PHARMACY

STUDIES ON ANTISICKLING EFFECTS OF GRIFFONIN

A. K. Abaitey, BPharm MPharm PhD MPSG and
Christiana S. Atuobi, BPharm MPharm MPSG

ABSTRACT

Results obtained from *in vitro* haematological studies show that griffonin causes sickled red blood cells to revert to the normal shape. The antisickling effect of griffonin was dose-and time-dependent. There was no difference between the antisickling effect of griffonin on red blood cells containing the various types of abnormal haemoglobin, namely, haemoglobins SS, AS and SC. On weight basis griffonin was more potent antisickling agent than urea.

Keywords: Griffonin,red blood cell, sickle-cell anaemia, haemoglobin deoxyhaemoglobin, antisickling.

INTRODUCTION

The plant *Griffonia simplicifolia* (Baill), family Caesalpinaceae, is used for various ailments. The leaves are fed to sheep and goats to stimulate reproduction, and the juice of the leaves is used as an enema for the treatment of kidney troubles. A decoction of the leaves is used as an antiseptic in the healing of suppurating wounds and also to stop vomiting, diarrhoea and to treat congestion of the pelvis [7]. A decoction of the roots is reported to be used for the control of sickle-cell crisis in Nigeria [5].

The main constituent of the roots, griffonin, a nitrile glycoside, was isolated by Dwuma-Badu, Watson, Copalakirshina, Okarter, Knapp, Schiff, Jr. and Slatkin in 1976 [6]. Preliminary *in vitro* haematological studies by Aloka [2] showed that griffonin has antisickling effect on sickled red blood cells but it had no effect on normal blood cells. Griffonin has also been shown to be a muscarinic drug devoid of nicotinic properties [1].

Sickle-cell anaemia is a hereditary and familial form of chronic haemolytic anaemia essentially peculiar to Negroes. It is characterized clinically by symptoms of anaemia, rheumatoid manifestations, leg ulcers and acute attacks of pain (crisis).

In S haemoglobin, the sixth amino acid of the beta chain, glutamic acid, has been replaced by valine. Sickle-cell anaemia is found in people with homozygous sickling gene, i. e. HbSS, but the trait is found in those with the heterozygous gene, HbAS. A clinical picture closely resembling that of sickle-cell anaemia is produced by simultaneous heterozygocity for the sickle-cell gene and the genes responsible for thalassaemia, haemoglobin C or haemoglobin D. The most common sickle-cell diseases found in Ghana are the HbSS and HbSC anaemias. HbS-thal is not very common in Ghana [11]. The sickle-cell crisis may develop without a precipitating cause or may follow infection or even blood transfusion. It has been reported that malaria accounts for 16% of painful crises requiring hospital admission in Accra [9].

Treatment of sickle-cell disease may be aimed at maintenance of good health and avoidance of crises or complications. Prophylactic treatment of infections and malaria should be maintained and established infections should be treated promptly.

In recent times, treatment aimed at preventing sickling by chemically reversing the binding of HbS molecules has generated great interest [4]. Examples of drugs used for this purpose include urea, sodium cyanate and carbamyl phosphate. Some agents may inhibit sickling through membrane modifications, for example, zinc and procaine hydrochloride [14]. Other agents like cetiedil inhibit sickling by modifying erythrocyte sodium or potassium movements in a manner that would increase cell water content and thus dilute cell haemoglobin [3].

The aim of the present study is to re-examine the antisickling properties of griffonin, a nitrile glycoside, isolated from *Griffonia simplicifolia* .

MATERIALS AND METHODS

Identification of Haemoglobin Types in Blood Samples

Fresh blood samples obtained from subjects at the Komfo Anokye Teaching Hospital, Kumasi, were used.

The blood sample was washed with normal saline to get rid of the plasma. This was done by adding normal saline to the blood sample and centrifuging at speed 2 with the M. S. E. Minor Centrifuge for 3 minutes and

Dr. A.K. Abaitey
Dept. of Pharmacology
Faculty of Pharmacy
UST, Kumasi, Ghana

Christiana S. Atuobi (Mrs.)
Krisam Chemists
Cantoments, Accra, Ghana

decanting the supernatant solution. This procedure was repeated two times.

A few drops of distilled water were added to the blood cells, shaken and then a few drops of carbon tetrachloride were added. The blood mixtures were again centrifuged and the supernatant solution (i.e. the haemolysate) was collected and used to spot Whatman's No. 3mm electrophoretic paper using a capillary tube.

The electrophoretic paper was soaked in Tris-EDTA-boric acid buffer (1-10 dilution), pH 8. 6, and hung in the electrophoretic tank containing barbitone buffer, pH 8. 6 and ionic strength 0. 05. The voltage was kept at 300 mV and the duration of electrophoresis was 8 hours.

Microscopic Examination of Blood Samples

Blood samples containing haemoglobin AS, SC, SS and AA were collected from outpatient subjects at Komfo Anokye Teaching Hospital, Kumasi, and placed into bottles containing 5% sodium citrate solution to prevent coagulation.

One millilitre of the blood-citrate mixture of blood samples containing either haemoglobin AS, SC or SS was pipetted into a tube containing one millilitre of 2% w/v freshly-prepared sodium metabisulphite solution and incubated at 37°C for one hour or until sickling was observed in about 95% of the cells. One millilitre of freshly-prepared 1% w/v aqueous solution of griffonin in normal saline was added to the blood-citrate-sodium metabisulphite mixture and incubated again at 37°C for one hour. Then the cells were observed under the microscope using the dry high power objective. The mixture was incubated for another hour and the cells observed again under the microscope.

The procedure was repeated using 2% w/v griffonin solution and 2% w/v urea, a known antisickling drug.

To haemoglobin AA, one millilitre of normal saline and an equal volume of griffonin or urea solution were added and the cells observed under the microscope. To serve as a positive control, one millilitre of normal saline was added to an equal volume of sickle-cell blood sample. One drop of this mixture was put on a slide mixed with one drop 2% w/v sodium metabisulphite solution and observed under the microscope. The pH of the various mixtures was checked to find out if there were any changes.

RESULTS

Identification of Haemoglobin Types in Blood Samples

Electrophoresis utilises the charge on the haemoglobin which determines the rate of migration of the haemoglobin molecule under the influence of electric current. The bands of the various haemoglobins, AA, AS, SS and SC, are shown in Fig. 1.

The bands obtained with blood samples 1, 2, 3, 6, 7, 8, 10 and 11 moved the same distance from the origin, and they were made up of two spots. The haemoglobin type was AS and these bands moved faster than band 4, which was also made up of two spots representing haemoglobin SC. Haemoglobin AA was represented by one spot, band 5, which moved faster than haemoglobin S. Band 9, a single spot, representing haemoglobin SS moved to a distance between bands 4 and 5 representing haemoglobins SC and AA respectively.

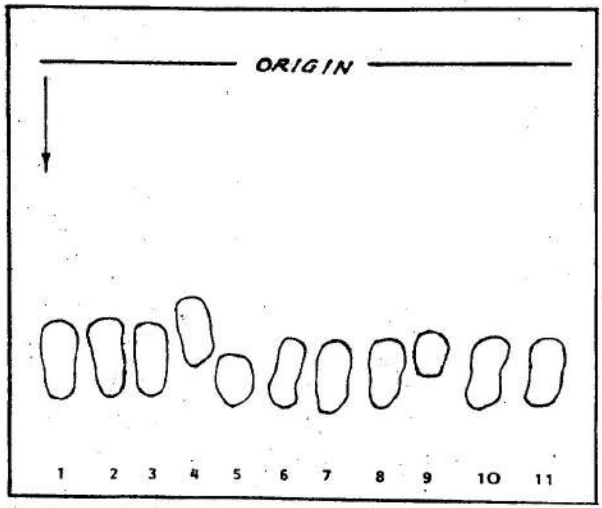

Fig. 1: Paper electrophoresis of normal haemoglobin AA (Band 5); sickle-cell trait haemoglobin AS (Bands 1, 2, 3, 6, 7, 8, 10, 11); sickel-cell anaemia haemoglobin SS (Band 9) and sickle-cell haemoglobin C disease, SC (Band 4) using barbitone buffer, pH 8.6.

Microscopic Examination of Blood Samples

The proportions of 500 red blood cells which sickled in blood samples containing haemoglobins AS, SS and SC after one hour and two hours of incubation with griffonin (1% w/v and 2% w/v) and urea (2% w/v) in five different fields on the slide were estimated. The results are shown in Tables 1 and 2.

The effects of griffonin and urea on the erythrocytes were dose-related and time-dependent irrespective of the sickle haemoglobin type used. Griffonin (1% w/v) reverted 58% of the erythrocytes in the blood sample containing haemoglobin AS to the normal spherical biconcave shape after one hour of incubation and reverted 73% of the cells back to the normal shape after two hours incubation. The corresponding values for 2% w/v griffonin were 73% and 93% in one hour and two hours respectively whilst those for urea (2% w/v) were 62% and 70% [See Tables 1 and 2 and Figs. 2, 3 and 4].

Statistical analysis using the Student's T-test showed that there were no significant differences between the effects of griffonin and urea on the various types of sickle-cell haemoglobin (i.e. AS, SC and SS) used.

There was no change in the pH of the incubated blood-griffonin-sodium metabisulphite mixtures used.

Table 1: *Antisickling effects of griffonin and urea. The values are the mean percentages ±s.e. of cells that were unsickled after one hour of incubation in five experiments. The cells were pretreated with 2% w/v sodium metabisulphite solution.*

Haemoglobin Type	Griffonin 1% w/v	Griffonin 2% w/v	Urea 2% w/v	Normal Saline
HbAS	58.0±0.65	73.6±0.60	62.5±0.58	3.8±0.46
HbSS	57.7±0.37	68.4±0.28	61.3±0.35	1.9±0.21
HbSC	60.0±0.43	65.4±0.39	63.2±0.45	5.0±0.23
HbAA	100	100	100	100

Table 2: *Antisickling effects of griffonin and urea. The values are the mean percentages ± s.e. of cells that were unsickled after two hours of incubation in five experiments. The cells were pretreated with 2% w/v sodium metabisulphite solution.*

Haemoglobin Type	Griffonin 1% w/v	Griffonin 2% w/v	Urea 2% w/v	Normal Saline
HbAS	73.4±0.39	93.2±0.15	70.3±0.31	4.2±0.28
HbSS	72.0±0.28	92.5±0.14	75.4±0.18	2.4±0.26
HbSC	73.7±0.22	93.5±0.21	70.9±0.18	3.8±0.15
HbAA	100	100	100	100

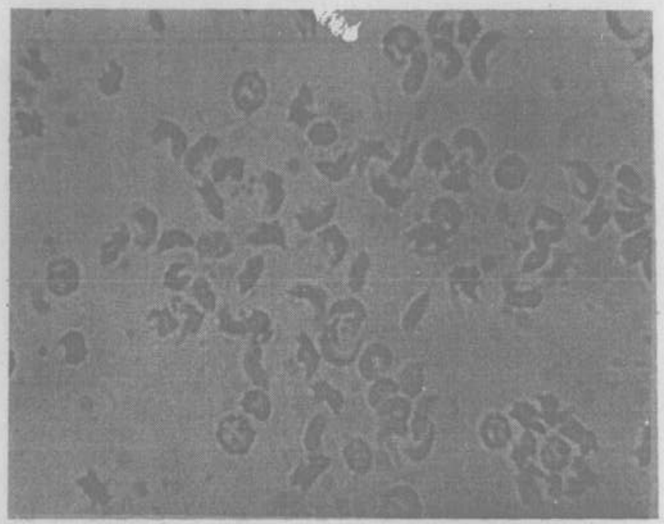

Fig. 2: Photomicrograph of red blood cells of sickle-cell trait haemoglobin AS treated with 2% w/v sodium metabisulphite solution. Mag. X 320.

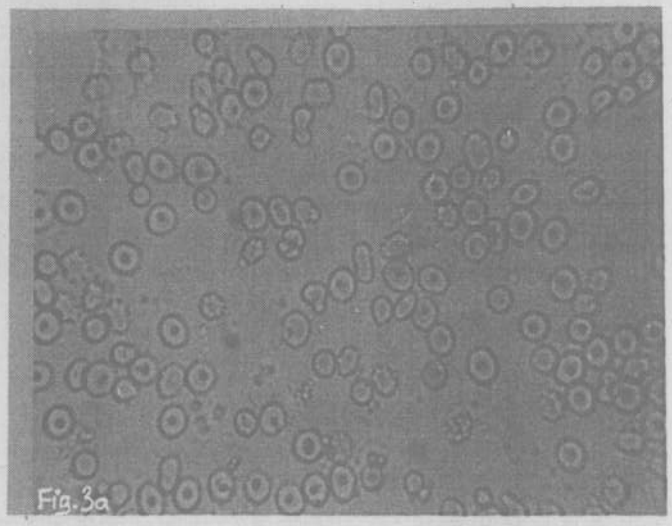

Fig. 3a

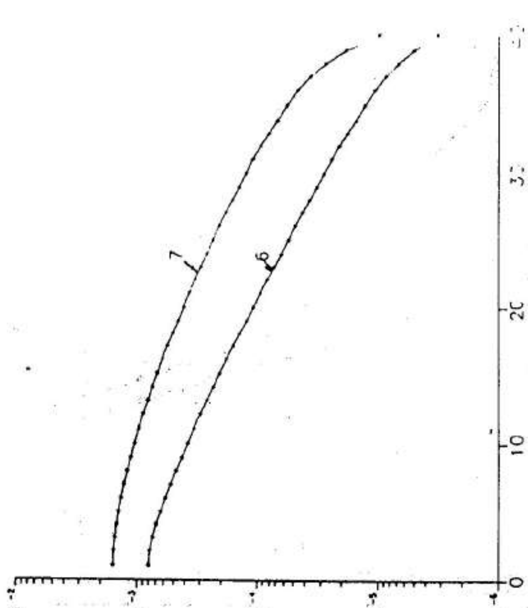

Fig.2: Attenuation of Epithermal Neutrons for Ghanaian Anum Serpentine Shield

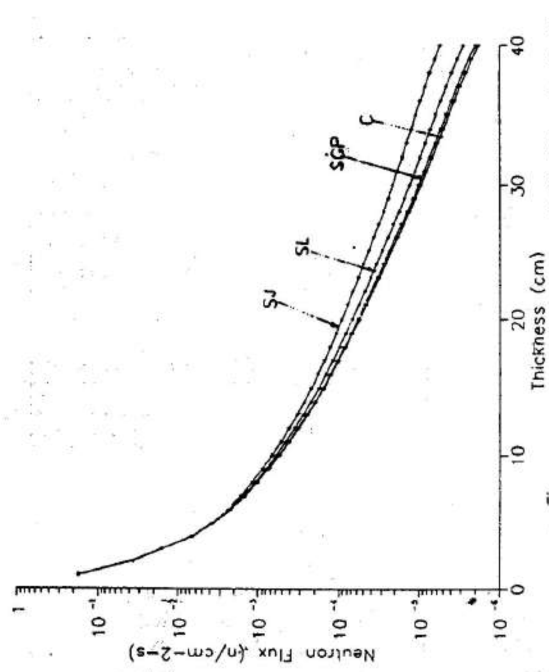

Fig. 4: Intensity of Group 1 Neutrons Behind Ghanaian Peki, Japanese, Libyan and Concrete Shields

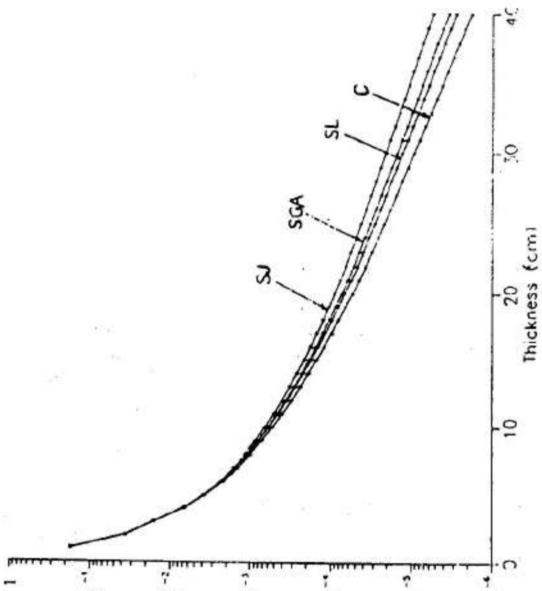

Fig. 3: Intensity of Neutrons Behind Anum, Japanese Libyan and Concrete Shields

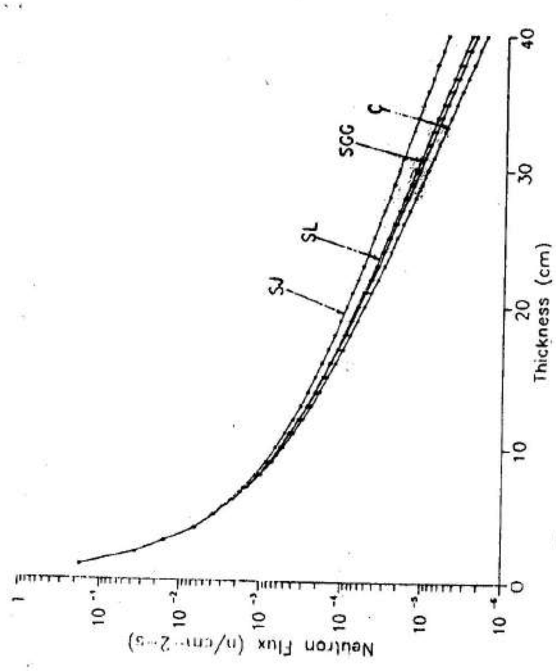

Fig.5: Intensity of Group 1 Neutrons Behind Ghanaian Golokwati, Japanese, Libyan and Concrete Shields

variation of fast neutron flux with thickness of Ghanaian serpentine from Anum (SGA) is presented in Fig. 1 and for the two epithermal groups (6 and 7) in Fig. 2. It can be noticed that the flux intensity decreases at different rates for different neutron energy groups. However, the rates of depression in flux intensity for fast groups 1,2 and 3 are higher at the first thickness than that at deep depression. From these plots it can be noticed that for layer thickness less than 10 cm the attenuation behaviour shows a slight build-up effect starting from group 4 and becomes more pronounced for the epithermal neutrons of groups 6 and 7. The build-up effect could be attributed to scattered neutrons. Similar trend of behaviour was noticed for all tested shields.

Comparison is made of the rates of attenuation of group 1 fast neutrons with those from ordinary concrete and foreign serpentine in Figs. 3-5. It can be observed from those plots that concrete is the most efficient shield, followed by the Ghanaian Peki-Dzake serpentine (SGP), then the Golokwati one (SGG) which is also better than the Libyan one (SL) which exhibits better attenuation of neutrons than the Anum (SGA) shield. The least in attenuation is the foreign serpentine from Japan (SJ) [8].

Based on the fact that the decrease of flux intensities over all thickness is not exponential, relaxation lengths of the shields were calculated using the relationship [9]

$$\frac{1}{\lambda} = \frac{-d\ln\phi(x)}{dx} = -\frac{1}{\phi(x)}\,\frac{d\phi(x)}{dx} \qquad (7)$$

where λ is the relaxation length, $\phi(x)$ is the flux at a selected thickness x.

The computed relaxation lengths at 30cm for the shields for energy group 1 are listed in Table 4.

Table 4: Relaxation lengths for different shields at 30 cm thickness for group 1

Shield	Relaxation Length (cm)
C	6.072
SGP	6.151
SGG	6.349
SL	6.532
SGA	6.811
SJ	7.427

Table 5: Neutron Attenuation Factors for Different Shields

Shield	Albedo	Absorption	Transmission
C	6.522E-01	3.219E-01	2.589E-02
SGP	7.663E-01	2.054E-01	2.827E-02
SGG	7.337E-01	2.319E-01	3.438E-02
SL	7.574E-01	2.022E-01	4.046E-02
SGA	7.527E-01	1.963E-01	5.105E-02
SJ	6.966E-01	2.224E-01	8.105E-02

The results indicate that concrete with the least value of λ is the best of all the shields. Among the serpentine shields the SGP is the most effective followed by SGG, next SL, then SJ.

In Table 5 we report the "absorption", "albedo" and "transmission" of neutrons obtained in the various serpentine shields in comparison to values for ordinary concrete. The smaller the value of transmission the better is the material for neutron radiation shielding. From the listed values one observes that the concrete is the best of the tested shields. The superiority of SGP over all the other serpentine shields is again noticed, followed by the SGG one which is also more effective than SL, then SGA and finally SJ as the least.

The different rates of neutron attenuation could be explained in terms of the concentration of the various elements in the shields. The combined effect of them play a major role in the bahaviour of the attenuation. The higher contents of Ca, Mg, Al and Mn in the two local serpentine from Peki-Dzake (SGP) and Golokwati (SGG) as compared to the foreign ones caused them to exhibit better attenuation properties. The local SGP is the best among the serpentines due to its higher content of water. It is likely that the Anum serpentine (SGA) is inferior to the Libyan one because it has lower contents of Si and Fe in it. The two shields have almost the same contents of H and Ca. The effect from low mass number elements of Na and K with low concentrations in the Libyan serpentine is insignificant. Despite the fact that the Anum one has higher contents of Mn and Al the combined effect of neutron attenuation due to the elements Si and Fe in the Libyan serpentine dominated over the effect produced by Al and Mn in the local shield. The serpentine from Japan has the least number of elements and most of them are of very low concentrations which is the cause of its inability to attenuate neutrons better than any shields investigated.

CONCLUSIONS

The attenuation properties of neutrons in the local serpentinite rocks were studied and their suitability for radiation shielding was determined by comparing their characteristics with those of foreign serpentines and concrete presently employed for shielding. From the trends of attenuation and computed factors such as relaxation lengths and transmission for the shields it can be concluded that all the Ghanaian sepentine shields possess good shielding properties to neutrons of fast energies. The serpentines from Peki-Dzake and Golokwati are more effective than any of the foreign serpentines from Libya and Japan. The least among the local serpentines which is the Anum, one although inferior to the Libyan one, it is superior to the Japanese serpentine which is already used for shielding of a nuclear reactor. In comparison with ordinary concrete their effectiveness to neutron attenuation is less. However, the attenuation of the local shield from Peki-Dzake, the best among the local shields is slightly lower than that of concrete. Its ability to exhibit good attenuation to fast neutrons can be attributed to its large water content of 15%.

In an earlier investigation the local shields were found suitable for shielding of photons [2]. Since this study has also proved their capability of attenuating neutrons, it will be expected that they will be safer than ordinary concrete used adjacent to the pressure vessel of the reactor. They can retain the water of crystalisation in them even at elevated temperatures up to 480°C. The use of them in radiation shielding will avoid expensive cooling system with sophisticated design.

It is expected that the addition of ferric oxide and boron-10 to the local serpentines will enhance their shielding properties to both photons and neutrons and this could make them superior to the ordinary concrete.

Experimental results using γ-emitting sources such as Co-60 and Cs-137 etc are needed to validate results of photon attenuation. Neutron sources from Cf-252 and research reactors may be used to check the results of attenuation of neutrons.

ACKNOWLEDGEMENTS

We are pleased to acknowledge the vital assistance of the Dr.K.O. Kessey of Geological Survey Department of Ghana for providing us with the chemical analysis of the local serpentinite rocks. We are also indebted to Dr. Enrico Satori of NFA Data Bank, Saclay for the coupled neutron-gamma library and the code ANISN/PC.

REFERENCES

1. ***Assay Report 1096,*** *Ghana Geological Survey Department Laboratory Report, 1987.*
2. ***Akaho E.H.K., Emi-Renolds G., Darko E,.O.,*** *A Theoretical Investigation of Ghanaian Serpentines for Shielding Gamma Sources, Tech. Report, GAEC-NNRI-RT-6, 1990.*
3. ***Zindah E.M.,*** *Concrete from Libyan Materials for Radiation Shielding Rart 1, Secretariat of Atomic Energy, SPLAJ, 1982.*
4. ***Vasilev, G.A.,*** *The Attenuation of Reactor Radiation by Serpentine Concrete,m J. Nucl. Energy, Parts A & B, Vol. 20, 1966.*
5. ***Engle Jr. W.W.,*** *A Users Manual for ANISN, a one Dimensional Discrete Ordinate Transport Code with Anisotropic Scattering, Union Carbide Corp., Nuclear Diversion K- 25, Report, K-1693, 1967.*
6. ***Marashi, M.K. Maiorino J.R. Mendonca A.G. and Santos, A.,*** *IRAN-LIB (Improved Range of ANISN/PC Library) : A P-3 Coupled Neutron-Gamma Crossection Libraries in ISOTXS Format to be used by ANSIN/PC (CCC-0501/02), 1990.*
7. ***Fayers, F.J., Davidson, W., George, C.H. and Halsaal, M.J.,*** *LWR-WIMS, A Modular Computer Code for the Evaluation of Light Water Reactor Lattices - Part I : Description of Methods, UKAEA Report AEEW-R 785, Winfrith, 1992.*
8. ***Yamajia, M.,*** *Shielding Modification Design of the N.S. MATSU., Japan Nuclear Ship Research and Development Agency Minato-Ku, Proceedings of the 6th International Conference on Radiation Shielding, Tokyo, Japan, 1983.*
9. ***Gladstone, S. and Sesonske, A.,*** *Nuclear Reactor Engineering, van Nostrand Reinhold Co., 1967.*