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Rafael Silva Nunes

Internal standard for amorphous pharmaceuticals products quantification and an application of parametric Rietveld refinement using time, temperature and relative humidity as 'non-crystallographic' parameters

Padrão interno para quantificação de fármacos amorfos e uma aplicação do refinamento de Rietveld paramétrico usando tempo, temperatura e umidade relativa como parâmetros "não cristalográficos"

Thesis submitted to Institute of Chemistry, of Paulista State University, as part of the requirements for obtaining the PhD degree in Chemistry

Supervisor: Carlos de Oliveira Paiva Santos

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Complementary Education

2013 - 2013	Short Term Course in: Local structure of crystalline materials using PDF. The University of Warwick, Warwick, England Scholarship from: Fundação de Amparo à Pesquisa do Estado de São Paulo
2013 - 2013	Short Term Course: International Workshop on Powder & Electron Crystallography (Protein). University of Patras, Rio, Greece Scholarship from: Fundação de Amparo à Pesquisa do Estado de São Paulo
2012 - 2012	Short Term Course: Powder Diffraction & Rietveld Refinement School. Durham University, Durham, England Scholarship from: Fundação de Amparo à Pesquisa do Estado de São Paulo

2011 - 2011	Short Term Course: 7th TOPAS Course, Bruker Corporation, Bruker, Germany Sydney, Australia Scholarship from: Fundação de Amparo à Pesquisa do Estado de São Paulo
2011 - 2011	Short Term Course in: Aplicações da teoria de grafos à cristalografia. Universidade de São Paulo, Sao Paulo, Brazil
2010 - 2010	Short Term Course: Workshop on Representation Theory of Space Groups. Universidade de la Republica do Uruguai, Montevideo, Uruguay Scholarship from: Fundação de Amparo à Pesquisa do Estado de São Paulo
2010 - 2010	Short Term Course: International School on Fundamental Crystallograph. Universidad de la Republica Uruguay, Montevideo, Uruguay Scholarship from: Fundação de Amparo à Pesquisa do Estado de São Paulo

Professional Experience

Universidade Estadual Paulista Júlio de Mesquita Filho

2011 - 2011	Contract: Bolsista Didático , Position: Bolsista , Working hours (weekly): 4, Schemes of job: Part-time
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Projects

2012	Preparation of a standard material for quantification of amorphous in crystalline drug without knowledge of the crystal structure. Description: Research propose to Brazilian Synchrotron (LNLS), Centro Nacional de Pesquisa em Energia e Materiais (CNPEM). Members: Rafael Silva Nunes, Fabiano Yokaichiya, Margareth Kazuyo Kobayashi Dias Franco, Fabio Furlan Ferreira, Carlos O. Paiva Santos (responsible).

2013	Characterization of compounds with transition metals triethanolamine. Description: Research propose to Brazilian Synchrotron (LNLS), Centro Nacional de Pesquisa em Energia e Materiais (CNPEM). Members: Rafael Silva Nunes, Selma Gutierrez Antonio, Carlos O. Paiva Santos, Stanlei Ivair Klein (responsible).

Other activities

2010 a 2011	Alternate student representative of Board of Departamento de Físico-química, Universidade Estadual Paulista, Araraquara, Brazil.

Scientific production

Papers published in journals

1. Nunes, R. S., Fontoura, A. F. M., Canova, H. F., Yokaichiya, F., Franco, M. K. K. D., Paiva-Santos, Carlos O., Evans, John S. O. TUCANO: *in situ* experiments using x-ray powder diffraction at LNLS with temperature and relative humidity control, Journal of Synchrotron Radiation

2. Nunes, R. S., Evans, J. S. O., Paiva-Santos, C. O. Internal standard for pharmaceuticals products I: amorphous quantification at room temperature, Journal of Applied Crystallography

3. Nunes, R. S., Eliziario, S. A., Paiva-Santos, C. O., Evans, J. S. O. Internal standard for pharmaceuticals products II: *in situ* amorphous quantification, Journal of Applied Crystallography

4. Nunes, R. S., Paiva-Santos, C. O., Evans, J. S. O. Internal standard for pharmaceuticals products III: relative humidity application, Journal of Applied Crystallography

Published works in events

1. Nunes, R. S., Fontoura, A. F. M., Evans, J. S. O., Canova, H. F., Scherer, J. A., Paiva-Santos, C. O., Yokaichiya, F., Franco, M. K. K. D. TUCANO: *in situ* temperature- humidity-controlled experiments using powder X-ray diffraction at LNLS. In: 24th Annual Users Meeting of LNLS/CNPEM, 2014, Campinas, Brazil.

2. Nunes, R. S., Evans, J. S. O., Paiva-Santos, C. O. Internal standard for amorphous quantification of pharmaceuticals. In: 28th European Crystallography Meeting, 2013, Warwick, United Kingdom.

3. Nunes, R. S., Sabino, J. R., Lima, E. C. O., Franco Junior, A. Application of Rietveld analysis to determine cations distribution in cobalt ferrite by X-ray dispersion effects. In: 21st Annual Users Meeting of LNLS/CNPEM, 2011, Campinas, Brazil.

4. Nunes, R. S., Paiva-Santos, C. O. Aplicar ou não aplicar a correção de Brindley? In: I Encontro dos Usuários de Técnicas de Difração da CEM, Santo Andre, Brazil.

5. Nunes, R. S., Sabino, J. R., Lima, E. C. O., Franco Junior, A. Cations distribution in ferrites by Rietveld analysis. In: Australian X-ray Analytical Association Workshops, Conference and Exhibition, 2011, Sydney, Australia.

6. Nunes, R. S., Antonio, S. G., Maia, N., Paiva-Santos, C. O. Lithium carbonate as internal standard. In: Australian X-ray Analytical Association Workshops, Conference and Exhibition, 2011, Sydney, Australia.

7. Nunes, R. S., Antonio, S. G., Maia, N., Paiva-Santos, C. O. Standard material for quantification of amorphous in pharmaceuticals. In: 20th Reunião da Associação Brasileira de Cristalografia, 2011, Campinas, Brazil.

8. Nunes, R. S., Bezzon, V. D. N., Ruiz, M. Inserção do software Mercury no ensino de cristalografia como recurso didático motivador. In: IX Evento de Educação em Química, 2011, Araraquara, Brazil.

9. Nunes, R. S., Sabino, J. R., Lima, E. C. O., Franco Junior, A. Application of Rietveld analysis to determine cations distribution in cobalt ferrite and magnesium ferrite. In: International School on Fundamental Crystallography, 2010, Montevideo, Uruguay.

Presentation of lectures

1. Nunes, R. S., Evans, J. S. O., Paiva-Santos, C. O. Internal standard for amorphous quantification of pharmaceuticals; European Young Crystallographers Satellite Meeting, 2013. University of Warwick, Warwick, United Kingdom.

2. Nunes, R. S. Além das aplicações clássicas da difração de raios x por pó, Seminário Geral, 2012. Universidade Estadual Paulista, UNESP, Araraquara, Brazil.

Participation in events

24th Annual Users Meeting of LNLS/CNPEM, March 11th-12th 2014, Campinas, Brazil.

28th European Crystallography Meeting, August 25th-29th 2013, Warwick, United Kingdom.

1st European Young Crystallographers Satellite Meeting, August 25th 2013, Warwick, United Kingdom.

22nd Annual Users Meeting of LNLS/CNPEM, February 28th-29th 2012, Campinas, Brazil.

I Encontro dos Usuários de Técnicas de Difração da CEM, December 7th-8th 2011, Santo Andre, Brazil.

20th Reunião da Associação Brasileira de Cristalografia, February 24th-25th 2011, Campinas, Brazil.

21st Annual Users Meeting of LNLS/CNPEM, February 22nd-23rd 2011, Campinas, Brazil.

Australian X-ray Analytical Association Workshops, Conference and Exhibition, February 6th-11th 2011, Sydney, Australia.

RAFAEL SILVA NUNES

Tese apresentada ao Instituto de Química, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Química.

Araraquara, 25 de setembro de 2014.

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"Man is the measure of all things: of things which are, that they are,

and of things which are not, that they are not."

Protagoras (490 BC – 420 BC)

Abstract

Recent studies on solid polycrystalline drugs by x-ray powder diffraction (XRPD) in Brazil, has demonstrated how important this technique can be, especially joined to Rietveld method, for structural understanding of these materials. Amorphous material has a higher internal energy than crystalline materials, what alters their therapeutic effects and it makes of amorphous quantification an important study. As an internal standard with international recognition for this purpose, such as NIST SRM-676a (Al_2O_3), can be economically unviable, a systematic characterization, mainly by XRPD, of a cheap material with liner coefficient absorption of same order of this organic products, to use as internal standard for quantitative phase analysis was made. Good results obtained with LiF, as internal standard, for amorphous pharmaceutical quantification at room temperature, shows its potential for a large-scale application (when compared to results obtained using NIST standard SRM-676a, considering microabsorption effect). Other important focus of this work was i*n situ* XRPD applied to pharmaceutical products and had as highlights: a chamber called TUCANO, to use relative humidity in experiments of *in situ* XRPD, developed with cooperation of Brazilian Synchrotron Light Source (LNLS); the Parametric Rietveld refinement, pioneered applied in Brazil; the first use of relative humidity as 'non-crystallographic' parameter in this refinement; and the application of internal standards (SRM-676a and LiF) during experiments in function of time (at constant temperature), temperature and relative humidity (RH). Norfloxacin (NF, $C_{16}H_{18}FN_3O_3$), has a high structural susceptibility to RH and after crystallization of Norfloxacin sesquihydrate form (the only with known crystal structure) starts, at high RH, the behaviour of its cell parameters were parameterized to obtain a smoothly behaviour between all powder patterns collected during RH variation (0% < RH < 96%), consequently, it was possible to correct the value of the relative humidity really felt by the drug during the experiment and the behaviour of this RH correction seems to fit well to a natural logarithm function. To Mebendazole (MBZ, $C_{16}H_{13}N_3O_3$), also used during amorphous quantification at room temperature, a kinetic study, using Arrhenius plot, from powder patterns collected in function of time at different temperatures between 130°C and 160°C, compare the activate energy calculated, for

transformation of Form C to Form A, using sequential Rietveld refinement and Parametric Rietveld refinement, with and without amorphous quantification (LiF as internal standard). This comparison demonstrates the better efficiency of Parametric refinement and shows how can be the influence of consider amorphous quantification during a kinetic study of a phase transition, which for MBZ a small difference was calculated. A recrystallization of Mebendazole Form B in Ethyl acetate, described in literature, allowed a better exploration of its structural behaviour as a function of temperature and a new crystal structure, crystallized from Form B transformation, was observed. Because of this new crystal structure and due amorphous generation during Form C transformation, new possibilities of MBZ polymorphic map were proposed.

Resumo

Os estudos recentes sobre fármacos sólidos por difração de raios X por pó (DRXP) no Brasil vem demonstrando o quão importante pode ser esta técnica, principalmente aliada ao método de Rietveld, para a compreensão estrutural destes materiais. Materiais amorfos tem uma energia interna maior que a energia interna de materiais cristalinos. Isto pode influenciar diretamente o efeito terapêutico de um medicamento, e isto faz da quantificação de amorfo um estudo importante. Como a utilização de um padrão com reconhecimento internacional, como o padrão NIST SRM-676a (Al_2O_3), pode ser economicamente inviável, foi feito uma caracterização sistemática, principalmente por DRXP, de um material barato e com coeficiente de absorção linear dos raios X da ordem destes produtos orgânicos, a ser usado como padrão interno em análises quantitativas de fases. Os bons resultados obtidos com LiF, como padrão interno, na quantificação de amorfo à temperatura ambiente, mostrou seu potencial para uma aplicação em larga escala (quando comparados aos obtidos pelo padrão NIST SRM-676a, considerando o efeito de microabsorção). Outro importante foco deste trabalho foi a DRXP *in situ* aplicada aos produtos farmacêuticos e teve como destaques: uma câmara chamada TUCANO, para aplicar umidade relativa em experimentos de difração de raios x por pó *in situ*, desenvolvida em colaboração com o Laboratório Nacional de Luz Síncrotron (LNLS); a aplicação, pioneira no Brasil, do refinamento de Rietveld Paramétrico e o primeiro uso da umidade relativa como parâmetro "não cristalográfico" neste refinamento; e aplicação dos padrões internos (SRM-676a e LiF) em experimentos em função do tempo (com temperatura constante), temperatura e umidade relativa (UR). Norfloxacino (NF, $C_{16}H_{18}FN_3O_3$), possui uma alta suscetibilidade estrutural à umidade relativa e depois da cristalização da forma sesquihidratada do Norfloxacino (única estrutura cristalina conhecida) começa, em alta UR, o comportamento dos seus parâmetros de rede foram parametrizados para obter um comportamento suave entre todos os difratogramas coletados durante a variação da UR ($0\% < UR < 96\%$), consequentemente, foi possível corrigir o valor da umidade relativa realmente sentida pelo fármaco durante o experimento e o comportamento desta correção da UR parece se ajustar bem à uma função de logaritmo natural. Para o Mebendazol (MBZ, $C_{16}H_{13}N_3O_3$), também utilizado na quantificação

de amorfo à temperatura ambiente, um estudo cinético, usando a equação de Arrhenius, a partir dos difratogramas coletados em função do tempo em diferentes temperaturas entre 130°C e 160°C, compara a energia de ativação calculada para transformação da Forma C em Forma A, usando o refinamento de Rietveld sequencial e o refinamento de Rietveld Paramétrico, com e sem quantificação de amorfo (LiF como padrão interno). Esta comparação demostra a melhor eficiência do refinamento Paramétrico e mostra como pode ser a influência de considerar a quantificação de amorfo durante um estudo cinético de transição de fase, que para o MBZ uma pequena diferença foi calculada. Uma recristalização do Mebendazol Forma B em acetato de etila, descrito na literatura, permitiu uma melhor exploração do seu comportamento estrutural em função da temperatura e uma nova estrutura cristalina foi observada. Por causa desta nova estrutura cristalina e devido a formação de amorfo durante a transformação da Forma C, novas possibilidades de mapa polimórfico do MBZ são propostos.

Chapter 1
Motivation, Introduction and Goals

Crystallographic and Crystalline Computational Analysis Laboratory (LabCACC), funded by Carlos Paiva in 1993, has focus on pharmaceutical products since 2007. Since then, one Thesis and three Dissertations were published at UNESP library with titles:

"Application of XRPD and Rietveld refinement in crystalline pharmaceutical polymorphs" (Antonio, 2010);

"Polymorphism in generic and similar drugs" (Salvi, 2012);

"Limits for identification and quantification of finasteride polymorphs by XRPD" (Bezzon, 2013);

"Polymorphic characterizations of tablets from public distribution by XRPD" (Tita, 2014).

This thesis, supported by FAPESP (process numbers: 2010/08789-4; 2012/09935-0), explored points of interest for pharmaceutical industries, as previous group's works. The thesis presentation is the union of four main chapters, where each chapter represents a paper to be published in journals of International Union of Crystallography[1-4].

Chapter 2[2] presents a comparison between two potential materials, LiF and Li_2CO_3, for amorphous pharmaceutical quantification at room temperature with linear absorption coefficient (μ) of same order of organic compounds. Beyond the importance of have a material for amorphous quantification in large-scale, once their higher internal energy than for

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[1]NUNES, R. S.; FONTOURA, A. F. M.; CANOVA, H. F.; YOKAICHIYA, F.; FRANCO, M. K. K. D.; PAIVA-SANTOS, C. O.; EVANS, J. S. O. TUCANO: in situ experiments using x-ray powder diffraction at LNLS with temperature and relative humidity control. **Journal of Applied Crystallography**.

[2]NUNES, R. S.; EVANS, J. S. O.; PAIVA-SANTOS, C. O. Internal standard for pharmaceuticals products I: amorphous quantification at room temperature. **Journal of Applied Crystallography**.

[3]NUNES, R. S.; ELIZIARIO, S. A.; PAIVA-SANTOS, C. O.; EVANS, J. S. O. Internal standard for pharmaceuticals products II: in situ amorphous quantification. **Journal of Applied Crystallography**.

[4]NUNES, R. S.; ANTONIO, S. G.; PAIVA-SANTOS, C. O.; EVANS, J. S. O. Internal standard for pharmaceuticals products III: relative humidity application. **Journal of Applied Crystallography**.

crystalline material, this chapter shows an important microabsorption study between LiF and Al_2O_3, where the Brindley analysis (Brindley, 1945), a mathematical method for microabsorption correction implemented in TOPAS-Academic, does not work (see appendix A). Chapter 2 also shows the applicability of LiF tested during Mebendazole (MBZ, $C_{16}H_{13}N_3O_3$) amorphous quantification (see appendix B).

Polymorphism is a very important crystallographic property of pharmaceuticals products. The high number of phase transition possibilities, depending on external parameters (temperature, relative humidity, pressure, etc.), represents an abundant area to explore, but a concerned more for pharmaceutical industry.

The stability of a medicine, according World Health Organization (WHO), needs to be tested on different environments conditions (temperature + relative humidity) and subdivided the planet into four zones, as showed in Table 1.1 (Brazil is zone IVB):

Table 1.1: World subdivided by WHO into four zones according temperature and relative humidity.

Climatic zone	Definition	Conditions
I	Temperate climate	21°C/ 45% RH
II	Subtropical and Mediterranean climate	25°C/ 60% RH
III	Hot and dry climate	30°C/ 35% RH
IVA	Hot and humid climate	30°C/ 65% RH
IVB	Hot and very humid climate	30°C/ 75% RH

Source: Created by the author.

In fact, pharmaceutical products with hydrate forms can be very susceptible to relative humidity. However, as the dehydration/rehydration is a reversible process *ex situ* XRPD measurements can be not effective. There was already a small list of chambers with temperature- humidity-controlled to proceed this *in situ* x-ray powder diffraction, but not in Brazil. Therefore, a project in collaboration with Brazilian Synchrotron (LNLS) developed a furnace called TUCANO.

Chapter 3[1] contains a technical presentation and the limits determination for temperature and relative humidity application of TUCANO. It allows applying temperature variation, at N_2 atmosphere, from room temperature up to 250°C and with relative humidity control around 90% from room temperature up to 50°C. In this chapter the temperature applied is corrected by

Parametric Rietveld refinement showing a good control of temperature up to 180°C (see annex C).

Chapter 4[4] shows an application of relative humidity to a drug. Norfloxacin (NF, $C_{16}H_{18}FN_3O_3$), initially present in Form B, transforms into a hydrate form, after exposed to a high RH, and the structural behaviour of this Norfloxacin sesquihydrate analysed in function of RH variation. A peak at 6.5° was observed and its presence depends if the RH is high (appears) or low (disappears). This 'reversible peak' demonstrates the importance of *in situ* experiments.

From four different experiments (RH variation), chapter 4 explores internal standard application, RH variation influence and the behaviour of an API in a medicine form (with excipients), however, the highlight of this chapter is the first parameterization, in function of RH applied, of NF sesquihydrate cell parameters (see annex D). The correction applied to the external parameter fits well to a natural logarithm function, resulted by difficult control of gas flux.

Several pharmaceutical products have one or more phase transitions up to 250°C and amorphous quantification during a phase transition process, induced by temperature, is other interesting point.

Chapter 5[3] quantified the Mebendazole amorphous for each temperature (between 130°C and 160°C), using LiF as internal standard, after an interaction test from thermal analysis (TG and DSC). A simple kinetic study from *in situ* x-ray powder diffraction, using Arrhenius plot, compares the influence of amorphous quantification on activation energy in transformation process of MBZ Form C to MBZ Form A. The quantitative phase analysis, made from sequential Rietveld refinement and Parametric Rietveld refinement, demonstrate the better efficiency of Parametric refinement, mainly when the presence of MBZ Form C is very low (see comparison between cell behaviour with and without correction in annex E).

The recrystallization of Mebendazole Form B in Ethyl acetate, in chapter 5, brings a polemic scientific discussion about its true powder pattern. For this material, assumed in this work as Form B, three possibilities of indexation were tested (see annex F) and its structural behaviour explored as a function of temperature. A new crystal structure, crystallized from Form B transformation, was observed. Because of this new crystal structure and due amorphous generation during Form C transformation, new possibilities of MBZ polymorphic map were proposed.

Chapter 6
Final considerations and perspectives

This project explored different points past four years. The characterization of an internal standard showed how important is to consider the microabsorption effect between two materials with very different linear coefficient absorption. The successful amorphous quantification with LiF as internal standard demonstrate that it is as possible as cheap to add the method to a large-scale application.

TUCANO chamber, that applies different environment conditions (temperature and relative humidity control), developed in collaboration with LNLS, can be used to explore drug's crystal structure under variation of these two parameters. Parametric Rietveld refinement was applied with temperature as 'non-crystallographic' parameter to correct the sample temperature during heating process.

Norfloxacin sesquihydrate form had its crystal structure behaviour, in function of relative humidity, analysed by Parametric Rietveld refinement. This was the first time that RH is used as a 'non-crystallographic' parameter and showed a coherent correction fitted by a natural logarithm. However, there are some points to better explore, as the reason of a natural logarithm function to describe RH felt by the sample, resulting this 'RH of transition', or why each experiment had different coefficients of RH equation correction. Molecular dynamics simulation, for example, can be used to compare expected cell parameters behaviour.

The kinetic study made by *in situ* XRPD experiment demonstrated the better efficiency of Parametric Rietveld refinement in comparison with a simple sequential Rietveld refinement. To verify the difference between activation energy considering or not, the amorphous content, for Mebendazole, the same study will be proceed to a sample SRM-676a/MBZ.

Form B, recrystallized in Ethyl acetate solution and its phase transition, need to be explored by complementary spectroscopic and thermic techniques to determine molecular

properties of this initial crystalline phase (called Form B) and the second unknown crystalline phase that appears during the *in situ* XRPD experiment.