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EDITORIAL NOTE

JMS Journal of Medical Science

Final ORBIS project conference

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We are pleased to present this Journal of Medical Sciences issue, entirely dedicated to the final ORBIS (Open Research Biopharmaceutical Internships Support) project conference.

The Final ORBIS Conference took place on 5–6 July 2023 at Poznan University of Medical Sciences (Poznan, Poland). The conference summarized the activities of the five-year ORBIS project (**e936**), which received funding of EUR 2.27 million under the Marie Sklodowska-Curie Actions, Research Innovation Staff Exchange of the Horizon 2020 framework program (H2020-MSCA-RISE, no. 778051).

Poznan University of Medical Sciences coordinated this international and cross-sectoral project with fourteen academic institutions and pharmaceutical companies from eight countries. The goal of ORBIS was research and training cooperation between universities and the industry sector to develop better, more effective, modern drugs. ORBIS implemented these ideas through international internships of scientists and PhD students to partner institutions (450 months in total).

The Final ORBIS Conference was an opportunity to celebrate this success. During the conference, scientists and specialists from European and American centers were given plenary lectures on the most current topics of modern pharmaceutical sciences, including preformulation (**e878**, **e908**, **e901**) and formulation studies (**e893**, **e859**, **e914**, **e926**) as well as challenges in the new therapies (**e925**, **e907**, **e903**) and regulatory policies (**e890**). There were also speeches presenting the scientific effects of completed internships, delivered by participants from the project consortium.

After the conference, the scientific committee invited all lecturers to submit their papers to this Journal of Medical Sciences issue. This issue includes all submitted and accepted papers, each providing substantial new material beyond the original conference version.

We thank the authors and reviewers of these papers for their efforts in producing and reviewing these papers within the strict time limits imposed by this issue's publication constraints. We also gratefully acknowledge the support of the JMS editors-in-chief (Prof. Jarosław Walkowiak and Prof. Adrianna Mostowska) and the editorial office.

Guest Editors
Marcin Skotnicki and Janina Lulek

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REVIEW PAPER

JMS Journal of Medical Science

INVITED EDITORIAL

ORBIS project – where have we arrived?

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ABSTRACT

The Open Research Biopharmaceutical Internships Support project (ORBIS) was a response to the scientific, economic, and social challenge of increasing the effectiveness and productivity of the drug development process, both for innovative and (super)generic drugs. The overarching objective of the ORBIS project was to form a transnational and intersectoral cooperation network of academic and industrial organisations delivering a joint research programme. The research aimed at improving the preclinical pathway of drug development and manufacturing, focusing on technological and methodological improvements of the existing processes. The participating staff from all institutions have developed new skills, were exposed to new work and research environments, and have significantly broadened their career perspectives. More than 450 months of secondments were completed, and over 175 early-stage and experienced researchers participated in the exchange. This review aims to present some aspects of the scientific, training, and organisational activities of the consortium, bringing together representatives of both the academic sector as well as small and medium-sized pharmaceutical enterprises.

Introduction

When the Open Research Biopharmaceutical Internships Support project (ORBIS) received funding of 2,268,000 EUR in 2018 (Grant Agreement no. 778051), it was the second largest project under the Research and Innovation Staff Exchange (RISE) call of Marie Skłodowska-Curie

Actions (MSCA), Horizon 2020 programme (H2020-MSCA-RISE-2017). The main objective of this project was to promote international and inter-sectoral collaboration through research and innovation staff exchanges as well as two-way knowledge and/or idea exchange between the academic and industrial sectors. The concept of the project, detailed research objectives, and project plan were described in our previous articles [1,2].

Initially, the implementation of the ORBIS project was planned for 48 months. Unfortunately,

Table 1. Institutions involved in the ORBIS project during the period 2018–2023.

Institution name and acronym	Role in the project	Participation time	City and country
Poznan University of Medical Sciences (PUMS)	Coordinator, Beneficiary	1st March 2018 – 31st August 2023	Poznań, Poland
APC Ltd. (APC)	Beneficiary	1st March 2018 – 31st August 2023	Dublin, Ireland
Celon Pharma S.A. (CLN)	Beneficiary	23th September 2021 – 31st August 2023	Łomianki/ Kielpin, Poland
JSC Farmak (FMK)	Beneficiary	1st March 2018 – 31st August 2023	Kyiv, Ukraine
Łukasiewicz Research Network – former Pharmaceutical Research Institute (PRI)	Beneficiary	1st March 2018 – 22nd October 2020	Warsaw, Poland
Physiolution GmbH (PHY)	Beneficiary	1st March 2018 – 31st August 2023	Greifswald, Germany
Poznan University of Technology (PUT)	Beneficiary	13th March 2019 – 31st August 2023	Poznań, Poland
University of Central Florida (UCF)	Third-Party (Partner Institution)	1st October 2021 – 31st August 2023	Orlando, FL, US
University of Chemistry and Technology Prague (UCTP)	Beneficiary	27th September 2021 – 31st August 2023	Prague, Czech Republic
University of Ljubljana (UL)	Beneficiary	28 September 2021 – 31st August 2023	Ljubljana, Slovenia
University of Helsinki (UH)	Beneficiary	1st March 2018 – 31st August 2023	Helsinki, Finland
Trinity College Dublin (TCD)	Beneficiary	1st March 2018 – 31st August 2023	Dublin, Ireland
Zentiva (ZNT)	Beneficiary	1st March 2018 – 31st August 2023	Prague, Czech Republic
Rutgers, the State University of New Jersey (RUTG)	Third-Party (Partner Institution)	1st March 2018 – 31st August 2023	Piscataway, NJ, US

unforeseen circumstances affected the execution of the project. However, a change in the status of one of the beneficiaries, a break of 18 months due to a global pandemic and some restrictions due to the ongoing war in Ukraine did not stop the ORBIS project. After a significant reduction in the number of planned secondments by two beneficiaries and quitting the consortium by a key partner, project tasks were continued with new beneficiaries from Europe and a new partner institution from the USA. At the end of the project, the consortium comprised thirteen beneficiaries and partners, including eight academic institutions and five pharmaceutical companies located in eight countries (**Table 1**).

The overarching objective of the ORBIS project was to form a transnational and intersectoral cooperation network of academic and industrial organisations delivering a joint research programme. The research aimed at improving the preclinical pathway of drug development and manufacturing, focusing on technological and methodological improvements of the existing processes. The participating staff from all institutions have developed new skills, were exposed to new work and research environments, and have significantly broadened their career perspectives. More than 450 months of secondments were completed, and over 175 early-stage and experienced researchers participated in the exchange. This review aims to present some aspects of the scientific, training and organisational activities of the consortium, bringing together representatives of both the academic sector as well as small and medium-sized pharmaceutical enterprises.

ORBIS research activities

TThe primary objective of work package WP1 (*Drug substances and pharmaceutical preformulation*) was to translate the discovery synthesis of a drug substance (active pharmaceutical ingredient, API) into technology development and to investigate the solid-state physicochemical properties of APIs. It aimed to improve unfavourable biopharmaceutical properties (e.g. solubility/dissolution) such actives and develop a strategy to enhance poor solubility and/or permeability of Biopharmaceutics Classification System (BCS) class II and IV APIs. A number of studies were carried out in WP1, including the synthesis of drug substances and their derivatives (Sidoryk *et al.* 2022), process scale-up and continuous processing development, investigations into intrinsic and derived solid state physicochemical properties of selected pharmaceuticals, preparation of crystalline/co-crystalline and/or amorphous/co-amorphous forms of selected API(s) [3,4] and correlation of physicochemical properties of APIs/excipients with their formulability, manufacturability and biopharmaceutical performance. The advances were facilitated by a variety of analytical techniques, such as thermal analysis (thermogravimetric analysis, differential scanning calorimetry and temperature-modulated differential scanning calorimetry), X-ray diffraction (single crystal and/or powder), spectroscopic methods (Fourier-transform infrared and Raman spectroscopy, nuclear magnetic resonance etc.), dynamic vapour sorption, microscopy, solubility and dissolution testing or chromatography to mention a few. Furthermore, the team from the ORBIS project participated in public consultations regarding the *Pharmaceutical strategy for Europe* in the area of drug synthesis [5].

The purpose of WP2 (*Dosage forms and drug delivery systems*) was to design, develop and test new drug carriers and dosage forms for oral and topical delivery of APIs. Experimental work aimed to optimise manufacturability and/or maximise the efficacy of drug delivery by formulating advanced drug delivery systems, such as nanoparticles [6], minitablets, self-(micro)emulsifying drug delivery systems, mesoporous silica as an API carrier and mucoadhesive systems [7, 8]. Also, novel analytical techniques for characterisation of drug delivery systems were developed, such as powder flowability test for small volume, texture analysis, quantitative image analysis of drugs in the solid dosage preparations by Raman mapping etc. Quality-by-Design tools such as Process Analytical Technologies (PAT) were employed for the optimisation of manufacturing operations, e.g. high shear wet granulation [9].

As regards topical drug delivery, the research focused on the assessment and improvement of

selected drug penetration across individual layers of the skin, including transdermal transport. The studies were conducted in relation to different groups of drugs, for example, non-steroidal anti-inflammatory drugs, antifungal agents and also photosensitizing dyes. The investigated delivery systems and dosage forms included liposomes, transferosomes, nanoparticles and microemulsions [10], semi-solids (hydrogels, organogels, creams) [11–13] and adhesives (patches). *In vitro* and *ex vivo* drug permeation from the developed formulations was tested using animal and human skin samples. The skin was also the focus of investigations of cellular senescence [14].

In WP3 (*Biopharmaceutical evaluation of dosage forms and drug delivery systems*), novel bioanalytical methods were developed, validated [15] and applied to real samples, e.g., HPLC-FLD (high-performance liquid chromatography - fluorescence detection) determination of pregabalin in human serum [16] or methods for UPLC-MS/MS (ultrahigh-pressure liquid chromatography - tandem mass spectrometry) for determination of antibiotics and antitubercular drugs in human plasma or fat tissue [17]. The European Medicines Agency (EMA) and the United States Food and Drug Administration (FDA) bioanalytical method validation recommendations were compared, and a novel statistical model for incurred sample reanalysis was developed [18,19].

Another area of WP3 research was the development of biorelevant/biopredictive *in vitro* methods for evaluating drug products. Food interaction with bisphosphonates was studied. The effect of different media buffers on API release from a formulation with a microenvironmental pH was also evaluated [20], and *in vitro* release testing methods aiming at predicting the *in vivo* behaviour of extended-release tablets were developed [21,22].

WP3 team also contributed to methodological improvements through theoretical research linking bioanalytical and pharmacokinetic topics, e.g. on toxicology [23], improving the clinical performance of drugs [24] or design of clinical trials [25]. WP3 team participated in public consultation of the EMA document on pharmacokinetics and pharmacodynamics in the obese population.

ORBIS training activities

The primary goal of WP4 (*Training*) was to support the career development and employability of academic and industrial staff by enhancing their research and transferrable skills as well as increasing the competence of the pharmaceutical R&D sector. Each secondee had an opportunity to learn new processes, methods, and techniques specific to the work package in which they participated. The international and intersectoral mobility was an invaluable experience, especially for early-stage researchers, who learned to work in a multidisciplinary and multicultural environment. ORBIS exchange was an opportunity for researchers to develop a wide range of transferrable skills in terms of science, communication, career management, as well as sector-specific knowledge (**Figure 1**).

In addition to the training activities integrated into the secondments, the consortium organised four summer schools and three workshops (**Figure 2**), which attracted 255 participants.

The 1st School was organised by Trinity College Dublin, APC and Farmak and the primary scientific topic was related to WP1. It encompassed lectures on solid-state pharmaceutical materials, continuous processing, fundamentals and application of preformulation and PAT. The workshop presented practical approaches and real cases established at GMP manufacturing facilities, such as API process development and transfer of technologies from laboratory to pilot and industrial scales. The 2nd School (organised by the University of Helsinki and Zentiva) was related to WP2 and focused on challenges and perspectives in the development of oral dosage forms and advanced emerging technologies, with the workshop activities on spray drying for the development of microparticles, compression of minitablets and application of coherent anti-Stokes Raman spectroscopy in drug characterisation. The 3rd School, arranged by Poznan University of Medical Sciences with Rutgers University, presented state-of-the-art and recent progress in the development of topical and transdermal delivery systems (e.g. microemulsions, transdermal patches, microneedles) and their characterisation methods, followed by demonstrations of *in vitro* permeation testing using Franz cells and

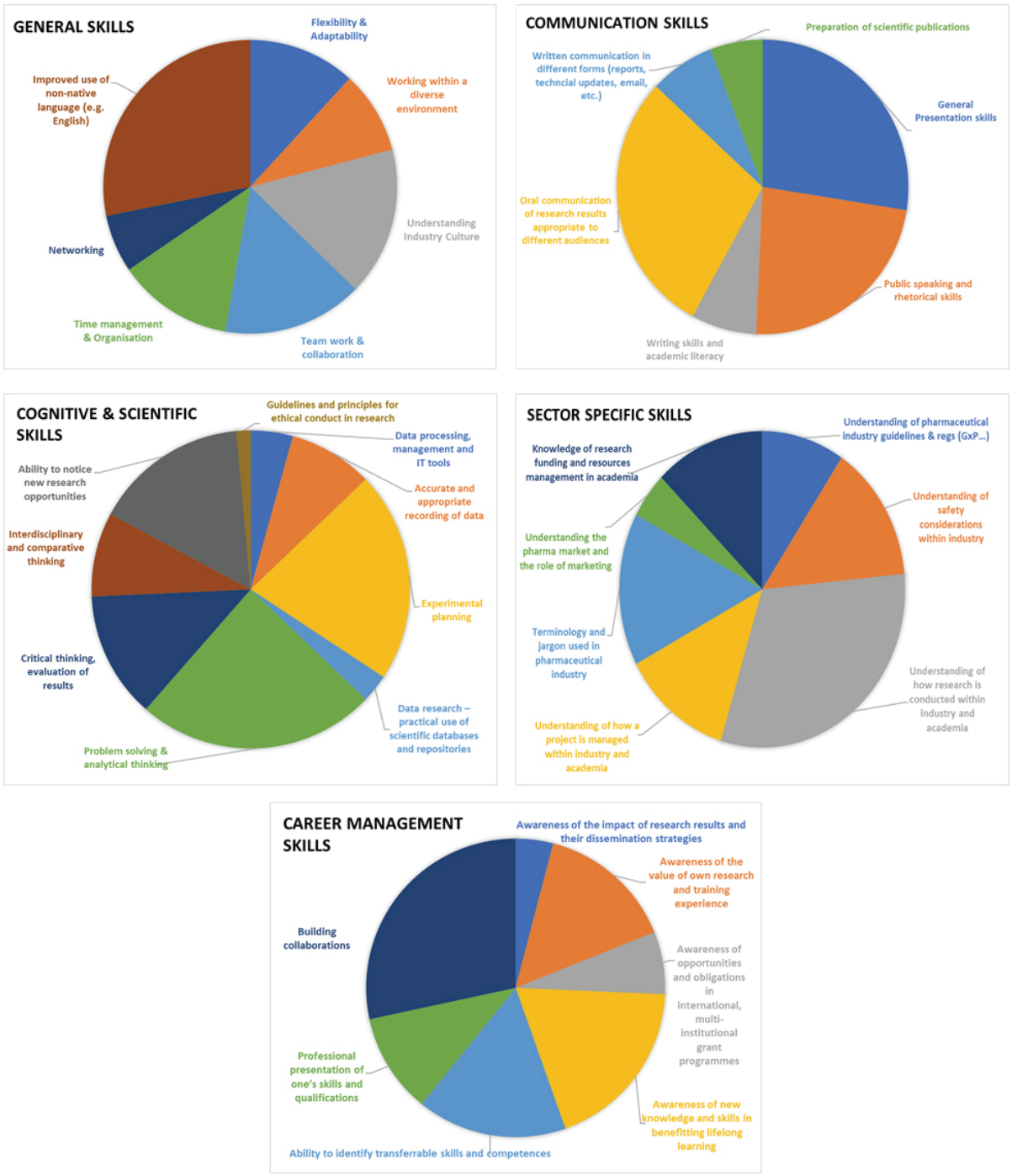

Figure 1. Summary of main transferrable skills identified and developed by the ORBIS secondees (self-reported; *n* = 48).

texture analysis of semisolid preparations. The 4th School (organised by Zentiva, Physiolution, University of Chemistry and Technology Prague) covered the WP3 topics of biopharmaceutics and novel techniques, drug delivery systems with increased bioavailability, in *vitro/in vivo* relationship, bioanalytical methods as well as non-traditional routes of drug absorption. The workshop concerned novel dissolution methods and *in situ* imaging techniques.

Additionally, consortium members had a unique opportunity to participate in a series of webinars created for ORBIS by a business coaching company DRevolve (Switzerland). The participants developed skills in networking, social media channels, and communica-

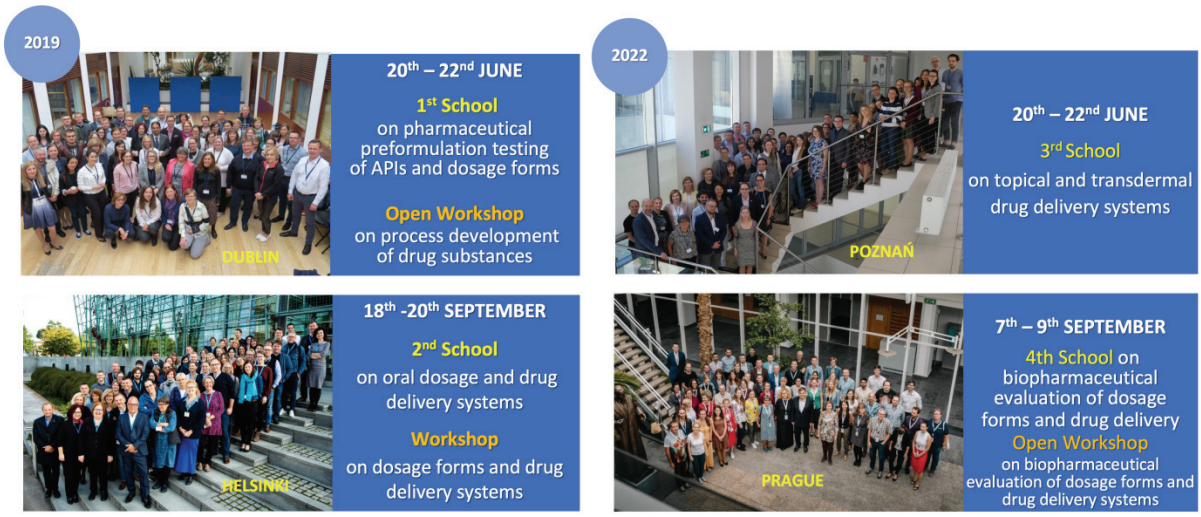

Figure 2 Overview of ORBIS Summer Schools and Workshops.

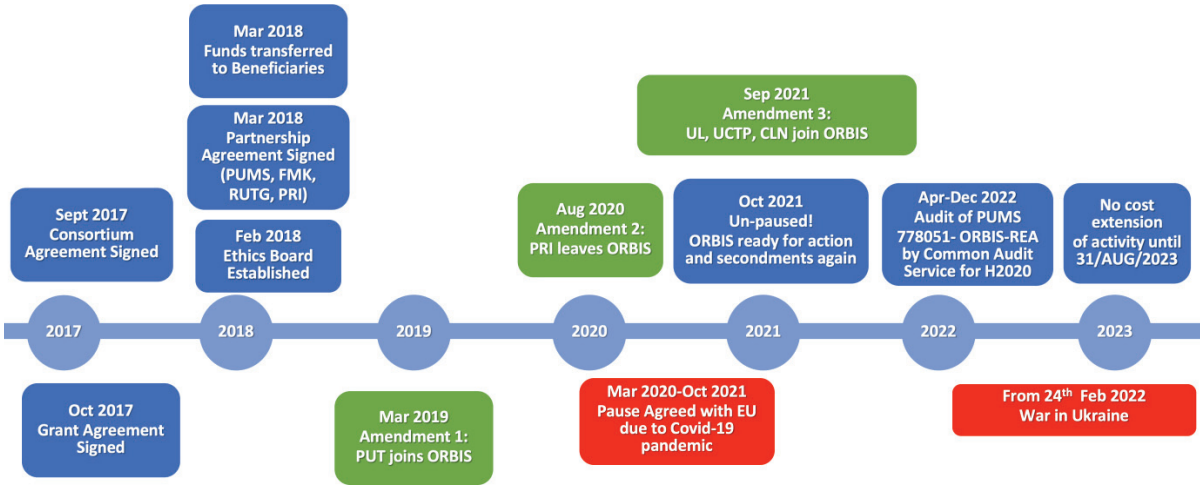

Figure 3. Project management timeline.

tion, as well as learned about cultural differences and the distinction between science and business approaches.

ORBIS management and dissemination activities

During the five years of the project, ORBIS faced many obstacles, including the unprecedented COVID-19 pandemic and the war in Ukraine. The project management team, headed by Janina Lulek (the project coordinator) and Bożena Raducha (the project manager), worked tirelessly to overcome the challenges, which required amending the grant agreement thrice (Figure 3). Day-to-day management by the General Assembly, Training Committee and Steering Committee supported by the Ethics Advisory Board, involved the control of secondments realisation, monitoring and submission of deliverables and milestones, communication with the EU Project Officer, evaluation and reporting of the project's research progress, as well as monitoring of ethical aspects.

Moreover, PUMS was responsible for organisation of the three meetings, i.e. Kick-off Meeting (12th–13th April, 2018, Poznan), Mid-Term Meeting (25th–26th September 2019, Poznan) and the Final Meeting (7th July, 2023, Poznan).

Project results were continuously disseminated in the form of peer-reviewed, open access

publications, presentations at seminars, conferences, and lectures. The ORBIS Final Conference, held on 5–6 July 2023 in Poznan, Poland, was a great success with nearly 200 participants. The project website www.orbisproject.eu, social media channels on Facebook, Twitter, YouTube and LinkedIn were active and updated throughout the project.

For example, communication and outreach activities were disseminated as lectures during the European Researchers Night in 2021 (https://www.youtube.com/watch?v=358a9Zvubas), scientific papers (26), notes and news pieces featured on various websites such as sectoral news channels e.g. https://maltabusinessweekly.com/zentiva-advances-pharmaceutical-research-through-orbis-project/16693/ or the local media channels (e.g.: https://radiopoznan.fm/informacje/pozostale/nie-byloby-innowacyjnych-terapii-gdyby-nie-wspolpraca-naukowcow-z-branza-farmaceutyczna), ensuring wide reception of the project's goals and achievements.

Conclusions

Despite the difficulties, the ORBIS project finished and became a massive success for all the consortium members, fulfilling all the required deliverables and milestones and realising all the planned research and training objectives. It is a testimony to the quality of the international and intersectoral collaboration established between the partners. Also, despite the substantial size of the consortium and the unexpected challenges (COVID-19 pandemic and war in Ukraine) the success rate of secondment completion was over 90%. The impact of the ORBIS project will last beyond those five years and ripple beyond the inner circle of participants. Over 175 researchers have been upskilled thanks to international and intersectoral mobility, improving their employability in academic and pharmaceutical sectors. The ORBIS project strengthened the European human capital in pharma research and innovation and has formed the cornerstone for future cooperation on innovative science for the advancement of medicines, strengthening Europe's intellectual potential in the R&D sector.

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Conflict of interest statement
The authors declare no conflict of interest.

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ORIGINAL PAPER

JMS Journal of Medical Science

New therapies targeting aging cells in the skin

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ABSTRACT

Senescence is accompanied by numerous processes that lead to alterations in cell metabolism, cell cycle arrest, and, increased production and secretion of senescence-associated secretory phenotype (SASP). Consequently, signaling pathways cascades are activated, leading to inflammation that can trigger multiple disorders, including cancer. Recently, a novel therapeutic approach was proposed based on targeting senescent cells using senolytics. This group of biologically active compounds includes fisetin, quercetin, dasatinib, and others. These compounds were shown to affect laboratory animals (rodents) by improving the quality of life and significantly increasing the length of life by reducing senescent cells pool in different organs. Based on these findings, we decided to evaluate the potential of these compounds in targeting senescent cells in human skin using in vitro model based on human-derived keratinocytes (HEKa) and

fibroblasts (HDFa). Cytotoxicity assay revealed that the activity of the compounds was time- and dose-dependent as well as cell-type dependent. Further studies were performed to reveal the mechanistic aspect of these observations including assessment of the senescence marker, namely p16. However, it requires clarification before entering clinical trials to provide not only efficient but, first of all, safe application of senolytics to human skin.

Introduction

The accumulation of senescent cells within the skin contributes to age-related skin damage, which can manifest in the depletion of skin functions, especially disturbed epidermal barrier, skin neoplasms and autoimmune skin diseases development [1,2]. Senescence-associated secretory phenotype (SASP), being a set of proinflammatory chemokines, cytokines, growth factors, lipids, and proteases produced and secreted by senescent cells, induces chronic inflammation and tissue changes in all skin layers, including epidermis (keratinocytes) and dermis (fibroblasts). The use of a strategy that eliminates senescent cells (senolytics) or neutralizes SASP components (senostatics) represents promising option for delaying skin aging and treatment of age-related skin diseases [1,2]. There is convincing evidence that flavanols, initially found in fruits and vegetables, can target cellular pathways crucial for clearing senescent cells and reducing the SASP (3). Several biologically active compounds, including fisetin, quercetin, and curcumin, have been reported to trigger positive effects against skin cellular senescence *in vivo* and *in vitro* [1,3]. Notably, these natural plant-derived compounds were reported safe. Fisetin was shown to inhibit PI3K/AKT/mTOR pathway, topoisomerase, and TNF-α-induced inflammation and oxidative damage in human keratinocytes. Applied topically in hairless mice prevented UVB-induced skin damage and restored epidermal function by increased expression of filaggrin and aquaporins [1,4]. Another flavanol, quercetin, was shown to decrease the number of stress-induced senescent cells, while curcumin led to the selective elimination of senescent cells by inducing apoptosis [3]. Combination of two senolytics was also evaluated, showing promising results [5]. In this study we aimed to assess the biological activity of fisetin and quercetin with dasatinib on skin cell metabolism, including survival and senescent cells elimination.

Materials and methods

Permeability of fisetin by Raman spectroscopy in an *in vitro* model

Skin penetration of fisetin (3,7,3',4'-tetrahydroxyflavone) was examined using Confocal Raman spectroscopy. The commercially available fisetin (Indofine, Hillsborough, NJ, USA) was used to prepare the 3% fisetin cream in multi-component medium with a pH 5.5 Lekobaza Pharma Cosmetic base (Fagron, Kraków, Poland).

Raman spectroscopy was performed using a WITec Alpha 300 spectrometer equipped with a confocal microscope (WITec alpha300 R, Ulm, Germany), TrueSurface attachment, and an electron-multiplying CCD (EMCCD) camera using previously described protocol [6]. Skin layer permeability analysis was performed on cross-sections through layers of the skin after incubation with the samples. Skin samples were obtained from excess skin during abdominoplasty from healthy middle-aged females. Samples (three repeats) prepared in a 6-well plate containing PBS to maintain hydration were incubated for 6 hours in 3% fisetin cream and then mounted on slides for Raman spectroscopy. The biophysical skin parameters (e.g. TEWL) must be evaluated in further detailed studies.

Cell culture

Adult human keratinocytes cell line (HEKa) and adult human fibroblasts cell line (HDFa) were purchased from ATCC (American Type Culture Collection) and cultured according to ATCC guidelines. Cells were studied in varied intervals including passages 2 to 6 to verify potential association with senescence status.

Viability test

MTT test was used to assess changes in cells viability as previously described [7]. HEKa or HDFa cells were plated into each well of 96-well plate at a density of 3 x 10^3 cells per well for 24h incubation (for cell adhesion) and then cells were treated for 24 or 72h with: fisetin solution (0.1, 1, 5, 10, 20, 100 or 200 µM) or Quercetin/Dasatinib combination (0.1 µM/1 nM, 1 µM/10 nM, 2.5 µM/25 nM, 5 µM/50 nM, 10 µM/100 nM, 25 µM/250 nM or 50 µM/500 nM). Results were presented relative to control cells (DMSO).

Assessment of senescence marker, p16

For the senescence marker assessment 3 x 10^5 HEKa or HDFa cells were plated into 60mm Petri dish for 24h (for cells adhesion) as previously described [8]. After 72 h treatment cells morphology was assessed using microscope (photos at 400x magnification) and then cells were lysed (lysates were stored in -80°C). ELISA test was performed according to manufacturer guidelines (Abcam, as previously described [9]). For absorbance measurement multimode plate reader EnSpire (Perkin Elmer) was used.

Statistical Analysis

Results were expressed as mean ± SD. All statistical analyses were carried out using GraphPad Prism (GraphPad Software, San Diego, CA, USA). Differences were assessed for statistical significance using repeated-measures ANOVA, followed by post-hoc the Dunnett's test method. All experiments were performed in triplicates unless specified otherwise. The threshold for significance was defined as $p < 0.05$ and are indicated by the (*) symbol.

Results

Permeability assessment

Skin penetration assessment was performed with fisetin (3,7,3',4'-tetrahydroxyflavone) using Confocal Raman spectroscopy showed significant penetration of the compound through the skin up to at least 610 µm up to 1330 µm (**Table 1**).

Biological potential of senolytics

Evaluation of HDFa cells viability after 2 passages showed that fisetin alone provoked significant decrease of cell viability starting from 10 or 20% reduction (for 24 and 72h, respectively) at 0.1 µM up to 50 and 70% reduction (for 24 and 72h, respectively) at 200 µM (**Figure 1A**). Cells subjected to further passaging (i.e. four passages) also showed significant reduction in metabolic activity and with almost 20 and 30% up to 30 and 50% reduction (for 24 and 72h, respectively) in cell survival at 0.1 µM and 200 µM, respectively (**Figure 1B**). HDFa cells evaluated after 6 passages demonstrated reduced cell viability by 10 and 30% at 0.1 µM (24 and 72 h respectively) up to 40 and 50% at 200 µM (24 and 72h respectively) (**Figure 1C**).

Assessment of the biological activity of fisetin in HEKa cells showed cytotoxic effect of the compound after both time intervals but with a different pattern. Treatment of cells after 2 passages showed decreased viability of HEKa cells by 20%

Table 1. Skin penetration of fisetin. The analysis was performed using Confocal Raman spectroscopy. Skin layer permeability analysis was performed on cross-sections through layers of the skin (obtained from excess skin during abdominoplasty from healthy females) after incubation with the test compound.

Biological replicates (donors)	Technical repeat	Penetration rate [µm]	Average value±SD [µm]
1	1	1480	1313.33 ± 294.09
	2	1560	
	3	900	
2	1	1840	1330 ± 365.79
	2	1150	
	3	1000	
3	1	540	610 ± 92.01
	2	550	
	3	740	

at 10 μM for 24h treatment while 72h-treatment revealed reduced cell viability by 15% at 0.1 μM. Further increase of the fisetin concentration led to more efficient inhibition of metabolic activity of studied cells, up to 80% decrease of viability at 200 μM when cells were treated for 24h and 90% decrease of viability at the same concentration when cells were treated for 72h (**Figure 1D**). In turn, treatment of cells after 4 passages led to a significant decrease of their survival by 5% when 1μM fisetin was applied (24h) and by 10% when 0.1 μM fisetin was used (72h) (**Figure 1E**). Further increase of the fisetin concentration led to more efficient inhibition of metabolic activity of studied cells up to almost 80% decrease at 200 μM for 24h and 95% when 72h treatment with 200 μM fisetin was applied (**Figure 1E**). When experiments were performed with the use

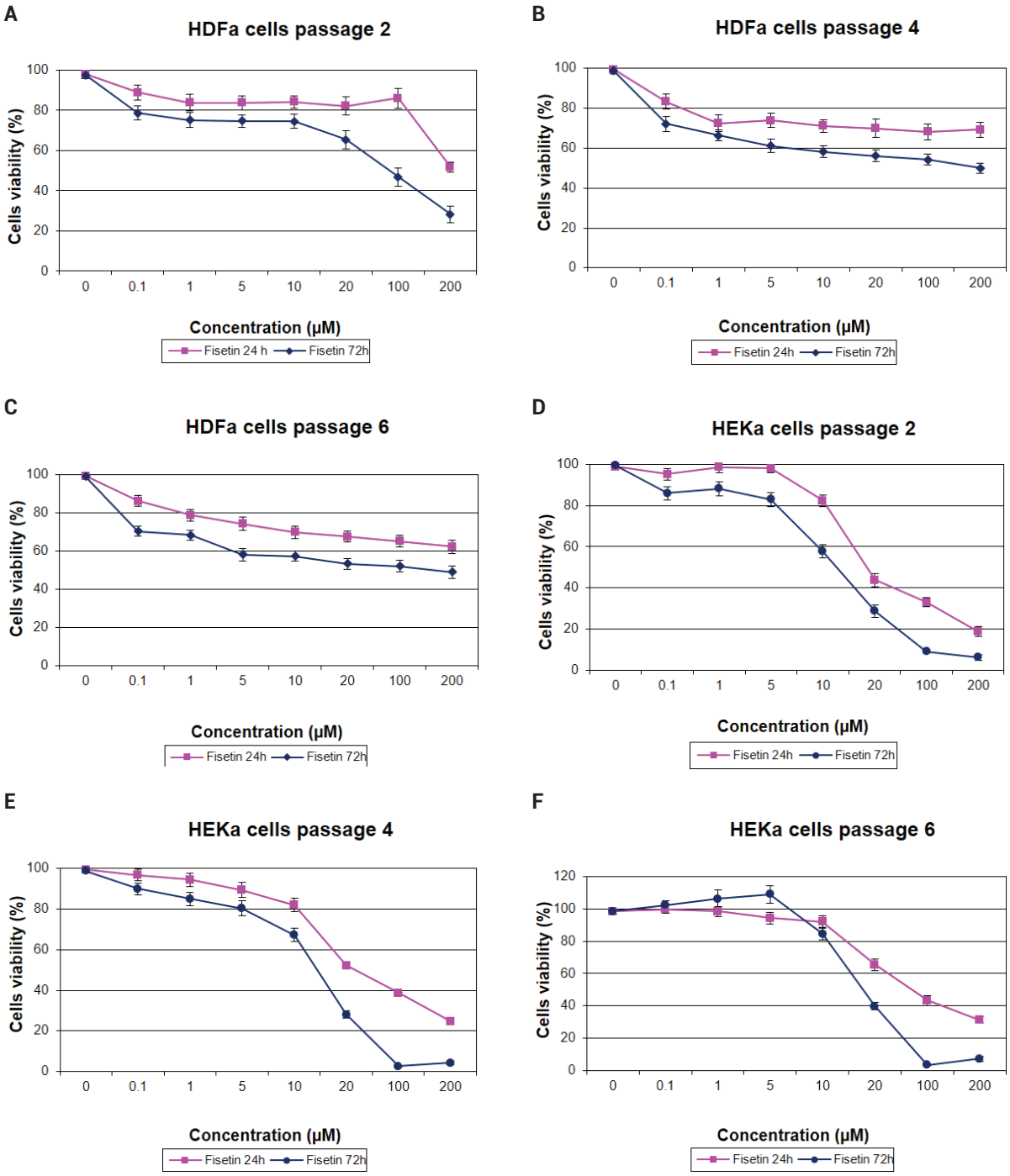

Figure 1. Cytotoxicity assessment of fisetin in human skin fibroblasts and keratinocytes.

of HEKa cells after 6 passages, significant viability decrease was observed at the concentration of 10 μM for both time intervals. Further concentration increases provoked significant reduction of cell survival up to 70 and 95% for 24 and 72h treatment with 200 μM fisetin, respectively (**Figure 1F**).

Biological potential of quercetin and dasatinib

HDFa cells were subjected to the quercetin/dasatinib (Q+D) combination (0.1 μM/1 nM, 1 μM/10 nM, 2.5 μM/25 nM, 5 μM/50 nM, 10 μM/100 nM, 25 μM/250 nM or 50 μM/500 nM). As demonstrated, the youngest cells (passage 2, **Figure 2A**) showed relatively low sensitivity to Q+D combination showing significant viability decrease at 2.5 μM/25 nM for both time intervals (10 and 15% survival reduction for 24 and 72h treatment, respectively). Increased concentration of Q+D provoked further reduction of HDFa cells viability up to 20 and 40% reduction at 50 μM/500 nM for 24 and 72h treatment time, respectively. When experiments were performed for cells after 4 passages, a more significant reduction of cell survival was observed, i.e. irrespectively to the treatment time, viability of cells was reduced by 10% at the lowest applied concentration (0.1 μM/1 nM) up to almost 80% reduction at 50 μM/500 nM (**Figure 2B**). Very similar results were observed when cells after 6 passages were studied with no relation to treatment time (**Figure 2C**).

Simultaneous assessment of p16 showed that its relative level was significantly reduced in HDFa cells after 72h treatment with studied compounds but only in cells treated with Q+D at 5 μM/50 after 4 (10%, p = 0.05) and 6 passages (30%, p = 0.05) with no significant alterations when lower concentration was applied (1 μM/10 nM) or in younger cells (i.e. after 2 passages) irrelevant to the concentration applied (**Figure 2D**). Interestingly, treatment of HDFa cells with fisetin alone did not show any significant alterations in p16 accumulation (**Figure 2D**).

Similarly, HEKa cells were subjected to the same concentrations of studied compounds. When cells after 2 passages were subjected to

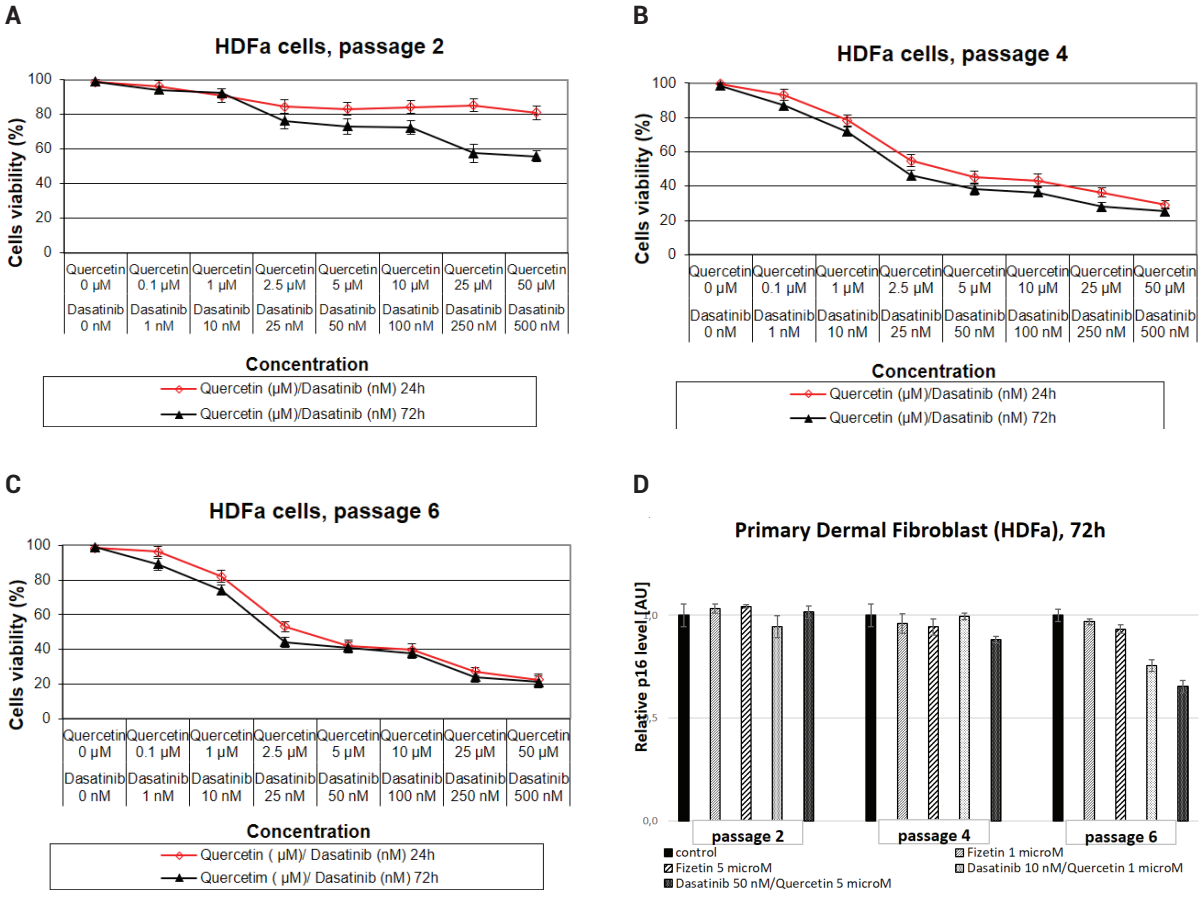

Figure 2. Cytotoxicity assessment of fisetin, dasatinib and quercetin in human skin fibroblasts.

(Q+D) combination a significant viability decrease was observed at concentration 5 μM/50 nM (20% at 24h) and 0.1 μM/1 nM (15% at 72h). Further concentration increase led to more efficient metabolism inhibition i.e. up to 20% for 24h and 90% for 72h treatment, respectively (both at 50 μM/500 nM, **Figure 3A**). Evaluation of cells after 4 passages showed more than 10% cell viability inhibition at 2.5 μM/25 nM for 24h treatment and also 10% viability reduction but at 0.1 μM/1 nM for 72h treatment (**Figure 3B**). Further concentration increase led to higher cytotoxicity up to 50 and 70% at 50 μM/500 nM for both time intervals, respectively. Assessment of cell survival after 6 passages and treatment with Q+D combination showed higher cytotoxicity of the compounds starting from 1 μM/10 nM for 24h (5% decrease) and 0.1 μM/1 nM for 72h (15% decrease) (**Figure 3C**). Higher concentrations led to more efficient metabolic activity inhibition up to 70 and ca 80% decrease at 50 μM/500 nM for 24 and 72h, respectively.

Verification of p16 accumulation showed significant decrease of the protein accumulation after 2 passages when cells were treated with 5 μM/50 (**Figure 3D**), while in cells after 4 passages, a significant reduction of this protein was observed in cells treated with 5 μM fisetin, and 1 μM/10 nM or 5 μM/50 nM Q+D. Interestingly, older cells (6 passages) did not show any significant alterations in p16 levels when Q+D were involved but fisetin alone (1 μM) led to a significant reduction of p16 level (**Figure 3D**).

Discussion

Cellular senescence represents the major cellular mechanism associated with aging and age-related dermal diseases [1,2]. Better understanding of the molecular mechanism and impact of these cells on skin conditions during aging including inflammation, metabolic alterations, carcinogenesis induction etc. will help to develop better therapeutic approaches by prevention and/or blocking the senescence pathway. Such approach can be provided by different senolytics including fisetin or dasatinib with quercetin.

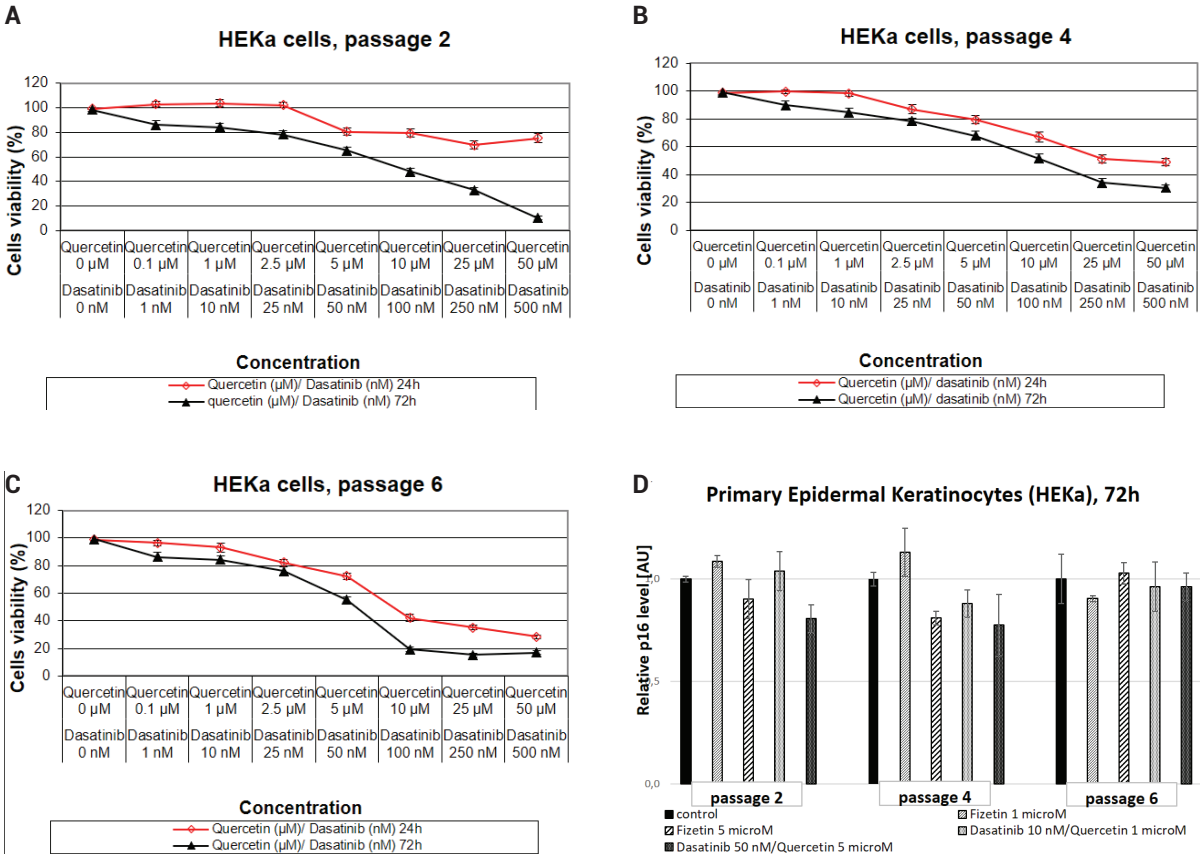

Figure 3. Cytotoxicity assessment of fisetin, dasatinib and quercetin in human skin keratinocytes.

The latter two compounds were shown by Kirkland lab to target senescent cells *in vitro* and *in vivo* in different organs in mice and humans by oral treatment [10]. Since the activity of dasatinib and quercetin (DQ) was already demonstrated we used them as a reference sample and focused on fisetin. First, we evaluated the ability of fisetin to penetrate skin tissue. As demonstrated, the skin was permeated for more than one millimeter (1330μm) in depth indicating that not only keratinocytes but also skin fibroblasts can be affected. Following our observation we completed cytotoxicity assessment of selected senolytics and their effect on senescence marker accumulation, p16 gene using *in vitro* human skin model based on human-derived keratinocytes (HEKa) and fibroblasts (HDFa). In both cell types, DQ showed gradually higher cytotoxic potential in older cells. Interestingly, in fibroblasts treated with DQ we observed lowered accumulation of p16 relative to control, untreated cells suggesting high efficiency in targeting and eliminating senescent cells. Additionally, cytotoxicity assay revealed that the activity of fisetin was time- and dose-dependent as well as cell-type dependent. As demonstrated, it was more efficient in attenuation of the metabolic activity of keratinocytes than fibroblasts. Additionally, fisetin was more efficient in the elimination of younger keratinocytes (passage 2 and 4), especially after a long-time exposure (72 h) at 0.1–20 μM while higher concentrations (100–200 μM) was even more potent (up to 90% reduction). In turn, fisetin was more efficient in reducing the metabolism of fibroblasts when elder cells were studied (passages 4 and 6) compared to younger cells indicating more selective targeting of senescent rather than healthy, non-senescent cells. Most of current clinical trials focus on oral administration of fisetin so it is difficult to compare to any other studies [11,12]. Thus our initial study shows the need to better understand the potential of topical treatment since the mechanism and metabolic effects of fisetin in skin cells is not known yet.

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Conflict of interest statement
The authors declare no conflict of interest.

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REVIEW PAPER

JMS Journal of Medical Science

Emerging Technologies Transforming Therapy

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ABSTRACT

The advancement of healthcare therapies is under constant development due to changing demographics and evolving disease-states. To ensure continuous furtherance of the healthcare system capacity to treat such ailments, emerging technologies (ETs) are coming to the forefront of medicine. It's the hope that ETs are capable of covering a broad scope of therapeutic treatment areas, enabling novel pharmaceutical pathways to be established. Highlighted in this mini review are examples of focus ET areas, including additive manufacturing (AM), microfluidics (MFs), microelectromechanical systems (MEMS) and machine learning (ML), that have shown promising qualities and should be targeted further to improve patient outcomes.

Introduction

ETs have developed greatly over the last decade, opening new manufacturing channels within pharmaceutical industry. Although many of their potentials are far from being actualised, their development is likely to affect the future of therapeutic treatment methods. Included in this mini review are some of the most promising technologies that are being used for novel healthcare applications, with the aim of sustaining an efficacious level of medical management for future treatments. A brief mention of related advantages and disadvantages is discussed also for each ET.

Additive Manufacturing and 4D Printing

AM, known commonly as 3D printing (3DP), allows for the in-house production of highly-customised drug delivery systems and medical devices, marshalling towards an era of feasible personalised-healthcare. The development of new printing technologies has allowed for a huge range of materials to be manipulated with this technology, ranging from metals to living tissues [1]. Developing areas of AM could bring about huge advancements for healthcare, for example bioprinting and 4D printing using "smart" polymers, which could

negate the requirement for allograft/xenograft tissue donation whilst simultaneously reducing the likelihood of immune rejection [2]; **Figure 1**.

Research into the AM of personalised drug-eluting scaffolds and Microneedles (MNs), used for sustained drug delivery and wound repair, has seen a rise in interest, allowing for the treatment of complex diseases such as various cancers (e.g., breast), diabetes and HIV [3,4]. The production of specialist medical devices, including personalised pieces, is possible using AM, which has been used for purposes including surgery, prosthetics and pre-operative planning [5]. Custom printing inks are routinely used for pharmaceutical AM, for example the use of drug-incorporated filaments for fused deposition modelling (FDM) printing [6].

The availability of 3DP has increased greatly, to the point that it's not uncommon for households to own a printer. Whilst this may differ from pharmaceutical printing, it's important to consider the impact that will be experienced for the technology as a whole due to the increase of technology users, as it could help fast track innovation being developed in the area. A skilled operator will have the capacity to optimise the print design, with a view about the chosen material's strengths and weaknesses.

4D Printing (4DP) is a further extension of AM, which incorporates smart materials capable of possessing a level of responsiveness to external and internal stimuli. The material responses often include shape and property changes, including chemical, structural and size alteration [7]. This adaptiveness of the materials can lead to more efficient medical treatment, for example allowing concise control of active pharmaceutical ingredient (API) release [8], occupying void space [9] or controlling the swelling potential of a hydrogel [10]. Polymers are a common choice of material used in 4DP due to their printable nature, modifiable structure and biocompatibility. Smart polymers often used for 4DP include carboxymethyl cellulose sodium (CMC) [11], polylactic acid (PLA) and polycaprolactone (PCL) [12]. Elastomers, a subsection of polymers and shortened from the name "elastic polymer", are also often used for shape responsive 4DP. 4DP allows for the adaptability of the medical device post-administration, which is a key advantage over 3DP. For 3DP the printed device won't alter its mechanism of action as a response to its environment due to a lack of smart material sensitivity.

Advantages of AM include the ability to produce individualised treatment options on-site, using a wide range of compatible materials. Current disadvantages include the risk of print failures and the fact that AM can be a time-consuming process for large or complex prints.

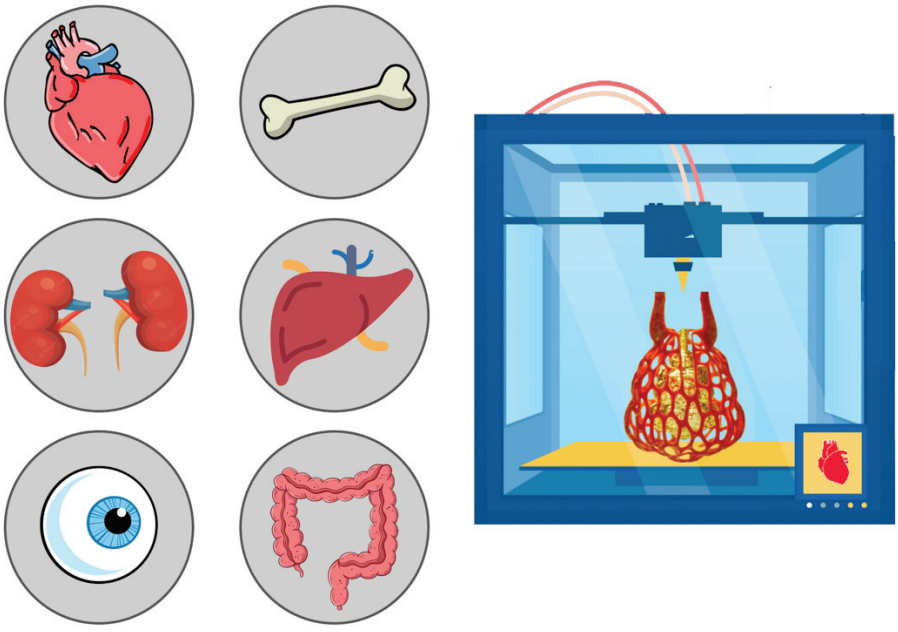

Figure 1. Graphic depicting the potential for the additive manufacturing of tissues and organs via bioprinting.

Microfluidics & Lab-on-a-chip

MFs has seen a great resurgence in popularity, owed largely to its capacity for high-quality formulation. MFs has quickly adopted processes that allow for the self-assembly of materials into nano/microparticles, allowing for the concise control of particle characteristics [13]. As evident during the COVID-19 pandemic [14,15], the use of nanoparticles (NPs) for therapy is an effective and sought-after approach. MFs can act as a platform for chemical synthesis (**Figure 2**), enabling reactions to occur in a time efficient manner with high atom economy [16], following closely with the guidance of the 12 principles of green chemistry [5,17].

The customisability of the MF system lies at the forefront of the attractiveness of the technology, whether it's required for individual sample preparation, or for high-throughput functionality. MF chip designs can vary widely, permitting various applications, other than formulation, for example, the micro-scaling of laboratory production, often referred to as lab-on-a-chip (LoC). The propensity of MFs for analysis and monitoring too has become an area of interest. A few therapy areas that have evolved thanks to this include point-of-care (PoC) analysis in combination also with biological microelectromechanical systems (BioMEMS [18]), disease-state progression monitoring [19] and closely mimicking *in vivo* conditions (Organ-on-a-chip) [20], as seen in **figure 2**. A common barrier to MFs has often been deemed to be its industrial scalability, however, solutions to this are currently being investigated, aiming towards increasing flow outputs whilst maintaining process quality [21].

Advantages of this technology include high levels of control of experimental conditions, coupled with high degrees of accuracy and low volumes of reagent needed. MFs and LoC are highly customisable technologies that allow for a wide range of experimental procedures. Disadvantages of the technologies include barriers to scalability, potential high costs and issues with device-reagent compatibility.

Microelectromechanical Systems

Closely coupled with LOC, MEMS devices are expanding treatment areas via coupling electrical components with a mechanical response. Integration of MEMs within various technologies e.g., MFs, has given rise to highly responsive systems capable of providing accurate therapeutic care. A poignant example of effective coupling of MEMS with other ETs comes with the use of

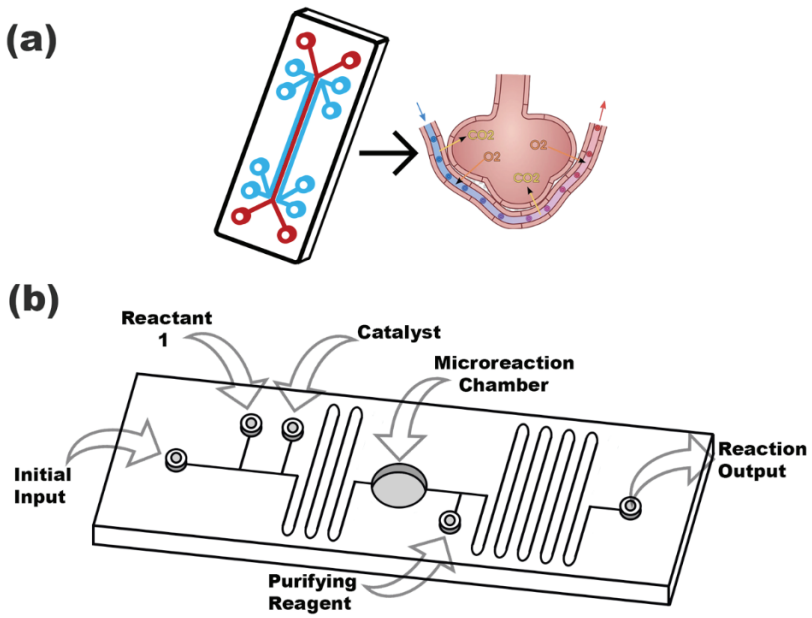

Figure 2. (a) Organ-on-a-chip technology, allowing *in vitro* mimicking of lung function oxygen transfer between vasculature and alveoli; (b) Example of the propensity of microfluidics for lab on a chip technology.

MNs for non-invasive prolonged transdermal API delivery and real-time monitoring have seen healthcare therapy in this area transform.

The combination of MN technology with BioMEMS ushers the possibility of real-time detection followed by subsequent automated pharmaceutical intervention. A simple feedback loop for insulin detection coupled with a syringe pump via a MN-MEMS array can allow for accurate detection and delivery of insulin (**Figure 3**), improving the quality of life of a diabetic patient [22].

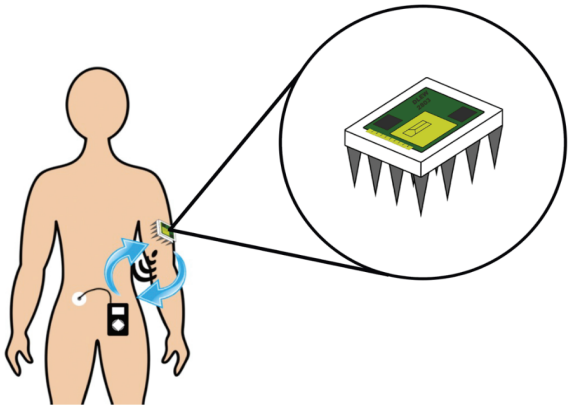

Figure 3. Graphical representation for a wirelessly communicating MN-MEMS device exchanging feedback with an insulin pump device.

MEMS devices have advantages such as portability, high sensitivity and high customisability, however, suffer from drawbacks mostly linked to economic reasons due to the high costs of research and device fabrication.

Machine Learning

Existing as a subsection of artificial intelligence (AI), ML has become an integral part of design of experiment (DoE) approaches, as well as experimental innovation [23]. ML has been used extensively for data analysis within the pharmaceutical sector [24], providing in-depth examination of big data within a reduced amount of time (**Figure 4**).

The applications of ML for pharmaceutical development include bettering experimental design, such as complimenting computer-aided design (CAD) processes, in-line analytical monitoring applications [25] and predictive modelling [24]. ML can also be viewed as improving the

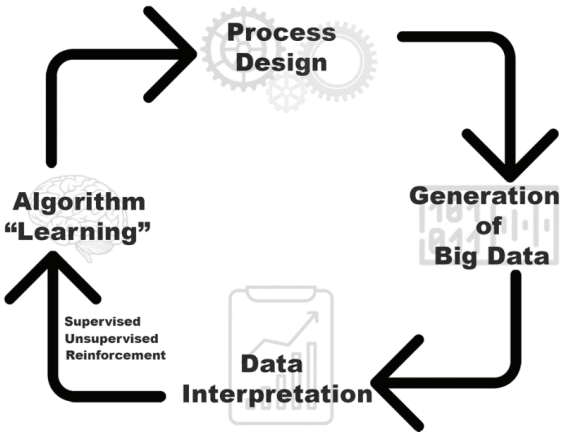

Figure 4. A typical design pathway for the active enrolment of ML within an exploratory system.

sustainability of many approaches, by helping decrease the environmental and economic burden of a technology due to pre-process analysis [17]. The recent influx of AI services on a commercial level, e.g., ChatGPT (OpenAI), is likely to open access and understanding to a wider population base, allowing for the next generation of scientists to have a greater understanding of the potential held by ML for pharmaceutical applications.

ML can process large datasets in a relatively short duration of time, however its major pitfall is the requirement for the initial production of large datasets for input, as well as the stipulation for high core-processing levels.

Conclusions

The mentioned ETs show great promise over a wide range of therapy areas. It is yet to be mentioned in this review how frequently the technologies are inter-compatible, leading to a fortification in functionality; for example, the use of AM to print highly-customised MF devices. An attribute common to all the technologies in this mini review is the high degree of customisability, which again is bridging the gap towards the final goal of a more individualised level of healthcare. The issues relating to ETs lies in their novelty, meaning that their true potential is far from being actualised, including optimisation of process parameters and key applications. For example, a highlighted goal for future healthcare is the capacity to provide individualised patient care on a wide-scale. To address this goal, factors

such as print speed for AM, or cost reduction for MEMS technology must be addressed. Scalability is also an issue associated with current healthcare technologies and especially some ETs, such as MFs. Future directions to enhance the scalability of these technologies is an issue that must be investigated, to allow technologies to truly benefit the area of healthcare. It's clear that ETs are beginning to have an impact felt by the wide scale medical field, however, further work is still needed to be performed.

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REVIEW PAPER

JMS Journal of Medical Science

Real-time quality control for chemical and biotechnological processes: a brief review

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ABSTRACT

Monitoring critical process parameters of chemical and biotechnological processes is an essential tool at every stage of drug manufacturing technology. The aim of Process Analytical Technology (PAT) is to provide effective tools, such as multidimensional data analysis, modern analytical methods, and monitoring tools, for the continuous improvement of process understanding and knowledge. Among the methods of wide interest are optical and spectroscopic techniques that can be used in the control of chemical and biotechnological processes. The selection of the appropriate method is crucial and depends on many factors, including the nature of the process, the number of variables, and analytical limitations. This review focuses on a brief and precise characterization of spectroscopic and optical methods that can be applied to monitoring and control of chemical and biotechnological processes.

Introduction

The chemical and pharmaceutical industry plays an important role in human life. In the traditional approach, the production process was controlled according to an approved protocol. Then, in order to check the quality of the completed process, compliance with the requirements of the final product was ensured by a pre-approved quality of the product itself [1,2]. The identification and monitoring of critical parameters of both chemical and biotechnological processes is an essential tool at every stage of drug manufacturing technology. The Process Analytical Technology (PAT) strategy, as defined by the Food and Drug Administration (FDA), is based on real-time process quality control. As a result, risk analysis is not limited to analysis of the final product and takes into account the variability of raw materials, materials, and apparatus during the process [3].

Process analysis technology has been defined as a mechanism for designing, analyz-

ing, and controlling pharmaceutical production processes by measuring critical process parameters that affect product quality [4]. PAT checks the quality of raw materials both physically and chemically. PAT can be employed in the transition from checking the quality of raw materials to the quality of products, by testing the chemical and biotechnological process at several intermediate stages. PAT offers a significant reduction in time and cost spent on product sampling and analysis. The main goal of PAT is to provide effective tools, such as multidimensional data analysis, modern process analyzers or analytical methods, monitoring of final processes, and control tools for continuous improvement of knowledge [5].

This review focuses on a brief and precise characterization of spectroscopic and optical methods that allow the monitoring and control of the chemical process. In the next two chapters, the various optical and spectroscopic methods are described.

The optical methods used in process analytical methods

The production of active pharmaceutical ingredients (APIs) faces various problems, including batch inconsistency in terms of particle crystal size, number of crystal particles produced, and purity profile (residual impurities in crystals or incorrect polymorphic or chiral purity). This can have a significant impact on both product quality and downstream operations of the process unit, including filtration, drying, milling and formulation of the product [1,2]. Particle size analyzers play an important role during process development and quality control of particle systems in order to develop efficient processes and achieve high final product quality. In the traditional approach, product inspection involves sampling and off-line particle size analysis. Laser diffraction, dynamic light scattering, microscopy, or sedimentation methods are used for this purpose. The application of optical PAT techniques drives efficiency in the sampling and analysis process. The ability to measure naturally occurring particles in the process improves the ability to understand, optimize, and control particle and droplet systems. Understanding precisely how process parameters impact concentration, size, shape, and structure of particles enables the scientist to make better decisions, eliminate process risks, and solve problems faster [3,4]. Process analytical technology uses different optical methods that allow the chemical process to be carried out and avoid the above-mentioned problems. The following are two most common methods used to determine particle properties.

The first one is Focused Beam Reflectance Measurement (FBRM), a technology that measures particle counts and sizes in real time based on laser light backscattering. This technique allows to determine the properties of particles in suspension, emulsion, and crystallization and to monitor the changes that occur during this process (for example from solution stage to crystal formation) [6–9]. The size, distribution, shape, and growth behavior of particles in the granulation and crystallization processes can be evaluated using the FBRM method. Due to the FBMR technique, the particle properties of the finished product can be controlled and monitored in real time [10]. For example, Sorota et al. [11] outlined the development of two robust crystallization methods that deliver purified islatravir with a controlled particle size distribution (PSD) and impurity profile. In turn, Muhaimin et al. [12] use FBRM measurements to investigate the effects of the polymer type and compare the size distributions with those obtained using other sizing methods, such as optical microscope and laser diffraction. The FBRM device is equipped with a probe that is inserted directly into the process stream at a suitable angle so that particles can easily flow through the probe window where the measurement takes place (**Figure 1**). The laser beam is guided along the probe's lead through an optical system that focuses it to a small spot on the sapphire window. The optical system rotates at a constant speed, causing the beam to scan quickly the particles passing by the window. As the particle system is scanned by the focused beam, single particles or particle structures cause laser light to be backscattered towards the detector. Clearly occurring pulses of backscattered light are detected and counted, and the duration of each pulse is multiplied by the scan rate to calculate the length of the segment that intersects each particle. This quantity is called the chord length – the basic measure of a particle related to its size. Within seconds, thousands of particles

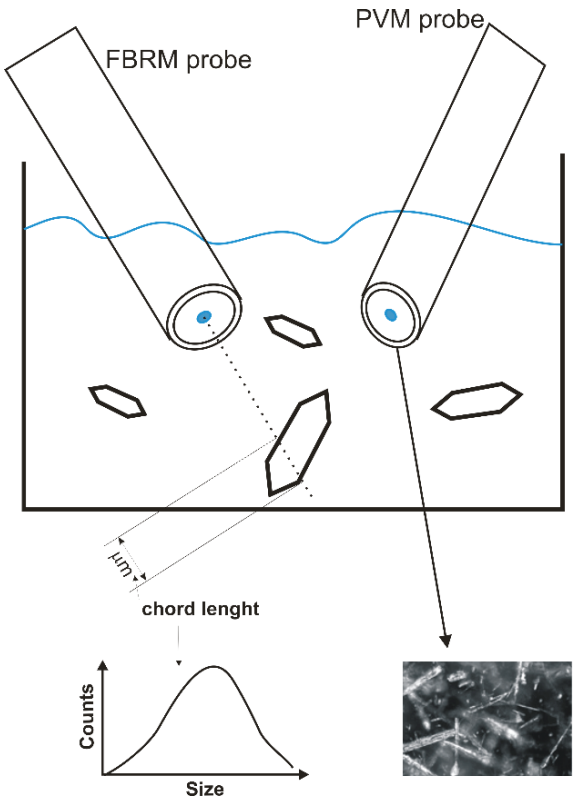

Figure 1. General schematic of the measurement using the Focused Beam Reflectance Measurement (FBRM) and Particle Video Microscope (PVM) techniques.

are counted and measured, resulting in a precise and sensitive measurement of chord length distribution, reported in real time. The chord-length distribution informs about changes in the size and number of particles from the beginning to the end of the process. Statistics on each chord-length distribution, such as the number of particles classified as fine or large, can be analyzed in terms of time trends. By varying operating conditions and tracking particles and their structures as they occur in the process, particle systems can be better understood, optimized, and controlled [13, 14].

PVM (Particle Video Microscope) is a type of online micro camera that can visually track nucleation, crystal growth, polymorphic transformation, blending, and fragmentation during a chemical process [4,9,15] or biotechnological process [16] in real time. In addition, color-related changes can be detected by endoscope probes. This kind of technology was used by Su et al. [17] to track the polymorphic transformation from the α to the β form of mannitol. Whereas Liu et al. [18] applied the PVM technique to study crystal growth and carbamazepine transformations.

The PVM-based instrument has a probe to visualize particles and particle mechanisms in real time. High-resolution images were obtained under different process conditions without the need for sampling or off-line manual analysis [9,15]. The sensitivity of the process trends to changes in particle size and concentration is automatically combined with the most relevant images (**Figure 1**), ensuring that all experiments are a simple and reliable method that combines the complete understanding of the process. High-resolution in-real-time particle imaging allows one to determine the impact of process parameters on the size, number, and shape of the particles. Particles can be designed to be predictable when key parameters change during development, scaling, and manufacturing. In addition, this fast and reliable method reduces the time, total production costs, and efforts necessary to fully understand the complex particle system and process [13,15].

In summary, it should be stated that the use of tools based on optical methods facilitates real-time process monitoring. Thanks to the FBRM and PVM methods, it is possible to track changes in the size and shape of particles during a given chemical (or biotechnological) process. This allows for control of certain processes during the crystallisation stage and introduces changes so that the production goes in the right direction.

The spectroscopic methods used in process analytical methods

Spectroscopy is a broad non-destructive analytical field that is based on the analysis of the interaction between an electromagnetic wave and an analyte via adsorption, emission, or scattering. Depending on the wavelength, spectroscopic methods can be classified as Ultraviolet-Visible (UV/Vis), Near Infrared (NIR), Mid Infrared (MIR), Far Infrared (FIR), Raman, and Nuclear Magnetic Resonance (NMR) [19]. Spectroscopic sensors integrated into upstream and downstream unit processes in *in-line*, *on-line*, or *at-line* mode enable real-time process monitoring and control. These tools are particularly important in biotechnological processes because biological drugs have a more complex structure, a less predict-

able conversion pathway, and a more dynamic nature than their small molecule counterparts [20]. Compared to optical techniques, an important aspect is also the possibility of using spectroscopic methods not only for qualitative analyzes, but also for quantitative measurements of parameters such as glucose, ammonium, lactate, and glutamate concentration, the amount of biomass or optical density [16].

NIR spectroscopy works on the principle that the atoms of molecules are in constant motion, vibrate at specific frequencies, and directly depend on the mass of each atom in the molecule and the strength of the chemical bond. Compared with IR, which detects basic vibrations, NIR measures higher-energy waves that create combined vibrations and allows the detection of weak absorbance bands where samples do not require a dilution for measurement. The advantageous high sensitivity of NIR generates spectral complexity, which is associated with the need to use multivariate statistical models to extrapolate data based on various parameters [16]. In the MIR method, the spectrum obtained can be used as a direct measurement of the components in the solution, while the disadvantage of this technique is the high interference of water, which is present in most test samples and limits accurate calibration. Raman spectroscopy provides characteristic information on molecular vibrations for analytes ranging from small molecules to biological compounds and cells. Compared to NIR or MIR, the Raman method has the advantage that water produces only a weak signal and does not overlap with peaks of interest for common analytes [21]. As a result, the Raman spectroscopic method is indicated in the literature as a potential analytical solution due to the possibility of measuring small-sized liquids, solids and gaseous samples, without prior preparation and destruction of the sample, carried out directly in reactors and bioreactors [22]. In process control, inline Raman spectroscopy in combination with multivariate statistical analysis has been identified as a potentially useful tool for advanced chemical and bioprocess development, although it has not been widely applied in industrial R&D. A solution suggested in the literature, based on the combination of Raman and IR spectroscopy as complementary methods, having different sensitivity to different functional groups, may offer an enhanced PAT approach [22,23].

In the work of Talicska et al. [24] real-time NMR analysis was utilized to characterize the reaction components in the continuously stirred tank reactor and determine the kinetics of the Grignard reaction to monitor the bromide starting material and the Grignard reagent. The possibility of using FTIR support in the hydrogenation reaction carried out in a gas-liquid flow reactor was also demonstrated. In the case of bioprocesses, NIR and Raman methods are sensitive to the content of e.g. glucose, lactate and ammonia while cell density can be determined by MIR or UV-Vis [19].

The analysis of spectra obtained by spectroscopic methods compares control samples with test samples and the identification of regions where peak changes are observed. Spectral signals are complex and require calibration to specific process conditions. At the initial stage, it is

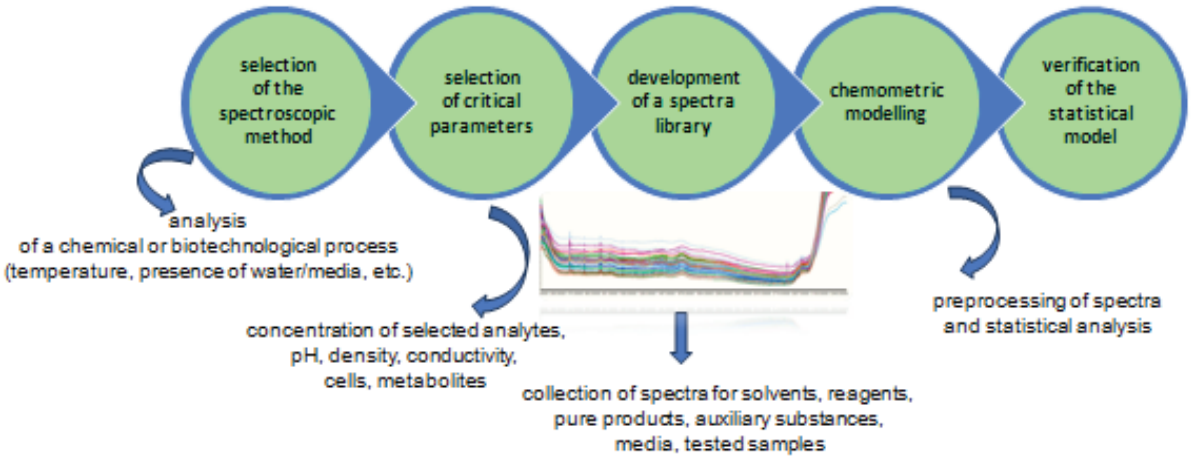

Figure 2. Scheme of PAT implementation stages using spectroscopic methods.

important to select the critical parameters (**Figure 2**). The generated spectra require preprocessing to reduce noise and other data interference. Spectral analysis can be performed using multivariate analysis methods including principal component analysis (PCA), hierarchical cluster analysis (HCA), and partial least squares (PLS). In the final stage, the developed model requires verification and calibration [25]. PAT implementation and real-time prediction of changes in critical parameters by integrating *in-line* process monitoring technology and chemometric analysis can be an important tool not only for batch processes, but also for flow systems, including reactions carried out in plug flow reactors (PFRs) and continuous stirred tank reactors (CSTRs) [24].

Conclusions

The ability to control individual operations with simple and reliable analytical tools is an important aspect in any field of technological processes. Advanced Quality by Design (QbD) tools can be used to assess risk early and predict critical product properties and process factors to improve process development and reduce costs. The emphasis on the development of control of critical process parameters is also visible in the pharmaceutical sector, where process analysis technology is an essential tool in chemical and biotechnological processes. The analysis of the literature indicates the wide possibilities of implementing optical and spectroscopic techniques as PAT tools. In addition, it is important to identify critical parameters and develop appropriate statistical models to implement accurate calibration.

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The authors declare no conflict of interest.

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REVIEW PAPER

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Energy efficient smart manufacturing of pharmaceutical solid oral dosage forms

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ABSTRACT

The global pharmaceuticals market is a trillion-dollar industry which grows more than 5% annually. However, in comparison to other manufacturing industries (e.g., oil refining, automotive), the pharmaceutical sector lags in manufacturing innovation and automation. In the production of pharmaceutical solid dosage forms, the use of energy utilization as a performance measure of production efficiency has neither been implemented extensively, nor been optimized to maximize efficiency. This study will focus on the development and implementation of a smart manufacturing platform to optimize energy productivity whilst maintaining tablet quality via the consideration of different manufacturing scenarios.

This study will consider three main unit operations (wet granulation, drying and milling) which are relatively more energy intensive in pharmaceutical downstream processing, used to produce solid dosage forms, such as tablets. Four case-studies will be considered, which are 1: baseline batch, 2: baseline continuous, 3: optimized batch and 4: optimized continuous. Smart manufacturing is implemented to present optimized cases 3: and 4: Improvements in the energy and performance metrics are quantified and compared to the baseline cases.

The smart manufacturing platform used in this study, integrates advanced process model development, optimization, technoeconomic analysis and data integration. The utilization of this framework contributed to a ~70% and ~80% improvement in energy utilization in the optimized batch and continuous cases, respectively, when compared to the baseline batch case. In the optimized cases, tablet quality was maintained within targeted specifications and was comparable to the baseline batch case. This smart manufacturing framework can be generalized for drug product manufacturing and other particulate-based industries such as food, agriculture, and fine chemicals.

Introduction & objectives

The pharmaceutical industry is currently undergoing a paradigm shift in manufacturing practices, going from batch to continuous, where improvements in product quality and healthcare are largely anticipated [1–3]. The impact of continuous manufacturing (CM) is evidenced by data

showing that on an average, product development is 3 months faster for time to approval and 4 months faster for time to market. This translates to ~$171-$537M USD in early revenue [4]. Data also shows that there are no substantial regulatory barriers to the transition from batch to CM, in terms of pre-approval inspections [4]. It should be noted that there could be additional energy consumption costs for scale-up of batch process operations which could be significant compared to continuous process operations which typically not require substantial scale-up. For optimal operation of CM processes, advanced process modeling, optimization, and smart manufacturing (SM) capabilities need to be integrated into the manufacturing platform [5–7]. The benefits of such an integrated system includes faster time-to-production of high-quality tablets which can be manufactured with minimal energy utilization [8]. The overall impact would be the development of an agile and flexible manufacturing process that is robust to market changes [1]. The overall objective of this study is to adopt a SM framework in process development phase via the use of advanced process models, sensors and data integration architecture and optimization of key pharmaceutical performance metrics such as energy and quality. The development of an advanced process modeling framework that is integrated with a smart manufacturing platform capable of data integration will ultimately lead to process intensification of production which could lead to overall reductions in cost and carbon footprint. This will be accomplished via an advanced process modeling framework that is integrated within a smart manufacturing platform.

Methods

Solid oral drug product manufacturing involves a sequence of unit operations that transforms powders into tablets. These processes start from material (active pharmaceutical ingredient and excipient) feeding and ends with tablet compaction and coating, where the final drug form is tested for its dissolution performance. The four major routes in solid drug product manufacturing are direct compaction, dry granulation, wet granulation, and spray drying (**Figure 1**). For this study, the wet granulation route was chosen due to its higher energy utilization compared to the other routes, due to the presence of larger amounts of motors, compressors and heating elements which are highly energy intensive [9]. Within the wet granulation configuration, the unit operations of granulation, drying and milling were focused on as case-studies to improve energy efficiency.

Four manufacturing cases were established for this study, all of which were performed at Rutgers University. These were cases 1: baseline batch, 2: baseline continuous, 3: optimized batch and 4) optimized continuous. Cases 1: and 2: represent the current state-of-the-art batch and continuous manufacturing processes in the pharmaceutical industry, respectively. These cases represent typical scenarios whereby the processes development is focus on achieving product quality compliance with minimal regard to any other performance metric such as energy. Cases 3: and 4: represent optimized versions of cases 1: and 2: respectively, which implemented the smart manufacturing framework involving advanced process modeling, optimization, techno-economic analysis, and data integration [5, 8]. Here we will focus on developments in advanced process modeling and data integration in a smart manufacturing platform.

Figure 2 demonstrates the advanced process modeling framework which was developed. Levels 1, 2, and 3 represents the incorporation of process parameters, material attributes and design properties in the model equations, respectively. In this study, we will implement a Level 1–2 model representation of the granulation, drying and milling unit operations.

The framework combines a modular development of intermediate, output, process and product models that are ultimately integrated to simulate product performance at each stage of the unit operations. Intermediate models are model representations that provide a quick estimation of output that is usually not measured and is only an indirect and/or partial indicator of product quality. Output models are model representations that take input from intermediate models and predict process outputs that can be measured but are not a descriptor of product quality. Product models are model representations that take input from output models and predict metrics that are direct indicators of product quality.

Subsequently, a smart manufacturing compliant data management framework (**Figure 3**) was developed. Different types of data were first collected using on/in/at/off-line methods, at the process and analytical equipment level and spectral data were pre-preprocessed via statistical algorithms to provide product quality data. These data were then sent to either an electronic laboratory notebook (ELN) or a data historian via a control platform, depending on the method of data acquisition. All data

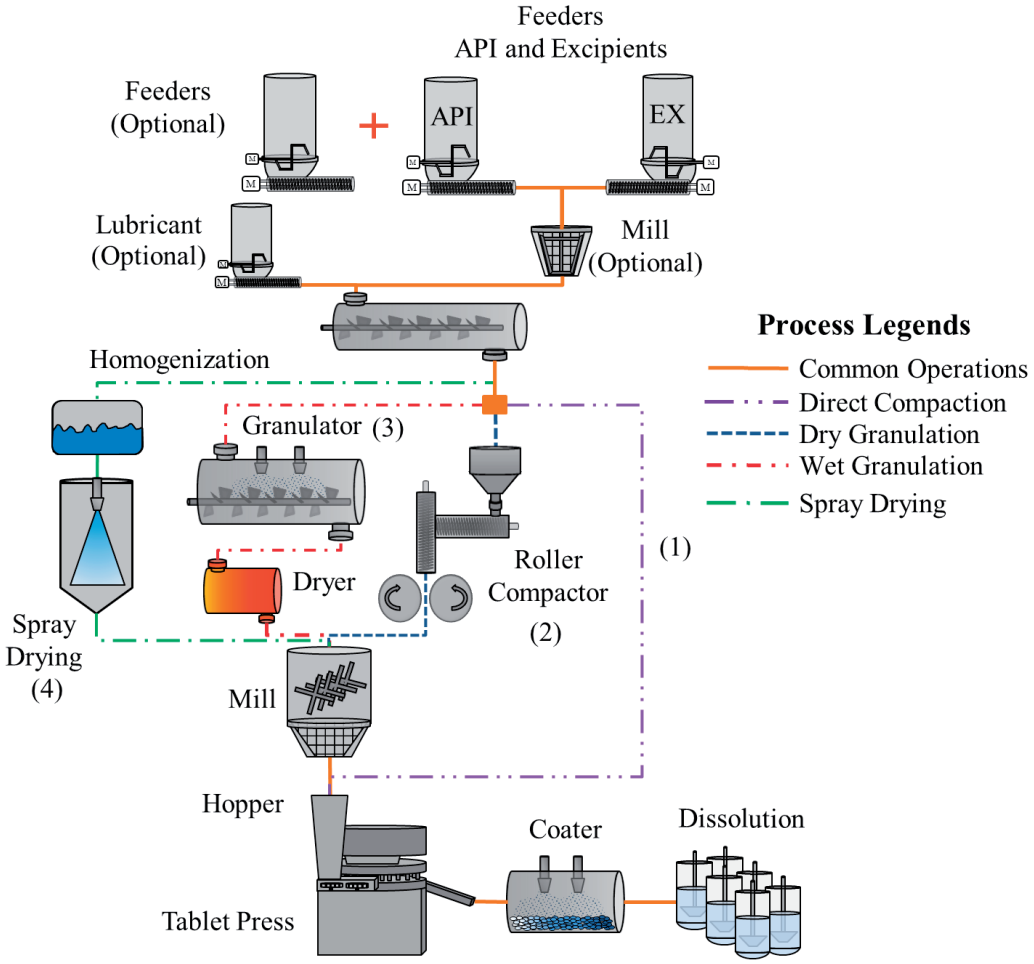

Figure 1. Schematic of drug product manufacturing routes.

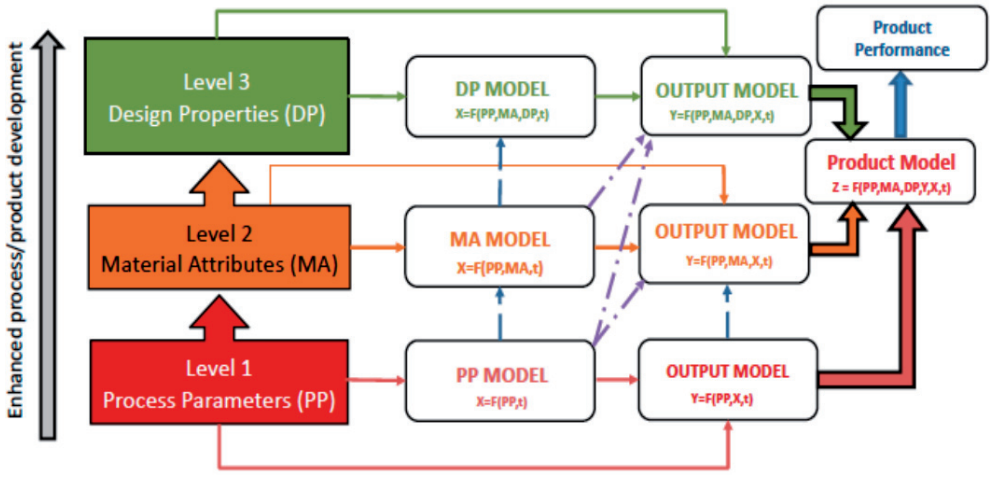

Figure 2. Advanced modeling framework.

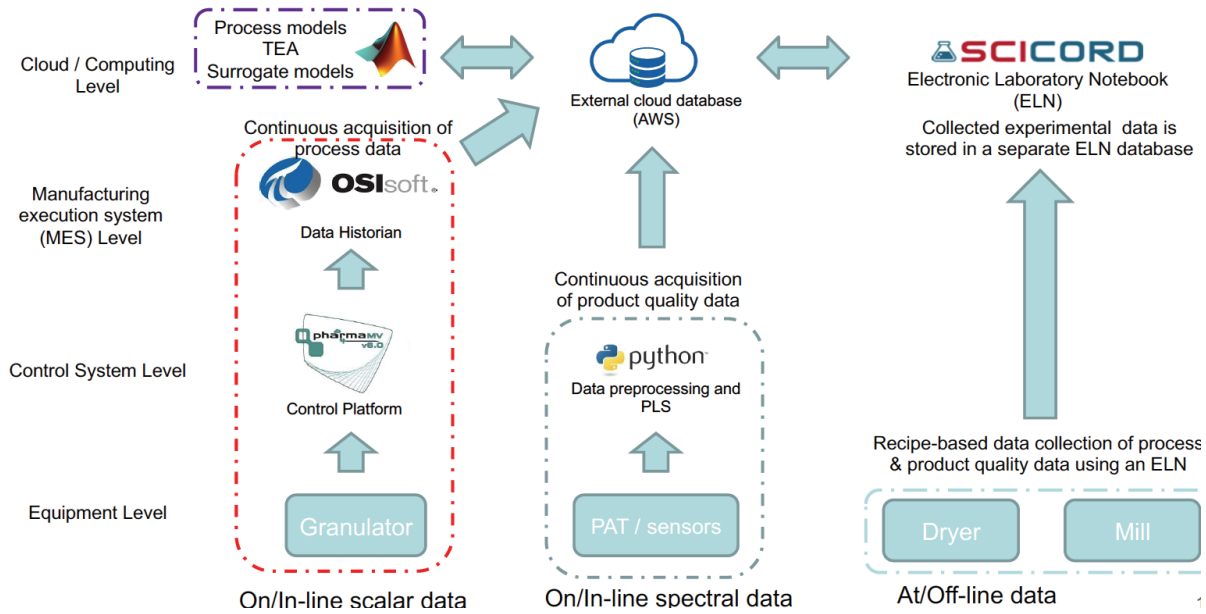

Figure 3. Smart manufacturing compliant data management framework.

was then made accessible through a cloud-based repository whereby the model, optimization and techno-economic algorithms were also implemented and deployed. In such a manner, the framework is bi-directional and can utilize in-process data for model calibration, verification, refinement etc., and the model can then be optimized. Real-time data can be accessed through the cloud, to be used for model predictions and optimization, which can provide updates to process for any necessary course correction required to continue production of tablets within desired specifications.

Results and conclusions

Results from technoeconomic analysis [5, 10] confirmed that the energy requirements for a baseline batch case is ~6K GJ/year. With the use of advanced model development and data integration framework from which optimization was performed, improvements to the energy utilization were made. The experimentally verified advanced process models, which contained both product quality and energy models, provided a framework for optimization, where the optimal operating conditions for the processes that led to energy reduction was identified. These include a 25.6% energy reduction from baseline batch to baseline continuous, a 71.7% energy reduction from baseline batch to optimized batch and an 83.3% energy reduction from baseline batch to optimized continuous. In all cases, the product yield and drug release kinetics of the tablets (demonstrated through USP standard dissolution testing) produced were similar, confirming that tablet quality was maintained. Other metrics such as time-to-market of key therapeutic drugs will also be minimized Such innovations can also be adapted to other similar manufacturing industries such as biologics, food, and fine chemicals.

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REVIEW PAPER

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Insights into solid dosage forms with nonlinear optical imaging

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ABSTRACT

Microscopic chemical and solid-state structures and their changes in solid drugs and dosage forms can profoundly affect pharmaceutical performance and patient safety. Despite this, their detailed spatially-resolved analysis can be difficult or impossible with established analytical technologies. Multimodal non-linear optical imaging presents opportunities for sensitive and specific chemical and solid-state pharmaceutical imaging. Non-linear optical imaging encompasses several nonlinear optical phenomena, including coherent anti-Stokes Raman scattering (CARS), stimulated Raman scattering (SRS), and sum frequency/second harmonic generation (SFG/SHG). Imaging in 3D with (sub)micron resolution is rapid, non-destructive, possible *in situ* in aqueous media, and generally does not require prior sample preparation. This mini-review explores several applications of non-linear optical imaging for solid drug and dosage form analysis.

Introduction

Most medicines are marketed as solid dosage forms comprised of an active pharmaceutical ingredient (API) and excipients. Their optimal therapeutic performance and safety requires understanding of numerous critical quality attributes, such as particle size and morphology, solid-state form, stability and drug release. Characterizing these attributes is comparatively straightforward with individual APIs and excipients, but much more challenging once the components are combined into a dosage form. Traditional pharmaceutical analysis techniques are often not sufficiently sensitive and/or specific for critical quality attribute analyses in solid dosage forms. One emerging analytical approach to help address this issue is nonlinear optical imaging (NLO). NLO offers chemical and solid-state specific imaging of solid dosage forms in a non-destructive, rapid,

and label-free manner, with (sub-)micron 3D resolution, either in dry or aqueous environments. In this mini-review, NLO is briefly introduced in the context of solid dosage forms and some possible related applications are considered.

Brief introduction to nonlinear optics

In general, two or more photons interact in an NLO process to create a new photon of a different frequency carrying information on the physical properties of the medium. The interaction scales nonlinearly with the incident light intensity, implying inherent confocality as any significant nonlinear process can take place only at tight focal point of a laser beam, and requiring the use of high peak power and ultrashort laser pulses in the femtoseconds to nanoseconds regime. In the sum frequency generation (SFG))/second harmonic (SHG) process, the frequency of the generated photon the sum of two incident ones and can only take place in non-centrosymmetric crystal structures, which represents a significant proportion of new small-molecule drug candidates [1]. In coherent Raman scattering, including coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS), vibrational Raman modes of the sample molecules are probed by tuning the frequency difference of two laser beams to match the desired wavenumber. Hence, the technique, with spectra exhibiting both chemical and solid-state specificity, is applicable to many sample types and is perfectly complemented by SFG/SHG. **Table 1** compares the characteristics of NLO microscopy to confocal Raman microscopy (with spontaneous Raman scattering).

Solid dosage form applications

Chemical imaging

Coherent Raman microscopy, including both CARS and SRS, are well suited to qualitative 2D and 3D imaging of component distribution in solid dosage forms such as tablets [2,3], dry powder inhalation mixtures [4], solid dispersions [5] and microparticles/granules [6–8]. As an example, the distributions of an active ingredient and the excipients at the surface of a tablet are presented in **Figure 1**, together with their SRS spectra. The spectral data was collected in approximately 3 minutes and analyzed with classical least squares to classify the pixel spectra into different components. Quantitative analysis of coherent Raman data may be performed through two general routes: analysis of the images or the coherent Raman spectra. The former is most suited to determine the particle/domain size of different components or quantification in a mixture, for example, as demonstrated by Francis et al (2018) [5]. For the latter, SRS has the advantage over CARS data of a signal intensity that is theoretically linear in the concentration. A review considering the potential, benefits and pitfalls of quantitative SRS has recently been published [9].

Solid-state imaging

Solid-state forms of the API and excipients have been imaged using both SFG/SHG and CARS/

Table 1. Spontaneous Raman and nonlinear optical imaging modalities most relevant to solid dosage form analysis

Microscopy Technique	Confocal spontaneous Raman	Coherent Raman (CARS and SRS)	Second harmonic/Sum frequency generation (SHG/SFG)
Spectral resolution	~1–4 cm^{-1}	~5–20 cm^{-1}	NA
Spatial resolution (lateral)	Variable (sub-micron to 20 mm)	Intrinsically confocal (sub-micron)	Intrinsically confocal (sub-micron)
Aquisition time	Slow, typically hours per image	Fast (video rate if single wavenumber measured)	Fast (video rate)
Chemical information content	High (whole spectrum can be recorded)	Moderate (whole spectrum can be recorded, but it is still technologically challenging)	Low (classifies crystals to symmetric/ non-centrosymmetric)
Challenges	Sample burning, fluorescence interference (sample dependent)	Sample burning, limited fluorescence interference (sample dependent)	Sample burning, limited fluorescence interference (sample dependent)

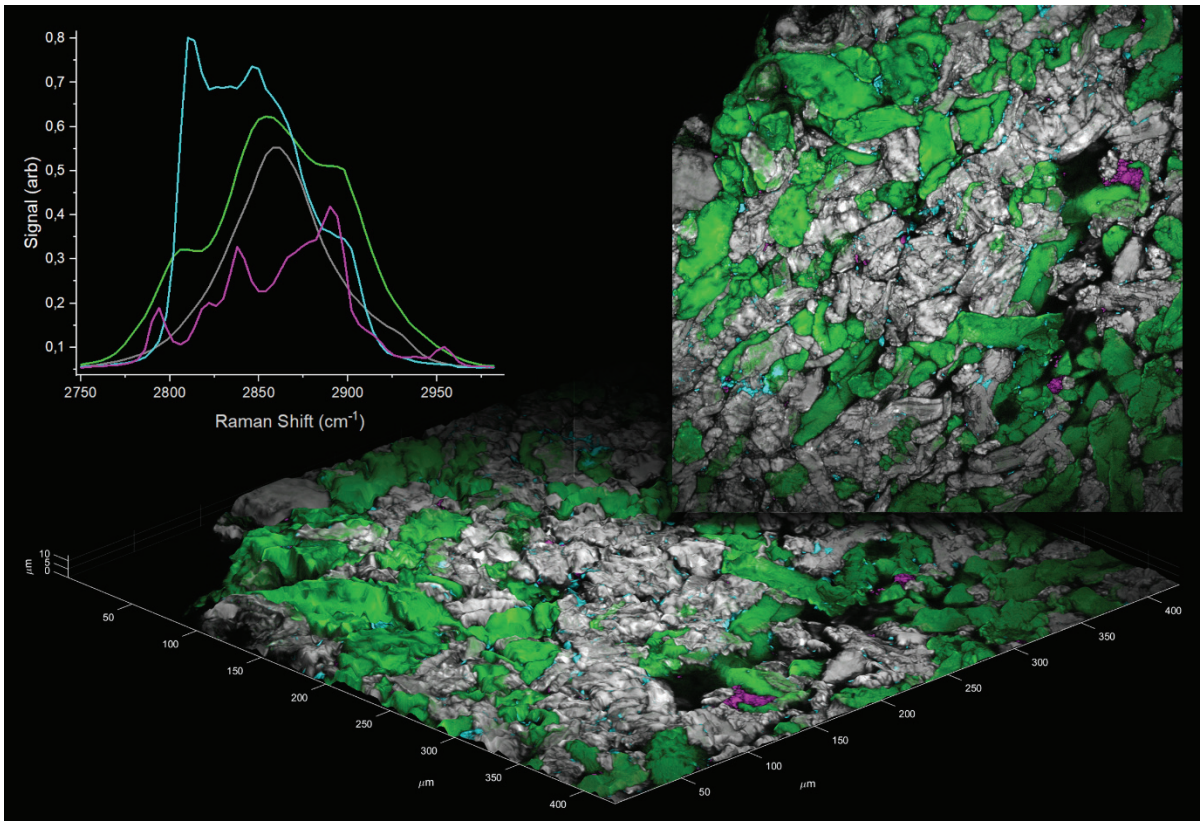

Figure 1. Example of SRS imaging of component distribution at the surface of a pharmaceutical tablet with a low-dose API. Component SRS spectra employed for classical least squares (CLS) analysis (top left), 2D composite projection (top right) and 3D projection (bottom). Green: hypromellose, grey: microcrystalline cellulose, cyan: magnesium stearate, magenta: API.

SRS. SHG imaging, which generally involves technically simpler instrumentation than CARS/SRS, has been used to detect and image crystallinity in otherwise amorphous powders and formulations subjected to different manufacturing processes [10–13]. The technique can be extremely sensitive to trace crystallinity (on the order of parts-per-million). The Simpson laboratory at Purdue University, in particular, has investigated and developed SHG imaging for solid-state pharmaceutical analysis [1,14–16]. Combining SFG/SHG with CARS/SRS can increase solid-state specificity and confidence in the analyses. For example, simultaneous multimodal CARS and SFG imaging have been used to simultaneously resolve the amorphous form and up to four crystalline forms indomethacin in compacted mixtures [17,18]. Trace levels of the forms were detectable below the sensitivity limit of x-ray powder diffraction, and infrared and Raman spectroscopies. By virtue of the chemical/solid-state specificity, rapid measurements and sub-micron spatial resolution, NLO imaging is highly suited for detecting trace levels of, for example, solid-state forms of low dose APIs in formulations.

Stability analysis

NLO imaging is well suited to the analysis of (surface) transformations in pure drugs and excipients, as well as in formulations. SHG/SFG and CARS/SRS can be used to detect subtle solid changes at an earlier stage than conventionally applied solid-state analysis methods, and in principle can detect in the parts-per-million range [16–19]. By virtue of both the spectral and spatial resolutions, crystallization of multiple solid-state forms may be efficiently detected in a single sample, especially when multimodal non-linear optical imaging is employed. For example, Novakovic *et al* detected the simultaneous crystallization of amorphous indomethacin into four different polymorphs [18].

Drug release and dissolution

A great advantage of NLO imaging in the context of drug release analysis, is the ability to rap-

idly and non-destructively image formulations *in situ* in aqueous environments. This benefit has allowed, for example, *in situ* non-linear optical imaging of drug release from sustained release formulations [5] and solid-state changes in dissolution media [20,21]. The speed of NLO imaging (video-rate to minutes) means there is tremendous potential to further explore important and otherwise hard to detect physicochemical phenomena that occur in solid dosage forms and the surrounding media upon drug release.

Conclusions

NLO imaging is well suited to elucidating solid dosage form structure and behaviors. Potentially valuable applications include high-resolution chemical- and solid-state imaging of low-dose formulations, detection of trace- and surface components/changes as a function of production and storage, and *in situ* examination of physicochemical phenomena upon drug release. Increased availability of the technique and awareness of its analytical possibilities and pitfalls, will help its potential to be realized in pharmaceutical industry and academia.

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REVIEW PAPER

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Recent advances in drug substance development – prodrug strategies for enhancing the bioavailability and potency of antiviral nucleosides

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ABSTRACT

Bioavailability is a prerequisite for drug activity. *In vivo* bioavailability (intestinal permeability), linked to drug substance solubility and drug product dissolution, became the basis of Gordon L. Amidon's Biopharmaceutical Classification System. One method of improving the drug substance's bioavailability is to modify its structure chemically, leading to increased lipophilicity and the ability to penetrate the phospholipid bilayer of the cell membrane. These modifications, known as prodrug strategies, involve derivatizing the drug substance by introducing substituents that reduce the hydrophilicity of the molecule. The present mini-review outlines the examples of Christopher McGuigan's prodrug strategies used to obtain antiviral nucleosides with enhanced bioavailability and activity. These strategies primarily involve forming and optimizing the structure of esters and amino acid esters, phosphoramidates, octadecyl phosphates, and *bis*-pivaloxymethyl phosphates. The review discusses the optimization of the phosphoramidate prodrug moiety of the SARS-CoV-2 antiviral nucleoside remdesivir in detail. It presents the resulting improvement in bioavailability and antiviral activity. Moreover, it focuses on the modern prodrug strategy as one of the major recent advances in drug substance development. This strategy effectively optimized physicochemical properties and improved the functional activity of the existing drug substances and drug substance candidates for the first time.

Introduction

Modern drug research has allowed the development of safe low-molecular compounds with high activity and selectivity [1]. However, several new compounds do not show the appropriate physicochemical properties [2] required for active drug substances. The high effectiveness of these substances *in vitro* is limited *in vivo* by their low solubility and bioavailability [3] and, thus, their poor

ability to penetrate the phospholipid bilayer of cell membranes. In addition to special pharmaceutical formulation techniques [4], the unfavorable physicochemical properties of these substances are currently eliminated through their chemical modifications [5]. These analogs are called prodrugs [6], as inactive derivatives of drug substances to be activated by endogenous enzymes. Until recently, the modifications have involved the conversion of drug substances into simple salts of inorganic or carboxylic acids [7], which improved solubility without affecting their activity.

The development of new drugs remarkably accelerated during the SARS-CoV-2 pandemic [8]. The search for new antiviral drugs has so far focused on derivatives of natural nucleosides [9] as potential inhibitors of replication [10] of the viral nucleic acids. As a result of the repositioning of these substances, synthetic nucleosides active also against the SARS-CoV-2 virus were rapidly obtained [11]. As highly polar compounds, however, they did not penetrate sufficiently cell membranes. It was, therefore, necessary to modify their structures to ensure adequate lipophilicity while maintaining antiviral activity.

New prodrug strategies, especially for polar substances with low lipophilicity, such as nucleosides, developed within the last three years, allowed not only a significant improvement in physicochemical properties but also a significant increase in their activity [12] for the first time. Therefore, the present mini-review focuses on the selected aspects of the modern prodrug strategy as one of the significant recent advances in drug substance development.

Amidon's Biopharmaceutics Classification System (BCS)

In his BCS concept (**Figure 1**), Gordon Amidon from the College of Pharmacy, the University of Michigan, proposed correlating *in vitro* drug product dissolution and *in vivo* bioavailability [13]. The correlation was based on recognizing that drug dissolution and gastrointestinal permeability (GI) are the fundamental parameters controlling the rate and extent of drug absorption. He used a transport model and human permeability to estimate *in vivo* drug absorption. BCS became widely accepted in the academic, industrial, and

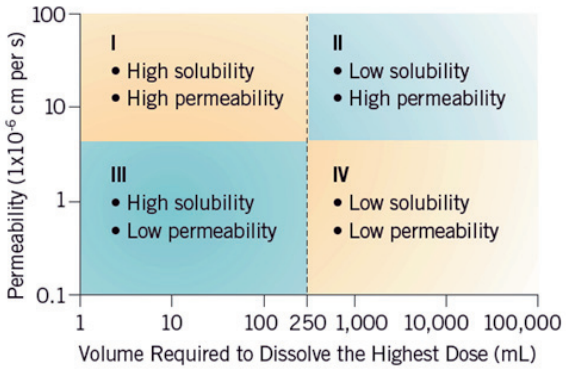

Figure 1. Amidon's Biopharmaceutics Classification System (BCS).

regulatory areas [14]. The major challenge is now moving a drug substance from the BCS Class IV of low solubility and low permeability to at least the BCS Class II of low solubility but high permeability. One of the chemical approaches to achieve this aim is briefly discussed below.

McGuigan's ProTide strategy for nucleoside prodrugs

ProTide procedure, developed by Christopher McGuigan from the School of Pharmacy of the University of Cardiff (**Figure 2**), is a prodrug approach for efficient intracellular delivery of synthetic nucleoside monophosphates and monophosphonates [15]. In this approach, the hydroxyl groups of the monophosphate or monophosphonates are masked by the aromatic group and the ester group of the amino acid [16]. The

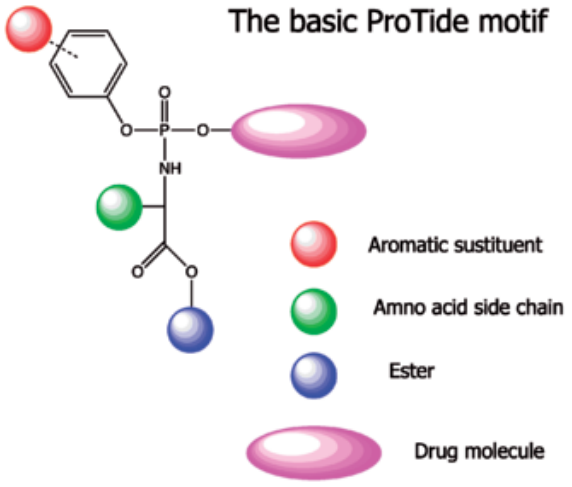

Figure 2. McGuigan's ProTide strategy for nucleoside prodrugs.

masking is designed to increase the lipophilicity of the synthetic nucleoside to enhance its penetration through the phospholipid double layer of the cell membrane. The masking groups are enzymatically cleaved inside the cells to release free nucleoside monophosphates and monophosphonates. The procedure represents the culminating achievement of medicinal chemistry research in the past few decades. The nucleoside drug substances must be converted by endogenous enzymes into the respective triphosphates for further incorporation into the viral nucleic acids. However, the first phosphorylation step is very slow, resulting in a low potency of the drug substance [17]. Protected phosphoramidates already introduce a phosphate moiety into the structure of a nucleoside to avoid the slow step. Other groups attached to the phosphate neutralize the two negative charges of the phosphate group, thus facilitating its permeability through cell membranes and oral bioavailability. Removal of the masking groups exposes the nucleotide phosphate to the next two phosphorylation. Since its discovery, the technology has been widely used in drug research. Most recently, it has led to the discovery of two FDA-approved ProTide antiviral drug substances, vis., sofosbuvir [18], and tenofovir alafenamide [19] (**Figure 3**). ***Sofosbuvir*** is a defective substrate of NS5B protein, inhibiting the viral RNA polymerase. It is orally used against the hepatitis C virus (HCV). ***Tenofovir alafenamide***, a PROTIDE analog of tenofovir, is an orally active hepatitis B virus (HBV) nucleotide reverse transcriptase inhibitor for therapy in HIV/AIDS and chronic hepatitis B. It is more antivirally active and better distributed into lymphoid tissues than the previously developed tenofovir disoproxil. ***Remdesivir*** is a phosphoramidate analog of a derivative of adenosine nucleoside [20]. It is a PROTIDE product readily diffusing into the cell. There, it is converted into monophosphate by CES1 and CTSA esterases and HINT1 phosphoramidase. As other nucleoside phosphoramidates, it is further phosphorylated to the respective triphosphate by nucleoside-phosphate kinases. *In vitro*, remdesivir penetrates the cell in its original form of phosporamidate. However, the pharmacokinetic analysis revealed premature serum hydrolysis of remdesivir *in vivo*, contributing to its relatively low antiviral potency. ***Dioxolanyl brivuridine phosphoramidates*** (DBPs) are derivatives of antiviral brivudine [21], an analog of

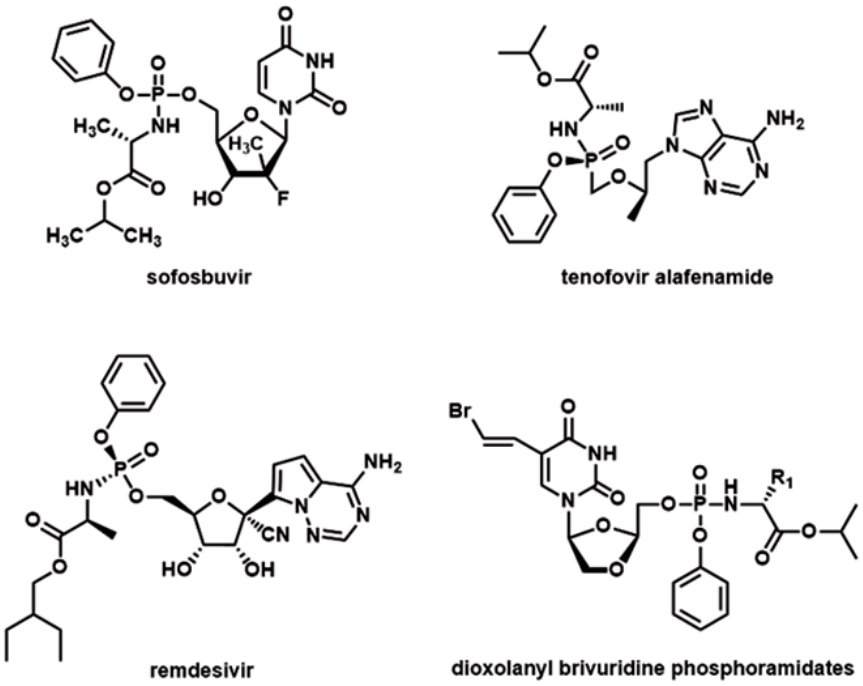

Figure 3. ProTide antiviral drug substances.

thymidine nucleoside. Its active metabolite, brivudine triphosphate, is incorporated into the viral DNA, inhibiting viral DNA polymerases. Brivudine is used orally against herpes zoster. The bromovinyl hydroxymethyl dioxolanyl analog of brivudine, **L-BHDU** is significantly active against the varicella-zoster virus. It was further structurally optimized by introducing various prodrug moieties.

Current phosphoramidate prodrug strategy

Unfortunately, **L-BHDU** is insufficiently lipophilic to penetrate nerve cells. Thus the very recent optimization of the nucleoside prodrug structures [21] included not only phosphoramidates of bromovinyl hydroxymethyl dioxolanyl uracil (L-BHDU, dioxolanyl analog of brivuridine) but also long-chain phospholipids and phosphate esters (**Figure 4**). *In vitro*, most of these prodrugs exhibited significant anti-varicella zoster virus (VZV) activity. Monophosphate ester prodrugs (POM-L-BHDU-MP and POC-L-BHDU-MP) and long-chain phospholipids (octadecyloxyethyl ODE-L-BHDU-MP) were antivirally active similarly as the starting L-BHDU. However, pharmacokinetics revealed that monophosphate ester prodrugs (POM-L-BHDU-MP) exhibited a 2.2-fold increase in oral absorption/availability compared to the parent L-BHDU. Long-chain monophosphate phospholipids (ODE-L-BHDU-MP) and monophosphate ester prodrugs (POM-L-BHDU-MP and POCL-BHDU-MP) are potentially antivirally active. Therefore, they are examined as novel anti-VZV agents. Current studies of these monophosphate prodrugs are directed to clinically relevant systems to select potential clinical candidates.

The premature hydrolysis of ***remdesivir*** resulted in limited efficacy and low accumulation in the lungs as the target tissue, encouraging further structure optimization of the remdesivir ProTide moiety. The structure of both substituents at the phosphate group was optimized using various aryl groups and long lipid chain substituted at the amino acid protecting group [22]. The lead analog **MMT5–14** (drawn below) resulting from this study showed reduced premature hydrolysis in plasma. That resulted in a 2- to 7-fold higher antiviral activity in four variants of SARS-CoV-2. The optimized MMT5–14 was 3- to 8-fold more stable than remdesivir in the plasma and liver microsome. It showed 200- to 300-fold increased prodrug concentration in the plasma and lungs, 5-fold enhanced lung accumulation of the active metabolite (remdesivir-TP), and 4- to 25-fold increased intracellular uptake and activation in lung epithelial cells. The optimized analog is a new potential antiviral drug to treat COVID-19 patients with severe symptoms.

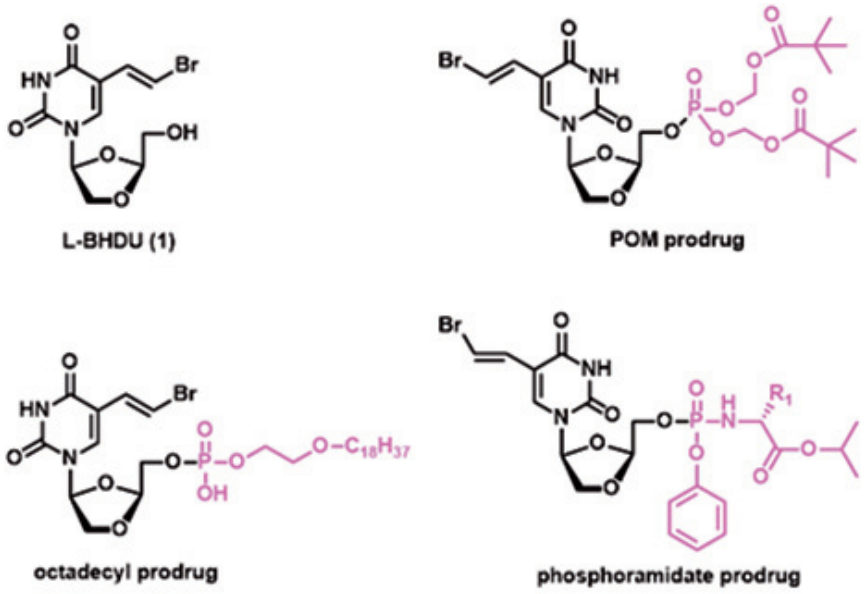

Figure 4. Nucleoside prodrug structures.

Summary and future directions

The prodrug strategy for nucleosides (PROTIDE) uses an inactive derivative (***phosphoramidate***) to ensure oral bioavailability and improved pharmacokinetics of a drug substance. The first two phosphoramidate nucleosides (sofosbuvir and tenofovir) have already been introduced to antiviral therapy. Optimization of the phosphoramidate moiety of remdesivir resulted in a more hydrolysis-resistant analog showing much higher antiviral activity, fundamentally increased prodrug concentration in the plasma and lungs, and enhanced accumulation of the active metabolite in the target tissue. The optimized analog is considered a new potential antiviral drug to treat COVID-19 patients with severe symptoms. Not only phosphoramidates but also other monophosphate esters were successfully introduced as prodrugs of therapeutic nucleosides and showed improved pharmacokinetics and antiviral potency. Considering the current progress in this field, prodrug strategy is among the most important achievements in drug substance development. The development of a new drug substance might not be sometimes necessary, as the prodrug strategy already improves the physicochemical properties, plasma stability, functional activity, and target tissue concentration.

Footnote

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Conflict of interest statement
The authors declare no conflict of interest.

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REVIEW PAPER

JMS Journal of Medical Science

Effect of moisture on solid state stability

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ABSTRACT

Water is omnipresent during pharmaceutical product manufacturing and may interact with the drug substance, excipients, and the drug product in either solvent or vapor form, resulting in several physico-chemical changes, ultimately affecting product performance. Therefore, understanding the mechanisms behind such moisture-induced changes is necessary at every stage of pharmaceutical development and manufacturing to obtain the target formulation.
Characterization tools, such as water sorption, spectroscopy, thermal analysis, diffraction, and more sophisticated approaches like simulations and PAT techniques, can help in the selection of the appropriate solid form, manufacturing method, excipients, and storage conditions, enabling the manufacturing of a stable drug product formulation.

Introduction

Water plays a critical role in the development, manufacturing, and storage of drugs. It influences the physicochemical and microbiological properties of the active drug substance, excipients, and the final drug product. The physical impacts of these interactions are: (i) changes in molecular properties, (ii) changes in bulk properties, and (iii) variations in final product performance. Additionally, water may contribute to changes in chemical stability, such as degradation and the formation of impurities. Consequently, all these factors can shorten the shelf life of pharmaceutical products. Therefore, it is absolutely necessary to understand the fundamentals of such interactions, which is imperative for manufacturing a robust drug product [1].

Zografi and co-workers [2] have shown how different physical states of water in solids, ranging from sorbed water to bulk water, affect their physicochemical properties. Consequently, not all water molecules in the solid should be treated as equivalent, as they do not affect the physicochemical properties of the solid in the same manner, rate, and extent.

Figure 1 below summarizes the main potential interactions of water with APIs, excipients, and drug products [1].

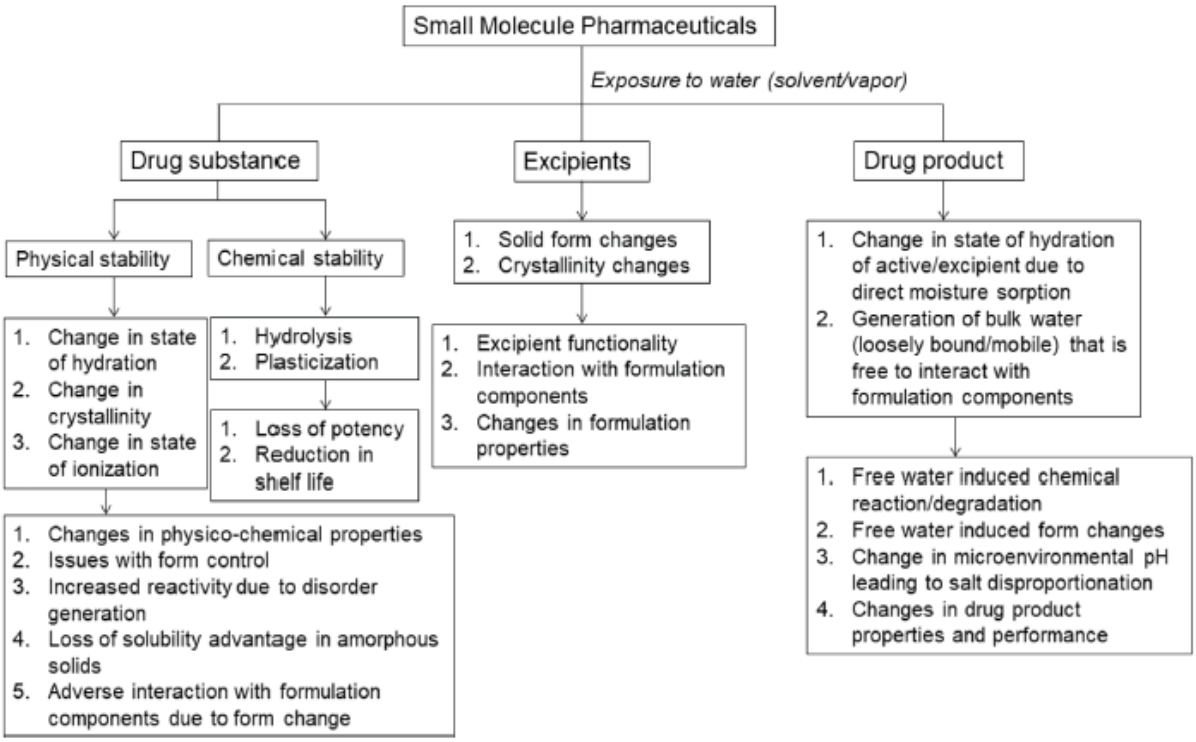

Figure 1. Water-solid interactions encountered during the drugs manufacturing [1].

Water-solid interactions in active pharmaceutical ingredient (API)

2.1. Chemical stability

Moisture-mediated chemical reactions, leading to the decomposition, particularly of active ingredients, and subsequently shortening the shelf life, are among the primary concerns during drug product development. Water not only acts as a reactant but can also participate as a reaction medium, catalyst, or plasticizer by enhancing the molecular mobility of the compounds, making them more reactive. It is essential to note that only mobile water (high water activity, a_w), present during processing or released inside the formulation components, is responsible for degradation reactions [3].

In a study [4] assessing the chemical stability of Levothyroxine Sodium Pentahydrate (LSP) tablets under increased temperature and humidity conditions, it was observed that hygroscopic excipients (e.g., povidone, crospovidone) were not suitable. These excipients release the sorbed water, leading to extensive LSP degradation.

Highly soluble crystalline salts are susceptible to chemical instability caused by deliquescence, i.e., the transition from the solid to the liquid state when the ambient relative humidity (RH) exceeds a critical threshold value (RH_0). Such salts, e.g., ranitidine hydrochloride, exhibit higher degradation rates when stored at RH > RH_0. Furthermore, including deliquescent actives and/or excipients with low RH_0 in the formulation can lead to the condensation of water vapours during storage, initiating or accelerating the degradation of actives [5]. Acetylsalicylic acid (Aspirin) is another moisture-sensitive drug susceptible to hydrolysis from the free water released by some excipients, like microcrystalline cellulose or dibasic calcium phosphate dihydrate [6].

Physical changes

The interactions change in physical stability typically refers to a modification in the solid form:

i) Change in the state of hydration: hydrate formation can affect solubility, dissolution rate, surface area and its energy, and mechanical properties. This may alter in-vivo exposure and bioavailability of APIs [7]. For example, it was shown that the formation of theophylline monohydrate upon storage led to the dissolution failure of tablets due to a lower dissolution rate of the hydrate [8].

ii) Change in crystallinity: in an effort to improve solubility and dissolution rate, and so the bioavailability, actives are increasingly prepared in the amorphous state. The essential property of this energetically higher state is to convert back to the more stable the crystalline and loss of all amorphous state benefits.

As an example, when amorphous (glassy) griseofulvin [9] gained only 0.75% of water via ad(ab)sorption, it transformed into the crystalline phase within 12 hours (**Figure 2**) [9].

iii) Change in the state of ionization: APIs exist either as weak acids or bases and are, however, often delivered as salts to improve solubility, dissolution, and physicochemical stability. In these salts is a risk of disproportionation, i.e. the conversion of the ionized form to the neutral form via proton transfer. This results in the loss of the ionized state benefits. Such conversions, can be triggered by small amount of water. Exposure to the moisture is possible during manufacturing, storage, and shipping and represents a concern for the quality of the drug product.

David A. Hirsh et al. [10] reported about such conversion with pioglitazone HCl (PiogHCl) in mixtures with metallic stearate excipients. ^{35}Cl solid-state nuclear magnetic resonance was used to detect the conversion's degree in formulations. Mixtures with Na-St and Mg-St, at 75% RH and 40 °C for 5 days only, produced the conversion of PiogHCl into Piog for about 7% and 15%, respectively [10, 11].

Water-solid interactions during the formulation process and in the formulation

During tableting, various defects may be present in the ejected tablets. To investigate the ability of formulations to consolidate under pressure, some equations, such as Heckel, have been developed. These equations are valuable for preparing optimal formulations; however, they do not provide insight into the phenomena occurring in the powder during compaction. To address this, simulation models based on the finite element method (FEM) have been developed to analyse the powder behaviour during compaction, allowing for a better understanding of the compression.

In the study [16], the authors investigated the sticking phenomena during the compaction of powder by simulating powder temperature and moisture behaviour/change. The simulation of 3D powder temperature distribution showed that, at the end of compaction, a similar temperature (36–40°C) was observed at the tablet's interfaces, but the core's temperature remained higher (about 46°C) [16]. Due to this temperature gradient, water migrates from the core to the tablet surfaces. The simulation of this is shown in **Figure 3**.

The tablet edges appear moister because of the higher powder density, which slows down the diffusion of water molecules into the surrounding area. Additionally, at the gaps between the compression tools and the tablet, capillary conden-

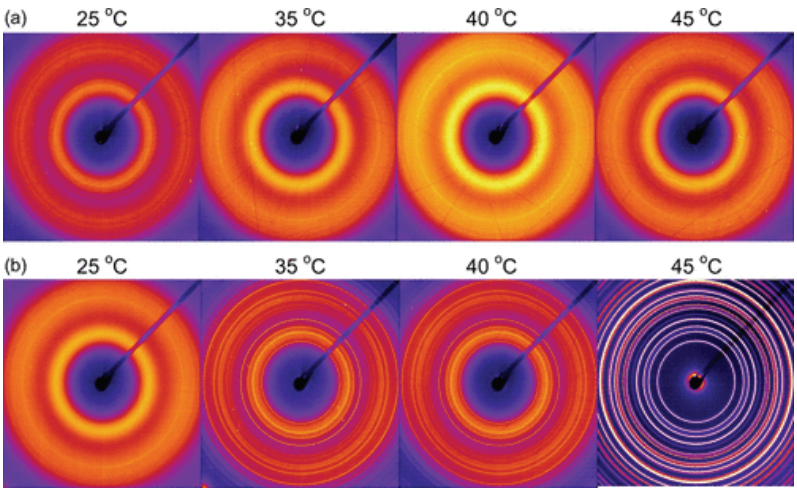

Figure 2. Synchrotron XRD patterns of amorphous griseofulvin after 12 h of storage at the indicated temperature. (a) Dry powder and (b) sample containing only 0.75% w/w water [9].

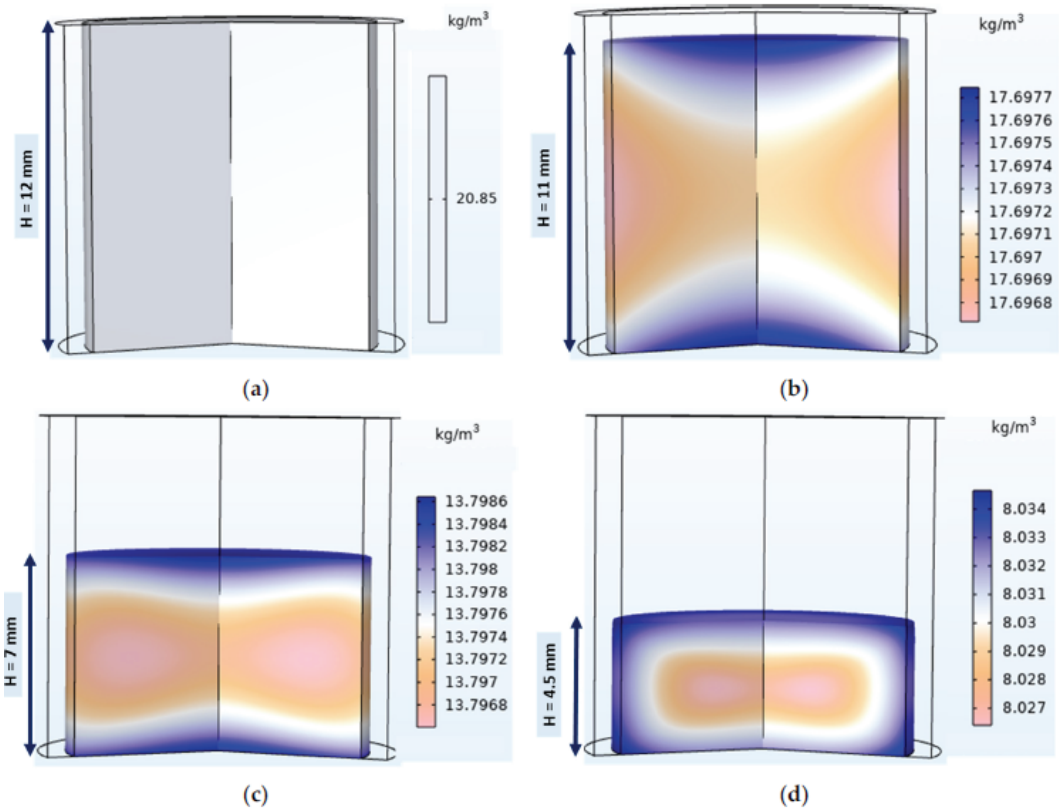

Figure 3. 3D view of the simulated moisture distribution during compaction of model powder (MCC VIVAPUR PH101) with flat-faced punches. The compaction process progresses from (a–d) [16].

sation occurs. All of these factors might be the source of serious problems: sticking, chipping, cracking, and capping [16].

The simulation results were validated using a thermal infrared camera (to monitor temperature evolution) and NIR sensors (for water distribution) [16].

Conclusion

Water molecules, either as a liquid or vapor, interact with solids in many ways, affecting their physico-chemical properties. Many of these interactions are often unknown and unpredictable; however, they are important for the drug product quality.

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Conflict of interest statement
The authors declare no conflict of interest.

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REVIEW PAPER

JMS Journal of Medical Science

Impact of ORBIS on public policies – open consultations of draft regulatory documents and the Pharmaceutical Strategy for Europe

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ABSTRACT

Public policies and regulations strongly influence research and manufacturing in pharmaceutical sector. Therefore, it is of critical importance that these policies and regulations are of high quality as well as appropriately balanced between general rules and detailed solutions. The process of public consultations prolongs adoption of novel documents. On the other hand, comments from different stakeholders like academia, industry, public administration and patients allow 360-degree critical evaluation of the document and a better understanding of the topic.

This mini-review summarizes the contributions of numerous members of ORBIS project team in open consultations of draft regulatory documents published by European Medicines Agency (EMA) and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ORBIS project feedback on the Pharmaceutical Strategy for Europe is also presented.

ORBIS project members contributed to open consultations of two ICH draft guidelines, and three EMA draft documents. ORBIS project was also active during the European Commission's efforts to develop Pharmaceutical Strategy for Europe.

The interaction between representatives of academic and industrial sectors allowed to form balanced comments. We hope that this paper will inspire more researchers to participate in future open consultations on public policies.

Introduction

Public policies and regulations strongly influence our lives, including research and manufacturing in pharmaceutical sector. Therefore, it is of critical importance that these policies and regulations are of high quality as well as appropriately balanced between general rules and

detailed solutions. The well-established democratic approach in Europe promotes discussion over enforcing rules by the empowered person or institution like it is practiced in authoritarian countries. The democratic approach is at its full display during the open consultations when different stakeholders – like academia, industry, public administration and citizens – may express their opinion. The 360-degree critical evaluation leads to a substantially better understanding of the topic. The public consultation process is definitely more time-consuming than the authoritarian approach, but allows to form wise and practical documents which stay up-to-date for a long period of time. And not only in the case of pharmacy, these documents form a stable base for the development of the research areas and industrial sectors.

This mini-review summarizes the contributions of numerous members of the ORBIS project team – listed in **Table 1** – in the open consultations of the European and international draft regulatory documents. Most of them are associated with biopharmacy (ORBIS Work Package 3): bioanalysis, pharmacokinetics and bioequivalence [1-5]. The list also includes the Pharmaceutical Strategy for Europe [6] developed by the European Commission. More information about ORBIS may be found at project website at https://orbis-project.eu.

Members of the ORBIS project team contributed to open consultations by the European Medicines Agency (EMA), the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the European Commission (EC) as specified in **Table 1**.

Table 1. Listing of documents under public consultations and authors contributing to ORBIS comments.

Ref.	Topic	ORBIS members involved*	Year
[1]	EMA Pharmacokinetics and pharmacodynamics in the obese population	G. Garbacz (PHY) F.K. Główka (PUMS) E. Gniazdowska (PRI) M. Karaźniewicz-Łada (PUMS) P.J. Rudzki (PRI) E. Szałek (PUMS)	2018
[2]	EMA Quality and equivalence of topical products	M. Kaza (PRI) B. Michniak-Kohn (RUTG) B. Milanowski (PUMS) T. Osmałek (PUMS) E. Pesta (PRI) P.J. Rudzki (PRI)	2019
[3]	EMA Lapatinib product-specific bioequivalence guidance	E. Gniazdowska (PRI) P.J. Rudzki (PRI)	2018
[4]	ICH guideline M10 on bioanalytical method validation	K. Buś-Kwaśnik (PRI) E. Gniazdowska (PRI) M. Karaźniewicz-Łada (PUMS) M. Kaza (PRI) P.J. Rudzki (PRI)	2019
[5]	ICH guideline M13A on bioequivalence for immediate-release solid oral dosage forms	O. Czerepow-Bielik (CLN) D. Danielak (PUMS) A. Gierczak-Pachulska (CLN) I. Grabnar (UL) M. Romański (PUMS) P.J. Rudzki (CLN)	2023
[6]	Pharmaceutical Strategy for Europe	A. Dumicic (ZNT) J. Lulek (PUMS) P.J. Rudzki (PRI) L. Tajber (TCD) A. Voelkel (PUT)	2020

* listed alphabetically
CLN – Celon Pharma SA (Poland), PHY – Physiolution GmbH (Germany), PRI – Pharmaceutical Research Institute (Poland), PUMS – Poznan University of Medical Sciences (Poland), PUT – Poznań University of Technology (PUT), RUTG – Rutgers, the State University of New Jersey (US), UL – University of Ljubljana (Slovenia), TCD – Trinity Collage Dublin (Ireland), ZNT – Zentiva a.s. (Czechia)

Pharmacokinetics and pharmacodynamics in the obese population [1]

As most pharmacokinetic studies are conducted in healthy subjects of normal weight, we agreed that more pharmacokinetic data is needed for rational decision-making and optimized therapy in the obese population. We have drawn attention to:

- inclusion of obese subjects in a bioequivalence study, especially for drugs intended for the obese population;
- studying pharmacokinetics and pharmacodynamics only in the obesity class III subjects and extension of the study to classes I and II in the case of significant differences in relation to normal weight;
- addition of a decision scheme to determine the need to study pharmacokinetics and pharmacodynamics in obese patients;
- providing well-defined criteria for the degree of obesity in pediatric populations.

We proposed expanding information on oral drug interactions and gastrointestinal transit conditions with emphasis on gastric emptying, concomitant pharmacotherapy, and neuropathies affecting pharmacokinetics in the obese population.

Quality and equivalence of topical products [2]

We welcomed the idea of guideline on equivalence of topical products as specific regulatory procedures for these products are lacking or are dispersed in other documents. We have commented on:

- dermal microdialysis as a possible replacement of clinical equivalence studies for topical products due to its similarity to bioequivalence with pharmacokinetic endpoints;
- lack of recommendations regarding homogeneity testing, nanoparticles-loaded products, virtual bioequivalence approach and reporting;
- need for specific recommendations and methodology of the rheological properties comparison between comparator and test products as well as the equipment/instrumentation used for dissolution testing and *in vitro* drug release testing.

Product-specific bioequivalence guidance for lapatinib [3]

This was limited to only one ORBIS beneficiary. The comments were related to evaluating bioequivalence in both fasting and fed conditions [7].

ICH guideline M10 on bioanalytical method validation [4]

This guideline was a long-awaited step towards the global unification of bioanalytical regulatory recommendations. Numerous recommendations were appreciated [8]:

- extrapolating stability at -20°C to lower temperatures for small molecules;
- detailed description for endogenous compounds;
- table specifying reporting.

We proposed specific comments regarding the matrix effect and incurred sample reanalysis in line with ORBIS publications [9, 10].

ICH guideline M13A on bioequivalence for immediate-release solid oral dosage forms [5]

Harmonization of bioequivalence recommendations by the ICH is an important step to increase the supply of high-quality medicines for the global community of patients. Our comments underscored the significance of:

- minimizing the number of bioequivalence studies to avoid the unnecessary exposure of healthy subjects to clinical trials;
- implementing decision schemes to establish a standardized interpretation;
- providing clarifications, well-defined criteria and resolving terminology discrepancies to achieve clarity and accuracy;
- adding recommendations on sampling points, data exclusion criteria, and statistical analysis methods.

Our collective comments and recommendations will be presented in a future paper.

Pharmaceutical Strategy for Europe [6]

We agreed that the provision of safe and affordable medicines to European patients is of critical importance and highlighted the following topics [11]:

- better cooperation between the industrial and academic sectors;
- manufacturing of active pharmaceutical ingredients (APIs) for essential generic medicines in Europe;
- antimicrobials need public intervention;
- environmental risks, including many current technologies used in the production of APIs and drug products rely on toxic intermediates and inefficient processes that result in unnecessary pollution of the environment.

We were pleased for the opportunity to provide ORBIS feedback on important documents for the pharmaceutical sector. The interaction between representatives of academic and industrial sectors allowed to form balanced comments. We hope that this mini-review will inspire more researchers to participate in future open consultations on public policies.

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The views expressed in this paper are those of the authors and do not necessarily reflect the European Union's, the Polish Ministry of Science and Higher Education or the affiliated institutions position on the subject.

Conflict of interest statement
P.J. Rudzki and O. Czerepow-Bielik are full-time employees of Celon Pharma S.A. The authors declare no conflict of interest.

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Conceptualization: P.J. Rudzki, O. Czerepow-Bielik and M. Karaźniewicz-Łada; methodology: P.J. Rudzki, O. Czerepow-Bielik and M. Karaźniewicz-Łada; writing original draft preparation: P.J. Rudzki, O. Czerepow-Bielik and M. Karaźniewicz-Łada.

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THOUSAND WORDS ABOUT...

JMS Journal of Medical Science

Senescent cells as new pharmacological targets for age-related diseases and anti-aging therapy

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Keywords: aging, senescence, senolytics

ABSTRACT

Aging is a natural process leading to decline in physical function, reducing ability to adjust to everyday organismal stress and increased frailty. Recent studies of the mechanism of aging have brought attention to naturally occurring senescent cells in different organs throughout the body. This natural process of senescence is caused by cell cycle arrest due to cellular damage, which protects cells from apoptosis, while stimulating the production and secretion of different senescent associated secretory phenotypes (SASPs) causing low grade chronic inflammation. Emerging studies show that by targeting and eliminating these cells with a new class of senolytic drugs in old animals we can improve a variety of health conditions including reduction of inflammation, improvement of insulin sensitivity and metabolic status, increase of bone mineral density and enhanced physical function together with extended overall longevity. Ongoing clinical trials using Desatanib and Quarcetin (D+Q) and other classes of senolytic drugs indicate high translational potentials in targeting and clearing senescent cells to cure some age-related diseases; however, more in depth studies have to be completed to incorporate these therapies in general healthy elderly populations for safe anti-aging intervention.

For centuries, people searched for the cure of aging and secrets of longevity with hopes for immortality and youthful lives. The last few decades of scientific research have established multiple theories of aging including (i) programmed aging, which contains neuroendocrine theory of aging, finite cell division, immune theory of aging, and (ii) wear and tear theory including free radicals, DNA damage, rate of living, error catastrophe and glycosylation theory. Through investigating these different topics using different biological aging models researchers found some overlapping mechanisms as well as some contradicting regulations still lacking consensus about the detailed mechanism of biological aging. Importantly, the newest trends in aging research focus on the process of cellular senescence. Cellular senescence is a defense mechanism to arrest proliferation of damaged cells. Through this mechanism potential cancerous cell stops dividing, but at the same time are protected from apoptosis. This "zombie" like cell keeps produc-

ing and secreting SASPs, mainly consisting of pro-inflammatory cytokines, chemokines, miRNAs, and variety of proteins and enzymes [1]. This pro-inflammatory activity sends localization signals attracting immune cells and activating immune response potentially suppressing tumor development and growth. Aging-associated increased accumulation of senescent cells shifts the beneficial function of these cells into a detrimental one through increased chronic inflammation, thus promoting tumor progression, tissue disfunction and acceleration in the development of varied age-related diseases. More importantly, Xu at al. showed that transplanting mice with senescent preadipocytes, thus increasing number of these cells in the organism, significantly shortens the overall survival of these mice when comparing with control animals with transplanted healthy cells [2].

Based on this knowledge, researchers are trying to develop novel senolytic therapies selectively targeting and eliminating senescent cells from human bodies in hopes to delay the aging phenotype and increase health span and lifespan [3–6]. There are several promising pharmacological agents already tested in vitro, in animal models as well as in humans [1, 7–9]. These treatments have been already reported to reduce the onset of aging-related diseases and increase the lifespan [2, 10].

One of the earliest senolytic cocktails developed and studied is the combination of dasatinib and quercetin (D + Q). This cocktail was shown to be effective in killing and eliminating senescent cells. Importantly, the study by Xu and colleagues showed that treating 20-months old mice with D+Q for 4 months significantly improved several physical functions in these old mice including maximum speed, grip strength, hanging endurance etc. However, the most significant observation was a successful lifespan extension in mice treated with D+Q [2]. However, present approaches with senolytic drugs represent hit and miss targeted therapy, which depends on providing a rather high dosage of the drug for 2–4 days with 2–4 weeks intervals. This regimen targets senescent cells in the whole body, yet there is a need to better understand possible side effects or consequences when therapy could be started too early. Based on this, scientists are trying to investigate the impact of senolytic therapy in different organs and varied diseases. The study by Saccon at al. indicated that treatment of old mice with D+Q also have a positive impact on eliminating senescent cells from intestinal tissues including the colon and cecum with a concomitant shift in intestinal and fecal microbiota promoting bacterial diversity and maintenance of good versus bad bacteria, thus reducing inflammation in the gut [4]. Unfortunately, there are some factors accelerating the accumulation of senescent cells which include high fat diet induced obesity, metabolic syndrome and diabetes. Different studies showed that obesity driven accumulation of adipose tissue is associated with increased number of senescent cells in fat tissue as well as other organs. This implies that obesity and/or metabolic associated chronic inflammation might be related to increased accumulation of senescent cells and higher production of SASPs. The study by Hanse at al. showed that obesity causes increased accumulation of these "zombie" cells in ovarian tissue potentially increasing the risk of infertility, ovarian cancer and decline of female health due to accelerated process of menopause. However, subjecting obese induced female mice to D+Q successfully targeted and cleared senescent cells from the ovaries indicating the potential of improving overall health and fertility through senolytic therapies [8]. Furthermore, other studies have demonstrated that mice exposed to irradiation damage, which caused impairment in physical capacity, had significant functional improvement after treatment with D+Q, that in an old or transgenic atherosclerosis mice model this senolytic intervention improved cardiac function [11, 12], and that D+Q senescent cell clearance improves bone health and metabolic functions by targeting adipose tissue [13, 14]. Lastly, there is also growing evidence of the role of senescent cells in brain function and Alzheimer's disease [15, 16]. However, the most critical question we can ask is the translational potential for senolytic therapies for human aging and age-related diseases. Several completed small trials or ongoing clinical testing showed potential for using senolytic therapy to eliminate senescent cells, reduce inflammation and attenuate frailty in humans. The first in human, open-label and then follow-up phase I, single-blind, randomized and placebo-controlled pilot trial demonstrated improved physical function, feasibility and toler-

ability after D+Q intervention in patients affected by idiopatic pulmonary fibrosis [7, 9]. Yet another clinical trial with D+Q showed reduced senescent cells accumulation in adipose tissue biopsies collected from patients diagnosed with diabetic kidney disease [17]. Importantly, due to high potential in targetting senescent cells in aging and age-related diseases there is growing interest in the develement of new senolytic drugs to improve efficiency and specificity thus reducing any potential toxicity to healthy cells.

However, at this point more studies and clinical trials are necessary to better understand this complex mechanism and to validate the safety and/or necessity to start senolytic therapies outside of pending clinical trials to prevent or delay aging and age-assocoiated disease in generally healthy elderly populations.

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THOUSAND WORDS ABOUT...

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Future Opportunities in the Field of Drug Delivery Research

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ABSTRACT

Much of the priorities in drug delivery research are focused on targeted drug delivery for cancer therapies and a wide range of controlled drug release systems for commonly used active pharmaceutical ingredients (APIs). In this "thousand words article" we highlight some of the emerging health threats and future opportunities for drug delivery research.

Important emerging health threats include viral pandemics beyond COVID, antibiotic-resistant pathogens, the need for new antifungal therapies, and emerging diseases caused by increasing pollution and climate change. Fundamentally new drugs may be needed. For example, one little known research effort focuses on the development of new antibiotics based on metal-organic frameworks. Finally, new delivery approaches will be needed. This is illustrated by the development of a topical peptide delivery system as a wound dressing for burn patients, combining biotechnology (a new peptide) with polymer science (a new topical delivery system) to address a medical need (burn injury) for which there is currently no effective treatment. Another important trend is the shift in our collective understanding of impact, moving away from "counting papers" to considering the societal benefit of the research including its potential for commercialization. To remain relevant in the coming decade, we need to anticipate and embrace future challenges. This is particularly important for younger scientists.

Important emerging research opportunities are related to a number of new, global health threats, including (but not limited to) viral pandemics beyond COVID, the development of antibiotic-resistant pathogens, the growing number of life-threatening fungal infections, and the health effects of climate change and pollution. To address these challenges, we anticipate significant changes in the way drug delivery research is being conducted.

This article is based on extended literature searches, discussions with numerous biomaterials scientists from the USA and Europe, and the preferential selection of topics relevant to the members of the ORBIS project.

Nanotechnology has greatly contributed to the development of mRNA vaccines and rapid detection kits for the diagnosis of COVID infections [1]. This line of research offers ample room for further innovation and improvement. It is likely that nanotechnology-inspired drug delivery systems will dominate most aspects of drug delivery research in the future [2]. This is an important change from the focus on controlled release formulations and drug-delivery implants that dominated drug delivery research in the past.

The next pandemic could be caused by a virulent and highly transmissible bacterium. Scientists have warned repeatedly of the potential threat of antibiotic resistant pathogens [3,4]. There is a need to think beyond our current small molecule-based approaches. For example, silver and iodine are exceptionally powerful antimicrobial agents that are difficult to deliver and have therefore not reached their full clinical potential [5,6]. Another innovative material approach is based on metal-organic frameworks. The work of Jaros et al. [7] has recently demonstrated significant promise and could open a new research direction for the development of metal-organic based antibiotics. One of the promising features of this approach is that these agents show both antibacterial and antiviral activity, in addition to activity against bacteria with multiple drug resistance [8].

Most people are unaware of the serious threat associated with fungal pathogens. According to the US Government Center for Disease Control, the human mortality rate from invasive fungal infections is over 50% [9]. While fungal infections are increasing world-wide, there are only 4 classes of antifungal agents in clinical use: polyenes, azoles, allylamines and echinocandins. Creating a new antifungal agent is much more complicated and time consuming than developing an antibiotic since fungal cells are more similar to mammalian cells than bacterial cells. Therefore, developing innovative delivery platforms for antifungal agents that increase and broaden their activity spectrum is as important as racing to develop new antifungal agents [10]. Another useful strategy is preventing the spread of fungal infections. There are powerful surface coating strategies to prevent fungal attachment and biofilm formation, but only a few laboratories focus on this important global threat. The most innovative approach is the development of intrinsically antifungal polymers as coatings [11]. While 1 billion people currently experience food insecurity, fungi destroy 30% of the global food supply. Thus, intrinsically anti-fungal polymers may also find wide applications as crop-protecting agents in agriculture.

We assume that we will see an accelerated shift away from conventional, small-molecule drugs and towards biological agents such as peptides, proteins, RNA and DNA [12]. These biologics will require new and different drug delivery systems.

Some of the future trends in drug delivery research are illustrated by the development of a peptide-delivery system to treat burn injuries. This research project features the use of engineered peptides as drug candidates (an aspect of biotechnology), the development of electrospun fiber mats as the peptide delivery platform (an aspect of innovative biomaterials) and addresses an important medical need (burn injury progression) for which there is currently no satisfactory treatment.

Burns are categorized by severity. 1st and 2nd degree burns usually heal without complications and without scarring. 3rd degree burns require skin grafts, lead to scarring and result in significant patient morbidity and mortality. Some 2nd degree burns progress to become 3rd degree burns about 48 hours after the actual burn injury [13]. "Burn injury progression" is a major complication, negatively affecting patient outcomes and increasing treatment costs.

Work by Clark et al. identified several fibrinogen-derived peptides, referred to as P12, cP12 and cNP8 [14,15]. These engineered peptides were shown to reduce burn injury progression when administered by intravenous injection within 24 hours after the burn injury occurred. cNP8 is the most advanced drug candidate since it is resistant to degradation by elastase, an enzyme present in wound sites.

From a clinical perspective, intravenous injection of these peptides is not a viable option. Therefore, to advance this research activity into

clinical use, a suitable delivery system had to be developed. The researchers focused on the development of new, rapidly degradable, biocompatible polymers that could (i) be formulated as a burn wound dressing, (ii) be loaded with hydrophilic, cationic fibrinogen-derived peptides such as P12, cP12 and cNP8, and (iii) release the peptide in a controlled fashion over the course of a few days [16].

In this project the utility of tyrosine-derived polycarbonate [17] electrospun fiber mats was explored. "Ultrafast" and "fast"-eroding polymer compositions were identified that eroded completely in about 24 h and 7 days respectively [16]. Accordingly, the release of the test peptide (P12) from "Ultrafast" fiber mats was controlled by polymer erosion, while the release of P12 from the "fast"-eroding fiber mats was controlled by diffusion of the peptide. A porcine excisional wound model was used to confirm the biocompatibility of these fiber mats in vivo [16]. These results provided the basis for an attempt to develop a clinically useful, new therapy for the treatment of burn injury. These efforts are currently on-going under funding from the US Department of Defense provided to a pharmaceutical company.

Drug delivery research has new challenges and new opportunities. There seems to be a shift away from the traditional macroscopic implants to nanotechnology, a shift away from traditional small molecule drugs to biologics, a shift away from predominantly serving the needs of chronic diseases in affluent countries to looking at emerging global health issues. As a consequence, drug delivery research will continue to be a central discipline in shaping clinical practice in the future.

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Author Contributions
This brief review was jointly conceptualized and written by Joachim Kohn and Bozena Michniak-Kohn.

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Overcoming the barrier of skin to drug permeation for localized dermatological therapies

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ABSTRACT

The skin's uppermost layer, the stratum corneum is a very effective barrier against the penetration of compounds including pharmaceuticals and cosmetic actives. To deliver higher amounts of drugs into the skin layers or to deliver drugs deeper into the skin (e.g., into the dermis), several enhancement techniques have been established. These techniques include chemical penetration enhancers as well as physical techniques such as iontophoresis and microneedles. In addition, one of the newer approaches includes the use of nano-based carriers such as metallic nanoparticles and polymeric self-assembling nanospheres.

This mini-review explores this new approach of using nano-based drug carriers for skin penetration enhancement. In particular we will explore the use of gold nanoparticles as well as biocompatible tyrosine-derived polymeric nanoparticles known as Tyrospheres.

The most investigated carriers in the class of metallic carriers are gold nanoparticles that can be used for both medical as well as diagnostic uses. Many investigators have reported that gold nanoparticles are able to enhance the skin transport and delivery of *macromolecular and hydrophilic drugs*. Meanwhile, for challenging *highly lipophilic and/or unstable compounds* such as adapalene and Vitamin D3 packaging them into polymeric nanocarriers such as Tyrospheres enables drug delivery to hair follicles, significantly increased aqueous solubility and resulted in elevated amounts of drug in targeted skin layers.

The relatively new approach of using nanotechnological approaches as a way of enhancement of drug delivery to skin shows significant promise over some other established techniques such as the addition of chemical penetration enhancers to formulations used for topical/transdermal uses.

Introduction

The skin consists of several layers which include (from top downwards into the body): the epidermis, dermis and hypodermis. By the mid 1800s scientists were aware that the top layer of the skin (consisting of the stratum corneum, stratum granulosum, stratum spinosum, and stratum

basale) was more impermeable to penetration of compounds than the lower layer (dermis) and by early 1900s we knew that skin was more permeable to lipophilic compounds than hydrophilic ones.

As electron microscopy techniques improved, scientists were able to to finally recognize the existence of a thin acellular layer 10–15 microns thick on top of the skin-this was the stratum corneum. By the 1940s scientists realized that this rather thin and potentially fragile topmost layer of the skin was freely permeable to water and dissolved substances when the stratum corneum (SC) was removed by sand-papering. Finally, scientists have realized that this layer cannot be regarded as totally "dead" and was an important tissue made up of sturdy anucleated cells named corneocytes, that are composed of lipids, water and proteins. These cornecytes produced a well-organized lipid structure which provides the barrier function to the skin. This layer can protect the body from potentially harmful external stimuli- microorganisms, chemical compounds, radiation, heat, electrical barrier, mechanical shock.

This article summarizes selected approaches to overcoming the barrier of skin to the penetration of drugs using skin penetration strategies including the use of nanotechnology systems that enhance transport of actives through the skin barrier.

Nanotechnology systems which enhance drug transport into skin

To overcome the barrier properties of the stratum corneum of the skin, nanocarrier mediated drug delivery systems have been utilized successfully in many cases and provide an alternative to the more traditional chemical and physical approaches [1,2]. The older strategies utilize chemical penetration enhancers (CPEs) such as surfactants, laurocapram-derivatives, terpenes, cyclodextrins and others as well as physical approaches such as iontophoresis, sonophoresis and others [3–5]. However, some of these methods do produce skin sensitization and sometimes irritation in a segment of the patient population [6]. In recent years alternative enhancement methods have been extensively explored and include utilizing various nanotechnology-based systems. They have been proposed as formulations for both pharmaceutical as well as cosmetic applications [7–11]. The main classes of these nano systems are: liposomes, nanoemulsions, lipid nanocapsules, metallic nanocarriers, solid lipid nanoparticles, polymeric nanoparticles/micelles and nanogels [2,12]. For the purposes of this review we will consider a subset of these delivery systems and specifically, metallic nanocarriers as well as polymeric nanoparticles/micelles.

Metallic nanocarriers

Metallic nanoparticles have some advantages over other carriers in the fact that they are very stable, possess a narrow particle size, and the ability to have their surface functionalized. All these features make them attractive carriers for topical formulation uses [13,14]. The most investigated carriers in this class are the gold nanoparticles for both medical as well as diagnostic uses [15,16]. These gold particles have low toxicity, a large surface area for functionalization, can be fabricated in various shapes and possess sizes in the range of 1–100 nm.

It has been demonstrated that gold nanoparticles (GNs)are able to enhance the skin transport and delivery of *macromolecular and hydrophilic drugs*. For example, Safwat et al. showed that 5-fluorouracil was better delivered in GNs than controls into mouse skin resulting in an improved anti-cancer effect [17,18]. The GNs were capped with cationic ligands which were able to load the negatively charged 5-fluorouracil under a pH of above 8.5 through the ionic interaction. Then the interaction with the positive charge of the nanocarrier and the skin may have been the main reason for the improved anti-cancer effects which were observed.

Koushki et al. used a dendritic cell-specific aptamer for the modification of allergen loaded GNs. These authors reported improved immunoregulation compared to non-targeted controls and even higher effects when skin penetrating peptides were also used [19]. GNs can also be combined with other nanomaterials and also with physical enhancement techniques such as iontophoresis and microneedles (hollow, coated or dissolvable) [2,20].

Polymeric nanoparticles/ micelles

For the delivery of challenging drugs that possess very low aqueous solubility and are *highly lipophilic or are unstable* another skin delivery approach can be taken. This is one using the various polymeric nanoparticles/micelles and in the example below, specifically biocompatible tyrosine-derived polymeric nanoparticles known as Tyrospheres [21, 22]. The chemical structure of these copolymers is composed of hydrophobic B-block i.e., oligomers of desaminotyrosyl-tyrosine ester (DTR) and diacid and hydrophilic poly(ethylene glycol) (PEG) A-blocks. These PEG-*b*-oligo(DTR-XA)-*b*-PEG triblock copolymers undergo self-assembly in an aqueous environment to form polymeric micelles referred to as TyroSpheres. These were used by Ramezanli et al. to load adapalene, a lipophilic drug with a logP of 8.04 and low aqueous solubility for delivery into hair follicles for the treatment of acne [23]. It was found that the Tyrospheres were significantly more effective than controls in a clinical mouse acne model [24].

Vitamin D3 (VD3) is very hydrophobic (log P of 9) and sensitive to many environmental factors (e.g., moisture, heat and light), which can induce isomerization or oxidation of its structure and adversely affecting its bioactivity. VD3-TyroSpheres were fabricated by Ramezanli et al.and characterized for their size, binding and loading efficiencies, stability, drug release and permeation in human cadaver skin samples [25]. TyroSpheres were able to substantially enhance the aqueous solubility of VD3 without affecting its activity. These biocompatible nanocarriers form a protective layer around the lipophilic drug that can protect it against environmental-induced degradation. Moreover, the skin delivery efficiency of TyroSpheres was found to be higher than some other dermal penetration enhancers, such as Transcutol. This study provided evidence of TyroSpheres' significant potential for targeted delivery of hydrophobic actives to skin layers.

Conclusion

In conclusion this article provides examples of how nanocarrier-mediated approaches as illustrated by gold nanoparticles and polymeric nanospheres (Tyrospheres) are able to provide enhanced transport of various challenging compounds past the skin barrier stratum corneum and into the skin layers below. The applications are broad for hydrophilic and lipophilic compounds in both the pharmaceutical as well as the cosmetic/personal care sectors.

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Professor Dr. Bozena B. Michniak-Kohn is a full-time employee of Rutgers, the State University of New Jersey, Piscataway, NJ 08854, USA. The author declares no conflict of interest. Joachim Kohn declares no conflict of interest.

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Conceptualization: B. Michniak-Kohn and J. Kohn equally; methodology: B. Michniak-Kohn; writing original draft preparation: B. Michniak-Kohn and J. Kohn equally.

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Abstract
The abstract should not exceed 250 words and should be structured into separate sections: Background, Methods, Results and Conclusions. It should concisely state the significant findings without reference to the rest of the paper. The abstract should be followed by a list of 3 to 6 Key words. They should reflect the central topic of the article (avoid words already used in the title).

The following categories of articles can be proposed to the Journal of Medical Science:

ORIGINAL RESEARCH

Original articles: Manuscripts in this category describe the results of original research conducted in the broad area of life science and medicine. The manuscript should be presented in the format of Abstract (250-word limit), Keywords, Introduction, Material and Methods, Results, Discussion, Perspectives, Acknowledgments and References. In the Discussion section, statements regarding the importance and *novelty of the study* should be presented. In addition, the limitations of the study should be articulated. The abstract must be structured and include: Objectives, Material and Methods, Results and Conclusions. Manuscripts cannot exceed 3500 words in length (excluding title page, abstract and references) and contain no more than a combination of 8 tables and/or figures. The number of references should not exceed 45.

Brief Reports: Manuscripts in this category may present results of studies involving small sample sizes, introduce new methodologies, describe preliminary findings or replication studies. The manuscript must follow the same format requirements as full length manuscripts. Brief reports should be up to 2000 words (excluding title page, abstract and references) and can include up to 3 tables and/or figures. The number of references should not exceed 25.

REVIEW ARTICLES

Review articles: These articles should describe recent advances in areas within the Journal's scope. Review articles cannot exceed 5000 words length (excluding title page, abstract and references) and contain no more than a combination of 10 tables and/or figures. Authors are encouraged to restrict figures and tables to essential data that cannot be described in the text. The number of references should not exceed 80.

A THOUSAND WORDS ABOUT... is a form of Mini-Reviews. Manuscripts in this category should focus on *latest achievements of life science and medicine.* Manuscripts should be up to 1000 words in length (excluding title page, abstract and references) and contain up to 5 tables and/or figures and up to 25 most relevant references. The number of authors is limited to no more than 3.

OTHER SUBMISSIONS

Invited Editorials: Editorials are authoritative commentaries on topics of current interest or that relate to articles published in the same issue. Manuscripts should be up to 1500 words in length. The number of references should not exceed 10. The number of authors is limited to no more than 2.

Images in Clinical Medicine: Manuscripts in this category should contain one distinct image from life science or medicine. Only original and high-quality images are considered for publication. The description of the image (up to 250 words) should present relevant information like short description of the patient's history, clinical findings and course, imaging techniques or molecular biology techniques (e.g. blotting techniques or immunostaining). All labeled structures in the image should be described and explained in the legend. The number of references should not exceed 5. The number of authors is limited to no more than 5.

The Rationale, Design and Methods of New Studies: Manuscripts in this category should provide information regarding the grants awarded by different founding agencies, e.g. National Health Institute, European Union, National Science Center or National Center for Research and Development. The manuscript should be presented in the format of Research Project Objectives, Research Plan and Basic Concept, Research Methodology, Measurable Effects and Expected Results. The article should also contain general information about the grant: grant title, keywords (up to five), name of the principal investigator and co-investigators, founding source with the grant number, *Ethical Committee permission number,* code in clinical trials (if applicable). Only grant projects in the amount over 100,000 Euro can be presented. Manuscripts should be up to 2000 words in length (excluding references) and can include up to 5 tables and/or figures. The abstract should not exceed 150 words. The number of authors is limited to the Principal Investigator and Co-investigators.

Acknowledgements

Under acknowledgements please specify contributors to the article other than the authors accredited. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.). Also acknowledge all sources of support (grants from government agencies, private foundations, etc.). The names of funding organizations should be written in full.

References

All manuscripts should use the 'Vancouver' style for references. References should be numbered consecutively in the order in which they appear in the text **and listed at the end of the paper.** References cited only in Figures/Tables should be listed in the end. Reference citations in the text should be identified by Arabic numbers in square brackets. Some examples:

This result was later contradicted by Smith and Murray [3].
Smith [8] has argued that...
Multiple clinical trials [4–6, 9] show...

Journal names should be abbreviated according to Index Medicus. If available alwaysprovide Digital Object Identifier (DOI) or PubMed Identifier (PMID) for every reference.

Some examples

Standard journal articles

1. Petrova NV, Kashirskaya NY, Vasilyeva TA, Kondratyeva EI, Marakhonov AV, Macek Jr M, Ginter EK, Kutsev SI, Zinchenko RA. Characteristics of the L138ins (p.Leu138dup) mutation in Russian cystic fibrosis patients. JMS [Internet]. 2020 Mar 31;89(1):e383. doi: 10.20883/medical.383.

Books

Personal author(s)

1. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburgh: Churchill Livingstone; 2003.

Editor(s) or compiler(s) as authors

2. Beers MH, Porter RS, Jones TV, Kaplan JL, Berkwits M (editors). The Merck manual of diagnosis and therapy. 18th ed. Whitehouse Station (NJ): Merck Research Laboratories; 2006.

Chapter in the book

1. Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465–478.

TABLES: Tables should be typed on sheets separate from the text (each table on a separate sheet). They should be numbered consecutively with Arabic numerals. Tables should always be cited in text (e.g. table 2) in consecutive numerical order. Each table should include a compulsory, concise explanatory title and an explanatory legend. Footnotes to tables should be typed below the table body and referred to by superscript lowercase letters. No vertical rules should be used. Tables should not duplicate results presented elsewhere in the manuscript (e.g. in figures).

FIGURES: All illustrations, graphs, drawings, or photographs are referred to as figures and must be uploaded as separate files when submitting a manuscript. Figures should be numbered in sequence with Arabic numerals. They should always be cited in text (e.g. figure 3) in consecutive numerical order. Figures for publication must only be submitted in high-resolution TIFF or EPS format (*minimum 300 dpi resolution).* Each figure should be self-explanatory without reference to the text and have a concise but descriptive legend. All symbols and abbreviations used in the figure must be defined, unless they are common abbreviations or have already been defined in the text. Figure Legends must be included after the reference section of the Main Text.

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SUPPLEMENTARY ONLINE MATERIAL: Authors may submit supplementary material for their articles to be posted in the electronic version of the journal. To be accepted for posting, supplementary materials must be essential to the scientific integrity and excellence of the paper. The supplementary material is subject to the same editorial standards and peer-review procedures as the print publication.

Review Process

All manuscripts are reviewed by the Editor-in-Chief or one of the members of the Editorial Board, who may decide to reject the paper or send it for external peer review. Manuscripts accepted for peer review will be blind reviewed by at least two experts in the field. After peer review, the Editor-in-Chief will study the paper together with reviewer comments to make one of the following decisions: accept, accept pending minor revision, accept pending major revision, or reject. Authors will receive comments on the manuscript regardless of the decision. In the event that a manuscript is accepted pending revision, the author will be responsible for completing the revision within 60 days.

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