African Journal of Biotechnology Vol. 10(48), pp. 9939-9950, 29 August, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2614
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Immunogenicity of novel sulfadimethoxide conjugates

Lei Chen[1], Jianyu Su[2]*, Xiangzhai Zhang[3], Lin Li[2] and Xiaowei He[2]

[1]Institute for Agro-product Processing, Anhui Academy of Agricultureal Sciences, Hefei, 230031, China.
[2]School of Light Industry and Food Sciences, South China University of Technology, Guangzhou, 510640, China.
[3]Key Laboratory of Hebei Province Preventive Veterinary Medicine, Hebei Normal University of Science and Technology, Qinhuangdao, 066004, China.

Accepted 6 May, 2011

Sulfadimethoxine (SDM) is an antibiotic commonly added to animal feeds. Anti-SDM antibodies are useful for the detection of residual SDM in foods, feeds and biological fluids by ELISA. In this study, we show that SDM is immunogenic in rabbits when it is conjugated with soy 11S globulin or with β-amylase. Rabbit anti-SDM antibodies obtained by immunization with SDM-11S and SDM-beta-amylase displayed low cross-reactivity against other sulfonamides, including sulfamethazine (SM2), sulfasulfonamides (SN), sulfadiazine (SD), sulfamethoxypyrazine (SMP), sulfalene (SMZ), sulfaquinoxaline (SQX). Thus, soy 11S globulin and β-amylase are suitable carriers for the induction of anti-SDM antibodies and have the advantage of being cheaper that BSA.

Key words: Sulfadimethoxine (SDM), sulfasulfonamides (SN), sulfadiazine (SD), sulfamethazine (SM2), sulfamethoxypyrazine (SMP), sulfalene (SMZ), sulfaquinoxaline (SQX), rabbit anti-SDM antibodies, soy 11S globulin, SDM-beta-amylase.

INTRODUCTION

Sulfadimethoxine (SDM) is an artificial antibiotic commonly used as feed additive added broadly to animal feeds. Because of its speed and high specificity, competitive indirect enzyme-linked immunosorbent assay (ELISA) is useful for the detection of residual sulfamido medicine in foods and feeds (Deborah et al., 1988). The development of an immunoassay requires the production of antibodies for the analyte. Sulfadimethoxine are small molecules and sulfadimethoxine derivatives, namely haptens, must being synthesized and coupled to a carrier to induce antibody production. Currently, the common hapten carriers used in ELISA are bovine serum albumin (BSA), human serum albumin (HSA) and keyhole limpet hemocyanin (KLH). However, when these proteins are used as the immunogen carrier, the affinity of the corresponding antibodies is poor (Hochul and Jun, 2000). Additionally, KLH and HSA are also relatively expensive and not easily obtainable. The objective of this study was to find a suitable replacement for BSA, KLH and the like. The ideal carrier should have a moderate price, a comparatively stable structure and strong water-solubility. The carrier needs to be able to undergo cross-linking with hapten under organic solvent circumstances while retaining solubility.

Sulfadimethoxine conjugated with ovalbumin served as the coating antigen. In this study, sulfadimethoxine was conjugated with α-amylase, β-amylase, soy 11S globulins, lysozy, soy 7S globulins, poly-l-lysine and BSA. The immunogenicity of the conjugates was tested in rabbits and the specificity of anti-ADM antisera was test-ed by competitive ELISA.

Soy 11S globulin is an inhomogeneous protein whose molecular weight ranges from 340 to 375KDa (Deak et al., 2007; Peng et al., 1984; Wolf, 1997). Soy 7S globulin is a major soybean antigen protein which has the 43% ratio in the soybean protein. Soy 7S globulin is an

*Corresponding author. E-mail: jysu@scut.edu.cn. Tel: +86-02-87113252. Fax: +86-02-87113252.

Abbreviations: **SDM,** Sulfadimethoxine; **SN,** sulfasulfonamides; **SD,** sulfadiazine; **SM2,** sulfamethazine; **SMP,** sulfamethoxypyrazine; **SMZ,** sulfalene; **SQX,** sulfaquinoxaline; **DNP,** 2,4-dinitrophenyl; **BPO,** benzylpenicilloyi; **BaA,** bacterial α-amylase; **TAA,**taka-amylase A; **BSA,** bovine serum albumin; **HSA,** human serum albumin; **KLH,** keyhole limpet hemocyanin

Table 1. Dosages in the preparation of immunogens by glutaraldehyde method and the coupling ratios of sulfadimethoxine with the carriers.

Carrier	11S	7S	α-Amylase	β-Amylase	poly-l-lysine	lysozy	BSA
Carrier (mg)	15	7	7	30	9	21.49	50
SDM (mg)	9	15	34.63	8	15	10.75	14.29
Glutaraldehyde (µl)	20	30	30	21	30	30	30
Couple ratio	18	10	13	15	9	11	14

alkaline globulin whose molecular weight ranges from 57 to 42 kDa. Its isoelectric point is 9.06 to 9.26. Soy 7S is polymeric, composed of four subunits bonded by disulfide bonds (Hiroyuki et al., 1987; Wolf, 1997). Though researchers have long been focused on soy allergizationin immature animals,soy 11S globulins and soy 7S globulins have never been used as hapten carriers before α-Amylase contains 526 amino acids and has a molecular weight of 58.6 kDa. Bacterial α-amylase (BaA), Taka-amylase A (TAA) have been conjugated with 2,4-dinitrophenyl (DNP) and benzylpenicilloyi (BPO) groups and used as haptenic determinants (Hamaoka et al., 1971). Lymphoid cells obtained from mice primed with these hapten-protein conjugates were stimulated *in vitro* with various DNP-and BPO-proteins as the second antigen and transferred into X-irradiated recipients.

β-Amylase is a glucoamylase that can be also called saccharifies enzyme. The difference between α-amylase and β-amylase is that β-amylase may cut-out α-1,4 glucoside keys from non-reducibility termination in starch, generate glucose one by one. It may also hydrolyze α-1,6 glucoside key of amylopectin bifurcation. β-Amylase exist in the mold, bacterium, yeast. While β-amylase is commonly used to saccharify amylum candy and other starchiness materials in fermentation industries, it has never been used as a hapten carrier (Masakazu et al., 2009; Hirata et al., 2004.)

Poly-l-lysine is a homo-polymer composed of 25 to 35 L-lysines with acidamide bonds between alpha-amidocyanogen and the epsilon-carboxyl. It has a molecular weight ranging from 500 to 4,500 kDa and favorably dissolves in water (Hiraki et al., 2003). It has been demonstrated that double-stranded RNA polyinosinic: polycytidylic acid can be used effectively as an endogenous interferon in rabbit and the rodent. Levy (1975) reported that poly-l-lysine dissolved in 0.5% cellulose can improve resistance to natural RNase in the human blood plasma by 5 to 10 times. Li Min (1999) used sodium alginate-poly-L-lysine-sodium alginate to capsulate cattle pheochromocyte and influxion rachnoidea spinalis, which reached more effective immune isolation. Fan (1984) used poly-L-lysine in enzyme linked immunosorbent assay (ELISA) to package T2 poison inoculated with rabbit T2 antibody and determined the antibody quantity based on specific antigen-antibody reaction.

Lysozyme, also known as muramidase, contains a single polypeptide chain of 129 amino acids. Its molecular weight is 14.4 KDa. The isoelectric point is 10.5 to 11.0. Lysozyme exists in the human being, animal, plants, microbe and aves albumen.

MATERIALS AND METHODS

Bovine serum albumin (BSA, 66.0 kDa), chicken egg ovalbumin (OVA, 45.0 kDa), goat anti-rabbit immunoglobulin G peroxidase, complete and incomplete Freund adjuvants were purchased from Sigma Chemical Co. Glutaraldehyde, defatted soybean seeds, lysozyme, β-amylase, poly-l-lysine, α-amylase were purchased from Guangzhou, Chemical Co. (Guangdong, China). Crystalline bacterial a-amylase (BaA) derived from *Bacillus subtilis* and crystalline Taka-amylase A (TAA) prepared from Taka-diastase Sankyo were kindly supplied by Dr T. Ikenaka, Faculty of Science, Osaka University. Sulfamethazine (SM2), sulfasulfonamides (SN), sulfadiazine (SD), sulfamethoxypyrazine (SMP), sulfalene (SMZ) and sulfaquinoxaline (SQX) were obtained from Jiangsu Biochemistry Research Institution (Jiangsu, China). Rabbits (New Zealand White does) were obtained from the Jiufu animal nursery (Guangdong, China). All the inorganic and organic reagents were of reagent grade.

Preparation of soy 11S globulins and soy 7S globulins

Soy 11S globulins and soy 7S globulins were isolated according to a new method (Deak et al., 2007; Nicolas et al., 2007), which is a modification of the procedure of Nagano et al.

Preparation of coating antigen

Since sulfadimethoxine has reactive group for coupling reactions, it was conjugated with ovalbumin (OVA) as an ELISA solid phase through diazonium salt coupling (Chemistry of the Diazonium and Diazo Groups).

Preparation of immunogen

Since sulfadimethoxine has reactive group for coupling reactions, it was then conjugated with BSA as an immunogen, by the glutaraldehyde method (Lau et a1., 1981). Dosages in the preparation of immunogens by glutaraldehyde method are shown in Table 1.

UV spectra

Sulfadimethoxine, carrier (α-amylase, β-amylase, soy 11S globulins, soy 7S globulins, lysozy, poly-l-lysine) and sulfadimethoxine

conjugates were dissolved in phosphate buffer (pH 7.2, 0.05 M) and then monitored from 190 to 400 nm with 1 cm path length on an ultraviolet spectral visible light photometer (Hitachi, U-3010) (Chemistry of the Diazonium and Diazo Groups).

Fluorescence spectra

Fluorescence measurements were performed on an F-4500 spectrophotometer (HITACHI, Japan) equipped with a 150 W xenon lamp and 5 nm slit width and 1.00 cm quartz cell at a scanning speed of 1200 nm min^{-1}, the temperature was controlled by digital aqueous thermostat (Shanghai, China). Sulfadimethoxine and sulfadimethoxine conjugates were dissolved in phosphate buffer (pH= 7.2, 0.05 M). The influence of the various component ratios on the formation of SDM conjugates has been considered (Chemistry of the Diazonium and Diazo Groups).

Rabbit immunization

Three rabbits [A(1,2,3),B(1,2,3),C(1,2,3),D(1,2,3),E(1,2,3),F(1,2,3), G(1,2,3)] were injected intradermally with SDM-BSA, SDM-α-amylase, SDM-β-amylase, SDM-11S, SDM-lysozy, SDM-7S, SDM-poly-l-lysine (2 mg) dissolved in 1 ml of saline and emulsified with 1 ml of Freund complete adjuvant at 30 to 40 sites on a shaved back area (Nuria et al., 2004). The other three rabbits (H-1, H-2 and H-3) were injected intradermally with sulfadimethoxine-BSA (2 mg) dissolved in 1 ml of saline and emulsified with 1 ml of Freund complete adjuvant. A booster of conjugate (0.5 mg) in 2 ml of saline-Freund complete adjuvant (1:1) was injected intramuscularly at day 1 and boosters of conjugate (0.5 mg) in 2 ml of saline-Freund incomplete adjuvant (1:1) were injected intramuscularly at day 14 and 28. Blood samples were drawn via marginal ear vein at regular intervals.

Antibody titration by indirect competitive ELISA

With the checkerboard procedure, the appropriate concentrations of coating antigen were prepared by serial dilutions from 1 to 4 µg/ml of SDM-OVA in the coating solution and primary sulfadimethoxine antibody (serially diluted purified sulfadimethoxine antiserum from 1:200 to1:256,000). The titration of sulfadimethoxine antibody was evaluated by indirect ELISA as described by Cazemier (2000). Sulfadimethoxine antibody titers are shown in Table 2.

Specificity of sulfadimethoxine antibody by indirect competition ELISA

To ascertain the specificity of the sulfadimethoxine antibody, the inhibition ratio of sulfadimethoxine with sulfadimethoxine antibody and the cross reactivity of the sulfadimethoxine antibody with other sulfonamides were calculated by a competitive indirect ELISA method. A competitive indirect ELISA was carried out by simultaneously incubating sulfadimethoxine with an appropriate dilution of rabbit sulfadimethoxine antiserum over a sulfadimethoxine ovalbumin solid phase (the appropriate concentration was 2 µg/ml) and then determining bound rabbit antibody with a goat anti-rabbit peroxidase conjugate (Muldoon et al., 2004). Plates were coated with an appropriate concentration of SDM-OVA in coating buffer by incubation at 4°C overnight.

50 ul/well of sulfadimethoxine solution or sulfonamides [Sulfamethazine(SM2), sulfasulfonamides(SN), sulfadiazine(SD), sulfamethoxypyrazine(SMP), sulfalene(SMZ), sulfaquinoxaline (SQX)], followed by 50 ul/well of SDM antibody solution at twice the desired assay concentration was added. Inhibition standard curve was prepared by serial dilutions from 1 ng/ml to 4 ug/ml of sulfadimethoxine solution. Competitive immunological reaction was allowed to take place for 1 h and then plates were washed as before. Afterwards, a 1/1000 dilution of goat anti-rabbit IgG-HRP in dilution solution was added and the reaction lasted for 30 min. After washing, the retained peroxidase activity was determined as indicated earlier. The absorbance was immediately read at 492 nm with a reference wavelength at 630 nm (Korpimaki et al., 2004).

The resulting curves were fitted with a four parameter logistic equation to determine the IC50, which is defined as the concentration of inhibitor required to inhibit color development by 50% compared with control wells containing no competitor. The cross reaction rate is defined as the concentration of other sulfonamides inhibitor required to inhibit color development by 50% compared with control wells containing sulfadimethoxine.

RESULTS

Ultraviolet absorption spectrogram analysis

Ultraviolet absorption spectrogram of sulfadimethoxine, neotype carrier and sulfadimethoxine conjugates dissolved in phosphate buffer (pH= 7.2, 0.05 M) are shown in Figures 1 to 6. The coupling ratios of sulfadimethoxine with carriers were also determined (Thouvenot and Morfin, 1983) and shown in Table 1. The ultraviolet absorption intensity of sulfadimethoxine conjugates increase at the basic of carriers (soy 11S globulins, lysozy, soy 7S globulins and poly-l-lysine) and were between the intensity of carrier and sulfadimethoxine. This result suggests that sulfadimethoxine conjugates were prepared successfully. As shown in the figures, carriers of the higher molecule ratios when sulfadimethoxine were conjugates with carriers were soy 11S globulins, β-amylase, α-amylase, lysozy, soy 7S globulins and poly-l-lysine by turns. The coupling ratios of sulfadimethoxine with BSA/OVA were 14 and 12.

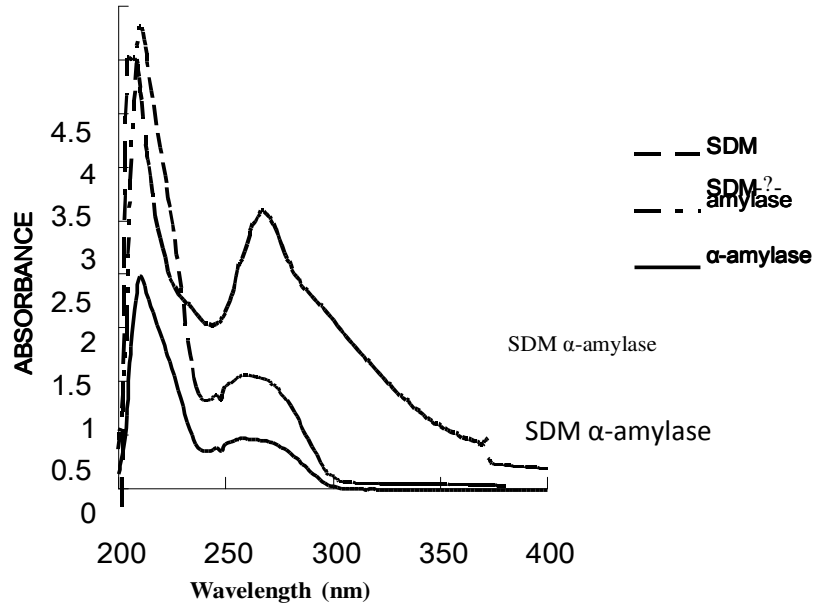
Fluorescence emission evidence of association process of sulfadimethoxine and carriers (11S, 7S)

Another argument in favor of the association of sulfadimethoxine with carriers comes from the appearance of the weak fluorescence of carriers by addition of SDM. The fluorescence spectra of sulfadimethoxine conjugates are shown in Figures 7 to 11 at different ratios. For example, in Figure 8, the characteristic fluorescence peak of 11S occurs at 350 nm. The blend of sulfadimethoxine to 11S not only results in the decrease of fluorescence intensity of 11S, but also leads to the red shift of maximum emission wavelength from approximately 350 to 370 nm. In Figure 9, the blend of sulfadimethoxine to 7S leads to the red shift of maximum emission wavelength from approximately 460 to 480 nm. It can be seen that the fluorescence intensities of the conjugates decrease as the ratio increases. In the mentioned systems, the intrinsic fluorescence of carriers

(11S, 7S) are quenched. Apparently, sulfadimethoxine

Table 2. Sulfadimethoxine antiserum titers in rabbits immunized.

Days after immunization	SDM-BSA A-1	SDM-α-amylase B-2	SDM-β-amylase C-3	SDM-11S D-1	SDM-lysozy E-3	SDM-7S F-2	SDM-poly-l-lysine G-1
7	6,400	3,200	3,200	6,400	3,200	3,200	6,400
21	25,600	6,400	6,400	12,800	6,400	6,400	25,600
35	51,200	25,600	25,600	25,600	12,800	12,800	50,000
42	102,400	51,200	51,200	51,200	25,600	25,600	80,000
53	50,000	80,000	100,000	102,400	51,200	60,000	51,200
62	25,600	12,800	25,600	51,200	12,800	25,600	25,600
69	6,400	6,400	6,400	12,800	6,400	6,400	6,400
76	3,200	3,200	3,200	6,400	3,200	3,200	3,200



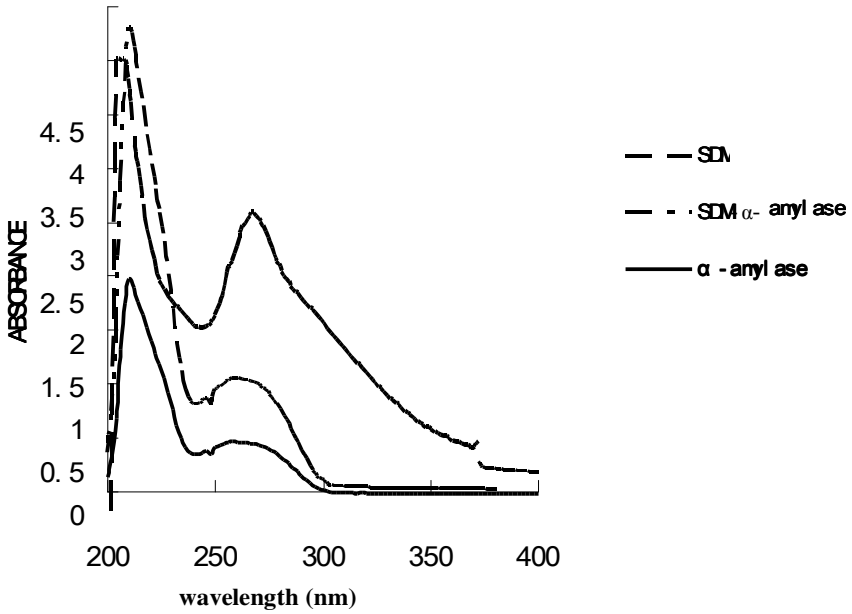


Figure 1. Ultraviolet absorption spectrogram of α-amylase, SDM and SDM-α-amylase conjugate.

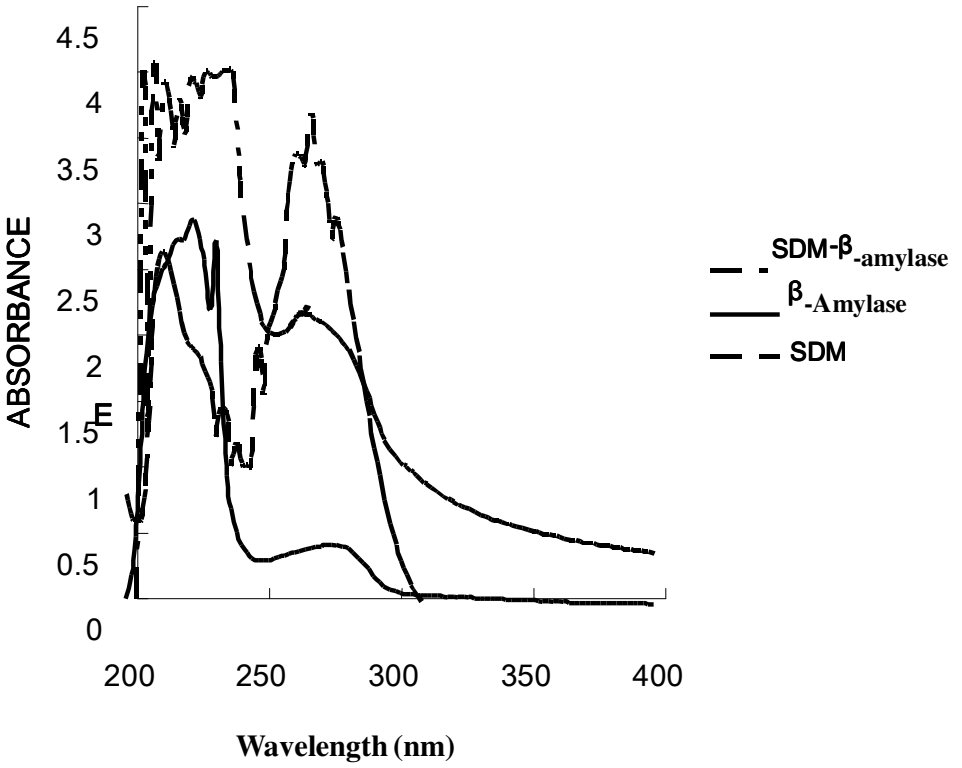


Figure 2. Ultraviolet absorption spectrogram of β-amylase, SDM and SDM-β-amylase conjugate.

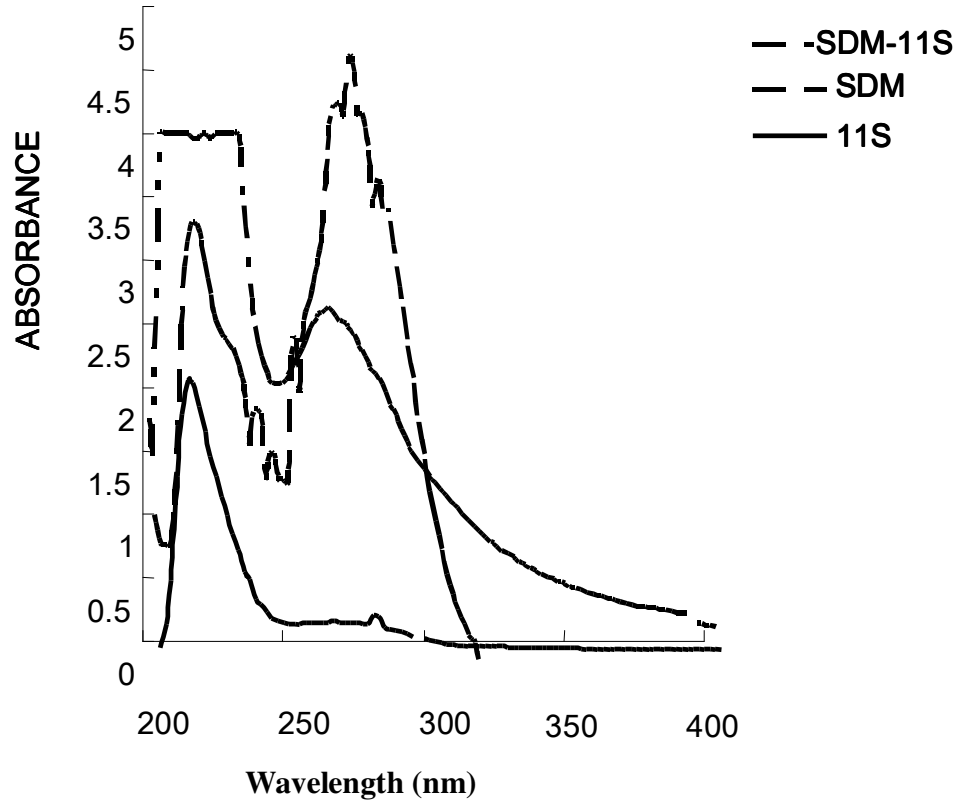


Figure 3. Ultraviolet absorption spectrogram of 11S, SDM and SDM-11S conjugate.

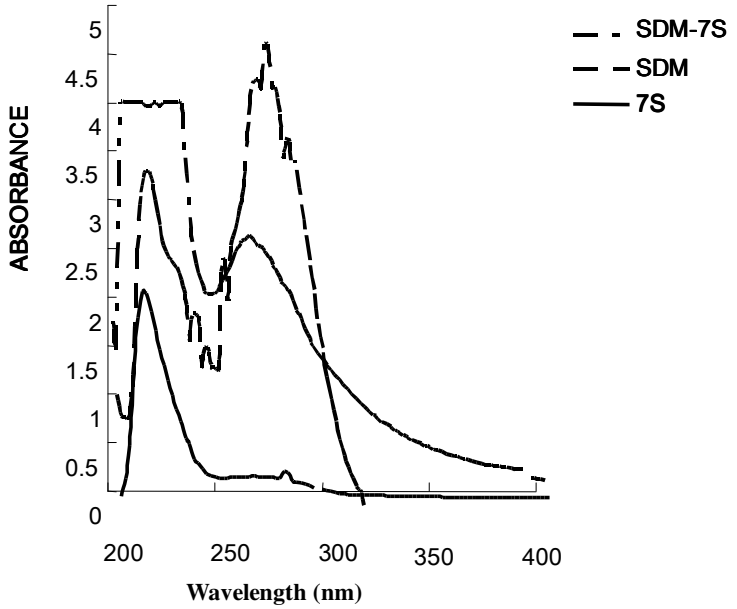


Figure 4. Ultraviolet absorption spectrogram of 7S, SDM and SDM-7S conjugate.

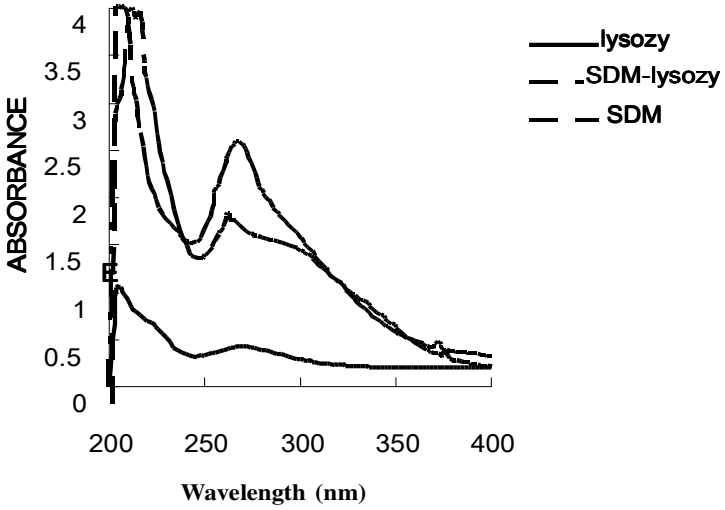


Figure 5. Ultraviolet absorption spectrogram of lysozy, SDM and SDM-lysozy conjugate.

have a quench for the fluorescence of carriers and there are interactions and energy-transfer happening among sulfadimethoxine and carriers. It effectively suggests that there is association process between sulfadimethoxine and carriers. It proved that sulfadimethoxine conjugate was prepared successfully.

Production of antiserum against sulfadimethoxine conjugate

An indirect ELISA was devised to monitor titers of sulfadimethoxine antiserum wherein rabbit antiserum was incubated over a micro titer plate solid phase coated with sulfadimethoxine ovalbumin (the appropriate concentration was 2 ug/ml).The total bound antibodies were subsequently detected with goat anti-rabbit peroxidase conjugate. The serum dilution visually distinct in color from preimmune serum control at the same dilution was arbitrarily designated as the titer. A summary of sulfadimethoxine antiserum titers against the seven immunogens over 76 days is shown in Table 2. ELISA titration

of rabbit anti-sulfadimethoxine antiserum is shown in Figure 7. The antiserum titers of SDM-α-amylase,

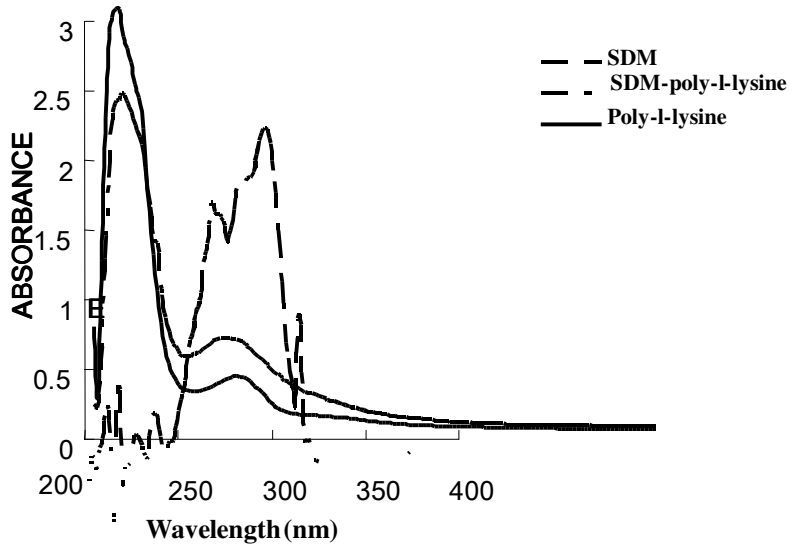


Figure 6. Ultraviolet absorption spectrogram of poly-l-lysine,SDM and SDM-poly-l-lysine conjugate.

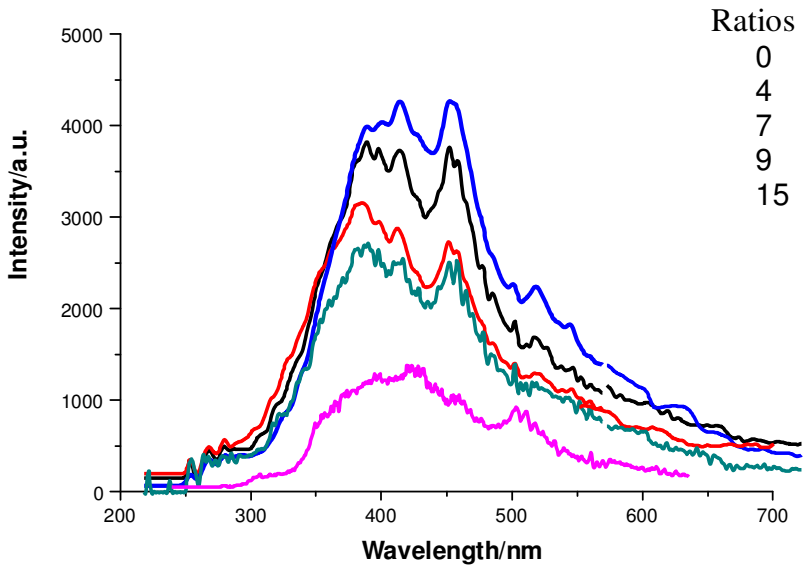


Figure 7. Fluorescent spectrum of SDM -β-amylase at different ratios.

SDM-β-amylase, SDM-11S, SDM-poly-l-lysine were all higher than 100,000. The antiserum which had higher titers was SDM-11S antiserum (102, 400, at days 53), SDM-β-amylase antiserum (100,000, at days 53), SDM-α-amylase antiserum (80,000, at days 53), SDM-poly-l-lysine antiserum (80,000, at days 42). The titer of SDM-BSA antiserum was 102, 400 at days 42.

Specificity of sulfadimethoxine antibody

Standard curve of antibody against SDM immunogen (SDM-BSA, SDM-11S, SDM-β-amylase, SDM-poly-l-lysine) reacting with SDM is shown in Figure 13. The competition effect of sulfadimethoxine with sulfadimethoxine antibody presented linear correlation when the density of sulfadimethoxine ranged from 32 ng/ml to 10 μg/ml.

Typical competition curve and IC50 (concentrations which resulted in 50% inhibition) are shown in Table 3. The SDM-11S globin antibody had the IC50 of 56.5135 ng/ml; the SDM-β-amylase had the IC50 of 79.4328

ng/ml. The SDM-BSA antibody had the IC50 of 137.9461 ng/ml.

Table 3. Typical competition curve and IC50.

Parameter	SDM-BSA	SDM-11S	SDM-β-amylase	SDM-poly-l-lysine
IC50	137.9461	56.5135	79.4328	144.5439
Standard curve	y=-0.2133x+0.9564 R^2=0.9925	y=-0.1424x+0.7495 R^2=0.9799	y=-0.1137x+0.7109 R^2=0.9816	y=-0.1224x+ 0.7642 R^2=0.9885
Blockade ELISA representative inhibition curve	y=-0.2133x+0.6413 R^2=0.9937	y=-0.1406x+0.5386 R^2=0.9834	y = -0.121x + 0.582 R^2= 0.986	y=-0.0757x + 0.4681 R^2=0.9814

Table 4. Cross reactivity of the polyclonal antibody with other sulfonamides.

Cross reactivity	SMZ	SD	SMP	SQX (%)	SM_2	SN
SDM-11S PAb				<0.012		
SDM-β-amylase PAb				<0.01		
SDM- poly-l-lysine PAb				<0.02		
SDM-BSA PAb				<0.015		

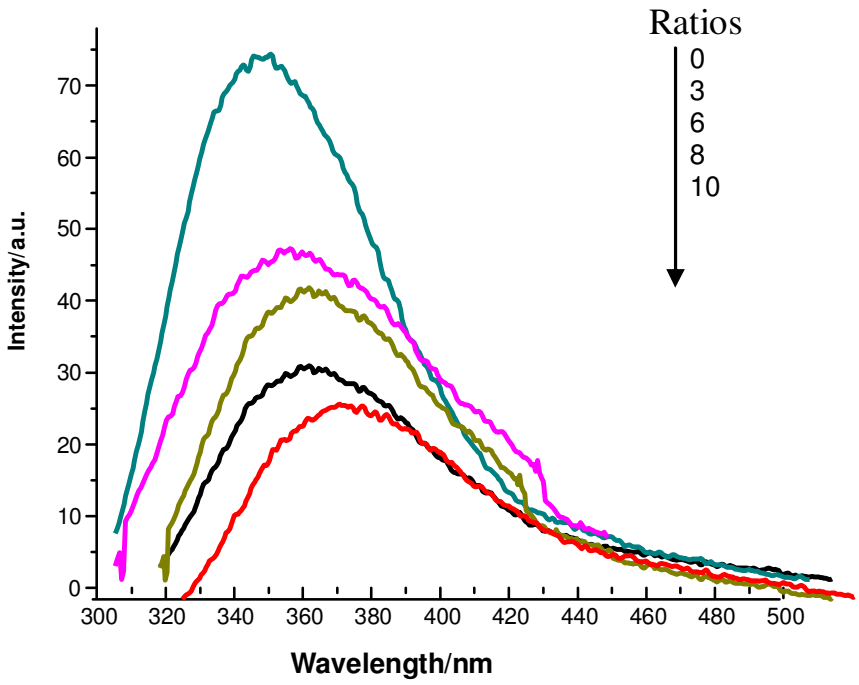


Figure 8. Fluorescent spectrum of SDM-11S at different ratios.

Table 4 shows the cross reactivity of the sulfadimethoxine antibody with other sulfonamides (SMZ, SD, SMP, SQX, SM2 and SN). Cross-re-activities of the polyclonal antibody with other sulfonamides were all below 0.05% and reactivity was highest with sulfamerazine which had the most similar structure to sulfadimethoxine.

The three polyclonal antibody (β-amylase, soy 11S globulins, poly-l-lysine) had high specificity to sulfadimethoxine. β-Amylase was the immunogen carrier whose cross-reactivity was the lowest. Blockade ELISA representative inhibition curve of SDM immunogen (SDM-BSA,

SDM-11S, SDM-β-amylase, SDM-poly-l-lysine) antiserum reacting with SDM is shown in Figure 14.

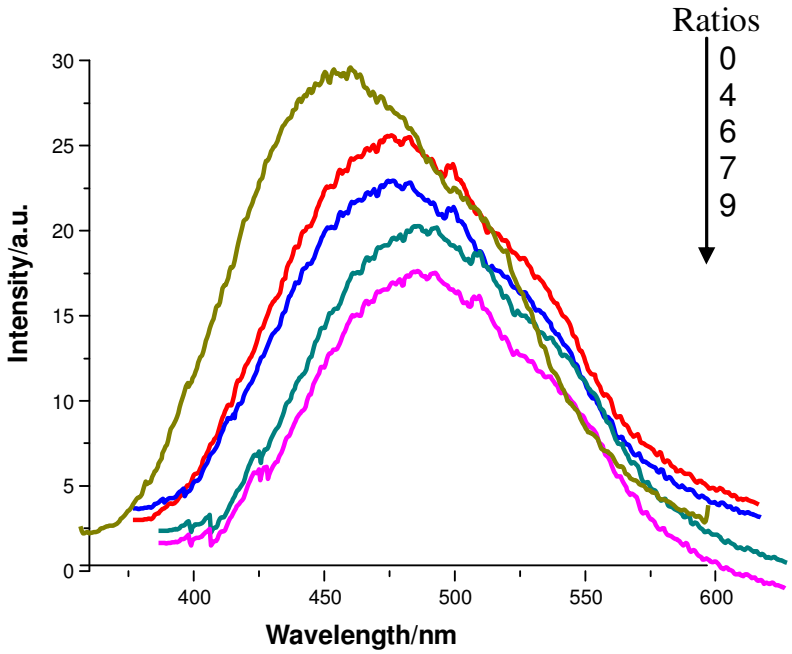


Figure 9. Fluorescent spectrum of SDM-7S at different ratios.

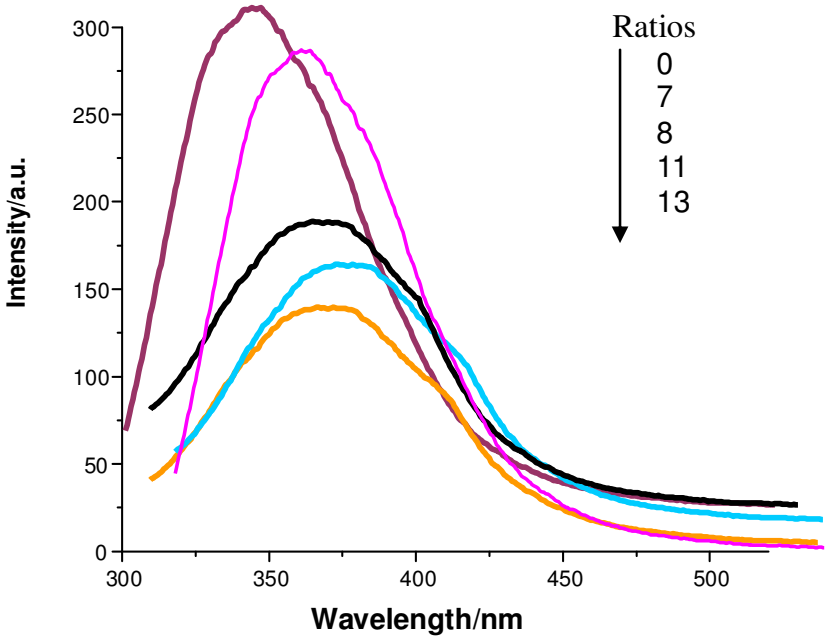


Figure 10. Fluorescent spectrum of SDM-poly-l-lysine at different ratios.

DISCUSSION

Though, procedures have been established for the production of sulfadimethoxine-BSA/OVA conjugates and antibodies to sulfadimethoxine-BSA/OVA in rabbits (Kamps et al., 1993) and these antibodies have been used in ELISAs for the detection of sulfadimethoxine in foods, feeds and biological fluids (Littlefield et al.,1990), this study is the first report of sulfadimethoxine being conjugated with other carriers (α-amylase, β-amylase,

and the production of antibodies to these immunogens in

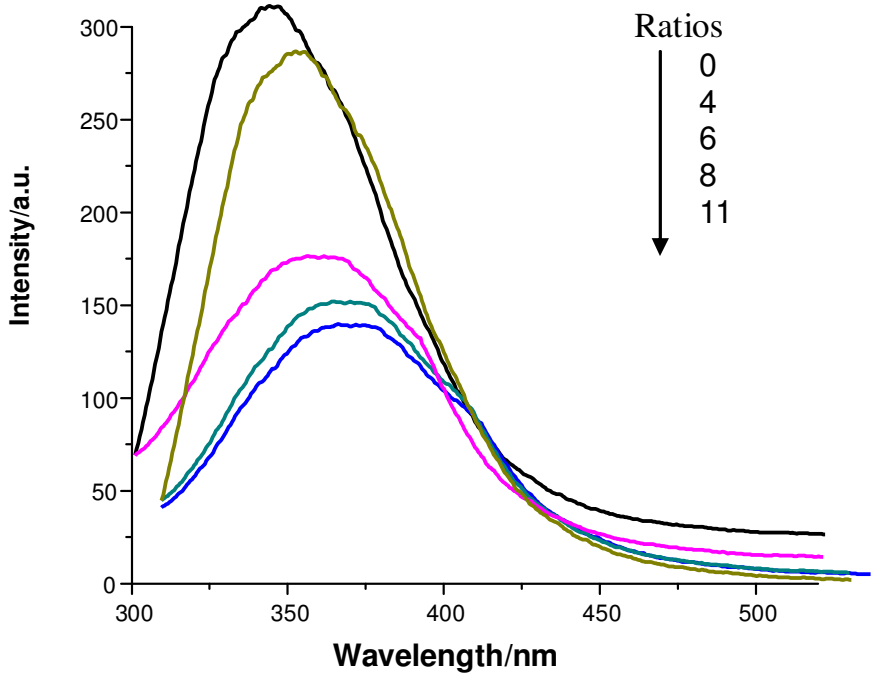


Figure 11. Fluorescent spectrum of SDM-α-amylase at different ratios.

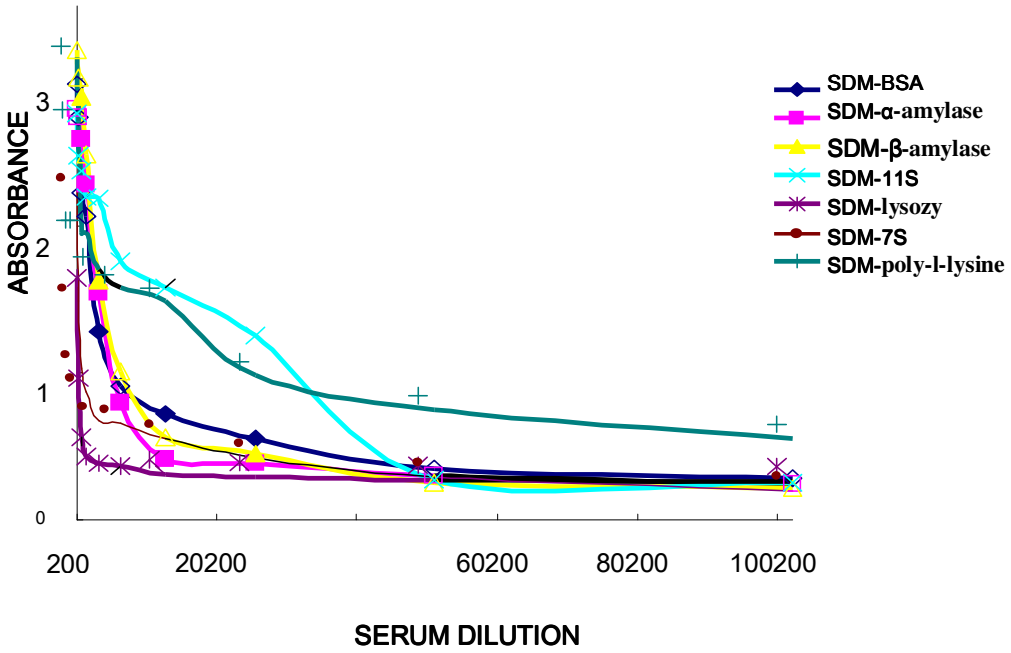


Figure 12. ELISA titration of rabbit anti-sulfadimethoxine antiserum.

rabbits. Our success in producing sulfadimethoxine antibodies in rabbits while others have failed might be attributable to several factors. Firstly, we utilized a glutaraldehyde conjugation procedure to conjugate sulfadimethoxine with other carriers. Secondly, we used an ammonium sulfate precipitation purification step in our rabbit serum which should have greatly decreased rabbit serum albumin present in the ELISA. Additionally, the use of 1% ovalbumin as a blocking protein might have improved ELISA results.

The sulfadimethoxine conjugates were prepared successfully. The key was that sulfadimethoxine were

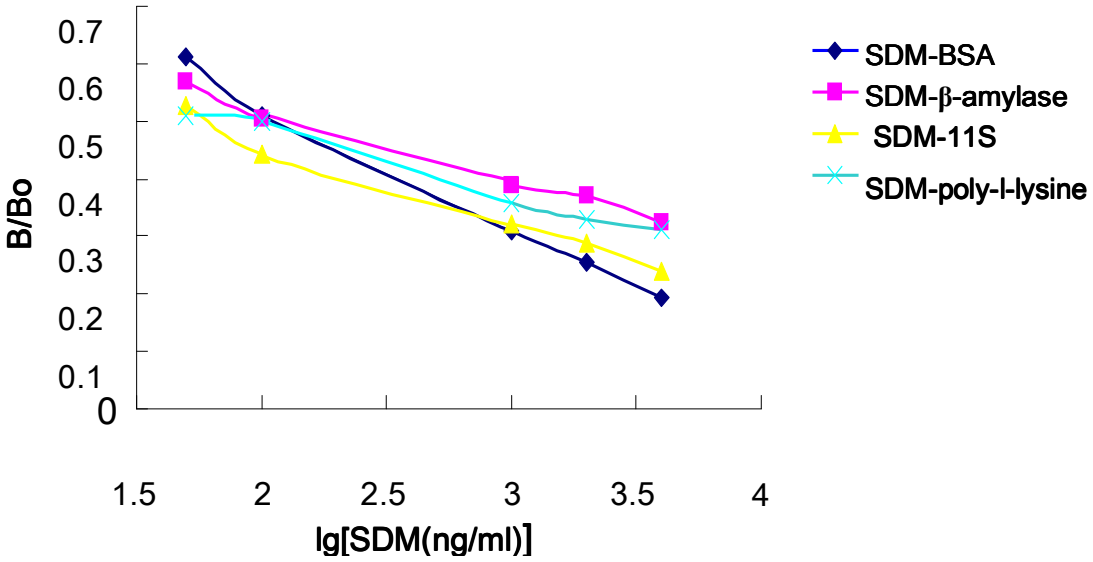


Figure 13. Standard curve of antibody against SDM immunogen reacting with SDM

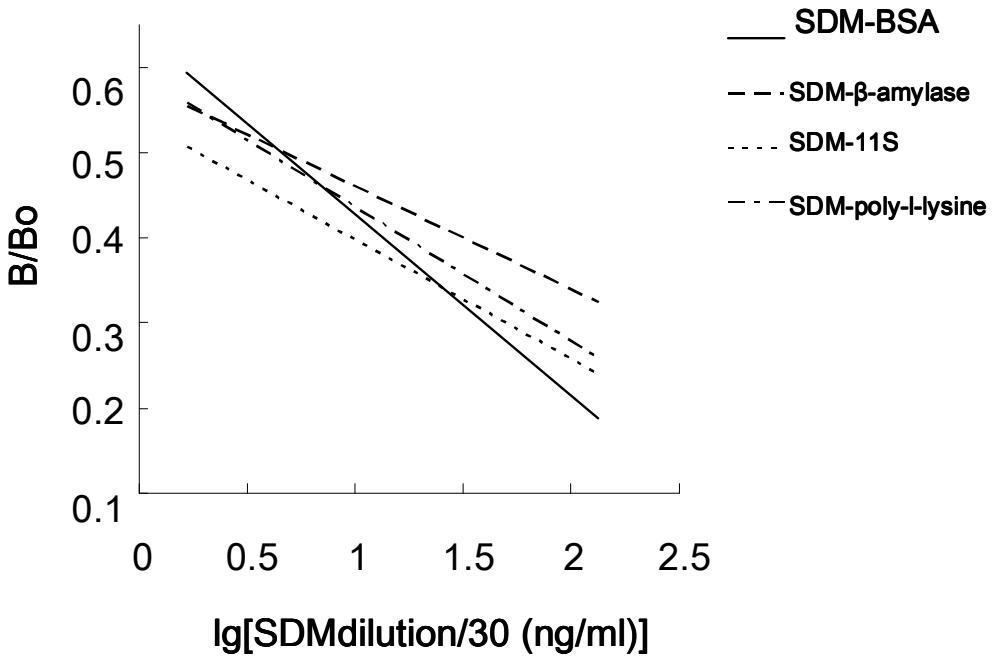


Figure 14. Blockade ELISA representative inhibition curve of SDM immunogen antiserum reacting with SDM.

conjugated with the carriers by the glutaraldehyde method rather than by other methods such as diazonium salt coupling which has been proven unsuitable after several attempts (unpublished observations). Both direct and indirect competitive ELISAs have been used in sulfadimethoxine detection (Sherma, 1997). The antisera of SDM-11S, SDM-β-amylase, SDM-poly-l-lysine had high inhibition ratio to SDM. Carriers which had better immunogenicity were β-amylase, soy 11S globulins and poly-l-lysine. SDM-amylase was not further discussed in

this study, as the data would appear in another study. Compared with BSA, sulfadimethoxine-soy 11S globulin

and β-amylase conjugate had similar, if not better immunogenicity. The anti-sulfadimethoxine antisera obtained with conjugates based on soy 11S globulin and β-amylase had higher specificity and better affinity than antibodies obtained using BSA or poly-l-lysine as a carrier as evidenced by a lower IC50 in inhibition experiments.

Soy 11S globulin could be isolated from defatted soybean seeds easily. Soybean seeds are cheap in China. β-Amylase of technical grade is cheaper in China than BSA and the required amount of β-amylase in the coupling reaction is low. Soy 11S globulin and β-amylase have stable structure and strong water-solubility. They both are capable of cross-linking with sulfadimethoxine under organic solvent circumstances and they confer immunogenicity to sulfadimethoxine in rabbits.

Our data showed that soy 11S globulin and β-amylase are suitable carriers for sulfadimethoxine in immunization experiments and represent a cheaper alternative to BSA as a carrier.

ACKNOWLEDGEMENTS

We thank the Fundamental Research Funds for the Central Universities (2011ZM0099) , for support of this work and this manuscript.

REFERENCES

Cazemier G, Haasnoot W, Du P (2000). Sulfonamide antibodies:from speific polyclonals to generic monoclonals. Food Agric. Immunol.12(1): 15-30.

Chemistry of the Diazonium and Diazo Groups: Part 1. S. Patai, Ed. 1978 Wiley-Blackwell. ISBN 0471994928. Chemistry of the Diazonium and Diazo Groups: Part 2. S. Patai, Ed. 1978 Wiley-Blackwell. ISBN 0471994936.

Dixon-Holland DE, Katz SE (1988). Competitive direct enzyme-linked immunosorbent assay for detection of sulfamethazine residues in swine urine and muscle tissue J. Assoc. Official Analytical Chemists. 71(6): 1137-1140.

Fan TS L, Zhang GS, Chu FS (1984). An indirect enzyme-linked immunosorbent assay for T-2 toxin in biological fluids. J. Food Prot. 47: 964-967.

Hamaoka T, Takatsu K, Kitagawa M (1971). Antibody production in Mice. Immunology, 21: p. 259.

Hara M, Sawada T, Ito A (2009). A major b-amylase expressed in radish taproots. Food Chem. 114: 523-528.

He LM, Xue YL, Li L, Zhang L, Wang ZF, Li XJ, Cui X, Li L S, Wang J, Zhu JH (1999). A study on analgesia induced by microencapsulated bovine chromaffin cell implantation to the spinal subarachnoid space. Medical J. Chinese People's Liberation Army, 24: 248-250.

Hirata A, Adachi M, Sekine A, Kang YN, Utsumi S, Mikami B (2004). Structural and enzymatic analysis of soybean b-amylase mutants with increased pH optimum. J. Biol. Chem. 279: 7287-7295.

Kagawa H, Yamauchi F, Hirano H (1987). Soybean basic 7S globulin represents a protein widely distributed in legume species. Fed. Eur. Biochem. Soc. 61: 480-489.

Kamps HC,Carlin RJ, Sheffield C (1993). Analysis of hapten-carrier protein conjugates by non denaturing gel electrophoresis. J. Immunol. Methods, 164: 245-253.

Ko E, Song H, Park JH (2000). Direct competitive enzyme-linked immunosorbent assay for sulfamethazine. J. Vet. Med. Sci. 62(10): 1121-1123.

Korpimaki T, Brockmann E-C , Kuronen O (2004). Engineering of a broad specificity antibody for simultaneous detection of 13 sulfonamides at the maximum residue level [J]. J. Agric. Food Chem. 52: 40-47.

Lau HP, Gaur PK, Chu FS (1981). Preparation and characterization of aflatoxin B,-hemiglutarate and its use for the production of antibody against aflatoxin B. J. Food Saf. 3: 1-13.

Levy HB, Baer G, Baron S, Buckler CE, Gibbs CJ, Iadarola MJ, London WT, Rice J (1975). A modified polyriboinosinic- polyribocytidylic acid complex that induces interferon in primates. J. Infect. Dis. 132: 434-439.

Littlefield N A, Sheldon W G, Allen R (1990). Chronic toxicity/carcinogenicity studies of sulphamethazine in Fisher 344/N rats:two-generation exposure. Food Chem. Toxical. 28: 157-167.

Long AR, Hsieh LC, Malbrough MS, Short CR, Barker SA (1990). Multiresidue method for the determination of sulfonamides in pork tissue. J. Agric. Food Chem. 38(2): 423-426.

Muldoon MT, Font IA, Beier RC (1999). Development of a crossreactive monoclonal antibody to sulfonamide antibiotics:evidence for structural conformation-selective hapten recognition. Food Agric. Immunol.11(2): 117-134

Murakami M , Jigami Y ,Tanaka H (1986). Expression of synthetic human lysozyme gene in Escherichia coli. Agric. Biol. Chem.50: p. 713.

Nicolas A, Deak Patricia A, Murphy Lawrence A (2007). Characterization of Fractionated Soy Proteins Produced by a New Simplified Procedure. J. Am. Oil Chem. Soc. 84: 137-149.

Nuria PN,Cristina GB,Angel M (2004). Specificpolyclonal-based immunoassays for sulfathiazole, Analytical Biochem.379(7): 1088-1099.

Patricia C.Eric C.Haasnoot W (2003). Generation of group-specificantibodies against sulfonamides. J. Agric. Food Chem. 51(20): 5835-5842.

Peng IC, Quass DW, Dayton WR (1984). The physicochemical and functional properties of soybean 11S globulin a review. Cereal Chem. 61: 480-489.

Sherma J (1997) Current status of pesticide residue analysis. J Assoc. Official Analytical Chemists. Int. 80(4): 283-287.

Sionf I, Bruce D, Hammock (1984). Comparision of coating and immunizing antigen structure on the sensitivity and specificity of immunoassay for benzylphenylurea insecticide[J].J. Agric. Food Chem. 32: 1294-1301.

Sospedra P, Nagy I B, Haro I, Mestres C, Hudecz F, Reig F (1999). Physicochemial behavior of polylysine-[HAV-VP3peptide] constructures at the air-water interrace. Langmuir, 15: 5111-5117.

Thouvenot DR, Morfin RF (1983). Radioimmunoassay for zearalenone and zearalanol in human serum: production,properties, and use of porcine antibodies. Appl. Environ. Microbiol. 45: 16-23.

Wolf WJ (1997). Physical and chemical properties 0f soybean proteins.Am Oil Chemist's Soc. 54: 112a-117a.