Application of Poly (aspartic acid) and its Derivatives in Medicine and Pharmacy

Marek Piątkowski[1*], Julia Radwan-Pragłowska[1], Konstantin Raclavský[2]

[1] Cracow University of Technology, Faculty of Chemical Engineering and Technology,
Department of Biotechnology and Physical Chemistry,
24 Warszawska Street, 31-155 Krakow, Poland

[2] VŠB-Technical University of Ostrava, ENET Centre,
17th November Street, 708 33 Ostrava-Poruba, Czech Republic

[*]*Corresponding author's email: mpiatkowski [AT] indy.chemia.pk.edu.pl*

ABSTRACT---- ***Poly(aspartic acid) (PASA) is a linear polyamide. Due to its chemical structure this polymer is repeatedly getting more application in medicine and pharmacy. It can be used as a biomaterial in biomimics, as well as a component in drug delivery systems or systems of controlled drug release. Because of carboxylic functional groups it is possible to create amphiphilic copolymers that enables preparation of micelles. Poly(aspartic) acid is characterized by biocompatibility, biodegrabability, biotolerance and not causing pyrogenic effect. PASA has hygroscopic properties and is well soluble in water. It can be obtained by hydrolysis of polysuccinimide, polycondensation of L-aspartic acid or ammonium salt of maleic acid using phosphoric acid as catalyst. It can also be prepared by microwave synthesis without any excipient what enables obtaining polymer which can be directly used in biomedicine. Applications include systems for controlled delivery of cancer drugs which must be modified in advance before being placed into the body where there is a hydrophilic environment. Creating micelles using PASA enables the delivery of hydrophobic drugs directly to the lesion site. It is also possible to obtain larger systems, which allows the encapsulation of biomolecules such as proteins. Poly(aspartic acid) is a good material as a component in theranostics. With its usage, quantum dots can be obtained from what gives the possibility of imaging cells. When used as a biomaterial in the composite of hydroxyapatite PASA increases the efficiency of bone tissue regeneration. Additionally, it can serve as a material for creating smart hydrogels.***

Keywords--- poly(aspartic acid), nanoparticles, biomaterials, drug delivery systems, controlled drug release

1. INTRODUCTION

Taking place in recent years is the rapid development of medicine caused by the need to look for ways to manufacture innovative biomaterials, which currently include mainly polymers. However, they must meet a number of different conditions, depending on the future application. For this reason macromolecular compounds have gained a lot of interest, but only those which are characterized by biocompatibility or lack of pyrogenicity. This group includes inter alia poly(aspartic acid) (PASA) and its derivatives. Because of biodegradability it can be used as a component in both biomimetics, tissue engineering as well as controlled release systems or the delivery of drugs, especially anti-cancer ones. Therefore, poly(aspartic acid) is generating more and more interest and number of applications in medicine keeps increasing. PASA is a linear polyamino acid. It is characterized by hygroscopicity, solubility in water and non-toxicity. Invoking pyrogenicity or hemolysis, biocompatibility, high biotolerance by the human body, rapid biodegradability, it is a frequently used polymer in medicine and pharmacy as a biomaterial or component of nanosystems. Methods for its preparation include poly(succinimide) hydrolysis, which can be obtained by thermal bulk polymerization of L-aspartic acid or ammonium salt of maleic acid. Another method is the polycondensation of L-aspartic acid in the presence of phosphoric acid which is not necessary when using microwave radiation during synthesis [1-3]. Therefore, the microwave synthesis of PASA is a method allowing preparation of the most pure polymer since the product obtained is not contaminated by the residues of the catalyst or any other additional substance. That is why this last method is a leading one for biomedical and pharmaceutical application as only biomaterials with the highest degree of purity can be used in treating human body [4].

1.1 Poly(aspartic acid) as a Component of Drug Delivery and Controlled Drug Release Systems and Nanosystems

Poly(aspartic acid), by having in its structure carboxyl groups, has the ability to ionize in a suitable medium. It may also be combined with another molecule of a hydrophobic or amphiphilic properties to create a micelle, wherein the drug is placed. Therefore more and more copolymers of hydrophilic PASA are currently being synthesized. Depending on the ratio of monomers used, it is possible to obtain particles with different properties. This can be multiparticulate structure sensitive to pH changes, which can help deliver the drugs of hydrophobic nature directly to the diseased sites of body without harming healthy cells, tissues or blood components. This type of copolymer-based micro- and nanoparticles also protect the active substance, that is being transported, against the low pH prevailed in stomach or digestive enzymes that may damage its structure. This therefore prevent the degradation of the drug by oxidation, deamination or hydrolysis of proteins is obtained [5]. This is particularly important when oral administration of anticancer drugs is used, which have mainly lipophilic character. Preparation of micelles with the appropriate properties can increase the solubility of the active substance and the reduction in the intake of that would minimize side effects occurrence. The specific role of PASA and its derivatives is very important while creating drug responsible for the treatment of bone tissue, due to the fact that the poly(aspartic acid) has the ability to bind to hydroxyapatite - basic bone building material [6].

A good example is a copolymer of poly(aspartic acid), poly(ethylene glycol) and the hydrophobic polyphenylalanine. The presence of both polar and non-polar ends of the chains had allowed to produce stable micelles containing inside water-soluble cationic drug. By varying the mutual ratio of the monomers used and the pH value, it has been shown that different micelles can be prepared by varying the size, sensitivity to the environment, the hydrophobicity of the core, quantity of drug placed and the profile of its release [7]. Zhang et al. synthesized a new kind of nanoparticles based on another complex - chitosan and sodium salt of poly(aspartic acid). Its ionic character was anionic or cationic, depending on the composition. With the selection of appropriate monomer ratio, a hydrophilic anticancer drug, 5-fluorouracil, which is commonly used in cancer therapy was immobilized inside by mixing or absorption method. Studies performed in mice inoculated with gastric carcinoma cells revealed that the chitosan complex of sodium salt of PASA with encapsulated 5-fluorouracil not only extended drug release time and reduced the amount of side effects but it has also reduced the bone marrow suppression that had been an effect of indirect drug activity. Further, the degree of necrosis of tumour cells was significantly greater than in the case of administration of the non-modified drug, which resulted from the increase in the pharmacodynamics of the active ingredient [8,9]. PASA derivatives of amphiphilic character provide even more opportunities for drug delivery systems. Preparation of copolymer based on polyaspartyl-hydrazide, palmitic acid and monomethoxy poly(ethylene glycol) 2,000 is capable of self-assembly in an aqueous medium due to the presence of hydrophobic alkyl chains that are able to close another anticancer drug, this time with a hydrophobic character. The micelles rich in non-polar chains of palmitic acid are also capable of dissolving significant amounts of tamoxifen, as well as inducing 300-fold increase in hydrosolubility. Cytotoxicity studies have shown that tamoxifen-filled micelles have inhibitory effect on the growth of cancer cells. Micro and nanostructures formed from a copolymer comprising a derivative of PASA probably also cause an increase in the amount of permeation of the active substance into pathological cell [10].

Anti-cancer drugs colloidal solutions consisting of magnetite nanoparticles are also being used. To ensure the safety of the patient in order to reduce its genotoxicity in a physiological medium, magnetite particle must be charged to a special coating, usually polymeric. Studies have demonstrated that poly(aspartic acid) used for this purpose allowed the preparation of biocompatible, biodegradable and non-toxic systems presenting anticancer activity, which is further enhanced by PASA coating. It appears that it is possible due to the inhibitory effect of PASA on the growth of pathologically changed cells and synergistic effect to the therapeutic result of active substance used [11].

Much more difficult is the production of particles having satisfactory properties with nanometric size intended to serve for closing proteins, hormones and enzymes due to difficulty in maintaining their native form, unfavorable pharmacokinetics and the poor immunological properties. These types of compounds easily lose their stability during micelle formation or after too long time of staying in it. They also tend to leave the micelles in a rapid and incomplete manner. Using as a substrate, poly(aspartic acid) or a derivative thereof, can create other stable systems of larger sizes, which can reduce or eliminate obstructions of nanosystems. For example, it can be a derivative of PASA having in its structure components of both hydrophilic and hydrophobic character, that is poly(hydroxyethyl aspartamide). Its structure allows to entrap the specific compounds such as cytokines or hormones characterized by a hydrophobic surface of the particles and special defects in their structure that determine the interactions with membrane receptors. The use of poly(hydroxyethyl aspartamide) grafted with hexadecylalkylamine and poly(ethylene glycol) which has amphiphilic character, allowed to produce the supramolecular arrangement in which the human growth hormone has been closed. It should be noted that affinity of the proteins to the compounds of the obtained copolymer plays a key role in these cases. The higher it is, the longer the release time and the amount of protein contained in the drug. At the same time there is a reduction of its bioactivity, and the release rate is too slow and may cause passivation and degradation of biomolecules [12]. Another example is a system of nanocapsules obtained using "layer-by-layer" method of blending two oppositely charged polyaspartamides, in which the bovine serum albumin was then inclosed using electrostatic adsorption. Also the release of the protein has been later examined giving a positive effect. Nanocapsules, during the test of releasing the

model compound in the buffer solution, showed a response to fluctuations in pH, which in the case of a change from the physiological pH results in acceleration of precipitation at acidic secretion of the protein [13].

By combining PASA with imitating elastin peptide, it is also possible to create nanoparticles protein which will have a strong dependence on temperature. In this case, the corresponding value will generate the formation of the nanosystem (approx. 37 °C) or its breakdown, whereas degree of sensitivity to changes in temperature can be regulated by the chain length of the peptide which imitates elastin. Nanoproteins of this type may find application in creating controlled drug delivery systems to locations in the body where there has been an increase in temperature as a result of inflammation [14].

An interesting use of poly(aspartic acid) in controlled drug delivery systems is the formation of complexes with aminoglycoside gentamicin, which is used in the treatment of cystic fibrosis due to its ability to partially restore the expression and function of proteins. In the case of self-application of this drug it exhibits a high nephropathy and ototoxicity effects. By complexation with PASA gentamicin reduced cytotoxicity by preventing aminoglycoside combination with the acidic groups of the phospholipids, as well as increased efficiency of the healing substance. It has been proved that gentamicin in the presence of PASA had a tendency to produce more of the CFTR protein, which was probably caused by slowing down the release process of aminoglycoside from the cytosol [15].

Poly(aspartic acid) plays a very important role in the creation of drugs on bone diseases such as osteoporosis and osteosarcoma. Most likely, this is because of the fact that there is a binding which is being formed due to ionic interaction between the negatively charged PASA and Ca^{2+} ions present in the structure of hydroxyapatite. Moreover, the presence of negative charge on the chains of the poly(amino acid) causes repulsion of the negatively charged surface of the cell membrane [6].

Of equal importance as the delivery of drugs to appropriate diseased locations in the body, is therapeutic substance release in sufficient quantities for a given period of time. This is particularly difficult in the case of delivery of DNA or RNA in gene therapy. Here poly(aspartic acid) was also applied. For example, when creating block of catiomers capable of anchoring both the DNA and endosomal release (that takes place later) a PASA addition increases the transfection efficiency and significantly enhances the stability of polyplex micelles [16].

Studies also show that poly(aspartic acid) in combination with different types of polysaccharides is a very good material for the genes carriers [17,18]. Gene therapy requires a suitable vector which will be characterized by the lack of cytotoxicity and high degree of transfection at the same time. Currently, most of them are based on viral systems, but their use is associated with immunogenicity. Synthetic vectors not only have this disadvantage eliminated, but they can also be produced on a larger scale. Recently positively charged polysaccharides, which possess the valuable ability to permeate through the cell membrane have been used for this purpose. Unfortunately they cannot serve as the vector alone, as they exhibit poor solubility in water at physiological pH. To obtain a fully effective carrier polysaccharides should be combined with cationic compounds which will exhibit similar chemical structure to the polypeptides present in the cell membrane. For this purpose, aminolysed poly(aspartic acid) appeared to be perfect. Thus prepared synthetic vector as a combination of polysaccharides and aminolysed poly(aspartic acid) has the ability to condense the DNA while maintaining the biodegradability or no toxicity and prevents DNA from being digested by the enzymes [19]. Equally efficient at delivering genes are polyionic complexes of poly (L-aspartic acid) and doxorubicin (cytostatic) or poly(L-aspartic acid) and the protamine sulfate which undergo self assembly by electrostatic attraction. Studies have shown that the complexes conjugate to chemotherapeutic agents when in contact with cancer cells and quickly release the drug into their interior. Moreover complexes consisting only of doxorubicin and PASA effectively inhibit cells' proliferation [18].

Due to its structure, acid derivatives can be coupled with other various substances that are important from a medical point of view, e.g. the main human neurotransmitter, γ-amino acid, butyric acid (GABA), which in the future may allow the production of modern neurological drugs [20].

1.2 Medicinal Diagnostics

Many substances used in medical diagnosis to be introduced into the body and provide accurate information on the health status of the patient must be modified before applying. Examples are already mentioned herein, manganese oxide nanoparticles used in clinical magnetic resonance for example of the liver. Due to the hydrophobic nature, prior to use, they must be coated with a hydrophilic substance which will improve their behavior in aqueous media. The perfect solution appears to be a crust of poly(aspartic acid), when its used as a coating causes a more efficient exchange of water [21].

A relatively new trend in medicine is theranostics, that is personalized therapy, which involves special medications, which act as both imaging and therapeutics. Applied nanosystems after introduction into the body, not only provide the active drug delivery, but also are able to diagnose the disease, as well as follow the therapeutic effect of the active substance. Poly(aspartic acid) is also being widely applied in this area.

The core of the leading examples of theranostics nanosystems due to its unique magnetic properties, consists of materials constituting of iron oxide nanocrystals. To enable them to provide information about the affected areas, they must remain in forms of stable colloidal systems in aqueous solutions. To achieve this, the cover of poly(aspartic acid) is used, due to its properties which allows for the preparation of thermodynamically stable mixture. At the same time, due to the presence of amino or carboxyl group, it allows attachment of other biologically active molecules. Poly(amino acid) is very well suited also for covering nanoparticles of other metals, e.g. gold or silver [22].

Another tool increasingly used in medical diagnostics are quantum dots, which set a new direction alter alia in imaging of living cells in the body. Currently used for imaging tissue or labeling cells quantum dots are based on organic fluorophores or proteins that exhibit fluorescence. Recently, considerable interest earned colloidal inorganic systems, nanocrystals of semi-conductive nature, which also have the ability to emit light [23]. For example, using poly(aspartic acid) scientists succeeded in producing stable and resistant to environmental conditions quantum dots which had a cysteine in its structure. Aminoacid presence enabled binding of, for example, silver or gold nanoparticles, wherein the core was a mixture of cadmium and selenium, and zinc sulfide coating. A key property of the polymer used was the presence of carboxyl groups that allowed the attachment of biomolecules responsible for targeting to connect with the desired biomolecules. In this study derived from PASA carboxyl groups allowed moiety to produce a covalent bond with antibodies, so that the quantum dots were capable of selective detection of proteins. Application to the polymer matrix also contributed to a significant reduction in the case of non-selective interaction incubation with cell cultures [24].

1.3 Poly(aspartic acid) as a Biomaterial

Both calcium phosphates and octacalcium phosphates are widely used in biomaterials, biomimetics, and tissue engineering as they are considered to be precursors of osteogenesis. These compounds, however, have one major drawback, i.e. they have a tendency to uncontrolled growth and aggregation of created nanocrystals, which are undesirable in biomedicine as their sharp ends may destroy healthy parts of the body. To eliminate it, researchers often use poly(aspartic acid). In vitro studies have demonstrated that PASA has an inhibitory effect on the mineralization of the solution, but when it is attached to a special type of calcium phosphate it even catalyzes the process. Thus, by appropriate selection of the amount of poly(aspartic acid) and mineralization conditions, it is possible to influence the size, shape and structure of the crystals that are forming these calcium compounds [25,26]. An example of the PASA addition effect may be the production of porous beads with octacalcium phosphate that are composed of phosphate nanocrystals, poly(aspartic acid) and other trace elements. The use of PASA helped to obtain beads of suitable size and structure of pores that have physicochemical properties allowing it to retain its original shape. Whilst being biodegradable and biocompatible, it also exhibit catalytic effect on the early stages of bone mineralization. Due to the presence of pores in the future, they may also be used as drug carriers, allowing for the development of more effective methods of regenerating bone tissue [27].

Poly(aspartic acid) derivatives are also added to the $Ca_3(PO_4)_2$ composite, which covers bone implants in order to accelerate bone formation [28]. Another example of application was the production of composites that simulated the bone tissue. The use of the sodium salt of poly(aspartic acid) during the mineralization of collagen sponge provoked the process that was much more effective than in its absence, and the resulting material was characterized by a much greater similarity in the construction of the fiber to the natural bone [29]. Studies have shown that poly(aspartic acid) increases the efficiency of the mineralization process of silk, allowing the formation of dense fibers of the most mineralized structure of β-harmonica and with density fluctuations at very low levels. Therefore, PASA plays an important role in the formation of scaffolds of silk fibers, which then acts as a substrate to produce a synthetic bone of the jaw [30].

Poly(aspartic acid) was also used to develop a double cross-linked, smart hydrogels which volume change is a result of cleavage of one of the bindings. Depending on the amount, cross-linking agents may be used to control the swelling ratio and the modulus. In the future smart hydrogels may be used as matrices or materials for urging the cell [31].

2. SUMMARY

Poly(aspartic acid) due to its special properties like biodegradability, biocompatibility, biotolerance and being non-toxic is known to be a very good water-soluble biomaterial for biomedicine and pharmacy. It can be obtained in different ways, however, preparation of the PASA with the highest purity index requires the application of the method without additional substances, e.g. microwave synthesis from L-aspartic acid. Presence of both amino and carboxylic functional groups enables attachment of other chemical compounds of hydrophobic or amphiphilic character. As a result it is possible to create non-pyrogenic micelles and structures for drug delivery and controlled drug release systems. PASA-containing complexes can not only encapsulate medication but can also enhance its biological activity and minimize side effects like in case of mixing poly(aspartic acid) with aminoglycoside gentamicin used for the treatment of cystic fibrosis. Depending on different size of created systems or micelles, various biomolecules can be encapsulated starting from lipophilic anticancer drugs ending with hormones or peptides. Poly(aspartic acid) can also be used in theranostics when complexed with magnetite nanoparticles or as a substrate for inorganic quantum dots. Its hydrophilic character enables vectors creation consisting of PASA and polysaccharides for gene therapy that does not induce an unwanted

immune response. Due to its chemical structure poly(aspartic acid) can be a template for the development of many innovative drugs. PASA is also a great co-substrate in case of inorganic biomaterials. It is proven that poly(aspartic acid) catalyzes the formation of hydroxyapatite crystals and helps not only to obtain crystals of desired shape and size but also enhances bone tissue regeneration. That makes it possible to create innovative drugs and biomaterials which can be used to induce or accelerate osteogenesis. PASA is also a good material for smart cross-linked hydro-gels that can be used as a different type of matrices. So many applications of poly(aspartic acid) and its unique properties gives a great number of possibilities in creation of innovative medications, drug carriers biomaterials and hydro-gels that is why further studies on this polymer can lead to acceleration and enhancement of medicine and pharmacy development.

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