PII: S0043-1354(99)00046-9

Wat. Res. Vol. 33, No. 15, pp. 3373–3378, 1999

Printed in Great Britain
0043-1354/99/$ - see front matter

PERGAMON
www.elsevier.com/locate/watres

RESEARCH NOTE

IMPROVEMENT OF EXTRACTION METHOD OF COAGULATION ACTIVE COMPONENTS FROM MORINGA OLEIFERA SEED

TETSUJI OKUDA*, ALOYSIUS U. BAES, WATARU NISHIJIMA and MITSUMASA OKADA

Department of Environmental Science, Faculty of Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashi-Hiroshima, Hiroshima, 739-8527 Japan

(First received 1 September 1998; accepted in revised form 1 January 1999)

Abstract—A new method for the extraction of the active coagulation component from *Moringa oleifera* seeds was developed and compared with the ordinary water extraction method (MOC–DW). In the new method, 1.0 mol l^{-1} solution of sodium chloride (MOC–SC) and other salts were used for extraction of the active coagulation component. Batch coagulation experiments were conducted using 500 ml of low turbid water (50 NTU). Coagulation efficiencies were evaluated based on the dosage required to remove kaolinite turbidity in water. MOC–SC showed better coagulation activity with dosages 7.4 times lower than that using MOC–DW for the removal of kaolinite turbidity. MOC–SC could effectively coagulate more than 95% of the 50 NTU initial kaolin turbidity using only 4 ml l^{-1}, while 32 ml l^{-1} of MOC–DW could only remove about 78% of the same kaolin turbidity. The improvement of coagulation efficiency by NaCl is apparently due to the salting-in mechanism in proteins wherein a salt increases protein–protein dissociations, leading to increasing protein solubility as the salt ionic strength increases. There was no difference in the coagulation efficiency observed for extracts using any of four 1:1 salts (NaCl, KNO_3, KCl and $NaNO_3$) in our study. Purification and isolation of the active component confirmed that the active component of MOC–SC was mainly protein. © 1999 Elsevier Science Ltd. All rights reserved

Key words—coagulation, kaolinite, low turbid water, *Moringa oleifera*, natural coagulant, water treatment

INTRODUCTION

Many coagulants are widely used in conventional water treatment processes for tap water production. These coagulants can be either inorganic coagulants (e.g. aluminum sulfate and polyaluminum chloride), synthetic organic polymers (e.g. polyacrylamide derivatives and polyethylene imine) or naturally occurring coagulants (e.g. chitosan and microbial coagulants). These coagulants are used for various purposes depending on their chemical characteristics. An inorganic salt, alum (aluminum sulfate), is the most widely used coagulant in water treatment because of its proven capability and lower cost. Some synthetic organic polymers are also equally effective and relatively inexpensive. Recently, significant amounts of synthetic organic polymers are widely used for water treatment.

However, some studies (Crapper *et al.*, 1973; Martyn *et al.*, 1989), have reported that aluminum which is the major component of alum and polyaluminum chloride, may induce Alzheimer's disease. It was also reported that monomers of some synthetic organic polymers such as acrylamide have neurotoxicity and strong carcinogenic properties (Mccollister *et al.*, 1964). On the other hand, naturally occurring coagulants are biodegradable and are presumed safe for human health. Some studies on natural coagulants have been carried out and various natural coagulants were produced or extracted from microorganisms, animals or plants.

Moringa oleifera is known as a plant containing an active coagulating compound. Several studies have been done on the performance of *M. oleifera* seeds as an alternative coagulant or coagulant aid. *M. oleifera*, a tropical plant, belongs to the family *Moringaceae* that is a single genus family of shrubs. Earlier studies (Schulz and Okun, 1983; Olsen, 1987; Jahn, 1988) recommended the use of *M.*

*Author to whom all correspondence should be addressed [Tel./fax: +81-824-22-7009; e-mail: tetsujio@ipc.hiroshima-u.ac.jp].

oleifera seed extracts as coagulant for water treatment in African and South Asian countries where the plant is considered indigenous. If *M. oleifera* coagulant (MOC) is proven to be active, safe and inexpensive, it is possible to use MOC widely for drinking water and waste water treatment in other countries as well. *M. oleifera* may become one of the cash products bringing more economic benefits for the producing countries.

Turbidity removal by MOC as primary coagulant were up to 80–99%, both for raw waters and synthetic turbid waters (Muyibi and Okuofu, 1995; Ndacigengesere *et al.*, 1995; Muyibi and Evison, 1996). However, Muyibi and Evison (1995) found that the residual turbidity of samples increased with the decrease in initial turbidity at optimum dosage of MOC. This indicates that MOC may not be an efficient coagulant for low turbid water. MOC may be used for primitive treatment, whereas its use for drinking water treatment may not be appropriate since turbidity of raw water for drinking water is usually low. It is necessary to improve the coagulation efficiency, possibly by improving the extraction method for MOC, for its wide use not only in wastewater treatment but also in drinking water treatment. The active components in *Moringa* seeds were found to be soluble cationic proteins having molecular weight of about 13 kDa and isoelectric pH value of 10 and 11 (Ndacigengesere *et al.*, 1995). The amino acid sequences of this protein had already been determined by Gassenschmidt *et al.* (1991, 1995). It is well known that solubility of proteins increase with salt concentration at low salt ionic strength (White *et al.*, 1968; Voet and Voet, 1990). This salting-in phenomenon is due to the decrease in mutual association of protein molecules by shielding of salt. Since the active component for coagulation in MOC is protein, it is probable to increase its solubility by increasing salt concentration. The increase in solubility of the active component will improve coagulant ion efficiency.

The objectives of this study is to improve the extraction efficiency of the active component from *M. oleifera* seed by using salt solution. Aqueous solutions of sodium chloride, sodium nitrate, potassium chloride and potassium nitrate were individually tested for extraction of the active component from *M. oleifera* seed.

MATERIALS AND METHODS

Preparation of M. oleifera seed extracts

The *M. oleifera* seeds used in this study were obtained from Los Baños, Laguna in the Philippines. Only seeds of dry pods were used. The seeds were removed from the pods, and stored at our laboratory at room temperature. The winged seed cover was shelled just before the extraction. The kernel was ground to a fine powder by using a mortar and pestle and 5.0 g of the seed powder was mixed with 500 ml of the extractant. The extractants tested were solutions of NaCl, KNO_3, KCl and $NaNO_3$ and distilled water. The suspension was stirred using a magnetic stirrer for 10 min to extract the active component. The solution was then filtered through a filter paper (pore size = *ca.* 7 μm). The filtered solutions (MOC) were used for coagulation experiments either immediately or after storing of up to 3 days at room temperature.

Preparation of synthetic turbid water

In all coagulation experiments, samples of turbid water were prepared by adding kaolin into tap water. Ten grams of kaolin (CP grade, Katayama Chemical) was added to one litre of water. This suspension was stirred for 1 h for uniform dispersion of kaolin particle and then stood for 24 h to allow for complete hydration of the particle. This suspension was used as the stock solution. Immediately before the coagulation experiments, 2.5 ml of this suspension was diluted to 500 ml using tap water. The initial pH of the suspension was 7.1 $\pm$ 0.1.

Coagulation test

Jar tests were carried out by using a jar tester (Miyamoto Riken) to evaluate coagulation activity at several dose levels of MOC. Four 500-ml beakers filled with 500 ml of kaolin suspension were placed in the slots of the jar tester. Then, the suspensions were agitated at 150 rpm. During this agitation, various amounts of MOC was added to each beaker and agitated for 2 min at 150 rpm. The mixing speed was reduced to 30 rpm and was kept for 30 min for slow mixing. After sedimentation for 1 h, 5 ml of the sample was collected from the middle of the beaker and residual turbidity of each coagulated water sample was measured using a turbidimeter (ANA-148, Tokyo Photoelectric Co.).

Purification of active components

The active component of MOC–SC was purified by using Sevag's method (Sevag, 1934) and dialysis. A mixture of chloroform and butanol (5:1) was added to MOC–SC (at a ratio of five times the MOC–SC volume) and then mixed for 30 min in a rotary shaker to remove the inactive components (gel). The aqueous layer was separated from the gel and from the organic solvent layer by centrifugation. This process was repeated four times for the aqueous layer until no gel was formed.

For dialysis, seamless cellulose tubings (UC36-32-100, Viskase Sales Corp) with molecular weight cutoff from 12 to 14 kDa were used to remove impurities with low molecular weights. Distilled water was used for the external solution of the tube and was changed eight times during the 24-h dialysis period.

Analytical methods

The pH was measured using a pH meter (F-8, Horiba). Protein and sugar were analyzed by the standard Lowry method (Clark and Switzer, 1977) and the phenol–sulfuric acid method (Dubois *et al.*, 1956) respectively. Total organic carbon (TOC) was determined using a total organic carbon analyzer (TOC-500, Shimadzu).

RESULTS AND DISCUSSION

Figure 1 shows turbidity removal by coagulation with each MOC. The coagulant dosage is expressed in ml l^{-1} throughout this study. The residual turbidity using the coagulant extracted by ordinary solvent, distilled water (MOC–DW) was 11.8 NTU at its optimal dosage of 32 ml l^{-1}. On the other hand, MOC extracted by 1.0 mol l^{-1} NaCl solution (MOC–SC), gave much lower residual turbidity. It

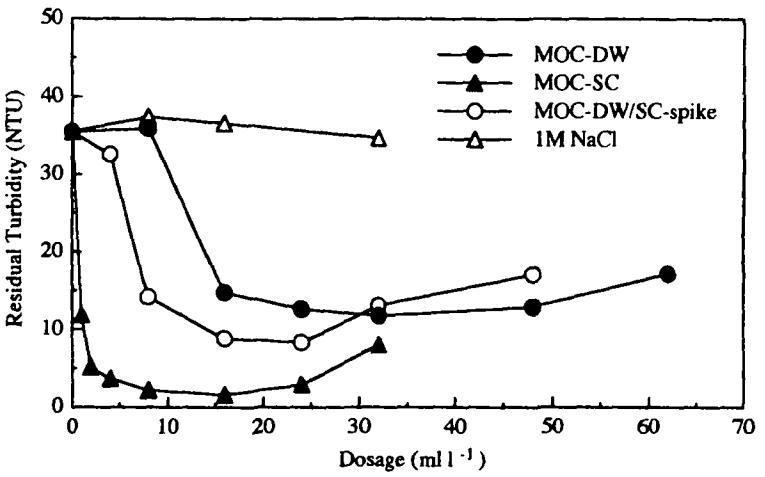

Fig. 1. Coagulation of kaolin suspension with *Moringa oleifera* coagulant extracted by distilled water (MOC-DW) and NaCl (MOC-SC), and with MOC-DW spiked with NaCl (MOC-DW/SC-spike) and 1 mol l^{-1} NaCl solution (1 M NaCl).

was 1.6 NTU at the optimal dosage of 16 ml l^{-1}. The efficiency of MOC-SC in terms of residual turbidity was 7.4 times better than that of MOC-DW.

A smaller amount of MOC-SC was necessary to give the same level of residual turbidity compared to MOC-DW. For example, 32 ml l^{-1} of MOC-DW was necessary to achieve 11.8 NTU of residual turbidity, while only 1 ml l^{-1} of MOC-SC was necessary to achieve the same residual turbidity. The advantage of MOC-SC over MOC-DW in terms of the amount of dosage is 32 times. Based on the increased coagulation efficiency, the salt extraction was proved to be more economical. Coagulation by 1.0 mol l^{-1} solution of NaCl are also shown in Fig. 1. The 1.0 mol l^{-1} NaCl did not show any coagulation activity indicating that coagulation by MOC-SC is be due to NaCl.

To study the improvement of extraction efficiency, the coagulation by water extract MOC-DW spiked with NaCl (1.0 mol l^{-1}, MOC-DW/SC-spike) was compared with others. Although NaCl concentration was same between MOC-DW/SC-spike and MOC-DW/SC during coagulation experiment, MOC-DW/SC-spike showed poor performance than MOC-SC (Fig. 1). NaCl, therefore, did not improve coagulation but extraction efficiency. However, MOC-DW/SC-spike showed better performance than MOC-DW. The improvement may be due to the loosening-up of protein associations leading to more soluble and coagulation active species in solution. This mechanism is similar to that of the increase in protein solubilities with the addition of 1:1 salts or the salting-in effect cited in earlier references (White *et al.*, 1968; Voet and Voet, 1990). The suggested mechanism of enhancing the breaking of protein associations, leading to the increased solubilities by salt addition is responsible for the improved activity by MOC-SC. The 1.0 mol l^{-1} NaCl solution acted

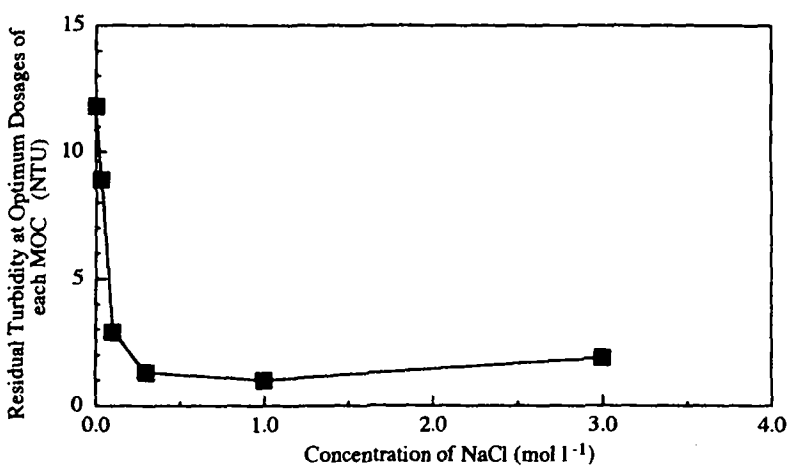

Fig. 2. Coagulation activities of six MOCs extracted by different concentrations of NaCl solution. Activities are represented by residual turbidity at optimum dosage of each MOC.

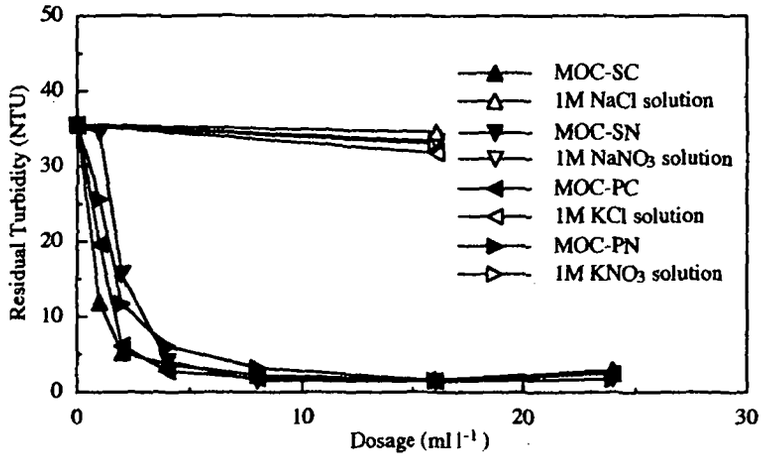

Fig. 3. Coagulation of kaolin suspension with *Moringa oleifera* coagulant extracted by NaCl solution (MOC–SC), KNO_3 solution (MOC–PN), KCl solution (MOC–PC), $NaNO_3$ solution (MOC–SN), and with four 1 mol l^{-1} solvents.

as a better solvent than pure water in breaking protein–protein or protein–polysaccharide or other associations in the seed powder, which led to increase protein solubility in the salt solution.

The effect of NaCl concentration on the improvement of extraction efficiency were studied. Figure 2 shows residual turbidities at each optimum dosage. The coagulation activity increased with the increase in the concentration of NaCl until 1.0 mol l^{-1}. The decrease in coagulation activity at 3.0 mol l^{-1} could be due to salting-out. The phenomena that solubility of proteins decrease with salt concentration at high salt ionic strength is also well known as well as salting-in (White *et al.*, 1968; Voet and Voet, 1990).

Figure 3 shows coagulation by other MOC seed extracts using KNO_3, KCl and $NaNO_3$ solution as solvent. There was not significant differences in the coagulation efficiency of the various salt extracts. This indicates that NaCl is not a unique salt to enhance solubility of the active component in *M. oleifera* seeds. Ionic strength, however, could be the main factor for the improvement of extraction efficiency rather than specific chemical characteristics of the 1:1 salt ions.

The effect of ionic strength on the coagulation efficiency was further studied using a dialysis membrane. The removal of NaCl by dialysis for MOC–SC resulted in the formation of white precipitates in the dialyzed solution (inside the cellulose tubing). The suspension of the white gel without NaCl did not show any coagulation activity. However, the addition of 1.0 mol l^{-1} NaCl to this suspension dissolved the white precipitates and recovered coagulation activity. These results confirm our previous suggestion that the presence of salt (NaCl) enhances the solubility of the active component that leads to a better coagulation

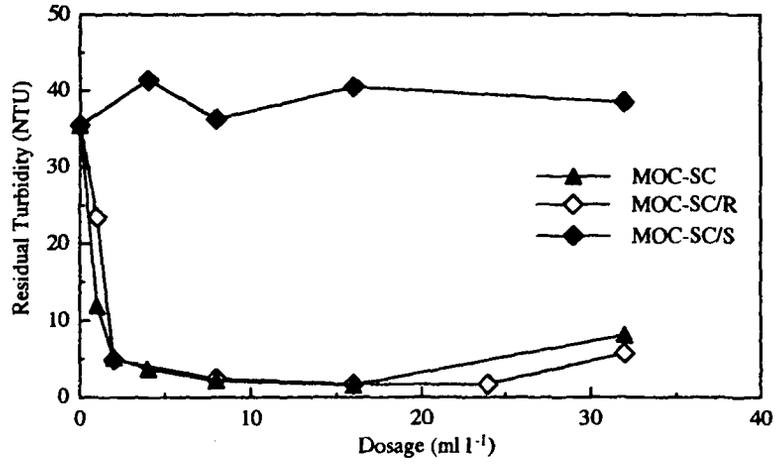

Fig. 4. Coagulation of kaolin suspension with *Moringa oleifera* coagulant extracted by NaCl solution (MOC–SC), the precipitates were separated from the dialyzed suspension by centrifugation and the redissolved residue of dialyzed MOC–SC into 1 mol l^{-1} NaCl (MOC–SC/R) and NaCl added supernatant solution of dialyzed MOC–SC with 1 mol l^{-1} NaCl (MOC–SC/S).

efficiency of its solution. The white precipitates formed after the removal of NaCl confirmed our earlier assumption that protein–protein association (or whatever other associations are present) prevents the active component from exerting its coagulation activity.

To confirm that the white precipitates are the active components, the precipitates were separated from the dialyzed suspension by centrifugation. The white residue was redissolved into 1.0 mol l^{-1} NaCl ('MOC–SC/R' in Fig. 4). Also, NaCl was added to the supernatant to obtain a solution with 1.0 mol l^{-1} NaCl ('MOC–SC/S' in Fig. 4). As expected, the solution with redissolved white residual showed coagulation activity, but the supernatant contained NaCl did not show any activity as shown in Fig. 4.

Purification experiments were carried to isolate the active component. Initially, the method of Sevag (1934), a common purification procedure for proteins, was used to remove inactive proteins, polysaccharides and lipids from MOC–SC. The coagulation activity of the Sevag treated MOC–SC did not lose the activity. Dialysis was done to remove components with molecular weight smaller than 12–14 kDa. The white precipitate which formed upon dialysis of the Sevag treated MOC–SC was separated from the suspension by centrifugation. The white solid, as discussed earlier, contained the active component. The precipitates were redissolved into 1.0 mol l^{-1} NaCl solution and the resulting solution exhibited the same coagulation activity as the previous ones. This suggests that the active component in *M. oleifera* probably have molecular weights greater than 12 kDa similar to the results of previous studies using water as extracts (Ndacigengesere *et al.*, 1995).

TOC, sugar (polysaccharide) and protein concentrations were determined during purification and are given in Table 1. Although the MOC–SC before purification contained sugar, no sugar was noted in the purified solution. The relative protein content of the purified solution increased considerably compared to that before purification as shown by the relatively high ratio of protein to TOC (2.84). The results confirm that proteins are the main active components in the seed extract which is in agreement with the results of previous studies (Ndacigengesere *et al.*, 1995) using distilled water as extractant.

Table 1. Characteristics of MOC–SC before and after purification. Concentration of total organic carbon (TOC) and proteins and sugars

	TOC (mg carbon l^{-1})	Proteins (mg BSA l^{-1})[a]	Sugars (mg glucose l^{-1})
Before purification	1735	3166	928
After purification	260	739	0

[a]BSA means bovine serum albumin.

CONCLUSIONS

The purpose of this study was to develop an improved method for the extraction of the active coagulation component from *M. oleifera* seeds. The specific conclusions derived from this study are as follows:

1. MOC–SC extracted by 1.0 mol l^{-1} NaCl solution showed better coagulation activity with dosages 7.4 times lower than that using MOC–DW extracted by distilled water for the removal of kaolinite turbidity. MOC–SC could effectively coagulate more than 95% of the 50 NTU initial kaolin turbidity using only 4 ml l^{-1}, while 32 ml l^{-1} of MOC–DW could remove only 78% of the same kaolin turbidity.
2. The improvement of coagulation efficiency by NaCl solution as extractant is apparently due to the salting-in mechanism in proteins wherein a salt increases protein–protein dissociations and protein solubility as the salt ionic strength increases. No difference in the coagulation efficiency was observed for extracts using any of the four 1:1 salts (NaCl, KNO_3, KCl and $NaNO_3$).
3. Purification and isolation of the active component confirmed that the active component of MOC–SC was mainly protein.

Acknowledgements—The authors would like to thank Dr Eiji Shoto, head of the Institute of Wastewater Treatment, Hiroshima University where this study was conducted, for his reliable advice and assistance. The authors also appreciate the assistance of Dr Maxima Flavier of the Division of Analytical and Environmental Chemistry, UPLB during the preliminary experiments in the Philippines.

REFERENCES

Clark Jr. J. M. and Switzer R. L. (1977) *Experimental Biochemistry*, 2nd edn. Freeman, New York.

Crapper D. R., Krishnan S. S. and Dalton A. J. (1973) Brain aluminum distribution in Alzheimer's disease and experimental neurofibrillary degeneration. *Science* **180**, 511–513.

Dubois M., Gilles K. A., Hamilton J. K., Rebers P. A. and Smith F. (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**, 350–356.

Gassenschmidt U., Jany K.-D. and Tauscher B. (1991) Chemical properties of flocculant: active proteins from *Moringa oleifera* Lam. *Biol. Chem. Hopper-Seyler* **372**, 659.

Gassenschmidt U., Jany K. D., Tauscher B. and Niebergall H. (1995) Isolation and characterization of a flocculating protein from *Moringa oleifera* Lam. *Biochim. Biophys. Acta* **1243**, 477–481.

Jahn S. A. A. (1988) Using *Moringa* seeds as coagulants in developing countries. *JAWWA* **80**, 43–50.

Mccollister D. D., Oyen E. and Rowe V. K. (1964) Toxicology of acrylamide. *Toxicol. Appl. Pharmacol.* **6**, 172–181.

Martyn C. N., Barker D. J. P., Osmond C., Harris E. C., Edwardson J. A. and Lacey R. F. (1989) Geographical relation between Alzheimer's disease and aluminium in drinking water. *Lancet* **1**, 59–62.

Muyibi S. A. and Evison L. M. (1995) Optimizing physical parameters affecting coagulation of turbid water with *Moringa oleifera* seeds. *Water Res.* **29**, 2689–2695.

Muyibi S. A. and Evison L. M. (1996) Coagulation of turbid water and softening of hardwater with *Moringa oleifera* seeds. *Int. J. Environ. Stud.* **49**, 247–259.

Muyibi S. A. and Okuofu C. A. (1995) Coagulation of low turbidity surface water with *Moringa oleifera* seeds. *Int. J. Environ. Stud.* **48**, 263–273.

Ndacigengesere A., Narasiah K. S. and Talbot B. G. (1995) Active agents and mechanism of coagulation of turbid water using *Moringa oleifera*. *Water Res.* **29**, 703–710.

Olsen A. (1987) Low technology water purification by bentone clay and *Moringa oleifera* seed flocculation as performed in Sudanese villages: effects on *Schistosoma mansoni*, Cercariae. *Water Res.* **21**, 517–522.

Schulz C. R. and Okun D. A. (1983) Treating surface waters for communities in developing countries. *JAWWA* **75**, 212–223.

Sevag M. G. (1934) Eine neue physikalische Enteiweißungsmethode zur Darstellung biologisch wirksamer Substanzen. *Biochem. Z.* **273**, 419–429.

Voet D. and Voet J. G. (1990) *Biochemistry*. John Wiley & Sons, New York.

White A., Handler P. and Smith E. L. (1968) *Principles of Biochemistry*, 4th edn. McGraw-Hill, New York.