvimento do sistema com multisensores, foi fundamental para aplicá-la na pesquisa farmacêutica. A análise do processo de desintegração de comprimidos fortaleceu-se com a possibilidade de obter imagens magnéticas, introduzindo um novo conceito em imagens biológicas (Capítulo 1). Associando-se a BAC com metodologias específicas foi possível realizar uma análise mais acurada dos parâmetros físicos envolvidos no processo de desintegração de comprimidos (Capítulo 2). A BAC permitiu, também, avaliar a motilidade gastrintestinal e a desintegração de cápsulas de hidroxipropilmetilcelulose (HPMC) no cólon humano (Capítulo 3). Além disso, possibilitou a investigação da influência do estado prandial no esvaziamento gástrico e no trânsito gastrintestinal de um sistema multiparticulado magnético enfocando a liberação colônica (Capítulo 4).

Esses trabalhos mostraram que a BAC é um método capaz de prover os requisitos necessários para monitorar diferentes processos farmacêuticos visando uma análise mais detalhada dos complexos parâmetros fisiológicos e farmacêuticos conhecidos por influenciarem a liberação e a absorção de drogas.

1. A farmacotécnica e o trato gastrintestinal humano

Ao administrar um fármaco devem ser considerados fatores como a via de administração que será mais efetiva e melhor aceita pelo paciente, além da forma farmacêutica apropriada (Ansel et al., 2000). A administração de drogas por via oral é amplamente utilizada, sendo o trato gastrintestinal (TGI) o principal acesso à circulação sistêmica. Apesar de apresentar algumas desvantagens, essa via é a preferida por oferecer maior comodidade e permitir o estabelecimento de esquemas terapêuticos fáceis de serem cumpridos (Jivraj et al., 2000; Sastry et al., 2000).

Em se tratando da administração por via oral, as formas farmacêuticas sólidas são muito utilizadas devido à relativa facilidade de obtenção, ao custo reduzido e à estabilidade (Jivraj et al., 2000). No entanto, os parâmetros relacionados ao TGI interagem com a forma farmacêutica e, conseqüentemente, influenciam os processos de liberação, absorção e biodisponibilidade do fármaco (Rouge et al., 1996; Martinez & Amidon, 2002). Assim, compreender como esses parâmetros e variáveis fisiológicas podem alterar a performance de uma forma farmacêutica *in vivo* é fundamental para o desenvolvimento de produtos com maior eficácia terapêutica e menor incidência de efeitos colaterais (Zahirul & Khan, 1996).

Para a absorção de um fármaco administrado por via oral são considerados processos que incluem desde a liberação da droga, por meio da desintegração da forma farmacêutica, até sua dissolução no meio, de com propriedades físico-químicas como solubilidade e coeficiente de partição (Lipka & Amidon, 1999). Além disso, as características fisiológicas do TGI, como tamanho da superfície de absorção, perfil do pH nas diferentes regiões, as taxas de esvaziamento gástrico e trânsito intestinal e a motilidade gastrintestinal são os principais fatores que influenciam diretamente a biodisponibilidade do fármaco e podem, ainda, limitar a fração da dose que será absorvida (Rouge et al., 1996).

O TGI humano é um meio complexo, que apresenta diferenças regionais bastante acentuadas as quais devem ser completamente investigadas para o desenvolvimento de um produto farmacêutico, seja para exercer um efeito local ou sistêmico.

1.1 Estômago, intestino delgado e cólon

O estômago humano é um órgão subdivido em dois compartimentos funcionais: enquanto a região proximal atua como reservatório para acomodar o conteúdo ingerido, a região distal é responsável pela trituração desse conteúdo e sua mistura com as secreções gástricas (Camilleri, 2006). No entanto, o processo de absorção no estômago é limitado, pois apresenta uma camada mucosa bastante espessa e uma superfície reduzida (Hörter & Dressman, 2001). Assim, a ação coordenada das regiões gástricas contribui para a otimização do aproveitamento dos alimentos reduzindo os alimentos sólidos a pequenas partículas e regulando precisamente a velocidade de transferência para o intestino delgado (Hasler, 1999). Por outro lado, o intestino delgado possui uma superfície extremamente ampla cujas propriedades fisiológicas facilitam a absorção de nutrientes e, conseqüentemente, de muitos fármacos (Hörter & Dressman, 2001; Masaoka et al., 2006).

No cólon proximal, e em menor grau nos demais segmentos, os padrões motores estão amplamente vinculados à sua função de propulsão, absorção de água e eletrólitos. Esse segmento apresenta movimentos lentos e coordenados que facilitam a absorção, além de propiciar o crescimento, no lúmen colônico, de microorganismos capazes de facilitar a absorção de certos nutrientes para os próprios colonócitos (Christensen, 1987; Camilleri & Ford, 1998). Até há pouco tempo, essas eram as únicas funções atribuídas a esse segmento. Atualmente, esse órgão vem ganhando destaque como um local específico para liberação de fármacos, proteínas e peptídios com potencial terapêutico (Chourasia & Jain, 2003; Shareef et al., 2003; Freire et al., 2006a). Comparando-se com o intestino delgado, o cólon tem uma superfície de absorção menor, fato que é compensado pelo trânsito mais lento, o que proporciona uma excelente oportunidade para a absorção de drogas e outros materiais.

1.2 Variáveis fisiológicas e farmacêuticas

A taxa e a extensão da absorção de fármacos administrados por via oral são determinadas por parâmetros relacionados ao TGI e à forma farmacêutica. Dentre os parâmetros fisiológicos, destacam-se a motilidade gastrintestinal, o estado prandial, o esvaziamento gástrico, o trânsito intestinal e a variação do pH ao longo do TGI (Singh, 1999; Kimura & Higaki, 2002; Martinez & Amidon, 2002). Além dos fatores fisiológicos, a solubilidade do fármaco e o tamanho das partículas, bem como as características da forma farmacêutica, como friabilidade, dureza, tipo de revestimento e densidade também podem interferir no processo de liberação e absorção de drogas (Jenquin et al., 1990; Hancock et al., 1997; Jain, 1999).

A motilidade do trato gastrintestinal humano

Dentre as propriedades funcionais do TGI destaca-se a motilidade, que é a capacidade de contrair e relaxar a sua musculatura para misturar e propelir o material ao longo do seu comprimento (Nguyen et al., 2007). A motilidade gastrintestinal é organizada, basicamente, de acordo com o estado prandial (Quigley, 1996; Camilleri, 2006). Com o término do processo digestivo tem início uma atividade motora cíclica denominada Complexo Motor Migratório (CMM), que alterna ciclos de atividade contrátil e quiescência (Fig. 1).

A fase I do CMM é caracterizada por um período de quiescência motora; a fase II apresenta atividade de contração fásica irregular e a fase III caracteriza-se por um período de intensas contrações rítmicas, conhecidas por “ondas de limpeza” (*housekeeper waves).* A fase IV representa a transição entre o período de atividade mais intensa e o de completa quiescência, onde o ciclo recomeça. As ondas contráteis do CMM propagam-se distalmente, do estômago ao íleo terminal, e são interrompidas com a ingestão de alimentos.

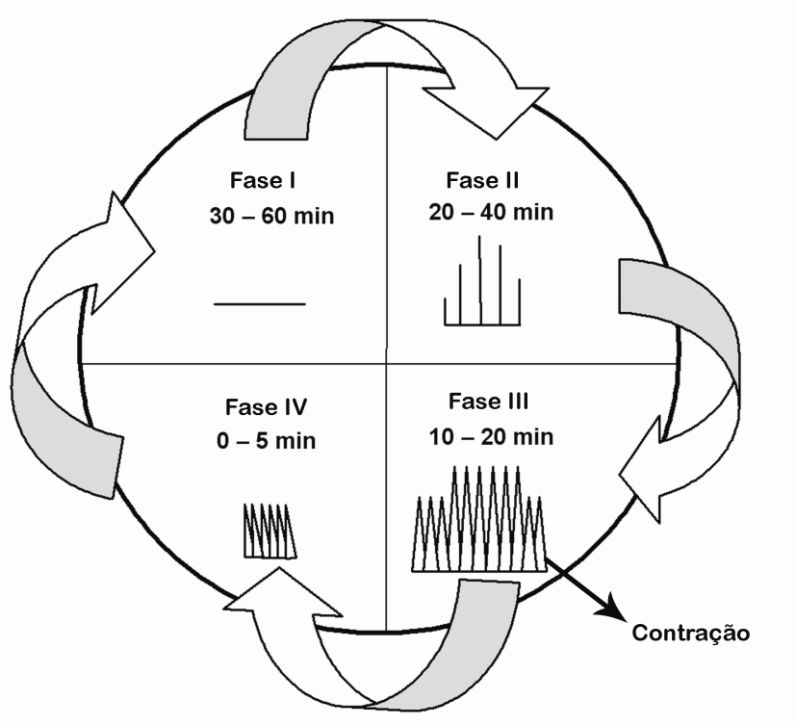

Fig. 1- Motilidade gastrintestinal no período interdigestivo – Complexo Motor Migratório (CMM) (*Modificado de Chawla et al., 2003*).

Com a ingestão de alimentos inicia-se o período pós-prandial, em que a freqüência e a amplitude da atividade de contração variam de acordo com o segmento do TGI, sendo que este período persiste até que o estômago esvazie completamente seu conteúdo (Hasler, 1999). Considerando que apenas durante a fase III ocorre o esvaziamento gástrico de partículas sólidas indigeridas, é a motilidade do TGI humano que determina o tempo de trânsito e de retenção de formas farmacêuticas sólidas em diferentes segmentos, interferindo desse modo, no processo de liberação e absorção do fármaco.

Esvaziamento gástrico

O esvaziamento gástrico é controlado por mecanismos coordenados que promovem alterações no tônus e na peristalse, sendo influenciado pela viscosidade, conteúdo calórico e volume da refeição ingerida (Simonian et al., 2004; Burton et al., 2005; Hellström et al., 2006).

Quando um líquido pouco calórico é ingerido, ocorre sua distribuição nas duas regiões gástricas e o esvaziamento inicia-se imediatamente, sendo descrito por uma exponencial de primeira-ordem, que é diretamente proporcional ao volume. Por outro lado, o esvaziamento de partículas sólidas é caracterizado por uma fase de atraso (*lag phase)*, na qual essas partículas ingeridas são redistribuídas para serem trituradas em partículas menores e propelidas em direção ao duodeno durante uma fase linear. Essa natureza bifásica caracteriza o esvaziamento gástrico de sólidos (Holt et al., 1982; Siegel et al., 1988; Ziessman et al., 1996).

Neste contexto, o esvaziamento gástrico tem um papel fundamental na determinação do tempo de retenção de uma forma farmacêutica sólida no estômago. As características físicas da forma farmacêutica, como a densidade e o tamanho, a ingestão de alimentos ou a administração concomitante de drogas que afetam a motilidade, além de fatores biológicos como idade, postura, índice de massa corporal, atividade física e algumas doenças constituem os principais parâmetros que alteram o esvaziamento gástrico (Davis et al., 1986; Dressman et al., 1993; Kuo et al., 2008).

Um comprimido com revestimento gastro-resistente pode permanecer retido no estômago durante todo o período digestivo, sendo que seu esvaziamento ocorrerá apenas durante a fase III do CMM. Por outro lado, sistemas multiparticulados podem ser esvaziados do estômago gradualmente, independente do CMM. Prolongar o tempo de retenção gástrica de uma forma farmacêutica constitui uma excelente abordagem para a absorção de drogas, especialmente para aquelas que são melhor absorvidas no TGI superior (Chawla et al., 2003; Talukder & Fassihi, 2004; Davis, 2005; Streubel et al., 2006).

Trânsito intestinal e côlonico

O trânsito em diferentes segmentos do TGI determina quanto tempo a forma farmacêutica permanece em contato com a superfície absortiva. A propulsão do conteúdo do intestino delgado depende do tônus da parede intestinal e da amplitude das contrações (Quigley, 1996). Similar ao esvaziamento gástrico, o trânsito intestinal também é influenciado pela atividade motora do CMM e pode ser dependente, também, do tipo de forma farmacêutica administrada e do estado prandial (Davis et al., 1986; Coupe et al., 1991).

O trânsito colônico, por sua vez, mostra-se significativamente variável sendo influenciado por fatores como a dieta e determinadas doenças (Frexinos & Delvaux, 1993; Price et al., 1993), além do tipo de forma farmacêutica. Em relação à forma farmacêutica, há uma vantagem em formular sistemas multiparticulados em detrimento dos monolíticos quando o alvo para liberação de drogas for o cólon, pois o trânsito de péletes é mais lento, assegurando que toda a droga será liberada e absorvida (Wilding et al., 2000; Asghar & Chandran, 2006; Freire et al., 2006b).

pH e fluidos gastrintestinais

A absorção da droga bem como a resposta clínica, depende da sua solubilidade nos fluidos gastrintestinais e, também, da superfície de absorção. A solubilidade, o pKa do fármaco, o pH do meio, a concentração do fármaco e a área da superfície de absorção são os principais fatores que influem na absorção de fármacos (Dressman et al., 1998). A taxa de dissolução de uma droga é uma função da superfície, do coeficiente de difusão e dos componentes do meio de dissolução (Corrigan et al., 2003; Azarmi et al., 2007). A variabilidade observada nos constituintes do fluido gastrintestinal tais como eletrólitos, enzimas e ácidos biliares, podem afetar a dissolução e, conseqüentemente, a absorção e biodisponibilidade de um fármaco (Lindahl et al. 1997).

Como a variação do pH ao longo do TGI humano também interfere com a solubilidade das drogas, pode ser explorada como uma alternativa à liberação de drogas de uma maneira controlada (Dittgen et al., 1997; Badawy & Hussain, 2007). A dieta, algumas doenças, ácidos graxos e outros produtos da fermentação colônica são responsáveis por uma expressiva variação de pH inter e intra- indivíduos (Evans et al., 1988; Dressman et al., 1990).

2. Formas Farmacêuticas Sólidas

Do ponto de vista tecnológico, as formas farmacêuticas sólidas como cápsulas, comprimidos e péletes, revestidos ou como matrizes hidrofílicas, são comumente utilizadas em detrimento de outras vias de administração (Pezzini et al., 2007).

Comprimidos são formas farmacêuticas sólidas convencionais que podem ser obtidos por granulação ou compressão direta, sendo a escolha do método dependente das características do princípio ativo que será utilizado (Ansel et al., 2000). A compressão direta consiste na mistura e compactação dos pós que, por sua vez, implica na redução do volume e no aumento da força mecânica, devido às interações entre as partículas (Ansel et al., 2000). Caracteriza-se por ser um método simples e econômico, pois requer menos tempo para o preparo da formulação, visto que envolve um menor número de etapas e unidades operacionais (Jivraj et al., 2000). Embora os princípios que governam a compressão direta sejam conhecidos há anos, apenas recentemente a técnica tornou-se mais estabelecida. Isso ocorreu devido à introdução de excipientes especificamente desenvolvidos, os quais apresentam, essencialmente, fluidez e compressibilidade, características exigidas para a obtenção de comprimidos por este método (Jivraj et al., 2000, Pifferi & Restani, 2003).

Cápsulas são formas farmacêuticas sólidas onde uma ou mais substâncias medicinais ou inertes são acondicionadas em um invólucro à base de gelatina ou derivados da celulose, como a hidroxipropilmetilcelulose (Ogura et al., 1998; Ansel et al., 2000). As cápsulas são bastante versáteis, possuem diversos tamanhos e podem ser preenchidas por uma grande variedade de produtos como grânulos, pós e péletes.

São formas farmacêuticas comuns na administração oral de medicamentos e apresentam como vantagens, em relação aos comprimidos, o processo de fabricação mais simples e com um menor número de etapas envolvidas. Assim como ocorre na produção de comprimidos, os excipientes são constituintes essenciais na obtenção do produto encapsulado, pois o princípio ativo e os excipientes devem constituir uma mistura homogênea e compatível (Ansel et al., 2000).

Péletes apresentam diversas formas e tamanhos e podem ser produzidos por diferentes processos que incluem a granulação, extrusão/esferonização ou revestimento de núcleos inertes, sendo que a seleção do método depende de fatores como custo, perfil de liberação desejado e propriedades do fármaco (Gandhi et al., 1999; Asghar and Chandran, 2006; Pezzini et al., 2007). Apesar da complexa produção e do alto custo, sistemas multiparticulados apresentam vantagens tecnológicas e biofarmacotécnicas que merecem consideração. Dentre essas vantagens, permitem veicular substâncias incompatíveis e dosagens diferentes para um mesmo produto. Além disso, observa-se menor variabilidade intra e inter-indivíduos, com risco reduzido de irritação da mucosa do TGI e menor flutuação na concentração plasmática (Asghar and Chandran, 2006). Associados às cápsulas gelatinosas duras ou de hidroxipropilmetilcelulose, esses péletes oferecem uma solução altamente flexível para tratamento específico, pois são passíveis de revestimento não apenas para a modulação da liberação, mas também para a proteção de fármacos instáveis (Pezzini et al., 2007).

A liberação do princípio ativo contido em uma forma farmacêutica sólida ocorre por meio do processo de desintegração (Fig. 2). A desintegração é caracterizada como um processo tempo-dependente que ocorre sob ação de um desintegrante e promove a fragmentação da forma farmacêutica em partículas passíveis de serem dissolvidas e absorvidas (Lowenthal, 1972; Melia & Davis, 1989). Se este processo for lento ou incompleto, a biodisponibilidade da droga será comprometida, portanto, a escolha dos excipientes apropriados é fundamental durante o desenvolvimento da formulação (Lipka & Amidon, 1999).

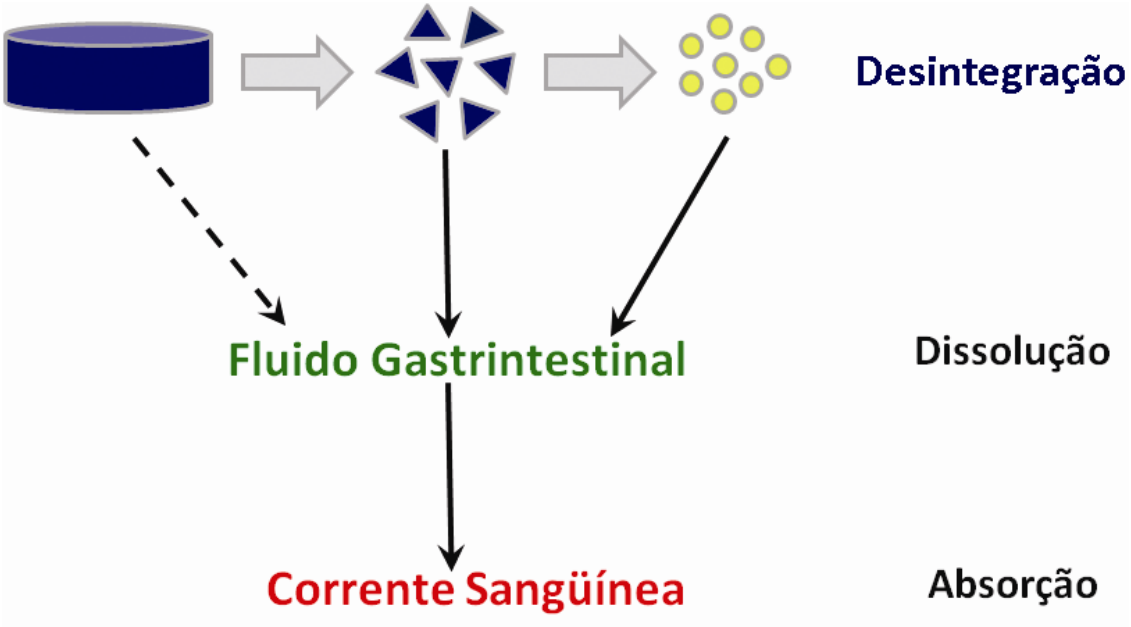

Fig. 2- Etapas envolvidas na biodisponibilidade de uma droga a partir da administração oral de uma forma farmacêutica sólida. A desintegração promove a fragmentação em partículas que serão dissolvidas no meio e absorvidas pela mucosa do TGI. (*Modificado de Melia & Davis, 1989*).

Como o processo de desintegração está intimamente relacionado com a liberação da droga, é imprescindível que o processo de desintegração ocorra numa razão tal que possibilite a dissolução do ativo no meio, bem como sua absorção. Atualmente, a indústria farmacêutica dispõe de desintegrantes cada vez mais efetivos e que desempenham um papel fundamental no controle de qualidade de seus produtos. Esses desintegrantes são importantes excipientes na obtenção de formas farmacêuticas sólidas, sobretudo os comprimidos, pois são compostos insolúveis com alta capacidade de intumescimento, decorrente da hidrofilidade, (Zhao & Augsburger, 2005) e atuam por diferentes mecanismos, incluindo a expansão das partículas, efeitos de capilaridade, interações partícula-partícula e desintegração por camadas (Lowenthal, 1972; Rudinic et al., 1982; Schmidt & Zessin, 1997; Zhao & Augsburger, 2005). Para a maioria dos desintegrantes a penetração de água na forma farmacêutica é um fator determinante para um bom desempenho, visto que resulta no intumescimento das partículas e no desenvolvimento da força que auxilia o processo de desintegração (Colombo et al., 1984; Van Kamp et al., 1986; Caramella et al., 1988; Massimo et al., 2000).

Ainda em relação ao desenvolvimento das formas farmacêuticas, um dos maiores progressos alcançados foi a possibilidade de controlar ou modificar a liberação de drogas no TGI humano (Ranade, 1991; Urquhart, 2000). As formas farmacêuticas convencionais são desenvolvidas para liberar o fármaco rapidamente após a administração. Por outro lado, formas farmacêuticas de liberação modificada são produzidas para modularem a liberação do fármaco, prolongando ou retardando sua dissolução (Pezzini et al., 2007). De um modo geral, essas formas farmacêuticas promovem a liberação do fármaco gradualmente e, assim, possibilitam a manutenção da mesma concentração terapêutica no plasma reduzindo flutuações, ou seja, evitam níveis sub-terapêuticos ou tóxicos, além de propiciarem uma redução na freqüência de administração, facilitando a adesão ao tratamento (Li et al., 1987; Kannan et al., 2003).

A qualidade de um produto será assegurada se houver um equilíbrio entre a escolha dos excipientes, do método de produção e dos perfis de liberação e dissolução do fármaco. O maior desafio é desenvolver uma forma farmacêutica cuja liberação e resposta clínica do fármaco possa ser monitorada por meio do estabelecimento de uma correlação *in vitro-in vivo* (Emami, 2006).

3- Métodos para avaliar formas farmacêuticas *in vivo*

Apesar dos diferentes métodos de análise consolidados pelas principais farmacopéias, nenhum deles é capaz de simular *in vitro* um meio tão complexo quanto o TGI humano (Zahirul & Khan, 1996; Lipka & Amidon, 1999). Assim, houve a necessidade de implementar métodos de análise capazes de caracterizar o comportamento dessas formas farmacêuticas *in vivo* por meio do desenvolvimento de técnicas não invasivas.

A Cintilografia sempre foi considerada como a técnica padrão para monitorar formas farmacêuticas sólidas no TGI humano (Wilding et al., 2001). Essa técnica fornece informações sobre o trânsito gastrintestinal de comprimidos, cápsulas, sistemas de liberação controlada de drogas sendo, também, associada à farmacocinética para avaliar o perfil de liberação de drogas (Kenyon et al., 1997;

Wilding et al., 2000; Brunner et al., 2003). Basicamente, essa técnica envolve a marcação da forma farmacêutica com um radionuclídeo que emite radiação gama, sendo seu acompanhamento realizado pela gama-câmara (Wilding et al., 2001). Além da radiação ionizante, outras desvantagens da Cintilografia incluem a complicada preparação e o custo dos radiofármacos utilizados nessas formulações e a impossibilidade de fornecer a localização anatômica precisa da forma farmacêutica.

A implementação de métodos fundamentados no Biomagnetismo para monitorar de maneira não invasiva formas farmacêuticas sólidas constitui, atualmente, uma alternativa à medicina nuclear para a pesquisa farmacêutica (Corá et al., 2008a). Há sensores magnéticos altamente sensíveis capazes de medir os campos magnéticos resultantes da atividade elétrica associada aos movimentos dos íons ou dos materiais magnéticos em resposta a um campo magnético aplicado externamente (Williamson & Kaufman, 1981). Freqüentemente, os materiais magnéticos, quando empregados em medidas biomédicas, são agrupados em traçadores ou marcadores magnéticos, de acordo com a sua forma de apresentação. Os traçadores magnéticos são definidos como partículas do material magnético dispersas em um meio, enquanto nos marcadores as partículas estão contidas em uma forma farmacêutica sólida (Américo, 2008). Geralmente, as ferritas e magnetitas são materiais magnéticos muito utilizados por serem inertes e inócuos ao indivíduo (Bahadur & Giri, 2003). Dentre as técnicas que utilizam esses princípios, destacam-se: os Dispositivos Supercondutores de Interferência Quântica (*SQUID*), os Sensores Anisotrópicos Magneto-resistivos (*AMR*), a Ressonância Magnética (*MRI*) e a Biosusceptometria de Corrente Alternada (*BAC*).

Empregando-se o SQUID é possível determinar a localização, a orientação e a evolução temporal do marcador magnético, com informações sobre o tempo de trânsito gastrintestinal da forma farmacêutica (Weitschies et al., 2005a). Por ser um método altamente sensível, o SQUID tem como principal desvantagem o alto custo de manutenção, além de ser pouco viável para estudos de desintegração.

O efeito magneto-resistivo baseia-se na alteração da resistividade elétrica de um material provocada pela aplicação de um campo magnético (Kwiatkowski & Tumanski, 1986). Os sensores AMR foram utilizados para monitorar o trânsito gastrintestinal de marcadores magnetizados, bem como o processo de desintegração de comprimidos (Weitschies et al., 2005b). No entanto, como a desintegração

promove a perda do momento magnético do marcador, nenhuma informação adicional pode ser adquirida pelos sensores AMR após o processo.

Recentemente, a MRI foi introduzida na pesquisa farmacêutica em alguns poucos estudos envolvendo a caracterização de novas formulações (Richardson et al., 2005) e monitoramento de um sistema de liberação de drogas baseado na propriedade de gastro-retenção em humanos (Steingöetter et al., 2003). Apesar das imagens de altíssima resolução, a MRI apresenta alguns inconvenientes como a dificuldade de posicionamento dos voluntários e a alta incidência de artefatos de movimento. Além disso, o alto custo de aquisição e manutenção do equipamento restringe sua utilização na pesquisa básica.

Biosusceptometria AC

Nos últimos anos, a Biosusceptometria de Corrente Alternada (BAC) despontou como uma técnica alternativa para estudos enfocando a motilidade gastrintestinal (Baffa et al., 1995; Miranda et al., 1997; Romeiro et al., 2006; Américo et al., 2007) bem como a pesquisa farmacêutica (Corá et al., 2005a). Essa técnica utiliza bobinas de indução para registrar a variação temporal do fluxo magnético obtida como resposta de um material ferromagnético. Esse material tem como principal característica uma alta susceptibilidade magnética (χ) e, por isso, produz uma resposta intensa quando um campo magnético é aplicado ao meio biológico.

Essencialmente, a BAC é constituída por um conjunto de sensores que apresentam dois pares de bobinas de indução separadas por uma linha de base fixa, sendo cada par de bobinas composto por uma bobina de excitação e uma bobina de detecção (Fig. 3).

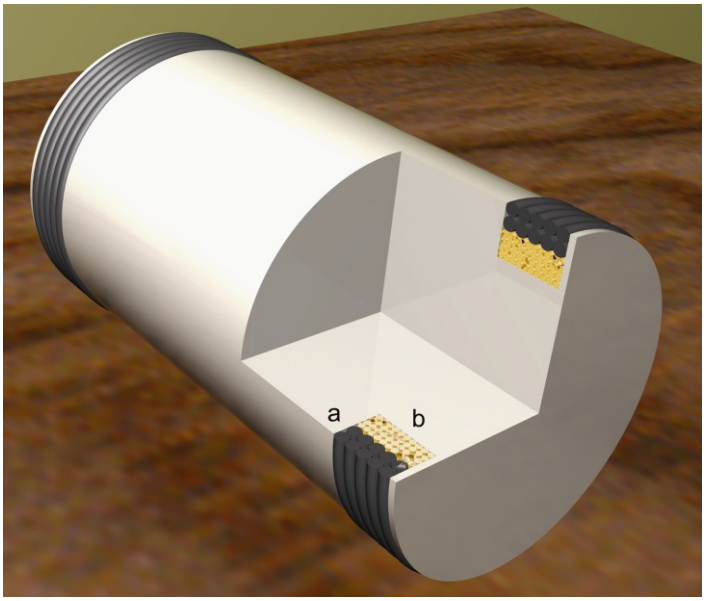

Fig. 3- Sensor magnético constituído por dois pares de bobinas de indução. (a) Bobina de excitação externa e (b) bobina de detecção interna.

Essas bobinas de detecção estão arranjadas em uma configuração gradiométrica de primeira-ordem e dispostas em um arranjo coaxial, ou seja, a bobina de excitação é externa, enquanto a bobina de detecção é interna. Essa configuração consiste no uso de duas bobinas em série, enroladas em sentidos contrários, de modo que, os fluxos magnéticos concatenados em cada bobina sejam subtraídos, eliminando os ruídos ambientais e tornando-as mais sensíveis (Miranda et al., 1992).

Portanto, o sensor é montado como um transformador duplo de fluxo magnético, com núcleo de ar, sendo que o par de bobinas (excitação/ detecção) localizado mais distante do material magnético atua como transformador de referência e o par mais próximo do material como transformador de medida (Fig. 4). A bobina de excitação induz fluxo magnético na bobina de detecção e, ao aproximar esse par do material magnético, ocorre um desbalanceamento na voltagem. Desse modo, a diferença de fluxo magnético entre as bobinas de detecção pode ser monitorado. O sinal detectado depende da área das bobinas de detecção, do número de voltas, da freqüência de excitação, da intensidade do campo magnético aplicado, da quantidade de material magnético e da distância entre o sensor e o material.

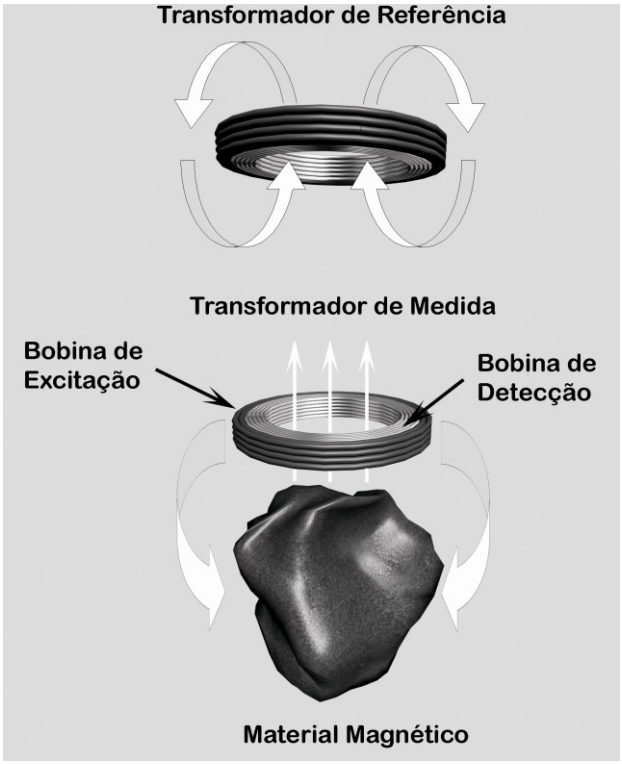

Fig. 4- Esquema de funcionamento do sensor magnético. A bobina de excitação induz fluxo magnético na bobina de detecção que ao ser aproximada do material magnético promove um desbalanceamento no fluxo magnético entre as bobinas, permitindo seu monitoramento.

Estes conceitos foram utilizados no desenvolvimento de um sistema de Biosusceptometria AC com multisensores, cuja finalidade foi aumentar a resolução espacial e a sensibilidade para aplicações farmacêuticas. O sistema com multisensores possui um par de bobinas de excitação e sete pares de bobinas de detecção para aquisição dos sinais em pontos distintos (Fig. 5). Esse arranjo foi fundamental para monitorar o processo de desintegração tomando por princípio a transição de um marcador para um traçador magnético. Quando a forma farmacêutica sólida é caracterizada como um marcador, os sinais registrados pela BAC são pontuais, concentrados apenas nos sensores que estiverem mais próximos. A desintegração promove o espalhamento do material magnético e uma distribuição desses sinais que será, a partir desse momento, detectado por todos os outros sensores (Corá et al., 2003). Além da resolução espacial, o sistema com multisensores também proporcionou um aumento na sensibilidade.

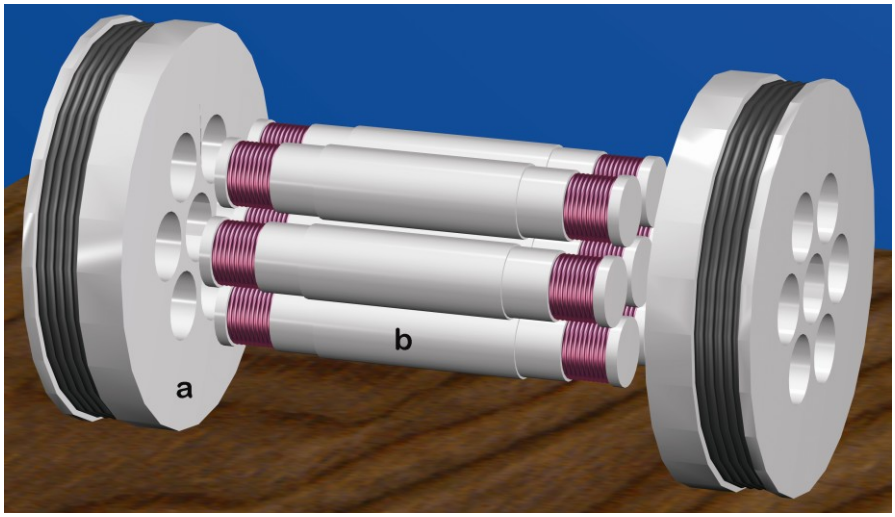

Fig. 5- Sistema de Biosusceptometria AC com multisensores mostrando o par de bobinas de excitação (a) e os sete pares de bobinas de detecção (b).

A BAC aplicada à pesquisa farmacêutica proporcionou outra abordagem referente ao processo de desintegração de comprimidos e cápsulas *in vitro* e no TGI humano (Corá et al., 2003; 2006a,b). Refletindo seu constante aperfeiçoamento, a BAC também demonstrou um grande potencial para obtenção de imagens magnéticas destas formas farmacêuticas, introduzindo um novo conceito em imagens dos sistemas biológicos (Corá et al., 2005b). Os sinais registrados pela BAC são representados por matrizes temporais e utilizando-se ferramentas matemáticas, é possível obter imagens seqüenciais provenientes de intervalos de tempo pré-definidos (3 segundos cada um). Uma vez calculadas, essas seqüências de imagens são submetidas ao processamento digital para subtração de *background,* ajustes de brilho e contraste e segmentação. Por meio da segmentação das imagens é possível quantificar, em número de pixels, a variação temporal da área da imagem magnética no decorrer do processo, possibilitando a análise de processos como a desintegração e o espalhamento de material magnético em uma determinada região do TGI.

Diante dos bons resultados, novas perspectivas ao estudo de formas farmacêuticas sólidas permitiram iniciar um trabalho cujo foco principal foi o controle de qualidade na indústria farmacêutica. De maneira inédita, a BAC foi associada a dois métodos de análise convencionais para investigar a influência da força de compressão na penetração de água e desenvolvimento de força durante a

desintegração de comprimidos efervescentes (Corá et al., 2008b). Além disso, essa técnica também foi utilizada para monitorar o esvaziamento gástrico e o trânsito intestinal de um sistema multiparticulado alterando-se o estado prandial do voluntário (Miranda et al., submetido para publicação). Além de ser um método não invasivo e livre de radiação ionizante, a BAC não requer ambiente magneticamente blindado e possui um custo de implementação relativamente baixo, comparando-se com as outras técnicas biomagnéticas.

Considerando o desenvolvimento de um novo produto ou o refinamento de uma forma farmacêutica já existente, torna-se imperativo dispor de uma técnica como a BAC com versatilidade para avaliar sua performance. A análise dos resultados obtidos nos trabalhos que serão apresentados permitiu demonstrar que a BAC é um método capaz de monitorar diferentes processos farmacêuticos *in vivo* e *in vitro,* corroborando seu potencial inovador para aplicações farmacêuticas.

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Objetivos

"Quando você tem uma meta, o que era um obstáculo passa a ser uma etapa de um dos planos." Gerhard Erich Boehme

Objetivos

Essa tese incorporou quatro artigos que utilizaram a BAC para avaliar diferentes parâmetros farmacêuticos em comprimidos, cápsulas e sistemas multiparticulados. Todos esses trabalhos empregaram a BAC tendo como objetivo principal:

- Obter imagens da desintegração de comprimidos *in vitro* e no estômago humano;

- Avaliar a influência da força de compressão em comprimidos para validar a técnica biomagnética para o controle de qualidade em processos farmacêuticos;

- Determinar o tempo de trânsito gastrintestinal em humanos de cápsulas de hidroxipropilmetilcelulose (HPMC) revestidas e quantificar o processo de desintegração na região ileocolônica;

- Avaliar um sistema multiparticulado sob influência do estado prandial no esvaziamento gástrico e trânsito intestinal.

Capítulo 1

Magnetic images of the disintegration process of tablets in the human stomach by AC Biosusceptometry

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Magnetic images of the disintegration process of tablets in the human stomach by ac biosusceptometry

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Abstract

Oral administration of solid dosage forms is usually preferred in drug therapy. Conventional imaging methods are essential tools to investigate the *in vivo* performance of these formulations. The non-invasive technique of ac biosusceptometry has been introduced as an alternative in studies focusing on gastrointestinal motility and, more recently, to evaluate the behaviour of magnetic tablets *in vivo*. The aim of this work was to employ a multisensor ac biosusceptometer system to obtain magnetic images of disintegration of tablets *in vitro* and in the human stomach. The results showed that the transition between the magnetic marker and the magnetic tracer characterized the onset of disintegration (t_{50}) and occurred in a short time interval (1.1 ± 0.4 min). The multisensor ac biosusceptometer was reliable to monitor and analyse the *in vivo* performance of magnetic tablets showing accuracy to quantify disintegration through the magnetic images and to characterize the profile of this process.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Medical imaging methods are essential to evaluate anatomically and functionally internal structures of the human body. Imaging techniques also play an important role in pharmaceutical research since they provide valuable information on the *in vivo* performance for any dosage forms (Wilson *et al* 1997, Singh and Waluch 2000, Newman *et al* 2003).

Oral administration is the most popular method for drug therapy and the active substances are conveniently administered in a solid form (Sastry *et al* 2000). Drug delivery occurs by the disintegration of the solid dosage form and promotes drug release to be absorbed in the gastrointestinal tract (Melia and Davis 1989). Physiological factors and the formulation design

have significant influences on the disintegration and drug absorption and therefore in the safety and efficacy of the drug product (Dressman *et al* 1993, Lipka and Amidon 1999).

For these reasons, imaging modalities introduced a new perspective for the *in vivo* investigation of drug delivery. γ -scintigraphy is the standard method widely used to assess complex interactions between the drug, the dosage form and the gastrointestinal physiology (Wilding *et al* 2001). The main drawbacks of this method are the exposure of patients to the ionizing radiation, precluding repetitive assays with a single subject and the complicated and expensive preparation of the radiopharmaceuticals.

The development of radiation-free techniques provides a non-invasive approach to acquire information about the *in vivo* performance of oral dosage forms within the gastrointestinal tract. Magnetic resonance imaging (MRI) was recently employed to monitor the intragastric course of a labelled and gastric-retentive tablet (Steingöetter *et al* 2003a, 2003b). Although there are favourable advantages for this purpose, including high temporal and spatial resolution, the use of MRI in studying oral delivery systems is restricted due to the cost of equipment and limitations in positioning of the patients, since up to now most MRI units do not operate with the subject in an orthostatic position.

Biomagnetic methods represent a feasible and promising alternative in clinical, physiological and pharmaceutical research. Magnetic fields associated with the flow of electrical activity or as a result of ingestion of a magnetically labelled dosage form are detectable by multichannel SQUID (superconducting quantum interference device) systems (Weitschies *et al* 1997, 2001,Hu *et al* 2000).

Whereas the SQUID has been developed to detect extremely weak magnetic fields, the need for a magnetically shielded environment and an expensive operational cost limit its use on a wide scale.

In the past few years, alternating current biosusceptometry (ACB) has been becoming an interesting and valuable tool in gastroenterology research. ACB uses induction coils for recording the magnetic flux variation obtained from the response of a magnetic material ingested (Miranda *et al* 1992). This material has a high magnetic susceptibility that produces a strong response when an alternating magnetic field is applied in a biological system containing small amounts of ferrite. ACB showed accuracy to evaluate, physiologically, gastric emptying (Baffa *et al* 1995, Oliveira *et al* 1996) and gastric motility in humans (Miranda *et al* 1997) and dogs (Moraes *et al* 2003) as well as colonic motility (Ferreira *et al* 2004). ACB was also employed to obtain magnetic imaging of ferromagnetic tracers *in vitro*, introducing a novel concept in imaging of biological systems (Moreira *et al* 2000).

Recently, a new instrumental arrangement comprising a multisensor system (multisensor ACB) was implemented and proposed for the first time to characterize the disintegration process of tablets *in vitro* and in the human stomach, through the acquisition of magnetic signals (Corá *et al* 2003). The results obtained were very satisfactory and emphasized the importance of studying how pharmaceutical forms behave in the human gastrointestinal tract (Corá *et al* 2005).

The aim of this investigation was to employ multisensor ACB to image the disintegration of tablets *in vitro* and in the human stomach.

2. Materials and methods

2.1. Fundamentals

The ac biosusceptometer consists of two pairs of coils separated by a fixed distance (baseline), where each pair of coils is composed of an excitation coil (outer) and a detection coil (inner), in a first-order gradiometric configuration (figure 1).

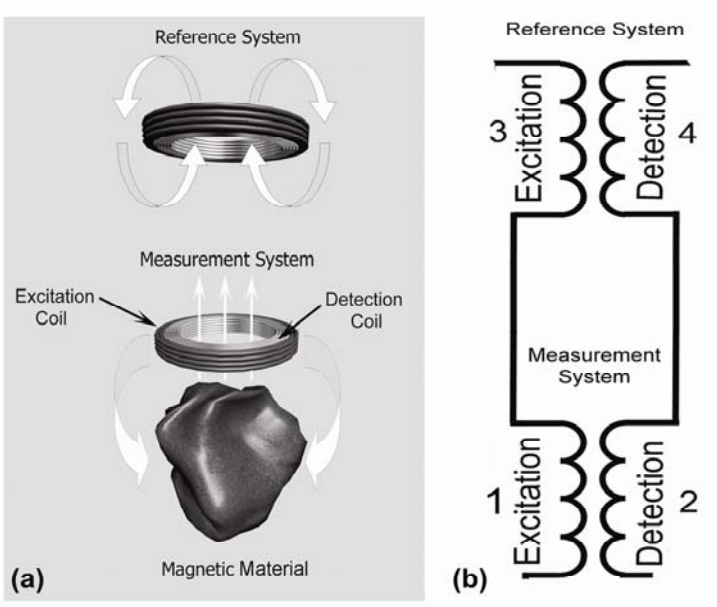

Figure 1. (a) Functional diagram of the single sensor ac biosusceptometer. The proximity of a magnetic material to the measurement system causes imbalance in the magnetic flux and the response is monitored. (b) Schematic diagram of the single sensor showing the pairs of excitation/detection coils in a coaxial arrangement.

This system is based on a couple of magnetic flux transformers with an air nucleus in which the pair (excitation/detection) located more distant from the magnetic material (ferrite) acts as a reference transformer (Miranda *et al* 1992, 1997,Baffa *et al* 1995, Oliveira *et al* 1996) and the pair closer to the sample as a measuring transformer.

Bastuscheck *et al* (1985), in accordance with the reciprocity theorem, evaluated the magnetic flux for the susceptometric magnetometer. From these findings, Baffa *et al* (1995) demonstrated that the output voltage (V_d) from the detection coils when an alternating current with frequency ω (10 kHz) is applied to the excitation coils can be written as equation (1):

$$V_d = (\Delta M)\frac{dI_e}{dt} + RI \tag{1}$$

where $\Delta M = M_{12} - M_{34}$ is the difference between the mutual inductance for the pair of excitation/detection coils, R is the electric resistance in the detection coil, I_e is the current supplied for the excitation coils and I is the current fed to the amplifier. The excitation coils induce equal magnetic flux in the detection coils which are arranged in a first-order gradiometric configuration, to minimize the output signal when there is no magnetic material near the detection system. With the proximity of a magnetic material an imbalance voltage in (V_d) occurs, due to the change in the differential flux between the detection coils. The gradiometric system detects the time variation of the magnetic flux between the detection coils as an electromotive force (emf) equal to ε, according to equation (2).

$$\varepsilon = -\frac{d\Delta\phi}{dt} = M' \tag{2}$$

where M' is the mutual inductance between the magnetic material and the detection coil and it is assumed that the magnetic material behaves as a magnetic dipole.

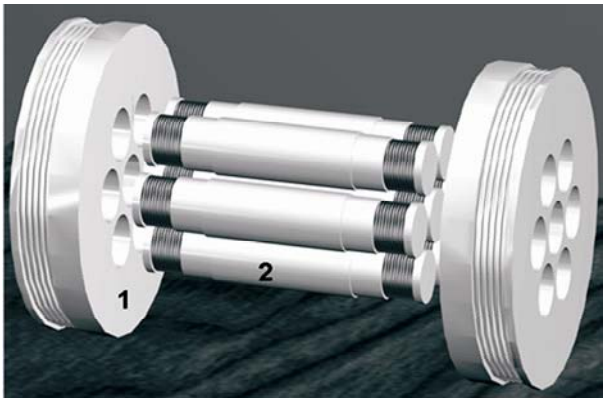

Figure 2. Multisensor ac biosusceptometer system. (1) A pair of excitation coils and (2) seven pairs of detection coils with hexagonal symmetry.

The multisensor ACB system employed in this study possesses only one pair of excitation coils ($\phi = 11$ cm) and seven pairs of detection coils ($\phi = 2$ cm) with 14 cm of baseline in a coaxial arrangement (figure 2). The first-order detection coils were arranged in a hexagonal configuration having 4cm separation between the centre of each gradiometer. This biomagnetic system is mounted in an adjustable vertical support that allows the acquisition of magnetic signals in distinct points distributed on the abdominal surface (Corá *et al* 2003).

2.2. Experimental protocol

2.2.1. Tablet preparation. In this study, magnetic tablets (10 mm diameter, 1.54 g weight) were prepared by direct compression from 1.00 g of ferrite ($MnFe_2O_3$), 0.50 g of microcrystalline cellulose (Merck, Germany), 0.01 g of magnesium stearate (Merck, Germany) and 0.10 g of an effervescent mixture (SmithKline, Brazil), coated by spray drying with a solution of a gastrosoluble polymer-Eudragit® E100 (Rohm, Germany). The ferrite is an inert material that is not absorbed by the GI tract, harmless to the organism and, therefore without biological side effects (Forsman 1998).

2.2.2. In vitro study. For the *in vitro* study, one coated magnetic tablet was used. A square glass vessel containing 1.5 l of acidic solution (pH = 1.2; 0.1 eq l^{-1} HCl; 37°C) was positioned in front of the multisensor ACB system. A digital camera was used to obtain images of the tablets in the solution. The magnetic tablet was introduced in the solution, simulating the ingestion by the volunteer, and video and magnetic signals were acquired simultaneously.

2.2.3. In vivo study. The *in vivo* study was carried out in nine healthy volunteers, both genders and ages ranging from 21 to 41 years, that had no history of gastrointestinal symptoms or abdominal surgery. Written informed consent for the participation in the study was obtained. The *in vivo* investigation was approved by the Ethics Committee in Research of the Medical School, Universidade Estadual Paulista (UNESP). All volunteers fasted at least 12 h prior to the administration of magnetic tablets. Each volunteer, in an orthostatic position in the measurement system, swallowed a magnetic tablet with 200 ml of water. The multisensor ACB system was positioned on the abdominal surface (figure 3), and the magnetic signals were acquired concomitantly for 15 min. The magnetic signals were acquired continuously through lock-in amplifiers (Stanford Research Systems) and the lock-in output was sampled at 10 Hz in accordance with previous studies of gastrointestinal motility (Miranda *et al* 1997,

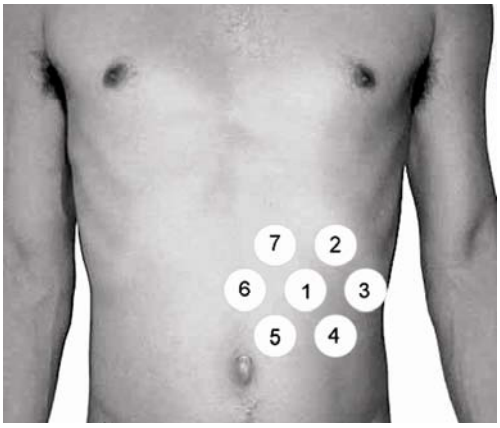

Figure 3. Positioning of the multisensor ac biosusceptometer in the gastric projection according to the external anatomic references.

Moraes *et al* 2003) and the disintegration of magnetic tablets (Corá *et al* 2003). This sampled frequency is enough to record the GI motility with frequencies below 20 contractions per minute (0.30 Hz) and allows enough time resolution to follow the disintegration process.

2.3. Magnetic images

As employed in our previous studies (Corá *et al* 2005), the lock-in amplifier and a magnetic phantom were used to calibrate and to adjust the intensity (mV) for each sensor, aiming to minimize the differences in the signal acquired, attributed to the geometric arrangement of the multisensor system.

The magnetic signals recorded by the multisensor ACB system are represented by a time series matrix. From these signals, stored in ASCII format, a seven-point matrix was calculated. Every data point in this initial matrix was obtained by computing an average in a 3 s time interval of the signal acquired, in order to obtain 30 matrices for each measurement (imaging sampled frequency at 0.33 Hz). The initial matrix corresponds to the configuration of the multisensor ACB system (figure 3).

To construct the imaging matrix the sensitivity profile of the multisensor ACB system was taken into account. Figure 4 shows the transversal sensitivity profile obtained for the central sensor for distinct distances between the sensor and a magnetic tablet. It can be observed that for distances greater than 25 mm, the variation rate of intensity (mV) in the magnetic signal is constant and practically null and, therefore, the points located laterally at 30 mm from the external detection coils were considered null, since $\frac{\partial \Phi}{\partial x} = \frac{\partial \phi}{\partial y} = 0$. The other argument for using this condition is that the system is sensitive only to near sources, thus for a near field approximation the magnetic fields are essentially axial, supporting this condition.

Nevertheless, sensitivity contours for different susceptometers were obtained by Carneiro *et al* (2002), showing regions that contribute positively and negatively to the magnetic flux. For the first-order gradiometers, like this multisensor ACB system, the negative magnetic flux is detected only for distances smaller than 6 mm (figure 4).

According to these characteristics and establishing that the points located laterally to the external detection coils are zero, a square matrix (7 × 7) equivalent to the area of the detector system (16 × 16 cm) was calculated by fitting the data of each sensor to the sensitivity profile. The square matrices were then interpolated (256 × 256) by the spline method and appropriate

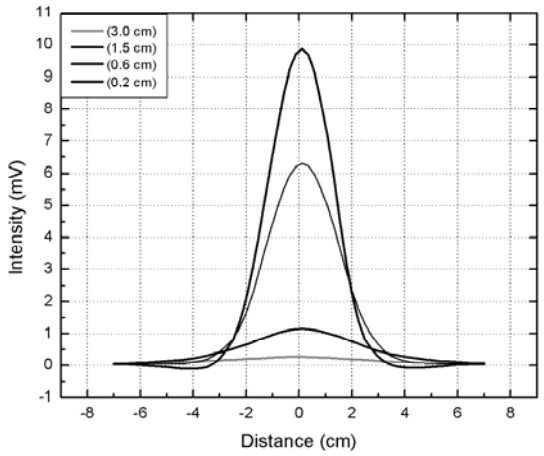

Figure 4. Transversal sensitivity profile of the multisensor ac biosusceptometer system. The variation rate of intensity (mV) in the magnetic signal was obtained for the central sensor for distinct distances between the sensor and a magnetic tablet.

routines to obtain the degraded images of the magnetic tablets *in vitro* and *in vivo* were applied. Further image processing for quantification included background subtraction, brightness and contrast adjustment and segmentation. The segmentation was used to quantify, in number of pixels, the imaging area. All the routines were implemented in MatLab® (Mathworks, Inc.).

The disintegration process is characterized by the transition of a magnetic marker, MM (non-disintegrating tablet) to a magnetic tracer, MT (disintegrating tablet). In the magnetic images, the MM was clearly delineated and the MT showed the spreading of the magnetic material *in vitro* and in the stomach. Therefore, the onset of the disintegration process (t_{50}) was calculated from the 50% increase of pixels in the imaging area (Perkins *et al* 2001).

3. Results

Figure 5(a) shows a series of photographs of a tablet in the acidic solution. In the instant t_1, the tablet arrived in the solution and the dissolution of the coating layer initiates (t_2). During this period, there is no occurrence of ferrite release, indicating a lag time until the onset of disintegration. When reducing the coating layer, the disintegration process (t_3) initiates and it is intensified from the instant t_4, due to the action of the excipients that promotes the spreading of the magnetic material in the glass vessel (t_5). The complete disintegration is shown in the instant t_6. The segmented area outlined in the photographs was used to calculate the spreading of the magnetic material from the number of pixels in that area (figure 5(b)).

For the same instants shown in the photographs, the magnetic images of the disintegration process of a tablet in the acidic solution (figure 6(a)) were obtained. In the image shown in t_1, the tablet can be observed as a MM. The onset of the disintegration process occurs from the instant t_3, with a gradual increase of the imaging area due to the spreading of the magnetic material. The instant t_6 represents the complete disintegration. Figure 6(b) shows the number of pixels contained inside a delineated area (spreading of the magnetic material) and its time variation ('velocity of disintegration').

The intragastric performance of the tablet for a volunteer is shown in the image sequence of figure 7(a). The expected stomach profile was delineated according to the external

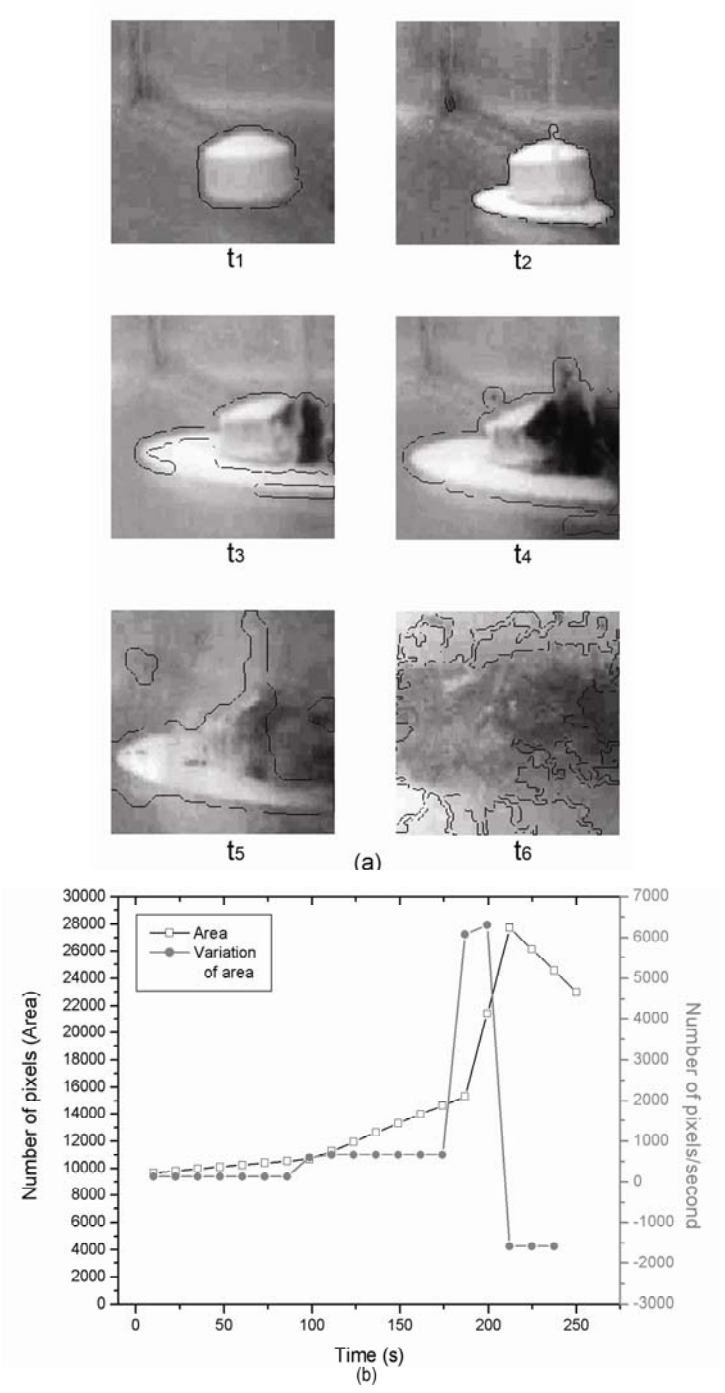

Figure 5. (a) Photographs of a tablet in the acidic solution to illustrate the disintegration process. The onset of disintegration (t_{50}) occurs in the instant t_3. (b) Spreading of the magnetic material and the time variation in the number of pixels in the segmented area of the photographs.

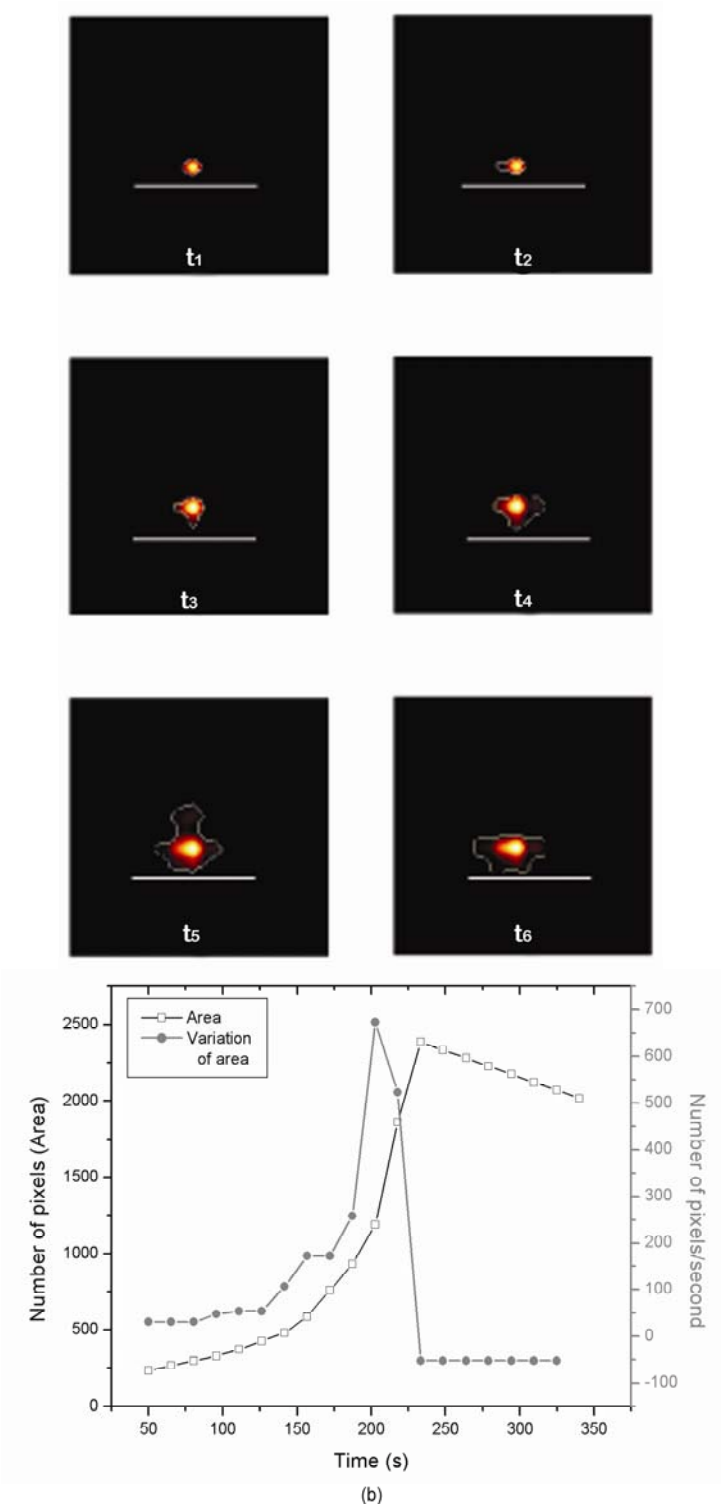

Figure 6. (a) Magnetic images of the disintegration process of a tablet *in vitro*. The onset of disintegration (t_{50}) occurs in the instant t_3. The gradual increase of the imaging area characterizes the spreading of the magnetic material. (b) *In vitro* spreading of the magnetic material and the time variation of the number of pixels contained inside a delineated area showing the 'velocity of disintegration'.

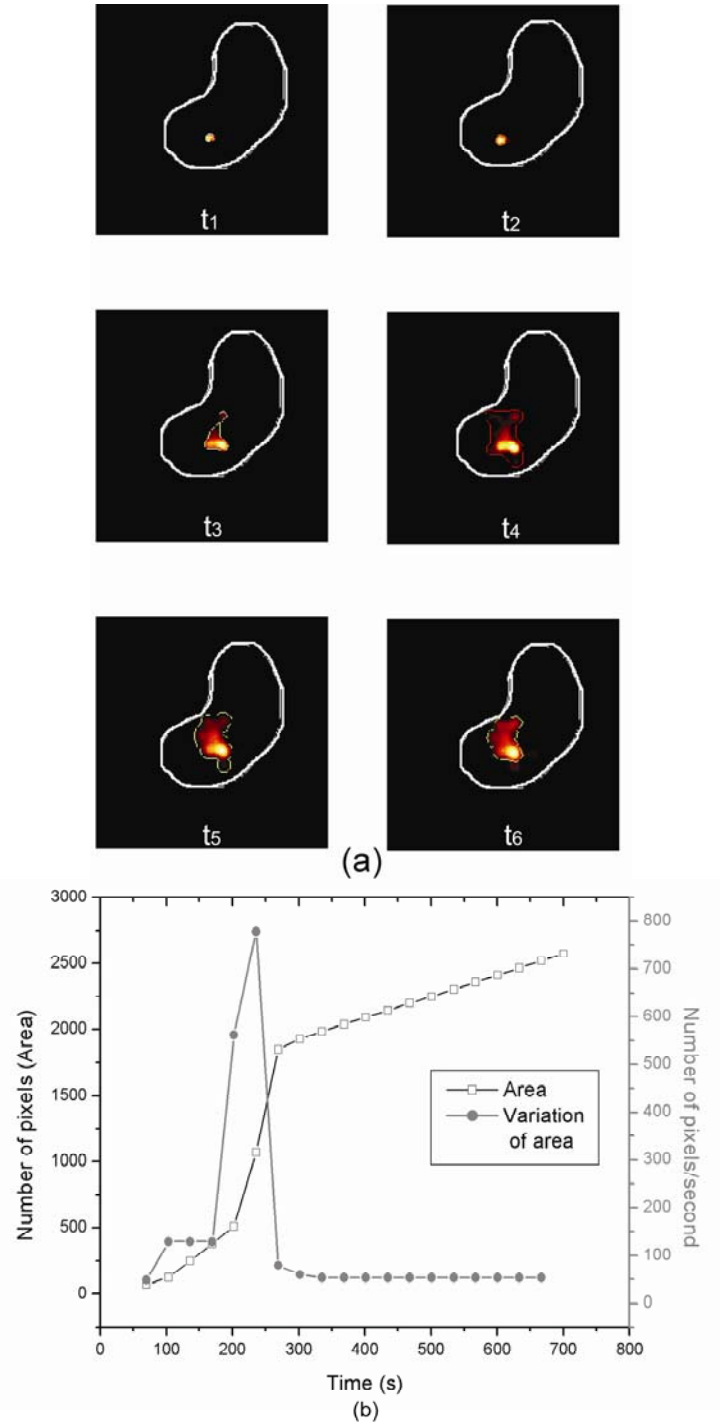

Figure 7. (a) Magnetic images of the disintegration process of a tablet in the human stomach at t_1–t_6. 50% disintegration (t_{50}) is located between t_i and t_{i+1}.(b) *In vivo* spreading of the magnetic material, as number of pixels, in the segmented area and its rate of change.

anatomic references and the positioning of the sensors in the abdominal surface (figure 3). In these images, the arrival of the tablet in the distal stomach can be observed (instant t_1). The onset of disintegration occurred in the instant t_2. After t_3, a gradual increase in the imaging area can be verified, characterizing the spreading of the magnetic material within the organ. The complete disintegration is represented in the instant $t6$.

Figure 7(b) shows the number of pixels present inside a delineated area and its time variation ('velocity of disintegration'). The onset of disintegration (t_{50}) of the tablets in the stomach ranged from 0.5 to 2.1 min (mean 1.1 ± 0.4).

4. Discussion

Oral administration of solid pharmaceutical forms is a common practice in drug therapy and the imaging methods represent important tools to provide more reliable information about their performance in the human gastrointestinal tract.

In a recent study, single-sensor ACB was successfully used to generate images of ferromagnetic phantoms (Moreira *et al* 2000). Nevertheless, the development of a multisensor ACB system improved the spatial resolution allowing us to monitor the disintegration process of tablets and simultaneous gastrointestinal motility under physiologic conditions (Corá *et al* 2003, 2005).

In addition, multisensor ACB showed sensitivity and temporal resolution to obtain magnetic images. This system remains over the area of interest during all the recording time, not requiring repositioning and, consequently, there is no noise from vibrations. These are the important features of the multisensor ACB system that allows evaluation of the dynamic process that occurs in brief periods of time.

In order to obtain a profile of the disintegration process of tablets an *in vitro* study was carried out aiming to qualitatively compare the photographs and the corresponding magnetic images. Analysing the image sequences shown in the figures 5 and 6, it was possible to verify the MM in the initial instances, while the tablet remains intact during the dissolution of the coating layer by the action of the acidic solution. When the coating layer was reduced the onset of disintegration (from instant t_3) could be observed, continuously, until the spreading of the magnetic material in the glass vessel (instant t_6).

This study was focused on the investigation of the disintegration process of tablets in the human stomach through magnetic imaging. The disintegration (figure 7(a)) was visualized solely by the magnetic method. By segmentation of the imaging area, it was possible to quantify the spreading of the magnetic material to characterize the transition between the MM and MT due to the disintegration process. Our data demonstrated that multisensor ACB was capable of identifying differences in the profile of the disintegration process.

From the results presented, it was observed that the onset of disintegration occurred in a short time interval (1.1 ± 0.4 min), indicating that this process once initiated promotes the dispersion of the ferrite continuously.

Pharmacoscintigraphy is an important method to investigate the gastrointestinal performance of pharmaceutical dosage forms and to provide information about the release and subsequent drug absorption (Wilding *et al* 2001). As an alternative to scintigraphy, biomagnetic methods have become feasible to monitor the dosage forms in the human gastrointestinal tract (Weistchies *et al* 2001). Although this multisensor ACB does not have sensitivity, the spatial resolution of the SQUID systems with a larger number of detectors was able to characterize efficiently the disintegration of tablets through magnetic images (Corá *et al* 2005). More reliable data will be obtained in combination with pharmacokinetics studies, since the magnetic material is devoid of harmful effects.

Despite the difficulties, the multisensor ACB system was able to obtain images with reasonable quality. However, the blurring in the magnetic images due to the differences in the sensitivity profile could be corrected by applying the point-spread function for each sensor. Moreover, the application of restoration techniques could improve image quality and suppress noise simultaneously (Kondo *et al* 1977, Gravel *et al* 2004).

More extensive studies are required to obtain a comprehensive knowledge of the behaviour of pharmaceutical forms in the human gastrointestinal tract. Moreover, it is essential that the development of sophisticated and specified delivery systems can improve and control the bioavailability and effectiveness of administered drugs. In the future, ACB with a larger number of channels as a non-invasive and radiation-free imaging tool might achieve the same importance as other techniques in pharmaceutical and physiological research. In summary, our study showed that multisensor ACB, a completely safe and harmless device, demonstrated enough sensitivity and spatial resolution to evaluate pharmaceutical dosage forms in the human gastrointestinal tract.

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Capítulo 2

Influence of compression forces on tablets disintegration by AC Biosusceptometry

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Research Paper

Influence of compression forces on tablets disintegration by AC Biosusceptometry

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Abstract

Analysis of physical phenomena that occurs during tablet disintegration has been studied by several experimental approaches; however none of them satisfactorily describe this process. The aim of this study was to investigate the influence of compression force on the tablets by associating the AC Biosusceptometry with consolidated methods in order to validate the biomagnetic technique as a tool for quality control in pharmaceutical processes.

Tablets obtained at five compression levels were submitted to mechanical properties tests. For uncoated tablets, water uptake and disintegration force measurements were performed in order to compare with magnetic data. For coated tablets, magnetic measurements were carried out to establish a relationship between physical parameters of the disintegration process. According to the results, differences between the compression levels were found for water uptake, force development and magnetic area variation measurements. ACB method was able to estimate the disintegration properties as well as the kinetics of disintegration process for uncoated and coated tablets. This study provided a new approach for in vitro investigation and validated this biomagnetic technique as a tool for quality control for pharmaceutical industry. Moreover, using ACB will also be possible to test these parameters in humans allowing to establish an in vitro/in vivo correlation (IVIVC).

Keywords: AC Biosusceptometry; Compression force; Disintegration force; Water uptake; Magnetic tablets; Disintegration

1. Introduction

Despite increasing interest in modified release systems, conventional tablets are still the most popular solid dosage forms due to ease of manufacture, convenience of dosing and stability [1,2].

Drug release from tablets occurs by disintegration process promoting a fast fragmentation of the dosage form under the action of the disintegrant [3]. If this process is slow or incomplete the bioavailability of a drug will be inadequate. Appropriate choice of a disintegrant and its consistency of performance have critical importance to the formulation development [4].

Disintegration of compressed tablets is an important quality parameter and it is strongly influenced by the properties of the excipients, such as particle size distributions and the compression force [5,6]. It is well established that the compression force is essential for the tablet manufacturing process since an increase in the compression force causes a reduction of tablet porosity and, as a consequence, a linear increase of the disintegration time [7,8].

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In experimental determination of tablet disintegration an official in vitro apparatus is used; however, it does not describe satisfactorily the disintegration properties. Notwithstanding, tablet disintegration has been studied by several research groups by developing novel experimental approaches for analysis of physical phenomena occurring during this process [9–11].

A mathematical model based on measurements of the disintegration force developed as a result of water uptake provided an interesting parameter to quantify and to compare the efficiency of disintegrants [12–15]. Moreover, it can be useful to determine the better relationship between the compression force and the force developed during the disintegration process.

In the past few years, Alternate Current Biosusceptometry (ACB) has been innovative in this application field and has become an alternative method for pharmaceutical research. Disintegration of compressed magnetic tablets has been evaluated *in vitro* and *in vivo* through signals and images employing a multisensor ACB system [16–20]. Biosusceptometry demonstrated versatility not only for human studies but also the capability to be used as a tool in quality control for pharmaceutical products.

The aim of this study was to investigate the influence of compression force on the tablets by associating the AC Biosusceptometry with consolidated methods, water uptake and disintegration force, in order to validate the biomagnetic technique as a tool for quality control in pharmaceutical processes.

2. Materials and methods

2.1. Materials

Materials used in this study were ferrite powder ($MnFe_2O_4$;80–125μm) as the magnetic marker (Thornton, Brazil), effervescent mixture (SmithKline Beecham, Brazil), microcrystalline cellulose PH101 (Valdequímica, Brazil), Eudragit® E100 (Röhm GmbH, Germany), magnesium stearate (Valdequímica, Brazil), talc (Valdequímica, Brazil), titanium dioxide (Valdequímica, Brazil), triethyl citrate (Scandiflex, Brazil), and isopropyl alcohol (Sigma– Aldrich, Brazil).

2.2. Preparation of tablets

Tablets were directly compressed on a single punch tablet machine (Marconi, MA-098/1CPE, Brazil) at five different force levels (10, 20, 30, 40, and 50 kN), using 11 mm concaved punches. The tablets had the following composition: 71% ferrite, 21.5% microcrystalline cellulose, 7% effervescent mixture, 0.5% magnesium stearate. Sample tablets at each compression force were taken and were stored in glass bottles before physical tests. A chemical characterization for magnetic material has been provided, as described previously [19].

Hardness testing of 10 tablets at each compression force was determined with a model THB 220 (Erweka Hardness Testers, Brazil). Friability of the tablets was determined using an Automated Friabilator EF-2 (Electrolab, Brazil) at 25 rpm/min for4min. The tablets were weighed and loss in weight (%) was calculated.

Coating dispersion was prepared by dissolving 6% (w/w) Eudragit E100, 2% (w/w) talc, 1% (w/w) triethyl citrate, 1% (w/w) magnesium stearate, and 2% (w/w) titanium dioxide in 88% isopropyl alcohol. Coating was performed with a coating machine (PCCA, Brazil) under the following conditions: spray air pressure, 1.5 mg/cm^2; inlet temperature, 40–45 °C; rotating speed, 20 rpm. Acid-soluble coating dispersion was applied to 16g of tablets at each compression force.

2.3. AC Biosusceptometry

AC Biosusceptometry bases its functioning on induction coils for recording the magnetic flux variation obtained from the response of a magnetic material when an alternating magnetic field is applied.

Essentially, the multisensor ACB system has one pair of excitation coils (ϕ = 11 cm) and seven pairs of detection coils (ϕ =2cm) separated by a fixed distance (baseline), coaxially arranged in a first-order gradiometric configuration for acquisition of magnetic signals in distinct points [18]. The sensor is mounted as a couple of magnetic flux transformers with an air nucleus in which the pair (excitation/ detection) that is located more distant from the magnetic material that will be detected acts as a reference transformer and the pair closest of the sample as a measurement transformer.

The excitation coils induce equal magnetic flux in the detection coils, hence, when a magnetic sample is nearest of the measurement system an imbalance in the voltage occurs, due to the change in the differential flux between the detection coils. Consequently, the gradiometric system detects the magnetic flux variation between the detection coils. Magnetic signals have been acquired employing lock-in amplifiers (Stanford Research Systems, Inc., USA), digitized by an A/D board of 16 bits (PCIMIO16XE-10, National Instruments Inc., Austin, TX, USA) and stored in the computer for further analysis.

The multisensor ACB system has been developed to improve spatial resolution and sensitivity for pharmaceutical applications [16–20].

2.4. Water uptake and disintegration force measurements

Water uptake and disintegration force measurements were carried out using an apparatus modified from Catellani et al. [14]. A glass container filled with 80 ml of distilled water and covered by a quantitative filter paper was positioned on an electronic precision balance. A force transducer (Model CI-6746, PASCO® Scientific, USA) was connected to the upper side of a cylindrical frame passing

through a slide guide locked by an arm which assured that the set always stopped at a fixed level. Samples of uncoated tablets at each compression force were then placed into the lower side of this slide guide. A schematic representation of the measurement apparatus is shown in Fig. 1.

When the tablet started absorbing water it was pressed against the cylindrical frame allowing to monitor the force developed during liquid uptake. Water uptake data corresponded to the weight decreases recorded by the precision balance. Water uptake and disintegration force data were acquired (sample rate at 10 Hz) and stored in a personal computer for analysis.

2.5. Magnetic measurements

Experimental determination of disintegration process was performed using a glass vessel positioned in front of the multisensor ACB system, as shown in Fig. 2. For uncoated tablets, the apparatus without the precision balance and the force transducer was positioned in front of the multisensor ACB system in order to acquire the magnetic signals during water uptake by the tablet.

As regards the relevance of coating process on drug delivery, the disintegration of magnetic coated tablets has also been evaluated. The recipient was filled with 900 ml of fasted state simulated gastric fluid without pepsin (0.1 N HCl; pH 1.2) prepared according to the USP XXII method. A tablet was added in the recipient test and the magnetic signals were acquired simultaneously for at least 20 min. The solution was replaced between each measurement.

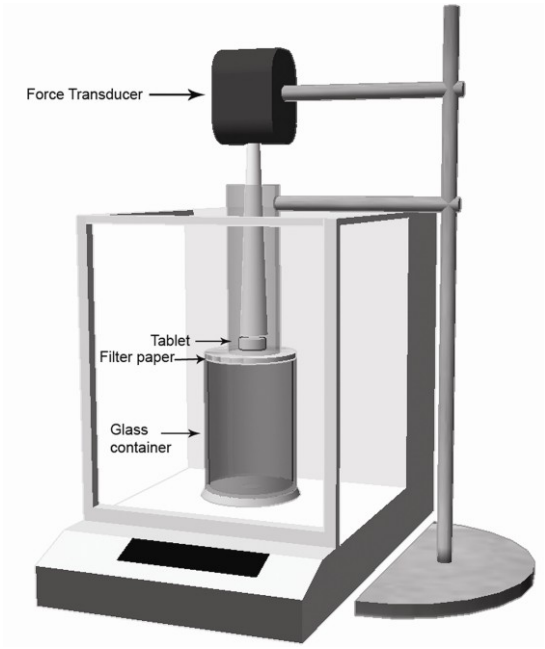

Fig. 1. Water uptake and disintegration force apparatus. When water was taken up by the uncoated tablet, the force developed was measured by the transducer placed on the upper side of the cylindrical frame.

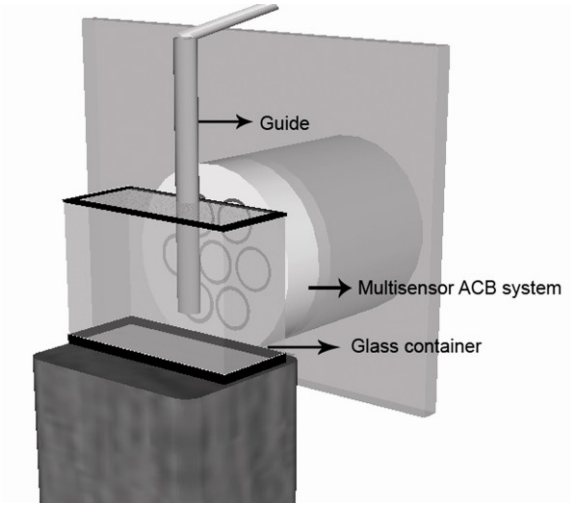

Fig. 2. Magnetic apparatus mounted for disintegration measurement. A coated tablet was inserted by a guide in the glass container placed in front of the multisensor AC Biosusceptometry system. The disintegration process was monitored in real time by seven magnetic sensors.

The disintegration process may be characterized by the transition of a magnetic marker, MM (non-disintegrated tablet), to a magnetic tracer, MT (disintegrated tablet). Hence, when the tablet is a MM, the magnetic signals were detected with high and located intensity values. As soon as the tablet started absorbing water the disintegration process occurred and the magnetic signals detected a distribution of the intensity values. For coated tablets, the interval between the transition has been delayed by the coating layer.

Magnetic signals were acquired with a sample rate of 10 Hz/channel and were stored as ASCII format for analysis.

2.6. Magnetic images

A detailed description about the principles of biomagnetic images from ACB for pharmaceutical applications was reported by Corá et al. [17]. Briefly, magnetic signals recorded by the multisensor ACB system are represented by a time series matrix. From these signals, imaging sequences have been calculated by computing an average in regular time interval of 3 s (sample rate at 0.33 Hz). Image processing techniques had included background subtraction, brightness and contrast adjustment. Once processed, the images were submitted to a segmentation process aiming to quantify, in the number of pixels, the magnetic area on each time interval.

2.7. Data analysis

In order to investigate the relationship between the magnetic area variation and the disintegration process, data have been correlated with water uptake and disintegration force. Water uptake *versus* time, time and magnetic area *versus* time profiles were fitted using the classical exponential Weibull distribution [21] modified from Pena Romero et al. [15], as shown by Eq. (1).

$$F = F_{max}\left(1 - e^{-\left(\frac{t-t_0}{t_{63.2}}\right)^{\beta}}\right) \quad (1)$$

where F is the force developed (N) at time t (min), F_{max} is the maximum force developed, t_0 is the lag time, $t_{63.2}$is the time needed to reach 63.2% of the maximum force developed, and $\beta$is the shape parameter. In analogy, Q_{max} and A_{max} were used to calculate the maximum amount of water uptake (mg) and the maximum magnetic area variation (pixel), respectively. This model allowed plotting a set of parameters involved in the overall tablet disintegration and the coefficient of determination (R^2) was the statistical parameter established to assure the integrity of fit.

For uncoated tablets, magnetic imaging area variation (A_{max}) was calculated during the water uptake aiming to establish a correlation with the force measured. All of these analyses were performed using Origin® (OriginLab Corporation, Northampton, MA, USA).

To evaluate the disintegration process of coated tablets, the following parameters were considered: coating dissolution time (CDT) was the time interval between the arrival of the magnetic tablet into the solution until its initial disintegration time; initial disintegration time (t_{10}) represented the 10% increase of pixels in the imaging area; complete disintegration time (t_{90}) was the time needed for calculating the 90% increase of pixels in the imaging area; disintegration time (DT) was calculated by subtracting t_{90} from t_{10}. DT was defined according to previous quantification parameters for *in vivo* measurements [16]. Plotting imaging area values *versus* time and particularly the first derivative of the curve was calculated and it was used for describing the disintegration kinetics.

Similarly, imaging area *versus* time profiles were fitted using Weibull distribution and the sigmoidicity of curves and time parameters ($t_{63.2}$ and DT) were also evaluated. Magnetic signals and images were processed and analyzed using MatLab® (Mathworks, Inc, Natick, MA, USA) according to procedures described previously [17].

All graphs plotted represent the mean value for the 10 tablets at each compression force applied without error bars for the sake of clarity.

3. Results and discussion

This study showed that the AC Biosusceptometry associated with conventional analysis methods could be proposed as a novel approach to investigate some physical parameters involved in the phenomenon of tablet disintegration. Hence, by using an alternative disintegrant, different compression force levels were chosen to investigate the disintegration properties of uncoated as well as coated magnetic tablets.

As expected, tablet hardness andfriability were typically compression force dependent. It was observed that at higher compression forces the hardness of the tablets increased (ranging from 104 to 487 N) and the friability decreased (ranging from 2.4% to 0.02%). Increases in the hardness hasten the disintegration time, since the tablets become harder and, consequently, less friable.

Hardness and friability are strictly related to the liquid penetration into solid dosage forms. Water uptake promotes the development of a force inside the tablet responsible for its disintegration and the rate of this process may be related to the rate of liquid penetration into the dosage form [22]. Therefore, there is a substantial relationship between formulation parameters and the disintegration efficiency since the force developed depends on the water uptake, the presence of the disintegrant and the compression force applied to the tablets.

Combined measurements of water uptake and force development have been extensively exploited to provide parameters to quantify the disintegration process of tablets [23,24]. ACB technique was proposed to investigate the relationships among these parameters and disintegration properties of tablets aiming to verify its ability to quantifying the efficiency of an effervescent disintegrant transforming liquid uptake into force through the magnetic image area.

Fig. 3 shows profiles of water uptake, disintegration force and magnetic area obtained for measurements using uncoated tablets at different compression forces. It may be observed that for different compression forces tablets differ more on the amount of water uptake (a), than on the disintegration force developed (b) and also on the magnetic area variation (c) during liquid penetration.

In order to demonstrate the performance of ACB technique to measure the physical parameters of the disintegration process, examples of magnetic area variation, water uptake and disintegration force developed profiles obtained for a tablet compressed at 30 kN are shown in Fig. 4.

Individual plots (Fig.4a) indicated that there was a similarity particularly evident in the magnetic and force developed data. A linear relationship between magnetic area against disintegration force (Fig. 4b) at a higher significance level (R= 0.987) has been found. Despite a short non-linear region between these parameters that could be explained by a preload force applied to the tablet due to the positioning of transducer, the results could suggest that the area variation may be related to force measurements.

Plotting magnetic area variation against water uptake and disintegration force (Fig.4c) was possible to observe that despite absorbing a substantial amount of water, the magnetic area as well as disintegration force remained constant when they reached the maximum value.

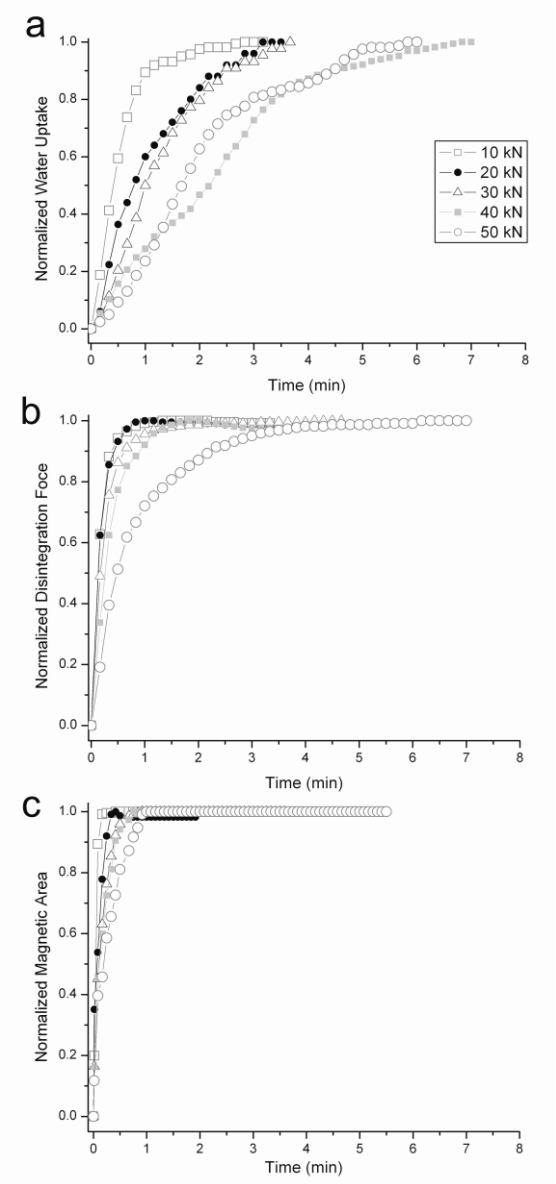

Fig. 3. Time-dependent profiles of water uptake (a), disintegration force (b) and magnetic area variation (c) for uncoated tablets at different compression forces.

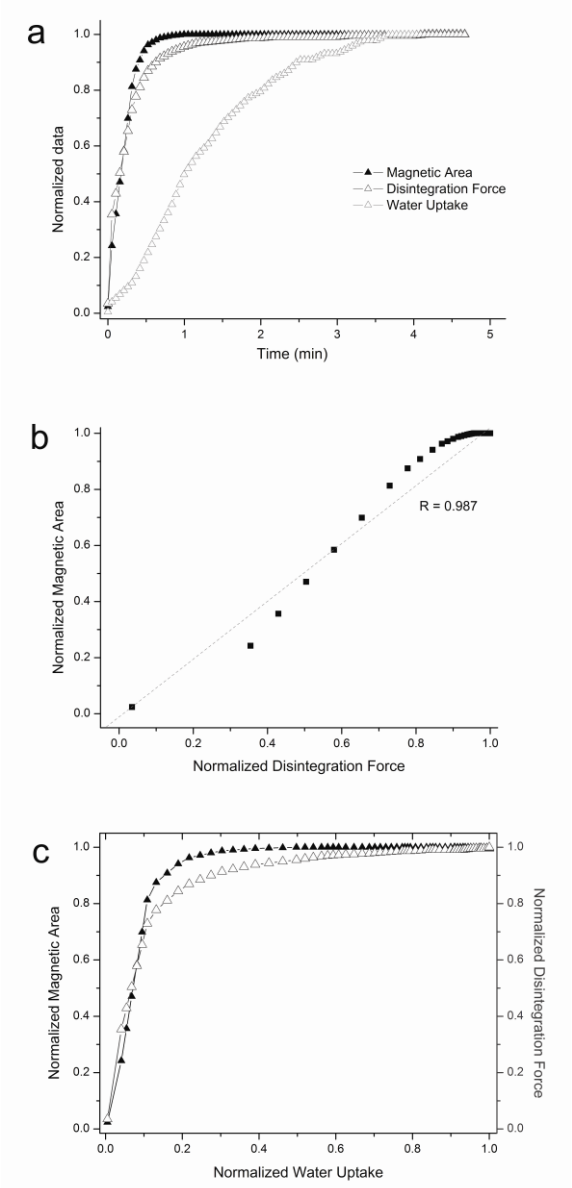

Fig. 4. Plots of physical parameters of the disintegration process for tablets compressed at 30 kN. (a) Temporal magnetic area variation and disintegration force developed during water uptake. (b) Correlation between magnetic area and force developed (R= 0.987). (c) Relationship between magnetic area, water uptake and disintegration force.

The most relevant water uptake, force developed and magnetic area data are summarized in Table 1. As expected, the time interval needed to achieve 63.2% of the total water amount (Q_{max}), of the maximum force developed (F_{max}) and of the maximum area variation (A_{max}) was directly influenced by compression force applied to the tablets. In general, tablets obtained at lower compression force developed a high disintegration force as well as an expressive magnetic area variation and disintegrated in a shorter time.

Several studies investigated the relationship between the liquid penetration rate and the disintegration force and had concluded that the force increases linearly with the amount of water absorbed and, as a result, contributes to the disintegration of tablets with swelling disintegrants [9,13,23].

Table I
Fit parameters of water uptake, disintegration force and magnetic area profiles for uncoated tablets according to Weibull model, expressed as mean±SD

Compression force (kN)	Water uptake (mg)			Disintegration force (N)			Magnetic area (pixel)		
	$t_{63.2}$ (min)	β	Q_{max} (mg)	$t_{63.2}$ (min)	β	F_{max} (N)	$t_{63.2}$ (min)	β	A_{max} (pixel)
10	0.55 ± 0.14	1.23 ± 0.11	1.60 ± 0.05	0.17 ± 0.04	1.00 ± 0.12	16.20 ± 0.6	0.06 ± 0.012	1.43 ± 0.1	1150 ± 98
20	1.13 ± 0.13	0.92 ± 0.13	1.57 ± 0.04	0.18 ± 0.03	1.13 ± 0.10	13.30 ± 0.9	0.11 ± 0.014	1.73 ± 0.15	1118 ± 108
30	1.39 ± 0.19	1.27 ± 0.15	1.62 ± 0.06	0.26 ± 0.04	0.88 ± 0.11	13.03 ± 1.1	0.16 ± 0.012	0.99 ± 0.16	1011 ± 88
40	2.02 ± 0.26	1.51 ± 0.09	1.61 ± 0.08	0.34 ± 0.08	1.03 ± 0.15	11.82 ± 1.8	0.20 ± 0.024	0.97 ± 0.11	975 ± 112
50	2.65 ± 0.35	1.67 ± 0.12	1.65 ± 0.05	0.77 ± 0.07	0.77 ± 0.09	11.5 ± 0.9	0.30 ± 0.022	1.03 ± 0.09	885 ± 76

Regarding our data, in general, the maximum liquid penetration occurred at a constant rate; however the disintegration force developed, magnetic area variation and the time interval defined as $t_{63.2}$ were clearly dependent on the compression force applied(Table1).

It could be partially explained by the disintegrant used, since effervescent tablets disintegrate by means of a reaction that promotes the disruption of the tablet due to the pressure of the gas formed [6,22]. The concept of effervescence is utilized in several dosage forms and the efficiency of this reaction depends on several factors, including the quantity of the disintegrant and its efficiency to react to water.

In addition, the shape of curves represented by β parameter seems to be related to the disintegration process of tablets since it provides information on compression behavior [10]. For magnetic tablets, different shapes characterized the curves evaluated, however, a certain discrepancy has been found and it may be related to the kind of disintegrant used. Further relationship between shape parameters and disintegration properties of swelling materials might be investigated by ACB method.

Whereas the time needed to absorb the maximum amount of water, to develop the maximum disintegration force and the maximum magnetic area variation, may be observed differences in relation to the compression force applied, as illustrated in Fig. 5. Comparing the curves, it was observed that for a same level of compression force a higher time interval ($t_{63.2}$) was needed for the amount of water absorbed to be able to promote the development of a force as well as a variation in the magnetic area resulting in the disintegration of tablet. Meanwhile, despite absorbing water continuously, not even the disintegration force or magnetic area had been affected which could be mainly attributed to the effervescent property. In our data this phenomenon continues to be evident while the compression forces increase confirming that the compression forces clearly exerted an expressive effect on several formulation parameters.

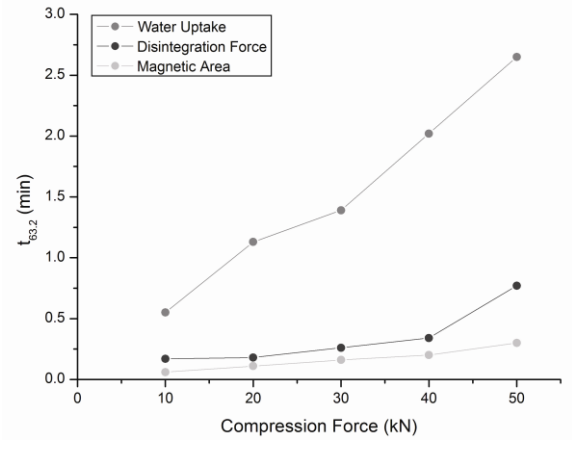

Fig. 5. Relationship between compression force and $t_{63.2}$ parameter. The time interval needed for the maximum amount of water to be able to promote the development of disintegration force and magnetic area was higher for a same level of compression force.

Aiming to establish a possible relationship between compression force, disintegration time and magnetic area variation, measurements having been performed for coated tablets. Nowadays, film coatings are important pharmaceutical excipients to control the drug release and, despite film-forming polymers have been widely used for film coating of solid oral dosage forms, there are few techniques able to verify in vitro and in vivo its uniformity properties. Typical examples of such curves are given in Fig. 6.

As discussed early, magnetic area variation was markedly influenced by compression force applied to the tablets (Fig. 6a). As expected, tablets obtained with higher compression forces tend to develop a slower area variation which could be explained by the decrease in porosity that would contribute to delay the water uptake and, consequently, to the development of a strong disintegration force.

The overall kinetics of tablet disintegration has been evaluated through the first derivative of magnetic area variation curves (Fig. 6b). This parameter, called input [10], was determined at time t63.2 of area curves and allowed describing the maximum capability of the effervescent agent to promote the disintegration of the tablets. In fact, input depends on the compression forces used: when the tablets became more resistant the variation rate was smaller than for the tablets prepared at lower compression levels. A magnetic measurement device for the in vitro determination of tablet disintegration kinetics has been previously employed [25].

The sigmoidicity of curves and time parameters of the magnetic measurements are reported in Table 2. The time

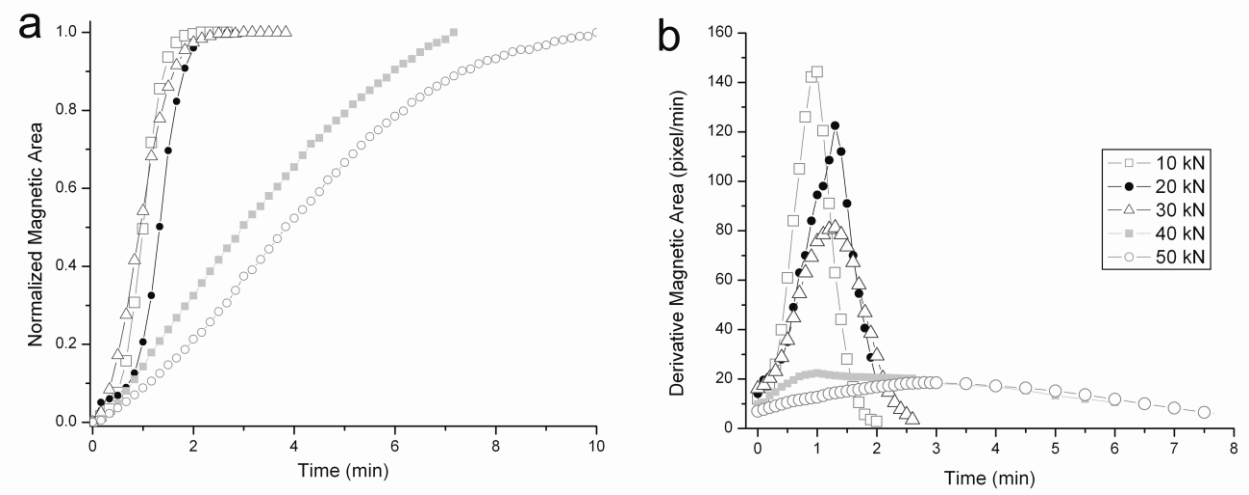

Fig. 6. Magnetic data profiles for coated tablets. (a) Temporal effect of compression force on the magnetic area variation. (b) First derivative of magnetic area variation curves for compression force with respect to time.

Table 2
Fit parameters of magnetic area profile for coated tablets according to Weibull model, expressed as mean ±SD

Compression force (kN)	Magnetic data					
	$t_{63.2}$ (min)	β	A_{max} (pixel)	DT (min)	CDT (min)	Input (pixel/min)
10	1.11 ± 0.13	3.57 ± 0.22	755 ± 35	1.24 ± 0.10	0.78 ± 0.23	120 ± 25
20	1.48 ± 0.11	4.46 ± 0.11	732 ± 29	1.69 ± 0.53	1.54 ± 0.47	103 ± 29
30	1.65 ± 0.19	2.32 ± 0.32	715 ± 42	2.33 ± 0.48	1.05 ± 0.30	61 ± 17
40	3.82 ± 0.28	1.31 ± 0.18	690 ± 33	3.85 ± 0.31	1.50 ± 0.55	17 ± 12
50	4.82 ± 0.26	1.39 ± 0.26	665 ± 46	5.26 ± 0.64	2.88 ± 0.56	14 ± 7

parameters ($t_{63.2}$ and DT) as well as A_{max} and input values were dependent on the compression forces. An inverse relationship among A_{max} and time parameters was found, since tablets showing higher A_{max} values exhibited shorter disintegration times. A similar behavior has been observed for uncoated tablets (Table 1) which can also be attributed to compression force used for tablet preparation.

Indeed, for uncoated tablet $t_{63.2}$ related to A_{max} values was faster than for coated tablets at each compression force applied. ACB may be useful to characterize the functionality of coating systems, since it has been demonstrated as appropriate to determine the performance for the product.

Concerning the shape parameter (β) it can be observed that the values calculated were higher than 1 and could indicate the presence of an initial obstacle to water penetration linked to the surface conditions of the coated tablet. However, as discussed above, shape parameters need further investigation.

Compression force plays an important role for tablet manufacturing process, since it is a well-known parameter influencing the disintegration time. Disintegration time of coated tablets ranged from 0.7 to 5.2 min. It has been reported that compression forces are related to the porosity of tablets [8], therefore, when the compression force was increased, the hardness increased resulting in tablets of low porosity. Besides compression forces, the coating layers had also interfered in the water uptake and, consequently, delaying the disintegration time.

4. Conclusions

The ACB technique associated with standard methods allowed evaluating the relationship between compression forces and magnetic area on the disintegration process of tablets. The parameters evaluated showed that ACB technique satisfactorily was able to estimate the disintegration properties as well as the kinetics of disintegration process for uncoated and coated tablets. Thus, this study was able to provide an alternative approach to investigate *in vitro* disintegration and also to validate a low-cost magnetic method as a tool for quality control for pharmaceutical industry.

On the basis of this study, further characterization of functionality of superdisintegrants can be made in predicting the mechanisms of action on the basis of compression data.

Regarding the importance of physiological parameters on the pharmaceutical processes, attention has been focused on dosage forms that have been tested in humans. In this research field, ACB technique has demonstrated promising results and has become an alternative to the conventional methods.

In summary, AC Biosusceptometry might be able to characterize a number of parameters related to drug delivery, deserving the same importance as conventional techniques for pharmaceutical research. Furthermore, ACB method will be especially powerful when combined with classical pharmacokinetic data (''magnetopharmacokinetic'') allowing establishing an in vitro/in vivo correlation (IVIVC).

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Capítulo 3

Enteric coated magnetic HPMC capsules evaluated in human gastrointestinal tract by AC Biosusceptometry

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Research Paper

Enteric Coated Magnetic HPMC Capsules Evaluated in Human Gastrointestinal Tract by AC Biosusceptometry

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Purpose. To employ the AC Biosusceptometry (ACB) technique to evaluate *in vitro* and *in vivo* characteristics of enteric coated magnetic hydroxypropyl methylcellulose (HPMC) capsules and to image the disintegration process.

Materials and Methods. HPMC capsules filled with ferrite (MnFe2O4) and coated with Eudragit® were evaluated using USP XXII method and administered to fasted volunteers. Single and multisensor ACB systems were used to characterize the gastrointestinal (GI) motility and to determine gastric residence time (GRT), small intestinal transit time (SITT) and orocaecal transit time (OCTT). Mean disintegration time (t_{50}) was quantified from 50% increase of pixels in the imaging area.

Results. *In vitro* and *in vivo* performance of the magnetic HPMC capsules as well as the disintegration process were monitored using ACB systems. The mean disintegration time (t_{50}) calculated for in vitro was 25 ± 5 min and for in vivo was 13 ± 5 min. In vivo also were determined mean values for GRT (55 ± 19 min), SITT (185 ± 82 min) and OCTT (240 ± 88 min).

Conclusions. AC Biosusceptometry is a non-invasive technique originally proposed to monitoring pharmaceutical dosage forms orally administered and to image the disintegration process.

KEY WORDS: biosusceptometry; colonic drug delivery; gastrointestinal motility; HPMC capsules; magnetic images.

INTRODUCTION

Colon-specific delivery has renewed interest in the development of therapeutic agents for treating colonic diseases because it maximizes its effectiveness and provides systemic absorption of drugs susceptible to enzymatic digestion in upper gastrointestinal (GI) tract (1,2).

Many colon-specific dosage forms have been developed for oral or rectal administration. However, oral route is preferred since rectal dosage forms have limited action and variability in distribution of the drug (2).

A common strategy to achieve colon specificity is the coating of oral solid dosage forms employing polymers with a pH-dependent solubility. The majority of enteric and colon delivery systems are based on coated tablets or conventional hard gelatin capsules (3). Nevertheless, capsules made from hydroxypropyl methylcellulose (HPMC) have been successfully manufactured as an alternative to gelatin (4). HPMC capsules have several technical advantages over gelatin capsules including a more irregular surface that provides a strongly adhesion and an excellent compatibility with the polymer (3,4).

Coated dosage forms designed for oral colon-specific drug delivery must overcome several physiological barriers that include motility patterns, GI transit and difference between the luminal pH (5). Moreover, the disintegration of the solid dosage form must be taken into consideration since this process provides the drug release for the absorption (6,7). For this reason in vitro tests are needed although the results are not fully comparable to the physiological conditions (8). More reliable data are obtained when human studies are carried out, since the bioavailability of drugs from colonic dosage forms is dependent on gastric emptying, small intestinal transit time and drug release profile (9).

Imaging techniques play an important role for monitoring of pharmaceutical dosage forms in human GI tract (10). The g-scintigraphy is the method of choice for this purpose, despite exposure of the patient to ionizing radiation and the complicated and expensive preparation of radiopharmaceuticals (11).

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On the other hand, radiation-free modalities introduced a new perspective for the *in vivo* investigation of drug delivery. Magnetic Resonance Imaging (MRI) has been employed to monitor solid pharmaceutical forms in animals and healthy subjects (12-14). Regardless of widespread use in clinical and inherent advantages, MRI has limited application in pharmaceutical research due to the high cost, the positioning of the subject during the exposure and the contrast agents do not represent an ideal drug model.

Biomagnetic methods are feasible alternative in clinical, physiological and pharmaceutical research and the multichannel SQUID (*Superconducting Quantum Interference Device*) devices are employed for the measurement of the magnetic field, after ingestion of a magnetically marked dosage form (15). This system is able to detect the extremely weak biomagnetic fields generally in a magnetically shielded environment. However, SQUID has an expensive operational cost, which limits its use in a wide scale.

Alternating Current Biosusceptometry (ACB) has been introduced as a valuable tool in gastroenterology (16) and pharmaceutical research (17). ACB uses induction coils for recording the magnetic flux variation obtained from the response of a magnetic material ingested (18). The ACB showed accuracy to evaluate physiologically different parameters of GI tract (16,19-22) as well as to obtain the magnetic images *in vitro* (23).

A multisensor ACB system was implemented to characterize the disintegration process of tablets *in vitro* and in the human stomach, through the acquisition of magnetic signals (17). In addition, this system was also employed to monitor magnetic tablets in GI tract and to image the disintegration process, introducing a novel technique in imaging of the biological systems (18,24,25).

Following these initial proposals, the aim of this work was to employ the single and multisensor ACB systems to determine the gastrointestinal transit time of enteric coated magnetic HPMC capsules and to image the disintegration process of these formulations in human ileocolonic region.

METHODS AND MATERIALS

Fundamentals

The single sensor ACB has two pairs of coils (ϕ = 3.0 cm) separated by a fixed distance (baseline), where each pair of coils are composed of an excitation coil (external) and a detection coil (internal), in the first-order gradiometric configuration (Fig. 1). This system working as a double magnetic flux transformer with an air nucleus, in which the pair (excitation/detection), located more distant from magnetic material (ferrite), acts as reference. Due to this configuration, when no magnetic material is near to the measurement system, the output signal is minimized. When there is an approximation of a magnetic mass, an unbalancing in the magnetic flux of the gradiometric system occurs, and the magnetic material is monitored (18,22,25).

The multisensor ACB has only a pair of excitation coil (ϕ = 11 cm) and seven pairs of detection coils (ϕ = 2.9 cm), coaxially arranged (Fig. 2). This system is fixed in a vertical support to be positioned on the abdominal surface and to acquire the magnetic signals at different points (17,18,24,25).

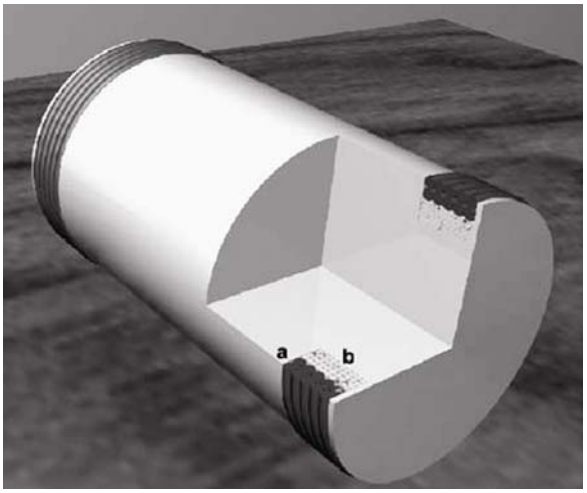

Fig. 1. Single sensor AC Biosusceptometer. (a) Excitation coil and (b) detection coil in the first-order gradiometric configuration.

The magnetic signals are acquired through "lock-in" amplifiers (Stanford Research Systems) digitalized by A/D board of 16 bits (PCI-MIO-16XE-10, National Instruments Inc.) and stored in a microcomputer.

Capsule Preparation

Size 00 capsules made from hydroxypropyl methylcellulose (Vcaps®, Capsugel Division of Pfizer Inc.) without colouring agent were filled with 1.20 g of ferrite ($MnFe_2O_4$-Ferroxcube, USA) into which had been mixed 0.30 g of sodium starch glycolate-Explotab® (Penwest, USA).

Ferrite is a ferromagnetic material with general composition $MeFe_2O_4$, where Me represents a divalent transition metal such as manganese (Mn). Ferrite was described as a contrast material or magnetic medicinal preparations (26,27). This material presents good mixing with the GI secretions, absence of toxicity, and lack effects on the digestive tract.

To provide a more objective chemical characterization for this material, the concentrations of iron ions in the dissolution medium were prepared according to USP XXII method (pH 1.2, pH 6.0 and pH 7.4) and were determined by FAAS (Flame Absorption Atomic Spectrometry) using Spectrophotometer SHIMADZU AA-6800. Standard solutions contained 0.50, 1.00, 2.00, 4.00 and 5.00 mg l^{-1} of Fe(III) ions in HCl 0.01 mol l^{-1} medium and were used in the calibration of spectrometer (according to the manufactures standard guidelines). No measured iron ion was detected in the samples collected at 0(control), 6, 12, 24 and 48 h, suggesting that the ferrite ($MnFe_2O_4$) is a stable molecule and is not absorbed by GI mucosa.

Commercially available system for colon specific drug delivery was used in this study. Eudragit® S 100 (Röhm, Pharma Polymers) is a methacrylic acid methylmethacrylate co-polymer, soluble above pH 7, making it particularly suitable for delivery into the colon (28).

Excipients used for the coating dispersions were triethyl citrate (Citroflex® 2 -Morflex Inc., USA) as a plasticizer, magnesium stearate as a lubricant, titanium dioxide employed as a pigment, Polysorbate 80 as an emulsifier and glycerol monostearate (Imwitor® 900 K Sasol, German) as a

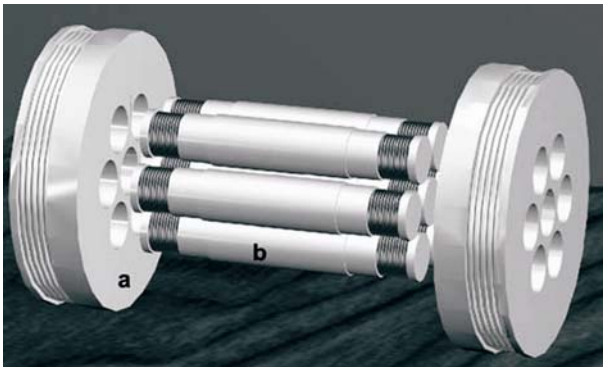

Fig. 2. Multisensor AC Biousceptometer system. (a) Pair of excitation coils and (b) seven pairs of detection coils c oaxially arranged with a hexagonal symmetry.

glidant. Capsules were sealed before coating. The polymer dispersion was prepared according to manufacturer's technical information (29) and sprayed at temperatures of 25 to 27°C.

In-vitro Test

In order to simulate the pH changes along the gastrointestinal tract, three dissolution media (at 37 ± 0.5°C, volume 500 ml), prepared according to the USP XXII method, with pH 1.2, pH 6.0, and pH 7.4 were sequentially used. The magnetic formulations was first placed in a pH 1.2 medium for 2 h and after that in pH 6.0 dissolution medium. After 3 h, the formulation was placed in a square glass vessel containing the pH 7.4 dissolution medium that was positioned in front of the multisensor ACB system.

A digital camera was used to obtain images of the enteric coated magnetic HPMC capsule in the solution. When the formulation was introduced in the last dissolution medium video and magnetic signals were acquired simultaneously until complete ferrite release in the solution. *In vitro* disintegration process analysis was accomplished through the magnetic images obtained from the signals, as demonstrated in our previous study (24).

Subjects and Study Protocol

GI performance of enteric coated HPMC magnetic capsules was evaluated in ten healthy volunteers, both genders (age: 20-32 years; BMI: 20.11 ± 0.6 kgm^{-2}). All volunteers had no history of gastrointestinal symptoms or abdominal surgery. Written informed consent of participation in the studies had been obtained. The *in vivo* investigation was approved by the Ethic Committee in Research of the Medical School-Universidade Estadual Paulista (UNESP), in accordance with the Declaration of Helsinki, promulgated in 1964.

The enteric coated magnetic HPMC capsules were administered with the volunteers in an upright position in front of the multisensor ACB system. After an overnight fast, all subjects swallowed a capsule with 200 ml of water and the magnetic signals were recorded during 20 min. The lower tip of the sternum and the umbilicus were the anatomic references (Fig. 3a).

After that, a mapping from abdominal surface was carried out every 10 min employing the single-sensor ACB. This procedure aimed to locate the magnetic formulation to determine the Gastric Residence Time (GRT), the Small Intestinal Transit Time (SITT) and the Orocaecal Transit Time (OCTT). Eating or drinking was allowed after gastric emptying of the capsule. The subjects remained moderately active during the study period.

An initial square matrix (9 x 9), corresponding to an area of 12 x 12 cm, was drawn in ileocolonic region of the volunteers. The McBurney's point and iliac right crest were the anatomic references (Fig. 3b). With the arrival of enteric coated magnetic HPMC capsule in ileocolonic region, the single-sensor ACB was used to scan this delimited area. Scanning at least for 2 min and was performed at approximately 10 min intervals until 120 min post-ileocolonic arrival.

Magnetic Data Analysis

The performance of HPMC magnetic capsules along the GI tract was monitored using the single and multisensor

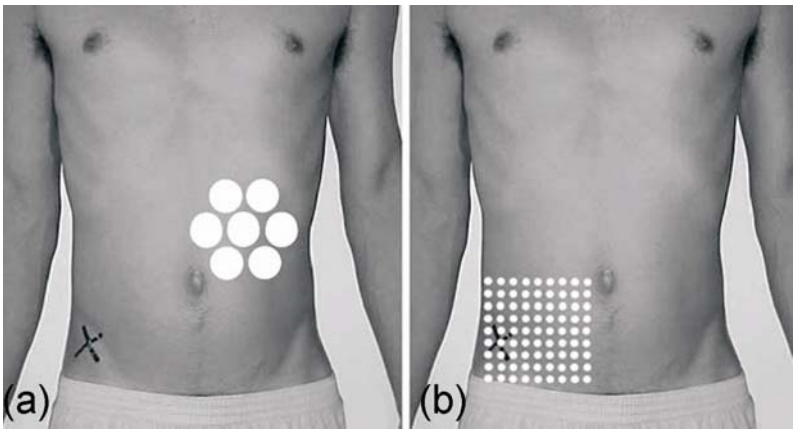

Fig. 3. (a) Positioning of the multisensor AC Biosusceptometer system on the abdominal surface. (b) Square matrix (9 x 9) draws on the ileocolonic region. The xiphoid process and the McBurney's point were the external anatomic references, respectively.

ACB systems. Initially, the magnetic signals were recorded from multisensor ACB with acquisition frequency of 10 Hz/ channel and stored in ASCII format. The signal processing included bi-directional Butterworth low-pass filter with cutoff frequency of 0.2 Hz and Fast Fourier Transform (FFT) and allowed to characterize the gastric activity contraction (GAC) in the interdigestive period.

The Gastric Residence Time (GRT) was defined as the time interval between the arrival of the enteric coated magnetic HPMC capsule in the stomach and its gastric emptying. The Orocaecal Transit Time (OCTT) was calculated by determining the time between the intake of capsule and the location in the ileocolonic region. The Small Intestinal Transit Time (SITT) was obtained by subtracting the GRT from the OCTT.

The square matrices (9 x 9) were interpolated (256 x 256) by the *spline* method and appropriate routines to obtain the degraded images of the enteric coated magnetic HPMC capsules in vivo were applied (25). Further image processing for quantification included: background subtraction, brightness and contrast adjustment and segmentation. The segmentation was used to quantify the spreading of the magnetic material and the velocity of disintegration (24). All the routines were implemented in MatLab® (Mathworks, Inc.).

The disintegration process for *in vitro* and *in vitro* measurements was characterized by the transition of a magnetic marker - MM (non-disintegrated capsule) to a magnetic tracer - MT (disintegrated capsule). In the magnetic images, the MM was clearly delineated and the MT showed the spreading of the magnetic material in the ileocolonic region. Disintegration process was calculated as the mean time disintegration (t_{50}) after reaching the ileocolonic region, and was obtained from the 50% increase of pixels in the imaging area (24,25).

RESULTS

Figure 4(a) illustrates a series of photographs of an enteric coated magnetic HPMC capsule in the phosphate buffer dissolution medium (pH 7.4). Instant t_1 represents 10 min of measurement and there was no occurrence of ferrite release. When the coating layer is reduced, the disintegration process (instant t_2) initiates and it is intensified due to the action of the excipients that promotes the spreading of the magnetic material in the glass vessel (instant t_3). The segmented area outlined in the photographs was used to calculate the mean time disintegration (t_{50}) from the 50% increase of pixels in the imaging area (Fig. 4b).

For the same instants shown in the photographs, the magnetic images were obtained from a capsule in dissolution medium and showed the disintegration process (Fig. 4c). In the instant t_1 the capsule can be observed as a MM. The onset of the disintegration process occurred in the instant t_2, with a gradual increase of the imaging area due to the spreading of the magnetic material (instant t_3). Fig. 4(d) shows the number of pixels contained inside a delineated area (spreading of the magnetic material) and its time variation ('velocity of disintegration'). The mean disintegration time (t_{50}) for *in vitro* measurements was 25 ± 5 (mean ± SD) min.

Gastric activity contraction (GAC) was recorded in real time by the multisensor ACB, concomitantly to the ingestion of the HPMC capsule is showed in Fig. 5a. The variation of intensity and the basal level of the magnetic signals acquired by the sensors located more distally showed that the enteric

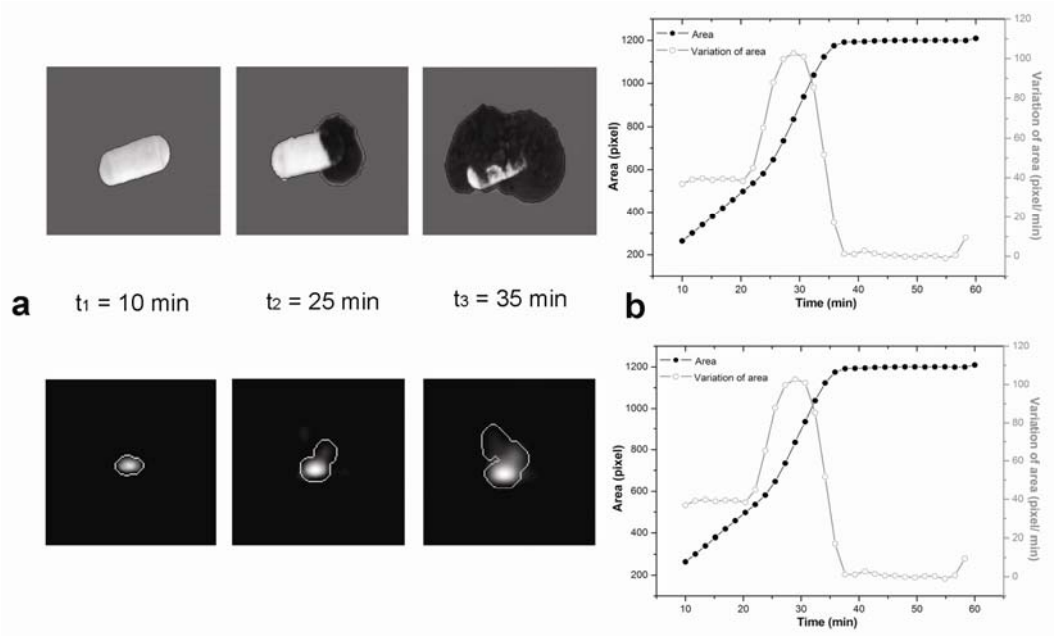

Fig. 4. *In vitro* characterization of an enteric coated magnetic HPMC capsule. (a) Photographs and corresponding magnetic images of the disintegration process of a capsule in the phosphate buffer. Mean disintegration time (t_{50}) occurred in the instant t_2. (b) Spreading of the magnetic material and the time variation of the number of pixels contained inside a delineated area showing the velocity of the disintegration.

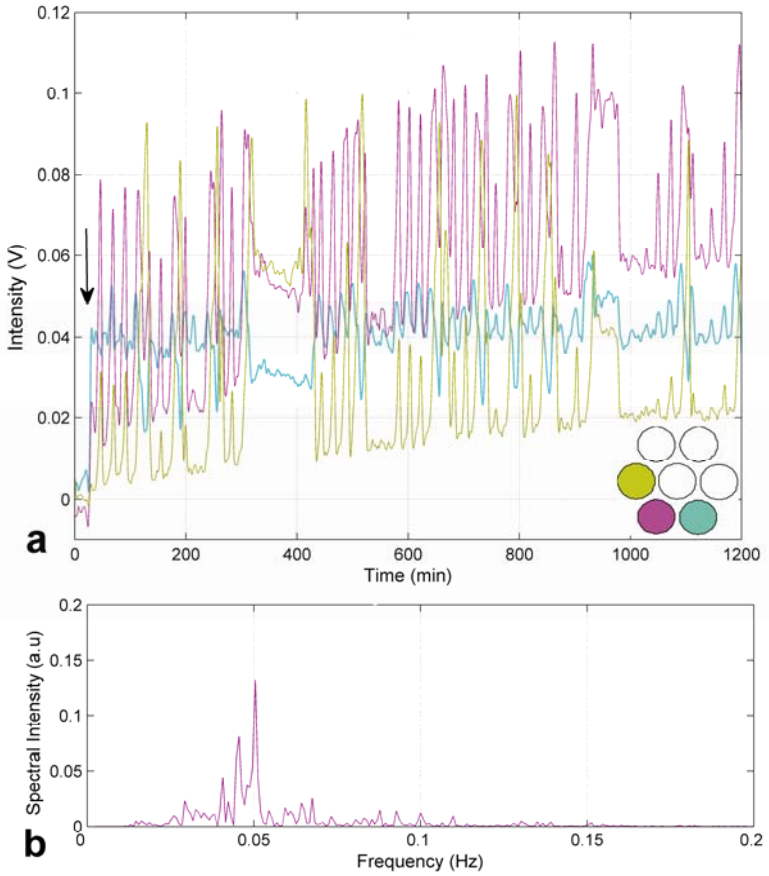

Fig. 5. Magnetic signals recorded concomitantly to the ingestion of the enteric coated HPMC capsule. (a) Intense contractile waves of the gastric activity recorded by the sensors located distally. (b) FFT showing the frequency peak of 0.05 Hz (3 cycles/minute).

coated magnetic HPMC capsule arrived on distal stomach (arrow).Typical frequency pattern around three cycles *per* minute (0.05 Hz) could be observed (Fig. 5b).

Table I. Gastrointestinal Transit Time and Mean Disintegration Time (t_{50}) for Magnetic Enteric Coated Magnetic HPMC Capsules

Volunteer	Time (min)			
	GRT	SITT	OCTT	t_{50}
1	60	190	250	10
2	40	190	230	10
3	40	90	130	20
4	50	380	430	10
5	50	150	200	10
6	80	140	220	10
7	60	210	270	10
8	70	230	300	20
9	20	100	120	20
10	80	170	250	10
X	55	185	240	13
SD	19	82	88	5
CV (%)	35	45	36	38

X Mean, *SD* standard deviation, *CV (%)* coefficient of variation.

The Gastric Residence Time (GRT) ranged from 20 to 80 min (mean 55 ± 19). Small Intestinal Transit Time (SITT) ranged from 90 to 380 min (mean 185 ± 82 min). Orocaecal Transit Time (OCTT) ranged from 120 to 430 min (mean 240 ± 88 min) (Table I).

Magnetic images of the disintegration process of magnetic HPMC capsules in the ileocolonic region for two volunteers are illustrated in Fig. 6a. The external anatomic references were delineated according to the positioning of the square matrix drawn on the abdominal surface (Fig. 3b).
In instant t_1, the ileocolonic arrival of the HPMC capsule can be observed. The onset of disintegration occurred in the instant t_2.

After t_3, a gradual increase in the imaging area can be verified, characterizing the spreading of the magnetic material within the organ.

The release of the magnetic material filled in the capsule occurred in the initial instants from the ileocolonic arrival. The number of pixels interpolated contained inside a segmented area and its time variation ("velocity of disintegration") is shown in Fig. 6b. After the onset of disintegration, the spreading of the magnetic material was relatively constant and significant variation in the image area was not observed. The *mean disintegration time* (t_{50}) was 13 ± 5 (mean ± SD) min.

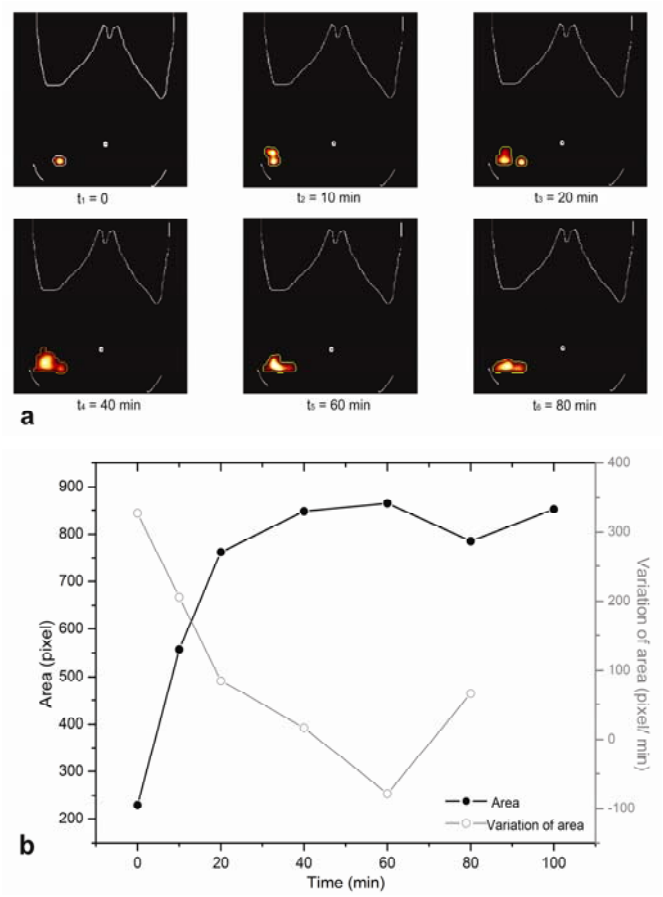

Fig. 6. (a) Magnetic images of the disintegration process of an enteric coated HPMC capsule in the ileocolonic region. The instant t1 shows the arrival of the capsule; from t_2 occurred a gradual increase in the image area that characterized the spreading of the magnetic material. (b) Spreading of the magnetic material in number of pixels in the segmented area showing the velocity of the disintegration process.

DISCUSSION AND CONCLUSION

An ideal technique to provide more reliable data about pharmaceutical drug product performance in humans should be harmless, noninvasive and have low cost. Thus, AC Biosusceptometry has gained importance in the pharmaceutical research for evaluating successful magnetic solid dosage forms in human GI tract (17,18,25).

A variety of coated forms have not been developed with significant therapeutic advantages for the local treatment of colonic diseases (2,31). The use of coated dosage forms for oral colon specific drug delivery, allowing to develop enteric coated of HPMC capsules that appears as an industrially viable process, resulting from improved coating technologies and flexibility in their design (3,38).

The polymers used for colon targeting exploit the generally accepted fact that pH of the human GI tract increases progressively from the stomach at the distal ileum (30). Therefore, should be able to withstand the lower pH values of the stomach and of the proximal part of the small intestine and also be able to disintegrate at the neutral to slightly alkaline pH of the terminal ileum or at the ileocecal junction (5,28).

In order to characterize the disintegration profiles from the magnetic HPMC capsules coated with Eudragit® S 100 an *in vitro* study was performed. None of the capsules released ferrite in acid medium or at pH 6.0, showing integrity of the coating layer under simulated gastric and small intestine medium.

Aiming to compare quantitatively the disintegration profiles, photographs and the corresponding magnetic images were analyzed (Fig. 4). The capsule was suitable to release rapidly the magnetic material at pH 7.4 as shown in the instant t_2. Once initiated, the disintegration promotes the dispersion of the ferrite continuously (instant t_3).

Magnetic images are different from the photographs since the field-of-views are not the same because they were obtained at distinct angles and distances. Despite this, employing ACB

it was possible to characterize the disintegration process by comparing the similar profiles with those obtained by photographs (Fig. 4). *In vitro* disintegration of the capsules suggests good performance of ACB and ferrite release only in the distal ileum or the proximal colon.

Although disintegration process can be studied *in vitro*, the interaction between physiological parameters and the solid dosage forms affect drug delivery profile and the reproducibility of drug release (8). If the intended site of drug release is the colon, it must be taken into consideration the prandial state and gastric emptying of dosage forms.

ACB system was able to record in real-time the gastric motility during the interdigestive period (Fig. 5), characterized by a cyclical motor pattern so-called myoelectric migrating complex (MMC) (32). Phase III contractions promote the emptying of indigestible materials, including solid pharmaceutical forms (33,34). In the presented study, all volunteers fasted prior to the administration of the magnetic formulation, allowing inferring that the capsules had been emptied from stomach during this period of activity.

Gastric residence time (GRT) for enteric coated magnetic HPMC capsules was obtained from the arrival in the stomach until its emptying (Table I). The mean GRT was 55 min showing an important intersubject variation despite of experimental protocol had been designed to minimize the influence of any external factor in the gastric emptying of the magnetic formulation.

The results from our investigation (Table I) showed that the SITT presented a significant intersubjects variation (mean 185 ± 82 min), however are within the normal ranges obtained in previous studies (33,37). As reported by other studies, it is generally accepted that small intestinal transit time (SITT) is not affected by the digestive state or by the nature of the pharmaceutical form (33,35,36).

Orocaecal transit time (OCTT) occurred on average at 240 ± 88 min (Table I). The variation observed can be attributed to the GRT and SITT, since these parameters showed significant intersubject differences as discussed earlier. Not surprisingly, GI transit for enteric coated magnetic tablets (25) compared with magnetic HPMC capsules was not significantly different for the GRT and SITT.

Mean disintegration time for magnetic capsules occurred in a short time interval (mean 13 ± 5 min), when compared with the disintegration of the magnetic tablets (mean 90 ± 40 min). Indeed the observed difference could be attributed to the kind of pharmaceutical form, since the powder filled into the capsule was not compressed. It is well known that the compression force is a very important parameter for the tablet manufacturing process, particularly for the development of the time-controlled disintegration (39).

Our findings about disintegration time of magnetic capsules contrasts sharply with those obtained by recent reports (3,4,38). The discrepancy might be due to the pharmaceutical strategies to achieve drug release in the colon (pH-sensitive or enzyme-controlled release), coating thickness and excipients used. Moreover, the criteria adopted for the analysis considered the mean time disintegration started after reaching the ileocolonic region, instead of the complete process.

Magnetic images constitute an innovative approach to characterize the disintegration of pharmaceutical dosage forms in human GI tract (24). The segmentation of the imaging area allowed quantifying the spreading of the magnetic material to characterize the transition between the MM to MT provided by the disintegration process. Although these images presented reasonable quality, the application of restoration techniques could improve image quality and suppress noise simultaneously.

Unfortunately, based on the characteristics of the magnetic formulation, it remains impractical to compare directly our findings with the results obtained from standard imaging techniques. However, great effort has been made to improve the biomagnetic systems and to reduce the amount of ferrite in the magnetic formulation similar to a conventional dosage form. Thereby, this magnetic method could be associated with pharmacokinetic parameters ("magnetopharmacokinetics")to predicting the drug absorption in a specific site of GI tract for optimized pharmacotherapy (40).

In summary, AC Biosusceptometry systems are completely safe and harmless devices, able to evaluate accurately solid dosage forms in human GI tract. Additionally, these systems represent a novel imaging tool to characterize diverse parameters related to drug delivery, thus deserving the same importance as conventional techniques in pharmaceutical research.

ACKNOWLEDGMENTS

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Capítulo 4

AC Biosusceptometry to evaluate the gastrointestinal transit of pellets under influence of prandial state

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AC Biosusceptometry to evaluate the gastrointestinal transit of pellets under influence of prandial state

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Abstract

Multiparticulate dosage forms have been proposed when distal regions of gastrointestinal tract are desirable as target of drugs. Nevertheless, as physiological parameters might interfere with the processes related to the drug delivery and absorption, it is essential to evaluate the behavior of these systems *in vivo*. The aim of this study was to propose the AC Biosusceptometry (ACB) as a noninvasive and radiation free technique to evaluate the gastrointestinal transit of a magnetic multiparticulate dosage form in healthy volunteers under fasting and fed conditions. Magnetic pellets were prepared by the powder layering method of ferrite on nonpareils sugar beads which have been coated by using Eudragit®. Our data showed that ACB was able to monitoring the gastrointestinal transit of pellets and resulted in similar profiles as demonstrated by standard techniques. Food intake has markedly influenced the gastric emptying as well as the colon arrival and the small intestine transit of magnetic pellets. This biomagnetic method showed a number of advantages over existing methodologies and deserves the same importance for this kind of analysis.

Keywords: *Gastrointestinal transit, pellet, prandial state, magnetic techniques, Biosusceptometry*

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1. Introduction

Controlled drug delivery systems are designed to achieve more predictable bioavailability of drugs either to increase efficacy or minimize adverse effects. Several approaches have been proposed, and multiparticulate dosage forms seem to have better performance when distal regions of gastrointestinal tract are desirable as target of drugs (Ranade, 1991; Gandhi et al., 1999).

Human colon has been proposed as the specific targeting of drugs for the topical treatment of intestinal diseases as well as for the delivery of therapeutic peptides and proteins (Yang et al., 2002; Chourasia and Jain, 2003; Shareef et al., 2003). For this purpose, the development of multiparticulate systems has been emphasized due to its advantages over single-unit dosage forms that include the release of drugs at optimal rate, reduced dosing frequency and constant blood levels (Asghar and Chandran, 2006).

Experimental determination of drug release may be assessed by well-established *in vitro* methodologies. However, these tests are often not predictive of the more complex *in vivo* behavior of drug products (Zahirul and Khan, 1996). Physiological parameters as motility, gastric emptying time, intestinal transit time, and pH are involved in the rate and extent of drug absorption (Dressman et al., 1993). Therefore, an understanding of the factors involved in the drug absorption and how these parameters can interfere with this process is crucial to develop more reliable drug delivery systems.

Scintigraphy has been extensively used as an imaging tool to monitoring the in vivo performance of drug delivery systems (Wilding et al., 2001); however the ionizing radiation exposure is its major drawback.

Biomagnetic as well as radiotelemetric methods have advantages over scintigraphy due to the non-invasivity and radiation-free features. SQUIDs (Superconducting Quantum Interference Devices) have been employed to investigate the behavior of solid dosage forms in the human gastrointestinal tract (Weitschies et al., 2005). Heidelberg Radiotelemetry Capsule is a device which has been used to monitor gastrointestinal pH and the gastric residence time in both humans and animals (Mojaverian, 1996).

Alternate Current Biosusceptometry (ACB) has been becoming a promising method for pharmaceutical research. AC Biosusceptometry was originally proposed as a method to investigate the gastrointestinal motility in humans (Miranda et al., 1997; Romeiro et al., 2006; Américo et al., 2007) and animals (Moraes et al., 2003; Andreis et al., 2007), regarding several aspects on physiology and clinical researches. Continuous improvements allowed enhancing the spatial resolution and the sensitivity of the device for pharmaceutical applications. It was initially proposed to monitor the disintegration process of magnetic tablets in vitro and in human stomach (Corá et al., 2003). The potential demonstrated allowed applying this method to investigate the behavior of enteric-coated dosage forms and to quantify the gastrointestinal transit time as well as the disintegration time in human colon (Corá et al., 2006a,b). Moreover, ACB method has been proposed as a new tool for imaging of pharmaceutical processes (Corá et al., 2005) and, more recently, as a tool for quality control for pharmaceutical products (Corá et al., 2008).

The aim of this study was to propose the AC Biosusceptometry as an alternative method to investigate the magnetic multiparticulate delivery system under influence of prandial state on the gastric emptying and intestinal transit.

2. Materials and methods

2.1. Materials

Ferrite powder ($MnFe_2O_4$; 80-125μm) was purchased from Thornton, Brazil and was used as the magnetic marker. The enteric polymer used was the methacrylate copolymer (Eudragit® S100) as a gift from Röhm GmbH, Germany. Other excipients used for coating were of standard pharmaceutical grade: magnesium stearate (Valdequímica, Brazil), talc (Valdequímica, Brazil), titanium dioxide (Valdequímica, Brazil), triethyl citrate (Scandiflex, Brazil), and isopropyl alcohol (Sigma-Aldrich, Brazil). The excipients used to prepare pellets and for coating were of standard pharmaceutical grade and all chemical reagents used were of analytical grade.

2.2. Preparation of pellets

Pellets were prepared by a powder layering method of ferrite on nonpareils sugar beads (inert core; ϕ = 1.70 mm) in a coating machine (PCCA, Brazil). This coating machine has the same principle for pan coat that uses a rotating drum and pressurized air. The introduction of the air evaporates the solution and dries the coating. The binder solution was added until the required magnetic material thickness was achieved. Binder solution was prepared by dissolving Eudragit S100 in isopropyl alcohol with magnetic stirring and it has been continuously sprayed on the moving nonpareils. The ferrite powder addition was started after a 1-min lag time of the binder solution. At regular intervals, amounts of the ferrite were layered onto the particles. The ferrite-loaded pellets were dried at 40°C after which sieve analysis was done and the fraction of 2.40–3.50 mm was separated for coating. Ferrites ($MnFe_2O_4$) are inert ferromagnetic materials which can be incorporated into the dosage forms since they present absence of toxicity and lack effects on the gastrointestinal tract (Frei et al., 1968; Corá et al., 2006a).

Coating dispersion were prepared by suspending 6% (w/w) Eudragit S100, 2% (w/w) talc, 1% (w/w) triethyl citrate, 1% (w/w) magnesium stearate, and 2% (w/w) titanium dioxide in 88% isopropyl alcohol. Coating was performed in the coating machine under the following conditions: spray air pressure, 1.50 mg/cm^2; inlet temperature, 40–45ºC; rotating speed, 20 rpm. The enteric-soluble coating dispersion was applied to each 10.0 g of pellets.

The potential of the prepared coated pellets to delay ferrite release in the physiological environment of the stomach and the small intestine was assessed by conducting dissolution tests according to standard methods. Dissolution media were prepared according to USP XXII method: initial release studies were conducted in 900 ml of simulated gastric fluid without pepsin (0.1 N HCl, pH 1.2, 37°C, 100 rpm) for 2 h. Then, pellets were transferred to 900 ml of simulated intestinal fluid without enzymes (pH 7.2) and had remained for 3 h.

Multiparticulate dosage form consisted of 1000 mg of coated pellets, with approximately 600 mg of ferrite, filled into a size 00 uncoated hard hydroxypropylmethycellulose capsules (Vcaps®, Capsugel, Brazil). Capsules had disintegration time of less than 5 min in 0.1 N HCl as tested by the dissolution test described above.

2.3. AC Biosusceptometry

A detailed description about the principles of AC Biosusceptometry (ACB) technique was reported by Corá et al. (2005a). Briefly, AC Biosusceptometry bases its functioning on induction coils for recording the magnetic flux variation obtained from the response of a magnetic material when an alternating magnetic field is applied. This study was carried out using a single-sensor ACB system which has a pair of excitation and detection coils (ϕ = 3.0 cm) coaxially arranged in a first-order gradiometric configuration (Fig. 1). The pair (excitation/detection) that is located more distant from the magnetic material that will be detected acts as a reference coil and the pair closest of the sample as a measurement coil.

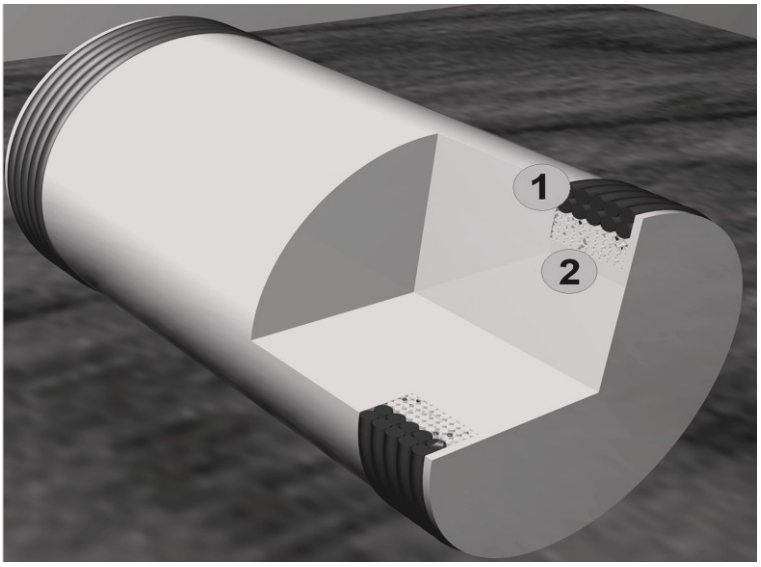

Fig. 1- Single-sensor AC Biosusceptometry system with the pair of excitation (1) and detection coils (2) coaxially arranged in a first-order gradiometric configuration.

The excitation coils induce equal magnetic flux in the detection coils, hence, when a magnetic sample is nearest of the measurement system an imbalance in the voltage occurs, due to the change in the differential flux between the detection coils. Magnetic signals from single-sensor ACB has been acquired employing a lock-in amplifier (Stanford Research Systems, Inc., USA).

2.4. Subjects and study protocol

Nine healthy volunteers (5 male, 4 female; age, 22-30 years; weight, 50-75 kg) participated in the study. The study was approved by the Ethical Committee of Medical School - Sao Paulo State University and the trial was conducted in accordance with the Declaration of Helsinki (1964) and its revisions. Each subject provided written informed consent to participate in the study.

The study consisted of two phases: on one occasion, the multiparticulate dosage form was administered after an overnight fasted (*Fasted phase*), and on another occasion following the standard breakfast (*Fed phase*) described above. The two phases of the study were carried out at least 1 week apart.

The Fasted phase consisted of the administration of the multiparticulate dosage form with 200 ml of water to volunteers who fasted for at least 12 h before dosing. The Fed phase consisted of the administration of the multiparticulate dosage form with 200 ml of water 10 min after the standard breakfast (comprising two slices of bread, two slices of ham, two slices of cheese and 180 g of yogurt) with energy content of 502 Kcal. Further drinks or meals were not allowed until the magnetic pellets had left the stomach.

2.5. Magnetic measurements

A square point matrix (5x5) was drawn around the gastric (lower tip of sternum and the umbilicus as anatomical references) and colonic (McBurney's point and iliac right crest as anatomical references) regions (Fig. 2). The ACB sensor was attached to a computer controlled x-y scanning stage and the measurements were performed. From each scanner, it was generated the magnetic field maps which indicated the intensity values distributed on gastric or colonic region. Those values were corrected for basal measurements prior the administration of the dosage form. Each magnetic monitoring had 120 s duration and was recorded at 10 min intervals over 7 h. This monitoring was performed with the volunteers in orthostatic position. Acquisition of the magnetic signals was carried out from the lock-in amplifier (Stanford Research Systems, Inc., USA) and the signal processing was done using MatLab® (Mathworks, Inc., Natick, MA, USA).

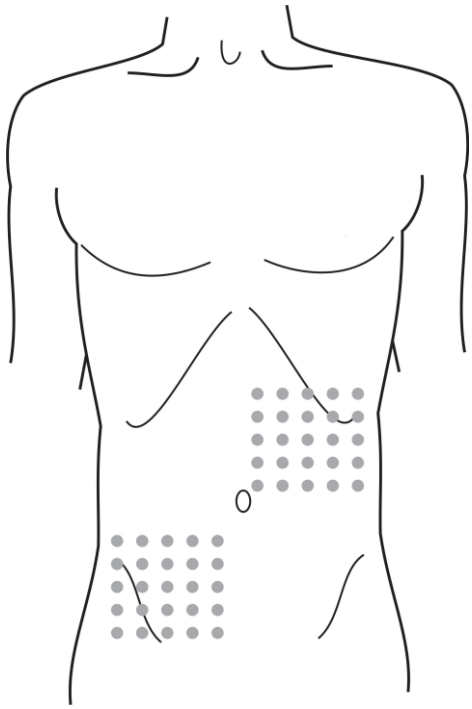

Fig. 2- Schematic representation of square point matrix (gray circles) drawn around gastric and colonic regions.

2.6. Analysis and quantification of magnetic data

Magnetic images were obtained and processed as reported by previous studies performed by our group (Corá et al., 2005b). The square matrices (25 points) were interpolated to obtain the degraded images (256×256). Thereafter, these images were processed for background subtraction, brightness and contrast adjustments and segmentation. The segmentation was the procedure used to find edges in the magnetic images. Thereafter, it was estimated the area of all pixels in the delimited image by summing the areas of each pixel in the image. The magnetic image area decreased if the pellets had emptied from stomach and it increased if the pellets had arrived in another region, as occurred when the pellets arrived on the proximal colon.

As occurs in the disintegration process (Corá et al., 2003, 2008), the pellets distribution was characterized by the transition between a magnetic marker (pellets inside the capsule) to a magnetic tracer (pellets outside the capsule). Hence, when the multiparticulate dosage form has been ingested by the volunteer, the magnetic signals were detected with high and located intensity values. As soon as the pellets started spreading from the capsule, the magnetic signals could be detected as a distribution

on the intensity values. The gastric emptying as well as the colonic arrival has been characterized by measuring the magnetic image area at different time intervals. For gastric emptying, the decrease in the area was calculated as the % in relation to the initial area, i.e., by measuring the number of pixels inside the delimited magnetic image area. For colon arrival, it was calculated the increase of the number of pixels inside the delimited magnetic image area. To obtain more accurate evaluation, those curves were interpolated for 60 points by *spline* method which has improved the temporal resolution.

As reported by a previous study (Podczeck et al., 1995), the parameter t_{50} is not reliable to quantify the gastric emptying mainly due to the irregular shaped profiles which may hinder the correct interpretation of the whole process. Hence, the gastric emptying and colonic arrival of magnetic pellets also were evaluated by applying statistical moments. The Mean Gastric Emptying Time (MGET) was described as amount of pellets emptied at time t; the Mean Colon Arrival Time (MCAT) was represented as amount of pellets arrived at time t; Mean Small Intestinal Transit Time (MSITT) also was quantified as being the difference between MCAT and MGET. MGET and MCAT, as well as their variances (VGET and VCAT, respectively) have been calculated using the simple trapezoidal rules, as proposed by Podczeck et al. (1995).

All the results are expressed as mean ± standard deviation (SD). In order to investigate the relationship between both analyses, the values of, MGET, MCAT and MSITT obtained under fasted and fed conditions were correlated. By, using paired *t*-test statistically significant difference was considered at $p < 0.01$.

3. Results and discussion

Concerning the retention time in the stomach and the transit through the small intestine, it is interesting that the start of drug release could be controlled by pH-dependent polymer dissolution (Leopold, 1999). An essential prerequisite for a delivery system for colon targeting it is to prevent the drug release until the dosage form reaches the colon. Hence, the polymer used in this study has been sufficiently able into assure the magnetic material release solely after arrival in the colon since none of the magnetic coated pellets showed ferrite released during the dissolution test performed at simulated gastric fluid.

The gastrointestinal transit times of the coated pellets for fasted and fed subjects are summarized in Table 1. As expected, gastric emptying time was markedly different under fasted and fed conditions. MGET values quantified for both fasted and fed conditions were 34 ± 14 and 125 ± 46, respectively. Statistically significant differences were obtained between the fed and fasted values of MGET ($p < 0.01$). Although a different method was employed, the observed delayed emptying of magnetic pellets is consistent with previously reported results that showed the gastric emptying time of solid dosage forms increases under fed conditions (O'Reilly et al., 1987; Wilding et al., 1991; Choe et al., 2001).

Gastric emptying plays an important role in determining the retention of oral dosage forms; then, delayed gastric emptying might be exploited as an approach to enhance the absorption of drugs with an absorption window in the upper gastrointestinal tract (Marathe et al., 2000).

Table 1

Gastric emptying, colon arrival and small intestinal transit time for multiparticulate formulation administered to fasted and fed volunteers characterized using Statistical Moments

Subjects	Stomach		Colon		Small Intestine
	MGET (min)	VGET (min^2)	MCAT (min)	VCAT (min^2)	MSITT (min)
Fasted					
1	28	305	218	5794	190
2	37	422	244	10675	207
3	22	129	133	1920	111
4	34	352	201	4664	167
5	34	489	195	5103	161
6	42	798	176	3431	134
7	30	329	160	9574	130
8	16	126	127	10893	111
9	66	1414	269	14300	203
Mean	34	485	191	7372	157
SD	14	402	48	4129	38
Fed					
1	172	9961	336	15894	164
2	92	2687	369	17978	277
3	90	3337	296	12356	206
4	120	8924	346	11045	226
5	210	7756	412	17452	202
6	150	7521	354	13066	204
7	131	5336	335	11056	204
8	62	5292	321	13482	259
9	98	6284	337	15664	239
Mean	125	6344	345	14221	220
SD	46	2441	32	2619	34

MGET is the mean gastric emptying time; MCAT is the mean colon arrival time; MSITT is the mean small intestinal transit time; VGET and VCAT are the variances for gastric emptying and colon arrival, respectively.

For pharmaceutical purposes, the transit of a dosage form through the gastrointestinal tract determines how long a compound remains in contact with its absorptive site. The bioavailability of a drug can be affected by factors that change gastrointestinal transit. Several publications have related that the pellets which had spread in small intestine tend to regroup in the ileocaecal junction for an undetermined period before reaching the colon and the passage across the junction can occurs quickly or for many hours (Coupe et al., 1991 ; Wilding et al., 1991; Clark et al., 1995; Wilding et al., 2000). In the present study, arrival of pellets in the colonic region seemed to occur in bolus, after a stagnation period which might be characterized by a signal with a punctual intensity, i.e., it would be detected none or a minimal spreading of the material. However, further investigation would be needed to elucidate this behavior.

MCAT values for fasted and fed condition were 191 ± 48 and 345 ± 32, respectively, also presented a significant increase ($p < 0.01$). The mean values of MSITT on both fasted and fed state were 157 ± 38 and 220 ± 34, respectively. Statistically significant differences were obtained between the fed and fasted values of MSITT ($p < 0.01$). As reported by others, although small intestinal transit time of pharmaceutical dosage forms in humans seems to be relatively constant and appears to be independent of both the type of dosage form and prandial state, it can be observed a considerable inter-subjects variability (Hardy et al., 1985; Davis et al., 1986; Coupe et al., 1991); moreover, colon arrival may be notably influenced by the gastric emptying. The spreading of pellets in the colon after emptying from the stomach was evaluated by compute the variance of the distributions for stomach (VGET) and colon (VCAT).

Figure 3 are the representative magnetic images of the multiparticulate system were taken from different time intervals, illustrating key stages of the gastrointestinal transit for a fasted subject. On all occasions, pellets had fully dispersed from the HPMC capsule within 5 min. In the subsequent post-dose magnetic images, spreading of the pellets could be observed in the stomach. Gastric emptying was characterized as a decrease in the segmented image area; meanwhile, the colon arrival was evidenced by the gradual increase in the image area.

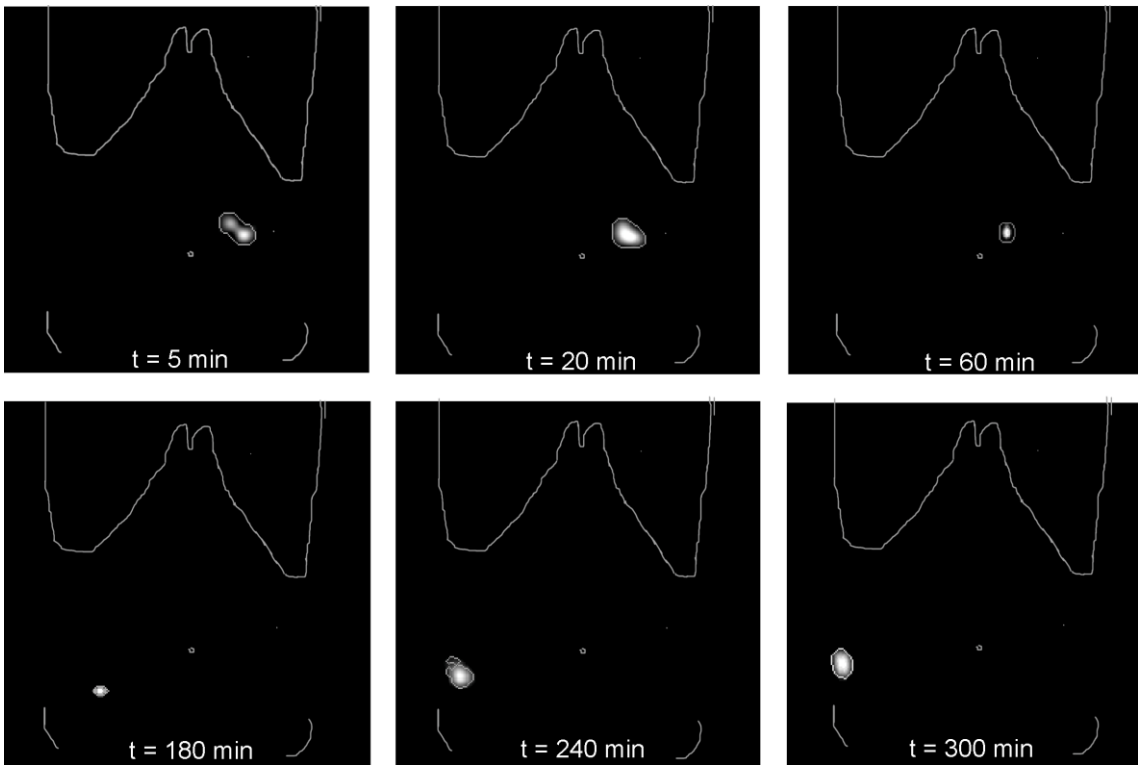

Fig. 3- Sequence of magnetic images of the multiparticulate system showing the pellets dispersion from the HPMC capsule in the stomach and its spreading in the colonic ion region for a fasted volunteer. White outlines are the ribs on top and the iliac crests on the bottom.

Imaging techniques could provide more reliable *in vivo* data than *in vitro* dissolution studies since they are able to demonstrate how solid dosage forms behave in human GI tract. These techniques are especially interesting into demonstrate whether the dosage form is delivering the drug to the target region at the expected time. Thereby, the ability to visualize the delivery process in a non-invasive manner becomes the AC Biosusceptometry as an elegant and innovative method to study a variety of pharmaceutical processes.

Gastric emptying and colon arrival profiles obtained for one subject who received the multiparticulate system on fasted and fed conditions are illustrated in the Fig. 4. In this study, the GI transit of the pellets has been expressed as individual profiles instead of as a single mean profile, because this approach could mask important patterns (Coupe et al., 1993). It was observed that gastrointestinal transit has been typically dependent of the prandial state. The pellets administered before standard meal exhibited an exponential pattern of gastric emptying, while for those

administered after the meal the emptying was characterized by a significant delay. In addition, an interesting profile for intestinal transit was found showing that even before the complete gastric emptying, when the formulation was administered to fed subjects, magnetic pellets had been detected on colonic region.

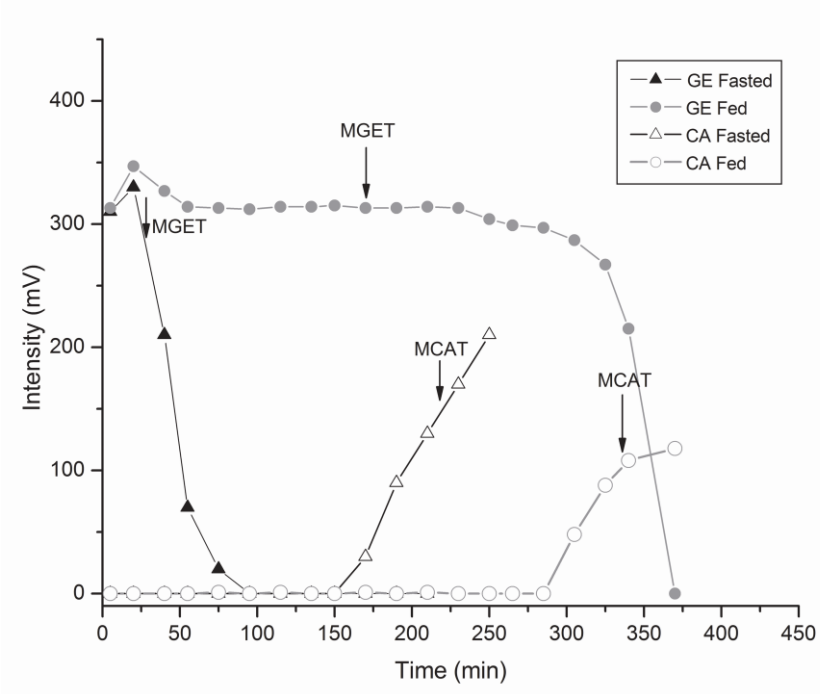

Fig. 4- Example of gastric emptying (GE) and colon arrival (CA) profiles on fasted and fed conditions for subject number 1. The arrows indicate the parameters measured.

4. Conclusion

The results demonstrated that AC Biosusceptometry technique was able to evaluate gastric emptying as well as the gastrointestinal transit of a multiparticulate dosage form under fed and fast conditions. Regarding the importance of physiological parameters on the fate of dosage forms in humans, it is essential the development of noninvasive methods to characterizing delivery systems *in vivo.* Currently, the main limitation of ACB into evaluating solid dosage forms in human gastrointestinal tract is the amount of magnetic material used in the formulations. However, it could be possible to decrease the amount of ferrite by improving the sensitivity and signal-to-noise ratio of the magnetic sensor, to becoming the "magnetic dosage form" in a more

conventional dosage form. Nevertheless, the development of more sensitive sensors will allow overcoming depth effects providing more accurate location of the dosage forms.

In recent years, continuous studies employing the ACB allowed characterizing a number of parameters related to solid dosage forms. It is reasonable to assume the potential of ACB as an alternative tool for pharmaceutical purposes. Furthermore, this technique will be especially valuable when associated with clinical pharmacokinetics to provide more predictable data on bioavailability of drugs correlated to physiological properties.

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Considerações Finais

"Vá tão longe quanto possa ver. Quando chegar lá, você poderá ver ainda mais longe." Thomas Carlyle

Considerações Finais

A indústria farmacêutica investe continuamente na pesquisa e desenvolvimento de produtos não apenas de liberação alvo-específica, como também de maior eficácia terapêutica. Nesse sentido, o advento de excipientes e sistemas de revestimento mais efetivos, possibilitou a obtenção de formas farmacêuticas capazes de controlar a liberação de um ativo no local de ação, visando um efeito mais prolongado ou ainda, a ação imediata e menos errática.

A qualidade de um produto será assegurada pelo equilíbrio entre a escolha dos excipientes, do método de produção e dos perfis de liberação e dissolução do fármaco. Portanto, a liberação do ativo constitui um fator limitante para sua absorção e, desse modo, qualquer fator que interfira nesse processo poderá alterar cineticamente a biodisponibilidade do fármaco administrado.

Sendo conhecido que o trato gastrintestinal humano influencia significativamente os parâmetros relacionados com a liberação e absorção de drogas, houve o interesse no desenvolvimento ou aprimoramento de métodos capazes de avaliar a performance de um produto *in vivo.* Nesse sentido, merece destaque a Biosusceptometria AC, uma técnica cujas características e versatilidade permitiram inseri-la na pesquisa farmacêutica como uma alternativa aos métodos tradicionais.

Diante dos resultados apresentados, a BAC demonstrou potencial como um método capaz de prover os requisitos necessários para monitorar diferentes processos farmacêuticos não apenas *in vitro*, como também no TGI humano. Permitiu, em um primeiro momento, a obtenção de imagens do processo de desintegração de comprimidos tanto *in vitro* quanto no estômago humano. Esse trabalhou inseriu a BAC como um novo método de imagem e introduziu outra perspectiva na análise do processo de desintegração, visto que até esse momento, era caracterizado apenas como alterações no nível de intensidade do sinal magnético detectado.

Ainda em relação ao processo de desintegração, a penetração de água na forma farmacêutica é um fator determinante para um bom desempenho, visto que resulta no intumescimento das partículas e no desenvolvimento de uma força que auxilia a desintegração. Então, o nível de compressão aplicado também é um

parâmetro fundamental no que concerne à obtenção de um comprimido, visto que na medida em que a compressão aumenta, ocorre uma diminuição na porosidade da forma farmacêutica que, por sua vez, interfere com a capacidade de penetração de líquido e, conseqüentemente, com a força desenvolvida durante a desintegração. Esses parâmetros puderam ser avaliados pela BAC em um estudo cujo foco principal foi demonstrar que os resultados fornecidos pela técnica magnética podem ser correlacionados com aqueles obtidos por metodologias específicas, garantindo uma análise mais acurada dos parâmetros físicos envolvidos com a desintegração de comprimidos.

Além de comprimidos, cápsulas gelatinosas duras ou aquelas constituídas por hidroxipropilmetilcelulose (HPMC) são formas farmacêuticas sólidas muito utilizadas quando se objetiva a administração oral de drogas. A possibilidade de utilizar cápsulas de HPMC revestidas visando a liberação colônica foi demonstrada em diversos estudos, visto que o cólon humano é um órgão-alvo para a liberação de drogas de ação local ou sistêmica. Nesse sentido, utilizando-se a BAC também foi possível avaliar o trânsito gastrintestinal e o processo de desintegração de cápsulas revestidas no cólon humano. Além da desintegração, também foi caracterizado o perfil da motilidade, o tempo de retenção e o trânsito intestinal da forma farmacêutica.

Considerando o desenvolvimento de novas formulações, um dos maiores progressos alcançados foi a possibilidade de controlar ou modificar a liberação de drogas no trato gastrintestinal humano e, desse modo, os sistemas multiparticulados foram propostos devido às vantagens biofarmacotécnicas e terapêuticas que apresentam. A BAC foi empregada com o intuito de monitorar um sistema multiparticulado magnético e avaliar a influência do estado prandial em parâmetros fisiológicos como esvaziamento gástrico e trânsito intestinal.

Este trabalho demonstrou que, apesar das suas limitações, a BAC tem potencial para avaliar diferentes formas farmacêuticas, inserindo-se como um método alternativo na pesquisa farmacêutica. Desse modo, seu constante aperfeiçoamento, aliado à necessidade de estabelecer uma análise mais acurada no que concerne à liberação não apenas do material magnético, como também de um princípio ativo, a BAC terá como foco principal avaliar a qualidade do produto farmacêutico proposto e comparar os perfis de dissolução de um medicamento

referência *in vitro,* seguindo as análises preconizadas pela Farmacopéia Brasileira, com os resultados obtidos por meio das medidas empregando-se esse método magnético. Desse modo, serão utilizados sistemas de liberação modificada de drogas baseados em matrizes hidrofílicas e sistemas multiparticulados em comprimidos. Além disso, a BAC será associada com análises farmacocinéticas ("magnetofarmacocinética"), no intuito de estabelecer a correlação *in vitro-in vivo* (IVIVC) para o fármaco utilizado.