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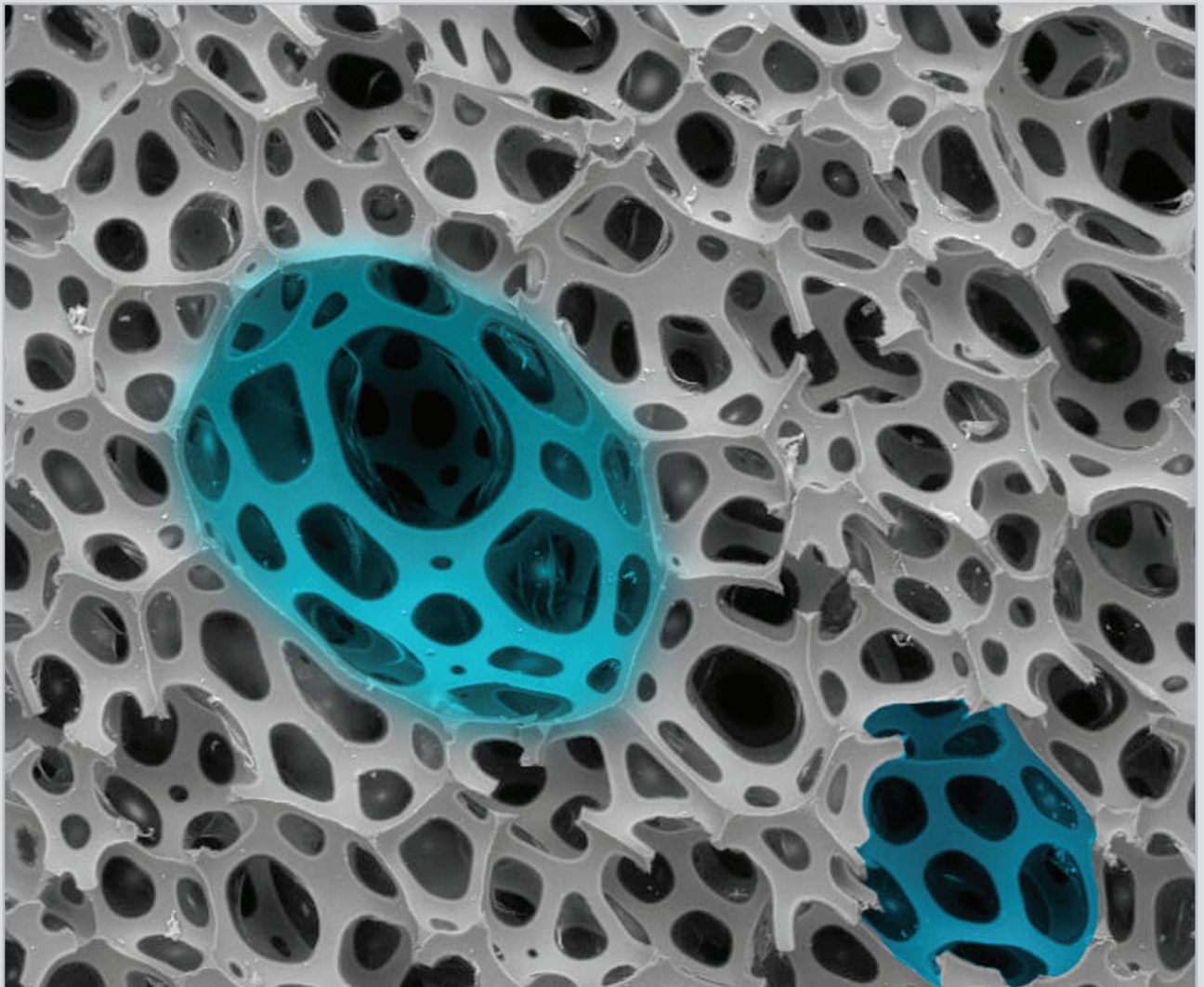
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of the Hungarian Society  
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## foams







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2019

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**Contents**

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Romána Zelkó: Editorial	3
Dóra Farkas, Nikolett Kállai-Szabó, István Antal: Foams as carrier systems for pharmaceuticals and cosmetics	5
Istvan Vincze, Laszlo Endrenyi, Laszlo Tothfalusi: Bioequivalence Metrics for Absorption Rates: Linearity, Specificity, Sensitivity	17
Márió Gajdács: A bibliometric analysis of Acta Pharmaceutica Hungarica (1965-2018)	23
Márk Fábián, Balázs Balogh, Zsófia Czudor, László Órfi: New computational studies to support cyclin-dependent kinase 9 inhibitor screening and design	31

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## EDITORIAL

On behalf of the Editorial Board of *Acta Pharmaceutica Hungarica*, its authors and readers, I would like to thank the Hungarian Society for Pharmaceutical Sciences, for the significant efforts to advance the profile of the prestigious Hungarian pharmaceutical journal and made it open access and internationally available.

I am honoured to assume the responsibilities of Editor-in-Chief of *Acta Pharmaceutica Hungarica*. The future success of *Acta Pharmaceutica Hungarica* is due to the efforts of my predecessors and the internationally diverse team of editors, reviewers, contributors and the supporting staff. I anticipate considerable challenges in this dynamic era of the excessive number of scholarly journals, and I am committed to improve the quality of publications, increase its professional impact and keep the journal relevant in the field of pharmaceuticals.

Our mission is to provide good submission experience for the authors with aiming at a shorter submission to decision time while keeping the professional level, and we will respond to any au-



thors' concern in a timely manner.

We warmly welcome submissions from the scientific community and industry partners.

I am sure that the new enthusiasm and the support from the Hungarian Society for Pharmaceutical Sciences will allow the journal to expand in scope and depth, attract both traditional and emerging collaborative researchers from the multidisciplinary fields of pharmaceutical sciences to improve the journal recognition and quality further.

As an Editor-in-Chief, I am committed to supporting access to this journal among the practising pharmacists as well.

I look forward to working with all the authors, reviewers or editors to bring about new achievements for *Acta Pharmaceutica Hungarica*. As an Editor-in-Chief, I welcome suggestions, discussions and thoughts from all the contributors to help us to understand and rectify any concern with the Journal.

Romána Zelkó



Acta Pharmaceutica Hungarica

## Foams as carrier systems for pharmaceuticals and cosmetics

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### Abstract

Foams are becoming more and more popular in several areas of our lives, including pharmaceuticals and cosmetics. They are colloid systems where gas is dispersed in a liquid phase. Active ingredient bearing pharmaceutical foams are traditionally applied topically (dermal, local rectal, vaginal), but formulations for other delivery routes (e.g. nanosystems parenterally, solid foams orally) are also available. Numerous advantages are attached to foams when compared to traditional vehicles, resulting in increased patient compliance. Amongst others, the suitable composition contributes to quick, oily residue free and convenient application even on large or hairy areas, as well as to good drug transfer rate.

**Keywords:** foam classification and characterization, formulation, excipients, image analysis

### Introduction

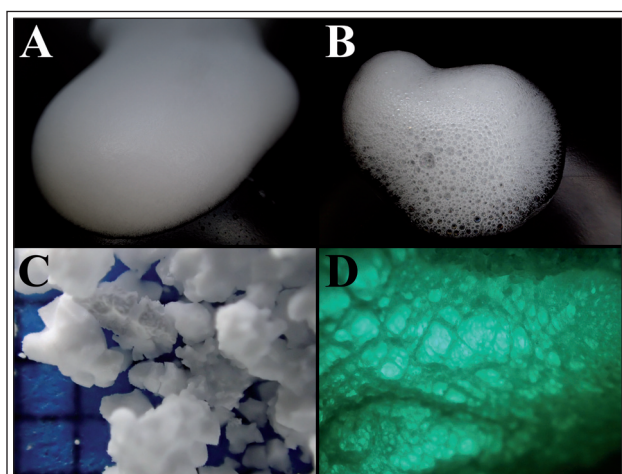
According to the conventional classification of colloid systems, foams (*Figure 1*) are defined as discontinuous gas bubbles dispersed in a continuous phase (liquid or solid). Solid foams include thin polymer walls, while traditional liquid foams contain thin liquid films separating gas bubble agglomerations [1]. Foams can also be described as deformed (laminar difform) lyosols because the dispersing phase is a colloid. The thickness of the lamellae separating the bubbles is in the micrometric range [2].

There are non-aqueous foams too, which with-

out the increase in the liquid phase viscosity, gelling, or solidification caused by cooling or polymerization are only stable for a short time. Due to their low density, good heat-insulating ability, and great mechanical solidity despite the small weight, these solid foams (xerosols, xerogels) are of great industrial significance in several areas, including pharmaceutical industry [2]. Gelatine or collagen-based solid foams, or sponges, containing antibiotic or steroid active ingredients are often used as primary wound dressings [3]. The mutual attribute of all foams that distinguishes them from sponges, irrespectively of the state of the film, is the enclosed bubbles and the lack of connection with the surrounding bubbles, so the gas phase is discontinuous while the liquid or solid phase is continuous [1].

### Foam formation

Foams can be produced by two basic methods. The more common, mechanical one is by dispersing the gas phase in the liquid with beating or shaking [2]. The other way is by the gas-supersaturation of the liquid. The gas can be dissolved under pressure that is later released or can be formed in situ [4]. In both cases, the lifetime of the foams made of pure liquids is quite short [2]. For an enduring dispersion, the presence of surface active agents absorbing spontaneously onto the interface is essential. These amphipathic molecules have polar groups that contact with the water and also a hy-



**Figure 1** Aerosol foam (A); propellant-free foam (B); solid polypropylene foam particles (C); structure of a gum based solid foam (D)

dophobic part that orients towards the gas and so creating a monomolecular layer. Surfactants reduce the energy required for foam formulation by decreasing the surface tension. They also foster the creation of an elastic boundary layer thus increasing the lamella stability by inhibiting their tapering. This progress can be hindered in different ways: by the presence of hydration shell of the surfactant molecules or by the repulsion of the ionic surfactant's electric charges. Another way is by the bulking of partially solvating particles, that reduce the system's free energy with the amount of adhesion work ( $W_a$ ), at the interface [2,5].

$$W_a = \gamma \cdot a^2 \cdot \pi \cdot [1 \pm \cos \theta]^2 \quad (1)$$

where  $\gamma$  is the surface tension,  $a$  is the radius of the particle and  $\theta$  is the contact angle [2].

Aerosol foams are formed due to the overpressure (usually 2-4 bars) when upon actuation the propellant evaporates quickly from the liquid. Delayed foam formulation can be reached by using a propellant with a high boiling point [6].

### Foam stability

Due to the excess interfacial Helmholtz free energy, the foams are thermodynamically unstable but can have kinetic stability in case of hampered phase disunion [5].

Ensuring good stability is essential for effective foam forming. Without decent firmness, the structure is to break down partially resulting in increased bubble sizes. There are several events occurring simultaneously in foams that influence their stability.

Liquid evaporation itself can destroy the foam structure. The type of the surfactant used can influence the speed by altering the nature of the monolayer and result in reduced evaporation [7].

It is known, that the smaller volume phase has higher vapor pressure and better solubility. As a result of the difference in the vapor pressure of various sized bubbles, the smaller ones disappear gradually. This is called Ostwald ripening or disproportionation and it can also be affected by the nature of the monolayer or the type of gas used. The more water-soluble gas results in less stable foams, since it is transported quicker across the films [7,8].

Owing to buoyancy force, in a few minutes after foam forming a creamy layer is observable on top of the liquid phase. Creaming is slower in concen-

trated dispersions.

Creaming and drainage lead to the break-down of foams. Curved liquid surface results in lower Laplace pressure (curvature pressure, capillary pressure) in the middle than around. This capillary downdraft results in

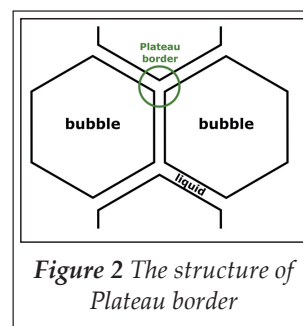


Figure 2 The structure of Plateau border

decreased water volume in the foam and causes the thinning of the liquid film. When the dispersed volume fraction is about 60%, the bubbles deform each other resulting in a polyhedral foam. The lamellae meet each other at 120-degree angles, forming a channel called Plateau border (Figure 2) [2,4,7].

The lifetime of foams can be elongated by increasing the viscosity of the liquid phase by adding e.g. polymers [2]. It is known, that even thick foam films can be stabilized by a low concentration polymer-surfactant complex. Given higher concentrations, uneven film thickness can be observed due to the aggregated complexes [9].

### Medicated foams

The European Pharmacopoeia defines medicated foams (*musci medicati*) as preparations for skin or mucosal applications that consist of large volumes of gas dispersed in a liquid. They usually contain one or more active ingredients, surfactants for foam formation and other excipients. The liquid formulation is filled into a pressurized container that is equipped with a valve suitable for the ex tempore foam formation at the site of application [10].

The composition of the foam formulation, the type and amount of propellant highly influence the properties of the foams. The properties of an ideal medicated foam from the perspective of the patient:

- the easy application even on bigger, hirsute or sensitive, inflamed areas,
- stable and does not collapse, or drain for a short period post-expelling,
- persist while manipulation – e.g. lifting off a surface with an applicator for application on the skin,
- good spreadability – low shear is enough to destroy, thus slight rubbing is sufficient,
- drug delivery ability at least as good as conventional transdermal formulations,
- less oily residues than creams, ointments, [11]
- easy removal, in case it is needed,

Table I Some examples of medicated foam formulations

Active pharmaceutical ingredient	Indication	Product examples
azelaic acid	mild to moderate rosacea	Finacea® Foam (Bayer)
betamethasone valerate	corticosteroid sensitive scalp dermatosis	Luxiq® (Stiefel Laboratories Inc.). Betamethasone valerate 0.12% Topical Foam (Mylan N.V.)
budesonide	colitis ulcerosa	Budenofalk® (Dr. Falk Pharma GmbH), UCERIS® Rectal Foam (Salix Pharmaceuticals Inc.)
calcipotriene	plaque psoriasis (adults)	Sorilux® (Mayne Pharma)
calcipotriol, betamethasone	psoriasis (adults)	Enstilar® (LEO Pharma)
clindamycin	acne vulgaris (above 12 years)	Evoclin® (Stiefel Laboratories Inc.)
clobetazol propionate	plaque psoriasis (above 12 years)	Olux® (Stiefel Laboratories Inc.)
common witch hazel extract, $\alpha$ -bisabolol	decubitus	TEVACARE antidecubitus foam spray (Laboratorium Dr. Deppe)
desonide	atopic dermatitis	Verdeso® (Aqua Pharmaceuticals)
dexpanthenol, bisabolol, Vitamin A, E and F	sunburn, skin irritation	Panthenol Foam (Dr. Müller Pharma)
dexpanthenol, lidocaine, sodium sulfosalicylate samarate	superficial burns, running sores	Phlogosam (PannonPharma Kft.)
hydrocortisone	colitis ulcerosa	Colifoam® (Meda Pharmaceuticals)
hydrocortisone acetate	adjuvant therapy of ulcerative proctitis	Cortifoam® (Alaven Pharmaceutical LLC)
hydrocortisone, pramoxine	inflamed, itchy manifestations of corticosteroid sensitive dermatosis	EpiFoam® (Alaven Pharmaceutical LLC)
ketoconazole	seborrheic dermatitis (above 12 years)	Extina® (Stiefel Laboratories Inc.), Ketoconazole Foam 2% (Perrigo Company Plc)
minoxidil	alopecia	Minoxidil Topical (Publix Super Markets Inc)
pramoxine, hydrocortisone	temporary relief of rectal inflammation, itching, pain, and swelling associated with hemorrhoids, fissures, cryptitis, proctitis	ProctoFoam®-HC (Alaven Pharmaceutical LLC)
sodium fluoride	caries protection	Oral-B® Minute-Foam® (Procter & Gamble Co.)
sodium sulfacetamide	seborrheic dermatitis, seborrhea sicca	Ovace® Plus (Espada Dermatology)
tolnaftate	tinea pedis, tinea corporis	FungiFoam® (The Tetra Corporation)
2QR bio-active bacterial blocker	chicken pox	PoxClin® (TRIMB HealthCare)
—	cleansing, restoring and protecting skin	Tena Wash Mousse (Essity Hygiene and Health AB)

– non-irritant, non-toxic, non-allergenic, pharmacologically inert vehicle.

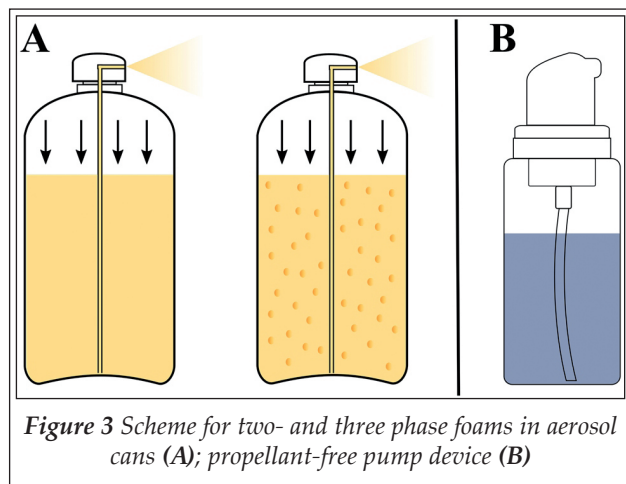
The drug transfer rate of foams is influenced by the significant physicochemical changes that the formulation undergoes while and after actuation from the container. When topically applied, the quick evaporation of the propellant causes increased active ingredient concentration, in some cases even supersaturation, that increases the absorption rate of foams [12].

A couple of pharmaceutical foams are available in Hungary and foreign countries and their indications for use are listed in [Table I](#) [11,12].

### Classification of foams

In the field of pharmaceuticals and cosmetics, the most used foams belong to aerosol foams, that are pressurized semi-solid formulations in aerosol cans.





**Figure 3** Scheme for two- and three phase foams in aerosol cans (A); propellant-free pump device (B)

Foams can be classified in several ways. We can differentiate aqueous, hydroethanolic and emollient foams as well as petrolatum-, oil- or other solvent-based foams too [13].

Emollient foams, as traditional creams or lotions, are emulsion-based so have a soothing, moisturizing effect. O/W or W/O emulsions can be used for the formulation, where the oil phase consists of mineral oil, triglyceride, fatty acid esters, such as isopropyl myristate, or isopropyl palmitate, or essential oil. Omega-3 and 6 oils can also be used for therapy, or silicone oils for their protective nature. Rarely petrolatum is used, but for its greasy, cloth-staining properties. Emollient foams are complicated systems, even a small change in the composition can lead to the destabilization of the foam [14]. At body temperature, they are relatively stable, but the easy application on large target area is guaranteed by shear force breakability.

Foams formed from nanoemulsions (droplet size between 20 and 200 nm) are promising formulations given their ability to increase the bioavailability and efficacy of hard-to-dissolve active ingredients by solubilizing them.

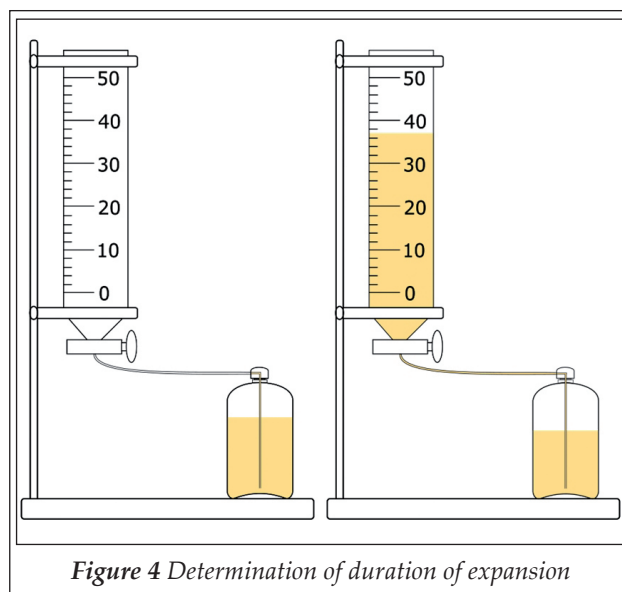
Hydroalcoholic (hydroethanolic) foams contain about 60% ethanol amongst others. Alcohol promotes better skin penetration of active ingredients compared to other vehicles. It alters the barrier properties of stratum corneum reversibly thus enhancing the penetration. The quick evaporation of alcohol from the skin results in fast drying of the foam, therefore it leaves a less unpleasant sticky feeling behind. Rapid evaporation also contributes to the thermolabile property of hydroalcoholic foams, discouraging dispensing foam onto the hands instead of directly to the target area. Owing to the undesired skin-drying property of alcohols,

the application of these foams is limited. In addition, skin irritation was reported repeatedly. These side effects lead to the creation of a foam that contains alcohol and still has an emollient effect (e.g. Scytera™ prepared coal tar 2% w/w foam).

Water-free foams that enable water-insoluble or unstable active ingredients to be used in the form of foams are also under development. The formulation would prevent microorganism growth, making preservatives unnecessary and could maximize the emollient effect [13].

Foam formulations containing aprotic, polar solvents, as dimethyl sulfoxide (DMSO) are under investigation. DMSO is a potent solvent, used as a penetration enhancer as it carries drugs through membranes without damage. According to laboratory studies, DMSO blocks peripheral nerve C fibers; thus the analgesic effect is attributed to it. Anti-inflammatory, antioxidant and membrane stabilizing properties promote DMSO-containing foams beneficial in the treatment of numerous diseases [15].

Amongst aerosol foams two- and three-phase foams can be differentiated (Figure 3A). By the fore-mentioned, the liquefied propellant is dissolved under high pressure in the mixture of the solvent, the surfactant, and the stabilizing agent. In three-phase foams, the propellant is dissolved in the inner phase of an oil-in-water type emulsion. In this case, the emulsifier and foaming agent could be the same, and vaporized propellant acts as the third phase. Shaking before use is required in the course of both the two- and three phase foams [6].



**Figure 4** Determination of duration of expansion

**Table II** Excipients for liquid foams

<b>Solvent</b>	distilled water, ethanol, isopropanol, glycerine, propylene glycol, dimethyl isosorbide, DMSO
<b>Foaming agent</b>	cetyl alcohol, cetyl stearyl alcohol, sodium docecyl sulfate, sodium oleate, sodium stearate, stearic acid, polysorbate 20
<b>Foam stabiliser</b>	xanthan gum, guar gum, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose methyl cellulose, agar-agar, alginates, sodium lauryl sulfate, lauryl acid, palmitic acid, stearic acid, coconut oil, tragachant gum, gelatine, glycerine
<b>Hydrophobic component, emollient</b>	mineral oils, plant oils, esters (e.g. isopropyl myristate), essential oils, petrolatum, omega-3 polyunsaturated oil, silicon oil
<b>Absorption promoter</b>	ethanol, fatty acids, fatty alcohols
<b>Foam breaker</b>	alkyl polysiloxanes, oils, alcohols, fatty alcohols, acetone
<b>Antifoaming agent</b>	silicone oil, glycerides, polyamide

### Excipients

Various functional excipients (summarized in [Table II](#)) are necessary for the preparation of medicated foams. The use of *foaming agents*, or *stabilizers*, that can be surface active agents, macromolecular substances that are easily solvated or partially wetting powders is inevitable. [2]. When deciding on the surfactant several factors have to be considered. Although ionic agents are effective they are known skin irritants; consequently, the use of non-ionic surfactants is suggested, especially when inflamed areas are to be treated [14].

Pharmaceutically acceptable oils, like mineral oils, plant oils or triglycerides are mostly chosen as *hydrophobic component*. Skin protective silicone oils are also preferred, while the greasy petrolatum is less used. Omega-3, Omega-6 polyunsaturated oils or other therapeutically beneficial oils are proven to work even well [14].

### Tests, examinations

The European Pharmacopoeia lists three tests for medicated foams: relative foam density, duration of expansion and sterility. The first one is determined by the *m/e* ratio, where *m* is the mass of the foam in grams and *e* is the mass of the same volume water in grams. It is sufficient if from three measurements the deviation of the values not exceeds 20% from the mean value.

Duration of expansion is determined in a burette with specified parameters ([Figure 4](#)). After thorough shaking the volume of the actuated ca. 30 ml foam is read every 10 seconds until it reaches the maximum volume. It is sufficient if from three measurements none exceeds 5 minutes [10].

Information from the quality of foam can be gained by examining its temporal behavior

through time. The reduction of the foam volume can be measured as well as the half lifetime, that is the time it takes the volume of the foam to be halved. Other than these static methods, stationary ones can also be used. In the latter case, it is possible to characterize the foaming agent and the foam itself together. For this a specific device is needed – foaming is induced by streaming gas with constant speed through a perforated membrane and the stationary foam volume is measured when the speed of foam forming and breakdown is equal [2].

There are several tests beyond the pharmacopoeial ones. The circumstances of use are imitated, so the tests are carried out with the lack of propellant, as it evaporates quickly after actuation [13].

Examining the physical properties of foams, like macroscopic appearance, bubble size, foam viscosity and elasticity, is inevitable.

A stereo microscope with digital ocular enables the observation of bubble size and the structure of the foam. With image analysis bubble size distribution, as well as several descriptive parameters (like Feret diameter, area, circularity or roundness) of individual bubbles, like the number of bubbles can be determined. Texture analysis is another method to gain information from the foam structure.

For basic evaluation of foams several methods can be used, including the determination of foam expansion (*FE* [%]), foam liquid stability (*FLS* [%]), foam volume stability (*FVS* [%]) and gas fraction (*GF* [ml]). These tests provide data on foamability and foam stability [6]. It is important to note, that there is no explicit connection between these two parameters [3].

The following parameters can be determined by filling up a glass cylinder with the foam and recording the initial and the aged volume as well as the volume of drained liquid over time.

$$FE(\%) = \frac{V_{foam} - V_{formulation}}{V_{formulation}} \cdot 100\% \quad (2)$$

where  $V_{formulation}$  is the volume of the formulation [ml] required to produce  $V_{foam}$  [ml]. A linear relationship can be found between  $FE$  and good foamability.

Foam liquid stability can be calculated as follows:

$$FLS(\%) = \frac{V_{liquid,30min}}{V_{formulation}} \cdot 100\% \quad (3)$$

where  $V_{liquid,30min}$  is the volume of drained liquid after 30 min [ml]. Lower  $FLS$  value refers to better stability.

Foam volume stability can be determined by using equation (4).

$$FVS(\%) = \frac{V(foam_{30min})}{V_{foam}} \cdot 100\% \quad (4)$$

where  $V_{foam,30min}$  is the foam volume after 30 min [ml]. There is an inverse relationship between  $FVS$  and foam stability [6].

Gas fraction, as well as bubble size, vary in wide range depending on the way of application [3]. For the calculation of  $GF$ , the following equation is to be used:

$$GF = V_{foam} - V_{formulation} \quad (5)$$

Turbiscan method, based on Faraday-Tyndall effect, provides another method for the determination of foam stability. The light scattering of colloid solutions is measurable by measuring the intensity of transmitted and backscattered light, that are influenced by the amount of air in the foam. Important to note, that the values vary in time due to foam destabilization.

Carrying out rheological measurements on foams is also circumstantial as for their instability. Oscillatory mode enables the determination of the film elasticity [6].

After dispensing foams, over time, they tend to collapse. Analyzing photos taken periodically can provide information about the time it takes the height of the foam to be halved. This foam collapse time is to be determined at 36 °C. It should take more than 1 minute, so proper application is possible, but 2-3 minutes is ideal [13].

Characterizing quick-breaking foams, due to their short lifetime, with the above-mentioned methods is complicated. In these cases, cryo-SEM

(cryogenic scanning electron microscopy) is an alternative method for quantitative foam analysis. Information can be gained aside from morphological parameters, also from bubble size, and bubble size distribution. For these examinations, the foam has to be quickly frozen with liquefied nitrogen and fractured by a precision rotary knife [16].

Krüss GmbH. has developed a dynamic foam analyser (DFA100) for the scientific analysis of liquid foams. The instrument precisely measures the height of foams, and with the help of the software (ADVANCE, Krüss GmbH.) data on foam stability, foamability, as well as decay characteristics can be easily obtained. It also enables the quick analysis of bubble size and bubble size distribution by measuring the liquid content. These features ease the optimization of all kinds of liquid foams, including pharmaceutical ones [17].

### Dermal foams

Dermatological preparations on the market are mainly creams, gels or ointments. However, in the last decades the interest in new vehicles, including foams, has significantly increased. This promising formulation is the objective of numerous patents, so it is highly likely that in a couple of years a growing number of foam preparations will be found in the market amongst the traditional pharmaceutical forms [16].

The first known dermatological application of foams belongs to Woodford and Barry. They examined the therapeutic advantages of a quick-breaking hydroethanolic foam containing betamethasone benzoate compared to traditional semi-solid formulations. They reported it effective in psoriasis treatment [18]. Another study reported that a calcipotriol and betamethasone dipropionate-bearing foam reduced the symptoms significantly better than the ointment studied [19].

Based on several other studies foams are proven to be at least as effective and safe as other dermal dosage forms with similar compositions. Patients – regardless of sex, age or ethnicity – considering other properties also prefer foams over traditional formulations [13,18–21].

In case of active ingredients with poor water solubility, foam formulations are advantageous, because with dissolving them in the oil phase of an emulsion foam, higher bioavailability can be reached [13].

Dermal foams, usually containing topical corticosteroids, are mainly used in the treatment of

skin and scalp dermatoses, like eczema, psoriasis or seborrhoea [12]. According to pharmacopoeial directions, foams have to be sterile when they are used on severely injured skin areas or open wounds [10].

In community pharmacies in Hungary two relevant medicated foams for dermal use are available. Phlogosam (PannonPharma Kft.), sold without prescription, containing dexpanthenol, lidocaine and sodium samarium disulfosalicylate anhydrate for first and second-degree superficial burn, dermatitis solaris or arteficialis and eczema [22].

TEVACARE antidecubitus foam spray is an emulsion foam containing common witch-hazel extract and  $\alpha$ -bisabolol. In addition to the skin nourishing and regenerating properties, it has cleansing and deodorizing effect. The foam layer helps in even distribution of the mechanical pressure thus reducing decubitus prevalence [23].

### Rectal foams

In the European Pharmacopoeia, the definition of rectal preparations is 'intended for rectal use in order to obtain a systemic or local effect, or they may be intended for diagnostical purposes' [24]. Local administration is for the adstringent or desinfectant effect, but more often steroid-bearing foams are used for the treatment of inflammatory rectal diseases (Chron disease, colitis ulcerosa). Beyond the advantages of rectal administrations, rectal foams have several other favourable properties. On the contrary to suppositories, active ingredient liberates without a latency time from the foam. As they gently fill the rectum, they provide a larger area for absorption. When applied no irritation is experienced derived from the solidity and shape of suppositories. Compared to rectal solutions (*klysmæ*), the application is significantly easier and more convenient, backflow is less likely.

Foam formulations provide good alternative for the rectal application of light and/or oxidation-sensitive active ingredients, as can be stored under pressure in a dark container.

As a disadvantage, their low density can be mentioned, for the reason that large API quantities cannot be administered due to the limited volume of rectum [25,26].

In 2009, Dr. Falk Pharma GmbH released Budenofalk® 2 mg rectal foam. This budesonide-containing preparation for the treatment of colitis ulcerosa, is the first and so far the only rectal foam on the market in Hungary [22].

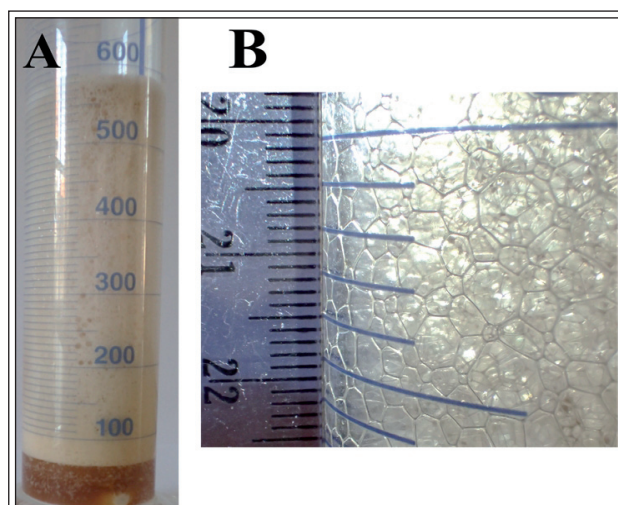


Figure 5 Foam expansion from effervescent intrauterine tablet (A) and the structure of the foam formed (B)

### Vaginal and intrauterine foams

Vaginal foams containing spermicides are used as local anticoncipients. There are also vaginal tablets and ovulum, that foams when in contact with cervical mucus. All three compositions are administered precoital. These contraceptive methods are preferred for their effectivity and the lack of contraindications, but in some cases, local irritation was reported. The inconvenience of application is also disadvantageous [27–29].

Antibiotic-bearing foams are also patented for the treatment of vaginal infections [30].

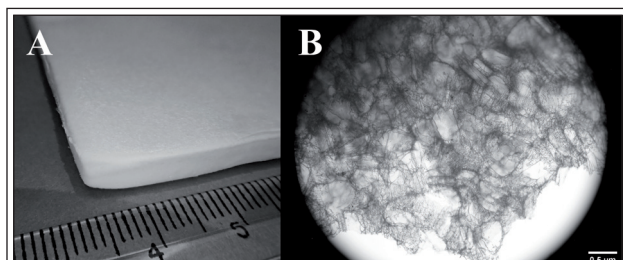
Intrauterine foams containing antibiotics or disinfectants are used in veterinary therapy of endometritis. The gas produced by the effervescent reaction (Figure 5), that helps the homogenous distribution of the active ingredient, as large volume of foam is formed [31].

### Dry foams

In 1974 the effect of a gentamicin-bearing anti-infective dry foam was investigated on *Pseudomonas aeruginosa* bacteria. Compared with an ointment, they were found to have the same efficacy [32].

Dry foam technology was developed as an alternative method to improve the solubility and bioavailability of active ingredients. A foam formulated from the suspension of the drug was dried under determined circumstances and tableted after granulation. This method was proven to have better solubility and bioavailability compared to direct compress tablets or wet granulated tablets [33,34].





**Figure 6** Gentamycin containing foam wound dressing (A) and its microscopic structure (B)

### Foam dressings

Over the years, a wide variety of wound dressings appeared on the market for the different wound types. An ideal dressing helps rapid healing while conveniently worn by the patient.

Foam dressings, made of porous polyurethane, have high moisture vapour transmission rate and provide moist, warm circumstances ideal for wound healing. Due to the foam structure, foam dressings have good absorbent properties that enable their use for heavily exuding wounds (Figure 6) [35,36].

In a broad sense, some surgical implants can also be categorised as foams. There are collagen-based gentamicin-impregnated foam implants that can promote the healing of chronic and post-operative wounds by encouraging the wound healing process of the human organism. They ensure local antibiotic concentration higher than the MBC (minimal bactericide concentration) while keeping the systemic level below toxic [36,37].

### DepoFoam

DepoFoam<sup>®</sup> is a sustained-release, lipid-based multivesicular drug delivery system applied in the form of injections. The name is based on structural similarities with foams, only here the particles contain several internal aqueous chambers separated by a non-concentric continuous network of biocompatible and biodegradable lipid membranes. This unique structure results in high aqueous-volume – liquid ratio (95:5). Subsequently, high drug loading and encapsulation capacity for therapeutic proteins, peptides and water-soluble drugs are provided.

Compared to traditional liposomes DepoFoam<sup>®</sup> particles are larger. The great size (1100 μm) inhibits quick clearance by tissue macrophages and results in depot formation, from where the active ingredient release is sustained without a ‘burst’ ef-

fect. The release profile can be altered by changes in the composition.

The technology was successfully applied with several active ingredients, like interleukins, IGF, insulin, colony stimulating factors and different peptides. DepoCyt<sup>®</sup> is a clinically investigated intrathecal injection containing cytarabine for the treatment of malignant lymphomatous meningitis, while DepoDur<sup>™</sup> is a morphine-bearing epidural injection for reducing postoperative pain [38–41].

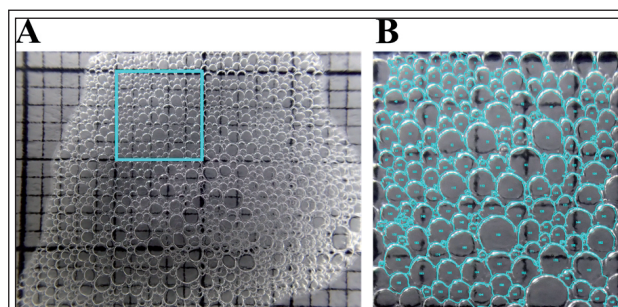
### Advantages

Foams offer a modern alternative in topical treatment. Owing to their good spreadability, fast absorption and easy application (even on large or hirsute areas), foams are preferred to creams or ointments. As rubbing in is not required, the application on sore, inflamed skin areas is painless. Furthermore, the emulsion-based emollient foams can also assist to the hydration of the skin without leaving oily residues and greasy feeling. These contribute to patient acceptance and enhanced compliance and adherence [12,13,42].

### Image analysis of foams

With picture analysis software (ImageJ, public domain program inspired by NIH, US) from photos or microscopic images, data can be gained from the shape, size and size distribution of the bubbles in foams. It enables the examination of the effect of the composition on the structure of the foam, or to follow the structural changes in time.

Figure 7A shows a picture (Olympus Stylus TG-4 digital camera, Olympus Corp., Japan) of a foam consisting of 5% w/w Labrasol<sup>®</sup> (Gattefossé, France) and distilled water (DW) formed with a propellant-free foam pump device (Figure 3 B, 100 ml, PET from Nordtek Imexco Kft., Hungary). Im-



**Figure 7** Photo of 5% w/w Labrasol<sup>®</sup> – DW foam before (A) and after analysis (B)



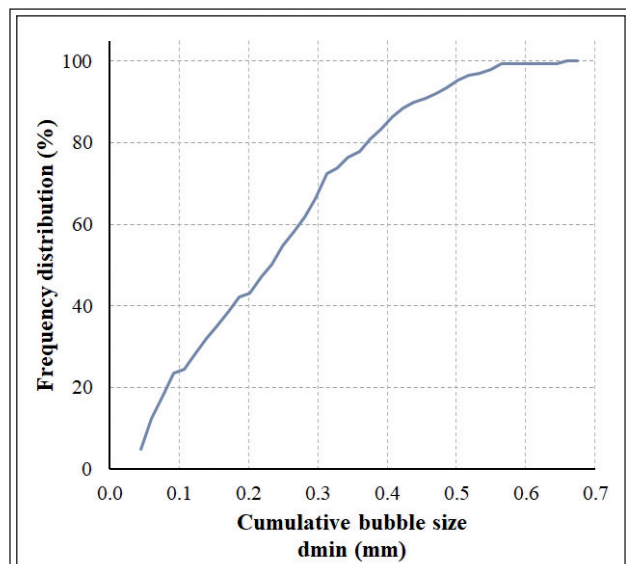


Figure 8 Cumulative bubble size distribution of the 5% Labrasol® – DW foam

Table III Image analysis data characterizing sample formulation

5% w/w Labrasol® – DW foam	
No.	199
Sum A	15.686 mm <sup>2</sup>
Mean A	0.079 mm <sup>2</sup> ± 0.077
Min A	0.003 mm <sup>2</sup>
Max A	0.401 mm <sup>2</sup>
D <sub>v,0.1</sub>	0.068 mm
D <sub>v,0.5</sub>	0.248 mm
D <sub>v,0.9</sub>	0.411 mm
Span	0.343

age analysis was accomplished from a 0,5x0,5 mm photo section (Figure 7 B). The results are shown in Table III Spanvalue was calculated as follows:

$$Span = \frac{D_{v,0.9} - D_{v,0.1}}{D_{v,0.5}} \quad (6)$$

where D<sub>v,0.9</sub>: 90% of the particles are under this diameter, D<sub>v,0.1</sub>: 10% of the particles are under diameter, D<sub>v,0.5</sub>: 50% of the particles are under this diameter.

The bubble size data gained from image analysis

enables the determination of bubble size distribution. Drawing frequency distribution (%) over the cumulative bubble size diameter (d<sub>min</sub>; mm) results in the cumulative bubble size distribution graph (Figure 8).

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## Bioequivalence metrics for absorption rates: linearity, specificity, sensitivity

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### Abstract

**Aims:** In order to ensure the therapeutic equivalence of generic products, it would be important to contrast measures additional to  $C_{max}$  in order to assess differences in absorption rates. Our aim was to compare partial AUC (PAUC), Swing, and PTF to  $C_{max}$  in terms of sensitivity, specificity and linearity under identical kinetic conditions.

**Methods:** Single-dose and multiple-dose concentration curves were generated assuming one-compartment models. Kinetic sensitivity curves were obtained by gradually changing the absorption rate constant and keeping all other parameters fixed.

**Results:** Ideally, a metric should reflect specifically the investigated kinetic feature (e.g., the rate of absorption), be linearly related to it, and should exhibit high kinetic sensitivity.  $C_{max}$  is related nonlinearly to the rate of absorption, is nonspecific to it (reflects also the extent of absorption as well as the rates of disposition processes), lacks kinetic sensitivity even following a single administration. Compared to  $C_{max}$ , PAUC was always more sensitive under every investigated condition. Swing and PTF showed high kinetic sensitivity but, in contrast to PAUC, they could be evaluated only in multiple-dose studies.

**Conclusion:** Under identical conditions, different metrics provide widely differing point estimates. Differences in kinetic sensitivity among bioequivalence metrics should be accounted for when results of different metrics are compared.

**Keywords:** Bioequivalence, Generic drugs, Partial AUC, Pharmacokinetics

### Introduction

There is general consensus on the criteria for evaluating the equivalence of the extent of absorption in two drug products which exhibit simple linear kinetics, following their single administration to individuals [1]. In contrast, there is no agreement on testing parameters for absorption rates. Major regulatory agencies apply different criteria. For example, the US Food and Drug Administration has identical expectations for judging the equivalence of two  $C_{max}$  values (maximum plasma concentration) and two AUC values (area under the curve contrasting plasma concentration with time). The European Community considers that  $C_{max}$  has generally larger variability than AUC and allows a more relaxed criterion to the former. The Canadian Health Protection Branch requires, in view of the prevailing uncertainty about the variability of  $C_{max}$ , that only the ratio of AUC values, recorded

for the two formulations, should be between pre-set limits [2-5].

A source of uncertainties is that  $C_{max}$  is only an indirect measure of absorption rates and absorption rate constants, as are other indices. Thus, these metrics may have limited specificity, sensitivity and precision. Explorations have been conducted for an appropriate metric testing the equivalence of absorption rates. For example, Endrenyi et al. [6,7] suggested that  $C_{max}/AUC$  should be used as an indirect index of absorption rates and proposed regulatory criteria based on the computed variability of  $C_{max}/AUC$  and  $C_{max}$ . Bois et al. [8] and Reppas et al. [9] compared several indices under various modeling assumptions but have not reached decisive conclusions. Studies of the effective evaluation of bioequivalence have considered mostly the single administration of drugs which follow uncomplicated linear kinetics. Information on the quantitative features of procedures and in-



dices for testing bioequivalence in the steady state is more limited.

Therefore, the present communication has two main goals: (1) To develop principles by which metrics characterizing pharmacokinetic features, especially those involving bioavailability and bioequivalence, can be evaluated; (2) to illustrate the application of the principles following single and repeated dose administration.

### Useful Features of Metrics Characterizing Bioavailability and Bioequivalence

Studies were performed [8-10] which recorded difficulties and complications in evaluating indirect metrics which characterize absorption rates, and evaluated their comparisons. Therefore, criteria for assessing features of such metrics will be discussed.

An ideal metric used for the evaluation of bioavailability and bioequivalence should reflect specifically the underlying kinetic parameter, be related linearly to it, have high kinetic sensitivity, and exhibit high statistical insensitivity. Direct metrics possess these features; the performance of AUC reflecting the extent of drug absorption serves as an example.  $C_{max}$  is an indirect metric for the rate of absorption; its general characteristics and some of the difficulties noted after the single administration of drug products will be illustrated in this section.

#### *Specificity*

A good metric should represent solely, specifically the investigated kinetic feature. For example, AUC is specific since it reflects only, in conjunction, plasma clearance and the extent of absorption; it does not depend on other kinetic parameters. In contrast,  $C_{max}$  reflects nonspecifically the rate of absorption; it depends also on the rates of disposition processes as well as the extent of absorption.  $C_{max}/AUC$  is more specific than  $C_{max}$  since it does not reflect the extent of absorption [6]. However, this parameter still varies along with the rates of disposition processes.

#### *Linearity*

The relationship between the applied metric and the investigated kinetic feature is characterized by a mathematical relationship. In the simplest case, the relationship is linear. For example, the mea-

sured AUC depends linearly on the extent of absorption. On the other hand, AUC is related reciprocally to plasma clearance. Consequently, the logarithm of AUC is connected linearly to the logarithms of both the extent of absorption and plasma clearance.

In contrast,  $C_{max}$  is related nonlinearly to  $k_a$  (the absorption rate constant). As discussed in the next subsection, the lack of kinetic sensitivity is a consequence of nonlinearity. Moreover, the observed variation of nonlinear metrics depends not only on the intraindividual but also intersubject variability of the underlying parameter(s). In addition, criteria for corresponding, parallel regulatory conditions depend on the levels of the sources of variation.

#### *Kinetic Sensitivity*

If a change in an investigated kinetic parameter elicits an identical change in the measured metric, then the latter can be said to exhibit full kinetic sensitivity. Full kinetic sensitivity prevails with respect to the extent of absorption: a 25% change in the latter elicits a 25% change in AUC. If, however, a change in the parameter gives rise to a much smaller change in the metric then, the metric lacks full kinetic sensitivity to the parameter.

Kinetic sensitivity can be evaluated, approximately, from the slopes of relationships between the metrics and the investigated parameters. Use of a double-logarithmic relationship is convenient since both kinetic metrics and parameters have multiplicative character.

However, studies of the effective evaluation of bioequivalence have usually considered single-dose administration scenarios for drugs with uncomplicated linear kinetics. There are substantially fewer studies in the steady state. Even though it is generally recognized that  $C_{max}$  is an insensitive metric [6-9] much less is known about its behavior at steady state, and how  $C_{max}$  sensitivity compares to other steady-state metrics proposed in the literature. Furthermore, results obtained from single-dose studies cannot be directly compared with the steady-state results unless the kinetic conditions are identical. Partial AUC (PAUC) is a recently introduced early exposure measure defined as the area under the curve until the time of maximum concentration ( $T_{max}$ ) of the reference formulation [10]. Its kinetic properties have been investigated following single-dose administration but not under steady-state conditions.

Therefore, we set the goal to compare the kinetic sensitivities of C<sub>max</sub> and the proposed alternative metrics, including PAUC, following single and repeated dose administrations under identical conditions.

### Methods

The kinetic model in the calculations and simulations assumed single exponential terms for absorption and disposition. Consequently, the time (t) course of concentrations (C) was described by the expression following single administration:

$$C(t) = \frac{F * Dose}{Vd} \frac{ka}{ka - \frac{Cl}{Vd}} \left( e^{-\frac{Cl}{Vd}t} - e^{-ka*t} \right) \quad [1]$$

and at steady state:

$$C(t) = \frac{F * Dose}{Vd} \frac{ka}{ka - \frac{Cl}{Vd}} \left( \frac{e^{-\frac{Cl}{Vd}t}}{1 - e^{-\frac{Cl}{Vd}T}} - \frac{e^{-ka*t}}{1 - e^{-ka*T}} \right) \quad [2]$$

where F is the fraction of dose absorbed into the systemic circulation, Vd is the apparent volume of distribution, T is the maintenance time interval, Cl is the drug clearance and ka is the absorption rate constant. The condition of single drug administration is approached as T is increased.

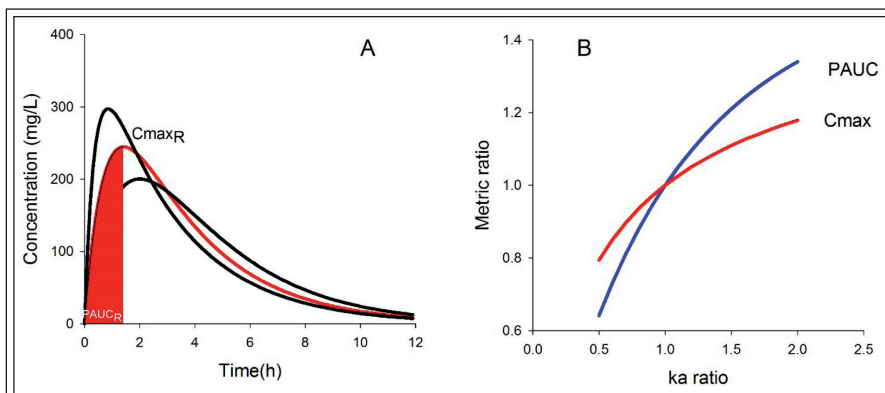
The application of several metrics has been proposed [9] for repeated administration bioequivalence studies. They include the maximum and minimum concentration (C<sub>max</sub> and C<sub>min</sub>), and the peak-trough fluctuation (PTF) and Swing which are defined as:

$$PTF = C_{max}/C_{min}$$

$$Swing = (C_{max} - C_{min})/C_{min}$$

where C<sub>max</sub> and C<sub>min</sub> are the maximum and minimum concentrations, respectively, during a dosing interval.

The true average parameters for the reference product, drug R, were arbitrarily set to ka = 1.39h<sup>-1</sup>, Vd = 1 L, Cl = 0.347 L/h, Dose = 400 (in arbitrary units).



**Figure 1** Metric definitions and metric sensitivity following a single oral dose. (A) Concentration-time profiles of the Reference (R) product in red and two Test (T) formulations, in black, following a single dose. Absorption rate constants are compared indirectly, by comparing numerical characteristics called bioequivalence metrics from the observed concentrations. The left panel of the figure illustrates the two regulatorily accepted bioequivalence metrics for absorption rates: C<sub>max</sub> and PAUC (shaded area). The bioequivalence decision is based on the statistical comparison of the appropriate metrics such as C<sub>max,T</sub> versus C<sub>max,R</sub>. (B) Calculated kinetic sensitivity curves contrasting the metric ratio with the corresponding ka, absorption rate constant, ratios.

<sup>1</sup>, Vd = 1 L, Cl = 0.347 L/h, Dose = 400 (in arbitrary units).

The kinetic equations were evaluated using Matlab (ver 2017b, MathWorks, USA).

### Results

#### Single-Dose Administration

Figure 1A shows concentration profiles of a reference (R) and a test (T) drug product which differ in their rate constants but not in their extents of absorption. For the quantitative comparison of the curves appropriate single-number characteristics are needed. These are called bioequivalence metrics. Figure 1A illustrates the definitions of two commonly used metrics, C<sub>max</sub> and PAUC. The figure shows in red the concentration profile for the reference product with the stated kinetic parameters. The concentration of two simulated sub-

**Table I** Sensitivity of absorption metrics following a single dose administration. The sensitivity of a bioequivalence metric is defined as the slope of the curve contrasting logarithmic absorption rate constants and logarithmic metric ratios. Sensitivities were calculated in two different kinetic conditions, when the elimination rate is relative fast to that of absorption (ka/ke=4) and when it much slower (ka/ke = 12)

Metrics	Sensitivity	
	ka/ke ratio = 4	ka/ke ratio = 16
C <sub>max</sub>	0.269	0.123
PAUC	0.498	0.358

jects are also shown in black whose absorption rate constants are different from that of the reference formulation but who have the same extent of absorption, and therefore the same AUC.

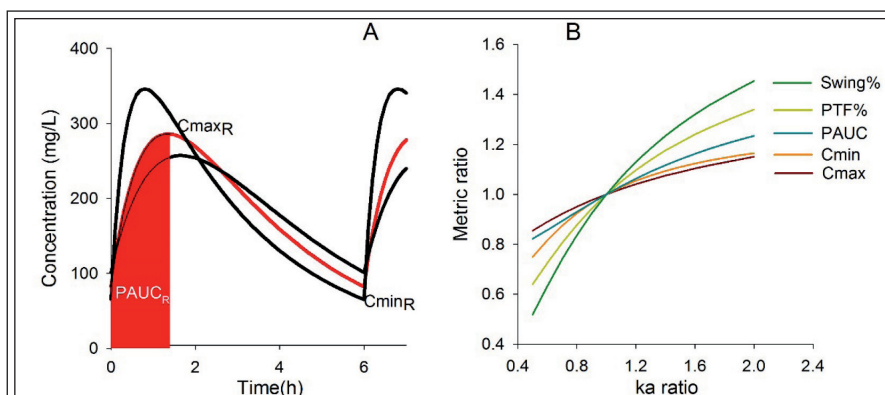
As the absorption rate constant of the test formulation gradually increases (or decreases) relative to the reference product so do the corresponding metrics. The quantitative relationships between absorption rate constants and the metrics with default kinetic parameters are displayed in Figure 1B. The slope between the logarithmic metric and  $k_a$  ratios can be used for numerical comparison. Slopes approximating 1.0 suggest satisfactory, high sensitivity while small values indicate low sensitivities. Table I shows that the slopes of  $C_{max}$  and PAUC are below 1. Therefore, compared to AUC,  $C_{max}$  is not a sensitive metric. Compared to  $C_{max}$ , PAUC is a more sensitive measure. However, neither indirect metric is specific, the slopes depend on the  $k_a/k_e$  ratio (Table I).

#### Repeated Drug Administration

The kinetic parameters in Figure 2A are the same as in Figure 1A but now the concentration profiles represent the steady-state condition.  $C_{max}$  and PAUC increase with rising absorption rate constant, relative to the reference product, while  $C_{min}$  (the concentration just before the next dose) decreases. Therefore, for easy visual comparison for  $C_{min}$  we computed the  $C_{min_R}/C_{min_T}$  ratio instead of  $C_{min_T}/C_{min_R}$ . Figure 2B shows that, at steady state, Swing is the most sensitive parameter

**Table II** Pharmacokinetic sensitivities of 5 metrics estimating absorption rates in the steady state. Under both kinetic conditions the between dose interval ( $T$ ) equals with the drug half-life.

Metrics	Sensitivity	
	$k_a/k_e$ ratio = 4	$k_a/k_e$ ratio = 16
<b><math>C_{max}</math></b>	0.213	0.079
<b><math>C_{min}</math></b>	0.287	0.062
<b>PTF</b>	0.499	0.138
<b>Swing</b>	0.673	0.360
<b>PAUC</b>	0.307	0.122



**Figure 2** Metric definitions and metric sensitivity following repeated dosing. (A) Concentration-time profiles of the Reference (R) and two Test (T) formulations in steady state. The left panel shows three directly calculated metrics for the reference drugs: the maximum concentration ( $C_{max_R}$ ), the minimum concentration ( $C_{min_R}$ ), and the area until  $T_{max}$  ( $PAUC_R$ ). (B) Calculated kinetic sensitivity curves contrasting the metric ratio with the corresponding  $k_a$ , absorption rate constant ratios. PTF and Swing are two additional, derived metrics defined as  $C_{max}/C_{min}$  and  $(C_{max}-C_{min})/C_{min}$ , respectively.

followed by PTF and PAUC. Compared to these metrics,  $C_{max}$  and  $C_{min}$  are much less sensitive (Table II). Just as in the case of single dose administration, all slopes are smaller than 1 and they decrease in parallel with  $k_a/k_e$  ratio. The slopes are higher in Table I than in the corresponding entries of Table II.

#### Discussion

We computed and compared kinetic sensitivities of absorption metrics following single and repeated dosing in two different kinetic conditions. Even though the numerical details were different, the relative ranking remained the same. PAUC was always more sensitive than  $C_{max}$  and metrics estimated from single dose studies were more sensitive than their steady-state counterparts. However, considerations for assessing comparative absorption rates are much more difficult and complicated in the steady state than following the single administration of drugs. The decrease in sensitivity was expected since the estimations generally involve the determination of the maximum concentration or a quantity related to it. The net height of concentration peaks (the fluctuation of concentrations) is lower in steady state than following a single administration and they further decrease with increasing accumulation. Nevertheless, Swing and PTF at steady state were more sensitive than the metrics applied following a single dose. Therefore, it is possible construct sensitive metrics that can be applied under steady-state

conditions. This is important because in certain cases bioequivalence should be demonstrated in the steady state possibly because single-dose studies cannot be performed at all. Additional multiple-dose bioequivalence studies are needed for prolonged-release formulations [11]. Multiple-dose bioequivalence studies are also accepted if a single-dose study cannot be conducted in healthy volunteers due to tolerability reasons. Multiple-dose bioequivalence studies are recommended with drugs with dose- and time-dependent kinetics such as strong enzyme inductors. In the latter case, the sensitivity can be even higher at steady state compared to a single dosing [12]. The reason is that due the enhanced elimination rate the  $k_a/k_e$  ratio is smaller and, as we showed, the kinetic sensitivity of the absorption metrics negatively correlates with the  $k_a/k_e$  ratio (Tables I and II).

### Conclusion

Under identical conditions different metrics provide differing point estimates. Differences in kinetic sensitivities among bioequivalence metrics should be accounted for when results of different metrics are compared. The kinetic analysis of bioequivalence metrics helps also not only to design new, sensitive metrics but also to identify clinical situations when the results of standard single-dose bioequivalence studies cannot be easily extrapolated to clinical settings.

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Acta Pharmaceutica Hungarica



# A bibliometric analysis of Acta Pharmaceutica Hungarica (1965-2018)

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## Abstract:

**Aims:** The aim of this study is present a general overview of the journal from 1965 to 2018 using bibliometric indicators.

**Methods:** The data analyzed in this study, was extracted from the Scopus database. The information was exported in RIS file format to the Harzing's Publish or Perish and VOSviewer software for data analysis.

**Results:** The number of documents published in APH during the study period was 1868, the most frequently published document type was „original article”. Articles published APH were cited 2555 times overall, averaging 1.37 citations per each paper. The highest number of articles published in collaboration was in 2007 and 2018 (16.7%). Overall, the period between 1981-2000 was the most fruitful, while the following years saw a decline in the number of articles. Clusters and co-occurrence networks of the authors keywords of the published documents were generated.

**Conclusion:** APH manages to reach a very wide audience of the Hungarian scientific community of researchers. Additionally, an evolution on the research topics covered by the journal could be observed, providing a contemporary overview of the pharmaceutical sciences. The submission of additional articles to the journal is highly encouraged to widen the reach of this journal towards international audiences.

**Keywords:** Acta Pharmaceutica Hungarica, bibliometric analysis, pharmaceutical science, citation, cluster analysis

## 1. Introduction

Acta Pharmaceutica Hungarica (APH) was founded by the Hungarian Society for Pharmaceutical Sciences (MGYT) together with its sister journal, *Gyógyszerészet (Pharmacy)*. While the latter is a monthly publication, which aims to assist pharmacists in education, continuous professional development, highlighting relevant literature and to inform about professional affairs, both in Hungary and on an international scale, APH is quarterly, research-oriented journal, reporting on the experimental results of laboratories involved in pharmaceutical science [1,2]. Bibliometrics is an area of science, which is concerned with the qualitative and quantitative assessment of research, that is often used to evaluate the impact of journals, institutions, study groups or individual scientists [3,4]. The bibliometric analysis of a given journal may be important for various reasons; university libraries often evaluate these characteristics before including journals in their collection. Furthermore, authors study commonly known bibliometric parameters (e.g., number of publications per year, impact factor, CiteScore, h-index) before submitting a manuscript to a journal [5,6]. Hence, the

present study aims to give a general bibliometric overview of APH in a 53-year period (1965-2018), since the journal was listed by international content indexing services.

## 2. Materials and methods

The data analyzed in this study, dating between 1965 and 2018 was extracted from the Scopus database on the 15<sup>th</sup> of October 2018. The information was exported in RIS (Research Information Systems, Incorporated) file format to the Harzing's Publish or Perish 6.35 software for data analysis [7,8]. In addition, VOSviewer 1.6.9 software was used to generate the collaboration and word co-occurrence networks from the recovered data [9].

## 3. Results

The total number of documents published in APH during the study period (1965-2018) was 1868, the number of papers published since 2001 decreased by 46.31-47.11% compared to the two previous periods (Table I) The most frequently published document type indexed by Scopus was „original article” (n=1710, 91.54%), followed by „review” (n=114, 6.10%;

Table I General bibliometric characteristics of APH between 1965-2018

Publication years	1965-1980	1981-2000	2001-2018	Overall
Number of papers	743 (39.77%)	732 (39.19%)	393 (21.04%)	1868
Citations on the papers during the study period (1965-2018)	600 (23.48%)	1463 (57.26%)	492 (19.26%)	2555
Cites per year	11.32	39.54	28.94	48.21
Cites per paper	0.81	2.00	1.25	1.37
Authors per paper	2.36	3.06	3.47	2.87
h-index	8	14	10	14

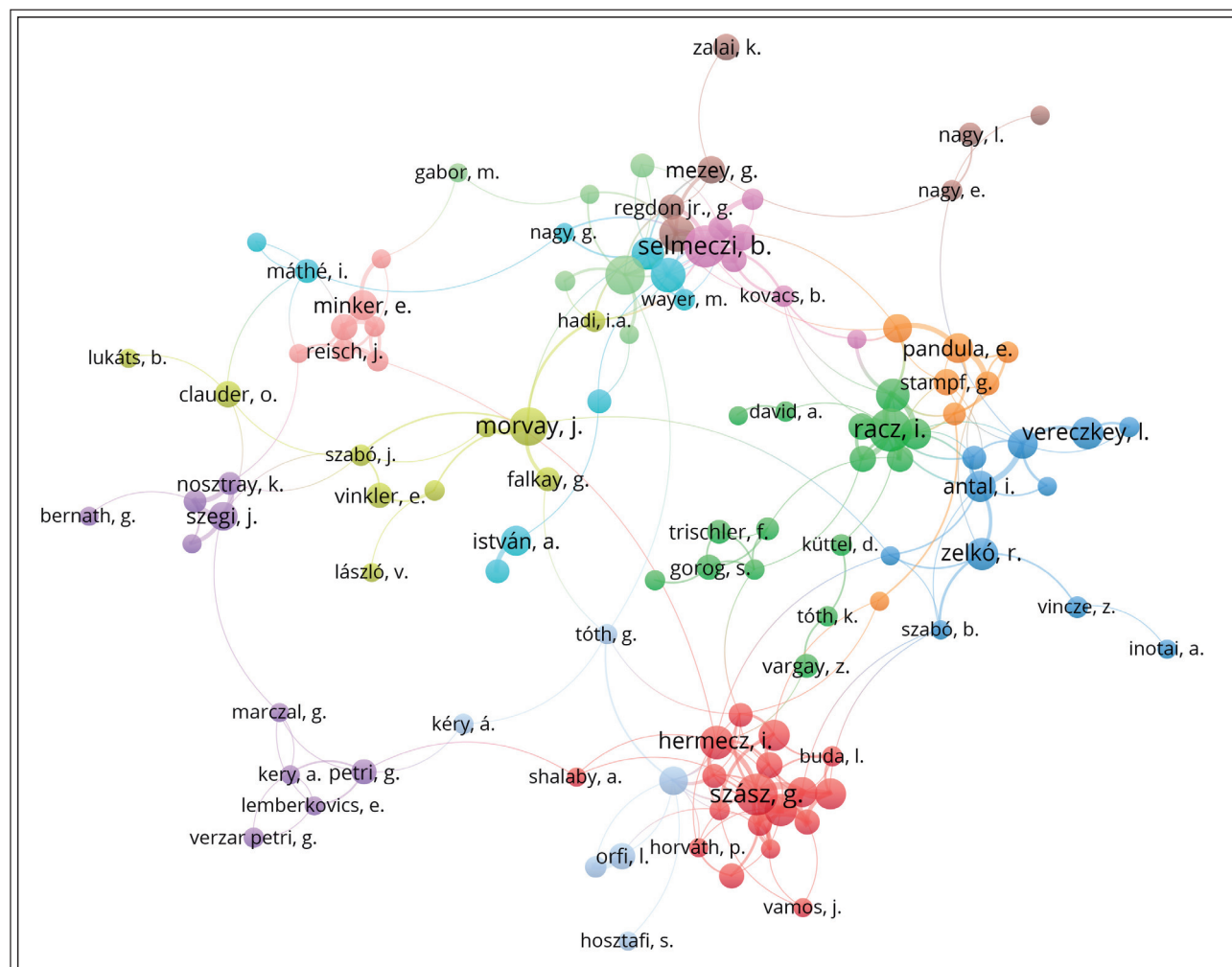


Figure 1 Co-author collaboration network from documents published in APH between 1965-2018

first occurrence in 1991), „short survey” (n=24, 1.28%; first occurrence in 1990) and 18 „conference paper” (n=18, 0.96%; first occurrence in 1989). There was only one paper classified as an „editorial” (in 2011 [10]) and an „erratum” (in 2003), respectively. The average amount of authors per paper increased consistently throughout the years (2.36-3.47; Table I).

Regarding the impact of papers in APH, articles published between 1965 and 2018 were cited 2555 times overall, averaging 1.37 citations per each pa-

per, with more than 57% of overall citations on articles published in the period between 1981-2000. The average ratio of self-citations was 26.76% (0-69.23%; highest in 2001). The papers with high amount of citations (defined as 15 or more cites) correspond to 12.13% of total citations (8.86 citations per year, 140.99 cites/author) for APH (articles listed in Table II). The journal is associated with two subject categories, namely *Medicine (miscellaneous)* and *Pharmaceutical sciences*. Based on the in-





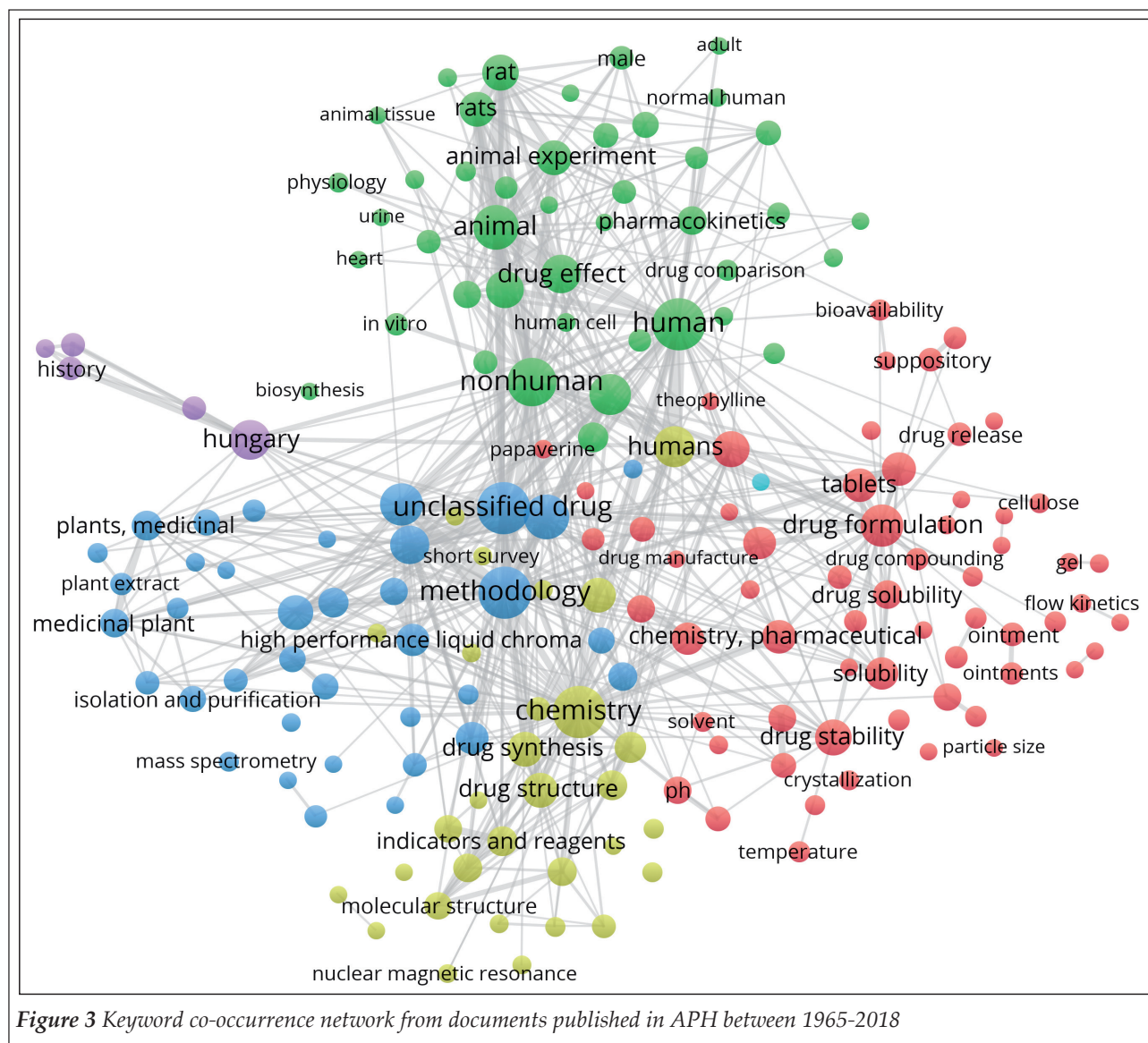


Figure 3 Keyword co-occurrence network from documents published in APH between 1965-2018

#### 4. Discussion

The aim of this study was to assess APH using bibliometric methods in the time period between 1965-2018, in which indexing data was available from the Scopus database. From an institutional perspective, most of the papers originated from the four universities in Hungary (Budapest, Debrecen, Pécs and Szeged) where there is a Faculty of Pharmacy or the training of pharmacists takes place. In addition, the Budapest University of Technology (BME) and some industrial institutions (e.g., Gedeon Richter Ltd.) were also important contributors. Citations are generally considered an important parameter to assess the quality of papers published in any given journal, however, the absolute number of citations is not always informative (self-citations or “negative citations” that call attention to flawed

papers may distort this bibliometric indicator). The average ratio of self-citations for APH was 26.76%, which can be considered a good result, compared to other journals published in Hungary. Overall, the 20-year period between 1981-2000 was the most fruitful for this journal, based on the number of published articles and the amount of citations, while the following years between 2001 and 2018 saw a decline in the number of articles by almost 50% and the number of citations by nearly 300%, whereas the ratio of papers reporting on the research of international collaboration was variable [25-27]. The reason for this decrease may be attributable to the increasing pressure to publish in international journals with (preferably high) impact factors, because this has become a pre-requisite for the awarding of doctoral (PhD) degrees and for successful application for research grants.

Table II Publications from APH with the most amount of citations

Publication	Year	Type	Citations	Cites/year
Kiss, B., Kárpáti, E.: <i>On the mechanism of action of vinpocetine</i> [11]	1996	Review	35	1.59
Fejes, S., Kéry, Á., Blázovics, A., Lugasi, A., Lemberkovics, É., Petri, G., Szőke, É.: <i>Investigation of the in vitro antioxidant effect of Petroselinum crispum (Mill.) Nym. ex A. W. Hill.</i> [12]	1998	Article	29	1.45
Medhin, D.G., Hadházy, P., Bakos, P., Verzár-Petri, G.: <i>Hypotensive effects of Lupinus termis and Coriandrum sativum in anaesthetized rats. A preliminary study</i> [13]	1986	Article	29	0.91
Matus, Z., Molnár, P., Szabó Gy.: <i>Main carotenoids in pressed seed (Cucurbitae semen) of oil-pumpkin (Cucurbita pepo convar. pepo var. styriaca)</i> [14]	1993	Article	26	1.04
Császár, J., Morvay, J.: <i>Characteristics of Schiff bases derived from salicylaldehyde and sulphonamides. Spectral and antibacterial studies</i> [15]	1983	Article	25	0.71
Budai, M., Szógyi, M.: <i>Liposomes as drug carrier systems. Preparation, classification and therapeutic advantages of liposomes</i> [16]	2001	Article	23	1.35
Issopoulos, P.B.: <i>Analytical investigations of <math>\beta</math>-lactam antibiotics in pharmaceutical preparations. IV. Sensitive colorimetric determination of five cephalosporins of the first generation</i> [17]	1991	Article	23	0.85
Kéry, A., Horváth, J., Nász, I., Verzár-Petri, G., Kulcsár, G., Dán, P.: <i>Antiviral alkaloid in Chelidonium majus L.</i> [18]	1987	Article	20	0.65
Lemberkovics, É., Kéry, Á., Marczal, G., Simándi, B., Szőke, É.: <i>Phytochemical evaluation of essential oils, medicinal plants and their preparations</i> [19]	1998	Short survey	18	0.90
El-Brashy, A.M.: <i>Titrimetric determination of captopril in dosage forms</i> [20]	1995	Article	18	0.78
Issopoulos, P.B.: <i>A sensitive spectrophotometric determination of acetaminophen</i> [21]	1992	Article	17	0.65
Sarg, T.M., Ateya, A.M., Farrag, N.M., Abbas, F.A.: <i>Constituents and biological activity of Bidens pilosa L. grown in Egypt</i> [22]	1991	Article	16	0.59
Kata, M., Schauer, M.: <i>Increasing the solubility characteristics of albendazole with dimethyl-<math>\beta</math>-cyclodextrin</i> [23]	1991	Article	16	0.59
Szántay Cs. Jr., Bihari, M., Brlik, J., Csehi, A., Kassai, A., Aranyi, A.: <i>Structural elucidation of two novel ergot alkaloid impurities in <math>\alpha</math>-ergokryptine and bromokryptine</i> [24]	1994	Article	15	0.63

Table III Quartile ranking of APH between 2001-2018

Category	2001	2002	2003	2004	2005	2006	2007	2008	2009
Medicine (miscellaneous)	Q3	Q3	Q2	Q3	Q3	Q3	Q3	Q3	Q4
Pharmaceutical sciences	Q3	Q3	Q2	Q3	Q4	Q3	Q3	Q4	Q4
	2010	2011	2012	2013	2014	2015	2016	2017	2018
Medicine (miscellaneous)	Q3	Q4	Q4	Q4	Q4	Q4	Q4	Q4	Q4
Pharmaceutical sciences	Q3	Q4	Q4	Q4	Q4	Q3	Q4	Q4	Q4

Table IV Articles published in international collaboration in APH between 2001-2018

	2001	2002	2003	2004	2005	2006	2007	2008	2009
Precentage	0%	0%	6.1%	3.7%	4.4%	8.3%	16.7%	10.5%	5.0%
	2010	2011	2012	2013	2014	2015	2016	2017	2018
	0%	6.7%	0%	6.7%	5.6%	0%	15.8%	0%	16.7%





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Acta Pharmaceutica Hungarica

## New computational studies to support cyclin-dependent kinase 9 inhibitor screening and design

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### Abstract:

**Aims:** Cyclin-dependent kinase 9 (CDK9) plays a major role in the regulation of transcription. Its overexpression – which occurs in several types of cancer – increases the levels of certain antiapoptotic proteins that can lead to tumorigenesis, therefore the identification of new, more potent and more selective inhibitors is essential.

**Methods:** In this study we present a computational approach, which can facilitate lead selection and optimization.

**Results:** First, a pharmacophore hypothesis based on the active compounds has been developed to identify the key features for the ligand-target interaction. This was followed by the docking of the compounds into the active site of CDK9, the poses and interactions with the amino acids were compared with those of the co-crystallized ligand. The mode of their binding further explained the characteristics of these inhibitors while the docking scores can be a factor in the selection of active compounds in the future. Finally, a field-based QSAR model was also created, to predict the activity of inhibitor candidates.

**Conclusion:** With our current work we deepened our knowledge about the interactions between CDK9 and its inhibitors, which can contribute to the discovery of novel CDK9 inhibitors.

**Keywords:** CDK9 inhibitor, docking, QSAR, pharmacophore model

## 1. INTRODUCTION

Cyclin-dependent kinases (CDK) are characterized as Ser/Thr kinases. Twenty members of this group have been discovered so far [1]. In most cases their activity depends on cyclins, which bind to them and act as regulatory subunits to control kinase activity and substrate specificity. These CDK-cyclins complexes are usually involved in cell cycle and transcription regulation, therefore their overexpression or amplification can easily lead to improper operation of cell cycle or transcription, which may cause uncontrolled proliferation and thus tumor formation [2].

CDK9 can be expressed in two isoforms, a lighter 42 kDa protein and a heavier 55 kDa protein [3]. Both are capable of forming complexes with cyclin T1, T2a, T2b and K [4]. The structure of CDK9 shows typical features of its class. There is an N-terminal lobe (residues 16-108), which contains five segments of  $\beta$ -sheet completed with a single  $\alpha$ -helix, while the C-terminal lobe (residues 109-330) is comprised of four  $\beta$ -strands and seven main  $\alpha$ -helices [5]. The ATP binding site is located in a cleft between the two lobes of the protein and

there is also a loop behind them called 'hinge'. All these three parts are involved in the ATP binding. The binding pocket has a characteristic sickle-like shape, with a volume of approximately 360 Å<sup>3</sup> (calculated by Schrödinger's SiteMap application) [6, 7]. It is relatively narrow and flat with a hydrophobic region in the middle. The structure of CDK9 is very similar to that of CDK2, as they have 40% sequence identity [5].

The determination of its crystallographic structure became possible only 14 years after the discovery of CDK9 [5]. Sixteen crystal structures of it were deposited in the RCSB database until present day. For our project we retrieved one from the Protein Database (PDB ID: 4BCF). It is in complex with cyclin T and what is more important with a 2-amino-4-heteroaryl-pyrimidine inhibitor. We have selected this structure, because the co-crystallized inhibitor was similar to our ligands, so it probably binds with similar interactions to the protein.

Physiologically, cyclin T is needed for the activation of the enzyme by the phosphorylation of Thr<sup>186</sup>. After that the complex can fulfill its role [8], which is the following: CDK9/cyclin T is a part of

a larger protein complex called positive transcription elongation factor b (P-TEFb) in which the CDK9 can phosphorylate the C-terminal domain (CTD) of RNA polymerase II (RNAPII). This is a key regulatory mechanism during elongation [4]. CDK9 mediated phosphorylation of the CTD in RNAPII increases the levels of antiapoptotic proteins such as Mcl-1 and XIAP. If CDK9 is overexpressed or amplified, this process can lead to tumorigenesis [9, 10]. Additionally, through the hexamethylene bisacetamide-inducible protein 1 (HEXIM1), CDK9 can be associated with the replication of Human Immunodeficiency Virus (HIV) [11]. Therefore, CDK9 inhibitors could be used in HIV and cancer therapy as well.

Several CDK and CDK9 inhibitors have been developed so far. Roscovitine (seliciclib) was the first CDK inhibitor to enter clinical trials [12], but palbociclib was the first that obtained FDA approval [13, 14]. As for CDK9, one of the most significant inhibitors is dinaciclib that reached the most advanced clinical phase [13, 15]. One of the main problems regarding the current CDK9 inhibitors is the lack of selectivity, which may lead to severe side effects during therapy [16]. Therefore, the need for more selective CDK inhibitors has been emerged. Our work aims to provide a useful method in compound pre-screening to reduce the time and costs of lead selection and optimization.

## 2. MATERIALS AND METHODS

### 2.1 Data set

All models we created were based on a set of 41 CDK9 inhibitors that was synthesized by our research group [17]. We kept the numbering of the compounds as it is in the above-mentioned paper. The structures and  $IC_{50}$  values of the compounds are listed in [Table I](#).

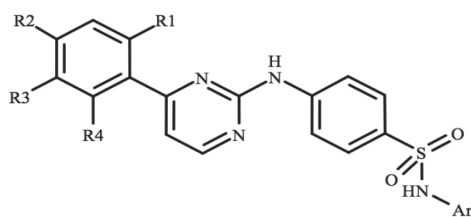
During our work we used Schrödinger's Maestro software [18] to run calculations and create models.

### 2.2 Protein and ligand preparation

The first task was to prepare the crystal structure of 4BCF with Protein Preparation Wizard [19], which included preprocessing, H-bond assessment and restrained minimi-

zation. Default settings were used in all parts of the preparation. Ligand structures were drawn with Schrödinger's Maestro graphical user inter-

**Table I** Structure and CDK9 inhibitory activity of *N*-aryl pyrimidinamines [17].



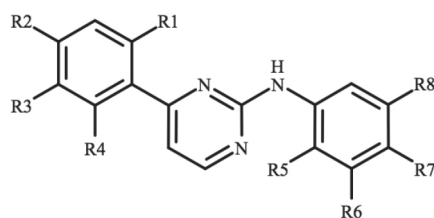
ID	R1	R2	R3	R4	Ar	IC <sub>50</sub> (nM)
21	MeO	H	H	H	3,4-dimethyl-isoxazole	3
22	MeO	F	H	H	3,4-dimethyl isoxazole	2
23	MeO	H	F	H	3,4-dimethyl-isoxazole	9
24	MeO	H	H	F	3,4-dimethyl-isoxazole	1
25	MeO	F	F	H	3,4-dimethyl-isoxazole	8
26	MeO	H	F	F	3,4-dimethyl-isoxazole	1
27	MeO	CL	H	H	3,4-dimethyl-isoxazole	12
28	MeO	H	H	Cl	3,4-dimethyl-isoxazole	25
29	MeO	MeO	H	H	3,4-dimethyl-isoxazole	9
30	EtO	F	H	H	3,4-dimethyl-isoxazole	8
31	EtO	H	H	F	3,4-dimethyl-isoxazole	2
32	EtO	Cl	H	H	3,4-dimethyl-isoxazole	17
33	iPro	F	H	H	3,4-dimethyl-isoxazole	49
34	H	H	iPro	F	3,4-dimethyl-isoxazole	458
35	MeS	F	H	H	3,4-dimethyl-isoxazole	13
36	Me	F	H	H	3,4-dimethyl-isoxazole	6
37	F	F	H	H	3,4-dimethyl-isoxazole	11
38	F	MeO	H	F	3,4-dimethyl-isoxazole	21
39	Cl	F	H	H	3,4-dimethyl-isoxazole	55
40	MeO	Cl	H	H	3,4-dimethyl-isoxazole	50
41	MeO	H	H	H	4,5-dimethyl-isoxazole	4
42	MeO	F	H	H	4,5-dimethyl-isoxazole	3
43	MeO	F	H	H	benzo[d]isoxazole	5
44	MeO	H	H	H	thiazole	33
45	MeO	F	H	H	thiazole	122
46	MeO	H	H	F	thiazole	2
47	EtO	F	H	H	thiazole	210
48	EtO	Cl	H	H	thiazole	378
49	MeO	H	H	H	*	12
50	MeO	F	H	H	*	6
51	MeO	H	F	H	*	9
52	MeO	H	H	F	*	2
53	MeO	F	F	H	*	10
54	MeO	H	F	F	*	0.4
55	MeO	MeO	H	H	*	14
56	EtO	F	H	H	*	2
57	EtO	Cl	H	H	*	5

\*: 2-methyl-[1,3,4]thiadiazol

Continuation on next page



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ID	R1	R2	R3	R4	R5	R6	R7	R8	IC <sub>50</sub> (nM)
58	MeO	H	H	H	F	H	MeO	***	1576
66	MeO	H	H	H	H	**	H	H	3
67	MeO	F	H	H	H	**	H	H	4
68	MeO	H	H	F	H	**	H	H	2

\*\*: 1H-benzimidazole-1-yl-methyl

\*\*\*: carboxamide

face (GUI), then the LigPrep module was used to generate energy-minimized 3D conformations of each compound. To perform this, the optimized potential for liquid simulations 2005 (OPLS-2005) force field [20] was adopted. One stereoisomer per ligand was studied. As for ionization forms, we generated the most possible state of each molecule with Ionizer at target pH of 7.4. The negative logarithm values ( $pIC_{50}$ ) of the previously published inhibitory activities were calculated and used for the model building [17]. Flexible Ligand Alignment procedure of the Maestro GUI was utilized for the superposition of the ligands. The best alignment was received by using the 'Common scaffold alignment' with 'fuzzy matching' option.

### 2.3 Docking

Schrödinger's Grid-based Ligand Docking with Energetics (GLIDE) was used in the docking studies. Firstly, we created the grid box for the docking. The co-crystallized ligand was used to determine the place of the binding pocket and after measuring the size of our ligands we decided to

set the size of the grid box for 20×20×20 Å to surely have enough space in it for all ligands. The co-crystallized ligand was re-docked with both the 'score in place' and the standard precision (SP) procedures in order to validate our model. Then all the examined ligands were docked via both the SP and the extra precision (XP) methods. Flexible ligand sampling was used with the standard settings during all docking procedures.

### 2.4 Pharmacophore Hypothesis

Schrödinger's Phase module [21] was used for pharmacophore model development. We developed two kinds of pharmacophore hypotheses. For the first one, we used the previously aligned ligands and defined entries with  $pIC_{50}$  value above 7.0 as active. Then several hypotheses were developed. As for the second kind, we used the XP docked ligand conformations for the model, thus there was no need to generate conformers during the development of these pharmacophore hypotheses. In both cases there were 4-7 features in the hypotheses, which matched 66%, 75% or 90% of the active ligands. Hereby we present one model, which we found the best based on Survival score and compatibility with our data from docking and pharmacophore hypothesis.

### 2.5 Field-Based QSAR

Our Quantitative Structure-Activity Relationship (QSAR) models were developed with Schrödinger's Field-based module. The previously aligned 41 ligands were used for this part. The activity property was  $pIC_{50}$ . The data set was randomly divided into training (66 %) and test sets (33%). Partial least squares regression (PLS) method with up to 6 factors with standard settings was used, except that

**Table II** Statistical parameters of the final field-based QSAR model

Number of PLS factors	4
Standard deviation of the regression (SD)	0.3847
Training set regression ( $R^2$ )	0.8046
Cross-validated $R^2$ ( $R^2$ -CV)	0.4567
$R^2$ with scrambled activities ( $R^2$ scramble)	0.4154
Stability of the model predictions to changes in the training set composition (maximum value is 1, meaning stable)	0.799
F – variance ratio (large values indicate a more statistically significant regression)	23.7
P – significance level of variance ratio (smaller values indicate a greater degree of confidence)	7.19E-08
Test set regression ( $Q^2$ )	0.5329

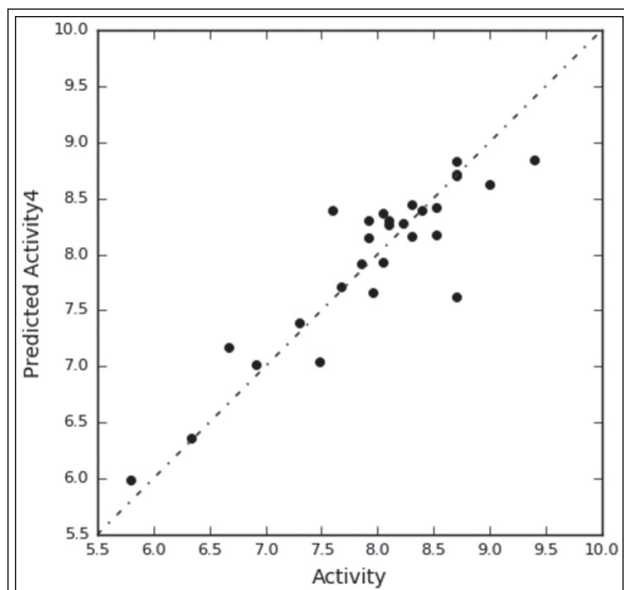


Figure 1 QSAR – Scatter plot of the training set

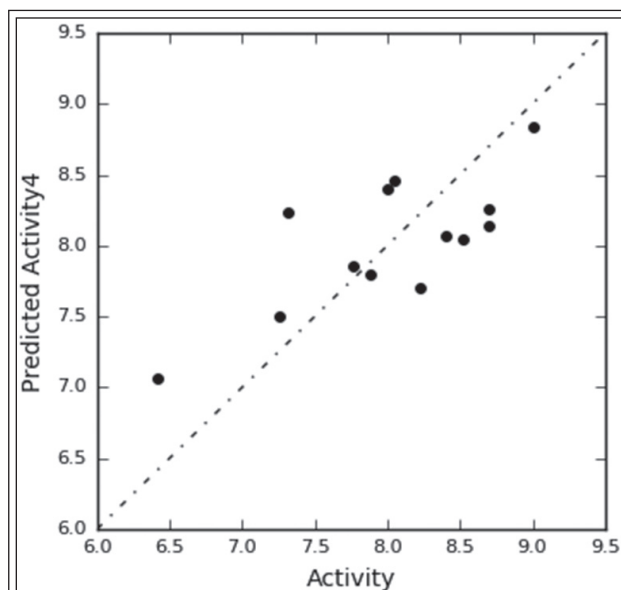


Figure 2 QSAR – Scatter plot of the test set

we eliminated variables with  $|t\text{-value}| < 2$ . The model with the best statistical attributes was selected as the final one.

### 3. RESULTS AND DISCUSSION

#### 3.1 Field-Based QSAR

As shown in [Table II](#), the model we found best, offered good predictive power and descriptive capability. The  $R^2$  value for the regression of the training set was above 0.8 and 0.53 for the test set, the cross-validated  $R^2$  and scrambled  $R^2$  values were also within the range of acceptance. These data together with the Stability and F and P values were indicating the reliability and robustness of our model. The graphical representation of the  $R^2$  ([Figure 1](#)) and  $Q^2$  ([Figure 2](#)) also indicated a good alignment with only a few outliers. Moreover, after visualization of the model, we could get more valuable information about CDK9 inhibitors ([Figure 3](#)). As it is illustrated, generally,

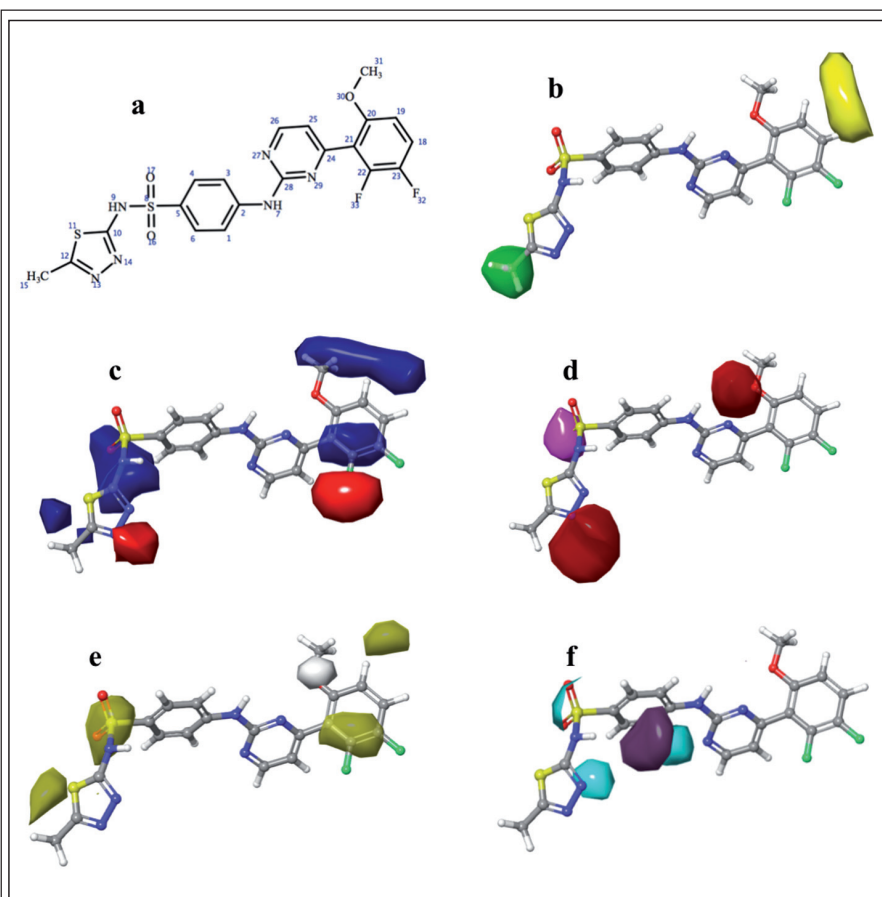


Figure 3 QSAR Visualization via Contour maps using compound 54 as an example: a: numbering of compound 54, b: Gaussian Steric Force Field, green: favorable, yellow: unfavorable c: Gaussian Electrostatic Force Field, blue: favorable, red: unfavorable d: Gaussian H-bond Acceptor, red: favorable, magenta: unfavorable e: Gaussian Hydrophobic, yellow: favorable, white: unfavorable f: Gaussian H-bond Donor, purple: favorable, cyan: unfavorable

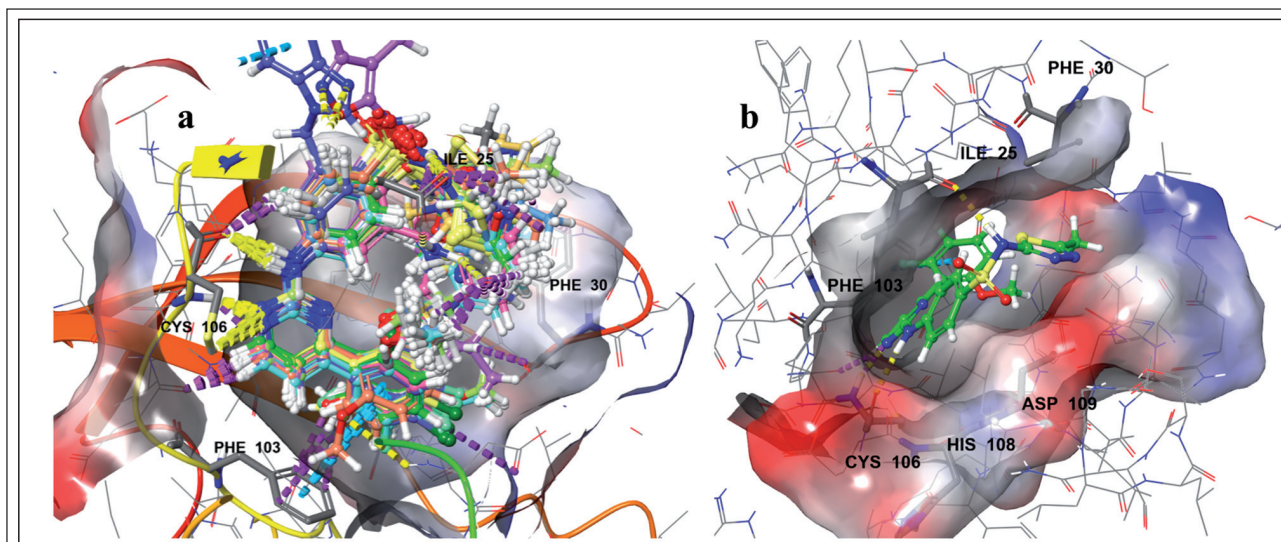


Figure 4 XP docking results a: side view of the site with all docked ligands included b: top view of the site, which reveals the characteristic narrow, flat shape of the pocket (together with compound 54 as an example of ligand positioning)

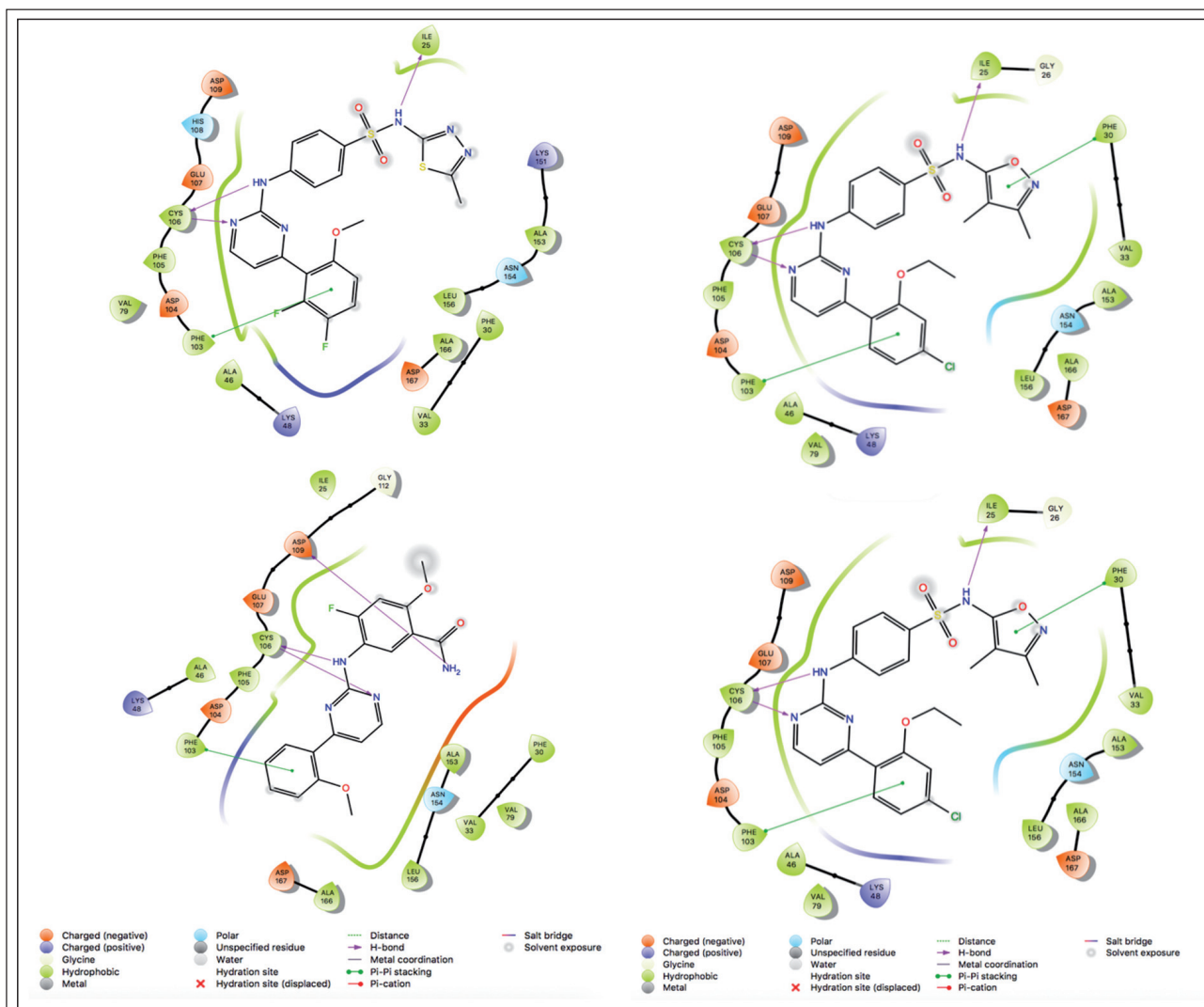


Figure 5 Ligand interaction diagrams showing the most common interactions between the inhibitors and the binding site

Table III Docking scores

ID	pIC <sub>50</sub>	XP docking score	SP docking score
54	9.398	-11.387	-9.498
25	8.097	-11.184	-9.245
32	7.770	-11.134	-8.702
35	7.886	-11.046	-9.294
33	7.310	-11.026	-9.435
28	7.602	-10.943	-8.737
27	7.921	-10.942	-9.366
42	8.523	-10.928	-9.257
30	8.097	-10.914	-9.326
68	8.699	-10.912	-9.891
40	7.301	-10.911	-9.158
37	7.959	-10.911	-9.453
26	9.000	-10.897	-9.740
67	8.699	-10.883	-9.250
53	8.000	-10.855	-9.208
22	8.699	-10.850	-9.248
24	9.000	-10.833	-9.610
34	6.339	-10.830	-9.195
46	8.699	-10.815	-9.120
23	8.046	-10.812	-8.876
39	7.260	-10.757	-9.265
52	8.699	-10.753	-9.447
36	8.222	-10.751	-9.288
50	8.222	-10.715	-9.113
51	8.046	-10.700	-9.225
56	8.699	-10.690	-9.373
43	8.301	-10.676	-9.136
48	6.423	-10.657	-8.986
47	6.678	-10.653	-9.389
21	8.523	-10.632	-9.262
49	7.921	-10.572	-9.092
57	8.301	-10.522	-9.082
45	6.914	-10.491	-9.155
44	7.481	-10.462	-9.225
66	8.523	-10.311	-9.472
31	8.699	-10.265	-9.659
38	7.678	-10.135	-9.140
58	5.802	-10.012	-8.837
29	8.046	-9.936	-8.947
42	8.398	-9.907	-8.795
55	7.854	-9.749	-9.238

introducing various substituents at the ends of the molecule can lead to changes in activity. The steric contour map (Figure 3b) suggested that a bulky group at C-12 on the thiadiazole ring could be a

good way to improve binding, while substitution at C-18 may be disadvantageous. From the electrostatic field contour map (Figure 3c) we could conclude that electrostatic groups at C-12, 19 and 20 may increase the activity while at C-22 an electro-negative group could lead to a similar result. Judging from Figure 3d, the introduction of a hydrogen bond acceptor moiety near C-20 and N-13 could also possibly improve the inhibition of CDK9. After having a look at Figure 3e it is easy to see that a hydrophobic substituent at C-19, 22 and 23 could be desirable in order to increase the inhibitor activity.

### 3.2 Docking

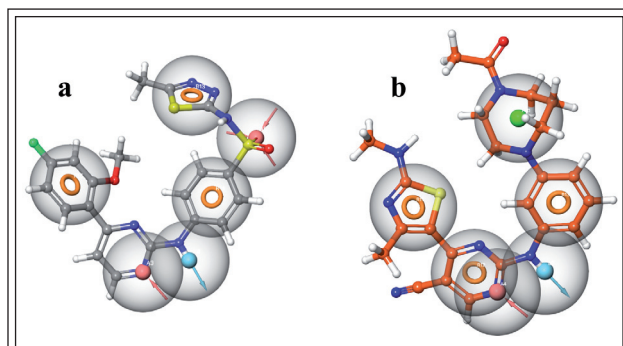
Each ligand of our compound set was docked into the binding pocket of 4BCF. As expected, XP scores were better than SP scores, however, no real correlation with the biological data was found as some lower activity compounds (eg. 34, 47, 48 and 58) were overscored (especially in terms of the XP method), which may be caused by the relatively low resolution of the X-ray structure.

As it can be seen in Figure 4a, the docked ligands are very well aligned and fit in the binding pocket quite easily. Every ligand formed two H-bonds with Cys106 (both as a hydrogen bond donor and acceptor), while Ile25 usually acted as a hydrogen bond acceptor. Phe30 often had a  $\pi$ - $\pi$  stacking with heterocyclic rings such as thiazoles or isoxazols, while Phe103 was often involved in a  $\pi$ - $\pi$  stacking interaction with one of the aromatic rings. In some cases His108 and Asp109 also formed secondary bonds with the inhibitors. Similarly to the co-crystallized inhibitor, the conformation of our compounds was bent and usually parts like the sulfonamide group, triazole and isoxazole rings were solvent exposed as they were (at least partially) positioned outside of the binding pocket granting more conformational freedom for these moieties, but as a result they were less likely to establish connections with the protein. Although they are not shown in this figure, there was a significant amount of Van der Waals bonds between the ligands and the protein, which we believe plays a significant role in the binding process, which is in line with our knowledge about the hydrophobic nature of this binding site.

### 3.3 Pharmacophore Hypotheses

Our best pharmacophore model hypotheses





**Figure 6** Comparison of pharmacophore models a: Our best (AADRRR) pharmacophore hypothesis superimposed on compound 54 b: Pharmacophore hypothesis developed from the 4BCF co-crystallized ligand

were obtained using the superposition of the conformations obtained from the XP docking poses. The knowledge we got from the docking experiments was also taken into consideration regarding the selection of pharmacophoric groups. Our best model, AADRRR can be characterized with a Survival score of 6.21 and a Selectivity score of 2.14. The Fitness of compound 54 in this hypothesis was 2.633653. This model contained a hydrogen bond donor (D) and an acceptor (A) at exactly the same position we could observe in the docking part. There were also two rings (R), which were also observed as  $\pi$ - $\pi$  interaction participants with the protein. There was another hydrogen bond acceptor (A), namely an oxygen atom in the sulfonamide group. As we saw earlier, this part of the molecule is usually out of the binding pocket, thus less likely forms any bonds. The high scores may indicate that this unique model could be suitable for the identification of new, potentially CDK9 selective inhibitors. As a comparison, a hypothesis based on the co-crystallized ligand was also developed. This model consisted of three aromatic rings (R), one hydrogen bond donor (D) and one acceptor (A) and also a hydrophobic region (Figure 6b). It must be pointed out, that despite the structural differences of the inhibitors, these hypotheses showed some significant similarities (four out of eight groups had a perfect match), which further indicates the validity of our model.

#### 4. Conclusions

Based on a previously published CDK9 X-ray structure and 41 CDK9 inhibitor compounds, a docking study was completed. Our investigations

also included ligand-based pharmacophore-, and QSAR modeling studies. Judging from our best pharmacophore hypothesis and the docking poses, a hydrogen bond donor and acceptor at close proximity are essential in the binding, because they both interact with the backbone atoms of Cys106. These interactions presumably help to fasten the compound in the binding pocket. According to our opinion, the orientation and presence of the aromatic rings could also be important, because they are able to form  $\pi$ - $\pi$  interactions with Phe30 and Phe103. The importance of the sulfonamide group lies in the fact, that its NH group is able to form a hydrogen bond with Ile25, while other components of the inhibitors like thiazole- and dimethylisoxazole groups are usually unbound so their orientations were varied in the poses. The bent conformation of the ligands and the shape and surface of the binding pocket are also key factors that determine the nature of the interactions between CDK9 and its inhibitors. To sum up, we generated a pharmacophore hypothesis and determined the main features of these molecules that play a role in their activity as inhibitors. Then we docked all of these compounds to deepen our understanding of the binding, and reveal new interactions and connections, and finally we built a robust field-based QSAR model with a strong predictive power, which might be able to predict the activity of inhibitor candidates in the future. If we apply the three above detailed methods in the right order, it gives us a powerful tool that can facilitate our CDK9 inhibitor research. We could screen databases and compound libraries by using our pharmacophore hypothesis. After that we could dock the selected ligands to compare their ability to bind to CDK9 and finally test them with a QSAR model to predict their activities. We are confident that all the models and knowledge that we gathered by this project can improve the CDK9 inhibitor design and can lead to new and – after completing the protocol with further targets – probably more selective inhibitors. We also believe that our method can reduce time and costs of CDK9 inhibitor drug development and can assist in lead selection and optimization.

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