International Journal of Pharmaceutics 456 (2013) 340–352

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Review

On the ubiquitous presence of fractals and fractal concepts in pharmaceutical sciences: A review

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ARTICLE INFO

Article history:
Received 14 June 2013
Received in revised form 26 August 2013
Accepted 28 August 2013
Available online 8 September 2013

Keywords:
Fractal analysis
Morphology
Advanced Drug Delivery nano Systems (aDDnSs)
Nanotechnology
Biopharmaceutics
Pharmacokinetics

ABSTRACT

Fractals have been very successful in quantifying nature's geometrical complexity, and have captured the imagination of scientific community. The development of fractal dimension and its applications have produced significant results across a wide variety of biomedical applications. This review deals with the application of fractals in pharmaceutical sciences and attempts to account the most important developments in the fields of pharmaceutical technology, especially of advanced Drug Delivery nano Systems and of biopharmaceutics and pharmacokinetics. Additionally, fractal kinetics, which has been applied to enzyme kinetics, drug metabolism and absorption, pharmacokinetics and pharmacodynamics are presented. This review also considers the potential benefits of using fractal analysis along with considerations of nonlinearity, scaling, and chaos as calibration tools to obtain information and more realistic description on different parts of pharmaceutical sciences. As a conclusion, the purpose of the present work is to highlight the presence of fractal geometry in almost all fields of pharmaceutical research.

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Abbreviations: FBS, fetal bovine serum; PBS, phosphate buffer saline; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol); DODAP, 1,2-dioleoyl-3-dimethylammonium-propane; DLA, Diffusion Limited Aggregation; RLCA, Reaction Limited Cluster Aggregation; MLCRSs, Modulatory Liposomal Controlled Released Systems; aDDnSs, advanced Drug Delivery nano Systems; ADME, absorption, biodistribution, metabolism and excertion; PK, pharmacokinetics; PD, pharmacodynamics.

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http://dx.doi.org/10.1016/j.ijpharm.2013.08.087

1. Introduction

The structural complexity of most objects found in nature cannot be completely described by the Euclidean geometry. The irregularity in shape, of common objects and materials with rough surfaces, as well as the cells of living organisms are considered as natural tracks that need further studies and holographic approaches in order to really disclose and understand their multifunctionality. Structure often possesses invariance under changes of the scale of magnification, which can be captured well by the fractal geometry. Fractal geometry is an extension of the conventional Euclidean geometry that allows the measures to change in a non-integer or fractional way when the unit of measurement changes. This characteristic can be described by assigning a fractional number – a fractal dimension – to the dimension of the object. Fractal analysis has proven to be a useful tool in quantifying the structure of a wide range of both idealized and naturally occurring objects, extending from pure and applied mathematics, physics and chemistry, to biology and medicine. Mandelbrot was the first to model this irregularity mathematically. In various fields of research, e.g. physics, chemistry and physiology, scientists are increasingly finding that the nonlinear phenomena control the processes; physical or physiological heterogeneity are everywhere while heterogeneous conditions prevail in numerous physical, physiological, biophysical and biochemical processes. Today's science shows that the real word is mostly nonlinear and, therefore, the techniques of nonlinear dynamics are required to analyze the nonlinear phenomena. In parallel, structural and functional heterogeneities can be described and understood with the concept of fractals. In essence, fractals are complex geometric patterns, however, they are used more often than many people are aware of. Fractals are being used in applications from image compression to movement of particles in physics to biological analysis. Until the development of fractal dimension, scientists were only capable of observing the nonlinear dynamical character of the natural structures and processes. Nowadays, they have mathematical tools, which describe, explain and prove the chaotic properties of nature, such as the weather. Computers became an integral part of this scientific revolution, allowing simulations with nonlinear dynamical systems and visualizing the results over time; scientists were able to watch the chaotic evolution of a naturally-occurring phenomenon on a computer's screen. Historically, mathematical 'strange' structures existed before fractals. They were characterized as 'pathological' since they did not fit the patterns of Euclid Exotic shapes, irregular geometrical objects and extraordinary figures emerged, revealing a world where mathematical objects (graphic representations of algorithmic processes) implied the real world. The late Benoit Mandelbrot was the first mathematician to shape this new area into an individual self-standing theory, which instantly became the most popular of all. He introduced the neologism *fractal* to unite all these strange objects under one term. "I coined *fractal* from the Latin adjective *fractus*. The corresponding Latin verb *frangere* means 'to break'; to create irregular fragments. It is therefore sensible and how appropriate for our needs- that, in addition to 'fragmented' (as in *fraction* or *refraction*), *fractus* should also mean "irregular", both meanings being preserved in "*fragment*".

Pharmaceutical *Nano*technology and Pharmacokinetics are important elements in pharmaceutical sciences that could act complementary in order to develop innovative drugs. The inspired approach of Mandelbrot to disclose the geometric reality in nature's objects and the appropriate procedures resulted in new approaches in the science and technology of drug delivery. The fractal approach can be characterized as the driving force to explore new paths for developing bio-inspired drug delivery systems (i.e. liposomes, dendrimers, polymerosomes, etc.), which are fractal objects (Table 1) that can be able to deliver pharmacomolecules to the specific sites of the organism. The design characteristics of nanotechnological formulations were recently outlined by fractal geometry, while biological systems are nowadays comprehensively understood as described by the fractal approach (Table 1). Additionally, fractal kinetics has been applied to enzyme kinetics, drug metabolism and absorption, pharmacokinetics and pharmacodynamics. The concepts delineated above are quoted in Table 1. The theory of nonlinear dynamical systems (chaos theory), which deals with deterministic systems that exhibit a complicated, apparently random-looking behavior, has formed an interdisciplinary area of research and has affected almost every field of science in the last 30 years. This is referred to as *chaotic* behavior, a specific subtype of nonlinear dynamics, which is the science dealing with the analysis of dynamical systems.

Table 1
Fractals and fractal concepts in pharmaceutical sciences.

Origin	Examples
Geometry	Arterial, venular networks Dendrimers, liposomes, chimeric systems Pharmaceutical Systems
Structure (conceived)	Volume of distribution Liver
Kinetics in fractal or in disordered systems	Time-dependent kinetics Fractional Kinetics
Dynamics	Attractor of heart dynamics Attractor of secretion of hormones

This review article deals with the potential benefits of using fractal analysis along with considerations of nonlinearity, scaling, and chaos as tools to obtain information and more realistic explanations on different parts of pharmaceutical sciences.

2. Fractals

Fractal geometry has become something of a buzzword since its inception by Mandelbrot (1982). Its popularity rests in the promise of a deeper understanding of complex, chaotic and disordered systems, which have resisted conventional geometrical attempts to model them. In Fig. 1, classic examples of fractals are presented; Cantor's set, Koch's snowflake and Sierpinski triangle. As it applies to the characterization of fine particles, the essentially useful feature of fractal geometry is the recognition of dilatational symmetry or scale invariance, as a measure to characterize structures and morphologies. The expression which embodies the basic concept of the fractal structure of aggregates is very simple:

$$M \sim R^{d_f} \quad (1)$$

where M is the mass of particles, R is a linear measure of size and d_f is the mass fractal dimension, which is a measure of the scaling. This expression can be used to communicate a couple of different concepts: M can represent the mass and R the radius of a particular aggregate such that a collection of aggregates of different sizes collectively display fractal scaling; or on a scale much smaller than an individual aggregate R can represent an imaginary spherical boundary centered on one primary particle and M then means the amount of mass contained within that sphere. The constant of proportionality will be different but the fractal scaling in the two cases is the same (Chu and Liu, 2000; Bushell et al., 2002; Kamiya and Takahashi, 2007; Link et al., 2011).

Since the bulk of the literature on this subject is concerned with the structure of aggregates formed from monodisperse spherical particles, the distinction between mass and number is rarely made.

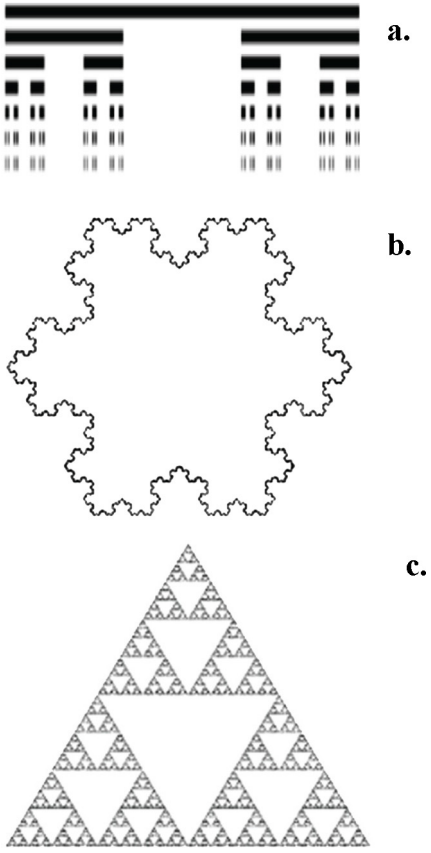

Fig. 1. Examples of fractals: (a) Cantor's set, (b) Koch's snowflake and (c) Sierpinski's triangle.

It is quite usual for authors to write an expression like Eq. (1) in terms of number of particles N and refer to the quantity d_f as the mass fractal dimension, although this is a rather small point of semantics because the mass fractal dimension and number fractal dimension are obviously the same thing when the particles are monodisperse. To be more specific Eq. (1) can be written in terms of overall aggregate scaling; it is often written as:

$$N = k_g\left(\frac{R_g}{r_0}\right)^{d_f} \tag{2}$$

where R_g is the radius of gyration of the aggregate, r_0 is the radius of the primary particles and k_g will be referred to in this paper as the structure prefactor. The subscript g is added to the structure prefactor here to clearly associate it with linear aggregate size defined in terms of the radius of gyration, which is the root-mean-square distance of the mass elements from their centre of mass. One can just as easily define the linear size in terms of an 'external radius' R_c and an associated power law prefactor k_g or indeed any other linear measure of size.

Furthermore, one of the simplest non-equilibrium growth processes that generates branched and open structures in colloidal systems characterized by a fractal dimension (d_f), which is synonymous to mass fractals, different from the Euclidean dimensionality (d), is the Diffusion-Controlled or Limited Aggregation process introduced by Witten and Sander (Witten and Sander, 1981) and the Diffusion-Controlled Deposition introduced by Racz and Vicsek (1983). It should be noted that in these models every collision between particles results in the formation of a permanent contact and the fractal dimensionality is insensitive to the sticking probability (Meakin, 1986, 1983a,b). On the other hand, several other aggregation mechanisms have been reported in the literature, for example, ballistic aggregation, chemically limited aggregation and aggregation with restructuring apart from Diffusion-Limited Aggregation (Jullien, 1987).

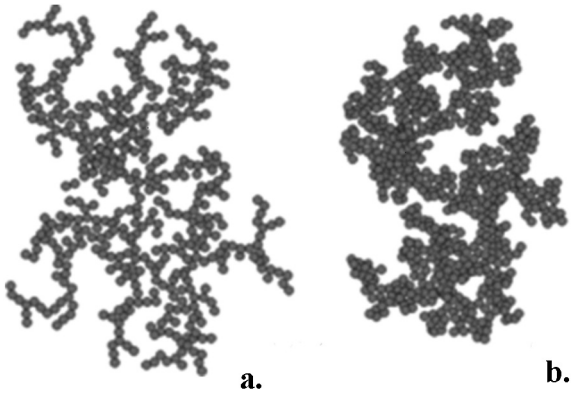

Fig. 2. Examples of (a) DLCA and (b) RLCA.

Witten and Sander (1981, 1983) have investigated the colloidal aggregation from a theoretical point of view (Witten and Sander, 1981, 1983). Diffusion-Limited Aggregation (DLA) is a model where particles are added one at a time to a growing aggregate of particles via random walk trajectories. The determination of fractal dimension has been carried out with this model (Tang et al., 2000; Thill et al., 1998).

The Diffusion-Limited Cluster Aggregation was improved by Meakin (Meakin, 1983b) and is an extension of DLA. In this model, the cluster's growth is controlled by diffusion, the sticking probability is equal to one, all collisions between particles are effective and the aggregates are open and branched in their structure. The fractal dimension was estimated to be 1.8 in three dimensions from computer simulations. DLCA is a fast aggregation process, following a power law for the average radius of gyration, R_g:

$$R_g \sim t^{1/d_f} \tag{3}$$

where t is the time and d_f the fractal dimension (Forrest and Witten, 1979). On the other hand, Reaction Limited Cluster Aggregation (RLCA) or Eden model is the slow aggregation process. The Eden model is a simple lattice model for the growth of the clusters, in which the particles are added one at a time at random to sites adjacent to occupied sites. The growth is limited by reaction kinetics due to the presence of an energy barrier to aggregation. The sticking probabilily is smaller than one because a large number of collisions are needed before the particles bind. The fractal dimension was estimated to be 2.1 in three dimensions from computer simulations and the aggregates are more compact and dense than DLCA clusters (Fig. 2). The kinetics of RLCA are characterized by a power law for the average radius of gyration, R_g:

$$R_g \sim e^{at} \tag{4}$$

where a is a constant. The value of a depends on the sticking probability. The surface fractal dimension is d_s and the expresssion which decribes the surface fractals is:

$$d_f = 6 - d_s \tag{5}$$

From an experimental point of view it is difficult to find aggregation phenomena described by the concept of surface fractals. Roldán-Vargas et al., reported the first experimental observation of a transition from surface fractals to mass fractal structures in a suspension of aggregating lipid vesicles (Roldán-Vargas et al., 2008). It must be noted that these two limiting regimes of irreversible growth of aqueous colloidal aggregates are universal. The "universality" of these aggregation phenomena was supported by Lin and co-workers (Lin et al., 1989).

Additionally, kinetics in *fractal-like environments* (usually named "fractal kinetics") characterizes processes taking place at or in the vicinity of fractal boundaries. In classical kinetics in homogeneous and well-stirred media, rate coefficients are independent of time, while reaction orders are equal to molarity and expressed as integers (Kopelman, 1988). However, the concept of classical reaction kinetics is unsatisfactory when the reactants are spatially constrained at the microscopic level, for instance by walls or phase boundaries. On the other hand, in fractal kinetics, rate coefficients are time dependent, usually expressed as power laws of the form:

$$k' = k \times t^{-h} \quad (6)$$

where k' is the fractal rate coefficient, k is the classical rate constant (i.e. when $h = 0$), and t is time; h is a heterogeneity component which can interpreted to result from irregular boundaries which slow down the reaction (Kopelman, 1988).

In fact, the fractal objects have multiscale properties, i.e. they continue to exhibit detailed structure over a large range of scales as the above Eqs. described. Consequently, the multiscale properties of fractal objects or processes depend on the spatial or temporal characteristic scale measurement (ruler size) used (Macheras and Iliadis, 2006). The physiological implications of the fractal mathematical concepts are serious since fractal structures, morphologies, processes and kinetics are ubiquitous in pharmaceutical research. However, real physical objects typically exhibit only limited scale-invariance within a given range, whereas mathematical fractals show scale-invariance to infinity.

3. Application of fractal geometry in the pharmaceutical sciences

The extension of the concepts of fractal geometry toward life and bio- sciences has led to significant progress in understanding complex functional properties and structural features characterizing cells and tissues during ontogenesis and both normal and pathological development processes. Additionally, a line of study has demonstrated that scale invariance is a common characteristic of biological systems, ranging from tissues to cultured cells, nucleus and chromatin (Mazzola, 2003; Kimori et al., 2011; Moreno et al., 2011; Mirny, 2011; Bancaud et al., 2012; Albrecht-Buehler, 2012). Fractal analysis can characterize the apparently irregular and complex structures in terms of a single parameter, the fractal dimension, which in turn can be related to the overall functional, pathological, or physiological status of the tissue or cells under study. For instance, it has been shown retrospectively that the composite fractal dimension of malignant mammographic cell nuclei is less than that of benign cell nuclei, suggesting the potential in developing objective and accurate cytologic diagnostic methods of breast cancer.

The following complication provides a selection of examples illustrating the practical value of fractal geometry, percolation theory and chaos theory in various branches of the pharmaceutical sciences. It is, however, not our aim to present a complete review of all literature references in this subject. This report intends only to bring to the attention of the reader on the usefulness of these concepts in this area.

From single atoms and molecules to the highest organized multicellular living systems, almost everywhere fractal geometry has found its application. The appearance of most materials is fractal, the irregularity of the surface or the lacunarity of the whole structure can be characterized by a single parameter, the fractal dimension, d_f. Avnir et al. (1984), listed an extensive compilation of materials with fractal dimensions of $2 < d_f < 3$.

In the laboratory, on the other hand, under experimental conditions, beautiful ramified ("multifractal") crystal aggregates can be obtained. Diffusion-limited dendritic crystal growth from supersaturated solutions yields irregular patterns of highly imperfect crystal clusters with fractal geometry. Fractal analysis is most often used in the study of the ruggedness of pharmaceutical solids. Thibert et al. (1988) compared the fractal dimension of a series of granules and excipients prepared by different techniques, and they found close correlation between the physical behavior of these granules and their surface geometry. A comparison of the fractal dimension of granules and excipients prepared by different techniques revealed a possible correlation between the physical behavior of these granules and their surface geometry (Brittain et al., 1991; Tromelin et al., 2001).

On the other hand, some authors (Koch, 1993) criticized the method of estimating the fractal dimension of rough particles from photomicrographs since the projection of the cross-sectional images can be misleading in that the observation can be a function of the orientation of the solid particle while Avnir et al. (1984) suggested to probe surfaces by gas absorption.

The spray–drying process is widely used in pharmaceutical, food and chemical industries to obtain powder products with "instant solubility". This means that the spongy granules easily dissolve in the appropriate liquid. Such products are regularly found as pharmaceutical formulations (e.g. plant extracts), food products (e.g. instant coffee), detergent powders, and the like. It appears likely that fractal description of powder grains will be predictive of both the flow and packing behavior of the powder, for instance in a tablet-making machine, as well as descriptive of the bioavailability of the active drug substance made in the spray dried formulation. The availability of a drug in the patient's body depends on the disintegration and/or dissolution rate in the body fluids, and this can be modified rather conveniently with such delivery systems (Burnett et al., 2011).

Spherical aggregates, historically termed "micelles", sometimes form clusters that are fractal with respect to their geometrical structure. For instance, casein micelles resulting from condensation of milk proteins when milk is heated, show raspberry-like aggregates on electron micrographs with a fractal dimension of $d_f = 1.86$. The structure of the aggregates, micellar casein particles, which are characterized as submicelles, is studied using light scattering and cryo-electron microscopy. It is found that the aggregates have a self-similar structure with dimension 2 (Panouillé et al., 2005; de Kruif et al., 2012). On the other hand, aggregations of casein micelles with particulate disordered structures having a fractal dimensionality of $d_f = 2.3$ were observed (Chardot et al., 2002; Panouillé et al., 2005; de Kruif et al., 2012).

3.1. Pharmaceutical technology

The most interesting pharmaceutical application of the concept of fractals may be found in drug dosage formulation. Fractal geometry and percolation theory can provide new insight into well-known problems in pharmaceutical technology (and in biopharmaceutics) in those cases where the classical approach cannot lead to an optimal resolution of the problem.

3.1.1. Dosage form characterization

Examples for successful application of fractal geometry have been presented in the areas of dosage form design, characterization of dosage forms, unit operations in production, and drug release properties.

For instance, the presence of a self-similar particle size distribution is a prerequisite for fractal patterns in pharmaceutical dosage forms. Fractals have been observed in powdered drug substances, in excipients, and in their mixtures, as well as in semifluid dosage forms like gels and emulsions. The percolation threshold dramatically changes the properties of tablets like crushing

strength, friability, disintegration time, dissolution rate, and others. The study of these phenomena can provide an interesting new tool for a more rational dosage form design, especially for fast and slow release solid dosage forms. Thibert et al. (1988) demonstrated that the degree to which a powder can consolidate, the compressibility ratio, of a series of materials is a function of the corresponding roughness factor "a" which equals $1 - d_f$. It was found that the compressibility ration is not dependent on the actual, true density of the material, but rather on its particle size and shape (Thibert et al., 1988; Fini et al., 2008). The granulation process in tablet manufacture is clearly a way to increase the fractal dimension of the granules, as compared with the original crystals of the neat drug substance (Abdullah et al., 2013).

As soon as the component with the lower mechanical stability is percolating the powder system, tablet hardness is controlled entirely by this component. This percolation threshold is a function of the geometrical arrangement of the particles in the compressed powder system (Esquena et al., 2000). The concepts of fractal geometry and percolation theory permit new insight into the physics of tablet compaction and the properties of tablets. The results obtained so far are promising and should stimulate further research in this field. According to Janssen and Stenull (2000, 2009), percolation clusters are random fractals whose geometrical and transport properties can be characterized with the help of probability distribution functions.

Size and shape of particles generally contribute to macroscopic pharmaceutical properties of drug dosage forms. The effect of surface morphology of steatic acid on the lubricative effectiveness of a series of samples was investigated, noting that only rounded flakes, but not angular flakes, were good lubricants. Solid dosage forms with a porous, sponge-like structure behave like a percolating pore-system. Furthermore, the fractal dimension of a series of matrix tablets was determined (with caffeine in ethyl cellulose) which became porous during the drug release. The pressure intrusion data of mercury porosimetry analysis were used to determine the pore diameter as well as the pore size distribution for the leached tablets, while the Menger sponge with a three-dimensional network of $d_f = 2.72$ was the mathematical basis for the calculation. The fractal dimensions of the pore system in the leached tablets ranged between 2.670 and 2.837, depending on the particle size distribution of the water-soluble drug substance and the fraction of drug released, respectively. The system that contained a broad fraction of particles yielded $d_f = 2.734$ and was closest to the dimension of the Menger sponge. The rheological behavior of suspensions in heavy liquid paraffin was studied of different types of microspheres having a variable degree of surface roughness (Nyström et al., 2010). It was found that the viscosity of the suspensions increased when the surface of the particles showed higher irregularity. The effect is attributed to the resistance of flow due to internal friction between the microspheres within the suspension. An approach based on percolation theory to study the viscosity of concentrated suspensions was proposed (Koch, 1993). A rheological approach, namely the fractal concept of flocculation as a novel analytical tool to thoroughly characterize the drug as well as its aggregated particle structure in suspension, was recently introduced to lipid-based pharmaceutical suspensions for filling of capsules (de Kruif et al., 2013). The geometrical distribution of the suspended spherical fillers which form microscopic clusters of fractal appearance is representative for the characteristics properties of such suspensions.

3.2. The fractal hologram in pharmaceutical nanotechnology

3.2.1. Self-assembly of fractal nanomaterials

In life sciences, knowledge of the interaction mechanism at short distances between the biomolecules immersed in an electrolyte solution is very important to elucidate the essential biological processes involved in many cellular phenomena like membrane fusion and these processes reflect to their functionality. Furthermore, self-assembly is one of the most interesting properties that arise in nano-scale or mesoscopic level.

Molecular self-assembly is a nano-manufacturing strategy that implies molecular and over-molecular design, in a way that complementarily brings to aggregation in the desired structure. This building mechanism brings different advantages: it realizes the most difficult steps in nano-manufacturing, that is, the ones involving atomic-level matter modifications, in a clean way, and it tends to produce stable and relatively perfect structures, since it requires the output structures to be the most thermodynamically stable (Whitesides and Grzybowski, 2007). Additionally, soft nanotechnology is a relatively young scientific and technological discipline with soft nanomaterials (Hamley, 2003; Nayak and Lyon, 2005; Whitesides and Lipomi, 2009). These materials have uniquely functional properties at nanometer-scale dimensions that, if harnessed effectively, could lead to novel engineering systems with highly useful characteristics. On the other hand, biomaterials are highly organized from the molecular to the nano- and macroscales, often in a hierarchical manner, with intricate nano-architectures that ultimately make up a myriad of different functional soft and hard tissues. Most of the natural objects and systems encountered in the real word are built in a completely irreversible and disordered fashion. In irreversible processes the details of growth mechanism seem to play a key role in determining the resulting shapes of the objects.

3.2.2. The application of fractal geometry in pharmaceutical nanotechnology

Physical properties at nanoscale scale are different from the ones at macro-scale giving new and more exciting possibilities of developing novel and useful applications. On the other hand, nanotechnology is essentially related to the problem of predicting properties of matter on the nanometer length scale. This problem is characterized by the so called $(N+1)$ problem: the properties of a system with N particles may be largely different and have widely different properties from those of a system with $(N+1)$ properties (Cerofini et al., 2008). Additionally, nanotechnology is a technology concerning processes which are relevant to physics taking place at a length scale of one divided by 100 million of a meter. According to M. Saladin El Naschie (2006) nanotechnology is: "*a technology applied in the grey area between classical mechanics and quantum mechanics*".

Pharmaceutical nanotechnology is a promising multidisciplinary scientific field which covers issues from molecular biology and biochemistry, colloid science, and medicine (Hughes, 2005; Marcato and Durán, 2008; Ravichandron, 2009; Mishra et al., 2010; Souza et al., 2010). One of the promising categories in pharmaceutical drug delivery nanocarriers are liposomes which belong to the class of bio-colloidal nanoparticles (Bangham et al., 1965; Gregoriadis et al., 1974). Small unilamellar as well as large multilamellar liposomes, made up from phosphoslipids and other constituents, remarkably influence the reactivity of chemicals enclosed in these vesicles. This can be described by equations involving the fractal dimension of the reaction medium during a study using the luminescence decay technique in various types of such vesicles. The viscosity of the fluid phase, which can be increased by adding cholesterol of sphingomyelin, is the critical parameter for chemical reactivity of inclusion compounds, while increased rigidity of the vesicles probably decreases their polydispersity. Nanocarriers produced by the combination of liposomes and polymers, are considered as a new class of soft drug delivery nanocarriers and were recently characterized as Modulatory

Liposomal Controlled Released Systems (MLCRSs) (Papagiannaros et al., 2005; Demetzos, 2010a,b, 2011).

Recently investigators from Prof. Demetzos' laboratory classified the MLCRS nanocarriers in *hybrid* and *chimeric* and they are characterized as advanced Drug Delivery nano Systems (aDDnSs) (Demetzos, 2010a,b, 2011; Gardikis et al., 2010a,b, 2011). Preclinical studies have shown that aDDnSs can affect the pharmacokinetic profile of the incorporated bioactive molecule and, consequently, alter its ADME (Absorption, Distribution, Metabolism and Excertion) profile (Gardikis et al., 2010a,b, 2011; Kontogiannopoulos et al., 2012). aDDnSs can be characterized as mixed nanosystems due to the combination of different in nature bionanomaterials and are used for biomimetic delivery (Balmert and Little, 2012). The interest in such systems stems from the possibilities for basic understanding of biological behavioral motifs, since biological systems extensively uses mixed materials to create multi-functional self-assembled nanostructures, too (Pispas and Sarantopoulou, 2007; Pispas, 2011a,b).

Pharmaceutical nanotechnology is basically oriented in the area of drug delivery, especially in colloidal dispersions (Cerofini et al., 2008; de Villires et al., 2009; Mishra et al., 2010; Crommelin and Florence, 2013). Nanocarriers provide opportunities in the area of drug delivery with higher ratio of surface area to volume. For this reason, they improve the solubility of hydrophobic compounds and increase the stability of wide variety of therapeutic agents and biomolecules like peptides. They also can improve the pharmacokinetic characteristics of the incorporated biomolecules, minimize their toxicity and improve the risk-to-benefit ratio. Colloidal nanocarriers like liposomes, polymeric nanoparticles, etc., can be classified in a more general category of "*soft matter*", which includes a wide range of materials, that cannot classified as solid or liquid and that can deform easily because their molecules simply rearrange (Bonacucina et al., 2009). In their recent paper, Liu et al. (2012) discuss recent progress on the design and fabrication of nanoparticles of various shapes and their unique delivery properties. The shapes of these drug carriers play an important role in therapeutic delivery processes, such as particle adhesion, biodistribution and cell internalization (Gaumet et al., 2008).

Fractal geometry is the tool to describe systems and devices in nanoworld and the kinetics of physical phenomena like aggregation of liposomal and polymeric nanoparticles and membrane fusion in this scale (Lattuada et al., 2001, 2003a,b; Sabín et al., 2007a,b; Roldán-Vargas et al., 2008, 2009; Crivoi and Duan, 2012; Pippa et al., 2012a,b,c; Wu et al., 2012; Hadjidemetriou et al., 2013; Gasilova et al., 2013; Meng et al., 2013). Additionally, the fractal analysis is used to determine the morphology of colloidal nanostructures other than liposomes. Fractal growth of poly(amidoamine) {PAMAM} dendrimers aggregates was observed in aqueous medium due to their self-assembling (Metulio et al., 2004; Jasmine and Prasad, 2010), while polymeric growth process and kinetics of gold nanoparticle aggregation were described by fractal geometry in order to better understand their physical behavior (Ogasawara et al., 2000; Sotiriou et al., 2007; Kim et al., 2008). The nanotechnology arena allows scientists from different fields to formulate alternative hypotheses for experimental observations, which lead to more realistic explanations compared to the traditional approaches, especially in nano-scale. The delivery of biomolecules can be improved by advanced drug delivery systems such as previously described, using therapeutic colloidal nanocarriers, including polymeric nanoparticles, micelles (Torchilin, 2007), polymeric micelles (Torchilin, 2004), nanotubes (Li et al., 2010) and nanostructured lipid vectors, as well as nanorobotics (Kostarelos, 2010; Igbal et al., 2012).

Additionally, we have to note that the definition on what nanotechnology is, based on the dimensionality of nanoparticles, can affect the manufacturing process because the manufactures have to meet the most stringent criteria and regulations in order to distribute to the broadest market way (Wagner et al., 2006; Caruthers et al., 2007). The driving force for producing innovative nanoscaled material is the technology (Wagner et al., 2006). In drug delivery, the ability of nanoparticles encapsulating biomolecules to pass across the *intra* and *extra* cellular biological membranes or to improve the solubility of not soluble in aqueous media pharmacological active molecules, offer an attractive area for research and for defining the term 'material' and especially the term '*bio*-material'. This is important, not only from the scientific point of view but to effectively apply the new *bio*-materials in drug delivery or in MRI or as implants by increasing their biocompatibility. However, the structural properties, the assembly and the sculpturity of the molecules in the atomic level at a given medium by proportional conditions, overcome the conventional principles and laws in mathematics and physics. We do believe that manufacturer can embraces new ideas on what *bio*-materials can offer in medical needs, by studying their unique properties at nanoscale level using new tools for identifying their properties.

Table 2
Physicochemical characterization of nanopharmaceuticals (adapted from de Villires et al., 2009).

Parameter	Conventional methods
Particle size, distribution, and particle morphology*	Dynamic light scattering Photon correlation spectroscopy Scanning electron microscopy* Transmission electron microscopy* Atomic force microscopy*
Surface area, porosity	Gas adsorption
Surface charge, hydrophilicity	Electrophoresis Zeta potential Laser Doppler anemometry
Surface property	Static secondary ion mass spectroscopy Mössbauer spectroscopy
Surface element analysis	X-ray photoelectron spectroscopy
Density	Pycnometer
Molecular weight	Gel or size–exclusion chromatography
Purity	Spectroscopy, FTIR, NMR
Polymorphism, crystalline form	X-ray diffraction Differential Scanning Calorimetry
Residual solvents	Gas chromatography
Drug release	Release testing Turbidity

* The nanoparticles' morphology evaluated only by electron microscopy, which could not be able to *quantify* their morphological properties and consequently their functionality.

3.2.3. *The fractal morphology of nanoparticles*

The development process seems to be conventional in terms of the properties of nanoparticles which are required to pass the regulatory criteria before approved for the market. Although they exist and apply several validated techniques for characterizing nanoparticles such as light scattering techniques, X-ray diffraction, microscopic techniques (Table 2) among chromatographic and spectroscopic techniques, there is not still established a "*gold standard*" method analysis for characterizing the morphology of nanoparticles. It should be noted that validated assays and techniques are important for detecting and quantifying nanoparticles and nanopharmaceuticals in tissues and advanced medical products and how physicochemical characteristics may impact product quality and performance. All the issues are critical for demonstrating control of a production process and for justifying drug release profile and parameters as well as bioequivalence testing

approaches. From the Euclidean point of view, the default shape for nanopharmaceuticals is thought to be spherical, but cylinders, disks, cubes, needles and random shapes do exist. On the other hand, nanotechnology allows scientists to design a vast array of bionanomolecules with distinct physical, chemical and biological characteristics, each with a specific size, charge, hydrophilicity, shape, and flexibility. Additionally, spherical nanoparticles has resulted in valuable insights, but many naturally occurring objects are non-spherical, and biological processes typically occur under dynamic conditions in which the motion of spherical and non-spherical vesicles or supramolecular aggregates will differ.

It is well established that the control of the nanosized *bio*-materials like liposomes, nanosized polymers, dendrimers, fullerenes, nanotubes is very complex and needs further discussions by the regulatory committees, while the role of experts in the field of Pharmaceutical Nanotechnology is considered to be crucial. There are several nanoscaled products in the market among them liposomes which are considered to be the most widespread nanoparticles for medical care.

By combining liposomes with amphiphilic copolymers, more stable systems could be obtained, usually known as Stealth or Sterically Stabilized liposomes (Cattel et al., 2004; Immordino et al., 2006; Mufamadi et al., 2011; Pippa et al., 2013a,b,c). These systems are characterized as mixed systems and belong to Modulatory Controlled Release Drug Delivery nano Systems (MCRDDnSs). The quality control of such nanosized systems is a challenge and novel advance approaches are needed in order to realize their behavior and functionality. The well-established techniques for measuring the particle size and the particle size distribution, the surface charge, the rigidity of bilayers, the interactions between *bio*-materials, the conjugations with plasma proteins, stability studies in different media and temperatures, as well as the shape, the surface characteristics based on their chemistry, thermodynamics and physical characteristics, are considered to create a very complex environment for characterization and for scaling up by the manufacturers. Furthermore, the chemical composition, the size and size distribution, the surface modification (surface charge and PEGylation) and targeting ligand functionalization are the crucial factors that have been shown to substantially affect the ADME profile of the circulating nanocarrier (Owens and Peppas, 2006; Alexis et al., 2008; Amoozgar and Yeo, 2012; Yoo et al., 2010). According to the recent literature, molecular parameters, such as shape and flexibility of nanoparticles, especially the interdependence of particle size and shape, effect on biological and pharmacokinetic processes, too (Champion and Mitragotri, 2006; Champion et al., 2007; Canelas et al., 2009; Tomalia, 2009; Longmire et al., 2011). The shape of the nanoscale drug vectors plays a significant role in therapeutic delivery processes, such as particle adhesion and biodistribution (Liu et al., 2012). The interactions of nanoparticles with cells are known to be influenced by the shape of nanocarriers (Gratton et al., 2008; Lacerda et al., 2008; Huang et al., 2010; Wang et al., 2011).

3.2.4. *Examples of fractal analysis as a method for the liposomal nanocarriers' characterization*

It is of interest to point out that controlling the morphology of nanoparticles is advantageous for exploiting their functionality and their properties in several emerging technologies. Fractal analysis (using light scattering techniques) has recently been used to describe the morphology of liposomal nanoparticles (Lattuada et al., 2001; Lattuada et al., 2003a,b; Sabín et al., 2007a,b; Roldán-Vargas et al., 2008, 2009; Pippa et al., 2012a,b,c) and it has been considered as a complementary analytical tool (Kanniah et al., 2012). Table 3 presents fractal dimensions of liposomal nanoparticles. Fractal analysis is also used in molecular biology (Mirny, 2011; Bancaud et al., 2012) and applied chemistry (Harshe et al., 2010; Harshe and Lattuada, 2012; Schaeublin et al., 2012). Previous investigations from Prof. Demetzos and colleagues were related to the physicochemical characterization (size, polydispersity, ζ-potential) of liposomes composed of Dipalmitoylphosphatidylcholine (DPPC), DPPC: cholesterol (chol) (9:1 molar ratio) liposomes, DPPC:DPPG (9:1 molar ratio) and DPPC:DODAP (9:1 molar ratio) liposomes and the determination of their fractal dimension (mass fractal (d_f) and surface fractal (d_s)), in an aqueous (HPLC grade water) and in a biological (Fetal Bovine Serum-FBS) medium have been recently published (Pippa et al., 2012a,b). Dynamic and static light scattering techniques were used to elucidate the structure and physicochemical parameters of liposomes in an ageing study in two different media, as well as their structural response in changes in concentration and temperature (Pippa et al., 2012a,b). Aggregation of DPPC liposomes in aqueous medium was observed, while d_f remained unchanged. The existence of Lateral Cluster-Cluster Aggregation could be a possible explanation for the observed behavior (Grogan et al., 2011). Physicochemical stability was observed for the DPPC:clolesterol (9:1) liposomes in the two dispersion media. The structural properties of DPPC liposomes in aqueous medium are quite different from those in FBS, as demonstrated from fractal analysis, especially for liposomes without cholesterol. The physical theories, which were developed for aggregation phenomena, include the fractal formalism for elucidating the shape of the resulting liposomal aggregates and structures, as mentioned above. The fractal dimensionality of DPPC liposomes was decreased while for DPPC: cholesterol (9:1) it remained unaffected in the two dispersion media. The structure of the liposomal systems, the process kinetics, and the fractal dimension are consistent with the Diffusion-Limited Cluster Aggregation (DLCA) and Reaction-Limited Cluster Aggregation (RLCA) models. DPPC:DPPG (9:1) and DPPC:DODAP (9:1) liposomes in HPLC-grade water were found to retain their original physicochemical characteristics at least for the time period that they were studied. The liposomal stability indicates that electrostatic repulsion should be responsible for keeping the liposomes far enough to avoid aggregation or fusion. The fractal dimension, d_f, was found equal to 1.8 for reconstituted DPPC:DPPG (9:1) and DPPC:DODAP (9:1) liposomes in aqueous media. Aggregation of reconstituted DPPC:DPPG (9:1) and DPPC:DODAP (9:1) liposomes in FBS was observed. Their fractal dimensions were 1.46 and 2.45, respectively (Table 3).

Table 3
The aggregation kinetics and the fractal dimension of liposomal aggregates (R_h: Hydrodynamic radius) (Pippa et al., 2012b,c).

Liposomal composition	Dispersion medium	Fractal dimension
DPPC	HPLC water	2.50
DPPC	FBS	1.80
DPPC: cholesterol (9:1 molar ratio)	FBS	2.45
DPPC:DODAP (9:1 molar ration)	PBS	1.80
DPPC:DPPG (9:1 molar ratio)	FBS	1.46

According to the literature, shape is crucial to the nanoparticle's mechanism of cell internalization and the release rate and profile of the therapeutic cargo (Champion and Mitragotri, 2006; Goldberd et al., 2007; Gratton et al., 2008). On the contrary, shape effects on biological processes and mechanisms are still not fully understood, particularly at the nanoscale, primarily due to past limitations in the control of nanoparticle fabrication (Goldberd et al., 2007). It should be noted that certain shape specific delivery systems have demonstrated advantages over spherical nanosystems (Wang et al., 2011). Furthermore, filamentous nanoparticles can help evade macrophage uptake, and shape-specific microparticles and/or nanoparticles can enhance vascular adhesion.

However, it is obvious that the regulatory framework in the European Union is considered to be extremely complicated and needs specific regulations to assure that the final product meets with the requirements concerning the pharmacokinetic

parameters (active substance release, biodistribution, tissue accumulation, biodegradability, and clearance). The subject of the requirements concerning the pharmacokinetic and pharmacodynamic properties of the nanoscaled products still remains under discussion. The literature points out that the terms 'size' and 'nanomaterial', should be kept in to account in the discussion process to disclaim the regulatory guidelines (Vamvakas et al., 2011; Rowland et al., 2012). However, the up and down limits of the term 'size', was not able to conclude base on the available scientific reports.

There is a large number of techniques available for the characterization of the structure of aggregates formed from suspensions and dispersions of particles in micro and nano scale and for the determination of their fractal dimension (Gregory, 2009).

The size distribution of nanoparticles, their physicochemical characterization, their stability in diverse media including biological, cell distribution, immune system effects, genotoxicity etc are considered to be crucial for proposing the regulatory framework for nanoproducts. Scientific challenges arise from the limitations of current testing methods and the unknown reliability of novel ones, because of the 'nanosize' and the unique behavior of such nanosystems in biological structures. Further scientific research will be needed to provide a sound scientific basis for an adequate evaluation of the quality, safety and efficacy of emerging nanomedicines. The precise role of nanoparticles morphology and shape in drug delivery has not been fully elucidated, most likely due to the lack of easy-to-use methods and techniques available to control nanoparticles shape. Certainly, as it was mentioned above, shape, along with size distribution and surface chemistry, is a critical feature of nanoparticles. Particle size, measured simply by hydrodynamic radii for imaginary Euclidean spheres, must be redefined since non-spherical but fractal nanovectors exist (Lattuada et al., 2001, 2003a,b; Sabín et al., 2007a,b; Roldán-Vargas et al., 2008, 2009; Pippa et al., 2012a,b,c). Manipulation of the biophysical properties of aDDnSs, especially for *chimeric* systems, provides improved control over the pharmacokinetics (PK) and pharmacodynamics (PD) of the encapsulated drugs relative to free drugs (Moghimi and Szebeni, 2003; Allen et al., 2006). It is well established in the literature, that the regulatory considerations are of great importance aiming at providing proofs concerning not only the design and preparation of drug delivery systems but also the final formulation's physicochemical and morphological characteristics (Chen, 2008). From experimental point of view, the determined fractal dimension illustrates in a comprehensive way the self-assembly and the morphological complexity of liposomal nanocarriers (Pippa et al., 2012b,c). Additionally, fractal dimension plays an important role for the elucidation of morphological characteristics, while size and/or size distribution of nanoparticles did not change by the change of colloidal parameters, like temperature and concentration (González et al., 2002; Pippa et al., 2012b,c). This in turn could be a useful tool for the development of innovative nanocarriers for drug or gene delivery with complete knowledge of their structural and morphological characteristics. The techniques for estimating the fractal dimension of nanovectors are those that are already reported in the literature and in Table 4.

Furthermore, during the past years microemulsions gained fundamental importance as drug delivery systems with interesting properties. Microemulsions are ternary or quaternary systems of the oil-in-water type containing four or five components. Besides water and oil, they require addition of a surfactant, a cosurfactant, and possibly a salt. However, the droplets are so small that microemulsions appear macroscopically as single-phase systems. Recently, it was shown that simultaneous variations of conductivity, dielectric relaxation, and dynamic viscosity of microemulsions can be interpreted within the framework of percolation theory, since the variations of these quantities are influenced by the fractal structure of the dispersed matter (Pinfield et al., 1997; Manoj et al., 2000; Helgeson et al., 2012). Wu et al. (2006) claimed that the diffusion of multiparticle clusters at oil-water interfaces is a strong function of cluster size and oil-phase viscosity and can be quantitatively related to fractal dimension while their decay profiles have been studied also in microemulsions, as it was done earlier with larger vesicles. It was found that the fractal modeling can be applied to describe the reaction in microemulsions, too, when they allow quencher exchange between the droplets.

Table 4
The techniques for the determination of fractal dimension.

Technique	Reference
Static Light Scattering (SLS)	Chu and Liu, 2000; Bushell et al., 2002; Gregory, 2009; Link et al., 2011
Small-Angle-X-Ray Scattering	Chu and Liu, 2000; Bushell et al., 2002; Gregory, 2009; Link et al., 2011
Small-Angle neutron scattering	Chu and Liu, 2000; Bushell et al., 2002; Gregory, 2009; Link et al., 2011
Dynamic Light Scattering	Chu and Liu, 2000; Bushell et al., 2002; Gregory, 2009; Link et al., 2011
Wide-Angle X-ray Diffraction	Chu and Liu, 2000; Bushell et al., 2002; Gregory, 2009; Link et al., 2011
Dynamic Rheological measurements	Khan and Zoeller, 1993
Sedimentation	Tang et al., 2000
Confocal Scanning Laser Microscopy	Thill et al., 1998
Electron microscopy	Kimori et al., 2011; Iezzi et al., 2011

3.3. Biopharmaceutics and pharmacokinetics

Next to pharmaceutical technology, biopharmaceutics, including pharmacokinetics, appears the most promising area where the concept of fractals could bring about a lot of fruitful new developments. Part of it, as well as a few suggestions for further studies, will be introduced in these sections. These sections consider the potential benefits of using fractal analysis along with considerations of nonlinearity as tools to obtain information and more realistic explanations on drug dissolution and release, as well as drug absorption, disposition, metabolism and excretion.

3.3.1. Drug dissolution and release

The bioavailability of drug depends to a great degree on its dissolution behavior. The dissolution process, both *in vitro* and *in vivo*, is determined by various properties of the drug compound, such as solubility; the formulation characteristics, such as particle size, size distribution, particle shape and morphology, surface area, degree of porosity, etc.; and the conditions of the environment such as pH, composition, stirring and motility. The kinetics of dissolution is governed by the so-called dissolution law, as expressed in the Noyes-Whitney equation or the Hixson-Crowell cube root equation and the modifications thereof.

Farin and Avnir (1992) have included the surface roughness of drug particles, which is fractal in many cases, into the Noyes-Whitney and Hixson-Crowell equations. The classical dissolution rate constant holds only for particles with a smooth surface. Particles with irregular surface, having a characteristic fractal dimension, do not follow the original Noyes-Whitney or the Hixson-Crowell equation, respectively. The authors have presented explicit equations, including the fractal dimension, d_f, of the drug particles into the original equation for the dissolution law (Fini et al., 1996, 1997, 2002; Tromelin et al., 1996). The modified equation is said to better describe the kinetic profile of the dissolution of powdered drug substances of this kind. The general form of the fractal dissolution law has the following form:

$$-\frac{dw}{dt} = K\left(w_e^{d_f/3} - w_0 + w_t\right) \quad (7)$$

where w_e, w_0 and w_t are the weight of the drug necessary to saturate the solution, the initial weight, and the weight of the dissolved drug after time t (Fini et al., 1996, 1997, 2002; Tromelin et al., 1996).

Later, by Macheras and colleagues many articles were published for the modeling of drug stochastic processes; dissolution and drug release (Macheras et al., 1996; Macheras and Dokoumetzidis, 2000; Dokoumetzidis et al., 2005, 2007, 2010a,b; Dokoumetzidis and Macheras, 2011). The concept of fractal geometry can be applied to describe the complexity of the heterogeneous nature of drug processes in the human body and the dissolution of bioactive compounds (Dokoumetzidis et al., 2004; Dokoumetzidis et al., 2008; Pereira, 2010; Dokoumetzidis and Macheras, 2011).

The first simulation studies of drug release were reported by Bunde et al. (1985). Extensive simulation techniques for the study of Fickian diffusion of drug release both in Euclidean and fractal spaces were applied several years later (Kosmidis et al., 2003a,b; Martínez et al., 2009). These studies demonstrated that the kinetics of drug release from Euclidean or fractal matrices can be described with the Weibull function. Most importantly, the value of the time exponent was found to be an indicator of the mechanism of drug transport through the matrix (Papadopoulou et al., 2006). Besides, Monte Carlo simulations were used for the study of drug transit, dissolution and uptake in the heterogeneous tube model of the GI tract (Kalampokis et al., 1999a,b).This model of oral drug absorption was based on a random, dendritic-type internal structure representing the villi of the GI tract; the intestinal transit flow was simulated using two diffusion models, namely, the blind ant and the myopic ant models.

3.3.2. Drug absorption, disposition, metabolism and excretion

Fractal geometry is used to mathematically explain the allometric 3/4 power model (West et al., 1997, 1999). The fractal approach offers the possibility to determine hepatic blood flow heterogeneity in perfused livers and to evaluate the functional implications (Weiss et al., 2012; Weiss, 2013). In contrast to classical pharmacokinetics in which a compartmental system made of a finite number of compartments, each being homogeneous and well mixed and interacting by exchanging material, the essential materials and drug in the human body are transported through space filling fractal networks of branching tubes while the internal surface areas of organisms for material exchange are “maximally fractal”, affectively introducing a fourth dimension which explains the 3/4 allometric law (West et al., 1997, 1999). Indeed, in all species, studied, including humans, the log of basal metabolic rate plotted against the log of body weight produces a straight line with a slope of 3/4. Also, drug clearance has been found to scale as a 3/4 power law with respect to body weight (Anderson and Holford, 2009).

Fractal volume of drug distribution scales proportionally to mass and this confirms the theoretically expected relationship between volume and mass in mammalian species (Karalis et al., 2001). The effective exchange area in the internal structure of the body was linked to the so-called “fractal volume of drug distribution”, which has been associated with the body surface area used in dosage regimen design (Laffon et al., 2006; Pereira, 2010). The relevant concept of “fractal clearance” has been also developed (Karalis and Macheras, 2002). Particularly in the pharmacokinetics field, Prof. Macheras and his colleagues were among the first to document real fractal pharmacokinetic applications (Macheras, 1995, 1996; Macheras and Argyrakis, 1997; Dokoumetzidis and Macheras, 2003). These articles demonstrate that either the time course of drugs in body fluids and tissues may show a fractal pattern or the fractal geometry of the vascular tree can be used to develop physiologically based PK models.

Furthermore, a fractal approach to enzyme kinetics has been proposed. The concept of fractal dimension facilitates the understanding of phenomena showing deviation from the classic Michaelis - Menten model. Fractal kinetics assumes that enzymatic reactions on heterogeneous media occur within a non-Euclidian space characterized by a certain fractal dimension; this fractal dimension gives the dependence on time of the kinetic coefficients (Aranda et al., 2006; Macheras and Iliadis, 2006). In such situations, heterogeneous or fractal kinetics can be encountered: rate coefficients become time-dependent, and reaction orders may be given by fractional powers.

Enzyme catalysis may follow more complex mechanisms than expressed in mathematical terms by the classic Michaelis–Menten equation which is valid in an Euclidean medium with non-fractal energy profile. The kinetic constant, however, could assume a fractal behavior. It has then the dimension of a rate constant multiplied by $(\text{concentration})^{d_f-1}$. Fruite et al. explored the ramifications of the fractal geometry of the key organ for drug elimination, the liver, on pharmacokinetic data analysis. Marsh and Tuszyński (2006) have demonstrated that a steady state fractal Michaelis-Menten equation best describes the elimination of the drug mibefradil from dogs. According to Kosmidis et al. (2004), the modified Michaelis-Menten equation can describe the pharmacokinetics of mibefradil as it is able to capture the heterogeneity of the enzymatic reaction in disordered media and under spatially constrained conditions. The profile of potential energy (U) against the reaction coordinate for different resolving power in complex (i.e. fractal) mechanisms shows a “rugged” appearance when it gets more and more resolved. The original (i.e. Michaelis-Menten) kinetic constant is replaced by K^{eff} which is fractal. The expression replacing the original Michaelis-Menten equation is as follows (Marsh and Tuszyński, 2006):

$$V = \frac{V_{\max[S]}^{\text{eff}}\, 2-\text{df}}{K_M^{\text{eff}} + [S]} \tag{8}$$

Further, besides time dependent coefficients an alternative representation of kinetics in fractal media is given by fractional order differential equations. These are differential equations, where the derivatives are of non-integer order (e.g. a one-half derivative). The latter has been applied in pharmacokinetics, pharmacodynamics and GI drug absorption (Dokoumetzidis and Macheras, 2009; Dokoumetzidis et al., 2010a,b; Verotta, 2010).

3.4. Pharmacology and biosciences

The fractal system of geometry overcomes the limitations of the Euclidean geometry for such objects and measurement of the fractal dimension gives an index of their space-filling properties (Losa and Nonnenmacher, 1996; Cross, 1997; Dey, 2005). The concept of fractals and the non-linear dynamics provide sensitive ways to characterize dysfunction resulting from disease and/or drug toxicity, as well as many phenomena encountered in pharmacology. For example, fractal dimension analysis of blood vessels may be a very efficient tool which enables comparison of shape and distribution of vessels on the surface (Jurczyszyn et al., 2012). Also, the equivalent of a steady state, in chaotic systems, often exhibits a non-periodic trajectory of a fractal dimension, which is called a strange attractor (Table 1). This is in contrast with the usual types of a steady state, which can be a fixed point or a periodic trajectory, etc.

The surface membranes of various cell types were digitized from electron micrographs and the fractal dimension for each of them calculated. The values range between $d_f = 1.02$ and $d_f = 1.34$ independent of magnification. Fractal analysis has been also used for evaluation of ultrastructural changes during early stages of apoptosis (Smith et al., 1996; Pantic et al., 2012).

Chaotic dynamics and fractal geometry also play a significant role in brain structure and in neural function at all levels of organization, down to the molecular and quantum levels, and in central

cognitive process (Jelinek and Fernandez, 1998; Fernández and Jelinek, 2001; Eke et al., 2002; King et al., 2010).

Additionally, the application of nonlinear dynamics to brain electrical activity offered new information about the dynamics of the underlying neuronal networks and formulated the brain disorders on the basis of the qualitative dynamics (Mackey and AnDerheiden, 1984; Stam et al., 1994; Milton and Black, 1995; Accardo et al., 1997; Jeong et al., 1998; Ehlers et al., 1998; Silva et al., 1999; Dokoumetzidis et al., 2001). Numerous applications of nonlinear dynamics and chaos theory to cardiac physiology and pharmacology have been published, too (Garfinkel et al., 1992; Wangner and Person, 1998; Geneser et al., 2008; Swenson et al., 2011).

Furthermore, one of the physiological processes where nonlinear dynamics is believed to play a significant role is the secretion of hormones (Tolic et al., 2000; Topp et al., 2000; Dokoumetzidis et al., 2002; IIias et al., 2002). The application of fractal analysis to cancer as a morphometric tool for diagnostic and prognostic purposes is well established in the recent literature (Baish and Jain, 2000; Brú et al., 2003; Janecka, 2007; Huang et al., 2009; McNally and Mazza, 2010; Tambasco et al., 2010; Streba et al., 2011).

Applications of fractals and nonlinear dynamics in biosciences is a wide field, ranging from atomic distances up to those found in macromolecules and large aggregates of matter of any kind. Fractal geometry has found application in catalysis and electrochemistry which deal with surfaces that are rough, in polymers that twist and turn in space, in colloid aggregates, and in porous media. Chaotic time series also have fractal phase portraits. Some of these phenomena pertaining to the pharmaceutical field have already been mentioned. Aon et al. (2000) attempted to analyze the coupling between the dynamics of biochemical reactions (especially chaotic dynamics), and the geometry of cytoarchitecture (especially fractal ultrastructure), because of its importance and consequences for the ultradian dynamic behavior of cells. According to Brodsky (2006), recent data concerning ultradian (circahoralian) intracellular rhythms are used to assess the biochemical mechanisms of direct cell-cell communication due to the fractal nature of ultradian rhythms and their self-organization. Fractal geometry in intracellular macromolecular assemblies suggests that chaotic dynamics occur during their organization. Non-linear interactions in and between spatial and temporal domains and over wide ranges of scales underlie the emergent properties of complex biological systems.

Aon et al. (2004) highlighted the links between fractals and scaling in cells and explore the kinetic consequences for biochemical reactions operating in fractal and heterogeneous media based on the proposal that the cytoskeletal architecture is organized as a percolation lattice, with clusters emerging as fractal forms. A key consequence of this spatiotemporal cytoplasmic organization is that enzyme reactions following Michaelis-Menten or allosteric type kinetics exhibit higher rates in fractal media (for short times and at lower substrate concentrations) at the percolation threshold than in Euclidean media, as mentioned above. As a result, considerably faster and higher amplification of enzymatic activity could be obtained (Aon et al., 2004).

4. Conclusions

The fractal objects, systems and processes in time have multiscale properties and self-similar patterns are frequently observed in nature, especially in nanoscale. The physiological implications of the fractal concepts are serious since fractal structures, systems and processes are ubiquitous in living things, e.g. the lung, neural networks, ion channel kinetics, the convoluted surface of the brain, and the distribution of blood flow through the blood vessels.

This review describes approaches to the analysis of fractal properties of observations in the different scientific parts of Biosciences and Pharmaceutical Sciences, from technological to clinical aspects. Fractals are useful to describe the natural irregularity of physiological systems because their irregularity is not truly random and can be demonstrated to have spatial or temporal correlation. The concepts of fractal analysis are introduced from intuitive, visual, and mathematical perspective. As it was discussed extensively in this review paper, fractal geometry applied as a frequent mathematical formalism in several areas of the pharmaceutical research and practice (from the formulation of drugs to *in vitro* and *in vivo* studies). Finally, the authors pointed out the ubiquitous existence of fractals in pharmaceutical sciences.

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