

## The Importance of Solubility for New Drug Molecules

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**A more pronounced concern for pharmaceutical companies to detect and develop of new drug molecules is observed and is expected. This is due, on the one hand exit under patent protection of new drug molecules, and on the other hand for to counteract competition from generic manufacturers. Subsequently synthesis of new chemical entities with therapeutic action out several studies are needed in design, development, characterization and optimization of pharmaceutical dosage forms thereof. When developing new drug molecules systems and optimizing pharmaceutical formulations, it is essential to determine the physical and chemical properties of the new drug molecules used and other derived properties. This review aims to highlight the role of the solubility of new drug molecules to achieve an effective formulation.**

**Keywords:** Dissolution; New Drug Molecules; Optimizing Pharmaceutical; Solubility.

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Pharmaceutical companies will focus on coordinating and financing their acquired skills and competences. The vision is supported by the apparent instability of the "blockbuster model", rising research-development costs, the very small number of innovative products with potential success, and the forces that reduce the profit of this type of product. This type of strong market reaction to marginal adjustments to public expectations demonstrates the pressure under which large pharmaceutical companies operate<sup>1</sup>. The review addresses the biopharmaceutical dependence modelling of hydrophobic drug molecules incorporated in solid and semisolid forms by pharmaceutical technology factors, the correlation between predictive in vitro release assays and biopharmaceutical properties for the

development of a methodology for correlating the performance of modern pharmaceutical or alternative pathways with the new bioethics safety and efficacy criteria<sup>2</sup>. The development of predictive models of in vivo biopharmaceutical behavior in the chemical structure, correlated with dissolution and diffusion tests, will be pursued to support the reduction of in vivo experiments in the design of new drug molecules (NDM) in pharmaceutical forms<sup>3</sup>. Supporting the development of a new methodology for the assessment of biopharmaceutical performance for current therapist systems will be represented by previous experience of the members of the working group in the field of bio-pharmaceutics and pharmacokinetics, respectively in-vivo evaluation<sup>4</sup>.



## Background

Designing, designing and building a new drug molecule is a laborious process that stretches on average over a minimum of 10 years<sup>5</sup>. Any medicinal product is sustained in its two-stage existence:

- Manufacturing the NDM starting from the active substance, excipients and adjuvants characterized physico-chemically and pharmacologically, known as preformulation of NDM, followed by formulation and preparation in a pharmaceutical form or therapeutic system by appropriate technology, packaging and storage;
- Obtaining the therapeutic action after administration of the NDM through an absorption and bioavailability consistent with the objectives set in the formulation and preparation phase<sup>6</sup>.

Preformulation involves the realization, on the basis of a structured program of the physicochemical, mechanical, biological, of the NDM, the evaluation of possible reciprocal influences or interactions with a number of excipients used in the formulation, the assessment of stability, etc<sup>7</sup>. In preformulation studies, particularly for a newly synthesized NDM or in the case of new therapeutic uses of known drugs, two fundamental properties are particularly important: intrinsic solubility and dissociation constant. These will indicate if it is necessary to identify ways to improve bioavailability, which can sometimes be reduced due to the low solubility of the NDM<sup>8</sup>. Preformulation studies of NDM have a role in anticipating problems and providing logical ways to solve the formation of various liquid, semisolid and solid medicinal products with systemic or local action<sup>9</sup>. The basic characteristics pursued in the preforming phase are related to: solubility in water or other solvents, pH influence, salt formation, lipid / water partition coefficient, chemical structure-pharmacological activity relationship, stability of drug active substance in solution and in solid state<sup>10</sup>. The physicochemical properties of the active substance, excipients and NDM formulation can greatly affect the bioavailability and, implicitly, absorption, therapeutic efficacy and stability<sup>11</sup>.

### Specific Physico-Chemical Factors in Preformulation

#### Solubility of Drug Substances. Dissolution Speed

Among the factors that greatly influence

bioavailability are water solubility, dissolution rate and NDM permeability<sup>12</sup>. Therefore, improving the solubility and dissolution rate of NDM is one of the main aspects that need to be investigated in the development of dosage forms, particularly those intended for oral or transdermal administration. At present, solving problems related to the convenient presentation of the drug and identifying suitable carriers is the subject of numerous researches<sup>13</sup>.

The solubility and dissolution rate of a drug substance in a pharmaceutical form are parameters that decisively influence the absorption and bioavailability of the NDM<sup>14</sup>. Solubility indicates the degree to which a pure substance can be dissolved in a solvent, forming a homogeneous solution, where the distribution of atoms, molecules, ions is uniform. Solubility is an important parameter because it depends on reaching the desired concentration in systemic circulation and obtaining an optimal therapeutic response<sup>14</sup>.

Disaggregation of the pharmaceutical form and dissolution of the drug are processes necessary for its absorption to be convenient and, respectively, to achieve the desired clinical response. NDM delivery of the pharmaceutical form, also called pharmaceutical availability, can be significantly influenced by the physicochemical properties of the drug substance and pharmaceutical form<sup>15</sup>. For in vitro determination of drug availability in pharmaceutical forms, tests that have the advantage of being more easily determinable and more reproducible than in vivo tests allow for the study of factors that can influence physicochemical processes in the absence of physiological variables. These determinations are necessary because the bioavailability depends on the physical properties of the NDM (particle size, crystalline form, method of manufacture of pharmaceutical forms, excipients used), pH of the solvent, etc. All of these factors influence dissolution in vitro<sup>16</sup>.

The solubility of a substance describes the maximum amount of the substance that dissolves in a certain amount of solvent at a certain temperature. Quantitatively, the solubility is equal to the amount of substance that dissolves at a certain temperature in 100 g of solvent. Solubility can therefore be understood as being the property of a substance to dissolve under certain conditions in a solvent or mixture of solvents resulting in a homogeneous

mixture<sup>17</sup>. It may be the factor that condition the formulation of a drug and its future therapeutic efficacy. NDM with a good water solubility usually do not raise formulation problems and show good bioavailability for almost any route of administration. Hardly soluble substances are those that raise formulation problems because both dissolution, bioavailability and therapeutic effect are dependent on this parameter<sup>18</sup>.

### **Solubilization of New Drug Molecules. Basic Principles and Methods of Solubilization**

Preformulation and optimization of pharmaceutical systems in the form of solutions with poorly soluble bioactive compounds is currently a challenge in pharmaceutical research. Ideally, a “well-balanced” drug molecule must be sufficiently hydrophilic to be soluble in aqueous biological liquids and buffer solutions but also sufficiently lipophilic to penetrate through biological membranes<sup>19</sup>.

A polar substance is soluble, but it lacks liposolubility to be permeable through the membranes. Also, the presence of an ionic charge causes poor absorption despite good dissolution<sup>20</sup>. On the other hand, liposoluble substances have difficulty in dissolving in the aqueous medium but have no problems in penetrating through the absorption membranes<sup>21</sup>. Today, research focuses on increasing the number of drugs that have a solubility deficiency in both apolar and lipophilic solvents. It requires guidance for finding the best pharmaceutical formulations that will provide the end product with all the qualities to maintain and adapt its clinical utility<sup>22</sup>. Of course, it is not always easy to intervene in the preform and formulation respectively. Often the possibilities of intervention in the formulation phase and the final presentation of the drug are often overestimated<sup>23</sup>. Although new pharmaceutical technologies (such as complexation with cyclodextrins, micro and nano-emulsions or suspensions, micro- and nano-encapsulation, nanoparticulate forms, solid/dispersed phase, solubilization with cosolvents, etc.) can combat certain inconveniences, successfully applied or are too expensive and often replaced by simple, easily accessible, reproducible procedures that ensure both therapeutic efficiency and economic profitability<sup>24</sup>.

In formulating a new drug molecule, the main concern is not related to a certain degree of

solubility of the drug substance or other aspects of its release in the body. In search of highly biologically active NDM, low solubility is very rarely an obstacle because in vitro testing seeks to obtain as low a concentration as possible, of micromolar, but even better, nanomolar<sup>25</sup>. However, problems arise when in vivo tests, and in particular in animal tests that need to be performed under safe conditions, large amounts of medication to be administered are required. This is the time when the formulator has the task of optimizing the solubility for the dosage forms of the new drug<sup>26</sup>. Thus, two main interests arise: obtaining for the parenteral route an injectable form, preferably in the form of a solution, while for the oral route it is intended to obtain pharmaceutical forms with dissolution and rapid absorption of the NDM dose. However, many of the drug substances do not have good solubility in water, so several solubilization techniques have been developed<sup>27, 9</sup>.

Solubilization is the operation by which insoluble substances are introduced into the solution in ways that facilitate dissolution<sup>28</sup>. This term “solubilization” was introduced by Mc. Bain and Hutchinson, which referred to micellar solubilization (that is, the possibility of passing insoluble substances into the solution by obtaining a colloidal solution)<sup>29</sup>. Later the term gained a wider meaning, including other methods of passing insoluble substances into solution<sup>30, 16</sup>. Some authors, however, consider that solubilization can only refer to the use of surfactants for this purpose, the other methods being only applications of classical dissolution. The molecular level of dispersion is the ideal degree of dispersion of a drug in the body. If the properties of the substance do not allow a complete dissolution of the NDM dose in body fluids or if it is required to obtain a certain concentration of an aqueous solution, other heterogeneous dispersion grades and systems are required<sup>31, 19</sup>. Solubility, the phenomenon of dissociation of the solute in the solvent in order to obtain a homogeneous system, is one of the most important parameters in order to achieve the desired plasma concentrations of the NDM in the systemic circulation, with significant results in achieving the pharmacological target pursued<sup>32</sup>. Reduced solubility in aqueous media is the main problem encountered when new chemical entities

develop (over 40 % of which are practically insoluble in water)<sup>33, 25</sup>.

Since it is preferable that any NDM to be absorbed be in the form of a solution at the site of absorption, solubilization becomes a major challenge in the formulations developed by the research teams. Various techniques have been developed to improve the solubility of these chemical entities or plant active principles, otherwise poorly soluble in aqueous media<sup>34, 28</sup>. These techniques include changes in the physical and /or chemical nature of the NDM, complexation, crystalline engineering, particle size reduction, solid dispersions, soluble salt formation, surfactant use and others<sup>35</sup>. Selecting the optimal method to improve this parameter takes into account the properties of the NDM, the site of absorption, the dose to be administered, the formulation, the route of administration, the stability of the drug, and obviously also economic considerations<sup>36-32</sup>.

### **There are Three Main Ways to Improve Solubility**

#### **Physical methods**

(Cosolvent solubilisation, various complex pharmaceutical systems, etc.). In recent years, nano- and bio-technologies have been extensively used in almost all areas of activity and seem to provide means to achieve otherwise inaccessible goals. In the medical field, this includes both the improvement of diagnostic means and the formulation in pharmaceutical practice<sup>33</sup>. The challenge of developing new therapeutic systems that improve transport, and in particular the release or even vectorization of the NDM, must combine excellent engineering / formulation with the extensive knowledge of biological knowledge. Based on these considerations and their benefits, a series of biocompatible nanostructures have recently been developed, which have recently changed their status from a subject of laboratory research into a powerful industrial instrument, the distance between their ideal characteristics and technical feasibility is shrinking continuously<sup>34-36</sup>.

#### **Physicochemical Methods**

Consisting in obtaining salts with a higher solubility than the substance itself. This method is only applicable to substances containing ionizable groups. Among the physicochemical methods for improving the solubility important in the

pharmaceutical field, solubilization occurs through salt formation and complexation<sup>37-39</sup>.

#### **Synthesis methods**

Aiming at the introduction into the molecule of functional groups that significantly improve solubility. Prodrugs, frequently developed for pharmaceutical applications, seek to modify the structure of the substance by covalent bonds made between appropriate functional groups. These bonds need to be hydrolytically or enzymatically broken so that the active substance is released into the body at the right place after the prodrug has passed through the absorption pathways<sup>40-42</sup>.

#### **Techniques for improving solubility can be systematized depending on the changes they make:**

##### **Physical Changes**

- Reduction of particle size (micronization, nanosuspension, sonocrystallisation, supercritical fluidization);
- Changing the crystalline state (polymorphism, pseudopolymorphism);
- Dispersion of substances into “carriers” (eutectic mixtures, solid dispersions, solid solutions);
- Complexing – refers to the use of complexing agents, especially those intended to obtain inclusion complexes<sup>43-45</sup>.

##### **Chemical Modifications**

(Solubilization with surfactants, formation of complexes or molecular associations, formation of salts, introduction of hydrophilic groups into molecules)<sup>46,47</sup>.

Other Methods for Increasing Solubility (Co-crystallization, hydrotrophy, nanotechnologies, selective adsorption or use of insoluble transporters, use of cosolvents, use of functional polymers, use of soluble prodrugs)<sup>48</sup>.

In order to provide a desired therapeutic effect, the formulator can optimize bioavailability by judicious choice of the final pharmaceutical form, recommendation of a particular route of administration, through a careful combination of the physicochemical parameters characteristic of the active substance as well as of the pharmaceutical and technological ones<sup>49,50</sup>. The formulator must, however, be aware of the potential for interaction of the components of the pharmaceutical formulation, it must be remembered that certain modifications to the NDM or formulation itself may affect the safety,

stability and, last but not least, the therapeutic efficacy of the NDM<sup>51</sup>.

### Conclusions and Remarks

Solubility may be the factor that condition the formulation of a NDM and its future therapeutic efficacy. Improving this parameter and determining the influence it exerts on the level of absorption, bioavailability, stability and therapeutic effect are major goals that need to be properly researched in the design and development of NDM.

Although the forces of change, both technological and demanded by the market, seem overwhelming, it is often the mistake of predicting prematurely the disappearance of large pharmaceutical companies. For example, in the 1990s, it was argued that large banks, through massive changes due to new regulations and the implementation of new technologies, would be assimilated by opportunists, so that survival became problematic. On the contrary, the big banks have not only survived, but they have probably strengthened their position on the financial services market, and many opportunists have withdrawn from the market. Therefore, irrespective of the structure of the pharmaceutical industry or health services, it appears that major pharmaceutical companies will have an important role to have in shaping this structure, locally and globally.

In addition to innovation, high-standard production, market policy and efficient marketing, pharmaceutical companies also need strategies that can cope with apparent contradictions, convergence and divergence, centralization and involution, global and local, focus and freedom, domestic production and external supply, property and alliances, networks and hierarchies, science or market orientation, all of which are part of the essence of a profitable and expanding pharmaceutical company.

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### REFERENCES

1. Rashed KN, Butnariu M. 2014. Isolation and antimicrobial and antioxidant evaluation of bioactive compounds from *Eriobotrya japonica* *Advanced Pharmaceutical Bulletin*. 4:75–81.
2. Hariri Moghadam F, Khalghani J, Moharrampour S, Gharali B, Mostashari Mohasses, M. Investigation of the induced antibiosis resistance by zinc element in different cultivars of sugar beet to long snout weevil, *Lixus incanescens* (Col: Curculionidae), *Banat's Journal of Biotechnology* 9(17), 5–12 (2018).
3. Barazesh F Oloumi H, Nasibi F, Kalantari KM. Effect of spermine, epibrassinolid and their interaction on inflorescence buds and fruits abscission of pistachio tree (*Pistacia vera*), "Ahmad-Aghai" cultivar, *Banat's Journal of Biotechnology* 8(16): 105–115 (2017).
4. Ghaderinia P, Shapouri R. Assessment of immunogenicity of alginate microparticle containing *Brucella melitensis* 16M oligo polysaccharide tetanus toxoid conjugate in mouse, *Banat's Journal of Biotechnology* 8(16), 83–92 (2017).
5. Aramesh M, Ajoudanifar H. Alkaline protease producing *Bacillus* isolation and identification from Iran, *Banat's Journal of Biotechnology* 8(16), 140–147 (2017).
6. Butnariu M, Sarac I, Samfira I. Spectrophotometric and chromatographic strategies for exploring of the nanostructure pharmaceutical formulations which contains testosterone undecanoate. *Scientific Reports*. 10: 3569 (2020). <https://doi.org/10.1038/s41598-020-60657-4>.
7. Ouis N, Hariri A. Antioxidant and antibacterial activities of the essential oils of *Ceratonia siliqua*, *Banat's Journal of Biotechnology* 9(17): 13–23 (2018).
8. Hariri A, Ouis N, Bouhadi D, Benatouche Z. Characterization of the quality of the steamed yoghurts enriched by dates flesh and date powder variety H'loua, *Banat's Journal of Biotechnology*, 9(17), 31–39 (2018).
9. Jahan S, Chowdhury SF, Mitu SA, Shahriar M, Bhuiyan, MA. Genomic DNA extraction methods: a comparative case study with gram-negative organisms, *Banat's Journal of Biotechnology* 6(11): 61–68 (2015).
10. Righi K, Assia Righi F, Boubkeur A, Boungab K, Elouissi A, Djendara AC. Toxicity and repellency of three Algerian medicinal plants against pests of stored product: *Ryzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) *Banat's Journal of Biotechnology* 9(17): 50–59 (2018).
11. Dadkhah A, Rad AHE, Azizinezhad R. Effect of pumpkin powder as a fat replacer on rheological properties, specific volume and moisture content of cake, *Banat's Journal of Biotechnology* 8(16), 116–126 (2017).
12. Georgieva N, Kosev V. Adaptability and Stability

- of White Lupin Cultivars, *Banat's Journal of Biotechnology* 9(18), 65–76 (2018).
13. Kumar A, Senapati BK. Genetic analysis of character association for polygenic traits in some recombinant inbred lines (ril's) of rice (*Oryza sativa*) *Banat's Journal of Biotechnology* 6(11), 90–99 (2015).
  14. Rahimian Y, Akbari SM, Karami M, Fafghani M. Effect of different levels of Fenugreek powder supplementation on performance, Influenza, Sheep red blood cell, New Castle diseases anti-body titer and intestinal microbial flora on Cobb 500 broiler chicks, *Banat's Journal of Biotechnology* 9(18): 29–37 (2018).
  15. Butnariu M. Samfira I; Sarac I; Negrea A; Negrea P. Allelopathic effects of *Pteridium aquilinum* alcoholic extract on seed germination and seedling growth of *Poa pratensis*. *Allelopathy journal*. 35(2), 227–236.
  16. Vasileva V. Root biomass accumulation in vetch (*Vicia sativa*) after treatment with organic fertilizer, *Banat's Journal of Biotechnology* 6(11), 100–105 (2015).
  17. Olufeagba SO, Okomoda, VT, Okache W. Growth performance of all male tilapia (*Oreochromis niloticus*) fed commercial and on-farm compounded diet, *Banat's Journal of Biotechnology* 7(13), 70–76 (2016).
  18. Marinova DH, Ivanova II, Zhekova ED. Evaluation of Romanian alfalfa varieties under the agro-environmental conditions in northern Bulgaria, *Banat's Journal of Biotechnology* 9(18), 56–64 (2018).
  19. Ouis N, Hariri A. Phytochemical analysis and antioxidant activity of the flavonoids extracts from pods of *Ceratonia siliqua* *Banat's Journal of Biotechnology* 8(16), 93–104 (2017).
  20. Bakari M, Yusuf HO. Utilization of locally available binders for densification of rice husk for biofuel production, *Banat's Journal of Biotechnology* 9(18), 47–55 (2018).
  21. Jasim, RK. Isolation and molecular characterisation xylanase produced by sporolactobacilli, *Banat's Journal of Biotechnology* 7(14), 30–37 (2016).
  22. Nikolova I, Georgieva N. Effect of biological products on the population of aphids and chemical components in alfalfa, *Banat's Journal of Biotechnology* 9(18), 38–46 (2018).
  23. Eed AM, Burgoyne AH. Tissue culture of *Simmondsia chinensis* (Link) Schneider, *Banat's Journal of Biotechnology* 6 (11), 45–53 (2015).
  24. Hassan SA, Soleimani T. 2016. Improvement of artemisinin production by different biotic elicitors in *Artemisia annua* by elicitation-infiltration method, *Banat's Journal of Biotechnology* 7(13), 82–94.
  25. Zerkaoui L, Benslimane M, Hamimed A. 2018. The purification performances of the lagooning process, case of the Beni Chougrane region in Mascara (Algerian N.W.), *Banat's Journal of Biotechnology* 9(18), 20–28.
  26. Saidi A, Eghbalnegad Y, Hajibarat Z. 2017. Study of genetic diversity in local rose varieties (*Rosa spp.*) using molecular markers, *Banat's Journal of Biotechnology* 8(16), 148–157.
  27. Belkhodja H, Belmimoun A, Meddah B. 2017. Chemical characterization of polyphenols extracted from different honeys, *Banat's Journal of Biotechnology* 8(15), 78–82.
  28. Hariri A, Ouis N, Bouhadi D, Yerou KO. 2017. Evaluation of the quality of the date syrups enriched by cheese whey during the period of storage, *Banat's Journal of Biotechnology* 8(16), 75–82.
  29. Bozhanska T. 2018. Botanical and morphological composition of artificial grassland of bird's-foot-trefoil (*Lotus Corniculatus*) treated with lumbrical and lumbrex, *Banat's Journal of Biotechnology* 9(18), 12–19.
  30. Butu A; Rodino S; Golea D; Butu M; Negoescu C; Dinu-Pirvu CE. Butnariu M. Liposomal nanodelivery system for proteasome inhibitor anticancer drug bortezomib. *Farmacia*. 63. (2), 224–229.
  31. Nair MSV, Williams ES. 2015. Comparative study of 2-phenoxy ethanol and clove oil on its efficiency as anesthetics in anesthetizing *Hypselobarbus Kurali*, *Banat's Journal of Biotechnology* 6(12), 15–22.
  32. Mahmoodi M, Afshari KP, Seyedabadi HR, Aboozari M. 2018. Sequence analysis of 12S rRNA and 16S rRNA mitochondrial genes in Iranian Afshari sheep, *Banat's Journal of Biotechnology* 9(19), 5–11.
  33. Ayadi Hassan S, Belbasi Z. 2017. Improvement of hairy root induction in *Artemisia annua* by various strains of agrobacterium rhizogenes, *Banat's Journal of Biotechnology* 8(15), 25–33.
  34. Satimehin FP, Tiamiyu LO, Okayi RG. 2017. Proximate and phytochemical changes in hydrothermally processed rubber (*Hevea brasiliensis*) leaf meal, *Banat's Journal of Biotechnology* 8(16), 12–17.
  35. Danilchuk YV. 2016. Selective crystallization of maltose by isopropanol and acetone from glucose-maltose syrups, *Banat's Journal of Biotechnology* 7(14), 120–125.
  36. Menkovska M, Damjanovski D, Levkov V, Gjorgovska N, Knezevic D, Nikolova N, Stanoev V. 2017. Content of B-glucan in cereals grown by organic and conventional farming, *Banat's*

- Journal of Biotechnology 8(16), 39–47.
37. Samfira I; Rodino S; Petrache P; Cristina RT; Butu M; Butnariu M. Characterization and identity confirmation of essential oils by mid infrared absorption spectrophotometry. *Digest journal of nanomaterials and biostructures*. 10(2), 557–565.
  38. Semnani SN, Hajizadeh N, Alizadeh H. 2017. Antibacterial effects of aqueous and organic quince leaf extracts on gram-positive and gram-negative bacteria, *Banat's Journal of Biotechnology* 8(16), 54–61.
  39. Dilali B, Ahmed H, Zouaoui B, Fatima S, Karima OY. 2017. Kinetic of batch production of lactic acid from carob pods syrup, *Banat's Journal of Biotechnology* 8(15), 57–65.
  40. Rezaei A, Akhshabi S, Sadeghi F. 2016. Evaluation of exon 17 of insulin receptor (INSR) gene and its relationship with diabetes type 2 in an Iranian population, *Banat's Journal of Biotechnology* 7(13), 61–69.
  41. Egu UN, Okonkwo JC. 2017. Effect of gonadotrophin (diclair (R)) on semen characteristics, hormonal profile and biochemical constituents of the seminal plasma of mature *Balami rams*. *Banat's Journal of Biotechnology* 8(15), 90–97.
  42. Ghasemi E, Kohnhrouz BB. 2016. Cloning the cotton *rrn23–rrn5* region for developing a universal interfamily plastidial vector, *Banat's Journal of Biotechnology* 7(14), 81–88.
  43. Basuny AMM, Al Oatibi HH. 2016. Effect of a novel technology (air and vacuum frying) on sensory evaluation and acrylamide generation in fried potato chips, *Banat's Journal of Biotechnology* 7(14), 101–112.
  44. Ruchin AB. 2017. The effects of illumination on the early development of tailed and *Tailless Amphibians*, *Banat's Journal of Biotechnology* 8(15), 113–118.
  45. Ojogu NA, Annune PA, Okayi GR. 2017. Toxicological effects of aqueous extract of *piptadeniastrium africanum* bark on *Clarias gariepinus juveniles*, *Banat's Journal of Biotechnology* 8(15), 123–135.
  46. Ould Yerou K, Meddah B, Touil AT, Sarsar F. 2017. *Laurus nobilis* from Algeria and immune response, *Banat's Journal of Biotechnology* 8(15), 119–122.
  47. Butnariu M Sarac I. Pentea M. Samfira I. Negrea A. Motoc M. Buzatu AR. Ciopec M. 2016. Approach for Analyse Stability of Lutein from *Tropaeolum majus*. *Revista de chimie*. 67(3), 503–506.
  48. Zarkani AA. 2016. Antimicrobial activity of *Hibiscus sabdariffa* and *Sesbania grandiflora* extracts against some G–ve and G+ve strains, *Banat's Journal of Biotechnology* 7(13), 17–23.
  49. Bhattacharya A, Sadhukhan AK, Ganguly A, Chatterjee PK. 2016. Investigations on microbial fermentation of hemicellulose hydrolysate for xylitol production, *Banat's Journal of Biotechnology* 7(14), 13–23.
  50. Salajegheh Ansary MM, Ahmadimoghadam A, Mirtadzadini SM. 2017. Distribution of cyanobacteria in two sirsch hot springs with regards to the physicochemical traits of water, *Banat's Journal of Biotechnology* 8(15), 83–89.
  51. Idris A. 2016. Comparative analysis of 16SrRNA genes of *Klebsiella* isolated from groundnut and some american type culture collections, *Banat's Journal of Biotechnology* 7(13), 34–40.