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Desenvolvimento Tecnológico e Avaliação In Vitro de Matrizes Hidrofílicas de Norfloxacino Contendo Polioxietileno e Hidroxipropilmetilcelulose

Paulo Renato de Oliveira

Florianópolis
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Desenvolvimento Tecnológico e Avaliação In Vitro de Matrizes Hidrofílicas de Norfloxacino Contendo Polioxietileno e Hidroxipropilmetilcelulose

por

Paulo Renato de Oliveira

Tese apresentada ao Programa de Pós-Graduação em Farmácia da Universidade Federal de Santa Catarina como requisito parcial à obtenção do grau de Doutor em Farmácia.

Orientador: Prof. Dr. Marcos A. Segatto Silva

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"Desenvolvimento e avaliação in vitro de matrizes hidrofílicas de Norfloxacino contendo polioxietileno e hidroxipropilmetilcelulose"

POR

Paulo Renato de Oliveira

Tese julgada e aprovada em sua forma final pelo Orientador e membros da Banca Examinadora, composta pelos Professores Doutores:

Banca Examinadora:

Profa. Dra. Silvia Cuffini (CEPROCOR – Membro Titular)

Profa. Dra. Clarice Madalena Bueno Rolim (UFSM – Membro Titular)

Profa. Dra. Nadia Maria Volpato (UFRGS – Membro Titular)

Prof. Dr. Flávio Henrique Reginatto (UFSC – Membro Titular)

Profa. Dra. Elenara Lemos Senna (UFSC – Membro Titular)

Prof. Dra. Marcos Antônio Segatto Silva (UFSC – Orientador)

Prof. Dr. Eloir Paulo Schenkel
Coordenador do Programa de Pós-Graduação em Farmácia da UFSC

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"Um homem precisa viajar. Por sua conta, não por meio de histórias, imagens, livros ou TV. Precisa viajar por si, com seus olhos e pés, para entender o que é seu. Para um dia plantar as suas próprias árvores e dar-lhes valor. Conhecer o frio para desfrutar o calor. E o oposto. Sentir a distância e o desabrigo para estar bem sob o próprio teto. Um homem precisa viajar para lugares que não conhece para quebrar essa arrogância que nos faz ver o mundo como o imaginamos, e não simplesmente como é ou pode ser. Que nos faz professores e doutores do que não vimos, quando deveríamos ser alunos, e simplesmente ir ver"

Amyr Klink

RESUMO

O norfloxacino é um fármaco antimicrobiano amplamente utilizado para o tratamento de infecções do trato urinário. A posologia indicada normalmente é de 400 mg a cada 12 horas. Este trabalho teve como objetivo desenvolver formulações de liberação prolongada contendo norfloxacino para administração em dose única diária, com conseqüente melhora na terapêutica. Durante a etapa de pré-formulação, estudos de compatibilidade entre fármaco e excipientes não demonstraram mudança significativa no perfil termoanalítico dos compostos, sugerindo ausência de incompatibilidade. Metodologia analítica por cromatografia líquida de alta eficiência foi desenvolvida e validada para o doseamento e avaliação da estabilidade das formulações. Os comprimidos foram obtidos através da compressão direta utilizando dois tipos de polímeros hidrofílicos: hidroxipropilmetilcelulose (HPMC) ou polioxietileno (POE), de diferentes massas moleculares, e em duas concentrações (20 e 30%), foram utilizados para obter as matrizes hidrofílicas. Através dos estudos de estabilidade foi possível verificar que o teor não foi influenciado pela temperatura e umidade, porém o revestimento ou emblistagem das formulações em material adequado é necessário para impedir a fotodegradação do fármaco. Os estudos de dissolução demonstraram que conforme o aumento da massa molecular e da concentração do polímero na formulação, o sistema hidrofílico apresenta um mecanismo de liberação que muda de Super Caso-II (mais dependente do relaxamento das cadeiras poliméricas e erosão da matriz) para Transporte Anômalo (dependente da difusão do fármaco e relaxamento/erosão matricial). As formulações que apresentaram melhor desempenho in vitro foram HPMC K100 LV 30%, HPMC K4M 20%, POE N60K 20% e POE N60K 30%. Os sistemas Dome Matrix® acoplados na configuração "void" demonstraram capacidade de flutuação in vitro por aproximadamente 240 min, indicando possível liberação prolongada e local-específica (estômago) in vivo. Este resultado é de grande relevância terapêutica, uma vez que o fármaco apresenta maior solubilidade em meio ácido. O trabalho desenvolvido demonstra que os sistemas matriciais podem apresentar melhor *performance* in vivo em comparação aos comprimidos convencionais de liberação imediata disponíveis atualmente no mercado.

Palavras-chave: norfloxacino; liberação prolongada; comprimido matricial; hidroxipropilmetilcelulose; polioxietileno; Dome Matrix®

ABSTRACT

Norfloxacin is an antibacterial drug mainly used for the treatment of urinary tract infections. The recommended dosage is 400 twice daily. The aim of this research was to develop extended-release tablets for once-a-day administration, with subsequent improvement of therapeutics. Compatibility studies between the drug and excipients did not evidenced difference in thermo-analytical profile of compounds, this way suggesting the absence of incompatibility. A stability-indicating liquid chromatographic method was developed and validated for the assay of formulations. The matrix tablets were successfully obtained by direct compression. Two polymers: hydroxypropylmethylcellulose and poly(ethylene oxide), with different molecular weights, and in two concentrations (20 and 30%) were used to obtain the formulations. Stability studies showed that the assay was not influenced by temperature and humidity, however tablet film-coating or opaque blistering are necessary to ensure the photostability. Dissolution studies showed that with the increase in molecular weight and concentration of polymer in the formulation, the dissolution mechanism changed from Super Case-II (a more polymer relaxation or erosion dependent) to Anomalous Transport (drug-diffusion and polymer relaxation/erosion dependent). The formulations containing HPMC K100 LV 30%, HPMC K4M 20%, PEO N60K 20%, and PEO N60K 30% demonstrated a better in vitro dissolution profile. Dome Matrix® systems assembled in "void" configuration were able to float in vitro for up to 240 min, indicating a possible in vivo extended and site-specific drug delivery system. This result is of great therapeutic interest since norfloxacin is more soluble in acid medium. The developed research demonstrated that matrix systems could provide a better in vivo performance compared to the norfloxacin conventional immediate-release tablets available in the market.

Keywords: norfloxacin; extended-release; matrix tablet; hydroxypropylmethylcellulose; poly(ethylene oxide); Dome Matrix®

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LISTA DE ABREVIATURAS

µg	Micrograma
ASC	Área sob a curva da concentração plasmática versus tempo
bioMEMS	Sistemas bio-micro-eletro-mecânico
C_{max}	Concentração plasmática máxima
Da	Daltons
DNA	Ácido desoxirribonuclêico
DPR	Desvio padrão relativo
DRIFT	Infravermelho com transformada de Fourier de reflexão difusa
DSC	Calorimetria exploratória diferencial
h	Hora
HPMC	Hidroxipropilmetilcelulose
ITU	Infecção do trato urinário
mg	Miligrama
mL	Mililitro
POE	Polioxietileno
SEM	Microscopia eletrônica de varredura
$t_{½}$	Tempo de meia-vida
TG	Termogravimetria
t_{max}	Tempo para atingir a concentração plasmática máxima
XRPD	Difração de raio-x de pó

INTRODUÇÃO

O norfloxacino pertence ao grupo das quinolonas, sendo um agente antibacteriano de amplo espectro contra patógenos Gram-positivos e Gram-negativos. É indicado terapeuticamente principalmente para o tratamento de infecções do trato urinário. Seu mecanismo de ação está baseado na inibição da enzima DNA girase e da topoisomerase IV, inibindo a replicação do DNA bacteriano. A posologia recomendada normalmente é de 400 mg a cada 12 horas e a duração do tratamento varia de acordo com o diagnóstico (MANDELL, 1988; VAN BAMBEKE et al., 2005; RANG et al., 2008; BOLON, 2009).

Recentemente a ANVISA, com intuído de diminuir o uso indiscriminado de antibióticos e o surgimento de microorganismos resistentes, determinou que a venda de medicamentos a base de antimicrobianos somente poderá ser efetuada mediante receita de controle especial, ficando uma via retida na farmácia (BRASIL, 2010). O estudo de formulações contendo antibióticos que objetivam o aumento da aderência do paciente ao tratamento, a manutenção da eficácia terapêutica minimizando ou evitando concentrações plasmáticas sub ou supraterapêuticas e a diminuição do surgimento de microorganismos resistentes é de grande relevância, uma vez que infecções bacterianas são bastante comuns e atingem milhões de pessoas anualmente. Em termos mercadológicos, os comprimidos representam a forma farmacêutica mais consumida, além disso, é a mais adequada para produção em escala industrial possibilitando menor custo efetivo (ALLEN; POPOVICH; ANSEL, 2007).

O estudo de propriedades no estado sólido e de compatibilidade fármaco-excipientes são pré-requisitos no desenvolvimento racional de medicamentos, pois diferentes formas cristalinas podem exibir distintas propriedades físico-químicas, alterando a biodisponibilidade e conseqüentemente a terapêutica (GIRON, 2002; AALTONEN et al., 2009). O desenvolvimento e a validação da metodologia analítica utilizada fornecem dados para assegurar a confiabilidade dos resultados obtidos (SHABIR, 2003; BRASIL, 2003; ICH, 2005; STÖCKL, 2009). Posteriormente estudos de perfil de dissolução e estabilidade são realizados para avaliar a liberação do fármaco e a qualidade do produto em desenvolvimento ou acabado.

As formas farmacêuticas de liberação prolongada objetivam o aumento do tempo de ação farmacológica de substâncias ativas, a diminuição de reações adversas, a redução da administração diária, a

manutenção da eficácia terapêutica, a otimização da aderência ao tratamento e a não permissão ou minimização do aparecimento de flutuação nas concentrações plasmáticas (AULTON, 2005; ALLEN; POPOVICH; ANSEL, 2007; HOFFMAN, 2008). Possibilitam, também, obter a mesma eficácia clínica com o decréscimo dos custos do tratamento, sendo uma alternativa econômica quando comparada com formas de dosagem de liberação imediata (SAKS; GARDNER, 1997).

O desenvolvimento de sistemas matriciais é ainda o modelo de referência para inovações em liberação prolongada de fármacos na industria farmacêutica devido ao fato de serem considerados confiáveis em termos de liberação e facilidades de formulação e fabricação (COLOMBO et al., 2009). Nestes sistemas, o mecanismo de liberação do fármaco e sua cinética podem ser governados pelo intumescimento e difusão do fármaco ou pelo intumescimento e erosão do polímero, dependendo da porcentagem e da massa molecular do polímero utilizado e das características fisico-químicas do fármaco (COLOMBO et al., 2000; CONTI et al., 2007; OMIDIAN; PARK, 2008; PARK et al., 2010). Dos agentes poliméricos disponíveis para a obtenção de comprimidos matriciais, destacam-se o polioxietileno e a hidroxipropilmetilcelulose, pois apresentam, entre outras características, boa compressibilidade, boa capacidade de intumescência e atoxicidade.

Além da utilização de diferentes polímeros, o emprego de novas tecnologias de fabricação representa alternativa para obtenção de formas farmacêuticas diferenciadas, possibilitando melhor resultado terapêutico. Um exemplo de nova tecnologia é a Dome Matrix®, onde módulos individuais "male" e "female" são obtidos utilizando punções especiais, e posteriormente pode-se acoplar os mesmos em diversas combinações, formando inclusive sistemas flutuantes. Esta tecnologia tem como característica possibilitar uma grande flexibilidade na formulação e posterior liberação do fármaco (LOSI et al., 2006; STRUSI et al., 2008; STRUSI et al., 2010).

Neste contexto, o presente estudo propõe o desenvolvimento e avaliação *in vitro* de matrizes hidrofílicas e sistemas Dome Matrix® de liberação modificada contendo norfloxacino.

OBJETIVOS

Objetivo geral

Desenvolvimento tecnológico de comprimidos matriciais de liberação prolongada e sistemas Dome Matrix® flutuantes de norfloxacino contendo os polímeros hidrofílicos polioxietileno (POE) e hidroxipropilmetilcelulose (HPMC).

Objetivo específicos

- Verificar as características físico-químicas do norfloxacino e dos polímeros POE e HPMC, obtendo resultados que servirão de referência para avaliações da qualidade;
- Avaliar a compatibilidade entre o norfloxacino e diversos excipientes farmacêuticos para o desenvolvimento racional das matrizes;
- Desenvolver e validar metodologia indicativa da estabilidade por cromatografia líquida em fase reversa;
- Produzir matrizes hidrofílicas de liberação prolongada de norfloxacino através da compressão direta, utilizando polímeros de diferentes massas moleculares e em duas concentrações;
- Realizar revestimento e emblistagem, para avaliar a influência destes na estabilidade das matrizes;
- Obter módulos individuais Dome Matrix® "male" e "female" e formar sistema flutuante;
- Avaliação das propriedades físico-químicas (farmacopéicas) dos comprimidos;
- Caracterizar as formulações quanto aos seus perfis de dissolução;
- Comparar o modelo cinético de liberação do norfloxacino a partir dos comprimidos matriciais através da aplicação de modelos dependentes e independentes;
- Avaliar a relação entre os diferentes polímeros e concentrações com o mecanismo de liberação do fármaco;
- Estudar a estabilidade acelerada em câmara climática e fotoestabilidade das formulações desenvolvidas.

CAPÍTULO 1 – Revisão bibliográfica

1. NORFLOXACINO

A infecção do trato urinário (ITU) é afecção muito comum e caracteriza-se pela presença de microorganismos, principalmente bactérias, nas vias urinárias, seja na bexiga, próstata, sistema coletor ou rins. A incidência é maior em mulheres, principalmente devido às condições anatômicas como uretra mais curta e maior proximidade do ânus com a uretra e o vestíbulo vaginal, o que possibilita a colonização destes por enterobactérias (principalmente *Escherichia coli*) (KRIEGER, 2002; WAGENLEHNER; NABER, 2006). As ITU destacam-se não somente pela sua freqüência como também pela possibilidade de complicações graves, como a insuficiência renal e a septicemia. Aproximadamente 15% de todos os antibióticos prescritos nos Estado Unidos são dispensados para tratamento de ITU, com um custo estimado anual superior a 1 bilhão de dólares. Além disso, o custo direta e indiretamente associado a esta enfermidade está estimado em mais de 1,6 bilhões de dólares ao ano (WAGENLEHNER; NABER, 2006).

A classe de antibióticos das quinolonas, desde sua descoberta na década de 60, tem sido extensivamente estudada e utilizada clinicamente, pois apresenta uma série de vantagens, tais como combinação de alta potência e amplo espectro de ação, boa biodisponibilidade, formulações orais e intravenosas, altos níveis plasmáticos, grande volume de distribuição e relativamente baixos efeitos colaterais (ANDERSSON; MACGOWAN, 2003).

O norfloxacino (Figura 1), comercializado desde 1986, pertencente ao grupo das quinolonas, é um agente bactericida de amplo espectro contra patógenos Gram-positivos e Gram-negativos. Seu mecanismo de ação está baseado na inibição da topoisomerase II (uma DNA-girase bacteriana), enzima que produz um supernovelo negativo no DNA, permitindo sua transcrição ou replicação (Figura 2) (RANG et al., 2008). É indicado terapeuticamente principalmente para o tratamento de infecções do trato urinário (cistite, pielite, cistopielite, pielonefrite, prostatite crônica, epididimite). Também é indicado para o tratamento de gastroenterites bacterianas, uretrite, faringite, proctite ou cervicite gonocócicas, febre tifóide e na profilaxia da sepse em pacientes com neutropenia intensa e da gastroenterite bacteriana. A posologia recomendada normalmente é de 400 mg a cada 12 horas e a duração do tratamento varia de acordo com o diagnóstico (MANDELL, 1988; CHRISTIAN, 1996; EMMERSON; JONES, 2003).

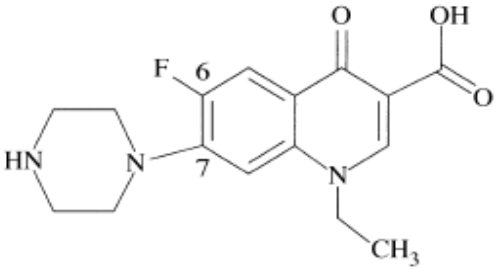


Figura 1. Estrutura química do norfloxacino.

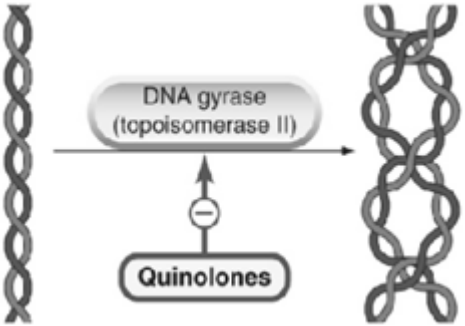


Figura 2. Diagrama simplificado do mecanismo de ação das fluorquinolonas (RANG et al., 2008).

Quimicamente, o norfloxacino é denominado como ácido 1-etil-6-fluor-1,4-diidro-4-oxo-7-(1-piperazinil)-3-quinolino carboxílico (Figura 1). É um pó cristalino branco a amarelo claro, pouco solúvel em água, metanol, etanol, acetato de etila e acetona, facilmente solúvel em ácido acético, ligeiramente solúvel em clorofórmio e insolúvel em éter etílico. Possui massa molecular de 319,34 Daltons, fórmula molecular $C_{16}H_{18}FN_3O_3$ (F. BRAS. IV, 2001).

A relação estrutura-atividade das quinolonas é bastante estudada, o átomo de flúor na posição 6 proporciona maior potência contra organismos Gram-negativos e o núcleo piperazínico na posição 7 é responsável pela atividade antipseudomonas e aumento da ação contra

Gram-positivos do norfloxacino (VAN BAMBEKE et al., 2005; BOLON, 2009).

O NFX, composto anfótero, apresenta dois sítios de protonação, o nitrogênio 4' do anel piperazinil (básico) e o grupo carboxila quinolona (ácido) (Figura 3), consequentemente apresenta duas constantes de equilíbrio, com valores de pKa de aproximadamente 8,5 e 6,5, respectivamente. De acordo com o pH, este fármaco apresentará diferentes formas. Em pH neutro será predominatemente um zwitterion (com o grupo carboxílico deprotonado e o nitrogênio 4' protonado). Em pH 10, mais de 90% estará na forma aniônica. A forma catiônica é obtida em pH igual ou inferior a 4,5 (YU; ZIPP; DAVIDSON, 1994; MUSA et al., 2009). O norfloxacino exibe maior solubilidade em pH inferior a 4,5 e superior a 8.

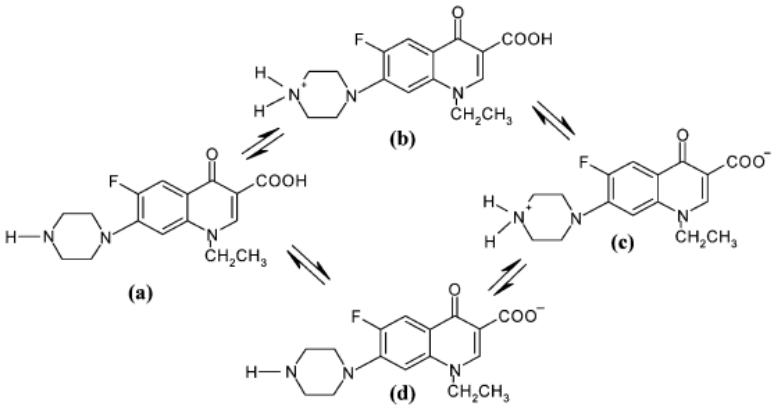


Figura 3. Diferentes protonações do norfloxacino dependentes do pH: (a) neutra, (b) cation, (c) zwitterion e (d) anion (MUSA et al., 2009).

Após administração oral de dose de 400 mg de norfloxacino, no mínimo 30-40% é absorvida, a concentração plasmática máxima atingida (C_{max}) é de aproximadamente 1,5 µg/mL sendo obtida após 1 hora (t_{max}). O tempo de meia-vida ($t_{½}$) é de 3-4 horas com excreção predominantemente renal através da filtração gromerular e secreção tubular. A área sob a curva da concentração plasmática versus tempo (ASC) é de 6,4 µg.h/mL. A administração concomitante com alimentos e antiácidos pode diminuir a absorção (GADEBUSH; SHUNGU, 1991; AL-RASHOOD et al., 2001).

O local preferencial de absorção do norfloxacino ou a proporção de fármaco absorvido em cada parte do trato gastrointestinal não estão descritos na literatura. Devido a sua alta solubilidade em meio ácido, a solubilização no estômago e absorção neste local ou na parte proximal do intestino delgado parecem ter grande influência para a biodisponibilidade e efeito farmacológico dos comprimidos de liberação convencional.

2. FORMAS FARMACÊUTICAS DE LIBERAÇÃO MODIFICADA

As formas farmacêuticas de liberação prolongada objetivam o aumento do tempo de ação farmacológica de substâncias ativas, a diminuição de reações adversas, a redução da administração diária, a manutenção da eficácia terapêutica, a otimização da aderência ao tratamento e não permissão ou minimização do aparecimento de flutuação nas concentrações plasmáticas (AULTON, 2005; ALLEN; POPOVICH; ANSEL, 2007; HOFFMAN, 2008). Possibilitam, também, obter a mesma a eficácia clínica com o decréscimo dos custos do tratamento, sendo uma alternativa econômica quando comparada com formas de dosagem de liberação imediata (SAKS; GARDNER, 1997).

Atualmente diversos sistemas de liberação modificadas de fármacos são estudados, desde os baseados em polímeros hidrofílicos biocompatíveis (mais relacionados aos processos "clássicos" de produção de medicamentos), passando pelos micro e nano-estruturados e chegando aos avançados sistemas bio-micro-eletro-mecânico (bioMEMS), onde sistemas microeletrônicos são produzidos com material biocompatível (por exemplo, polimetilmetacrilato) podendo ser programados para liberar o fármaco, contido em reservatório, de acordo com mudanças fisiológicas detectadas pelo sistema (COLOMBO et al., 2009).

Uma alternativa na otimização do esquema posológico é o desenvolvimento de formas de liberação modificadas, mais especificamente, de um sistema matricial monolítico. Este sistema tecnológico compreende a mistura comprimida de um fármaco e um polímero hidrofílico. A formulação de fármacos em sistemas matriciais inertes é um dos métodos de referência utilizados para o desenvolvimento de liberação prolongada de fármacos, principalmente devido a apresentar vantagens como: facilidade de manufatura e custo

reduzido de produção, uma vez que se utilizam equipamentos e processos convencionais (tais como a compressão direta e granulação), baixa influência das variáveis fisiológicas no processo de dissolução do princípio ativo, facilidade no controle de liberação, versatilidade na incorporação de substâncias ativas (inclusive em grandes quantidades) (LOPES; LOBO; COSTA, 2005; COLOMBO et al., 2009).

Resumidamente, após a dissolução do fármaco na superfície do comprimido na água ou no suco gástrico, o polímero hidrofílico hidrata-se e intumesce rapidamente, formando uma camada externa de gel, com propriedades ideais para controlar a liberação do fármaco. A camada de gel torna-se uma barreira à entrada de mais água e à transferência de fármaco, sendo que a liberação do fármaco ocorre na medida em que o polímero passa do estado vítreo (matriz seca) para o gelatinoso (COLOMBO et al., 1996; COLOMBO et al., 2000; SIEPMANN; PEPPAS, 2001; LOPES; LOBO; COSTA, 2005; OMIDIAN; PARK, 2008). A liberação do fármaco, se solúvel, ocorre por difusão pela camada de gel, e se for insolúvel, é liberado por erosão, seguida de dissolução. Após a erosão, a nova superfície torna-se hidratada e forma uma nova camada de gel (COLOMBO et al., 1996; BETTINI et al., 2001; CONTI et al., 2007). Em uma observação macroscópica do processo de intumescimento, podem ser verificadas três distintas áreas (frontes). O primeiro fronte, o de intumescimento, pode ser observado pela clara separação da região intumescida da região vítrea. O segundo fronte, chamado de fronte de erosão, é a delimitação externa do sistema. E o terceiro fronte, chamado de difusão, é caracterizado pela região compreendida entre os frontes de intumescimento e erosão (Figura 4) (COLOMBO et al., 1996; BETTINI et al., 2001; LOPES; LOBO; COSTA, 2005). A formação do gel é importante para a resistência da matriz e é controlada pela concentração, viscosidade e estrutura química do polímero no estado gélico. A rápida formação dessa camada externa é essencial para a estabilidade do sistema (SIEPMANN; PEPPAS, 2001; LOPES; LOBO; COSTA, 2005; CONTI et al., 2007; PARK et al., 2010).

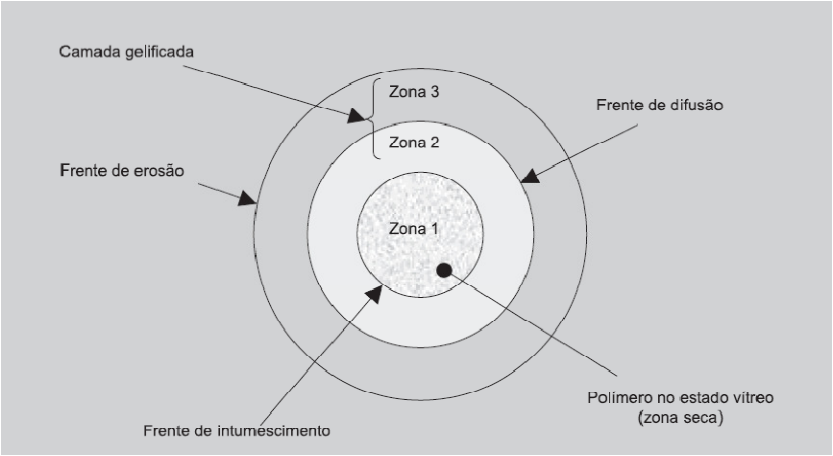


Figura 4. Diagrama dos diferentes frontes existentes em um sistema matricial hidratado (LOPES; LOBO; COSTA, 2005).

Dos agentes poliméricos disponíveis para a obtenção de comprimidos matriciais, o POE (Figura 5) é um polímero bastante utilizado devido à sua boa compressibilidade, não iônico, intumescível, e de fácil manipulação. Para a produção de comprimidos, POE pode ser usado como um excipiente até a concentração de 5-85% (HELLER et al., 2002; PETROVIC et al., 2009; ROWE; SHESKEY; QUINN, 2009). Devido a sua natureza não iônica, o pH do meio não exerce significativa influência na solubilidade e, consequentemente, no perfil de dissolução para o POE. Qualquer alteração pH dependente será devido ao fármaco ou outros componentes da formulação (KIM, 1995, DOW, 2002). O mecanismo de liberação pode ser governado pelo intumescimento e difusão do fármaco ou pelo intumescimento e erosão do polímero, dependendo da porcentagem e da massa molecular do POE usado. Os tipos de POE disponíveis encontram-se descritos na tabela 1. No que tange à sua concentração na formulação e sua massa molecular, os dados de literatura demonstram haver uma relação direta entre estes fatores e a liberação do fármaco a partir dos comprimidos (KIM, 1995; MAGGI et al., 2002). Geralmente, para produtos de baixa massa molecular o mecanismo que impera na liberação do fármaco é a razão de erosão do polímero, uma vez que, para massa molecular alta, o intumescimento do material polimérico é o passo dominante na cinética

de liberação controlada (APICELLA et al., 1993; MAGGI et al., 2002; JAMZAD; TUTUNJI; FASSIHI, 2005; JAMZAD; FASSIHI, 2006).

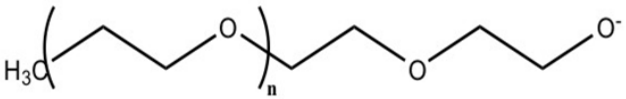
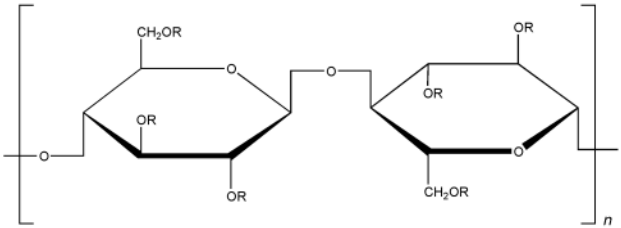


Figura 5. Estrutura química do polioxietileno.

Tabela 1. Tipos de polioxietileno disponíveis e respectivas massas moleculares aproximadas (ROWE; SHESKEY; QUINN, 2009).

Polioxietileno	Massa molecular (Da)
WSR N-10	100 000
WSR N-80	200 000
WSR N-750	300 000
WSR N-3000	400 000
WSR 205	600 000
WSR 1105	900 000
WSR N-12K	1 000 000
WSR N-60K	2 000 000
WSR 301	4 000 000
WSR Coagulant	5 000 000
WSR 303	7 000 000

A HPMC, conhecida também como hipromelose (Figura 6) é hoje o polímero mais comumente utilizado no desenvolvimento de sistemas matriciais de liberação de fármacos. A HPMC K, a mais amplamente utilizada como recurso para extensão da liberação de fármacos, está disponível em diferentes massas moleculares e é classificada de acordo com sua viscosidade (Tabela 2). Da mesma forma que o POE, a HPMC tem características não iônicas, não sofrendo influência significativa do pH do meio na solubilidade e viscosidade da camada gélica formada (DOW, 2000).



Onde R: H, CH_3, ou $CH_3CH(OH)CH_2$

Figura 6. Estrutura química da hidroxipropilmetilcelulose.

Tabela 2. Tipos de hidroxipropilmetilcelulose disponíveis e respectivas viscosidades (2%, p/v, 20 ºC) (ROWE; SHESKEY; QUINN, 2009).

Hidroxipropilmetilcelulose	Viscosidade (mPa s)
Methocel K3 Premium LV	3
Methocel K100 Premium LVEP	100
Methocel K4M Premium	4 000
Methocel K15M Premium	15 000
Methocel K100M Premium	100 000

Dois aspectos conferem uma adequada performance à HPMC em relação à extensão da liberação de fármacos, a rápida formação da camada gélica durante e hidratação e a viscosidade relacionada ao tipo de HPMC. Uma vez que a camada de gel é formada, a viscosidade dessa regula a razão da liberação do fármaco. Essa regulação esta relacionada principalmente à propriedade de dissolução e difusão do fármaco na camada hidratada do polímero, quando este tem características hidrofílicas. Por outro lado, fármacos com características lipofílicas terão sua liberação regulada preferencialmente pela erosão da matriz polimérica. Entretanto, sabe-se que ambos os mecanismos de liberação atuam sinergicamente, salvo as proporcionalidades decorrentes das características de solubilidade do fármaco em questão (SIEPMANN; PEPPAS, 2001; LOPES; LOBO; COSTA, 2005; OMIDIAN; PARK, 2008).

De uma maneira geral, o POE apresenta uma hidratação e geleificação mais rápida que a HPMC, sendo o fármaco mais facilmente dissolvido no meio e a matriz hidrofílica mais susceptível à erosão. Consequentemente, há redução da eficiência do POE em relação ao controle da liberação do fármaco, o que pode ser corrigido com a

quantidade e a massa molecular do POE utilizado (MAGGI; BRUNI; CONTE, 2000; JAMZAD; FASSIHI, 2006).

A tecnologia existente hoje permite modular a liberação de fármacos no trato gastro-intestinal a partir de sistemas farmacêuticos ao longo de períodos que podem chegar a 24 h. Porém, para alguns fármacos, prolongamento do tempo de retenção gástrica em determinadas circunstâncias pode ser útil para aumentar sua biodisponibilidade e efeito terapêutico. No caso de fármacos absorvidos principalmente no estômago ou na parte proximal do intestino delgado ou ainda no caso de fármacos que sejam degradados em pH alcalino as vantagens deste tipo de dispositivos tornam-se evidentes (BARDONNET et al., 2006; BARROCAS et al., 2007). De acordo com Bardonnet et al. (2006) estes sistemas podem ser classificados em: sistemas flutuantes, sistemas expansíveis, sistemas muco-adesivos e sistemas magnéticos.

Os sistemas flutuantes, onde a forma farmacêutica flutua no suco gástrico (figura 7), são muito promissoras, uma vez que nesta situação o sistema fica fisicamente afastado do piloro, dificultando seu esvaziamento gástrico. Várias estratégias podem ser adotadas para esta flutuação, por exemplo: sistemas hidrodinamicamente equilibrados, sistemas de baixa densidade, sistemas geradores de gás (principalmente CO_2) (BARDONNET et al., 2006) e mais recentemente, sistemas Dome Matrix®.

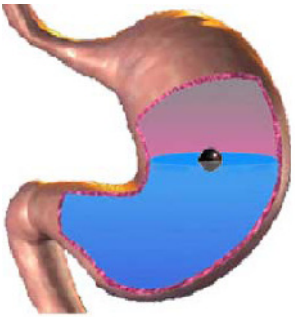


Figura 7. Representação de sistema flutuante (BARDONNET et al., 2006)

Em 2006 foi apresentada tecnologia inovadora para sistemas de liberação modificada, caracterizada por grande flexibilidade na produção, chamada Dome Matrix® (LOSI et al., 2006). Nesta tecnologia, módulos individuais são acoplados e a liberação será influenciada pela maneira com que estes módulos estão conectados entre si e pelas suas composições individuais. Cada módulo tem a forma de um disco com as bases curvas (uma côncava e outra convexa), conforme demonstrado na Figura 8. Neste artigo é apresentada a Dome Matrix® obtida com polímero hidrofílico HPMC. A forma não usual deste sistema foi projetada para permitir o encaixe de uma base côncava em uma convexa adjacente, formando desta maneira a configuração "piled". Outra forma possível de encaixe são dois módulos unidos pela base côncava, neste caso haverá um espaço interno vazio, esta configuração é denominada de "void". Desta forma a dose medicamentosa pode ser facilmente ajustada ou vários sistemas de liberação podem ser montados (STRUSI et al., 2010).

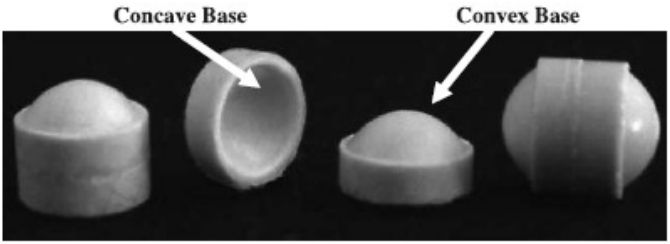


Figura 8. Módulos individuais da Dome Matrix e módulos acoplados nas configurações "piled", à esquerda e "void", à direita (LOSI et al., 2006).

Quando a configuração "void" é obtida, devido à cavidade vazia no interior do sistema matricial, é obtido um sistema flutuante. Nesta configuração a velocidade de dissolução do fármaco é significativamente menor em comparação com a liberação a partir de módulos individuais (não-unidos). Isto indica que existem diferenças biofarmacêuticas relevantes entres os módulos individuais e acoplados. Além disso, a geometria do sistema tem influência no intumescimento da matriz e consequentemente na liberação.

A presença de uma base côncava e uma convexa na matriz não altera totalmente a cinética de liberação em comparação com formulação de base lisa (convencional). A diferença na liberação também está relacionada à maior área superficial inicial da Dome Matrix®. Entretanto, quando as bases individuais são analisadas, a velocidade de liberação e a cinética são diferentes. O comportamento de intumescimento das bases curvas dá origem a diferentes velocidades e mecanismos de liberação. Como diferentes módulos podem ser combinados, incluindo aqueles com base lisa, o objetivo de aumentar a flexibilidade na produção de comprimidos pode ser atingido. Além disso, a velocidade de liberação pode ser modificada utilizando módulos fabricados com polímeros de diferentes massas moleculares, que resultam em matrizes com diferentes viscosidades.

O comportamento da configuração "void" na obtenção de uma matriz flutuante foi estudado *in vitro* e *in vivo* (STRUSI et al., 2008). A flutuação *in vitro* do sistema inicia imediatamente após imersão em água e é mantida por mais de 5 horas. Estudos *in vivo* confirmaram os dados *in vitro*, onde o sistema manteve-se no estomago de humanos por 214,5 ± 54,2 minutos. A flutuação *in vivo* está correlacionada com os dados obtidos *in vitro*, uma vez que o sistema somente é eliminado quanto todo o conteúdo estomacal é eliminado, e não porque o sistema perde sua capacidade de flutuação. De fato, a ingestão de alimentos e água prolonga o tempo de residência no estômago.

Dessa maneira os módulos Dome Matrix® acoplados na configuração "void" podem oferecer vantagens para fármacos que se beneficiem de um tempo prolongado de residência gástrica. Este tempo de permanência maior no estômago seria importante para uma formulação de liberação prolongada contendo norfloxacino, uma vez que ele é pouco solúvel em água, mas facilmente solúvel em soluções ácidas (F. BRAS. IV, 2001), o que pode favorecer a absorção e consequentemente a biodisponibilidade do medicamento, inclusive com possibilidade de redução da dosagem em cada sistema matricial.

Em relação ao tratamento utilizando antibióticos, a pesquisa de novas formas de liberação de fármacos é de grande importância, pois a resistência dos microorganismos a estes fármacos, seus efeitos adversos e a não aderência do paciente ao tratamento constituem problemas terapêuticos importantes. Na maioria dos tratamentos as concentrações plasmáticas sub e supraterapêuticas são responsáveis pela resistência microbiana e efeitos adversos, respectivamente. Assim, para que estas flutuações nas concentrações plasmáticas sejam minimizadas ou

eliminadas devem ser propostas alternativas terapêuticas, tais como, modificação da forma farmacêutica vinculada ao fármaco, que é o objeto de estudo deste projeto.

Algumas formulações farmacêuticas do norfloxacino em β-ciclodextrinas, dispersões sólidas de PEG-6000, nanopartículas, lipossomas, oligopeptídeos, sistema de liberação dérmica utilizando quitosana e complexo com alumínio foram estudadas (GUYOT et al., 1995; FAWAZ et al., 1996; MONTERO et al., 1996; COESSENS; SCHACHT; DOMURADO, 1997; JEON et al., 2000; ROSEEUW et al., 2003; DENKBAS et al., 2004; KAMAL et al, 2007; BREDA et al., 2009). Entretanto, não existem relatos de desenvolvimento de comprimidos matriciais de liberação prolongada contendo o antibiótico norfloxacino.

3. CARACTERIZAÇÃO E ESTABILIDADE

Utilizando métodos de análise térmica como a calorimetria exploratória diferencial (DSC) e termogravimetria (TG), é possível inferir sobre as características térmicas da amostra, assim como sobre a estabilidade, a identificação através da faixa de fusão e pureza pela respectiva entalpia de fusão. Além disso, observações referentes ao aparecimento, mudança ou desaparecimento de eventos endotérmicos ou exotérmicos característicos em misturas binárias homogêneas fármaco:excipiente (1:1, m/m) pode sugerir a interação entre os compostos e uma possível incompatibilidade. Desta forma a DSC é uma importante ferramenta para o estudo de pré-formulação de medicamentos (BUCKTON; RUSSEL; BEEZER, 1991; GIRON, 2002; LIZARRAGA; ZABALETA; PALOP, 2007). Os padrões de difração de raios-X de pó e/ou monocristal de uma substância permitem identificá-la, bem como fornecem informações sobre sua estrutura espacial, grau de cristalinidade e polimorfismo (STEPHENSON; FORBES; REUTZEL-EDENS, 2001; NEWMAN; BYRN, 2003; RODRÍGUEZ-SPONG et al., 2004; SHAH; KAKUMANU; BANSAL, 2006; AALTONEN, 2009). Métodos de avaliação microscópica como a microscopia eletrônica de varredura possibilitam a avaliação de fármacos através da observação da homogeneidade da amostra e determinação do tamanho e forma das partículas. O polimorfismo e a estabilidade do norfloxacino, bem como a estrutura cristalina do norfloxacino anidro, hidratos (dihidrato, sesquihidrato) e alguns co-

cristais estão descritos na literatura (ŠUŠTAR; BUKOVEC; BUKOVEC, 1993; FLORENCE et al., 2000; BARBAS et al., 2006; BASAVOJU; BOSTRÖM; VELAGA, 2006; ROY et al., 2008; BARBAS; PROHENS; PUIGJANER, 2007; PUIGJANER et al., 2010).

A cromatografia líquida tem sido empregada para determinação quantitativa de fármacos em matérias primas, formas farmacêuticas acabadas, estudos de dissolução, estudos de estabilidade e em matriz biológica, pois é uma técnica bastante conhecida e dominada, possuindo características de resolução, precisão e exatidão significativas. O desenvolvimento de métodos cromatográficos envolve a avaliação e otimização de condições, incluindo etapas de preparação da amostra, separação cromatográfica, detecção e quantificação. A validação é necessária para demonstrar, através de estudos experimentais, que o método atende às exigências das aplicações analíticas, assegurando a confiabilidade e reprodutibilidade dos resultados obtidos (SHABIR, 2003; BRASIL, 2003; ICH, 2005; AHUJA, 2007; BRASIL, 2008; STÖCKL; D'HONDT; THIENPONT, 2009; BOUABIDI et al., 2010).

Com relação ao norfloxacino, estão descritos na literatura metodologias para sua quantificação, incluindo algumas para avaliação da estabilidade, por exemplo: por cromatografia líquida (PARASRAMPURIA; GUPTA, 1990; CHEN; LIU; WU, 1993; HUSSAIN; CHUKWUMAEZE-OBIAJUNWA; MICETICH, 1995; RAO; NAGARAJU, 2004; MOHAMMAD et al., 2007), fluorimetria (STANKOV et al., 1993; DJURAJEVIC; JELIKIC-STANKOV; STANKOV, 1995; EL WALILY; BELAL; BAKRY, 1996), espectrofotometria (EL KHATEEB; RAZEK; AMER, 1998; EL WALILY et al., 1999; RAHMAN; AHMAD; AZMI, 2004), eletroforese capilar de zona e quimiluminescência (ALNAJJAR; ABUSEADA; IDRIS, 2007a; ALNAJJAR; IDRIS; ABUSEADA, 2007b; LIU et al., 2010), absorção atômica, condutimetria e colorimetria (RAGAB; AMIN, 2004).

A absorção de fármacos após administração oral depende da sua liberação da forma farmacêutica, da dissolução ou solubilização sob condições fisiológicas e de permeabilidade através do trato gastrintestinal. Os estudos de dissolução *in vitro* fornecem informações úteis tanto para a pesquisa e desenvolvimento quanto para a produção e controle de qualidade. A avaliação do perfil de dissolução permite a comparação de produtos de diferentes fabricantes, otimização de formulações e avaliação da influência de alterações realizadas na

formulação (MOORE; FLANNER, 1996; COSTA; LOBO, 2001; DOKOUMETZIDIS; MACHERAS, 2006; AZARMI et al., 2007).

A estabilidade é o período de tempo em que uma forma farmacêutica mantém suas propriedades dentro de limites pré-estabelecidos. O produto deve preservar suas características farmacotécnicas, organolépticas e microbiológicas, além de eficácia terapêutica e ausência de toxicidade. Todos os fármacos estão sujeitos a alguma forma de decomposição química ou física. Algumas classes químicas são mais vulneráveis e tendem a se decompor mesmo em condições brandas. A escolha dos excipientes também pode influenciar na estabilidade física, química e biodisponibilidade. Desta forma, deve-se respeitar a compatibilidade com os excipientes escolhidos, os quais são componentes importantes contidos nas formulações que podem significar melhorias das características, mas podem também reduzir a eficácia de algumas preparações.

A segurança de um fármaco e da forma farmacêutica sólida na qual está veiculado é afetada por vários fatores, como temperatura, umidade, luz e ar, que aumentam a velocidade das reações intrínsecas. O conhecimento do mecanismo de degradação é importante para definir prazo de validade e condições de armazenagem específicas. Para mimetizar o tempo de análise da degradação química e/ou física dos comprimidos, submete-os a condições típicas de estresse, denominado estudo acelerado de estabilidade. No Brasil, país classificado na região climática IV (quente e úmido), as formulações são avaliadas após permanecerem durante seis meses a 40 ± 2 ºC e 75 ± 5% de umidade relativa. Um medicamento é considerado estável se suas características físicas e químicas não sofrerem mudanças, restando acima de 90% da concentração inicial (ICH, 1996; BRASIL, 2005; WHO, 2009).

Na exposição prolongada à luz, alguns grupos funcionais do norfloxacino se decompõem (CÓRDOBA-BORREGO; CÓRDOBA-DÍAZ; CÓRDOBA-DIAZ, 1999; MUSA; ERIKSSON, 2009). Além disso, seu aquecimento prolongado em meio ácido produz um produto de degradação descarboxilado, que está relacionado a precipitações em formulações injetáveis e que também pode ser encontrado como impureza em algumas matérias-primas (EL KHATEEB; RAZEK; AMER, 1998; BORREGO; DIAZ; DIAZ, 1999; ALNAJJAR; IDRIS; ABUSEADA, 2007b). A partir dos resultados dos estudos de estabilidade, o formulador pode obter informações importantes sobre a necessidade do revestimento da forma farmacêutica, tipo de embalagem necessária para a comercialização do produto final, etc. Além disso, os

estudos de estabilidade podem comprovar uma incompatibilidade fármaco/excipiente sugerida no estudo de pré-formulação.

Dessa maneira, a completa caracterização das matérias-primas de fármacos e excipientes, o estudo minucioso do processo tecnológico empregado na obtenção de um comprimido de liberação prolongada, bem como a avaliação da qualidade do produto final, são etapas fundamentais para que o produto tenha características tais que seja viável sua produção em larga escala, com melhora na qualidade de vida do paciente.

CAPÍTULO 2 - Caracterização térmica e estudos de compatibilidade fármaco/excipiente.

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Thermal characterization and compatibility studies of norfloxacin for development of extended release tablets

P. R. Oliveira · L. S. Bernardi · F. S. Murakami · C. Mendes · M. A. S. Silva

INTRODUÇÃO

A caracterização no estado sólido de fármaco e excipientes é etapa essencial para o desenvolvimento racional de um novo medicamento. Os resultados obtidos por diversas técnicas servem como parâmetros para a aquisição de matérias-primas com adequada qualidade e para garantir a homogeneidade e reprodutibilidade na produção industrial da formulação final, assegurando sua eficácia, segurança e qualidade. Além disso, o polimorfismo e diferenças de cristalinidade de fármacos devem ser investigados, uma vez que diferentes formas cristalinas podem apresentar diferentes propriedades físicas e físico-químicas que alteram a biodisponibilidade e terapêutica. (BYRN et al., 2001; STEPHENSON; FORBES; REUTZEL-EDENS, 2001; GIRON et al., 2002).

No desenvolvimento de formulações é fundamental avaliar se há interação química e/ou física entre o princípio-ativo e excipientes, uma vez que estas interações podem afetar negativamente a estabilidade e a biodisponibilidade da formulação final. Não existe protocolo padrão determinando como realizar estudos de compatibilidade (KISS et al., 2006; BRUNI et al., 2010). Normalmente métodos termoanalíticos como DSC e TG são utilizados. Outras técnicas também podem ser aplicadas para melhor compreensão e confirmação dos resultados obtidos. De maneira geral, os estudos de compatibilidade são realizados comparando-se o perfil termoanalítico do fármaco com o obtido de uma mistura preparada na proporção 1:1 (p/p) fármaco:excipiente. O surgimento, mudança ou desaparecimento de eventos térmicos são considerados como interação, o que pode indicar uma possível incompatibilidade (BRUNI et al., 2002; MURA; GRATTERI; FAUCCI, 2002; BERNARDI et al., 2009; BRUNI et al., 2010).

Neste capítulo são apresentados resultados da caracterização do norfloxacino, POE e HPMC por DSC, TG, SEM, XRPD e DRIFT, bem como o cálculo da energia de ativação do fármaco por método isotérmico e não-isotérmico. Também são demonstrados estudos de compatibilidade entre o norfloxacino e alguns excipientes que poderiam compor a formulação matricial final.

Thermal Characterization and Compatibility Studies of Norfloxacin for Development of Extended Release Tablets

P. R. Oliveira*, L. S. Bernardi, F. S. Murakami, C. Mendes and M. A. S. Silva

Department of Pharmaceutical Sciences, Health Science Centre, Federal University of Santa Catarina, 88040-900, Florianópolis-SC, Brazil

*Author for correspondence: prenato.oliveira@gmail.com

Abstract

Norfloxacin (NFX) is a synthetic antibacterial drug. The development of extended release tablets improves the patients' comfort and compliance, resulting in lower discontinuation of the therapy; with consequently decrease in bacterial resistance. In the present work, the thermal behavior of NFX was investigated using TG and DSC techniques. Isothermal and non-isothermal methods were employed to determine kinetic data of decomposition process. Compatibility studies between NFX and pharmaceutical excipients, including three hydrophilic polymers were carried out in order to develop a new formulation of NFX to obtain extended release tablets with an approved quality.

Keywords: Norfloxacin, Thermal characterization, Kinetic Studies, Compatibility Studies

Introduction

Norfloxacin, chemically known as 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1-ethyl-fluoro-1.4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline-carboxylic acid (Fig. 1A) [1]. It is currently used as a broad spectrum antibacterial drug, being the firstly selected drug for the treatment of diseases caused by *Campylobacter, E. coli, Salmonella, Shigella and V. cholera* [2-3]. The drug is also used for the treatment of gonorrhoea as well as infection of eyes and urinary tract [2]. Resistance in Gram-negative bacteria has become common, making the therapeutic decisions more difficult. Increasing bacterial resistance to currently available quinolones has reduced their effectiveness and may compromise future use of this class of drugs [4-5].

The development of controlled-release formulations is a successful area in the pharmaceutical industry because expenses of new drug development are very high, and novel innovation is at an all-time low. Hydrophilic matrices are one of the most used controlled delivery systems in the world, due to the simple technology and low cost. Among the various hydrophilic polymers employed, hydroxypropyl methylcellulose (HPMC, Fig. 1B) is the most commonly used, due to its versatility, compatibility with many drugs and safety [6] Nevertheless, high molecular mass polyethylene oxides (PEOs, Fig. 1C) have been proposed as an alternative to HPMC [7].

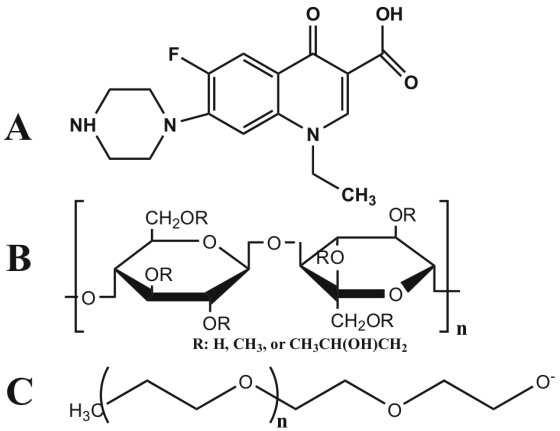

Figure 1. Chemical structure of NFX (A), HPMC (B) and PEOs (C).

Thermoanalytical techniques measure changes in physical and/or chemical properties of the sample as a function of temperature. There are many possible applications in pharmaceutical industry, for example, identification, characterization of active and inactive ingredients, routine analysis, quality control and stability study [8-9,]. Kinetic parameters (activation energy, frequency factor and reaction order) can be measured by thermoanalytical methods according to progress of reactions [10-12]. The successful formulation of a stable and effective solid dosage form depends on the careful selection of the excipients [8,13] and on the characterization of solid-state properties using appropriate analytical methodologies [14-16]. The compatibility studies using thermal analysis present advantageous to readily available knowledge of any physical and chemical interactions between drugs and excipients which might give rise to changes in chemical nature, stability, solubility, absorption and therapeutic response of drugs [12]. In particular differential scanning calorimetry has been proposed as a rapid method for evaluating physicochemical interactions between components of the formulation through comparison of thermal curves of pure substances with curve obtained from a 1:1 mixture, and therefore selecting excipients with suitable compatibility [10,15,17-21].

The aim of this study was to perform the physicochemical solid-state characterization of norfloxacin and different polymers, to analyze the kinetic parameters under isothermal and non-isothermal conditions, and to carry out compatibility studies, to begin the development of a new formulation of NFX extended release tablets.

Experimental

Materials

Norfloxacin (NFX) bulk material was kindly donated by União Química Farmacêutica Nacional (Embu-Guaçu, SP, Brazil). The polymers tested were: Polyox WSR N80 NF, Polyox WSR 301 NF, and Methocel K100 Premium LV CR (all from Colorcon do Brasil, São Paulo, Brazil). The pharmaceutical excipients tested were microcrystalline cellulose, magnesium stearate, colloidal silicon dioxide, lactose monohydrated and Opadry II White.

Methods

Differencial Scanning Calorimetry (DSC) and Thermogravimetric (TG) Analysis

The DSC curves were obtained on Shimadzu DSC-60 cell (Kyoto, Japan) using aluminum crucibles with about 1.5 mg of samples. The temperature range was from 30 to 500 ºC at a heating rate of 10 ºC min^{-1} in dynamic N_2 atmosphere with the flow rate of 50 mL min^{-1}. The DSC equipment was preliminarily calibrated with standard reference of indium (m.p. 156.6 ^{o}C; ΔH_{fus}=-28.54 J g^{-1}) and zinc (m.p. 419.5 ^{o}C). The compatibility studies were performed with binary mixtures of NFX and each excipient (1:1; *m/m*). TG experiments were measured on Shimadzu thermobalance model TGA-50 (Kyoto, Japan) in the temperature range of 30–800 °C, using platinum crucibles with approximately 4 mg of samples, under dynamic N_2 atmosphere (50 mL min^{-1}) at a heating rate of 10 °C min^{-1}. The equipment was preliminarily calibrated with standard reference of calcium oxalate. Non-isothermal kinetic investigation of NFX was performed from TG data by application of Ozawa's method [22]. The graph of mass loss versus temperature of five TG curves was obtained at different heating rates (2.5, 5.0, 10, 15, and 20 °C min^{-1}), under N_2 atmosphere. For isothermal method, the temperature was from 230 to 270 °C, with 10 °C temperature increment, in N_2 atmosphere. A graphic of ln*t vs 1/T* (K^{-1}) was plotted and linear regression was applied.

X-ray powder diffraction (XRPD)

For characterization of crystallinity, X-ray diffraction patterns were obtained on a Siemens diffractometer model D 5000, with tube of CuKα, voltage of 40 kV and current of 40 mA, in the range of 3–40 (2θ) with a pass time of 1 second.

Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT)

The DRIFT spectra were measured in a Shimadzu spectrophotometer (Prestige), in a scan range of 400 - 4000 cm^{-1} with an average of over 32 scans at a spectral resolution of 4 cm^{-1} in KBr. A background spectrum was obtained for each experimental condition.

Scanning Electron Microscopy (SEM)

The photomicrographs of NFX and the polymers were observed by using a Phillips scanning electron microscope, model XL30. Samples

were mounted onto metal stubs using double-side adhesive tape, vacuum-coated with gold (350 Å) in a Polaron E 5000 and directly analyzed under SEM (N=50, 200, and 1000).

Results and discussion

DSC curve of norfloxacin (Fig. 2A) showed a sharp endothermic event (T_{peak}) at 219.4 °C (ΔH_{fusion} = -101.51 J g^{-1}) corresponding to melting point followed by an exothermal event. The decomposition was defined in two major endothermic stages. This was confirmed by TG/DTG curves that indicated thermal decomposition in the following temperature range: 330–376 °C (Δm=47.4 %) and 421-455 °C (Δm=27.8 %). The DSC curve of Polyox WSR 301 (Fig. 2B) and Polyox WSR N80 (Fig. 2C) showed sharp endothermic peaks at 69.3 °C (ΔH_{fusion} = -162.70 J g^{-1}) and 65.3 °C (ΔH_{fusion} = -180.36 J g^{-1}), respectively, corresponding to melting event. The TG/DTG curves indicated one thermal decomposition step in the temperature range of 397–433 °C (Δm=95.9 %) for Polyox WSR 301 and 394–432 °C (Δm=95.8 %) for Polyox WSR N80. The similarity of these thermal decomposition profiles can be explained by the same polymer chemical structure; the only difference is the molecular mass, 4,000,000 Da (Polyox WSR 301) and 200,000 Da (Polyox WSR N80). Different molecular masses usually are tested in the development of extended release tablets because its increase leads to an increase in gel strength, which tends to decrease the diffusion of the drug from the matrix. DSC curve of Methocel K100 LV (Fig. 2D) showed a broad endothermic event between 93-140 °C (ΔH = -120.97 J g^{-1}) and TG/DTG curves indicated thermal decomposition in the temperature range of 360–394°C (Δm=83.6 %).

Based on the photomicrographs obtained from scanning electron microscopy, orthorhombic crystals were observed for NFX (Fig. 3A). A particle size variation can be visualized for both Polyox samples (Fig. 3B and 3C). An amorphous characteristic was observed for Methocel K100 (Fig. 3D).

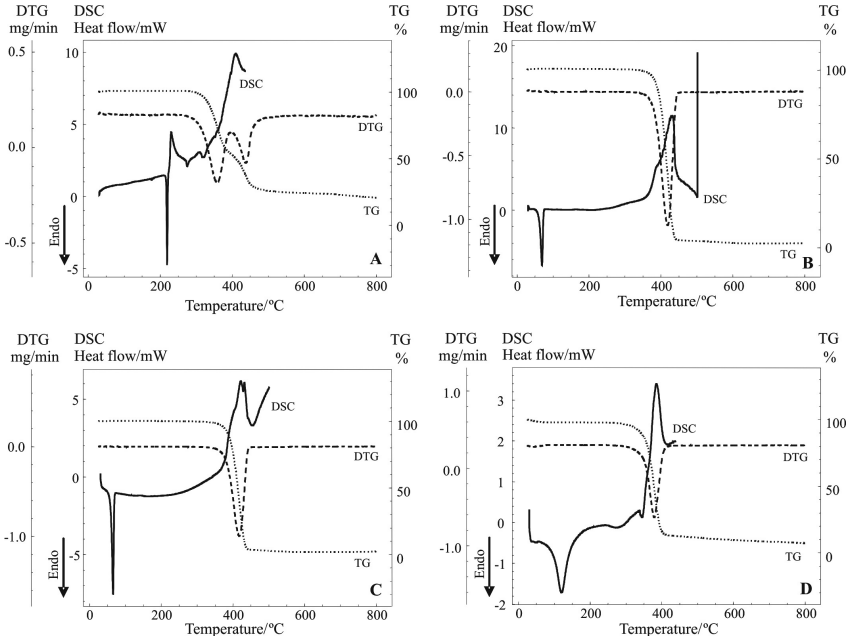

Figure 2. DSC and TG/DTG curves of norfloxacin (A), Polyox WSR 301 (B), Polyox WSR N80 (C), and Methocel K100 LV CR (D) in dynamic nitrogen atmosphere (50 mL min $^{-1}$) and heating rate of 10 ºC min $^{-1}$.

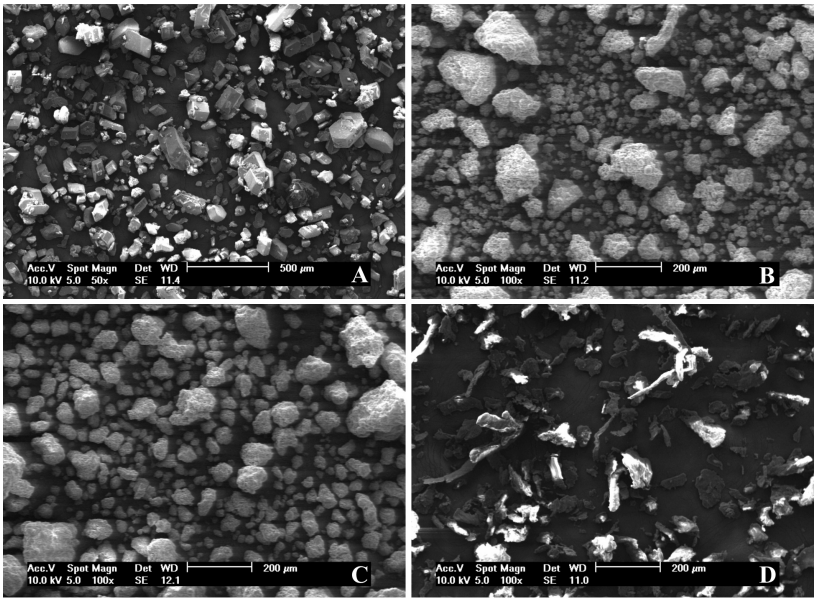

Figure 3. SEM of NFX (A), Polyox WSR 301 (B), Polyox WSR N80 (C), and Methocel K100 LC CR (D). The photomicrograph A was taken at a magnification of 50x, and B, C and D of 100x.

X-ray powder diffraction studies were performed in order to obtain more information about the crystalline characteristics. The 2θ values of the diffraction peaks (Fig. 4) for NFX were 2θ = 7.87, 9.93, 10.63, 11.98, 13.38, 14.98, 16.08, 18.88, 20.78, and 25.08. For both Polyox polymers, only two intensive peaks were observed: 2θ = 19.13 and 23.33. For Methocel K100, only two broad peaks, with low intensity, between 2θ = 5.7-13.3 and 15.53–25.5, were observed, indicating an amorphous state for this polymer.

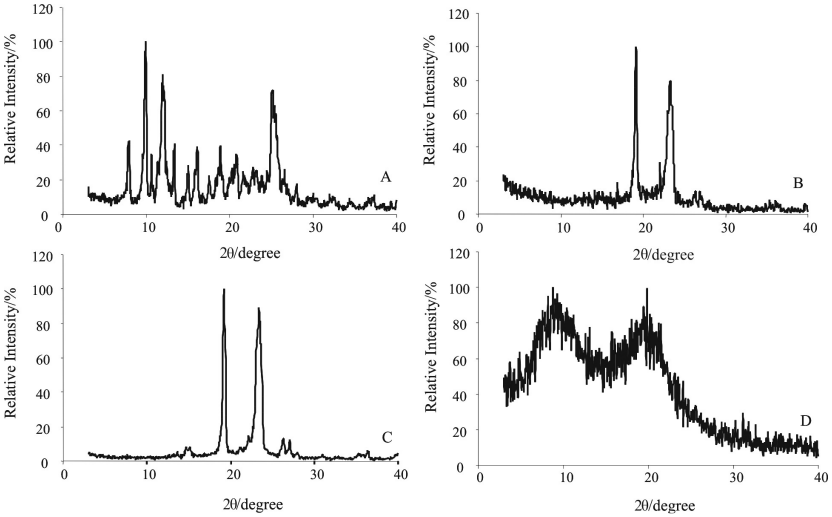

Figure 4. X-ray diffraction spectra of NFX (A), Polyox WSR 301 (B), Polyox WSR N80 (C), and Methocel K100 LC CR (D).

The IR spectra of quinolones are more representative in the region 1800–1300 cm^{-1} [23]. The IR spectrum (Fig. 5) of NFX exhibits a stretching vibration band at about 1716 cm^{-1} (–COOH stretching) and 1631 cm^{-1} (pyridone keto). For Polyox WSR 301 and N80, it was observed bands in 2915 and 1465 cm^{-1} (streching $–CH_2$-) and intense bands in 1150 – 1085 cm^{-1}, which is attributed to asymmetric axial deformation C–O–C, characteristic of aliphatic ethers, confirming the identification of the polymers. Methocel K100 IR spectra showed absorption bands at 3440 cm^{-1} (O-H stretching), 2904 cm^{-1} (C-H stretching), 1643 cm^{-1} (C=O), and 1066 cm^{-1} (C-O-C).

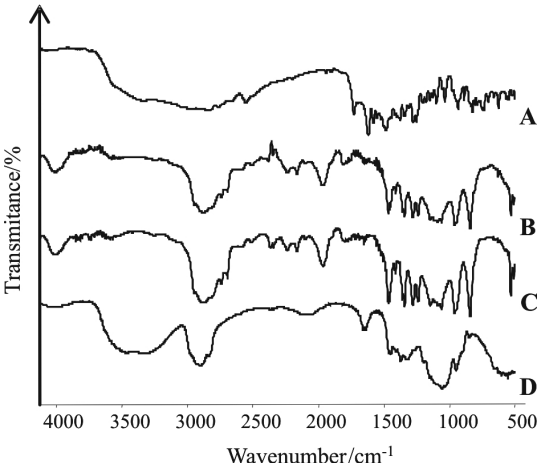

Figure 5. DRIFT spectra of Norfloxacin (A), Polyox WSR 301 (B), Polyox WSR N80 (C), and Methocel K100 LC CR (D).

For non-isothermic study, the superposition of the TG curves of NFX is shown in Fig. 6A. Ozawa's method was applied in order to determine the activation energy (*E*a), Arrhenius frequency factor (A) and order of reaction at the beginning of first thermal decomposition step at around 300 to 350 °C. The *E*a, calculated was 126 kJ mol $^{-1}$, the Arrhenius frequency factor was 4.029 x 10^{9} min^{-1} and order of reaction followed a zero order reaction ($n = 0$).

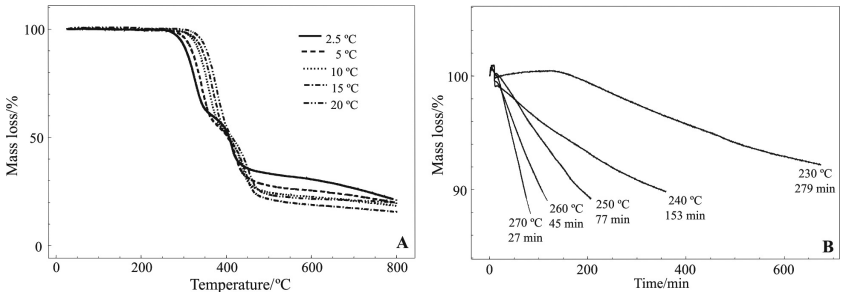

Figure 6. (A) TG curves obtained for the non-isothermic study of NFX at 2.5, 5, 10, 15, and 20 ºC min^{-1}. (B) Isothermal TG curves of NFX obtained between 230 and 270 ºC, with a temperature increment of 10 ºC.

The isothermal TG curves of NFX are illustrated in Fig. 6B. These curves were used to obtain a graphic of ln*t vs*. the reciprocal of temperature 1/*T* (K^{-1}). From this linear regression method, the equation obtained was *y*= *–16.098x* + 26.382 (*R*=0.9982). The activation energy calculated from the product of 16.098 with the molar gas constant (*R*=8.314) and was *E*a=134 kJ mol^{-1}. This result is in agreement with the value obtained from the dynamic method. The selection of adequate excipients for a new formulation is based on the characteristics of the drug and its compatibility with other components. Moreover, excipients can influence the dissolution profile affecting the bioavailability of the drug. The results from the compatibility studies between NFX and excipients are shown in Fig. 7, where the DSC curves can be considered as superposition of the curves of pure compounds indicating that there is no interaction, and therefore no physical-chemical incompatibility.

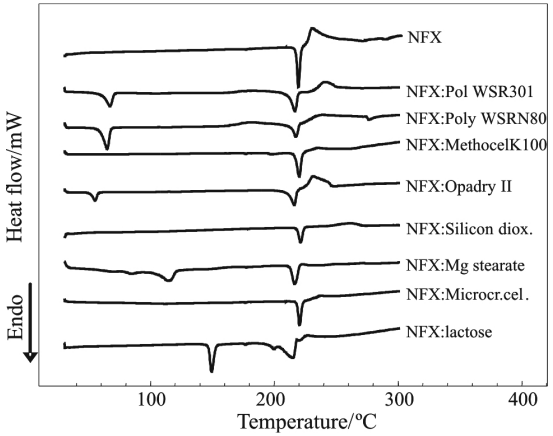

Figure 7. DSC curves of NFX and excipients obtained in dynamic nitrogen atmosphere (50 mL min^{-1}) and heating of rate 10 °C min^{-1}.

Conclusion

The thermal behaviour and the solid-state characterization of NFX and the polymers were carried out by means of TG, DSC, DRIFT, SEM, and XRPD. The obtained isothermal and non-isothermal kinetic parameters can be used as reference values for the routine quality control of NFX. The results demonstrated the applicability of DSC as a

fast screening tool for selection of adequate excipients at the early stages of pre-formulation studies. No interaction was observed for NFX and the tested excipients, making feasible the development of a high quality formulation of NFX extended release tablets.

Acknowledgments

The authors wish to thank CNPq and CAPES for the support.

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CAPÍTULO 3 – Desenvolvimento e validação de metodologia analítica.

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Liquid Chromatographic Determination of Norfloxacin in Extended-Release Tablets

Paulo R. Oliveira[1,*], Larissa S. Bernardi[1], Cassiana Mendes[1], Simone G. Cardoso[1], Maximiliano S. Sangoi[2], and Marcos A. S. Silva[1]

[1] Department of Pharmaceutical Sciences, Health Science Centre, Federal University of Santa Catarina, 88040-900, Florianópolis-SC, Brazil; [2] Faculty of Pharmacy, Federal University of Rio Grande do Sul, 90610-000, Porto Alegre-RS, Brazil

INTRODUÇÃO

A análise do fármaco é necessária nas diversas fases do desenvolvimento farmacêutico, como estudos de formulação e controle de qualidade da forma farmacêutica. Após o desenvolvimento analítico a validação da metodologia é necessária para atestar, de forma documentada, que o procedimento fornece resultados reprodutíveis, precisos, exatos, específicos e confiáveis, adequados aos fins pretendidos (SHABIR, 2003; BRASIL, 2003; ICH, 2005; STÖCKL, 2009).

Para atingir o objetivo de se avaliar a estabilidade de formulações farmacêuticas, o método analítico deve demonstrar adequada capacidade de separar e, se possível, identificar os produtos de degradação formados. Para tanto, submete-se o fármaco a diversas condições de stress, tais como: hidrólise ácida, hidrólise básica, fotólise, oxidação, temperatura (ICH, 2005; BRASIL, 2005; ALSANTE et al., 2007; BRASIL 2008). Desta forma, procura-se demonstrar que se alguma substância for formada, o método será capaz de separar e até quantificar.

O presente capítulo tem como objetivo demonstrar os resultados do desenvolvimento e validação de metodologia analítica para quantificação de norfloxacino em sistemas matriciais de liberação prolongada. Com o objetivo de avaliar se o método é adequado para os ensaios de estabilidade (em câmara climática, fotoestabilidade) foi realizado estudo de especificidade em condições de stress e a produção de um produto de degradação (norfloxacino descarboxilado) de acordo com procedimento descrito na literatura (EL KHATEEB; RAZEK; AMER, 1998).

Liquid Chromatographic Determination of Norfloxacin in Extended Release Tablets

Paulo R. Oliveira*[a], Larissa S. Bernardi[a], Cassiana Mendes[a], Simone G. Cardoso[a], Maximiliano S. Sangoi[b], and Marcos A. S. Silva[a]

a Department of Pharmaceutical Sciences, Health Science Centre, Federal University of Santa Catarina, 88040-900, Florianópolis-SC, Brazil.
b Faculty of Pharmacy, Federal University of Rio Grande do Sul, 90610-000, Porto Alegre-RS, Brazil.

* Corresponding author: prenato.oliveira@gmail.com

Abstract

A stability indicating reversed-phase liquid chromatography method is developed and validated for the determination of norfloxacin in a new formulation of extended release tablets. The LC method is carried out on a Luna C_{18} column (150 x 4.6 mm), maintained at 40 ºC. The mobile phase is composed of phosphate buffer 0.04 M, pH 3.0 /acetonitrile (84:16, v/v), run at a flow rate of 1.0 mL/min, and detection at 272 nm. The chromatographic separation was obtained within 10 min and it is linear in the concentration range of 0.05-5 µg/mL. Validation parameters such as the specificity, linearity, precision, accuracy, and robustness were evaluated, giving results within the acceptable range. Moreover, the proposed method was successfully applied for the assay of norfloxacin in the developed formulations.

Keywords: Norfloxacin, Extended release, Liquid chromatography, Method validation.

Introduction

Norfloxacin, chemically known as 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1-ethyl-fluoro-1.4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline-carboxylic acid (Figure 1) (1), is currently used as a broad spectrum antibacterial drug, being the first choice drug for the treatment of diseases caused by *Campylobacter, E. coli, Salmonella, Shigella and V. cholera* (2,3). The drug is also used for the treatment of gonorrhea as well as infection of eyes and urinary tract (2). Resistance in Gram-negative bacteria has become common, making the therapeutic decisions more difficult. Increasing bacterial resistance to currently available quinolones has reduced their effectiveness and may compromise future use of this class of drugs (4,5).

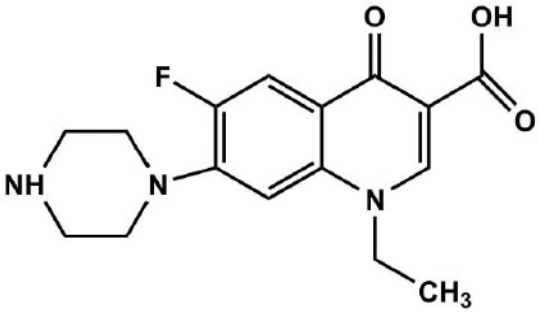

Figure 1. Chemical structure of norfloxacin.

The development of controlled-release formulations is a successful area in the pharmaceutical industry because expenses of new drug development are very high, and true innovation is at an all-time low. Hydrophilic matrices are one of the most used controlled delivery systems in the world, due to the simple technology and low cost. Among the various hydrophilic polymers employed, hydroxypropyl methylcellulose (HPMC) is the most commonly used, due to its versatility, compatibility with many drugs and safety (6). Nevertheless, high molecular weight polyethylene oxides (PEOs) have been proposed as an alternative to HPMC (7). The correct choice of the hydrophilic polymer, molecular weight and quantity in the matrix formulation can

provide an appropriate combination of swelling, dissolution or erosion mechanisms to control drug release kinetics (8,9).

Moreover, drug release at a constant rate is often desirable, to maintain the plasmatic levels of the drug in the therapeutic range, thereby avoiding the peak and valley profile characteristics of conventional dosage forms in a multidose regime (10), which, for antibiotics, is very important, mainly in combination with a better patient compliance to the therapy, to avoid the discontinuation of the treatment and the development of resistant microorganisms.

Analytical methods have been published for the determination of NFX in pharmaceuticals by means of spectrophotometry, liquid chromatography (LC) and capillary electrophoresis, and some of them are listed in the references (11-16).

As the excipients (including polymeric ones) and the technological process can affect the stability of the active pharmaceutical ingredient and the evaluation of the stability-indicating capability of LC methods becoming mandatory by the surveillance agencies (17,18), the aim of the present work was to develop and validate a stability-indicating method for the quality assessment of the new formulation of norfloxacin extended release tablets.

Experimental

Chemical and reagents

Norfloxacin reference standard was kindly provided by Brazilian Pharmacopeia and norfloxacin raw material was from União Química Farmacêutica Nacional (Embu-Guaçu, SP, Brazil). The polymers used in this study, Polyox® WSR N80, Polyox® WSR 301, Polyox® WSR 303, Polyox® N60K, Methocel® K100 LV, Methocel® K100M, and Methocel® K4M were kindly provided by Colorcon do Brazil Ltda (São Paulo, SP, Brazil). Others excipients used were: microcrystalline cellulose (Microcel® 102, Blanver, Itapevi, SP, Brazil), magnesium stearate (M. Cassab, São Paulo, SP, Brazil), and colloidal silicon dioxide (Aerosil®, Labsynth, Diadema, SP, Brazil). HPLC-grade acetonitrile was purchased from Tedia (Fairfield, OH, USA). For all the analyses, ultrapure water was purified using a Milli-Q Gradient System (Millipore, Bedford, USA).

Samples

The composition of each NFX tablet formulation was: NFX (700 mg), polymer (20 or 30%), magnesium stearate (1%), colloidal silicon dioxide (0.5%), and microcrystalline cellulose (qs 1,07 g). For the production of the extended release tablets, NFX and excipients were mixed for 10 min and then compressed by direct compression (Fellc compressing model F-10/8, São Paulo, SP, Brazil).

Methods

Liquid chromatography (LC)

A Shimadzu LC system (Shimadzu, Kyoto, Japan) was used equipped with a SCL-10A$_{VP}$ system controller, LC-10 AD pump, DGU-14A degasser, CTO-10AS$_{VP}$ column oven, SPD-10A$_{VP}$ UV detector, and a SPD-M10A$_{VP}$ photodiode array detector. The detector was set at 272 nm and peak areas were integrated automatically by computer using a Shimadzu Class VP® V 6.12 software program. The experiments were carried out on a reversed-phase Phenomenex (Torrance, USA) Luna C_{18} column (150 mm x 4.6 mm I.D., with a particle size of 5 µm and pore size of 100 Å). A security guard holder (4.0 mm x 3.0 mm I.D.) was used to protect the analytical column. The LC system was operated isocratically at 40 ºC using a mobile phase of phosphoric acid 0.04 M, pH 3.0/acetonitrile (84:16, v/v). This was filtered through a 0.45 µm membrane filter and run at a flow rate of 1.0 mL/min. The injection volume was 20 µL for both standard and samples.

Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT)

The DRIFT spectra were measured in a Prestige spectrophotometer (Shimadzu, Kyoto, Japan), in a scan range of 400 - 4000 cm^{-1} with an average of over 32 scans at a spectral resolution of 4 cm^{-1} in KBr. A background spectrum was obtained for each experimental condition.

Mass spectrometry (MS)

The MS experiments were performed on a triple quadrupole mass spectrometer (Micromass, Manchester, UK), model Quattro LC, equipped with an electrospray ionization (ESI) source in positive mode, set up in scan mode, using a Masslynx (v 3.5) software program. The

samples were introduced into the mass spectrometer by direct infusion at 10 µL/min, diluted in mobile phase. The best response for NFX was obtained with electrospray capillary potential of 3 kV, cone voltage of 30 V, RF lens voltage of 0.3 V, source temperature of 120 ºC, and ESI probe temperature of 400 ºC. The mass spectrometry data were acquired in the m/z range between 100 and 550 amu.

Procedure

Preparation of reference solutions

The stock solutions of norfloxacin were prepared by weighing 50 mg, transferred to individual 50 mL volumetric flasks, dissolved with 0.2 mL of acetic acid glacial, and diluted to volume with mobile phase, obtaining a concentration of 1 mg/mL. The stock solutions were stored at 2-8 °C protected from light. Working standard solutions were prepared daily by diluting the stock solutions to an appropriate concentration in mobile phase.

Preparation of decarboxylated norfloxacin (DCN)

The hydrolysis of NFX during prolonged heating of its acid solution yields a decarboxylated degradant (DCN), which was prepared based on a described procedure (11). 250 mg of NFX was refluxed with 70 ml of hydrochloric acid 2 M at 150 °C for 48 h, protected from light. Then, the solution was cooled and adjusted to pH 7.5 with sodium hydroxide 2 M. After that, the solution was evaporated under vacuum to dryness. The residue was extracted with ethanol and filtered. To verify the identity of the obtained product, a sample was analyzed by means of DRIFT, LC-MS, and the proposed LC method.

Preparation of sample solutions

To prepare the sample stock solution, the obtained extended release tablets were crushed to a fine powder. An appropriated amount was transferred into an individual 50 mL volumetric flask, dissolved with 0.2 mL of acetic acid glacial, and diluted to volume with mobile phase, obtaining a concentration of 1 mg/mL of the active pharmaceutical ingredient. This solution was stored at 2-8 °C protected from light. Working sample solutions were prepared daily by diluting the sample stock solutions to an appropriate concentration in mobile phase.

Validation of the method

Analytical method development and validation play a major role in the discovery, development, and manufacture of pharmaceuticals (19). The International Conference on Harmonization (ICH) (20) requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. A stability-indicating method is the one that quantifies the drug and also resolves its degradation products (17,18,21). The method was validated to quantify NFX in a new formulation of extended release tablets by the determination of the following parameters: specificity, linearity, accuracy, precision, robustness, and quantitation and detection limits.

Specificity

In order to determine the specificity of the method, a placebo solution was analyzed to evaluate the absence of interference from the formulation excipients (including the polymers) on the NFX peak. Moreover, the specificity was determined by subjecting a sample solution (1 mg/mL) to accelerated degradation by acidic, basic, neutral, oxidative, and photolytic conditions. After the procedures, the samples were diluted in mobile phase to a final concentration of 1 µg/mL. A sample solution in 5 M hydrochloric acid and 5 M sodium hydroxide, both refluxed at 100 ºC for 24 h, were used for the acidic and basic hydrolysis, respectively. The oxidative degradation was induced by storing the sample solution in 30% hydrogen peroxide, at ambient temperature for 24 h, protected from light. Photodegradation was induced by exposing the samples to 200 watt hours/square meter of near ultraviolet light. Then, the specificity of the method was established by determining the peak purity of norfloxacin in degradated samples using a PDA detector.

Linearity and range

Linearity was determined by constructing three independent calibration curves. For the construction of each calibration curve seven standard concentrations of NFX in the range of 0.05–5 µg/mL were prepared in mobile phase. Three replicates of 20 µL injections were made for the standard solution to verify the repeatability of the detector response at each concentration. The peak areas of the chromatograms were plotted against the concentrations of NFX to obtain the calibration curve. The seven concentrations of the standard solutions were

subjected to regression analysis by the least squares method to calculate calibration equation and correlation coefficient.

Precision and accuracy

The precision of the method was determined by repeatability and intermediate precision. Repeatability was examined by six evaluations of the same concentration sample, on the same day, under the same experimental conditions. The intermediate precision was assessed by carrying out the analysis on three different days (inter-days) and also by other analysts performing the analysis in the same laboratory (between-analysts). The accuracy was evaluated by the recovery of known amounts (0.3, 0.5 and 0.7 µg/mL) of the reference substance added to a sample solution (containing 0.50 µg/mL of NFX and tablet excipients) to obtain solutions with final concentrations of 0.80, 1.0, and 1.2 µg/mL, corresponding to 80, 100, and 120% of the nominal analytical concentration, respectively. The accuracy was calculated as the percentage of the drug recovered from the formulation matrix.

Limits of quantitation (LQ) and detection (LD)

The LQ was taken as the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy, and the LD was taken as the lowest absolute concentration of analyte in a sample that can be detected but not necessarily quantified. The LD and LQ were calculated from the slope and the standard deviation of the intercept of the mean of three calibration curves, determined by a linear regression model, as defined by ICH.

Robustness

Two approaches are possible to evaluate robustness, either an one-variable-at-a-time (OVAT) procedure or an experimental design procedure. The OVAT procedure varies the levels of one factor while keeping the other factors at nominal levels, to evaluate the effect of this former factor on the method response(s). When applying an experimental design, the effect of a given factor is calculated at several level combinations of the other factors. Thus, in an experimental design, a reported factor effect is an average value for the whole domain, and it represents more globally what is happening around the nominal situation (22-24).

The robustness was determined by analyzing the same samples under a variety of conditions of the method parameters, such as: flow rate, column temperature, changing the mobile phase composition and pH. The response surface method (RSM) design was applied to evaluate the relationships between one or more measured responses. Moreover, the D-optimal criteria was used to select design points to minimize the variance associated to the estimates of specified model coefficients, with a low number of experiments.

System suitability

The system suitability was carried out to evaluate the resolution and reproducibility of the system for the analysis to be performed, using six replicate analyses of the drug at a concentration of 1 μg/mL. The parameters evaluated were peak area, retention time, theoretical plates, and asymmetry.

Analysis of the extended release tablets

For the quantitation of NFX in the extended release tablets, twenty tablets of each batch were separated, accurately weighed and crushed to a fine powder. An appropriate amount of each tablet was transferred into an individual 50 mL volumetric flask, dissolved with 0.2 mL of acetic acid glacial, sonicated for 15 min, diluted with mobile phase (sonicated again for 15 min), and diluted to volume, obtaining the NFX final concentration of 1 mg/mL (stock solutions). For the analysis, the stock solutions were diluted to appropriate concentrations with mobile phase. An aliquot of 20 μL was injected for the analysis and the amount of each drug per tablet calculated against the respective reference standard.

Results and discussion

To obtain the best chromatographic conditions, the mobile phase was optimized to provide adequate peak symmetry and sensitivity. Potassium phosphate, sodium phosphate, sodium acetate, formic acid, and phosphoric acid buffers were tested. Methanol was tested as the organic solvent; however a broad and non-symmetric peak was obtained. The use of phosphoric acid 0.04 M (pH 3.0) in combination with acetonitrile, which was optimized to 16%, at 40 ºC, resulted in a relatively short retention time of 6.6 min, better peak

symmetry (1.11), and a simple mobile phase (without salt buffer addition). For the selection of the best wavelength detection a PDA detector was used. The optimized conditions of the LC method were validated for the analysis of NFX in the developed tablets and a typical chromatogram obtained by the proposed LC method is shown in Figure 2A.

A stability-indicating method is defined as an analytical method that accurately quantitates the active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities. Forced degradation studies should be the first step in method development. The presence of degradants and impurities in pharmaceutical formulations can result in changes in their chemical, pharmacological and toxicological properties affecting their efficacy and safety. Therefore, the adoption of stability-indicating methods is always required to control the quality of pharmaceuticals during and after the production. This greatly contributes to the possibility of improving drug safety (17,18, 25,26).

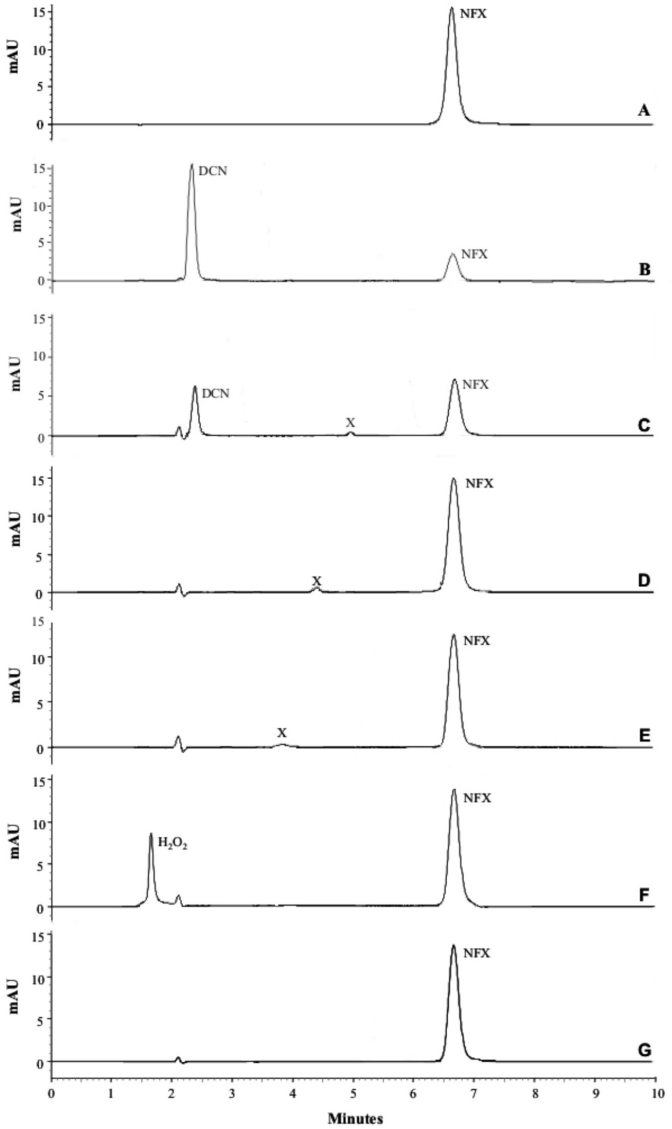

Figure 2. LC chromatograms of norfloxacin (A) developed formulation (1 µg/mL). (B) Decarboxylated degradant. (C) After acidic condition. (D) After basic condition. (E) After photolytic condition. (F) After oxidative condition. (G) After neutral condition. Chromatographic conditions: Luna C_{18} column (150 x 4.6 mm, 5 µm), 40 ºC; mobile

phase: phosphate buffer 0.04 M, pH 3.0 /acetonitrile (84:16, v/v); flow rate: 1.0 mL/min; detection: 272 nm.

For NFX, its decarboxylated degradant has a particular significance since the pharmacological activity of the drug depends on the carboxylic group (27). On the other hand, this degradant was recorded as impurity in the bulk form and precipitate in the injection formulation (11). The prepared degradant (DCN) and the intact drug were submitted to FTIR, MS and LC analysis. The spectrum scan of NFX standard exhibited a strong stretching vibration band at 1715 cm^{-1} together with a broad band around 2500–3500 cm^{-1} characterizing carbonyl and hydroxyl moieties of the carboxylic group, respectively. These two bands, in the spectrum of DCN, corresponded in position to that obtained for NFX standard, but the relative intensity decreased, indicating decarboxylation. The mass spectra obtained is shown in Figure 3A, with characteristic signal on m/z 320 amu for NFX[H^+] and m/z 361 amu for NFX[CH_3CN-H^+], which is an adduct produced with NFX and acetonitrile (41 Da) from the mobile phase. Adduct is defined as an ion formed through the interaction between two species, usually an ion and a molecule, containing all the atoms of one specie plus one or several atoms of the other (28). In Figure 3B is shown the signal on m/z 276 amu for DCN[H^+], supporting the identity of the degradation product. Moreover, the degraded sample was analyzed by the proposed LC method (Figure 2B) and its retention time was used to assume the identity of this product in the samples subjected to stress studies.

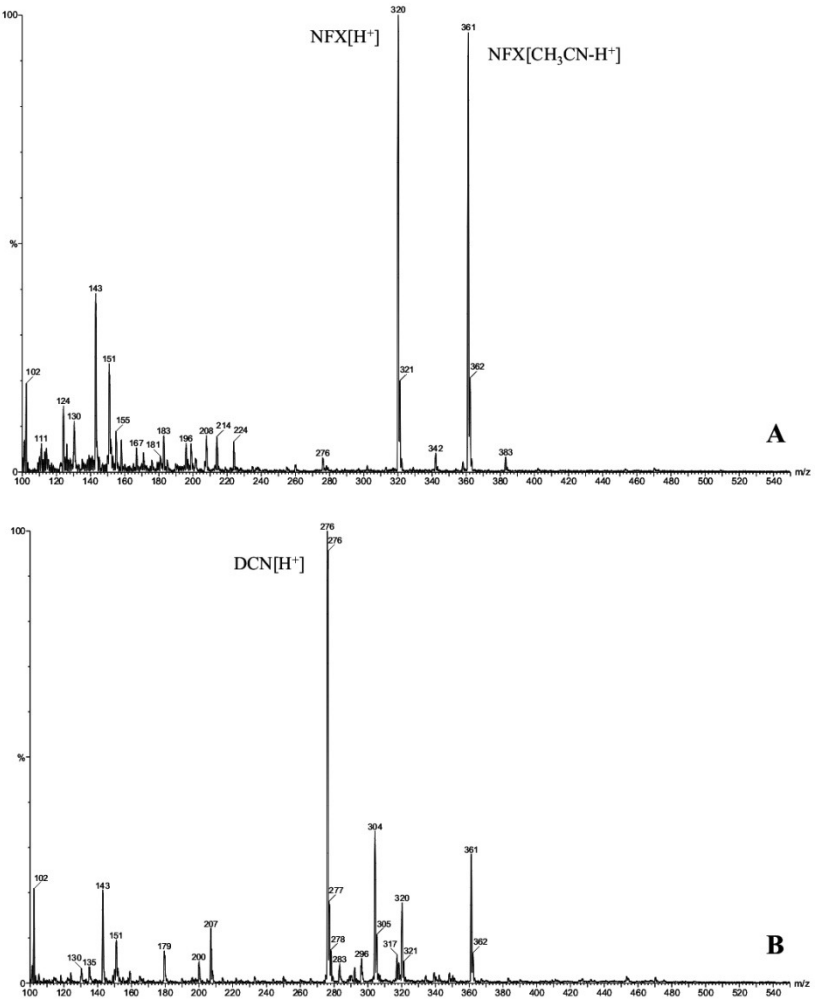

Figure 3. Mass spectra for (A) norfloxacin and (B) decarboxylated degradant.

The specificity of the analytical method for NFX was indicated in Figure 2, where the excipients did not interfere on NFX peak. Under acidic condition, one additional peak was observed with the same retention time of DCN, thus confirming its identity. The basic and photolytic conditions generated one additional peak. Under oxidative

and neutral conditions there was no change in the area and no additional peak was detected. The studies with the PDA detector showed that the norfloxacin peak was free from any coeluting peak, with values of peak purity index higher than 0.9999, thus demonstrating that the proposed method is specific.

The calibration curves constructed for norfloxacin were found to be linear in the 0.05–5 µg/mL range. The value of the determination coefficient calculated (r^2=0.9999, y=186846 ± 1960x – 951.8 ± 890.6, where, x is concentration and y is the peak absolute area) indicated the linearity of the calibration curve for the method. The validity of the assay was verified by means of ANOVA, which demonstrated significant linear regression and non-significant linearity deviation (P < 0.01).

The precision evaluated as the repeatability of the method was studied by calculating the relative standard deviation (RSD) for six determinations of the concentration of 1 µg/mL performed on the same day and under the same experimental conditions. The RSD value obtained was 0.98%.

The intermediate precision was assessed by analyzing two samples of the pharmaceutical formulation on three different days (inter-day); the RSD values obtained were 0.59% and 0.39%. Between-analysts precision was determined by calculating the RSD for the analysis of two samples of the pharmaceutical formulation by three analysts; the values were found to be 0.05% and 0.28% (Table 1).

Table 1. Inter-day and between-analysts precision data of the method.

Sample	Inter-day			Between-analysts		
	Day	Recovery[a] (%)	RSD[b] (%)	Analysts	Recovery[a] (%)	RSD[b] (%)
1	1	99.99	0.59	A	100.00	0.05
	2	101.07		B	99.91	
	3	100.96		C	100.00	
2	1	99.06	0.39	A	101.72	0.28
	2	99.63		B	101.15	
	3	98.88		C	101.46	

[a] Mean of three replicates
[b] RSD = Relative standard deviation

The accuracy was assessed from three replicate determinations of three different added standard solutions containing 0.3, 0.5 and 0.7 µg/mL of NFX. The results are shown in table 2, with a mean value of 99.90% and RSD of 0.97%, demonstrating that the method is accurate within the desired range.

Table 2. Accuracy of the method.

Added Concentration (µg/mL)	Mean concentration found[a] (µg/mL)	RSD[b] (%)	Accuracy (%)
0.30	0.30	1.09	100.48
0.50	0.50	1.16	100.23
0.70	0.69	0.40	98.70

[a] Mean of three replicates
[b] RSD = Relative standard deviation

For the calculation of the LD and LQ, the calibration equation for NFX was generated by using the mean values of the three independent calibration curves. The mean of the slope and the standard deviation of the intercept of the independent curves were 186846 and 951.8 respectively. The values calculated for the LD and LQ were 0.01 and 0.05 μg/mL, respectively. The LQ evaluated experimentally for NFX was also 0.05 μg/mL and was included in the calibration curve of the method.

To evaluate the robustness of an analytical method usually the OVAT approach is applied, however it is not recommended. The most important reason is that when the factors are examined in given intervals, the effects are estimated for a smaller domain around the nominal levels with the OVAT than with the experimental design approach. Moreover, the OVAT approach requires more (too many) experiments, especially when the number of examined factors becomes larger, and secondly, the importance of factor interactions cannot be taken into account (22,23). The experimental ranges of the selected variables evaluated are given in Table 3. The analysis of variance ANOVA was performed and the model terms (variables) were not significant ($P > 0.05$). The normal plot of residuals and outlier T for the responses evaluated are shown in Figure 4, and the results demonstrated that the method was robust. Moreover, the stability of the analytical solution was analyzed and it was found to be stable up to 48 h (99.65%, assay).

Table 3. Chromatographic conditions and range investigated during robustness testing.

Experi-mental	Factors				Responses[a]		
	ACN (%)	Flow (ml/min)	pH	Temp (^{o}C)	RSD (%)	Assay (%)	Peak Symmetry
1	18.00	1.00	2.7	45.00	0.43	99.78	1.09
2	16.00	1.20	3.3	35.00	0.31	100.48	1.10
3	16.00	1.20	2.7	45.00	0.35	99.84	1.09
4	14.00	1.00	3.3	45.00	0.35	100.23	1.10
5	16.00	1.20	3.3	45.00	0.49	99.99	1.09
6	14.00	1.00	2.7	35.00	0.54	100.17	1.11
7	14.00	0.80	3.0	35.00	0.41	100.05	1.11
8	14.00	0.80	2.7	40.00	0.28	100.56	1.10
9	14.00	1.20	3.0	35.00	0.89	100.02	1.10
10	18.00	0.80	3.0	35.00	0.52	100.41	1.10
11	18.00	0.80	3.3	40.00	0.17	99.61	1.09
12	16.00	1.20	2.7	35.00	0.44	100.41	1.10
13	14.00	1.00	3.3	45.00	0.22	99.76	1.08
14	18.00	1.00	2.7	45.00	0.31	100.12	1.09
15	14.00	0.80	3.0	35.00	0.70	100.34	1.10
16	16.00	0.80	2.7	35.00	0.63	99.90	1.09
17	18.00	1.00	2.7	35.00	0.38	100.37	1.09

[a] Mean of three replicates

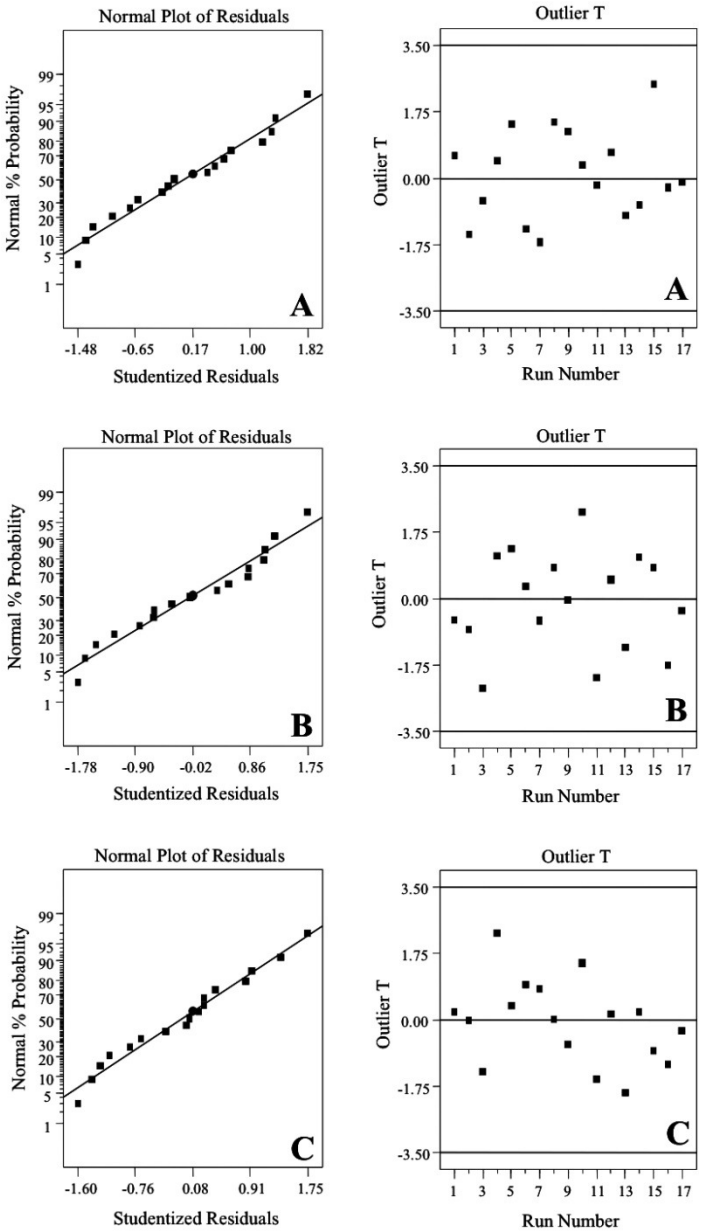

Figure 4. Normal plot of residuals and outlier T values for responses (A) peak area RSD%, (B) assay, and (C) peak symmetry.

The system suitability results showed that the parameters were within the suitable range. The RDS values calculated for peak area and retention time were 0.98 and 0.15%, respectively. The mean asymmetry and theoretical plates ± RSD were 1.11 ± 0.34% and 8226 ±1.3%, respectively.

The LC method validated in this paper was applied for the determination of norfloxacin in the new extended release formulations, without prior separation of the excipients. The values obtained ranged from 99.43 to 102.35%.

Conclusion

The results of the validation studies showed that the LC method is specific, accurate and possesses significant linearity and precision characteristics without any interference from the formulation excipients and degradation products. Moreover, the proposed method was successfully applied for the quantitative analysis of norfloxacin in the developed extended release dosage forms.

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CAPÍTULO 4 – Formulação, estabilidade e avaliação in vitro de comprimidos de liberação prolongada contendo Norfloxacino.

Publicação científica: Oliveira, P.R.; Bernardi, L.S.; Mendes, C.; Silva, M.A.S. Formulation, stability and in vitro dissolution studies of norfloxacin extended-release matrix tablets. Manuscrito em preparação.

INTRODUÇÃO

A tecnologia de produção de sistemas matriciais de liberação prolongada é relativamente simples, possibilitando inclusive sua obtenção por compressão direta (COLOMBO et al., 2009). O estudo do tipo de polímero utilizado, da sua massa molecular e concentração na formulação é etapa fundamental para o desenvolvimento destes sistemas, objetivando uma liberação adequada in vivo (LOPES; LOBO; COSTA, 2005; JAMZAD; FASSIHI, 2006).

A estabilidade de produtos farmacêuticos depende de fatores ambientais como temperatura, umidade, luz e de outros relacionados ao próprio produto como propriedades físicas e químicas do fármaco, além do processo de fabricação. A estabilidade acelerada é realizada para estudar a degradação química e/ou mudanças físicas de um produto farmacêutico, utilizando condições forçadas de armazenamento. Como o Brasil está classificado em zona IV (clima quente e úmido), este estudo é realizado em câmara climática em temperatura de 40 ± 2 ºC e umidade relativa de 75 ± 5% (BRASIL, 2005; WHO, 2009). A estabilidade da formulação frente à luz UV também deve ser avaliada (ICH, 1996; BRASIL, 2008). Os resultados obtidos são utilizados para estabelecer prazo de validade e recomendar condições de armazenamento. Neste capítulo, além dos comprimidos, avaliou-se a influência do revestimento e da embalagem na estabilidade.

O ensaio de perfil de dissolução é de fundamental importância no desenvolvimento de comprimidos de liberação prolongada (JORGENSEN; BHAGWAT, 1998; CHEN et al., 2010; MOURÃO et al., 2010), pois através dele pode-se avaliar o resultado das alterações realizadas na formulação e planejar a etapa seguinte. A partir da analise dos perfis é possível saber a cinética e o mecanismo de liberação do fármaco a partir da matriz, bem como a relação destes com a composição da formulação.

As equações utilizadas nesta etapa para avaliação da cinética foram: zero-ordem (COSTA; LOBO, 2001), primeira-ordem (COSTA; LOBO, 2001), Higuchi (HIGUCHI, 1963; COSTA; LOBO, 2001) e Korsmeyer-Peppas (COSTA; LOBO, 2001; KORSMEYER et al., 1983). Além disso, através da equação de Korsmeyer-Peppas pode-se calcular o mecanismo de liberação predominante: difusão fickiana, transporte Caso-II (relaxamento e erosão das cadeias poliméricas), transporte anômalo (combinação dos dois mecanismos citados) ou

transporte super Caso-II (aumento da plasticidade das cadeias poliméricas, relaxamento e erosão poliméricas).

Formulation, stability and in vitro dissolution studies of norfloxacin extended-release matrix tablets

Paulo Renato Oliveira*, Larissa Sakis Bernardi, Cassiana Mendes, Marcos A. Segatto Silva.

Department of Pharmaceutical Sciences (J/K - 207), Health Science Centre, Federal University of Santa Catarina, 88040-900, Florianópolis-SC, Brazil.

* Corresponding author: prenato.oliveira@gmail.com

Abstract

The aim of this research was to develop a new hydrophilic matrix system containing norfloxacin (NFX) and to carry out stability and in vitro release studies. Extended-release tablets are usually intended for once-a-day administration with benefits to the patient and lower discontinuation of the therapy, which for antibacterial drugs is very important since it can result in a decrease of bacterial resistance. Formulations were developed with hydroxypropylmethylcellulose (HPMC) and poly(ethylene oxide) (PEO) as hydrophilic polymers, with different molecular weights (MWs) and concentrations (20 and 30%). The tablets were found to be stable (6 months at 40 ± 2 ºC and 75 ± 5% relative humidity) and the film-coating process is recommended to avoid NFX photodegradation. The dissolution profiles demonstrated an extended-release of NFX for all developed formulations, however for those containing high MW polymers, at the end of the analysis, the drug was not completely released. Dissolution curves analyzed using the Korsmeyer exponential equation showed that drug release was controlled by both drug diffusion and polymer relaxation or erosion mechanisms. A more erosion controlled system was obtained for the formulations containing lower MW and amount of polymer. With the increase in MW and amount of polymer in the formulation, the gel layer became stronger and the dissolution was more drug-diffusion dependent (with decreasing in exponent *n* values). Formulations containing intermediate MW polymers or high concentration (30%) of low MW polymers demonstrated a combination of extended and complete in vitro drug release. This way, these formulations could provide an increased bioavailability in vivo.

Keywords: Norfloxacin; hydroxypropylmethylcellulose; poly(ethylene oxide); hydrophilic polymers; extended-release; dissolution studies.

1. Introduction

Hydrophilic matrix tablets are among the most popular orally administered controlled release systems. Despite having been around since four decades, matrices are still the reference starting point for innovations in drug delivery. It can be due to the fact that they are considered quite reliable in terms of drug delivery, simple technology and low-cost of manufacture. Moreover, matrices can be continuously innovated as new materials for formulation become commercially available [1-5].

The matrix tablets are usually composed of active pharmaceutical ingredients (APIs) and hydrophilic swellable polymers. When the system is exposed to the aqueous medium, water will be absorbed and a gel layer will be formed. This viscous gel layer may hinder water penetration and become the rate-controlling step during gel formation. The gel strength is important in the matrix performance and is dependent on the chemical structure, concentration and viscosity of the polymer used. Depending on the mechanical properties of the gel layer, drug release is controlled by different mechanisms and kinetics. Polymer swelling, drug dissolution, drug diffusion, and matrix erosion are the basic phenomena leading to the drug release from swellable matrices [6-12]. Additionally, drug load and solubility can influence the release mechanism and kinetics.

Hydroxypropylmethylcellulose (HPMC) is a propylene glycol ether of methylcellulose and is widely used as a matrix former in oral controlled release tablet formulations [1]. One of its most important characteristics is the high swellability, which has a significant effect on the release kinetics of an incorporated drug. Furthermore, HPMC is compatible with numerous drugs, accommodates high levels of drug loading and can be easily incorporated to form matrix tablets by direct compression or granulation [9,13-16]. The availability of a wide range of viscosity grades also allows the formulator to modify the release of drugs from HPMC matrix tablets according to therapeutic need.

High molecular weight poly(ethylene oxide) (PEOs) have been proposed as an alternative to HPMC in controlled release dosage forms [17]. They are important polymers for the pharmaceutical industries mainly because of their non-toxicity, high water-solubility and swellability, insensitivity to the pH of the biological medium and ease of production. PEOs swell and form a compact gel layer on the surface of

the tablet which is responsible for the controlled drug release [17-22]. They are also available in a wide range of molecular weights, thus allowing the formulator to control the mechanism of drug release to achieve the therapeutic goal.

Norfloxacin (NFX) is a synthetic broad spectrum antibacterial drug being the firstly selected drug for the treatment of diseases caused by *Campylobacter, E. coli, Salmonella, Shigella and V. cholera* [23,24]. The drug is also used for the treatment of urinary tract infections as well as gonorrhoea and infection of eyes [23]. The recommended dosage is usually 400 mg twice daily. The half-life of NFX in serum and plasma is 3-4 hours and only approximately 30-40% of an oral dose is absorbed [25,26]. Increasing bacterial resistance to currently available antibiotics, including the quinolone class, has reduced their effectiveness, making the therapeutic decisions more difficult and may compromise future use of this class of drugs [27-31].

The development of an extended release formulation that could improve the bioavailability of NFX and reduce the administration schedule may improve the patients' comfort and compliance, resulting in lower discontinuation of the therapy; with consequently decrease in bacterial resistance. The correct choice of the hydrophilic polymer, molecular weight and quantity in the matrix formulation can provide an appropriate combination of polymer swelling, erosion or drug diffusion mechanisms to control drug release. Thus, the aim of this work was to develop and carry out stability and in vitro dissolution studies of a new formulation of norfloxacin extended-release tablets.

2. Materials and Methods

2.1 Materials

Norfloxacin (NFX) was purchased from Zhejiang Neo-Dankong pharmaceutical (Zhejiang, China). Hydroxypropylmethylcellulose (HPMC) K100 LV (apparent viscosity: 100 mPa s, 2% in water at 20 ºC), HPMC K4M (4 000 mPa s), HPMC K100M (100 000 mPa s), and poly(ethylene oxide) (PEO) N60K (2 000 kDa), PEO 301 (4 000 kDa) and PEO 303 (7 000 kDa) were kindly donated by Colorcon (São Paulo, Brazil). The pharmaceutical excipients used were: microcrystalline cellulose (Microcel 102, Blanver, Itapevi, Brazil), magnesium stearate (M. Cassab, São Paulo, Brazil), and colloidal silicon dioxide (Aerosil®, Labsynth, Diadema, Brazil).

2.2 Methods

2.2.1 Preparation of matrix tablets

A powder blend containing NFX, polymer and microcrystalline cellulose was prepared and mixed for 15 min, followed by addition of magnesium stearate and colloidal silicon dioxide with a further 5 min mixing. The modules having the composition reported in Table 1 were prepared by direct compression using a 19 x 8 mm punch set. (Fellc compressing model F-10/8, São Paulo, Brazil).

Table 1. Composition of tablets containing hydroxypropylmethyl cellulose (HPMC) or poly(ethylene oxide) (PEO).

Composition	For one tablet	For one tablet
Norfloxacin	700 mg	700 mg
Polymer	20 %	30 %
HPMC K100 LV		
HPMC K4M		
HPMC K100M		
PEO N60K		
PEO WSR 301		
PEO WSR 303		
Magnesium stearate	1 %	1 %
Colloidal silicon dioxide	0.5 %	0.5 %
Microcrystalline cellulose	q.s.	q.s.
Total weight	1.07 g	1.07 g

2.2.2 Characterization of tablet formulation

Tablets were characterized by weight, hardness, friability, dimension, and loss on drying according to pharmacopeial limits [33,34]. The average weight was obtained for at least 20 units. Hardness was determined for at least 10 tablets using a Hardness Tester (298-AT, Nova Ética, Vargem Grande Paulista, Brazil), and adopting a minimum hardness of 3 kgf as the acceptance criterion. For each formula, friability was evaluated for a sample of 20 tablets, using the acceptance criterion of a maximum loss of 1.5% of the initial weight. Dimension was evaluated measuring 10 tablets with a paquimeter. Loss on drying was carried out with 2 g of sample, in vacuum, at 105 ºC for 2 h.

2.2.3 Tablet coating and blistering

A tablet coating solution was formed by adding 30 g of Opadry II White (Colorcon, São Paulo, Brazil) to 120 g of purified water and stirring for 2 min. An amount corresponding to 50% of each formulation batch was placed in a Rama Cota RD conventional coating machine. Tablets were preheated until the bed temperature reached 45 °C. Pan rotation was set to 40 rpm and tablets were coated using a Binks Model 460 spray gun operating at 2 Bar. The coating solution was pumped at a rate of 5.9-9.6 g/min using a peristalic pump. Tablet bed temperature was maintained between 42-45 °C during the spray coating process. After coating, an amount of coated and uncoated tables were blistered in transparent PVC blister and sealed with an aluminium foil.

2.2.4 NFX tablets assay

NFX quantification assay was carried out according to a previously validated method [35]. Briefly, the LC system was operated isocratically at 40 ºC using a mobile phase composed by phosphoric acid 0.04 M, pH 3.0/acetonitrile (84:16; v/v), eluted at a flow rate of 1.0 mL/min. A reversed-phase Phenomenex (Torrance, USA) Luna C_{18} column (150 mm x 4.6 mm I.D., with a particle size of 5 µm and pore size of 100 Å) was used and the detector was set at 272 nm. The injection volume was 20 µL.

To prepare the sample stock solution, the manufactured extended-release tablets were crushed to a fine powder. An appropriated amount was transferred into an individual 50 mL volumetric flask, dissolved with 0.2 mL of glacial acetic acid, and diluted to volume with mobile phase, obtaining a concentration of 1 mg/mL of the API. The NFX standard stock solutions were prepared by weighing 50 mg, transferred to 50 mL volumetric flasks, dissolved with 0.2 mL of acetic acid glacial, and diluted to volume with mobile phase, obtaining a concentration of 1 mg/mL. Both sample and standard stock solutions were stored at 2-8 °C protected from light. Working solutions were prepared daily by diluting the stock solutions to an appropriate concentration in mobile phase.

2.2.5 Stability tests

The manufactured tablets were submitted to accelerated stability test. Samples of each batch (non-coated, coated, with and without blister) were maintained for 6 months in a accelerated stability chamber (420 CLD, Nova Ética, Vargem Grande Paulista, Brazil) at 40

± 2 ºC and 75 ± 5% relative humidity [36,37]. For photostability tests, samples were exposed to an overall illumination of not less than 1.2 million lux [38]. The illumination was measured with a Digital Lux Meter (MLM-1011, Minipa, São Paulo, Brazil). Protected samples (wrapped in aluminium foil) were used as dark controls to evaluate the contribution of thermally induced change to the total observed change.

2.2.6 Drug release study

Drug release studies were performed based on pharmacopeial methods using USP apparatus II Vankel 7000 dissolution tester (Varian Technology Group, Cary, USA), with paddle rotation of 75 rpm, in 900 ml of buffer pH 4.0 at 37.0 ± 0.5 °C [33,34]. At specified time intervals, 5 mL samples were withdrawn, filtered and quantified in a UV spectrophotometer (Varian Cary 50 bio, Cary, USA) at the wavelength 278 nm.

2.2.7 Analysis of drug release

The analysis of the values obtained in dissolution tests is easier when mathematical formulas that express the dissolution results as a function of some of the dosage forms characteristics are used. NFX release kinetic was evaluated according to the following models: zero order, first order, Higuchi, and Korsmeyer-Peppas. Additionally, the difference factor ($f1$) and similarity factor ($f2$) were used to compare the dissolution profiles.

2.2.7.1 Zero-order model

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly (assuming that area does not change and no equilibrium conditions are obtained) following a 'steady-state release' can be represented by the following equation (eq. 1) [39]:

$$Q_t = Q_o + k_o t \qquad \text{(eq. 1)}$$

Where Qt is the fraction of drug released at time t, Q_0 is the initial amount of drug in the solution (most times $Q_0 = 0$); k_0 is the zero-order release constant. The pharmaceutical dosage forms following this profile release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a pharmacological prolonged action.

2.2.7.2 First-order model

The drug dissolution is assumed to decline exponentially and the release rate is proportional to the residual amount of drug in the dosage form (eq. 2) [39]:

$$\log Q_t = \log Q_0 + \frac{k_1 t}{2.303} \qquad \text{(eq. 2)}$$

Where Qt is the fraction of drug released at time t, Q_0 is the initial amount of drug in the solution; k_1 is the first-order release constant. The pharmaceutical dosage forms following this dissolution profile release the drug by unit of time in a way that is proportional to the amount of drug remaining in its interior.

2.2.7.3 Higuchi model

The most widely used model to describe drug release from matrices, derived from Higuchi for a planar matrix. It describes the drug release mechanism as a diffusion process based on Fick's law, dependent on the square root of time (eq. 3) [39,40].

$$Q_t = K_H \sqrt{t} \qquad \text{(eq. 3)}$$

Where Qt is the fraction of drug released at time t and K_H is the Higuchi dissolution constant.

2.2.7.4 Korsmeyer-Peppas model

This model is generally used to analyze the release of pharmaceutical polymeric dosage forms when the release mechanism is not well known or when more than one type of release phenomena could be involved (eq. 4) [39,41].

$$\frac{M_t}{M_\infty} = kt^n \qquad \text{(eq. 4)}$$

where $Mt/M\infty$ is the fraction of drug released, k is the kinetic constants characteristic of the drug/polymer, n is the diffusional exponent for drug release. Dissolution values in the range of 5 – 60% were used to fit release data.

2.2.7.5 Difference factor ($f1$) and similarity factor ($f2$)

The relevance of the difference between the release curves were assessed using difference factor $f1$ and similarity factor $f2$, calculated by eq. (5) and (6), respectively [42,43]:

$$f1 = \left\{ \sum_{i=1}^{n} (Rt - Tt) / \sum_{i=1}^{n} Rt \right\} .100 \quad \text{(eq. 5)}$$

$$f2 = 50.\log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{i=1}^{n} (Rt - Tt)^2 \right]^{-0.5} .100 \right\} \quad \text{(eq. 6)}$$

where Rt and Tt are the percentages released at each time point.
An $f1$ value up to 15 (0-15) and $f2$ value between 50 and 100 implies similarity between two release profiles. Only one more point after the 85% of drug has released was used for the equation.

3. Results and Discussion

Norfloxacin matrix tables were successfully obtained by direct compression (Fig. 1). Different polymers and molecular weights did not interfere in the technological process. The pharmacopeial characteristics of the manufactured tablets are summarized in Table 2. These results demonstrated that the tablets were reliable on hardness and friability, which are important characteristics for the further step of coating.

Consistent hardness of the tablet surface enables the coating to "lock" into the surface. If the surface is too soft, the impingement of the solution can erode the tablet. Too hard a surface will not allow the solution to impinge and adhere, and the coating will peel away. Both of these coating defects can also occur by over- or under-applying the coating solution or by applying the coating with too much or too little force [44-47]. The film-coating (Opadry II) applied on the NFX tablets surface is non-functional, however it can improve the final quality by protecting the hygroscopic polymer from absorbing humidity and preventing photodegradation of the drug. NFX coated tablets showed an uniform, smooth and shiny surface, without coating defects (Fig. 1). From Table 2, it can be observed that the weight and hardness increased about 3% and 9%, respectively, demonstrating the influence of the coating process. The loss on drying analysis (Table 2) showed that the

coated tablets have a lower amount of volatile matter, probably due to the loss of water absorbed during the coating process at 42-45 °C.

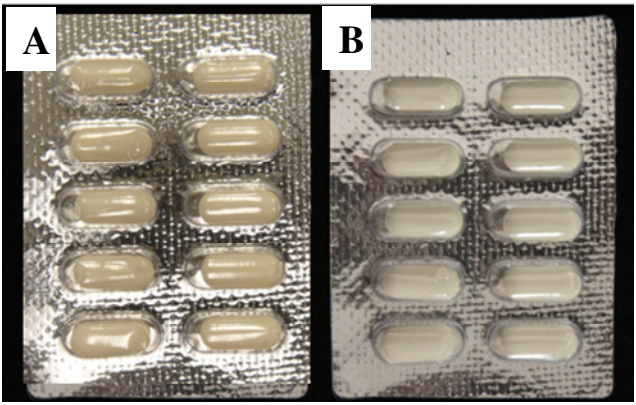

Figure 1. Norfloxacin blistered matrix tablets: uncoated (A) and coated (B).

Table 2. Pharmacopeial characteristcs of norfloxacin matrix tablets.

Formulation	Weight (g)[a]		Hardness (KgF)[b]		Water Loss (%)		Friability (%)[a]
	Uncoat.	Coated	Uncoat.	Coated	Uncoat	Coated	Uncoat.
HPMC K100LV 20%	1.1154	1.1472	16.3	17.0	7.75	7.29	0.021
HPMC K100LV 30%	1.1025	1.1321	14.8	16.4	7.93	7.60	0.014
HPMC K4M 20%	1.1033	1.1314	13.1	15.1	7.08	6.60	0.034
HPMC K4M 30%	1.0795	1.1119	13.0	14.5	7.29	7.11	0.018
HPMC K100M 20%	1.1048	1.1351	17.3	17.9	6.68	5.85	0.022
HPMC K100M 30%	1.0967	1.1277	14.3	16.2	7.02	7.21	0.024
PEO N60K 20%	1.0858	1.1193	14.2	15.7	7.02	5.08	0.017
PEO N60K 30%	1.0854	1.1131	16.9	17.7	6.77	5.64	0.021
PEO 301 20%	1.0831	1.1162	16.6	18.8	7.16	6.91	0.018
PEO 301 30%	1.1014	1.1306	16.0	17.5	7.09	7.01	0.023
PEO 303 20%	1.0748	1.1090	14.4	15.6	6.13	5.55	0.024
PEO 303 30%	1.0734	1.1077	14.6	15.8	5.51	4.22	0.019

[a] mean of twenty determinations; [b] mean of ten determinations

The assay determination of NFX demonstrated that all formulations were in the range from 99.43 to 102.35% (Table 3). Therefore, the coating process did not influence on the assay of the drug.

Table 3. Assay results of accelerated stability test.

Formulation	Time zero		After 6 months			
			Blister		Without blister	
	Uncoat (%)	Coated (%)	Uncoat (%)	Coated (%)	Uncoat (%)	Coated (%)
HPMC K100LV 20%	101.98	101.09	101.16	101.51	102.03	101.18
HPMC K100LV 30%	101.47	101.81	101.87	103.01	100.87	101.74
HPMC K4M 20%	101.45	100.42	100.44	102.98	101.92	101.08
HPMC K4M 30%	100.88	99.31	99.12	99.45	99.91	98.09
HPMC K100M 20%	102.35	101.74	99.14	101.25	103.22	102.80
HPMC K100M 30%	102.25	101.58	99.66	99.31	99.02	101.80
PEO N60K 20%	102.06	101.72	102.89	98.47	101.44	100.26
PEO N60K 30%	99.43	99.92	98.49	99.05	99.44	99.03
PEO 301 20%	99.48	99.35	101.24	97.51	101.75	100.31
PEO 301 30%	99.71	100.50	99.03	100.49	100.61	100.36
PEO 303 20%	99.98	99.50	99.79	97.11	100.62	97.36
PEO 303 30%	99.95	99.36	99.16	97.78	99.10	99.35

Accelerated stability testing was carried out to provide evidence of how the quality of the manufactured tablets may change with time under the influence of environmental factors such as temperature and humidity. Brazil, being considered with hot and humid climate is classified in the region IV [36]. According to this classification, the accelerated stability study was carried out for 6 months in a climatic chamber at 40 ± 2 ºC and 75 ± 5% relative humidity. The obtained results are shown in Table 3. All formulations were considered stable since after 6 months a change from the initial assay of 5% or more was not observed [36]. The presence of coating and/or blister did not influence in the stability of the developed tablets. Additionally, the chromatographic profiles did not show any additional degradation peak.

Light testing should be an integral part of stress testing and recommends evaluation of the photostability of a formulation to demonstrate that light exposure does not result in unacceptable changes

[36,38]. For this study, the following formulations were selected: HPMC K100 LV (20 and 30%) and PEO N60K (20 and 30%). At the end of the exposure period (about five days), equivalent of not less than 1.2 million lux, samples were examined for changes in appearance and for assay. It was observed a color change from pale-yellow to dark-yellow in NFX raw material and uncoated tablets. The transparent blister (primary packing) did not have any protecting influence in the formulations (Fig. 2). Prolonged exposure of NFX bulk drug, tablets and specially in solution under direct sunlight or fluorescent light results in the formation of ethylenediamine degradation product [48,49]. Since the chromatograms did not show additional peaks and a significant decrease of drug content was not observed (Table 4), it seems that the ethylenediamine degradant requires an exposure time and/or intensity higher than the used in this research to be significantly formed. Nonetheless, to prevent drug exposure to light and degradation, it would be recommended the coating process or light-protective blister for the formulations.

Table 4. Assay results (%) of photostability test.

Formulation	Blister		Without blister	
	Uncoated (%)	Coated (%)	Uncoated (%)	Coated (%)
HPMC K100LV 20%	100.80	99.22	100.30	99.71
HPMC K100LV 30%	100.25	100.36	99.96	100.60
PEO N60K 20%	98.78	100.35	99.54	99.26
PEO N60K 30%	98.17	100.79	99.36	99.06

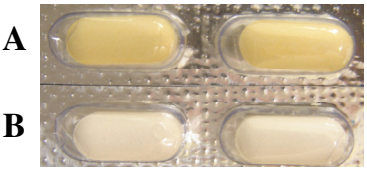

Figure 2. Norfloxacin blistered matrix tablets after photostability study: uncoated (A) and coated (B).

Two concentrations (20 and 30%) of different MWs HPMC or PEO polymers were used to manufacture the NFX matrix tablets used in this study (Table 1). The dissolution test was carried out under sink conditions, defined as the volume of medium being at least three times higher than that necessary to obtain a saturated solution of the drug [33]. Samples were withdrawn from the dissolution medium at the following times: 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 h. The first time point at 0.5 h was included to study if the product presents a burst effect (with an excessive early drug release), while the final time point shows whether or not the intended dose is fully delivered.

NFX release profiles are shown in Fig. 3 – Fig. 8. The polymers used have different average MWs and therefore they differ in controlling drug release from matrix tablets. An extended-release of NFX was obtained for all formulations manufactured, demonstrating that the mechanical strength of the viscous-gel layer was strong enough to maintain its integrity and drug release. Faster dissolution was obtained for formulations containing lower MW polymer and concentration (20%) (Fig. 3, Fig. 6). The tablets containing HPMC K100 LV showed the fast dissolution profile, with complete drug release at about 6 – 8 h (Fig. 3).

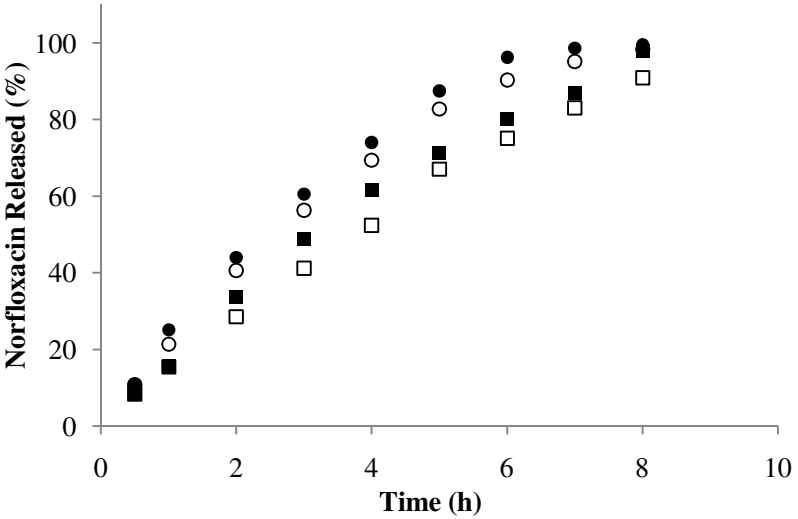

Figure 3. Norfloxacin released vs. time of matrix tablets containing HPMC K100 LV: 20% uncoated(●); 20% coated (○); 30% uncoated (■); 30% coated (□).

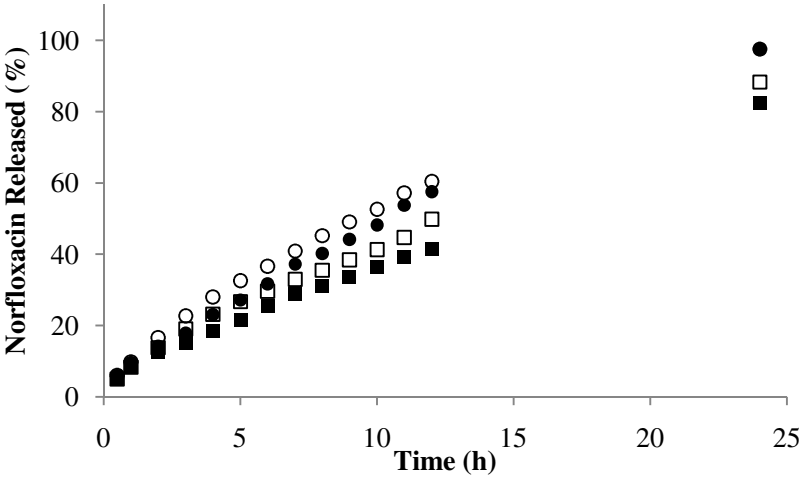

Figure 4. Norfloxacin released vs. time of matrix tablets containing HPMC K4M: 20% uncoated(●); 20% coated (○); 30% uncoated (■); 30% coated (□).

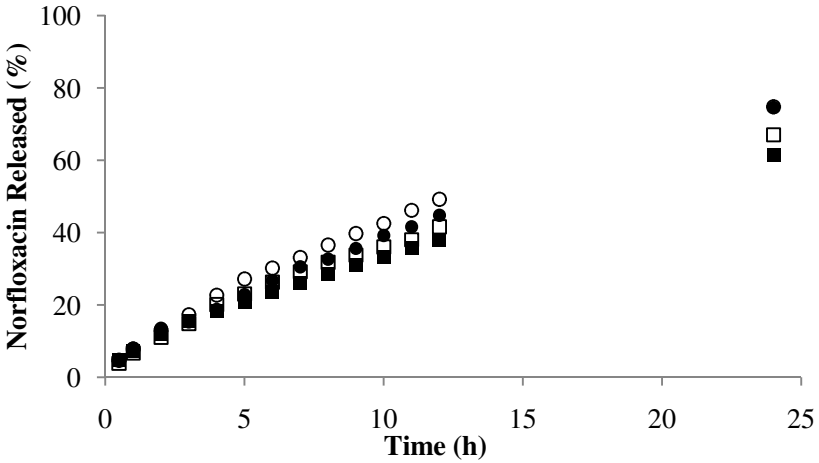

Figure 5. Norfloxacin released vs. time of matrix tablets containing HPMC K100M: 20% uncoated(●); 20% coated (○); 30% uncoated (■); 30% coated (□).

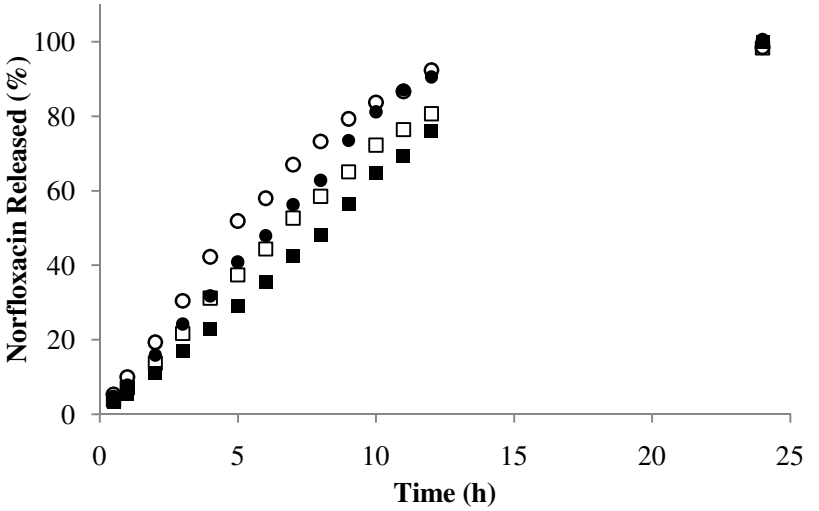

Figure 6. Norfloxacin released vs. time of matrix tablets containing PEO N60K: 20% uncoated(●); 20% coated (○); 30% uncoated (■); 30% coated (□).

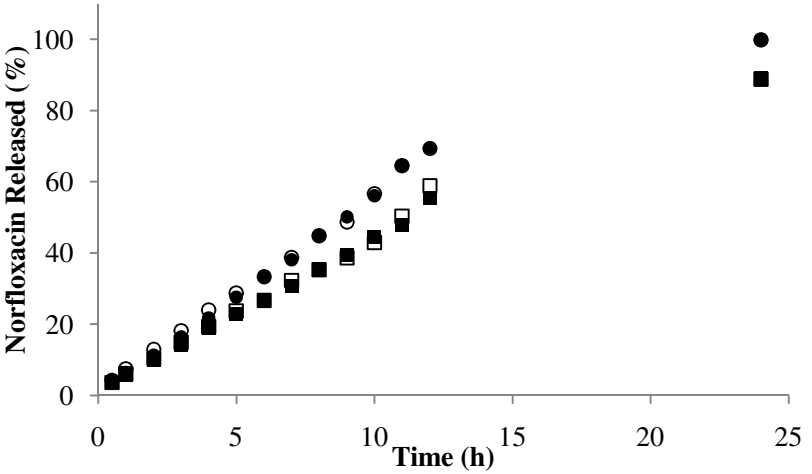

Figure 7. Norfloxacin released vs. time of matrix tablets containing PEO 301: 20% uncoated(●); 20% coated (○); 30% uncoated (■); 30% coated (□).

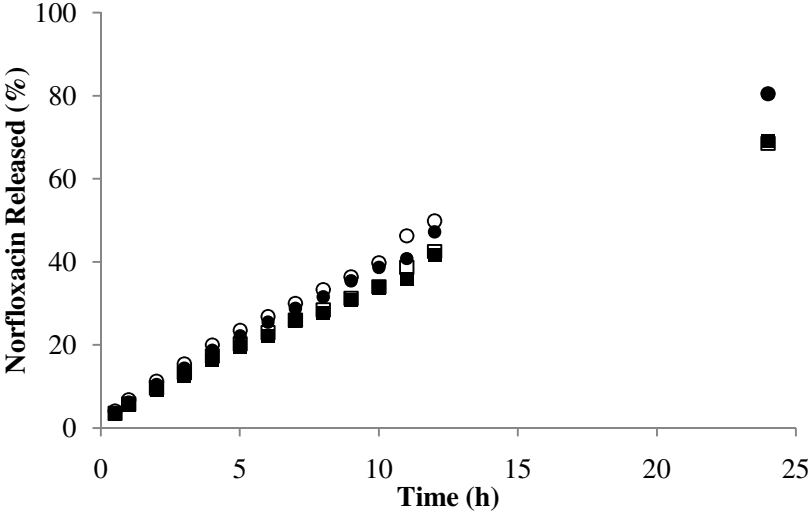

Figure 8. Norfloxacin released vs. time of matrix tablets containing PEO 303: 20% uncoated(●); 20% coated (○); 30% uncoated (■); 30% coated (□).

For the formulations containing HPMC K100M (20 and 30%, Fig. 5) and PEO 303 30% (Fig. 8), the NFX release was not complete at 24 h. Due to the high MW and/or concentration of polymer in the formulations, the swelling was too slow and the gel strength was very high, resulting the central part of the tablet not being fully wetted or hydrated (a "dry core"), with incomplete drug release. Probably, these formulations would exhibit an inadequate performance in vivo, since an incomplete polymer gelification and drug release would be obtained while passing through stomach and small intestine.

It seems that the coating process somehow influenced the NFX dissolution profile (Fig. 3 – Fig. 8), and a relation with the polymer MW could be suggested. In general, coated formulations exhibited faster drug release than uncoated ones. The faster NFX release may be due to the coating process temperature that resulted in lower residual humidity tablets (Table 2) and consequently a faster water uptake and polymer swelling in the dissolution medium.

For HPMC K100 LV formulations, due to the lower MW, water uptake, polymer hydration and gelification is faster than dissolution of the coating film. In this case, the coating may have worked as a "barrier", and drug release was delayed. For PEO 301 and PEO 303 the dissolution profiles were overlapped, demonstrating no influence of the coating. It can be explained since high MW polymers forms a stronger gel layer, with lower water uptake rate and drug release, hence influencing drug diffusion and dynamics of matrix erosion. However, the influence of the coating process was not relevant based on the difference ($f1$) and similarity ($f2$) parameters calculated (Table 5).

Table 5. Difference factor ($f1$) and similarity factor ($f2$) calculated for uncoated and coated norfloxacin matrix tablets.

Formulation	$f1$	$f2$
HPMC K100LV 20%	5.47	71.72
HPMC K100LV 30%	8.50	62.32
HPMC K4M 20%	9.22	70.03
HPMC K4M 30%	14.23	66.36
HPMC K100M 20%	9.22	73.89
HPMC K100M 30%	9.27	77.02
PEO N60K 20%	13.77	56.35
PEO N60K 30%	14.66	57.81
PEO 301 20%	2.00	91.85
PEO 301 30%	3.12	88.95
PEO 303 20%	4.90	83.40
PEO 303 30%	2.76	93.30

Dissolution profiles were analyzed for zero-order, first-order and Higuchi models with the equations up to 12 h of drug release, except for HPMC K100 LV 20 and 30% formulations where the equations were analyzed for up to 6 and 8 h, respectively. The analysis according to Korsmeyer-Peppas was carried out with the diffusional exponential equation up to 60% of drug released [41]. Calculation of the exponent n identifies the prevalent mechanism of release. For cylindrical systems, n = 0.45 indicates diffusion-controlled (Fickian) drug release and n = 0.89 indicates swelling/erosion-controlled drug release (Case-II transport). Values of n between 0.45 and 0.89 can be regarded as an indicator for the superposition of both phenomena, indicating that the drug delivery was not controlled only by diffusion, but also by significant polymer relaxation or erosion mechanisms (anomalous transport). The n > 0.89 values reveals a super case-II transport. This mechanism could result from an increased plasticization at the relaxing boundary (gel layer) and is also related to polymer relaxation and erosion mechanisms [41,50].

In general, data of all matrices provided better fit to Korsmeyer-Peppas model (Table 6 and Table 7). None formulation fitted to Higuchi equation, this way demonstrating that NFX release mechanism was not only a diffusion process dependent on the square root of time.

Table 6. Coefficients of determination (r^2) obtained from dissolution of norfloxacin uncoated formulations according to different mathematical models.

Formulation	Zero order	First order	Higuchi	Korsmeyer-Peppas	
	r^2	r^2	r^2	r^2	*n*
HPMC K100LV 20%	0.9794	0.9247	0.9685	0.9952	0.9623
HPMC K100LV 30%	0.9794	0.8538	0.9681	0.9985	0.9761
HPMC K4M 20%	0.9943	0.9916	0.9604	0.9963	0.7115
HPMC K4M 30%	0.9834	0.9954	0.9801	0.9986	0.6593
HPMC K100M 20%	0.9866	0.9962	0.9744	0.9978	0.6838
HPMC K100M 30%	0.9759	0.9909	0.9878	0.9995	0.6422
PEO N60K 20%	0.9976	0.9302	0.9372	0.9994	0.9485
PEO N60K 30%	0.9978	0.9513	0.9127	0.9990	1.0027
PEO 301 20%	0.9978	0.9591	0.9180	0.9978	0.8900
PEO 301 30%	0.9978	0.9795	0.9308	0.9971	0.8771
PEO 303 20%	0.9935	0.9934	0.9598	0.9982	0.7863
PEO 303 30%	0.9932	0.9931	0.9589	0.9979	0.7866

Table 7. Coefficients of determination (r^2) obtained from dissolution of norfloxacin coated formulations according to different mathematical models.

Formulation	Zero order	First order	Higuchi	Korsmeyer-Peppas	
	r^2	r^2	r^2	r^2	*n*
HPMC K100LV 20%	0.9827	0.9719	0.9649	0.9990	0.9283
HPMC K100LV 30%	0.9898	0.9571	0.9588	0.9999	0.8867
HPMC K4M 20%	0.9833	0.9981	0.9807	0.9998	0.7270
HPMC K4M 30%	0.9784	0.9921	0.9821	0.9984	0.7041
HPMC K100M 20%	0.9829	0.9973	0.9794	0.9994	0.7404
HPMC K100M 30%	0.9755	0.9917	0.9825	0.9980	0.7406
PEO N60K 20%	0.9759	0.9716	0.9685	0.9975	0.9892
PEO N60K 30%	0.9955	0.9766	0.9468	0.9992	1.0019
PEO 301 20%	0.9970	0.9593	0.9277	0.9979	0.8522
PEO 301 30%	0.9911	0.9586	0.9216	0.9917	0.8550
PEO 303 20%	0.9934	0.9889	0.9553	0.9968	0.7803
PEO 303 30%	0.9924	0.9898	0.9538	0.9975	0.7757

The formulations containing PEO demonstrated also a good fit to zero-order kinetics. The exponent *n* calculated (Table 6, Table 7) for Korsmeyer-Peppas equation confirmed this to PEO N60K (*n* between 0.94 - 1.0) and to PEO 301 (*n* about 0.87), indicating super case-II and case-II transport mechanism, respectively, as also evidenced by quasi-linear release profiles (Fig. 6 and Fig. 7). It can be due to the lower MW of these polymers in comparison to PEO 303. In the case of low viscosity gelling agents, erosion of the swollen polymer is the major release factor, generally leading to zero-order release kinetics [13]. For the tablets containing PEO 303, the exponent *n* obtained (about 0.78) indicated that drug delivery was controlled by diffusion and polymer relaxation/erosion mechanisms. Moreover, the dissolution kinetic equation fit was very similar to zero and first-order release.

HPMC K100LV formulations demonstrated a similar release profile to PEO N60K, where a super case-II transport mechanism was obtained due to the dissolution of polymeric matrix and relaxation of the polymer chain, with zero-order release. Most of matrix tablets containing HPMC K4M and HPMC K100M fitted better to first-order than to zero-order kinetic (Table 6 and Table 7). Moreover, the *n* exponent calculated (between 0.64 – 0.74) indicated the significant influence of both drug diffusion and polymer relaxation/erosion to drug release. It can be explained base on the fact that high viscosity polymers can form a mechanically stable gel and polymer dissolution/disintegration will be lower [13]. Therefore, the diffusion-controlled mechanism will have more influence on drug release from the swollen matrix.

Based on the dissolution profiles, HPMC K100 LV 30%, HPMC K4M 20%, PEO N60K 20%, and PEO N60K 30% matrices presented a combination of polymer type, MW, concentration, and complete drug release that could result in a formulation able to resist to the destructive forces within the gastro-intestinal tract, providing a superior in vivo performance. In fact, the results obtained confirm that gels showing lower strength and texture, usually derived from low MW polymers, have lower resistance to the fluid erosion action and the release of the active molecule is mainly due to polymer relaxation and chains disentanglement, leading to drug delivery kinetic towards an erosion/relaxation mechanism, with exponent $n \geq 0.89$. On the other hand, when the MW or polymer concentration is increasing, the gel layer formed will be concomitantly characterized by higher strength and consistence, being less susceptible to erosion and chains

disentanglement, with drug release mechanism tending to diffusion (with decreasing exponent *n* values).

4. Conclusions

In this study the development of a stable extended-release dosage form containing norfloxacin was demonstrated. The film-coating of tablets was necessary to avoid a photo-induced color changing of the active pharmaceutical ingredient. The dissolution studies showed that according to the increase in polymer molecular weight and concentration, the matrix changed from a more erodible system (with zero-order release) to a system with dissolution controlled by drug diffusion and polymer relaxation/erosion mechanisms. The formulations containing intermediate molecular weight HPMC or PEO or high concentration (30%) of low molecular weight polymers (HPMC K100 LV 30%, HPMC K4M 20%, PEO N60K 20%, and PEO N60K 30%) are more promising, since a combination between gel structure and complete in vitro drug release was obtained. This prolonged and complete in vitro release profile is expected to lead to an increased bioavailability, however in vivo studies are necessary to confirm this possibility. Based on an improved bioavailability combined with a reduced frequency of administration, an improved patient compliance and decreased bacterial resistance could be achieved.

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CAPÍTULO 5 – Desenvolvimento de sistemas Dome Matrix® de Norfloxacino.

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Assembled modules technology for site-specific prolonged delivery of norfloxacin

Paulo Renato Oliveira[a], Larissa Sakis Bernardi[a], Orazio Luca Strusi[b], Salvatore Mercuri[b], Marcos A. Segatto Silva[a], Paolo Colombo[b], Fabio Sonvico[b,*]

[a] Department of Pharmaceutical Sciences, Federal University of Santa Catarina, Quality Control Laboratory, J/K 207, 88040-900 Florianópolis-SC, Brazil
[b] Department of Pharmacy, University of Parma, Viale G.P. Usberti 27/A, 43125 Parma, Italy

INTRODUÇÃO

Dentre os sistemas gastro-retentivos de fármacos, os sistemas flutuantes apresentam a vantagem de manter a forma farmacêutica fisicamente afastada do piloro dificultando seu esvaziamento gástrico (BARDONNET et al., 2006). Um sistema com esta característica foi desenvolvido pelo grupo do Prof. Paolo Colombo, na Università degli Studi di Parma – Italia. Este sistema, denominado Dome Matrix®, basicamente consiste em dois comprimidos com uma base côncava e uma convexa ligados entre si de forma que exista uma câmara com ar no seu interior, sendo esta responsável pela flutuação (LOSI et al., 2006).

O norfloxacino apresenta uma maior solubilidade em meio ácido (F. BRAS. IV, 2001) consequentemente, uma maior permanência no estômago pode resultar em maior biodisponibilidade para o fármaco. Esta representa uma outra estratégia para melhorar a resposta in vivo alterando a formulação farmacêutica, sem alterar quimicamente o fármaco. Com este sistema, uma maior biodisponibilidade para o norfloxacino pode ser obtida a partir de uma permanência do sistema no estômago (maior solubilidade do fármaco no meio ácido), combinada a uma liberação prolongada, obtida através da utilização de polímeros hidrofílicos (HPMC e POE) na formulação.

Neste estudo avaliou-se a dissolução de módulos individuais e na configuração flutuante "void" em fluido gástrico simulado, bem como estudos de flutuação in vitro, já prevendo o comportamento in vivo destas formulações.

Assembled Modules Technology for Site-specific Prolonged Delivery of Norfloxacin

Paulo Renato Oliveira[a], Larissa Sakis Bernardi[a], Orazio Luca Strusi[b], Salvatore Mercuri[b], Marcos A. Segatto Silva[a], Paolo Colombo[b], Fabio Sonvico[b*].

[a] Department of Pharmaceutical Sciences, Federal University of Santa Catarina, Quality Control Laboratory, J/K 207, 88040-900, Florianópolis-SC, Brazil.
[b] Department of Pharmacy, University of Parma, Viale G.P. Usberti 27/A, Parma, Italy

* Corresponding author. Dipartimento Farmaceutico. Università degli Studi di Parma, Viale G.P. Usberti 27/A, 43125, Parma, Italy. Tel.: +39 0521905086; fax: +39 0521905006.

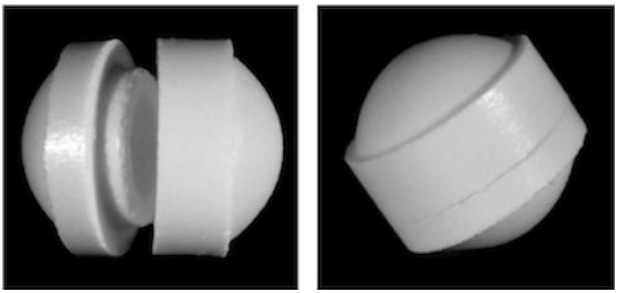
E-mail address: Fabio.sonvico@unipr.it

Abstract

The aim of this research was to design and study norfloxacin (NFX) release in floating coditions from compressed hydrophilic matrices of hydroxypropylmethylcellulose (HPMC) or poly(ethylene oxide) (PEO). Module assembling technology for drug delivery system manufacturing was used. Two differently cylindrical base curved matrix/modules, identified as female and male, were assembled in void configuration by friction interlocking their concave bases obtaining a floating release system. Drug release and flotation behavior of this assembly was investigated. Due to the higher surface area exposed to the release medium, faster release was observed for individual modules compared to their assembled configuration, independently on the polymer used and concentration. The release curves analyzed using the Korsmeyer exponential equation and Peppas & Sahlin binomial equation showed that the drug release was controlled both by drug diffusion and polymer relaxation or erosion mechanisms. However, convective transport was predominant with PEO and at low content of polymers. NFX release from PEO polymeric matrix was more erosion dependent than HPMC. The assembled systems were able to float in vitro for up to 240 min, indicating that this drug delivery system of norfloxacin could provide gastro-retentive site-specific release for increasing norfloxacin bioavailability.

Keywords
Norfloxacin; Dome Matrix®; Release Modules; Floating dosage form

Graphical Abstract



1. Introduction

Modified-release formulations are valuable developments in pharmaceutical industry since, if compared to the new drug application expenses, product innovation remains affordable. Among the different approaches adopted for oral prolonged-release dosage forms, hydrophilic matrices are the most used delivery systems, due to the simple technology and manufacturing [1-4].

An innovative drug delivery platform based on hydrophilic matrices, named module-assembling technology (Dome Matrix®), has been presented [5]. In this technology, release modules made as swellable polymeric matrices are fixed together in a firm structure forming the drug delivery system. The individual module in its typical shape is a cylindrical tablet having one concave and one convex base, designed for allowing their assembling by inserting the convex into concave base. In dependence on modules assemblage, different system configurations can be made. Piled configurations are obtained by stacking two or more modules convex base into concave base. A peculiar assembly obtained by sticking the concave base of one module to the concave base of another module made feasible the construction of floating systems intended to keep the drug release into the stomach. This configuration, named "void", is characterized by an inner empty space that makes buoyant the assembly [6]. Referring to the module assembling for delivery system manufacturing, two differently shaped matrix/modules, identified as female and male, have been constructed. The friction interlocking of the complementary concave bases of these modules drives their assemblage in void configuration.

Using this technology, the individual dose administered can be easily adjusted or, if the composition of modules is different, multiple release kinetics can be achieved. In addition, module assemblage can allow the delivery of two drugs in a single unit at a specific time and at a proper rate and duration, characterizing the flexibility of the Dome Matrix® technology [7].

Norfloxacin (NFX) is a synthetic broad-spectrum antibacterial drug firstly selected for the treatment of diseases caused by *Campylobacter, E. coli, Salmonella, Shigella and V. cholera* [8, 9]. The drug is mainly used for the treatment of urinary tract infections. [8]. Development of bacterial resistance to currently available antibiotics, due to the lack of patient compliance, suggests an appropriate dosing of quinolonic drugs [10-14]. NFX is very slightly soluble in water;

however, its solubility increases sharply at pH below 4.0 and above 10.0, due to the amphoteric nature of the drug [15, 16]. The recommended dosage is usually 400 mg twice daily. The half-life of NFX in serum and plasma is 3-4 hours; only approximately 30-40% of an oral dose is absorbed and the fecal recovery accounts for 30% of the administered dose [15].

Since NFX is more soluble in acidic media and better absorbed from the upper part of the gastrointestinal tract, a prolonged gastric residence of dose form is expected to lead to an increased dissolution rate. Dome Matrix® modules assembled in void configuration floated for up to 4 hrs on gastric content (6). Based on the gastro-retention evidence, the development of a gastro-retentive site-specific drug delivery system of NFX could improve the drug bioavailability and simplify the administration schedule. This would favor the patient convenience and compliance and result in less erratic absorption, hampering bacterial resistance development.

Thus, the aim of this work was to design and study a floating norfloxacin prolonged delivery system manufactured with Dome Matrix® modules. In this paper, the formulations and the performances of the NFX non-assembled modules made with two different polymers (HPMC and PEO) at two concentrations (20 and 30 % w/w) and the corresponding void configurations have been examined. In vitro studies on floatation behavior and the release profile of the system have been carried out.

2. Materials and Methods

2.1 Materials

Norfloxacin was purchased from Zhejiang Neo-Dankong Pharmaceutical (Zhejiang, China). Hydroxypropylmethylcellulose (Methocel K4M, viscosity 2% (p/v) solution 4000 mPa s) and poly-(ethylene oxide) (Polyox N60K, MW 2 x 10^6 Da, viscosity 2% (p/v) solution 3060 mPa s) were kindly donated by Colorcon (Gallarate, Italy). Magnesium stearate (Eigemann & Veronelli S.p.A., Milan, Italy), colloidal silicon dioxide (Aerosil®, Evonik Degussa S.p.A., Ravenna, Italy), and talc (A.C.E.F., Fiorenzuola D'Arda, Italy) were pharmacopoeia grade. The compatibility of NFX with excipients was described elsewhere [17]. NaCl anhydrous (A.C.E.F., Fiorenzuola

D'Arda, Italy), HCl 37% and NaOH anhydrous (Carlo Erba S.p.A., Milan, Italy) were used to prepare the simulated gastric fluid.

2.2 Methods

2.2.1 Matrix modules preparation

NFX, hydrophilic polymer and talc were blended in a Turbula® mixer (WAB, Basel, Switzerland) for 15 min, followed by the addition of magnesium stearate and colloidal silicon dioxide with a further 5 minutes mixing. The modules composition is reported in Table 1. Male and female modules were prepared by direct compression in a single punch tableting machine (EKO Korsch, Berlin, Germany) equipped with special sets of cylindrical punches of 7.4 mm diameter, having the tip surface concave or convex. In the assembling procedure, one male and one female module were manually interlocked concave-to-concave bases to give rise to a void configuration system (Figure 1).

Table 1. Composition of the norfloxacin male and female modules

Composition	Polymer 20% mg	Polymer 30% mg
Norfloxacin	100	100
HPMC K4M or PEO N60K	20	30
Talc	4.8	5.2
Magnesium stearate	1.2	1.3
Colloidal silicon dioxide	0.6	0.7
Total weight	126.6	137.2

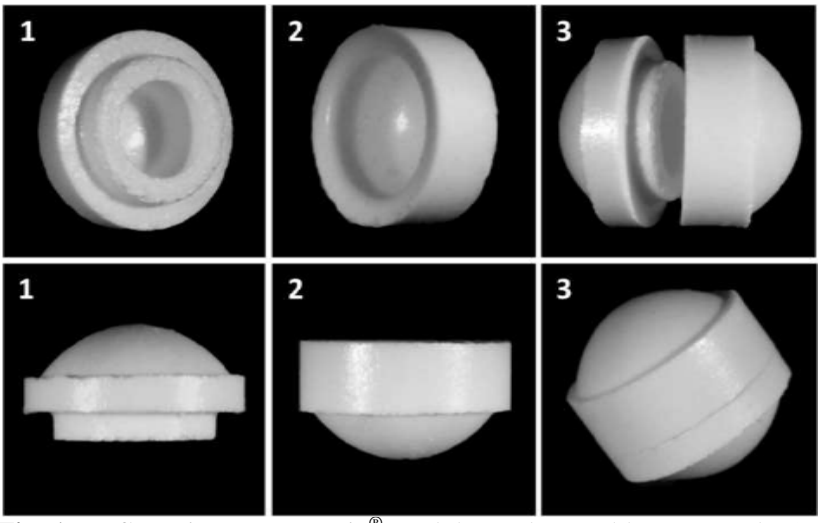


Fig. 1 Norfloxacin Dome Matrix® modules and assemblage: 1- Male. 2- Female. 3- Void configuration assembled modules.

2.2.2 In vitro drug release

Drug release studies were performed using USP apparatus II (Erweka DT6R, Heusenstamm, Germany) with paddle rotation of 50 rpm, in 900 ml of simulated gastric fluid without pepsin (USP 29) at 37.0±0.5 °C. At specified time intervals, 5 mL samples were withdrawn, filtered and quantified by a validated UV spectrophotometric method (Jasco V530, Tokyo, Japan) at the wavelength 278 nm.

Drug release data were analyzed according to Korsmeyer Eq. (1) [18] and Peppas and Sahlin Eq. (2) [19]:

$$\frac{M_t}{M_\infty} = kt^n \tag{1}$$

$$\frac{M_t}{M_\infty} = k_d t^m + k_r t^{2m} \tag{2}$$

where M_t/M_∞ is the fraction of drug released, k is the kinetic constants characteristic of the drug/polymer combination, n is the diffusional exponent for drug release, k_d and k_r are diffusion and relaxation rate constants, respectively, and m is the purely Fickian diffusion exponent for a device of any geometrical shape that exhibits controlled release. The value of 0.425 was used in this analysis according to the aspect ratio of the matrix. These mathematical models are capable of describing the solute release kinetics and mechanism from polymeric hydrophilic matrices and were used to fit release fractions in the range of 5–60%. The Peppas and Sahlin equation allows the calculation of the fraction of drug released due to Fickian mechanism, F, as in Eq. (3):

$$F = \frac{1}{1+\frac{k_r}{k_d}t^m} \tag{3}$$

Release curves were compared using difference factor $f1$ and similarity factor $f2$, calculated by Eq. (4) and (5), respectively [20, 21]:

$$f1 = \left\{ \sum_{i=1}^{n} (Rt - Tt) / \sum_{i=1}^{n} Rt \right\}.100 \tag{4}$$

$$f2 = 50.\log\left\{ \left[1+\left(\frac{1}{n}\right)\sum_{i=1}^{n}(Rt-Tt)^2 \right]^{-0.5}.100 \right\} \tag{5}$$

where Rt and Tt are the percentages released at each time point. $f1$ value up to 15 (0-15) and $f2$ value between 50 and 100 implies similarity between two release profiles.

2.2.3 Floatation behavior

The flotation characteristics of the void assembled modules were assessed using an apparatus constructed according to Timmermans and Moes [22]. A digital camera pictured the swollen system during the

immersion in the medium for determining the volume of the floating object. The resultant-force due to the sinking of the system in simulated gastric fluid without enzyme (USP 29) at 37 ± 0.5 °C was measured during time by the weighing part of the apparatus (precision 0.1 mg). The resultant-force is the difference between the buoyancy and gravity forces both acting on the system submerged in water, according to the following equation Eq. (6):

$$F_{result} = (d_f - d_s)\, g.V \qquad (6)$$

where F_{result} is the resultant force, d_f is the density of the medium in which the object is sunk, d_s is the apparent density of the solid, g is the gravity acceleration and V the volume of the object. This force was measured as weight by the balance inserted in the apparatus.

3. Results and discussion

3.1 In vitro drug release

The modules have been designed to allow their assemblage by interlocking the curved bases of disc. Two differently shaped Dome Matrix® modules were manufactured in this study to obtain NFX prolonged release floating drug delivery systems i.e., a "female" module and "male" module (Figure 1). In particular, as the picture shows, the protrusion on the concave base rim of male module , was designed in order to fit the concavity on the concave base of the module without protrusion on the rim, i.e., female. Thus, facing the concave base of male module to the concave base of the female one and exerting a light pressure, the two modules interlock by clicking giving rise to one-piece assembled system characterized by the presence of an empty internal space.

The norfloxacin release profiles of the Dome Matrix® modules and their assemblies are shown in Figures 2-5. The release of NFX observed for the female modules, independently on the polymer used and its concentration, was faster compared to the male modules. However, the curves were found pretty similar based on the difference ($f1$) and similarity ($f2$) parameters calculated (Table 2). This difference between the two shapes of modules was already observed with other

drugs having different solubility and formulated as hydrophilic matrices with HPMC [7, 23]. Moreover, even when inert polymers or compounds were used to obtain the modules (such as Tapioca starch derivatives [23]), the female module showed a faster release profile than the male. Apart the different initial surface area, the release difference has to be also assigned to the swelling kinetics of the two modules. The male and female modules have different concavity size due to the protrusion on the rim of male concave base. We observed that the swelling determined the fill-up with jellified polymer of the concavity of the male module but not of the female module that has a larger concavity. As a consequence, the female module erodes/dissolves more quickly than the male one. Finally, the individual modules exhibited no floatation.

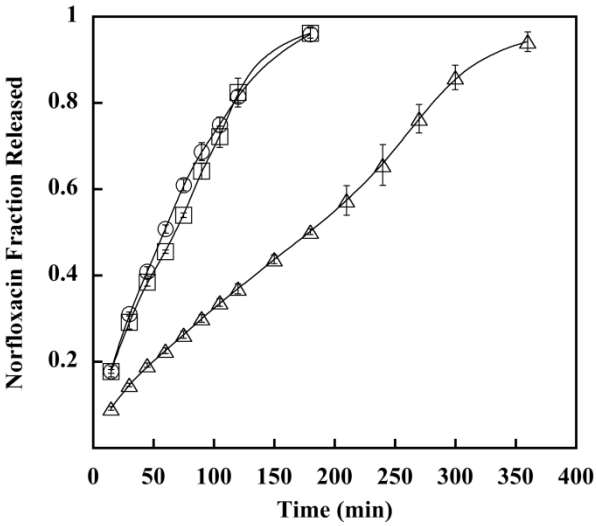


Fig. 2 Norfloxacin fraction released vs. time of Dome Matrix® modules containing 20% of HPMC: female module (O); male module (□); void configuration (△) (mean values ± standard deviation, n=6)

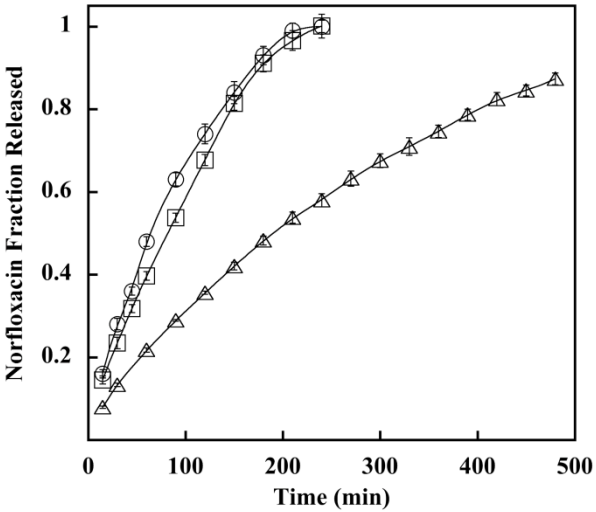


Fig. 3 Norfloxacin fraction released vs. time of Dome Matrix® modules containing 30% of HPMC: female module (O); male module (□); void configuration (△) (mean values ± standard deviation, n=6)

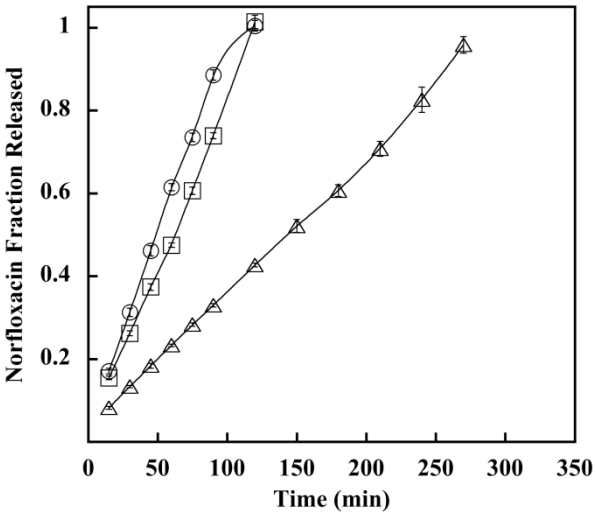


Fig. 4 Norfloxacin fraction released vs. time of Dome Matrix® modules containing 20% of PEO: female module (O); male module (□); void configuration (△) (mean values ± standard deviation, n=6)

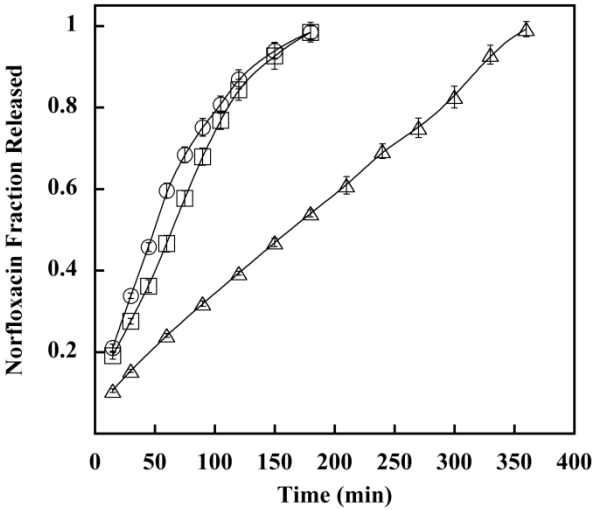


Fig. 5 Norfloxacin fraction released vs. time of Dome Matrix® modules containing 30% of PEO: female module (O); male module (□); void configuration (△) (mean values ± standard deviation, n=6)

Table 2. Difference factor ($f1$) and similarity factor ($f2$) calculated for the male and female modules

Formulation	$f1$	$f2$
HPMC K4M 20%	4.84	71.41
HPMC K4M 30%	6.37	64.96
PEO N60K 20%	13.86	50.18
PEO N60K 30%	7.54	58.54

The release studies of the void configuration demonstrated that NFX release rate was significantly slowed down in comparison to the release of individual modules, independently on the polymer and concentration. The void assembled modules containing 20% HPMC, released about 80% of drug in 270 min maintaining the floatation up to 240 min (Figure 2). In correspondence of this last time, assembled modules disintegrated completely with the consequent impairment of floatation capacity. This was evidently reflected by the release profile that in between 200-250 minutes had a sudden increase of fraction

release rate. The significant increase in the NFX release values standard deviations after 200 minutes indicates the variability of the void configuration disintegration time. The floating capability of the Dome Matrix® in void configuration was already studied in humans, showing that the system remained in the stomach after a light standard meal for 214.5 ± 54.2 min [6]. The prolonged gastric residence could be beneficial for NFX absorption that due to the favored dissolution in acid can be quickly absorbed in stomach or in the first intestinal tract [24, 25].

The assembled modules containing 30% HPMC had a different performance in terms of release and floatation. The void configuration system remained floating until 480 minutes, the end of the release experiment (Figure 3). At 210 min, which corresponded to the average time of void configuration gastro-residence, about 55% of NFX was released. The 80% release was achieved at about 400 min; at this time, the system could already be eliminated from the stomach.

High molecular weight poly-(ethylene oxide) have been proposed as an alternative to HPMC. The correct choice of this hydrophilic polymer molecular weight and quantity in the matrix formulation can provide an appropriate combination of swelling, dissolution or erosion mechanisms to control drug release kinetics [26-29]. For the formulations containing PEO, the drug release rate of individual modules was faster than the correspondent modules made with HPMC. The same was also observed with the void configurations in comparison to the release of the HPMC assembled modules (Figures 4 and 5).

However, similarly to HPMC system, the void assembled modules made with 20% PEO maintained the floating characteristic up to 240 min. After this time, assembled systems completely disintegrated with impairment of floating capacity. Despite the different polymer, also with this polymer the disintegration of the assembled system was reflected by an evident slope increase in the profile fraction released versus time after 200 min (Figure 4). Similarly, the increase in standard deviation of NFX release values due to the variability of disintegration time can be observed. The floatation time corresponded to about 80% NFX released.

Compared to HPMC composition, the Dome Matrix® modules containing 30% PEO (Figure 5) showed faster release rate for both the modules than for the assembly. The floating time was prolonged until

330 min by the higher polymer concentration. At 210 min, about 60% of NFX was released and the 80% release was achieved at about 300 min.

3.2 Analysis of drug release

The release profiles were analyzed with the Korsmeyer diffusional exponential equation up to 60% of drug released [18]. Release data analysis was carried out also with the Peppas and Sahlin equation in order to calculate the Fickian fraction released of each profile [19]. Parameter values are listed in Table 3. Calculation of the exponent n identifies the prevalent mechanism of release. For planar systems, n = 0.5 indicates diffusion-controlled (Fickian) drug release and n = 1.0 indicates swelling/erosion-controlled drug release (Case-II transport). Values of n between 0.5 and 1.0 can be regarded as a superposition of both phenomena, indicating that the drug delivery was not controlled only by diffusion, but also significantly by polymer relaxation or erosion mechanisms (anomalous transport). In general, the data of all release systems studied provided good fit to the different models (Table 3), supporting the adaptability of both models to fit the release data with the systems and polymers studied.

Table 3. Mathematical modeling and drug release kinetics from Dome Matrix® modules using Korsmeyer et. al and Peppas and Sahlin equations (mean values ± SD; n=6).

Modules	Korsmeyer et. al equation		Peppas and Sahlin equation		
	n ± 95% CI	r^2	$k_d \times 10^3$	$k_r \times 10^3$	r^2
HPMC K4M 20%					
Male	0.686 ± 0.044	0.9994	29 ± 7.6	9.1 ± 1.2	0.9992
Female	0.758 ± 0.021	0.9996	17 ± 3.6	13 ± 0.8	0.9995
Void	0.688 ± 0.011	0.9986	14 ± 3.4	4.5 ± 0.5	0.9996
HPMC K4M 30%					
Male	0.767 ± 0.040	0.9983	16 ± 6.9	9.4 ± 1.3	0.9999
Female	0.732 ± 0.049	0.9998	15 ± 5.6	12 ± 1.2	0.9981
Void	0.718 ± 0.014	0.9998	14 ± 1.8	4.2 ± 0.3	0.9995
POE N60K 20%					
Male	0.841 ± 0.025	0.9977	-4.8 ± 3.4	16 ± 0.7	0.9979
Female	0.921 ± 0.029	0.9995	-17 ± 5.2	22 ± 1.0	0.9993
Void	0.816 ± 0.014	0.9993	-0.4 ± 2.0	7.4 ± 0.3	0.9997
POE N60K 30%					
Male	0.679 ± 0.015	0.9911	18 ± 3.4	11 ± 0.7	0.9940
Female	0.742 ± 0.024	0.9975	15 ± 6.1	15 ± 1.3	0.9983
Void	0.670 ± 0.013	0.9982	15 ± 1.4	4.9 ± 0.2	0.9996

In the case of HPMC modules, the n values from Korsmeyer equation were in the range 0.686 - 0.767 indicating anomalous (non-Fickian) transport as evidenced by the quasi-linear release profiles [33]. The k_d and k_r values for male and female modules revealed the relevance of drug diffusion or polymer relaxation and erosion. Calculations showed that the fraction of drug released due to Fickian mechanism was the lowest for the female module compared to the male module and void assembly (Figure 6). Fickian fraction released was very similar for formulations containing 20 and 30% of HPMC assembled in void configuration. This result fits well with the similar n exponents obtained with Korsmeyer equation (Table 3). The contribution of Fickian diffusion to NFX release was around 50% for the assembled system at the beginning of the release experiment and tended to decrease with time. The percentages of drug released by

Fickian mechanism for individual modules, were lower than 40% with the exception of the male module containing 20% of HPMC that behaved similarly to the assembled system. Considered that the difference between the assembled system and the individual modules is the non-accessibility of the concave bases to the release medium after the assemblage, it seems straightforward to consider that solvent penetration, chain disentanglement, build-up of gel-layer thickness and erosion of the matrix occurred differently affecting the balance between Fickian and relaxation release mechanism. These results corresponded to a relevant anomalous Fickian release typical of swellable matrices due to an important contribution of the polymer swelling and erosion mechanisms to drug delivery.

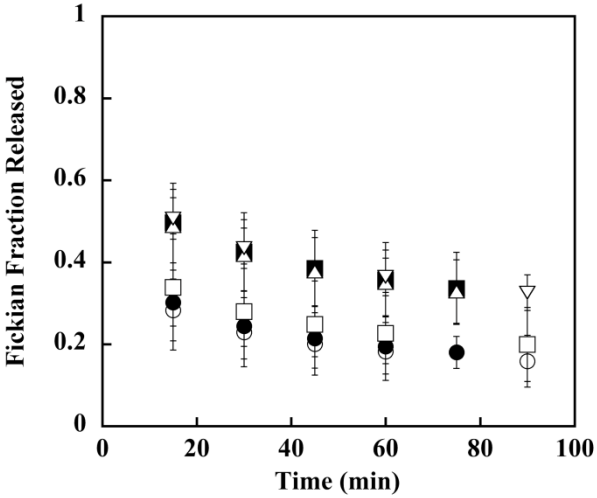


Fig. 6 Norfloxacin Fickian released fraction vs. time of Dome Matrix® modules containing 20% of HPMC: female module (●); male module (■); void configuration (△) and 30% of HPMC: female module (○); male module (□); void configuration (▽) (mean values ± standard deviation, n=6)

In the case of PEO polymer, n diffusional exponents for the PEO concentration of 20% were in the range 0.816 - 0.921, indicating a release mechanism very close to Case II transport. The negative values of k_d and the high values of k_r in Peppas and Sahlin equation indicate

that the drug release is predominantly controlled by polymer relaxation or erosion [30]. Due to the negative values of k_d obtained, the Fickian release fraction was not calculated in this case. Likely, the PEO gel layer was weaker in comparison to HPMC and could be more rapidly removed by the dissolution medium; therefore, NFX/PEO matrix system is more susceptible to the erosion process. In general, PEO polymer is considered more soluble than HPMC, but, its molecular weight and amount in the formulation allow tailoring the drug release kinetics [26, 27, 29, 31].

For the 30% PEO concentration, the diffusional exponents between 0.670 - 0.742 indicated anomalous Fickian transport profiles [32, 33]. The similarity between k_d and k_r values revealed that the drug release was controlled by drug diffusion and polymer relaxation and erosion mechanisms. Figure 7 showed that also in this case the fraction of drug released due to Fickian mechanism was the lowest for the female module. Fickian fraction release values for void configuration were very similar to those obtained for 20 and 30% of HPMC assembled in the same configuration, confirming also the n diffusional exponents obtained (Table 3). As observed for HPMC, the NFX release due to Fickian contribution initially was about 50% and decreased with time (Figure 7). It was impressive to see the linearity of the release profiles obtained with this polymer considered that a quasi-linear dissolution profile (Fig. 4 and Fig. 5) could be observed up to 80% of NFX released.

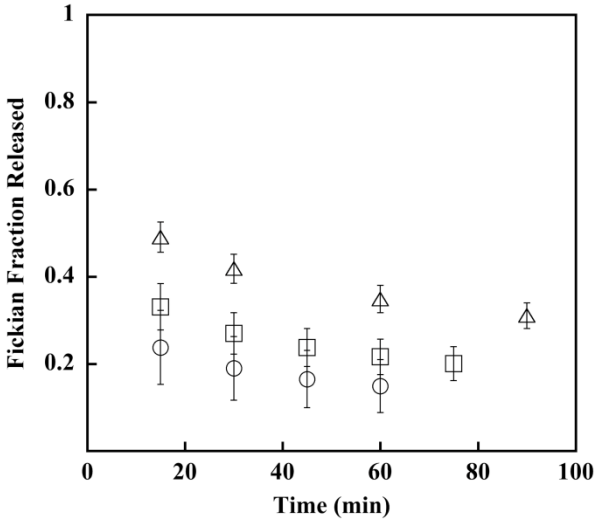


Fig. 7 Norfloxacin Fickian released fraction vs. time of Dome Matrix® modules containing 30% of PEO: female module (O); male module (□); void configuration (△) (mean values ± standard deviation, n=6)

3.3 In vitro floatation behavior of assembled modules

Originally, the dome-shaped modules were designed for facilitating their assembly by insertion of the convex base into the concave in the aim to build up a pile. The possibility to proceed to a different module assembly was discovered later when glue or ultrasounds were used to firmly attach the modules by creating links between the flat surfaces of the concave base rims held in contact. In this study, two new types of dome modules described elsewhere [6], having the rim of concavity modified in order to favor a firm concave-to-concave assembly by simple interlocking were used (see Figure 1). The floatation behavior of the void assembled NFX system, made of two swellable modules, was studied in vitro. In particular, it was determined the force required to hold the system submerged in water and its variation over time, according to [6, 22]. The individual male and female modules never floated.

Based on the floating time observed in the dissolution profiles, two formulations (20% HPMC and 20% PEO) were selected for this study. The floatation behavior, expressed as resultant-weight variation versus time, is shown in Figure 8. The profiles show that the systems did

not float immediately but it started to float between 5-10 min after submersion in medium; at 10 min a positive resultant-weight of approximately 4 and 2 mg was measured for PEO and HPMC, respectively. Then, the resultant-weight values increased attaining peak values of approximately 42 mg for HPMC and 45 mg for PEO, both at 125 min. After this peak, the resultant-weight had a continuous slow decrease for HPMC, while an abrupt decrease was observed for PEO. This PEO profile could anticipate the beginning of disintegration system manifested by a partial exit of air bubbles from the inside of the void module. The buoyancy variation during time can be explained as the volume and weight change of the immersed system due to water uptake and swelling. Therefore, the augmentation of resultant-weight over time was due to the swelling of the system, which reinforced the capability of the system to float. The gastric residence time demonstrated in vivo (214.5 ± 54.2 min) was derived from an experiment after a standard meal without any additional food [6]. In case where a prolonged gastric residence time is requested and the food intake after drug administration is allowed, a more durable Dome Matrix® system could be constructed. In this case, formulations containing higher amount of polymers (HPMC or PEO) should be studied.

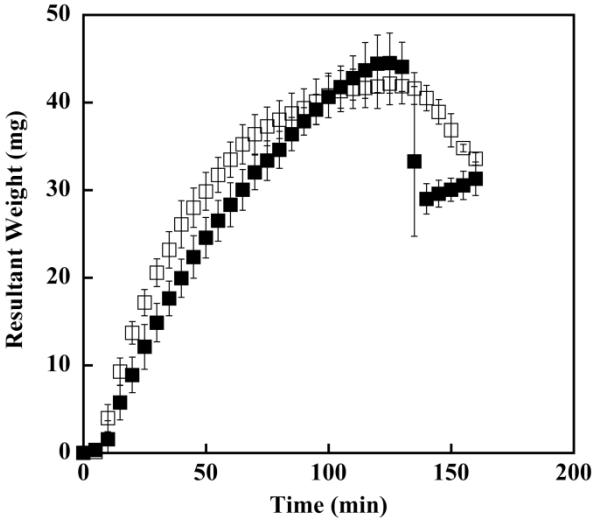


Fig. 8 Resultant weight vs. time of Dome Matrix® 20% HPMC K4M (□) and 20% PEO N60K (■) modules assembled in void configuration (mean values ± standard deviation, n=3)

4. Conclusion

This study revealed a prolonged linear release of norfloxacin in simulated gastric fluid when the drug is formulated in Dome Matrix® modules with hydroxypropylmethylcellulose but, in particular, with poly(ethylene oxide). Two modules, "male and female" have been manufactured and were interlocked to form the "void" configuration. This assembly exhibited in vitro floatation up to 240 min, while the individual male and female modules never floated. The mechanism of NFX release from Dome Matrix® modules and void assemblies was mainly governed by the swelling and erosion of the polymer matrix. The diffusive contribution to drug release was lower than 50% and decreased during release time. In conclusion, a site-specific prolonged drug delivery system of norfloxacin was achieved when the modules were assembled in the void configuration; the release kinetics was strongly linear, independently on floating conditions. However, in vivo studies are necessary to confirm the possibility for this linear release gastro-retentive system to increase norfloxacin bioavailability and reduce dose administration.

Acknowledgments

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DISCUSSÃO GERAL

O norfloxacino é um agente antibacteriano amplamente utilizado para o tratamento de infecções no trato urinário. Normalmente a posologia indicada é de 400 mg duas vezes ao dia (MANDELL, 1988; CHRISTIAN, 1996; EMMERSON; JONES, 2003) e não se encontra disponível comercialmente formulação de liberação prolongada, para administração em dose única diária. Para o desenvolvimento racional de medicamentos, as etapas de caracterização das matérias-primas e estudos de pré-formulação são de fundamental importância, pois através da investigação da compatibilidade entre fármaco-excipiente é possível selecionar excipientes adequados para proceder com o desenvolvimento farmacotécnico (BRUNI et al., 2002; GIRON et al., 2002; BRUNI et al., 2010).

A caracterização do norfloxacino e excipientes, bem como os estudos de compatibilidade, foram apresentados na forma de publicação científica no **capítulo 2**. Através de DSC pode-se avaliar que o fármaco apresenta ponto de fusão de aproximadamente 219 ºC, seguido de evento exotérmico, sem perda de massa (observado por TG). Como não há evento endotérmico antes da fusão, pode-se inferir que a matéria-prima é a forma anidra e não dihidrato ou sesquihidrato. A degradação do fármaco ocorre em duas etapas sucessivas em temperaturas entre 330–376 ºC e 421–455 ºC. Através das imagens obtidas por SEM e dos padrões de XRPD demonstrou-se alto grau de cristalinidade para a amostra, o que é importante para o desenvolvimento de comprimidos, umas vez que substâncias amorfas tendem a ser menos estáveis em relação às cristalinas. O polímero hidrofílico POE também demonstrou características cristalinas, com reflexões bem definidas, e a HPMC mostrou-se amorfa.

O norfloxacino apresenta três formas anidras (A, B e C) e suas temperaturas de fusão são 219 ºC (A), 212 ºC (B) e 207 ºC (C) (BARBAS; PROHENS; PUIGJANER, 2007). A estrutura cristalina da forma A foi determinada por difração de raio-x de monocristal, sendo triclínica, grupo espacial *P*-1 (BASAVOJU, S.; BOSTRÖM, D.; VELAGA, 2006; PUIGJANER et al., 2010). Baseando-se principalmente no ponto de fusão (219 ºC) e no perfil termoanalítico pode-se inferir que a forma utilizada nesta tese é a forma A.

Para o estudo de compatibilidade, uma vez que não se encontram relatos de interação/incompatibilidade do norfloxacino, selecionaram-se os excipientes farmacêuticos que possivelmente comporiam a formulação final. Utilizou-se mistura física fármaco-excipiente na proporção 1:1 (p/p) e analisou-se através de DSC. As

curvas obtidas foram consideradas como superposição das curvas individuais dos compostos da mistura, não sendo observados deslocamentos ou desaparecimento do ponto de fusão do norfloxacino, demonstrando ausência de incompatibilidade.

A cromatografia líquida possibilita a separação e quantificação de diferentes componentes de uma formulação farmacêutica através da escolha adequada dos parâmetros do sistema como colunas, fase móvel e método de detecção. Apesar de existir método farmacopeico para comprimidos de norfloxacino de liberação imediata (F. BRAS. IV, 2001), este requer um tempo de estabilização da coluna analítica por 8 horas e não pode ser utilizado para estudos de estabilidade sem prévia avaliação. Além disso, como se trata de nova formulação, é necessário método analítico adequado. Desta maneira, desenvolveu-se e validou-se procedimento para a determinação de norfloxacino nos comprimidos matriciais conforme demonstrado no **capítulo 3**. A metodologia consistiu na utilização de coluna C_{18} mantida a 40 ºC, detecção no UV a 272 nm, fase móvel composta por tampão fosfato 0,04 M (pH 3,0) e acetonitrila na proporção 84:16 (v/v), com vazão de 1,0 mL/min.

Na avaliação da especificidade foi possível a identificação de um composto de degradação, o norfloxacino descarboxilado. Além disso, nos cromatogramas obtidos, os picos dos produtos de degradação encontraram-se resolvidos em relação ao pico do norfloxacino, demonstrando a especificidade e que o procedimento também pode ser usado como indicativo da estabilidade. O método apresentou-se linear na faixa de 0,5-5 μg/mL (r^2 = 0,9999). Os dados obtidos para a repetibilidade (DRP $\leq$ 0,98), precisão intermediária (DRP $\leq$ 0,59) e exatidão (99,90% ± 0,97%) estão dentro dos valores preconizados. A avaliação da robustez através da superfície de resposta demonstrou que mudanças pequenas e deliberadas em diversos fatores não influenciaram significativamente a metodologia. Desta forma, demonstrou-se que o método proposto cumpre os requisitos preconizados pela literatura, podendo ser empregado para a análise pretendida.

O desenvolvimento, estudos de estabilidade e avaliação in vitro de formulações com tecnologia de liberação prolongada contendo norfloxacino foi apresentado como publicação científica no **capítulo 4**. A partir do estudo de pré-formulação, além dos polímeros hidrofílicos POE e HPMC, foram selecionados como excipientes a celulose micro-cristalina (diluente), o estearato de magnésio (lubrificante) e o dióxido de silício coloidal (deslizante, absorvente).

Para o desenvolvimento das formulações avaliou-se o processo de granulação via seco e via úmida, porém não se observou diferença entre estes e a compressão direta na obtenção dos comprimidos. Dessa forma, optou-se pelo processo de compressão direta, devido a maior simplicidade e rapidez. Devido ao fato da liberação prolongada ser obtida através dos polímeros hidrofílicos, foram avaliados dois tipos (POE e HPMC) de diversas massas moleculares e duas concentrações (20 e 30%) nas formulações. Os valores de concentração foram selecionados pois estão na faixa normalmente utilizada na indústria farmacêutica para a fabricação de comprimidos de liberação prolongada. Dessa forma a obtenção dos comprimidos por compressão direta e a quantia de polímero na formulação levaram em conta uma possível transposição para escala industrial.

O equivalente a 50% do total de comprimidos produzidos foi submetido ao processo de revestimento. Para estudar a influencia da presença de blister, realizou-se a emblistagem de comprimidos revestidos e não revestidos. A avaliação da qualidade foi determinada segundo as características farmacopéicas de variação de peso, dureza, friabilidade e perda por dessecação e os resultados obtidos estão de acordo com os valores preconizados.

O estudo de estabilidade acelerada, realizado em condições recomendadas para zona climática IV (40 ± 2 ºC e 75 ± 5% umidade relativa) por 6 meses (BRASIL 2005, WHO, 2009) demonstrou que não houve alteração na aparência ou teor de principio ativo. Porém, na avaliação da fotoestabilidade, foi evidenciada a necessidade do revestimento dos comprimidos ou a presença de blister opaco, uma vez que houve alteração na coloração dos comprimidos.

Os perfis de dissolução foram realizados utilizando método baseado nas farmacopéias brasileira e americana, utilizando tampão pH 4 (F. BRAS. IV, 2001; USP 30, 2007). Os resultados in vitro demonstraram que conforme aumentava-se a massa molecular e a concentração do polímero hidrofílico, a liberação do fármaco tornava-se mais lenta, inclusive com algumas formulações apresentando incompleta dissolução após 24 horas.

Através da análise dos perfis pela equação de Korsmeyer-Peppas (KORSMEYER et al., 1983) pode-se concluir que a liberação do norfloxacino foi controlada pela difusão do fármaco e intumescimentos/erosão do sistema matricial. Quando o comprimido continha menor concentração e/ou menor massa molecular do polímero o mecanismo de liberação predominante, indicado pelo valor do

expoente $n \geq 0,89$, era o Caso-II ou Super Caso-II (intumescimento do polímero, relaxamento da matriz ou liberação mediante erosão). Quando aumentava-se a massa molecular e/ou a concentração de polímero na matriz o mecanismo de liberação mudava para transporte anômalo ($0,45 < n > 0,89$), mostrando crescente influência da difusão do fármaco na dissolução.

As formulações contendo POE ou HPMC de massas moleculares intermediárias (HPMC K4M 20%, POE N60K 20% e POE N60K 30%) ou alta concentração de baixa massa molecular (HPMC K100 LV 30%) foram consideradas mais promissoras para futuros testes in vivo, pois resultaram em combinação entre dissolução total do norfloxacino e estrutura da camada gelatinosa formada. Baseado na liberação prolongada e total do fármaco, uma melhor biodisponibilidade é esperada. Para melhor compreensão e correlação com o que poderá ocorrer in vivo, estudos em diferentes pHs ou em fluidos gástrico e intestinal simulados estão sendo planejados como continuação desta tese. Dependendo dos resultados obtidos as formulações poderão ser otimizadas, inclusive com a possibilidade da utilização de polímeros de diferentes massas moleculares na mesma formulação para obtenção do perfil de liberação adequado.

Além da fabricação de sistemas matriciais utilizando compressoras com punções convencionais, a inovação tecnológica permite a renovação e a otimização destas técnicas clássicas. Um exemplo disso foi o desenvolvimento dos sistemas de acoplamento Dome Matrix®. Baseado nesta tecnologia, foi estudada a possibilidade de obter sistema flutuante para liberação prolongada e local específica de norfloxacino, conforme descrito no **capítulo 5**. Utilizou-se duas concentrações, 20 e 30% de HPMC e, pela primeira vez, POE. Módulos individuais "male" e "female" foram obtidos por compressão direta utilizando punções especiais (LOSI et al., 2006). O acoplamento destes permitiu a obtenção da configuração flutuante "void", devido a presença de ar no interior do sistema.

Além das formulações demonstradas neste capítulo (contendo HPMC K4M e POE N60K, ambos a 20 e 30%) foram produzidas outras (contendo 20 e 30%) de: HPMC K100 LV, HPMC K15M, POE N12K e POE 301. Para os polímeros de baixa massa molecular (HPMC K100 LV e POE N12K) obteve-se dissolução in vitro muito rápida e pouco tempo de flutuação. Ao contrário, com os polímeros de massa molecular mais alta (HPMC K15M e POE 301) a velocidade de dissolução era demasiada lenta, tanto para os módulos individuais como para o sistema

flutuante. A dosagem de 200 mg para a configuração "void" é inferior à recomendada normalmente para adultos de 400 mg. Porém, devido a esta tecnologia estar ainda em desenvolvimento, outros punções com capacidade superior (e inferior) de carga estavam sendo desenvolvidos quando da realização desta etapa. Diferentes processos tecnológicos como granulação a seco e via úmida não resultaram em melhora em comparação à compressão direta.

Nos estudos de dissolução observou-se maior velocidade de liberação do norfloxacino nos módulos individuais em relação à configuração flutuante, o que pode ser explicado devido à maior área superficial em contato com o meio. Esta característica já havia sido observada com outros fármacos de diferentes solubilidades e formulados como matrizes hidrofílicas (CASAS et al., 2010; STRUSI et al., 2010) Os perfis de dissolução analisados de acordo com as equações de Korsmeyer-Peppas e Peppas-Sahlin demonstraram que a liberação era controlada tanto pela difusão do fármaco como pelo intumescimento e erosão da matriz. Da mesma forma que para os comprimidos descritos no capítulo 4, os sistemas Dome Matrix® fabricados com POE e menor concentração de polímero apresentam mecanismo de liberação mais dependente da erosão, o que pode ser evidenciado pelos perfis de dissolução quase lineares e nos maiores valores do expoente *n* obtidos.
A HPMC K4M apresenta uma viscosidade média de 4000 mPa s (2%; p/v) que pode ser considerada semelhante a do POE N60K (3060 mPa s, 2%, p/v). Dessa forma fica evidente o caráter mais erodível do POE em relação à HPMC, o que está de acordo com o descrito por Jamzad e Fassihi (2006). A contribuição da difusão de fick para a liberação do norfloxacino era inicialmente menor que 50% e diminuía de acordo com o tempo.

Os módulos na configuração "void" iniciaram a flutuar em menos de 10 minutos e mantiveram esta capacidade por pelo menos 240 minutos, o que está de acordo com o tempo médio de permanência estudado in vivo de 214, 5 ± 54,2 minutos (STRUSI et al., 2008). Com o aumento da concentração de polímero na formulação, aumenta-se a capacidade flutuante do sistema. A flutuação in vitro obtida sugere um sistema gastro-retentivo in vivo, o que poderia aumentar a biodisponibilidade do norfloxacino, uma vez que este fármaco é mais solúvel em meios ácidos, com conseqüente redução da posologia.

CONCLUSÕES

- ✓ As matérias-primas de norfloxacino e polímeros (HPMC e POE) foram caracterizadas e os resultados obtidos podem ser utilizados como parâmetros de controle de qualidade para futuros estudos;

- ✓ Estudos de pré-formulação foram realizados e não observou-se incompatibilidade entre fármaco e excipientes;

- ✓ O método desenvolvido e validado, por cromatografia líquida em fase reversa, foi utilizado para análise de norfloxacino nas matrizes desenvolvidas e estudos de estabilidade;

- ✓ Comprimidos matriciais foram obtidos por compressão direta e cumpriram com as características farmacopéicas preconizadas;

- ✓ O processo de revestimento tem influencia sobre o perfil de dissolução dos comprimidos, porém esta não foi considerada biofarmaceuticamente relevante;

- ✓ Os estudos de estabilidade sugerem que as formulações são estáveis frente à temperatura e umidade, porém o revestimento e/ou presença de blister opaco deve ser recomendado para prevenir a fotodegradação do princípio-ativo;

- ✓ As formulações contendo POE ou HPMC de massas moleculares intermediárias (HPMC K4M 20%, POE N60K 20% e POE N60K 30%) ou alta concentração de baixa massa molecular (HPMC K100 LV 30%) apresentaram melhor perfil de liberação in vitro;

- ✓ O mecanismo de liberação do norfloxacino depende da concentração e massa molecular do polímero utilizado;

- ✓ Sistemas Dome Matrix® acoplados na configuração "void" permitiram flutuação in vitro, sugerindo liberação local específica (gastro-retentiva) in vivo para o fármaco;

- ✓ Estudos in vivo são necessários para confirmar a possibilidade de aumento na biodisponibilidade e redução do regime posológico do norfloxacino através do uso de sistemas matriciais e Dome Matrix®.

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